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Estabilidad del mutante *sugary1* en maíz dulce

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Dedicatorias

Esta tesis representa la culminación
del soporte y el ánimo
que mi madre me ha prodigado a lo largo
de mi formación

A mi hijo

RESUMEN

El conocimiento de la viabilidad de un mutante es crucial para estudios evolutivos y genéticos. Esto es especialmente cierto para mutantes de importancia económica como *sugary1* (*su1*) en el maíz (*Zea mays* L.). Los objetivos del presente trabajo son: 1) evaluar los efectos de diversos fondos genéticos del maíz en la viabilidad de *su1* y estimar los efectos genéticos que controlan esta viabilidad, 2) determinar los efectos de la introducción de *su1* en el valor agronómico y los caracteres más limitantes para la viabilidad, y 3) identificar regiones cromosómicas asociadas a la viabilidad de *su1*. Estos objetivos han sido abordados utilizando dos sistemas basados en medias de generaciones autofecundadas separadamente durante cinco años consecutivos y evaluados en campo y en condiciones controladas. El primer sistema está constituido por dos líneas puras provenientes de dos fondos genéticos diferentes (EP42 y A661), mientras que el segundo sistema está constituido por dos líneas puras de fondos genéticos similares (A619 y A632). Los padres (P_1 y P_2), F_1s, F_2s y los retrocruzamientos se han cruzado con la línea P39 como donante de *su1*, y los 12 cruzamientos se han autofecundado durante cinco años. La regulación genética de la viabilidad de *su1* se ha estudiado en dos poblaciones de líneas recombinantes (RIL) provenientes de B73 × P39 y B73 × Il14h). Para ambas poblaciones se ha utilizado un mapa de 1106 marcadores de polimorfismo de un nucleótido (SNP). Además, se ha utilizado (A619 y P39) F_2 para identificar QTL que controlen la viabilidad de *su1*. Esta población se ha caracterizado con 295 marcadores microsatélites (SSR).

El control genético de la viabilidad de *su1*, así como los efectos de su introducción en los caracteres agronómicos están fuertemente influidos por la naturaleza del fondo genético y las condiciones ambientales. La frecuencia del alelo *su1* disminuyó a lo largo de las generaciones de autofecundación. Nuestros resultados confirman que la viabilidad de *su1* está controlada genéticamente, con efectos aditivos significativos debidos, probablemente, a la contribución de genes con efectos menores. Nuestros resultados sugieren que caracteres como el contenido de las hojas en clorofila o la longitud de mazorca tienen un papel fundamental en el control de la viabilidad de *su1*. La detección de regiones genómicas que controlan la viabilidad de *su1* ha estado muy influida por la naturaleza del fondo genético. Un gran bloque de ligamiento ha sido detectado alrededor del locus *Su1* en las dos poblaciones RIL. Por otra parte, regiones genómicas afectando la viabilidad han sido detectadas a lo largo de todo el genoma. Se han encontrado QTL con efectos menores que controlaban varios caracteres en la F_2. Algunas regiones genómicas controlan simultáneamente la viabilidad y la fertilidad por medio, probablemente, de efectos pleiotrópicos.

En conclusión, sugerimos que la viabilidad del mutante *su1* depende de múltiples genes con efectos menores distribuidos a lo largo de todo el genoma. Estos genes, que están fuertemente influidos por la naturaleza del fondo genético y por las condiciones ambientales, controlan varios caracteres ligados a la viabilidad del mutante *sugary1*.

SUMMARY

The fitness of a mutant is crucial from both evolutionary studies and breeding. This is particularly so for economically important mutants, as the sweetcorn mutant *sugary1* (*su1*) in maize (*Zea mays* L.). Our objectives were 1- to evaluate the effect of diverse maize genetic backgrounds on *sugary1* fitness and to estimate the genetic effects on *sugary1* fitness, 2- to know the effect of the introgression of *su1* in the genetic regulation of agronomic performance and to determine the most limiting factors for *su1* fitness under natural and controlled conditions, and 3- to identify the genes or genomic regions affecting the genetic regulation of *su1* viability. These objectives were studied with five successive selfing cycles in two separated mean generation designs in the field. The first design involved two unrelated inbreds (EP42 and A661), while the second design involved two inbreds with similar genetic backgrounds (A619 and A632). Parents, F_1s, F_2s, and backcrosses were crossed to P39 as the donor of *su1* and the 12 crosses were successively self-pollinated for 5 years. Moreover, the genetic regulation of *su1* was examined in two recombinant inbred lines (RILs) released from crosses between the maize inbred B73 and two sweetcorn lines (P39 and Il14h) belonging to the Nested Association Mapping (NAM) design that were genotyped with 1106 single nucleotide polymorphisms (SNPs). Finally, an F_2 population derived from the cross between A619 and P39 was characterized with 295 SSRs in order to identify QTLs for *su1* fitness.

The genetic regulation of *su1* fitness and its genic effects on agronomic traits are strongly dependent on specific sweet corn × field corn interaction and environmental conditions. The *su1* frequency decreased linearly across selfing generations in both designs. Our results confirm that the fitness of *su1* is under genetic control, with significant additive effects due to minor contributions of multiple genes. Moreover, various works showed that both emergence and early vigour are important traits determining *su1* viability. In our research, we studied the effect of the introgression of *su1* along the growth cycle of the plant. We found that some traits such as chlorophyll content, stand, silking date, plant height and ear length were also of great importance in *su1* fitness. The detection of genomic regions involved in *su1* fitness regulation is highly influenced by the genetic background. A large linkage block was detected around *su1* in the RILs. Furthermore, significant genomic regions associated with *su1* fitness were detected along the 10 maize chromosomes. Quantitative trait loci (QTLs) with effects in multiple traits related to *su1* fitness were detected in the F_2 in diverse chromosomes and with small weight. An important consideration is the presence of genomic regions that control both viability and fertility factors which suggests pleiotropic effects on mutant fitness.

It can be concluded that *su1* fitness depends on many genes with small effects on a variety of fitness-related traits throughout the genome that are significant or not depending on both genetic background and environmental conditions.

RESUM

El coneixement de la viabilitat d'un mutant és crucial en estudis evolutius i genètics. Això és especialment important en mutants amb importància econòmica com ara el mutant *sugary1* (*su1*) del blat de moro (*Zea mays* L.). Els objectius d'aquest treball són: 1) avaluar els efectes de diversos fons genètics del blat de moro sobre la viabilitat de *su1* alhora que estimar els factors genètics que afecten aquesta variabilitat, 2) aprofundir en el nostre coneixement dels efectes de la introducció de *su1* en el valor agronòmic i, així doncs, determinar els caràcters més limitants per a la seva viabilitat 3) i identificar gens o regions cromosòmiques que afecten la regulació genètica de la viabilitat de *su1*. Aquest objectius s'han abordats utilitzant dos sistemes basats en mesures de generacions auto-fecundades separadament durant cinc anys consecutius. El primer sistema està constituït per dues línies pures provinents de dos fons genètics diferents (EP42 i A661), mentre que el segon sistema està constituït per dues línies pures de fons genètics similars (A619 i A632). Els pares (P_1 i P_2), F_1s, F_2s i els 12 creuaments productius s'han fecundat durant cinc anys. La regulació genètica de la viabilitat del mutant *su1* s'ha estudiat en dues poblacions de línies recombinants (IRL) provinents de B73 × P39 i B73 × Il14h Per a ambdues poblacions s'ha fet servir un mapa de 1106 marcadors de polimorfisme d'un sol nucleòtid (SNP). A més a més, s'ha utilitzat una F_2 derivada del creuament de les línies A619 i P39 per a identificar QTL que controlin la viabilitat de *su1*. Aquesta població s'ha caracteritzat amb 295 marcadors microsatèllits (SSR).

El control genètic de la viabilitat de *su1* així com els efectes de la seva introducció en el caràcters agronòmics estan fortament influïts per la naturalesa dels fons genètic i les condicions ambientals. La freqüència de l'al·lel *su1* va disminuir al llarg de les generacions autofecundades. Els nostres resultats confirmen que la viabilitat de *su1* està controlada genèticament, amb efectes additius significatius deguts, probablement, a la contribució de diversos gens amb efectes menors. En aquest treball hem han mostrat els efectes de la introducció de *su1* en el valor agronòmic dels caràcters al llarg del creixement de la planta. Caràcters com són el vigor i longitud de la panotxa semblen tenir un paper fonamental en el control de la viabilitat de *su1*. La detecció de regions genòmiques que controlen la viabilitat de *su1* ha estat molt influïda per la naturalesa del fons genètic. Un gran bloc de lligament ha estat detectat al voltant del locus S*u1* en les dues poblacions RIL. D'altra banda, diverses regions genòmiques que afecten la viabilitat han estat detectades al llarg de tot el genoma. S'han trobat QTL amb efectes menors que controlen diversos caràcters a la F_2. Algunes regions genòmiques controlen simultàniament la viabilitat i fertilitat mitjançant, probablement, efectes pleiotròpics.

En conclusió, suggerim que la viabilitat de *su1* depèn de múltiples gens amb efectes menors distribuïts al llarg de tot el genoma. Aquests gens, que estan fortament influïts per la naturalesa del fons genètic i per les condicions ambientals, controlen diversos caràcters lligats a la viabilitat del mutant *su1*.

Chapter 1

Introducción general

Las mutaciones representan la materia prima para la evolución de las especies a lo largo del tiempo. La selección natural depende en gran medida de la fitness de la mutación en cuestión y de su viabilidad. El ambiente y el fondo genético en que se produce la mutación son los dos factores principales que limitan la estabilidad de una mutación y su propagación.

El maíz (*Zea mays* L. 2n=20) presenta una gran variabilidad genética, morfológica y fisiológica, lo que favorece su cultivo tanto en las regiones tropicales como en las zonas templadas (Buckler *et al*. 2009; Ganal *et al*. 2011). Este cultivo se extiende desde la latitud 40° Sur, en Argentina y Sudáfrica, hasta la latitud 58° Norte, en Canadá, y llega hasta los 3000 m de altitud en la región de los Andes (Buckler *et al*. 2009). Esta gran variabilidad se refleja también en las diferencias morfológicas de la mazorca y en las características del endospermo del grano.

Actualmente el maíz es uno de los cereales más importantes desde el punto de vista de su utilización gracias a su gran diversidad fenotípica y genética. La diversidad molecular del maíz es aproximadamente de 2 a 5 veces mayor que la de los demás cereales domésticos (Buckler *et al.* 2009). Con un genoma de casi 2,3 Giga pares de bases (Gpb) (Hansey *et al*. 2012) y más de 59000 genes (Yan *et al*. 2009), los mejoradores tienen grandes posibilidades para explotar esta enorme diversidad genética (Flint-Garcia *et al.* 2005). En general, el maíz presenta tres tipos de uso: alimentación animal, alimentación humana (Ganal *et al.* 2011) y bioenergía (Torney *et al.* 2007; Ganal *et al.* 2011).

El almidón es la principal sustancia de reserva sintetizada por los vegetales superiores. Constituye una fuente energética indispensable en la alimentación de los seres vivos y es la base de varias aplicaciones industriales (James *et al.* 2003). En el endospermo del maíz el almidón representa casi el 73% del peso total del grano (Whitt *et al.* 2002). El almidón se compone de dos sustancias: la amilopectina, que representa el 75%, y la amilosa, que representa el 25%.

La síntesis de amilopectina conlleva la intervención de varias isoformas de enzimas de síntesis de almidón (Starch Synthetase, SS), que favorecen la elongación de las cadenas lineales mediante uniones de tipo α-(1-4), y de enzimas ramificadoras del almidón (Starch Branching, BE) que catalizan las uniones del tipo α-(1-6) (Schultz & Juvik 2004). Además de SS y BE, Dinges *et al.* (2001) han puesto en evidencia, gracias a estudios genéticos y bioquímicos, el papel de enzimas desramificadoras de almidón (Starch Debranching, DBE) en la biosíntesis de la amilopectina en los cereales (Whitt *et al.* 2002). Numerosas mutaciones han sido descritas en la literatura como responsables de la producción de polisacáridos solubles en agua (Water Soluble Polysaccharides, WSP) o fitoglucógeno, en lugar de almidón. La acumulación de fitoglucógeno ha sido observada por Mouille *et al.* (1996); Nakamura *et al.* (1996); Zeeman *et al.* (1998) et Kubo *et al.* (1999) en *Arabidopsis Taliana* y *Chlamydomonas*. En cada especie, la acumulación de fitoglucógeno estaba correlacionada con una reducción de la actividad DBE de tipo isoamilasa (Rahman *et al.* 1998; Mouille *et al.* 1996; Zeeman *et al.* 1998).

En el maíz, hay varios mutantes que tienen una gran importancia económica porque produce cambios químicos, morfológicos y fisiológicos en el almidón. Es el caso de la mutación recurrente *sugary1* (*su1*) situada en el cromosoma 4, Bin 4.05 (James *et al.* 1995; Tracy *et al.* 2006), y que es el origen del fenotipo conocido como maíz

dulce o sweetcorn (homocigoto *su1su1*). El gen *su1* es el responsable de la acumulación de oligosacáridos y WSP que se traducen en una reducción de la amilopectina y, por tanto, de la cantidad de almidón en el grano (Dinges *et al.* 2001; James *et al.* 1995). El alelo *sugary1*, contrariamente a lo que sugiere su nombre, no aumenta excepcionalmente la cantidad de azúcar en el grano sino que incrementa mucho la de WSP. Este aumento de la cantidad de WSP confiere al endospermo homocigoto *su1su1* una textura lisa y cremosa (Marshall & Tracy 2003) característica del maíz dulce normal.

El gen *su1* está entre las primeras mutaciones estudiadas en el maíz por Correns en 1901 (Tracy *et al.* 2006). Como en el caso del maíz grano, el origen de la diversidad en el maíz dulce ha sido sujeto de varios estudios. Galinat (1971) y Mangelsdorf (1974) propusieron, basándose en datos morfológicos, que el alelo *su1* ha sido seleccionado en primer lugar en los Andes peruanos y, a continuación, introducido en maíz local. En esta hipótesis, Mangelsdorf (1974) proponen que la raza peruana *Chullpi* sea la fuente y el ancestro de la raza *Maiz Dulce*, originaria de México. La raza *Maíz Dulce*, por su parte, fue cruzada con maíz de palomitas *Reventador* para producir *Dulcillo del Noroeste*, al noroeste de México. A partir de *Dulcillo del Noroeste*, de acuerdo con la hipótesis de Mangelsdorf, el alelo *su1* fue introducido en la raza Northern Flint, que es el ancestro de todo el maíz dulce comercial actual (Revilla & Tracy 1995b). En 2006, Tracy y sus colaboradores estudiaron el origen del maize dulce con 57 poblaciones de maize dulce colectadas en seis áreas geográficas de los EE.UU., México y Perú. Los resultados moleculares contradicen la hipótesis de Mangelsdorf ya que han revelado varios orígenes independientes del maíz dulce. El material del Noreste de EE.UU. tiene una substitución del aminoácido triptófano a arginina justo en el residuo 578 (W578R). Este material representa el progenitor del maíz dulce comercial actual. Algunas poblaciones del *Dul-*

cillo del Noroeste tienen una substitución del aminoácido asparraguina a serina (N561S), lo que indica el segundo origen. Otro origen se encontró en una raza del *Maíz Dulce,* un transposon de 1.3 Kbp situado en el exon1 del gen *Su1*. El maize dulce que proviene de la zona central del Norte de EE.UU. muestra una substitución del aminoácido arginina al cisteína (R504C), lo que determina otro origen.

En los programas de mejora genética, el maíz dulce, contrariamente al maíz grano (*Su1Su1*) presenta dos grandes limitaciones. Por una parte, la base genética del maíz dulce utilizada actualmente en los programas de mejora genética es relativamente estrecha (Tracy 1994; Revilla & Tracy 1995*a*) comparada a la del maíz grano. En general, la mayor parte de las líneas de maíz dulce provienen de tres cultivares de polinización libre: Golden Bantam, Stowell's Evergreen y Country Gentleman (Tracy 1994). Por otra parte, los grupos heteróticos no están bien definidos en el maíz dulce (Revilla & Tracy 1997; Revilla *et al.* 2006*b*). Los programas de mejora no están, por tanto, basados en grupos heteróticos como en el maíz grano cuando se trata de obtener nuevos híbridos comerciales. Por ello, la elaboración y la creación de grupos heteróticos es de gran importancia para mejorar el comportamiento agronómico y ampliar el ámbito de adaptación del maíz dulce. Además, los mejoradores de maíz dulce deben ser conscientes de los riesgos de agotamiento de la heterosis si se recombinan sistemáticamente las mismas líneas sin introducir nuevas combinaciones genéticas (Revilla & Tracy 1997; Revilla *et al*. 2000*b*).

Para paliar estos problemas, se han realizado numerosos trabajos utilizando maíz grano (*Su1Su1*) como un recurso para ampliar la base genética del maíz dulce en los EE.UU. (Haber 1954; Tracy 1994; Davis *et al.* 1988). El desarrollo de Stowell's Evergreen (cruzamiento entre la variedad Menomony de la raza Southern Dent y maíz dulce)

y Golden Bantam (cruzamiento entre Northern Flint y maíz dulce) son los mejores ejemplos del éxito de la utilización de maíz grano en programas de mejora genética de maíz dulce (Tracy 1994; Revilla & Tracy 1997). Según Tracy (1994), la población de maíz dulce de polinización libre "Spanish Gold" fue creada por Jones y Singleton en 1931 mediante el cruzamiento de maíz grano *Su1Su1* de tipo liso precoz llamado Cinquantino, de origen español, y de diversas variedades de maíz dulce. Haber (1954) utilizó líneas de maíz tipo Corn Belt Dent para mejorar el vigor y la resistencia a enfermedades del maíz dulce tratando de mantener las cualidades organolépticas del maíz dulce durante la selección. Utilizando maíz grano de tipo Corn Belt Dent y tropical, Tracy (1994) constató que el maíz grano no solamente aumenta el vigor y el comportamiento de los componentes del rendimiento (número de mazorcas, peso de mazorca y tamaño de mazorca) sino también mejora caracteres ligados a la calidad (aspecto y forma de la mazorca). Por otra parte, el vigor temprano y la germinación representan los dos problemas principales de la adaptación del maíz dulce al clima atlántico europeo, así como a otras regiones que presenten primaveras frías y una corta estación de cultivo (Ordás *et al*. 1994). Los investigadores del grupo de la Misión Biológica de Galicia, Pontevedra, también han utilizado el maíz grano *Su1Su1* para aumentar la tolerancia al frío y, en general, la adaptación del maíz dulce (Cartea *et al.* 1996*a*, *b*; Malvar *et al.* 1997*a*, *b*; Revilla *et al.* 1998; Revilla *et al.* 2000*b*).

Los mejoradores de maíz dulce frecuentemente han intentado introducir el alelo *su1* en líneas *Su1Su1* en sus programas de mejora para ensanchar la base genética del maíz dulce. No obstante, a pesar del éxito de algunos programas de mejora como los citados arriba, los resultados de la utilización de maíz grano *Su1Su1* son frecuentemente indeseables, de modo que la nueva línea producida no suele ser viable como resultado

de la incorporación de algunos factores genéticos en la nueva línea de maíz dulce producida (Tracy 1990). El éxito de tales introducciones depende fundamentalmente de la viabilidad del alelo *su1* en el nuevo fondo genético *Su1* (Revilla *et al.* 2006*a*).

El alelo *su1* está considerado como un mutante letal o semiletal cuando se introduce en algunos fondos genéticos de maíz grano *Su1Su1*, donde se puede mantener únicamente en estado heterocigoto (Tracy 1994). Revilla *et al.* (2000*a*, 2006*a*, 2010) han constatado que la viabilidad o la fitness del alelo *su1* depende principalmente de los padres donante y receptor del mutante y de la interacción entre los fondos genéticos *su1su1* × *Su1Su1*. No obstante, las causas genéticas de tales variaciones en la viabilidad y la fitness del alelo *su1* en función de los genotipos implicados como padres son aún desconocidas.

Se han llevado a cabo varias investigaciones para conocer los mecanismos responsables de la variación de la fitness del alelo *su1*. Martins & Da Silva (1998) descubrieron una selección direccional contra el alelo *su1* en cruzamientos entre *su1su1* y S*u1Su1*. Revilla *et al.* (2000*a*) estudiaron la viabilidad del alelo *su1* en cruzamientos entre poblaciones de maíz grano y de maíz dulce y constataron que la frecuencia del alelo *su1* disminuía a lo largo de generaciones de recombinación y que el cruzamiento entre Corn Belt Dent (maíz grano) y Stowell's Evergreen (maíz dulce) era la combinación más favorable para el mantenimiento del alelo *su1*. Investigaciones más recientes han revelado que la disminución de la frecuencia de *su1* depende más de la interacción específica entre las líneas de maíz dulce y de maíz grano que de los grupos heteróticos involucrados (Revilla *et al.* 2006*a*).

La selección negativa contra un mutante puede generarse por dos mecanismos: la reducción de la viabilidad y la reducción de la fertilidad (Falconer 1981). Siguiendo

el ciclo de desarrollo de la planta, la selección contra el alelo *su1* comienza primeramente a nivel de caracteres de viabilidad como germinación y vigor temprano (Ordas *et al.* 2010); después se puede producir reducción de caracteres de fertilidad como fecundación o formación de grano (Cisneros-Lopez *et al.* 2010; Zhang *et al.* 2011). La viabilidad de *su1* ha sido ampliamente estudiada en las primeras fases del desarrollo de la planta. La germinación es considerada como el primer factor limitante para el desarrollo del maíz dulce, siendo el vigor temprano el siguiente factor (Martins & Da Silva 1998; Revilla *et al.* 2000*a*; Gad & Juvik 2002; Juvik *et al*. 2003; Revilla *et al.* 2006*b*). Por el contrario, pocos trabajos se han publicado sobre fases posteriores de desarrollo de la planta. Recientemente, Revilla *et al.* (2010) han comparado el comportamiento de plantas de maíz dulce y de maíz grano obtenidas a partir de cruzamientos entre tres poblaciones de maíz grano y tres de maíz dulce y no encontraron diferencias significativas para el porcentaje de plantas que llegan a estado adulto (stand), mazorcas por planta o rendimiento. Estos autores concluyeron que la humedad de grano, la altura de la planta, la longitud de la mazorca y el número de granos por mazorca podía ser considerados como caracteres asociados a la fitness del alelo *su1*.

La viabilidad del alelo *su1* depende principalmente de la interacción entre genotipos *su1su1* × *Su1Su1*. En consecuencia, tanto el padre donante de maíz dulce como el padre receptor de maíz grano deben ser escogidos con precaución para tener éxito en la conversión de maíz grano a maíz dulce. Todas las investigaciones anteriormente citadas sugieren que la fitness de *su1* está genéticamente controlada (Revilla *et al.* 2006*b*). Por otra parte, las posibilidades de que una mutación sea fijada en una población depende grandemente del fondo genético donde se encuentre y de su interacción que se traduce en efectos epistáticos entre genes (Le Gac & Doebeli 2010). En efecto, Butler (1977)

mostró en el tomate que la variación de las tasas de viabilidad de las mutaciones en función del fondo genético donde se encuentran está probablemente causada por sus asociaciones con otros genes.

Recientemente algunos investigadores han utilizado *Drosophila* como modelo genético para comprender los diferentes mecanismos que controlan el comportamiento de una mutación en una población. Yamamoto *et al.* (2009) y Magwire *et al*. (2010) han constatado que la supervivencia y la conservación de mutaciones a partir de un mismo gen dependen específicamente del fondo genético, por una parte, y de factores ambientales, por otra parte. Morton *et al.* (2006) estudiaron la variación en la dinámica de las mutaciones en maíz a nivel molecular con más de 10472 marcadores de tipo SNP (polimorfismo de un nucleótido). Estos autores han mostrado que el comportamiento de una mutación está influido por la composición en nucleótidos que la franquean. Recientemente, en maíz, y con el propósito de comprender las bases moleculares de las variaciones fenotípicas, McMullen *et al.* (2009) han creado 25 poblaciones tipo RIL (Recombinant Inbred Line) cruzando 25 líneas puras con la línea pura B73 para construir un sistema de poblaciones de mapeo de asociación anidado conocido como NAM (Nested Association Mapping). En este panel, dos poblaciones RIL se han creado a partir de dos líneas de maíz dulce: la línea P39 y la línea Il14h. Estos investigadores han observado una gran distorsión de la segregación a nivel del cromosoma 4, especialmente en el entorno del locus *Sugary1*.

A partir de todos estos trabajos de investigación mencionados anteriormente, hemos constatado que la reducción de la viabilidad y de la fitness en general no son únicamente el resultado de la mutación en si misma sino que también puede haber un control genético sobre el efecto de las mutaciones. A pesar de todo, la información dis-

ponible actualmente sobre los diferentes mecanismos genéticos y las variables agronómicas que controlan la dinámica de la mutación *su1* sigue siendo limitada. Por tanto, otros trabajos de investigación son necesarios para profundizar el conocimiento teórico. Para ello, varias cuestiones se han planteado en este trabajo: ¿Por qué la dinámica de estas mutaciones cambia en función del fondo genético? ¿Cuáles con los genes o los factores genéticos que controlan la fitness de la mutación? ¿Cuáles son los efectos genéticos de la introducción del mutante *su1* en el desarrollo y el comportamiento de la planta a lo largo de su ciclo de desarrollo? Y por último ¿Cuáles son los caracteres agronómicos limitantes para la fitness del mutante *su1*?

