Physiological, agronomic and molecular changes for early and late senescence maize inbred lines under abiotic stresses

Nadia Chibane

Universitat de Lleida

TESI DOCTORAL

Physiological, agronomic and molecular changes for early and late senescence maize inbred lines under abiotic stresses

Nadia Chibane

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
Programa de Doctorat en Ciència i Tecnologia Agrària i Alimentàri

Director/a
Ordás López Bernardo
Revilla Temiño Pedro

Tutor/a
Romagosa Clariana Ignacio

2021

Acknowledgements

I would like to express my deepest gratitude to my supervisors, Dr. Bernardo Ordás López and Dr. Pedro Revilla Temiño, for their good guidance, encouragement and immense knowledge that they have provided throughout my PhD, whilst giving me the space to work in my own way. I am very grateful for all yours help, and invaluable advices.

I would like to give my sincere thanks to all the group of Maize Genetics and Breeding of the Misión Biológica de Galicia, especially to Amando Ordás, Rosa Ana Malvar, Ana Butrón and Rogelio Santiago for all his helps and advice during my thesis.

My sincere gratitude goes to the Ministry of Science, Innovation and Universities for providing me with the opportunity to benefit from 4-years scholarship, within the project "Maize breeding for sustainability and added value (AGL2016-77628-R)".

I thank the Misión Biológica de Galicia and the Spanish National Research Council (CSIC) for their support all these years, and for managing all my needs for the good development of this work. To the University of Lleida for accepting my registration and orientation during all these years, I also want to thank my tutor Ignacio Romagosa, for his help within the university, and to be always present to answer my questions.

Special thanks to Marie-Laure Martin-Magniette and Véronique Brunaud of Genomic Networks group (IPS2) for the five-month staying at the Agronomic Research Institute of France (INRAE), who helped me in RNA sequencing data analysis, for all support and scientific discussions during my staying, and I am thankful for all GNet group for their kindness during the most complicated situation with covid-19, they do not let me feel alone in these difficult times.

I also thank my colleagues and friends of the MBG for their generosity and encouragement: Nieves Santamaria, Ana Carballeda, Ana López, Juan Carlos Pazos Pazos, David Figueroa, Rocío Campañó, Amanda Verde Rodriguez, Noemí Gesteiro Portas, Fernando Gonzalez Rios, Diego Cacheda Guillén, Qiang Yi, Marlon Caicedo, Nuria Lopez Aruajo, Sol Campañó, Marwa Dhogri and Rami Reddy Yannam Venkata to share all these moments with me, and to be so compressive and nice during all these years.

To my best friends Celia Oukassi, Celia Ait Gherbi, and Chine Lina.

All in all, thanks to everyone who helped and supported me in this project as well as life.

Dedication

This work is dedicated to my beloved parents for all their support throughout my life. No word in this world can express my appreciation and love to them. Thank you so much for everything!!! My sisters, to be always in my side and support me.

To all my maternal family uncles and aunts;

To my engaged Said for his moral support and the good times that he lets me live,

To the memory of my best Abderahmane teacher and brother Djemel, who was always wanted to see me finishing this thesis, but life wants something else, that he rests in peace.

Abstract

Senescence is the final stage of leaf development and leads to its death. Senescence can be induced prematurely by abiotic stresses. Early senescence or induced senescence by abiotic stresses can be undesirable, affecting the growth and yield of plants. There is considerable genetic variation in the patterns of senescence in maize. The stay-green (SG) is a secondary trait that enables crop plants to maintain their green leaves and photosynthesis capacity for a longer time after silking. The objectives of this thesis were divided into two main studies. Firstly, evaluate the effect of SG phenotype in maize (*Zea mays* L.) phenological, physiological, and agronomic characters; and assess how abiotic stresses affect these traits. The second objective was to identify genes differentially expressed (DEGs) during senescence for contrasting SG phenotype in inbred lines and to show how their expression changes under abiotic stresses. The first objective was made with eight inbred lines with contrasting SG phenotypes. The experiments were carried out in 2018 and 2019 at two locations, with two repetitions per trial. The eight genotypes were evaluated under two water levels, with water stress and optimum water conditions; three nitrogen levels, N1 (0U), N2 (30U), and N3 (90U); and two plant densities of plants, high plant density (80000 plant ha^{-1}) and low plant density (50000 plant ha^{-1}). For the second objective, we used two representative genotypes from the complete set of genotypes used in the first objective, one with SG phenotype and the other with early senescence rate. RNA-seq analysis was made for different samples collected during different senescence stages, starting from silking to support the objective. For the first objective, the result shows that SG genotypes have better performance for most measured traits. Drought and nitrogen are the most critical stresses that negatively affect plant physiological activity and yield and accelerate leaf senescence. Plant density has a positive effect on maximal biomass and grain yield. However, it can reduce the individual plant yield and affect grain quality. For the second objective of genes' expression, the results reveal that several genes are activated or repressed during the senescence period. Those genes were activated or repressed earlier for early senescence genotype, and these expressions were delayed for the stay-green line. We also identified the expression of some specific genes corresponding to each abiotic stress or combined stress. Down-regulated genes were mainly involved in photosynthesis, different processes of biosynthesis and metabolism. In contrast, the upregulated genes were involved in the degradation and catabolism processes, and for responses to abiotic stresses. Furthermore, during the senescence process and under different

abiotic stresses, we showed the expression of different transcription factors related to senescence and response to abiotic stress. From the previous results of these studies, we conclude that leaf senescence was under genetic control. It can be affected by different abiotic stresses, which can negatively affect plant physiology and yield. Delaying leaf senescence can be useful to maintain plant physiological activity for a long time in order to increase biomass and grain yield.

Key words: Maize (*Zea mays* L.); Leaf senescence; Stay-green; Abiotic stresses; physiological and agronomic traits; differentially expressed genes (DEGs).

Resum

La senescència és l'etapa final del desenvolupament de la fulla i condueix a la mort. La senescència pot ser induïda prematurament per estrès abiòtic. La senescència primerenca o la senescència induïda per estrès abiòtic poden ser indesitjables i poden afectar al creixement i rendiment de les plantes. Hi ha una variació genètica considerable en els patrons de senescència del blat de moro. La senescència tardana o "stay-green" (SG) és un tret secundari que permet a les plantes de cultiu mantenir les seves fulles verdes i la seva capacitat de fotosíntesi durant més temps després de la floració. Els objectius d'aquesta tesi es van dividir en dos, el primer es l'avaluació de l'efecte del fenotip SG en els caràcters fenològics, fisiològics i agronòmics del blat de moro (*Zea mays* L.) i la avaluació de com els estressos abiòtics afecten a aquests trets. El segon objectiu es identificar gens diferencialment expressats (DEGs) durant la senescència de diverses línies pures de blat de moro contrastant pel fenotip SG, i mostrar com canvia la seva expressió sota estrès abiòtic. El primer objectiu consisteix en una avaluació de vuit línies pures de blat de moro amb fenotip SG contrastant. L'experiment es va realitzar durant dos anys consecutius: 2018 i 2019. L'avaluació es va realitzar en dos ambients, amb dues repeticions en cada ambient per a cada any d'experiment. Els vuit genotips van ser avaluats sota dos nivells d'aigua (amb estrès hídric i condicions hídriques òptimes) i tres nivells de nitrogen: N1 (0U), N2 (30U) i N3 (90U). Després, l'últim factor estudiat va ser la densitat de plantes, amb dos nivells de densitat, alta densitat de plantes (80000 plant ha^{-1}) i baixa densitat de plantes (50000 plant ha^{-1}). A més, per al segon objectiu utilitzem dos genotips representatius del total de genotips utilitzats en el primer objectiu, un amb fenotip SG i un altre amb senescència primerenca. Per respondre a aquest objectiu, es va realitzar una anàlisi de RNAseq per a diferents mostres recollides durant diferents temps de senescència, a partir de la floració. Pel que fa al primer objectiu, els resultats mostren que els genotips SG tenen una millor resposta per a la majoria dels trets mesurats. La sequera i el nitrogen són els factors estressants més importants que afecten negativament a l'activitat fisiològica de la planta i al seu rendiment, i tenen un efecte més gran per a promoure la senescència de les fulles. La densitat de la planta té un efecte positiu per a la biomassa màxima i per el rendiment de gra, però, pot reduir el rendiment de la planta individual i afectar la qualitat del gra. Per al segon objectiu de l'expressió de gens, el resultat revela que diversos gens s'activen o reprimeixen durant el període de senescència. Aquests gens, s'activen o reprimeixen abans per al genotip de senescència primerenca i van retardar aquesta expressió per als genotips amb

senescència tardana. També es va identificar l'expressió d'alguns gens específics corresponents a cada estrès abiòtic o estressos combinats. Els gens que van reprimir la seva expressió estaven involucrats principalment en la fotosíntesi, en diferents processos de biosíntesi i en el metabolisme. Mentre que els gens que van augmentar la seva expressió participaven en processos de degradació i catabolisme, i en diferents processos d'estímul davant estrès abiòtic. A més, durant el procés de senescència i sota diferents estressos abiòtics, vam mostrar l'expressió de diferents TF relacionats amb la senescència i la resposta a l'estrès abiòtic. Del resultat d'aquests estudis podem concloure que la senescència foliar està sota control genètic. Es pot veure afectat per diferents estressos abiòtics, que poden afectar negativament a la fisiologia i el rendiment de la planta. No obstant això, retardar la senescència de les fulles pot ser una característica més útil per mantenir l'activitat fisiològica de la planta durant més temps que per augmentar la biomassa i el rendiment de gra.

Paraules clau: Blat de moro (*Zea mays L* .); Senescència foliar; *Stay-green*; Estrès abiòtiques; trets fisiològics i agronòmics; gens expressats diferencialment (DEG).

Resumen

La senescencia es la etapa final del desarrollo de la hoja y conduce a su muerte. La senescencia puede ser inducida prematuramente por estrés abiótico. La senescencia temprana o la senescencia inducida por estrés abiótico pueden ser indeseables y pueden afectar el crecimiento y rendimiento de las plantas. Existe una variación genética considerable en los patrones de senescencia del maíz. La senescencia tardía o "stay-green" (SG) es un rasgo secundario que permite a las plantas mantener sus hojas verdes y su capacidad de fotosíntesis durante más tiempo después de la floración. Los objetivos de esta tesis se dividieron en dos estudios principales, en primer lugar, la evaluación del efecto del fenotipo SG en los caracteres fenológicos, fisiológicos y agronómicos del maíz (*Zea mays* L.); y evaluar cómo los estreses abióticos afectan estos caracteres. El segundo objetivo fue identificar genes diferencialmente expresados (DEGs) durante la senescencia para líneas puras de maíz contrastantes para el fenotipo SG, y mostrar cómo cambia su expresión bajo estrés abiótico. El primer objetivo consiste a una evaluación de ocho líneas puras de maíz con diversa expresión de fenotipo SG. El experimento se realizó en 2018 y 2019 en dos locaslidades, con dos repeticiones por ensayo. Los ocho genotipos fueron evaluados bajo dos niveles de agua, con estrés hídrico y condiciones hídricas óptimas; tres niveles de nitrógeno, N1 (0U), N2 (30U) y N3 (90U). El último factor estudiado fue la densidad de plantas, con alta (80000 plant ha^{-1}) y baja densidad de plantas (50000 plant ha^{-1}). Además, para el segundo objeto utilizamos dos genotipos representativos de los genotipos utilizados en el primer objetivo, uno con fenotipo SG y otro con senescencia temprana. Para abordar este objetivo, se realizó un análisis de RNAseq para diferentes muestras recolectadas durante diferentes tiempos de senescencia, a partir de la floración. Para el primer objetivo, el resultado muestra que los genotipos SG tienen un mejor comportamiento para la mayoría de los caracteres medidos. La sequía y el nitrógeno son los factores estresantes más importantes que afectan negativamente la actividad fisiológica de la planta y el rendimiento y promueven la senescencia de las hojas. La densidad de la planta tiene un efecto positivo para la biomasa máxima y el rendimiento de grano, sin embargo, puede reducir el rendimiento de la planta individual y afectar la calidad del grano. En el segundo objetivo de la expresión de genes, el resultado revela que varios genes se activan o reprimen durante el período de senescencia. Estos genes fueron activados o reprimidos antes para el genotipo de senescencia temprana, y retrasaron esta expresión para el genotipo con senescencia tardía. También identificamos la expresión de algunos genes específicos correspondientes a cada estrés abiótico o

estreses combinados. Los genes que retrasan su expresión estaban involucrados principalmente en la fotosíntesis, diferentes procesos de biosíntesis y metabolismo. Mientras que los genes que incrementaron su expresión participan en el proceso de degradación y catabolismo, y en diferentes procesos de estímulo bajo estrés abiótico. Además, durante el proceso de senescencia y bajo diferentes estreses abióticos, se detectó la expresión de diferentes factores de transcripción relacionados con la senescencia y la respuesta al estrés abiótico. Del resultado anterior de estos estudios, podemos concluir que la senescencia foliar estaba bajo control genético y puede verse afectada por diferentes estreses abióticos, que pueden afectar negativamente la fisiología y el rendimiento de la planta. Retrasar la senescencia de las hojas puede ser una característica útil para mantener la actividad fisiológica de la planta durante más tiempo para aumentar la biomasa y el rendimiento de grano.

Palabras clave: maíz (*Zea mays* L.); Senescencia foliar; ”Stay green”; Estreses abióticos; caracteres fisiológicos y agronómicos; genes expresados diferencialmente (DEG).

Table of content

List of abbreviations

ASI: Anthesis Silking Interval

BL: Black layer or physiological maturity

C: Carbon

Chla: Chlorophyll a

Chlb: Chlorophyll b

CM: Cobs moisture

CY: Cobs yield

DAS: days after silking

DEGs: Differentially expressed genes

DM: dry matter

DNA: Deoxyribonucleic acid

FF: Female flowering

GO: Gene ontology

GY: Grains yield

KM: Kernel moisture

N: Nitrogen

NSG: Non stay-green

PSII: Quantum efficiency of photosystem II

RNAseq: Ribonucleic acid sequencing

ROS: Reactive oxygen species

SAGs: Senescence Associated Genes

SG: Stay-green

TFs: Transcriptions factors

INDEX OF TABLES

INDEX OF FIGURES

Annexes

Chapter 1: General introduction

I. Chapter 1: General introduction

1.1. General overview: Senescence, maize uses, and stay-green phenotype

Senescence is a natural phenomenon characterized by a reduction in leaf functionality and identified by changes in leaf color (Luoni et al., 2019). Leaf senescence is a complex physiological process involving chlorophyll catabolism, leading to a decline in photosynthesis, protein and nucleic acid degradation, molecular metabolism, and cell death (Koyama, 2014). Senescence typically occurs in mature cells of tissues, after their growth phase has ceased, to enable efficient recycling of nutrients to new growing sinks or seeds (Thomas, 2013). Plants have developed various strategies to respond efficiently to the changing environment. Under optimal conditions, the onset of leaf senescence depends mainly on the ontogeny of the plant. This process, however, can be induced prematurely by endogenous and exogenous stimuli, like biotic or abiotic stresses, to accelerate the remobilization of nutrients (Buchanan-Wollaston et al., 2003). Of these limiting factors, the most important is the increasing drought and infertile soils due mainly to nitrogen deficiency (Meseka et al., 2008).

Maize (*Zea mays* L.) is very demanding at the post-anthesis stages when nutrients are remobilized mainly to maximize the number of reproductive structures and to improve seed development (Borrell et al., 2001). Maize belongs to the grass family *Poaceae*, and originated from teosinte (*Zea mays ssp. parviglumis*) in Mexico and Guatemala. Maize is a cereal crop with wide environmental adaption (Ishola, 2016). The agronomic importance of maize as a food crop throughout the world is an undeniable fact, motivating investigation to obtain more efficient maize production (Chetty 2004). It is used for human consumption, animal feed, starch industry, pharmaceutical industry and oil production (Amin et al., 2007). Furthermore, maize serves as a source of raw material for industrial use (Crow and Kermicle, 2002). Maize is used mainly as an energetic plant species, but specialized versions for protein, fat, and starch are widespread (Turi et al., 2007). Maize hybrids have many vital uses in food, medicine, beverages, ethanol and industrial applications, amounting to an average annual utilization of about 23% of the world's annual grain market (Watson, 2003). The productivity of maize increased from 255 million tons in 1968 to 1,134 million tons in 2017 representing an average annual growth of 3.46%

(https://knoema.com/atlas/World/ topics/ Agriculture/ Crops-Production-Quantity-tones/Maize-production) (Kimotho et al., 2019).

However, successful maize production is dependent on the influence of both biotic and abiotic factors, which constitute an extensive range of production constraints playing a pivotal role in determining the success of maize production (Ishola, 2016). Nitrogen is particularly essential for corn grain development. Root uptake of the nitrogen and its leaves relocation directly impact grain quality (Woli et al., 2019). Natural leaf senescence is a genetically controlled process that influences nutrient recycling during reproductive growth stages. Under drought stress conditions, the senescence program may be accelerated (Yang et al., 2019). Recent studies have increased our understanding of the senescence process, particularly at the molecular level (Buchanan-Wollaston et al., 2003; Guo et al., 2004). Some senescence-associated genes (SAGs) have been identified in various plants species at the transcriptional level (Breeze et al., 2011 ; Wu et al., 2016b). For instance, almost one-fourth of the Arabidopsis genes associated with senescence, as assessed by transcriptome analyses (Zentgraf et al., 2018). Delayed leaf senescence or stay-green phenotype has been studied in maize for several decades (Tollenaar et al., 2004). The genotypes with the SG characteristic maintain greenness during the final stage of leaf development due to coordinated genetic mechanisms that regulate the transition from nutrient assimilation to nutrient remobilization (Aasen et al., 2018). Some stay-green hybrids delay leaf senescence, which results in crop yield earnings, especially under drought conditions (Bekavac et al., 2007). Regarding nitrogen availability, Ma and Dwyer (1998) demonstrated that stay-green varieties had a higher nitrogen use efficiency than the conventional hybrids. Transcriptional studies performing RNAseq profiles under abiotic factors, like drought, have been evaluated and associated with leaf senescence and the stay-green trait in maize (Li et al., 2017).

1.2. Senescence and crop breeding

Leaves are the primary photosynthetic organs in plants, and as reproductive growth proceeds, the photosynthetic system declines, and leaves enter the last stage called senescence (Quirino et al., 2000). Leaf senescence occurs alongside color changes in leaves, and it is an easily visible phenomenon in the life cycle of a plant (Koyama, 2014). A change of leaf color from green to yellow due to chlorophyll degradation is the first visible indication of senescence (Mattila et al.,

2018). It is also characterized by disintegration of the photosynthetic organs, which is the main characteristic of leaf senescence (Erley et al., 2010).

Senescence plays a crucial role in the adaptability of plants. Effective senescence can enhance the adaptation of plants to the environment (Schippers, 2015). In crops, leaf senescence is an important agronomic trait that affects crop yield and crop quality (Distelfeld et al., 2014). The increased catabolic activity is responsible for converting the cellular materials of leaves' growth phase into exportable nutrients supplied to developing seeds or other growing organs (Asad et al., 2019). It has been estimated that more than 70% of the leaf nitrogen is exported from the senescing leaves during the grain filling stage of annual crops (Hollmann et al., 2014). In maize, grain filling depends on the amount of green leaf area, which takes an active part in the photosynthesis and subsidizes the total photosynthetic level after silking; as a result, the proportion of green leaf area is correlated with grain yield (Yamori et al., 2010). Leaf senescence affects the photosynthetic activity and hence affects the grain filling process, biomass accumulation, and yield in maize (Liang et al., 2018). Various factors participate in triggering and modulating the senescence process, including nutrient availability (Diaz et al., 2006), and abiotic and biotic stresses (drought, low nitrogen, high temperature, pathogen attack, and others).

Senescence is an active process regulated by interaction between developmental and environmental signals, and it requires the involvement of numerous senescence-associated genes (SAGs) (Lim et al., 2003). Nevertheless, the imposition of abiotic or biotic stresses can accelerate leaf senescence, possibly as an adaptive response to allow the plant's survival as a whole (Kanojia and Dijkwel, 2018).

1.2.1. Physiological changes during senescence

1.2.1.1. Photosynthetic activity

Photosynthetic pigments (such as chlorophyll a and b, carotenoids, and lutein) will be degraded (Jyothsna and Murthy, 2016). In higher plants, studies have shown that the loss of chlorophyll is greater than the loss of carotenoids, which causes senescent leaves to appear yellow (Jyothsna and Murthy, 2016). Chlorophyll is the primary photosynthetic pigment that enables carbohydrate assimilation through photosynthesis by effectively utilizing solar energy (Lodish et al., 2007). After the silking stage, the gradual loss in chlorophyll content and active photosynthetic green leaf area leads to leaf senescence (Erley et al., 2010; Ahmad et al., 2019). The grain-filling process in maize depends on the active photosynthetic leaf area. The contribution of the net

photosynthetic rate after silking is more than 90%, resulting in a higher grain yield (Yamori et al., 2010). Therefore, reducing the degradation of photosynthetic pigments and extending the photosynthetic duration during the grain-filling stage is crucial for obtaining a higher grain yield in maize (Ahmad et al., 2019). However, reduced photosynthetic activity causes accelerated senescence (Quirino et al., 2000). Among several strategies contributing to increases in crop biomass and yield, extending the duration of photosynthesis is one of the most effective ways (Richards, 2000). Because the extended foliar greenness or delayed senescence maintains the leaves photosynthetically active for a long time after silking (Thomas and Ougham, 2014).

1.2.1.2. Chlorophyll content and quantum efficiency of photosystem II (PSII)

Chlorophyll degradation is one of the potential indicators of leaf senescence, and any effect on chlorophyll degradation may be directly related to leaf senescence (Mattila et al., 2018). The decomposition of chlorophyll leads to the yellowing of leaves, which is the most obvious symptom of chlorophyll decomposition during senescence (Sakuraba et al., 2015). A pathway for chlorophyll degradation consists of several reaction steps catalyzed by enzymes (Takamiya et al., 2000). In the process of chlorophyll degradation, the decomposition products of two chlorophylls, “chl a” as well as “chl b” are derived as the final decomposition product (Christ and Hörtensteiner, 2014). These degradation products are transferred to the cell vacuole (Sarwat et al., 2013). The visible manifestation of senescence results from chlorophyll breakdown during chloroplast disassembly (Quirino et al., 2000). Chloroplasts are the major cellular organelles in a photosynthetic cell, and up to 80% of total leaf nitrogen is reserved in the chloroplasts. At the same time, Rubisco (D-ribulose-1, 5-bisphosphate carboxylase/ oxygenase) represents up to 50% soluble proteins. Hence, efficiently achieving chloroplast breakdown and Rubisco and chlorophyll degradation is crucial for nutrient recycling (Wu et al., 2012). Visible yellowing is the most visible senescence phenotype caused by the ordered dismantling of chloroplasts and the breakdown of Chl during the early stages of senescence (Hörtensteiner and Feller, 2002). Other metabolic changes include increased oxidation and hydrolysis of macromolecules, such as proteins, lipids, and nucleic acids. These hydrolyzed molecules are remobilized into developing seeds (Munné-Bosch, 2008). Thus, leaf senescence is a genetically controlled developmental process that was evolutionarily acquired for higher fitness and survival (Kim et al., 2016). Therefore, the protection of the photosynthetic apparatus of chloroplasts, such as the maintenance

of photosystem II (PSII) and control of the content of reactive oxygen species, was also indicated as a major contribution to slowing the degeneration of tissues in wheat genotypes (Luo et al., 2006).

1.2.1.3. Nitrogen assimilation and remobilization

The status of nitrogen is closely related to leaf senescence. The senescence program is often associated with the degradation of chloroplasts and reutilization of nitrogen present in the chloroplast proteins. Rubisco, the central enzyme in the dark reaction of photosynthesis, is the largest source of leaf nitrogen (Distelfeld et al., 2014). Within senescing leaves, rubisco breaks down into amino acids, which are then reused as nitrogen supplements for grains (Masclaux-Daubresse et al., 2008). Hence, small-grained cereals like barley, wheat, and rice may mobilize up to 90% of the nitrogen from the vegetative plant parts to the grain, while in maize, 35–55% of the grain nitrogen is derived from soil uptake after anthesis (Gregersen et al., 2008). In general, there is a close relationship between the level of leaf nitrogen and senescence (Moschen et al., 2016). Plants assimilate carbohydrates and nitrogen in vegetative organs and remobilize them to newly developing tissues during development or to reproductive organs (Zhang et al., 2019). Currently, a broadly accepted viewpoint is that higher leaf nitrogen levels are associated with delayed leaf senescence, which confers drought tolerance (Sade et al., 2018**).** Also, Gregersen et al.,(2013) show that increasing source strength in cereal crops leads to higher grain yield**.**

On the other hand, Jagadish et al., (2015) estimate that optimal N concentrations stimulate foliage greenness and growth, which in turn remobilize N that otherwise would require degradation of chloroplast protein to release molecules of N. Deficient conditions precipitate senescence remobilization of C and N from “green” tissues to fasten grain-filling. These physiological changes alter C and N metabolism by impairing translocation mechanisms leading to a source-sink unbalanced distribution (Munaiz et al., 2020). Relocation of nutrients through the leaf senescence process increases the productivity of significant cereal grains, such as rice, maize, and wheat. Enhancing the efficiency of nutrient remobilization during leaf senescence directly affects grain yield in cereal crops (Distelfeld et al., 2014). N supplies prolong leaf greenness during the reproductive growth stage, while shortages of N induce early leaf senescence (Gully et al., 2015).

1.2.2. Molecular changes during senescence

Leaf senescence is a primary physiological process that affects plants' vegetative and productive developmental processes, and delayed senescence can extend the leaf life and increase seed yield (Khan et al., 2014). In order to clarify the molecular mechanisms of leaf senescence, genome-wide transcriptome analysis has been widely used in the past decades to determine the critical regulators of leaf senescence in different plant species (Breeze et al., 2011; Zhang et al., 2014; Xu, 2020). The transition from leaf maturity to senescence is complex, and it is related to changes in gene expression levels across the genome. Several SAGs have been found in many plant species (Li et al., 2014). Approximately 5,356 SAGs were identified in 44 species, being ~69.89% found in *Arabidopsis thaliana*.

The first transcriptome analysis of leaf senescence in cereal species was performed in flag leaves of wheat (*Triticum aestivum* L.), grown in the greenhouse, using DNA microarray technology (Gregersen and Holm, 2007). The changes in global gene expression of wheat flag leaves were studied during the period from ear emergence until 50% yellowing of harvested leaf samples. Considerable overlap has been observed between DEGs in wheat flag leaves and leaves of other species during senescence; this provides strong evidence that leaf senescence processes of monocot and dicot are highly conserved (Kim et al., 2016).

In Arabidopsis, it has been found a large number of differentially expressed genes (DEGs) are expressed during developmental leaf senescence. The down-regulated genes are overrepresented for genes involved in anabolic processes (including photosynthetic activity, carbon fixation, and amino acid metabolism). In contrast, up-regulated genes are involved in the degradation of proteins, lipids, and nucleotides (Breeze et al., 2011; Xu, 2020). Early senescence was induced in the inbred line B73 by preventing pollination (Ceppi et al., 1987). In addition, with the development of genome sequencing and global gene expression profiling tools, several studies have evaluated global transcriptomic reprogramming during natural and induced senescence (Breeze et al., 2011; Wu et al., 2017).

It is believed that the transcriptional control mechanisms that lead to differential genes expression play an essential role in coordinating the senescence process (Balazadeh et al., 2008). Different experimental methods, including microarray-based expression profiling and suppression subtractive hybridization, had revealed that hundreds of genes change their expression during developmentally-regulated leaf senescence in Arabidopsis or when senescence was artificially

induced through prolonged dark incubation or leaf detachment (Buchanan-Wollaston et al., 2003; Guo et al., 2004b).

1.2.2.1. Gene expression

Senescence is a physiological process in which nutrient reserves are mobilized to fruits and seeds. This translocation leads to a decrease in RNA synthesis, resulting from changes in gene expression, thereby reducing protein synthesis, resulting in decreased photosynthetic capacity and cell division, leading to plant death (Luche et al., 2015). The primary purpose of senescence is remobilization and recycling so that developing tissues can be used, thereby damaging the senescent tissues (Buchanan-Wollaston et al., 2003). Genes encoding proteins with functions related to the photosynthetic mechanism constitute some of the oldest senescence processes conserved in multiple clades of plants. Proteins related to the regulation of senescence processes and their integration with developmental and stress signal networks constitute some of the latest discovered proteins (Thomas et al., 2009). In Arabidopsis, nearly 20% of genes change their expression during natural senescence (Zentgraf et al., 2004). These genes are involved in different molecular, biochemical, morphological, and physiological events that contribute to the senescence phenotype (Luoni et al., 2019)**.**

In Zhang et al. (2014) study, RNA-seq technology is used to examine the global gene-expression profile of maize leaves at early and late senescence stages during developmental leaf senescence. The GO analysis of 4522 DEGs divides these DEGs into biological processes such as protein metabolism, transporters, and signal transduction. Further analyses of 263 transporter genes showed that the genes encoding ABA (Abscisic acid) and sugar transporters were significantly up-regulated in the later stages of leaf senescence. This suggests that these transporters may be involved in nutrient remobilization, mainly at this stage. Comparison of transcriptome data of maize and Arabidopsis by Breeze et al. (2011) found that about 30% of DEGs in maize are also present in Arabidopsis during the developmental leaf senescence process photosynthesis, lipid metabolism, and protein degradation of these conservative DEGs are enriched. This means that these two species' molecular mechanisms of leaf senescence shared some similarities (Kim et al., 2016)**.**

Recently, several omics analyses have been performed to identify senescence-associated genes (Zhang et al., 2014), miRNAs (X. Wu et al., 2016), and proteins (Wei et al., 2015) in maize.

Although many genes have been screened, only ZmSnRK1s and knotted1 have been experimentally verified (Wang et al., 2019). Therefore, manipulating the senescence process of maize can help to achieve high grain yield and quality.

1.2.2.2. Transcription factors (TFs)

TFs are composed of sequence-specific DNA binding domains. They activate or repress the activity of RNA polymerase, thereby regulating gene expression. TFs can be divided into 40–60 families (Yilmaz et al., 2009) based on their DNA-binding domain (Riechmann et al., 2000). The presence or absence of transcription factors, activators, and inhibitors that regulate target gene transcription usually involves the entire signal transmission cascade determined by tissue type, developmental stage, or environmental condition (Wyrick and Young, 2002). The regulation of leaf senescence requires TFs combined with specific motifs in the regulatory region of its target genes. The transcription profiling analysis of TFs differentially expressed at different developmental stages or under various environmental stresses provides a global picture of the gene regulatory network of Arabidopsis leaf senescence (Breeze et al., 2011). Buchanan-Wollaston et al., (2003) reported that 96 putative TFs genes (within 827 up-regulated genes) increased their transcript abundance during developmental leaf senescence. These include WRKY, NAC, MYB, bZIP, and AP2/EREBP (AP2/ERF) TFs families.

According to previous reports, bZIP TFs are involved in developmental and physiological processes and biotic/abiotic stress responses under normal and stressful growth conditions. Therefore, they are essential for plants to withstand adverse environmental conditions (Wang et al., 2011). The bZIP TFs play crucial roles in organ and tissue differentiation (Shen et al., 2007), cell elongation (Fukazawa et al., 2000), nitrogen/carbon, and energy metabolism (Baena-González et al., 2007), and other metabolic processes. On the other hand, bZIP TFs also respond to various abiotic stresses such as drought, high salinity, and cold stresses(Baloglu et al., 2014**).** Another plant-specific TFs family that regulates leaf senescence is the WRKY superfamily (Guo et al., 2004). In addition to playing an essential role in regulating leaf senescence and hormone pathways, TFs in this family also participate in plant defense response and respond to various biotic and abiotic stresses (Chen et al., 2012). The AP2/ERF domain was identified in proteins that bind to ethylene-responsive gene promoters (Ohme-takagi and Shinshi, 1995). However, subsequent studies have shown that TFs of the ERF family play an active role in all aspects of

plant growth, development and physiology, floral organ abscission, lipid metabolism, alkaloid biosynthesis, and responses to environmental stress (Iwase et al., 2011). By identifying and characterizing many SAGs and senescence-related in many plant species, many advances have been made in understanding leaf senescence at the molecular level, including plants such as *Arabidopsis thaliana*, *Oryza sativa*, and *Medicago truncatula* (Desclos et al., 2009). In these SAGs, many TFs such as NAC, WRKY, MYB (Balazadeh et al., 2008), signal transduction-related proteins, and metabolic regulators are all involved in regulating leaf senescence, which indicates that senescence is a comprehensive response to many signals which are controlled by highly complex transcriptional regulatory networks**.** NAC proteins are plant-specific TFs which function concerning plant development and also for abiotic and biotic stress responses (Nakashima et al., 2012). TFs of homeodomain-leucine zipper (HDZip) families I and II contribute to the plasticity of plant growth and are responsible for modulating plant development in response to environmental stimuli (Agalou et al., 2008). MYB TFs are involved in ABA signaling pathways in response to drought stress (Baldoni et al., 2015). They also play a crucial role in enhancing the tolerance of plants against stresses via biotic and abiotic stresses (Javed et al., 2020).

1.2.2.3. Phytohormones modulated leaf senescence

The role of hormones involves the process of signal transduction (Wang and Irving, 2011). Plant hormones have essential roles in both age-dependent and stress-induced senescence, and their signaling pathways show both similarities and differences (Xu, 2020). The initiation and progression of senescence are under hormonal control (Thomas and Ougham, 2014). Under stress conditions, plants rapidly regulate their physiology through the biosynthesis of plant hormones, promoting stress resistance or premature senescence (Luoni et al., 2019). Plant hormones such as ethylene, abscisic acid (ABA), jasmonic acid (JA), auxin (AX), and salicylic acid (SA) can promote senescence. At the same time, cytokinin (CE) and gibberellin (GA) can delay senescence (Luoni et al., 2019).

Cytokinins are a class of plant hormones essential to promote cell division, growth and differentiation, and leaf senescence (Haberer and Kieber, 2002). Cytokinins delay leaf senescence in several plant species (Peleg and Blumwald, 2011). In transgenic tobacco plants that are

induced to produce high cytokinins, the link between higher hormone content and higher chlorophyll and nutrient content and the maintenance of photosynthesis in older tissues reduce the degenerative effects of aging (Jordi et al., 2000)**.**

Auxin is an essential hormone for plant growth and development. It is synthesized by actively growing tissues such as meristems, leaf primordia, young leaves, developing seeds, fruits, and pollen. Auxins regulate many biological processes: cell division, cell expansion, root germination, ethylene production, fruit development, and other morphological and molecular processes (Wang and Irving, 2011). Auxin is called a plant developmental hormone and plays an essential role in senescence (Kim et al., 2011). Several studies have shown a significant correlation between auxin levels and senescence. In plants, auxins may delay or accelerate senescence (Ellis et al., 2005). Therefore, auxin is considered a negative regulator of leaf senescence, where its expression delays leaf senescence (Mueller-Roeber and Balazadeh, 2014).

Gibberellin is a pentacyclic diterpene that participates in plant development processes, such as cell elongation, seed germination, dormancy, reproductive growth, senescence, and tolerance to various environmental stresses (Rodrigues et al., 2012). Gibberellin is an hormone that delays senescence (Schippers et al., 2007). It has been proposed that GA can antagonize the effect of ABA by inhibiting the senescence of the leaves of *Aesculus paris* (Jyothsna and Murthy, 2016).

The accumulation of abscisic acid (ABA) plays a vital role in abiotic stress signal and transduction pathways, mediating many responses (Wasilewska et al., 2008)**.** A significant effect of ABA is to cause stomata to close and prevent water loss due to transpiration (Grill and Himmelbach, 1998). In addition to the stress response characteristics, ABA is also related to normal physiological operations, such as compound storage, dehydration in late embryogenesis, seed maturation, dormancy formation, and shedding. ABA is a carotenoid derivative produced in chloroplasts and other plastids, and its their production increases under drought or other abiotic stresses (Wasilewska et al., 2008).

Ethylene is a gas hormone that can activate fruit ripening, stimulate germination, accelerate senescence, and cause cell death. Ethylene levels may also increase or decrease in response to abiotic and biotic stresses (Kulaeva and Prokoptseva, 2004). Senescence is related to the balance between hormones (such as cytokinin and ethylene). The overexpression or inhibition of these hormones shows changes in senescence time, thereby accelerating and delaying the senescence process (Buchanan-Wollaston et al., 2003). For jasmonic acid is derived from the modulator of

linolenic acid (Chen et al., 2005). The content of jasmonic acid is the highest inactive areas, such as stem tips, young leaves, immature fruits, and root tips (Arteca, 1996). Jasmonic acid has a variety of functions in plants. Such as, inhibiting the formation of roots and tubers; on the other hand, jasmonic acid may be related to leaf senescence.

1.3. Delayed leaf senescence

Stay-green is the term assigned to genotypes, where the senescence is delayed compared with the standard reference genotype (Thomas and Howarth, 2000)**.** Choosing to maintain the SG genotype can help increase crop yields to meet the expected increase in population, especially under stress conditions (Kamal et al., 2019). The characteristic of stay-green is that the green state of the plant is longer in the later stage of grain filling (Silva et al., 2008)**.** On the other hand, as demonstrated in a previous study, delaying leaf senescence and extending the duration of effective photosynthesis may significantly increase the photoassimilate source, thereby increasing grain yield (Richards, 2000). As described by Davies et al., (2011), many green plants that show delayed leaf senescence have multiple beneficial effects, including promoting more root growth and providing more carbon. Therefore, the onset of leaf senescence is very important for crop yield (Wu et al., 2012). SG maize hybrids have late-senescing leaves and can produce higher grain yield (GY), especially in the case of climate warming (Xiao and Tao, 2016). This is beneficial for post-silking dry matter accumulation (PostDM) and post-silking nitrogen uptake (PostN), which improves GY (Valentinuz and Tollenaar, 2004) (Figure 1). SG maize hybrids can accumulate more than 10% DM and N during grain filling (Rajcan and Tollenaar, 1999). A contradictory results reported by Kosgey et al. (2013) from field experiments indicated that SG hybrids have no higher GY and accumulated less N in grain. It was found that the genotypic differences in delayed and reduced leaf senescence rate were due to differences in specific leaf nitrogen and nitrogen uptake during grain filling (Borrell and Hammer, 2000). Reduced CO_2 assimilation caused by reduced stomatal conductance, reduced concentrations and activities of photosynthetic enzymes, chlorophyll, and N loss, among other factors, consequently limiting photosynthates' availability and partitioning into grain filling (Galyuon et al., 2019).

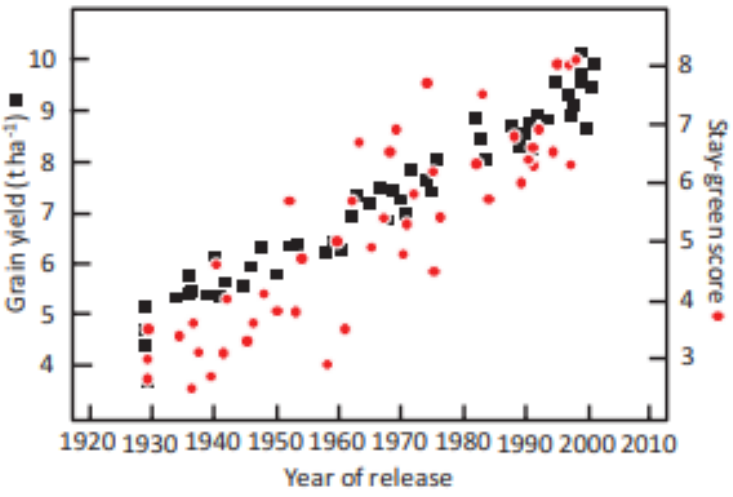

Figure 1: Progressive increases in yields and stay-green scores of modern maize varieties since 1930 (Duvick et al., 2004).

1.3.1. Definition, types, and estimation of SG trait

Stay-green phenotype can be classified into two major categories, functional and non-functional or cosmetic (Hörtensteiner, 2009). The cosmetic phenotype is a phenotype in which the chlorophyll pigment is retained, but the plant loses its photosynthetic capacity. This is because in the first step of chlorophyll degradation, the ring is not broken, and the green color is retained, but the chlorophyll has no function. On the contrary, the functional stay green is a plant that continues photosynthesis for a long period, and the entire senescence process is delayed or slowed down (Myers et al., 2018). Therefore, plant breeders mainly rely on functional SG to increase plant yield or stress resistance (Munaiz et al., 2020). Functional stay-green mutants are of great agricultural and economic importance because they seem to positively impact crop yields by delaying leaf senescence and maintaining photosynthetic capacity (Hörtensteiner, 2009). For example, Thomas and Howarth, (2000) found that the highest maize yield was obtained from the FS854 variety with stay-green character. Only functional stay-green is of interest for crop improvement. Functional stay-green can be achieved by varying leaf-greenness dynamics in several different ways (Thomas and Howarth, 2000). SG plants may be greener around anthesis before initiation of senescence, commence senescence later, or senesce slowlier than NSG plants (Harris, 2007).

From these two categories of functional and non-functional SG, five types of SG plants can be distinguished. Type A occurs when the leaves and stems maintain the activity of their photosynthetic zone for a long time, leading to delayed plant senescence. For type B, senescence occurs in the standard period of plant development, but the rate of occurrence is relatively slow. For type C, pigment accumulates on the organ's surface, giving the impression that senescence is reduced. However, the degradation rate of protein and chlorophyll usually occurs below the green surface. Type D repeatedly appears in the freezing herbs and vegetables, in which the green color is maintained with leaf death via freezing, boiling, or drying. Type E is considered to have the highest chlorophyll content in photosynthetic tissues, and that increased concentration results in a delay in yellowing of leaves and stems (similar to type A) and maintaining the green tissue, even with the reduced ability to fix carbon dioxide (Luche et al., 2015; Kamal et al., 2019).

Several techniques have been used to evaluate SG traits in the field, for example, rating the senescence of the whole plant (Jordan et al., 2012), or counting the number of green leaves per stem (Haussmann et al., 1999). More objective measures of greenness have been taken for individual leaves with a SPAD meter which measures chlorophyll content (Harris, 2007). Measurements of photosynthesis, transpiration, and stomatal conductance as a gas exchange function can also be used to identify the SG phenotype. These equipments are open systems that carry an infrared sensor helpful in the analysis of gas exchange (IRGA), which means that photosynthetic measurements are based on the differences of CO_2 and H_2O in an airstream that flows through a chamber closed where the sheet is to be analyzed (Caicedo, 2018). When photosynthesis is measured with an IRGA, a net photosynthetic value is obtained, that is, the balance between fixed and emitted CO_2 (Varela et al., 2010). More recently, the canopy with normalized difference vegetative index (NDVI)-based methods have opted (Christopher et al., 2014).

1.3.2. Application of the stay-green character in plant breeding

1.3.2.1. Improvement of physiological traits

Based on the increase of grain filling ability and improvement of required traits, it is believed that SG can increase yield. Yield gains are the result of increased photosynthetic rate and efficiency of the photosyntetic system, making SG an important tool (Parry et al., 2011). In addition, SG

genotypes constitute a potential germplasm resource for crop breeding programs aiming at improvingresponse to abiotic stresses. Even under stress conditions, SG can maintain the photosynthetic activity of the leaves and improve the grain filling process (J. Zhang et al., 2019). In addition, a strong association of chlorophyll content in leaves and late senescence with high grain yield performance was found in maize recombinant inbred lines and other segregant populations especially under restrictive water supply conditions (Câmara et al., 2007).

Previous results published by Caicedo (2018) revealed a progressive decrease in the chlorophyll content and photosynthetic rate of the SG genotypes; on the contrary, the NSG lines showed early drying compared to the previous ones. Accordingly, other authors also reported a decrease in chlorophyll content, photosynthetic rate and even efficiency of the photosynthetic system when analyzing lines of maize, sorghum and cotton (Wu et al., 2016b; Lin et al., 2015).

1.3.2.2. Increase biomass, grain yield, and other agronomic traits

SG has been identified as an essential part of the genetic improvement of several crops to promote stress tolerance and increase yield (Luo et al., 2006). Positive correlations between SG and desired traits have been reported, such as a higher number of grains per ear (Luche et al., 2013), higher industrial quality (Silva et al., 2004), or tolerance to abiotic and biotic stresses (Kassahun et al., 2010). It has been observed that greater grain filling capacity can maintain the photosynthetic tissues of the SG wheat genotypes, increasing the average grain weight (Silva et al., 2003). SG is also considered the main factor in increasing the average grain weight of durum wheat mutants, which is due to the expansion of the production capacity of photo-assimilates to the end of maturity (Spano et al., 2003). Maintenance of grain filling in the last stage of plant maturity has been considered key to stay-green genotypes' success (Luche et al., 2015). In addition to grain yield enhancements, SG phenotypes are interesting for enhancing biomass, especially in bioenergy crops (Munaiz et al., 2020). it has been shown that delaying leaf senescence is crucial in increasing the total biomass of new hybrids (Richards, 2000). If senescence is synchronized with seasonal growth, the biomass production of biofuels in woody plants can be maximized (Jackson, 2009). Sorghum and many other kinds of grasses are considered biofuel crops with high potential in the future (Calviño and Messing, 2012), and leaf senescence management is essential to achieve high biomass (Robson et al., 2012). In sorghum, maintaining green traits is closely combined with drought tolerance after flowering to achieve

high biomass (Harris, 2007). The SG lines presented higher grain yield than the NSG, and they also showed high grain moisture; Also. the results of Caicedo (2018) suggest that highe grain and biomass yield and the high values of grain moisture and biomass are associated with the SG character.

1.3.2.3. Nitrogen assimilation and remobilization

Plant growth and grain development require a lot of nutrients, especially nitrogen (N) (Xu et al., 2012). Nitrogen constitutes the main factor determining yield and is an essential nutrient for plant growth and development. The nitrogen in the soil provide a source of nitrogen for amino acids, nucleic acids, chlorophyll, and ATP (adenosine triphosphate) (Lam et al., 1996). The progression of leaf senescence is very important for crop yield. This is due to the control of the remobilization of post-anthesis photoassimilates (Thomas and Howarth, 2000). This is best reflected in nitrogen utilization efficiency, which involves nitrogen uptake and remobilization (Hirel et al., 2007). The transition from C capture to that of N remobilization corresponds to the functional initiation of senescence (Thomas and Ougham, 2014). Crop grain yield depends on pre-anthesis nitrogen uptake and post-anthesis remobilization during seed maturation (Masclaux-Daubresse et al., 2008). There is a complex relationship between the onset of leaf senescence and nitrogen use efficiency (Masclaux-Daubresse and Chardon, 2011). Early leaf senescence could decrease crop yields in general but increase pre-anthesis nitrogen use efficiency under low nitrogen conditions (Gregersen et al., 2008). Furthermore, delaying leaf senescence may lower the nitrogen use efficiency, increasing the final yield (Masclaux-Daubresse and Chardon, 2011).

Leaf nitrogen (N) and photosynthesis are connected as most of the N in leaves are associated with photosynthetic machinery (Yang et al., 2015). In contrast to a non-stay-green cultivar, the stay-green cultivar maintained more reduced nitrogen, chlorophyll content, and higher nitrate reductase and carboxylase enzyme activities, contributing to the accumulation of additional nutrients photosynthetic products during the grain-filling period (Crafts-Brandner et al., 1984). In particular, the yield-increasing potential of the SG grain was more evident under the condition of N-deficiency stress (Christopher et al., 2016). Functional SG genotypes in which the C–N transition point is delayed, or the transition occurs on time, but subsequent yellowing and N remobilization run slowly (Thomas and Howarth, 2000).

1.3.3. Agronomic problems associated with the stay-green trait

1.3.3.1. High seed moisture

A significant variation exists among genotypes for grain moisture when the black layer is entirely developed, representing their physiological maturity (Carter and Poneleit, 1973). After grain filling, a period of drying in the field or drying down is necessary to reduce the humidity of the grain at harvest to reduce post-harvest costs. Therefore, the moisture content of the grain during ripening and post-ripening are significant factors that influence the harvest and post-harvest management (Maiorano et al., 2014). Drying during the harvest of grain corn is also ideal because too much water remaining in the buds can block the cutting mechanism of the combined harvester. Fast field dries down can reduce growers' production costs related to artificial grain drying and economic losses due to delayed harvesting (Yang et al., 2010). The SG lines have a higher percentage of moisture in the grain at harvest concerning the NSG lines, which would imply additional post-harvest activities for drying, with the consequent increase in production costs (Caicedo, 2018).

1.3.3.2. Long phenological cycle

Grain filling is the ultimate growth stage of cereal caryopse formation when the final kernel weight is established, contributing significantly to grain productivity (Borrás et al., 2003). For some cultivated species, the long-term C capture period and the preservation of the dense green canopy may have serious adverse effects on crops, damaging crop nutrients and water economy (Thomas and Ougham, 2014). The stay-green trait can increase crop yield; however, unfavorably prolonged delayed leaf senescence results in a low grain filling rate, a low nitrogen use efficiency, and a low grain protein content, creating a dilemma for using the stay-green trait as a selection criterion in breeding (Gong et al., 2005). The effect of delaying leaf senescence on grain yield and grain protein concentration relies on nitrogen availability during the post-anthesis period (Bogard et al., 2011). Hence, post-anthesis leaf senescence should be under tight genetic and management control (Wu et al., 2012).

1.3.4. Senescence and abiotic stresses

The senescence of plant organs can be prematurely induced by a range of post-harvest abiotic stresses (Liebsch and Keech, 2016). It is a protective mechanism, leading to decreased yield and

quality in crop plants by limiting the growth phase (Hörtensteiner and Feller, 2002). Abiotic stresses are the major yield-limiting factors for crop plants (Zörb et al., 2019). Different factors like extreme temperatures, drought, flooding, salinity, and others may affect crop plants' growth and yield formation (Vaughan et al., 2018). Approximately 90% of arable lands are susceptible to one or more of the above mentioned stresses (dos Reis et al., 2012), which cause up to 70% yield losses in major food crops (Mantri et al., 2012). Based on comprehensive estimates of climate change and crop yield models, it is predicted that the productivity of major crops, including rice, wheat, and maize, will further decline, which may have severe consequences for food security (Tigchelaar et al., 2018). Moreover, a more remarkable ability to tolerate different abiotic stresses was identified in stay-green genotypes due to the protection of photosynthetic activity (Tian et al., 2013). Stay-green and stress response traits are closely associated.

1.3.4.1. Drought stress

Drought stress is one of the most important abiotic stresses that limit crop production. The effect of drought is manifested at morphological, cellular, physiological, biochemical, metabolic, and genetic levels (Rafique, 2020)**.** Drought effect on maize can be seen at different developmental stages, starting from seedling emergence or establishment to grain filling. The physiological responses of maize to drought stress are complex and often unpredictable (Moreno et al., 2005)**.** Drought affects various morpho-physiological processes including development of plant biomass, root length, shoot length, photosynthesis, water use efficiency (WUE), and leaf water content (Abdul Jaleel et al., 2007). A maize plant's productivity depends upon the presence or absence of drought stress at three critical developmental periods—the first being crop establishment, followed by flowering phase, and lastly, grain filling phase. However, the yield is most severely affected when drought stress strikes during the flowering and grain filling period (Bänziger et al., 2000). Drought stress induces a decrease in photosynthesis, loss of canopy area, and reduction in carbon assimilation (Yang et al., 2018). During reproductive growth stages, drought stress may cause premature senescence. The translocation of carbon and nitrogen molecules between the source and sink is also affected by drought (Li et al., 2016).

Carbohydrates are important metabolic regulators of drought-induced leaf senescence as they are involved in various responses for adaptation to drought (Tang et al., 2015)**.** The stay-green phenotype increases drought resistance. For example, Rivero et al., (2007) engineer drought

tolerance by delaying drought-induced senescence via up-regulation of isopentenyl transferase gene involved in cytokinin biosynthesis in tobacco.

Plants can also adapt to stress conditions by changing the expression of stress-responsive genes. Diverse sets of genes related to response to drought stress have been identified (Ingram and Bartels, 1996). Among the many families of TFs that regulate the expression of many other downstream genes and gene clusters, they have an essential role in drought tolerance in wheat plants (Baloglu et al., 2014)**.** Maize responds to drought by launching leaf senescence as a strategy to avoid drought by reducing canopy size and mobilizing nutrients to support the growth of the upper younger leaves and grains (Leta et al., 2016). This regulation of leaf senescence has an obvious adaptive value in wild plants allowing them to complete their life cycle even under stressful conditions. In crop plants, drought-induced leaf senescence is often associated with reduced grain yield (Gungula et al., 2005), and causing premature death of photosynthetically active leaves (Leta et al., 2016).

1.3.4.2. Low nitrogen stress

Nitrogen (N) is a primary plant nutrient that plays a crucial role in determining plant growth and productivity. Plants require nitrogen to synthesize vital molecules, such as proteins, nucleic acids, and chlorophyll (Goel and Singh, 2015). Most plants take up nitrogen mainly in inorganic forms, as nitrate (NO_3^-) and ammonium (NH_4^+) (Hessini et al., 2019). The nitrogen assimilation involves the reduction of nitrate to ammonium which is finally incorporated into amino acids by ammonia assimilation (Goel and Singh, 2015). Nitrogen supply is one of the main constituents of leaf cell components, particularly those associated with the photosynthetic apparatus, including carboxylation enzymes and membrane proteins (Pandey et al., 2000). N deficiency inhibits plant growth and development, especially in the older leaves near the plant base, and ultimately they turn yellow and fall off under severe N deficit (Sen et al., 2016). In plants, several processes, including N uptake and assimilation, are adversely affected by abiotic stresses (Goel and Singh, 2015)**.**

Maize growth is susceptible to soil nitrogen variation. Nitrogen stress reduces photo-assimilates production in the leaf via a reduction in leaf chlorophyll, leaf area, an increased rate of senescence. Nitrogen plays a significant role in leaf chlorophyll formation and, hence, determines the plant's photosynthetic efficiency. This indicates that nitrogen is a determinant factor of yield

(Bänziger et al., 2000). When N stress occurs during grain filling, it increases the rate of leaf senescence through remobilization and reduces the rate of photoassimilate production and kernel weight. According to Bänziger et al. (2000), the senescence program is often associated with the degradation of chloroplasts and reutilization of nitrogen present in the chloroplast proteins. Rubisco, the central enzyme in the dark reaction of photosynthesis, is the largest source of leaf nitrogen (Distelfeld et al., 2014). With senescing of leaves, rubisco breaks down into amino acids, which are then reused as nitrogen supplements for grains (Masclaux-Daubresse et al., 2010). In crops, there is a close relationship between the level of leaf nitrogen and senescence (Moschen et al., 2016). Currently, a broadly accepted viewpoint is that leaf nitrogen levels are associated with leaf senescence (Sade et al., 2018). Senescence can be accelerated under situations of low nitrogen supply (SCHULZE et al., 1994), or it can be delayed or even reversed by excess nitrogen supply (Schildhauer et al., 2008).

1.3.4.3. High planting density

Planting density is one of the most critical factors that affect the grain yield of maize, being possible to increase maize yield, water use efficiency (WUE), and average grain-filling rate (Duvick, 2005; Testa et al., 2016). It has been shown that varying the maize planting density significantly affects the grain-filling process, yield, and yield components (Sangoi et al., 2002). The grain-filling rate of maize significantly decreases with increasing the density of plantation significantly (Jia et al., 2018). For instance, in the United States, the planting density of maize has been increasing from 60,000 ha^{-1} to more than 70,000 ha^{-1} plants from the 1990s to the end of the 20th century. There are usually about 100,000 ha^{-1} plants in high-yield fields (Xu et al., 2019). However, too high planting density will reduce the yield of a single plant, and improper control will even reduce the yield (Ren et al., 2017). Research conducted on maize yield and senescence physiology under different planting densities shows that, as maize planting density increases, light transmission within the canopy decreases and competition of light in canopy increases, accelerate senescence, the grain number per spike and the 100-grain weight decrease, and the lodging rate increases (Cao et al., 2013). High plant density can lead to weak stems and lodging in maize, partly due to the fast remobilization of DM and N at the early post-silking stage (Rajcan and Tollenaar, 1999). Planting density significantly affects the leaf area index, plant height, ear length, number of grains per ear, weight per ear, 1000-grain weight, and grain yield (Shafi et al.,

2012). In addition, high plant density could significantly affect the grain-filling process and result in lower maximum and average maize grain-filling rates (Novacek et al., 2013). Population yield increases with increasing density within a specific density range, and rational close planting is a vital cultivation practice for achieving high yields (Zhang et al., 2006). If the planting density is too high, it will reduce the ability of light to penetrate the lower canopy (Liu et al., 2014), resulting in premature senescence of the lower leaves (Borras et al., 2003). Ultimately, this significantly reduces maize crop yield and yield components (Sangoi et al., 2002).

Conversely, the use of high-density populations induces undesirable phenotypes such as apical dominance, barrenness, and decreased numbers of ears per plant and kernels set per ear (Sangoi et al., 2000). Optimum density varies depending on climatic factors and soil fertility, hybrid selection, planting date, planting pattern, and harvest time (Burken et al., 2013). The grain yield of an individual maize plant decreases as the plant density increases, and competition for photosynthate may lead to ear and grain abortion during the flowering phase (Andrade et al., 2002).

1.3.4.4. Combined stresses

The significant abiotic constraints that plants face are drought, waterlogging, low nutrient availability, high temperatures, and salinity during their lifespan (Rafique et al., 2019). Plants have developed several mechanisms to detect environmental changes and respond with different abiotic stress or a combination of stresses (Rafique et al., 2019). They respond to these abiotic stresses either by escaping, i.e. completing the life cycle before the onset of s, tress or avoidance and tolerance through, morphological alterations and changes in their physiological processes (Foulkes et al., 2009). Many physiological or biochemical traits associated with improved drought tolerance have been identified (Foulkes et al., 2009). However, most plant × environment interaction studies focused on single stress (Mittler, 2006). Although, tolerance to two different abiotic stresses has been emphasized in the breeding strategy for maize and some other crops (Jiang and Huang, 2001). Water and nitrogen affect crop growth, development, and production either separately or in combination. Humbert et al., (2013) observed the physio-molecular changes in response to water and nitrogen. Finally, they concluded that the responses of plants to the combination of these two stresses might cause additional effects that were different from the individual effects, and hence, cannot be inferred from the results obtained from different stresses applied individually. Drought affects maize grain yield to some degree at almost

all growth stages, but the crop is most susceptible during flowering (Grant et al., 1989). N availability affects assimilate partitioning between vegetative and reproductive organs and N metabolism in young ear shoots (Czyzewicz and Below, 1994). Therefore, the timing and intensity of stress determine yield reduction either due to source or sink limitations (Rafique, 2020)**.**

Additionally, germplasm selected for tolerance to drought also shows resistance to low-N stress. Hence there is spillover from drought to low-N tolerance in maize genotypes (Zaidi et al., 2008). Recent evidence shows that plants respond to multiple stresses differently from individual stresses (Atkinson and Urwin, 2012). Plants activate a specific and unique stress response when subjected to the combination of multiple stresses (Rizhsky et al., 2004). They modify their response according to multiple stress conditions and show several unique and expected responses. Therefore, combined stress factors on crops depend on the nature of interactions between the stresses (Ramu et al., 2016).

1.3.5. Stay green phenotype and abiotic stresses

The contributions of the SG phenotype have been reported in several crops, and its employment has increased yield grain, establishing tolerance to abiotic stresses. SG plants are more resistant to pathogens and less susceptible to lodging (Silva et al., 2005). Significant correlations between grain yield and maintaining green leaf area at maturity (0.75) and leaf senescence rate (-0.74) were reported under stress conditions, showing the superiority of SG hybrids (Borrell et al., 2000). A strong association of chlorophyll content in leaves and senescence retardation with high grain yield was found in maize recombinant inbred lines and other segregating populations, especially under restrictive water supply conditions (Câmara et al., 2007). In addition, the analysis of 936 wheat lines resulted in a significant association between stress tolerance to high temperatures and SG character, finding high positive correlations between the delay of senescence and tolerance to high temperatures (r=0,90) and with grain yield (r=0.89) in wheat genotypes (Kumari et al., 2007). Abiotic stress tolerance is a significant feature of SG genotypes, giving stability to grain yield even in unfavorable environmental conditions (Silva et al., 2008). The superiority of grain yield in SG lines was predominantly expressed in stressed environmental conditions such as low rainfall at the end of the cycle (Luche et al., 2013). Delayed leaf senescence in the SG phenotype can enhance crop yields by remobilizing nutrients from the source to sink under various stresses and nutrient-limited conditions (Munaiz et al., 2020)**.**

The SG phenotype has been linked to improved yield stability in several cereal crop species, particularly under terminal drought stress (Gregersen et al., 2013). Many drought-resistant sorghum cultivars stay-green until harvest and the SG trait has been used for years by breeders as a measure of post-flowering drought tolerance (Jordan et al., 2012). The trait is characterized by retaining green stems and green upper leaves, even under severe post-flowering drought stress. It is associated with the maintenance of grain fill, reduced lodging, high stem carbohydrate content, and resistance to charcoal stem rot under such conditions (Borrell et al., 2014). Thus, delaying leaf senescence is an effective strategy for increasing cereal production under water-limited conditions (Mahalakshmi and Bidinger, 2002).

Thesis objectives

SG genotypes constitute a potential germplasm source for the genetic improvement of essential crops to mitigate several stresses. SG is considered an important agronomic trait that allows plants to maintain their leaves photosynthetically active and improve the grain-filling process even under stress conditions (Zhang et al., 2019). Functional SG varieties perform photosynthesis and can potentially incorporate C and N during a lengthy period (Swanckaert et al., 2017), which could be positive for several traits such as grain yield, silage yield and quality, stress resistance, and many more. (Reguera et al., 2013). Several environmental factors promote leaf senescence, such as drought, nutrient starvation, high plant density, inhibited pollination, salinity stress, and biotic stresses (Schippers, 2015). Maize hybrid has a long active photosynthetic period mainly achieved by having higher chlorophyll content during senescence or maintaining a higher photosynthetic activity level during chlorophyll loss, increasing grain yield. Maize is frequently impacted by different biotic and abiotic stresses, like drought, high salinity, high plant density, and low-temperature yield (Wu et al., 2016).

As already mentioned, the delay in senescence (SG) is a desirable trait for crop production and is associated with biomass production, resistance to lodging, and yield. Furthermore, there is likely a relationship between senescence and abiotic stresses; as drought, low nitrogen, and high plant density.

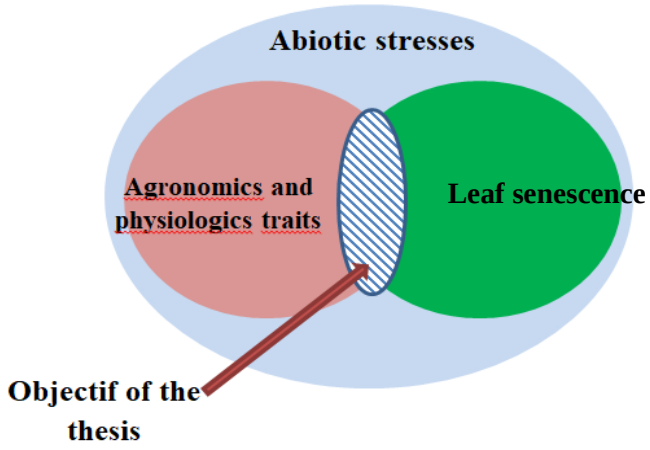

Figure 2: Venn diagram representing the content of this thesis**.**

The general objective of this thesis, represented by the Veen diagram (Figure 2), was to investigate the process of leaf senescence in maize and its relationship with different traits under various levels of abiotic factors. The general objective is articulated in two specific objectives

a- To study the influence of senescence and a combination of abiotic factors (water, nitrogen, and density) on agronomic traits and the absorption and recycling of nitrogen.

b- To study the change in gene expression during senescence under different levels of abiotic factors (water, nitrogen, and density) using RNA-Seq, which in turn will serve to identify genes associated with senescence under diverse environmental conditions.

Thesis outline

Chapters one and two have given a broad overview and a comprehensive basis of senescence in plants, how the abiotic stresses can affect plants during grain filing time, and how genotypes with delayed leaf senescence can provide better yield and tolerance to abiotic stresses. Then we explain the different materials and methods used to carry out this work.

In **Chapter 3**, we aim to answer the objective of studying physiological and agronomic traits measured in SG and NSG genotypes evaluated in trials conducted in two years under control and abiotic stresses.

Chapter 4 aims to answer objective b, identifying different genes expressed during senescence and the difference between SG and NSG genotype for genes expression under different environmental conditions.

Chapter 2: Material and methods

II. Chapter 2: Material and methods

2.1. Experimental site

The study was conducted in two locations, Tomeza "TM" (latitude 42.40°N and longitude 8.63°W) in the province of Pontevedra, and Xinzo "XZ" (latitude 42.07N and longitude 7.73°W) in the province of Ourense. The experiments were repeated for two years 2018 and 2019.

2.2. Germplasm

Eight maize inbred lines were used in this study, including 4 stay green lines (PHW79, PHW52, PHP38, PHBW8), and 4 non-stay green lines (PHBB3, B73, PHT11, PHM10) (Table 1). These lines were selected from 197 inbred lines evaluated in the Misión Biológica de Galicia for senescence related traits under optimal water and nitrogen conditions (Caicedo, 2018; Chibane et al., 2021). Except B73, all lines belong to two heterotic groups widely used nowadays (White et al., 2020; Mikel and Dudley, 2006). B73 is the most important line in the history of temperate maize breeding which belongs to the Stiff Stalk Synthetic (BSSS) heterotic group.

Table 1: Stay-green phenotype, heterotic groups and origin of the eight inbred lines of maize used in this study.

Genotypes	Stay green	Heterotic groups	Origin
PHBW8	SG	Amargo (PHG39)	Pioneer ExPVP
PHW52	SG	Oh07-Midland (PH595)	Pioneer ExPVP
B73	NSG	Stiff stalk	Iowa State University
PHW79	SG	Oh07-Midland (PH595)	Pioneer ExPVP
PHP38	SG	Amargo (PHG39)	Pioneer ExPVP
PHT11	NSG	Amargo (PHG39)	Pioneer ExPVP
PHM10	NSG	Amargo (PHG39)	Pioneer ExPVP
PHBB3	NSG	Amargo (PHG39)	Pioneer ExPVP

(White et al., 2020; Mikel and Dudley, 2006)

2.3. Experimental design

The experimental layout in each location was a split plot design with two replications and three factors: water, nitrogen, and planting density (Figure 3).

