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Genetic stability of the sweet corn mutant *sugary1*

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Abstract

Maize has numerous starch mutants that affect kernel composition and quality, including the *sugary* mutants which are responsible for the production of popular sweet corn varieties. *Sugary* maize was originally due to the recessive allele *sugary1* (*su1*). Sweet corn breeders frequently search sources of stress tolerance and agronomic performance in field corn genotypes for broadening the narrow genetic base of sweet corn. To do so, they have to deal with the reduced viability of *su1* plants within some field corn genetic backgrounds. Both emergence and seedling vigor are the most critical traits affecting the viability of *su1* plants. In the two populations of RILs involving sweet corn inbred lines (B73×P39 and B73×IL14h) used in the present study, a net natural selection was revealed acting against the *su1* allele. The *su1* viability is under genetic and environmental controls with significant additive effects that are probably due to multiple genes with minor contribution. Previous reports have hypothesized that there are specific genes that are associated to *su1* viability. Our results confirm the hypothesis that there are specific genes involved in mutant viability and these genes depend not only on the mutant but also on the genetic background where the mutant is introduced. The quantitative trait loci (QTLs) identified in this study could be used by sweet corn breeders by combining the most favorable alleles associated to *sugary1* viability in breeding new genotypes from field × sweet corn crosses.

Resumen

En el maíz hay numerosos mutantes que afectan a la composición y calidad del endospermo, incluyendo los mutantes « *sugary* » que son responsables de la producción del popular maíz dulce. El maíz dulce inicial era debido al mutante recesivo *sugary1* (*su1*). Los mejoradores de maíz dulce buscan habitualmente fuentes de tolerancia a estrés y de valor agronómico en el maíz grano para ampliar la estrecha base genética del maíz dulce. Para ello, tienen que afrontar la reducida viabilidad de las plantas *su1* en algunos entornos genéticos. Los caracteres más críticos que afectan a la viabilidad de *su1* son la nascencia y el vigor temprano. En las dos poblaciones de líneas recombinantes (RIL) usadas en este estudio (B73×P39 y B73×IL14h), se observó una clara selección natural contra el alelo *su1*. La viabilidad de *su1* está controlada tanto por el genotipo como por el ambiente con efectos aditivos significativos probablemente debidos a múltiples genes con pequeñas contribuciones. Las publicaciones anteriores plantean la hipótesis de la existencia de genes específicos asociados a la viabilidad de *su1*. Nuestros resultados confirman esta hipótesis, demostrando que hay genes específicos implicados en la viabilidad del mutante y que estos genes dependen no sólo del mutante sino también del entorno genético en el que se introduce el mutante. Los genes implicados en la regulación de caracteres cuantitativos (QTL) identificados en este estudio podrían servir para que los mejoradores combinasen los alelos más favorables asociados a la viabilidad de *sugary1* para obtener nuevos genotipos a partir de cruzamientos entre maíz dulce y maíz grano.

Résume

Chez le maïs doux, il y a plusieurs mutants altérant la composition et la qualité de l'endosperme, particulièrement les mutants « *sugary* » qui sont responsables de la production du populaire maïs doux. Le maïs doux originel était produit par le mutant récessif *sugary1* (*su1*). Les améliorateurs de maïs cherchent normalement des sources de tolérance aux stresses ou de valeur agronomique chez le maïs grain pour augmenter l'étroite base génétique du maïs doux. Pour cela, ils doivent faire face à la faible viabilité des plantes *su1* dans certains génotypes. Les caractères les plus critiques affectant la viabilité de *su1* sont l'émergence et la vigueur précoce. Chez les deux populations de lignées recombinantes (RIL) étudiées ici (B73×P39 et B73×IL14h), on a observé une nette sélection naturelle contre l'allèle *su1*. La viabilité de *su1* était contrôlée autant par le génotype que par l'environnement, ayant effets additifs significatifs probablement causés par plusieurs gènes à faible effet. Les publications précédentes posent l'hypothèse de l'existence de gènes spécifiques associés à la viabilité de *su1*. Nos résultats confirment cette hypothèse en démontrant qu'il y a de gènes spécifiques impliqués en la viabilité du mutant et que ces gènes dépendent aussi du mutant que de l'environnement génétique où le mutant est introduit. Les gènes impliqués dans la régulation des caractères quantitatifs (QTL) identifiés dans cette étude pourront être utilisés par les améliorateurs en combinant les allèles plus favorables associés à la viabilité de *sugary1* afin d'obtenir de nouveaux génotypes à partir de croisements entre le maïs doux et le maïs grain.