OBJETIVOS

Los objetivos del presente trabajo son:

1. Evaluar los efectos de diversos fondos genéticos del maíz en la viabilidad de *su1* y estimar los efectos genéticos que controlan esta viabilidad

2. Profundizar nuestro conocimiento sobre los efectos de la introducción de *su1* en el valor agronómico y determinar los caracteres más limitantes para la viabilidad bajo condiciones ambientales naturales y controladas

3. Identificar genes o regiones cromosómicas que afecten a la regulación genética de la viabilidad de *su1*.

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Chapter 2

Genetic effects on fitness of the mutant *sugary1* in wild type maize

Short title: *Genetic effects of mutant fitness*

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SUMMARY

Knowing the genetic regulation of fitness is crucial for using mutants in breeding programmes, particularly when the mutant is deleterious in some genetic backgrounds, as happens with the sweetcorn mutant *sugary1* (*su1*) in maize (*Zea mays* L.). The fitness and genetic effects of maize mutant *su1* were monitored through five successive selfing generations in two separated mean generation designs. The first involved two inbreds with similar genetic backgrounds, while unrelated inbreds were used for the second design. Parents, F_1s, F_2s, and backcrosses were crossed to P39 as the donor of *su1* and the 12 crosses were successively self-pollinated for 5 years. The *su1* frequency decreased linearly across selfing generations in both designs. Additive effects were significant for *su1* seed viability. However, dominance effects were of higher magnitude than additive effects, even though the dominance effects were not significant. Genetic effects depended on genotypes and environments. Therefore, the fitness of *su1* is under genetic control, with significant additive effects due to minor contributions of multiple genes. The fitness of *su1* is strongly affected by maize genotypic background and environment. It is hypothesized that genotypes could have evolutionary potential for modulating the fitness of single mutations.

INTRODUCTION

The fitness of a mutant is crucial from both theoretical and practical perspectives. Indeed, knowing the fitness of a mutant allows prediction of its prevalence during a species' evolution. Similarly, for economically important mutants, breeders need to know its seed viability in order to design plant breeding programmes adequately. In maize, several mutants have economic importance because they produce chemical, morphological, and physiological changes in the seed endosperm. Such is the case of the recurrent mutant *sugary1* (*su1*), located in chromosome 4, the primary gene for sweetness in maize (Tracy *et al*. 2006). The *sugary1* gene codes for an isoamylase affecting starch synthesis in maize endosperm (Rahman *et al*. 1998). Homozygous *su1* increases levels of the water soluble polysaccharides (WSP) that give *su1* endosperm the smooth texture and creaminess characteristic of traditional sweetcorn varieties (James *et al*. 2003). Sweetcorn varieties are cultivated all over the world with some restrictions in areas with cold springs and short summers. However, sweetcorn is expected to expand its cultivated area due to climate change (Ceccarelli *et al.* 2010).

Sweetcorn has some limitations from a maize breeding perspective. First, the narrow genetic base of sweetcorn limits its improvement (Haber 1954; Tracy 1990). Additionally, heterotic groups are not well-defined in sweetcorn (Revilla & Tracy 1997; Revilla *et al*. 2006*b*). The usefulness of field maize genotypes (*Su1*) to broaden sweetcorn genetic base and to improve its performance has been the focus of several theoretical studies (Haber 1954; Tracy 1990; Cartea *et al*. 1996*a*, *b*; Malvar *et al*. 1997*a*, *b*; Revilla *et al*. 1998). However, practical results can be disappointing because undesirable genetic factors could be incorporated into the new sweetcorn genotype (Tracy 1990; Revilla *et al*. 2000, 2006*a*, 2010).

The allele *su1* can be lethal or near lethal when it is introgressed into some field maize genetic backgrounds, being maintained only in heterozygotes, but it survives well enough in other genetic backgrounds. Sweetcorn lines homozygous for this allele often have nearly 1.00 germination (Tracy 1990).

The gene and genotype frequencies of *su1* and *su1 su1* individuals, respectively, were monitored throughout five successive generations of *Su1* × *su1* by Martins & Da Silva (1998); the gene frequency of *su1* was steadily reduced across generations, indicating a directional selection against the allele. Revilla *et al.* (2000) studied seed viability of *su1* in crosses between *Su1* and *su1* populations; *su1* frequency was reduced across recombinations in all crosses and interaction of S*u1* and *su1* genetic backgrounds affected seed viability of *su1* significantly: Corn Belt Dent *Su1* × Stowell's Evergreen *su1* was the most favourable combination. According to Revilla *et al.* (2006*b*), the reduction of *su1* gene frequency depends on specific sweetcorn × field maize interaction but is not related to the field maize heterotic groups.

The mutants whose seed viability shows great heterogeneity and whose seed viability values show both excess and deficiency are probably being influenced by genetic background (Butler 1977). The opportunity for a mutation to invade a population can vary dramatically depending on the context in which this mutation occurs (Le Gac & Doebeli 2010). Recent studies were carried out to understand the factors that affect variation of mutant fitness in *Drosophila*. Magwire *et al.* (2010) reported that mutations in the same gene can be associated with either an increase or a decrease in *Drosophila* life span, depending on genetic background and environmental factors. Furthermore, Yamamoto *et al.* (2009) confirmed that the size of the genetic effects in wild backgrounds was highly correlated with the size of the main effects of mutations, indicating

evolutionary potential for enhancing or suppressing effects of single mutations. These studies demonstrate that the chance of mutant seed viability can only be understood in the light of its genetic and environmental interactions.

The previous works mentioned above suggest that seed viability is not solely a function of the mutant gene but it is probably affected by genetic background effects. The genetic regulation of mutant fitness is still poorly understood, and further research should be carried out in order to identify the genetic factors underlying *su1* fitness. The aims of the present research were: 1) to evaluate the effect of diverse field maize genetic backgrounds on *sugary1* fitness, and 2) to estimate the genetic effects on *sugary1* fitness.

MATERIALS AND METHODS

Four field maize inbred lines were used that differentially affected seed viability of *su1* individuals (Revilla *et al*. 2006*b*): A632 and EP42 are field maize parents previously identified as having higher seed viability of *su1* than A661 and A619. Inbred lines belong to diverse heterotic groups (Table 1).

Two separate designs of mean generation analyses were developed to analyse genetic effects (Mather & Jinks 1982). One of the designs involved two inbreds from the same genetic background (Corn Belt): A619 and A632, while unrelated inbreds were used for the second design: A661 (Corn Belt) and EP42 (European Flint). Crosses between each pair of inbred lines started in 2001. Crosses were self-pollinated and backcrossed, obtaining six generations per design: the two parents, F_1, F_2, BC_1, and BC_2. The 12 entries were crossed with P39 as donor of *su1* allele.

All crosses to P39 were successively selfed in 2006, 2007, 2008, 2009, and 2010 in Pontevedra, Spain (42° 24', 8' 38'N, 20 m asl), a location in the northwest of Spain where annual rainfall is in the range of 1600–1700 mm. Around 100 plants from each of the 12 entries were self-pollinated by hand, harvested, and conserved in bulk. From each bulk, a sample of 150 kernels was sown in blocks 3 m long, spaced 0.8 m apart; plants within the row were 0.20 m apart. The self-pollination generations were repeated up to 5 years.

The frequency of mutant *su1* kernels was determined in three samples of 500 kernels from each bulk. The allelic frequency *q* of *su1* was calculated as the square root of the frequency of the homozygote kernels in the first selfed generation, while for the other generations the frequency was calculated as follows:

$$q= [(N\times2) + C\times(1500-N)]/3000$$

where N is the observed number of *su1* kernels in the total of 1500 kernels, C is the proportion of individuals with one *su1* among the non- *su1* kernels (2/5, 2/9, 2/17 and 2/33 for the 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} selfed generations, respectively).

Linear and quadratic regressions of the gene frequency on the number of selfed generations were computed for each cross. The coefficients of regression (*b*) were tested for homogeneity. For each selfed generation, the expected number of *su1* kernels was calculated from the number of the *su1* kernels in the previous generation and was compared to the observed number using the χ^2 test. In order to estimate fitness, the selective value (*s*) of *su1* was calculated as 1 minus the proportion of the contribution of offspring to the next generation (1 – *s*) (Falconer 1981). In order to estimate the effects of the proportion of unfavourable genotypes on the fitness of *su1*, A619 × A632 and

EP42 × A661, one unfavourable and one favourable parent were defined within each cross, concerning the effects on the fitness of *su1* when this mutant is introduced through backcrosses. From these crosses the respective F_1, F_2, BC_1, and BC_2 were developed, which carried different proportions of the genome of the unfavourable parent: P_1=1, P_2= 0, F_1=0.50, F_2= 0.50, BC_1= 0.75 and BC_2= 0.25. Regression analyses of the selective value (*s*) along the successive generations on the proportion of unfavourable genotypes were carried out for each design and across designs.

For the mean generation analyses, the coefficients of regression were used as an estimator of *su1* seed viability and each design was considered a separate experiment. Data from each generation were subjected to regression analyses. Adjustment of the generation means to a genetic model was tested with a χ^2 test. The test was applied to the simplest model and, if it revealed a lack of fit, the next model was tried. The models considered were the following: a model with only the mean, an additive model, an additive-dominance model, an additive-dominance model with epistasis, an additive-dominance model with environmental effects and interactions and, finally, an additive-dominance model with both epistasis and environmental effects and interactions. The genetic parameters estimated were m = mean, a = additive effect, d = dominance effect, aa = additive × additive effect, ad = additive × dominance effect, and dd = dominance × dominance effect (Mather & Jinks 1982; Kearsey & Pooni 1996).

The variance of the generation means (s^2_i) are not the same; this heterogeneity among variances was adjusted in the analyses by weighting the means differently; these weights being the reciprocals of the squared standard errors (Mather & Jinks 1971). In addition, weight was taken into account when solving for m, a, d, aa, ad and dd. For the six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) the solution is obtained in the form of

a matrix by the SAS (SAS Institute 2005) statistical package using PROC IML as follows:

$$X= (C' \times W \times C)^{-1} \times (C' \times W \times Y)$$

where Y = 6 × 1 vector of generation means, C = 6 × z matrix of coefficients, z depending on the genetic model, W = 6 × 6 diagonal matrix weight, C' = the transpose of C matrix and $^{-1}$ represents the inverse of a matrix.

RESULTS

In both designs, A661 × EP42 (Table 2) and A619 × A632 (Table 3), the reduction of *su1* frequency across self-pollination generations was less important in the first generation. In the first design, the frequencies in all generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) decreased linearly and showed a continuous and pronounced fall from 0.534 to 0.270 and from 0.491 to 0.161 in A661 and EP42, respectively (Fig. 1*a*). The same tendency was observed for the second design, i.e. there was a reduction of *su1* frequency from 0.466 to 0.051 in A619 and 0.504 to 0.171 in A632 (Fig. 1*b*). The χ^2 test for comparison between observed and expected number of *su1* kernels revealed significant differences for almost all crosses across self-pollination generations (data not shown).

For both designs, the coefficients of regression were all negative and significantly different from zero. The coefficients of determination were above 0.90, except for EP42 and the segregating population F_2 involving A619 and A632. The quadratic regression was not significant in any case (data not shown). The reduction on frequency of *su1* for EP42 fluctuated more across years than for A661. However, the behaviour of A619 and A632 concerning *su1* fitness was more stable across years.

The coefficients of regression for each generation in the first design were not homogeneous; seed viability of *su1* mutant was higher for A661 than that for the backcross to EP42 with $b = –0.065$ and $b = –0.097$, respectively. Regression coefficients for EP42, F_1, F_2 and the backcross of A661 were not heterogeneous; however, the backcross of A661 had the most unfavourable coefficient ($b = –0.086$) for *su1* seed viability. In addition, the coefficients of regression for F_1 ($b = –0.078$) and F_2 ($b = –0.075$) were higher than the coefficients for both parents.

The coefficients of regression in the second design were not heterogeneous; therefore, the frequency of the *su1* mutant was similarly reduced across the five self-pollination generations. All genotypes have negative selection against *su1* and the highest reduction was for A619 ($b = –0.109$). The comparison between the four inbred lines employed in the present work show that the parent least favourable for *su1* seed viability was A619 and the best was A661.

The selective value *s* of *su1* is also used as an estimator of seed viability and differed between parents and derived populations. The selection against *su1* occurs in the same direction in both designs (Table 2, Table 3). The coefficient of selection *s* varied from 0.130 to 0.397 in the first design and from 0.193 to 0.519 in the second design.

The selection effect against *su1* depended on the field maize genotype. EP42 ($s = 0.235$) had a larger negative selection effect against s*u1* than A661($s = 0.130$), but differences were not significant. However, the selection effects in F_1, F_2, BC_1, and BC_2 were above the values in the parents. For the second design, A619 ($s = 0.519$) was worse than A632 ($s = 0.213$).

Mean generation analysis was used to determine the genetics effects and their type of action on *su1* fitness using the coefficients of regression as parameters of seed viability. As shown above, the differences among generations were significant only in the first design (A661 × EP42) and, for this reason; only the genetic parameters were estimated for this cross. The mean generation analysis has shown that the additive model explained adequately the variation observed ($\chi^2_{(4)} = 6.75$, with $P = 0.149$) with m = –0.08±0.004 and a = 0.013±0.005.

The coefficient of selection against the mutant showed a tendency to increase along the selfed generations. The combined-over-designs regression analysis and both individual analyses for each design showed that the coefficient of selection of the fifth generation of inbreeding (*s*5) was consistently significant. In addition, coefficients of selection were significantly affected by the contribution of unfavourable genotypes, particularly in the second self-pollination generation (*s*2) in the combined analysis and in the first design (Table 4).

DISCUSSION

It is generally assumed that most mutants are less fit than the wild type. Accordingly, the *su1* frequency was reduced steadily across five self-pollination generations in both designs. The present results confirm previous reports showing that *su1* allele is less viable than the wild type (Tracy 1990; Martins & Da Silva 1998; Revilla *et al*. 2000).

The tendency of *su1* gene frequency reduction occurred in both designs but with different intensities depending on the field maize inbred lines involved, as previously reported by Revilla *et al*. (2006*a*, 2010). Three background types were used in the present study; A619 and A632 were released from Lancaster and Reid (Corn Belt Dent race), while A661 was released from Corn Belt germplasm different from Reid or Lan-

caster and EP42 comes from a European flint population genetically distinct from the American germplasm pools. Seed viability of *su1* mutant was lower in the Reid and Lancaster inbreds than in the cross involving inbreds with different genetic backgrounds, A661 and EP42. These dynamic aspects of the *su1* gene frequency have been reported by Revilla *et al*. (2006*b*, 2010), who concluded that seed viability of s*u1* gene depends on specific *su1* × field maize interactions. Sweetcorn breeders are aware of the unfavourable effects of base germplasm on *su1* seed viability (Tracy 1990) and the present results suggest that it could be worthwhile searching for favourable field maize genotypes for sweetcorn breeding outside the current sources, for example, in hard endosperm maize (Alonso Ferro *et al.* 2008). However, previous results are not encouraging, at least for European germplasm (Cartea *et al*. 1996*a*, *b*; Malvar *et al*. 1997 *a*, *b*; Revilla *et al*. 1998)

A net selection was revealed acting against *su1*, with the fitness of the mutant allele highly dependent of the field maize inbred line, being A619 less unfavourable than the other field maize inbred lines. Selection against *su1* may operate firstly through factors related to seed viability (germination, early vigour, etc.) (Ordas *et al.* 2010) and after that, by factors related to pollination and grain formation or fertility in general (Cisneros-López *et al.* 2010; Zhang *et al.* 2011). Martins & Da Silva (1998) found that germination and gametophytic factors might be involved in the reduction of the fitness of *su1*. In the present experiment, all plants in the first generation were heterozygous (*Su1*/*su1*) and there was no selection in favour or against *su1* until the production of gametes, because all plants had wild phenotype. For that reason, the change in frequency in the first generation of selfing was only due to fertility factors. It was found that the reduction of *su1* frequencies in the first generation was not important and for some gen-

otypes *su1* frequency was above the expected value, which indicates that gametophytic or fertility factors were probably of small importance for the fitness of the *su1* allele in the present experiment. Therefore, the important reduction of the mutant frequency that was found after the first generation was probably due to a lower seed viability of the mutant allele compared to the wild type.

The coefficient of selection (*s*) against the *su1* mutant increased along the selfing generations, i.e. selection intensity was lowest in the first generation and highest in the last. This can be attributed to the increased homozygosity. The fitness of *su1* was probably variable due to environmental factors. All results suggest that fitness depends on environmental circumstances but these effects do not hide the genetic effects. Although it is not possible to state unequivocal conclusions concerning the environmental effects on *su1* fitness, the present results and previous observations suggest that there is an important genotype × environment interaction on *su1* fitness.

Mean generation analysis was carried out to identify genetic effects on the fitness of *su1*. The additive effect was the most important for *su1* seed viability in the crosses involving A661 and EP42, which belong to different groups of germplasm. In contrast, genetic effects were not significant for the genotypes involving the Corn Belt inbred lines A619 and A632. This material manifests a more consistent and stable behaviour than the first design on *su1* fitness probably due to the lack of genetic variation between the two inbred lines because of the similar genetic origin.

In congruence with the additive effects of the genotypes on the fitness of *su1*, it was found that the variation of the proportion of the unfavourable genotype explains a considerable part of the variation observed for the selective value (*s*), particularly in the last generation where the mutant is expected to have the highest exposure as a conse-

quence of homozygosity advance. These results suggest that the fitness of a mutant depends on the genetic background in general rather than on single genes. Magwire *et al.* (2010) used *Drosophila* as a model system to provide an explanation of genetic and environmental factors that affect variation in life span and senescence, concluding that variations observed were especially due to genetic background and epistatic effects. The present research also confirms the suggestion of Yamamoto *et al.* (2009) that the variation in the magnitude of the genetics effects among the wild genetic backgrounds could have evolutionary implications. Furthermore, the ability of genotypes for moderating the fitness costs of new genetic variants has been hypothesized by Raymond *et al*. (2011). Altogether, these evidences and the current data suggest that genotypes could modulate the fitness of new mutations.

In conclusion, the present results confirm that the fitness of *su1* is under genetic control with significant additive effects which are probably due to minor contributions of multiple genes. It is proposed that the interaction of genetic backgrounds with alleles could have evolutionary implications by increasing or decreasing the change of mutant fixation.

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Table 1. Pedigree and germplasm types of the field maize inbred lines homozygous for *Su1* and the sweetcorn genotype used as donor of *su1*.

Genotype	Pedigree	Germplasm type
Sweetcorn		
P39	Golden Bantam	Golden Bantam
Field maize		
EP42	Tomiño	Northern Spain
A619	(A171 × Oh43)Oh43	Lancaster
A632	(Mt42 × B14)B14^{3}	Reid
A661	AS-A	U.S.A. synthetic variety

Table 2. Frequencies of *su1* through five selfing generations of crosses between the *su1* inbred P39 and six basic generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from crosses between two field maize inbred lines, coefficient of regression (b ± s.e.), coefficient of determination (R^2), and selective value (s ± s.e.).

Generations	A661	EP42	F_1	F_2	BC_1	BC_2
1	0.534	0.491	0.458	0.474	0.458	0.503
2	0.454	0.353	0.423	0.354	0.319	0.420
3	0.368	0.370	0.256	0.237	0.250	0.284
4	0.328	0.308	0.253	0.234	0.170	0.176
5	0.270	0.161	0.150	0.155	0.099	0.137
b	–0.065±0.004	–0.07±0.015	–0.08±0.011	–0.08±0.012	–0.09±0.007	–0.10±0.009
R^2	0.97 ($P < 0.001$)	0.83 ($P = 0.019$)	0.91 ($P < 0.01$)	0.90 ($P < 0.01$)	0.97 ($P < 0.001$)	0.96 ($P < 0.001$)
s	0.130±0.0071	0.24±0.118	0.27±0.098	0.29±0.095	0.40±0.087	0.29±0.071

Table 3. Frequencies of *su1* through five selfing generations of crosses between the *su1* inbred P39 and six basic generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from crosses between two field maize inbred lines, coefficient of regression (b ± s.e.), coefficient of determination (R^2), and selective value (s ± s.e.).

Generations	A619	A632	F_1	F_2	BC_1	BC_2
1	0.466	0.504	0.476	0.453	0.479	0.483
2	0.365	0.452	0.466	0.416	0.423	0.397
3	0.190	0.380	0.310	0.240	0.310	0.250
4	0.106	0.188	0.277	0.176	0.261	0.159
5	0.051	0.171	0.218	0.158	0.131	0.149
b	–0.109±0.011	–0.09±0.014	–0.07±0.011	–0.08±0.014	–0.09±0.007	–0.09±0.013
R^2	0.95 ($P < 0.01$)	0.91 ($P < 0.01$)	0.90 ($P < 0.01$)	0.88 ($P = 0.011$)	0.97 ($P < 0.001$)	0.91 ($P < 0.01$)
s	0.519±0.113	0.21±0.104	0.19±0.071	0.29±0.081	0.28±0.083	0.29±0.096

Table 4. Significant regressions (b ± s.e.) of the selective value (*s*) on the proportion of the unfavourable genotype in six basic generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) across five selfing generations of crosses between the *su1* inbred P39 and two pairs of field maize inbred lines.

Analysis	Parameter	*b*	Adjusted R^2
Combined	*s*1	0.17±0.068 (*P* = 0.032)	0.32
	*s*2	0.24±0.118 (*P* = 0.069)	0.22
	*s*5	0.53±0.106 (*P* < 0.001)	0.68
EP42 x A661	*s*2	0.33±0.151 (*P* = 0.095)	0.43
	*s*5	0.37±0.060 (*P* < 0.001)	0.88
A619 x A632	*s*5	0.69±0.174 (*P* = 0.016)	0.75
	s (averaged)	0.24±0.110 (*P* = 0.094)	0.43

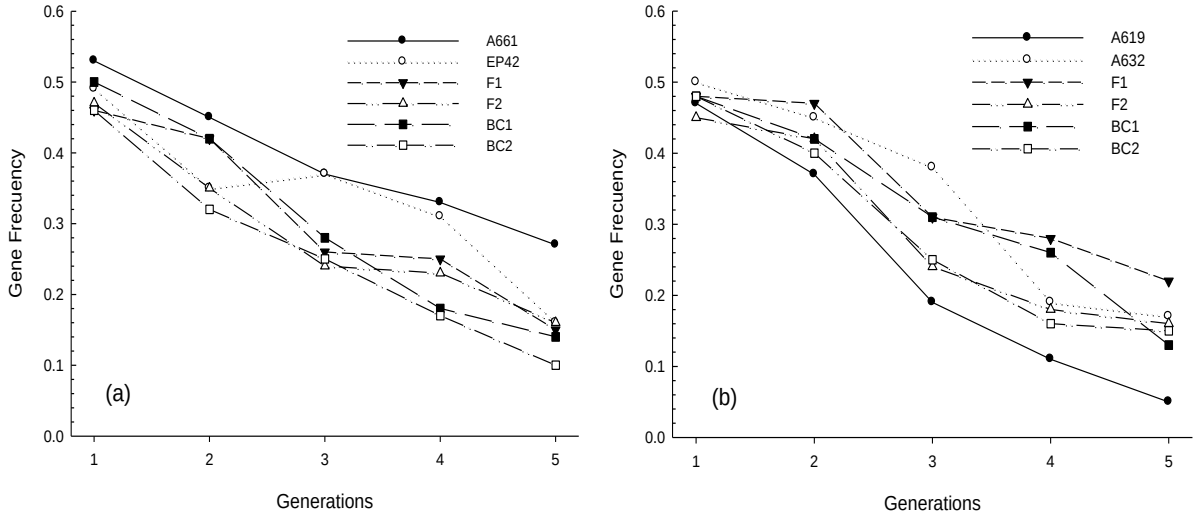

Figure 1. Change of gene frequency across five selfing generations of crosses between the *su1* inbred P39 and six basic generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from crosses between two pairs of field maize inbred lines: (a) First design, (b) Second design.

CHAPTER 3

Genetic effect of the introgression of *su1* in the genetic regulation of agronomic traits

3.1. Genic effects of the critical factors of *sugary1* fitness

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SUMMARY

Emergence and seedling vigor are the most important agronomic traits in determining the *sugary1* (*su1*) fitness. This study was designed to estimate the effects of the mutant *su1* on the genetic regulation of both fitness-related traits when introgressed into field corn backgrounds. Estimated genetic effects of agronomic traits in *Su1 vs*. *su1* plants were monitored in two separated mean generation designs. The first involved two Corn Belt inbred lines A619 and A632, while unrelated inbred lines EP42 and A631 were used for the second design. Parents, F_1s, F_2s, and backcrosses were crossed to the *su1* inbred P39 as the donor of *su1* and the 12 crosses were successively self-pollinated for 5 years. For each cross, *Su1* and *su1* kernels were evaluated separately in the growth chamber under controlled environmental conditions following a randomized complete block design. In addition, the genotypes were evaluated in field trials in 10 × 10 triple lattice designs. The performance of *su1* plants was lower when compared to the *Su1* plants for all traits evaluated in both designs and across environments. The estimates of genetic effects of *Su1 vs*. *su1* plants were strongly affected by genes and environments. The results suggest that, independent on specific sweet x field corn interaction, seedling vigor, and particularly chlorophyll content (CCM), was the most critical trait in determining *su1* plants viability. However, the complexity of the genetic regulation of emergence and the great heterogeneity of environmental conditions in the field evaluation prevent the estimation of its genetic regulation on *su1* fitness.

INTRODUCTION

Sweetcorn (*Zea mays* L.) has some limitations from a maize breeding perspective. First, the narrow genetic base of sweetcorn limits its improvement (Haber 1954; Tracy 1990*b*). Additionally, heterotic groups are not well-defined in sweetcorn (Revilla & Tracy 1997; Revilla *et al*. 2006*b*). Field maize genotypes (*Su1*) have been frequently used for broadening sweetcorn genetic base and for improving its agronomic performance, particularly the fitness limiting traits emergence and early vigor (Haber 1954; Tracy 1990*b*; Cartea *et al*. 1996*a*, *b*; Malvar *et al*. 1997*a*, *b*). However, practical results can be disappointing because undesirable genetic factors could be incorporated into the new sweetcorn genotypes (Tracy 1990*a*; Revilla *et al*. 2000, 2006*a*, 2010). The importance of the field corn background effect on *su1* fitness is the lethality or near lethality when it is introgressed into some field corn germplasm (Tracy 1990a). Revilla *et al*. (2000, 2006*a*, 2010) also reported that the viability of *su1* mutant depends on specific sweet corn x field corn genotype interaction and genetic background plays a major role in the viability of this mutant. However, little is known about the genetics causes of the differences in the fitness rates of *su1* when different field corn genotypes are used as recurrent parents in breeding programs. Djemel *et al*. (2011, 2012) reported that fitness of *su1* was under genetic control and depends on many genes with small effects on a variety of viability-related traits throughout the genome.