- Water factor with two irrigation levels (optimal and reduced). It was irrigated weekly in optimal irrigation and every 15 days, with half of the amount of water, in the reduced level.
- The nitrogen factor at 3 levels of nitrogen fertilization (N1: without nitrogen; N2: low nitrogen and N3: optimal nitrogen), Low and optimal nitrogen evaluation was achieved by fertilizing at the rate of 30 and 90 kg ha^{-1}, respectively.
- The plants density factor has 2 levels (high density of 80.000 plants ha^{-1} and low density of 50.000 plants ha^{-1}).

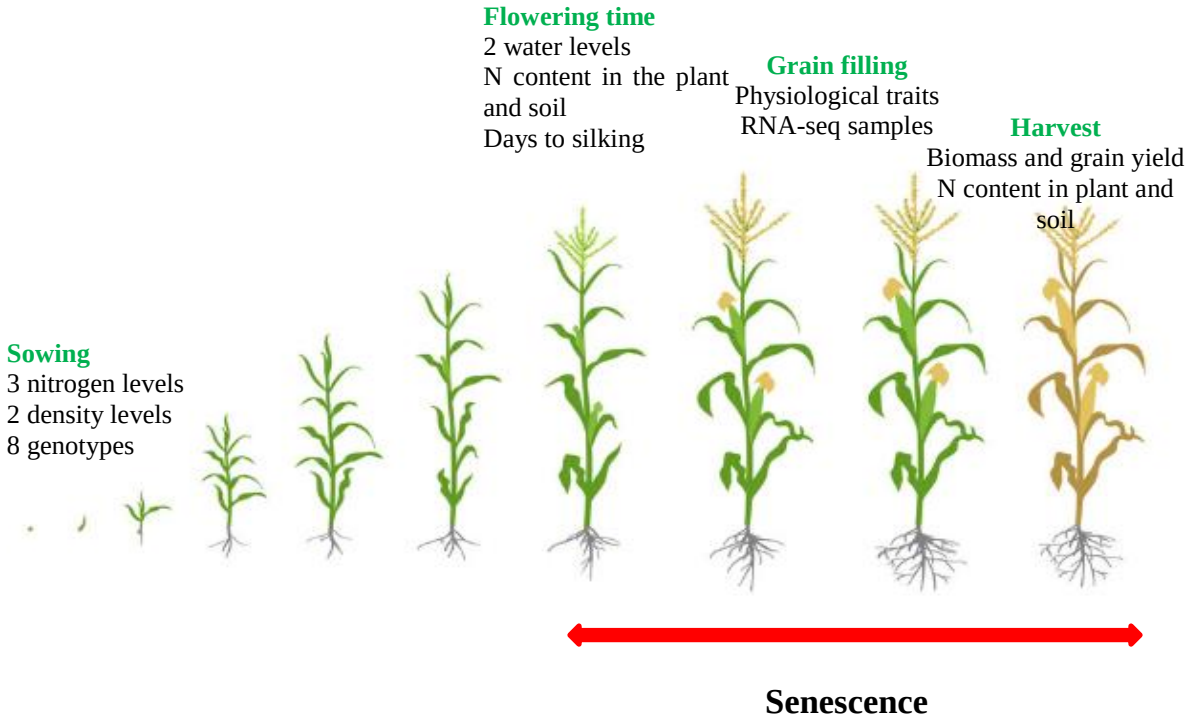

Figure 3: Experimental design and post-flowering measurements for eight maize inbred lines evaluated in two locations for stay-green trait under abiotic stress.

2.4. Field Experiment

Each experimental plot consisted of two rows, each row with 13 double-kernel hills planted manually, each block being 26.6 × 3.25 m, spacing between rows was 0.8 m and between consecutive hills 0.16 or 0.25 with final density of 80000 and 50000 plants ha^{-1}, respectively. For the first year 2018, the sowing was made the 21st of May in Tomeza, and the 23rd of May in Xinzo; for the second year 2019, the sown was made the 16th of May in Tomeza, and the 23rd of May in Xinzo. The fertilizers were applied during land preparation using standard agricultural procedures. The trials were kept weed free and different insect attacks were controlled with the application of herbicides (Pendimentalina 33% and Sulcotriona 30%), and insecticides (Lambda cihalotrin 10%). At each location of the experiment for 2018, we made a previous analysis of nitrogen and carbon content in the soil. Soil samples from 0 to 30 cm soil layer for each location were collected before planting, and were analyzed in the laboratory of the University of Vigo. The content of various nutrient elements, such as nitrogen fraction (NO_3^-, NH_4^+, N organic), and C (mg kg^{-1}) were measured, with the method of Houba et al. (2000). For the second year 2019, we could not make previous analysis of the soil because the fertilization was made before taking the samples. The results of soils analysis, show that there is a difference between soils nitrogen availability between both locations. Nitrogen content was generally lower in both locations, where the NO_3 content had low value (Table 2). This result was similar to the result found by Angle et al. (1993), who found that under no fertilizer soils, the NO_3 content change between 2.5 to 9.1 mg ha^{-1}.

Table 2: Soil analysis before sowing for both locations TM and XZ of Galicia region for the experiment made in 2018**.**

Elements	Total nitrogen (mg kg^{-1})	NO_3 (mg kg^{-1})	NH_4 (mg kg^{-1})	C (mg kg^{-1})
Tomeza	3.96	7.55	5.33	26.36
Xinzo	1.55	4.26	3.35	11.71

During the growing season of both years 2018 and 2019, meteorological data were downloaded from (http://meteogalicia.es). The data included the monthly average temperature (Tavg), maximum temperature (Tmax), minimum temperature (Tmin) and precipitation.The result show some variations for precipitation distribution between both locations and for both years, and TM show high precipitation quantity during growing season compared to XZ. In addition, the first year trial 2018 was drier than 2019 (Figure 4).

The mean temperature was similar for both location, but for Xinzo, the minimal temperature was below zero for several months and for both years (Figure 4).

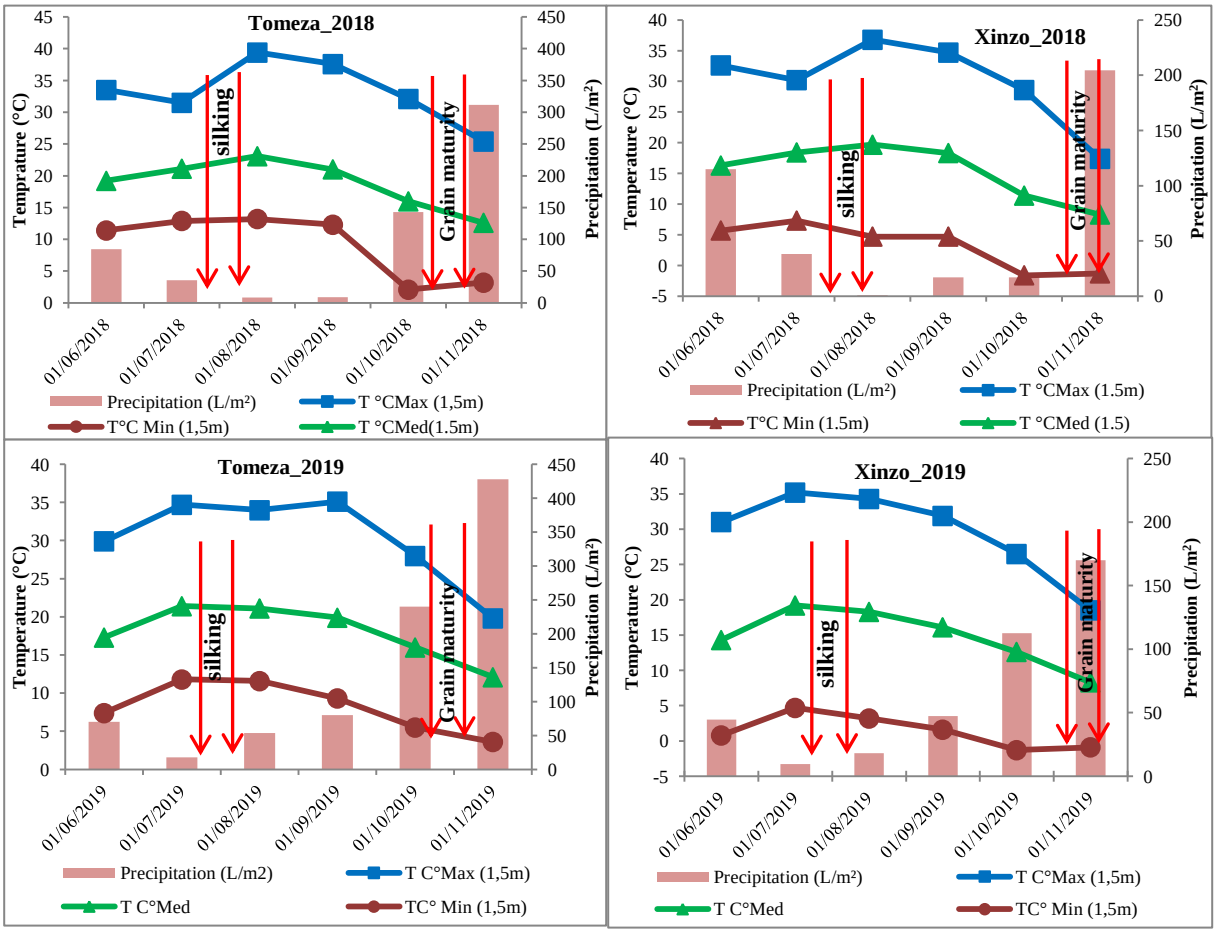

Figure 4: Temperature and precipitation data during both growing season 2018 and 2019 in both locations (Tomeza and Xinzo).

2.5. Data collection

2.5.1. Physiological data

During both growing seasons of 2018 and 2019, several agronomic and physiological data were collected to better study the senescence process under different treatments. From silking time to total senescence of leaf, in both locations net photosynthetic rate, and conductance were measured by using a LI-6400 photosynthesis system (USA), chlorophyll concentration was measured by using a SPAD portable system (CCM-200), and a portable fluorometer OS-30p was used for estimating quantum efficiency of PSII (F_v/F_m). Photosynthetic rate was computed by measuring the rate of change of CO_2. The leaf below the principal ear was adequately dark-adapted for 20 min with the use of tweezers before measurements of F_v/F_m. Measurements of Chlorophyll content, F_v/F_m and photosynthetic rate have been done at silking, 30, 45 and 60 days after flowering (DAS) for two genotypes, SG (PHW79) and NSG (B73). Measurments were taken in the ear leaf of five plants per plot. For photosynthetic activity, measurements were taken in two plants per plot.

2.5.2. Phenological data

Days to silking were recorded, as the number of days from planting to the date when 50% of the plants had emerged silks, and days to anthesis, when 50% had shed pollen. Moreover, we estimated days to physiological maturity from silking time based on the presence of black layer. It was detected using at least 5 ears per plot and identified by visual analysis of a thin black layer observed in the seed base, according de Daynard and Duncan (1969).

2.5.3. Agronomic data

All the agronomic data were taken from the eight genotypes. At physiological maturity, we estimated different agronomic traits of stover and grain yield. 10 plants were harvested randomly from each plot. Then we estimate different yield parameters: weight of 1000 grains (g), cobs weigh (Kg ha^{-1}), and stover yield (Kg ha^{-1}).

At flowering time, 5 plants harvested randomly for each line were weighted (fresh weight). Then, a sample of crushed plants of each line were weighted before and after drying to measure fresh

and dry stover weight (SYFT), the same operation was at harvest time (SYHT). The biomass yield was estimated from the formula presented below:

$$\textbf{Biomass yield (Kg ha}^{-1}\textbf{) = PD*(PFP*(1-\%M))/PN}$$

PD: plants density

PFP: fresh weight of harvested plants (kg)

(1-%M) (Humidity percentage) = 1- (Stover sample dry weigh/stover fresh weight)

PN: Harvested plants number.

The amount of stover yield that is not remobilized (SYNR) (Kg ha^{-1}) is directly the weight of the stover at harvest (SYHT), while the stover yield remobilized (SWR) (kg ha^{-1}) is the difference between the weight of the stover at flowering and harvest.

$$\textbf{SYR}(\textbf{kg ha}^{-1}) = \textbf{SYFT} - \textbf{SYHT}$$

To estimate stover moisture, we took the fresh weight and dry weight (60 °C for 5 days) of 5 random plants of each plot at silking and harvest time. The same process was used to estimate grain moisture. We calculated the percentage of moisture with the formula:

$$\textbf{Moisture}(\%) = \left(1 - \frac{\textbf{Dw}}{\textbf{Fw}}\right) \times 100$$

Dw: dry weigh (g $plants^{-1}$)

Fw: fresh weight (g $plants^{-1}$)

2.5.4. Nitrogen content and remobilization

Data of total nitrogen content and nutrient (NO_3 and NH_4) in soil were taken only in six genotypes for experiment 1 and 2 (3 SG, and 3 NSG); however, at the second year trial we only measured total nitrogen in two representative genotypes (1 SG, and 1 NSG). Samples of 5 random plants were harvested for each plot at silking and harvest time. Data of total nitrogen in plant and grain were estimated in six genotypes for 4^{th} experiments. This was due to laboratory cost and availability due to covid-19 restrictions.

Nitrogen and carbon concentration were measured at flowering and harvest time in the plant stover (leaves and stem) and in the kernels using the elemental analysis (Flash EAI112 series). Then, other variables were estimated from those basic values. The following variables related to the N in the whole plant were calculated: *total N* (TN) (g kg^{-1}) which is the total amount of N uptake by the whole plant and was estimated as the sum of the stover and kernel N at harvest; *N uptake until flowering (SN_UF)* (g kg^{-1}) is the content of N in the plant stover at flowering; *N uptake after flowering (SN_AF)* (g kg^{-1}) is the difference between the total N and the N uptake until flowering. For the variables related to stover, we estimate the *N of the stover not remobilized to the grain* (SN_NR) (g kg^{-1}) is the N content of the stover at harvest; *N of the stover remobilized to the grain (SN_R)* (g kg^{-1}) is the difference between the N content at flowering and the N content of the stover at harvest. The percentage of N of the stover remobilized *(SN_R%)* and no remobilized (SN_NR%) to the grain with respect to the N content of the stover at flowering was calculated. Finally, regarding the kernels, the percentage of N of the kernel that derived from remobilized N (KN_R %) was estimated as the N of the stover remobilized to the grain divided by the N content of the kernel; the percentage of N of the kernel that derived from N uptake after flowering (KN_UpAF%) was estimated as the N uptake after flowering divided by the N content of the kernel. While for soil nitrogen content, we take samples at silking and harvest. The analyses of soil total nitrogen, carbon and nitrogen assimilable by plants (NO_3 and NH_4) content were done using elemental analysis (Flash EAI112 series) (Krotz and Giazzi, 2000).

2.6. Statistical Analyses

2.6.1. Physiologic data analysis

For physiological data, we use a statistical model for repeated measures with Proc GLIMMIX of SAS statistical package (SAS studio). The lines and the study factors (water, nitrogen and density) were considered as fixed effects, and environment and repetitions (environment) as random effects. For this analysis we consider the study factors water condition and nitrogen levels to combined a factor called treatment. This is to reduce the number of factors of the model and the number of interactions. And we use the factor time to mark each senescence time.

The model used for this analysis was:

$$Y_{ijk}=\mu + \alpha + b_{ij} + \Upsilon_k + \alpha\Upsilon_{ik} + w_{ijk}$$

Where terms are defined as follows:

- $\mu_{ik}= \mu + \alpha_i + \Upsilon_k + \alpha\Upsilon_{ik}$: mean treatment *i* at time k, containing effects for treatment, time, and treatment ×time interaction.
- b_{ij} : the between-subjects effect for the j^{th} subject assigned to treatment *i*.
- w_{ijk} : within-subjects effect for time k on the ij^{th} subject.

We have different times interval, for this we have different variances and the covariance different from zero: $Var[e_{ijk}] = \alpha^2_k$ and $Cov[e_{ijk}, e_{ijk'}] = \sigma_{kk'}$

In other words, we allow the variance of e_{ijk} to depend on the measurement time k, and the covariance between the errors at two times k and k', for the same subject, depends on time. In the language of GLIMMIX procedure this is called compound symmetry model, or type = CS in SAS syntax. With the residual parameter $Var[e_{ijk}] = \sigma^2_e$ and covariance parameter, $Cov[e_{ijk}, e_{ijk'}] = \sigma_{cs}$.

CS covariance assume that time has no impact on either variance or within-subject correlation.

2.6.2. Agronomic data and nitrogen content analysis

For each studied character, a combined analysis of variance was performed for both years and locations, with the mixed models procedure (PROC MIXED) of the SAS statistical package (SAS studio). For the analysis of variance, the lines and the treatments (water, nitrogen and density) were considered as fixed effects; environment, and repetitions (environment) as random effects. Each environment is represented by one location in one year. So for two years trials we have 4 environments with two repetitions in each environment. Comparisons between means were made using Fisher's protected least significant difference (LSD) at 5% probability.

To fit the linear mixed model

$$Y_{ij} = \mu + \alpha_i + b_j + e_{ij}$$

Where μ and α_i represent fixed factors: intercept and the treatments (water and nitrogen conditions, plant density and SG phenotype), respectivly.

b_j and e_{ij} are random factors (environment, and repetitions (environment)) and the error, respectively.

We assume that the random effects b_j, are independently and identically distributed with mean zero and variance αb2. Additionally, we assume that the residual effects e_{ij}, are independently and identically distributed with mean zero and variance α2. The covariance assumed with this model equal zero.

2.7. Molecular data (RNAseq analysis)

To estimate the senescence process at molecular level, we have made an RNAseq study where two inbred lines with distinct leaf senescence characteristics, early leaf senescence B73, and stay-green or delayed leaf senescence, PHW79 were used.

2.7.1. Sampling in field

From both genotypes, we took leaf samples at four moments (M1, M2, M3 and M4) that corresponded to flowering time, 30, 45, and 60 days post-silking time, respectively. A leaf sample was collected from each line in the two replications at each moment. Approximately 10 cm^2 were taken from the central part of the ear leaf (in three randomly chosen plants) and immediately frozen in liquid nitrogen and stored at -80 °C in a deep-freezer.

2.7.2. RNA preparation, library construction, and Illumina NextSeq500 sequencer

A quantity of 100 mg of fresh tissue belonging to each sample were taken for total RNA extraction, using the Maxwell® 16 LEV Plant RNA kit (Promega) in a Maxwell® 16 Instrument (AS2000) and following the technical instructions suggested by the manufacturer. For homogenization, the tissue was placed in a microtube (QIAGEN catalog no. 19560) with 600 µl of cold homogenization solution / thioglycerol and a 3 mm tungsten ball (QIAGEN catalog no. 69997). Homogenization was carried out in a TissueLyser mill (QIAGEN) during two 2-minute grinding shifts at 30 HZ. Samples were mixed for 30-60 seconds and placed on ice. 400 µl of the homogenate was transferred to an Eppendorf using a cut tip. 200 µl of Lysis buffer was added to the homogenate and mixed vigorously for 15 seconds. Incubated at room temperature for 10 min,

then centrifuged at full speed for 2 min. The cartridges in the rack were prepared by removing the protective paper, adding 5 µl of DNase to the wells in position 4 of each of the cartridges and placing a tip in well 8 of said cartridges. The supernatant collected after centrifugation was transferred to well number one of each cartridge, trying not to transfer any solid material. The rack with the cartridges was placed in the Maxwell® 16 Instrument and the RNA method "Simply RNA" was selected.

The construction of mRNA libraries was made by the external service of Cornell University that sent us back the raw data. The 3'RNA-Seq libraries were prepared from ~500ng of total RNA per sample using the Lexogen QuantSeq 3'mRNA-Seq Library Prep Kit FWD for Illumina (https://www.lexogen.com/quantseq-3mrna-sequencing/) with 13 PCR cycles. The libraries were quantified on a Molecular Devices Spectra Max M2 plate reader (with the intercalating dye QuantiFluor) and pooled accordingly for maximum evenness. The pool was quantified by digital PCR and sequenced on 1 lane of an Illumina NextSeq500 sequencer, single-end 1x86bp, and de-multiplexed based upon six base i7 indices using Illumina bcl2fastq software (version 2.18; Illumina, Inc., San Diego, CA). For this project, a total of 192 RNA samples were sequenced (2 genotypes x 4 moments x 2 locations x 2 nitrogen levels x 2 water conditions x 4 replicates). For genotype B73, we have sampled only at M1, M2 and M3; at M4 we could not take sample, because it was completely dry in the first location. While, in XZ we take samples only at M1 and M2 for B73, and at M1, M2, and M3 for PHW79.

2.7.3. Quality control and read mapping

The maize genome and gene information were downloaded from the maize genomic database (http://www.maizesequence.org/index.html). We got the clean reads after removing the adaptor sequences and low quality sequences for which the quality score < 20. The STAR (v2.7) software was used to map the clean reads to the maize genome. The single end RNA-Seq reads sequences are stored in compressed FASTQ files. Before preceding the statistical analysis, we checked first reads quality: they are aligned to a reference genome and counted into annotated genes. In our case the reference genome and annotated genes come from the reference maize B73 genome version 4 ("Zea_mays_AGPv4.dna.toplevel.fa" and "Zea_mays_AGPv4.gtf file").

For quality analyses we used fastqc and multiqc. The second step is the mapping (alignment) of the reads to a reference genome with STAR software and the counts of reads associated at each gene. For each read, all the multi-hits are removed and only the best score of mapping is considered. The result of quality control and mapping alignment presented in the Table 3 and Figure 5.

From the table 3 of the mapping count genome, we can conclude that the median of reads number was around 4 million reads, with 73% of the total reads samples. From the median reads sequence, we can estimate 89.62% of the total reads was mapped to the reference genome B73v4. We have just 1.53% of the ambiguous reads, and 14% of the reads can't be aligned to the reference genome.

Table 3: Summary results of mapping count genome results of RNAseq samples, with the maximum, minimum and median reads genes, the percentage of mapping and counted reads, no feature, ambiguous and removed reads after mapping.

	Reads_ parsed	**Mapping_ reads%**	**Reads_ counted %**	**No_ feature %**	**Ambiguous%**	**Multihits_ removed count%**
min	57282.00	38.28	31.43	6.24	0.61	5.88
max	36300846.00	96.60	80.19	17.21	2.28	21.23
median	**3949658.00**	**89.62**	**73.77**	**14.26**	**1.53**	**13.07**

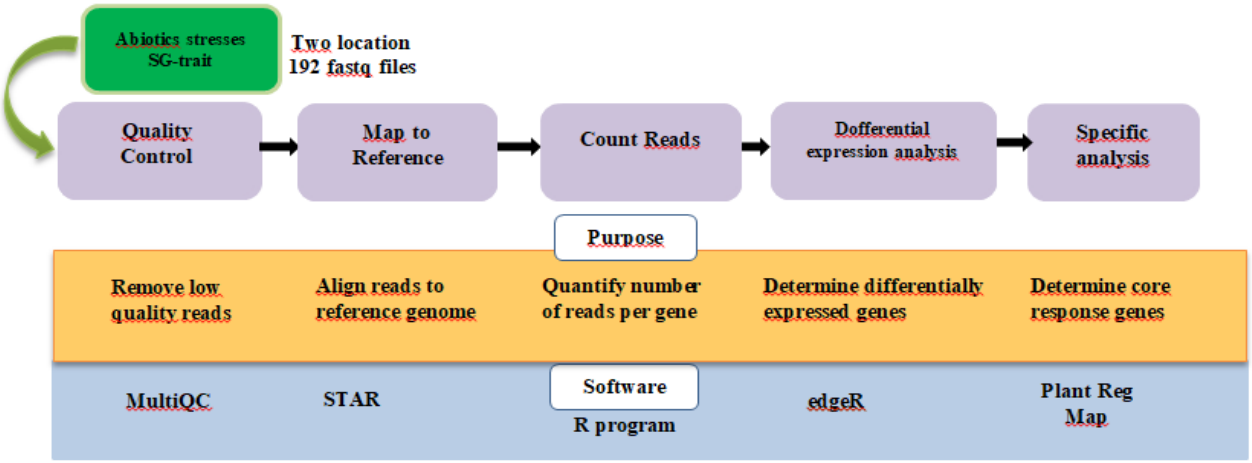

Figure 5. Schematic diagram represented the summary of complete process to prepare RNAseq data analysis with preliminary quality control.

2.7.4. Gene expression quantification, differential expression analysis and function enrichment

We started the analysis by filtering not expressed genes and those with low counts. We used the Counts per Million (CPM) method and kept genes with at least 1 cpm in each sample. We choose the default method TMM to normalize the RNAseq libraries. The TMM method available in the package EdgeR, estimates scale factors between samples that are incorporated into the generalized linear model used for the differential analysis. For the differential analysis, we use EdgeR function from R packages. This function estimates the parameters of the GLM and performs differential analysis for all contrasts. First data were filtered and normalized, then parameters of the GLM was estimated, and a likelihood ratio test (LRT) was performed for each contrast. The probabilities of significance (p-values) generated by the LRT are adjusted by the Benjamini-Hochberg procedure (BH), False Discovery Rate (FDR) <0.05, and p-value<0.05.

The changes in expression were considered significant if the absolute log2 fold change was greater than 0.3 and the propability of FDR adjusted $p \leq 0.05$. We use different contrasts to analyze the difference of genes expression under each specific or combined condition. We compare the change of gene expression between two senescent moments (M2vsM1, M3vsM2 and M4vsM3). The gene ontology (GO) classification of DEGs was performed using PlantRegMap

platform. This platform adopts topGO and Fisher's exact tests to find the significantly over-represented GO terms in our input gene set. By default, all genes in maize will be used as the background (http://plantregmap.gao-lab.org/go.php). We select from different contrasts the specific genes active (up or down regulated) for the chosen treatments. For example, when we search genes active only for N1 stress, we select genes active only for N1, and not active for N3. Also, when we search genes active in both stresses (SN1), we select genes active only for SN1 and not active for SN3, ON1, and ON3. For the early senescence genes, we select genes active at [M1_M2] for the genotype B73, and at [M2_M3] for the genotype PHW79. Then, for the late senescence genes, we select genes active at [M2_M3] for B73 genotype, and during [M3_M4] for PHW79 genotype. When we obtain the selected genes of each treatment, we use the PlantRegMap platform to see the genes ontology (GO terms) of this specific group of genes. The result on gene ontology will be represented in three categories of biological process, molecular function, and cellular component.

2.7.5. TF Identification and Analysis

To identify the transcription factors (TFs) expressed in our study, we used 3308 transcription factors (TFs) annotated in maize genome. The transcription factors (TFs) list was downloaded from the transcription factors database for *Zea mays*, version 4 (PlantTFDB v4.0), and classified within 56 families that were compared with all the genes DEGs during senescence period using the R program**.**

2.7.6. RNAseq statistical Analyses

For the second part of molecular analysis, the statistical model used to study the expression has to be formulated based on the experimental design that, as explained above, contains:

- Three biological factors:
  - Treatment (T1): Optimal water_high nitrogen level (ON3), T2: optimal water_ low_nitrogen level (ON1), T3: water stress_high nitrogen level (SN3), T4: water stress_low nitrogen level (SN1)).
  - Moment (M1, M2, M3, M4).
  - Density (1: H; 2: R).
  - Genotypes (1:B73; 2:PHW79)
- One technical factor:
  - Repetitions (1,2)

Let Y_{rdtlm} denote the expression of a given gene in the "r" replicate of the line "l" at moment m when the density is "d" and the treatment "t" and the general proposed model for the log of the averaged expression is:

$$\log(EY_{rdtlm}) = \log(N_{rdtlm}) + \log(\lambda_{rdtlm})$$

Where:

- $\log(N_{rdtlm})$ is an offset calculated during the normalization step. N_{rdtlm} denotes the library size of the sample described by the indexes (rdtlm)

- $\log(\lambda_{rdtlm})$ is the proportion of reads mapped on the gene under study in the sample described by the indexes {rdtlm}.

According to the experimental design, we assumed that the model contains all the biological and technical factors of our experiment, and the possible interactions between the three biological factors.

$$\log(\lambda)\{rdtlm\} = \text{Intercept} + \text{Replicate}_r + \text{Density}_d + \text{Treatment}_t + \text{Moment}_m + \text{Line}_l + (\text{Treatment}_t \times \text{Moment}_m) + (\text{Moment}_m \times \text{Line}_l) + (\text{Treatment}_t \times \text{Line}_l) + \text{Line}_l \times \text{Treatment}_t \times \text{Moment}_m.$$

Chapter 3: Field evaluation of different agronomic and physiological traits related to senescence under abiotic stresses

III. Chapter 3: Field evaluation of different agronomic and physiological traits related to senescence under abiotic stresses

3.1. Introduction

As global climate change and population growth lead to increasing expectations of crop yield losses, there is an urgent need to accelerate plant breeding for discovering new characteristics to increase yield potential and better adaptation to abiotic stresses to ensure food availability and to satisfy future demand of agricultural production (Abdelrahman et al., 2017). Based on this, selection of stay-green (SG) genotypes can be an important strategy for increasing crop yield to meet expected population growth demands, particularly under abiotic stresses conditions (Kamal et al., 2019).

SG genotypes are characterized by delayed senescence and loss of chlorophyll (Chl) compared with the NSG genotypes (Kamal et al., 2019). For this reason, SG phenotype was considered an important agronomic trait, which enables plants to maintain the photosynthetic activity even under stress conditions, and subsequently improves the grain-filling process (Zhang et al., 2019; Clay et al., 2009). There are two types of SG, functional and non-functional. Functional SG genotypes are agronomically important because they can maintain photosynthetic capacity for a longer period than non-NSG genotypes (Kamal et al., 2019).

Maize is one of the three major cereal crops. It is not only a staple food, but also a raw material for feed and bioenergy. In a constantly changing world, increasing the yield potential, stability and performance of maize is of paramount importance for global food security (Wang et al., 2016). Delayed senescence of SG maize hybrids can lead to higher dry mater accumulation, compared with NSG hybrids (Pommel et al., 2006).

In addition to the beneficial effects of SG trait in post-silking dry matter accumulation and post-silking nitrogen (N) uptake (PostN), SG improves grain yield (Borrell and Hammer, 2000). Modern maize hybrids can accumulate more than 10% of dry matter and nitrogen during grain filling than older hybrids (Rajcan and Tollenaar, 1999). Delayed leaf senescence allows the source tissues to continue to produce, recycle, and remobilize photosynthetic products for a longer period of time, ultimately helping to increase grain yield and quality (Gregersen et al., 2008).

Nitrogen plays an important role in plant nutrition, and it can also be combined with various abiotic stresses like salinity or drought (Fahad et al., 2016), but this mineral element is usually deficient in cultivated soil. Although the demand for nitrogen is the largest among all mineral elements, its deficiency limits growth and development of plants (Fahad et al., 2016). There is a strict regulation of carbon and nitrogen metabolism in photosynthesis and N uptake (Gutierrez et al., 2008). In Addition to nitrogen deficit, water deficit is the most detrimental environmental stress that adversely affects maize productivity (Rafique, 2020). Finally, increasing plant density is an important strategy to increase maize yield (Duvick, 2005). Even, when plant density is too high, it can reduce individual plant production, and improper control may even reduce yields (Ren et al., 2017).

Some previous studies discussed the relationship between maize SG phenotype and some agronomic and physiological traits under individual abiotic stress. However, there is limited information on the effects of combined abiotic stresses on the different agronomic and physiological traits of SG and NSG genotypes. In our research, we focus on the relationship between stay green and several physiological and agronomic traits related to senescence under drought, low nitrogen and high plant density stresses. This was made with two-years field trial conducted at two locations.

3.2. Results

The analysis of variance and means' comparison was made with data obtained from trials in two locations and two years, where each location of each year was considered as an environment with two repetitions in each environment (Exp1: Tomeza 2018; Exp 2: Xinzo 2018; Exp 3: Tomeza 2019; and Exp 4: Xinzo 2019). In order to organize the presentation of the results, all analyzed features will be divided in four groups: (i) Physiological traits related to senescence including chlorophyll content, photosynthetic rate, quantum efficiency of photosystem II (F_v/F_m) and stomatic conductance; (ii) phenological and stover traits, including days to silking (FF), anthesis silking interval (ASI), and physiological maturity or black layer, stover yield and moisture at silking and at harvest time, and stover remobilization and uptake after silking; (iii) Ear related traits including weight of 1000 grains, grain moisture, cob yield and moisture (Tables 4 and 5)**;** (iv) Nitrogen uptake and remobilization in plant and soil. For agronomic traits (stover and grains

yield), the results were obtained from eight genotypes, 4 SG and 4 NSG. The results of physiological traits were obtained from two genotypes, namely PHW79 with SG phenotype, and the NSG genotype B73. However, for nitrogen assimilation and remobilization the results were obtained from six genotype 3 SG and 3 NSG.

3.2.1. Effect of abiotic stresses in the physiological activity for SG and NSG genotypes

3.2.3.1. Physiological activity and SG phenotype

To compare between SG and NSG genotypes for their physiological activity during different senescence times; repeated measures of different physiological traits for chlorophyll content, quantum efficiency of photosystem II (F_v/F_m), photosynthetic rate and stomatic conductance were taken at silking, 30, 45 and 60 days after silking (DAS). The maximum value was recorded at silking stage for both genotypes, then declined to attain minimal value or zero at 60 DAS.

For all physiological traits, we found a significant decrease during senescence period; but the magnitude of decrease was lower for SG genotype PHW79; in opposite to NSG genotype B73, where the decrease was more expressed. The variation of chlorophyll content during senescence was significantly different for the time × genotype interaction, which mean the behavior of each genotype during senescence was different. The decrease of chlorophyll content was significantly different between SG and NSG genotypes. From silking to complete plant senescence, SG genotype had higher value of chlorophyll content, and the decrease was more consistent during senescence to attain the minimal value at 60 DAS. For the NSG genotype, the decrease of chlorophyll content after silking was faster to attain 0 SPAD at 60 DAS (Figure 6, Annex 3: Table 1).

Photosynthetic rate ($\mu mol.CO_2m^{-2}S^{-1}$) was significantly different between B73 and PHW79. B73 had higher photosynthetic rate during silking, compared to PHW79; but, after silking, B73 lose their photosynthetic rate fast to attain zero at 60 DAS. Conversely, PHW79 showed a slower decline of their photosynthetic rate during successive senescence times to attain the minimal value of 5 $\mu mol.CO_2m^{-2}S^{-1}$ at 60 DAS (Figure 7, Annex 3: Table 3).

For quantum efficiency of photosystem II (F_v/F_m) ($\mu mol.m^{-2}.s^{-1}$), the same trend was obtained compared to chlorophyll content, where the difference between both genotypes was significant. From silking to 30 DAS, both genotypes had the maximal value; then, after 30 DAS, SNG genotype loss their quantum efficiency F_v/F_m fastly to reach zero at 60DAS, while SG genotype show a slow decline at 30 DAS, to reach a value of 0.6 $\mu mol.m^{-2}.s^{-1}$ at 60DAS (Figure 8, Annex 3: Table 2).

Finally, for stomatic conductance (mmol $H_20.m^{-2}.s^{-1}$) the time × genotype interaction was not significant. Nevertheless, after 30 DAS, PHW79 maintain their stomatic conductance relatively high until 45 DAS, but still had a value different from zero at 60 DAS. While, in B73 the decline was started at 30 DAS to attain a zero value at 60 DAS (Figure 9, Annex 3; Table 4).

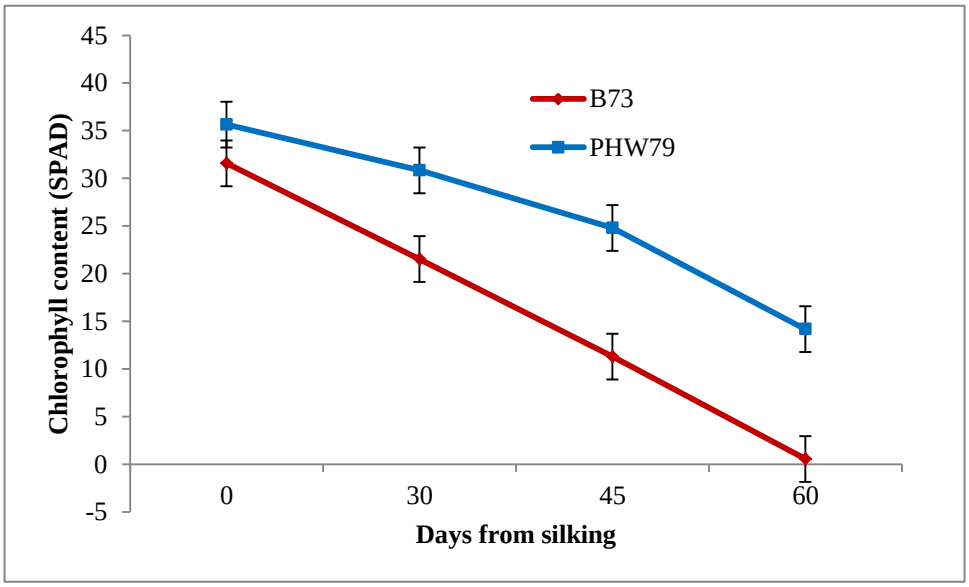

Figure 6. Means of chlorophyll content and standard error of two maize inbred lines with opposite characteristic for SG phenotype, evaluated for two years from silking to sixty days after silking under abiotic stresses of drought, low nitrogen and high plant density.

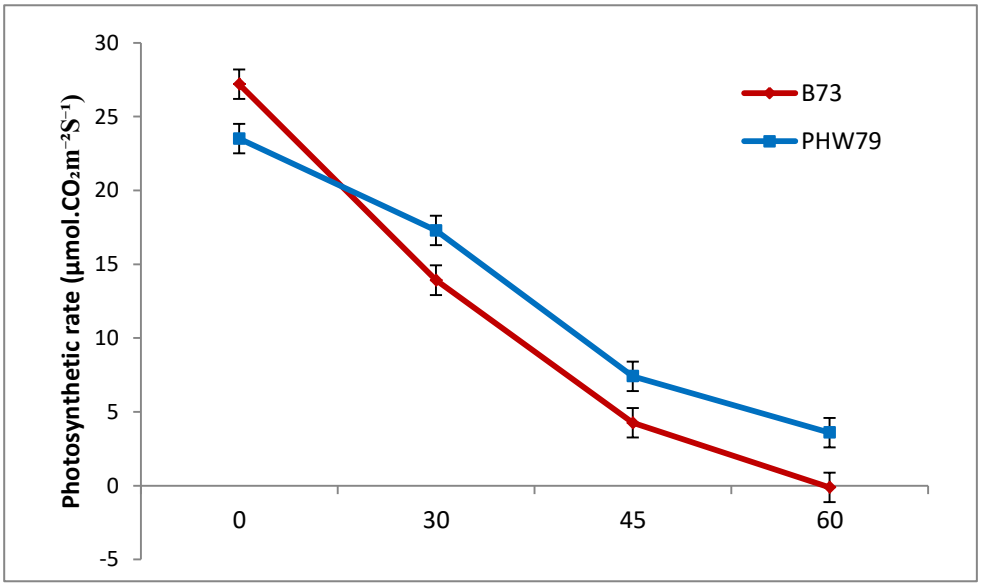

Figure 7. Means of photosynthetic rate (µmol.$CO_2m^{-2}S^{-1}$) and standard error of two maize inbred lines with opposite characteristic for SG phenotype, evaluated for two years from silking to sixty days after silking under abiotic stresses of drought, low nitrogen and high plant density

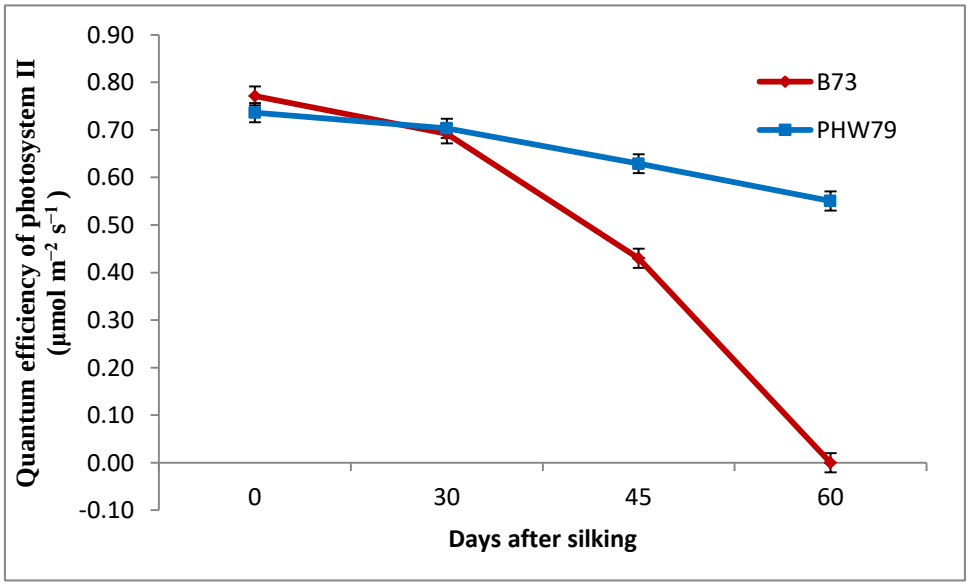

Figure 8. Means of quantum efficiency of photosystem II (F_v/F_m) (µmol.m^{-2}.s^{-1}) and standard error of two maize inbred lines with opposite characteristic for SG phenotype, evaluated from silking to sixty days after silking under abiotic stresses of drought, low nitrogen and high plant density.

.

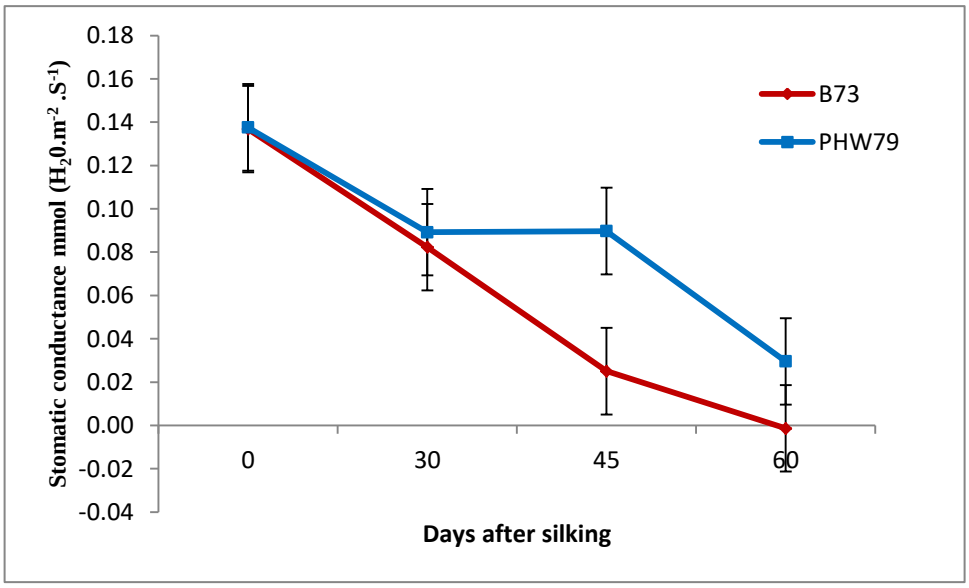

Figure 9. Means of stomatic conductance (mmol $H_20.m^2.s^{-1}$) and standard error of two maize inbred lines with opposite characteristic for SG phenotype, evaluated for two years from silking to sixty days after silking under abiotic stresses of drought, low nitrogen and high plant density.

3.2.3.2. Physiological activity and abiotic stresses

3.2.3.2.1. Drought and nitrogen stress

For all physiological traits of the SG and NSG genotype evaluated in 4 trials, the results show significant differences between different treatments of both genotypes along senescence times; only for stomatic conductance, differences were not significant between treatments (Annex 3: Table 1, 2, 3, and 4).

For chlorophyll content, the highest values were detected under optimum water (ON3, ON2, and ON1) condition compared to water stress conditions (SN3, SN2, and SN1). Among nitrogen levels, the highest value of chlorophyll showed under N3, and lower under N2 and N1. During all senescence times, ON3 treatment shows the maximal value of chlorophyll content (Figure 10).

For F_v/F_m, the maximum value of F_v/F_m was obtained under optimum water conditions at 30 and 45 DAS. For nitrogen level, the highest value was found at N3 under optimum water condition and N2 under water stress. The treatment ON3 had the highest value at 45 DAS (Figure 11).

Finally, concerning the effect of nitrogen and drought on photosynthetic rate, both stresses had a large effect after silking time. The photosynthetic rate was lower under water stress compared to optimum water conditions. A similar pattern was found for nitrogen levels, where the highest value was shown in N3 under both water conditions. During silking and at 30 DAS, the maximal value of photosynthetic rate was shown under ON3 treatment. After 45 DAS, when the plant starts to loss their photosynthetic activity, the effect of both stresses was not significant, and no differences were detected between treatments (Figure 12).

For all physiological traits, our results indicate that both nitrogen and drought stresses have a negative effect on physiological activity of both genotypes; though their effects were more expressed for NSG genotype.

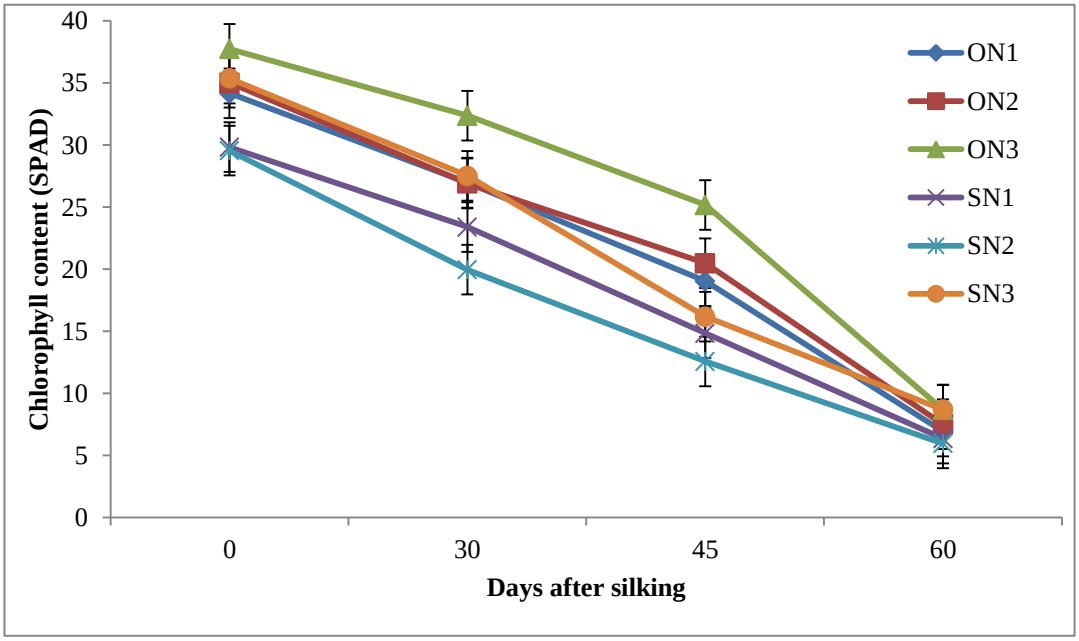

Figure 10. Means comparison for changes in chlorophyll content (SPAD) for both genotypes during senescence period for different treatments of water and nitrogen level (ON1: optimum water and low N (0U) condition; ON1: optimum water and medium N (30U) condition; ON3: optimum water and higher N (90U) condition; SN1: water stress and low N (0U); SN2 water stress and medium N(30U) condition; SN3: water stress and higher N(90U) condition)

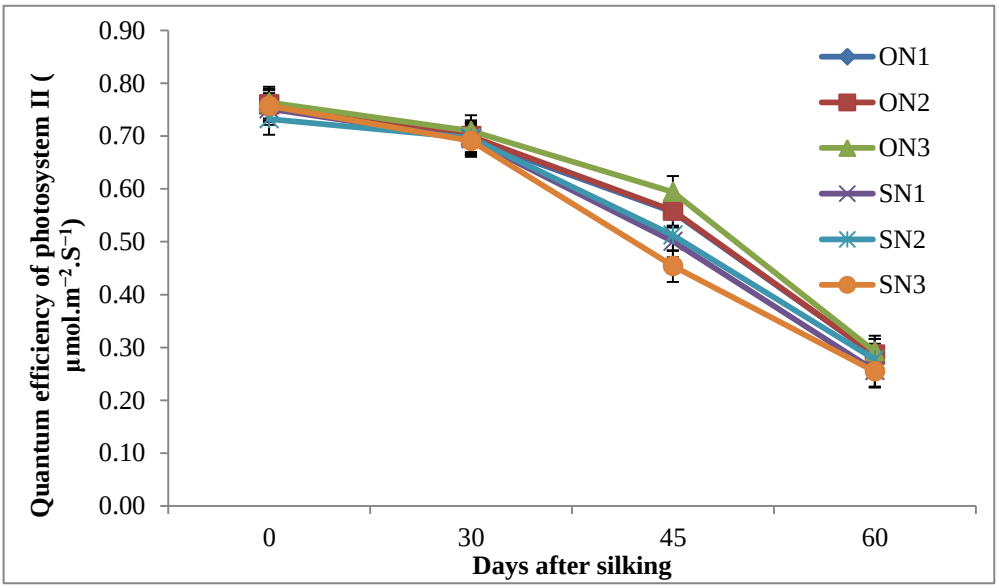

Figure 11. Means comparison of change in Quantum efficiency of photosystem II (F_v/F_m)(µmol.m^{-2}.s^{-1}) for both genotypes during senescence period for different treatments of water and nitrogen level (ON1: optimum water and low N (0U) condition; ON1: optimum water and medium N (30U) condition; ON3: optimum water and higher N(90U) condition; SN1: water stress and low N (0U); SN2 water stress and medium N (30U) condition; SN3: water stress and higher N (90U) condition).

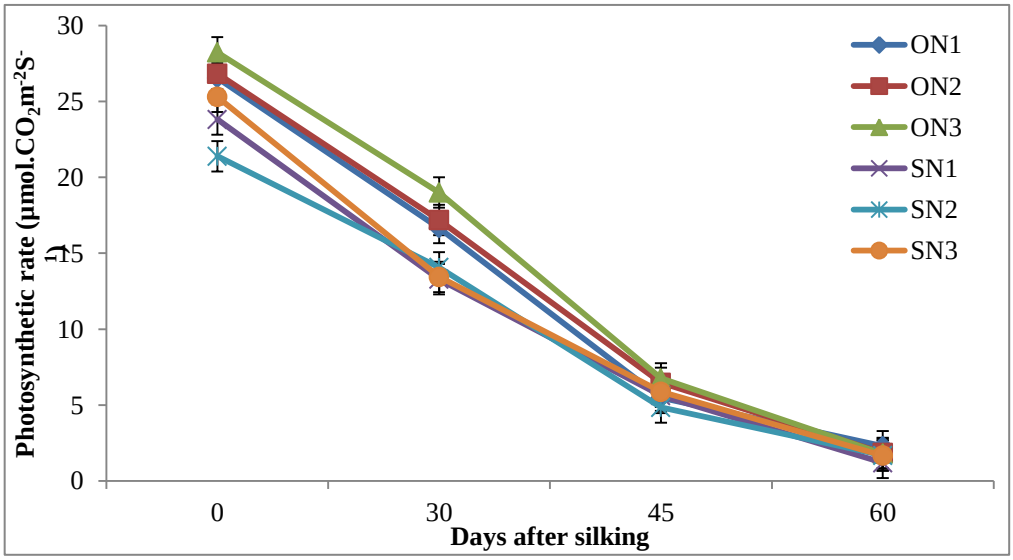

Figure 12. Means comparison of change in Photosynthetic rate (µmol.CO^2.m^{-2}.S^{-1}) for both genotypes during senescence period for different treatments of water and nitrogen level (ON1: optimum water and low N (0U) condition; ON1: optimum water and medium N (30U) condition; ON3: optimum water and higher N(90U) condition; SN1: water stress and low N(0U); SN2 water stress and medium N(30U) condition; SN3: water stress and higher N(90U) condition).

3.2.3.2.2. Plant density

For plant density, no significant difference was found between both densities of planting for most physiological traits; except for chlorophyll content, where the result showed significant differences between both densities during senescence times (Annex 3: Table 1, 2, 3, and 4). During silking, 30 and 45 DAS, the chlorophyll content was higher under low plant density compared to high plant density, which mean high plant density caused plants competition, and accelerate chlorophyll loss in plants (Figure 13).

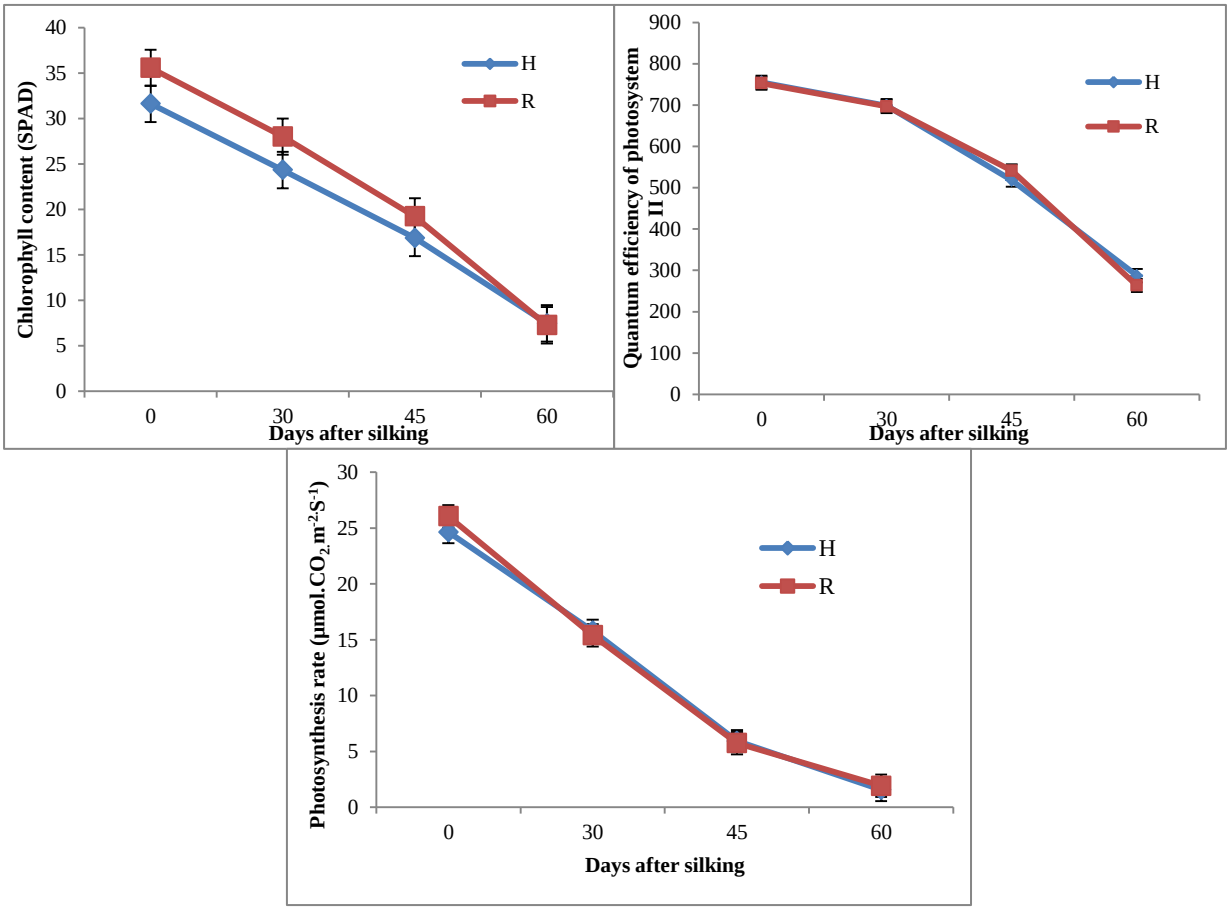

Figure 13. Means comparison of change in different physiological traits of both genotypes during senescence period under two plant density level (H: high plant density; R: low plant density).

3.2.4. Effect of abiotic stresses for phenological and stover yield of SG and NSG inbred lines during senescence

For phenological data, for days to silking (FF) and ASI, where the ASI is defined as the interval from the tassel shedding pollen to the emergence of silk over the husks (Oury et al., 2016), the results show no significant difference between SG and NSG genotypes; which is around 86 days for FF and 3 days for ASI (Table 4, Annex 1: Table 1 and 2).

For the black layer (BL) or the physiological maturity, which was measured as the days numbers form silking time to the presence of black layer in the kernel, there were significant differences between SG and NSG genotypes. The SG genotypes need more time to reach physiological maturity (80.4 days) compared to NSG genotypes with 77.7 days (Table 4; Annex 1: Table 3).

Nitrogen level and plant density had significant effects on days to silking and ASI, and water conditions also affected ASI significantly. Under water stress, FF and ASI were 86 and 3.1 days, respectively; and under optimum water FF and ASI were 85.5 and 2.4 days, respectively. For different nitrogen levels, FF and ASI were 85.2 and 2.3 days in N3, 86 and 3 in N2, and 86.1 and 3 days in N1, respectively; where the nitrogen level N3 differed significantly from N2 and N1 for both traits. Finally for plant density, FF and ASI varied significantly from 85.3 and 2.4 under lower plant density to 86.3 and 3.1 days under high plant density, respectively (Table 4, Annex 1: Table 1 and 2).

In the current study, there were significant differences between water stresses and optimum water conditions for BL trait; under water stress condition, BL was reduced to 78 days, compared to optimum condition 80 days, which mean that drought can accelerate senescence. Conversely, nitrogen and plant density stress, or the interaction water conditon×nitrogen level have no significant difference for BL trait (Table 4, Annex 1: Table 3).

The combined analysis of variance for two years trials showed significant differences between SG and NSG genotypes for stover yield and stover moisture at silking and harvest time, and also for stover yield remobilized from silking to harvest (Table 4, Annex 1: Table 4, 5, and 6). At silking, NSG genotypes presented higher stover yield and moisture compared to SG genotypes. However, at harvest, SG genotypes showed higher stover yield and moisture (9690 kg ha^{-1}, 69.3%), respectively; compared to NSG (9061 kg ha^{-1} and 67.5%), respectively (Table 4, Annex 1: Table 4, 5, and 6).

The comparison between SG and NSG genotypes for stover remobilization, which is the difference between stover yield at silking and harvest time, showed a significant difference for the remobilization, where the NSG remobilized more biomass (5589 kg ha^{-1}) from silking to harvest, compared to the SG genotype, which remobilized just 3519 kg ha^{-1}. The NSG genotypes remobilize 21% from total stover yield at silking; while, the SG remobilized only 11% of the total stover yield at silking, which, is about half quantity of the biomass remobilized by the NSG genotypes (Figure 14 and Table 4, Annex 1: Table 11).

The analyses of variance for all abiotic stresses included in this study (drought, low nitrogen and high plant density) and combined stresses showed significant effects of all stresses on stover yield and moisture. However, the result of different stresses interactions were not significant for most studied traits, except the water control × nitrogen level interaction that showed significant differences for most studied traits. At silking time, stover yield under both water conditions and different nitrogen levels have no significant differences. While, the water condition × nitrogen levels interaction was highly significant for stover yield at silking, where, optimum water_N2 and optimum water_N3 represent the highest stover yield, with 16282 kg ha^{-1} and 15022 kg ha^{-1}, respectively. Optimum water × N1 represent the lowest one with 11885 kg ha^{-1}. Finally, for plant density, our result show that stover yield (SYF) was higher under high plant density (16177 kg ha^{-1}), compared to lower plant density (11738 kg ha^{-1}) (Table 4, Annex 1: Table 4, 5, and 6); however, the specific analysis for stover production for individual plant under both plant densities show higher stover yield under lower plant density in both silking and harvest times (235.5 and 157.2 g $plant^{-1}$, respectively), compared to high plant density (201.9 and 137.2 g $plant^{-1}$, respectively) (Annex 2).

All stresses had significant effects for stover yield at harvest time, which represent the stover yield non_remobilized. The stover yield was higher under optimal conditions compared to stress conditions. For water conditions, the stover yield was 9952 kg ha^{-1} under optimum water condition compared to 8799 kg ha^{-1} under water stress conditions. For nitrogen levels, the highest stover yield was for N3 with 9981 kg ha^{-1}, while N2 and N1 yielded 9124 kg ha^{-1} and 9022 kg ha^{-1}, respectively. The water condition × nitrogen levels interaction was also significant, where the highest stover yield was found under optimum water × N3 (10700 kg ha^{-1}) and the lowest one under optimum water × N1 and water stress × N1 (8905 and 9138 kg ha^{-1}, respectively) (Table 4).

The stover remobilization from silking to harvest was significantly affected by plant density. Under high plant density, the remobilization was higher (5290 kg ha^{-1}) than under low plant density (3818 kg ha^{-1}) (Table 4). The interaction water condition × nitrogen levels were significant for stover remobilization. The highest level of remobilization was shown under optimum water_N2 and water stress_N1, and lowest value under optimum water_N1.

Water stress reduces stover moisture at silking and harvest (82.4% and 68% at silking and harvest time, respectively), compared to optimum water conditions (83.5 and 68.8% at silking and harvest, respectively). Stover moisture at silking was lower under N1 (82.7%) and N2 (82.6%), compared to N3 (83.5%). The water condition × nitrogen levels interaction had a significant effect on stover moisture at silking, where the highest value of stover moisture was under optimum water × N3 (84.0%), and the lowest under water stress × N2 (81.7%). At harvest time nitrogen levels, plant density and the interaction water condition × nitrogen levels have not a significant effect on stover moisture (Table 4, Annex 1: Table 5 and 10).

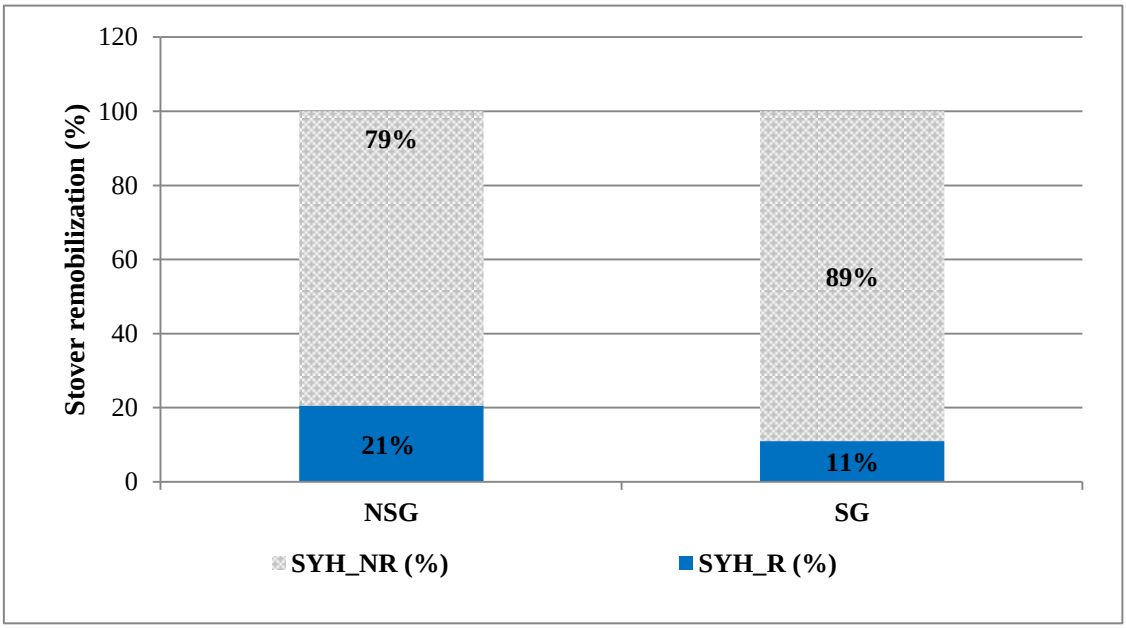

Figure 14. Percentages of remobilized or non-remobilized Stover yield for SG and NSG genotypes at harvest time, evaluated in 2018 and 2019 in two locations in Galicia. SYH_NR: stover yield non-remobilized at harvest; SY_RH: Stover yield remobilized at Harvest; SG: stay-green genotypes. NSG: non-stay-green genotypes.

Table 4. Means[1], standard errors, and comparison between SG[2] and NSG for stover production at silking and harvest time under different conditions of water, nitrogen and plant density evaluated in 2018 and 2019 in two locations in Galicia.