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I. Introduction

I. Introduction

Maize

1- Maize and its diversity

Maize (*Zea mays L. ssp. mays*), along with wheat and rice, is one of the most important crop plants of the world. Unlike wheat and rice, which are mainly consumed as human food, maize is a multipurpose crop that can be eaten by humans, fed to animals, or used as a raw input into industry.

Maize is a highly adaptable crop because it has high genetic, morphological and physiological variability; as a result it is being widely grown around the world in tropical and temperate climatic zones. It has been cultivated from latitude 40° South, Argentina and South Africa, to latitude 58° North, in Canada, and reaches up to 3000 m altitude in the Andes region (Buckler et al., 2009).

Maize in traditional populations is a highly heterozygous plant that displays an extremely high level of phenotypic and genotypic diversity. Its molecular diversity is approximately 2 to 5 times more than the other domesticated cereals (Buckler et al., 2009). Maize genome has 2.3 Gbp which makes it comparable in terms of size to the human genome (Ganal et al., 2011). The frequency of nucleotide polymorphism observed when comparing the genomes of two modern maize inbred lines is equivalent to the sequence diversity between chimpanzees and humans (Buckler et al., 2006).

This extraordinary level of genotypic diversity has been exploited in modern breeding programs. The large genetic diversity of the maize genome is

exploited in association mapping strategies, wherein correlations between phenotypic and genotypic diversity are identified in analysis of natural populations (Yu and Buckler 2006). Such studies have identified candidate genes associated with complex traits, such as flowering time (Thornsberry et al., 2001), starch biosynthesis (Wilson et al. 2004), and kernel carotenoid content (Harjes et al., 2008), which can be manipulated by breeders for agronomic and nutritional improvement of maize varieties.

2- Starch and maize kernel

Maize kernel is composed of several chemicals of commercial value. It consists of tip cap, pericarp, germ (embryo) and endosperm as prominent botanical tissues. The mature kernel is composed of 70 to 75% starch, 8 to 10% protein, and 4 to 5% oil (Earle et al., 1946; Whitt et al., 2002). The two major structures of the kernel, the endosperm and the germ, constitute approximately 80 and 10% of the mature kernel dry weight, respectively (Boyer and Hannah, 2001). Mature endosperm, which is the most important major constituent of the kernel, is a rich source of starch (approaching 90%). The germ contains high levels of oil (30%) and protein (18%) (Boyer and Hannah, 2001). Immature kernels contain relatively high levels of sugars and lesser amounts of starch, protein and oil, which accumulate during development (Boyer and Shannon, 1982). Starch is the main reserve substance synthesized by higher plants and an essential energy source in the diet of living things and is the basis for various industrial applications (James et al., 2003).

Carbohydrates enter the maize kernel at its base as sucrose, and are converted to UDP-glucose and fructose by the enzyme sucrose synthase. Each of these

monosaccharides is then converted to ADP-glucose, which serves as the basic building block for starch synthesis (Schultz and Juvik, 2004). Starch synthesis in maize occurs within amyloplasts of endosperm cells during kernel development via the concerted actions of ADP-Glucose pyrophosphorylase, starch synthases (SSs), branching enzymes (BEs) and debranching enzymes (DBEs) (Martin and Smith, 1995; Nelson and Pan, 1995; Preiss and Sivak, 1996; Smith et al., 1996).

Starch comprises of two homopolymers of glucose, amylose and amylopectin. Amylose, constituting approximately 25% of maize kernel starch, is predominantly a linear molecule in which glucosyl units are joined by α1-4 linkages. Amylopectin makes up the remaining 75% of the starch, by contrast, is more branched polysaccharide in which approximately 5% of the glycosyl units are joined by α1-6 linkages (branch linkages). Linear glucosyl linkages in both amylose and amylopectin are formed by the actions of several informs of starch synthases (SSs), and branch linkages are introduced via various isoforms of branching enzymes (BEs) (Schultz and Juvik 2004).