In the areas with cool and humid springs and relatively short summers such as the North Atlantic areas of Europe and America, the cultivation of sweet corn is limited by their reduced emergence and poor seedling vigor (Cartea *et al.* 1996*a*, *b*, Malvar *et al.* 2007*a*, *b*). When the recessive mutant *su1* is segregating in a maize breeding population, the selection against mutant's acts first through viability (germination, early vigor) (Ordás *et al*. 2010), then

through fertility (mating ability and grain formation) (Cisneros-López *et al.* 2010; Zhang *et al.* 2011). The viability of *su1* plants has been investigated mainly at first stages of plants growth. The germination is the first limiting factor and the next important factor is early vigor (Martins & Da Silva 1998; Revilla *et al*. 2000; Gad *et al*. 2002; Juvik *et al.* 2003; Revilla *et al*. 2006*a*). Revilla *et al*. (2010) compared in different maize genetic backgrounds the performance of plants carrying *su1* in order to choose the most appropriate field corn varieties for improving the agronomic performance of sweet corn. In this study, early vigor was the most limiting factor for *su1* plant development. However, little is known about the genetic effect of *su1* in the genetic regulation of both germination and early vigor when introgressed in field corn. Tracy (2001) also reported that these two characters are affected by genetic factors in sweet corn, both at planting and during seed production.

All of the previous works mentioned above suggest that *su1* viability is not solely a function of the mutant *su1* but it is probably under genetic regulation and both germination and early vigor were of great importance in conditioning the *su1* fitness. The aims of the present research were: (1) to investigate the effect of the introgression of *su1* in the genetic regulation of these fitness-limiting factors and (2) to determine the effect of germination and early vigor on *su1* fitness.

MATERIALS AND METHODS

Plant Material

Two separate designs of mean generation analyses were developed to analyze genetic effects (Mather & Jinks 1982). One of the designs involved two field corn (*Su1Su1*) inbreds from the same genetic background (Corn Belt): A619 and A632, while unrelated field corn inbreds were used for the second design: A661 (Corn Belt) and EP42 (European Flint).

Crosses between each pair of inbred lines started in 2001. Crosses were self-pollinated and backcrossed, obtaining six generations per design: the parents P_1 and P_2, F_1, F_2, BC_1, and BC_2. The 12 entries were crossed with the sweet corn (*su1su1*) inbred P39 as donor of *su1* allele. All crosses to P39 were successively selfed in 2006, 2007, 2008, 2009 and 2010; producing 30 genotypes for each design. To investigate the effect of the *su1* introgression on the genetic regulation of fitness-limiting factors (emergence and early plant development); all these materials cited above were evaluated for both traits in controlled growth chamber under both cold and standard conditions. In addition, the genotypes were evaluated in the field in order to confirm the results from the growth chamber.

Growth chamber trial

For each genotype, the kernels were randomly sampled, and *su1* and *Su1* kernels were separated for further evaluation in the growth chamber. Therefore, 60 genotypes were produced for each design, consisting in 30 *su1* genotypes and 30 *Su1* genotypes. The 120 entries plus the parents' inbred lines involved in the two designs were evaluated in plastic multi-cell seed trays (each seed tray consists of 104 alveoli or 8 x 13 mm) in 2011 at Pontevedra (Spain). The experimental design in each of the two experiments (standard and cold treatments) consisted on 15 multi-cell seed trays followed a randomized complete block with three replications (5 multi-cell on each replication). In the growth chamber, the evaluations were carried out for a period of 30 days. Each experimental plot consisted of 4 kernels per row and one kernel per alveoli. Sowing depth was approximately 2 cm. All trays were watered with 12 liters every week. The two designs (EP42 × A661 and A619 × A632) were evaluated in a growth chamber under standard conditions and a posterior evaluation was made in the same growth chamber but applying cold temperature. The conditions were set at

14 h with light (provided by seven very high-output (VHO) fluorescent lamps with a photosynthetic photon flux (PPF) of 228 µmol/m^2/s) at 14 °C and 10 h without light at 8 °C for cold treatment. However, under standard conditions, the temperatures were 25 °C and 18 °C in light and dark, respectively. The following data were recorded: proportion of emergence, and three vigor-related traits: early vigor (taken on the five-leaf stage with a visual scale from 1= weak plant to 9= strong plant); leaf chlorophyll content measured at the three-leaf stage using a hand-held Chlorophyll Content Meter, CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA), and finally, plant weight was taken at the end of each experiment.

Combined analyses of variance of the data set were conducted using the General Linear Model (GLM) procedure of SAS (SAS Institute, 2005). The sources of variations were divided into effect due the environments, treatments (years of selfing, generation, gene and the respective interactions) and the environments × treatments interactions. The sums of squares for each partition of the environments x treatments interactions were initially tested with the sum of squares of the pooled error, if the *F-test* was significant, all of the sums of squares of the treatments were tested with their corresponding environments x treatments interaction; if not, the sums of squares were tested with the sum of squares of the pooled error (Cochran & Cox, 1992).The analyses of variance were carried out using SAS (SAS Institute 2005).

Field trial

For each genotype (except those produced in 2010), the *su1* and *Su1* kernels were separated for further evaluation in the field as mentioned above. The field trials were sown in 2010 and 2011 at Pontevedra (42° 24’N, 8’ 38’W, altitude 20 m a.s.l.), a location in the

northwest of Spain where annual rainfall is in the range of 1600–1700 mm; and in 2010 at Algiers (36 º 47 ' N, 2 º 03' E, altitude 32 m a.s.l.), located in the sub humid North of Algeria with 900 mm of annual rainfall. Each experimental plot consisted of two rows with 25 kernels per row and one kernel per hill. The rows were spaced 0.80 m apart, and the hills were spaced 0.12 m apart. The hills were thinned after emergence to obtain a final density of 75 000 plants ha^{-1}. Appropriate techniques for cultivation were carried out according to local practices. Harvesting was manual in both locations. The following data were recorded: proportion of emergence, and three vigor-related traits: early vigor (taken on the five-leaf stage on a visual scale from 1= weak plant to 9= strong plant), was taken on each plot at Pontevedra and Algiers; leaf chlorophyll content measured at vegetative stage using a hand-held Chlorophyll Content Meter, CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA) and plant weight (g) at the five-leaf stage were taken only at Pontevedra.

Combined analyses of variance were made using the adjusted means obtained from the lattice analysis (*Proc lattice* procedure) of each environment, when the efficiency of the lattice design was above 105%, otherwise, the traits were analyzed as randomized complete block designs. The sources of variations were divided into effect due the environments, treatments (year of selfing, generation, gene and the respective interactions) and the environments x treatments interactions as mentioned above (see growth chamber trial).

Generation mean analysis

For both growth chamber and field trials, the mean generation analyses were carried out by using the adjusted means. Generation mean for each trait, showing significant differences among generations in the analysis of variance, was calculated from each year of

selfing individually. Each design was considered a separate experiment. The equations for the generation means' analysis of the various generations employed in this study are:

P_1=m + a + aa,

P_2=m – a + aa,

F_1=m + (½)^n d,

F_2=m + ½(½)^n d,

BC_1= m + ½ a +½(½)^{n}d + (¼) aa + ¼ (½)^{n}ad,

BC_2= m - ½ a +½(½)^n d+ (¼) aa - ¼ (½)^{n}ad ,

Were n= the number of year of selfing.

The adjustment of the generation means to a genetic model was tested with a χ^2 test. The test was applied to the simplest model and, if the χ^2 revealed lack of fit, the next model was tried (Mather & Jinks 1982; Kearsey & Pooni 1996). The models considered were: a model with only the mean, an additive model, an additive-dominant model, an additive-dominant model with epistasis additive × additive, and an additive-dominant model with epistasis additive × dominance. The genetic parameters estimated were m=mean value, a=additive effects, d=dominance effects, aa= additive × additive epistatic effects and ad=additive x dominance effects (Mather & Jinks 1982). For the six basic generations (P_1, P2, F_1, F_2, BC_1 and BC_2) the solution is obtained in the form of a matrix by using PROC IML of SAS (SAS Institute, 2005) as follows:

$X = (C' \times C)^{-1} \times (C' \times Y)$

Where Y= 6×1 vector of generation means,

C= the matrix (6×z), when z depends on the genetic model,

C′ is the transpose of C matrix and $^{-1}$ represents the inverse of a matrix. The standard error associated to each estimate of a genetic effect was obtained as the diagonal items in the

variance-covariance matrix $(C' \times C)^{-1}$. Significance of each genetic effect estimate was evaluated as described by Kearsey & Pooni (1996), utilizing a *t-test*; if the χ^2 of the model was not significant the *t-test* = |estimate of a genetic effect| / (their corresponding standard error). The standard error was multiplied by the square of the χ^2 / (degrees of freedom) if the $X^2$of the model was significant.

RESULTS

In the growth chamber, there were not significant differences between generations excepted for CCM in the first design. However, all traits except plant weight showed significant differences between generations in the second design (Table 1). The differences between genes were highly significant for all traits in both designs. The differences between years of selfing were significant for most traits excepted for emergence in the first design and CCM in the second one. In both designs, differences between environments were highly significant for all traits. The environment × treatment interactions were seldom significant except for environment × gene. The interactions were more important in the first design (EP42 × A661) than in the second design (A619 × A632). The environment × treatment interactions were often of rank rather than of magnitude (see supporting information) and the interactions involving the genes were the most important.

In the field trials, both designs behaved diversely in the combined analyses of variance. Indeed, only CCM was significant between generations for the first mean generation design (EP42 × A661) while only early vigor was not significant for the second design (A619 × A632) (Tables 2 and 3). Moreover, differences between years of selfing were only significant for CCM in the first design and for all traits except CCM in the second design. Contrarily, genes (*Su1 vs*. *su1*) differed significantly for emergence and plant weight in both

designs. In general, the environments × treatment interactions were seldom significant and were more important in both designs for emergence and early vigor.

Estimates of genetic effects from generation mean analysis according to a 5-parameter model and the chi-square (χ^2) values are presented separately for each environment and year of selfing. Only traits showing significant differences between generations or environment × generation interaction were used for calculating genetic effects. In addition, the environment × gene interaction was almost significant for all traits in both designs, and for those reasons we analyzed environments separately. In general, the performance of the *Su1* plants was higher than that of the *su1* plants in both chamber and the field trials (see supporting information).

The observed values of chi-square (χ^2) values and the estimates of genetic effects for both designs evaluated in the growth chamber are given in Table 4 and 5. The χ^2 values for early vigor and plant weight were not significant and the "m model" fit adequately to the data in all environments (data not shown). Almost all emergence data fits the additive - dominance model with both epistatic interactions. However, the estimates of genetic effects and their significance were strongly affected by gene, year of selfing and environment for CCM. All models: "m", "m + a", "m + a + d", "m + a +d + aa" and "m + a +d + aa + ad" were detected in both designs. In general, the absolute values of dominance (d) gene effects were much higher than the additive (a) gene effects. Moreover, the absolutes values of the epistatic "ad" gene effects were largest than the "aa" epistatic effect. CCM exhibited a significant genetic effects and this variation was strongly affected by genes (*Su1 vs. su1*). In the first design, data were adjusted to the additive × dominance model with both epistatic interactions in the plants carrying the *su1* allele, compared to the data of plants carrying the wild type that were adjusted to the "m" model or to the additive model (in the first, third and fifth year of selfing

in the cold treatment, and, in the second year of selfing in the case of standard treatment). Moreover, in the second design, we found the same behavior in the genetic regulation of CCM in the second and third year of selfing under standard treatment. However, the data of plants carrying the wild type were adjusted to the additive × dominance model with epistatic interactions and the data of the plants carrying the *su1* allele fitted the "m" model in the third and fifth year of selfing in the cold treatment.

The observed values of chi-square (χ^2) and the estimates of genetic effects for the first design (EP42 × A661) in the field are given in table 6. For all years of selfing; the χ^2 values for early vigor and plant weight were not significant and the "m model" fit adequately to the given data in all environments as in the growth camber (data not shown). Besides additive and dominance effects, both digenic epistatic interactions were presented for emergence and CCM. Moreover, the χ^2 values were significant or not, depending on genes (*Su1 vs*. *su1*) and environments. All data were adjusted to the additive or additive × dominance model with both epistatic interactions except in the fourth year of selfing, which data fit the additive-dominance model interactions.

The relative magnitudes of dominance (d) gene effects were in general much higher than those of additive (a) gene effects. The absolute and the relative magnitudes of the epistatic "ad" gene effects are variable depending on the traits and are largest than the "aa" epistatic effect. However, the magnitude of the total epistatic effects was more important than the additive or dominance effects. Moreover, the negative or positive effects of "a", "d" and "aa" were highly depending on gene (*Su1 vs*. *su1*) and environments.

The observed values of χ^2 and the estimates of genetic effects for the second design (A619 × A632) are given in table 7. As expected in the first design, early vigor and plant weight data were adjusted to the "m" model (data not shown). The remaining traits

(emergence and CCM) data fit the additive-dominance model with both epistatic interactions. The additive and dominance effects were significant in all cases. However the positive or negative signs of the effects and their significance were strongly affected by gene, year of selfing and environment. Both epistatic gene effects were detected in almost all environments and genes and were significant in almost all cases. The absolute and relative magnitudes of the dominance effects were more important than the absolute and relative of the additive effects, respectively.

DISCUSSION

The performance of *su1* plants differed from that of *Su1* plants mainly at the first stages of development. Both emergence and seedling vigor are the most limiting agronomics traits for *su1* plants viability (Martins & Da Silva 1998; Revilla *et al.* 2000, 2006*a*) and Tracy (2001) also reported that these traits are affected by genetic factors, both at planting and during seed production. The *su1* fitness is under both genetic and environmental control and, understanding the genetic and environmental factors that affect variation in the fitness of *su1* mutation and of mutations at large is of major interest for breeding programmers and evolutionary biology.

The performance of *su1* plants was lower than that of the *Su1* plants for all trait evaluated in both designs and across environments. The significant differences between *su1* and the wild type allele *Su1* for all traits in growth chamber and, for both emergence and plant weight, in field trial confirms that emergence and vigor-related traits (early vigor score, plant weight and CCM) were of great importance in determining the *su1* fitness. These results agree with previous reports showing that *su1* is less viable than the wild type (Martins & Da Silva 1998; Revilla *et al.* 2000). The lack of significant differences between generations and

between genes (*su1 vs. Su1*) for early vigor in the field can be attributed to the high environmental effect.

Environmental interactions, especially with genes, were important and this is the second reason why environments were analyzed individually. Although variations depended on the design, environments and traits, in most cases the variation in the generation means did not fit a simple epistatic model (additive-dominance and digenic epistatic model), which indicated that the genetics underlying both emergence and CCM traits was complex and others parameters are involved. This was true for all environments and for both genes (*Su1 vs. su1*). Furthermore, both emergence and CCM traits are of great importance on *su1* fitness and viability. Early vigor and plant weight fitted the "m" model in both designs, and this suggests that both traits did not explain *su1* fitness in our present study. However, Revilla *et al.* (2000, 2010) concluded that early vigor is one of the fitness-related traits in the crosses between field and sweet corn.

Epistatic effects were also important in the basic genetic regulation of both fitness-related traits (emergence and CCM Traits). For other types of maize, Alonso Ferro *et al.* (2008) reported that the epistatic effects are not important for agronomic and quality traits. However, the magnitude and significance of the estimates for "aa" and "ad" were strongly affected by environments and genes (*Su1 vs. su1*). Furthermore, the introgression of *su1* increased the importance of epistatic effects compared to the wild type.

The effects of *su1* vs. *Su1* on genetic effects were strongly influenced by environments. In almost all cases, the estimated effects of emergence fit the epistatic model in both controlled environments and field. However, the estimated effects of CCM were the only stable across environments in field trials. These results suggest that the genetic regulation of emergence is more complex than that of CCM and uniformity of environmental conditions

were of great importance in order to estimate the genetic effect of *su1 vs. Su1* on agronomic traits.

The variation of *su1* gene frequency have been reported by Djemel *et al.* (2011) who concluded that the fitness of *su1* was under genetic control with significant additive effects probably due to minor contributions of multiple genes and was strongly depended on specific sweet × field maize interactions. Some changes were shown in the genetic control of fitness-related traits when *su1* was incorporated into field corn. Only the genetic regulation of CCM was showed strongly altered in both designs and in both growth chamber treatments (cold and standard). Differences between *Su1 vs. su1* for genetic regulation suggest that CCM has consistent significant effects on *su1* fitness. These results have not been previously reported.

The variation of the significance and magnitude of the genetic effect across years of selfing depended on environments and genes (*Su1 vs. su1*). The influence of the *Su1* and *su1* genes on the estimation of the genetic effects was similar for emergence both in the growth chamber and in the field, and for CCM in the field. Moreover, in order to release sweet corn genotypes with high agronomic performance at early stages of development, breeders should chose the combination of sweet corn and field corn genotypes as donors and receptors, respectively, of *su1* which interaction produces the highest *su1* performance particularly at earlier stages of growth. Our results suggest that independently on specific sweet corn × field corn interactions, seedling vigor, and particularly chlorophyll content, are critical traits for *su1* plants viability. However, the complexity of the genetic regulation of emergence and the great heterogeneity of environmental conditions in the field evaluation prevent the estimation of its genetic regulation on *su1* fitness.

We hypothesize that these conclusions can be generalized for other defective mutants that have important roles in plant breeding and evolution at large. Therefore, donor × receptor

interactions are crucial for mutant fitness and the fitness-limiting traits should be searched among those biological processes affected by the defective mutant, for example germination and seedling vigor in defective endosperm mutant of maize.

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Table 1. Mean squares from the combined analysis of variance for two generation means designs (produced from EP42 × A661 and A619 × A632, respectively) evaluated under cold and standard conditions in a growth chamber.

Source of variation		(EP42 × A661)				(A619 × A632)			
	df [a]	Emergence	Early vigor	Plant weight	CCM	Emergence	Early vigor	Plant weight	CCM
Environments (E)	1	49816.93***	747.96***	32.30***	4513.55***	19304.58***	689.96***	26.71***	5913.37***
Treatments									
Year of selfing (S)[b]	4	377.83	5.78***	0.56***	21.38**	1977.95***	13.09***	0.57***	8.94
Generation (G)[c]	5	197.98	1.91	0.04	11.49*	1044.68*	2.71*	0.08	18.63*
Gene (*su1* and *Su1*)	1	12133.77***	90.61***	4.42***	90.68***	21178.03***	77.80***	5.80***	354.24***
S x Gene	4	365.41	1.31	0.05	10.93	476.32	0.61	0.05	10.01
G x Gene	5	309.04	1.25	0.04	18.70**	1054.44*	2.42*	0.08	5.31
S x G	20	376.75	0.69	0.03	7.40	615.61*	0.65	0.03	7.73
S x G x Gene	20	253.41	1.08	0.05	5.82	543.85	0.80	0.05	8.01
E x Treatments									
E x S	4	307.07	0.62	0.10*	2.69	713.52	0.94	0.09	5.37
E x G	5	183.40	0.96	0.01	4.31	288.09	1.19	0.01	7.38
E x Gene	1	5128.82***	16.37***	1.32***	160.85***	242.03	18.21***	1.73***	174.27***
E x S x Gene	4	606.14	1.33	0.05	8.30	632.46	0.66	0.05	26.14**
E x G x Gene	5	387.06	1.48	0.01	1.76	346.58	0.89	0.01	20.25*
E x S x G	20	334.55	1.09	0.04	5.88	252.62	0.79	0.04	6.36
E x S x G x Gene	20	365.38	1.25	0.06*	6.66	232.26	0.54	0.02	6.68
Error	df	230	230	230	230	230	230	230	230
	ms [d]	404.21	1.08	0.03	5.07	370.92	0.93	0.03	6.76

*, ** and *** Significant at the level of probability 0.05, 0.01 and 0.001, respectively.

[a] degrees of freedom.

[b] Number of years of selfing.

[c] The six basic generations: P_1,P_2 F_1, F_2, BC_1, and BC_2 .

[d] Mean squares.

Table 2. Mean squares from the combined analysis of variance for two generation means designs (produced from EP42 × A661 and A619 × A632, respectively) evaluated in two locations (Pontevedra, Spain, and Alger, Algeria).

Source of variation	df [a]	First design (EP42 × A661)		Second design (A619 × A632)	
		Emergence	Early vigor	Emergence	Early vigor
Environments (E)	2	24449.36***	107.18***	25249.28 ***	80.13***
Treatments					
Year of selfing (S)[b]	3	320.12	26.21	3332.65***	42.18***
Generation (G)[c]	5	267.91	3.40	647.64***	3.75
Gene (*su1* and *Su1*)	1	33774.81*	90.31	21486.29***	61.18
S × Gene	3	2600.31***	5.06*	1086.96***	1.58
G × Gene	5	664.77***	2.12	1056.95***	1.56
S × G	15	132.39	0.56	302.12**	2.46
S × G × Gene	15	211.96	2.02	194.66	1.44
E × *Treatments*					
E × S	6	205.72	5.58**	182.25	3.47
E × G	10	94.40	1.68	174.69	1.71
E × Gene	2	1194.63***	10.46**	124.79	11.17**
E × S × Gene	6	187.97	1.95	191.68	0.40
E × G × Gene	10	259.47	1.09	93.52	1.16
E × S × G	30	144.45	1.93	142.40	1.27
E × S × G × Gene	30	92.61	1.28	127.44	1.43
Pooled error	Df	531	531	531	531
	ms [d]	143.28	1.74	143.28	1.74

*, ** and *** Significant at the level of probability 0.05, 0.01 and 0.001, respectively.

[a] degrees of freedom.

[b] Number of years of selfing.

[c] The six basic generations: P_1,P_2 F_1, F_2, BC_1, and BC_2.

[d] Mean squares.

Table 3. Mean squares from the combined analysis of variance for generation means designs (produced from EP42 × A661 and A619 × A632, respectively) evaluated two years in Pontevedra, Spain.

		First design (EP42 × A661)		Second design (A619 × A632)	
Source of variation	df [a]	Plant weight	CCM	Plant weight	CCM
Environments (E)	1	22218.05***	5859.84***	11855.48***	5087.88***
Treatments					
Year of selfing (S)[b]	3	11266.04	826.79***	10280.63***	72.33
Generation (G)[c]	5	790.79	209.56***	1028.82***	1399.51***
Gene (*su1* and *Su1*)	1	8839.55***	13.27	16035.88***	3.63
S × Gene	3	1657.14*	78.58	1308.82*	40.08
G × Gene	5	514.57	82.94	323.62	136.85**
S × G	15	210.83	51.64	374.20	74.91
S × G × Gene	15	638.36	86.51**	459.48	51.81
E × *Treatments*					
E × S	3	2418.58**	22.05	242.52	44.43
E × G	5	671.91	83.85	242.31	19.42
E × Gene	1	100.91	0.06	290.36	8.55
E × S × Gene	3	371.41	7.40	736.92	69.29
E × G × Gene	5	597.90	56.48	308.47	33.58
E × S × G	15	543.13	27.64	221.56	91.29**
E × S × G × Gene	15	443.00	30.26	450.60	61.13
Pooled error	df	301	360	301	360
	ms [d]	446.32	38.61	446.32	38.61

*, ** and *** Significant at the level of probability 0.05, 0.01 and 0.001, respectively.

[a] degrees of freedom.

[b] Number of years of selfing.

[c] The six basic generations: P_1,P_2 F_1, F_2, BC_1, and BC_2.

[d] Mean squares.

Table 4. Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from five years of selfing of the first design (produced from EP42 × A661) evaluated under cold and standard conditions in a growth chamber.

Traits	Gene effects	Cold treatment		Standard treatment	
		Su1	*su1*	*Su1*	*su1*
First year of selfing					
Emergence %	m	62.44±2.08***	75.11±2.080***	80.88±2.080***	85.00±2.080***
	a	-4.50±0.71***	4.00±0.71***	4.00±0.71***	0
	d	38.07±5.50***	-66.81±5.50***	36.14±5.50***	-2.66±5.50**
	aa	24.29±2.24***	-5.25±2.24***	14.59±2.24***	7.33±2.24***
	ad	-46.00±6.32***	-132.00±6.32***	-84.00±6.32***	36.00±6.32***
	χ^2 (df [a])	**31.13***(1)**	**71.18***(1)**	**184.03***(1)**	**5.77*(1)**
CCM	m	4.11±0.41	7.81±2.08***	14.86±0.41***	11.16±0.41***
	a		0.06±0.71ns [b]	3.04±0.63***	1.76±0.63**
	d		-6.66±5.50ns		
	aa		-3.28±2.24ns		
	ad		24.64±6.32***		
	χ^2 (df)	**8.67ns(5)**	**1.25ns(1)**	**6.27ns(4)**	**5.24ns(4)**
Second year of selfing					
Emergence %	m	85.00±2.08***	68.44±2.08***	103.00±2.08***	94.77±2.08***
	a	0	4.00±0.71***	-4.00±0.71***	-12.50±0.71***
	d	-5.33±11.10**	-94.51±11.10***	-98.66±11.10***	-7.40±11.10**
	aa	7.33±2.24***	-17.03±2.24***	-9.33±2.24***	-14.81±2.24***
	ad	-200.00±12.65***	-32.00±12.65***	-32.00±12.65*	100.00±12.65***
	χ^2 (df)	**6.00*(1)**	**362.96***(1)**	**289.66***(1)**	**12.22***(1)**
CCM	m	4.26±0.41	4.32±0.41***	13.48±0.41	9.68±0.21***
	a		2.07±0.63**		-1.18±0.71ns
	d				5.78±11.10ns
	aa				0.28±2.25sn
	ad				34.08±12.65**
	χ^2 (df)	**7.91ns(5)**	**9.43ns(4)**	**10.23ns(5)**	**3.05ns(1)**
Third year of selfing					
Emergence %	m	64.66±2.08***	96.66±2.08***	109.77±2.08***	104.44±2.08***
	a	10.50±0.71***	-12.50±0.71***	0	0
	d	140.44±22.20***	-207.40±22.20***	-137.48±22.20***	-109.03±22.20***
	aa	12.44±2.24***	-53.40±2.24***	-9.48±2.24***	-13.03±2.24***
	ad	-424.00±25.30***	600.00±25.30***		0
	χ^2 (df)	**8.16**(1)**	**220.01***(1)**	**5.24ns(2)**	**21.09***(1)**
CCM	m	4.52±0.41***	5.09±2.08***	13.37±0.41	10.27±0.41
	a	1.45±0.63*	0.39±0.71ns		
	d		-2.08±22.20ns		
	aa		-2.12±2.24**		
	ad		16.16±25.30*		
	χ^2 (df)	**8.85ns(4)**	**9.98**(1)**	**10.86ns(5)**	**9.91ns(5)**
Fourth year of selfing					
Emergence %	m	64.66±2.08***	48.55±2.08***	104.11±2.08***	75.00±2.08***
	a	-10.5±0.71***	-4.50±0.71***	8.50±0.71***	-12.50±0.71***
	d	24.88±44.40**	186.07±44.40***	-214.51±44.40***	277.33±44.40***
	aa	12.44±2.24***	15.03±2.24***	-13.25±2.24***	12.66±2.24***
	ad	336.00±50.59***	-144.00±50.59***	272.00±50.59***	688.00±50.59***
	χ^2 (df)	**8.16**(1)**	**64.46***(1)**	**20.85***(1)**	**1.72ns(1)**

Table 4 (Cont.). Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from five years of selfing of the first design (produced from EP42 × A661) evaluated under cold and standard conditions in a growth chamber.