Factors	levels	FF (Days)	ASI(Days)	BL (days)	SYF (Kg ha^{-1})	SMF (%)	SYH_NR (kg ha^{-1})	SYH_R (kg ha^{-1})	SMH (%)
WC	Opti	85.5 ± 4.2a^{ns}	2.4 ± 0.8a**	79.9 ± 2a**	14396 ± 6309a^{ns}	83.5 ± 0.8a***	9952 ± 2562a***	4406 ± 3866a^{ns}	68.8 ± 2.5a*
	WS	86.0 ± 4.2a	3.1 ± 0.8b	78.3 ± 2b	13519 ± 6309a	82.4 ± 0.8b	8799 ± 2562b	4702 ± 3866a	68.0 ± 2.5a
NL	N3	85.2 ± 4.2a*	2.3 ± 0.8a*	79.5 ± a2^{ns}	14484 ± 6309a^{ns}	83.5 ± 0.8a***	9981 ± 2562a**	4508 ± 3866a^{ns}	68.2 ± 2.5a^{ns}
	N2	86.0 ± 4.2b	3.0 ± 0.8b	79.2 ± 2a	14266 ± 6309ab	82.6 ± 0.8b	9124 ± 2562b	5002 ± 3866a	68.5 ± 2.5a
	N1	86.1 ± 4.2b	3.0 ± 0.8b	78.6 ± 2a	13123 ± 6309b	82.7 ± 0.8b	9022 ± 2562b	4151 ± 2553a	68.44 ± 2.5a
PD	R	85.3 ± 4.2a**	2.4 ± 0.8a**	79.2 ± 2a^{ns}	11738 ± 6309a***	83.1 ± 0.8a^{ns}	7907 ± 2562a***	3818 ± 3866a**	68.2 ± 2.5a^{ns}
	H	86.3 ± 4.2b	3.1 ± 0.8b	78.9 ± 2a	16177 ± 6309b	82.8 ± 0.8a	10844 ± 2562b	5290 ± 3866b	68.6 ± 2.5a
WC × NL	Opti_N3	85.1 ± 4.2a^{ns}	2.1 ± 0.8a^{ns}	80.4 ± 2a^{ns}	15022 ± 6309ab***	84.0 ± 0.8a***	10700 ± 2562a ***	4239 ± 3866bc**	68.8 ± 2.5a^{ns}
	Opti_N2	85.4 ± 4.2a	2.4 ± 0.8ab	80.2 ± 2a	16282 ± 6309a	83.6 ± 0.8a	10252 ± 2562a	5895 ± 3866a	68.6 ± 2.5a
	Opti_N1	86.2 ± 4.2ab	2.8 ± 0.8abc	79.0 ± 2ab	11885 ± 6309d	82.9 ± 0.8bc	8905 ± 2562 b	3085 ± 3866c	69.0 ± 2.5a
	WS_N3	85.2 ± 4.2a	2.5 ± 0.8ab	78.5 ± 2b	13945 ± 6309bc	83.1 ± 0.8b	9262 ± 2562b	4778 ± 3866ab	67.7 ± 2.5a
	WS_N2	86.7 ± 4.2b	3.6 ± 0.8c	78.2 ± 2b	12250 ± 6309cd	81.7 ± 0.8d	7997 ± 2562c	4110 ± 3866bc	68.4 ± 2.5a
	WS_N1	86.0 ± 4.2ab	3.2 ± 0.8cb	78.2 ± 2b	14362 ± 6309b	82.5 ± 0.8c	9138 ± 2562b	5217 ± 3866 a	67.9 ± 2.5a
SGT	NSG	85.9 ± 4.2a^{ns}	2.6 ±0.8a^{ns}	77.7 ± 2a***	14725 ± 6309a**	83.6 ± 0.8a***	9061 ± 2562a**	5589 ± 3866a***	67.5 ± 2.5a***
	SG	85.6 ± 4.2a	2.9 ± 0.8a	80.4 ± 2b	13190 ± 6309a	82.3 ± 0.8b	9690 ± 2562b	3519 ± 3866b	69.3 ± 2.5b

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; SG: Stay-green genotypes; NSG: non-stay-green genotypes; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively.; Opti_N3, Opti_N2, Opti_N1, WS_N3, WS_N2, WS_N1: interaction between nitrogen and water conditions; FF: silking days; SYF(kg ha^{-1}): stover yield at flowering (kg ha^{-1}); SMF: stover moisture at flowering (%); SYH_NR(kg ha^{-1}): stover yield at harvest time (kg ha^{-}1); SMH: stover moisture at harvest (%); SYH_R (kg ha^{-1}) : Stover yield remobilized from silking to harvest. a; b; and c: different groUps of significant traits within each factor of study.
*, ** and *** Significant effect of each factor for each character at p = 0.05, p = 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

3.2.5. Ear related traits for SG and NSG inbred lines under abiotic stresses

There were significant differences between SG and NSG genotypes for cobs yield and moisture, weight of 1000 grains (1000KW), and grains moisture (Table 5, Annex 1:Table 12, 13, 14 and 15).

The SG genotypes had higher cobs yield (1232.2 kg ha^{-1}), compared to NSG (978. 2 kg ha^{-1}). A similar pattern was observed for kernel weight (1000KW) as the SG genotypes reached the greatest 1000KW with 278.9 g; while the NSG genotype had significantly lower kernel weight (239.6 g). Significant differences were also found between SG and NSG genotypes for cobs and grain moisture; in both cases, SG genotypes showed high moisture value 56.3% and 32.4% for CM and KM, respectively; compared to NSG genotypes (54.1 and 30.8%) (Table 5, Annex 1: Table 12, 13, 14 and 15).

CY and 1000KW under water stress condition were 1015 kg ha^{-1} and 252 g, respectively; compared to 1194 kg ha^{-1} and 266.5 g under optimum water conditions, and those differences were statistically significant. For nitrogen level, there was a significant differences for CY; but not significant for 1000KW. The value of CY under N3 (1169 kg ha^{-1}) was higher than the value of, N2 (1048 kg ha^{-1}) and N1 (1098 kg ha^{-1}). The interaction water conditions × nitrogen levels was significant for CY and 1000KW. The maximum CY and 1000KW was obtained under optimum water × N3 (1253.0 kg ha^{-1} and 268.3g); and the lowest CY and 1000KW were obtained under water stress_N2 (883.9 kg ha^{-1} and 243.6g, respectively). Finally, for plant density the highest value of CW was observed under high plant density (1303.5 kg ha^{-1}), compared to 906.8 kg ha^{-1} for low plant density. For 1000KW, the highest value was obtained under low plant density with 262.1 kg ha^{-1}, compared to high PD (256.4 kg ha^{-1}). Our results show that abiotic stresses did not have generally a clear effect for moisture content in cobs and grains. Effects were significant only for CM under nitrogen level, plant density and nitrogen level × water condition (Table 5, Annex 1: Table 13 and 15).

Table 5. Means[1], standard errors, and comparisons between SG[2] and NSG for grain yield under different conditions of water, nitrogen and plant density evaluated in 2018 and 2019 in two locations in Galicia.

Factors	levels	CY (kg ha^{-1})	CM (%)	1000KW (g)	KM (%)
WC	Opti	1194 ± 175a***	55.1 ± 5a^{ns}	266.5 ± 17a***	31.2 ± 5a^{ns}
	WS	1015 ± 175b	55.3 ± 5a	252.0 ± 17b	32.1 ± 5a
NL	N3	1169 ± 175a***	54.4 ± 5ab*	262.5 ± 17a^{ns}	31.4 ± 5a^{ns}
	N2	1048 ± 175b	56.3 ± 5a	255.7 ± 17b	31.8 ± 5a
	N1	1098 ± 175b	55.0 ± 5b	259.5 ± 17ab	31.7 ± 5a
PD	R	906.8 ± 175a***	54.4 ± 5a*	262.1 ± 17a*	31.4 ± 5a^{ns}
	H	1303.5 ± 175b	56.0 ± 5b	256.4 ± 17b	31.8 ± 5a
WC * NL	Opti_N3	1253.0 ± 175a***	54.5 ± 5b**	268.3 ± 17a*	31.1 ± 5a^{ns}
	Opti_N2	1211.8 ± 175 a	54.9 ± 5b	267.8 ± 17a	31.1 ±5a
	Opti_N1	1118.1 ± 175b	56.0 ±5ab	263.3 ± 17ab	31.4 ± 5a
	WS_N3	1085.5 ± 175b	54.2 ± 5b	256.7 ± 17b	31.8 ± 5a
	WS_N2	883.9 ± 175c	57.7 ± 5a	243.6 ± 17c	32.5 ± 5a
	WS_N1	1078.8 ± 175b	53.9 ± 5b	255.7 ± 17b	31.9 ± 5a
SGT	NSG	978.2 ± 175a***	54.1 ± 5a***	239.6 ± 17a***	30.8 ± 5a***
	SG	1232.2 ± 175b	56.3 ± 5b	278.9 ± 17b	32.4 ± 5b

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; SG: Stay-green genotypes; NSG: non-stay-green genotypes; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively.; Opti_N3, Opti_N2, Opti_N1, WS_N3, WS_N2, WS_N1: interaction between nitrogen and water conditions; CY: cobs yield ((kg ha^{-1}); CM: cobs moisture (%); 1000KW:kernel weight of 1000 grains; KM: kernel moisture. a; b; and c: different groups of significant traits within each factor of study.

*, ** and *** Significant effect of each factor for each character at p = 0.05, p = 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

3.2.6. Effect of abiotic stresses in Nitrogen assimilation and remobilization in soil and plant for SG and NSG genotypes

For better understanding of nitrogen assimilation and remobilization in the plant, we measured nitrogen availability in soil and plant at silking, and then compared it to N availability and remobilization at harvest time. All the analyses of soil and plant were done in 6 genotypes, three with SG phenotype, and the others three with early leaf senescence.

3.2.4.1. Nitrogen and carbon content in soil

For total nitrogen availability in the soil, at first we analyzed 6 genotypes for the first year 2018 (Table 6; Annex 4a: Table 1, 5, 9, 10, 11, and 12), then we carried out the analyses only in two genotypes, PHW79 and B73, for both years; this is for the availability of two genotypes during the second year of trials 2019 (Table 7, Annex 4a: 3, and 7). For both analyses of first or both years, we did not find significant differences between SG and NSG genotypes. For the effect of abiotic stresses in the nitrogen availability at silking and harvest time; we can show that for the first year 2018 of field experiment in both locations there is a significant effect of water conditions and water condition × nitrogen levels interaction at silking time. At silking time, the total nitrogen availability was higher under optimum water (1.5 g kg^{-1}) compared to water stress (1.4 g kg^{-1}). For the water condition × nitrogen levels interaction the highest values were shown under optimum water_N3 (1.6 g kg^{-1}) and optimum water_N1 (1.6 g kg^{-1}); and the lowest value was found under water stress_N3 (1.3 g kg^{-1}). However, at harvest time, we detected a significant effect for nitrogen level and for the water conditions × nitrogen levels interaction. At harvest time, the highest value was found under N3 and N2 (1.7 g kg^{-1}), compared to N1 (1.5 g kg^{-1}).

The analysis of the fourth experiments for both genotypes, the results show that only the effect of water control was significant at silking time; where under optimum water the total nitrogen content was 1.7 g kg^{-1} compared to 1.5 g kg^{-1} under water stress (Table 7).

NO_3 results for Expe1 and Exp 2 show that, the value of N-NO_3 availability in the soil at silking time was higher than harvest time for all treatments. The result of NO_3 shows significant differences under drought and nitrogen stress in both silking and harvest time. At silking time, the maximum quantity of NO_3 was found under optimum water conditions (21.8 g kg^{-1}), compared to water stress 18.7 g kg^{-1}. For nitrogen level, our results show that the highest value of NO_3 was obtained under N3 (23.8 g kg^{-1}), and N2 (20.9 g kg^{-1}), compared to N1 (16.2 g kg^{-1}). At harvest

time, the availability of NO_3 under optimum water was lower (5.9 g kg^{-1}), than under water stress (8.8 g kg^{-1}) (Table 6). For NH_4 assimilate, no significant differences were recorded between genotypes, and between different abiotic stresses. This may be due to the lower rate of assimilation of this nitrogen form (NH_4) compared to the assimilation rate of NO_3. Our result shows also that plant density do not have a significant effect for total nitrogen or nutrients availability in soil (Table 6, Annex 4a: Table 9, 10, 11 and 12).

For the carbon content in soil at silking and harvest time for both analysis of Exp1 and 2 or all 4^{th} experiment, the result show no significant differences between SG and NSG genotypes. However, at the Exp 1 and 2 we detected significant water conditions × nitrogen levels interaction at silking time. While, for the analysis of all 4 Exp was no significant for carbon content under different abiotic stresses (Tables 6 and 7; Annex 4a: Table 2, 4, 6, 8).

Table 6. Means[1], standard errors, and comparison between six SG[2] and NSG for nitrogen content in soil at silking and harvest time under different conditions of water, nitrogen and plant density evaluated in 2018 in six locations in Galicia.

Factors	levels	TNS (g kg^{-1})	TNH(g kg^{-1})	TCS (g kg^{-1})	TCH(g kg^{-1})	NO_3_S(mg kg^{-1})	NH_4_S(mg kg^{-1})	NO_3_H(mg kg^{-1})	NH_4_H(mg kg^{-1})
WC	Opti	1.5± 0.4a**	1.5 ± 0.05a^{ns}	18.3 ±7.6a^{ns}	18.2 ± 0.7a^{ns}	21.8 ± 10a*	7.9 ± 5.4a^{ns}	5.9 ± 2.3a***	9.1 ± 1.4a^{ns}
	WS	1.4 ± 0.4b	1.6 ± 0.05a	18.6 ±7.6a	18.7 ± 0.7a	18.7 ± 10b	9.2 ± 5.4a	8.8 ± 2.3b	8.1 ± 1.4a
NL	N3	1.5 ± 0.4a^{ns}	1.7 ± 0.05a*	18.2 ± 7.5a^{ns}	19.5 ±0.8a*	23.78 ± 10a***	8.2 ± 5.4a^{ns}	7.9 ± 2.3a**	9.1 ± 1.4a^{ns}
	N2	1.5 ± 0.4a	1.7 ± 0.05a	18.7 ± 7.5a	19.1 ± 0.8a	20.9 ± 10a	8.9 ± 5.4a	7.8 ± 2.3a	7.6 ± 1.4a
	N1	1.5 ± 0.4a	1.5 ± 0.05b	18.4 ± 7.5a	16.8 ± 0.8b	16.2 ± 10b	8.5 ± 5.4a	6.4 ± 2.3b	9.2 ± 1.4a
WC x NL	Opti_N3	1.6±0.4a***	1.6 ±0.05ab**	18.8 ± 7.5ab**	19.2 ± 1.1ab**	24.2 ±10a^{ns}	6.7 ± 5.4a	5.5± 2.3c**	9.8 ±1.6a
	Opti_N2	1.4±0.4bc	1.5 ±0.05bc	17.7 ± 7.5b	16.9 ± 1.1 bc	23.4 ±10a	7.7 ± 5.4ab	6.7± 2.3bc	8.8 ±1.6ab
	Opti_N1	1.6±0.4a	1.6 ±0.05ab	18.3 ± 7.5ab	18.6 ± 1.1ab	17.8 ±10b	9.3 ± 5.4ab	5.5± 2.3c	8.8 ±1.6ab
	WS_N3	1.3±0.4c	1.7 ±0.05ab	17.7 ± 7.5 b	19.8 ± 1.1ab	23.4 ±10a	9.7 ± 5.4ab	10.2± 2.3a	8.6±1.6ab
	WS_N2	1.5 ±0.4ab	1.8 ±0.05a	19.6 ± 7.5a	21.4 ± 1.1a	18.3 ±10b	10.2 ± 5.4b	8.9± 2.3a	6.3±1.6b
	WS_N1	1.4 ±0.4bc	1.3 ±0.05c	18.5 ± 7.5ab	15.0 ± 1.1c	14.6±10b	7.8 ± 5.4ab	7.4± 2.3b	9.5±1.6a
PD	R	1.5 ± 0.4a^{ns}	1.6 ± 0.05a^{ns}	18.4± 7.5a^{ns}	18.6 ± 0.7a^{ns}	19.1 ± 10a^{ns}	8.8 ± 5.4a^{ns}	7.5 ± 2.3a^{ns}	8.7 ± 1.4a^{ns}
	H	1.5 ± 0.4a	1.6 ± 0.05a	18.5 ± 7.5a	18.4 ± 0..7a	21.5 ± 10a	8.3 ± 5.4a	7.3 ± 2.3a	8.6 ± 1.4a
SGT	NSG	1.5 ± 0.4a^{ns}	1.5 ± 0.05a^{ns}	18.4± 7.5a^{ns}	18.3 ± 0.6a^{ns}	19.3 ± 10a^{ns}	9.2 ± 5.4a^{ns}	7.4 ± 2.3a^{ns}	8.3 ± 1.4^{ns}
	SG	1.5 ± 0.4a	1.6 ± 0.05a	18.5± 7.5a	187 ± 0.6a	21.3 ± 10a	7.9 ± 5.4a	7.3 ± 2.3a	8.9 ± 1.4

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; SG: Stay-green genotypes; NSG: non-stay-green genotypes; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively.; TNS (g kg^{-1}): total N content in soil at silking time; TNH(g kg^{-1}): total N content in soil at harvest time; TCS (g kg^{-1}): total C content in soil at silking time; TCH(g kg^{-1}): total C content in soil at harvest time; NO_3_S (mg kg^{-1}): soil content of NO_3 at silking time; NH_4_S (mg kg^{-1}): soil content of NH_4 at silking time; NO_3_H (mg kg^{-1}): soil content of NO_3 at harvest time; NH_4_H (mg kg^{-1}): soil content of NH_4 at harvest time. a; b; and c: different groUps of significant trait within each factor of study.

* ,** and *** Significant effect of each factors for each character at p = 0.05, 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

Table 7. Means[1], standard errors, and comparison between two SG[2] and NSG for nitrogen content in soil at silking and harvest time under different conditions of water, nitrogen and plant density evaluated in 2018 and 2019 in two locations in Galicia.

Factors	levels	TNS (g kg^{-1})	TNH(g kg^{-1})	TCS (g kg^{-1})	TCH(g kg^{-1})
WC	Opti	1.7 ± 0.3a**	1.6 ± 0.2a^{ns}	19.0 ± 4.5a^{ns}	18.6 ± 3.1a^{ns}
	WS	1.5 ± 0.3b	1.7 ± 0.2a	18.5 ± 4.5a	18.7 ± 3.1a
NL	N3	1.6 ± 0.3a^{ns}	1.7 ± 0.2a^{ns}	19.2 ± 4.5a^{ns}	19.2 ± 3.1a^{ns}
	N2	1.6 ± 0.3a	1.7 ± 0.2a	18.7 ± 4.5a	18.9 ± 3.1a
	N1	1.6 ± 0.3a	1.6 ± 0.2a	18.4 ± 4.5a	17.9 ± 3.1a
WC x NL	Opti_N3	1.7 ± 0.3a^{ns}	1.7± 0.2a ns	19.9 ± 4.5a^{ns}	19.4 ± 3.1a^{ns}
	Opti_N2	1.6 ± 0.3ab	1.6± 0.2a	18.7 ± 4.5a	17.8 ± 3.1a
	Opti_N1	1.6 ± 0.3ab	1.7± 0.2a	18.5 ± 4.5a	18.5 ± 3.1a
	WS_N3	1.5 ± 0.3b	1.7± 0.2a	18.5 ± 4.5a	19.0 ± 3.1 a
	WS_N2	1.5 ± 0.3b	1.7± 0.2a	18.7 ± 4.5a	19.9 ± 3.1a
	WS_N1	1.5 ± 0.3b	1.6± 0.2a	18.4 ± 4.5a	17.3 ± 3.1a
PD	R	165 ± 0.3a^{ns}	1.7 ± 0.2a^{ns}	18.6 ± 4.5a^{ns}	18.8 ± 3.1a^{ns}
	H	1.6 ± 0.3a	1.6 ± 0.2a	19.0 ± 4.5a	18.5 ± 3.1a
SGT	NSG	1.6 ± 0.3a^{ns}	1.7 ± 0.2a^{ns}	18.9 ± 4.5a^{ns}	18.8 ± 3.1a^{ns}
	SG	1.6 ± 0.3a	1.6 ± 0.2a	18.6 ± 4.5a	18.5 ± 3.1a

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; SG: Stay-green genotypes; NSG: non-stay-green genotypes; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively.; TNS (g kg^{-1}): total N content in soil at silking time; TNH(g kg^{-1}): total N content in soil at harvest time; TCS (g kg^{-1}): total C content in soil at silking time; TCH(g kg^{-1}): total C content in soil at harvest time. a; b; and c: different groUps of significant trait within each factor of study.

* ,** and *** Significant effect of each factors for each character at p = 0.05, 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

3.2.4.2. Nitrogen uptake and remobilization in plant

The total nitrogen content in plant, i.e. the total nitrogen at harvest in stover and grains, was not significantly different between SG and NSG genotypes, and also differences were not significant between the levels of water and density. However, the differences were significant between the level of N where the nitrogen content show the higher value under N3 (25.8 g kg^{-1}), then N2 (24.6 g kg^{-1}) and N1 (25.5 g kg^{-1}). For nitrogen up-take until and after silking time by stover (TN_UF and TN_AF, respectively), the difference between SG and NSG genotypes were not significant (Table 8, Annex 4b: 1, 3, 9, and 11**)**.

For the effect of abiotic stresses for stover N at silking and after silking, our result shows a significant difference between nitrogen levels at silking time, where N3 had the highest value of stover N (15.2 g kg^{-1}), then N2 (13.4 g kg^{-1}) and N1 (13.1 g kg^{-1}), while for water and density level at silking, the difference was not significant. After silking time, the result show a significant effect of water condition for N assimilation, where the higher value of N obtained under water stress condition (12.1 g kg^{-1}), and no significant difference obtained for nitrogen and density level (Table 8; Annex 4b: 1, 3, 7, 9, and 11).

For nitrogen remobilization from stover to the reproductive organs, the result show no significant difference between genotypes; while for the percentage of N remobilization, the difference was significant, where the NSG genotype remobilize 29% of N content, compared to 24% for SG genotypes (Figure 15). For N non_remobilized or N at harvest time, the result showed significant difference between SG and NSG genotypes, where SG genotypes had high value of SN_NR (10.1 g kg^{-1}), which represent 76% of total N in the stover. However, the NSG genotypes had 9.4 of stover N non-remobilized, which represent 71% pf total N in the stover (Figure 15, Table 8; Annex 4b: 13, 15, 17, and 19).

For the effect of abiotic stresses for remobilized and non-remobilized N, the result show significant difference for nitrogen and drought stresses, and for the interaction of nitrogen and drought stresses was significant. However, no significant effect was observed for high plant density stress for both stover N remobilized and non-remobilized. Under water condition, the remobilization of N was higher under optimum condition (5.1 g kg^{-1}), then water stress (3.0 g kg^{-1}). While, for stover N non-remobiliezd to stover higher value was observed under water stress

(10.4 g kg^{-1}) (Table 8; Annex 4b: 13, 15, 17, and 19). For nitrogen levels, the stover N remobilized was higher under N3 level (4.8 g kg^{-1}), then N2 (3.5 g kg^{-1}), and N1 (3.9 g kg^{-1}). The same trend was observed for stover N non-remobilized, where N3 had the highest value (10.3 g kg^{-1}). For the interaction of both stresses, the higher value of stover N remobilized was obtained under optimum water conditions for all nitrogen levels; however, for stover N non-remobilized the higher value was obtained under water stress condition for all nitrogen levels (Table 6, Table 7 ; Table 8; Annex 4b: 13, 15, 17, and 19).

Regarding kernel N content, NSG genotypes had higher kernel N content (16.7 g kg^{-1}), than SG genotypes (15.8 g kg^{-1}). The percentage of kernel N remobilized from stover was significantly higher for NSG genotypes (32%), than for SG ones (25%). Whereas, for the percentage of kernel N uptake after silking show high value for SG genotypes (75%), and 68% for NSG genotypes (Figure 15, Table 8, Annex 4b; Table 3, and 5). Both drought and nitrogen stresses had a significant effect on kernel N content. The higher value was observed under water stress condition (16.7g kg^{-1}). This result was opposite for stover N remobilized, where the remobilization was higher under optimum condition (Figure 15, Table 8, and Annex 4b: Table 3 and 5).

For the analysis of carbon content in the plant and kernel, and carbon remobilization, there were no significant differences between SG and NSG genotypes for most of the measured traits. For the effect of abiotic stresses, drought stress had a significant effect for all traits, except stover C remobilized and non-remobilized (Table 9, Annex 4b: 4, 6). The higher value for total C, kernel C, total C until silking and total C after silking was found under optimum water condition. For nitrogen and plant density stresses, the difference was not significant for all measured traits (Table 9, Annex 4b: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20).

Table 8 : Total N content[1] at silking time and physiological maturity of plant stover (leaf and stem), N-content in grain, and N remobilization and Uptake by grain; evaluated in six maize inbred lines under different conditions of water, nitrogen and plant density during two years 2018 and 2019 in two locations in Galicia.

Factors	Levels	TN[2] (g kg^{-1})	KN (g kg^{-1})	TN_UF (g kg^{-1})	TN_AF (g kg^{-1})	SN_R (g kg^{-1})	SN_NR (g kg^{-1})
WC	Opti	24.56. ± 2.1a^{ns}	15.8 ± 1a***	14.3 ± 1a^{ns}	10.7 ± 2.7a**	5.1 ± 2a***	9.1 ± 1a***
	WS	25.1 ± 2.1a	16.7 ± 1b	13.8 ± 1a	12.1 ± 2.7b	3.0 ± 2 b	10.4 ± 1b
NL	N3	25.8 ±2.1a*	16.3 ± 1a^{ns}	15.2 ± 1a ***	11.0± 2.7a^{ns}	4.8 ± 2 a**	10.3 ± 1a***
	N2	24.6 ± 2.1b	16.3 ± 1a	13.4 ± 1b	11.4 ± 2.7a	3.5 ± 2 b	9.9 ± 1a
	N1	24.5 ± 2.1 b	16.2 ± 1a	13.1 ± 1b	11.8± 2.7a	3.9 ± 2 b	9.0 ± 1b
WC x NL	Opti_N3	25.2± 2.1ab^{ns}	15.9± 1a^{ns}	15.5 ± 1a^{ns}	10.2± 2.7b^{ns}	5.4 ± 2 a**	9.9± 1a**
	Opti_N2	24.0± 2.1b	15.9± 1a	14.2 ± 1b	10.5 ± 2.7b	5.4 ± 2ab	8.6 ± 1b
	Opti_N1	24.5± 2.1b	15.8± 1a	13.2 ± 1c	11.2 ± 2.7b	4.4 ± 2 abc	8.7 ± 1b
	WS_N3	25.5 ± 2.1a	16.8± 1b	15.0 ± 1ab	11.7± 2.7ab	4.2 ± 2 bc	10.7 ± 1c
	WS_N2	24.9 ± 2.1b	16.6± 1b	13.2 ± 1c	12.4± 2.7b	1.6 ± 2 c	11.2 ± 1c
	WS_N1	24.6 ± 2.1b	16.6± 1b	13.2 ± 1c	12.3± 2.7b	3.3 ± 2 d	10.7 ± 1ab
PD	R	25.2 ± 2.1a^{ns}	16.3 ± 1a^{ns}	14.3 ± 1a^{ns}	11.4 ± 2.7a ^{ns}	4.1 ± 2 a^{ns}	9.6 ± 1a^{ns}
	H	24.7 ± 2.1a	16.3 ± 1a	13.8 ± 1a	11.4 ± 2.7a	4.0 ± 2 a	9.8 ± 1a
SGT	NSG	24.9 ± 2.1a^{ns}	16.5 ± 1a**	14.0 ± 1a^{ns}	11.1 ± 2.7a^{ns}	4.4 ± 2 a^{ns}	9.4 ± 1a**
	SG	25.0 ± 2.1a	16.0 ± 1b	14.0 ± 1a	11.7 ±2.7a	3.7± 2 a	10.1 ± 1b

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; SG: Stay-green genotypes PHW79; NSG: non-stay-green genotypes B73; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively; TN (g kg^{-1}): N taken Up by the whole plant; KN (g kg^{-1}): TN_UF: N-stover content at flowering time which is the N Uptake until flowering; TN_AF: N Uptake after flowering by the whole plant; KN: N-kernel content at harvest time; SN_R: stover N remobilized to the grain; SN_NR: stover N non-remobilized to the grain.

*,** and *** Significant effect of each factor for each character at p = 0.05, 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

Table 9: Total carbon content[1] at silking time and physiological maturity of plant stover (leaf and stem), and C-content in grain, evaluated in two maize inbred lines under different conditions of water, nitrogen and plant density during two years 2018 and 2019 in two locations in Galicia.

Factors	levels	TC[2] (g kg$^{-1)}$	KC (g kg^{-1})	TC_UF (g kg^{-1})	TC_AF (g kg^{-1})	SC_R (g kg^{-1})	SC_NR (g kg^{-1})
WC	Opti	859 ± 9a***	430 ± 12a**	416 ± 6a*	449 ± 12a*	-9.2± 14a^{ns}	437± 6a^{ns}
	WS	823 ± 9b	427 ± 12b	413 ± 6b	420 ± 12b	-7.6± 14a	436± 6a
NL	N3	848 ± 9a^{ns}	428 ± 132ab^{ns}	416 ± 6a^{ns}	437 ± 12a^{ns}	-10.7± 14a^{ns}	436± 6a^{ns}
	N2	829 ± 9a	427 ± 12a	414 ± 6a	421 ± 12a	-9.8± 14a	437± 6a
	N1	845 ± 9a	430 ± 12b	413 ± 6a	445 ± 12a	-4.8± 14a	437± 6a
WC ×NL	Opti_N3	855± 9a*	430 ± 12a^{ns}	417.5 ± 6a^{ns}	451 ± 12a^{ns}	-12.4± 14a^{ns}	439± 6a^{ns}
	Opti_N2	865± 9a	428 ± 12ab	415.4 ± 6ab	449 ± 12a	-9.0 ± 14a	437± 6a
	Opti_N1	857± 9a	430 ± 12a	413.6 ± 6b	447 ± 12a	-6.4 ± 14a	437± 6a
	WS_N3	841± 9a	425 ± 12b	413.5 ± 6b	423 ± 12a	-8.9 ± 14a	435± 6a
	WS_N2	792 ± 9b	425 ± 12b	412.7 ± 6b	394 ± 12b	-10.7 ± 14a	436 ± 6a
	WS_N1	834 ± 9a	429 ± 12 a	413.2 ± 6 b	442± 12 ab	-3.3 ± 14a	338± 6a
PD	R	848 ± 9a^{ns}	428 ± 12a^{ns}	414 ± 6a^{ns}	439 ± 12a^{ns}	-8.4 ± 14a^{ns}	437± 6a^{ns}
	H	834 ± 9a	427 ± 12a	413 ± 6a	430 ± 12a	-8.5 ± 14a	436± 6a
SGT	NSG	837 ± 9a^{ns}	429 ± 12a^{ns}	412 ± 6a**	435 ± 12a^{ns}	-7.2 ± 14a^{ns}	437± 6a^{ns}
	SG	844 ± 9b	427 ± 12a	415 ± 6b	435 ± 12a	-9.6 ± 14a	437± 6a

[1] Means followed by the same letter, within the same column and factor, are not significantly different.

[2] WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait SG: Stay-green genotype PHW79; NSG: non-stay-green genotype B73; Opti: optimal water conditions; WS: water stress conditions; N3, N2, N1: nitrogen levels (0U), (30U) and (90U), respectively.; TC (g kg^{-1}): C fixed by the whole plant; TC_UF: C-stover content at flowering time which is the C Uptake until flowering; TC_AF: C Uptake after flowering by the whole plant; KC: C-kernel content at harvest time.

*,** and *** Significant effect of each factors for each character at p = 0.05, 0.01, and p = 0.001; respectively; ^{ns} : non-significant.

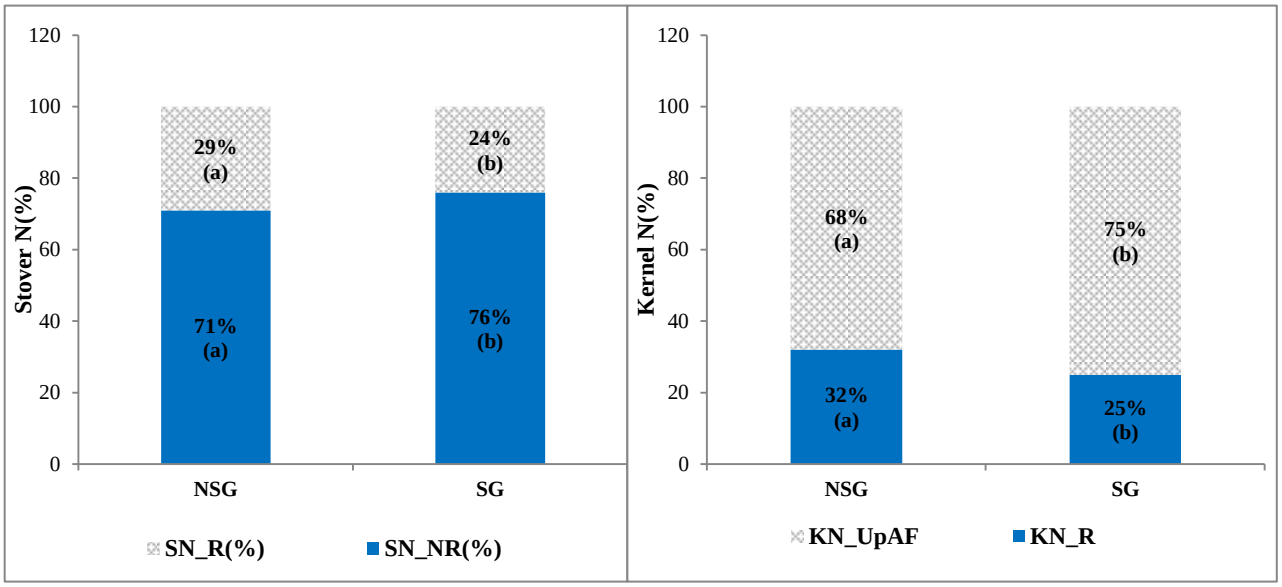

Figure 15. N remobilization from stover and uptake by grain of two maize inbred lines, evaluated in two maize inbred lines under different conditions of water, nitrogen and plant density during two years 2018 and 2019 in two locations in Galicia (SN_NR: percentage of stover N non_remobilized; SN_R: percentage of stover N remobilized; KN_UpAF percentage of Kernel N_Up take after silking; KN_R: percentage of Kernel N remobilized from stover).

3.3. Partial discussion of chapter three

3.3.1. Comparison between SG and NSG genotypes for physiological, agronomic and post-silking N uptake during senescence.

In this chapter different physiological, phenological and agronomic traits related to senescence were compared between SG and NSG genotypes. Also, to show if the SG phenotypes had an advantage with delayed grain filling period, and how this long period of grain filling affects N post-silking uptake and remobilization. The results show significant differences between SG and NSG genotypes for most physiological and agronomic characters. SG genotypes showed higher grain and stover yield, and maintained higher photosynthetic activity, quantum efficiency of photosystems II (F_v/F_m) and chlorophyll content for longer time than NSG genotypes.

For days to silking and ASI, the results show no significant differences between SG and NSG genotypes, which mean that all genotypes used in this study belong to the same flowering groups, and silking days and ASI did not have an effect on the difference obtained for the others traits.

The SG genotypes showed higher stover yield at harvest time compared to NSG genotypes. The results of Borrell et al., 2001, Pommel et al., 2006, Chen et al., 2014 and Chibane et al., 2021 found that SG trait was associated with high stover production. Conversely, Acciaresi et al., (2014) found no differences on grain yield and dry matter accumulation between earlier and late-senescing hybrids. Grain yield of cereals depends on two resources; the post-silking photo-assimilates directly transferred to the grain and assimilation redistributed from vegetative tissues during pre- or post-silking stages (Yang and Zhang, 2006). Leaves are the main photosynthetic organ, which can provide up to 50–80% photosynthetic material to meet the needs of grains (Kalt-Torres et al. 1987), while the pre-silking assimilation reserves in the stems and sheaths of cereal contribute only 10 to 40% of the final grain weight (Yang and Zhang, 2006; YE et al., 2020). Photosynthesis plays a decisive role in carbon fixation and biomass accumulation. In higher plants, the light reaction of photosynthesis is accomplished by the two photosystems PSI and PSII. These two photosystems work in series through the photosynthetic energy transport chain and are involved in the reactions of light-dependent carbon fixation (Gururani et al., 2015). In this study, NSG genotypes remobilize 21% of total stover yield at silking, compared to SG genotypes, where, the remobilization was only 11% from total stover yield at silking. This result is in concordance with Pommel et al. (2006), who found that delayed senescence in SG maize hybrids can result in a higher dry matter, which mainly accumulates in the stem rather than in the grain compared to NSG hybrids (Zhang et al., 2019**).** Ning et al. (2013) also showed that dry matter remobilization in stay-green cultivars was much less than the cultivars with fast leaf senescence. In the other hand, Masclaux-Daubresse et al. (2010) found that post-silking senescence is associated with the degradation and remobilization of leaf nitrogen.

Stay-green is considered in maize as one of the key traits in modern breeding for high grain yield (Mueller and Vyn, 2016; Lee and Tollenaar, 2007). The genetic gains in yield over the past decades involved the incorporation of delayed senescence or stay-green (Valentinuz and Tollenaar, 2004; Ding et al., 2005). In this study, SG genotypes have higher cobs yield and higher 1000KW. Chen et al. (2014), describe a strong relationship between grain dry weight and biomass accumulation after silking, which mean higher biomass yield produce higher grain

weight. The advantage of SG for increasing grain yield production has been described in many crops, including wheat, sorghum, barley, rice and maize (Kumari et al.. 2007; Gous et al.. 2016). In this sense, Silva et al.. (2003) and Chibane et al., (2021), found that SG genotypes have higher grain weight compared to NSG ones. While stover, cobs and grains moisture for SG genotypes were higher than moisture of NSG at harvest time. This can produce a problem for the farmer during the storage. This result was in agreement with Thomas and Smart (1993), who found that SG phenotypes is associated with greater moisture levels in the stover. Borrell et al. (2001) also found similar results, indicating that SG genotypes have higher stover production at physiological maturity; with higher humidity level. High stover and grain moisture was also found by previous studies of Bekavac et al.. 2007; Chapman et al., 2021 and Chibane et al., 2021.

After silking stage, the gradual loss in chlorophyll content and therefore active photosynthetic green leaf area leads to leaf senescence (Erley et al., 2010; Ahmad et al., 2019). The results reported by these authors were in concordance with our study, as we found for all physiological traits a significant decrease during senescence period, but the magnitude of the decrease was lower for the SG genotype PHW79; in opposite to the NSG genotype B73, where the decrease was fast. This result was consistent with the results found by Yang et al. (2017) and Chibane et al., (2021), who found that the decrease of chlorophyll content and photosynthetic activity was faster in the NSG genotypes than in the SG ones. The decrease was more pronounced after 30 DAS in both genotypes in agreement with results obtained by Ding et al. (2005), Caicedo (2018), Antonietta et al. (2014), and Chibane et al., (2021), where the difference between SG and NSG genotypes for photosynthetic rate was significant at the end of the grain filling period. This is also consistent with the results of Martin et al. (2018)**,** who reported that leaf photosynthesis diminished with the age of the leaf. Other authors have also associated the SG character with higher photosynthetic activity and chlorophyll content at later stages of the cultivation cycle (Yang et al., 2017; Zhang et al., 2012). In different studies, stay-green genotypes exhibited high photosynthetic activity, resulting in a subsequent improvement in grain weight (Dolferus. 2014; Jagadish et al.. 2015; Caicedo, 2018 ; Chibane et al., 2021, Silva et al.. 2003) which was in agreement with our results. In addition, Clay et al., (2009), show that maintenance of a high photosynthetic rate during grain-filling period is a major determinant of high grain yield in maize. The light reaction of photosynthesis is accomplished by the two photosystems PSI and PSII. These two photosystems work in series through the photosynthetic energy transport chain and are

involved in the reactions of light-dependent carbon fixation (Gururani et al., 2015; Yang et al., 2019). For quantum efficiency of photosystem II (F_v/F_m), our results showed the same trend of decrease than photosynthetic activity. There was significant difference between both genotypes for the decrease in F_v/F_m during senescence time which is in accordance with the previous studies of Yang et al. (2017); Caicedo (2018) and Chibane et al. (2021). Moreover, Zhang et al. (2012) found that the decrease is faster for a quick leaf senescence line HZ4, which lost the quantum efficiency of PSII (F_v/F_m) faster than SG genotypes after silking.

Photosynthetic rate during leaf senescence may be influenced by changes in stomatal aperture or conductance (Wong et al., 1985). The stomatal conductance declined after silking in parallel with photosynthetic activity, but the decline was faster for NSG line (B73) compared to SG line (PHW79). A similar result was found in the previous study of Chibane et al. (2021). Dai et al. (2004) described that the physiological changes in the plant itself such as senescence can affect the performance of plant photosystems. Stomatal conductance is responsible for controlling water loss to the atmosphere, however reducing stomatal opening also decreases CO_2 availability to the RuBisCO carboxylation sites, and thus C-assimilation (Buckley, 2019). Therefore, photosynthetic activity and stomatal conductance measurements can be used to distinguishing tolerant/susceptible genotypes to drought (Flexas et al., 2018).

For nitrogen availability in soil during silking and harvest time, our results show no significant differences between SG and NSG genotypes for total N, total C, and N assimilable by plants (NO_3 and NH_4). These results can be explained because it is difficult to estimate this variable in the field, with environmental conditions like rain that can wash assimilable nitrogen very fast. In this sense, Gnädinger, (2018) studied C and N content in soil, and he found that it is difficult to establish these measures under field conditions, where losses can be avoided, or with heavy rainfall that can wash out a nitrogen applied in the form of N enriched nitrate. Further they emphasize that nitrate and ammonium nitrogen are essential nutrients for successful crop production (Khan et al., 2017; Rafique, 2020). Increased C supply to the roots can increase N uptake during grain filling, which makes an important contribution to the total N uptake of the plant (Borrell et al., 2001; Li et al., 2019b). For nitrate content, our result show high content during silking time and low content at harvest time for all treatments. This is due to largest NO_3 uptake by the plant after silking time, and the leaching by rain or irrigation under optimum condition. This result is in agreement with results obtained by Friedrich et al., (1979), who

found that the rate of NO_3 uptake was largest during the period from silking time to three week after silking. (Ballabio et al., 2016) found that the potential of NO_3 leaching was high in regions dominated by sandy soils. Our results show also that soil NO_3 concentration increased with increasing fertilization, which is in agreement with the result of Angle et al. (1993).

Therefore, in this study, we concentrated only on the C and N accumulation and translocations within the above ground plant parameters stover and kernel. A strong dependency of nitrogen and carbon allocation was already previously demonstrated (Ciampitti and Vyn, 2011). Post-silking senescence is associated with the degradation and remobilization of leaf nitrogen (Masclaux-Daubresse et al., 2010; Yang et al., 2019). Most N accumulated in the grains was provided by remobilization of nitrogen absorbed in pre-silking period (Gallais et al., 2007). Delayed leaf senescence in SG genotype can enhance crop yields, by remobilizing nutrients from source to sink under various stresses, and nutrient limited conditions (Munaiz et al., 2020). For total nitrogen content in plant, we did not found significant difference between SG and NSG genotypes; but we found significant difference for total stover N content at harvest or N non_remobilized. This is due to stover nitrogen remobilization to the kernel; where, NSG remobilized higher part of total nitrogen in stover (29%), compared to SG genotypes (24%). This was in agreement with previous studies, where SG maize hybrids have lower remobilized N (Pommel et al.,2006). This result was interpreted by Tollenaar and Lee, (2006), who explain that SG hybrids require a large amount of applied N to maintain high foliar N levels, which is associated with chloroplasts integrity. Genotypes accumulating more N during the post-silking period would be able to meet N demand from kernel without remobilizing excessive amount of N from leaves, thereby delaying senescence (Subedi and Ma, 2005). This result is in concordance with previous studies of Rajcan and Tollenaar, (1999) and Chibane et al., (2021), who reported that NSG show fast recycling which involve biomass reduction. This result was reported by Subedi and Ma (2005), who described that SG have a direct consequence of improved N balance. This result was also supported by our result for kernel N content, where the NSG genotypes have higher value of N kernel content compared to SG genotypes. Chen et al. (2014) also found that 60-85% nitrogen, derived from nitrogen remobilization from silking to maturity can be found in maize, which explains the high importance of nitrogen remobilization within the plants. Thereby, reducing the remobilization of N from other plant organs such as leaves which may result in a longer maintained leaf area (Rajcan and Tollenaar, 1999).

Uhart and Andrade (1995) reported that the large portion of assimilates and N containing compounds are temporarily stored in the stem during the vegetative growth period and remobilized during the reproductive period in maize. Others studies found the positive effect of later senescence on both N uptake and yield reported (Yang et al., 2016; Mueller and Vyn, 2016; Chibane et al., 2021). Kosgey et al. (2013) found that SG genotypes incorporated more N into the vegetative tissues and the translocation rate was lower for SG genotypes than for NSG genotypes. In addition, NSG hybrids have limited post-silking N uptake from the soil, so the lack of nitrogen supply can be compensated by accelerated senescence and remobilization of N to the grain (Borrell et al., 2001). On the other hand, SG phenotype has higher N-uptake and accumulation of more biomass during the grain filling period (Borrell et al., 2001; Kitonyo et al., 2018)**.** This results was agree with Acciaresi et al., (2014), who reported that delayed-senescence may be associated with higher N retention in leaves but also with lower N concentration in kernels.

For C content in the stover and total plant, no difference obtained between SG and NSG genotypes. Whereas, remobilization of C from the leaves was very low or even negative, this resulted in an accumulation of C in the stover, which means that the photosynthetic activity can be maintained to guarantee plant growth (Gnädinger, 2018). This observation was confirmed by Wang et al. (2014) who discovered that N uptake was strongly driven by photosynthetic C assimilation. Consistent with previous studies of Ciampitti and Vyn (2011) and Pommel et al. (2006), where they observed that the carbon accumulated in the stems of late senescence varieties was higher, which can attribute to the overall biomass accumulation. In addition, SG cultivars accumulated higher root biomass (Gnädinger, 2018)**,** which makes it possible to maintain N uptake and remobilization from the roots during grain filling by providing the roots with carbohydrates.

3.3.2. Effect of abiotic stresses for different agronomic and physiological activity, and post-silking N uptake of SG and NSG maize genotypes during senescence.

Maize was originally derived from the tropics, and has been imported and cultivated in more temperate areas with higher geographic latitude. In the temperate regions, maize cultivation faces many abiotic stresses in the field, including water deficit, low and high temperature, and shading stress caused by increased plant density, all of these stresses can result in decreased maize yields

(Li et al., 2019). The present study examines how the magnitude of the physiological activity in post-silking period, and different agronomic traits of SG and NSG maize genotypes can be changed under different abiotic stresses: drought, low nitrogen, and high plant density.

3.3.2.1. Under drought stress

Drought stress is one of the most important abiotic stresses that limits crop production (Yang et al., 2019). During reproductive growth stages or grain filling stage, drought stress may cause premature senescence (Yang et al., 2019). In this study, drought stress showed a significant and negative effect for different agronomic and physiological trait. Drought has a negative effect and decrease stover and cobs yield, and 1000KW. Similar results were observed when maize plants subjected to drought during late growth stage, where post-silking drought reduced grain weight and number, resulting in grain yield loss (Luche et al., 2013; Li et al., 2018; YE et al., 2020). This was in agreement with the result of Li et al. (2018), who found that deficit of irrigation or drought stress reduces the plant biomass and grain yield of maize by reducing the photosynthesis activity and chlorophyll contents. Aydinsakir et al., (2013)**,** show that the reduction in the 1000 grain weight can be attributed to low level of available water causing low transition of photosynthesis matter and assimilates to kernels (Aydinsakir et al., 2013)**.**

Under water stress, we found a delay in silking days and an increase in anthesis silking interval compared to optimum water condition; however, increased ASI is a symptom rather than the direct mechanism that causes kernel abortion (Li et al., 2019)**.** Water stresses can accelerate the period of physiological maturity, which can reduce significantly the number of days to obtain black layer. In this sense, Rajcan and Tollenaar (1999) describe that senescence might be accelerated due to abiotic stress as drought or low nitrogen. Others studies in maize have shown that when the demand of water cannot be met due to insufficient rainfall, the balance of plant water relations is disturbed, resulting in a series of unfavorable changes such as reduced in photosynthetic rate and transpiration, and accelerated leaf senescence. Thereby affecting plant growth and development and leading to biomass and yield loss (Cairns et al., 2012; Ye et al., 2020)**.**

Drought stress reduces physiological activity of the plant during the grain filling period, and the loss of photosynthetic activity, chlorophyll content, stomatal conductance and quantum efficiency of the photosystem II (F_v/F_m) were faster under water stress condition compared to optimum

water condition. This is in accordance with previous studies that found that drought stress induces a decrease in photosynthesis, loss of canopy area, and reduction in carbon assimilation (Yang and Zhang, 2018; Yang et al., 2019). Yang et al. (2019) show the reduction in the chlorophyll content suggesting that leaf senescence was accelerated under drought stress. Chaves et al. (2009) found that photosynthetic activity is the main physiological process and it is highly sensitive to drought stress. In this sense, previous studies found that leaf chlorophyll decrease under drought and can thus be taken as proxies of drought stress degree for crop plants (Parajuli et al., 2018; Song et al., 2016).

Therefore, deficient irrigation or drought stress reduces the plant biomass and grain yield of maize by reducing the photosynthetic rate and chlorophyll content (Li et al., 2018**;** YE et al., 2020). This is also in concordance with results of Gnädinger (2018)**,** who found that under suboptimal growth conditions characterized by drought stress, the performance of the cultivars as evidenced by the reduction in dry weight biomass will be affected from flowering to late maturity. Accordingly, Cernusak et al. (2013) reported that environmental conditions such as drought stress potentially influence stomatal conductance and photosynthetic activity. Gnädinger (2018), show that only late senescence cultivars were able to better withstand drought stress and did benefit from the late senescence, which accumulates biomass until grain maturity.

The imbalance in carbon and nitrogen metabolism is one of the major consequences of drought (Yang et al., 2019). Drought stress can also affect N and C content in soil and plant, implying that N and C content in soil was higher under optimum water condition. This is similar also for NO3. This is because the availability of water on soil avoids plant assimilation. Our results are in agreement to the previous study of Gnädinger (2018)**,** who proved that drought stress reduce drastically the period of active C and N uptake in early maturing cultivars and lead to an interruption of N remobilization. This is in agreement with our result, where the N remobilization was reduced under drought stress. The translocation of carbon and nitrogen molecules between the source and sink is also affected (Chen et al., 2015; Li et al., 2016).

3.3.2.2. Under low nitrogen stress

Nitrogen fertilization exerted a significant influence on the performance of several physiological activities during grain filling and genotypes yields. Efeoğlu et al., (2009) show that any

remaining nitrogen could reactivate the photosynthetic activity again. However, low N availability is an important yield-limiting factor (Bänziger et al., 2000). In the other hand, the application of N can contribute to drought resistance to a certain extent in many plants (Wang et al., 2016). Under water deficits, N supplies can reduce drought effects by protecting photosynthetic apparatus, activating antioxidant defense systems and improving osmoregulation (Gou et al., 2017; Wang et al., 2020). The results of this study show that for most measured traits, the high nitrogen level has a positive effect and the low nitrogen level show the lowest yield. The low nitrogen level N1 shows the lowest value of stover and cobs yield; whereas, the highest value was obtained under N3. This result is in concordance with previous study of Gnädinger (2018), where biomass and kernel yield increased with increased nitrogen rates. Therefore, fertilization supply has a positive impact on biomass production and cannot be compensated by genetic improvement (Yan et al., 2014). Others studies show that nitrogen uptake ability affects maize dry matter accumulation by influencing leaf development, green leaf area maintenance, photosynthetic efficiency, and thus grain yield (Zhai et al., 2017; R. Li et al., 2019).

Nitrogen fertilization affect also silking date and anthesis silking interval. Similarly to drought stress, low nitrogen level increases the ASI and delays silking day. In addition, for all physiological traits, the highest value showed under N3, and the lowest one was found under N1. In this study, nitrogen fertilization has not significant effect in moisture percentage for stover and kernel, also was not related to physiological maturity. Nitrogen fertilization has a similar effect to drought stress for physiological activities. Under low nitrogen level, plants reduce all the physiological activities compared to high nitrogen level N3. The photosynthetic rate, chlorophyll content, and quantum efficiency of photosystem II showed highest values during silking period.

The nitrogen content in maize stover largely depends on the availability of soil nitrogen (Worku et al., 2007; Singh et al., 2021). In our study nitrogen content in soil was related to nitrogen fertilization. This is also related to N plant content and N uptake by the plant. Under low nitrogen fertilization N1 the plant N-uptake and remobilization was lower compared to N3, this is due to the availability of N for the plant under N3. For N remobilization, the plant remobilizes more N under N3, then N1 and N2. A similar pattern of nitrogen uptake was found in previous studies of Ciampitti and Vyn, (2012); Kiniry et al. (2001), who found that higher soil N can lead to a high proportion of N in plant biomass and vice versa. Nitrogen use efficiency is strongly influenced by

climate conditions, fertilization rates and genetic variety (Ciampitti and Vyn, 2012). Higher nitrogen application significantly increased nitrogen uptake during grain filling period (Gnädinger, 2018). Leaf N levels also reflect N availability in the soil (Xu and Zhou, 2006; Li et al., 2019b). However, kernel N remobilization was not dependent on nitrogen fertilization. Maybe because, this trait was related was more to capacity of each genotype to remobilize N to kernel after silking. In this study, nitrogen fertilization does not have a significant effect for carbon content and availability in soil, or carbon content and remobilized in plant.

3.3.2.3. Under high plant density

Planting density is one of the most important factors that affect grain yield of maize (Feng et al., 2014). It has been shown that varying the maize planting density greatly affects the grain-filling process, yield and yield components (Sangoi et al., 2002; Jia et al., 2018). Other studies have shown that increasing population density is an important method to achieve high yields (Roy et al., 2014; Tong et al., 2019). However, with high planting density, the individual plants will shade each other, which will deteriorate the permeability of the canopy, decrease the photosynthetic performance, increase the plant height and increase the risk of lodging (Sangakkara et al., 2012; Tong et al., 2019). Therefore, reduce individual plant production and improper control may even reduce yields (Andrade et al., 2002; Ren et al., 2017; Meng et al., 2020). Li et al., (2019b) found that high plant density caused significant reductions in grain number per ear and 1000KW, but increased the ear number ha^{-1}.

In this study, high plant density can delay silking day and the ASI. This was in concordance with result found by Ajayo et al., (2021), who found that ASI value increased significantly with increased plant density. Results from other researchers have consistently shown that increased ASI is associated with increased plant density due to the increased number of days to silking after anthesis (Al-Naggar and Atta, 2017), and previous study of Shrestha et al., (2018) found that silking date was delayed with increasing plant density. It can also increase the maximum yield of stover. However, the individual yield decrease with high planting density. Stover yields and 1000KW were lower under high plant density compared to low density. This result was in concordance with previous results, where increase plant density decrease the 1000-kernel weight (Borrás et al., 2003; Jia et al., 2018). High plant density can reduce the ability of light to penetrate the lower canopy (Liu et al., 2014), leading to premature senescence of the lower leaves (Borrás

et al., 2003). Ultimately, this will significantly reduce yield and yield components of maize crop (Borrás et al., 2003; Sangoi et al., 2002).

Conversely to drought and nitrogen stress, high plant density have not significant effects on most physiological activity of the plant, which differ from to the result of Sher et al., (2017), who found that increasing planting density, photosynthetic activity per plant is severely limited, which may enhance dry matter remobilization from stalk to the ear (Shao et al., 2021)**.** High plant density affects only the chlorophyll content in the plant. In addition, plant density have not a significant effect on nitrogen and carbon availability in soil, or nitrogen and carbon assimilation and remobilization in plant. These results are not in agreement with results of Ciampitti and Vyn, (2011), who found a possible relationship between plant density and nitrogen allocation in maize plants.

Chapter 4: RNA-Seq analysis reveals effect of leaf senescence on gene expression under abiotic stress of two maize inbred lines.

IV. Chapter 4: RNA-Seq analysis reveals effect of leaf senescence on gene expression under abiotic stress of two maize inbred lines.

4.1. Introduction

Leaf senescence is a major physiological process that affects vegetative and productive developmental processes in plants (Wu et al., 2016). Leaf senescence determines crop grain yield and biomass formation, which is a highly regulated, well-coordinated, and biologically active process that marks the end of the life cycle of the leaf and, ultimately the whole plant (Hollmann et al., 2014; Kohl et al., 2012). The color change of leaf plants is considered as the most common indicator of leaf senescence, with visual estimation (Buchanan-Wollaston et al., 2003) associated with a series of physiological processes, particularly chlorophyll breakdown, termination of photosynthesis, protein and nucleic acid degradation, molecular metabolism and nutrient transport decrease, and responses to cell death (Koyama, 2014). Delaying plant senescence can effectively prolong photosynthesis and increase the overall biomass of crops (Khan et al., 2014).

SG genotypes constitute a potential germplasm source for the genetic improvement of important crops to mitigate several stresses. SG is considered as an important agronomic trait that allows plants to maintain their leaves photosynthetically active and subsequently improve the grain-filling process even under stress conditions (Zhang et al., 2019). SG has two types, functional and non-functional. The functional SG genotypes are able to maintain their photosynthetic capacity for a longer time than the NSG genotypes. Conversely, in the non-functional or cosmetic SG genotypes, leaf greenness is maintained because of the failure of the chlorophyll (Chl) degradation pathway, with decline in photosynthetic capacity (Kamal et al., 2019). Several environmental factors can promote leaf senescence, such as drought, nutrient starvation, high plant density, inhibited pollination, salinity stress, and biotic stresses (Quirino et al., 2000; Schippers, 2015).

Maize is one of the most important crops in the world (Zhou et al., 2016). Hybrid maize has a long active photosynthetic period that is mainly achieved by having higher chlorophyll content during senescence, or by maintaining a higher photosynthetic activity level during chlorophyll loss, which increases grain yield. Maize is frequently impacted by different biotic and abiotic

stresses, like drought, high salinity, high plant density, and low temperature (Wu et al., 2016). Plants respond to abiotic stresses at the cellular and molecular levels, including stress perception, signal transduction to cellular components, gene expression, and metabolic changes (Agarwal et al., 2006**;** Shinozaki and Yamaguchi-Shinozaki, 2007)**.**

The transition from leaf maturation to senescence is complex and is related to changes in genes expression levels throughout the genome. Several senescence related genes (SAG) have been found in many plant species (Li et al., 2014). Approximately 3,356 SAGs were identified from 44 species, and ~69.89% was found in Arabidopsis. In addition, more than 100 transcription factors, such us NAM, ATAF and CUC (NAC), as well as WRKY, SQUAMOSA promotor binding protein (SBP), APETALA2, and MYB, are involved in the regulation of leaf senescence (Balazadeh et al., 2008; Guo et al., 2004). In this study, two inbred lines with distinct leaf senescence characteristics, early leaf senescence B73, and stay-green, or delayed-leaf-senescence, PHW79, were selected as the materials to determine target genes in leaf senescence and how is affected by combined abiotic stress (drought stress and low nitrogen). The information will increase our understanding of the molecular mechanisms of leaf senescence in response to abiotic stresses.

4.2. Results

The quality control of our data shows a significant difference for genes expression between both locations. The results were represented by a Principal Components Analysis plot (Figure 16). The result show significant difference between the two locations for different treatments and genotypes; where, we can observe clearly two distinct ellipses. For this we decide to make separately the analysis in each location then discuss the difference showed in both locations.

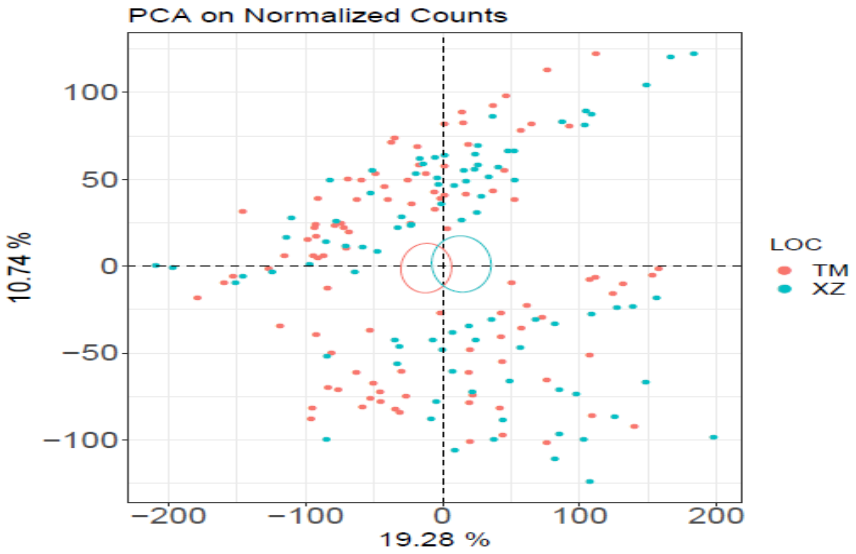

Figure 16. PCA of the normalized counts of two locations with quality control analysis. (TM: Tomeza; XZ: Xinzo)

4.2.1. Result of gene expression in Tomeza location

4.2.1.1. Gene expression, quantification and differential expression analysis

Among the 13516 expressed genes, 8583 and 4933 genes are differentially expressed during the senescence time [M1_M2] and [M2_M3], respectively. At [M3_M4], we estimate only 4440 Differentially Expressed Genes (DEG) for the SG genotype PHW79. The NSG genotype B73 loss this activity and was dry before M3, so we did not take samples after this time. The number of DEGs expressed during different senescence times differed between genotypes for each treatment. The NSG genotype B73 showed more DEGs for all treatment than the SG genotype PHW79. For different treatments, we show that the water stress treatment revealed more DEGs that optimum water. Also, the nitrogen N3 present higher number of DEGs than N1 (Figure 17).

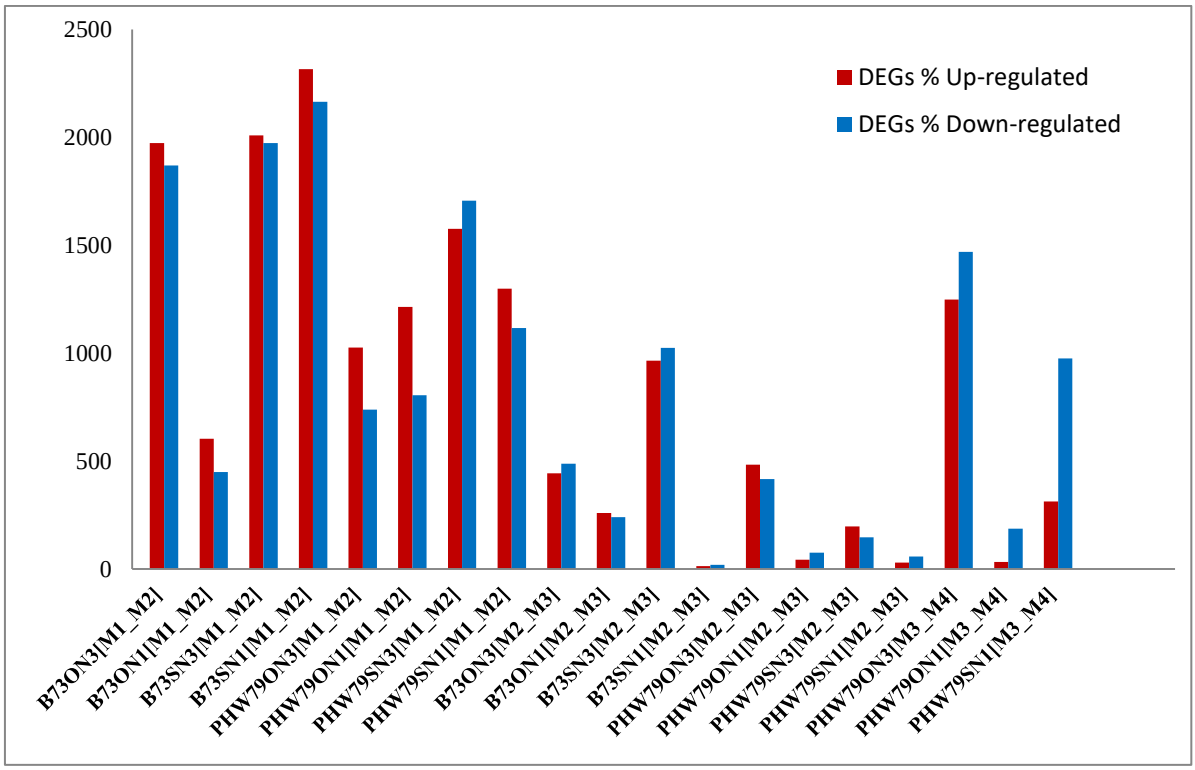

Figure 17. DEGs up and down-regulated, detected in each genotype during senescence time for different treatment in Tomeza (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively. B73: non stay green genotype; PHW79: stay green genotype).

4.2.1.2. Identification of differentially expressed genes (DEGs) between genotypes.

When we compare the number of DEGs between both genotypes, we show a high number of DEGs expressed between both genotypes. This difference varied also between treatment and senescence time. The highest number of DEGs was shown for water stress and N3 nitrogen level treatments at different times. The number of DGEs up-regulated was generally higher than the number of DEGs down-regulated for most treatments. Also, we can see that the numbers of DEGs were higher for M2 and M3 times than for M1 for all treatments (Figure 18).

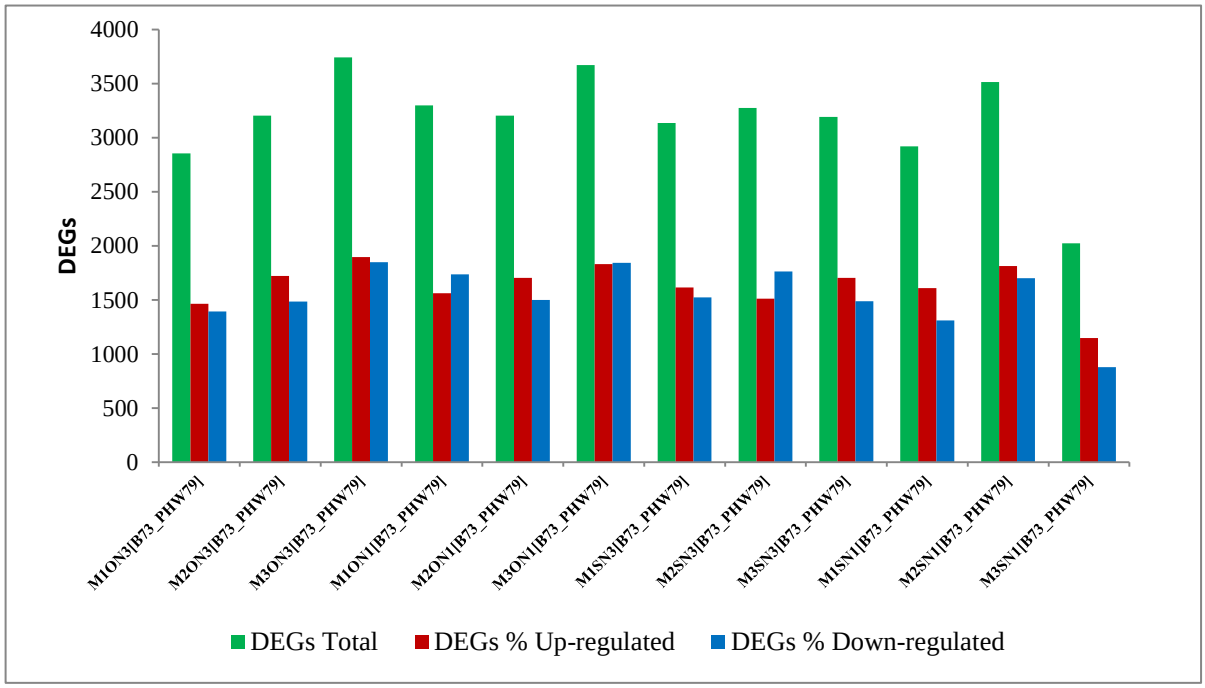

Figure 18: Differentially expressed genes (DEGs) between both genotypes at different senescence times for each treatment in Tomeza (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively. B73: non stay green genotype; PHW79: stay green genotype).