Debranching enzymes (DBEs) are necessary for the normal formation of crystalline starch granules (Dinges et al., 2001). The first genetic evidence for DBE involvement in starch biosynthesis came from mutations of the maize *sugary1* (*su1*) gene, described a century ago in the scientific literature (Correns, 1901). These mutations result in the accumulation of soluble sugars and a water-soluble polysaccharide (WSP) termed phytoglycogen in the kernel (Morris and Morris, 1939). The accumulation of phytoglycogen also has been reported in mutants of rice, Arabidopsis, and chlamydomonas (Mouille et al., 1996; Nakamura et al., 1996; Zeeman et al., 1998; Kubo et al., 1999). In each species, phytoglycogen accumulation correlates with a lack of DBE activity of

the isoamylase type (Mouille et al., 1996; Rahman et al., 1998; Zeeman et al., 1998; Beatty et al., 1999; Kubo et al., 1999).

As a result of increased demands on food production from increasing population growth and environmental degradation, there is interest in improved breeding strategies for crop plants. Progress in cereal starch production is especially important because these starches comprise 55-75% of daily human food intake and are the main source of food for domestic animals (Pan, 2000).

3- Mutations affecting maize kernel

Among cultivated allogamous species, maize is of particular interest due mainly to the genetic knowledge accumulated. Especially interesting are several endosperm mutants that are of great economic importance because they produce chemical, morphological, physiological and nutritional changes in the kernels (Martins & Da Silva 1998).

Maize kernel has been the focus of hundreds of genetic analyses of morphological and biochemical mutants. Mutations affecting the development of the embryo or the accumulation of storage proteins and starch in the endosperm are especially abundant, and have contributed to our understanding of developmental and biosynthetic pathways operating in the kernel (Laughnan 1953; Scanlon et al., 1994).

Maize has numerous starch mutants that affect kernel composition and quality, including *opaque2*, which increases lysine content (Mertz et al., 1964), amylose-free *waxy1* (*wx1*), important for many industrial applications (Lambert, 2001),

and the *sugary* mutants, responsible for the production of popular sweet corn varieties (Pan, 2000; Schultz and Juvik, 2004). Identifying those genes and alleles in maize that control traits such as kernel yield, starch concentration, and starch quantity is an important step in meeting the future goals of both agriculture and industry.

Sweet corn

1- Sweet corn and its importance

Sweet corn is a special type of maize used as a vegetable and is homozygous for one or more genes that increase sugar level in the endosperm (Revilla and Tracy 1995a). Primarily defined by the presence of the *su1* allele, located in the short arm of chromosome 4 (Coe et al., 1988). However, other alleles at other loci have been reported during the last few decades that alter the composition of the endosperm, producing a sweet corn phenotype. Among these new sweet genes, *shrunken2* (*sh2*) (Laughnan, 1953) is particularly important due to its widespread adoption (Tracy, 1997). Another important sweet corn gene is *sugary enhancer1* (*se1*) (Ferguson et al., 1978), which improves the flavor of *sugary1* (*su1*).

Sweet corn is originated through mutation and it is characterized by having at least one of these eight mutant genes. The main genes are: *sh2* on chromosome 3, *brittle* and *amylose extender* on chromosome 5, *se1*, *su1* and *brittle-2* (*bt2*) on chromosome 4; *du* on chromosome 10, and *wx1* on chromosome 9 (Tracy et al., 2006). These genes code for enzymes important in endosperm starch biosynthesis. The alleles important in sweet corn, *su1* and *sh2* reduce starch

synthesis and increase the concentration of sugar, phytoglycogen, or both in the endosperm (Boyer and Shannon., 1982).

Sweet corn is one of the most popular vegetables in the United States and Canada, and consumption is rapidly increasing in eastern Asia and parts of Europe (Tracy, 2001). It is second in per capita consumption among processed vegetables after tomatoes and ranks seventh among the fresh vegetables (U.S. Dept. of Agriculture, 1990). Among vegetables, sweet corn is one of the most important protein sources because of its high consumption and relatively high protein concentration (3.5 g protein/100 g edible portion; U.S. Dept. of Agriculture, 1975) (Goldman and Tracy, 1994). It is known that in the last few years the cultivation of vegetables has been affected mostly by climate change and has become dependent on high amounts of water to ensure good yields. As sweet corn is a relative drought-tolerant crop that is adapted to a wide range of climates (Bray, 1997), the production and cultivation of sweet corn is the most effective strategy when facing climate changes.