Traits	Gene effects	Cold treatment		Standard treatment	
		Su1	*su1*	*Su1*	*su1*
CCM	m	3.28±0.41	3.79±0.41	6.38±2.08**	11.80±2.08***
	a			-0.28±0.63ns	-1.24±0.71ns
	d			118.89±44.41**	-34.72±44.40ns
	aa			7.12±2.24**	-3.60±2.24ns
	ad				114.56±50.59*
	χ^2 (df)	**4.16 ns(5)**	**9.37 ns(5)**	**1.00 ns(2)**	**2.92 ns(1)**
Fifth year of selfing					
Emergence %	m	69.00±2.08***	70.11±2.08***	95.66±2.08***	128.88±2.08***
	a	-12.50±0.71***	-12.50±0.71***	4.50±0.71***	8.50±0.71***
	d	-64.00±88.82ns	-258.37±88.82 ***	92.44±88.82***	-1682.96±88.81***
	aa	6.00±2.24ns	-30.59±2.24***	-8.88±2.24***	-36.74±2.24***
	ad	240.00±101.19ns	-288.00±101.19***	-288.00±101.19***	-32.00±101.19ns
	χ^2 (df)	**1033.50***(1)**	**220.01***(1)**	**32.45***(1)**	**20.74***(1)**
CCM	m	3.52±0.41	10.18±2.08***	15.00±2.08***	13.55±2.08**
	a		-0.29±0.71*	-1.93±0.71**	1.86±0.71**
	d		-204.65±88.82 ***	-149.21±88.82 ns	-111.28±88.82 ns
	aa		-7.86±2.24***	-3.96±2.242ns	-1.26±2.24ns
	ad		-229.12±101.19***	254.72±101.19*	-357.44±101.19***
	χ^2 (df)	**3.27ns(5)**	**5.84*(1)**	**0.64ns(1)**	**1.04ns(1)**

ns, *, ** and ***, nor significant, significant at the level of probability 0.05, 0.01 and 0.001, respectively
[a] degrees of freedom.

Table 5. Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from five years of selfing of the second design (produced from A619 × A632) evaluated under cold and standard conditions in a growth chamber.

Traits	Gene effects	Cold treatment		Standard treatment	
		Su1	*su1*	*Su1*	*su1*
First year of selfing					
Emergence %	m	71.88±2.08***	41.55±2.08***	70.88±2.08***	102.11±2.08***
	a	0	2.50±0.71*	-4.00±0.71***	-9.50±0.71***
	d	14.81±5.50*	55.25±5.50***	61.48±5.50***	-34.81±5.50***
	aa	13.25±2.24***	41.03±2.24***	25.92±2.24***	-13.25±2.24***
	ad	-100.00±6.32***	22.00±6.32*	-16.00±6.32***	-30.00±6.32***
	χ^2 (df [a])	**249.18***(1)**	**456.46***(1)**	**32.37***(1)**	**149.03***(1)**
CCM	m	4.57±0.41	3.56±0.41	14.02±0.41	5.39±0.41
	a				
	d				
	aa				
	ad				
	χ^2 (df)	**10.62ns[b]**	**4.13 ns**	**3.42 ns**	**5.49 ns**
Second year of selfing					
Emergence %	m			74.33±2.08***	54.11±2.08***
	a			0	-34.50±0.71***
	d			-19.55±11.10**	146.37±11.10***
	aa			26.88±2.24***	4.74±2.24***
	ad			136.00±12.65***	340.00±12.65***
	χ^2 (df)			82.62***(1)	23.50***(1)
CCM	m			14.95±0.41	12.35±2.08***
	a				-3.78±0.71***
	d				-0.51±11.10ns
	aa				-3.41±2.24ns
	ad				32.84±12.65***
	χ^2 (df)			**7.31ns(5)**	**0.07ns(1)**
Third year of selfing					
Emergence %	m	68.66±2.08***	17.33±2.08***	96.44±2.08***	55.88±2.08***
	a	-4.50±0.71***	0	0	0
	d	119.11±22.20***	616.88±22.20***	18.96±22.20**	187.25±22.20***
	aa	19.11±2.24***	62.88±2.24***	2.96±2.24***	25.25±2.24***
	ad	40.00±25.30***	80.00±25.30***	-128.00±25.30***	528.00±25.30***
	χ^2 (df)	**4.16*(1)**	**416.66***(1)**	**20.65***(1)**	**185.66***(1)**
CCM	m	3.33±0.41	4.09±0.41	6.65±2.08***	16.25±2.08***
	a			-1.87±0.71***	0.67±0.63ns
	d			95.39±22.20***	-74.12±22.20***
	aa			7.45±2.24***	-8.62±2.24***
	ad			74.88±25.30***	
	χ^2 (df)	**5.39ns(5)**	**2.95ns(5)**	**13.07***(1)**	**2.11ns(1)**
Fourth year of selfing					
Emergence %	m	91.44±2.08***	39.33±2.08***	99.55±2.08***	112.22±2.08***
	a	0	-12.50±0.71***	4.00±0.71***	-8.00±0.71***
	d	-314.07±44.40***	476.44±44.40***	-165.92±44.40***	-845.03±44.40***
	aa	-1.03±2.24ns	16.22±2.24***	-4.96±2.24***	-36.51±2.24***
	ad	0	688.00±50.59***	160.00±50.59***	-544.00±50.59***
	χ^2 (df)	**136.96***(1)**	**60.16***(1)**	**104.58***(1)**	**20.84***(1)**

Table 5 (Cont). Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from five years of selfing of the second design (produced from A619× A632) evaluated under cold and standard conditions in a growth chamber.

traits	Gene effects	Cold treatment		Standard treatment	
		Su1	*su1*	*Su1*	*Su1*
CCM	m	8.67±2.08***	3.70±0.41	13.81±0.41***	12.88±2.08***
	a	0.06±0.632ns		-2.01±0.63***	-0.76±0.71***
	d	109.32±44.41*			-34.39±44.40***
	aa	-6.46±2.24**			-4.65±2.24***
	ad				115.68±50.59***
	χ^2 (df)	**5.25ns(2)**	**10.23ns(5)**	**1.67ns(4)**	**4.76*(1)**
Fifth year of selfing					
Emergence %	m	83.88±2.08***	46.00±2.08***	70.88±2.08***	93.44±2.08***
	a	-4.00±0.71***	-8.50±0.71***	4.00±0.71***	0
	d	-658.96±88.82 ***	789.33±88.82***	983.70±88.82***	414.81±88.82***
	aa	-14.74±2.24***	-14.66±2.24***	25.92±2.24***	-27.70±2.24***
	ad	-1344±101.19***	-2144.00±101.19**	-768.00±101.19***	0
	χ^2 (df)	**185.18***(1)**	**253.50***(1)**	**32.37***(1)**	**82.71***(1)**
CCM	m	7.49±2.08***	3.12±0.41	10.27±0.64***	13.37±2.08***
	a	0.77±0.71ns		-0.73±0.63ns	2.35±0.71**
	d	-182.94±88.82*		172.00±38.02***	-137.52±88.82 ns
	aa	-3.23±2.24ns			-2.14±2.24ns
	ad	-296.96±101.19**			-483.52±101.19***
	χ^2 (df)	**0.006ns(1)**	**0.61 ns(5)**	**7.65 ns(3)**	**2.17 ns(1)**

ns, *, ** and ***, nor significant, significant at the level of probability 0.05, 0.01 and 0.001, respectively

[a] degrees of freedom.

Table 6. Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from four year of selfing of the first design (produced from EP42 × A661) evaluated in two locations (Pontevedra, Spain, and Alger, Algeria).

Traits	Gene effects	Alger		Pontevedra 2010		Pontevedra 2011	
		Su1	*su1*	*Su1*	*Su1*	*Su1*	*su1*
First year of selfing							
Emergence %	m	72.45±2.08***	23.82±2.08***	72.40±2.08***	59.69±2.08***	74.89±2.08***	41.55±2.08***
	a	-2.10±0.71**	6.11±0.71***	-9.03±0.71***	1.53±0.71*	-0.33±0.71*	-5.66±0.71***
	d	-65.97±5.50***	60.92±5.50***	12.95±5.50*	23.16±5.50***	-23.41±5.50***	-2.94±5.50ns
	aa	-12.91±2.24***	20.89±2.24***	7.31±2.24**	1.65±2.24ns	-5.63±2.24***	5.48±2.24*
	ad	44.44±6.32***	-40.12±6.32***	104.58±6.32***	-46.22±6.32***	49.34±6.32***	-1.34±6.32ns
	χ^2 (df[a])	**0.009ns(1)**	**86.52***(1)**	**3.22ns(1)**	**1.05ns(1)**	**8.97**(1)**	**90.76***(1)**
CCM[b]	m			30.30±2.08***	49.59±2.08***	36.22±2.08***	65.40±2.08***
	a			8.01±0.71***	4.13±0.71***	2.96±0.71***	0.21±0.71ns
	d			20.11±5.50***	-25.80±5.50***	33.29±5.50***	-41.17±5.50***
	aa			13.87±2.24***	-3.69±2.24***	19.46±2.24***	-9.47±2.24***
	ad			-51.74±6.32***	-12.46±6.32***	-17.52±6.32***	40.04±6.32***
	χ^2 (df)			**16.32***(1)**	**42.25***(1)**	**71.59***(1)**	**36.50***(1)**
Second year of selfing							
Emergence %	m	69.65±2.08***	31.22±2.08***	97.82±2.08***	80.93±2.08***	70.73±2.08***	4.06±2.08ns
	a	-10.84±0.71***	-11.98±0.71***	-3.71±0.71***	-2.43±0.71***	-1.86±0.71***	2.00±0.71*
	d	-26.56±11.10ns	30.23±11.10ns	-58.21±11.10***	-65.67±11.10***	12.27±11.10***	143.86±11.10***
	aa	2.88±2.24ns	-6.05±2.24ns	-9.27±2.24***	-19.38±2.24***	-2.16±2.24**	34.28±2.24***
	ad	45.40±12.65**	205.28±12.65***	18.04±12.65***	87.32±12.65***	-21.36±12.65***	-16.00±12.65***
	χ^2 (df)	**259.09***(1)**	**370.51***(1)**	**11.86***(1)**	**179.92***(1)**	**18.11***(1)**	**291.02***(1)**
CCM	m			31.79±2.08***	30.63±2.08***	50.63±2.08***	41.98±2.08***
	a			6.24±0.71***	5.71±0.71***	4.73±0.63***	-1.85±0.71***
	d			42.08±11.10***	26.26±11.10***	-14.08±11.10ns	22.62±11.10***
	aa			1.61±2.24ns	9.89±2.24***	-6.82±2.24**	3.29±2.242***
	ad			-33.92±12.65**	-111.48±12.65***		24.28±12.649***
	χ^2 (df)			**0.99ns(1)**	**15.16***(1)**	**2.09ns(1)**	**10.41**(1)**

Table 6 (Cont.). Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from four year of selfing of the first design (produced from EP42 × A661) evaluated in two locations (Pontevedra, Spain, and Alger, Algeria).

Traits	Gene effects	Alger		Pontevedra 2010		Pontevedra 2011	
		Su1	*su1*	*Su1*	*Su1*	*su1*	*Su1*
Third year of selfing							
Emergence %	m	71.38±2.08***	26.98±2.08***	57.58±2.08***	75.94±2.08***	76.82±2.08***	42.37±2.08***
	a	-1.76±0.71ns	1.73±0.71***	1.19±0.71*	-0.47±0.71ns	-2.67±0.71***	4.66±0.71***
	d	-191.38±22.20***4	235.41±22.20***	244.05±22.04***	-38.70±22.20**	-71.94±22.20***	161.59±22.20***
	aa	-14.30±2.24***	17.95±2.24***	12.61±2.24***	-5.74±2.24***	-19.90±2.24***	-5.08±2.24*
	ad	-164.40±25.30***	210.24±25.30***	67.36±25.30***	-71.84±25.30***	32.16±25.30**	-106.64±25.30***
	χ^2 (df)	**298.53***(1)**	**33.41***(1)**	**81.25***(1)**	**53.52***(1)**	**45.04***(1)**	**0.13ns(1)**
CCM	m			47.78±2.08***	31.72±2.08***	53.41±2.08***	33.73±2.08***
	a			3.25±0.63***	2.31±0.71***	3.26±0.71***	3.05±0.71***
	d			-78.03±22.20***	21.97±22.20ns	-70.69±22.20***	82.65±22.20***
	aa			-11.77±2.24***	5.25±2.24**	-8.65±2.24***	13.77±2.24***
	ad				-137.84±25.30***	-71.44±25.30***	61.92±25.30*
	χ^2 (df)			**4.40ns(2)**	**84.60***(1)**	**8.03***(1)**	**1.80ns(1)**
Fourth year of selfing							
Emergence %	m	78.61±2.08***	28.11±2.08***	86.07±2.08***	61.45±2.08***	52.89±2.08***	36.74±0.64***
	a	1.56±0.71***	-2.96±0.71***	1.79±0.71*	-6.82±0.71***	-4.67±0.71***	-4.26±0.63***
	d	-632.48±44.40***	287.83±44.40***	-167.76±44.40***	179.33±44.40***	82.83±44.40***	107.91±19.01**
	aa	-19.32±2.24***	15.75±2.24***	-6.42±2.24**	1.46±2.24*	5.47±2.24***	
	ad	415.84±50.59***	443.52±50.59***	-181.12±50.59***	166.40±50.59***	-85.12±50.59***	
	χ^2 (df)	**25.88***(1)**	**113.08***(1)**	**3.47ns(1)**	**13.66***(1)**	**7.42**(1)**	**1.08ns(3)**
CCM	m			41.44±2.08***	17.90±2.08***	45.05±2.08***	44.84±0.64***
	a			-0.59±0.71ns	-2.12±0.71***	-0.61±0.71ns	4.17±0.63***
	d			-134.05±44.40**	323.14±44.40***	-22.00±44.40ns	-72.18±19.01***
	aa			-7.02±2.24**	18.19±2.24***	-3.02±2.24*	
	ad			-208.16±50.59***	65.12±50.59*	-100.32±50.59***	
	χ^2 (df)			**0.06ns(1)**	**49.67***(1)**	**34.03***(1)**	**3.24ns(3)**

ns, *, ** and ***, nor significant, significant at the level of probability 0.05, 0.01 and 0.001, respectively

[a] degrees of freedom.[b] CCM was not recorded in Alger

Table 7. Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from four years of selfing of the second design (produced from A619 × A632) evaluated in two locations (Pontevedra, Spain, and Alger, Algeria).

Traits	Gene effects	Alger		Pontevedra 2010		Pontevedra 2011	
		Su1	*Su1*	*Su1*	*su1*	*Su1*	*Su1*
First year of selfing							
Emergence %	m	80.15±2.08***	21.62±2.08***	97.12±2.08***	78.87±2.08***	60.51±2.08***	52.22±2.08***
	a	4.26±0.71***	1.60±0.71ns	-0.96±0.63ns	1.91±0.71**	-7.67±0.71***	-5.00±0.71***
	d	-41.55±5.50***	63.97±5.50***	-27.68±5.50***	-10.29±5.50ns	13.53±5.50***	-13.62±5.50***
	aa	-16.44±2.24***	26.41±2.24***	-11.59±2.24***	-9.35±2.24***	12.79±2.24***	0.15±2.24ns
	ad	16.58±6.32***	29.78±6.32**		-31.88±6.32***	54.68±6.32***	-57.32±6.32***
	χ^2 (df[a])	**34.99***(1)**	**361.38***(1)**	**3.01ns(2)**	**0.01ns(1)**	**5.11*(1)**	**26.71***(1)**
CCM[b]	m			55.34±2.08***	29.55±2.08***	37.26±2.08***	47.73±2.08***
	a			-2.60±0.71***	-11.07±0.71***	-5.85±0.71***	-8.21±0.71***
	d			-45.70±5.50***	35.37±5.50***	39.60±5.50***	6.98±5.50***
	aa			-18.41±2.24***	11.90±2.24***	13.13±2.24***	1.75±2.24**
	ad			27.08±6.32***	21.02±6.32**	13.98±6.32***	-16.30±6.3***
	χ^2 (df)			**4.31*(1)**	**2.42ns(1)**	**6.82**(1)**	**10.55**(1)**
Second year of selfing							
Emergence	m	18.73±2.08***	30.40±2.08***	45.30±2.08***	80.58±2.08***	50.65±2.080***	43.33±2.08***
	a	0.59±0.71ns	-11.52±0.71***	-2.12±0.71***	-10.98±0.71***	-5.00±0.71***	-20.33±0.71***
	d	177.92±11.10***	11.88±11.10ns	141.29±11.10***	-168.43±11.10***	-7.05±11.10ns	-16.00±11.10**
	aa	35.50±2.24***	11.27±2.24***	37.29±2.24***	-20.65±2.24***	17.78±2.24***	-17.33±2.24***
	ad	228.44±12.65***	32.40±12.65***	94.16±12.65***	75.76±12.65***	189.36±12.65***	178.64±12.65***
	χ^2 (df)	**0.43ns(1)**	**98.94***(1)**	**31.09***(1)**	**95.89***(1)**	**0.66ns(1)**	**53.96***(1)**
CCM	m			57.31±2.08***	43.38±2.08***	52.67±2.08***	56.43±2.08***
	a			-3.97±0.71***	-6.35±0.71***	-9.85±0.71***	-6.55±0.71***
	d			-59.97±11.10***	-14.08±11.10***	-11.40±11.10ns	-14.23±11.10***
	aa			-19.94±2.24***	-5.97±2.24***	-3.94±2.24ns	-12.14±2.24***
	ad			-23.32±12.65***	-51.60±12.65***	58.72±12.65***	-43.72±12.65***
	χ^2 (df)			**34.72***(1)**	**10.00**(1)**	**1.60ns(1)**	**25.36***(1)**

Table 7 (Cont.). Estimates ± standard error of mean (m), additive (a), dominance (d), and epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from four years of selfing of the second design (produced from A619 × A632) evaluated in two locations (Pontevedra, Spain, and Alger, Algeria).

Traits	Gene effects	Alger		Pontevedra 2010		Pontevedra 2011	
		Su1	*Su1*	*Su1*	*Su1*	*Su1*	*Su1*
Third year of selfing							
Emergence %	m	27.86±2.08***	59.87±2.08***	76.66±2.08***	77.55±2.08***	48.74±2.08***	74.60±2.08***
	a	2.48±0.71***	-9.13±0.71***	5.94±0.71***	-4.22±0.71***	-1.33±0.71*	-11.66±0.71***
	d	316.55±22.20***	-86.01±22.20***	52.17±22.20***	-55.60±22.20***	67.10±22.20***	-195.26±22.20***
	aa	25.35±2.24***	-10.49±2.24***	6.87±2.24***	-10.90±2.24***	8.48±2.24***	-20.50±2.24***
	ad	404.80±25.30***	-32.72±25.30ns	-5.68±25.30ns	196.32±25.30***	256.08±25.30***	218.64±25.30***
	χ^2 (df)	**0.001ns(1)**	**112.28***(1)**	**28.03***(1)**	**49.99***(1)**	**110.74***(1)**	**132.75***(1)**
CCM	m			32.72±2.08***	50.88±2.08***	35.66±2.08***	42.13±2.08***
	a			-9.94±0.71***	-2.83±0.71***	-9.93±0.71***	-8.23±0.71***
	d			94.05±22.20***	-81.28±22.20***	83.89±22.20***	89.59±22.20***
	aa			8.71±2.24***	-18.04±2.24***	16.77±2.24***	4.13±2.24***
	ad			33.84±25.30***	-247.44±25.30***	126.32±25.30***	42.00±25.30***
	χ^2 (df)			**29.49***(1)**	**8.42**(1)**	**4.28*(1)**	**32.00***(1)**
Fourth year of selfing							
Emergence %	m	60.52±2.08***	82.63±2.08***	85.47±2.08***	68.76±2.08***	67.41±2.08***	52.29±2.08***
	a	-5.45±0.71***	-8.21±0.71***	2.33±0.71***	-1.95±0.71***	4.33±0.71***	-3.67±0.71***
	d	-5.73±44.40ns	-674.95±44.40***	-15.32±44.40ns	-4.76±44.40ns	-185.89±44.40***	-244.93±44.40***
	aa	-11.48±2.24***	-45.87±2.24***	-1.32±2.24*	2.09±2.24*	-12.84±2.24***	-14.46±2.24***
	ad	416.80±50.59***	186.72±50.59***	45.44±50.59**	-539.04±50.59***	671.84±50.59***	309.44±50.59***
	χ^2 (df)	**0.58ns(1)**	**11.22***(1)**	**13.74***(1)**	**29.52***(1)**	**2.97ns(1)**	**36.93***(1)**
CCM	m			9.38±2.08***	22.77±2.08***	40.96±2.08***	35.30±2.08***
	a			-7.24±0.71***	-9.89±0.71***	-3.93±0.71***	-8.12±0.71***
	d			628.21±44.40***	368.32±44.40***	134.85±44.40**	385.71±44.40***
	aa			32.64±2.24***	19.74±2.24***	3.42±2.24ns	5.63±2.24*
	ad			310.88±50.59***	33.44±50.59ns	-132.80±50.59**	-107.20±50.59*
	χ^2 (df)			**80.49***(1)**	**49.24***(1)**	**0.57ns(1)**	**3.06ns(1)**

ns, *, ** and ***, nor significant, significant at the level of probability 0.05, 0.01 and 0.001, respectively

[a] degrees of freedom.[b] CCM was not recorded in Alger.

3.2. Influence of the *Sugary1* locus on the genetic effects of polygenic traits

Short title: *Sugary1 interaction with genetic effects*

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SUMMARY

Knowledge of genetic control of mutant viability is of great importance in maize breeding, particularly for mutants with deleterious effects. Little is known about the genetics of the viability of mutants and no previous report has been published concerning the genetic effects of the mutant *sugary1* on agronomic traits. Our objective was to study the effects of the sweetcorn mutant *sugary1* (*su1*) on the genetic effects of agronomic traits in two wild type corn backgrounds. Estimated genetic effects of agronomic traits in *Su1 vs*. *su1* plants were monitored through four successive selfpollination cycles in two separated mean generation designs. The first involved two Corn Belt inbred lines A619 and A632, while unrelated inbred lines EP42 and A631 were used for the second design. Parents, F_1s, F_2s, and backcrosses were crossed to the *su1* inbred P39 as the donor of *su1* and the 12 crosses were successively selfpollinated for 4 years. For each cross, *Su1* and *su1* seeds were separated and evaluated in 10 x 10 triple lattice designs. The *Su1* plants showed higher performance than the *su1* plants in almost all traits. The estimates of genetic effects of *Su1 vs*. *su1* plants were strongly affected by the *su1* × wild type corn interactions. The introgression of *su1* in wild type corn strongly affects the genetic effects of flowering time and, to a lesser stent, that of other plant or ear traits. Mutant viability is regulated by additive, dominance and digenic epistatic effects and the importance of those genetic effects depends on the genetic background and environmental conditions.

INTRODUCTION

Knowledge of the nature of gene viability involved in the expression of economically important traits is fundamental for optimizing breeding programs. In maize (*Zea mays* L.), many mutants are known to affect endosperm development. Such is the case of the sweetcorn mutant *sugary1* (*su1*), located in the chromosome 4, Bin 4.05 (James *et al.* 1995; Tracy *et al.* 2006). The recessive *su1* allele results in the accumulation of phytoglycogen rather than starch (James *et al.* 2003).

Compared to others types of maize, sweetcorn has a relatively restricted genetic base (Tracy 1990); this is the reason why Haber (1954), Tracy (1990), and other authors have used non-sweetcorn germplasm (*Su1*) to broaden the genetic base for breeding programs and to improve the agronomic performance of sweetcorn. However, the final results are sometimes undesirable or not viable for sweetcorn breeding because some undesirable genetics factors are incorporated into the new sweetcorn genotype (Revilla *et al.* 2000*a*, 2006, 2010). Revilla *et al.* (2000a, 2006, 2010) also reported that the viability of *su1* mutant depends on specific sweetcorn × wild type corn genotype interaction, genetic background playing a major role in the viability of this mutant. However, little is known about the genetics causes of the differences in the viability rates of *su1* when different wild type corn genotypes are used as recurrent parents in breeding programs.

Knowing the genetic effects of viability of *su1* allele is crucial for its future use in breeding programs, particularly when the mutant is deleterious in some genetic backgrounds. The viability and genetic effects of *su1* were monitored through five

successive selfpollination cycles in two separated mean generation designs by Djemel *et al.* (2011), who found that viability of *su1* is under genetic control with significant genetic effects that are probably due to minor contributions of multiple genes.

A number of questions concerning the viability of *su1* should be solved, e.g. from which agronomic traits can a new sweetcorn capitalize viability in order to produce stable and viable offspring? and what is the effect of the introgressed *su1* in the genetic effect of agronomic performance across plant growing cycle?