4.2.1.3. Gene function and enrichment analyses

To obtain the gene ontology of the specific genes active during senescence for each treatment, we select from different contrasts the specific genes group active only for the selected treatment that we search. The number of genes used for each treatment was presented below in the Table 10 for both locations. The number of genes expressed during the early senescence time was higher than the late senescence time. Under most treatments, the number of genes down regulated was higher than the number of up regulated. We can see also that the number of specific genes for each

treatment was higher in Tomeza than in Xinzo. This is due to early dry of B73 in Xinzo before 45 days, so we did not take sample after 30 DAS (Table 10).

Table 10: DEGs for each treatments and genotype analyzed with PlantRegMap for genes biological function during senescence process for two maize inbred lines at two locations.

Treatment	Genotype	Genes number			
		Tomeza		Xinzo	
		Down	Up	Down	Up
Early senescence genes		121	45	33	26
Late senescence genes		33	34	/	/
Drought and nitrogen stress (SN1)	B73	340	325	114	139
	PHW79	386	139	259	292
Optimal water and nitrogen stress (ON3)	B73	286	244	54	64
	PHW79	672	749	515	432
Nitrogen stress (N1)	B73	404	354	2	2
	PHW79	554	300	223	275
Optimal nitrogen level (N3)	B73	390	353	144	155
	PHW79	1377	1259	231	135
Optimal water condition (Opt)	B73	374	310	4	4
	PHW79	936	1016	352	283
Water stress condition (WS)	B73	1315	1107	260	220
	PHW79	1157	614	159	175

4.2.1.3.1. Core genes enrichment for early senescence genes

The result of gene ontology of the early senescence genes shows 14 and 72 enriched GO terms for the up and the down regulated genes, respectively. For the down regulated genes, many enriched GO terms were related to photosynthesis activity while many of the enriched GO terms for the up regulated genes were associated with cellular and thylakoid structure (Table 11, Annex 5: Table S1 and S2). Within the genes associated with cellular and thylakoid structure we found *Zm00001d001857* a chlorophyll A-B binding protein; *Zm00001d034179*, a putative component

PetM/VII of cytochrome b6-f complex; *Zm00001d043972*, a ribosomal protein L12-1 and *Zm00001d009877* RNA, transcription plastid transcriptionally active.

Table 11. Main biological process of the GO terms identified during early senescence time in Tomeza location, using the Plant Reg Map platform.

Aspect	GO.ID	Term	p-value
Down	GO:0015979	Photosynthesis	1.1e-09
	GO:0009765	Photosynthesis, light harvesting	5.2e-05
	GO:0019684	Photosynthesis, light reaction	6.8e-05
	GO:0009768	Photosynthesis, light harvesting in photosystem I	0.00035
	GO:1901566	Organo nitrogen compound biosynthetic process	0.00297
Up	GO:0044436	Thylakoid part	0.00036
	GO:0009579	Thylakoid	0.00101
	GO:0009535	Chloroplast thylakoid membrane	0.00146
	GO:0055035	Plastid thylakoid membrane	0.0015

4.2.1.3.2. Core genes enrichment for late senescence genes

For late senescence genes expressed, we detected fewer expressed genes compared to early senescence. The GO enriched terms show one up regulated term, and seven down regulated terms enriched. The up regulated term "GO:0005509" enriched for "calcium ion binding "and the down regulated terms enriched for different process of photosynthesis membrane, thylakoid part and membrane, plastid (Annex 5: Table: S3, S4). Also enriched for monocarboxylic acid metabolic process, which is marked by the expression of three genes "*Zm00001d053675*-jasmonic acid biosynthesis"; "*Zm00001d006886*-Indole-3-acetate biosynthesis II"; "*Zm00001d045919*-glycolysis IV (plant cytosol)" (https://www.maizegdb.org). All those genes were involved in biosynthesis processes (http://zzdlab.com/plad/maize_genedetail.php). That means that for the late core senescence genes, we have found a decrease in different biosynthetic processes.

4.2.1.3.3. Transcriptions factors (TF)

Transcription factors play critical roles in the onset of leaf senescence. In Tomeza location, 50 families of TF are active at different times during senescence [M1_M2], [M2_M3],and [M3_M4] were respectively: WRKY (16, 28 and 30%), bHLH (20, 30 and 32%), MYB (11, 18 and to 14%), NAC (24, 27 and 33%), C3H (47, 29 and 16%), BZIP (32, 25 and 43%), MYB-related (24, 21 and 24%), ARF (50, 20 and 15%), C2H2 (24, 25 and 26%), HD-ZIP (30, 22, and 6%), and TALE (51, 16 and 6%) families were the top 11 largest families active during leaf senescence, some of them are critical components of plant adaptive response to biotic, abiotic stresses and senescence (Lin et al., 2015) (Annex 5: Table S4). In particular, the BHLH, C3H, NAC, bZIP, and MYB-related transcription factor families had significantly differential expressions that were induced by senescence, with over 40% of the members of this family showing altered expression at various times during senescence. The highest rate of expression was detected during the senescence times [M1_M2] and [M2_M3] compared to [M3_M4]. Most of these transcription factor families have been identified as important leaf senescence regulators in Arabidopsis (Chai et al., 2019).

4.2.1.3.4. Genes enrichment for nitrogen and water stress

To investigate the response of both genotypes to water and nitrogen stresses and compare the change in genes expression under each stress or both stresses together, Gene Ontology (GO) analyses were made for different treatments during successive senescence times.

The results show that, for the genotype B73, the number of enriched GO terms was 145, 189, 118 and 332 for different treatments N1, N3, optimum and water stress, respectively (Annex 5: Table 12 and 13). For PHW79 genotype, the number of enriched GO terms was higher than B73 for all treatments; being 189, 363, 277, and 340 for N1, N3, optimum water, and water stress, respectively (Annex 5: Table S16 and S17).

From all these enriched GO terms for each treatment, we try to compare between different biological processes induced from each treatment for both genotypes. Furthermore, we compared if the same process was active in both genotypes under each treatment, or there was a difference which distinguishes between both genotypes. The biological functions most significant up and down-regulated were represented for the common function active under both stresses SN1 for

each genotype and under optimum conditions ON3 (Annex 5: Table S6, S7, S8, S9 and S11**)**. Also, we investigate the enriched GO terms under each stress for each genotype.

We found several enriched GO terms active in both genotypes under both stresses (SN1). The most significant down regulated GO terms common for both genotypes were "protein transmembrane" and "RNA interference". For the up-regulated DEGs, the GO terms "cellular response to stimulus", "cellular localization", and "response to abiotic stimulus". However, under optimum condition (ON3) both genotypes have the same response and we found similar terms active for both genotypes. The down-regulated GO terms enriched for molecular localization, transport, biosynthesis and metabolic processes. While the up-regulated GO terms enriched for photosynthesis and metabolic processes for B73 and for metabolic and biosynthesis processes and response to stimulus for the PHW79 (Figure 19 a).

For B73, the most enriched GO terms under SN1 for down-regulated DEGs were "Mitochondrial fission", "Regulation of cell shape", "Cell-cell signaling", and "protein targeting to membrane". And for the up-regulated enriched GO terms was "Response to stimulus", "polyamine metabolic process", "Response to oxygen containing compound", "Response to stress", "Response to chemical" and "Malate metabolic process" (Figure 19 b).

For PHW79, we have also shown the expression of different pathways involved for phytohormone expression, we have GO terms active for auxin, and cytokinine expression. And for GO term involved for ABA (abscisic acid), we show their expression in both genotypes; just their expression is down-regulated for SG genotype PHW79 and up-regulated in NSG genotype B73.

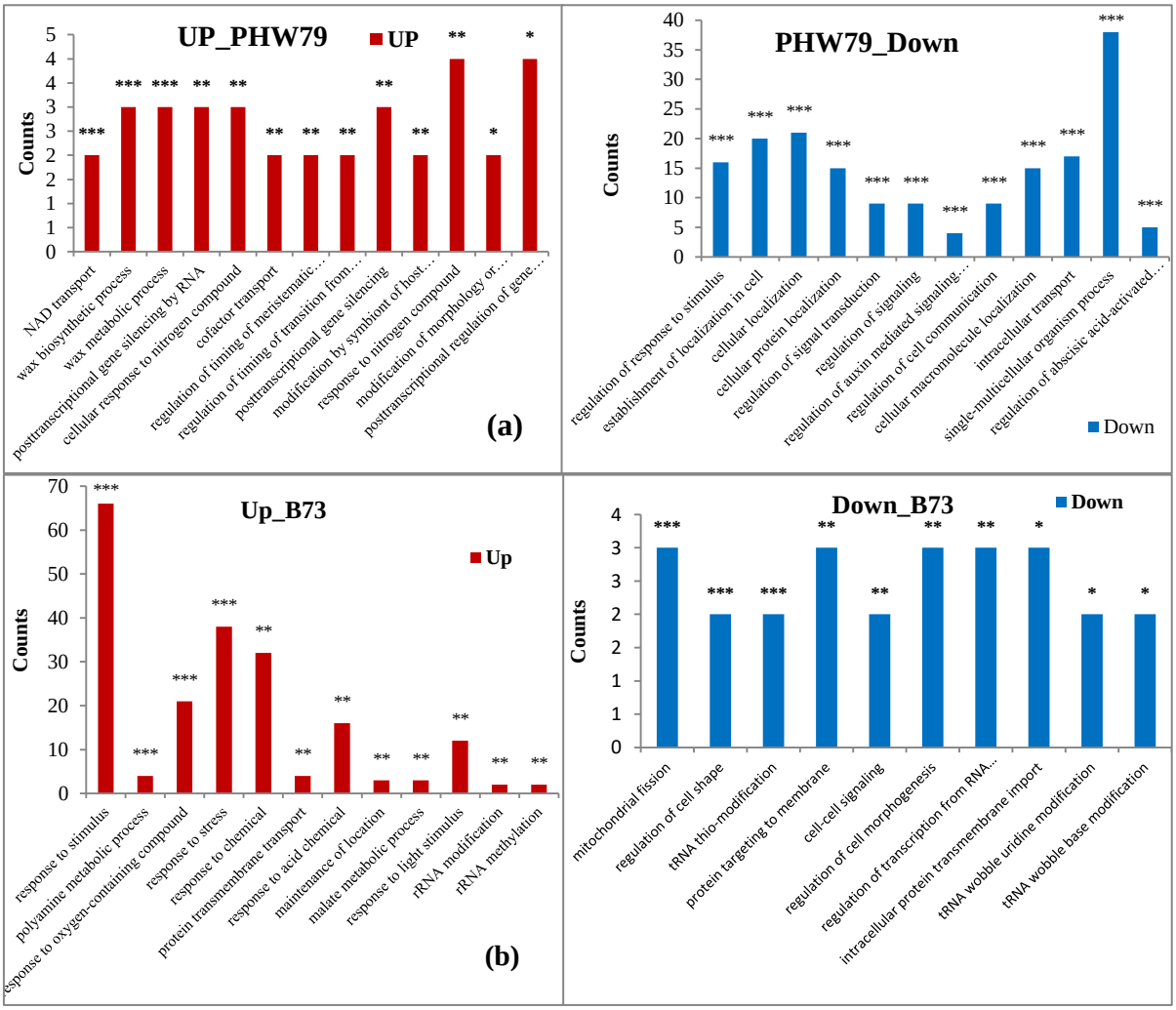

Figure 19: Biological Process GO terms exclusively enriched up and down-regulated for each genotype B73 and PHW79 under drought and nitrogen stress during senescence times in Tomeza location. Asterisk represented significance levels (*p-value<0.01; **p-value<0.005; ***p-value<0.001).

The analyses of specific genes expressed in each abiotic stress, show different enriched terms involved in several biological functions. The most significant ones were presented in Table 12 for nitrogen stress and Table 13 for water stress for each genotype.

For nitrogen treatment, we found that each genotype has some specific response to each nitrogen level. For N1 nitrogen level, the genotype B73 has enriched the GO terms “mitochondrial fission”, and different process of localization and transport for down-regulated GO terms. For the up-regulated GO terms, we have “Response to stimulus”, and different biosynthesis and

metabolic processes. While, PHW79 has enriched GO terms for wax biosynthetic and metabolic process for up-regulated terms, which is specific only for this genotype. For the down-regulated GO terms, we found different GO terms involved in transport and localization processes (Table 12; Annex 5: Table S12 and S16). For N3 nitrogen level, both genotypes have similar enriched up and down regulated GO terms. The most significant GO terms down-regulated for both genotypes were enriched for metabolic and catabolic processes. Whereas, the up-regulated GO terms were enriched for the biosynthetic and metabolic processes in both genotypes (Table 12; Annex 5: S17 and S13).

For water treatments, we found that under water stress condition, the most expressed GO terms for B73 were enriched for catabolic process for down-regulated GO terms and for different biosynthetic and metabolic processes for the up-regulated GO terms. While, for the PHW79 we show that the GO terms involved for different molecular localization and response to abiotic stimulus were down-regulated. However, GO terms involved for "amino acid activation", "tRNA amino acylation", and "Translation" was up-regulated (Table 13; Annex 5: Table S14, S18 and S19). Under optimum conditions, the most enriched GO terms for B73 involved for molecular localization for down-regulated GO terms, and enriched for photosynthesis process and response to abiotic stimulus for up-regulated GO terms. For PHW79, the up regulated GO terms under optimum water condition were involved for different development processes and response to radiation. While the down regulated GO terms were enriched for metabolic and biosynthesis processes (Table 13; Annex 5: Table S15, S20 and S21).

Our results show also that for the term enriched in Establishment of localization in cell was a common GO term for both stresses and both genotypes. We have two important genes active for this term. We have "*Zm00001d01835*-translocon at the inner envelope membrane of chloroplasts" have a direct effect for chloroplast and oxidation-reduction process; and "*Zm00001d007065*- Nucleoporin auto-peptidase", which is involved in mRNA export from nucleus

Table 12: Biological Process GO terms exclusively enriched in up and down-regulated DEGs for each genotype B73 and PHW79 under nitrogen stress during senescence times.

Genotype	AS	GO.ID	Terms down regulated	p-value	GO.ID	Terms up regulated	p-value
B73	N1	GO:0000266	mitochondrial fission	7.9e-05	GO:0050896	response to stimulus	7.8e-06
		GO:0051649	establishment of localization in cell	0.00023	GO:0006595	polyamine metabolic process	0.00021
		GO:0015031	protein transport	0.00025	GO:1901700	response to oxygen-containing compound	0.00086
		GO:0033036	macromolecule localization	0.00026	GO:0009416	response to light stimulus	0.00088
	N3	GO:0044265	cellular macromolecule catabolic process	1,00E-06	GO:0043043	peptide biosynthetic process	1.4e-08
		GO:0051603	proteolysis involved in cellular protein catabolic process	8.2e-06	GO:0006518	peptide metabolic process	2.3e-08
		GO:0030163	protein catabolic process	8.5e-06	GO:0043604	amide biosynthetic process	3.5e-08
		GO:0044257	cellular protein catabolic process	1,00E-05	GO:0006412	translation	3.6e-08
PHW79	N1	GO:0051641	cellular localization	7.5e-08	GO:0010035	response to inorganic substance	0.00052
		GO:0051649	establishment of localization in cell	1.2e-07	GO:0010025	wax biosynthetic process	0.00067
		GO:0046907	intracellular transport	1.4e-07	GO:0010166	wax metabolic process	0.00081
		GO:0034613	cellular protein localization	1.2e-06	GO:0009414	response to water deprivation	0.00089
	N3	GO:0043603	cellular amide metabolic process	2.5e-12	GO:0006518	peptide metabolic process	3.8e-16
		GO:0043604	amide biosynthetic process	7.9e-12	GO:0043043	peptide biosynthetic process	9.1e-16
		GO:0006518	peptide metabolic process	1.8e-11	GO:0006412	translation	1.1e-15
		GO:0006412	translation	4.1e-11	GO:0043603	cellular amide metabolic process	2.3e-15

AS: Abiotic stresses, N1, N2, N3: different nitrogen level 0U, 30U, and 90U; respectively.

Table 13: Biological Process GO terms exclusively enriched in up and down-regulated DEGs for each genotype B73 and PHW79 under drought stress during senescence times.

Genotype	AS	GO.ID	Down_Terms	p-value	GO.ID	Up_Terms	p-value
B73	**Water stress**	GO:0030163	protein catabolic process	1.8e-10	GO:1901566	organonitrogen compound biosynthetic process	1,00E-23
		GO:0070647	protein modification by small protein conjugation or removal	1.8e-09	GO:0006518	peptide metabolic process	2.9e-23
		GO:0016579	protein deubiquitination	2.7e-09	GO:0043043	peptide biosynthetic process	7.6e-23
		GO:0051603	proteolysis involved in cellular protein catabolic process	3.6e-09	GO:0043603	cellular amide metabolic process	8.4e-23
	Optimal water	GO:0033036	macromolecule localization	0.00016	GO:0009628	response to abiotic stimulus	0.00045
		GO:0008104	protein localization	0.00039	GO:0009416	response to light stimulus	0.00047
		GO:0051641	cellular localization	0.00042	GO:0009314	response to radiation	0.00066
		GO:0042147	retrograde transport, endosome to Golgi	0.0016	GO:0009765	photosynthesis, light harvesting	0.00104
PHW79	**Water stress**	GO:0033036	macromolecule localization	5.4e-07	GO:0043038	amino acid activation	0.00026
		GO:0051641	cellular localization	1,00E-06	GO:0043039	tRNA aminoacylation	0.00026
		GO:0070727	cellular macromolecule localization	3.6e-06	GO:0006412	translation	0.00034
		GO:0051716	cellular response to stimulus	3.8e-06	GO:0010608	posttranscriptional regulation of gene expression	0.00034
	Optimal water	GO:0043603	cellular amide metabolic process	4.1e-08	GO:0009314	response to radiation	1.7e-06
		GO:0043604	amide biosynthetic process	4.8e-08	GO:0009628	response to abiotic stimulus	2.1e-06
		GO:0006412	translation	1,00E-07	GO:0048507	meristem development	3.4e-06
		GO:0006518	peptide metabolic process	1.4e-07	GO:0009416	response to light stimulus	5.8e-06

AS: Abiotic stresses

4.2.2. Result of Gene expression in Xinzo

4.2.2.1. Gene expression quantification and differential expression analysis

In Xinzo, we detected higher number of DEGs for B73 during [M1_M2] for all treatments, compared to the genotype PHW79. The number of DEGs during [M1_M2] was lower than 300 genes. However, during [M2_M3], the genotype PHW79 showed an increase in the number of DEGs compared to [M1_M2] for all treatments. On the other hand, for the genotype B73 we could not make the extraction of RNA after 30 days from flowering; because the plant was dry (Figure 20).

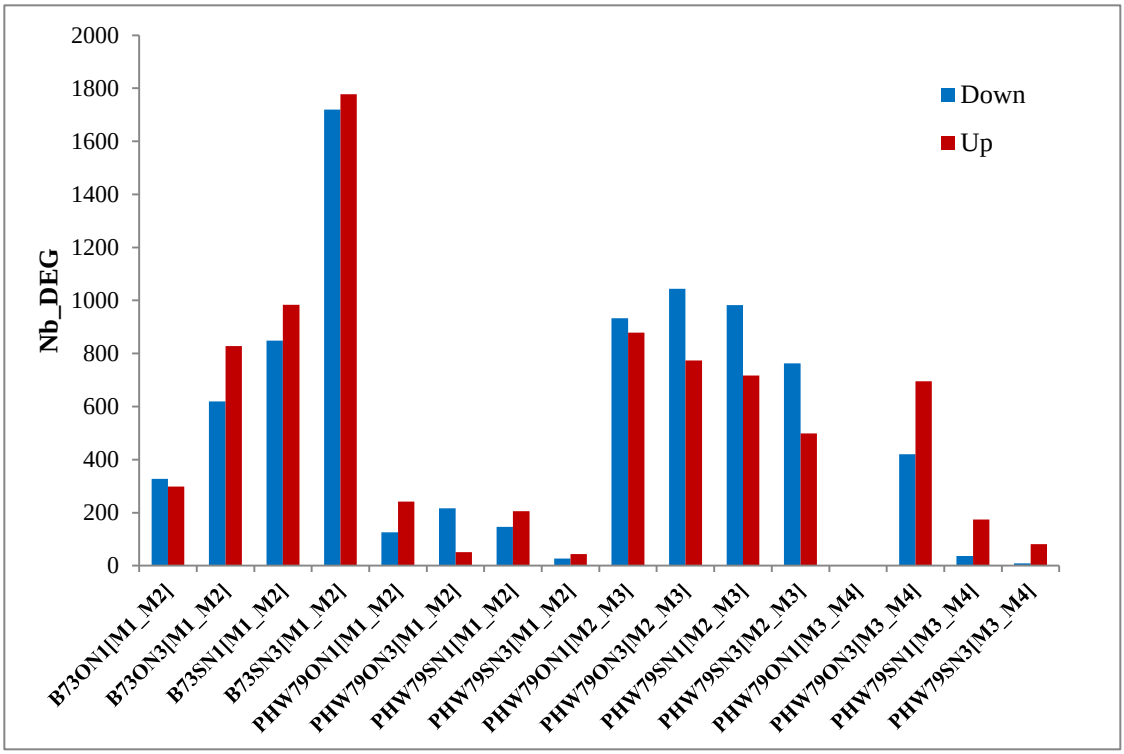

Figure 20. DEGs Up and Down-regulated detected in each genotype during senescence times for different treatments in Xinzo (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimal nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively; B73: non stay green genotype; PHW79: stay green genotype).

4.2.2.2. Identification of differentially expressed genes (DEGs) over genotypes

For the comparison between both genotypes in Xinzo, we compare ultil the senescence time M1 and M2, because we do not have more samples for the genotype B73 after M2. The result of the comparison shows that for all treatments the number of DEGs was higher at M2 than at M1, which means that senescence genes started their expression at M1 (Figure 21).

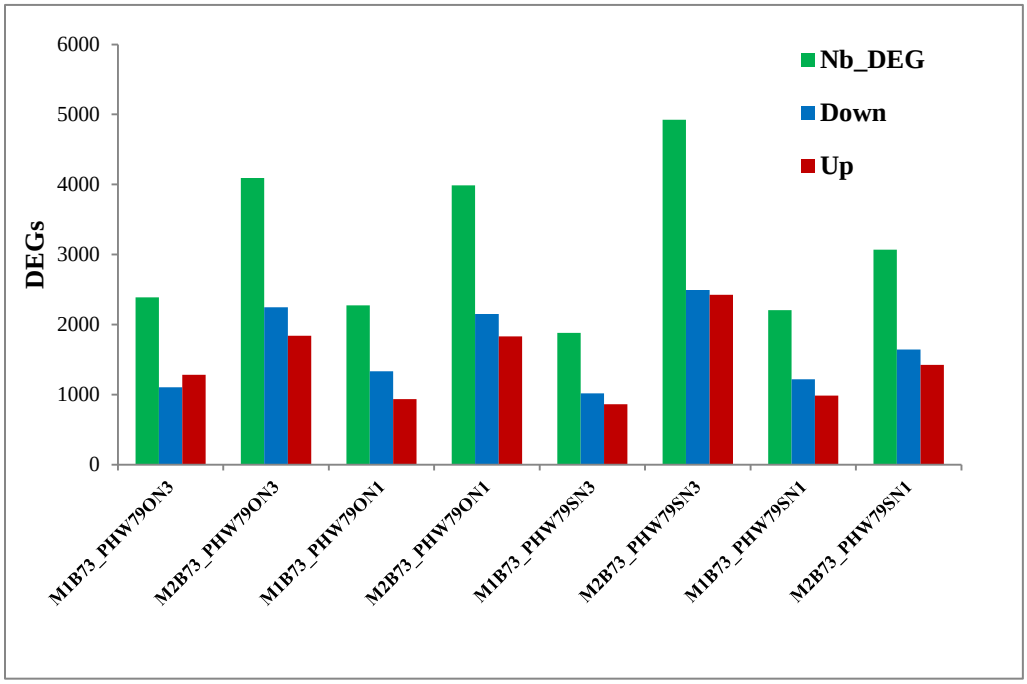

Figure 21: Differentially expressed genes (DEGs) between both genotypes at different senescence times for each treatment in Xinzo (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively; B73: non stay green genotype; PHW79: stay green genotype).

4.2.2.3. Gene function and enrichment analyses

4.2.2.3.1. Core genes enrichment for early senescence genes

The result of gene ontology for the specific genes enriched for early senescence in Xinzo show that, 18 and 4 GO terms were detected from up and down-regulated genes, respectively. Most up-regulated enriched GO terms during early senescence are related to the processes of cellular transport (ADP and ATP transport and also nucleotide, nucleoside and nucleotide transport) and some catabolic processes like cellular nitrogen compound catabolism. Down-regulated enriched GO terms are involved in photosynthesis but also protein folding and translational elongation (Table 14, Annex 6: Table S'1).

Table 14: Main biological process of the enrichment Go terms identified during early senescence in Xinzo, using the PlantRegMap platform.

Aspect	GO.ID	Terms	p-value
Up	GO:0015866	ADP transport	8.9e-05
	GO:0015867	ATP transport	0.00011
	GO:0015868	purine ribonucleotide transport	0.00016
	GO:0051503	adenine nucleotide transport	0.00016
Down	GO:0006457	protein folding	0.00045
	GO:0006414	translational elongation	0.00081
	GO:0019684	photosynthesis, light reaction	0.00234
	GO:0015979	photosynthesis	0.00973

4.2.2.3.2. Transcriptions factors (TFs)

In Xinzo, 49 families of TFs families had significant differential expression induced by senescence in the two studied inbred lines, most of them expressed during [M2_M3] (Annex 6: Table S'2). Regarding the families more active in maize leaf senescence according to Lin et al. (2015), all of them were highly expressed during senescence in Xinzo. As we can see, the percentage of expression involved was higher in [M2_M3] than in other senescence times; which mean that senescence in this location set up later after M2. The families related with senescence,

which had a higher significantly differential expressions were presented for each time interval, [M2_M1], [M3_M2], and [M4_M3], respectively: ARF (16, 39, and 13 %), NF-YA (32, 31 and 1%), C3H (2, 25 and 5%), bZIP (3, 22, 6%) and NF-YC (0, 20, and 8%). All of them started with a low percentage of TF expressed in [M1_M2] time, reached a peak at [M2_M3] and then decreased again in [M3_M4]. Note that there is an exception: NF-YB and NF-YC were not expressed in [M1_M2] time and the expression started in [M2_M3]. The rest of them were expressed since silking time (Annex 6: Table S'2).

4.2.2.3.3. Genes ontology for nitrogen and water stress

Under both abiotic stresses (SN1), the up-regulated GO terms for B73 were related to the regulation of autophagy but also with the localization and transport of some organic substances, for instance "protein localization" and "protein transport"; While the down-regulated GO terms were involved in "cellular nitrogen compound metabolic process" and other macromolecule metabolic processes like CTP and heterocycle. We show aslo the down-regulation of GO terms involved for nucleotides and nucleosides related metabolic process, especially pyrimidine nucleoside, ribonucleoside and ribonucleotide metabolic processes (Figure 22. (A), Annex 6: Table S'3, S'4).

For the genotype PHW79, under SN1 the up-regulated enriched GO terms were associated with protein deneddylation, COP9 signalosome assembly and lipid translocation, transport and distribution. It is related aslo with metabolic processes like nucleotides and nucleosides related metabolic process, especially pyrimidine nucleoside and ribonucleotides biosynthetic processes (Figure 22. (B) Annex 6: Table S'5 and S'6). However, the down-regulated GO terms for this genotype were related to gene expression, ribosome biogenesis, and metabolic processes like cofactor metabolic processes, "organonitrogen compound metabolic processes", and "nitrogen compound metabolic process", but, also with photosynthesis and chloroplast organization. Under optimum condition (ON3) we found up-regulation GO terms involved for metabolic process in both genotypes and, GO terms involved for biosynthesis process enriched only for B73. While, for the down-regulated GO terms we show GO terms involved for biosynthesis, transport and metabolic process enriched for the genotype PHW79. For B73, we found only one GO term enriched for mediator complex (Annex 6: Table S'57 and S8).

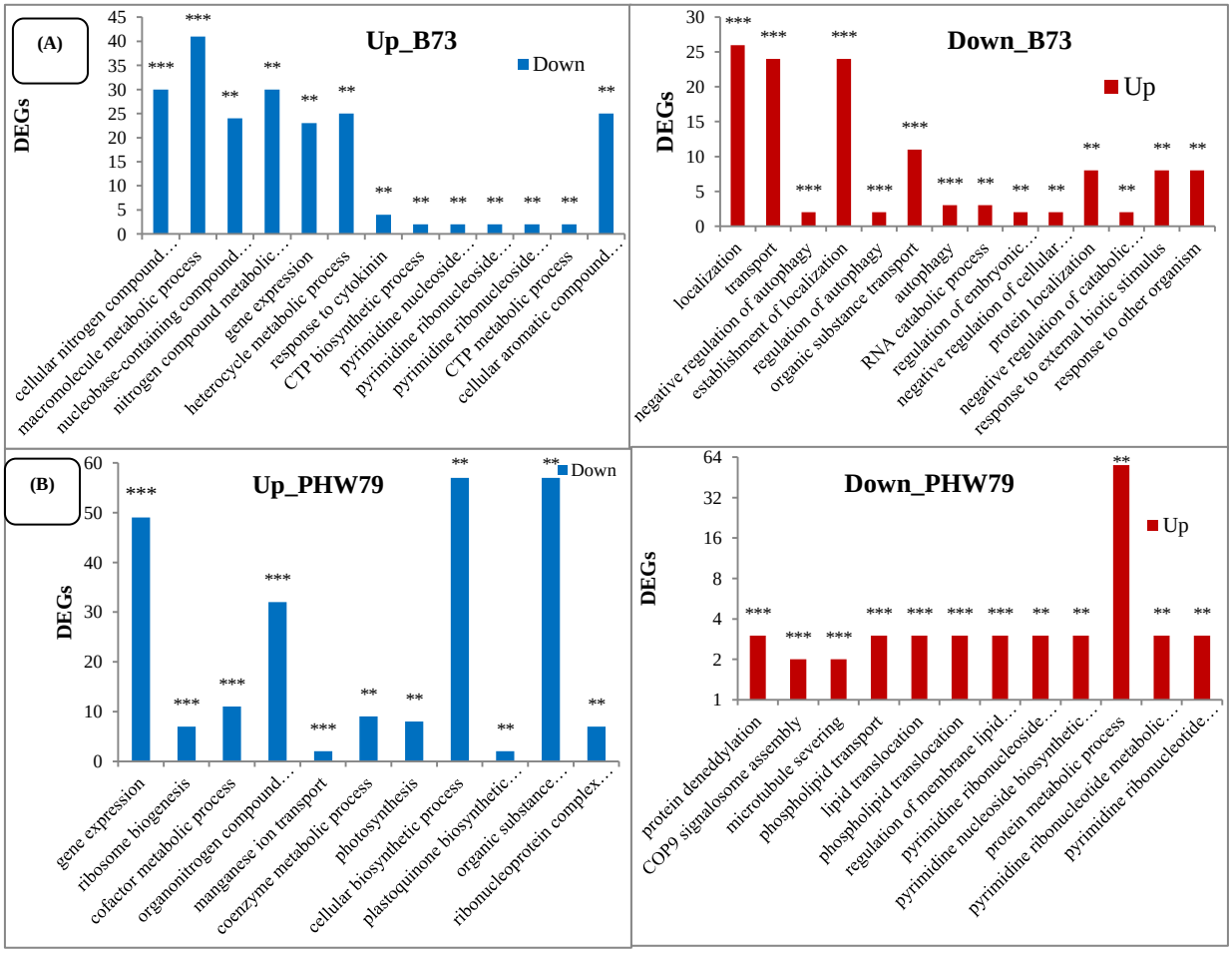

Figure 22. Differentially expressed genes (DEGs) up and down-regulated, detected in each genotype during senescence times for different treatments in Xinzo. B73: non stay green genotype; PHW79: stay green genotype.

For nitrogen treatments, when it comes to N3 condition for the B73 genotype, six GO terms were up-regulated. They were related to jasmonic acid biosynthetic process and also with other catabolic processes of organic and carboxylic acids. The same response was observed for the PHW79 for the up regulated terms under N3 conditions. The down-regulated GO terms for it were 10, and they were related to cellular organization of chloroplast, plastids and their fission and other biosynthetic processes like sucrose's biosynthesis (Table 15; Annex 6: Table S'10). For PHW79, the down-regulated GO terms were enriched for different metabolic and catabolic processes (Table 15; Annex 6: Table S'14). For the B73 genotype, there are not up or down-regulated enriched GO terms for N1 condition. PHW79 under nitrogen stress N1 had several up-

regulated enriched GO terms related to catabolic processes like “heterocycle catabolic process”, transport processes like “pyrimidine nucleobase transport” and “uracil transport” and response to stimulus. PHW79 had 30 down-regulated GO terms related with chloroplast and plastid organization and phosphatase activity (Annex 6: Table S’13).

When it comes to water treatment in Xinzo; under optimum water conditions the genotype B73 had no up or down-regulated genes. However, in water stress conditions, several enriched GO terms were up and down-regulated (Table 16; Annex 6: Table S’11). First, the up-regulated ones were associated especially with biological processes like transport and localization but also with acids catabolism, acids oxidation, acids transport and with ceramide biosynthesis. Second, the down-regulated enriched GO terms are related with photosynthesis (light harvesting and light reaction) and also with plastid and chloroplast organization and translation.

For PHW79 under optimum water conditions, the down-regulated GO terms were associated with the response to stimulus, especially abiotic stimulus and also GO terms related to photosynthesis (Table 15; Annex 6: Table S’17 and S’18). While, the up-regulated GO terms under optimum water conditions are strongly related with transport and localization processes (e.g. amino acid transport) and with the cellular homeostasis. Under water stress conditions, the down regulated terms were enriched for protein processing and maturation, and different metabolic processes, while the up-regulated terms were enriched for gene expression, chemical stimulus, and others processes of organization.

Table 15: Gene ontology (GO terms) up and down-regulated for each genotype B73 and PHW79 under nitrogen stress during senescence times in Xinzo.

Genotype	AS	GO.ID	Up_Terms	p-value	GO.ID	Down_Terms	p-value
B73	N3	GO:0009625	response to insect	0.0022	GO:0009657	plastid organization	2.7e-05
		GO:0009695	jasmonic acid biosynthetic process	0.0022	GO:0009658	chloroplast organization	0.00034
		GO:0009694	jasmonic acid metabolic process	0.0039	GO:0005986	sucrose biosynthetic process	0.00049
		GO:0016054	organic acid catabolic process	0.0067	GO:0006002	fructose 6-phosphate metabolic process	0.00288
PHW79	N1	GO:0046700	heterocycle catabolic process	2,00E-04	GO:0006457	protein folding	6.1e-07
		GO:0015855	pyrimidine nucleobase transport	0.00024	GO:0009658	chloroplast organization	3.4e-05
		GO:0015857	uracil transport	0.00024	GO:0009657	plastid organization	5.1e-05
		GO:0051716	cellular response to stimulus	0.00035	GO:0042254	ribosome biogenesis	0.00024
	N3	GO:0009867	jasmonic acid mediated signaling pathway	0.0032	GO:0000375	RNA splicing, via transesterification reactions	0.00051
		GO:0071395	cellular response to jasmonic acid stimulus	0.0032	GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.00051
		GO:0071310	cellular response to organic substance	0.008	GO:0046185	aldehyde catabolic process	0.00135
					GO:0044237	cellular metabolic process	0.00183

As: Abiotic stresses; N1, N2, N3: different nitrogen level 0U, 30U, and 90U; respectively.

Table 16 : Gene ontology (GO terms) exclusively enriched in up and down-regulated DEGs for each genotype B73 and PHW79 under drought stress during senescence times in Xinzo.

Genotype	AS	GO.ID	Up_Terms	p-value	GO.ID	Down_Terms	p-value
B73	**Water stress**	GO:0006810	transport	1.9e-05	GO:0015979	photosynthesis	2.8e-30
		GO:0051234	establishment of localization	2.3e-05	GO:0019684	photosynthesis, light reaction	1.7e-18
		GO:0051179	localization	3.5e-05	GO:0006091	generation of precursor metabolites and energy	4.6e-12
		GO:0046513	ceramide biosynthetic process	6.5e-05	GO:0009657	plastid organization	7.2e-11
PHW79	**Water stress**	GO:0040029	regulation of gene expression, epigenetic	0.0015	GO:0051604	protein maturation	0.00022
		GO:0016571	histone methylation	0.0021	GO:0016485	protein processing	0.00422
		GO:0016568	chromatin modification	0.0024	GO:0032270	positive regulation of cellular protein metabolic process	0.00422
		GO:0070887	cellular response to chemical stimulus	0.0025	GO:0051247	positive regulation of protein metabolic process	0.00516
	Optimal water	GO:0006865	amino acid transport	0.00077	GO:0009628	response to abiotic stimulus	9.2e-05
		GO:0044765	single-organism transport	0.00129	GO:0019684	photosynthesis, light reaction	0.00028
		GO:0050801	ion homeostasis	0.00165	GO:0050896	response to stimulus	0.00031
		GO:1902578	single-organism localization	0.00168	GO:0009266	response to temperature stimulus	0.00032

AS: abiotic stresses

4.2.2.3.4. Change in genes expression

To better understand the change in genes expression in each location during senescence, we take some studied genes related to senescence process. The specific genes represented with the stay-green gene "SGR1" "*Zm00001d006211*"; non yellow coloring "NYC1" "*Zm00001d039312*"; and the transcription factor: "TF-NAC" ("*Zm00001d022424*","*Zm00001d041472*"); "TF-HD-ZIP" ("*Zm00001d021934*"); and "TF-ERF" ("*Zm00001d016616*"). These genes had a specific catabolic path during senescence, where their expression was altered.

During the silking time M1, the expression of SGR1 for both was low. However, at M2, we show that the expression of SGR1 of genotype B73 is increased, but for genotype PHW79, they still maintain the same rate of their expression. In M3, we have the maximum expression of SG1 in both genotypes. The maximum expression log counts of B73 are 10, and the log counts of PHW79 are 8. In M4, we only have the expression of SGR1 in PHW79 genotype, and it remains stable with M3. We found that B73 has the same rate of expression of SGR1 in different treatments. However, for PHW79, the expression rate under stress conditions was higher than that under normal conditions for M3 time (Figure 23).

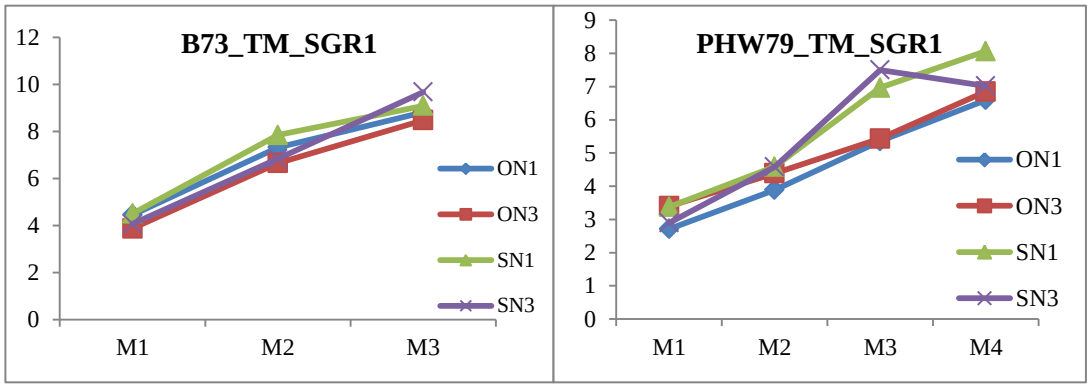

Figure 23. Expression of SGR1 in both genotypes of maize during senescence in Tomeza (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively).

For the non-yellow coloring "NYC1" "*Zm00001d039312*" gene, we have an up-regulated expression for both genotypes in both locations. This expression is stable across locations during M1 to M2, and then the expression rises until a maximal value at M3. Only for the genotype B73

in Xinzo, we show an increase of the expression of "NYC1" from M1 to M2 to maximal value. The rate of the expression was higher under stress condition compared to optimum condition in M3 for both genotypes in Tomeza location. However, in Xinzo we found the same rate of expression for different treatment in both genotypes. Even in Xinzo, the genotype B73 has dried before M3 or 45 days after silking period. For this we have only data of genes expression at M1 and M2 (Figure 24).

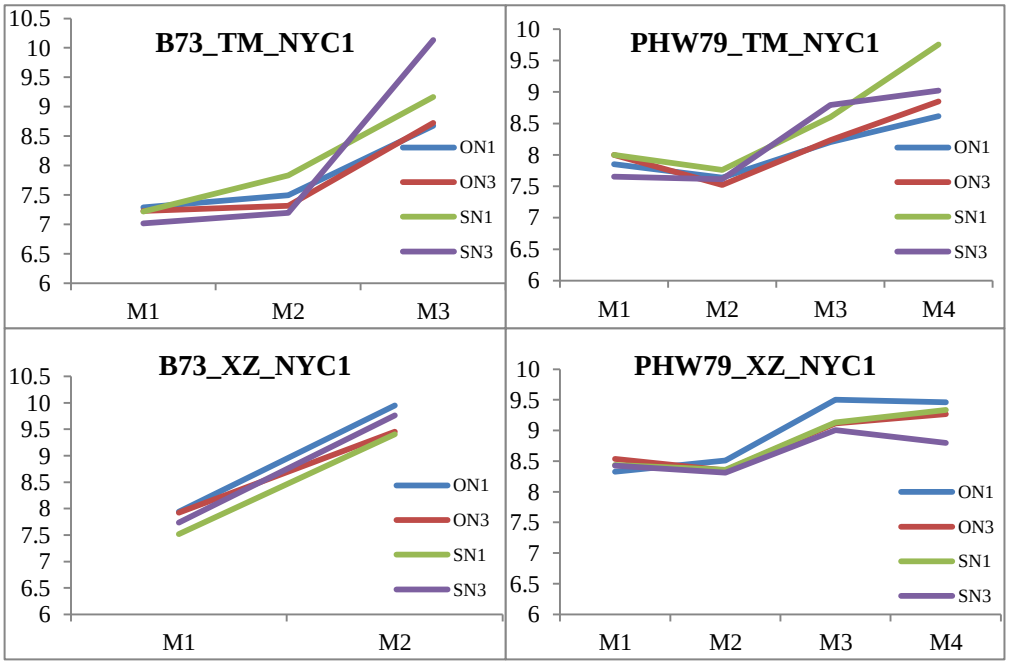

Figure 24. Expression of NYC1 in both genotypes of maize during senescence in Tomeza and Xinzo (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively).

For the transcription factor NAC, we identified the expression of two genes that regulated this TF: "*Zm00001d022424*", and "*Zm00001d041472*" expressed in both locations for both genotypes. For the first gene "*Zm00001d022424*", we found an up-regulated expression for both genotypes in both locations. At silking time M1, we found the minimal value of the expression of "*Zm00001d022424*" in all conditions and both locations. But, in Tomeza at silking, the genotype B73 have higher counts of genes expression (Log counts = 4 to 6) compared to PHW79 (log-counts = 3). In both locations we show the maximal expression of NAC TFs at M3 time for both genotypes. For the genotype PHW79, we show a stable rate or lower decreased of expression

after M3 time in both locations (Figure 25 (A)). For the second gene "*Zm00001d041472*" of NAC-TF family, the results show more variation in their expression for PHW79 compared to B73. The expression rate varied between 4 and 8.5 log-counts for PHW79 and, between 6 to 8 log counts for B73 (Figure 25 (B)). In the other hand, the expression of both genes of NAC-TF family is similar in each genotype for both locations. For PHW79 the maximum rate of genes expression showed at M3. After M3, we found a stable rate of expression. For B73 genotype, the maximum rate of expression was at M3 for Tomeza location and at M2 for Xinzo location.

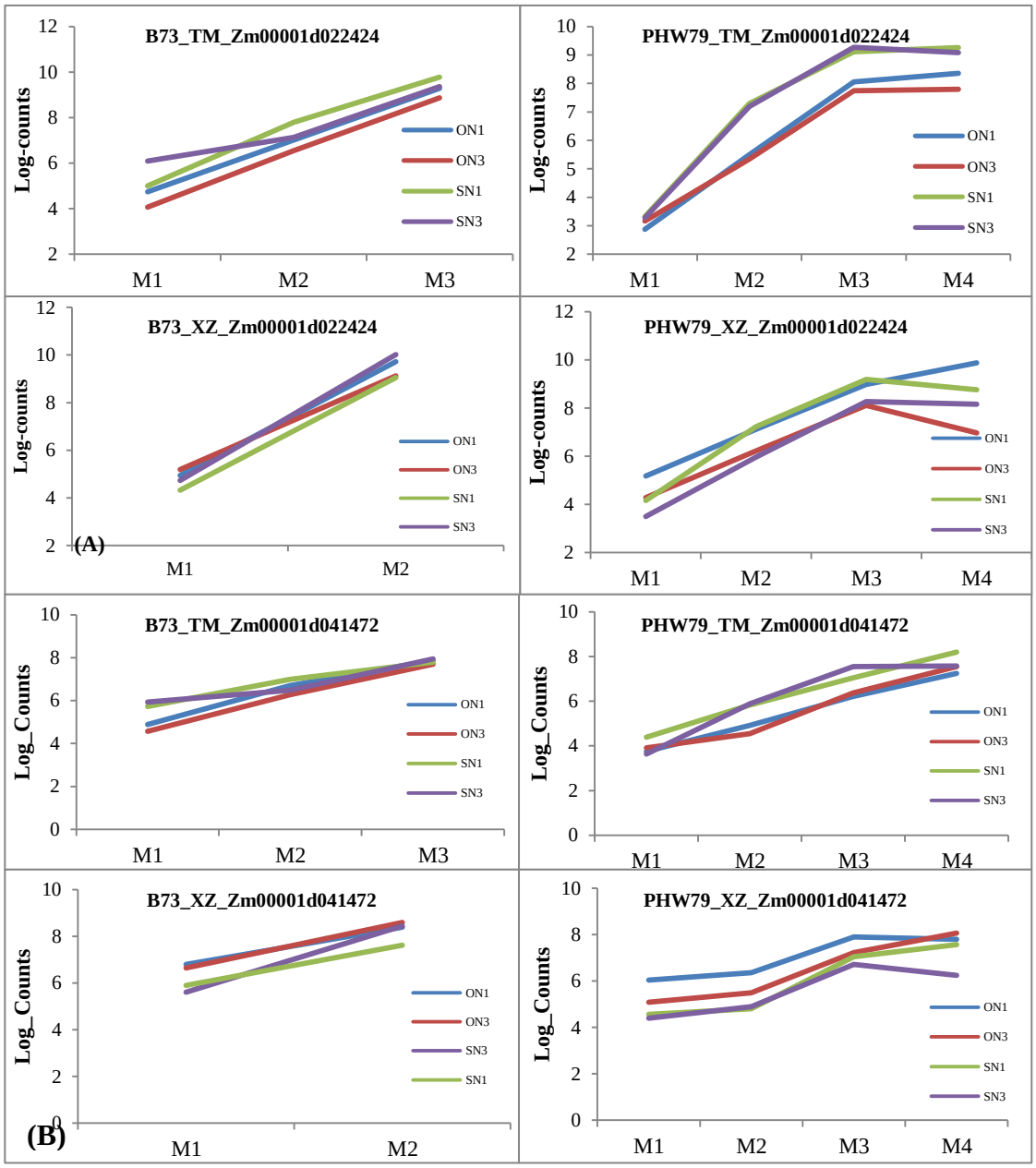

Figure 25. NAC transcription factor with two represented genes in both maize genotypes during senescence in Tomeza and Xinzo. ((A): "*Zm00001d022424*"gene in both location and genotypes, and (B): "*Zm00001d041472*" gene in both locations and genotype (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence times, flowering, 30, and 45 days after flowering, respectively).

Our results show that the TF-HD-ZIP has large variability of expression in both locations and genotypes. In Tomeza TF-HD-ZIP expressed only for PHW79 at M4. However, in Xinzo, we show their expression for both genotypes at different senescence times. This expression varied between and within genotypes. This variation is more expressed for PHW79 (Figure 26). For the TF-ERF, we found this expression only at Tomeza location at M4 senescence time for the genotype PHW79.

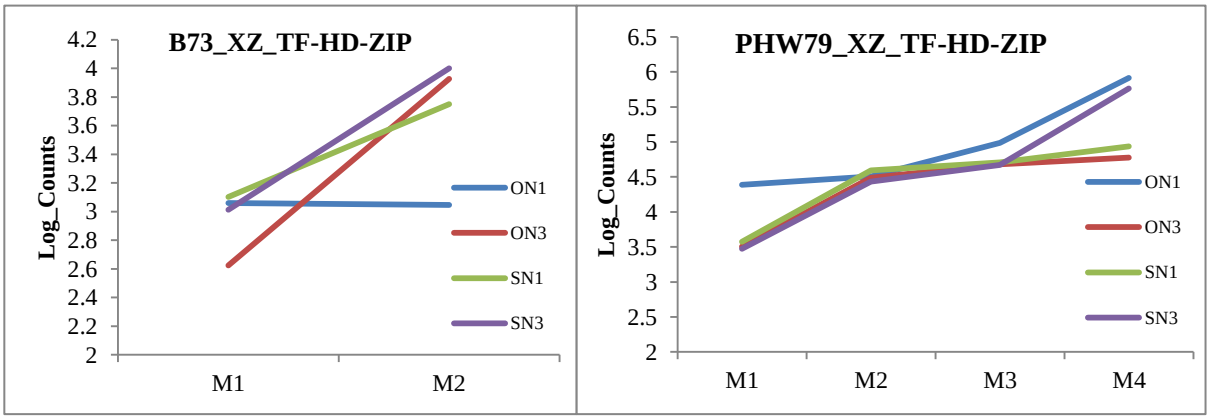

Figure 26. Expression of transcription factor "TF-HD-ZIP" ("*Zm00001d021934*") in both genotypes of maize during senescence at Xinzo (ON3: optimum water and nitrogen treatment; ON1: optimum water and low nitrogen level; SN3: stress water and optimum nitrogen level; SN1: low water and nitrogen level; M1, M2, M3: different senescence time, flowering, 30, and 45 days after flowering, respectively; B73: non stay green genotype; PHW79: stay green genotype).

4.3. Partial discussion of chapter four

Senescence, as the final step of plant growth and development is highly correlated with crop yield (Wu et al., 2012). During senescence, the metabolism of leaf cells changes. Specifically, assimilation decreases while catabolism is enhanced, e.g., chloroplast degradation occurs, the photosynthetic capacity decreases, and macromolecular material degrades (Lira et al., 2017). Furthermore, leaf senescence is affected by both internal and external factors (Zhang et al., 2018). The present study provides an overview enrichment of genes associated with leaf senescence under abiotic stresses in two maize inbred lines evaluated in two locations, through genomics interventions. We have identified a total of 12453 DEGs during senescence, where high numbers of DGEs were detected during [M1_M2] senescence time (8583 DEGs) compared to [M2_M3] senescence time (4933 DEGs).

Previous research considered leaf senescence as a complicated and highly regulated developmental process, and many senescence associated genes (SAGs) were identified in arabidopsis, wheat, rice, and maize (Li et al., 2014). They show also that, leaf senescence occurs via degradation of proteins, carbohydrates, lipids, and nucleic acids, and the mobilization of micronutrients (Chao et al., 2018). In addition, Eckardt (2009) stated that chlorophyll degradation is vital during leaf senescence and fruit ripening, as it allows recycling of nitrogen and other nutrients. The result of gene ontology (GO) test of early and late senescence genes for both genotypes in both locations, reveal that the genes involved in “photosynthesis”, “organonitrogen compound biosynthetic process”, and “metabolic process”, were down-regulated, which is consistent with decreasing in photosynthetic activity. This result agrees with the result reported by Wu et al. (2017), who found that genes involved in photosynthesis were down-regulated, and a decline in photosynthetic activity may trigger senescence. In addition, Gregersen et al. (2008) found that early leaf senescence caused by intrinsic or environmental factors results in a photosynthetic decline, which confirm our result. However, genes mainly encoding “thylakoid”, “thylakoid part”, and “chloroplast membrane” were up-regulated in Tomeza location. However, in Xinzo the up_regulated GO terms were enriched for ADP and ATP transport (GO: 0015866 and GO: 0015867, respectively), and catabolic processes. Both ADP and ATP were directly implicated in the ATP/ADP transport especially in the mitochondrial ADP/ATP carrier proteins as seen in *Solanum pennellii* (a wild tomato species) by D’Esposito et al. (2019).

Several studies were conducted to better understand leaf senescence process and for the identification of a number of transcription factors (TFs). Lin et al. (2015) found in his study that WRKY, bHLH, C3H and AP2 were the top TFs families active during senescence in cotton. WRKY have been reported to be important for senescence (Robatzek and Somssich, 2001; Miao et al., 2004). This is in accordance with our result, as we detected the expression of all this TFs during senescence in both locations. MYB proteins are responsible for controlling development and metabolism in plant, and they participate also in leaf senescence and in the defense and response to variable biotic or abiotic stress (Lin et al., 2013 ; Liu et al., 2004). bZIP proteins are one of the most diverse TFs, which can regulate plant development, physiological process, and biotic/abiotic stress response (Baloglu et al., 2014). Balazadeh et al. (2008) reported also, that TFs NAC, WRKY, MYB, C2H2, bZIP and AP2 have been identified as taking part in the regulation of leaf senescence progress, and Caicedo (2018) reported the over expression of this TFs during senescence. This is similar to our result for the TFs families' active during senescence in both locations. In this study, the expression of TFs related to abiotic stresses mean that both genotypes have a response to abiotics stresses, and they expressed different TFs to regulate the genes expression under those conditions of stresses.

Different environmental stresses can affect plants during senescence, and can limit crop yield. To endure those stresses, plants respond with coordinated changes in their transcriptome. A specific analysis was carried out for each genotype under both stresses, and then for each individual stress. For the first location Tomeza, the combined stresses SN1 in B73 have enriched GO terms involved for "Mitochondrial fission", "regulation of cell shape", "cell-cell signaling" and "protein targeting membrane" for the down-regulated genes. And it was enriched for "Response to oxygen containing compound", "response to stress", "and response to chemical" and, "malate metabolic process" for up-regulated genes. For the SG genotype PHW79, the most enriched GO terms were "NAD transport", "Wax biosynthetic process", "Wax metabolic process" and cofactor transport" for up-regulated genes; and enriched for "single organism development process", "developmental process", "multicellular organismal development", "system development", "shoot system development" for down-regulated genes. For Xinzo, we identified different GO terms enriched for each genotype. For PHW79 genotype, we detected the up-regulation of GO terms involved in "Protein deneddylation", "cop9 signalosome" and different GO terms involved in metabolism and transport. For B73, the up-regulated GO terms were enriched for different

processes of localization and transport. We found also the down-regulation of terms involved for "gene expression", metabolic and biogenesis processes, organo-nitrogen and nitrogen compound metabolic process, and photosynthesis and chloroplast organization, which is similar to B73. We can see that, both genotype in both location have answer to combined stress by different processes that can help the plant to finish the cecle or promote senescence, where the plant increases the expression of genes involved in wax biosynthesis, response to oxygen containing, response to stimulus and stresses, and reduce the expression of different process of biosynthesis, metabolism, and development process; this answer can limit plant yield loss and help to stresses tolerance.

During senescence, mitochondria provide energy and metabolites for degrading the cell components and relocating them to other younger parts of the plant (Ruberti et al., 2014; Chrobok et al., 2016). During leaf senescence in individually darkened leaves in Arabidopsis, the number of mitochondria decreases (Keech et al., 2007). Yoshinaga et al., (2005) has been reported that morphological changes in mitochondria are one of the features of cell death that is induced by reactive oxygen species in Arabidopsis. Moreover, it was shown that ablation of mitochondrial fission extends the life span of the two fungal species, *Podospora anserina* and *Saccharomyces cerevisiae* (Scheckhuber et al., 2007). "Mitochondrial fission" is the most up-regulated GO term for B73. Zottini et al. (2006), show that inhibition of mitochondrial fission *per se*, may be a primary cause for senescence-associated cellular changes and further suggest that dynamic mitochondrial fission is needed to prevent cells from undergoing senescence-associated phenotypic changes. We show also for B73 the high expression of terms involved for cellular nitrogen compound and nitrogen compound, which can be the explication for higher nitrogen remobilization showed in Chapter 3, and contribute to early senescence.

Previous study found that the accumulation of wax has a key role in limiting water losses from plants (Bartels and Nelson, 1994). In addition, drought stress can increase the amount of wax in several species (Kosma et al., 2009; Bondada et al., 1996), and this increase is associated with an improved drought tolerance (Islam et al., 2009). Also, wax protects plants against high temperature, strong UV radiation, bacterial and fungal pathogens as well as insects, increases plants' tolerance to high salinity and low temperature (Lee and Suh, 2015) . In addition, it was also found that cuticular wax is involved in the processes of plant morphology and development

through tight epidermal connections (Javelle et al., 2011). Cuticular wax plays an important role in crop yield, and the increase of wax content is associated with enhanced drought tolerance in many plants (Guo et al., 2016). Drought-tolerance and yield were higher in crops having more cuticular wax than those with less wax or non-waxy crops (Guo et al., 2016). According to our result, wax was the most expressed GO term under both nitrogen and drought stresses for the SG genotype PHW79.

For drought stress, we identified differences for genes expression between water levels for each genotype in each location. PHW79 under water stress was enriched for RNA interference, biosynthesis and metabolic process for up-regulated GO terms, and enriched for different processes of localization and transport, response to stimulus for down-regulated GO terms. While B73 was enriched for different catabolic processes for up-regulated genes, and for metabolic and biosynthetic processes for down regulated processes. In addition, the answer of both genotypes under well water condition was similar for most enriched down and up-regulated GO terms. In Xinzo, under water stress for B73, we show that the up-regulation of different GO terms were involved in transport and localization processes, and down regulation of processes involved in photosynthesis. Our findings were consistent with those of You et al. (2019), who showed that transcriptome data showed that the majority of DEGs during drought stress were enriched in biological process related to macromolecule metabolic process, nitrogen compound metabolic process, biosynthetic process, protein modification process and organelle organization (You et al., 2019). Chao et al. (2018) show that leaf senescence occurs via degradation of proteins, carbohydrates, lipids, nucleic acids and the mobilization of micronutrients.

For nitrogen levels, the most enriched GO terms under N1 in Tomeza for B73 were mitochondrial fission and response to stimulus for down and up-regulated GO terms, respectively. While, for PHW79, the most enriched GO terms were wax biosynthesis and metabolic process for up and down enriched GO terms, respectively. In Xinzo we did not detect any enriched GO term under nitrogen stress condition N1 for B73. However, for PHW79, the up-regulation of GO terms involved catabolic processes like “heterocycle catabolic process”, transport like “pyrimidine nucleobase transport” and “uracil transport” and response to stimulus. And the down-regulated terms were related with chloroplast and plastid organization and phosphatase activity. The difference between both nitrogen levels N1 and N3 was expressed for

the up regulated terms. Under N1 level for both genotypes we have the up-regulation of terms involved for response to stimulus, metabolic process, and localization. In addition to those terms, for PHW79 we found the expression of genes involved in wax biosynthesis and metabolic processes. For down regulated terms, we did not identify a clear difference between both genotypes for the answer to nitrogen levels.

The NAD GO term is the specific and most expressed (up_regulated) term under different stresses for PHW79. The assimilation of nitrogen is associated with high NADH/NADPH consumption (Xu et al., 2012); where, N absorption improves the photosynthetic system which is one of the biggest resources of NADPH production in plants (Evans, 1989). NADPH also acts as an electron donor in carbon dioxide fixation in the Calvin cycle (light-independent reactions) (Flood et al., 2011) and lipid biosynthesis (Ohlrogge and Browse, 1995). These results can explain that enriched GO terms for the genotype PHW79 and not enriched for B73, and vice versa may play a regulatory role during senescence, also the enriched GO terms expressed for the SG genotype PHW79 may play regulatory roles for abiotic stresses tolerance.

The specific comparisons of the change in genes expression rate during each senescence time show some differences between both locations. Both genes involved in chlorophyll degradation, SGR1 and NYC1, are expressed in Tomeza location. Whereas, in Xinzo only the NYC1 was expressed during different senescence times. Chlorophyll break down occurs in response to several abiotic and biotic stresses, in addition to senescence (Lim et al., 2007). While, NYC1 is thought to represent a “Chl b” reductase necessary for catalyzing the first step of “Chl b” degradation. NYC1 was found to be induced concomitant with chlorophyll a degradation by SGR expression (Sato et al., 2007). For the transcription factors, the specific analysis of expression rate of four TFs shows that for the TF-ERF, we show only this expression in Tomeza during M4, we detected the expression of TF-HD-ZIP only in Xinzo location and not in Tomeza, and we show the expression of NAC (“Zm00001d022424” and”Zm00001d041472”) in both locations. AP2/ERF TFs play a vital role in abiotic and biotic stresses endurance through different stress-mediated signal transduction pathways (Javed et al., 2020). And HD-Zip TFs play an important role in the regulation of development in response to changes in environmental conditions and hormonal stimuli, especially under water deficit stress and different light conditions (Harris et al., 2011). Zip protein family directly and positively regulates the expression

of several auxin biosynthesis, transport, and response genes (Huang et al., 2014). For the NAC TFs, which is one of the most important and largest family of plant-specific stress-responsive TFs (Jensen et al., 2010), we detected their expression in both locations for both genes. The *NAC* family has been found to function in various processes including leaf senescence (Breeze et al., 2011), and biotic and abiotic stress responses (Nakashima et al., 2012).

From this chapter we can conclude some principal result from this experiment like:

- Our results show that, during leaf senescence for both SG and NSG genotypes under different conditions and locations; genes enriched for the photosynthetic activity will be decreased during senescence.
- During Senescence, different transcriptions factors related to senescence were active in both locations; with some difference for the activation rate in each time.
- The SG genotype PHW79 showed the expression of different terms involved in delayed leaf senescence and abiotic stresses tolerance, like terms involved for wax and NAD expression; however the NSG genotype B73 showed the expression of terms that can promote leaf senescence, as nitrogen compound, and mitochondrial fission; which is the primary cause of leaf senescence. This difference may explain the difference between both genotypes for Stay-green phenotype.
- The difference between both nitrogen and water level were not very clear; but, generally, under abiotic stresses in both locations the plants increased their catabolic process, and localization of different elements remobilized to kernel. And decrease their photosynthetic activity, and different metabolic and biosynthetic process.

Chapter 5: General discussion

V. Chapter 5: General discussion

The stay-green is a secondary trait that enables crop plants to maintain their green leaves and photosynthetic capacity for a longer time after silking, especially under abiotic stresses (Zhang et al., 2019). Therefore, SG plants have a longer grain filling period and higher yield than NSG plants. Breeding for functional SG has contributed to increase crop yield, especially when it is combined with others useful traits (Kamal et al., 2019). Genetic dissection of target traits through mapping and transcript analysis is currently a powerful method for better understand of complex traits including delayed leaf senescence. It has been proved that stay-green is largely polygenic in nature and regulated by quantitative traits (You et al., 2016).

In our study we have used different methodologies to obtain a comprehensive analysis of the senescence process and their effect under abiotic stress for maize inbred lines with contrasting character for the SG trait. In this context, eight genotypes with contrasting expression of SG trait were used for the first objective in which we carried out physiological and agronomic evaluations in the field of different genotypes during senescence under different levels of abiotic factors (Chapter 3); and for the second objective, the analysis of genes differentially expressed during senescence under abiotic stresses, we opted for two representative genotypes (Chapter 4).

5.1. Evaluation of SG and NSG genotypes during senescence time

For obtaining a deeper understanding of the differences between SG and NSG genotypes, we have made a integrate discussion including both phenotypic and expression data. For both types of data we detected significant differences between SG and NSG genotypes. The comparison between SG and NSG genotypes for their physiological and agronomic traits showed that SG genotypes have higher performance than NSG genotypes for most traits. With respect to physiological traits, we found the loss of different activities, specifically photosynthesis activity, after silking time and consistently we found that several genes enriched for photosynthetic activity were down regulated. We found that some core genes down regulated during senescence for both types of genotypes were involved in photosynthetic activity, while different catabolic processes were up regulated. This can explain the decrease in photosynthetic activity, and the degradation of chlorophyll, and quantum efficiency of photosystem II observed during evaluation

of different physiological traits. As previously reported, leaf senescence occurs via degradation of proteins, carbohydrates, lipids, nucleic acids and the mobilization of micronutrients (Chao et al., 2018). Hörtensteiner and Feller (2002) found that senescing leaf begins primarily with protein degradation and nucleic acid catabolism. Wu et al. (2012) estimated that the genes involved in macromolecule degradation and nutrient recycling account for about 9% of the total genes expressed during senescence. On the other hand, Wu et al. (2012) showed that during senescence, plants activate a self-destructive program to degrade cell structure, and make final contribution to the plant by remobilizing the nutrients accumulated in the senescing leaf.

Our results show that the grain filling period is delayed for SG genotypes compared to NSG genotypes, which can be explained by the early expression of SAGs for the NSG genotype compared to the SG. The early expression of these genes accelerates the senescence process, which can affect biomass and grain yield. In addition, for the SG genotypes, the expression of NAD and different biosynthesis processes were up-regulated, which means that the plant continues its photosynthetic activity, and nitrogen assimilation. However, for NSG, we detected the early expression of genes involved in ROS, nitrogen compound and different processes of cellular degradation, which produce the early senescence and nitrogen remobilization. These results can explain our physiological and agronomic results, where SG genotypes have lower nitrogen remobilization than NSG genotypes. The recent focus on the breeding of specialized biofuel crops has stimulated research on biomass production and previous studies showed that in maize, delaying leaf senescence is a key component for increasing the overall biomass (Richards, 2000), and biomass production for biofuels can be maximized by delaying senescence (Wu et al., 2012). The molecular results showed the late up-regulation of different SAGs related to catabolism and cellular degradation for SG genotypes compared to NSG ones. He et al. (2002) found that senescence process involves the degradation of chloroplasts and release of nitrogen from leaves to other organs.

The up-regulation of catabolic and cellular degradation terms has an effect to accelerate senescence; whereas, for SG genotypes we found the expression of terms involved in delaying senescence, and delaying different processes related to the physiological activity of the plant. The same result was obtained with the field evaluation for different physiological and agronomic traits; where the SG loss their photosynthetic activity, chlorophyll content, and quantum efficiency of photosystem II more lately compared to NSG. Pinto et al., (2016) showed a similar

relationship between stay-green and agronomic traits, especially with yield and yield component traits.

Many transcriptional factors exhibit a senescence-associated pattern, including NAC, WRKY and MYB domains, indicating the importance of transcriptional regulation for senescence (Wu et al., 2012)**.** In this study, we identified TFs that change the expression with senescence we found that belong mainly to the famlies NAC, WRKY, MYB, bZIP and AP2, which was in agreement with Caicedo (2018). The expression of different TFs related to senescence can also justify our result for the decrease in different physiological activities of the genotypes during field evaluation. Furthermore, the TFs can play a role to increase tolerance to different abiotic stresses like ERF, WRKY, NAC and WRKY; these TFs are associated with stress and are the major regulatory factor during multiple stresses, and play critical roles in plants in response to biotic and abiotic stresses (Atkinson and Urwin, 2012; Tiwari et al., 2020).

In this study, SG genotypes have higher grain and stover yield, and maintain better physiological activity of the plant; however, SG genotypes have delayed grain filling period, and high grain and stover moisture; which can be a dilemma for the farmer because the harvest has to be delayed and there may be complications in preserving grain and stover during post-harvest storage due to the high moisture at harvest. In this context, Gong et al. (2005) noted that SG trait can increase crop yield; but, unfavorably prolonged delayed leaf senescence resulting in a low grain filling rate and a low grain protein content.

5.2. Effect of abiotic stresses for SG and NSG genotype during senescence

The evaluation in field of different physiological and agronomical traits shows that abiotic stresses have significant and negative effects for most traits during senescence. Other authors, for example Zhang et al. (2018) found that leaf senescence is affected by both internal and external factors. According to Rajcan and Tollenaar (1999) the senescence might be accelerated due to abiotic stresses, which drastically reduce the period of active C and N uptake in early maturing cultivars (Gnädinger, 2018).

An effective response to the environment is particularly important for plants. This means that cells have the ability to quickly sense signals from the surrounding environmental. System signals generated by the tissues exposed to abiotic and biotic stress coordinate and execute plant

stress responses in terms of metabolism and developmental adjustments (Piao et al., 2019)**.** Our results show that abiotic stress delay silking days and increase anthesis silking interval, which can produce pollen abortion and loss of grain yield. On the other hand, abiotic stresses produced early senescence and a reduced grain filling period. This can result in less biomass accumulation and less nitrogen assimilation after silking that can reduce biomass and grain yield. Moreover, the results show lower stover, cobs and 1000KW under abiotic stresses. Also, for different physiological traits, we found that all genotypes have better physiological activity under optimal condition compared to stress conditions.

Leaf senescence is an important life process that can be accelerated after stress (Hörtensteiner and Feller, 2002) that reduces crop yield and quality (Chao et al., 2018). These results coincide with our molecular results, where we identified various terms enriched for each stress or combined stresses. We detected the expression of the terms enriched for ROS, response to stress, response to chemical, transport, localization, and catabolic process, and response to stimulus. All these terms were up-regulated under abiotic stresses, which means that the plant respond to stress by reduction of their activity, and activate different processes of catalyzation, oxydation and degradation for different structures of the plant. Naika et al. (2013) found the same terms active under abiotic stresses, and they concluded that these terms can be associated with multiple stresses. We found also alteration of the expression of transport and localization processes after flowering allowing the remobilization from vegetative part to the kernel, and accelerate senescence. The expression of different processes related to stress as respond to chemicals and response to stimulus confirm that the plant reacted to stress and tried to defend itself.

In addition, we show the reduction of genes expression in terms involved in different processes of metabolism, cellular and organelle organization (chloroplast and thylakoid), and biosynthesis under stresses. The decrease in genes expression for these terms mean that the plant responds to different stresses by the reduction of different metabolic processes, and cell division or formation, and by the degradation of different tissues (chloroplasts and thylakoid). All these processes limit the activity of the plant and accelerate senescence rate. This result is in agreement with Guo et al. (2004) who estimate that the most notable characteristics of leaf senescence is the obvious metabolic transition from primary anabolism to catabolism. The number of catabolic genes highly expressed in senescing leaves is almost twice that of anabolic genes. In this sense, Tahmasebi et al. (2019) found that the gene families involved in cell wall showed various patterns of

expression under abiotic stresses. In other ways, Gregersen et al. (2013) show that accelerated senescence might reduce crop yield, when leaf senescence occurs during grain filling induced by environmental stresses such as drought or low soil nitrogen content.

From molecular analysis, we detected the activation of some specific terms in one genotype and not in other under abiotic stresses. For the SG genotype the most expressed terms were wax and NAD terms; being both of them up-regulated. The same terms were not expressed for NSG genotype. On the other hand we found some specific terms for the NSG genotype as mitochondrial fission and nitrogen compound. Previous results show that wax terms have an effect for abiotic stresses tolerance, and NAD terms permit to maintain photosynthesis activity. As previously noted, cuticular wax provides an essential barrier to protect plants from drought stress (Lee and Suh, 2015), and also serves as a barrier to restrain uncontrolled non-stomatal plant gas exchange (Xue et al., 2017). Xu et al. (2012) reveal that the assimilation of nitrogen is associated with high NADH/NADPH consumption (Evans, 1989), while mitochondrial fission has the effect of accelerating senescence, and nitrogen compound accelerate nitrogen remobilization to kernel. SG genotypes had better tolerance to abiotic stresses than NSG ones which is in accordance with the result of Thomas and Ougham (2014), who found that the SG phenotype is associated with heat and drought tolerance in several crop species. Similarly, Zheng et al. (2016) found that the SG phenotype exhibits a better drought resistance. In the same context, the development of SG genotypes has contributed to increased yield under stressful conditions in grasses, such as wheat, maize, rice, sorghum, and barley (Sade et al., 2018). For mitochondrial fission, a previous study showed that during leaf senescence the number of mitochondria decreases in Arabidopsis (Keech et al., 2007). These mitochondria are thought to provide energy and metabolites for degrading the cell components and relocating them to other younger parts of the plant (Keech et al., 2007; Chrobok et al., 2016).

We conclude also from our results that, drought and nitrogen stresses have an important effect for plant physiology and yield, compared to plant density stress. In this context, Yang et al. (2019) consider drought stress as one of the most important abiotic stresses that limit crop production. Plant density has a negative effect for individual plant yield (stover and 1000kw); but have no effect for general yield, this result was confirmed also by the molecular results, where we did not identify DEGs enriched for plant density.

Chapter 6: Conclusions and Perspectives

VI. Chapter 6: Conclusions and Perspectives

6.1. Conclusions

This study aimed to better understand the regulation of leaf senescence process under different abiotic stresses during grain filling period. The SG phenotype is an important trait to increase yield and face abiotic stresses in maize. This research was performed via a forward of physiological, agronomic and genetic approach. Here, I summarize the findings of this research with the comparison between genotypes with contrasting stay-green phenotype:

1- Regarding the relationship of senescence and agronomic traits, we found that the stay-green genotypes loss their photosynthetic activity during grain filling at slower rates compared to non-stay-green ones that translates into higher biomass and grain yield. On the other hand, the stay-green genotypes have higher stover and grain moisture, and a long grain filling period, which can be a problem for farmer storage, and can increase the costs of post-harvest management.

2- The stover nitrogen remobilization at maturity was lower for stay-green cultivars, which decreases the grain nitrogen content and produces low protein content in grain.

3- Drought and nitrogen stresses decreased different plant activities and yield, and promote senescence. High plant density has a positive effect in stover and grain yield per hectare, but a negative effect in individual plant production.

4- Nitrogen content in the plant depended on the availability of soil nitrogen, however kernel nitrogen remobilization was not dependent on nitrogen fertilization, but on genotypes capacity.

5- The senescence process is controlled by multiple genes repressed or activated, which can change their expression under abiotic stresses

6- The same senescence-associated genes expressed earlier for NSG genotype were delayed for SG ones; which results in delayed photosynthetic activity and increased the overall biomass and grain yield.

7- SG genotype increased the expression of genes responsible of the senescence delay and tolerance to abiotic stress like wax biosynthesis and metabolic process. Conversely, NSG

genotypes expressed some genes responsible of accelerating leaf senescence and cellular degradation, like the ROS and mitochondrial fission.

8- During senescence process, both genotypes increased the expression of transcriptions factors related to senescence and response to abiotic stresses.

9- Under abiotic stresses, both SG and NSG genotypes increased the expression of genes involved in catabolism and localization and decreased the expression of genes involved in metabolic and biosynthetic process.

6.2. Perpectives

Predictions of food stocks over the next 50 years indicate that a great challenge awaits us due to population growth (Tester and Langridge 2010). The increasing frequency of natural disasters and the unfavorable disturbances of the environment caused by climate change, as well as the search for alternative sources of biofuels, are adding even more pressure to agricultural production. Innovative approaches and new strategies have to be adopted to achieve further yield potential. The senescence of annual crops has been most intensively studied, and a delayed leaf senescence is the key component for the past yield gains in major crops. So, the exploitation of the control of leaf senescence, combined with efforts to increase the rate of photosynthesis and the ability to tolerate stresses, is essential for crop improvement to either achieve yield potential or to stabilize yield under stress conditions. In our studies for the present thesis, we evaluated physiological, agronomic, and molecular data in maize inbred lines, with contrasting SG phenotype under different abiotic stresses. So for the future works, it will be interesting focusing in:

- To extend the study of leaf senescence to all parts of the plant including roots
- To widen the study of senescence to other germplasm and type of materials, specifically hybrids
- To continue the analysis of senescence in combination with abiotic stresses to identify the optimum senescence for each environment using hyperspectral images that allow to include more genotypes and environments in the analysis

Bibliographic references

Bibliographic references

Bibliographic references

VII. Bibliographic references

Aasen, H., Honkavaara, E., Lucieer, A., Zarco-Tejada, P.J., 2018. Quantitative remote sensing at ultra-high resolution with UAV spectroscopy: A review of sensor technology, measurement procedures, and data correctionworkflows. Remote Sensing 10, 1–42. https://doi.org/10.3390/rs10071091

Abdelrahman, M., El-Sayed, M., Jogaiah, S., Burritt, D.J., Tran, L.S.P., 2017. The "STAY-GREEN" trait and phytohormone signaling networks in plants under heat stress. Plant Cell Reports 36, 1009–1025. https://doi.org/10.1007/s00299-017-2119-y

Abdul Jaleel, C., Manivannan, P., Kishorekumar, A., Sankar, B., Gopi, R., Somasundaram, R., Panneerselvam, R., 2007. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in Catharanthus roseus exposed to water deficit. Colloids and Surfaces B: Biointerfaces 59, 150–157. https://doi.org/10.1016/j.colsurfb.2007.05.001

Acciaresi, H.A., Tambussi, E.A., Antonietta, M., Zuluaga, M.S., Andrade, F.H., Guiamét, J.J., 2014. Carbon assimilation, leaf area dynamics, and grain yield in contemporary earlier- and later-senescing maize hybrids. European Journal of Agronomy 59, 29–38. https://doi.org/10.1016/j.eja.2014.05.007

Agalou, A., Purwantomo, S., Övernäs, E., Johannesson, H., Zhu, X., Estiati, A., De Kam, R.J., Engström, P., Slamet-Loedin, I.H., Zhu, Z., Wang, M., Xiong, L., Meijer, A.H., Ouwerkerk, P.B.F., 2008. A genome-wide survey of HD-Zip genes in rice and analysis of drought-responsive family members. Plant Molecular Biology 66, 87–103. https://doi.org/10.1007/s11103-007-9255-7

Agarwal, P.K., Agarwal, P., Reddy, M.K., Sopory, S.K., 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Reports 25, 1263–1274. https://doi.org/10.1007/s00299-006-0204-8

Ahmad, I., Kamran, M., Su, W., Haiqi, W., Ali, S., Bilegjargal, B., Ahmad, S., Liu, T., Cai, T., Han, Q., 2019. Application of Uniconazole Improves Photosynthetic Efficiency of Maize by Enhancing the Antioxidant Defense Mechanism and Delaying Leaf Senescence in Semiarid Regions. Journal of Plant Growth Regulation 38, 855–869. https://doi.org/10.1007/s00344-018-9897-5

Ajayo, B.S., Badu-Apraku, B., Fakorede, M.A.B., Akinwale, R.O., 2021. Plant density and nitrogen responses of maize hybrids in diverse agroecologies of west and central Africa. AIMS Agriculture and Food 6, 381–400. https://doi.org/10.3934/AGRFOOD.2021023

Al-Naggar, A., Atta, M., 2017. Elevated Plant Density Effects on Performance and Genetic Parameters Controlling Maize (Zea mays L.) Agronomic Traits. Journal of Advances in Biology & Biotechnology 12, 1–20. https://doi.org/10.9734/jabb/2017/31550

Amin, A.A., Rashad, E.M., Hassanein, M.S., Zaki, N.M., 2007. Response of Some White Maize Hybrids to Foliar Spray with Benzyl Adenine. Res. J. Agric. Biol. Sci. 3, 648.

Andrade, F.H., Calviño, P., Cirilo, A., Barbieri, P., 2002. Yield responses to narrow rows depend on increased radiation interception. Agronomy Journal 94, 975–980. https://doi.org/10.2134/agronj2002.0975

Angle, J.S., Gross, C.M., Hill, R.L., McIntosh, M.S., 1993. Soil Nitrate Concentrations under Corn as Affected by Tillage, Manure, and Fertilizer Applications. Journal of Environmental Quality 22, 141–147. https://doi.org/10.2134/jeq1993.00472425002200010018x

Antonietta, M., Fanello, D.D., Acciaresi, H.A., Guiamet, J.J., 2014. Senescence and yield responses to plant density in stay green and earlier-senescing maize hybrids from Argentina. Field Crops Research 155, 111–119. https://doi.org/10.1016/j.fcr.2013.09.016

Arteca, R.N., 1996. Plant growth substances: principles and applications. Chapman & Hall, New York, USA.

Asad, M.A.U., Zakari, S.A., Zhao, Q., Zhou, L., Ye, Y., Cheng, F., 2019. Abiotic stresses intervene with aba signaling to induce destructive metabolic pathways leading to death: Premature leaf senescence in plants. International Journal of Molecular Sciences 20, 23. https://doi.org/10.3390/ijms20020256

Atkinson, N., Urwin, P.E., 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany 63, 3523–3544. https://doi.org/10.1093/jxb/err313

Aydinsakir, K., Erdal, S., Buyuktas, D., Bastug, R., Toker, R., 2013. The influence of regular deficit irrigation applications on water use, yield, and quality components of two corn (Zea mays L.) genotypes. Agricultural Water Management 128, 65–71. https://doi.org/10.1016/j.agwat.2013.06.013

Baena-González, E., Rolland, F., Thevelein, J.M., Sheen, J., 2007. A central integrator of transcription networks in plant stress and energy signalling. Nature 448, 938–942. https://doi.org/10.1038/nature06069

Balazadeh, S., Riaño-Pachón, D.M., Mueller-Roeber, B., 2008. Transcription factors regulating leaf senescence in Arabidopsis thaliana. Plant Biology 10, 63–75. https://doi.org/10.1111/j.1438-8677.2008.00088.x

Baldoni, E., Genga, A., Cominelli, E., 2015. Plant MYB transcription factors: Their role in drought response mechanisms. International Journal of Molecular Sciences 16, 15811–15851. https://doi.org/10.3390/ijms160715811

Ballabio, C., Panagos, P., Monatanarella, L., 2016. Mapping topsoil physical properties at European scale using the LUCAS database. Geoderma 261, 110–123. https://doi.org/10.1016/j.geoderma.2015.07.006

Baloglu, M.C., Eldem, V., Hajyzadeh, M., Unver, T., 2014. Genome-wide analysis of the bZIP transcription factors in cucumber. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0096014

Bänziger, M., Edmeades, G.O., Beck, D., Bellon, M., 2000. Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice, Mexico, D.F.: CIMMYT. https://doi.org/633.1553

BARTELS, D., NELSON, D., 1994. Approaches to improve stress tolerance using molecular genetics. Plant, Cell & Environment 17, 659–667. https://doi.org/10.1111/j.1365-3040.1994.tb00157.x

Bekavac, G., Purar, B., Stojakovic, M., Jockovic, D.J., Ivanovic, M., Nastasic, A., 2007. Genetic Analysis of Stay-Green Trait in Broad-Based Maize Populations. cereal research communications 35, 31–41. https://doi.org/10.1556/CRC.35.2007.1.5

Bogard, M., Jourdan, M., Allard, V., Martre, P., Perretant, M.R., Ravel, C., Heumez, E., Orford, S., Snape, J., Griffiths, S., Gaju, O., Foulkes, J., Le Gouis, J., 2011. Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. Journal of Experimental Botany 62, 3621–3636. https://doi.org/10.1093/jxb/err061

Bondada, B.R., Oosterhuis, D.M., Murphy, J.B., Kim, K.S., 1996. Effect of water stress on the

epicuticular wax composition and ultrastructure of cotton (Gossypium hirsutum L.) leaf, bract, and boll. Environmental and Experimental Botany 36, 61–65. https://doi.org/10.1016/0098-8472(96)00128-1

Borrás, L., Maddonni, G.A., Otegui, M.E., 2003. Leaf senescence in maize hybrids: Plant population, row spacing and kernel set effects. Field Crops Research 82, 13–26. https://doi.org/10.1016/S0378-4290(03)00002-9

Borras, L., Westigate, M.E., Otegui, M.E., 2003. Control of Kernel Weight and Kernel Water Relations by Post-owering Source sink Ratio in Maize. Annals of botany 91, 857–867. https://doi.org/10.1093/aob/mcg090

Borrell, A., Hammer, G., Van Oosterom, E., 2001. Stay-green: A consequence of the balance between supply and demand for nitrogen during grain filling? Annals of Applied Biology 138, 91–95. https://doi.org/10.1111/j.1744-7348.2001.tb00088.x

Borrell, A., Hammer, G.L., 2000. Nitrogen Dynamics and the Physiological Basis of Stay-Green in Sorghum. crop 40, 1295–1307. https://doi.org/10.2135/cropsci2000.4051295x

Borrell, A.K., Hammer, G.L., Douglas, A.C.L., 2000. Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. Crop Science 40, 1026–1037. https://doi.org/10.2135/cropsci2000.4041037x

Borrell, A.K., Van Oosterom, E.J., Mullet, J.E., George-jaeggli, B., Jordan, D.R., Klein, P.E., Hammer, G.L., 2014. Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. New Phytologist 203, 817–830.

Breeze, E., Harrison, E., McHattie, S., Hughes, L., Hickman, R., Hill, C., Kiddle, S., Kim, Y.S., Penfold, C.A., Jenkins, D., Zhang, C., Morris, K., Jenner, C., Jackson, S., Thomas, B., Tabrett, A., Legaie, R., Moore, J.D., Wild, D.L., Ott, S., Rand, D., Beynon, J., Denby, K., Mead, A., Buchanan-Wollaston, V., 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. Plant Cell 23, 873–894. https://doi.org/10.1105/tpc.111.083345

Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T., Pink, D., 2003. The molecular analysis of leaf senescence--a genomics approach. Plant Biotechnol J 1, 3–22. https://doi.org/10.1046/j.1467-7652.2003.00004.x

Buckley, T.N., 2019. How do stomata respond to water status? New Phytologist 224, 21–36. https://doi.org/10.1111/nph.15899

Burken, D., Harding, J., McGee, A., 2013. Effects of Corn Hybrid, Plant Density, and Harvest Time on Yield and Quality of Corn Plants. Nebraska Beef Cattle Report 42–43.

Caicedo, B.M., 2018. Mejora Getética de Maiz para Senescencia Retrasada " STAY GREEN ." Thesis doctoral, Santiago de Compostela- Lugo.

Cairns, J.E., Sanchez, C., Vargas, M., Ordoñez, R., Araus, J.L., 2012. Dissecting Maize Productivity: Ideotypes Associated with Grain Yield under Drought Stress and Well-watered Conditions. Journal of Integrative Plant Biology 54, 1007–1020. https://doi.org/10.1111/j.1744-7909.2012.01156.x

Calviño, M., Messing, J., 2012. Sweet sorghum as a model system for bioenergy crops. Current Opinion in Biotechnology 23, 323–329. https://doi.org/10.1016/j.copbio.2011.12.002

Câmara, T.M.M., Bento, D.A.V., Alves, G.F., Santos, M.F., Moreira, J.U.V., Souza Júnior, C.L. de, 2007. Parâmetros genéticos de caracteres relacionados à tolerância à deficiência hídrica em milho tropical. Bragantia 66, 595–603. https://doi.org/10.1590/S0006-87052007000400009

Cao, D., Zhou, S., Niu, Z., 2013. Optimal combination of base station densities for energy-efficient two-tier heterogeneous cellular networks. IEEE Transactions on Wireless Communications 12, 4350–4362. https://doi.org/10.1109/TWC.2013.080113.121280

Carter, M.W., Poneleit, C.G., 1973. Black Layer Maturity and Filling Period Variation Among Inbred Lines of Corn (Zea mays L.) 1 . Crop Science 13, 436–439. https://doi.org/10.2135/cropsci1973.0011183x001300040014x

Ceppi, D., Sala, M., Gentinetta, E., Verderio, A., Motto, M., 1987. Genotype-Dependent Leaf Senescence in Maize. Plant Physiology 85, 720–725. https://doi.org/10.1104/pp.85.3.720

Cernusak, L.A., Ubierna, N., Winter, K., Holtum, J.A.M., Marshall, J.D., Farquhar, G.D., 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. New Phytologist 200, 950–965. https://doi.org/10.1111/nph.12423

Chai, M., Guo, Z., Shi, X., Li, Y., Tang, J., Zhang, Z., 2019. Dissecting the Regulatory Network of Leaf Premature Senescence in Maize (Zea mays L .) Using Transcriptome Analysis of ZmELS5 Mutant. Genes 10, 18.

Chao, Y., Xie, L., Yuan, J., Guo, T., Li, Y., Liu, F., Han, L., 2018. Transcriptome analysis of leaf senescence in red clover (Trifolium pratense L.). Physiology and Molecular Biology of Plants 24, 753–765. https://doi.org/10.1007/s12298-018-0562-z

Chapman, E.A., Orford, S., Lage, J., Griffiths, S., 2021. Delaying or Delivering: Identification of novel NAM-1 alleles which delay senescence to extend wheat grain fill duration. Experimental Biology 35.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany 103, 551–560. https://doi.org/10.1093/aob/mcn125

Chen, L., Song, Y., Li, S., Zhang, L., Zou, C., Yu, D., 2012. The role of WRKY transcription factors in plant abiotic stresses. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms 1819, 120–128. https://doi.org/10.1016/j.bbagrm.2011.09.002

Chen, Y., Xiao, C., Chen, X., Li, Q., Zhang, J., Chen, F., Yuan, L., Mi, G., 2014. Characterization of the plant traits contributed to high grain yield and high grain nitrogen concentration in maize. Field Crops Research 159, 1–9. https://doi.org/10.1016/j.fcr.2014.01.002

Chen, Y., Xiao, C., Wu, D., Xia, T., Chen, Q., Chen, F., Yuan, Y., Mi, G., 2015. Effects of nitrogen application rate on grain yield and grain nitrogen concentration in two maize hybrids with contrasting nitrogen remobilization efficiency. European Journal of Agronomy 62, 79–89. https://doi.org/10.1016/j.eja.2014.09.008

Chen, Y.F., Etheridge, N., Schaller, G.E., 2005. Ethylene signal transduction. Annals of Botany 95, 901–915. https://doi.org/10.1093/aob/mci100

Chetty, L., 2004. The impact of pollen movement on identity preservation of maize (Zea mays). Magister Scientiae. University of the Free State-South Africa.

Chibane, N., Caicedo, M., Martinez, S., Marcet, P., Revilla, P., Ordás, B., 2021. Relationship between

Delayed Leaf Senescence (Stay-Green) and Agronomic and Physiological Characters in Maize (Zea mays L.). Agronomy 11, 276. https://doi.org/10.3390/agronomy11020276

Christ, B., Hörtensteiner, S., 2014. Mechanism and Significance of Chlorophyll Breakdown. Journal of Plant Growth Regulation 33, 4–20. https://doi.org/10.1007/s00344-013-9392-y

Christopher, J.T., Christopher, M.J., Borrell, A.K., Fletcher, S., Chenu, K., 2016. Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. Journal of Experimental Botany 67, 5159–5172. https://doi.org/10.1093/jxb/erw276

Christopher, J.T., Veyradier, M., Borrell, A.K., Harvey, G., Fletcher, S., Chenu, K., 2014. Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics. Functional Plant Biology 41, 1035–1048.

Chrobok, D., Law, S.R., Brouwer, B., Lindén, P., Ziolkowska, A., Liebsch, D., Narsai, R., Szal, B., Moritz, T., Rouhier, N., Whelan, J., Gardeström, P., Keech, O., 2016. Dissecting the metabolic role of mitochondria during developmental leaf senescence. Plant Physiology 172, 2132–2153. https://doi.org/10.1104/pp.16.01463

Ciampitti, I., Vyn, T., 2011. A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. Field Crops Research 121, 2–18.

Ciampitti, I.A., Vyn, T.J., 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. Field Crops Research 133, 48–67. https://doi.org/10.1016/j.fcr.2012.03.008

Clay, S.A., Clay, D.E., Horvath, D.P., Pullis, J., Carlson, C.G., Hansen, S., Reicks, G., 2009. Corn response to competition: Growth alteration vs. yield limiting factors. Agronomy Journal 101, 1522–1529. https://doi.org/10.2134/agronj2008.0213x

Crafts-Brandner, S.J., Below, F.E., Wittenbach, V.A., Harper, J.E., Hageman, R.H., 1984. Differential Senescence of Maize Hybrids following Ear Removal. Plant Physiology 74, 368–373. https://doi.org/10.1104/pp.74.2.368

Crow, J.F., Kermicle, J., 2002. Anecdotal , Historical and Critical Commentaries on Genetics Oliver Nelson and Quality Protein Maize. Genetics 160, 819–821.

Czyzewicz, J.R., Below, F.E., 1994. Genotypic variation for nitrogen uptake by maize kernels grown in vitro. Crop Science 34, 1003–1008. https://doi.org/10.2135/cropsci1994.0011183X003400040032x

D'Esposito, D., Cappetta, E., Andolfo, G., Ferriello, F., Borgonuovo, C., Caruso, G., De Natale, A., Frusciante, L., Ercolano, M.R., 2019. Deciphering the biological processes underlying tomato biomass production and composition. Plant Physiology and Biochemistry 143, 50–60. https://doi.org/10.1016/j.plaphy.2019.08.010

Dai, J., Gao, H., Dai, Y., Zou, Q., 2004. Changes in Activity of Energy Dissipating Mechanisms in Wheat Flag Leaves During Senescence. Plant biology (Stuttgart, Germany) 6, 171–177.

Davies, W.J., Zhang, J., Yang, J., Dodd, I.C., 2011. Novel crop science to improve yield and resource use efficiency in water-limited agriculture. Journal of Agricultural Science 149, 123–131. https://doi.org/10.1017/S0021859610001115

Desclos, M., Etienne, P., Coquet, L., Jouenne, T., Bonnefoy, J., Segura, R., Reze, S., Ourry, A., Avice, J.C., 2009. A combined 15N tracing/proteomics study in Brassica napus reveals the chronology of

proteomics events associated with N remobilisation during leaf senescence induced by nitrate limitation or starvation. Proteomics 9, 3580–3608. https://doi.org/10.1002/pmic.200800984

Diaz, C., Saliba-Colombani, V., Loudet, O., Belluomo, P., Moreau, L., Daniel-Vedele, F., Morot-Gaudry, J.F., Masclaux-Daubresse, C., 2006. Leaf yellowing and anthocyanin accumulation are two genetically independent strategies in response to nitrogen limitation in Arabidopsis thaliana. Plant and Cell Physiology 47, 74–83. https://doi.org/10.1093/pcp/pci225

Ding, L., Wang, K.J., Jiang, G.M., Biswas, D.K., Xu, H., Li, L.F., Li, Y.H., 2005. Effects of Nitrogen Deficiency on Photosynthetic Traits of Maize Hybrids Released in Different Years. Annals of botany 96, 925–930. https://doi.org/10.1093/aob/mci244

Distelfeld, A., Avni, R., Fischer, A.M., 2014. Senescence , nutrient remobilization , and yield in wheat and barley. Journal of Experimental Botany 65, 3783–3798. https://doi.org/10.1093/jxb/ert477

Dolferus, R., 2014. To grow or not to grow: A stressful decision for plants. Plant Science 229, 247–261. https://doi.org/https://doi.org/10.1016/j.plantsci.2014.10.002

dos Reis, S.P., Lima, A.M., de Souza, C.R.B., 2012. Recent molecular advances on downstream plant responses to abiotic stress. International Journal of Molecular Sciences 13, 8628–8647. https://doi.org/10.3390/ijms13078628

Duvick, D.N., 2005. Genetic progress in yield of United States maize (Zea mays L.). Maydica 50, 193–202.

Duvick, D.N., Smith, J.S.C., Cooper, M., 2004. Long-term selection on a commercial hybrid maize breeding program. Plant Breeding Reviews 24, 109–151.

Eckardt, N.A., 2009. A new chlorophyll degradation pathway. Plant Cell 21, 700. https://doi.org/10.1105/tpc.109.210313

Efeoğlu, B., Ekmekçi, Y., Çiçek, N., 2009. Physiological responses of three maize cultivars to drought stress and recovery. South African Journal of Botany 75, 34–42. https://doi.org/10.1016/j.sajb.2008.06.005

Ellis, C.M., Nagpal, P., Young, J.C., Hagen, G., Guilfoyle, T.J., Reed, J.W., 2005. Auxin response FACTOR1 and auxin response FACTOR2 regulate senescence and floral organ abscission in Arabidopsis thaliana. Development 132, 4563–4574. https://doi.org/10.1242/dev.02012

Erley, G.S. auf'm, Ambebe, T.F., Worku, M., Bänziger, M., Horst, W.J., 2010. Photosynthesis and leaf-nitrogen dynamics during leaf senescence of tropical maize cultivars in hydroponics in relation to N efficiency in the field. Plant and Soil 330, 313–328. https://doi.org/10.1007/s11104-009-0205-9

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Division of Plant Industry 78, 9–19. https://doi.org/10.1109/LSP.2017.2723724

Fahad, S., Hussain, S., Saud, S., Hassan, S., Ihsan, Z., Shah, A.N., Wu, C., Yousaf, M., Nasim, W., Alharby, H., Alghabari, F., Huang, J., 2016. Exogenously applied plant growth regulators enhance the morpho-physiological growth and yield of rice under high temperature. Frontiers in Plant Science 7, 13. https://doi.org/10.3389/fpls.2016.01250

Feng, L., Mathis, G., Ritchie, G., Han, Y., Li, Y., Wang, G., Zhi, X., Bednarz, C.W., 2014. Optimizing irrigation and plant density for improved cotton yield and fiber quality. Agronomy Journal 106, 1111–1118. https://doi.org/10.2134/agronj13.0503

Flood, P.J., Harbinson, J., Aarts, M.G.M., 2011. Natural genetic variation in plant photosynthesis. Trends in Plant Science 16, 327–335. https://doi.org/10.1016/j.tplants.2011.02.005

Foulkes, M.J., Hawkesford, M.J., Barraclough, P.B., Holdsworth, M.J., Kerr, S., Kightley, S., Shewry, P.R., 2009. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Research 114, 329–342. https://doi.org/10.1016/j.fcr.2009.09.005

Friedrich, J.W., Schrader, L.E., Nordheim, E. V., 1979. N Deprivation in Maize During Grain-Filling. I. Accumulation of Dry Matter, Nitrate-N, and Sulfate-S 1 . Agronomy Journal 71, 461–465. https://doi.org/10.2134/agronj1979.00021962007100030020x

Fukazawa, J., Sakai, T., Ishida, S., Yamaguchi, I., Kamiya, Y., Takahashi, Y., 2000. Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. Plant Cell 12, 901–915. https://doi.org/10.1105/tpc.12.6.901

Gallais, A., Coque, M., Le Gouis, J., Prioul, J.L., Hirel, B., Quilléré, I., 2007. Estimating the proportion of nitrogen remobilization and of postsilking nitrogen uptake allocated to maize kernels by nitrogen-15 labeling. Crop Science 47, 685–693. https://doi.org/10.2135/cropsci2006.08.0523

Galyuon, I.K.A., Gay, A., Borrell, A.K., Howarth, C.J., 2019. Phsiological and biochemical basis for stay-green trait in Sorghum. African Crop Science Journal 27, 653–677.

Gnädinger, F., 2018. High-Throughput Phenotyping the Nitrogen and Carbon Dynamics in Maize at the Reproductive Phase. Doktorarbeit, Technischen Universität München zur Erlangung des akademischen Grades eines.

Goel, P., Singh, A.K., 2015. Abiotic stresses downregulate key genes involved in nitrogen uptake and assimilation in brassica juncea l. PLoS ONE 10, 1–17. https://doi.org/10.1371/journal.pone.0143645

Gong, Y.H., Zhang, J., Gao, J.F., Lu, J.Y., Wang, J.R., 2005. Slow export of photoassimilate from stay-green leaves during late grain-filling stage in hybrid winter wheat (Triticum aestivum L.). Journal of Agronomy and Crop Science 191, 292–299. https://doi.org/10.1111/j.1439-037X.2005.00173.x

Gou, W., Zheng, P., Tian, L., Gao, M., Zhang, L., Akram, N.A., Ashraf, M., 2017. Exogenous application of urea and a urease inhibitor improves drought stress tolerance in maize (Zea mays L .). Journal of Plant Research 130, 599–609. https://doi.org/10.1007/s10265-017-0933-5

Gous, P.W., Hickey, L., Christopher, J.T., Franckowiak, J., Fox, G.P., 2016. Discovery of QTL for stay-green and heat-stress in barley (Hordeum vulgare) grown under simulated abiotic stress conditions. Euphytica 207, 305–317. https://doi.org/10.1007/s10681-015-1542-9

Grant, R.F., Jackson, B.S., Kiniry, J.R., Arkin, G.F., 1989. Water Deficit Timing Effects on Yield Components in Maize. Agronomy Journal 81, 61–65. https://doi.org/10.2134/agronj1989.00021962008100010011x

Gregersen, P.L., Culetic, A., Boschian, L., Krupinska, K., 2013. Plant senescence and crop productivity. Plant Molecular Biology 82, 603–622. https://doi.org/10.1007/s11103-013-0013-8

Gregersen, P.L., Holm, P.B., 2007. Transcriptome analysis of senescence in the flag leaf of wheat (Triticum aestivum L.). Plant Biotechnology Journal 5, 192–206. https://doi.org/10.1111/j.1467-7652.2006.00232.x

Gregersen, P.L., Holm, P.B., Krupinska, K., 2008. Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biology 10, 37–49. https://doi.org/10.1111/j.1438-8677.2008.00114.x

Grill, E., Himmelbach, A., 1998. ABA signal transduction. Current Opinion in Plant Biology 1, 412–418. https://doi.org/10.1016/S1369-5266(98)80265-3

Gully, K., Hander, T., Boller, T., Bartels, S., 2015. Perception of Arabidopsis At Pep peptides, but not bacterial elicitors, accelerates starvation-induced senescence. Frontiers in Plant Science 6, 1–10. https://doi.org/10.3389/fpls.2015.00014

Gungula, D.T., Togun, A.O., Kling, J.G., 2005. The Influence of N Rates on Maize Leaf Number and Senescence in Nigeria International Institute of Tropical Agriculture (IITA), 1, 1–5.

Guo, M., Liu, J., Ma, X., Luo, D., Gong, Z., Lu, M., 2016. The Plant Heat Stress Transcription Factors (HSFs): Structure , Regulation , and Function in Response to Abiotic Stresses. frontiers in plant science 7, 1–13. https://doi.org/10.3389/fpls.2016.00114

Guo, Y., Cai, Z., Gan, S., 2004. Transcriptome of Arabidopsis leaf senescence. Plant, Cell and Environment 27, 521–549. https://doi.org/10.1111/j.1365-3040.2003.01158.x

Gururani, M.A., Venkatesh, J., Tran, L.S.P., 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. Molecular Plant 8, 1304–1320. https://doi.org/10.1016/j.molp.2015.05.005

Gutierrez, R.A., Stokes, T.L., Thum, K., Xu, X., Obertello, M., Katari, M.S., Tanurdzic, M., Dean, A., Nero, D.C., McClung, C.R., Coruzzi, G.M., 2008. Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene CCA1. Proceedings of the National Academy of Sciences of the United States of America 12, 4939_4944. https://doi.org/10.1073/pnas.70.12.3387

Haberer, G., Kieber, J.J., 2002. Cytokinins. New insights into a classic phytohormone. Plant Physiology 128, 354–362. https://doi.org/10.1104/pp.010773

Harris, J.C., Hrmova, M., Lopato, S., Langridge, P., 2011. Modulation of plant growth by HD-Zip class I and II transcription factors in response to environmental stimuli. New Phytologist 190, 823–837. https://doi.org/10.1111/j.1469-8137.2011.03733.x

Harris, K.R., 2007. Genetic Analysis Of The Sorghum Bicolor Stay-Green Drought Tolerance Trait. PhD thesis, Texas A&M University.

Haussmann, B.I.G., Obilana, A.B., Ayiecho, P.O., Blum, A., Schipprack, W., Geiger, H.H., 1999. Quantitative-genetic parameters of sorghum [Sorghum bicolor (L.) Moench] grown in semi-arid areas of Kenya. Euphytica 105, 109–118. https://doi.org/10.1023/A:1003469528461

He, P., Zhou, W., Jin, J., 2002. Effect of nitrogen application on redistribution and transformation of photosynthesized14C during grain formation in two maize cultivars with different senescence appearance. Journal of Plant Nutrition 25, 2443–2456. https://doi.org/10.1081/PLN-120014706

Hessini, K., Issaoui, K., Ferchichi, S., Saif, T., Abdelly, C., Siddique, K.H.M., Cruz, C., 2019. Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. Plant Physiology and Biochemistry 139, 171–178. https://doi.org/10.1016/j.plaphy.2019.03.005

Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. Journal of Experimental Botany 58, 2369–2387. https://doi.org/10.1093/jxb/erm097

Hollmann, J., Gregersen, P.L., Krupinska, K., 2014. Identification of predominant genes involved in regulation and execution of senescence-associated nitrogen remobilization in flag leaves of field grown barley. Journal of Experimental Botany 65, 3963–3974. https://doi.org/10.1093/jxb/eru094

Hörtensteiner, S., 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends in Plant Science 14, 155–162. https://doi.org/10.1016/j.tplants.2009.01.002

Hörtensteiner, S., Feller, U., 2002. Nitrogen metabolism and remobilization during senescence. Journal of Experimental Botany 53, 927–937. https://doi.org/10.1093/jexbot/53.370.927

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Communications in Soil Science and Plant Analysis 31, 1299–1396. https://doi.org/10.1080/00103620009370514

Huang, T., Harrar, Y., Lin, C., Reinhart, B., Newell, N.R., Talavera-Rauh, F., Hokin, S.A., Kathryn Barton, M., Kerstetter, R.A., 2014. Arabidopsis KANADI1 acts as a transcriptional repressor by interacting with a specific cis-element and regulates auxin biosynthesis, transport, and signaling in opposition to HD-ZIPIII factors. Plant Cell 26, 246–262. https://doi.org/10.1105/tpc.113.111526

Humbert, S., Subedi, S., Cohn, J., Zeng, B., Bi, Y.M., Chen, X., Zhu, T., McNicholas, P.D., Rothstein, S.J., 2013. Genome-wide expression profiling of maize in response to individual and combined water and nitrogen stresses. BMC Genomics 14, 13. https://doi.org/10.1186/1471-2164-14-3

Ingram, J., Bartels, D., 1996. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 377–403. https://doi.org/10.1146/annurev.arplant.47.1.377

Ishola, A.I., 2016. Diallel analysis of extra early maize (zea mays l .) inbred lines for drought and low nitrogen tolerance. Masters of philosophy,Kwame Nkrumah University Of Science And Technology Kumasi, Ghana.

Islam, M.A., Du, H., Ning, J., Ye, H., Xiong, L., 2009. Characterization of Glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. Plant Molecular Biology 70, 443–456. https://doi.org/10.1007/s11103-009-9483-0

Iwase, A., Mitsuda, N., Koyama, T., Hiratsu, K., Kojima, M., Arai, T., Inoue, Y., Seki, M., Sakakibara, H., Sugimoto, K., Ohme-Takagi, M., 2011. The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in arabidopsis. Current Biology 21, 508–514. https://doi.org/10.1016/j.cub.2011.02.020

Jackson, S.D., 2009. Plant responses to photoperiod. New Phytologist 181, 517–531. https://doi.org/10.1111/j.1469-8137.2008.02681.x

Jagadish, S.V.K., Murty, M.V.R., Quick, W.P., 2015. Rice responses to rising temperatures - challenges, perspectives and future directions. Plant, Cell and Environment 38, 1686–1698. https://doi.org/10.1111/pce.12430

Javed, T., Shabbir, R., Ali, A., Afzal, I., Zaheer, U., Gao, S.J., 2020. Transcription factors in plant stress responses: Challenges and potential for sugarcane improvement. Plants 9, 1–18. https://doi.org/10.3390/plants9040491

Javelle, M., Vernoud, V., Rogowsky, P.M., Ingram, G.C., 2011. Epidermis: The formation and functions of a fundamental plant tissue. New Phytologist 189, 17–39. https://doi.org/10.1111/j.1469-

8137.2010.03514.x

Jensen, M.K., Kjaersgaard, T., Nielsen, M.M., Galberg, P., Petersen, K., O'Shea, C., Skriver, K., 2010. The Arabidopsis thaliana NAC transcription factor family: structure–function relationships and determinants of ANAC019 stress signalling. Biochemical Journal 426, 183–196. https://doi.org/10.1042/BJ20091234

Jia, Q., Sun, L., Mou, H., Ali, S., Liu, D., Zhang, Y., Zhang, P., Ren, X., Jia, Z., 2018. Effects of planting patterns and sowing densities on grain-filling, radiation use efficiency and yield of maize (Zea mays L.) in semi-arid regions. Agricultural Water Management 201, 287–298. https://doi.org/10.1016/j.agwat.2017.11.025

Jiang, Y., Huang, B., 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop Science 41, 436–442. https://doi.org/10.2135/cropsci2001.412436x

Jordan, D.R., Hunt, C.H., Cruickshank, A.W., Borrell, A.K., Henzell, R.G., 2012. The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. Crop Science 52, 1153–1161. https://doi.org/10.2135/cropsci2011.06.0326

Jordi, W., Schapendonk, A., Davelaar, E., Stoopen, G.M., Pot, C.S., De Visser, R., Van Rhijn, J.A., Gan, S., Amasino, R.M., 2000. Increased cytokinin levels in transgenic P(SAG12)-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. Plant, Cell and Environment 23, 279–289. https://doi.org/10.1046/j.1365-3040.2000.00544.x

Jyothsna, P., Murthy, S.D.., 2016. A review on effect of senescence in plants and role of phytohormones in delaying senescence. International Journal of Plant, Animal and Environmental Sciences 6, 152–162.

Kamal, N.M., Gorafi, Y.S.A., Abdelrahman, M., Abdellatef, E., Tsujimoto, H., 2019. Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. International Journal of Molecular Sciences 20, 26. https://doi.org/10.3390/ijms20235837

Kanojia, A., Dijkwel, P.P., 2018. Abiotic Stress Responses are Governed by Reactive Oxygen Species and Age. Annual Plant Reviews online 1, 295–326. https://doi.org/10.1002/9781119312994.apr0611

Kassahun, B., Bidinger, F.R., Hash, C.T., Kuruvinashetti, M.S., 2010. Stay-green expression in early generation sorghum [Sorghum bicolor (L.) Moench] QTL introgression lines. Euphytica 172, 351–362. https://doi.org/10.1007/s10681-009-0108-0

Keech, O., Pesquet, E., Ahad, A., Askne, A., Nordvall, D., Vodnala, S.M., Tuominen, H., Hurry, V., Dizengremel, P., Gardeström, P., 2007. The different fates of mitochondria and chloroplasts during dark-induced senescence in Arabidopsis leaves. Plant, Cell and Environment 30, 1523–1534. https://doi.org/10.1111/j.1365-3040.2007.01724.x

Khan, A., Tan, D.K.Y., Munsif, F., Afridi, M.Z., Shah, F., Wei, F., Fahad, S., Zhou, R., 2017. Nitrogen nutrition in cotton and control strategies for greenhouse gas emissions: a review. Environmental Science and Pollution Research 24, 23471–23487. https://doi.org/10.1007/s11356-017-0131-y

Khan, M., Rozhon, W., Poppenberger, B., 2014. The role of hormones in the aging of plants - A mini-review. Gerontology 60, 49–55. https://doi.org/10.1159/000354334

Kim, J., Woo, H.R.R., Nam, H.G.G., 2016. Toward Systems Understanding of Leaf Senescence: An Integrated Multi-Omics Perspective on Leaf Senescence Research. Molecular Plant 9, 813–825.

https://doi.org/10.1016/j.molp.2016.04.017

Kim, J.I., Murphy, A.S., Baek, D., Lee, S.W., Yun, D.J., Bressan, R.A., Narasimhan, M.L., 2011. YUCCA6 over-expression demonstrates auxin function in delaying leaf senescence in Arabidopsis thaliana. Journal of Experimental Botany 62, 3981–3992. https://doi.org/10.1093/jxb/err094

Kimotho, R.N., Baillo, E.H., Zhang, Z., 2019. Transcription factors involved in abiotic stress responses in Maize (Zea mays L.) and their roles in enhanced productivity in the post genomics era. PeerJ 7, 1–46. https://doi.org/10.7717/peerj.7211

Kiniry, J.R., McCauley, G., Xie, Y., Arnold, J.G., 2001. Rice parameters describing crop performance of four U.S. cultivars. Agronomy Journal 93, 1354–1361. https://doi.org/10.2134/agronj2001.1354

Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2018. Nitrogen supply and sink demand modulate the patterns of leaf senescence in maize. Field Crops Research 225, 92–103. https://doi.org/10.1016/j.fcr.2018.05.015

Kohl, S., Hollmann, J., Blattner, F.R., Radchuk, V., Andersch, F., Steuernagel, B., Schmutzer, T., Scholz, U., Krupinska, K., Weber, H., Weschke, W., 2012. A putative role for amino acid permeases in sink-source communication of barley tissues uncovered by RNA-seq. BMC Plant Biology 12, 1–18. https://doi.org/10.1186/1471-2229-12-154

Kosgey, J.R., Moot, D.J., Fletcher, A.L., McKenzie, B.A., 2013. Dry matter accumulation and post-silking N economy of "stay-green" maize (Zea mays L.) hybrids. European Journal of Agronomy 51, 43–52. https://doi.org/10.1016/j.eja.2013.07.001

Kosma, D.K., Bourdenx, B., Bernard, A., Parsons, E.P., Lü, S., Joubès, J., Jenks, M.A., 2009. The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiology 151, 1918–1929. https://doi.org/10.1104/pp.109.141911

Koyama, T., 2014. The roles of ethylene and transcription factors in the regulation of onset of leaf senescence. Frontiers in Plant Science 5, 1–8. https://doi.org/10.3389/fpls.2014.00650

Krotz, L., Giazzi, G., 2000. Carbon/Nitrogen Ratio in Soils and Plants using The FLASH 2000 Organic Elemental Analyzer, Thermo Fisher Scientific.

Kulaeva, O.N., Prokoptseva, O.S., 2004. Recent Advances in the Study of Mechanisms of Action of Phytohormones. Biochemistry (Moscow), 69, 293–310.

Kumari, M., Singh, V.P., Tripathi, R., Joshi, A.K., 2007. Variation for Staygreen Trait and its Association with Canopy Temperature Depression and Yield Traits under Terminal Heat Stress in Wheat, in: Buck, H.T., Nisi, J.E., Salomon, N. (Eds.), Wheat Production in Stressed Environments. pp. 357–363.

Lam, H.M., Coschigano, K.T., Oliveira, I.C., Melo-Oliveira, R., Coruzzi, G.M., 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 569–593. https://doi.org/10.1146/annurev.arplant.47.1.569

Lee, E.A., Tollenaar, M., 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Science 47, 1–14. https://doi.org/10.2135/cropsci2007.04.0010IPBS

Lee, S.B., Suh, M.C., 2015. Advances in the understanding of cuticular waxes in Arabidopsis thaliana and crop species. Plant Cell Reports 34, 557–572. https://doi.org/10.1007/s00299-015-1772-2

Leta, T.B., Miccah, S.S., Steven, M.R., Wondyifraw, T., Charless, M., Clet, W.M., Richard, O.O., Eduardo, B., Francis, W., 2016. Drought tolerant tropical maize (Zea mays L.) developed through genetic transformation with isopentenyltransferase gene. African Journal of Biotechnology 15, 2447–2464. https://doi.org/10.5897/ajb2016.15228

Li, P., Cao, W., Fang, H., Xu, S., Yin, S., Zhang, Y., Lin, D., Wang, J., Chen, Y., Xu, C., Yang, Z., 2017. Stranscriptomic profiling of the maize (Zea mays L.) leaf response to abiotic stresses at the seedling stage. Frontiers in Plant Science 8, 1–13. https://doi.org/10.3389/fpls.2017.00290

Li, R., Liu, P., Dong, S., Zhang, J., Zhao, B., 2019. Increased maize plant population induced leaf senescence, suppressed root growth, nitrogen uptake, and grain yield. Agronomy Journal 111, 1581–1591. https://doi.org/10.2134/agronj2018.09.0554

Li, W.W.W., Hao, Z., Pang, J., Zhang, M., Wang, N., Li, X., Li, W.W.W., Wang, L., Xu, M., 2019. Effect of water-deficit on tassel development in maize. Gene 681, 86–92. https://doi.org/10.1016/j.gene.2018.09.018

Li, X., Kang, S., Zhang, X., Li, F., Lu, H., 2018. Deficit irrigation provokes more pronounced responses of maize photosynthesis and water productivity to elevated CO2. Agricultural Water Management 195, 71–83. https://doi.org/10.1016/j.agwat.2017.09.017

Li, Y., Wang, M., Zhang, F., Xu, Y., Chen, X., Qin, X., Wen, X., 2016. Effect of post-silking drought on nitrogen partitioning and gene expression patterns of glutamine synthetase and asparagine synthetase in two maize (Zea mays L.) varieties. Plant Physiology and Biochemistry 102, 62–69. https://doi.org/10.1016/j.plaphy.2016.02.002

Li, Z., Zhao, Y., Liu, X., Peng, J., Guo, H., Luo, J., 2014. LSD 2.0: An update of the leaf senescence database. Nucleic Acids Research 42, 1200–1205. https://doi.org/10.1093/nar/gkt1061

Liang, D., Shen, Y., Ni, Z., Wang, Q., Lei, Z., Xu, N., Deng, Q., Lin, L., Wang, J., Lv, X., Xia, H., 2018. Exogenous melatonin application delays senescence of kiwifruit leaves by regulating the antioxidant capacity and biosynthesis of flavonoids. Frontiers in Plant Science 9, 1–14. https://doi.org/10.3389/fpls.2018.00426

Liebsch, D., Keech, O., 2016. Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. New Phytologist 212, 563–570. https://doi.org/10.1111/nph.14217

Lim, P.O., Kim, H.J., Nam, H.G., 2007. Leaf Senescence. annual review of plant biology 58, 115–136.

Lim, P.O., Woo, H.R., Nam, H.G., 2003. Molecular genetics of leaf senescence in Arabidopsis. Trends in Plant Science 8, 272–278. https://doi.org/10.1016/S1360-1385(03)00103-1

Lin, M., Lai, D., Pang, C., Fan, S., Song, M., Yu, S., 2013. Generation and Analysis of a Large-Scale Expressed Sequence Tag Database from a Full-Length Enriched cDNA Library of Developing Leaves of Gossypium hirsutum L. PLoS ONE 8, 1–15. https://doi.org/10.1371/journal.pone.0076443

Lin, M., Pang, C., Fan, S., Song, M., Wei, H., Yu, S., 2015. Global analysis of the Gossypium hirsutum L. Transcriptome during leaf senescence by RNA-Seq. BMC Plant Biology 15, 1–18. https://doi.org/10.1186/s12870-015-0433-5

Lira, B.S., Gramegna, G., Trench, B.A., Alves, F.R.R., Silva, E.M., Silva, G.F.F., Thirumalaikumar, V.P., Lupi, A.C.D., Demarco, D., Purgatto, E., Nogueira, F.T.S., Balazadeh, S., Freschi, L., Rossi, M., 2017. Manipulation of a senescence-associated gene improves fleshy fruit yield. Plant Physiology 175, 77–91. https://doi.org/10.1104/pp.17.00452

Liu, J., Bu, L., Zhu, L., Luo, S., Chen, X., Li, S., 2014. Optimizing plant density and plastic film mulch to increase maize productivity and water-use efficiency in semiarid areas. Agronomy Journal 106, 1138–1146. https://doi.org/10.2134/agronj13.0582

Liu, Y., Schiff, M., Dinesh-Kumar, S.P., 2004. Involvement of MEK1 MAPKK, NTF6 MAPK, WRKY/MYB transcription factors, COI1 and CTR1 in N-mediated resistance to tobacco mosaic virus. Plant Journal 38, 800–809. https://doi.org/10.1111/j.1365-313X.2004.02085.x

Lodish, A., Berk, H., Krieger, M., Scot, P.., Bretsher, A., Ploegh, H., Matsudaira, P., 2007. Molecular cell Biology, Sixth Edit. ed.

Luche, H. de S., da Silva, J.A.G., Nörnberg, R., da Silveira, S.F.S., Baretta, D., Groli, E.L., da Maia, L.C., de Oliveira, A.C., 2013. Desempenho per se e parâmetros genéticos de linhagens de trigo com expressão do caráter "stay-green." Pesquisa Agropecuaria Brasileira 48, 167–173. https://doi.org/10.1590/S0100-204X2013000200006

Luche, H. de S., Silva, J.A.G. da, Maia, L.C. da, Oliveira, A.C. de, 2015. Stay-green: a potentiality in plant breeding. Ciência Rural 45, 1755–1760. https://doi.org/10.1590/0103-8478cr20140662

Luo, P., Ren, Z., Wu, X., Zhang, Huaiyu, Zhang, Huaiqiong, Feng, J., 2006. Structural and biochemical mechanism responsible for the stay-green phenotype in common wheat. Chinese Science Bulletin 51, 2595–2603. https://doi.org/10.1007/s11434-006-2175-0

Luoni, S.B., Astigueta, F.H., Nicosia, S., Moschen, S., Fernandez, P., Heinz, R., 2019. Transcription factors associated with leaf senescence in crops. Plants 8, 1–28. https://doi.org/10.3390/plants8100411

Ma, B.L., Dwyer, L.M., 1998. Nitrogen uptake and use of two contrasting maize hybrids differing in leaf senescence. Plant and soil 199, 283–291.

Mahalakshmi, V., Bidinger, F.R., 2002. Evaluation of stay-green sorghum germplasm lines at ICRISAT. Crop Science 42, 965–974. https://doi.org/10.2135/cropsci2002.9650

Maiorano, A., Fanchini, D., Donatelli, M., 2014. MIMYCS.Moisture, a process-based model of moisture content in developing maize kernels. European Journal of Agronomy 59, 86–95. https://doi.org/10.1016/j.eja.2014.05.011

Mantri, N., Patade, V., Penna, S., Ford, R., Pang, E., 2012. Abiotic Stress Responses in Plants: Present and Future, in: Ahmad, P., Prasad, M.N. V (Eds.), Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer New York, New York, NY, pp. 1–19. https://doi.org/10.1007/978-1-4614-0634-1_1

Martin, A.R., Hale, C.E., Cerabolini, B.E.L., Cornelissen, J.H.C., Craine, J., Gough, W.A., Kattge, J., Tirona, C.K.F., 2018. Inter-and intraspecific variation in leaf economic traits in wheat and maize. AoB PLANTS 10, 1–14. https://doi.org/10.1093/aobpla/ply006

Masclaux-Daubresse, C., Chardon, F., 2011. Exploring nitrogen remobilization for seed filling using natural variation in Arabidopsis thaliana. Journal of Experimental Botany 62, 2131–2142. https://doi.org/10.1093/jxb/erq405

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Annals of Botany 105, 1141–1157. https://doi.org/10.1093/aob/mcq028

Masclaux-Daubresse, C., Reisdorf-Cren, M., Orsel, M., 2008. Leaf nitrogen remobilisation for plant development and grain filling. Plant Biology 10, 23–36. https://doi.org/10.1111/j.1438-8677.2008.00097.x

Mattila, H., Valev, D., Havurinne, V., Khorobrykh, S., Virtanen, O., Antinluoma, M., Mishra, K.B., Tyystjärvi, E., 2018. Degradation of chlorophyll and synthesis of flavonols during autumn senescence-the story told by individual leaves. AoB PLANTS 10, 1–13. https://doi.org/10.1093/aobpla/ply028

Meng, Y., Liu, X.M., Gu, W.R., Wei, S., 2020. Effects of a chemical plant growth regulator and planting density on the leaf senescence and yield of spring maize in northeast china. Applied Ecology and Environmental Research 18, 3297–3311. https://doi.org/10.15666/aeer/1802_32973311

Meseka, S.K., Menkir, A., Ibrahim, A.E.S., 2008. Yield potential and yield stability of maize hybrids selected for drought tolerance. Science 3, 82–90.

Miao, Y., Laun, T., Zimmermann, P., Zentgraf, U., 2004. Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. Plant Molecular Biology 55, 853–867. https://doi.org/10.1007/s11103-004-2142-6

Mikel, M.A., Dudley, J.W., 2006. Evolution of North American dent corn from public to proprietary germplasm. Crop Science 46, 1193–1205. https://doi.org/10.2135/cropsci2005.10-0371

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science 11, 15–19. https://doi.org/10.1016/j.tplants.2005.11.002

Moreno, A., Lumbreras, V., Pagés, M., 2005. Drought tolerance in maize [Zea mays L.]. AGRIS 50, 549–558.

Moschen, S., Bengoa Luoni, S., Di Rienzo, J.A., Caro, M. del P., Tohge, T., Watanabe, M., Hollmann, J., González, S., Rivarola, M., García-García, F., Dopazo, J., Hopp, H.E., Hoefgen, R., Fernie, A.R., Paniego, N., Fernández, P., Heinz, R.A., 2016. Integrating transcriptomic and metabolomic analysis to understand natural leaf senescence in sunflower. Plant Biotechnology Journal 14, 719–734. https://doi.org/10.1111/pbi.12422

Mueller-Roeber, B., Balazadeh, S., 2014. Auxin and Its Role in Plant Senescence. Journal of Plant Growth Regulation 33, 21–33. https://doi.org/10.1007/s00344-013-9398-5

Mueller, S.M., Vyn, T.J., 2016. Maize Plant Resilience to N Stress and Post-silking N Capacity Changes over Time : A Review. frontiers in plant science 7, 1–14. https://doi.org/10.3389/fpls.2016.00053

Munaiz, E.D., Martínez, S., Kumar, A., Caicedo, M., Ordás, B., 2020. The senescence (Stay-Green)—an important trait to exploit crop residuals for bioenergy. Energies 13, 22. https://doi.org/10.3390/en13040790

Munné-Bosch, S., 2008. Do perennials really senesce? Trends in Plant Science 13, 216–220. https://doi.org/10.1016/j.tplants.2008.02.002

Myers, J.R., Aljadi, M., Brewer, L., 2018. The Importance of Cosmetic Stay-Green in Specialty Crops. Plant Breeding Reviews 42, 219–256. https://doi.org/10.1002/9781119521358.ch6

Naika, M., Shameer, K., Sowdhamini, R., 2013. Comparative analyses of stress-responsive genes in Arabidopsis thaliana: Insight from genomic data mining, functional enrichment, pathway analysis and phenomics. Molecular BioSystems 9, 1888–1908. https://doi.org/10.1039/c3mb70072k

Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki, K., 2012. NAC transcription factors in plant abiotic stress responses. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms 1819, 97–103. https://doi.org/10.1016/j.bbagrm.2011.10.005

Ning, P., Li, S., Yu, P., Zhang, Y., Li, C., 2013. Post-silking accumulation and partitioning of dry matter, nitrogen, phosphorus and potassium in maize varieties differing in leaf longevity. Field Crops Research 144, 19–27. https://doi.org/10.1016/j.fcr.2013.01.020

Novacek, M.J., Mason, S.C., Galusha, T.D., Yaseen, M., 2013. Twin rows minimally impact irrigated maize yield, morphology, and lodging. Agronomy Journal 105, 268–276. https://doi.org/10.2134/agronj2012.0301

Ohlrogge, J., Browse, J., 1995. Lipid Biosynthesis. American Society of Plant Physiologistsociety of Plant Physiologists 7, 957–970. https://doi.org/10.1002/9781119991311.ch2

Ohme-takagi, M., Shinshi, H., 1995. Ethylene-lnducible DNA Binding Proteins That lnteract with an Ethylene-Responsive Element. The Plant Cell 7, 173–182.

Oury, V., Tardieu, F., Turc, O., 2016. Ovary apical abortion under water deficit is caused by changes in sequential development of ovaries and in silk growth rate in maize. Plant Physiology 171, 986–996. https://doi.org/10.1104/pp.15.00268

Pandey, R.K., Maranville, J.W., Chetima, M.M., 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment II . Shoot growth , nitrogen uptake and water extraction. Agricultural Water Management 46, 15–27.

Parajuli, S., Ojha, B.R., Ferrara, G.O., 2018. Quantification of Secondary Traits for Drought and Low Nitrogen Stress Tolerance in Inbreds and Hybrids of Maize (Zea mays L.). Journal of Plant genetics and breeding 2, 106.

Parry, M.A.J., Reynolds, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.G., Price, G.D., Condon, A.G., Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany 62, 453–467. https://doi.org/10.1093/jxb/erq304

Peleg, Z., Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. Current Opinion in Plant Biology 14, 290–295. https://doi.org/10.1016/j.pbi.2011.02.001

Piao, W., Sakuraba, Y., Paek, N.C., 2019. Transgenic expression of rice MYB102 (OsMYB102) delays leaf senescence and decreases abiotic stress tolerance in Arabidopsis. BMB Reports 52, 653–658. https://doi.org/10.5483/BMBRep.2019.52.11.071

Pinto, R.S., Lopes, M.S., Collins, N.C., Reynolds, M.P., 2016. Modelling and genetic dissection of staygreen under heat stress. Theoretical and Applied Genetics 129, 2055–2074. https://doi.org/10.1007/s00122-016-2757-4

Pommel, B., Gallais, A., Coque, M., Quilleré, I., Hirel, B., Prioul, J.L., Andrieu, B., Floriot, M., 2006. Carbon and nitrogen allocation and grain filling in three maize hybrids differing in leaf senescence. European Journal of Agronomy 24, 203–211. https://doi.org/10.1016/j.eja.2005.10.001

Quirino, B.F., Noh, Y.S., Himelblau, E., Amasino, R.M., 2000. Molecular aspects of leaf senescence. Trends in Plant Science 5, 278–282. https://doi.org/10.1016/S1360-1385(00)01655-1

Rafique, S., 2020. Drought Responses on Physiological Attributes of Zea mays in Relation to Nitrogen and Source-Sink Relationships, in: IntechOpen (Ed.), Abiotic Stress in Plants. Open Access books,

pp. 1–23. https://doi.org/DOI: http://dx.doi.org/10.5772/intechopen.93747

Rajcan, I., Tollenaar, M., 1999. Source: Sink ratio and leaf senescence in maize: II. Nitrogen metabolism during grain filling. Field Crops Research 60, 255–265. https://doi.org/10.1016/S0378-4290(98)00143-9

Ramu, V.S., Paramanantham, A., Ramegowda, V., Mohan-Raju, B., Udayakumar, M., Senthil-Kumar, M.S.K., 2016. Transcriptome analysis of sunflower genotypes with contrasting oxidative stress tolerance reveals individual-And combined-biotic and abiotic stress tolerance mechanisms. PLoS ONE 11, 1–19. https://doi.org/10.1371/journal.pone.0157522

Reguera, M., Peleg, Z., Abdel tawab, Y.M., Tumimbang, E.B., Delatorre, C.A., Blumwald, E., 2013. Stress-Induced Cytokinin Synthesis Increases Drought Tolerance through the Coordinated Regulation of Carbon and Nitrogen Assimilation in Rice. americain society of plant biologists 163, 1609–1622. https://doi.org/10.1104/pp.113.227702

Ren, B., Liu, W., Zhang, J., Dong, S., Liu, P., Zhao, B., 2017. Effects of plant density on the photosynthetic and chloroplast characteristics of maize under high-yielding conditions. Science of Nature 104, 12. https://doi.org/10.1007/s00114-017-1445-9

Richards, R.A., 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51, 447–458. https://doi.org/10.1093/jexbot/51.suppl_1.447

Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C.Z., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K., Yu, G.L., 2000. Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science 290, 2105–2110. https://doi.org/10.1126/science.290.5499.2105

Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., Blumwald, E., 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proceedings of the National Academy of Sciences of the United States of America 104, 19631–19636. https://doi.org/10.1073/pnas.0709453104

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., Mittler, R., 2004. When defense pathways collide. The response of arabidopsis to a combination of drought and heat stress 1[w]. Plant Physiology 134, 1683–1696. https://doi.org/10.1104/pp.103.033431

Robatzek, S., Somssich, I.E., 2001. AtWRKY6 is associated with both senescence and defense related processes. The Plant Journal 28, 123–133.