Selection against a mutant may operate firstly through reduced viability or through reduced fertility (Falconer 1981). In the life cycle of plants, selection acts first through viability (germination, early vigor, etc.) (Ordás *et al.* 2010), then through fertility (mating ability and grain formation) (Cisneros-López *et al.* 2010; Zhang *et al.* 2011). The viability of *su1* plants has been investigated mainly at the first stages of plants development. Selection against *su1* alleles has been found in crosses between wild type corn and sweetcorn inbred lines. Germination is the first limiting factor and once germination has been accounted, the next important factor is early vigor (Martins & Da Silva 1998; Revilla *et al.* 2000*a*; Gad & Juvik 2002; Juvik *et al.* 2003; Revilla *et al.* 2006). Recently, Revilla *et al.* (2010) compared different wild type corn genetic backgrounds for performance of plants carrying *su1* in order to choose the most appropriate wild corn varieties for improving the agronomic performance of sweetcorn. Early vigor was the main limiting factor for *su1* plant development and the difference in performance between *Su1* and *su1* plants decreased with plant development. Indeed, as the growth cycle continues, stand, ears per plant, and yield

did not have significant effect on *su1* viability (Revilla *et al.* 2010). However, Revilla *et al.* (2000*b*) concluded that grain yield, grain moisture, plants height, ear length, and seed row number can be viability-related traits.

The magnitude of the genetic effects is influenced by the environment which is termed additive (or other genetic effect) × environment interaction. The magnitude of the genetic effects which depends on the segregating alleles could be affected not only by the environment, but also by the genetic background, that is, by other genes that are not segregating. However, little attention has been paid to the change in the genetic effects that can be produced by the introgression of a mutant and no previous report has been published concerning the effects of the mutant *sugary1* on the additive, dominance and epistatic genetic effects of agronomic traits. The aim of the present research was to estimate the change in the genetic effects produced by the introgression of the *su1* mutant of maize for several agronomic traits.

MATERIALS AND METHODS

We used mean generation analyses designs in order to estimate the effect of the *su1* allele on the genetic effects of several traits (Mather & Jinks 1982). This design allows the estimation of additive, dominance and epistatic genic effects for quantitative traits and, therefore, is appropriate for studying the type of heredity and the effects of a mutant in those components of the genetics underlying quantitative traits.

Two separate designs of mean generation analyses were developed. One of the designs involved two wild type (*Su1Su1*) corn inbreds from the Corn Belt genetic background: A619 (from the maize family Lancaster, which is an open pollinated cultivar that is the source of various inbred lines) and A632 (from the maize family Reid, which is an open pollinated cultivar and represent the source of Illinois inbred line and account for half of EE.UU. hybrid maize background). The Lancaster and Reid inbred lines constitute the most common heterotic pattern used in maize production. The second design for mean generation analyses was developed from unrelated wild type corn inbreds: A661 (Northern Corn Belt) and EP42 (European Flint). Corn Belt and European Flint constitute the second most commonly used heterotic pattern in Europe for maize production.

A632 and EP42 are wild type maize parents previously identified as having higher seed viability of *su1* than A661 and A619 (Revilla *et al.* 2006). Crosses between each pair of inbred lines started in 2001. Crosses were selfpollinated and backcrossed, obtaining six generations per design: the two parents P_1 and P_2, F_1, F_2, BC_1, and BC_2. The 12 entries were crossed with the sweetcorn (*su1su1*) inbred P39 as donor of *su1* allele. Around 100 plants from each of the 12 entries were selfpollinated by hand, harvested, and conserved in bulk. During the selfpollination no artificial selection was made. From each bulk, a sample of 150 seeds (maintaining the expected proportion of the *su1* allele in the absence of selection) was sown, the following year, in 10 blocks 3 m long, spaced 0.8 m apart; plants within the row were 0.20 m apart. The selfpollination cycles were repeated up to 4 years: 2006, 2007, 2008 and 2009 in

Pontevedra, Spain (42° 24′, 8′ 38′N, 20 m asl), a location in the northwest of Spain where annual rainfall is in the range of 1600–1700 mm. We obtained 24 different populations for each design, corresponding to the different combinations of generation (P_1, P_2, F_1, etc) and selfpollination cycle (first, second, etc). The differences between the means of the different generations, and the genetic effects due to polygenic traits, are assumed to remain unchanged after all generations were crossed to the same sweetcorn inbred line. For each population the seeds were randomly sampled, and *su1* and *Su1* seeds were separated for further evaluation in the field. Thus, for each of the six basic generations we had two subpopulations differing only for the allele *su1* vs. *Su1* at the *Su1* locus, one population with only the *su1* allele and other population with the *Su1* allele. This allows the estimation of the genetic effects from the basic generations with the *su1* allele and independently the estimation of the genetic effects from the basis generations with the *Su1* allele. The comparison between the effects estimated in the basic generations with the *su1* allele and the effects estimated in the basic generations with the *Su1* allele, will allow the estimation of the change in genetics effects due to the *su1* allele. Therefore, finally 48 populations were produced for each design, consisting of 24 populations (6 generations' × 4 selfpollination cycles) with the *su1* phenotype and 24 with the *Su1* phenotype.

The 96 entries plus the parental inbred lines involved in the two designs were evaluated in 10 × 10 triple lattice designs. The field trials were sown in 2010 and 2011 at Pontevedra (42° 24′N, 8′ 38′W, altitude 20 m), a location in the northwest of Spain

where annual rainfall is in the range of 1600–1700 mm; and in 2010 at Algiers (36 º 47 ' N, 2 º 03' E, altitude 32 m), located in the sub-humid North of Algeria with 900 mm of annual rainfall.

Each experimental plot consisted of two rows with 25 seeds per row and one seed per hill. The rows were spaced 0.80 m apart and the hills were spaced 0.12 m apart. The hills were thinned after emergence to obtain a final density of 75,000 plants ha^{-1}, or 18 plants per row. Appropriate techniques for cultivation were carried out according to local practices. Harvesting was manual in both locations.

The following data were taken on each plot: days to shedding pollen (from sowing to 50% of plants shedding pollen), days to silking (from sowing to 50% of plants silking), plant height (from the soil to the top of the tassel, cm), adult plant performance (taken on the full development stage of the plant on a visual scale from 1= weak plant to 9= strong plant), stand (plants that reached adult stage measured in a 0-1 scale), ear length (cm), ear row (number of seeds rows per ear), ear weight (kg), ear number (per plot), number of ears per plant, ear performance (rated on a nine point visual scale taking into account the shape and the health aspect) and 100-seed weight (g).

Combined analyses of variance were made using the adjusted means obtained from the lattice analysis in each environment when the efficiency of the lattice design was above 105%; otherwise, the traits were analyzed as randomized complete block designs. The sources of variations were divided into effects due to the environments, treatments (selfpollination cycle, generation, gene, and the respective interaction),

and the environments × treatments interactions. The sums of squares for each partition of the environments × treatments interactions were initially tested with the sum of squares of the pooled error. If the F-test was significant, all of the sums of squares of the treatments were tested with their corresponding environments × treatments interaction; if not, the sums of squares were tested with the sum of squares of the pooled error (Cochran & Cox 1992).The analyses of variance were carried out using SAS (SAS Institute 2005).

The adjusted means were used for the mean generation analyses. For those traits showing significant differences among generations in the analysis of variance the means of the populations were calculated. For each trait, and using the six basic generations, one independent mean generation analysis was carried out for each combination of environment, design, selfpollination cycle, and phenotype. Independent mean generation analyses were carried out for each environment because the analysis of variance showed that the interactions were significant for several traits. The equations for the generation means' analysis of the various generations employed in this study are:

$P_1 = m + a + aa,$

$P_2 = m - a + aa,$

$F_1 = m + (½)^n d,$

$F_2 = m + ½(½)^n d,$

$BC_1 = m + ½ a + ½(½)^n d + (¼) aa + ¼ (½)^n ad,$

BC_2= m - ½ a +½(½)^{n}d+ (¼) aa - ¼ (½)^{n}ad , were n= the number of selfpollination cycle.

The adjustment of the generation means to a genetic model was tested with a χ^2 test. The test was applied to the simplest model and, if the χ^2 test revealed lack of fit, the next model was tried (Mather & Jinks 1982; Kearsey & Pooni 1996). The models considered were: a model with only the mean, an additive model, an additive-dominant model, an additive-dominant model with epistasis additive × additive, and an additive-dominant model with epistasis additive × dominance. The genetic parameters estimated were m=mean value, a=additive effects, d=dominance effects, aa= additive × additive epistatic effects and ad=additive × dominance effects (Mather & Jinks 1982).For the six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) the solution is obtained in the form of a matrix by the SAS (SAS Institute 2008) statistical package using PROC IML as follows:

$$X = (C' \times C)^{-1} \times (C' \times Y)$$

Where Y= 6×1 vector of generation means,

C= the matrix (6×z), when z depending on the genetic model,

C′ is the transpose of the C matrix and $^{-1}$ represents the inverse of a matrix.

The standard error associated to each estimate of a genetic effect was obtained as the diagonal items in the variance-covariance matrix $(C' \times C)^{-1}$. Significance of each genetic effect estimate was evaluated as described by Kearsey & Pooni (1996), utilizing a t-test; if the χ^2 test of the model was not significant the t-test = |estimate of a genetic effect| / (their corresponding standard error). On the other hand, the

standard error would be multiplied by the square of the χ^2 / (degrees of freedom) if the χ^2 test of the model was significant.

RESULTS

The combined analysis of variance across environments showed highly significant differences among generations for most traits, except pollen date, silk date, adult plant performance, stand, and ear performance for the first mean generation design (EP42 × A661). However, the environments × generations interactions were significant for both flowering times (data not shown). The genes (*Su1 vs. su1*) differed significantly for more traits than the differences among selfpollination cycles. In the case of the second design, A619 × A632, there were significant differences between generations excepted for adult plant performance, ear performance and 100-seed weight. The differences between genes were important for traits related to the yield components (ear number, ear/plant, ear length, and seed row number), adult plant performance, and stand (data not shown). The differences between generations were significant for more traits than the differences between *Su1* and *su1*. In both designs, the effects of the environments were strongly significant for all traits, except 100-seed weight and seed row number for the first design. The interactions involving the components of "treatments" as sources of variations, except the double interaction (selfpollination cycle × generation × gene) were significant for almost all traits related to yield and plant growth. The interactions involving the components of environments × treatments as sources of variations were seldom significant. However, the differences were more important in

the second design (A619 × A632) than in the first design (EP42 × A661). The environments × treatments interaction was often of rank rather than of magnitude (data not shown) and the interactions involving selfpollination cycle, generations, and the genes were the most important. In general, for both designs, the interactions were often significant for the important traits related to plant growth and viability (flowering time, stand, ear weight, ear number, adult plant performance, and 100-seed weight).

Estimates of genetic effects from generation mean analysis according to a 5-parameter model and the chi-square (χ^2) values are presented separately for each environment and selfpollination cycle. Only traits showing significant differences between generations or environments × generations' interaction were used. For both designs, the performance of the *Su1* plants differed from that of *su1* plants. In general, the *Su1* plants were earlier than the *su1* plants for male and female flowering (data not shown). Non clear differences were shown for plant height and ear length, with the behavior of plants highly affected by environments. However, for stand, ear number, and 100-seed weight, the *Su1* plants showed a high proportion of adult plants that reached maturity and high yield production.

The observed values of chi-square (χ^2) for the models that best fit the data and the estimates of genetic effects for those models are given in tables 1-8. The models can be classified as simple (without genetic effects), genetic additive-dominant (only additive and/or dominant effects), genetic epistatic (additive, dominant and digenic epistatic effects) and complex in which the additive, dominant and digenic epistatic

effects are insufficient to explain the data. For several of the combinations of trait, genotype at the *Su1* locus, environment and cycle of selfpollination, the genetic models that best fit the data were the epistatics. For some combinations even the models with the digenetic epistatic parameters did not fit the data and complex models would be needed. On the contrary, for some of the combinations the simple model, without genetic parameters, adequately fitted the data.

The type of model that best fitted the data varied between traits, genotype at the *Su1* locus, environments and cycles of selfpollination. For plant height and ear number complex models were usually needed and most of the genetic parameters were significant. For the flowering traits the simple model usually fitted the data in the *Su1* genotypes, but in the *su1* genotypes the genetic models fitted the data in the last cycles of selfpollination in some of the environments. For stand and ear length there were significant genetic effects in the A619 × A632 design, but not in the EP42 × A661 design. On the contrary, for 100-seed weight there were genetics effects in EP42 × A661, but not in A619 × A632. The genetics effects of 100-seed weight were expressed in two of the environments, but the types of effects varied between cycles of selfpollination, environments, and genotype at the *Su1* locus.

DISCUSSION

The study of the genetic control of *su1* viability rate is of great importance for using this mutant in breeding programs. Actually, *su1* viability is under both genetic and environmental controls. Both emergence and seedling vigor are the most critical traits affecting the viability of *su1* plants (Revilla *et al.* 2000*a*, 2006). Tracy (2001) also

reported that these traits are affected by genetic factors, both at planting and during seed production.

For both designs, the performance of the *Su1* plants differed from that of *su1* plants. The *Su1* plants were earlier than the *su1* plants for both flowering times; however, these differences were small. Our results confirmed those observed by Revilla *et al.* (2010), who also reported that the small differences detected for earliness between *Su1* and *su1* should have minor contributions to the differences in performance. As the growth cycle continues and depending of the genetic background, successive traits are less important for the performance of *su1* plants. Moreover, differences for plant height and ear length were smaller than those reported by Revilla *et al.* (2000*b*). However, for stand and yield components the *Su1* plants showed higher performance than the *su1* plants. These results agree with previous studies reporting that sweetcorn has poorer agronomic performance than wild type corn (Tracy 1990; Revilla *et al.* 2010).

The differences between generation means for adult plant performance and ear performance were not significant. For ear weight and ear/plant, although the differences between generations were significant, the generation means fitted the simplest models with just a parameter, the mean, "m". Thus, for these traits (adult plant and ear performance and ear weight and ear/plant) the genetics effects are not important and are not probably involved in *su1* viability.

For those traits with significant genetic effects, the epistatic effects were generally important contributors to variation suggesting that epistatic gene effects

could be relevant in the basic genetic effects of the viability of *su1*. These results contrast with those reported for agronomic traits in wild type corn by Alonso Ferro *et al.* (2008) reporting that epistatic effects are not important for agronomic and quality traits. However, the magnitude and significance of the estimates for "aa" and "ad" epistatic effect were strongly affected by environments and genes (*Su1 vs. su1*). Although varying depending on the design, environments and traits, in several cases the variation in the generation means did not fit a epistatic model (additive-dominance and digenic epistatic model). Such complex effects of genes with genotypes and environments make more difficult the study of genetic effects involved on *su1* viability and suggest that there are other mechanisms involved in this issue. This is the case of 100-seed weight which fitted a epistatic model with the *su1* mutant, but the simplest model without genetic effects with the wild type in Pontevedra 2010 and the forth cycle of selfpollination. However, 100-seed weight fitted the simplest model with the *su1* mutant and the additive model with the wild type allele in Pontevedra 2011 and the forth cycle of selfpollination.

In spite of the variability produced by the environments, the cycles of selfpollination and the germplasm, some generalization arise from the analyses of some traits. Thus, the genetic of plant height and ear number was complex, involving additive, dominance, digenic epistatic effects and effects not included in the model. For these traits, the complexity of the genetic effects was consistently found in different environments, germplams and cycles of selfpollination. For stand the genetic effects were complex in one design and not important in the other, indicating

that, for this trait, the genetic effects depend on the particular germplasm. For interpreting the influence of the *Su1* locus on the genetic effects, we mainly will focus on the forth cycle of selfpollination because the intraloci allelic interactions of P39 with the wild type corn inbreds are expected to be low due to the fixation. According to our data, the locus *Su1* did not influence the genetic effects of ear number. However, for plant height the magnitude of the additive effects were lower in the sugary genotypes than in wild genotypes in the design derived from EP42 and A661. The additive effects had even different sign in the *sugary1* than the wild genotypes in one of the environments. In the EP42 × A661 design, the *Su1* locus also consistently altered the magnitude of the ad epistatic interactions in the three environments, and the "aa" epistatic effects and the dominance effects in two environments. For stand, the *su1* mutant also altered the magnitude of the additive effects in two of the environments, changing from zero (non significant) to negative (significant) and the magnitude of the "ad" epistatic interactions which were lower with the *su1* mutant than in the wild type (in A619 × A632). For ear length the genetics effects were not significant in the presence of the *su1* allele, but significant in the presence of the wild type allele in the last cycles of self-pollination (in A619 × A632). The differences between *Su1 vs*. *su1* in terms of genetic effects suggest that plant height, stand and ear length have significant effects on *su1* viability. Our results are in concordance with Revilla *et al.* (2000*b*) who found that plant height and ear length are viability-related traits. Revilla *et al.* (2000*a*, 2006) and Djemel *et al.* (2011) reported the dynamic aspects of the *su1* gene frequency decrease and also concluded that the viability of

su1 gene was under genetic control and strongly depended on specific *su1* × wild type maize interactions.

For silking date, the *Su1* locus had a strong effect affecting not only the magnitude, but also the nature of the genetic effects which were not significant in the *Su1* genotypes in any environment, except one and complex in the *su1* genotypes in two of the three environments in the forth cycle of selfpollination. This effect was consistently found in the two designs, that is, is seems independent of the germplam. The tendency to increase the complexity of the genetic effects also was observed in pollen date, but only in one of the designs. Accordingly, Ordás *et al.* (2010) reported that flowering is the only trait that changes when *su1* inbreds lines are converted to the *sh2* due a significant mutant × wild type corn interaction.

As conclusion, our data suggest that the introgression of *su1* in wild type corn affected the genetic effects of some traits as stand, silking date, plant height, and ear length. These traits could contribute to *su1* viability in addition to emergence and early seedling vigor which are the main traits determining *su1* plants viability in previous works.

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Table 1. Estimates of mean (m), additive (a), dominant (d), epistatic (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the first design (EP42 × A661) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra 2011 (MBG11).

Traits	Gene effect	Alger		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (d)	m	84.37±0.407***	79.55±2.080***	75.43±0.407	76.07±0.407	72.39±0.407	73.44±0.407
	a	-1.27±0.632*	0.79±0.632				
	d	-4.97±2.374*	10.51±5.550				
	aa		3.31±2.242				
	χ^2 (df †)	**7.05 (3)ns**	**5.69 (2)ns**	**1.41 (5)ns**	**1.84 (5)ns**	**2.98 (5)ns**	**10.35 (5)ns**
Silk date (d)	m	88.41±0.407***	86.85±0.407***	76.17±0.407	67.85±0.407	74.72±0.407	75.33±0.407
	a	0.27±0.632	1.38±0.632*				
	d	-7.4±2.374**					
	χ^2 (df)	**4.92 (3)ns**	**7.65 (5)ns**	**3.46 (5)ns**	**4.93 (5)ns**	**2.97 (5)ns**	**4.44 (5)ns**

Table 1 (cont.). Estimates of mean (m), additive (a), dominant (d), epistatic (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the first design (EP42 × A661) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Plant height (cm)	m	123.28±2.080***	174.20±2.080***	169.83±2.080***	196.79±2.080***	130.41±2.080***	157.91±2.080***
	a	-3.4±0.707***	5.20±0.707***	0.53±0.707	4.51±0.707***	4.83±0.707***	-11±0.707***
	d	102.98±5.50***	4.27±5.50	32.26±5.50***	-26.85±5.50***	37.68±5.50***	6.71±5.50
	aa	36.53±2.242***	-6.19±2.242**	22.78±2.242***	-4.58±2.242***	23.38±2.242***	9.07±2.242***
	ad	109.08±6.324***	70.12±6.324***	-16±6.324***	-76.34±6.324***	-26.00±6.324***	58.68±6.324***
	χ^2 (df)	**42.90 (1)*** **	**3.21 (1)ns**	**54.84 (1)*** **	**74.36 (1)*** **	**158.09 (1)*** **	**94.19 (1)*** **
Ear number	m	39.77±2.080***	42.93±2.080***	43.48±2.080***	35.15±2.080***	24.30±2.080***	25.52±2.080***
	a	-1.86±0.707***	-1.05±0.707***	-2.90±0.707***	7.15±0.707***	4.67±0.707***	3.17±0.707***
	d	-42.10±5.50***	-52.89±5.50***	0.01±5.50	11.25±5.50*	44.47±5.50***	8.61±5.50
	aa	-14.12±2.242***	-24.61±2.242***	-3.34±2.242***	5.03±2.242*	13.30±2.242***	7.22±2.242**
	ad	39.92±6.324***	23.24±6.324***	64±6.324***	-32.60±6.324***	-22.68±6.324***	17.96±6.324**
	χ^2 (df)	**4.62 (1)***	**15.96 (1)*** **	**11.07 (1)*** **	**3.25 (1)ns**	**19.80 (1)*** **	**3.45 (1)ns**

Table 1 (cont.). Estimates of mean (m), additive (a), dominant (d), epistatic (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the first design (EP42 × A661) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
100-kernel weight (g)	m	-	-	29.01±2.080***	20.80±0.407***	25.31±2.080***	25.21±2.080***
	a	-	-	1.35±0.707***	1.80±0.632**	1.16±0.707***	-1.12±0.707
	d	-	-	-9.8±5.50***		2.79±5.50***	-9.93±5.50
	aa	-	-	-5.37±2.242***		0.37±2.242	-6.84±2.242**
	ad	-	-	-2.5±6.324		13.62±6.324***	17.28±6.324**
	χ^2 (df)	**-**	**-**	**20.35 (1)*** **	**3.22 (5)ns**	**4.22 (1)***	**0.03 (1)ns**

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 2. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the second selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	84.05±0.407***	83.19±0.407	76.52±0.407	77.47±0.407	74.49±0.407	75.33±0.407
	a	-1.68±0.632**					
	χ^2 (df †)	**5.33 (4)ns**	**7.87 (5)ns**	**6.70 (5)ns**	**4.96 (5)ns**	**2.37 (5)ns**	**6.65 (5)ns**
Silk date (days)	m	85.79±0.407***	85.82±0.407	77.39±0.407	78.93±0.407	76.55±0.407	77.11±0.407
	a	-1.80±0.632**					
	χ^2 (df)	**5.15 (4)ns**	**6.22 (5)ns**	**5.53 (5)ns**	**3.22 (5)ns**	**1.47 (5)ns**	**4.38 (5)ns**
Plant height (cm)	m	167.28±2.080***	205.13±2.080***	166.36±2.080***	192.90±2.080***	181.91±2.080***	130.00±2.080***
	a	2.46±0.707***	11.17±0.707***	6.95±0.707***	20.29±0.707***	10.83±0.707***	1.67±0.707
	d	-48.56±11.101***	-250.45±11.101***	75.86±11.101***	-99.16±11.101***	-168.66±11.101***	32.87±11.101
	aa	-7.17±2.242***	-56.60±2.242***	14.62±2.242***	-16.87±2.242***	-34.04±2.242***	12.10±2.242***
	ad	56.6±12.649***	160.8±12.649***	40.16±12.649***	-105.96±12.649***	129.36±2.242***	54.64±±2.242**
	χ^2 (df)	**7.40 (1)****	**51.49 (1)*****	**17.77 (1)*****	**48.94 (1)*****	**7.64 (1)*****	**450.89 (1)*****

Table 2 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the second selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear number	m	26.22±2.080***	21.26±2.080***	53.97±2.080***	49.46±2.080***	48.10±2.080***	10.03±2.080***
	a	3.36±0.707***	0.44±0.707	1.15±0.707**	1.25±0.707*	2.33±0.707**	1.50±0.707**
	d	-5.05±11.101	-35.47±11.101***	-38.6±11.101***	-61.24±11.101***	-58.04±11.101***	47.02±11.101***
	aa	2.17±2.242	-6.67±2.242***	-9.81±2.242***	-15.28±2.242***	-14.14±2.242***	12.02±2.242***
	ad	-53.76±12.649***	20.00±12.649*	-58.00±12.649***	74.80±12.649***		-25.36±12.649**
	χ^2 (df)	**75.80 (1)*** **	**90.23 (1)*** **	**51.96 (1)*** **	**117.57 (1)*** **	**0.07 (2)ns**	**111.80 (1)*** **
100-kernel weight (gr)	M	-	-	24.06±0.407	11.84±2.080***	25.13±0.407	-34.94±2.080***
	a	-	-		2.05±0.707***		-0.43±0.707
	d	-	-		38.55±11.101***		378.22±11.101***
	aa	-	-		6.53±2.242***		49.85±2.242***
	ad	-	-		-22.72±12.649***		-1.16±12.649
	χ^2 (df)	**-**	**-**	**10.75 (5)ns**	**4.38 (1)***	**10.69 (5)ns**	**167.09 (1)*** **

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 3. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	83.15±0.407	83.46±0.407	78.43±0.407	78.79±0.407	76.38±0.407	79.11±2.080
	a						1.00±0.707
	d						-29.03±22.204
	aa						-2.37±2.242
	ad						-5.28±25.298
	χ^2 (df †)	**7.86 (5)ns**	**7.64 (5)ns**	**1.91 (5)ns**	**5.02 (5)ns**	**4.08 (5)ns**	**8.96 (1)***
Silk date (days)	m	82.63±2.080***	85.30±2.080***	79.43±0.407	82.73±2.080***	78.05±0.407	78.61±0.407
	a	2.25±0.707**	2.32±0.707**		-0.54±0.707**		
	d	32.86±22.204	-1.75±22.204		-34.64±22.204***		
	aa	4.49±2.242*	1.46±2.242		-3.18±2.242***		
	ad	-65.70±25.298*	-76.72±25.298**		22.24±25.298***		
	χ^2 (df)	**0.001 (1)ns**	**2.61 (1)ns**	**4.23 (5)ns**	**9.19 (1)****	**4.09 (5)ns**	**1.87 (5)ns**