Robson, P., Mos, M., Clifton-Brown, J., Donnison, I., 2012. Phenotypic Variation in Senescence in Miscanthus: Towards Optimising Biomass Quality and Quantity. Bioenergy Research 5, 95–105. https://doi.org/10.1007/s12155-011-9118-6

Rodrigues, C., Vandenberghe, L.P.D.S., De Oliveira, J., Soccol, C.R., 2012. New perspectives of gibberellic acid production: A review. Critical Reviews in Biotechnology 32, 263–273. https://doi.org/10.3109/07388551.2011.615297

Roy, A.K., Wagner-Riddle, C., Deen, B., Lauzon, J., Bruulsema, T., 2014. Conséquences du taux, du moment et des antécédents d'application des engrais azotés sur les émissions d'oxyde nitreux par le maïs (Zea mays L.). Canadian Journal of Soil Science 94, 563–573. https://doi.org/10.4141/CJSS2013-118

Ruberti, C., Barizza, E., Bodner, M., La Rocca, N., De Michele, R., Carimi, F., Lo Schiavo, F., Zottini,

M., 2014. Mitochondria change dynamics and morphology during grapevine leaf senescence. PLoS ONE 9, 1–12. https://doi.org/10.1371/journal.pone.0102012

Sade, N., Del Mar Rubio-Wilhelmi, M., Umnajkitikorn, K., Blumwald, E., 2018. Stress-induced senescence and plant tolerance to abiotic stress. Journal of Experimental Botany 69, 845–853. https://doi.org/10.1093/jxb/erx235

Sakuraba, Y., Park, S.Y., Paek, N.C., 2015. The divergent roles of STAYGREEN (SGR) Homologs in chlorophyll degradation. Molecules and Cells 38, 390–395. https://doi.org/10.14348/molcells.2015.0039

Sangakkara, R., Bandaranayake, S., Attanayake, U., Stamp, P., 2012. Impact of associated intercrops on growth and yield of maize (Zea mays L) in major seasons of South Asia. Maydica 57, 6–10.

Sangoi, L., Ender, M., Guidolin, A.F., Bogo, A., Kothe, D.., 2000. Disease incidence and severity of four maize hybrids grown at different plant densities. Cienc. Rural. 30, 17–21.

Sangoi, L., Luiz De Almeida, M., Da Silva, P.R.F., Argenta, G., 2002. Bases morfofisiológicas bases morfofisiológicas para maior tolerância dos híbridos modernos de milho a altas densidades de plantas. Bragantia, Campinas 61, 101–110.

Sarwat, M., Naqvi, A.R., Ahmad, P., Ashraf, M., Akram, N.A., 2013. Phytohormones and microRNAs as sensors and regulators of leaf senescence: Assigning macro roles to small molecules. Biotechnology Advances 31, 1153–1171. https://doi.org/10.1016/j.biotechadv.2013.02.003

Sato, Y., Morita, R., Nishimura, M., Yamaguchi, H., Kusaba, M., 2007. Mendel's green cotyledon gene encodes a positive regulator of the chlorophyll-degrading pathway. Proceedings of the National Academy of Sciences of the United States of America 104, 14169–14174. https://doi.org/10.1073/pnas.0705521104

Scheckhuber, C.Q., Erjavec, N., Tinazli, A., Hamann, A., Nyström, T., Osiewacz, H.D., 2007. Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. Nature Cell Biology 9, 99–105. https://doi.org/10.1038/ncb1524

Schildhauer, J., Wiedemuth, K., Humbeck, K., 2008. Supply of nitrogen can reverse senescence processes and affect expression of genes coding for plastidic glutamine synthetase and lysine-ketoglutarate reductase/saccharopine dehydrogenase. Plant Biology 10, 76–84. https://doi.org/10.1111/j.1438-8677.2008.00075.x

Schippers, J.H.M., 2015. Transcriptional networks in leaf senescence. Current Opinion in Plant Biology 27, 77–83. https://doi.org/10.1016/j.pbi.2015.06.018

Schippers, J.H.M., Jing, H.C., Hille, J., Dijkwel, P.P., 2007. Developmental and Hormonal Control of Leaf Senescence. Senescence Processes in Plants 26, 145–170. https://doi.org/10.1002/9780470988855.ch7

SCHULZE, W., SCHULZE, E. -D, STADLER, J., HEILMEIER, H., STITT, M., MOONEY, H.A., 1994. Growth and reproduction of Arabidopsis thaliana in relation to storage of starch and nitrate in the wild-type and in starch-deficient and nitrate-uptake-deficient mutants. Plant, Cell & Environment 17, 795–809. https://doi.org/10.1111/j.1365-3040.1994.tb00174.x

Sen, S., Smith, M, E., Setter, T., 2016. Effects of low nitrogen on chlorophyll content and dry matter accumulation in maiz. African Journal of Agricultural Research 11, 1001–1007. https://doi.org/10.5897/ajar2015.9673

Shafi, M., Bakht, J., Ali, S., Khan, H., Khan, M.A., Sharif, M., 2012. Effect of planting density on phenology, growth and yield of maize (Zea mays L.). Pakistan Journal of Botany 44, 691–696.

Shao, H., Shi, D., Shi, W., Ban, X., Chen, Y., Ren, W., Chen, F., Mi, G., 2021. The impact of high plant density on dry matter remobilization and stalk lodging in maize genotypes with a different stay-green degree. Archives of Agronomy and Soil Science 67, 504–518. https://doi.org/10.1080/03650340.2020.1737679

Shen, H., Cao, K., Wang, X., 2007. A conserved proline residue in the leucine zipper region of AtbZIP34 and AtbZIP61 in Arabidopsis thaliana interferes with the formation of homodimer. Biochemical and Biophysical Research Communications 362, 425–430. https://doi.org/10.1016/j.bbrc.2007.08.026

Sher, A., Khan, A., Cai, L.J., Irfan Ahmad, M., Asharf, U., Jamoro, S.A., 2017. Response of Maize Grown Under High Plant Density; Performance, Issues and Management - A Critical Review. Advances in Crop Science and Technology 05. https://doi.org/10.4172/2329-8863.1000275

Shinozaki, K., Yamaguchi-Shinozaki, K., 2007. Gene networks involved in drought stress response and tolerance. Journal of Experimental Botany 58, 221–227. https://doi.org/10.1093/jxb/erl164

Silva, A.S., De Carvalho, F.I.F., Nedel, J.L., Jacinto Cruz, P., Teichert Peske, S., Simioni, D., Cargnin, A., 2003. Enchimento de sementes em linhas quase-isogênicas de trigo com presença e ausência do caráter "stay-green." Pesquisa Agropecuaria Brasileira 38, 613–618. https://doi.org/10.1590/S0100-204X2003000500009

Silva, J.A.G. da, Carvalho, F.I.F. de, Hartwig, I., Oliveira, A.C. de, Bertan, I., Caetano, V. da R., Schmidt, D.A.M., Valério, I.P., Ribeiro, G., Busato, C.C., 2008. Caráter stay-green e produtividade de grãos em trigo. Bragantia 67, 161–167. https://doi.org/10.1590/s0006-87052008000100020

Silva, S.A., Carvalho, F.I.F. de, Nedel, J.L., Vasconcellos, N.J.S. de, Cruz, P.J., Simioni, D., Silva, J.A.G. da, 2004. Composição de subunidades de gluteninas de alto peso molecular (HMW) em trigos portadores do caráter "stay-green." Ciência Rural 34, 679–683. https://doi.org/10.1590/s0103-84782004000300005

Silva, S.A., Félix De Carvalho, F.I., Nedel, J.L., Cruz, P.J., González Da Silva, J.A., Da Rosa Caetano, V., Hartwig, I., Da Silva Sousa, C., 2005. Path analysis for the yield components of seeds in wheat. Bragantia 64, 191–196. https://doi.org/10.1590/s0006-87052005000200004

Singh, P., Tomar, R.S., Kumar, K., Kumar, B., Rakshit, S., Singh, I., 2021. Morpho-physiological and biochemical characterization of maize genotypes under nitrogen stress conditions. Indian Journal of Genetics and Plant Breeding 81, 1255–1265. https://doi.org/10.31742/IJGPB.81.2.8

Song, L., Huang, S.S.C., Wise, A., Castanoz, R., Nery, J.R., Chen, H., Watanabe, M., Thomas, J., Bar-Joseph, Z., Ecker, J.R., 2016. A transcription factor hierarchy defines an environmental stress response network. Science 354, 27. https://doi.org/10.1126/science.aag1550

Spano, G., Di Fonzo, N., Perrotta, C., Platani, C., Ronga, G., Lawlor, D.W., Napier, J.A., Shewry, P.R., 2003. Physiological characterization of "stay green" mutants in durum wheat. Journal of Experimental Botany 54, 1415–1420. https://doi.org/10.1093/jxb/erg150

Subedi, K.D., Ma, B.L., 2005. Nitrogen Uptake and Partitioning in Stay-Green and Leafy Maize Hybrids. crop science 45, 740–747. https://doi.org/10.2135/cropsci2005.0740

Swanckaert, J., Pannecoucque, J., Van Waes, J., Steppe, K., Van Labeke, M.C., Reheul, D., 2017. Stay-green characterization in Belgian forage maize. Journal of Agricultural Science 155, 766–776.

https://doi.org/10.1017/S002185961600085X

Tahmasebi, A., Ashrafi-Dehkordi, E., Shahriari, A.G., Mazloomi, S.M., Ebrahimie, E., 2019. Integrative meta-analysis of transcriptomic responses to abiotic stress in cotton. Progress in Biophysics and Molecular Biology 146, 112–122. https://doi.org/10.1016/j.pbiomolbio.2019.02.005

Takamiya, K.I., Tsuchiya, T., Ohta, H., 2000. Degradation pathway(s) of chlorophyll: What has gene cloning revealed? Trends in Plant Science 5, 426–431. https://doi.org/10.1016/S1360-1385(00)01735-0

Tang, G., Li, X., Lin, L., Guo, H., Li, L., 2015. Combined effects of girdling and leaf removal on fluorescence characteristic of Alhagi sparsifolia leaf senescence. Plant Biology 17, 980–989. https://doi.org/10.1111/plb.12309

Testa, G., Reyneri, A., Blandino, M., 2016. Maize grain yield enhancement through high plant density cultivation with different inter-row and intra-row spacings. European Journal of Agronomy 72, 28–37.

Thomas, H., 2013. Senescence, ageing and death of the whole plant. New Phytologist 197, 696–711. https://doi.org/10.1111/nph.12047

Thomas, H., Howarth, C.J., 2000. Five ways to stay green. Journal of Experimental Botany 51, 329–337.

Thomas, H., Huang, L., Young, M., Ougham, H., 2009. Evolution of plant senescence. BMC Evolutionary Biology 9, 1–33. https://doi.org/10.1186/1471-2148-9-163

Thomas, H., Ougham, H., 2014. The stay-green trait. Journal of Experimental Botany 65, 3889–3900. https://doi.org/10.1093/jxb/eru037

Thomas, H., Smart, C.M., 1993. Crops that stay green1. Annals of Applied Biology 123, 193–219.

Tian, F., Gong, J., Zhang, J., Zhang, M., Wang, G., Li, A., Wang, W., 2013. Enhanced stability of thylakoid membrane proteins and antioxidant competence contribute to drought stress resistance in the tasg1 wheat stay-green mutant. Journal of Experimental Botany 64, 1509–1520. https://doi.org/10.1093/jxb/ert004

Tigchelaar, M., Battisti, D.S., Naylor, R.L., Ray, D.K., 2018. Future warming increases probability of globally synchronized maize production shocks. Proceedings of the National Academy of Sciences of the United States of America 115, 6644–6649. https://doi.org/10.1073/pnas.1718031115

Tiwari, J.K., Buckseth, T., Zinta, R., Saraswati, A., Singh, R.K., Rawat, S., Dua, V.K., Chakrabarti, S.K., 2020. Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. Scientific Reports 10, 1–18. https://doi.org/10.1038/s41598-020-58167-4

Tollenaar, M., Ahmadzadeh, A., Lee, E.A., 2004. Physiological basis of heterosis for grain yield in maize. Crop Science 44, 2086–2094. https://doi.org/10.2135/cropsci2004.2086

Tollenaar, M., Lee, E.A., 2006. Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. Maydica 51, 399–408.

Tong, T., Gu, W.R., Liu, X.M., Li, C.F., 2019. Maize yield and leaf photosynthetic characteristics in response to planting densities and application of yuhuangjin, as a new plant growth regulator. Applied Ecology and Environmental Research 17, 10717–10730. https://doi.org/10.15666/aeer/1705_1071710730

Turi, N.A., Shah, S.S., Ali, S., Rahman, H., Ali, T., Sajjad, M., 2007. Genetic variability for yield parameters in maize (Zea mays L.) genotypes. Journal of Agricultural and Biological Science 2, 1–4.

Uhart, S.A., Andrade, F.H., 1995. Nitrogen and Carbon Accumulation and Remobilization during Grain Filling in Maize under Different Source/Sink Ratios. Crop Science 35, 183–190. https://doi.org/10.2135/cropsci1995.0011183X003500010034x

Valentinuz, O., Tollenaar, M., 2004. Vertical Profile of Leaf Senescence during the Grain-Filling Period in Older and Newer Maize Hybrids. Crop Science 44, 827.

Varela, S., Caballe, G., Curetti, M., Portela, J., 2010. Sistemas IRGA en la medición de intercambio gaseoso, Técnicas de Medición en Ecofisiología Vegetal. Instituto Nacional de Tecnología Agropecuaria-Argentina Técnicas.

Vaughan, M.M., Block, A., Christensen, S.A., Allen, L.H., Schmelz, E.A., 2018. The effects of climate change associated abiotic stresses on maize phytochemical defenses. Phytochemistry Reviews 17, 37–49. https://doi.org/10.1007/s11101-017-9508-2

Wang, J., Guan, H., Dong, R., Liu, C., Liu, Q., Liu, T., Wang, L., He, C., 2019. Overexpression of maize sucrose non-fermenting-1-related protein kinase 1 genes, ZmSnRK1s, causes alteration in carbon metabolism and leaf senescence in Arabidopsis thaliana. Gene 691, 34–44. https://doi.org/10.1016/j.gene.2018.12.039

Wang, J., Zhou, J., Zhang, B., Vanitha, J., Ramachandran, S., Jiang, S.Y., 2011. Genome-wide Expansion and Expression Divergence of the Basic Leucine Zipper Transcription Factors in Higher Plants with an Emphasis on Sorghum. Journal of Integrative Plant Biology 53, 212–231. https://doi.org/10.1111/j.1744-7909.2010.01017.x

Wang, X., Wang, L., Shangguan, Z., 2016. Leaf gas exchange and fluorescence of two winter wheat varieties in response to drought stress and nitrogen supply. PLoS ONE 11, 1–15. https://doi.org/10.1371/journal.pone.0165733

Wang, Y., Huang, Y., Fu, W., Guo, W., Ren, N., Zhao, Y., Ye, Y., 2020. E ffi cient Physiological and Nutrient Use E ffi ciency Responses of Maize Leaves to Drought Stress under. Agronomy 10, 1–14.

Wang, Y., Xu, J., Deng, D., Ding, H., Bian, Y., Yin, Z., Wu, Y., Zhou, B., Zhao, Y., 2016. A comprehensive meta-analysis of plant morphology, yield, stay-green, and virus disease resistance QTL in maize (Zea mays L.). Planta 243, 459–471. https://doi.org/10.1007/s00425-015-2419-9

Wang, Y.H., Irving, H.R., 2011. Developing a model of plant hormone interactions. Plant Signaling and Behavior 6, 494–500. https://doi.org/10.4161/psb.6.4.14558

Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frei Dit Frey, N., Leung, J., 2008. An update on abscisic acid signaling in plants and more ⋯. Molecular Plant 1, 198–217. https://doi.org/10.1093/mp/ssm022

Watson, S.A., 2003. Description, development, structure, and composition of the corn kernel., in: White, P.J., Johnson, L.A. (Eds.), Corn: Chemistry and Technology. pp. 69–106.

Wei, S., Wang, X., Zhang, J., Liu, P., Zhao, B., Li, G., Dong, S., 2015. The role of nitrogen in leaf senescence of summer maize and analysis of underlying mechanisms using comparative proteomics. Plant Science 233, 72–81. https://doi.org/10.1016/j.plantsci.2015.01.002

White, M.R., Mikel, M.A., de Leon, N., Kaeppler, S.M., 2020. Diversity and heterotic patterns in North

American proprietary dent maize germplasm. Crop Science 60, 100–114. https://doi.org/10.1002/csc2.20050

Woli, K.P., Sawyer, J.E., Boyer, M.J., Abendroth, L.J., Elmore, R.W., 2019. Corn era hybrid nutrient concentration and accumulation of secondary and micronutrients. Agronomy Journal 111, 1604–1619. https://doi.org/10.2134/agronj2018.09.0621

Wong, S.-C., Cowan, I.R., Farquhar, G.D., 1985. Leaf Conductance in Relation to Rate of CO(2) Assimilation: III. Influences of Water Stress and Photoinhibition. Plant physiology 78, 830–834. https://doi.org/10.1104/pp.78.4.830

Worku, M., Bänziger, M., Erley, G.S.A. m., Friesen, D., Diallo, A.O., Horst, W.J., 2007. Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Science 47, 519–528. https://doi.org/10.2135/cropsci2005.05.0070

Wu, Liancheng, Li, M., Tian, L., Wang, S., Wu, Liuji, Ku, L., Zhang, J., Song, X., Liu, H., Chen, Y., 2017. Global transcriptome analysis of the maize (Zea mays L .) inbred line 08LF during leaf senescence initiated by pollination-prevention. PLoS ONE 12, 1–23.

Wu, X.-Y.Y., Hu, W.-J.J., Luo, H., Xia, Y., Zhao, Y., Wang, L.-D.D., Zhang, L.-M.M., Luo, J.-C.C., Jing, H.-C.C., 2016. Transcriptome profiling of developmental leaf senescence in sorghum (Sorghum bicolor). Plant Molecular Biology 92, 555–580. https://doi.org/10.1007/s11103-016-0532-1

Wu, X., Ding, D., Shi, C., Xue, Y., Zhang, Z., Tang, G., Tang, J., 2016. microRNA-dependent gene regulatory networks in maize leaf senescence. BMC Plant Biology 16, 1–15. https://doi.org/10.1186/s12870-016-0755-y

Wu, X.Y., Kuai, B.K., Jia, J.Z., Jing, H.C., 2012. Regulation of Leaf Senescence and Crop Genetic Improvement. Journal of Integrative Plant Biology 54, 936–952. https://doi.org/10.1111/jipb.12005

Wyrick, J.J., Young, R.A., 2002. Deciphering gene expression regulatory networks. Current Opinion in Genetics and Development 12, 130–136. https://doi.org/10.1016/S0959-437X(02)00277-0

Xiao, D., Tao, F., 2016. Contributions of cultivar shift, management practice and climate change to maize yield in North China Plain in 1981–2009. International Journal of Biometeorology 60, 1111–1122. https://doi.org/10.1007/s00484-015-1104-9

Xu, G., Fan, X., Miller, A.J., 2012. Plant Nitrogen Assimilation and Use Efficiency. Annual Review of Plant Biology 63, 153–182. https://doi.org/10.1146/annurev-arplant-042811-105532

Xu, T.J., Lü, T.F., Chen, C.Y., Liu, Zhang, Y.T., Liu, X.Z., Zhao, J.R., Wang, R.H., 2019. Effects of plant density and plant growth regulator on stalk traits of maize and their regulation. Scientia Agricultura Sinica 52, 629–638. https://doi.org/10.3864/j.issn.0578-1752.2019.04.005

Xu, X., 2020. Regulation of postharvest inflorescence senescence in Arabidopsis thaliana. PhD thesis, Massey University, Palmerston North, New Zealand.

Xu, Z.Z., Zhou, G.S., 2006. Nitrogen metabolism and photosynthesis in Leymus chinensis in response to long-term soil drought. Journal of Plant Growth Regulation 25, 252–266. https://doi.org/10.1007/s00344-006-0043-4

Xue, D., Zhang, X., Lu, X., Chen, G., Chen, Z.H., 2017. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Frontiers in Plant Science 8, 1–12. https://doi.org/10.3389/fpls.2017.00621

Yamori, W., Noguchi, K., Hikosaka, K., Terashima, I., 2010. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. Plant Physiology 152, 388–399. https://doi.org/10.1104/pp.109.145862

Yan, P., Yue, S., Qiu, M., Chen, X., Cui, Z., Chen, F., 2014. Using maize hybrids and in-season nitrogen management to improve grain yield and grain nitrogen concentrations. Field Crops Research 166, 38–45. https://doi.org/10.1016/j.fcr.2014.06.012

Yang, J., Carena, M.J., Uphaus, J., 2010. Area under the dry down curve (AUDDC): A method to evaluate rate of dry down in maize. Crop Science 50, 2347–2354. https://doi.org/10.2135/cropsci2010.02.0098

Yang, J., Zhang, J., 2018. Approach and mechanism in enhancing the remobilization of assimilates and grain-filling in rice and wheat. Kexue Tongbao/Chinese Science Bulletin 63, 2932–2943. https://doi.org/10.1360/N972018-00577

Yang, J., Zhang, J., 2006. Grain filling of cereals under soil drying. New Phytologist 169, 223–236. https://doi.org/10.1111/j.1469-8137.2005.01597.x

Yang, L., Fountain, J.C., Ji, P., Ni, X., Chen, S., Lee, R.D., Kemerait, R.C., Guo, B., 2018. Deciphering drought-induced metabolic responses and regulation in developing maize kernels. Plant Biotechnology Journal 16, 1616–1628. https://doi.org/10.1111/pbi.12899

Yang, M., Geng, M., Shen, P., Chen, X., Li, Y., Wen, X., 2019. Effect of post-silking drought stress on the expression profiles of genes involved in carbon and nitrogen metabolism during leaf senescence in maize (Zea mays L.). Plant Physiology and Biochemistry 135, 304–309. https://doi.org/10.1016/j.plaphy.2018.12.025

Yang, W., Guo, Z., Huang, C., Wang, K., Jiang, N., Feng, H., Chen, G., Liu, Q., Xiong, L., 2015. Genome-wide association study of rice (Oryza sativa L.) leaf traits with a high-throughput leaf scorer. Journal of Experimental Botany 66, 5605–5615. https://doi.org/10.1093/jxb/erv100

Yang, Z., Li, X., Zhang, N., Wang, X., Zhang, Y., Ding, Y., Kuai, B., Huang, X., 2017. Mapping and validation of the quantitative trait loci for leaf stay-green-associated parameters in maize. Plant Breeding 136, 188–196. https://doi.org/10.1111/pbr.12451

Ye, Y. xiu, Wen, Z. rong, Yang, H., Lu, W. ping, Lu, D. lei, 2020. Effects of post-silking water deficit on the leaf photosynthesis and senescence of waxy maize. Journal of Integrative Agriculture 19, 2216–2228. https://doi.org/10.1016/S2095-3119(20)63158-6

Yilmaz, A., Nishiyama, M.Y., Fuentes, B.G., Souza, G.M., Janies, D., Gray, J., Grotewold, E., 2009. GRASSIUS: A platform for comparative regulatory genomics across the grasses. Plant Physiology 149, 171–180. https://doi.org/10.1104/pp.108.128579

Yoshinaga, M., Tanaka, S., Shimago, A., Sameshima, K., Nishi, J., Nomura, Y., Kawano, Y., Hashiguchi, J., Ichiki, T., Shimizu, S., 2005. Metabolic syndrome in overweight and obese Japanese children. Obesity Research 13, 1135–1140. https://doi.org/10.1038/oby.2005.134

You, C., Zhu, H., Xu, B., Huang, W., Wang, S., Ding, Y., Liu, Z., Li, G., Chen, L., Ding, C., Tang, S., 2016. Effect of removing superior spikelets on grain filling of inferior spikelets in rice. Frontiers in Plant Science 7, 1–16. https://doi.org/10.3389/fpls.2016.01161

You, J., Zhang, Yujuan, Liu, A., Li, D., Wang, X., Dossa, K., Zhou, R., Yu, J., Zhang, Yanxin, Wang, L., Zhang, X., 2019. Transcriptomic and metabolomic profiling of drought-tolerant and susceptible

sesame genotypes in response to drought stress. BMC Plant Biology 19, 1–16. https://doi.org/10.1186/s12870-019-1880-1

Zaidi, P.H., Yadav, M., Singh, D.K., Singh, R.P., 2008. Relationship between drought and excess moisture tolerance in tropical maize (Zea mays L.). Australian journal of crop science 1, 78–96.

Zentgraf, U., Doll, J., Riester, L., 2018. Live and Let Die: The Core Circadian Oscillator Coordinates Plant Life History and Pilots Leaf Senescence. Molecular Plant 11, 351–353. https://doi.org/10.1016/j.molp.2018.02.001

Zentgraf, U., Jobst, J., Kolb, D., Rentsch, D., 2004. Senescence-related gene expression profiles of rosette leaves of Arabidopsis thaliana: Leaf age versus plant age. Plant Biology 6, 178–183. https://doi.org/10.1055/s-2004-815735

Zhai, Lichao, Xu, P., Zhang, Z., Li, S., Xie, R., Zhai, Lifang, Wei, B., 2017. Effects of deep vertical rotary tillage on dry matter accumulation and grain yield of summer maize in the Huang-Huai-Hai Plain of China. Soil and Tillage Research 170, 167–174. https://doi.org/10.1016/j.still.2017.03.013

Zhang, J., Fengler, K.A., Van Hemert, J.L., Gupta, R., Mongar, N., Sun, J., Allen, W.B., Wang, Y., Weers, B., Mo, H., Lafitte, R., Hou, Z., Bryant, A., Ibraheem, F., Arp, J., Swaminathan, K., Moose, S.P., Li, B., Shen, B., 2019. Identification and characterization of a novel stay-green QTL that increases yield in maize. Plant Biotechnology Journal 17, 2272–2285. https://doi.org/10.1111/pbi.13139

Zhang, L. li, ZHOU, X. li, FAN, Y., FU, J., Hou, P., Yang, H. long, QI, H., 2019. Post-silking nitrogen accumulation and remobilization are associated with green leaf persistence and plant density in maize. Journal of Integrative Agriculture 18, 1882–1892. https://doi.org/10.1016/s2095-3119(18)62087-8

Zhang, Q., Xia, C., Zhang, L., Dong, C., Liu, X., Kong, X., 2018. Transcriptome analysis of a premature leaf senescence mutant of common wheat (Triticum aestivum L.). International Journal of Molecular Sciences 19, 18. https://doi.org/10.3390/ijms19030782

Zhang, W.Y., Xu, Y.C., Li, W.L., Yang, L., Yue, X., Zhang, X.S., Zhao, X.Y., Zhang, T., 2014. Transcriptional analyses of natural leaf senescence in maize. PLoS ONE 9, 1–31. https://doi.org/10.1371/journal.pone.0115617

Zhang, Y., Mao, S., Zhang, X., 2006. Study on close planting and nutrient improvement of maize effect of row distance to yield and nutrition of maize. Journal of Maize Sciences 14, 108.

Zhang, Z., Li, G., Gao, H., Zhang, L., Yang, C., Liu, P., Meng, Q., 2012. Characterization of photosynthetic performance during senescence in stay-green and quick-leaf-senescence Zea mays L. inbred lines. PLoS ONE 7, 1–10. https://doi.org/10.1371/journal.pone.0042936

Zheng, C., Wang, Y., Ding, Z., Zhao, L., 2016. Transcriptome Analysis of a Premature Leaf Senescence Mutant of Common Wheat (Triticum aestivum L.) Qiang. International Journal of Molecular Sciences Sci 19, 18. https://doi.org/10.3389/fpls.2016.01858

Zhou, W., Jia, C.G., Wu, X., Hu, R.X., Yu, G., Zhang, X.H., Liu, J.L., Pan, H.Y., 2016. ZmDBF3, a Novel Transcription Factor from Maize (Zea mays L.), Is Involved in Multiple Abiotic Stress Tolerance. Plant Molecular Biology Reporter 34, 353–364. https://doi.org/10.1007/s11105-015-0926-2

Zörb, C., Geilfus, C.M., Dietz, K.J., 2019. Salinity and crop yield. Plant Biology 21, 31–38.

https://doi.org/10.1111/plb.12884

Zottini, M., Barizza, E., Bastianelli, F., Carimi, F., Lo Schiavo, F., 2006. Growth and senescence of Medicago truncatula cultured cells are associated with characteristic mitochondrial morphology. New Phytologist 172, 239–247. https://doi.org/10.1111/j.1469-8137.2006.01830.x

Annexes

VIII. Annexes

Annex 1. Analysis of variance of different agronomic and physiologic trait

Table 1: Analysis of variance for Female flowering time in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	719	1.75	0.1869
NL	2	719	3.36	0.0353
PD	1	719	9.58	0.0020
SGT	1	719	0.92	0.3376
WC*NL	2	719	1.79	0.1671
WC*PD	1	719	1.35	0.2454
NL*PD	2	719	0.36	0.6966
WC*SGT	1	719	0.22	0.6415
PD*SGT	1	719	0.00	0.9657
WC*NL*PD	2	719	1.22	0.2944
NL*SGT	2	719	0.12	0.8848
WC*NL*SGT	2	719	0.05	0.9471
WC*PL*SGT	1	719	0.02	0.8961
NL*PD*SGT	2	719	0.16	0.8511
WC*NL*PD*SGT	2	719	0.12	0.8849

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; Num DF: is the number of degrees of freedom in the model; Den DF: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	73.1416	60.1158	1.22	0.1119	0.05	23.3476	1045.32
Rep(environment)	0.7729	0.6945	1.11	0.1329	0.05	0.2294	16.6723
Residual	19.9637	1.0529	18.96	<.0001	0.05	18.0507	22.1994

Table 2: Analysis of variance for anthesis silking interval (ASI) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	718	7.26	0.0072
NL	2	718	3.59	0.0281
PD	1	718	7.83	0.0053
SGT	1	718	1.31	0.2532
WC*NL	2	718	1.04	0.3550
WC*PD	1	718	0.12	0.7291
NL*PD	2	718	0.25	0.7797
WC*SGT	1	718	0.28	0.5967
PD*SGT	1	718	1.47	0.2263
WC*NL*PD	2	718	1.05	0.3507
NL*SGT	2	718	0.05	0.9481
WC*NL*SGT	2	718	0.37	0.6932
WC*PL*SGT	1	718	1.85	0.1747
NL*PD*SGT	2	718	1.00	0.3679
WC*NL*PD*SGT	2	718	0.24	0.7877

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; Num DF: is the number of degrees of freedom in the model; Den DF: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	2.6833	2.2368	1.20	0.1151	0.05	0.8468	40.7344
Rep(Environment)	0	.	.	.	.	.	.
Residual	10.8664	0.5719	19.00	<.0001	0.05	9.8272	12.0806

Table 3. Analysis of variance for black layer or physiologic maturity (days) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	696	10.58	0.0012
NL	2	696	1.11	0.3297
PD	1	696	0.40	0.5249
SGT	1	696	31.01	<.0001
WC*NL	2	696	0.65	0.5212
WC*PD	1	696	0.55	0.4602
NL*PD	2	696	0.25	0.7811
WC*SGT	1	696	0.88	0.3482
PD*SGT	1	696	0.25	0.6178
WC*NL*PD	2	696	1.33	0.2642
NL*SGT	2	696	1.21	0.2987
WC*NL*SGT	2	696	0.01	0.9906
WC*PL*SGT	1	696	1.82	0.1778
NL*PD*SGT	2	696	0.12	0.8861
WC*NL*PD*SGT	2	696	0.27	0.7658

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	14.6630	12.7055	1.15	0.1242	0.05	4.4839	265.02
Rep(Environment)	1.3303	1.2830	1.04	0.1499	0.05	0.3719	42.1755
Residual	42.4483	2.2756	18.65	<.0001	0.05	38.3189	47.2867

Table 4. Analysis of variance for stover dry weight at silking time (Kg ha^{-1}) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	717	2.80	0.0945
NL	2	717	2.60	0.0747
PD	1	717	71.72	<.0001
SGT	1	717	8.56	0.0035
WC*NL	2	717	12.85	<.0001
WC*PD	1	717	1.97	0.1612
NL*PD	2	717	0.03	0.9738
WC*SGT	1	717	1.07	0.3003
PD*SGT	1	717	4.39	0.0365
WC*NL*PD	2	717	0.46	0.6284
NL*SGT	2	717	0.26	0.7686
WC*NL*SGT	2	717	0.96	0.3844
WC*PL*SGT	1	717	0.00	0.9776
NL*PD*SGT	2	717	0.42	0.6544
WC*NL*PD*SGT	2	717	0.26	0.7746

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Estimation**	**Standard error**	**Z value**	**Pr > Z**	**Alpha**	**inferior**	**superior**
Environment	1.5869E8	1.2969E8	1.22	0.1106	0.05	50886214	2.2149E9
Rep(Environment)	24482	398229	0.06	0.4755	0.05	.	.
Residual	51345918	2711503	18.94	<.0001	0.05	46419914	57104107

Table 5. Analysis of variance for stover moisture at silking time (%) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	716	62.11	<.0001
NL	2	716	19.07	<.0001
PD	1	716	3.61	0.0580
SGT	1	716	88.27	<.0001
WC*NL	2	716	10.69	<.0001
WC*PD	1	716	0.26	0.6122
NL*PD	2	716	0.35	0.7062
WC*SGT	1	716	0.00	0.9998
PD*SGT	1	716	0.04	0.8385
WC*NL*PD	2	716	0.81	0.4438
NL*SGT	2	716	0.37	0.6879
WC*NL*SGT	2	716	0.02	0.9792
WC*PL*SGT	1	716	0.22	0.6427
NL*PD*SGT	2	716	0.28	0.7595
WC*NL*PD*SGT	2	716	0.65	0.5229

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Estimation**	**Standard error**	**Z value**	**Pr > Z**	**Alpha**	**inferior**	**superior**
Environment	2.7251	2.2536	1.21	0.1133	0.05	0.8656	39.9774
Rep(Environment)	0.03418	0.04977	0.69	0.2461	0.05	0.006626	51.9586
Residual	3.3290	0.1760	18.92	<.0001	0.05	3.0094	3.7027

Table 6. Analysis of variance for Stover dry weight at harvest time or SWNR (Kg ha^{-1}) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	720	24.58	<.0001
NL	2	720	6.85	0.0011
PD	1	720	159.43	<.0001
SGT	1	720	7.31	0.0070
WC*NL	2	720	9.90	<.0001
WC*PD	1	720	0.07	0.7899
NL*PD	2	720	0.95	0.3877
WC*SGT	1	720	0.23	0.6352
PD*SGT	1	720	0.35	0.5553
WC*NL*PD	2	720	1.21	0.2987
NL*SGT	2	720	0.04	0.9582
WC*NL*SGT	2	720	0.24	0.7837
WC*PL*SGT	1	720	0.04	0.8412
NL*PD*SGT	2	720	0.06	0.9405
WC*NL*PD*SGT	2	720	1.16	0.3130

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	26130640	21374601	1.22	0.1108	0.05	8373302	3.6604E8
Rep(Environment)	21938	93378	0.23	0.4071	0.05	1955.85	2.184E32
Residual	10142523	534596	18.97	<.0001	0.05	9171184	11277621

Table 7. Analysis of variance for Stover yield at harvest time or SYS (g.plant^{-1}) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	716	2.09	0.1484
NL	2	716	3.15	0.0432
PD	1	716	18.78	<.0001
SGT	1	716	7.14	0.0077
WC*NL	2	716	14.17	<.0001
WC*PD	1	716	1.19	0.2752
NL*PD	2	716	0.38	0.6839
WC*SGT	1	716	1.35	0.2456
PD*SGT	1	716	2.62	0.1059
WC*NL*PD	2	716	0.10	0.9088
NL*SGT	2	716	0.35	0.7049
WC*NL*SGT	2	716	0.91	0.4040
WC*PL*SGT	1	716	0.05	0.8280
NL*PD*SGT	2	716	0.47	0.6247
WC*NL*PD*SGT	2	716	0.08	0.9243

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	39307	32114	1.22	0.1105	0.05	12608	547829
Rep(Environment)	29.2588	103.37	0.28	0.3886	0.05	2.8089	3.88E20
Residual	11168	590.21	18.92	<.0001	0.05	10096	12422

Table 8. Analysis of variance for Stover dry weight at harvest time or SYNR (g.plant^{-1}) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	719	27.40	<.0001
NL	2	719	5.21	0.0056
PD	1	719	31.07	<.0001
SGT	1	719	5.66	0.0176
WC*NL	2	719	1.99	0.1374
WC*PD	1	719	2.74	0.0980
NL*PD	2	719	0.60	0.5489
WC*SGT	1	719	0.68	0.4106
PD*SGT	1	719	0.09	0.7627
WC*NL*PD	2	719	0.73	0.4835
NL*SGT	2	719	0.02	0.9832
WC*NL*SGT	2	719	0.24	0.7891
WC*PL*SGT	1	719	0.02	0.8857
NL*PD*SGT	2	719	0.26	0.7743
WC*NL*PD*SGT	2	719	0.23	0.7931

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	1133.14	3219.76	0.35	0.3624	0.05	124.44	1.954E15
Rep(Environment)	4541.80	3229.41	1.41	0.0798	0.05	1623.51	38166
Residual	2418.18	127.54	18.96	<.0001	0.05	2186.46	2689.00

Table 9. Analysis of variance of Stover dry weight at harvest time or SWNR (g.plant^{-1}) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	719	27.40	<.0001
NL	2	719	5.21	0.0056
PD	1	719	31.07	<.0001
SGT	1	719	5.66	0.0176
WC*NL	2	719	1.99	0.1374
WC*PD	1	719	2.74	0.0980
NL*PD	2	719	0.60	0.5489
WC*SGT	1	719	0.68	0.4106
PD*SGT	1	719	0.09	0.7627
WC*NL*PD	2	719	0.73	0.4835
NL*SGT	2	719	0.02	0.9832
WC*NL*SGT	2	719	0.24	0.7891
WC*PL*SGT	1	719	0.02	0.8857
NL*PD*SGT	2	719	0.26	0.7743
WC*NL*PD*SGT	2	719	0.23	0.7931

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	26130640	21374601	1.22	0.1108	0.05	8373302	3.6604E8
Rep(Environment)	21938	93378	0.23	0.4071	0.05	1955.85	2.184E32
Residual	10142523	534596	18.97	<.0001	0.05	9171184	11277621

Table 10. Analysis of variance of Stover moisture at harvest time (%) in 8 maize inbred lines in 4 experiments

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	721	3.82	0.0509
NL	2	721	0.15	0.8566
PD	1	721	0.99	0.3205
SGT	1	721	18.17	<.0001
WC*NL	2	721	0.49	0.6113
WC*PD	1	721	0.30	0.5811
NL*PD	2	721	0.02	0.9762
WC*SGT	1	721	0.01	0.9248
PD*SGT	1	721	0.15	0.6973
WC*NL*PD	2	721	1.22	0.2950
NL*SGT	2	721	2.46	0.0863
WC*NL*SGT	2	721	0.90	0.4059
WC*PL*SGT	1	721	0.63	0.4258
NL*PD*SGT	2	721	1.65	0.1925
WC*NL*PD*SGT	2	721	0.01	0.9883

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Estimation**	**Standard error**	**Z value**	**Pr > Z**	**Alpha**	**inferior**	**superior**
Environment	56.4711	46.7793	1.21	0.1137	0.05	17.9127	834.37
Rep(Environment)	1.2527	1.1546	1.08	0.1390	0.05	0.3639	30.8331
Residual	34.2538	1.8042	18.99	<.0001	0.05	30.9755	38.0843

Table 11. Analysis of variance for Stover dry weight remobilized at harvest time or SWR (Kg ha^{-1}) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	737	0.37	0.5411
NL	2	737	1.04	0.3525
PD	1	737	9.29	0.0024
SGT	1	737	18.35	<.0001
WC*NL	2	737	5.54	0.0041
WC*PD	1	737	1.05	0.3061
NL*PD	2	737	0.31	0.7352
WC*SGT	1	737	1.82	0.1779
PD*SGT	1	737	6.72	0.0097
WC*NL*PD	2	737	0.53	0.5911
NL*SGT	2	737	0.34	0.7129
WC*NL*SGT	2	737	0.90	0.4086
WC*PL*SGT	1	737	0.04	0.8397
NL*PD*SGT	2	737	0.51	0.5996
WC*NL*PD*SGT	2	737	0.11	0.8929

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Estimation**	**Standard error**	**Z value**	**Pr > Z**	**Alpha**	**inferior**	**superior**
Environment	59346430	48646608	1.22	0.1112	0.05	18984982	8.3865E8
Rep(Environment)	0	.	.	.	.	.	.
Residual	44792702	2327090	19.25	<.0001	0.05	40559994	49727946

Table 12. Analysis of variance for cobs dry weight (kg ha^{-1}) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	716	54.65	<.0001
NL	2	716	8.54	0.0002
PD	1	716	271.28	<.0001
SGT	1	716	110.93	<.0001
WC*NL	2	716	11.94	<.0001
WC*PD	1	716	5.37	0.0208
NL*PD	2	716	2.94	0.0534
WC*SGT	1	716	1.07	0.3016
PD*SGT	1	716	3.47	0.0628
WC*NL*PD	2	716	3.00	0.0504
NL*SGT	2	716	0.09	0.9118
WC*NL*SGT	2	716	0.38	0.6858
WC*PL*SGT	1	716	0.71	0.3988
NL*PD*SGT	2	716	0.16	0.8503
WC*NL*PD*SGT	2	716	0.96	0.3833

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	119493	98981	1.21	0.1137	0.05	37904	1765267
Rep(Environment)	2310.41	2453.39	0.94	0.1732	0.05	594.56	136754
Residual	108139	5715.34	18.92	<.0001	0.05	97757	120277

Table 13. Analysis of variance for cobs moisture (%) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	718	0.06	0.8111
NL	2	718	3.27	0.0384
PD	1	718	5.79	0.0163
SGT	1	718	12.11	0.0005
WC*NL	2	718	5.24	0.0055
WC*PD	1	718	0.07	0.7963
NL*PD	2	718	1.70	0.1827
WC*SGT	1	718	3.50	0.0616
PD*SGT	1	718	0.01	0.9195
WC*NL*PD	2	718	0.07	0.9360
NL*SGT	2	718	0.44	0.6450
WC*NL*SGT	2	718	0.47	0.6243
WC*PL*SGT	1	718	0.33	0.5687
NL*PD*SGT	2	718	0.40	0.6698
WC*NL*PD*SGT	2	718	0.30	0.7413

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	263.83	216.42	1.22	0.1114	0.05	84.3475	3740.11
Rep(Environment)	1.6594	1.7201	0.96	0.1673	0.05	0.4361	82.9584
Residual	73.9785	3.9043	18.95	<.0001	0.05	66.8853	82.2693

Table 14. Analysis of variance for weight of 1000 grains (g) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	701	28.55	<.0001
NL	2	701	2.11	0.1224
PD	1	701	4.52	0.0338
SGT	1	701	211.50	<.0001
WC*NL	2	701	3.31	0.0369
WC*PD	1	701	0.34	0.5575
NL*PD	2	701	0.20	0.8227
WC*SGT	1	701	1.08	0.2993
PD*SGT	1	701	0.04	0.8506
WC*NL*PD	2	701	0.70	0.4956
NL*SGT	2	701	1.13	0.3244
WC*NL*SGT	2	701	0.37	0.6906
WC*PL*SGT	1	701	0.81	0.3682
NL*PD*SGT	2	701	0.46	0.6312
WC*NL*PD*SGT	2	701	0.15	0.8571

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	1157.82	949.34	1.22	0.1113	0.05	370.31	16381
Rep(Environment)	0	.	.	.	.	.	.
Residual	1326.84	70.6705	18.78	<.0001	0.05	1198.53	1477.02

Table 15. Analysis of variance for grains moisture (%) in 8 maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	701	3.20	0.0742
NL	2	701	0.23	0.7941
PD	1	701	0.51	0.4752
SGT	1	701	11.59	0.0007
WC*NL	2	701	0.40	0.6704
WC*PD	1	701	0.82	0.3656
NL*PD	2	701	0.80	0.4481
WC*SGT	1	701	1.56	0.2115
PD*SGT	1	701	0.00	0.9769
WC*NL*PD	2	701	0.43	0.6490
NL*SGT	2	701	0.11	0.8922
WC*NL*SGT	2	701	0.04	0.9646
WC*PL*SGT	1	701	0.03	0.8697
NL*PD*SGT	2	701	0.03	0.9739
WC*NL*PD*SGT	2	701	0.23	0.7913

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Estimation	Standard error	Z value	Pr > Z	Alpha	inferior	superior
Environment	112.97	92.7626	1.22	0.1116	0.05	36.0882	1608.14
Rep(Environment)	0.8243	0.9200	0.90	0.1851	0.05	0.2030	71.1538
Residual	42.5236	2.2715	18.72	<.0001	0.05	38.4005	47.3519

Annex 2. Mean and standards deviation of stover yield at silking and harvest time (g/plant)

Factors	levels	SWF (g.plant^{-1})	SWH_NR (g.plant^{-1})
WC	Opti	224.3 ± 65.2^{ns}	156.6 ± 26.4***
	WS	213.1 ± 65.2	137.8 ± 26.4
NL	N3	227.1 ± 65.3*	151.4 ± 26.4**
	N2	224.0 ± 65.3	139.0 ± 26.4
	N1	205.1 ± 65.3	151.2 ± 26.4
PD	R	235.5 ± 65.2***	157.2 ± 26.4***
	H	201.9 ± 65.2	137.2 ± 26.4
WC * NL	Opti_N3	234.6 ± 65.6***	157.3 ± 26.4^{ns}
	Opti_N2	253.9 ± 65.6	153.3 ± 26.4
	Opti_N1	184.6 ± 65.6	159.2 ± 26.4
	WS_N3	219.6 ± 65.6	145.5 ± 26.4
	WS_N2	194.1 ± 65.6	124.7 ± 26.4
	WS_N1	225.6 ± 65.6	143.1 ± 26.4
SGT	NSG	229.1 ± 65.2**	142.9 ± 26.4*
	SG	208.3 ± 65.2	151.5 ± 26.4

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait; **Num DF**: is the number of degrees of freedom in the model; **Den DF**: is the number of degrees of freedom associated with the model errors.)

Annex 3. Analysis of variance for repeated measure during senescence period.

Table 1: Analysis of variance for chlorophyll content in two maize inbred lines in 4 experiments

a: fixed effects

Effect	DDL num.	DDL den.	Value F	Pr > F
Treat	5	30	16.07	<.0001
Time	3	18	308.36	<.0001
PD	1	6	14.61	0.0087
Genotype	1	6	4.50	0.0781
Treat*Time	15	90	1.36	0.1837
Treat*PD	5	30	0.61	0.6896
Time*PD	3	18	2.18	0.1254
Treat*genotype	5	30	0.41	0.8362
PD*genotype	1	6	0.51	0.5023
Treat*Time*PD	15	90	0.24	0.9985
Time*genotype	3	18	12.37	0.0001
Treat*Time*genotype	15	90	0.38	0.9809
Treat* PD*genotype	5	30	1.00	0.4361
Time* PD*genotype	3	18	0.39	0.7645
Treat*Time* PD*genotype	15	90	0.23	0.9988

(PD: plant density; Treat: treatment (water condition×nitrogen level (WC×NL)); Time: different moments from silking to harvest. DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Variable	Estimation	Standard error
CS	nomvar(Environment)	44.6856	26.2628
Residual		78.7376	4.3180

Table 2: Analysis of variance for Quantum efficiency of photosystem II in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DDL num.	DDL den.	Value F	Pr > F
Treat	5	30	3.28	0.0177
Time	3	18	627.07	<.0001
PD	1	6	0.02	0.8920
Genotype	1	6	25.78	0.0023
Treat*Time	15	90	1.14	0.3305
Treat*PD	5	30	0.92	0.4844
Time*PD	3	18	1.23	0.3288
Treat*genotype	5	30	0.80	0.5559
PD*genotype	1	6	1.38	0.2839
Treat*Time*PD	15	90	0.29	0.9953
Time*genotype	3	18	240.51	<.0001
Treat*Time*genotype	15	90	2.02	0.0221
Treat* PD*genotype	5	30	0.36	0.8717
Time* PD*genotype	3	18	0.40	0.7538
Treat*Time* PD*genotype	15	90	0.32	0.9925

(PD: plant density; Treat: treatment (water condition×nitrogen level (WC×NL)); Time: different moments from silking to harvest. DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Variable**	**Estimation**	**Standard error**
CS	nomvar(Environment)	2407.14	1474.75
Residual		14127	774.16

Table 3. Analysis of variance for Photosynthetic rate in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DDL num.	DDL den.	Value F	Pr > F
Treat	5	30	7.83	<.0001
Time	3	18	741.50	<.0001
PD	1	6	0.58	0.4759
Genotype	1	6	0.35	0.5780
Treat*Time	15	90	1.84	0.0410
Treat*PD	5	30	0.62	0.6882
Time*PD	3	18	1.09	0.3803
Treat*genotype	5	30	0.38	0.8611
PD*genotype	1	6	0.13	0.7259
Treat*Time*PD	15	90	0.28	0.9962
Time*genotype	3	18	21.19	<.0001
Treat*Time*genotype	15	90	0.49	0.9407
Treat* PD*genotype	5	30	0.26	0.9314
Time* PD*genotype	3	18	0.11	0.9521
Treat*Time* PD*genotype	15	90	0.31	0.9934

(PD: plant density; Treat: treatment (water condition×nitrogen level (WC×NL)); Time: different moments from silking to harvest. DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	**Variable**	**Estimation**	**Standard error**
CS	nomvar(Environment)	15.0980	8.8923
Residual		28.4753	1.5735

Table 4. Analysis of variance for stomatic conductance in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DDL num.	DDL den.	Value F	Pr > F
Treat	5	30	1.24	0.3132
Time	3	18	17.51	<.0001
PD	1	6	0.10	0.7610
Genotype	1	6	1.43	0.2767
Treat*Time	15	90	0.80	0.6730
Treat*PD	5	30	0.88	0.5038
Time*PD	3	18	0.56	0.6454
Treat*genotype	5	30	0.63	0.6750
PD*genotype	1	6	0.01	0.9374
Treat*Time*PD	15	89	0.96	0.5001
Time*genotype	3	18	1.36	0.2870
Treat*Time*genotype	15	90	1.12	0.3501
Treat* PD*genotype	5	30	1.09	0.3869
Time* PD*genotype	3	18	1.76	0.1911
Treat*Time* PD*genotype	15	89	0.87	0.6009

(PD: plant density; Treat: treatment (water condition×nitrogen level (WC×NL)); Time: different moments from silking to harvest. DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors.)

b: random effects

Covariance	Variable	Estimation	Standard error
CS	nomvar(Environment)	0.000665	0.000576
Residual		0.02869	0.001600

Annex 4. Analysis of variance of Nitrogen assimilation and remobilization in soil and plant

➢ **Annex 4.a: Nitrogen in the soil**

Table 1. Analysis of variance for soil nitrogen content at silking time (g kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	259	10.62	0.0013
NL	2	259	0.73	0.4820
PD	1	259	0.01	0.9385
SGT	1	259	0.01	0.9411
WC*NL	2	259	7.20	0.0009
WC*PD	1	259	0.27	0.6071
NL*PD	2	259	4.25	0.0153
WC*SGT	1	259	0.09	0.7674
PD*SGT	1	259	0.14	0.7117
WC*NL*PD	2	259	0.05	0.9529
NL*SGT	2	259	1.66	0.1914
WC*NL*SGT	2	259	1.00	0.3684
WC*PL*SGT	1	259	0.02	0.8824
NL*PD*SGT	2	259	0.26	0.7737
WC*NL*PD*SGT	2	259	1.09	0.3393

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0.3759	0.5344
Rep(Environment)	0.002644	0.004044
Residual	0.1007	0.008846

Table 2. Analysis of variance for soil carbon content at silking time (g kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	259	0.65	0.4195
NL	2	259	0.40	0.6683
PD	1	259	0.07	0.7961
SGT	1	259	0.11	0.7422
WC*NL	2	259	5.29	0.0056
WC*PD	1	259	0.16	0.6930
NL*PD	2	259	3.16	0.0442
WC*SGT	1	259	0.56	0.4560
PD*SGT	1	259	1.53	0.2174
WC*NL*PD	2	259	0.27	0.7612
NL*SGT	2	259	1.39	0.2510
WC*NL*SGT	2	259	1.62	0.2004
WC*PL*SGT	1	259	0.05	0.8300
NL*PD*SGT	2	259	0.07	0.9303
WC*NL*PD*SGT	2	259	1.02	0.3607

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	111.92	161.36
Rep(Environment)	4.1953	4.3378
Residual	10.1080	0.8882

Table 3. Analysis of variance for soil nitrogen content at silking time (g kg-1) in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	156	8.03	0.0052
NL	2	156	0.30	0.7435
PD	1	156	0.04	0.8334
SGT	1	156	0.35	0.5572
WC*NL	2	156	1.51	0.2246
WC*PD	1	156	0.71	0.4011
NL*PD	2	156	0.61	0.5446
WC*SGT	1	156	1.68	0.1962
PD*SGT	1	156	1.99	0.1605
WC*NL*PD	2	156	0.19	0.8300
NL*SGT	2	156	0.61	0.5440
WC*NL*SGT	2	156	0.12	0.8869
WC*PL*SGT	1	156	0.60	0.4380
NL*PD*SGT	2	156	0.18	0.8357
WC*NL*PD*SGT	2	156	0.65	0.5230

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0.3208	0.2741
Rep(Environment)	0.02441	0.02082
Residual	0.1147	0.01299

Table 4. Analysis of variance for soil carbon content at silking timetime (g kg-1) in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	156	1.03	0.3119
NL	2	156	0.78	0.4599
PD	1	156	0.47	0.4940
SGT	1	156	0.29	0.5890
WC*NL	2	156	0.83	0.4382
WC*PD	1	156	0.12	0.7331
NL*PD	2	156	0.76	0.4695
WC*SGT	1	156	0.43	0.5116
PD*SGT	1	156	0.02	0.8940
WC*NL*PD	2	156	0.63	0.5315
NL*SGT	2	156	0.67	0.5155
WC*NL*SGT	2	156	0.39	0.6793
WC*PL*SGT	1	156	0.09	0.7690
NL*PD*SGT	2	156	0.16	0.8484
WC*NL*PD*SGT	2	156	1.94	0.1473

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	77.6945	65.4437
Rep(Environment)	4.3558	3.4337
Residual	11.7450	1.3298

Table 5. Analysis of variance for soil nitrogen content at harvest time (g kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	259	0.67	0.4143
NL	2	259	3.49	0.0319
PD	1	259	0.00	0.9485
SGT	1	259	0.30	0.5824
WC*NL	2	259	6.11	0.0025
WC*PD	1	259	0.06	0.8102
NL*PD	2	259	8.16	0.0004
WC*SGT	1	259	0.13	0.7229
PD*SGT	1	259	0.83	0.3643
WC*NL*PD	2	259	0.19	0.8232
NL*SGT	2	259	0.34	0.7100
WC*NL*SGT	2	259	0.51	0.6033
WC*PL*SGT	1	259	0.35	0.5532
NL*PD*SGT	2	259	0.05	0.9526
WC*NL*PD*SGT	2	259	0.04	0.9570

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0	.
Rep(Environment)	0	.
Residual	0.4142	0.03619

Table 6. Analysis of variance for soil carbon content at harvest time (g kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	259	0.28	0.5997
NL	2	259	3.34	0.0370
PD	1	259	0.04	0.8423
SGT	1	259	0.18	0.6758
WC*NL	2	259	6.56	0.0017
WC*PD	1	259	0.00	0.9497
NL*PD	2	259	7.24	0.0009
WC*SGT	1	259	0.16	0.6928
PD*SGT	1	259	0.39	0.5353
WC*NL*PD	2	259	0.10	0.9065
NL*SGT	2	259	0.33	0.7190
WC*NL*SGT	2	259	0.36	0.6976
WC*PL*SGT	1	259	0.22	0.6398
NL*PD*SGT	2	259	0.04	0.9620
WC*NL*PD*SGT	2	259	0.07	0.9344

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0	.
Rep(Environment)	0	.
Residual	60.2052	5.2602

Table 7. Analysis of variance for soil nitrogen content at harvest time (g kg-1) in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	160	0.03	0.8580
NL	2	160	0.50	0.6079
PD	1	160	0.05	0.8177
SGT	1	160	0.11	0.7451
WC*NL	2	160	0.89	0.4119
WC*PD	1	160	0.49	0.4852
NL*PD	2	160	2.08	0.1279
WC*SGT	1	160	0.45	0.5036
PD*SGT	1	160	0.01	0.9274
WC*NL*PD	2	160	0.05	0.9540
NL*SGT	2	160	0.52	0.5970
WC*NL*SGT	2	160	0.66	0.5168
WC*PL*SGT	1	160	0.15	0.7013
NL*PD*SGT	2	160	0.07	0.9318
WC*NL*PD*SGT	2	160	0.23	0.7911

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0.2318	0.1933
Rep(Environment)	0	.
Residual	0.2413	0.02665

Table 8. Analysis of variance for soil carbon content at harvest time (g kg-1) in two maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	160	0.04	0.8466
NL	2	160	0.82	0.4402
PD	1	160	0.09	0.7604
SGT	1	160	0.09	0.7676
WC*NL	2	160	1.34	0.2653
WC*PD	1	160	0.21	0.6469
NL*PD	2	160	1.59	0.2065
WC*SGT	1	160	0.37	0.5412
PD*SGT	1	160	0.01	0.9410
WC*NL*PD	2	160	0.15	0.8629
NL*SGT	2	160	0.29	0.7513
WC*NL*SGT	2	160	0.54	0.5810
WC*PL*SGT	1	160	0.03	0.8561
NL*PD*SGT	2	160	0.06	0.9448
WC*NL*PD*SGT	2	160	0.16	0.8508

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	38.1134	31.7136
Rep(Environment)	0	.
Residual	34.9097	3.8551

Table 9. Analysis of variance for soil NO_3 content at silking time (mg kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	256	5.73	0.0174
NL	2	256	12.00	<.0001
PD	1	256	3.28	0.0713
SGT	1	256	2.52	0.1135
WC*NL	2	256	0.92	0.3995
WC*PD	1	256	0.01	0.9383
NL*PD	2	256	4.69	0.0100
WC*SGT	1	256	0.19	0.6599
PD*SGT	1	256	0.19	0.6625
WC*NL*PD	2	256	0.36	0.6973
NL*SGT	2	256	0.42	0.6569
WC*NL*SGT	2	256	0.40	0.6732
WC*PL*SGT	1	256	0.24	0.6247
NL*PD*SGT	2	256	0.65	0.5251
WC*NL*PD*SGT	2	256	0.11	0.8950

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	175.49	299.94
Rep(Environment)	68.5829	70.2400
Residual	117.08	10.3487

Table 10. Analysis of variance for soil NH_4 content at silking time (mg kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	256	2.04	0.1545
NL	2	256	0.19	0.8254
PD	1	256	0.44	0.5075
SGT	1	256	1.85	0.1745
WC*NL	2	256	2.41	0.0922
WC*PD	1	256	0.51	0.4745
NL*PD	2	256	0.59	0.5573
WC*SGT	1	256	0.40	0.5252
PD*SGT	1	256	5.54	0.0193
WC*NL*PD	2	256	1.24	0.2923
NL*SGT	2	256	1.49	0.2271
WC*NL*SGT	2	256	2.98	0.0526
WC*PL*SGT	1	256	1.56	0.2127
NL*PD*SGT	2	256	1.24	0.2901
WC*NL*PD*SGT	2	256	0.26	0.7700

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	37.3423	86.5015
Rep(Environment)	42.8640	43.6840
Residual	58.0294	5.1291

Table 11. Analysis of variance for soil NO_3 content at Harvest time (mg kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	256	53.99	<.0001
NL	2	256	5.37	0.0052
PD	1	256	0.19	0.6593
SGT	1	256	0.02	0.9018
WC*NL	2	256	4.99	0.0074
WC*PD	1	256	0.02	0.8990
NL*PD	2	256	4.13	0.0172
WC*SGT	1	256	2.39	0.1232
PD*SGT	1	256	0.00	0.9704
WC*NL*PD	2	256	2.13	0.1211
NL*SGT	2	256	0.01	0.9877
WC*NL*SGT	2	256	0.31	0.7355
WC*PL*SGT	1	256	0.14	0.7108
NL*PD*SGT	2	256	0.61	0.5460
WC*NL*PD*SGT	2	256	0.23	0.7929

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	10.0462	15.9703
Rep(Environment)	2.2686	2.4260
Residual	11.1783	0.9880

Table 12. Analysis of variance for soil NH_4 content at harvest time (mg kg-1) in six maize inbred lines for experiment 1 and 2.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	256	1.50	0.2226
NL	2	256	1.77	0.1728
PD	1	256	0.01	0.9327
SGT	1	256	0.62	0.4304
WC*NL	2	256	1.35	0.2602
WC*PD	1	256	0.05	0.8307
NL*PD	2	256	1.35	0.2623
WC*SGT	1	256	0.12	0.7253
PD*SGT	1	256	4.07	0.0448
WC*NL*PD	2	256	0.20	0.8192
NL*SGT	2	256	0.65	0.5230
WC*NL*SGT	2	256	0.29	0.7520
WC*PL*SGT	1	256	0.53	0.4658
NL*PD*SGT	2	256	1.03	0.3597
WC*NL*PD*SGT	2	256	0.03	0.9693

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0	.
Rep(Environment)	6.4277	5.7710
Residual	45.3853	4.0115

➢ Annex 4. b: Nitrogen in plant

Table 1. Analysis of variance for total nitrogen content in plant (g kg-1) for six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	537	2.86	0.0916
NL	2	537	4.14	0.0164
PD	1	537	1.11	0.2917
SGT	1	537	0.04	0.8505
WC*NL	2	537	0.62	0.5363
WC*PD	1	537	0	0.9675
NL*PD	2	537	0.04	0.9626
WC*SGT	1	537	0.14	0.7092
PD*SGT	1	537	0.89	0.3451
WC*NL*PD	2	537	1.79	0.1687
NL*SGT	2	537	0.03	0.9669
WC*NL*SGT	2	537	1.61	0.2005
WC*PL*SGT	1	537	0.05	0.8221
NL*PD*SGT	2	537	0.63	0.5324
WC*NL*PD*SGT	2	537	0	0.9992

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	16.7244	14.3874
Rep(Environment)	1.3602	1.2457
Residual	28.039	1.7112

Table 2. Analysis of variance for total carbon content in plant (g kg-1) for six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	531	15.29	0.0001
NL	2	531	1.71	0.1822
PD	1	531	2.42	0.1208
SGT	1	531	0.6	0.4398
WC*NL	2	531	3.79	0.0232
WC*PD	1	531	0	0.9682
NL*PD	2	531	2.05	0.1294
WC*SGT	1	531	0.78	0.3771
PD*SGT	1	531	0.4	0.527
WC*NL*PD	2	531	2.04	0.1305
NL*SGT	2	531	0.06	0.9453
WC*NL*SGT	2	531	0.4	0.6704
WC*PL*SGT	1	531	0.38	0.5379
NL*PD*SGT	2	531	0.31	0.7338
WC*NL*PD*SGT	2	531	0.28	0.7542

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	186.12	392.22
Rep(Environment)	317.71	351.36
Residual	12176	747.43

Table 3. Analysis of variance for nitrogen content in kernel (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	507	16.83	<.0001
NL	2	507	0.05	0.9515
PD	1	507	0.39	0.5328
SGT	1	507	7.4	0.0067
WC*NL	2	507	0.09	0.9102
WC*PD	1	507	0.01	0.9416
NL*PD	2	507	0.23	0.7971
WC*SGT	1	507	0.23	0.6328
PD*SGT	1	507	3.95	0.0475
WC*NL*PD	2	507	1.18	0.3076
NL*SGT	2	507	0.53	0.5885
WC*NL*SGT	2	507	1.03	0.3574
WC*PL*SGT	1	507	0.16	0.6852
NL*PD*SGT	2	507	0.14	0.869
WC*NL*PD*SGT	2	507	0.02	0.9755

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	3.8663	3.3766
Rep(Environment)	0.4488	0.3702
Residual	5.0347	0.3162

Table 4. Analysis of variance for carbon content in kernel (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	506	8.3	0.0041
NL	2	506	2.32	0.0996
PD	1	506	2.02	0.1561
SGT	1	506	2.14	0.1444
WC*NL	2	506	1.17	0.31
WC*PD	1	506	1.15	0.2842
NL*PD	2	506	1.05	0.3504
WC*SGT	1	506	0	0.9735
PD*SGT	1	506	3.88	0.0493
WC*NL*PD	2	506	1.3	0.2724
NL*SGT	2	506	0.32	0.7242
WC*NL*SGT	2	506	1.31	0.2715
WC*PL*SGT	1	506	0	0.9749
NL*PD*SGT	2	506	1.71	0.1814
WC*NL*PD*SGT	2	506	2.31	0.1008

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	578.59	483.63
Rep(Environment)	24.9097	19.2903
Residual	147.79	9.2919

Table 5. Analysis of variance for kernel nitrogen remobilized after flowering (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	506	20.79	<.0001
NL	2	506	3.4	0.0341
PD	1	506	0.26	0.6134
SGT	1	506	8.6	0.0035
WC*NL	2	506	2.6	0.0749
WC*PD	1	506	0.23	0.6353
NL*PD	2	506	0.51	0.6019
WC*SGT	1	506	0.19	0.6658
PD*SGT	1	506	0.39	0.5306
WC*NL*PD	2	506	0.22	0.8053
NL*SGT	2	506	0.03	0.9691
WC*NL*SGT	2	506	0.93	0.3951
WC*PL*SGT	1	506	0.05	0.8295
NL*PD*SGT	2	506	0.1	0.9089
WC*NL*PD*SGT	2	506	0.64	0.5274

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	641.26	528.04
Rep(Environment)	0	.
Residual	686.87	43.0139

Table 6. Analysis of variance for carbon remobilized to kernel (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	506	1.83	0.1764
NL	2	506	0.95	0.3888
PD	1	506	1.93	0.1649
SGT	1	506	0	0.9572
WC*NL	2	506	0.39	0.6752
WC*PD	1	506	1.83	0.1769
NL*PD	2	506	0.06	0.9432
WC*SGT	1	506	3.17	0.0755
PD*SGT	1	506	1.4	0.2374
WC*NL*PD	2	506	0.07	0.9335
NL*SGT	2	506	0.11	0.8983
WC*NL*SGT	2	506	1.48	0.2295
WC*PL*SGT	1	506	0.02	0.8827
NL*PD*SGT	2	506	0.72	0.4877
WC*NL*PD*SGT	2	506	0.15	0.8633

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	20.9027	18.8998
Rep(Environment)	0	.
Residual	294.84	18.4636

Table 7. Analysis of variance for Nitrogen Up-take after flowring by kernel (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	506	20.79	<.0001
NL	2	506	3.4	0.0341
PD	1	506	0.26	0.6134
SGT	1	506	8.6	0.0035
WC*NL	2	506	2.6	0.0749
WC*PD	1	506	0.23	0.6353
NL*PD	2	506	0.51	0.6019
WC*SGT	1	506	0.19	0.6658
PD*SGT	1	506	0.39	0.5306
WC*NL*PD	2	506	0.22	0.8053
NL*SGT	2	506	0.03	0.9691
WC*NL*SGT	2	506	0.93	0.3951
WC*PL*SGT	1	506	0.05	0.8295
NL*PD*SGT	2	506	0.1	0.9089
WC*NL*PD*SGT	2	506	0.64	0.5274

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	641.26	528.04
Rep(Environment)	0	.
Residual	686.87	43.0139

Table 8. Analysis of variance for carbon up take after flowering by kernel (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	506	1.83	1
NL	2	506	0.95	2
PD	1	506	1.93	1
SGT	1	506	0	1
WC*NL	2	506	0.39	2
WC*PD	1	506	1.83	1
NL*PD	2	506	0.06	2
WC*SGT	1	506	3.17	1
PD*SGT	1	506	1.4	1
WC*NL*PD	2	506	0.07	2
NL*SGT	2	506	0.11	2
WC*NL*SGT	2	506	1.48	2
WC*PL*SGT	1	506	0.02	1
NL*PD*SGT	2	506	0.72	2
WC*NL*PD*SGT	2	506	0.15	2

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	20.9027	18.8998
Rep(Environment)	0	.
Residual	294.84	18.4636

Table 9. Analysis of variance for nitrogen content in stover during silking time (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	3.63	0.0574
NL	2	518	23.21	<.0001
PD	1	518	3.11	0.0784
SGT	1	518	0.00	0.9785
WC*NL	2	518	1.03	0.3570
WC*PD	1	518	0.11	0.7387
NL*PD	2	518	1.18	0.3081
WC*SGT	1	518	0.01	0.9280
PD*SGT	1	518	1.53	0.2166
WC*NL*PD	2	518	0.51	0.5986
NL*SGT	2	518	0.14	0.8678
WC*NL*SGT	2	518	0.42	0.6547
WC*PL*SGT	1	518	0.63	0.4276
NL*PD*SGT	2	518	0.37	0.6896
WC*NL*PD*SGT	2	518	1.03	0.3574

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	3.5997	3.3782
Rep(Environment)	0.8979	0.7263
Residual	8.9934	0.5588

Table 10. Analysis of variance for carbon content in stover during silking time (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	5.92	0.0153
NL	2	518	1.65	0.1924
PD	1	518	0.37	0.5415
SGT	1	518	9.01	0.0028
WC*NL	2	518	1.22	0.2952
WC*PD	1	518	0.99	0.3203
NL*PD	2	518	2.13	0.1193
WC*SGT	1	518	0.27	0.6068
PD*SGT	1	518	0.42	0.5195
WC*NL*PD	2	518	3.85	0.0218
NL*SGT	2	518	2.22	0.1101
WC*NL*SGT	2	518	0.57	0.5640
WC*PL*SGT	1	518	0.13	0.7156
NL*PD*SGT	2	518	0.64	0.5279
WC*NL*PD*SGT	2	518	3.05	0.0482

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	147.23	121.89
Rep(Environment)	2.1775	2.8649
Residual	128.31	7.9727

Table 11. Analysis of variance for nitrogen up take after flowering time (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	536	8.18	0.0044
NL	2	536	0.91	0.4014
PD	1	536	0.02	0.8981
SGT	1	536	1.13	0.2874
WC*NL	2	536	0.23	0.7922
WC*PD	1	536	0	0.9449
NL*PD	2	536	0.42	0.6565
WC*SGT	1	536	0.2	0.6537
PD*SGT	1	536	0.29	0.5922
WC*NL*PD	2	536	0.3	0.7403
NL*SGT	2	536	0.04	0.9563
WC*NL*SGT	2	536	1.81	0.1651
WC*PL*SGT	1	536	0.08	0.7841
NL*PD*SGT	2	536	0.24	0.7838
WC*NL*PD*SGT	2	536	0.56	0.5734

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	28.4357	23.5593
Rep(Environment)	0.3059	0.5912
Residual	37.054	2.2636

Table 12. Analysis of variance for carbon nitrogen up take after flowering time (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	533	6.47	0.0112
NL	2	533	1.45	0.2353
PD	1	533	0.7	0.4046
SGT	1	533	0	0.9757
WC*NL	2	533	1.57	0.2081
WC*PD	1	533	0.36	0.5511
NL*PD	2	533	1.06	0.3464
WC*SGT	1	533	0.9	0.3422
PD*SGT	1	533	2.91	0.0886
WC*NL*PD	2	533	0.02	0.9784
NL*SGT	2	533	0.25	0.7778
WC*NL*SGT	2	533	1.44	0.2381
WC*PL*SGT	1	533	0.01	0.9343
NL*PD*SGT	2	533	0.37	0.6907
WC*NL*PD*SGT	2	533	0.12	0.8842

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	0	.
Rep(Environment)	570.08	453.53
Residual	18430	1129.22

Table 13. Analysis of variance for nitrogen remobilization from silking to harvest time in stover (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	532	32.51	<.0001
NL	2	532	4.76	0.0089
PD	1	532	0.02	0.8833
SGT	1	532	3.53	0.0609
WC*NL	2	532	5.99	0.0027
WC*PD	1	532	0.04	0.8376
NL*PD	2	532	0.14	0.8688
WC*SGT	1	532	0	0.9965
PD*SGT	1	532	1.22	0.2707
WC*NL*PD	2	532	0.69	0.501
NL*SGT	2	532	0.03	0.9687
WC*NL*SGT	2	532	0.75	0.4727
WC*PL*SGT	1	532	0.05	0.8202
NL*PD*SGT	2	532	0.13	0.8823
WC*NL*PD*SGT	2	532	0.95	0.3882

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	17.1014	14.1235
Rep(Environment)	0.1434	0.2758
Residual	17.5535	1.0762

Table 14. Analysis of variance for carbon remobilization from silking to harvest time in stover (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	533	1	0.3178
NL	2	533	0.43	0.6535
PD	1	533	0.74	0.3898
SGT	1	533	0.9	0.3429
WC*NL	2	533	0.68	0.507
WC*PD	1	533	0.82	0.3669
NL*PD	2	533	0.43	0.6516
WC*SGT	1	533	0.67	0.4147
PD*SGT	1	533	2.73	0.0989
WC*NL*PD	2	533	0.55	0.5745
NL*SGT	2	533	0.52	0.5957
WC*NL*SGT	2	533	1.39	0.2497
WC*PL*SGT	1	533	0.01	0.9256
NL*PD*SGT	2	533	0.04	0.9615
WC*NL*PD*SGT	2	533	0.11	0.8927

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	32.201	166.55
Rep(Environment)	168.8	191.73
Residual	6899.9	422.67

Table 15. Analysis of variance for percentage of nitrogen remobilization from silking to harvest time in stover (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	32.86	<.0001
NL	2	518	2.43	0.0895
PD	1	518	0	0.9675
SGT	1	518	5.5	0.0193
WC*NL	2	518	8.33	0.0003
WC*PD	1	518	0.67	0.4138
NL*PD	2	518	0.2	0.8181
WC*SGT	1	518	0.15	0.6973
PD*SGT	1	518	0.29	0.5878
WC*NL*PD	2	518	0.67	0.5114
NL*SGT	2	518	0.45	0.6387
WC*NL*SGT	2	518	1.71	0.1822
WC*PL*SGT	1	518	1.34	0.247
NL*PD*SGT	2	518	1.73	0.1792
WC*NL*PD*SGT	2	518	0.92	0.3996

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	886.91	751.7
Rep(Environment)	57.1624	46.831
Residual	633.11	39.339

Table 16. Analysis of variance for percentage of carbon remobilization from silking to harvest time in stover (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	0.15	0.6989
NL	2	518	0.13	0.8767
PD	1	518	0	0.9479
SGT	1	518	2.76	0.097
WC*NL	2	518	0.13	0.8804
WC*PD	1	518	0.02	0.9014
NL*PD	2	518	2.31	0.1002
WC*SGT	1	518	0.01	0.9141
PD*SGT	1	518	3.97	0.0469
WC*NL*PD	2	518	0.27	0.7643
NL*SGT	2	518	1.43	0.2411
WC*NL*SGT	2	518	0.23	0.7932
WC*PL*SGT	1	518	0.4	0.5248
NL*PD*SGT	2	518	0.74	0.4778
WC*NL*PD*SGT	2	518	0.24	0.7836

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	4.9495	9.9504
Rep(Environment)	9.7289	8.6062
Residual	159.13	9.8887

Table 17. Analysis of variance for nitrogen non-remobilized from silking to harvest time in stover (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	526	30.58	<.0001
NL	2	526	10.64	<.0001
PD	1	526	0.59	0.4411
SGT	1	526	8.56	0.0036
WC*NL	2	526	6.84	0.0012
WC*PD	1	526	0.14	0.7123
NL*PD	2	526	0.94	0.3912
WC*SGT	1	526	0.47	0.4951
PD*SGT	1	526	0.15	0.6964
WC*NL*PD	2	526	1.28	0.2783
NL*SGT	2	526	0.08	0.9245
WC*NL*SGT	2	526	0.85	0.4272
WC*PL*SGT	1	526	0.01	0.937
NL*PD*SGT	2	526	0.04	0.962
WC*NL*PD*SGT	2	526	0.27	0.7599

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	5.4033	4.51
Rep(Environment)	0.1171	0.1634
Residual	8.0492	0.4963

Table 18. Analysis of variance for carbon non- remobilized from silking to harvest time in stover (g kg-1) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	526	0.82	0.3649
NL	2	526	0.05	0.9545
PD	1	526	0.41	0.5246
SGT	1	526	0.09	0.7631
WC*NL	2	526	0.78	0.4609
WC*PD	1	526	0.45	0.5008
NL*PD	2	526	0.69	0.5026
WC*SGT	1	526	1.33	0.2488
PD*SGT	1	526	0.96	0.3288
WC*NL*PD	2	526	2.12	0.1215
NL*SGT	2	526	0.53	0.5867
WC*NL*SGT	2	526	0.17	0.8425
WC*PL*SGT	1	526	1.72	0.1901
NL*PD*SGT	2	526	1.39	0.2502
WC*NL*PD*SGT	2	526	1.03	0.3567

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	151.35	128.7
Rep(Environment)	6.7096	8.5556
Residual	385.75	23.7842

Table 19. Analysis of variance for percentage of nitrogen non- remobilized from silking to harvest time in stover (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	32.86	<.0001
NL	2	518	2.43	0.0895
PD	1	518	0	0.9675
SGT	1	518	5.5	0.0193
WC*NL	2	518	8.33	0.0003
WC*PD	1	518	0.67	0.4138
NL*PD	2	518	0.2	0.8181
WC*SGT	1	518	0.15	0.6973
PD*SGT	1	518	0.29	0.5878
WC*NL*PD	2	518	0.67	0.5114
NL*SGT	2	518	0.45	0.6387
WC*NL*SGT	2	518	1.71	0.1822
WC*PL*SGT	1	518	1.34	0.247
NL*PD*SGT	2	518	1.73	0.1792
WC*NL*PD*SGT	2	518	0.92	0.3996

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	886.91	751.7
Rep(Environment)	57.1624	46.831
Residual	633.11	39.339

Table 20. Analysis of variance for percentage of carbon non- remobilized from silking to harvest time in stover (%) in six maize inbred lines in 4 experiments.

a: fixed effects

Effect	DF Num	DenDF	F value	Pr>F
WC	1	518	0.15	0.6989
NL	2	518	0.13	0.8767
PD	1	518	0	0.9479
SGT	1	518	2.76	0.097
WC*NL	2	518	0.13	0.8804
WC*PD	1	518	0.02	0.9014
NL*PD	2	518	2.31	0.1002
WC*SGT	1	518	0.01	0.9141
PD*SGT	1	518	3.97	0.0469
WC*NL*PD	2	518	0.27	0.7643
NL*SGT	2	518	1.43	0.2411
WC*NL*SGT	2	518	0.23	0.7932
WC*PL*SGT	1	518	0.4	0.5248
NL*PD*SGT	2	518	0.74	0.4778
WC*NL*PD*SGT	2	518	0.24	0.7836

(WC: Water condition; NL: Nitrogen level; PD: Plant density; SGT: Stay-green trait); DDL **Num**: is the number of degrees of freedom in the model; DDL **Den** : is the number of degrees of freedom associated with the model errors).

b: random effects

Covariance	Variable	Estimation
Environment	4.9495	9.9504
Rep(Environment)	9.7289	8.6062
Residual	159.13	9.8887

Annex 5: Genes ontology (Go terms) for specific studied factors for experiment one.

Table S 1.. Main biological process of early senescence genes down-regulated in two inbred lines of temperate maize in Tomeza location.

GO.ID	GO Terms	p-value
GO:0015979	Photosynthesis	1.1e-09
GO:0018298	Protein-chromophore linkage	1.6e-05
GO:0009765	Photosynthesis, light harvesting	5.2e-05
GO:0019684	Photosynthesis, light reaction	6.8e-05
GO:0009628	Response to abiotic stimulus	8.8e-05
GO:0042742	Defense response to bacterium	0.00016
GO:0009416	Response to light stimulus	0.00017
GO:0009314	Response to radiation	0.00022
GO:0005985	Sucrose metabolic process	0.00023
GO:0005986	Sucrose biosynthetic process	0.00025
GO:0009768	Photosynthesis, light harvesting in photosystem I	0.00035
GO:0009617	Response to bacterium	0.00039
GO:0050896	Response to stimulus	7,00E-04
GO:0006950	Response to stress	0.00082
GO:0009645	Response to low light intensity stimulus	0.00126
GO:1901566	Organonitrogen compound biosynthetic process	0.00297
GO:0005984	Disaccharide metabolic process	0.0035
GO:0098542	Defense response to other organism	0.00353
GO:0010035	Response to inorganic substance	0.00469
GO:2000028	Regulation of photoperiodism, flowering	0.00471
GO:0006412	Translation	0.0049
GO:0043043	Peptide biosynthetic process	0.0054
GO:0009311	Oligosaccharide metabolic process	0.00551
GO:0006518	Peptide metabolic process	0.00618
GO:0009414	Response to water deprivation	0.00643
GO:0043604	Amide biosynthetic process	0.00694
GO:0009642	Response to light intensity	0.00703
GO:0009415	Response to water	0.00707
GO:0051247	Positive regulation of protein metabolic process	0.00718
GO:0048583	Regulation of response to stimulus	0.00902
GO:0043603	Cellular amide metabolic process	0.00917
GO:0001101	Response to acid chemical	0.00931

Table S 2. Main biological process of early senescence genes Up-regulated in two inbred lines of temperate maize in Tomeza location.

GO.ID	GO Terms	p-value
GO:0034357	photosynthetic membrane	3,00E-04
GO:0044436	thylakoid part	0.00036
GO:0009579	thylakoid	0.00101
GO:0009535	chloroplast thylakoid membrane	0.00146
GO:0055035	plastid thylakoid membrane	0.0015
GO:0042651	thylakoid membrane	0.0024
GO:0005840	ribosome	0.00312
GO:0009534	chloroplast thylakoid	0.0032
GO:0031976	plastid thylakoid	0.0032
GO:0010287	plastoglobule	0.00575
GO:0030529	ribonucleoprotein complex	0.00815
GO:0009523	photosystem II	0.0083

Table S 3. Main biological process of late senescence genes Up and Down-regulated in two inbred lines of temperate maize in Tomeza location.

GO.ID	GO Terms	p-value	Type
GO:0032787	monocarboxylic acid metabolic process	0.0077	Down
GO:0042651	thylakoid membrane	0.0065	Down
GO:0009507	chloroplast	0.0069	Down
GO:0034357	photosynthetic membrane	0.0077	Down
GO:0044436	thylakoid part	0.0085	Down
GO:0009536	plastid	0.0087	Down
GO:0005509	calcium ion binding	0.0054	Up

Table S 4: TF families and percentage of expression involved in each senescence moment of two maize inbred lines for Tomeza location.

TF Class	M1_M2		M2_M3		M3_M4	
	Expressed_	%TF_Express	Expressed_TF	%TF_Express	Expressed_TF	%TF_Express
AP2	13	25	10	10	53	3
ARF	31	50	20	20	62	15
ARR-B	3	23	6	6	13	3
B3	11	13	11	11	82	3
BBR-BPC	5	56	0	0	9	0
BES1	3	18	0	0	17	1
bHLH	61	20	30	30	298	32
bZIP	70	32	25	25	217	43
C2H2	42	24	25	25	176	26
C3H	52	47	29	29	111	16
CAMTA	6	60	3	3	10	4
CO-like	13	72	9	9	18	4
CPP	3	18	2	2	17	1
DBB	10	50	7	7	20	0
Dof	11	21	3	3	52	5
E2F/DP	2	8	2	2	24	2
EIL	1	11	0	0	9	0
ERF	14	7	8	8	205	9
FAR1	12	50	3	3	24	5
G2-like	16	18	12	12	90	18
GATA	20	37	22	22	54	13
GeBP	1	4	1	1	28	2
GRAS	5	5	5	5	102	4
GRF	2	6	0	0	32	1
HB-other	9	33	4	4	27	2
HB-PHD	0	0	0	0	4	0

HD-ZIP	29	30	22	22	97	6
HRT-like	0	0	0	0	2	0
HSF	19	39	22	22	49	8
LBD	0	0	0	0	63	0
LFY	0	0	0	0	4	0
LSD	14	70	11	11	20	0
M-type_MADS	4	9	2	2	45	1
MIKC_MADS	32	37	26	26	87	7
MYB	23	11	18	18	201	14
MYB_related	41	25	21	21	167	24
NAC	45	24	27	27	186	33
NF-X1	1	25	0	0	4	1
NF-YA	16	47	16	16	34	9
NF-YB	6	22	1	1	27	1
NF-YC	10	40	11	11	25	0
Nin-like	9	39	5	5	23	0
RAV	0	0	0	0	3	0
S1Fa-like	5	100	0	0	5	0
SBP	19	35	14	14	55	9
SRS	0	0	0	0	11	0
STAT	2	100	0	0	2	0
TALE	27	51	16	16	53	6
TCP	0	0	0	0	53	0
Trihelix	11	19	1	1	59	4
VOZ	8	80	0	0	10	0
Whirly	2	33	0	0	6	0
WOX	1	3	3	3	30	7
WRKY	25	16	28	28	160	30
YABBY	9	25	0	0	36	0
ZF-HD	0	0	0	0	26	0

Table S 5**:** Main biological process of SN1 (both stress) genes Down-regulated genes for B73 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0000266	mitochondrial fission	4.3e-05
GO:0008360	regulation of cell shape	0.00116
GO:0034227	tRNA thio-modification	0.00116
GO:0006612	protein targeting to membrane	0.0017
GO:0007267	cell-cell signaling	0.00173
GO:0022604	regulation of cell morphogenesis	0.00247
GO:0034470	ncRNA processing	0.00389
GO:0006357	regulation of transcription from RNA polymerase II promoter	0.0042
GO:0044743	intracellular protein transmembrane import	0.0055
GO:0002098	tRNA wobble uridine modification	0.00612
GO:0002097	tRNA wobble base modification	0.00856

Table S 6: Main biological process of SN1 (both stress) genes Up-regulated genes for B73 genotype in Tomeza location**.**

GO.ID	**B73 GO Terms_Up**	**p-value**
GO:0050896	response to stimulus	8.10E-06
GO:0006595	polyamine metabolic process	0.00014
GO:1901700	response to oxygen-containing compound	0.00049
GO:0006950	response to stress	0.00066
GO:0042221	response to chemical	0.00112
GO:0001101	response to acid chemical	0.00205
GO:0051235	maintenance of location	0.00206
GO:0006108	malate metabolic process	0.00236
GO:0009416	response to light stimulus	0.00291
GO:0045036	protein targeting to chloroplast	0.00344
GO:0072596	establishment of protein localization to chloroplast	0.00344
GO:0072598	protein localization to chloroplast	0.00385
GO:0009129	pyrimidine nucleoside monophosphate metabolic process	0.0051
GO:0009130	pyrimidine nucleoside monophosphate biosynthetic process	0.0051
GO:0051188	cofactor biosynthetic process	0.00522
GO:0009628	response to abiotic stimulus	0.00601
GO:0097305	response to alcohol	0.00697
GO:0044743	intracellular protein transmembrane import	0.00759
GO:0009240	isopentenyl diphosphate biosynthetic process	0.00767
GO:0046490	isopentenyl diphosphate metabolic process	0.00767
GO:0009737	response to abscisic acid	0.00802
GO:0042254	ribosome biogenesis	0.00819
GO:0065002	intracellular protein transmembrane transport	0.00825
GO:0032507	maintenance of protein location in cell	0.00913
GO:0045185	maintenance of protein location	0.00913

Table S 7: Main biological process of SN1 (both stress) genes Down-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms PHW79**	**p-value**
GO:0048583	regulation of response to stimulus	1.10E-05
GO:0051649	establishment of localization in cell	1.80E-05
GO:0051641	cellular localization	2.10E-05
GO:0034613	cellular protein localization	4.10E-05
GO:0009966	regulation of signal transduction	4.10E-05
GO:0023051	regulation of signaling	4.40E-05
GO:0010928	regulation of auxin mediated signaling pathway	5.00E-05
GO:0010646	regulation of cell communication	5.40E-05
GO:0070727	cellular macromolecule	6.10E-05

	localization	
GO:0046907	intracellular transport	6.10E-05
GO:0044707	single-multicellular organism process	0.00013
GO:0009787	regulation of abscisic acid-activated signaling pathway	0.00013
GO:1901419	regulation of response to alcohol	0.00013
GO:0007275	multicellular organismal development	0.00016
GO:0044767	single-organism developmental process	0.00018
GO:0006886	intracellular protein transport	2.00E-04
GO:0032502	developmental process	0.00027
GO:0009791	post-embryonic development	0.00029
GO:0009628	response to abiotic stimulus	3.00E-04
GO:0048585	negative regulation of response to stimulus	0.00035
GO:0008104	protein localization	0.00041
GO:0035265	organ growth	0.00044
GO:0048731	system development	0.00045
GO:0032501	multicellular organismal process	0.00057
GO:0033036	macromolecule localization	0.00059
GO:0048513	organ development	0.00072
GO:0032870	cellular response to hormone stimulus	0.00076
GO:0071495	cellular response to endogenous stimulus	0.00083
GO:0051716	cellular response to stimulus	0.00107
GO:0009755	hormone-mediated signaling pathway	0.0012
GO:0010587	miRNA catabolic process	0.00121
GO:0048467	gynoecium development	0.00143
GO:0016192	vesicle-mediated transport	0.00146
GO:0045184	establishment of protein localization	0.00194
GO:0010117	photoprotection	0.00199
GO:0071310	cellular response to organic substance	0.00203
GO:0051234	establishment of localization	0.00205
GO:0009738	abscisic acid-activated signaling pathway	0.00207
GO:0007165	signal transduction	0.00209
GO:0048608	reproductive structure development	0.00222
GO:0061458	reproductive system development	0.00222
GO:0044700	single organism signaling	0.0023
GO:0023052	signaling	0.00234
GO:0071365	cellular response to auxin stimulus	0.00282
GO:0097306	cellular response to alcohol	0.00286
GO:0044702	single organism reproductive process	0.00288
GO:0010586	miRNA metabolic process	0.00296
GO:0090503	RNA phosphodiester bond hydrolysis, exonucleolytic	0.00296
GO:0051179	localization	0.00306
GO:0048364	root development	0.00332
GO:0009408	response to heat	0.00334
GO:0022622	root system development	0.00342
GO:0050896	response to stimulus	0.00347
GO:0051241	negative regulation of multicellular organismal process	0.00372
GO:0070647	protein modification by small protein conjugation or removal	0.00382
GO:0044265	cellular macromolecule catabolic process	0.00393
GO:0034661	ncRNA catabolic process	0.0041
GO:0070370	cellular heat acclimation	0.0041
GO:0015031	protein transport	0.00431
GO:0051128	regulation of cellular component organization	0.00441
GO:0009414	response to water deprivation	0.00448
GO:0050793	regulation of developmental process	0.00483
GO:0006810	transport	0.00494
GO:0007154	cell communication	0.00499
GO:0003006	developmental process involved in reproduction	0.00504
GO:0071215	cellular response to abscisic acid stimulus	0.00524
GO:0009415	response to water	0.00528
GO:0000919	cell plate assembly	0.00541
GO:0051093	negative regulation of developmental process	0.00553
GO:0048856	anatomical structure development	0.00554
GO:0051239	regulation of multicellular organismal process	0.00588
GO:0048438	floral whorl development	0.00626
GO:0010286	heat acclimation	0.0066
GO:0032446	protein modification by small protein conjugation	0.0074
GO:0010033	response to organic substance	0.00757
GO:0048367	shoot system development	0.00815
GO:0071396	cellular response to lipid	0.00828
GO:0010375	stomatal complex patterning	0.00854
GO:0070887	cellular response to chemical stimulus	0.0089
GO:0006643	membrane lipid metabolic process	0.00898
GO:0009734	auxin-activated signaling pathway	0.00928
GO:0010154	fruit development	0.00941
GO:0040007	growth	0.00953

Table S 8: Main biological process of SN1 (both stress) genes Up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms PHW79_Up**	**p-value**
GO:0043132	NAD transport	2.8e-05
GO:0010025	wax biosynthetic process	7.8e-05
GO:0010166	wax metabolic process	9.4e-05
GO:0035194	posttranscriptional gene silencing by RNA	0.00254
GO:1901699	cellular response to nitrogen compound	0.00268
GO:0051181	cofactor transport	0.00282
GO:0048506	regulation of timing of	0.00363

	meristematic phase transition	
GO:0048510	regulation of timing of transition from vegetative to reproductive phase	0.00363
GO:0016441	posttranscriptional gene silencing	0.00403
GO:0044003	modification by symbiont of host morphology or physiology	0.00407
GO:1901698	response to nitrogen compound	0.00474
GO:0051817	modification of morphology or physiology of other organism involved in symbiotic interaction	0.00502
GO:0010608	posttranscriptional regulation of gene expression	0.00635

Table S 9: Main biological process of ON3 (optimal water and nitrogen codition) genes Down and Up-regulated genes for B73 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**	**Type**
GO:0033036	macromolecule localization	1.7e-05	Down
GO:0008104	protein localization	2.4e-05	Down
GO:0051641	cellular localization	6.2e-05	Down
GO:0016192	vesicle-mediated transport	0.00037	Down
GO:0015031	protein transport	0.00073	Down
GO:0042147	retrograde transport, endosome to Golgi	0.00077	Down
GO:0046907	intracellular transport	0.00083	Down
GO:0045184	establishment of protein localization	0.00088	Down
GO:0051649	establishment of localization in cell	0.0011	Down
GO:0034613	cellular protein localization	0.00176	Down
GO:0010337	regulation of salicylic acid metabolic process	0.00211	Down
GO:0030244	cellulose biosynthetic process	0.00222	Down
GO:0070727	cellular macromolecule localization	0.00223	Down
GO:0032271	regulation of protein polymerization	0.00256	Down
GO:0043254	regulation of protein complex assembly	0.00281	Down
GO:0051493	regulation of cytoskeleton organization	0.00364	Down
GO:0051179	localization	0.00445	Down
GO:0051274	beta-glucan biosynthetic process	0.0051	Down
GO:0016197	endosomal transport	0.00572	Down
GO:0009696	salicylic acid metabolic process	0.00663	Down
GO:0065003	macromolecular complex assembly	0.00697	Down
GO:0006461	protein complex assembly	0.00741	Down
GO:0071702	organic substance transport	0.00774	Down
GO:0070271	protein complex biogenesis	0.00821	Down
GO:0051258	protein polymerization	0.00834	Down
GO:0030243	cellulose metabolic process	0.00842	Down
GO:0006334	nucleosome assembly	0.00884	Down
GO:0034728	nucleosome organization	0.00935	Down
GO:0015979	photosynthesis	0.00011	Up
GO:0019684	photosynthesis, light reaction	0.00028	Up
GO:0009765	photosynthesis, light harvesting	0.00046	Up
GO:0006013	mannose metabolic process	0.00199	Up
GO:0043648	dicarboxylic acid metabolic process	0.00311	Up
GO:0010206	photosystem II repair	0.00317	Up
GO:0019318	hexose metabolic process	0.00399	Up
GO:0034250	positive regulation of cellular amide metabolic process	0.0046	Up
GO:0045727	positive regulation of translation	0.0046	Up
GO:0009628	response to abiotic stimulus	0.00464	Up
GO:0006091	generation of precursor metabolites and energy	0.00691	Up
GO:0009773	photosynthetic electron transport in photosystem I	0.00719	Up
GO:0006536	glutamate metabolic process	0.00817	Up
GO:0030091	protein repair	0.00817	Up
GO:0010629	negative regulation of gene expression	0.0095	Up

Table S 10: Main biological process of ON3 (optimal water and nitrogen codition) genes Down-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0043604	amide biosynthetic process	6.5e-06
GO:0043603	cellular amide metabolic process	1.9e-05
GO:0006412	translation	2.2e-05
GO:0043043	peptide biosynthetic process	3.1e-05
GO:0006518	peptide metabolic process	4.9e-05
GO:0015931	nucleobase-containing compound transport	5.8e-05
GO:0051641	cellular localization	8,00E-05
GO:0051649	establishment of localization in cell	0.00011
GO:0046907	intracellular transport	0.00011
GO:1901566	organonitrogen compound biosynthetic process	0.00018
GO:0071702	organic substance transport	3,00E-04
GO:0071705	nitrogen compound transport	0.00032
GO:0055062	phosphate ion homeostasis	0.00046
GO:0072506	trivalent inorganic anion homeostasis	0.00046
GO:0010966	regulation of phosphate transport	0.00059

GO:1903795	regulation of inorganic anion transmembrane transport	0.00059
GO:2000185	regulation of phosphate transmembrane transport	0.00059
GO:0006406	mRNA export from nucleus	0.00064
GO:0071427	mRNA-containing ribonucleoprotein complex export from nucleus	0.00064
GO:0016973	poly(A)+ mRNA export from nucleus	0.00073
GO:0051028	mRNA transport	0.001
GO:0071166	ribonucleoprotein complex localization	0.001
GO:0071426	ribonucleoprotein complex export from nucleus	0.001
GO:0015866	ADP transport	0.00107
GO:0072505	divalent inorganic anion homeostasis	0.00107
GO:0006405	RNA export from nucleus	0.00122
GO:0055081	anion homeostasis	0.00122
GO:1902582	single-organism intracellular transport	0.00141
GO:0015867	ATP transport	0.00151
GO:0022618	ribonucleoprotein complex assembly	0.00174
GO:0071826	ribonucleoprotein complex subunit organization	0.00174
GO:0009920	cell plate formation involved in plant-type cell wall biogenesis	0.00174
GO:0035435	phosphate ion transmembrane transport	0.00174
GO:0051179	localization	0.00185
GO:1901564	organonitrogen compound metabolic process	0.00199
GO:0055083	monovalent inorganic anion homeostasis	0.00203
GO:0006810	transport	0.00203
GO:0050657	nucleic acid transport	0.00211
GO:0050658	RNA transport	0.00211
GO:0051168	nuclear export	0.00211
GO:0051236	establishment of RNA localization	0.00211
GO:0006403	RNA localization	0.00248
GO:1902578	single-organism localization	0.00251
GO:0051234	establishment of localization	0.00258
GO:0015868	purine ribonucleotide transport	0.00266
GO:0051503	adenine nucleotide transport	0.00266
GO:0044267	cellular protein metabolic process	0.00324
GO:0006913	nucleocytoplasmic transport	0.00336
GO:0051169	nuclear transport	0.00336
GO:0015865	purine nucleotide transport	0.0034
GO:0009793	embryo development ending in seed dormancy	0.00342
GO:0048316	seed development	0.00348
GO:0008104	protein localization	0.00365
GO:0006862	nucleotide transport	0.00387
GO:0061025	membrane fusion	0.00387
GO:0044765	single-organism transport	0.00438
GO:0033036	macromolecule localization	0.00495
GO:1901607	alpha-amino acid biosynthetic process	0.00554
GO:0010021	amylopectin biosynthetic process	0.00561
GO:0051668	localization within membrane	0.00561
GO:0070676	intralumenal vesicle formation	0.00561
GO:1902591	single-organism membrane budding	0.00561
GO:2000896	amylopectin metabolic process	0.00561
GO:0010154	fruit development	0.00603
GO:0009790	embryo development	0.00667
GO:0015031	protein transport	0.00741
GO:0016192	vesicle-mediated transport	0.00743
GO:0006071	glycerol metabolic process	0.00828
GO:0006564	L-serine biosynthetic process	0.00828
GO:0034613	cellular protein localization	0.00882
GO:0015858	nucleoside transport	0.00886
GO:0045184	establishment of protein localization	0.00925

Table S 11: Main biological process of ON3 (optimal water and nitrogen codition) genes Up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0034660	ncRNA metabolic process	4,00E-06
GO:0006518	peptide metabolic process	8.5e-06
GO:0043043	peptide biosynthetic process	1.2e-05
GO:0048507	meristem development	1.2e-05
GO:0006399	tRNA metabolic process	1.4e-05
GO:1901566	organonitrogen compound biosynthetic process	1.6e-05
GO:0043603	cellular amide metabolic process	1.7e-05
GO:0006412	translation	1.9e-05
GO:0043604	amide biosynthetic process	2.9e-05
GO:0034470	ncRNA processing	2,00E-04
GO:0009888	tissue development	0.00046
GO:0007584	response to nutrient	5,00E-04
GO:0042372	phylloquinone biosynthetic process	5,00E-04
GO:0042374	phylloquinone metabolic process	5,00E-04
GO:0006396	RNA processing	0.00055
GO:0009416	response to light stimulus	0.00072
GO:0009628	response to abiotic stimulus	0.00073
GO:0006400	tRNA modification	0.00074
GO:0044237	cellular metabolic process	0.00075
GO:0042726	flavin-containing compound metabolic process	0.00089
GO:1901564	organonitrogen compound metabolic process	0.00101
GO:0006996	organelle organization	0.00102
GO:0018193	peptidyl-amino acid modification	0.00106
GO:0034641	cellular nitrogen compound metabolic process	0.00111
GO:0009314	response to radiation	0.00113
GO:0006450	regulation of translational fidelity	0.00117
GO:0007275	multicellular organismal	0.00118

	development	
GO:0044767	single-organism developmental process	0.00122
GO:0044707	single-multicellular organism process	0.00128
GO:0009657	plastid organization	0.00147
GO:0009637	response to blue light	0.00164
GO:0000413	protein peptidyl-prolyl isomerization	0.00165
GO:0010467	gene expression	0.00181
GO:0070681	glutaminyl-tRNAGln biosynthesis via transamidation	0.00184
GO:0008033	tRNA processing	0.00185
GO:0009987	cellular process	0.00188
GO:0009791	post-embryonic development	0.00191
GO:0032502	developmental process	0.00193
GO:0018208	peptidyl-proline modification	0.00195
GO:0043038	amino acid activation	0.00229
GO:0043039	tRNA aminoacylation	0.00229
GO:0032501	multicellular organismal process	0.00245
GO:0006807	nitrogen compound metabolic process	0.00274
GO:0003006	developmental process involved in reproduction	0.00294
GO:0015979	photosynthesis	0.00315
GO:0045036	protein targeting to chloroplast	0.00322
GO:0072596	establishment of protein localization to chloroplast	0.00322
GO:0051276	chromosome organization	0.00327
GO:0048573	photoperiodism, flowering	0.00347
GO:0009451	RNA modification	0.00354
GO:0009855	deGO Termsination of bilateral symmetry	0.00362
GO:0045038	protein import into chloroplast thylakoid membrane	0.00362
GO:0006448	regulation of translational elongation	0.00369
GO:0072598	protein localization to chloroplast	0.00373
GO:0010228	vegetative to reproductive phase transition of meristem	0.00396
GO:0044267	cellular protein metabolic process	0.00418
GO:0048731	system development	0.00449
GO:0009785	blue light signaling pathway	0.0046
GO:0030522	intracellular receptor signaling pathway	0.0046
GO:0035266	meristem growth	0.00465
GO:0010073	meristem maintenance	0.00504
GO:0043933	macromolecular complex subunit organization	0.00518
GO:0010449	root meristem growth	0.00558
GO:0001510	RNA methylation	0.00564
GO:0006771	riboflavin metabolic process	0.00565
GO:0009231	riboflavin biosynthetic process	0.00565
GO:0042727	flavin-containing compound biosynthetic process	0.00565
GO:0022613	ribonucleoprotein complex biogenesis	0.00569
GO:0006266	DNA ligation	0.00594
GO:0009799	specification of symmetry	0.00594
GO:0016144	S-glycoside biosynthetic process	0.00594
GO:0019758	glycosinolate biosynthetic process	0.00594
GO:0019761	glucosinolate biosynthetic process	0.00594
GO:0033273	response to vitamin	0.00594
GO:0044260	cellular macromolecule metabolic process	0.00618
GO:0009648	photoperiodism	0.00651
GO:0048638	regulation of developmental growth	0.00651
GO:0030488	tRNA methylation	0.00683
GO:0006259	DNA metabolic process	0.00694
GO:0043414	macromolecule methylation	0.00703
GO:0044763	single-organism cellular process	0.00742
GO:0009108	coenzyme biosynthetic process	0.00747
GO:1902589	single-organism organelle organization	0.00802
GO:0019538	protein metabolic process	0.00811
GO:0006741	NADP biosynthetic process	0.00876
GO:0016024	CDP-diacylglycerol biosynthetic process	0.00876
GO:0046341	CDP-diacylglycerol metabolic process	0.00876
GO:0071840	cellular component organization or biogenesis	0.00907
GO:0040008	regulation of growth	0.00923
GO:0042254	ribosome biogenesis	0.00945
GO:0006364	rRNA processing	0.00975
GO:0016568	chromatin modification	0.00987

Table S 12: Main biological process of N1 (low nitrogen stress) genes Down and Up-regulated genes for B73 genotype in Tomeza location.

GO.ID	GO Terms_N1_ Down	p-value	GO.ID	GO Terms_N1_ Up	p-value
GO:0000266	mitochondrial fission	7.9e-05	GO:0050896	response to stimulus	7.8e-06
GO:0051649	establishment of localization in cell	0.00023	GO:0006595	polyamine metabolic process	0.00021
GO:0015031	protein transport	0.00025	GO:1901700	response to oxygen-containing compound	0.00086
GO:0033036	macromolecule localization	0.00026	GO:0009416	response to light stimulus	0.00088
GO:0072594	establishment of protein localization to organelle	0.00026	GO:0042221	response to chemical	0.00097

GO:0034660	ncRNA metabolic process	0.00027	GO:0009314	response to radiation	0.00123
GO:0016482	cytoplasmic transport	0.00028	GO:0051235	maintenance of location	0.00281
GO:0071806	protein transmembrane transport	0.00029	GO:0006950	response to stress	0.00282
GO:0045184	establishment of protein localization	0.00032	GO:0071806	protein transmembrane transport	0.00303
GO:0006886	intracellular protein transport	0.00039	GO:0006108	malate metabolic process	0.00322
GO:0006605	protein targeting	0.00039	GO:0022613	ribonucleoprotein complex biogenesis	0.0034
GO:0008104	protein localization	0.00058	GO:0042254	ribosome biogenesis	0.00343
GO:0051641	cellular localization	0.00067	GO:0000154	rRNA modification	0.00375
GO:0033365	protein localization to organelle	0.00069	GO:0031167	rRNA methylation	0.00375
GO:0046907	intracellular transport	0.00093	GO:0090407	organophosphate biosynthetic process	0.00393
GO:0044743	intracellular protein transmembrane import	0.00094	GO:0009644	response to high light intensity	0.00448
GO:0034613	cellular protein localization	0.00095	GO:0045036	protein targeting to chloroplast	0.00467
GO:1902580	single-organism cellular localization	0.00104	GO:0072596	establishment of protein localization to chloroplast	0.00467
GO:0065002	intracellular protein transmembrane transport	0.00105	GO:0072598	protein localization to chloroplast	0.00522
GO:0071396	cellular response to lipid	0.00127	GO:0009628	response to abiotic stimulus	0.00598
GO:0070727	cellular macromolecule localization	0.00128	GO:0001101	response to acid chemical	0.00598
GO:0006298	mismatch repair	0.0014	GO:0009129	pyrimidine nucleoside monophosphate metabolic process	0.00631
GO:0008360	regulation of cell shape	0.00173	GO:0009130	pyrimidine nucleoside monophosphate biosynthetic process	0.00631
GO:0034227	tRNA thio-modification	0.00173	GO:0009165	nucleotide biosynthetic process	0.00655
GO:0009788	negative regulation of abscisic acid-activated signaling pathway	0.00195	GO:1901293	nucleoside phosphate biosynthetic process	0.007
GO:1901420	negative regulation of response to alcohol	0.00195	GO:0031328	positive regulation of cellular biosynthetic process	0.00778
GO:0097306	cellular response to alcohol	0.00202	GO:0043173	nucleotide salvage	0.00782
GO:0007267	cell-cell signaling	0.00258	GO:0051188	cofactor biosynthetic process	0.00929
GO:0006612	protein targeting to membrane	0.00301	GO:0009240	isopentenyl diphosphate biosynthetic process	0.00947
GO:0051302	regulation of cell division	0.00302	GO:0046490	isopentenyl diphosphate metabolic process	0.00947
GO:0007049	cell cycle	0.00359	GO:0006364	rRNA processing	0.00985
GO:1902582	single-organism intracellular transport	0.00364	GO:0009642	response to light intensity	0.00985
GO:0017038	protein import	0.00382			
GO:0022604	regulation of cell morphogenesis	0.00437			
GO:0071702	organic substance transport	0.00478			
GO:0007005	mitochondrion organization	0.00583			
GO:0001676	long-chain fatty acid metabolic process	0.00603			
GO:0006399	tRNA metabolic process	0.00617			
GO:0006357	regulation of transcription from RNA polymerase II promoter	0.00734			
GO:2001020	regulation of response to DNA damage stimulus	0.00747			
GO:0016192	vesicle-mediated transport	0.00774			
GO:0009620	response to fungus	0.00846			
GO:0002098	tRNA wobble uridine modification	0.00904			

Table S 13: Main biological process of N3 (optimal nitrogen level) genes Down and Up-regulated genes for B73 genotype in Tomeza location.

GO.ID	GO Terms_N3_Down	p-value	GO.ID	GO Terms_N3_Up	p-value
GO:0044265	cellular macromolecule catabolic process	1,00 E-06	GO:0043043	peptide biosynthetic process	1.4e-08
GO:0051603	proteolysis involved in cellular protein catabolic process	8.2e-06	GO:0006518	peptide metabolic process	2.3e-08
GO:0030163	protein catabolic process	8.5e-06	GO:0043604	amide biosynthetic process	3.5e-08
GO:0044257	cellular protein catabolic process	1,00 E-05	GO:0006412	translation	3.6e-08
GO:0070647	protein modification by small protein conjugation or removal	2.1e-05	GO:0043603	cellular amide metabolic process	9.8e-08
GO:0044248	cellular catabolic process	3.4e-05	GO:1901566	organonitrogen compound biosynthetic process	5.8e-07
GO:0016579	protein deubiquitination	7.1e-05	GO:1901564	organonitrogen compound metabolic process	8.8e-05
GO:1901575	organic substance catabolic process	7.7e-05	GO:0044267	cellular protein metabolic process	0.00016
GO:0009057	macromolecule catabolic process	0.00014	GO:0044271	cellular nitrogen compound biosynthetic process	0.00019
GO:0006511	ubiquitin-dependent protein catabolic process	0.00017	GO:0015804	neutral amino acid transport	5,00 E-04
GO:0019941	modification-dependent protein catabolic process	2,00 E-04	GO:0034645	cellular macromolecule biosynthetic process	0.00068
GO:0070646	protein modification by small protein	2,00 E-04	GO:0051172	negative regulation of nitrogen compound	0.00091
	removal			metabolic process	
GO:0043632	modification-dependent macromolecule catabolic process	0.00021	GO:0044249	cellular biosynthetic process	0.00102
GO:0009056	catabolic process	0.00037	GO:0009059	macromolecule biosynthetic process	0.00109
GO:0009737	response to abscisic acid	0.00083	GO:0045934	negative regulation of nucleobase-containing compound metabolic process	0.00141
GO:0031538	negative regulation of anthocyanin metabolic process	0.00123	GO:1901576	organic substance biosynthetic process	0.00158
GO:0033036	macromolecule localization	0.00171	GO:0009684	indoleacetic acid biosynthetic process	0.00164
GO:0005987	sucrose catabolic process	0.00203	GO:0019538	protein metabolic process	0.00207
GO:0097305	response to alcohol	0.00281	GO:0015979	photosynthesis	0.00208
GO:0000245	spliceosomal complex assembly	0.00301	GO:0019684	photosynthesis, light reaction	0.00236
GO:0006376	mRNA splice site selection	0.00301	GO:0044260	cellular macromolecule metabolic process	0.00241
GO:0008104	protein localization	0.00349	GO:0009683	indoleacetic acid metabolic process	0.00243
GO:0000956	nuclear-transcribed mRNA catabolic process	0.00427	GO:0010467	gene expression	0.00272
GO:0015031	protein transport	0.00464	GO:0070370	cellular heat acclimation	0.00338
GO:0016567	protein ubiquitination	0.00522	GO:0031327	negative regulation of cellular biosynthetic process	0.00347
GO:0046907	intracellular transport	0.00527	GO:0009058	biosynthetic process	0.00348
GO:0016071	mRNA metabolic	0.00539	GO:0031349	negative regulation of	0.00349

	process		24	cellular metabolic process	
GO:0034613	cellular protein localization	0.00546	GO:009890	negative regulation of biosynthetic process	0.00373
GO:0046352	disaccharide catabolic process	0.00551	GO:007252 5	pyridine-containing compound biosynthetic process	0.0045
GO:0045184	establishment of protein localization	0.0056	GO:003464 1	cellular nitrogen compound metabolic process	0.00481
GO:0009108	coenzyme biosynthetic process	0.006	GO:009851	auxin biosynthetic process	0.00569
GO:0033993	response to lipid	0.00686	GO:004427 3	sulfur compound catabolic process	0.00569
GO:0009313	oligosaccharide catabolic process	0.00702	GO:005160 7	defense response to virus	0.00615
GO:0031537	regulation of anthocyanin metabolic process	0.00702	GO:000202 6	microtubule cytoskeleton organization	0.00679
GO:0070727	cellular macromolecule localization	0.00703	GO:004599 5	regulation of embryonic development	0.00705
GO:0006402	mRNA catabolic process	0.0075	GO:000280	nuclear division	0.0076
GO:0006886	intracellular protein transport	0.00755	GO:004852 3	negative regulation of cellular process	0.00949
GO:0032446	protein modification by small protein conjugation	0.00779			
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.00869			
GO:0000288	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	0.00869			
GO:0044267	cellular protein metabolic process	0.00925			
GO:0006643	membrane lipid metabolic process	0.00926			

Table S 14: Main biological process of WS (water stress) genes Down and Up-regulated genes for B73 genotype in Tomeza location.

GO.ID	GO Terms_WS_Down	p-value	GO.ID	GO Terms_WS_Up	p-value
GO:0030163	protein catabolic process	1.8e-10	GO:1901566	organonitrogen compound biosynthetic process	1,00E-23
GO:0070647	protein modification by small protein conjugation or removal	1.8e-09	GO:0006518	peptide metabolic process	2.9e-23
GO:0016579	protein deubiquitination	2.7e-09	GO:0043043	peptide biosynthetic process	7.6e-23
GO:0051603	proteolysis involved in cellular protein catabolic process	3.6e-09	GO:0043603	cellular amide metabolic process	8.4e-23
GO:0070646	protein modification by small protein removal	5.7e-09	GO:0006412	translation	8.8e-23
GO:0044257	cellular protein catabolic process	5.8e-09	GO:0043604	amide biosynthetic process	1.2e-22
GO:0044265	cellular macromolecule catabolic process	7.1e-09	GO:1901564	organonitrogen compound metabolic process	1.2e-19
GO:0006511	ubiquitin-dependent protein catabolic process	2,00E-07	GO:0044271	cellular nitrogen compound biosynthetic process	9.3e-09
GO:0019941	modification-dependent protein catabolic	2.8e-07	GO:0044249	cellular biosynthetic process	2,00E-08

	process				
GO:0043632	modification-dependent macromolecule catabolic process	3.5e-07	GO:1901576	organic substance biosynthetic process	3.1e-08
GO:0009057	macromolecule catabolic process	7.3e-07	GO:0009058	biosynthetic process	7.2e-08
GO:0033036	macromolecule localizatior	8.6e-07	GO:0034641	cellular nitrogen compound metabolic process	1.2e-07
GO:0046907	intracellular transport	2.7e-06	GO:0006807	nitrogen compound metabolic process	1.5e-07
GO:0045184	establishment of protein localization	3.2e-06	GO:0010467	gene expression	2.7e-06
GO:0006914	autophagy	3.4e-06	GO:0034645	cellular macromolecule biosynthetic process	1.3e-05
GO:0051649	establishment of localization ir cell	3.5e-06	GO:0009059	macromolecule biosynthetic process	2.5e-05
GO:0015031	protein transport	4.5e-06	GO:0006741	NADP biosynthetic process	4.5e-05
GO:0008104	protein localization	5.2e-06	GO:0044267	cellular protein metabolic process	0.00014
GO:0044248	cellular catabolic process	7.5e-06	GO:1901293	nucleoside phosphate biosynthetic process	0.00018
GO:0016482	cytoplasmic transport	1.1e-05	GO:0019359	nicotinamide nucleotide biosynthetic process	4,00E-04
GO:0051641	cellular localization	1.7e-05	GO:0009165	nucleotide biosynthetic process	0.00047
GO:1902582	single-organism intracellular transport	1.8e-05	GO:0045036	protein targeting to chloroplast	0.00051
GO:0071702	organic substance transport	3.5e-05	GO:0072596	establishment of protein localization to chloroplast	0.00051
GO:0006886	intracellular protein transport	3.5e-05	GO:0019363	pyridine nucleotide biosyntheti	0.00055

				c process	
GO:0006605	protein targeting	4.9e-05	GO:0072525	pyridine-containing compound biosynthetic process	0.00064
GO:0032502	developmental process	6.4e-05	GO:0072598	protein localization to chloroplast	0.00064
GO:0044707	single-multicellular organism process	7.2e-05	GO:0018208	peptidyl-proline modification	8,00E-04
GO:0044767	single-organism developmental process	7.4e-05	GO:0051188	cofactor biosynthetic process	0.001
GO:0034613	cellular protein localization	9.2e-05	GO:0009108	coenzyme biosynthetic process	0.00101
GO:0072594	establishment of protein localization to organelle	9.4e-05	GO:0090407	organophosphate biosynthetic process	0.00112
GO:1902580	single-organism cellular localization	9.7e-05	GO:0009658	chloroplast organization	0.00152
GO:0009056	catabolic process	0.00011	GO:0044237	cellular metabolic process	0.00168
GO:0070727	cellular macromolecule localizatior	0.00017	GO:0071258	cellular response to gravity	0.0018
GO:0007275	multicellular organismal development	0.00018	GO:0019538	protein metabolic process	0.00187
GO:0006810	transport	2,00E-04	GO:0000413	protein peptidyl-prolyl isomerization	0.00249
GO:0051234	establishment of localization	2,00E-04	GO:0045037	protein import into chloroplast stroma	0.00303
GO:0032501	multicellular organismal process	0.00028	GO:0009628	response to abiotic stimulus	0.00319
GO:0017038	protein import	0.00029	GO:0009124	nucleoside monophosphate biosynthetic process	0.00377
GO:1901575	organic substance catabolic process	0.00032	GO:0070972	protein localization to endoplasmic reticulum	0.00428
GO:0051179	localization	0.00046	GO:1902580	single-organism	0.0044

				cellular localization	
GO:0033365	protein localization to organelle	5,00E-04	GO:0006353	DNA-templated transcription, GO Termsination	0.00526
GO:0006913	nucleocytoplasmic transpor	0.00056	GO:1901661	quinone metabolic process	0.00596
GO:0051169	nuclear transport	0.00056	GO:1901663	quinone biosynthetic process	0.00596
GO:0051170	nuclear import	0.00058	GO:0042726	flavin-containing compound metabolic process	0.00615
GO:0032446	protein modification by small protein conjugation	7,00E-04	GO:0009314	response to radiation	0.0066
GO:0006513	protein monoubiquitination	9,00E-04	GO:0055086	nucleobase-containing small molecule metabolic process	0.00684
GO:0007219	Notch signaling pathway	9,00E-04	GO:0042181	ketone biosynthetic process	0.00695
GO:0009846	pollen germination	0.00109	GO:0033365	protein localization to organelle	0.00708
GO:0048583	regulation of response to stimulus	0.00116	GO:0051494	negative regulation of cytoskeleton organization	0.00733
GO:0048518	positive regulation of biological process	0.00136	GO:0009651	response to salt stress	0.00743
GO:0009791	post-embryonic development	0.00146	GO:0019674	NAD metabolic process	0.00753
GO:0009314	response to radiation	0.0016	GO:0006310	DNA recombination	0.00757
GO:0006508	proteolysis	0.00167	GO:0009416	response to light stimulus	0.00762
GO:0009416	response to light stimulus	0.00176	GO:0065003	macromolecular complex assembly	0.00806
GO:000	regulation of	0.002	GO:003	cellular	0.00
8593	Notch signaling pathway	11	4614	response to reactive oxygen species	82
GO:0016487	farnesol metabolic process	0.00211	GO:0009894	regulation of catabolic process	0.0092
GO:0045747	positive regulation of Notch signaling pathway	0.00211	GO:2000022	regulation of jasmonic acid mediated signaling pathway	0.00977
GO:0097031	mitochondrial respiratory chain complex I biogenesis	0.00211	GO:1902600	hydrogen ion transmembrane transport	0.00993
GO:0006396	RNA processing	0.00224			
GO:0006606	protein import into nucleus	0.00226			
GO:0009896	positive regulation of catabolic process	0.00226			
GO:0044744	protein targeting to nucleus	0.00226			
GO:1902593	single-organism nuclear import	0.00226			
GO:0051716	cellular response to stimulus	0.00262			
GO:2000030	regulation of response to red or far red light	0.00276			
GO:0000266	mitochondrial fission	0.00294			
GO:0006542	glutamine biosynthetic process	0.00294			
GO:0006342	chromatin silencing	0.00299			
GO:0007049	cell cycle	0.00346			
GO:0071806	protein transmembrane transport	0.00363			
GO:0006743	ubiquinone metabolic process	0.00401			
GO:0006744	ubiquinone biosynthetic process	0.00401			
GO:003	protein	0.004			

4504	localization tc nucleus	01
GO:004 8522	positive regulation of cellular process	0.004 14
GO:001 0506	regulation of autophagy	0.004 54
GO:001 6567	protein ubiquitinatior	0.004 58
GO:002 2604	regulation of cell morphogenes s	0.004 97
GO:004 5036	protein targeting to chloroplast	0.004 97
GO:007 2596	establishment of protein localization tc chloroplast	0.004 97
GO:001 6072	rRNA metabolic process	0.005 24
GO:000 9640	photomorpho genesis	0.005 29
GO:004 5814	negative regulation of gene expression, epigenetic	0.005 5
GO:000 9108	coenzyme biosynthetic process	0.005 68
GO:001 6458	gene silencing	0.00 568
GO:005 1128	regulation of cellular component organization	0.00 574
GO:007 2598	protein localization tc chloroplast	0.00 592
GO:001 6093	polyprenol metabolic process	0.00 612
GO:003 2465	regulation of cytokinesis	0.00 612
GO:003 4660	ncRNA metabolic process	0.00 618
GO:004 8856	anatomical structure developme nt	0.00 626
GO:004 4702	single organism reproductiv e process	0.00 656
GO:004 8584	positive regulation of response	0.00 658
	to stimulus	
GO:000 1558	regulation of cell growth	0.00 721
GO:000 7031	peroxisome organizatio n	0.00 821
GO:000 9084	glutamine family amino acid biosynthetic process	0.00 821
GO:190 1661	quinone metabolic process	0.00 821
GO:190 1663	quinone biosynthetic process	0.00 821
GO:200 0377	regulation of reactive oxygen species metabolic process	0.00 821
GO:003 2989	cellular component morphogen esis	0.00 93
GO:000 3006	developme ntal process involved in reproductio n	0.00 931
GO:004 2181	ketone biosynthetic process	0.00 955
GO:000 9788	negative regulation of abscisic acid-activated signaling pathway	0.00 984
GO:190 1420	negative regulation of response to alcohol	0.00 984

Table S 15: Main biological process of Opti (optimal water) genes Down and Up-regulated genes for B73 genotype in Tomeza location.