Table 3 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Plant height (cm)	m	137.97±2.080***	137.30±2.080***	194.43±2.080***	169.51±2.080***	143.65±2.080***	112.55±2.080***
	a	1.23±0.707	6.83±0.707***	18.35±0.707***	8.22±0.707***	10.83±0.707***	3.67±0.707***
	d	246.11±22.204***	271.08±22.204***	-155.71±22.204***	-28.65±22.204***	117.25±22.204***	313.46±22.204***
	aa	21.21±2.242***	13.82±2.242***	-23.81±2.242***	9.06±2.242***	4.17±2.242*	28.14±2.242***
	ad	-0.16±25.298	16.00±25.298***	-216.16±25.298***	23.68±25.298*	-77.09±25.298***	111.84±25.298***
	χ^2 (df)	**474.47 (1)*** **	**17.63 (1)*** **	**201.57 (1)*** **	**23.20 (1)*** **	**151.26 (1)*** **	**4.72 (1)***
Ear number	m	24.41±2.080***	9.67±2.080***	35.70±2.080***	30.68±2.080***	60.52±2.080***	1.18±2.080
	a	2.11±0.707***	3.67±0.707***	5.40±0.707***	7.90±0.707***	8.83±0.707***	2.33±0.707***
	d	-35.11±22.204*	59.27±22.204***	41.49±22.204***	38.45±22.204***	-255.25±22.204***	294.12±22.204***
	aa	-5.14±2.242**	10.49±2.242***	1.39±2.242	6.85±2.242***	-28.55±2.242**	19.46±2.242***
	ad	-19.01±25.298	-52.80±25.298**	46.46±25.298***	-129.60±25.298***	71.51±25.298**	-186.64±25.298***
	χ^2 (df)	**91.28 (1)*** **	**102.45 (1)*** **	**19.35 (1)*** **	**22.94 (1)*** **	**1.97 (1)ns**	**25.80 (1)*** **

Table 3 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
100-kernel weight (gr)	m			23.22±0.407	17.43±0.407	22.27±2.080***	21.18±2.080***
	a					0.41±0.707	1.71±0.707***
	d					30.05±22.204	-14.11±22.204**
	aa					2.09±2.242	-4.09±2.242***
	ad					92.60±25.298***	-78.16±25.298***
	χ^2 (df)			**5.55 (5)ns**	**2.86 (5)ns**	**0.01 (1)ns**	**7.63 (1)****

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 4. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Silk date (days)	m	87.35±0.407	87.78±0.641***	80.34±0.407	86.63±2.080***	78.83±0.407	78.77±0.407
	a		-1.01±0.632ns		-1.29±0.632*		
	d		-83.85±19.01***		-134.23±44.40**		
	aa				-7.07±2.242**		
	χ^2 (df †)	**10.27 (5)ns**	**6.64 (3)ns**	**3.92 (5)ns**	**4.19 (2)ns**	**2.82 (5)ns**	**3.92 (5)ns**
Plant height (cm)	m	215.04±2.080***	116.07±2.080***	172.46±2.080***	139.32±2.080***	148.76±2.080***	148.06±2.080***
	a	10.16±0.707***	-10.23±0.707***	6.47±0.707***	4.18±0.707***	5.00±0.707***	1.33±0.707**
	d	-1246.30±44.40***	928.64±44.40***	-56.26±44.40***	572.95±44.40***	98.60±44.40***	-324.25±44.40***
	aa	-51.23±2.242***	44.02±2.242***	-1.73±2.242**	30.67±2.242***	-6.13±2.242***	-14.16±2.242***
	ad	-110.28±50.59***	635.68±50.59***	-866.24±50.59***	184.32±50.59***	-320.00±50.59***	405.44±50.59***
	χ^2 (df)	**3298.04 (1)*** **	**1.12 (1)ns**	**9.79 (1)** **	**1.89 (1)ns**	**7.42 (1)** **	**65.16 (1)*** **

Table 4 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the first design (EP42 × A661) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear number	m	34.04±2.080***	-2.59±2.080**	50.83±2.080***	33.53±0.407***	26.07±2.080***	18.75±2.080***
	a	1.36±0.707***	1.30±0.707***	3.50±0.707***	-1.52±0.632**	-1.83±0.707***	1.83±0.707***
	d	-268.72±44.40***	467.67±44.40***	-187.02±44.40***		91.73±44.40***	66.56±44.40***
	aa	-5.74±2.242**	18.07±2.242***	-6.71±2.242***		1.16±2.242*	2.04±2.242***
	ad	-52.96±50.59**	-240.16±50.59***	-412.8±50.59***		-143.84±50.59***	122.56±50.59***
	χ^2 (df)	**20.35 (1)*** **	**30.87 (1)*** **	**5.60 (1)****	**9.35 (4)ns**	**8.71 (1)****	**4.54 (1)***
100 kernel weight (gr)	m			23.16±0.407	18.69±2.080***	22.25±0.407***	18.39±0.407
	a				2.33±0.707**	2.82±0.632***	
	d				-26.33±44.40		
	aa				0.89±2.242		
	ad				-128.64±50.59*		
	χ^2 (df)			**10.19 (5)ns**	**2.19 (1)ns**	**7.35 (4)ns**	**0.25 (5)ns**

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 5. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	83.30±0.407	83.24±0.407	77.22±0.407	77.81±0.407	75.22±0.407***	76.88±0.407***
	a					-2.20±0.632***	-1.80±0.632**
	χ^2 (df †)	**8.28 (5)ns**	**3.08 (5)ns**	**4.45 (5)ns**	**6.16 (5)ns**	**6.04 (4)ns**	**4.05 (4)ns**
Silk date (days)	m	84.91±0.641***	86.12±0.407	78.31±0.407	79.29±0.407	78.77±0.407	79.55±0.407
	a	-0.18±0.632					
	d	6.25±2.374**					
	χ^2 (df)	**4.75 (3)ns**	**4.43 (5)ns**	**2.72 (5)ns**	**2.36 (5)ns**	**3.26 (5)ns**	**9.26 (5)ns**
Plant height (cm)	m	182.28±2.080***	178.19±2.080***	222.19±2.080***	199.34±2.080***	166.77±2.080***	189.07±2.080***
	a	-7.06±0.707***	7.30±0.707***	-5.63±0.707***	-6.52±0.707***	-6.73±0.632***	-6.50±0.707***
	d	-28.64±5.550**	-21.68±5.550*	-93.25±5.550***	4.30±5.550**	-9.46±5.550	-75.21±5.550***
	aa	-0.43±2.242	-5.64±2.242	-30.12±2.242***	-0.14±2.242	-7.91±2.242***	-32.84±2.242***
	ad	34.14±6.324***	16.12±6.324	32.94±6.324***	2.60±6.324		-54.00±6.324***
	χ^2 (df)	**479.62 (1)*** **	**566.94 (1)*** **	**1.62 (1)ns**	**12.88 (1)*** **	**0.11 (2)ns**	**8.70 (1)** **

Table 5 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Stand (%)	m	84.15±2.080***	43.18±2.080***	72.25±2.080***	62.24±2.080***	58.93±0.641***	65.03±2.080***
	a	-1.43±0.707*	-1.13±0.707	-2.30±0.707***	0.65±0.707	-1.33±0.632*	-5.00±0.707***
	d	-54.91±5.550***	13.48±5.550*	-1.14±5.550	1.00±5.550	-14.10±2.374***	-67.17±5.550***
	aa	-17.50±2.242***	9.34±2.242***	-0.36±2.242	-2.77±2.242		-23.30±2.242***
	ad	44.44±6.324***	61.74±6.324***	32.80±6.324***	-29.00±6.324***		-17.32±6.324**
	χ^2 (df)	**0.90ns**	**232.66*** **	**1.50ns**	**0.89ns**	**7.36ns**	**0.20ns**
Ear number	m	33.08±2.080***	29.31±2.080***	39.62±2.080***	42.01±2.080***	38.33±2.080***	43.91±2.080***
	a	-9.18±0.707***	-1.14±0.632	-0.85±0.707***	-3.35±0.707***	-8.50±0.707***	-8.00±0.707***
	d	-19.40±5.550***	-16.16±5.550**	5.43±5.550***	-3.08±5.550	8.01±5.550	-36.33±5.550***
	aa	-4.38±2.242***	-4.66±2.242*	3.34±2.242***	1.47±2.242	2.67±2.242	-10.04±2.242***
	ad	34.30±6.324***		-23.00±6.324***	-38.20±6.324***	39.32±6.324***	-20.00±6.324**
	χ^2 (df)	**47.76 (1)*** **	**0.73 (2)ns**	**12.79 (1)*** **	**0.97 (1)ns**	**1.50 (1)ns**	**0.82 (1)ns**

Table 5 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the first selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear length (cm)	m	14.89±0.407	14,96±0.407	24.64±2.080***	17.45±0.407	15.59±0.407	14.88±0.407
	a			0.90±0.632			
	d			-16.19±5.550**			
	aa			-5.77±2.242*			
	χ^2 (df)	**9.54 (5)ns**	**9.39 (5)ns**	**4.38 (2)ns**	**3.03 (5)ns**	**2.62 (5)ns**	**4.46 (5)ns**

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 6. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the second selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	83.77±0.407***	38.44±0.407	79.28±0.407	80.21±0.407	78.55±0.407	78.94±0.407
	a	-1.01±0.632					
	χ^2 (df †)	**9.01 (4)ns**	**6.19 (5)ns**	**2.97 (5)ns**	**2.14 (5)ns**	**4.13 (5)ns**	**9.00 (5)ns**
Silk date (days)	m	86.30±0.407	85.43±0.407	80.50±0.407	81.67±0.407	79.93±2.080***	82.28±0.407
	a					2.00±0.707**	
	d					-3.36±11.101	
	aa					1.28±2.242	
	ad					-32.0012.649*	
	χ^2 (df)	**6.50 (5)ns**	**10.84 (5)ns**	**6.28 (5)ns**	**3.99 (5)ns**	**2.38 (1)ns**	**7.20 (5)ns**

Table 6 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the second selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Plant height (cm)	m	148.77±2.080***	181.55±2.080***	176.61±2.080***	183.96±2.080***	132.71±2.080***	174.50±2.080***
	a	-4.33±0.707***	-10.00±0.707***	-9.74±0.707***	-18.65±0.707***	-10.00±0.707***	-6.33±0.707***
	d	108.35±11.101***	-13.76±11.101**	37.41±11.101***	17.38±11.101***	140.31±11.101***	-70.66±11.101***
	aa	6.48±2.242*	-16.76±2.242***	0.39±2.242	-8.96±2.242***	22.01±2.242***	-15.42±2.242***
	ad	-127.48±12.649***	26.72±12.649***	29.12±12.649***	92.84±12.649***	-10.64±12.649	-250.72±12.649***
	χ^2 (df)	**364.34 (1)*** **	**35.41 (1)*** **	**69.72 (1)*** **	**9.60 (1)** **	**139.07 (1)*** **	**63.72 (1)*** **
Stand (%)	m	24.37±2.080***	47.33±2.080***	34.32±2.080***	56.87±2.080***	47.92±2.080***	35.62±2.080***
	a	-6.26±0.707***	-9.40±0.707***	-175±0.707***	-11.75±0.707***	-6.33±0.707***	-18.66±0.707***
	d	167.77±11.101***	-69.79±11.101***	137.8±11.101***	-74.07±11.101***	-33.55±11.101***	17.04±11.101***
	aa	25.44±2.242***	-2.20±2.242*	34.41±2.242***	-5.48±2.242***	-1.85±2.242***	-12.68±2.242***
	ad	109.24±12.649***	33.60±12.649***	92.40±12.649***	86.80±12.649***	87.96±12.649***	80.04±12.649***
	χ^2 (df)	**111.12 (1)*** **	**56.48 (1)*** **	**25.14 (1)*** **	**28.43 (1)*** **	**10.08 (1)** **	**8.42 (1)** **

Table 6 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the second selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear number	m	8.59±2.080***	21.44±2.080***	21.07±2.080***	39.48±2.080***	33.74±2.080***	30.55±2.080***
	a	-3.34±0.707***	-3.33±0.707***	-9.35±0.707***	-9.55±0.707***	-10.16±0.707***	-15.00±0.707***
	d	51.57±11.101***	-40.35±11.101***	98.45±11.101***	-55.70±11.101***	-17.97±11.101***	-1.48±11.101
	aa	15.33±2.242***	0.30±2.242	21.31±2.242***	-2.40±2.242*	2.04±2.242***	-12.96±2.242***
	ad	59.96±12.649***	-91.64±12.649***	70.00±12.649***	-23.60±12.649***	41.32±12.649***	53.28±12.649***
	χ^2 (df)	**17.31 (1)*** **	**1.62 (1)ns**	**3.34 (1)ns**	**31.68 (1)*** **	**7.65 (1)****	**8.96 (1)****

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 7. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the second design (A619 × A632) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	83.26±0.407	82.72±0.407	79.83±0.407	77.03±2.080***	78.44±0.407	78.83±0.407
	a				-0.96±0.707***		
	d				46.71±22.204***		
	aa				3.56±2.242***		
	ad				26.71±25.298***		
	χ^2 (df †)	**4.63 (5)ns**	**4.14 (5)ns**	**3.05 (5)ns**	**6.68 (1)****	**5.96 (5)ns**	**10.83 (5)ns**
Silk date (days)	m	82.08±2.080***	85.94±2.080***	81.21±0.407	78.32±2.080***	79.88±0.407	74.01±2.080***
	a	-1.64±0.707***	-1.04±0.707**		-0.94±0.707***		1.19±0.632
	d	34.49±22.204***	12.34±22.204		48.16±22.204***		95.87±22.209***
	aa	5.92±2.242***	-2.40±2.242		3.86±2.242***		7.98±2.244***
	ad	42.13±25.298***	64.09±25.298***		42.07±25.298***		
	χ^2 (df)	**4.84 (1)***	**53.95 (1)*****	**3.45 (5)ns**	**7.47 (1)****	**5.96 (5)ns**	**1.50 (2)ns**

Table 7 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the second design (A619 × A632) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Plant height (cm)	m	124.83±2.080***	167.47±2.080***	171.65±2.080***	197.46±2.080***	142.32±2.080***	129.01±2.080***
	a	0.33±0.707	7.13±0.707***	-8.16±0.707***	-9.28±0.707***	-5.66±0.707***	-8.50±0.707***
	d	335.23±22.204***	28.31±22.204	36.99±22.204*	-286.89±22.204***	7.03±22.204	243.59±22.204***
	aa	44.40±2.242***	-2.89±2.242	1.86±2.242	-24.69±2.242***	5.28±2.242***	18.17±2.242***
	ad	-15.38±25.298	-72.51±25.298***	457.81±25.298***	386.58±25.298***	277.37±25.298***	141.82±25.298***
	χ^2 (df)	**266.66 (1)*** **	**1.30 (1)ns**	**85.98 (1)*** **	**39.36 (1)*** **	**60.82 (1)*** **	**43.87 (1)*** **
Stand (%)	m	40.90±2.080***	63.27±2.080***	71.10±2.080***	61.24±2.080***	22.96±2.080***	50.24±2.080***
	a	3.70±0.707***	-9.81±0.707***	5.45±0.707***	-7.05±0.707***	-4.66±0.707***	-8.33±0.707***
	d	109.23±22.204***	-91.83±22.204***	-59.27±22.204***	-29.08±22.204***	222.38±22.204***	-65.42±22.204***
	aa	12.64±2.242***	-10.82±2.242***	0.04±2.242	-9.58±2.242***	19.75±2.242***	-6.53±2.242***
	ad	35.53±25.298***	178.57±25.298***	-68.09±25.298***	31.15±25.298***	224.27±25.298***	111.81±25.298***
	χ^2 (df)	**27.41 (1)*** **	**0.20 (1)ns**	**19.64 (1)*** **	**12.87 (1)*** **	**20.54 (1)*** **	**21.42 (1)*** **

Table 7 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the third selfing generation of the second design (A619 × A632) in Alger (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear number	m	23.29±0.641***	30.36±2.080***	32.35±2.080***	56.39±2.080***	13.74±2.080***	51.61±2.080***
	a	2.66±0.632***	-7.83±0.632***	-5.30±0.707***	-5.39±0.707***	-10.66±0.707***	-12.00±0.707***
	d	-65.93±9.504***	-121.08±22.209***	69.00±22.204***	-169.09±22.204***	186.14±22.204***	-189.98±22.204***
	aa		-8.03±2.244***	12.08±2.242****	-23.36±2.242***	16.92±2.242***	-19.84±2.242***
	ad			38.51±25.298***	5.32±25.298	202.93±25.298***	69.13±25.298***
	χ^2 (df)	**5.85 (3)ns**	**5.18 (2)ns**	**17.13 (1)*** **	**14.28 (1)*** **	**52.59 (1)*** **	**17.40 (1)*** **
Ear length (cm)	m	15.01±0.641***	12.96±0.407	16.85±0.407***	15.01±0.407	14.02±0.407	12.92±0.407
	a	2.20±0.632***		2.12±0.632***			
	d	-19.27±9.504*					
	χ^2 (df)	**6.44 (3)ns**	**4.88 (5)ns**	**0.92 (4)ns**	**2.06 (5)ns**	**5.98 (5)ns**	**2.55 (5)ns**

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Table 8. Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Pollen date (days)	m	82.91±0.407	83.13±0.407	75.11±2.080***	75.50±2.080***	79.49±0.407	80.37±0.641***
	a			-1.77±0.632**	-2.20±0.707***		-0.86±0.632
	d			174.02±44.408***	158.02±44.408***		53.96±19.010**
	aa			6.17±2.242**	8.95±2.242***		
	ad				126.40±50.596***		
	χ^2 (df †)	**1.14 (5)ns**	**7.03 (5)ns**	**3.94 (2)ns**	**14.27 (1)*** **	**10.83 (5)ns**	**7.77 (5)ns**
Silk date (days)	m	85.67±0.407	86.12±0.407	82.14±0.641***	77.56±2.080***	80.94±0.407	82.45±2.080***
	a			-0.91±0.632	-1.49±0.707***		0.17±0.707
	d			48.35±19.010*	142.63±44.408***		30.70±44.408***
	aa				8.93±2.242***		2.73±2.242***
	ad				91.68±50.596***		112.00±50.596***
	χ^2 (df)	**3.91 (5)ns**	**8.22 (5)ns**	**4.65 (3)ns**	**15.09 (1)*** **	**7.84 (5)ns**	**5.35 (1)***

Table 8 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Plant height (cm)	m	234.41±2.080***	199.87±2.080***	163.41±2.080***	177.53±2.080***	150.56±2.080***	166.11±2.080***
	a	-17.03±0.707***	-10.93±0.707***	-0.77±0.707***	-18.53±0.707***	-9.33±0.707***	-8.66±0.707***
	d	-1623.91±44.408***	-1063.91±44.408***	195.12±44.408***	-302.13±44.408***	-208.79±44.408***	-399.39±44.408***
	aa	-70.23±2.242***	-41.51±2.242***	15.58±2.242***	-7.48±2.242***	-1.63±2.242***	-19.03±2.242***
	ad	607.04±50.596***	943.20±50.596***	359.20±50.596***	700.16±50.596***	255.84±50.596***	-288.16±50.596***
	χ^2 (df)	**74.43* (1)****	**665.70 (1)*****	**15.52 (1)*****	**160.51 (1)*****	**362.91 (1)******	**85.65 (1)*****
Stand (%)	m	72.82±2.080***	83.39±2.080***	68.12±2.080***	46.87±2.080***	46.82±2.080***	24.58±2.080***
	a	-8.85±0.707***	-6.59±0.707***	-0.15±0.707	-8.80±0.707***	0.33±0.707	-2.00±0.707***
	d	-445.62±44.408***	-673.03±44.408***	21.09±44.408	211.43±44.408***	-143.89±44.408**	256.88±44.408***
	aa	-21.11±2.242***	-34.91±2.242***	2.34±2.242	10.71±2.242***	-3.90±2.242	3.06±2.242***
	ad	645.28±50.596***	445.12±50.596***	168.00±50.596**	-124.80±50.596*	181.44±50.596***	106.56±50.596***
	χ^2 (df)	**0.46 (1)ns**	**10.02 (1)****	**4.45 (1)ns**	**0.005 (1)ns**	**0.40 (1)ns**	**5.57 (1)***

Table 8 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Ear number	m	38.71±2.080***	26.50±2.080***	31.87±2.080***	30.58±0.641***	39.25±2.080***	10.21±2.080***
	a	-4.95±0.707***	-6.26±0.707***	-5.30±0.707***	-8.22±0.632***	-7.67±0.707***	-2.00±0.707***
	d	-419.80±44.408***	-300.64±44.408***	182.63±44.408***	105.41±19.010***	-201.88±44.408***	431.49±44.408***
	aa	-22.60±2.242***	-6.54±2.242***	8.11±2.242***		-7.60±2.242***	4.70±2.242***
	ad	122.24±50.596***	417.76±50.596***	-105.60±50.596*		394.88±50.596***	-74.88±50.596*
	χ^2 (df)	**13.74 (1)*** **	**16.49 (1)*** **	**2.40 (1)ns**	**5.08 (3)ns**	**6.44 (1)***	**72.41 (1)*** **
Ear length (cm)	m	17.83±2.080***	11.50±0.407	16.85±0.407	15.00±0.407	13.86±0.407	12.70±0.407
	a	-1.55±0.707***					
	d	-99.35±44.408***					
	aa	-5.35±2.242***					
	ad	66.24±50.596***					
	χ^2 (df)	**8.51 (1)****	**4.85 (5)ns**	**1.60 (5)ns**	**6.63 (5)ns**	**3.32 (5)ns**	**7.66 (5)ns**

Table 8 (cont.). Estimates of mean (m), additive (a), dominance (d), epistasis (aa and ad) gene effects of *Su1* and *su1* plants, and χ^2 test from generation mean analysis derived from the fourth selfing generation of the second design (A619 × A632) in ALGER (ENSA), Pontevedra in 2010 (MBG10), and Pontevedra in 2011 (MBG11).

Traits	Gene effect	ENSA		MBG10		MBG11	
		Su1	*su1*	*Su1*	*su1*	*Su1*	*su1*
Kernel row no.	m	17.79±2.080***	13.50±0.407	14.15±0.407	14.25±0.407	14.07±0.407	13.71±0.407
	a	-2.53±0.707***					
	d	-90.38±44.408***					
	aa	-5.25±2.242***					
	ad	59.04±50.596***					
	χ^2 (df)	**5.43 (1)***	**1.50 (5)ns**	**7.57 (5)ns**	**8.90 (5)ns**	**4.78 (5)ns**	**4.58 (5)ns**

ns, *, ** and ***, not significant, or significant at the level of probability 0.05, 0.01, and 0.001, respectively.

† degrees of freedom.

Chapter 4

Genomic regions affecting fitness of the sweetcorn mutant *sugary1*

Short title: *Genomic regions of sugary1 fitness*

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SUMMARY

Mutants often reduce fitness when incorporated into some genotypes, as is the case of the mutant gene *sugary1* (*su1*) in maize (*Zea mays* L.). Understanding the genetic factors affecting variation in the fitness of a mutant is of major interest from a theoretical point of view and also from a breeder's perspective. The genetic regulation of *su1* behaviour was examined in two independent materials. First, populations of two recombinant inbred lines (RIL) were used, belonging to the Nested Association Mapping (NAM) design produced from crosses between the maize inbred B73 and two sweetcorn lines (P39 and Il14h) that were genotyped with 1106 single nucleotide polymorphisms (SNPs). These RILs had a group of lines with the *su1* allele and another group with the wild allele. At each marker, the allele frequencies of both groups of RILs were compared. Second, an F_2 population derived from the cross between A619 (a field maize inbred line) and P39 (a sweetcorn inbred line) was characterized with 295 SSRs. In addition, the population was phenotyped for several traits related to viability. A large linkage block was detected around *su1* in the RILs belonging to the NAM. Furthermore, significant genomic regions associated with *su1* fitness were detected along the 10 maize chromosomes, although the detected effects were small. Quantitative trait loci (QTLs) with effects in multiple traits related to *su1* fitness were detected in the F_2 population, for example at bin 5.04. Therefore, the present results suggest that the *su1* fitness depends on many genes of small effect distributed along the genome, with pleiotropic effects on multiple traits.

INTRODUCTION

Mutations are the raw material of evolution wherein the effect of natural selection depends strongly on the fitness of each mutant within a given environment and genetic background. To understand the nature of quantitative variation, and thus the potential and speed of adaptation of cultivars to different environments (Badu-Apraku *et al.* 2012), it is important to determine the positive or negative fitness effects of mutations. Indeed, most mutations affecting fitness and fitness components are harmful (Garcia-Dorado *et al*. 1998). Some mutants reduce fitness, as is the case of the recurrent mutant gene *sugary1* (*su1*) in maize (*Zea mays* L.) (Revilla *et al*. 2000, 2006) located on chromosome 4 (Tracy *et al*. 2006). Mutant seeds homozygous for the allele *su1* are deficient in the production of insoluble starch, but accumulate an increased proportion of soluble sugars during endosperm development (Schultz & Juvik 2004). Sweetcorn varieties are cultivars homozygous for *su1* (or some other endosperm mutant) and are one of the main products obtained from maize which, in addition to varieties used for cornflakes (Alonso Ferro *et al*. 2008), are directly used for human consumption in temperate areas.

The gene *su1* is considered lethal or semi-lethal when introduced in most field maize genetic backgrounds (Tracy 1990). Directional selection against *su1* has been reported by Martins & Da Silva (1998) in crosses between *Su1* and *su1* maize inbred lines. They also found that the reduced germination and smaller seedling vigour of a homozygous *su1* seed significantly affect gene frequencies. Revilla *et al*. (2000, 2006, 2010) and Ordás *et al.* (2010) also reported that the viability of the *su1* and *shrunken2* (*sh2*) mutants depend on specific sweetcorn × field maize genotype interaction, with genetic background playing a major role in the viability of those mutants. The same conclusions were reached by Yamamoto *et al*. (2009), Le Gac & Doebeli (2010) and Magwire *et al*. (2010) for the factors affecting variation of mutant fitness in *Drosophila melanogaster*. Butler (1977) reported that the mutants whose viability value showed great heterogeneity, with both excesses and deficiencies, were probably influenced by their linkage with other genes. Re-

cently, the genetic effects on the fitness of the *su1* allele in wild type maize were monitored through five successive generations of selfing in two separate designs of mean generation analyses by Djemel *et al*. (2011). Fitness of *su1* is under genetic control with significant additive effects that are probably due to multiple genes with minor contributions. This suggests that the interaction of genetic backgrounds with alleles could have evolutionary implications by increasing or decreasing the probability of mutant fixation. All the works previously mentioned suggest also that the viability of seeds homozygous for *su1* is not solely a function of the allele, but that it is also controlled by other genes.

In order to understand the molecular basis of phenotypic variation in maize, McMullen *et al*. (2009) crossed 25 diverse inbred lines with the reference inbred line B73 and obtained RIL populations to create the Nested Association Mapping population (NAM); the two sweetcorn lines (Il14h and P39) show distortion on chromosome 4 against the *su1* mutant. Numerous analyses have demonstrated that context dependency and dynamics variation of mutation across genomes can be attributed to the composition of the nucleotides flanking a mutation (Morton *et al*. 2006).