GO.ID	GO Terms_Opti Down	p-valu e	GO.ID	GO Terms_O pti_Up	p-valu e
GO:003 3036	macromolec ule localization	0.00 016	GO:000 9628	response to abiotic stimulus	0.00 045
GO:000 8104	protein localization	0.00 039	GO:000 9416	response to light stimulus	0.00 047

GO:0051641	cellular localization	0.00042	GO:0009314	response to radiation	0.00066
GO:0042147	retrograde transport, endosome to Golgi	0.0016	GO:0009765	photosynthesis, light harvesting	0.00104
GO:0016192	vesicle-mediated transport	0.00192	GO:0019684	photosynthesis, light reaction	0.00121
GO:0046907	intracellular transport	0.00192	GO:0009637	response to blue light	0.00267
GO:0070727	cellular macromolecule localization	0.00292	GO:0009987	cellular process	0.00323
GO:0051274	beta-glucan biosynthetic process	0.00331	GO:0015979	photosynthesis	0.00334
GO:0006334	nucleosome assembly	0.00332	GO:0006013	mannose metabolic process	0.00345
GO:0006888	ER to Golgi vesicle-mediated transport	0.00348	GO:0010206	photosystem II repair	0.00546
GO:0034728	nucleosome organization	0.00358	GO:0009639	response to red or far red light	0.00569
GO:0031497	chromatin assembly	0.00414	GO:0071214	cellular response to abiotic stimulus	0.00618
GO:0006167	AMP biosynthetic process	0.00438	GO:0071482	cellular response to light stimulus	0.0064
GO:0010337	regulation of salicylic acid metabolic process	0.00438	GO:0034250	positive regulation of cellular amide metabolic process	0.00789
GO:0046033	AMP metabolic process	0.00438	GO:0045727	positive regulation of translation	0.00789
GO:0048193	Golgi vesicle transport	0.00444	GO:0071478	cellular response to radiation	0.00789
GO:0006333	chromatin assembly or disassembly	0.00475	GO:0043648	dicarboxylic acid metabolic process	0.0083
GO:0015031	protein transport	0.00477	GO:0051716	cellular response to stimulus	0.00863
GO:0065004	protein-DNA complex assembly	0.00543	GO:0007165	signal transduction	0.00982
GO:0045184	establishment of protein localization	0.0057			
GO:0071824	protein-DNA complex subunit organization	0.00579			
GO:0044265	cellular macromolecule catabolic process	0.00597			
GO:0006323	DNA packaging	0.00616			
GO:0034613	cellular protein localization	0.00678			
GO:0032271	regulation of protein polymerization	0.00723			
GO:0043254	regulation of protein complex assembly	0.0079			
GO:0009057	macromolecule catabolic process	0.00827			
GO:0030244	cellulose biosynthetic process	0.00828			
GO:0051649	establishment of localization in cell	0.00873			
GO:1902582	single-organism intracellular transport	0.00943			

Opti: Optimal water

Table S 16: Main biological process of N1 (low nitrogen) genes Down and Up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	GO Terms_N1_Down	p-value	GO.ID	GO Terms_N1_Up	p-value
GO:0051641	cellular localization	7.5e-08	GO:0010035	esponse to norganic ubstance	0.00052
GO:0051649	establishment of localization in cell	1.2e-07	GO:0010025	vax biosynthetic rocess	0.00067
GO:0046907	intracellular transport	1.4e-07	GO:0010166	vax metabolic rocess	0.00081
GO:0034613	cellular protein localization	1.2e-06	GO:0009414	esponse to vater leprivation	0.00089
GO:0070727	cellular macromolecule localization	2.2e-06	GO:0009415	esponse to vater	0.00106
GO:0006886	intracellular protein transport	1.5e-05	GO:0043038	mino acid ctivation	0.00128
GO:0008104	protein localization	1.6e-05	GO:0043039	RNA minoacylation	0.00128
GO:0033036	macromolecule localization	2.4e-05	GO:0042221	esponse to hemical	0.00169
GO:0009628	response to abiotic stimulus	4.9e-05	GO:0010628	positive egulation of gene xpression	0.00256
GO:1902582	single-organism intracellular transport	6.1e-05	GO:0051716	ellular esponse to timulus	0.00301
GO:0048583	regulation of response to stimulus	6.2e-05	GO:0016246	RNA nterference	0.00355
GO:0044707	single-multicellular organism process	6.9e-05	GO:0050896	esponse to timulus	0.00418
GO:0007275	multicellular organismal development	6.9e-05	GO:0001101	esponse to cid chemical	0.00491
GO:0016482	cytoplasmic transport	8.6e-05	GO:0071310	ellular esponse to rganic ubstance	0.00517
GO:0044767	single-organism developmental process	8.7e-05	GO:0006470	rotein lephosphorylation	0.00572
GO:0032502	developmental process	0.00014	GO:0051254	positive egulation of RNA netabolic rocess	0.0062
GO:0048364	root development	0.00017	GO:0010604	positive egulation of nacromolecule netabolic rocess	0.00711
GO:0022622	root system development	0.00018	GO:0006418	RNA minoacylation or protein ranslation	0.00752
GO:0045184	establishment of protein localization	0.00018	GO:0031050	lsRNA ragmentation	0.00784
GO:0010928	regulation of auxin mediated signaling pathway	0.00018	GO:0043331	esponse to lsRNA	0.00784
GO:0032501	multicellular organismal process	0.00023	GO:0070918	roduction of mall RNA nvolved in ene silencing y RNA	0.00784
GO:0048731	system development	0.00027	GO:0071359	ellular esponse to lsRNA	0.00784
GO:0048513	organ development	0.00031	GO:0045935	positive egulation of ucleobase-ontaining ompound netabolic rocess	0.0081
GO:0009791	post-embryonic development	0.00032			
GO:0015031	protein transport	0.00038			
GO:0000919	cell plate assembly	0.00042			
GO:0016192	vesicle-mediated transport	0.00044			
GO:0044702	single organism reproductive process	0.00049			
GO:0009966	regulation of signal transduction	0.00053			
GO:0023051	regulation of signaling	0.00056			
GO:0009787	regulation of abscisic acid-activated signaling pathway	0.00063			
GO:1901419	regulation of response to alcohol	0.00063			
GO:0010646	regulation of cell communication	0.00067			
GO:0009414	response to water deprivation	0.00107			
GO:0035265	organ growth	0.00118			
GO:0007034	vacuolar transport	0.00132			
GO:0009415	response to water	0.00133			
GO:0071365	cellular response to auxin stimulus	0.00134			
GO:0048608	reproductive structure development	0.00161			
GO:0061458	reproductive system	0.00161			

	development	
GO:0009408	response to heat	0.00165
GO:0051234	establishment of localizatior	0.00166
GO:0051179	localization	0.00168
GO:0048856	anatomical structure development	0.00196
GO:0010154	fruit development	0.00201
GO:0044265	cellular macromolecul e catabolic process	0.00211
GO:0032870	cellular response to hormone stimulus	0.00223
GO:0051128	regulation of cellular component organization	0.00233
GO:0010587	miRNA catabolic process	0.00236
GO:0030259	lipid glycosylation	0.00236
GO:0070085	glycosylation	0.00239
GO:0071495	cellular response to endogenous stimulus	0.00244
GO:0048585	negative regulation of response to stimulus	0.00254
GO:0003006	developmenta process involved in reproduction	0.00282
GO:0048316	seed development	0.00289
GO:0051241	negative regulation of multicellular organismal process	0.00325
GO:0006810	transport	0.00339
GO:0048193	Golgi vesicle transport	0.00384
GO:0010117	photoprotectic n	0.00388
GO:0009266	response to temperature stimulus	0.0045
GO:0051716	cellular response to stimulus	0.0046
GO:0051239	regulation of multicellular organismal process	0.00482
GO:0050793	regulation of developmenta process	0.00513
GO:0051093	negative regulation of developmenta process	0.00515
GO:0051493	regulation of cytoskeleton organization	0.00516
GO:0010565	regulation of cellular keton metabolic process	0.00571
GO:0010586	miRNA metabolic process	0.00574
GO:0090503	RNA phosphodieste r bond hydrolysis, exonucleolyti	0.00574
GO:0071702	organic substance transport	0.00588
GO:0048467	gynoecium development	0.00613
GO:0006289	nucleotide-excision repai	0.00616
GO:2000242	negative regulation of reproductive process	0.0063
GO:0048581	negative regulation of post-embryonic development	0.00659
GO:2000026	regulation of multicellular organismal development	0.00676
GO:0050896	response to stimulus	0.00689
GO:0006099	tricarboxylic acid cycle	0.00693
GO:0009755	hormone-mediated signaling pathway	0.00714
GO:0006101	citrate metabolic process	0.00761
GO:0071310	cellular response to organic substance	0.00761
GO:0006486	protein glycosylation	0.00775
GO:0043413	macromolecul e glycosylation	0.00775
GO:0034661	ncRNA catabolic process	0.00793
GO:0070370	cellular heat acclimation	0.00793
GO:0070507	regulation of microtubule cytoskeleton organization	0.00793
GO:0051603	proteolysis involved in cellular	0.00817

	protein catabolic process				
GO:0030163	protein catabolic process	0.0082			
GO:0000911	cytokinesis by cell plate formation	0.00832			
GO:0040007	growth	0.00855			
GO:0048831	regulation of shoot system development	0.00862			
GO:0009738	abscisic acid-activated signaling pathway	0.00868			
GO:0032506	cytokinetic process	0.00908			
GO:1902410	mitotic cytokinetic process	0.00908			
GO:0044257	cellular protein catabolic process	0.00931			
GO:1902580	single-organism cellular localization	0.00987			

Table S 17: Main biological process of N3 (Optimal nitrogen level) genes down and up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	GOTerms_N3_Down	p-value	GO.ID	GOTerms_N3_Up	p-value
GO:0043603	cellular amide metabolic process	2.5e-12	GO:0006518	peptide metabolic process	3.8e-16
GO:0043604	amide biosynthetic process	7.9e-12	GO:0043043	peptide biosynthetic process	9.1e-16
GO:0006518	peptide metabolic process	1.8e-11	GO:0006412	translation	1.1e-15
GO:0006412	translation	4.1e-11	GO:0043603	cellular amide metabolic process	2.3e-15
GO:0043043	peptide biosynthetic process	8.8e-11	GO:0043604	amide biosynthetic process	9.6e-15
GO:1901566	organonitrogen compound biosynthetic process	1.4e-09	GO:1901566	organonitrogen compound biosynthetic process	8,00E-14
GO:1901564	organonitrogen compound metabolic process	3.6e-07	GO:1901564	organonitrogen compound metabolic process	3,00E-10
GO:1902582	single-organism intracellular transport	8.8e-07	GO:0006399	tRNA metabolic process	8.4e-07
GO:0046907	intracellular transport	2.5e-06	GO:0034660	ncRNA metabolic process	9.5e-06
GO:0006913	nucleocytoplasmic transport	4.1e-06	GO:0044267	cellular protein metabolic process	1,00E-05
GO:0051169	nuclear transport	4.1e-06	GO:0015979	photosynthesis	1.2e-05
GO:0009610	response to symbiotic fungus	5.8e-06	GO:0019538	protein metabolic process	1.5e-05
GO:0022618	ribonucleoprotein complex assembly	7.7e-06	GO:0046686	response to cadmium ion	4.3e-05
GO:0071826	ribonucleoprotein complex subunit organization	7.7e-06	GO:0009628	response to abiotic stimulus	5.2e-05
GO:0044267	cellular protein metabolic process	7.8e-06	GO:0009416	response to light stimulus	5.2e-05
GO:0022613	ribonucleoprotein complex biogenesis	8.6e-06	GO:0043038	amino acid activation	8.4e-05
GO:0006406	mRNA export from nucleus	8.6e-06	GO:0043039	tRNA aminoacylation	8.4e-05
GO:0071427	mRNA-containing ribonucleoprotein complex export from nucleus	8.6e-06	GO:0009314	response to radiation	0.00011
GO:0051641	cellular localization	1.1e-05	GO:0044237	cellular metabolic process	0.00012
GO:0051028	mRNA transport	2,00E-05	GO:0042372	phylloquinone biosynthetic process	0.00012
GO:0071166	ribonucleoprotein complex localization	2,00E-05	GO:0042374	phylloquinone metabolic process	0.00012
GO:0071426	ribonucleoprotein complex export from	2,00E-05	GO:0009987	cellular process	0.00015

	nucleus				
GO:0051649	establishment of localization in cell	2.1e-05	GO:0010038	response to metal ion	0.00018
GO:0009608	response to symbiont	2.8e-05	GO:0019684	photosynthesis, light reaction	0.00021
GO:0006405	RNA export from nucleus	3,00E-05	GO:0048573	photoperiodism, flowering	0.00025
GO:0033036	macromolecule localization	5.8e-05	GO:0009657	plastid organization	0.00041
GO:0010467	gene expression	6,00E-05	GO:0034641	cellular nitrogen compound metabolic process	0.00046
GO:0015931	nucleobase-containing compound transport	7.5e-05	GO:0009639	response to red or far red light	0.00049
GO:0050657	nucleic acid transport	8.4e-05	GO:0006400	tRNA modification	0.00064
GO:0050658	RNA transport	8.4e-05	GO:0010035	response to inorganic substance	0.00065
GO:0051168	nuclear export	8.4e-05	GO:0009648	photoperiodism	0.00072
GO:0051236	establishment of RNA localization	8.4e-05	GO:0010467	gene expression	0.00087
GO:0055083	monovalent inorganic anion homeostasis	1,00E-04	GO:0006418	tRNA aminoacylation for protein translation	0.00102
GO:0006403	RNA localization	0.00011	GO:0006807	nitrogen compound metabolic process	0.00106
GO:0071702	organic substance transport	0.00012	GO:0022613	ribonucleoprotein complex biogenesis	0.00115
GO:0070925	organelle assembly	0.00015	GO:1901661	quinone metabolic process	0.00124
GO:0019538	protein metabolic process	0.00016	GO:1901663	quinone biosynthetic process	0.00124
GO:0006606	protein import into nucleus	0.00026	GO:0006414	translational elongation	0.00143
GO:0044744	protein targeting to nucleus	0.00026	GO:0042181	ketone biosynthetic process	0.00151
GO:1902593	single-organism nuclear import	0.00026	GO:0016024	CDP-diacylglycerol biosynthetic process	0.00159
GO:0055081	anion homeostasis	3,00E-04	GO:0046341	CDP-diacylglycerol metabolic process	0.00159
GO:0008104	protein localization	0.00052	GO:0009637	response to blue light	0.00174
GO:0009937	regulation of gibberellic acid mediated signaling pathway	0.00057	GO:0009266	response to temperature stimulus	0.00175
GO:0034504	protein localization to nucleus	0.00057	GO:0048255	mRNA stabilization	0.00198
GO:0042255	ribosome assembly	0.00057	GO:0090231	regulation of spindle checkpoint	0.00198
GO:0030163	protein catabolic process	0.00059	GO:0090266	regulation of mitotic cell cycle spindle assembly checkpoint	0.00198
GO:0042254	ribosome biogenesis	0.00062	GO:1901976	regulation of cell cycle checkpoint	0.00198
GO:0045184	establishment of protein localization	0.00072	GO:1903504	regulation of mitotic spindle checkpoint	0.00198
GO:0051170	nuclear import	0.00079	GO:0006448	regulation of translational elongation	0.00202
GO:0065003	macromolecular complex assembly	0.00087	GO:0048507	meristem development	0.00211
GO:0015031	protein transport	0.00096	GO:0008033	tRNA processing	0.00215
GO:0000375	RNA splicing, via transesterification reactions	0.00137	GO:0042180	cellular ketone metabolic process	0.00236
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.00137	GO:0007584	response to nutrient	0.0027
GO:0034622	cellular macromole	0.00195	GO:0006415	translational GO	0.00298

	cular complex assembly			Termsination	
GO:0016482	cytoplasmic transport	0.00204	GO:1901068	guanosine-containing compound metabolic process	0.00298
GO:0000245	spliceosomal complex assembly	0.00213	GO:0034470	ncRNA processing	0.00321
GO:0006376	mRNA splice site selection	0.00213	GO:0000413	protein peptidyl-prolyl isomerization	0.00341
GO:0006396	RNA processing	0.00218	GO:0010228	vegetative to reproductive phase transition of meristem	0.00355
GO:1901607	alpha-amino acid biosynthetic process	0.00218	GO:0001510	RNA methylation	0.00398
GO:0043269	regulation of ion transport	0.00219	GO:0044763	single-organism cellular process	0.00399
GO:0010071	root meristem specification	0.00242	GO:0018208	peptidyl-proline modification	0.00414
GO:0010966	regulation of phosphate transport	0.00242	GO:0060966	regulation of gene silencing by RNA	0.00417
GO:1903795	regulation of inorganic anion transmembrane transport	0.00242	GO:0010109	regulation of photosynthesis	0.00438
GO:2000185	regulation of phosphate transmembrane transport	0.00242	GO:2000028	regulation of photoperiodism, flowering	0.00438
GO:0043933	macromolecular complex subunit organization	0.00279	GO:0042254	ribosome biogenesis	0.00443
GO:0034613	cellular protein localization	0.00286	GO:0009108	coenzyme biosynthetic process	0.0045
GO:0044257	cellular protein catabolic process	0.00313	GO:0009640	photomorphogenesis	0.0045
GO:0055062	phosphate ion homeostasis	0.00359	GO:0006396	RNA processing	0.00461
GO:0072506	trivalent inorganic anion homeostasis	0.00359	GO:0030488	tRNA methylation	0.00463
GO:1901605	alpha-amino acid metabolic process	0.00366	GO:0006091	generation of precursor metabolites and energy	0.00532
GO:0009740	gibberellic acid mediated signaling pathway	0.00374	GO:0015804	neutral amino acid transport	0.00577
GO:0006511	ubiquitin-dependent protein catabolic process	0.00387	GO:0043488	regulation of mRNA stability	0.00577
GO:0051049	regulation of transport	0.00394	GO:0043489	RNA stabilization	0.00577
GO:0016192	vesicle-mediated transport	0.00431	GO:0070681	glutaminyl-tRNAGln biosynthesis via transamidation	0.00577
GO:0070727	cellular macromolecule localization	0.00461	GO:1900368	regulation of RNA interference	0.00577
GO:0019941	modification-dependent protein catabolic process	0.00464	GO:0009409	response to cold	0.00578
GO:0022607	cellular component assembly	0.00501	GO:0006183	GTP biosynthetic process	0.00605
GO:0043632	modification-dependent macromolecule catabolic process	0.00521	GO:0006228	UTP biosynthetic process	0.00605
GO:0071705	nitrogen compound transport	0.00521	GO:0006450	regulation of translational fidelity	0.00605
GO:0016973	poly(A)+ mRNA export from nucleus	0.00553	GO:0046039	GTP metabolic process	0.00605
GO:0051225	spindle assembly	0.00553	GO:0046051	UTP metabolic process	0.00605

GO:0071329	cellular response to sucrose stimulus	0.00553	GO:0042221	response to chemical	0.00619
GO:0010476	gibberellin mediated signaling pathway	0.00557	GO:0009658	chloroplast organization	0.00658
GO:0006807	nitrogen compound metabolic process	0.00576	GO:0042548	regulation of photosynthesis, light reaction	0.00726
GO:0006886	intracellular protein transport	0.00577	GO:0042726	flavin-containing compound metabolic process	0.00726
GO:0000398	mRNA splicing, via spliceosome	0.00584	GO:0006413	translational initiation	0.00776
GO:0071370	cellular response to gibberellin stimulus	0.00668	GO:0046916	cellular transition metal ion homeostasis	0.00836
GO:0001522	pseudouridine synthesis	0.00668	GO:0010449	root meristem growth	0.00845
GO:0008219	cell death	0.00694	GO:0010608	posttranscriptional regulation of gene expression	0.00869
GO:0016265	death	0.00694	GO:0043467	regulation of generation of precursor metabolites and energy	0.00887
GO:0002238	response to molecule of fungal origin	0.00703			
GO:0009920	cell plate formation involved in plant-type cell wall biogenesis	0.00703			
GO:0035435	phosphate ion transmembrane transport	0.00703			
GO:0055064	chloride ion homeostasis	0.00703			
GO:0034765	regulation of ion transmembrane transport	0.00769			
GO:0009561	megagametogenesis	0.00771			
GO:0032879	regulation of localization	0.00787			
GO:0015866	ADP transport	0.008			
GO:0071324	cellular response to disaccharide stimulus	0.008			
GO:0072505	divalent inorganic anion homeostasis	0.008			
GO:0044265	cellular macromolecule catabolic process	0.00808			
GO:0034762	regulation of transmembrane transport	0.00881			

Table S 18:Main biological process of WS (Water stress) genes Down-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0033036	macromolecule localization	5.4e-07
GO:0051641	cellular localization	1,00E-06
GO:0070727	cellular macromolecule localization	3.6e-06
GO:0051716	cellular response to stimulus	3.8e-06
GO:0046907	intracellular transport	8.4e-06
GO:0034613	cellular protein localization	1.3e-05
GO:0008104	protein localization	1.4e-05
GO:0031669	cellular response to nutrient levels	1.4e-05
GO:0030163	protein catabolic process	1.4e-05
GO:0051649	establishment of localization in cell	1.6e-05
GO:0009267	cellular response to starvation	1.7e-05
GO:0045184	establishment of protein localization	2.4e-05
GO:0006396	RNA processing	3.5e-05
GO:0009408	response to heat	4.1e-05
GO:0009628	response to abiotic stimulus	4.2e-05
GO:0042594	response to starvation	4.6e-05
GO:0051239	regulation of multicellular organismal process	4.8e-05
GO:0050793	regulation of developmental process	5.8e-05
GO:0071702	organic substance transport	6.1e-05
GO:0044265	cellular macromolecule catabolic process	6.1e-05
GO:0048831	regulation of shoot system	7.6e-05

	development	
GO:0015031	protein transport	8.3e-05
GO:2000026	regulation of multicellular organismal development	9,00E-05
GO:0051234	establishment of localization	9.1e-05
GO:0051179	localization	9.4e-05
GO:0006886	intracellular protein transport	9.5e-05
GO:0031667	response to nutrient levels	9.9e-05
GO:0031668	cellular response to extracellular stimulus	9.9e-05
GO:0051603	proteolysis involved in cellular protein catabolic process	1,00E-04
GO:0033554	cellular response to stress	0.00012
GO:0044257	cellular protein catabolic process	0.00014
GO:0071496	cellular response to external stimulus	0.00015
GO:0050896	response to stimulus	0.00017
GO:0006810	transport	0.00021
GO:0048583	regulation of response to stimulus	0.00023
GO:0016036	cellular response to phosphate starvation	0.00023
GO:0010928	regulation of auxin mediated signaling pathway	0.00025
GO:0007154	cell communication	0.00026
GO:0031538	negative regulation of anthocyanin metabolic process	0.00027
GO:0009966	regulation of signal transduction	0.00027
GO:0043632	modification-dependent macromolecule catabolic process	0.00029
GO:0023051	regulation of signaling	3,00E-04
GO:0070925	organelle assembly	0.00033
GO:0009743	response to carbohydrate	0.00034
GO:0010646	regulation of cell communication	0.00039
GO:0010078	maintenance of root meristem identity	5,00E-04
GO:2000241	regulation of reproductive process	5,00E-04
GO:0009991	response to extracellular stimulus	5,00E-04
GO:1901700	response to oxygen-containing compound	0.00053
GO:0044767	single-organism developmental process	0.00055
GO:0009266	response to temperature stimulus	0.00055
GO:0006511	ubiquitin-dependent protein catabolic process	0.00059
GO:0032502	developmental process	0.00068
GO:2000024	regulation of leaf development	0.00068
GO:0019941	modification-dependent protein catabolic process	0.00072
GO:0006465	signal peptide processing	0.00075
GO:0035265	organ growth	0.00075
GO:0048731	system development	0.00086
GO:0007165	signal transduction	0.00112
GO:0044707	single-multicellular organism process	0.00116
GO:0007275	multicellular organismal development	0.00125
GO:1902582	single-organism intracellular transport	0.00126
GO:0048580	regulation of post-embryonic development	0.00129
GO:0044700	single organism signaling	0.00133
GO:0023052	signaling	0.00137
GO:0022618	ribonucleoprotein complex assembly	0.00138
GO:0071826	ribonucleoprotein complex subunit organization	0.00138
GO:0048518	positive regulation of biological process	0.00147
GO:0009744	response to sucrose	0.00151
GO:0009791	post-embryonic development	0.00156
GO:0032365	intracellular lipid transport	0.0017
GO:0043617	cellular response to sucrose starvation	0.0017
GO:0034285	response to disaccharide	0.00174
GO:0016192	vesicle-mediated transport	0.00178
GO:0015931	nucleobase-containing compound transport	0.00199
GO:0051168	nuclear export	0.00214
GO:0016482	cytoplasmic transport	0.0023
GO:0009909	regulation of flower development	0.00258
GO:0071310	cellular response to organic substance	0.0026
GO:0001510	RNA methylation	0.0026
GO:0006403	RNA localization	0.00261
GO:0008380	RNA splicing	0.00281
GO:0009787	regulation of abscisic acid-activated signaling pathway	0.00282
GO:1901419	regulation of response to alcohol	0.00282
GO:0006950	response to stress	0.00305
GO:0009737	response to abscisic acid	0.00312
GO:0022613	ribonucleoprotein complex biogenesis	0.00325
GO:0006777	Mo-molybdopterin cofactor biosynthetic process	0.00336
GO:0019720	Mo-molybdopterin cofactor metabolic process	0.00336
GO:0071329	cellular response to sucrose stimulus	0.00336
GO:0040007	growth	0.00348
GO:0071322	cellular response to carbohydrate stimulus	0.00377
GO:0048367	shoot system development	0.00405
GO:0044702	single organism reproductive process	0.00415
GO:0097305	response to alcohol	0.00439
GO:0010286	heat acclimation	0.00448
GO:0035195	gene silencing by miRNA	0.00448
GO:0033993	response to lipid	0.00481
GO:0009057	macromolecule catabolic process	0.00484
GO:0031537	regulation of anthocyanin metabolic process	0.00488
GO:0032324	molybdopterin cofactor biosynthetic process	0.00488

GO:0043545	molybdopterin cofactor metabolic process	0.00488
GO:0051189	prosthetic group metabolic process	0.00488
GO:0071324	cellular response to disaccharide stimulus	0.00488
GO:0044764	multi-organism cellular process	0.00493
GO:0036079	purine nucleotide-sugar transport	0.00497
GO:0046740	transport of virus in host, cell to cell	0.00497
GO:0055064	chloride ion homeostasis	0.00497
GO:1902586	multi-organism intercellular transport	0.00497
GO:0010033	response to organic substance	0.00501
GO:0042221	response to chemical	0.00511
GO:0008283	cell proliferation	0.00512
GO:0009416	response to light stimulus	0.00524
GO:0016485	protein processing	0.00527
GO:0040008	regulation of growth	0.00536
GO:0048856	anatomical structure development	0.00541
GO:0032501	multicellular organismal process	0.00597
GO:0048513	organ development	0.00601
GO:0031399	regulation of protein modification process	0.00621
GO:0043414	macromolecule methylation	0.00654
GO:0009908	flower development	0.00701
GO:0006913	nucleocytoplasmic transport	0.00715
GO:0051169	nuclear transport	0.00715
GO:0010467	gene expression	0.00726
GO:0022607	cellular component assembly	0.00732
GO:0032870	cellular response to hormone stimulus	0.00744
GO:0048608	reproductive structure development	0.00751
GO:0061458	reproductive system development	0.00751
GO:0009414	response to water deprivation	0.00774
GO:0034660	ncRNA metabolic process	0.0079
GO:0043933	macromolecular complex subunit organization	0.00795
GO:0001932	regulation of protein phosphorylation	0.00802
GO:0009894	regulation of catabolic process	0.00802
GO:0006405	RNA export from nucleus	0.00822
GO:0071495	cellular response to endogenous stimulus	0.00828
GO:0009314	response to radiation	0.0083
GO:0006643	membrane lipid metabolic process	0.00872
GO:0090351	seedling development	0.00882
GO:0006400	tRNA modification	0.00897
GO:0002098	tRNA wobble uridine modification	0.00901
GO:0048467	gynoecium development	0.00957
GO:0001934	positive regulation of protein phosphorylation	0.00966
GO:0007292	female gamete generation	0.00966
GO:0010587	miRNA catabolic process	0.00966
GO:0033674	positive regulation of kinase activity	0.00966
GO:0034969	histone arginine methylation	0.00966
GO:0042327	positive regulation of phosphorylation	0.00966
GO:0045860	positive regulation of protein kinase activity	0.00966
GO:0046786	viral replication complex formation and maintenance	0.00966
GO:0009415	response to water	0.00984
GO:0042325	regulation of phosphorylation	0.01

Table S 19:Main biological process of WS (Water stress) genes up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0043038	amino acid activation	0.00026
GO:0043039	tRNA aminoacylation	0.00026
GO:0006412	translation	0.00034
GO:0010608	posttranscriptional regulation of gene expression	0.00034
GO:0016246	RNA interference	0.00039
GO:0043043	peptide biosynthetic process	0.00045
GO:0006420	arginyl-tRNA aminoacylation	0.00053
GO:0006013	mannose metabolic process	0.00062
GO:0006518	peptide metabolic process	0.00065
GO:0043094	cellular metabolic compound salvage	0.00068
GO:0043604	amide biosynthetic process	0.00088
GO:0009313	oligosaccharide catabolic process	0.00091
GO:0043603	cellular amide metabolic process	0.00097
GO:0006418	tRNA aminoacylation for protein translation	0.0011
GO:0010206	photosystem II repair	0.00128
GO:0046185	aldehyde catabolic process	0.00128
GO:0006091	generation of precursor metabolites and energy	0.00133
GO:0031050	dsRNA fragmentation	0.00148
GO:0043331	response to dsRNA	0.00148
GO:0070918	production of small RNA involved in gene silencing by RNA	0.00148
GO:0071359	cellular response to dsRNA	0.00148
GO:0015979	photosynthesis	0.00149
GO:1901566	organonitrogen compound biosynthetic process	0.00152
GO:0044710	single-organism metabolic process	0.00165
GO:0044723	single-organism carbohydrate metabolic process	0.00179
GO:0030422	production of siRNA involved in RNA interference	0.00236
GO:0016441	posttranscriptional gene silencing	0.00247
GO:0006414	translational elongation	0.00269
GO:0019684	photosynthesis, light reaction	0.00271
GO:0006449	regulation of translational GO Termsination	0.00306
GO:0006452	translational frameshifting	0.00306
GO:0010031	circumnutation	0.00306
GO:0045901	positive regulation of translational elongation	0.00306
GO:0045905	positive regulation of translational	0.00306

	GO Termsination	
GO:0050879	multicellular organismal movement	0.00306
GO:0010035	response to inorganic substance	0.00332
GO:0007602	phototransduction	0.00362
GO:0009585	red, far-red light phototransduction	0.00362
GO:0044724	single-organism carbohydrate catabolic process	0.00405
GO:0006109	regulation of carbohydrate metabolic process	0.00412
GO:0009817	defense response to fungus, incompatible interaction	0.00412
GO:0006399	tRNA metabolic process	0.00425
GO:0009583	detection of light stimulus	0.00445
GO:0000023	maltose metabolic process	0.00502
GO:0010258	NADH dehydrogenase complex (plastoquinone) assembly	0.00502
GO:0015714	phosphoenolpyruvate transport	0.00502
GO:0019243	methylglyoxal catabolic process to D-lactate via S-lactoyl-glutathione	0.00502
GO:0044281	small molecule metabolic process	0.00519
GO:0010025	wax biosynthetic process	0.00538
GO:0030091	protein repair	0.00538
GO:0043255	regulation of carbohydrate biosynthetic process	0.00538
GO:0035194	posttranscriptional gene silencing by RNA	0.00613
GO:0010166	wax metabolic process	0.00642
GO:1901699	cellular response to nitrogen compound	0.00666
GO:0009814	defense response, incompatible interaction	0.00735
GO:0010257	NADH dehydrogenase complex assembly	0.00742
GO:0035436	triose phosphate transmembrane transport	0.00742
GO:0043243	positive regulation of protein complex disassembly	0.00742
GO:0009765	photosynthesis, light harvesting	0.00758
GO:1901564	organonitrogen compound metabolic process	0.00766
GO:0009147	pyrimidine nucleoside triphosphate metabolic process	0.00885
GO:0009581	detection of external stimulus	0.00885
GO:0009582	detection of abiotic stimulus	0.00885
GO:1901698	response to nitrogen compound	0.00899
GO:0009112	nucleobase metabolic process	0.00993

Table S 20: Main biological process of Opti (Optimal Water) genes Down-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0043603	cellular amide metabolic process	4.1e-08
GO:0043604	amide biosynthetic process	4.8e-08
GO:0006412	translation	1,00E-07
GO:0006518	peptide metabolic process	1.4e-07
GO:0043043	peptide biosynthetic process	1.7e-07
GO:1901566	organonitrogen compound biosynthetic process	8.7e-06
GO:0046907	intracellular transport	7.7e-05
GO:0051641	cellular localization	8.2e-05
GO:0015931	nucleobase-containing compound transport	9.4e-05
GO:0051649	establishment of localization in cell	0.00016
GO:1901564	organonitrogen compound metabolic process	0.00023
GO:1902582	single-organism intracellular transport	0.00026
GO:0051668	localization within membrane	0.00036
GO:0070676	intralumenal vesicle formation	0.00036
GO:1902591	single-organism membrane budding	0.00036
GO:0071705	nitrogen compound transport	0.00074
GO:0071702	organic substance transport	0.00086
GO:0010966	regulation of phosphate transport	0.00112
GO:1903795	regulation of inorganic anion transmembrane transport	0.00112
GO:2000185	regulation of phosphate transmembrane transport	0.00112
GO:0055062	phosphate ion homeostasis	0.00119
GO:0072506	trivalent inorganic anion homeostasis	0.00119
GO:0048316	seed development	0.00128
GO:0044267	cellular protein metabolic process	0.0014
GO:0006862	nucleotide transport	0.00183
GO:0000919	cell plate assembly	0.00186
GO:0016973	poly(A)+ mRNA export from nucleus	0.00186
GO:0022618	ribonucleoprotein complex assembly	0.00187
GO:0071826	ribonucleoprotein complex subunit organization	0.00187
GO:0000911	cytokinesis by cell plate formation	0.00202
GO:0009793	embryo development ending in seed dormancy	0.00209
GO:0006406	mRNA export from nucleus	0.00211
GO:0071427	mRNA-containing ribonucleoprotein complex export from nucleus	0.00211
GO:0032506	cytokinetic process	0.0023
GO:1902410	mitotic cytokinetic process	0.0023
GO:0010154	fruit development	0.0026
GO:1902578	single-organism localization	0.0026
GO:0006913	nucleocytoplasmic transport	0.00261
GO:0051169	nuclear transport	0.00261
GO:0015866	ADP transport	0.00272
GO:0072505	divalent inorganic anion homeostasis	0.00272
GO:0051179	localization	0.00298
GO:0051028	mRNA transport	0.00326
GO:0071166	ribonucleoprotein complex localization	0.00326
GO:0071426	ribonucleoprotein complex export from nucleus	0.00326

GO:0002238	response to molecule of fungal origin	0.0033
GO:0009920	cell plate formation involved in plant-type cell wall biogenesis	0.0033
GO:0035435	phosphate ion transmembrane transport	0.0033
GO:0006810	transport	0.0037
GO:0044765	single-organism transport	0.00377
GO:0015867	ATP transport	0.00378
GO:0006405	RNA export from nucleus	0.00396
GO:0055081	anion homeostasis	0.00396
GO:0043933	macromolecular complex subunit organization	0.00443
GO:0008104	protein localization	0.00457
GO:0000281	mitotic cytokinesis	0.00464
GO:0009790	embryo development	0.00471
GO:0051234	establishment of localization	0.00481
GO:0055083	monovalent inorganic anion homeostasis	0.00507
GO:0061640	cytoskeleton-dependent cytokinesis	0.00516
GO:0006996	organelle organization	0.00569
GO:0000910	cytokinesis	0.00571
GO:0033036	macromolecule localization	0.00624
GO:0002697	regulation of immune effector process	0.00645
GO:0009610	response to symbiotic fungus	0.00645
GO:0050688	regulation of defense response to virus	0.00645
GO:0015868	purine ribonucleotide transport	0.0066
GO:0051503	adenine nucleotide transport	0.0066
GO:0048545	response to steroid hormone	0.00669
GO:0050657	nucleic acid transport	0.00669
GO:0050658	RNA transport	0.00669
GO:0051168	nuclear export	0.00669
GO:0051236	establishment of RNA localization	0.00669
GO:0034762	regulation of transmembrane transport	0.0075
GO:0022613	ribonucleoprotein complex biogenesis	0.0078
GO:0006403	RNA localization	0.00782
GO:0015865	purine nucleotide transport	0.00836
GO:0034613	cellular protein localization	0.00842
GO:0015711	organic anion transport	0.00944

Table S 21: Main biological process of Opti (Optimal Water) genes Up-regulated genes for PHW79 genotype in Tomeza location.

GO.ID	**GO Terms**	**p-value**
GO:0009314	response to radiation	1.7e-06
GO:0009628	response to abiotic stimulus	2.1e-06
GO:0048507	meristem development	3.4e-06
GO:0009416	response to light stimulus	5.8e-06
GO:0043603	cellular amide metabolic process	8.8e-06
GO:0006518	peptide metabolic process	2.6e-05
GO:1901566	organonitrogen compound biosynthetic process	3.9e-05
GO:0042372	phylloquinone biosynthetic process	4.8e-05
GO:0042374	phylloquinone metabolic process	4.8e-05
GO:0043043	peptide biosynthetic process	5.9e-05
GO:0006412	translation	7.9e-05
GO:0043604	amide biosynthetic process	0.00015
GO:0006399	tRNA metabolic process	0.00018
GO:0034660	ncRNA metabolic process	2,00E-04
GO:0015979	photosynthesis	0.00022
GO:0009888	tissue development	3,00E-04
GO:0050896	response to stimulus	0.00036
GO:0009266	response to temperature stimulus	0.00053
GO:0018193	peptidyl-amino acid modification	0.00058
GO:0043933	macromolecular complex subunit organization	6,00E-04
GO:0009987	cellular process	0.00075
GO:0009657	plastid organization	0.00086
GO:0006996	organelle organization	0.00089
GO:0044237	cellular metabolic process	0.00103
GO:0019684	photosynthesis, light reaction	0.00104
GO:0034641	cellular nitrogen compound metabolic process	0.00132
GO:0007584	response to nutrient	0.00135
GO:1901564	organonitrogen compound metabolic process	0.00147
GO:0045036	protein targeting to chloroplast	0.00154
GO:0072596	establishment of protein localization to chloroplast	0.00154
GO:0006259	DNA metabolic process	0.00156
GO:0044763	single-organism cellular process	0.00166
GO:0007275	multicellular organismal development	0.00176
GO:0072598	protein localization to chloroplast	0.00185
GO:0009637	response to blue light	0.0019
GO:0006807	nitrogen compound metabolic process	0.00196
GO:0044707	single-multicellular organism process	0.00224
GO:0051276	chromosome organization	0.00236
GO:0009409	response to cold	0.00248
GO:0044767	single-organism developmental process	0.00253
GO:0010073	meristem maintenance	0.00266
GO:0000413	protein peptidyl-prolyl isomerization	0.00276
GO:0032502	developmental process	0.0028
GO:0010449	root meristem growth	0.00307
GO:0006450	regulation of translational fidelity	0.00307
GO:0042726	flavin-containing compound metabolic process	0.00309
GO:0018208	peptidyl-proline modification	0.00331
GO:0009658	chloroplast organization	0.00351
GO:0010165	response to X-ray	0.00359
GO:0010275	NAD(P)H dehydrogenase complex assembly	0.00359
GO:0070681	glutaminyl-tRNAGln biosynthesis via transamidation	0.00359
GO:0071483	cellular response to blue light	0.0038
GO:0043038	amino acid activation	0.00393

GO:0043039	tRNA aminoacylation	0.00393	
GO:0032501	multicellular organismal process	0.0041	
GO:0006400	tRNA modification	0.0041	
GO:0035266	meristem growth	0.0041	
GO:0010228	vegetative to reproductive phase transition of meristem	0.00422	
GO:0009414	response to water deprivation	0.00447	
GO:0009914	hormone transport	0.0046	
GO:0060918	auxin transport	0.0046	
GO:0034470	ncRNA processing	0.00492	
GO:0071840	cellular component organization or biogenesis	0.00509	
GO:0016043	cellular component organization	0.00527	
GO:0010207	photosystem II assembly	0.00555	
GO:0009415	response to water	0.00568	
GO:0009615	response to virus	0.00571	
GO:0009902	chloroplast relocation	0.00572	
GO:0019750	chloroplast localization	0.00572	
GO:0046836	glycolipid transport	0.00572	
GO:0051644	plastid localization	0.00572	
GO:0051667	establishment of plastid localization	0.00572	
GO:0042221	response to chemical	0.00587	
GO:0050793	regulation of developmental process	0.00631	
GO:0006108	malate metabolic process	0.0066	
GO:0002697	regulation of immune effector process	0.00702	
GO:0009610	response to symbiotic fungus	0.00702	
GO:0009855	deGO Termsination of bilateral symmetry	0.00702	
GO:0045038	protein import into chloroplast thylakoid membrane	0.00702	
GO:0050688	regulation of defense response to virus	0.00702	
GO:0080037	negative regulation of cytokinin-activated signaling pathway	0.00702	
GO:1902580	single-organism cellular localization	0.00852	
GO:1902589	single-organism organelle organization	0.00871	
GO:0018298	protein-chromophore linkage	0.00896	
GO:0006448	regulation of translational elongation	0.00942	
GO:0048731	system development	0.00947	
GO:0010467	gene expression	0.00954	
GO:0044699	single-organism process	0.00983	

Annex 6: Genes ontology (Go terms) for specific studied factors in experiment two

Table S' 1. Main biological process of early senescence genes down and Up -regulated in two inbred lines of temperate maize in Xinzo location.

GO.ID	Terms	p-value	Type
GO:0015866	ADP transport	8.9e-05	Up
GO:0015867	ATP transport	0.00011	Up
GO:0015868	purine ribonucleotide transport	0.00016	Up
GO:0051503	adenine nucleotide transport	0.00016	Up
GO:0015865	purine nucleotide transport	0.00019	Up
GO:0015858	nucleoside transport	0.00037	Up
GO:0006862	nucleotide transport	0.00085	Up
GO:0015711	organic anion transport	0.00102	Up
GO:1901264	carbohydrate derivative transport	0.0029	Up
GO:0015748	organophosphate ester transport	0.0035	Up
GO:0015931	nucleobase-containing compound transport	0.00362	Up
GO:0006081	cellular aldehyde metabolic process	0.00456	Up
GO:0006820	anion transport	0.00543	Up
GO:0009743	response to carbohydrate	0.00691	Up
GO:0016054	organic acid catabolic process	0.00762	Up
GO:0046395	carboxylic acid catabolic process	0.00762	Up
GO:0044270	cellular nitrogen compound catabolic process	0.00991	Up
GO:0046700	heterocycle catabolic process	0.00991	Up
GO:0006457	protein folding	0.00045	Down
GO:0006414	translational elongation	0.00081	Down
GO:0019684	photosynthesis, light reaction	0.00234	Down
GO:0015979	photosynthesis	0.00973	Down

Table S' 2: TF families and percentage of expression involved in each senescence moment of two maize inbred lines for Xinzo location.

TF Class	M1_M2		M2_M3		M3_M4	
	Expressed_	%TF_Express	Expressed_TF	%TF_Express	Expressed_TF	%TF_Express
AP2	0	0	11	21	3	6
ARF	10	16	24	39	8	13
ARR-B	0	0	3	23	0	0
B3	4	5	16	20	1	1
BBR-BPC	0	0	2	22	0	0
BES1	0	0	2	12	2	12
bHLH	6	2	47	16	15	5
bZIP	7	3	47	22	14	6
C2H2	3	2	20	11	17	10
C3H	2	2	28	25	6	5
CAMTA	1	10	4	40	3	30
CO-like	1	6	9	50	3	17
CPP	0	0	5	29	3	18
DBB	2	10	8	40	4	20
Dof	1	2	9	17	1	2

E2F/DP	0	0	1	4	0	0
EIL	0	0	0	0	0	0
ERF	4	2	7	3	1	0
FAR1	1	4	4	17	0	0
G2-like	16	18	23	26	12	13
GATA	4	7	19	35	7	13
GeBP	0	0	0	0	1	4
GRAS	2	2	14	14	5	5
GRF	4	13	1	3	4	13
HB-other	1	4	4	15	2	7
HB-PHD	0	0	0	0	0	0
HD-ZIP	9	9	18	19	2	2
HRT-like	0	0	0	0	0	0
HSF	6	12	15	31	8	16
LBD	0	0	0	0	0	0
LFY	0	0	0	0	0	0
LSD	8	40	6	30	8	40
M-type_MADS	1	2	4	9	2	4
MIKC_MADS	10	11	28	32	0	0
MYB	7	3	27	13	6	3
MYB_related	6	4	32	19	8	5
NAC	21	11	26	14	13	7
NF-X1	0	0	2	50	0	0
NF-YA	11	32	11	32	1	3
NF-YB	0	0	3	11	0	0
NF-YC	0	0	5	20	2	8
Nin-like	3	13	6	26	0	0
RAV	0	0	0	0	0	0
S1Fa-like	0	0	2	40	0	0
SBP	2	4	6	11	5	9
SRS	0	0	0	0	0	0
STAT	0	0	2	100	0	0
TALE	3	6	11	21	11	21
TCP	0	0	0	0	0	0
Trihelix	0	0	8	14	1	2
VOZ	0	0	7	70	0	0
Whirly	0	0	4	67	0	0
WOX	0	0	0	0	1	3
WRKY	5	3	32	20	14	9
YAB	0	0	0	0	0	0
BY ZF-HD	0	0	0	0	0	0

(L1 and L2: genotype NSG B73 and SG PHW79, respectively)

Table S' 3: Main biological process of SN1 (both stress) genes Down-regulated genes for B73 genotype in Xinzo location.

GO.ID	B73 Terms_Down	p-value
GO:0034641	cellular nitrogen compound metabolic process	0.00037
GO:0043170	macromolecule metabolic process	0.00066
GO:0006139	nucleobase-containing compound metabolic process	0.00144
GO:0006807	nitrogen compound metabolic process	0.00159
GO:0010467	gene expression	0.00178
GO:0046483	heterocycle metabolic process	0.00181
GO:0009735	response to cytokinin	0.00221
GO:0006241	CTP biosynthetic process	0.00222
GO:0009148	pyrimidine nucleoside triphosphate biosynthetic process	0.00222
GO:0009208	pyrimidine ribonucleoside triphosphate metabolic process	0.00222
GO:0009209	pyrimidine ribonucleoside triphosphate biosynthetic process	0.00222
GO:0046036	CTP metabolic process	0.00222
GO:0006725	cellular aromatic compound metabolic process	0.00238
GO:0009147	pyrimidine nucleoside triphosphate metabolic process	0.00248
GO:0044271	cellular nitrogen compound biosynthetic process	0.00259
GO:0046132	pyrimidine ribonucleoside biosynthetic process	0.00303
GO:0046134	pyrimidine nucleoside biosynthetic process	0.00303
GO:1901360	organic cyclic compound metabolic process	0.00312
GO:0090304	nucleic acid metabolic process	0.00323
GO:0009218	pyrimidine ribonucleotide metabolic process	0.00332
GO:0009220	pyrimidine ribonucleotide biosynthetic process	0.00332
GO:0044260	cellular macromolecule metabolic process	0.00333
GO:0006213	pyrimidine nucleoside metabolic process	0.00428
GO:0046131	pyrimidine ribonucleoside metabolic process	0.00428
GO:0044237	cellular metabolic process	0.00568
GO:0015995	chlorophyll biosynthetic process	0.00613
GO:0016070	RNA metabolic process	0.00727
GO:0044238	primary metabolic process	0.00758
GO:0042542	response to hydrogen peroxide	0.00972

Table S' 4: Main biological process of SN1 (both stress) genes Up-regulated genes for B73 genotype in Xinzo location.

GO.ID	Terms	p-value
GO:0010507	negative regulation of autophagy	2.7e-05
GO:0051179	localization	5,00E-05
GO:0006810	transport	0.00024
GO:0051234	establishment of localization	0.00028
GO:0010506	regulation of autophagy	0.00074
GO:0071702	organic substance transport	8,00E-04
GO:0006914	autophagy	0.00086
GO:0006401	RNA catabolic process	0.00101
GO:0045995	regulation of embryonic development	0.00117
GO:0031330	negative regulation of cellular catabolic process	0.00143
GO:0008104	protein localization	0.00159
GO:0009895	negative regulation of catabolic process	0.00171
GO:0043207	response to external biotic stimulus	0.00287
GO:0051707	response to other organism	0.00287
GO:0034655	nucleobase-containing compound catabolic process	0.00342
GO:0009607	response to biotic stimulus	0.00357
GO:0015031	protein transport	0.00424
GO:0045184	establishment of protein localization	0.0048
GO:0009605	response to external stimulus	0.00522
GO:0048574	long-day photoperiodism, flowering	0.00528
GO:0033036	macromolecule localization	0.006
GO:0000956	nuclear-transcribed mRNA catabolic process	0.00632
GO:0048571	long-day photoperiodism	0.00744
GO:0044248	cellular catabolic process	0.00745
GO:0006402	mRNA catabolic process	0.00928

Table S' 5: Main biological process of SN1 (both stress) genes Down-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	PHW79_Down	p-value
GO:0010467	gene expression	0.00036
GO:0042254	ribosome biogenesis	0.00044
GO:0051186	cofactor metabolic process	0.00052
GO:1901564	organonitrogen compound metabolic process	0.00057
GO:0006828	manganese ion transport	0.00089
GO:0006732	coenzyme metabolic process	0.00104
GO:0015979	photosynthesis	0.00114
GO:0044249	cellular biosynthetic process	0.00115
GO:0010236	plastoquinone biosynthetic process	0.00133
GO:1901576	organic substance biosynthetic process	0.00168
GO:0022613	ribonucleoprotein complex biogenesis	0.00175
GO:0006807	nitrogen compound metabolic process	0.00231
GO:1901661	quinone metabolic process	0.00237
GO:1901663	quinone biosynthetic process	0.00237
GO:0044237	cellular metabolic process	0.00247
GO:0034641	cellular nitrogen compound metabolic process	0.00261
GO:0042181	ketone biosynthetic process	0.00262
GO:0009658	chloroplast organization	0.00325
GO:0019684	photosynthesis, light reaction	0.00325
GO:0009657	plastid organization	0.00334
GO:0009753	response to jasmonic acid	0.00338
GO:0006412	translation	0.00385
GO:0006091	generation of precursor metabolites and energy	0.00396
GO:0034645	cellular macromolecule biosynthetic process	0.0043
GO:0043043	peptide biosynthetic process	0.00444
GO:1901566	organonitrogen compound biosynthetic process	0.00471
GO:0009058	biosynthetic process	0.00486
GO:0006518	peptide metabolic process	0.00542
GO:0009059	macromolecule biosynthetic process	0.00605
GO:0051188	cofactor biosynthetic process	0.0061
GO:0043604	amide biosynthetic process	0.00642
GO:0000041	transition metal ion transport	0.00657
GO:0042255	ribosome assembly	0.00882
GO:0043603	cellular amide metabolic process	0.00963
GO:0046496	nicotinamide nucleotide metabolic process	0.00997

Table S' 6: Main biological process of SN1 (both stress) genes Up-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms PHW79	p-value
GO:0000338	protein deneddylation	2.9e-05
GO:0010387	COP9 signalosome assembly	0.00054
GO:0051013	microtubule severing	0.00054
GO:0015914	phospholipid transport	0.00075
GO:0034204	lipid translocation	0.00075
GO:0045332	phospholipid translocation	0.00075
GO:0097035	regulation of membrane lipid distribution	0.00087
GO:0046132	pyrimidine ribonucleoside biosynthetic process	0.00101
GO:0046134	pyrimidine nucleoside biosynthetic process	0.00101
GO:0019538	protein metabolic process	0.00105
GO:0009218	pyrimidine ribonucleotide metabolic process	0.00116
GO:0009220	pyrimidine ribonucleotide biosynthetic process	0.00116
GO:0010388	cullin deneddylation	0.00133
GO:0006213	pyrimidine nucleoside metabolic process	0.0017
GO:0046131	pyrimidine ribonucleoside metabolic process	0.0017
GO:0006222	UMP biosynthetic process	0.00186
GO:0009173	pyrimidine ribonucleoside monophosphate metabolic process	0.00186
GO:0009174	pyrimidine ribonucleoside monophosphate biosynthetic process	0.00186
GO:0046049	UMP metabolic process	0.00186

GO:1901642	nucleoside transmembrane transport	0.00186
GO:0015748	organophosphate ester transport	0.00189
GO:0010100	negative regulation of photomorphogenesis	0.00246
GO:0009129	pyrimidine nucleoside monophosphate metabolic process	0.00314
GO:0009130	pyrimidine nucleoside monophosphate biosynthetic process	0.00314
GO:0051246	regulation of protein metabolic process	0.00442
GO:0016458	gene silencing	0.00463
GO:0031330	negative regulation of cellular catabolic process	0.00474
GO:0080188	RNA-directed DNA methylation	0.00474
GO:0006508	proteolysis	0.00498
GO:0009895	negative regulation of catabolic process	0.00565
GO:0006401	RNA catabolic process	0.00569
GO:0006221	pyrimidine nucleotide biosynthetic process	0.00612
GO:0006220	pyrimidine nucleotide metabolic process	0.00704
GO:0031047	gene silencing by RNA	0.00729
GO:0044267	cellular protein metabolic process	0.00745
GO:0043549	regulation of kinase activity	0.00753
GO:0045859	regulation of protein kinase activity	0.00753
GO:0040029	regulation of gene expression, epigenetic	0.00754
GO:0048519	negative regulation of biological process	0.00787
GO:0001932	regulation of protein phosphorylation	0.00804
GO:0009894	regulation of catabolic process	0.00804
GO:0042325	regulation of phosphorylation	0.00912

Table S' 7: Main biological process of ON3 (optimal water andnitrogen codition) genes Down and Up-regulated genes for B73 genotype in Xinzo location.

GO.ID	Terms	p-value	Type
GO:0051188	cofactor biosynthetic process	7,00E-04	Up
GO:1901564	organonitrogen compound metabolic process	8,00E-04	Up
GO:1901566	organonitrogen compound biosynthetic process	0.00092	Up
GO:0009108	coenzyme biosynthetic process	0.00242	Up
GO:0034660	ncRNA metabolic process	0.00364	Up
GO:0043603	cellular amide metabolic process	0.00405	Up
GO:0006790	sulfur compound metabolic process	0.0048	Up
GO:0006807	nitrogen compound metabolic process	0.00612	Up
GO:0006952	defense response	0.00638	Up
GO:0051186	cofactor metabolic process	0.00901	Up
GO:0034470	ncRNA processing	0.00915	Up
GO:0016592	mediator complex	0.0025	Down

Table S' 8: Main biological process of ON3 (optimal water and nitrogen codition) genes Down and Up -regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms	p-value	Type
GO:0046037	GMP metabolic process	0.002	Up
GO:0044260	cellular macromolecule metabolic process	0.002	Up
GO:0007623	circadian rhythm	0.0025	Up
GO:0048511	rhythmic process	0.0025	Up
GO:1901068	guanosine-containing compound metabolic process	0.0042	Up
GO:0016579	protein deubiquitination	0.0053	Up
GO:0010337	regulation of salicylic acid metabolic process	0.0054	Up
GO:0035437	maintenance of protein localization in endoplasmic reticulum	0.0054	Up
GO:0051220	cytoplasmic sequestering of protein	0.0054	Up
GO:0072595	maintenance of protein localization in organelle	0.0069	Up
GO:0008652	cellular amino acid biosynthetic process	7.8e-05	Down
GO:1901607	alpha-amino acid biosynthetic process	0.00014	Down
GO:0098656	anion transmembrane transport	0.00021	Down
GO:0090414	molybdate ion export from vacuole	0.00032	Down
GO:0015689	molybdate ion transport	0.00085	Down
GO:0034220	ion transmembrane transport	9,00E-04	Down
GO:0034486	vacuolar transmembrane transport	0.00095	Down
GO:0016311	dephosphorylation	0.00177	Down
GO:0051194	positive regulation of cofactor metabolic process	0.00188	Down
GO:1901403	positive regulation of tetrapyrrole metabolic process	0.00188	Down
GO:1901465	positive regulation of tetrapyrrole biosynthetic process	0.00188	Down
GO:1901605	alpha-amino acid metabolic process	0.00218	Down
GO:0009396	folic acid-containing compound biosynthetic process	0.00222	Down
GO:0016125	sterol metabolic process	0.00222	Down
GO:0006820	anion transport	0.00253	Down
GO:0006811	ion transport	0.00318	Down
GO:0006821	chloride transport	0.00382	Down
GO:0042559	pteridine-containing	0.00382	Down

	compound biosynthetic process		
GO:0015698	inorganic anion transport	0.00399	Down
GO:0071166	ribonucleoprotein complex localization	0.00447	Down
GO:0071426	ribonucleoprotein complex export from nucleus	0.00447	Down
GO:0042398	cellular modified amino acid biosynthetic process	0.00463	Down
GO:0016053	organic acid biosynthetic process	0.00476	Down
GO:0046394	carboxylic acid biosynthetic process	0.00476	Down
GO:0006520	cellular amino acid metabolic process	0.00497	Down
GO:0044711	single-organism biosynthetic process	0.00503	Down
GO:0006405	RNA export from nucleus	0.00519	Down
GO:0044763	single-organism cellular process	0.0057	Down
GO:0009067	aspartate family amino acid biosynthetic process	0.0061	Down
GO:0019632	shikimate metabolic process	0.00635	Down
GO:0043433	negative regulation of sequence-specific DNA binding transcription factor activity	0.00635	Down
GO:0048564	photosystem I assembly	0.00635	Down
GO:0050657	nucleic acid transport	0.00776	Down
GO:0050658	RNA transport	0.00776	Down
GO:0051168	nuclear export	0.00776	Down
GO:0051236	establishment of RNA localization	0.00776	Down
GO:0016126	sterol biosynthetic process	0.00836	Down
GO:0016925	protein sumoylation	0.00836	Down
GO:1901259	chloroplast rRNA processing	0.00836	Down
GO:0006403	RNA localization	0.00875	Down

Table S' 9: Main biological process of N1 (low nitrogen stress) genes Down and Up-regulated genes for B73 genotype in Tomeza location. (No function enriched for this factor)

Table S' 10: Main biological process of N3 (optimal nitrogen level) genes Down and Up-regulated genes for B73 genotype in Xinzo location.

GO.ID	Terms_N3_Down	p-value	GO.ID	Terms_N3_Up	p-value
GO:0009657	plastid organization	2.7e-05	GO:0009625	response to insect	0.0022
GO:0009658	chloroplast organizati	0.0034	GO:0009695	jasmonic acid biosynthe	0.0022
	on			tic process	
GO:0005986	sucrose biosynthetic process	0.0049	GO:0009694	jasmonic acid metabolic process	0.0039
GO:0006002	fructose 6-phosphate metabolic process	0.0288	GO:0016054	organic acid catabolic process	0.0067
GO:0010020	chloroplast fission	0.0288	GO:0046395	carboxylic acid catabolic process	0.0067
GO:0043572	plastid fission	0.0331	GO:0042537	benzene-containing compound metabolic process	0.0071
GO:0019359	nicotinamide nucleotide biosynthetic process	0.0377			
GO:0019363	pyridine nucleotide biosynthetic process	0.0426			
GO:0019674	NAD metabolic process	0.0531			
GO:0051701	interaction with host	0.0984			
GO:0072525	pyridine-containing compound biosynthetic process	0.0984			

Table S' 11: Main biological process of WS (water stress) genes Down and Up-regulated genes for B73 genotype in Xinzo location.

GO.ID	Terms_WS_Down	p-value	GO.ID	Terms_WS_Up	p-value
GO:0015979	photosynthesis	2.8e-30	GO:0006810	transport	1.9e-05
GO:0019684	photosynthesis, light reaction	1.7e-18	GO:0051234	establishment of localization	2.3e-05
GO:0006091	generation of precursor metabolites and energy	4.6e-12	GO:0051179	localization	3.5e-05
GO:0009657	plastid organization	7.2e-11	GO:0046513	ceramide biosynthetic	6.5e-05

				process	
GO:0018298	protein-chromophore linkage	4.2e-09	GO:0090414	molybdate ion export from vacuole	6.5e-05
GO:0009658	chloroplast organization	1.4e-08	GO:0072329	monocarboxylic acid catabolic process	0.00016
GO:0009765	photosynthesis, light harvesting	1,00 E-06	GO:0034486	vacuolar transmembrane transport	0.00019
GO:0010207	photosystem II assembly	2.3e-06	GO:0006865	amino acid transport	3,00 E-04
GO:0032544	plastid translation	5.8e-06	GO:0044765	single-organism transport	5,00 E-04
GO:0006518	peptide metabolic process	6.7e-06	GO:0006635	fatty acid beta-oxidation	0.00062
GO:0009668	plastid membrane organization	1.1e-05	GO:1902578	single-organism localization	0.00065
GO:0010027	thylakoid membrane organization	1.1e-05	GO:0098656	anion transmembrane transport	0.00082
GO:0006412	translation	1.2e-05	GO:0006811	ion transport	0.00083
GO:0044237	cellular metabolic process	1.2e-05	GO:0009062	fatty acid catabolic process	0.00093
GO:0043043	peptide biosynthetic process	1.5e-05	GO:0019395	fatty acid oxidation	0.00105
GO:0009773	photosynthetic electron transport in photosystem I	1.6e-05	GO:0034220	ion transmembrane transport	0.00151
GO:0009987	cellular process	1.6e-05	GO:0034440	lipid oxidation	0.00163
GO:0043603	cellular amide metabolic process	1.9e-05	GO:0006672	ceramide metabolic process	0.00177
GO:0009767	photosynthetic electron transport chain	2,00 E-05	GO:0010413	glucuronoxylan metabolic process	0.00177
GO:0043604	amide biosynthetic process	2.8e-05	GO:0010417	glucuronoxylan biosynthetic process	0.00177
GO:0042548	regulation of photosynthesis, light reaction	3.4e-05	GO:0003333	amino acid transmembrane transport	0.00198
GO:0009768	photosynthesis, light harvesting in photosystem I	4.1e-05	GO:0043547	positive regulation of GTPase activity	0.00201
GO:0043467	regulation of generation of precursor metabolites and energy	4.3e-05	GO:0043087	regulation of GTPase activity	0.00212
GO:1901564	organonitrogen compound metabolic process	4.4e-05	GO:0006820	anion transport	0.00238
GO:0044085	cellular component biogenesis	6,00 E-05	GO:0055085	transmembrane transport	0.00273
GO:0010109	regulation of photosynthesis	0.00013	GO:0051345	positive regulation of hydrolase activity	0.00276
GO:0033013	tetrapyrrole metabolic process	0.00033	GO:0019318	hexose metabolic process	0.0032
GO:0051186	cofactor metabolic process	0.00035	GO:0006012	galactose metabolic process	0.00341
GO:0043623	cellular protein complex assembly	4,00 E-04	GO:0015689	molybdate ion transport	0.00341
GO:0009628	response to abiotic stimulus	0.00041	GO:0071577	zinc II ion transmembrane transport	0.00341
GO:0008152	metabolic process	0.00042	GO:0044242	cellular lipid catabolic process	0.00384
GO:0071840	cellular component organization or biogenesis	0.00048	GO:0015849	organic acid transport	0.00386
GO:1901566	organonitrogen compound	0.00048	GO:0046942	carboxylic acid transport	0.00386

	biosynthetic process				
GO:0033014	tetrapyrrole biosynthetic process	0.00056	GO:0030148	sphingolipid biosynthetic process	0.00408
GO:0006432	phenylalanyl-tRNA aminoacylation	0.00067	GO:1903825	organic acid transmembrane transport	0.00412
GO:0009735	response to cytokinin	0.00076	GO:0016054	organic acid catabolic process	0.00442
GO:0009416	response to light stimulus	0.00089	GO:0046395	carboxylic acid catabolic process	0.00442
GO:0006828	manganese ion transport	0.00111	GO:0007034	vacuolar transport	0.00442
GO:0009052	pentose-phosphate shunt, non-oxidative branch	0.00111	GO:0006829	zinc II ion transport	0.00479
GO:0055114	oxidation-reduction process	0.00113	GO:0098661	inorganic anion transmembrane transport	0.00556
GO:0009314	response to radiation	0.00121	GO:0043085	positive regulation of catalytic activity	0.00826
GO:0042180	cellular ketone metabolic process	0.00147	GO:0030258	lipid modification	0.00917
GO:0006996	organelle organization	0.00147	GO:0044093	positive regulation of molecular function	0.00917
GO:0010236	plastoquinone biosynthetic process	0.00165			
GO:0009644	response to high light intensity	0.00183			
GO:0070271	protein complex biogenesis	0.00183			
GO:0044281	small molecule metabolic process	0.00185			
GO:0022900	electron transport chain	0.00214			
GO:0034660	ncRNA metabolic process	0.00237			
GO:0022607	cellular component assembly	0.00239			
GO:0009409	response to cold	0.0029			
GO:0042549	photosystem II stabilization	0.00304			
GO:0044267	cellular protein metabolic process	0.00335			
GO:0046496	nicotinamide nucleotide metabolic process	0.00339			
GO:0034470	ncRNA processing	0.00342			
GO:0006364	rRNA processing	0.00349			
GO:0019362	pyridine nucleotide metabolic process	0.00352			
GO:0019538	protein metabolic process	0.00355			
GO:0044802	single-organism membrane organization	0.00382			
GO:0016072	rRNA metabolic process	0.00399			
GO:0009266	response to temperature stimulus	0.00406			
GO:0042254	ribosome biogenesis	0.00419			
GO:0072524	pyridine-containing compound metabolic process	0.00464			
GO:0016043	cellular component organization	0.00475			
GO:0042742	defense response	0.00526			

	to bacterium	
GO:0050896	response to stimulus	0.00541
GO:0006733	oxidoreduction coenzyme metabolic process	0.00583
GO:0009772	photosynthetic electron transport in photosystem II	0.00585
GO:0006461	protein complex assembly	0.00585
GO:0044710	single-organism metabolic process	0.00607
GO:0071822	protein complex subunit organization	0.00625
GO:0000413	protein peptidyl-prolyl isomerization	0.00632
GO:0006739	NADP metabolic process	0.00659
GO:0034250	positive regulation of cellular amide metabolic process	0.00697
GO:0045727	positive regulation of translation	0.00697
GO:0018208	peptidyl-proline modification	0.00699
GO:0006732	coenzyme metabolic process	0.00766
GO:0043038	amino acid activation	0.0077
GO:0043039	tRNA aminoacylation	0.0077
GO:0042440	pigment metabolic process	0.00809
GO:0009645	response to low light intensity stimulus	0.00818
GO:0034622	cellular macromolecular complex assembly	0.0087
GO:0009636	response to toxic substance	p-value

Table S' 12: Main biological process of Opti (optimal water) genes Down and Up-regulated genes for B73 genotype in Xinzo location. **(No function enriched for this factor)**

Table S' 13: Main biological process of N1 (low nitrogen) genes Down and Up-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms_N1_Down	p-value	GO.ID	Terms_N1_Up	p-value
GO:0006457	protein folding	6.1e-07	GO:0046700	heterocycle catabolic process	2,00E-04
GO:0009658	chloroplast organization	3.4e-05	GO:0015855	pyrimidine nucleobase transport	0.00024
GO:0009657	plastid organization	5.1e-05	GO:0015857	uracil transport	0.00024
GO:0042254	ribosome biogenesis	0.00024	GO:0051716	cellular response to stimulus	0.00035
GO:0034047	regulation of protein phosphatase type 2A activity	0.00056	GO:0007186	G-protein coupled receptor signaling pathway	0.00097
GO:0009628	response to abiotic stimulus	0.00088	GO:1901361	organic cyclic compound catabolic process	0.00097
GO:0022613	ribonucleoprotein complex biogenesis	0.00099	GO:0015851	nucleobase transport	0.00118
GO:0043666	regulation of phosphopr	0.00113	GO:0044270	cellular nitrogen compoun	0.00159

	otein phosphatase activity			d catabolic process	
GO:0010921	regulation of phosphatase activity	0.00128	GO:0007205	protein kinase C-activating G-protein coupled receptor signaling pathway	0.00217
GO:0010608	posttranscriptional regulation of gene expression	0.00129	GO:0007165	signal transduction	0.00257
GO:0035303	regulation of dephosphorylation	0.00161	GO:0044700	single organism signaling	0.00277
GO:0035304	regulation of protein dephosphorylation	0.00161	GO:0023052	signaling	0.00281
GO:0009611	response to wounding	0.00166	GO:0007154	cell communication	0.0029
GO:0009668	plastid membrane organization	0.00179	GO:0070925	organelle assembly	0.0032
GO:0010027	thylakoid membrane organization	0.00179	GO:0080188	RNA-directed DNA methylation	0.0042
GO:0016441	posttranscriptional gene silencing	0.00182	GO:0006401	RNA catabolic process	0.00478
GO:0016559	peroxisome fission	0.00259	GO:0031047	gene silencing by RNA	0.00587
GO:0006950	response to stress	0.00345	GO:0031401	positive regulation of protein modification process	0.00588
GO:0032544	plastid translation	0.00466	GO:0052646	alditol phosphate metabolic process	0.00588
GO:0034250	positive regulation of cellular amide metabolic process	0.00466	GO:0042430	indole-containing compound metaboli	0.00634
				c process	
GO:0045727	positive regulation of translation	0.00466	GO:0050896	response to stimulus	0.00703
GO:0009415	response to water	0.00554	GO:0010928	regulation of auxin mediated signaling pathway	0.00782
GO:0050896	response to stimulus	0.0061	GO:0009072	aromatic amino acid family metabolic process	0.00787
GO:0042631	cellular response to water deprivation	0.00729	GO:1901605	alpha-amino acid metabolic process	0.00816
GO:0045037	protein import into chloroplast stroma	0.00729	GO:0044763	single-organism cellular process	0.00819
GO:0071462	cellular response to water stimulus	0.00729			
GO:0031668	cellular response to extracellular stimulus	0.00842			
GO:0006364	rRNA processing	0.00941			
GO:0071496	cellular response to external stimulus	0.00975			
GO:0035194	posttranscriptional gene silencing by RNA	0.00983			

Table S' 14: Main biological process of N3 (Optimal nitrogen level) genes Down and Up-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms_N3_Down	p-value	GO.ID	Terms_N3_Up	p-value
GO:0000375	RNA splicing, via transesterification reactions	0.00051	GO:0009867	jasmonic acid mediated signaling pathway	2.2e-05

GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.00051	GO:0071395	cellular response to jasmonic acid stimulus	2.2e-05
GO:0046185	aldehyde catabolic process	0.00135	GO:0071310	cellular response to organic substance	2.2e-05
GO:0044237	cellular metabolic process	0.00183			
GO:0016071	mRNA metabolic process	0.00371			
GO:0000398	mRNA splicing, via spliceosome	0.00379			
GO:0008380	RNA splicing	0.00394			
GO:0010467	gene expression	0.00429			
GO:0008152	metabolic process	0.00432			
GO:0006397	mRNA processing	0.00625			
GO:0044260	cellular macromolecule metabolic process	0.00934			

Table S' 15: Main biological process of WS (Water stress) genes Down-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms	p-value
GO:0051604	protein maturation	0.00022
GO:0016485	protein processing	0.00422
GO:0032270	positive regulation of cellular protein metabolic process	0.00422
GO:0051247	positive regulation of protein metabolic process	0.00516

Table S' 16: Main biological process of WS (Water stress) genes Up-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms	p-value
GO:0040029	regulation of gene expression, epigenetic	0.0015
GO:0016571	histone methylation	0.0021
GO:0016568	chromatin modification	0.0024
GO:0070887	cellular response to chemical stimulus	0.0025
GO:0044763	single-organism cellular process	0.0036
GO:1902275	regulation of chromatin organization	0.004
GO:1903308	regulation of chromatin modification	0.004
GO:0006479	protein methylation	0.0044
GO:0008213	protein alkylation	0.0044
GO:0071310	cellular response to organic substance	0.0048
GO:0006325	chromatin organization	0.007
GO:0007165	signal transduction	0.0077
GO:0044700	single organism signaling	0.0081
GO:0023052	signaling	0.0081
GO:0009630	gravitropism	0.0083
GO:0051716	cellular response to stimulus	0.0091
GO:0033044	regulation of chromosome organization	0.0097
GO:0016458	gene silencing	0.0099

Table S' 17: Main biological process of Opti (Optimal Water) genes Down-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms	p-value
GO:0009628	response to abiotic stimulus	9.2e-05
GO:0019684	photosynthesis, light reaction	0.00028
GO:0050896	response to stimulus	0.00031
GO:0009266	response to temperature stimulus	0.00032
GO:0006457	protein folding	0.00044
GO:0006534	cysteine metabolic process	0.00126
GO:0009408	response to heat	0.00131
GO:0015979	photosynthesis	0.00132
GO:0006396	RNA processing	0.00145
GO:0009735	response to cytokinin	0.00162
GO:0006950	response to stress	0.00201
GO:0006636	unsaturated fatty acid biosynthetic process	0.00213
GO:0033559	unsaturated fatty acid metabolic process	0.00213
GO:0001510	RNA methylation	0.00232
GO:0030422	production of siRNA involved in RNA interference	0.00334
GO:0009767	photosynthetic electron transport chain	0.00389
GO:0006775	fat-soluble vitamin metabolic process	0.00392
GO:0010189	vitamin E biosynthetic process	0.00392
GO:0042360	vitamin E metabolic process	0.00392
GO:0042362	fat-soluble vitamin biosynthetic process	0.00392
GO:0040029	regulation of gene expression, epigenetic	0.00457
GO:0016246	RNA interference	0.00463
GO:0009313	oligosaccharide catabolic process	0.005
GO:0005985	sucrose metabolic process	0.00512
GO:0009642	response to light intensity	0.00603

GO:0016441	posttranscriptional gene silencing	0.00607
GO:0042026	protein refolding	0.0062
GO:0046185	aldehyde catabolic process	0.0062
GO:0000375	RNA splicing, via transesterification reactions	0.00848
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.00848
GO:0006535	cysteine biosynthetic process from serine	0.00895
GO:0010267	production of ta-siRNAs involved in RNA interference	0.00895

Table S' 18: Main biological process of Opti (Optimal Water) genes Up-regulated genes for PHW79 genotype in Xinzo location.

GO.ID	Terms	p-value
GO:0006865	amino acid transport	0.00077
GO:0044765	single-organism transport	0.00129
GO:0050801	ion homeostasis	0.00165
GO:1902578	single-organism localization	0.00168
GO:0048878	chemical homeostasis	0.00182
GO:0009110	vitamin biosynthetic process	0.00194
GO:0006766	vitamin metabolic process	0.00223
GO:0000160	phosphorelay signal transduction system	0.00237
GO:0009737	response to abscisic acid	0.00276
GO:0006775	fat-soluble vitamin metabolic process	0.0029
GO:0010189	vitamin E biosynthetic process	0.0029
GO:0042360	vitamin E metabolic process	0.0029
GO:0042362	fat-soluble vitamin biosynthetic process	0.0029
GO:0010232	vascular transport	0.0037
GO:0010233	phloem transport	0.0037
GO:0003333	amino acid transmembrane transport	0.00403
GO:0007623	circadian rhythm	0.00495
GO:0048511	rhythmic process	0.00495
GO:0031050	dsRNA fragmentation	0.00665
GO:0043331	response to dsRNA	0.00665
GO:0070918	production of small RNA involved in gene silencing by RNA	0.00665
GO:0071359	cellular response to dsRNA	0.00665
GO:0097305	response to alcohol	0.00707
GO:0035556	intracellular signal transduction	0.0074
GO:0035670	plant-type ovary development	0.00771
GO:0048481	ovule development	0.00771
GO:1903825	organic acid transmembrane transport	0.00827
GO:0015849	organic acid transport	0.00931
GO:0046942	carboxylic acid transport	0.00931