There are several questions that must be answered in order to understand which genes or genetics factors are essential for a mutant viability. Because of the complexity of the multitude of biological processes required for a plant to grow, it is possible that a large and diverse set of genes are likely to be involved. Given the different mechanisms affecting mutant fitness and the need to understand the genetic networks underlying each mechanism, there is a clear need for genome screens to identify genes and genetic networks affecting mutant viability. The aims of the present study were to: (i) determine the size of the critical genomic regions that co-segregates with the *su1* allele and examine the existence of chromosomal regions that exhibit segregation distortion against the *su1* allele; and (ii) to identify the genes affecting the viability of lines containing the allele *su1*.

MATERIALS AND METHODS

Single nucleotide polymorphism (SNP) characterization in the RIL

In order to estimate the size of the chromosomal regions flanking the *su1* allele, the genetic map of two populations of recombinant inbred lines (RIL) derived from the cross between the reference inbred B73 (released from the Iowa Stiff Stalk Synthetic population) and two sweetcorn inbreds, namely P39 (developed from cvar Golden Bantam) and Il14h (developed from cvar Stowell's Evergreen), was used. Two other RIL populations derived from the cross of B73 with two field maize inbred lines (Oh43 and Tx303) were used as reference. All these materials were part of the NAM population, released and genotyped by the Maize Diversity Project (www.panzea.org; now known as Genetic Architecture of Maize and Teosinte; verified 9 March 2012).

The NAM genetic map consists of 1106 loci, with an average marker density of one marker every 1.3 centimorgans (cM) (McMullen *et al*. 2009). Because the *Su1* locus was not mapped in NAM, the B73 reference genome v2 (www.maizesequence.org; verified 9 March 2012) was used to estimate the exact coordinates of this locus and the position on NAM genetic map. The *Su1* locus is estimated between the positions 53.7 cM and 55.2 cM on the chromosome 4 and flanked by the markers PZA01751.2 and PZA00445.22. The RILs were classified into field maize or sweetcorn types when both flanking markers of *Su1* had the B73 or the alternative allele from the sweetcorn inbred line (P39 or Il14h), respectively. All intervals with missing values were excluded from the analyses.

Simple sequence repeat (SSR) characterization of the F_2 population

To identify the genetic factors controlling the viability of seeds containing different alleles, an F_2 population derived from the cross between the field maize inbred line A619 (Lancaster) and the sweetcorn inbred line P39 was employed; this population was used because A619 showed the highest negative selection intensity against *su1* in a previous study (Djemel *et al*. 2011).

Self-pollination of the F1 derived from the cross A619 × P39 yielded a F_2 population in Pontevedra (Spain, 42º 24' N, 8º 38' W, 20 m asl) in 2006. Six hundred $F_{2:3}$ seeds were sown in 2009. The distance was 0.80 m between rows and 0.21 m between plants for a planting density of *c*. 60 000 plants/ha. The F_2 plants were self-pollinated. At harvest, the total number of plants that survived was 488, of which 175 were heterozygous *Su1su1*. All analyses were carried out on heterozygous plants only.

Observations were made on individual plants of: early vigour (at the five-leaf stage by using a visual scale from 1 = poor to 9 = excellent), leaf chlorophyll content measured at vegetative and reproductive stage using a hand-held Chlorophyll Content Meter, the CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA), ear length (mm), ear weight (g), observed number of field maize and sweetcorn (*su1su1*) seeds and seed type (using a visual scale from 1 = dent to 4 = flint).

For each plant, 25 seeds from each heterozygote ear for each phenotype (sugary or non sugary) were germinated in Petri dishes in a growing chamber at 25 °C. Each Petri dish had 7 ml of distilled water added before closing with Parafilm. Characters related to germination were recorded 7 days later (proportion of sugary or non sugary seeds with roots and coleoptiles). The coefficient of selection against *su1* was calculated as the proportion of germination of sugary seeds relative to that from non-sugary ones.

Leaf tissue was collected from the fourth or fifth leaf for each heterozygous plant. DNA was extracted from leaf tissue according to Liu & Whittier (1994). SSR amplifications were performed as described by Butrón *et al.* (2003). SSR products were separated after amplification by electrophoresis on a 60 g/l (6 %) non-denaturing acrylamide gel (*c*. 250 V for 3 h) (Wang *et al*. 2003). A set of 295 SSR markers distributed along the genome were screened for polymorphism among parental inbred lines.

Data analyses

For the RILs populations, the proportion of expected segregation *Su1*:*su1* is 1:1 when there is no selection. The expected number of sweetcorn RILs was compared to the observed number using the chi-square goodness of fit test (χ^2) ($P < 0.05$). The maximum and the minimum of the genomic region flanking a *Su1* interval were defined as the length of the chromosome fragment (cM) conserving the B73 or the alternative alleles continuously at both sides of the *Su1* locus.

Moreover, to examine the existence of chromosomal regions that exhibited segregation distortion against the *su1* allele, SNPs variability was classified into two main types (sweetcorn and field maize) and, within each type, the number of RILs sharing the B73 alleles was compared with the number of RILs sharing the sweetcorn allele in other loci through the genome. The deviation from the expected number was tested with χ^2 at $P < 0.05$ and at P value with Bonferroni criterion ($P < 0.05/N$; N= number of SNP markers) using a contingency table (Steel *et al*. 1997). The *– log P* for the χ^2 value for segregation of B73 *vs.* the two sweetcorn parental alleles was calculated.

For each plant of the population (A619 × P39) F_2 the expected number of sugary seeds was calculated and compared with the observed number of sugary seeds using the χ^2 test at $P < 0.05$. The SSRs associated with *su1* viability were identified by using bulk segregant analysis of *su1* frequency (Quarrie *et al*. 1999). A total of 90 SSRs that showed polymorphism between both parental lines (A619 and P39) were used to genotype the heterozygous (*Su1su1*) plants that exhibited a lower *su1* frequency. The SSRs detected to be associated with low *su1* frequency were used to genotype all the heterozygous plants from the F_2 population in order to quantify the effects of each potential QTL on the fitness of *su1*. The PROC GLM program (SAS Institute 2008) was used to detect SSRs associated with the *su1* allele viability. Each marker locus was analysed for all previously mentioned traits. Since the number of plants within markers was not equal, the comparisons of means of the allelic classes were carried out using least squares means.

RESULTS

Genomics regions with segregation distortion against su1

In both RIL populations developed from crosses between B73 and sweetcorn inbreds (B73 × P39 and B73 × Il14h), a significant segregation distortion was identified for the B73: alternative allele (P39 or Il14h) for the SNPs flanking the *Sugary1* locus (Table 1). The segregation was skewed toward the B73 allele and the lowest number of sweet RILs was observed within the RIL released from B73 × Il14h. In contrast, the RILs from B73 × Oh43 and from B73 × Tx303 displayed the expected Mendelian distribution of B73 and the alternative allele with no significant skewness toward the genotype of Oh43 and Tx303, respectively. To better understand how the *su1* allele affects the genome, the SNP genotypes at the genomic regions flanking the *Su1* locus interval were analysed. No significant differences were noted in the size of the flanking fragments (data not shown) between all four populations.

Using a 0.05 experiment-wise threshold and applying Bonferroni's criterion, only the SNPs located on chromosome 4 showed deviations from the expected frequencies for the B73 or alternate allele in the sugary RIL and non-sugary RIL (Table 2). However, when a 0.05 comparison-wise threshold was applied without Bonferroni's criterion, 0.10 and 0.07 of the markers located outside the chromosome 4 in the B73 × P39 and B73 × Il14h, respectively, showed that the relative frequency of the B73 or alternate alleles in the sweetcorn RIL was different from the relative frequency of those same alleles in the non-sweet maize RIL (Fig. 1). In contrast, when the RILs developed from B73 × Oh43 and B73 × Tx303 are studied, only 0.01 and 0.05 respectively, of markers located outside the chromosome 4 showed deviations. Depending on the population, the chromosomes showing deviation from the random distribution of B73 and sweetcorn alleles were not the same; chromosomes 1, 2, 3, 5, 6, 9 and 10 showed it for B73 × P39 and chromosomes 1, 3, 6, 7 and 8 for B73 × Il14h. In the absence of both selection and linkage disequilibrium,

the segregation of all markers would be expected to follow normal Mendelian frequencies in both sweet and non-sweet RILs. However, all markers were skewed toward the B73 allele in the non sweetcorn RILs and toward the P39 or Il14h allele in the sweetcorn RILs. In both populations the maximum distortion was found in chromosome 4. The length of the linkage group of chromosome 4 was larger for Il14h than for P39 (from *c*. 19.4–90.7 cM and from 97.9–12.5 cM for Il14h, and from 21.3–97.9 cM for P39). The SNPs located in chromosomes 1 and 6 were shared by both Il14h and P39, while those in chromosomes 7 and 8 were exclusive to Il14h and those of chromosomes 2, 5, 9 and 10 were exclusive to P39. Only two regions on chromosome 6, i.e. bins 6.07 and 6.08, were common for both populations. All these markers were unevenly distributed over the chromosomes and were located in several regions along the chromosome. The size of these regions varied from *c*. 1–27 cM.

QTLs related to su1 viability

Bulk segregant analysis was employed in order to detect markers associated with the viability of the *su1* mutant along the genome. The χ^2 test detected only eight heterozygous plants that exhibited a lower *su1* allele frequency compared to expectations (data not shown). From the 90 SSRs markers used to genotype these plants, only eight were significantly associated with the viability of *su1* alleles: *phi 090* (Bin 2.08), *umc 1746* (Bin 3.01), *umc 2259* (Bin 3.03), *phi 029* (Bin 3.04), *umc 1102* (Bin 3.05), *umc 1221* (Bin 5.04), *phi 081* (Bin 6.05) and *umc 1309* (Bin 8.05) with frequencies that were 0.37, 0.30, 0.31, 0.31, 0.28, 0.31, 0.33 and 0.25 of the allele frequency of P39, respectively. All these SSRs markers were used to genotype all the heterozygous plants from the F_2 population to check this association. These analyses revealed significant associations among markers and diverse viability-related traits such as early vigour, ear length, ear weight, number of sugary seeds, seed type and number of seeds with roots and coleoptiles (Table 3). All significant markers explained only low proportions of variability for *su1*, with R^2 ranging from 0.040–0.102.

Five SSRs markers were positively associated with number of sugary seeds (*umc 1221*, R^2 = 0.048), ear length (*phi 081*, R^2 = 0.042), ear weight (*phi 029*, R^2 = 0.042), seed type (*umc 2259*, *phi* 029 and *umc 1221*, R^2 = 0.054, R^2 = 0.102 and R^2 = 0.059, respectively), early vigour (*phi 029* and *umc 1221*, R^2 = 0.040 and R^2 = 0.045, respectively), proportion of sugary seeds with root (*umc 1221*, R^2 = 0.056) and proportion of non-sugary seeds with coleoptiles (*umc 1309* and *phi 081*, R^2 = 0.079 and R^2 = 0.065, respectively) (Table 3).

Moreover, the loci *phi 029*, *umc 1221* and *phi 081* were associated with almost all traits evaluated in the F_2 population. The presence of A619 alleles had negative effects on early vigour, number of observed sugary seeds and root growth, while the P39 allele had negative effects for ear length and number of non-sugary seeds with coleoptile (Table 3). The coefficient of selection was calculated as an estimator of *su1* fitness; two SSRs markers *umc 1221* (R^2 = 0.057) and *phi 081* (R^2 = 0.051) were associated with this trait. In addition, coefficients of selection were significantly affected by the contribution of a parental allele of A619 (0.71 *vs.* 0.89 and 0.38 *vs.* 1.10 for root growth and coleoptiles growth, respectively).

DISCUSSION

Fitness is not solely a function of the mutant gene but is influenced by other genes. Furthermore, the behaviour of a mutant can vary depending on the context in which the mutation occurs. RILs are formed by crossing two inbred lines followed by repeated selfing to create a random set of inbred lines whose genome is a mosaic of the parental genomes (Yu *et al*. 2008). No selection other than natural is applied during the process of developing RILs.

In the two populations of RILs involving sweetcorn inbred lines (B73 × P39 and B73 × Il14h) used in the present study, a net natural selection was revealed acting against the *su1* allele, a fact previously reported by McMullen *et al*. (2009) (Table 1). The sweetcorn inbred Il14h brought about a higher reduction of the *su1* allele when crossed to B73 than the sweetcorn inbred P39

when crossed to the same inbred. The two non-sweet maize inbred lines used as references (Oh43 and Tx303) showed that the effect of selection was solely due to the *su1* allele, because the frequency of the alternative allele had a similar frequency as the B73 allele. Furthermore, the number of progenies with a normal segregation for the allele at the *Sugary1* locus was lower when the parents were sweetcorn lines than when they were not. These results confirm the general observation that the viability of sugary seeds was closely related to the specific sweet × field maize genotype interaction (Revilla *et al*. 2000, 2006, 2010; Djemel *et al*. 2011).

Fluctuation in the fitness value of genes can be caused by a closely-linked gene (Butler 1977). In order to understand the genetic regulation of the *su1* allele, the variation in the mutation fitness related to the genomic regions flanking the *su1* locus was examined. No significant relationship was found between mutation behaviour and size of flanking genome effect; this was probably due to the limited recombination caused by linkage disequilibrium in this region of chromosome 4 (Lu *et al*. 2002). Galinat (1978) also proposed block inheritance of the genes on chromosome arm *4S*, referring to this block as the 'chromosome 4 complex', which covers nearly all of 4S from the *Ph* position to the *su1* position. In both populations of the present study, the highest deviation from random distribution was observed in chromosome 4, probably due to the selection against the *su1* allele during the selfing generations that yielded the RILs. The *Su1* locus was mapped in the chromosome 4 at Bin 4.05 (James *et al*. 1995). McMullen *et al*. (2009) and Lu *et al*. (2002) reported that these regions were under higher selective pressure with a low recombination rate, so the Hill-Robertson effect (in a population of finite size which is subject to natural selection, random linkage disequilbria will occur, caused by genetic drift or by mutation, and they will tend to slow down the process of evolution) in a region under strong selection such as this could have increased the linkage block.

In a previous study with maize, a total of 18 chromosomal regions on the 10 maize chromosomes showed segregation distortion (Lu *et al*. 2002). In the present study, various SNP markers located outside the chromosome 4 in the two sweetcorn RILs populations showed non-random distribution of the allelic frequency of the B73 or alternate alleles in both the sweetcorn and the non-sweet maize RILs. These markers were skewed toward the B73 allele in the non-sweet maize RILs and toward the P39 or Il14h allele in the sweetcorn RILs. In the study of Eichten *et al*. (2011), two inbred lines parents (B73 and Mo17) were used to produce a set of near-isogenic lines (NILs). The 150 NILs produced were separated into two background: 100 lines with B73 as the recurrent parent and Mo17 as the donor parent (B73-like NILs), and 50 lines with Mo17 as the recurrent parent and B73 as the donor parent (Mo17-like NILs). Eichten *et al*. (2011) identified several regions with the opposing biases toward either the B73 or Mo17 parental allele in both background. The most likely explanation is that there are several loci for which there is a preferred allele with the ability to confer increased fitness. This result supports the present authors' suggestion that the alleles from the sweetcorn parent are required for guarantying the viability of the *su1* allele. All these SNPs were detected in the 10 chromosomes of maize, but they were unequally distributed depending of the RIL population (Fig. 1). Our results indicate that genetic factors for *su1* fitness exist on most chromosomes and also that the fitness of an allele depends on the specific genetic background (Revilla *et al*. 2006). Although the effects of a few genes of large effect on *su1* fitness could still be a reasonable hypothesis (Djemel *et al*. 2011), the results of the present paper suggest that there are probably also a lot of genes with minor effects affecting the diverse viability-related traits.

In order to check this hypothesis, bulk segregant analyses was used to detect markers associated with the *su1* mutant fitness along the 10 maize chromosomes in (A619 × P39)F_2 by using SSR markers. The selection against the mutant may operate either through viability at germination

or at the seedling growth stage (Falconer 1981; Martins & Da Silva 1998; Ordás *et al*. 2010) and then through reduced fertility (pollen and ovule production, pollination pattern and zygote development) (Clegg *et al*. 1978; Falconer 1981; Cisneros-López *et al*. 2010; Zhang *et al*. 2011).

In the present study, the SSR markers detected to be associated with *su1* fitness were chosen based on the low frequency of the P39 allele in the bulk segregant analysis. Only heterozygous plants (*Su1su1*) were used and the *su1* allele frequency was calculated after pollination. For this reason, the selection against the *su1* allele was probably due only to fertility factors. Significant genomic regions associated with *su1* fitness were detected (Table 3) and those markers were tested for the other viability traits. The SSR marker *umc 1221* (with R^2 = 0.048) located on chromosome 5 was strongly associated with the observed number of sugary seeds. This marker was also associated with other important traits: early vigour and proportion of sugary seeds with roots. Interestingly, the effect of the P39 allele was positive for all these traits. This region of the chromosome 5 (Bin 5.04) was also detected in the RIL population B73 × P39 and exhibited a net deviation from the random distribution of the P39 allele. The centromeric region of chromosome 5, as well as the other regions (Bin 6.07 and Bin 6.08) with significant deviations from the random distribution detected in both RILs populations, have all been reported as regions with major effects on grain yield (Graham *et al*. 1997; Schaeffer *et al*. 2006; McMullen *et al*. 2009; Schön *et al*. 2010), so these regions seem to be good candidates to contain genes involved in the regulation of *su1* fitness. Further research should be made to deepen knowledge on this matter.

Some other markers were significantly associated with several traits simultaneously. For example, marker *phi 029* was associated with early vigour, ear weight and seed type and marker *phi 081* significantly affected ear length and coleoptile development. However, some markers were only associated with one trait, for example *umc 2259,* that was only related to seed type, and *umc 1309* to non-sugary, related to seeds with coleoptile. No marker located on chromosome 4

was significantly associated with the low fitness of *su1*, probably due to the high linkage disequilibrium in the region neighbour to the s*u1* locus.

An important consideration is the presence of genomic regions that control both viability and fertility which is a clear evidence of pleiotropic effect on mutant fitness and the close relationship between the two factors of natural selection. Various authors studied the genetic control of germination and seedling as two factors limiting sweetcorn cultivation (Gad & Juvik 2002; Juvik *et al*. 2003). Tracy (2001) also reported that these two characters are affected by genetic factors, both at planting and during seed production. A powerful indication is the importance of the genetic background effect on the lethality or near-lethality of the *su1* allele when it is introduced into field maize. It is proposed that the pleiotropic effects of genes selected for their desired traits by humans probably have a role in this loss of fitness (Keightley & Hill 1990). The choice of field maize and sweetcorn parents affects the relative positive or negative effects of the alleles; indeed, A619 is not a vigorous inbred but has a better agronomic performance than P39; therefore, the allele from A619 had a negative effect on early vigour and coleoptile development, and a positive effect on ear weight and ear length. Furthermore, as was also observed for the P39 and Il14h RILs, each allele performed better in his original genetic background, i.e. the coleoptiles were more abundant when the sugary seeds had the P39 allele and when the non-sugary seeds had the A619 allele (Table 3). The potential QTLs with highest effect on any trait was *phi* 029 for seed type (R^2 = 0.102), but most QTLs had R^2 *c*. 0.05. The work of Rebourg *et al*. (2003), based on both molecular and historical data, revealed that the European maize was related to the Northern Flint material, the progenitor of the modern sweetcorn (Revilla & Tracy 1995). Malvar *et al*. (1997) reported that European flint inbreds offer new possibilities for improving the adaptation of sweetcorn to European conditions. All of these results suggest that the SSRs associated with seed type can be candidates for *su1* adaptation.

The genomic regions identified in the two RILs populations that showed segregation distortion against the *su1* allele can be potential candidates for QTLs of mutant fitness. However, the number of SSR markers detected to be associated with *su1* fitness in the F_2 population was low. The present study employed different marker types. Significant associations between markers and traits were detected with the SSRs, while linkage disequilibrium was detected with the SNPs. The combination of these two marker types in future works can enhance significantly the power to detect QTLs. The genetic regulation of mutant fitness is still poorly understood and further research should be carried out using larger samples and more markers.

It can be concluded that *su1* fitness depends on many genes with small effects on a variety of viability-related traits throughout the genome that are significant or not depending on the genetic background of the materials involved.

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Table 1. Segregation distortion at the SNPs flanking the *Sugary1* locus for B73: alternative genotype (P < 0.05) in four RILs populations obtained from crosses between the maize inbred line B73 and four diverse lines (DL).

Population	Progenies Number*	No. observed homozygotes		No. expected homozygotes	χ^2 value
		B73 allele	DL parent allele	For each phenotype	
B73 × P39†	155	107	48	77.5	22.45
B73 × Il14h†	157	132	25	78.5	72.92
B73 × Oh43‡	175	83	92	87.5	0.46 (ns§)
B73 × Tx303‡	171	74	97	85.5	3.09 (ns§)

* Excluding RIL with missing value at *su1* interval position
† Sweetcorn inbred line
‡ Field maize inbred line
§ Not significant at $P < 0.05$

Table 2. SNP markers showing segregation distortion in (B73 × P39) and (B73 × Il14h) populations for P39* and Il14h* parental alleles *vs*. B73 parental allele, χ^2 value and genetic position (cM) for each marker locus.

Population					
B73 × P39			**B73 × Il14h**		
Markers	**χ^2value†**	**cM‡**	**Markers**	**χ^2 value**	**cM**
PZA02138.1	26.0	33.8	PZA02385.6	25.3	39.2
PZA01122.1	28.7	33.9	PHM8527.2	25.3	40.4
PZA02385.6	45.0	39.2	PZA00139.4	27.1	43.9
PHM8527.2	45.0	40.4	PZA01422.3	37.7	47.7
PZA00139.4	56.1	43.9	PZA03048.18	42.7	48.3
PZA01422.3	71.4	47.7	PZA02457.1	58.9	49.4
PZA03048.18	83.0	48.3	PZA02002.1	53.0	49.4
PZA02457.1	101.7	49.4	PHM15427.11	109.4	52.1
PZA02002.1	95.9	49.4	PZA02705.1	109.4	52.1
PHM15427.11	115.5	52.1	PZA03247.1	129.7	52.8
PZA02705.1	115.5	52.1	PZA01106.3	114.7	52.8
PZA03247.1	130.8	52.8	PHM13623.14	114.7	52.8
PZA00541.1	119.3	52.8	PZA01713.4	114.7	52.8
PZA01106.3	116.3	52.8	PHM5572.19	114.7	52.8
PHM13623.14	116.3	52.8	PZA00541.1	114.7	52.8
PZA01713.4	116.3	52.8	PZA03385.1	129.0	53.4
PHM5572.19	116.3	52.8	PZA01751.2	129.0	53.7
PZA03385.1	134.5	53.4	PZA01759.1	162.0	55.4
PZA01751.2	155.0	53.7	PHM14055.6	162.0	55.4

Table 2 (cont.). SNP markers showing segregation distortion in (B73 × P39) and (B73 × Il14h) populations for P39* and Il14h* parental alleles *vs.* B73 parental allele, χ^2 value and genetic position (cM) for each marker locus.

Population					
B73 × P39			**B73 × Il14h**		
Markers	**χ^2value†**	**cM‡**	**Markers**	**χ^2 value**	**cM**
PZA01759.1	163.0	55.4	PZA00726.8/10	162.0	55.4
PHM14055.6	163.0	55.4	PHM1307.11	162.0	55.4
PZA00726.8/10	163.0	55.4	bt2.7/4	161.0	55.8
PHM1307.11	163.0	55.4	PZA03254.1	155.4	56.0
bt2.7/4	161.0	55.8	PZA03587.1	155.4	56.0
PZA03254.1	154.1	56.0	PZA03597.1	149.3	56.1
PZA03587.1	154.1	56.0	PZA03270.2	138.1	56.3
PZA03597.1	154.1	56.1	PZA00218.1	138.1	56.4
PZA03270.2	152.2	56.3	PZA02767.1	134.0	56.5
PZA00218.1	144.5	56.4	PZA03564.1	120.8	57.0
PZA02767.1	140.5	56.5	PZA03203.2	114.6	57.4
PZA03564.1	137.5	57.0	PZA00104.1	110.4	57.7
PZA03203.2	137.5	57.4	PZA03231.1	110.4	57.9
PZA00104.1	137.5	57.7	PZB00093.7	106.4	58.3
PZA03231.1	137.5	57.9	PZA03409.1	105.6	58.6
PZB00093.7	126.3	58.3	PZA00704.1	105.6	58.6
PZA03409.1	126.3	58.6	fea2.3	105.6	58.8
PZA00704.1	126.3	58.6	PZA02027.1	102.6	58.8
fea2.3	127.2	58.8	PZA03459.1	103.8	58.9

Table 2 (cont.). SNP markers showing segregation distortion in (B73 × P39) and (B73 × Il14h) populations for P39* and Il14h* parental alleles *vs.* B73 parental allele, χ^2 value and genetic position (cM) for each marker locus.

Population					
B73 × P39			**B73 × Il14h**		
Markers	**χ^2 value**	**cM**	**Markers**	**χ^2 value**	**cM**
PZA02027.1	127.2	58.8	PZA02147.1	95.0	60.6
PZA03459.1	126.3	58.9	PZA03152.3	89.5	61.8
PZA02147.1	105.9	60.6	PZA02982.7	89.5	61.8
PZA03152.3	105.0	61.8	PZA02992.15	89.5	62.0
PZA02982.7	105.0	61.8	PZA00057.2	80.4	65.9
PZA02992.15	104.2	62.0	PZA01926.1	80.4	69.8
PZA00057.2	83.8	65.9	PZA03116.1	31.9	75.3
PZA01926.1	83.8	69.8	PZA00453.2	29.0	76.2
PZA03116.1	34.7	75.3	PZA01289.1	24.4	77.2
PZA00453.2	33.1	76.2			
PZA01289.1	30.6	77.2			
PHM3155.14	23.3	78.4			

* *Su1* locus is estimated between position 53.7 cM and position 55.2cM on the NAM map
† χ^2 value with d.f. = 1 and *P* value adjusted with Bonferroni method
‡ Genetic position in the NAM map (cM)

Table 3. Significant main effect of all loci affecting the agronomic traits in the F_2 population, *P* value, coefficient of determination (R^2) and allelic classes' effect (mean±s.e.).

Traits	Marker	Bin*	*P* value	R^2	Mean of allelic classes of markers		
					P39	Het†	A619
Observed number of *su1* seeds	*umc 1221*	5.04	0.025	0.048	69.5±3.2	79.5±3.2	58.1±3.2
Ear length (mm)	*phi 081*	60.5	0.039	0.042	154.8±2.4	152.7±2.4	167.0±2.4
Ear weight (g)	*phi 029*	3.04	0.039	0.042	89.3±3.3	108.8±3.3	98.8±3.3
Seed type (Scale from 1= dent to 4= flint)	*umc 2259*	3.03	0.017	0.054	2.4±0.1	2.0±0.1	1.9±0.1
	phi 029	3.04	<0.001	0.102	2.5±0.1	1.9±0.1	2.0±0.1
	umc 1221	5.04	0.011	0.059	2.4±0.1	1.9±0.1	2.11±0.1
Early vigour (Scale from 1= poor to 9= excellent)	*phi 029*	3.04	0.045	0.040	5.0±0.1	4.9±0.1	4.6±0.1
	umc 1221	5.04	0.033	0.045	4.7±0.1	5.0±0.1	4.6±0.1
su1 seeds with roots (0-1 scale)	*umc 1221*	5.04	0.034	0.056	0.81±0.02	0.76±0.02	0.63±0.02
Su1 seeds with coleoptiles (0-1 scale)	*umc 1309*	8.05	0.017	0.079	0.42±0.03	0.42±0.03	0.60±0.03
	phi 081	6.05	0.019	0.065	0.38±0.03	0.46±0.03	0.60±0.03
Coefficient of selective of roots growth‡	*umc 1221*	5.04	0.033	0.057	0.89±0.03	0.86±0.03	0.71±0.03
Coefficient of selective of coleoptiles growth‡	*phi 081*	6.05	0.045	0.051	1.10±0.11	0.53±0.11	0.38±0.11

* Chromosome location.
† Heterozygote class.
‡ ratio of the *su1* endosperm relative to the *Su1* endosperm.

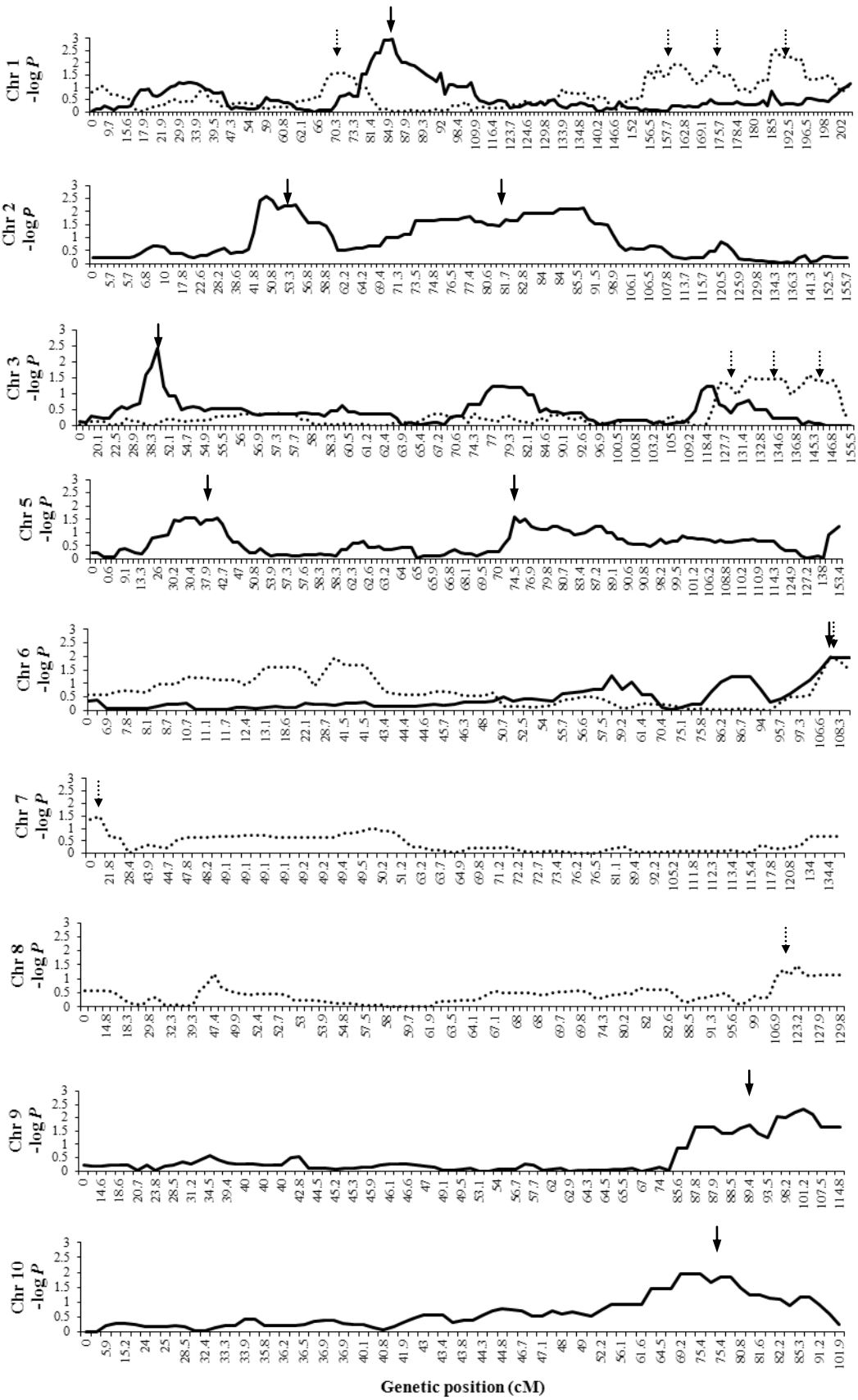
Chr 1 -log P
Chr 2 -log P
Chr 3 -log P
Chr 5 -log P
Chr 6 -log P
Chr 7 -log P
Chr 8 -log P
Chr 9 -log P
Chr 10 -log P
Genetic position (cM)

Figure 1. Segregation distortion across the maize chromosomes*. The solid line indicates the –log *P* for the χ^2 value for segregation of P39 parental allele versus B73 parental allele. The broken line indicates the –log *P* for the χ^2 value for segregation of Il14h parental allele versus B73 parental allele. The arrow indicates the position of the genomic regions with a significant segregation distortion.

* Chromosome 4 is not included because the whole chromosome showed segregation distortion.

GENERAL DISCUSSION

Mutations are the ultimate source of all genetic variation, and are essential for evolution under natural selection. Mutants are valuable resources for genetic studies (Carson *et al.* 2004). Many of the mutants identified in maize (*Zea mays* L.) were genes affecting kernels characteristics at chemical, morphological, or physiological levels. Such is the case of the recurrent mutant *sugary1* (*su1*), located in chromosome 4 at bin 4.05 (James *et al.* 1995; Tracy *et al.* 2006). In general, it is assumed that the s*u1* mutant is less fit that the wild type and is considered lethal or semi-lethal when introduced in most maize genetic backgrounds (Tracy 1990). Revilla *et al.* (2006) confirmed that the viability of *su1* depends on specific sweet × filed corn interactions and suggested that *su1* viability is not solely a function of the mutant gene performance but it is probably affected by their genetic association with other genes. However, our general knowledge of the genetic regulation of mutant viability is still poorly understood. According to previous studies, our hypothesis is that the viability of the mutant *sugary1* is genetically regulated by the maize genome and by specific genes that determine the mutant fitness and viability. Our general challenge in these researches was to elucidate and clarify the genetic mechanisms underlying the mutant viability control.

The first part of this thesis studied the effect of different maize genetic backgrounds on variation in gene frequency in sweet corn and the genetic control of the *sugary1* fitness under natural conditions. When the 12 genotypes derived from both mean generation designs and crossed to P39 were selfed successively, the frequency of *su1* was reduced across all selfing cycles in all crosses and followed a linear tendency in both designs. The coefficient of selection against *su1* mutant was lowest in the first selfing cycle and highest in the last one. This can be attributed to the increased homozygosity. Revilla *et al.* (2000*a*) suggested that the combination of genetic background of *Su1Su1* and *su1su1* inbred lines differ for viability of *su1*. The viability of *su1* was low-

er in the generations derived from the cross between the Corn Belt inbreds (A619 and A632) than in the cross involving inbreds with different genetic backgrounds A661 (Northern Corn Belt) and EP42 (European Flint), being A619 less favourable than the other field maize inbred lines for *su1* viability. Moreover, the reduction on frequency of *su1* for EP42 fluctuated more across years than for A661. However, the behaviour of A619 and A632 concerning *su1* fitness was more stable across years, suggesting that mutant stability across environments can also depend on genetic background. Revilla *et al.* (2000*a*) studied the viability of *su1* in crosses between *Su1* and *su1* populations; *su1* frequency was reduced across recombinations in all crosses and interaction of S*u1* and *su1* genetic backgrounds affected the viability of *su1* significantly: Corn Belt Dent *Su1* × Stowell's Evergreen *su1* was the most favourable combination. The effects of different maize genetic backgrounds on variation in *su1* frequency were investigated in the studies carried out by Revilla *et al.* (2006); the reduction of *su1* gene frequency depends on specific sweetcorn × field maize interaction but is not related to the field maize heterotic groups. Our results confirm the general observations that *su1* viability depends on specific *su1* × field maize interactions and that the *su1* viability is under genetic control (Revilla *et al*. 2000*a*, 2006). Selection against *su1* may operate firstly through factors related to seed viability (Ordás *et al.* 2010) and after that, by factors related to fertility (Cisneros-López *et al.* 2010; Zhang *et al.* 2011). In the first selfing cycle, all plants were heterozygous and no selection against *su1* was made until the production of gametes, because all plants had wild phenotype. The change in frequency in the first selfing cycle was not important, which indicates that fertility factors were probably of small importance for the fitness of the *su1* allele in the present study.

Our results and previous reports suggest that viability of *su1* depends on the sweet and field corn parent and is genetically controlled; and this is the reason why mean generation analyses were made to estimate the genetic effects on *su1* viability and to confirm this hypothesis.The coef-

ficients of regression of the gene frequency on the number of selfed generations were used as an estimator of *su1* viability and each design was considered a separate experiment. Data from each generation were subjected to regression analyses. Adjustment of the generation means to a genetic model was tested with a χ^2 test. Only significant additive effect (m = –0.08±0.004 and a = 0.013±0.005, $\chi^2_{(4)} = 6.75$, with $P = 0.149$) was detected in the experimental design involving the two unrelated inbreds: EP42 and A661. However, genetic effects were not significant in the experimental design involving A619 and A632, probably due to the lack of genetic variation between the two inbred lines because of the similar genetic origin (Corn Belt).Our results confirm that the *sugary1* viability was genetically controlled probably by multiple genes with minor contributions. However, this viability was probably variable due to environmental factors, but these effects do not hide the genetic effects.

Along the life cycle of plants, selection acts first through viability (germination and early vigor) (Ordás *et al.* 2010), and finally through fertility (mating ability and grain formation) (Cisneros-López *et al.* 2010; Zhang *et al.* 2011). The viability of *su1* plants has been investigated mainly at the first stages of plants development: germination and early vigor (Martins & Da Silva 1998; Revilla *et al*. 2000*a*; Gad & Juvik. 2002; Juvik *et al.* 2003; Revilla *et al.* 2006). However, as the growth cycle continues, little is known about the importance of the successive traits involved in *su1* viability. This second part of the thesis studied the change in the genetic effects produced by the introgression of the *su1* mutant into field corn for several agronomic traits along the growth cycle of plants. Estimated genetic effects of agronomic traits in *Su1 vs*. *su1* plants were monitored through five (controlled conditions) and through four (filed conditions) successive selfpollination cycles in the two mean generation designs (EP42 × A661 and A619 × A632). For each cross, *Su1* and *su1* seeds were separated and evaluated in 10 × 10 triple lattice designs.

The type of genetic model that best fitted the data means varied between traits, genotype, environments and cycles of selfpollination. For those traits with significant genetic effects, the epistatic effects were generally important contributors to variation suggesting that epistatic gene effects could be relevant in the basic genetic effects of the viability of *su1*. In spite of the influence of the environments, the cycles of selfpollination and the germplasm in the genetic effect estimation, some patterns were observed in the last cycle of selfpollination for some traits. Some changes were shown in the genetic control of fitness-related traits when *su1* was incorporated into field corn. The effects of *su1 vs*. *Su1* on genetic effects were strongly influenced by environments and genetic background for almost all traits. Emergence, and particularly chlorophyll content (CCM) exhibited a significant genetic effects and this variation was strongly affected by alleles (*Su1 vs*. *su1*). For CCM, the mean data were adjusted to the additive × dominance model with epistatic interactions in the plants carrying the *su1* allele in the first design, and in the plants carrying the wild type in the second design. Independently on specific sweet corn × field corn interactions, differences between *Su1 vs*. *su1* for genetic regulation suggest that chlorophyll content has consistent significant effects on *su1* fitness. However, the complexity of the genetic regulation of emergence and the great heterogeneity of environmental conditions in the field evaluation prevented the estimation of its genetic regulation on *su1* fitness. These results have not been previously reported and this is the first study that elucidates the effect of the *su1* introgression on genetic inheritance of traits. As the growth cycle continues, successive traits were less or more important for the *su1* viability. The differences between generation means for adult plant performance and ear performance were not significant. For ear weight and ear/plant, the differences between generations were significant, but the generation means fitted the simplest model with just a parameter, the mean "m". For these traits, the genetics effects were not important and were not probably involved in *su1* fitness viability. Stand and ear length had significant genetic effects in the A619 × A632 design, while 100-seed weight genetics effects

were only detected in EP42 × A661 design. The additive effects of plant height presented lower magnitudes and different sign in the *sugary1* genotypes than in wild type genotypes in the design derived from EP42 and A661. The *Su1* locus also consistently altered the magnitude of the additive × dominance epistatic interactions in the three environments, and the additive × additive epistatic effects and the dominance effects in both environments. For stand, the *su1* mutant also altered the magnitude of the additive effects in two of the environments, changing from zero (non significant) to negative (significant) and the magnitude of the additive × dominance epistatic interactions which were lower with the *su1* mutant than in the wild type in A619 × A632. For ear length, the genetic effects were not significant when *su1* was homozygous, but they were significant for the wild type allele in the last cycles of self-pollination in A619 × A632. However, 100-seed weight fitted the simplest model with the *su1* mutant and the additive model with the wild type allele but only in one environment (in Pontevedra 2011 and the forth cycle of selfpollination). Revilla *et al.* (2000*b*) reported significant specific heterosis effects for yield and other traits in crosses between sweet and field corn; plant height and ear length had positive heterosis for sweet × field corn crosses. Silking date data followed the simple model (with only the mean as significant parameter) in the *Su1* genotypes, but in the *su1* genotypes other genetic models fitted the data in the last cycles of selfpollination in some of the environments. The alteration of the combining abilities caused by the replacement of *su1* by *sh2* alleles has been studied by Ordás *et al.* (2010). They also reported that flowering was the only trait that changes when *su1* inbreds lines are converted to *sh2* due a significant mutant × wild type corn interaction. The differences between *Su1 vs*. *su1* combinations in terms of genetic effects suggest that silking date, plant height, stand and ear length have significant genetic effects on *su1* viability and can be considered as viability-related traits.

Fitness of *su1* is under genetic control with significant additive effects that control a number of viability related traits. The identification of genes affecting the *su1* viability is of great interest to broaden our knowledge in the dynamic of mutants' fitness. Two recombinant inbred lines (RILs) populations, produced from crosses between the maize inbred B73 and two sweet corn lines (P39 and Il14h) that were genotyped with 1106 single nucleotide polymorphisms (SNPs), and the F_2 population (A619 × P39) F_2 that was characterized with 295 SSRs. To examine the existence of chromosomal regions that exhibited segregation distortion against the *su1* allele, SNPs variability was classified into two main types (sweet corn and field maize) depending on the parent providing the allele for each locus and, within each type, the number of RILs sharing the B73 alleles was compared with the number of RILs sharing the sweetcorn allele in other loci throughout the genome.

A net natural selection was revealed acting against the *su1* allele, a fact previously reported by McMullen *et al*. (2009). The sweetcorn inbred Il14h brought about a higher reduction of the *su1* allele when crossed to B73 than the sweetcorn inbred P39 when crossed to the same inbred. The *Su1* locus was mapped in the chromosome 4 at Bin 4.05 (James *et al*. 1995). McMullen *et al*. (2009) and Lu *et al*. (2002) reported that these regions were under higher selective pressure with a low recombination rate; this is the reason why high linkage disequilibrium was detected in this region of chromosome 4. Galinat (1978) also proposed block inheritance of the genes on chromosome arm *4S*, referring to this block as the 'chromosome 4 complex', which covers nearly all *4S* from the *Ph* position to the *su1* position. Outside the chromosome 4 and depending on populations, various genomic regions showed segregation distortion against the *su1* allele and only two regions located in the chromosome 6 (Bin 6.07 and Bin 6.08) were in common between the two populations. All of these SNP markers located outside the chromosome 4 were skewed toward the B73 allele in the non-sweet maize RILs and toward the P39 or Il14h allele in the sweetcorn RILs.

In the study of Eichten *et al*. (2011), two inbred lines (B73 and Mo17) were used to produce a set of near-isogenic lines (NILs). The 150 NILs produced were separated into two backgrounds: 100 lines with B73 as the recurrent parent and Mo17 as the donor parent (B73-like NILs), and 50 lines with Mo17 as the recurrent parent and B73 as the donor parent (Mo17-like NILs). Eichten *et al*. (2011) identified several regions with the opposing biases toward either the B73 or Mo17 parental allele in both backgrounds. The most likely explanation is that there are several loci for which there is a necessary allele having the ability to confer viability to the mutant. Our results support the present authors' suggestion that the alleles from the sweet corn parent are required for guarantying the viability of the *su1* allele and indicate that genetic factors for *su1* fitness exist on most chromosomes and also that the fitness of an allele depends on the specific genetic background, as previously suggested by several authors (Lu *et al*. 2002; Revilla *et al*. 2006; Djemel *et al.* 2011). The SSRs associated with *su1* viability were identified by using bulk segregant analysis of *su1* frequency. The SSRs detected to be associated with low *su1* frequency were used to genotype all the heterozygous plants from the F_2 population in order to quantify the effects of each potential QTL on the fitness of *su1*. Various SSRs markers were detected to be associated with *su1* viability. The SSR marker *umc1221* (with R^2= 0.048) located on chromosome 5 was strongly associated with the observed number of sweet corn seeds. This marker was also associated with other important traits: early vigor and proportion of sugary seeds with roots. The effect of the P39 allele was positive for all these traits. This region of the chromosome 5 (Bin 5.04) was also detected in the RIL population B73 × P39 and exhibited a net deviation from the random distribution of the P39 allele. The centromeric region of chromosome 5, as well as the other regions (Bin 6.07 and Bin 6.08) with significant deviations from the random distribution detected in both RILs populations, have all been reported as regions with major effects on grain yield (Graham *et al*. 1997; Schaeffer *et al*. 2006; McMullen *et al*. 2009; Schön *et al*. 2010), so these regions seem to be good candidates to contain genes involved in the regulation of *su1* fitness. An important consideration is

the presence of genomic regions that control both viability and fertility which suggests pleiotropic effects on mutant fitness and the close relationship between the two factors of natural selection.

As general conclusion, we suggest that the viability of mutants is genetically regulated by a variable and large number of loci distributed through the genome with small effects on a variety of viability-related traits.

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CONCLUSIONES

El objetivo general del presente estudio era proporcionar información suplementaria para profundizar en el conocimiento de los factores genéticos que controlan la fitness de la mutación *su1* en el maíz en condiciones ambientales naturales y controladas. Para ello, nos hemos basado en cuatro líneas puras de maíz normal: EP42, A661, A619 y A632, y una línea de maíz dulce como padre donante del alelo *su1*. Hemos estudiado el efecto del fondo genético de las cuatro líneas de maíz normal sobre la viabilidad de *su1* gracias a dos dispositivos basados en las medias generacionales originadas a partir de EP42 × A661 y A619 × A632. En cada dispositivo, se produjeron las seis generaciones base P_1, P_2, F_1, F_2, BC_1 y BC_2. Cada uno de estos genotipos se cruzó con la línea pura P39 como donante del alelo *su1*. Estos doce genotipos se autofecundaron cinco veces. Para responder al objetivo general de este trabajo, se han abordado tres cuestiones principales:

1. ¿La viabilidad del alelo *su1* está bajo control genético?
2. ¿Cuáles son los efectos genéticos de la introgresión del alelo *su1* sobre el desarrollo y el comportamiento de la planta a lo largo de su ciclo de vida? ¿Cuáles son los caracteres agronómicos limitantes para la fitness del alelo *su1*?
3. ¿Cuáles son los genes o los factores genéticos que controlan la fitness de la mutación en cuestión?

En la primera parte del trabajo, hemos evaluado el efecto de los diferentes fondos genéticos sobre la viabilidad y la fitness del alelo *su1* en condiciones naturales. Sesenta genotipos provenientes de los dos dispositivos de medias generacionales fueron evaluados, llegando a las siguientes conclusiones:

- ✓ La frecuencia del alelo *su1* disminuyó a lo largo de la generaciones de autofecundación a causa de la selección natural.

- ✓ La viabilidad o la fitness del alelo *su1* depende de los genotipos receptores del alelo y del ambiente.
- ✓ La viabilidad de *su1* está controlada genéticamente con efectos aditivos significativos debidos, probablemente, a la contribución de múltiples genes con efectos menores.

En la segunda parte, hemos abordado la cuestión de los caracteres agronómicos críticos para la viabilidad de la mutación y de los efectos de la introgresión del alelo *su1* sobre el control genético de estos caracteres agronómicos. Se han realizado ensayos agronómicos en Argelia (Escuela Nacional Superior Agronómica, Argel) y en España (Misión Biológica de Galicia, Pontevedra) para responder a esta cuestión. Además de la germinación y el vigor temprano considerados como los dos factores principales limitantes de la fitness de *su1*, se han detectado otros factores. Las principales conclusiones de la segunda parte son:

- ✓ Los efectos de la introgresión del alelo *su1 versus* el alelo silvestre *Su1* son notablemente afectados por el fondo genético y el ambiente. Por otra parte, la introgresión del alelo *su1* genera una alteración del control genético de ciertos caracteres.
- ✓ Los caracteres contenido en clorofila de la hoja, proporción de plantas que alcanzan el estado adulto ("stand"), floración femenina, altura de planta y longitud de mazorca parecen tener un papel principal en el control de la viabilidad del alelo *su1*, pero la importancia de cada carácter está fuertemente influida por el fondo genético.

En el tercer capítulo, hemos realizado un estudio de las bases genéticas que controlan la viabilidad de la mutación *su1* a fin de poder determinar los genes o regiones genómicas responsables de la disminución de la fitness de este mutante. Tal estudio requiere útiles genéticos apropiados vista la complejidad del genoma del maíz así como la dependencia de la viabilidad de *su1* del fondo genético. Para afrontar estas dificultades, se han empleado dos poblaciones de líneas recombinantes 'RIL' (B73 × P39 y B73 × Il14h), con un mapa genético saturado por 1106

marcadores SNP. Además se ha empleado la población (A619 × P39) F_2 que presentó una fuerte disminución de la frecuencia del alelo *su1*. Los resultados obtenidos permiten concluir que:

- ✓ Las dos poblaciones RIL han mostrado una gran distorsión de segregación en el cromosoma 4, probablemente debida a una fuerte selección contra el alelo *su1*.
- ✓ Las regiones genómicas localizadas en los cromosomas 1 y 6, que generan una fuerte selección contra el alelo *su1* fueron detectadas en ambas poblaciones RIL. Mientras que las regiones genómicas detectadas en los cromosomas 7 y 8 únicamente fueron significativas en la población B73 × Il14h, y las localizadas en los cromosomas 2, 5, 9 y 10 fueron observadas únicamente en la población B73 × P39. Estos resultados refuerzan nuestra hipótesis según la cual la regulación genética de la viabilidad del mutante *su1* depende de interacciones específicas entre maíz dulce y maíz normal.
- ✓ Dos regiones situadas en el cromosoma 6 (Bin 6.07 y 6.08) fueron identificadas paralelamente en las dos poblaciones. Por otra parte, en (A619 × P39) F_2, el marcador umc1221 localizado en el cromosoma 5 (Bin 5.04) estaba significativamente asociado con el número de granos por mazorca, el vigor temprano y la proporción de granos *su1su1* con raíces. Esta región ha sido igualmente detectada en la población B73 × P39, donde genera una distorsión de segregación contra el alelo *su1*. Esta región genómica, así como las regiones de los Bin 6.07 y 6.08 han sido extensamente estudiadas en otros trabajos por sus efectos en el rendimiento
- ✓ La presencia de estas regiones genómicas que controlan al mismo tiempo la viabilidad (vigor temprano) y la fertilidad (número de granos) por medio de efectos pleiotrópicos indica una fuerte relación entre estos dos componentes de la selección natural.

GENERAL CONCLUSSIONS

1. There was a selection against *su1* with variable intensity depending on genetic background. The viability of *su1* was mainly under additive genetic control but, depending on genotypes and environments, dominance or epistatic effects were detected.
2. The effects of *su1* introgression on agronomic value and genetic regulation of diverse traits depended on genetic background and environment. The introgression of *su1* significantly modified the genetic regulation of several traits. The main limiting factors for *su1* viability were both vegetative (as chlorophyll content) and reproductive (as ear length).
3. A large segregation distortion was detected around the locus *Sugary1*, probably due to a strong selection pressure against the mutant *su1*. Moreover, genomic regions associated to *su1* viability were identified in several chromosomes, depending on the genetic background, although some QTLs were more consistent than others, e.g. 2 regions located in chromosome 6 (Bins 6.07 and 6.08), as well as a region located in chromosome 5 (Bin 5.04) that could have pleiotropic effects on several traits.

As final conclusion, the viability and fitness of the mutant *su1* were strongly associated and both were under the genetic control of multiple genes distributed throughout the genome with small effects on diverse vegetative and reproductive traits.