2- Origin of sweet corn

Alleles at *Su1* locus were among the first to be genetically characterized by Correns in 1901 following the rediscovery of Mendel. While *su1* was one of the earliest genes genetically characterized in maize, maize geneticists have long debated its origin (Tracy et al., 2006). Galinat (1971) and Mangelsdorf (1974) used morphological evidence to argue that the *su1* allele was selected once in the Peruvian Andes and then introgressed into local maize throughout the hemisphere. In this hypothesis (Mangelsdorf, 1974), the Peruvian race Chullpi was the original *su1* source and the progenitor of the race Maiz Dulce from

Jalisco, Mexico. Maiz Dulce was crossed to popcorn Reventador to produce Ducillo del Noroeste in northwest Mexico (Wellhausen et al., 1952). From Ducillo del Noroeste, *su1* was introgressed into northern races including Northern Flint originating Golden Bantam, the progenitor of modern commercial sweet corn (Revilla and Tracy, 1995b).

Tracy et al., (2006) studied the origin of the sweet corn. Seed from 57 *su1* accessions encompassing six geographic areas in the USA, Mexico, and Peru were obtained from the North Central Plant Introduction Center in Ames, IA, and the University of Wisconsin sweet corn breeding program. The survey revealed various independent origins in the history of sweet corn. The material of the northeastern USA cultivars sequenced had a tryptophan to arginine substitution at residue 578 (W578R). These are the progenitors of modern commercial sweet corn (Revilla and Tracy, 1995b; Gerdes and Tracy, 1994). These results are similar to those reported by Whitt et al., (2002), and this W578R allele was also one of two identified by Dinges et al., (2001). Some populations of Ducillo del Noroeste from northwest Mexico and accessions from southwestern USA had an asparagine to serine mutation (N561S) as a second origin. Another origin was confirmed by the 1.3-kbp transposable element in exon1 of the gene *Su1* found in Maiz Dulce from Guanajuato, Mexico (PI628428). This is the same transposable element previously observed in Mexican *su1* maize (Whitt et al., 2002). And Maiz Dulce from northcentral USA shows a substitution of arginine to cysteine mutation at residue 504 (R504C) as the other origin.

3- *Sugary1* sweet corn

Sugary maize was originally due to the recessive allele *su1* (Tracy, 1994). The allele *su1* is a recurrent mutation, located in chromosome 4, the primary gene for sweetness in maize (Tracy et al., 2006).The *su1* gene codes for an isoamylase affecting starch synthesis in maize endosperm (Rahman et al., 1998). Homozygous *su1* increases levels of water soluble polysaccharides (WSP) and decreases starch levels that give *su1* endosperm the smooth texture and creaminess characteristic of traditional sweet corn varieties (James et al., 2003; Schultz and Juvik, 2004).

The *su1* varieties, at immature milky stage, contain 10.2% of sucrose and 22.8% of water soluble polysaccharide, about 3 times the sugar and WSP contents of field corn, respectively (Creech 1965). The *sugary* varieties have good corn flavor but their kernels can lose their sucrose from 14.4% to 5.7% at room temperature 24 hours after harvest due to sucrose rapidly converting to starch (Garwood et al., 1976). These losses greatly affect the eating quality. As a result, the harvest and shortage periods for the *su1* varieties are short. These varieties are suitable for processing, canning and freezing.

4- Limitations of sweet corn

Sweet corn has limitations from a maize breeding perspective. Morphological and molecular variability in sweet corn is small compared to field corn (Revilla and Tracy, 1995a, b). The genetic base of sweet corn used presently in breeding programs is relatively narrow (Haber 1954; Tracy 1990), and genetically related inbreds are often crossed to meet strict requirements of market quality and appearance (Tracy 1994). Most sweet corn inbreds are descended from three

open pollinated cultivars: "Golden Bantam", "Stowell´s Evergreen", and "Country Gentleman" (Tracy 1993, 1994).

Additionally, heterotic groups among sweet corn inbreds are not well-defined (Revilla & Tracy 1997; Revilla et al., 2006b). Sweet corn breeders have not relied on heterotic patterns in the development of commercial hybrids. The development and creation of heterotic groups is of great importance for improving the agronomic performance and broaden the adaptation of sweet corn in new regions of production. In addition, sweet corn breeders should be aware of the risks of exhausting of heterosis if the same lines are systematically recombined without introducing new genetic combinations (Revilla and Tracy 1997; Revilla et al, 2000b).

5- Sweet corn breeding

As a result of the narrowness of the genetic base of sweet corn and the lack of defined heterotic groups, field corn has been used extensively to improve sweet corn and successful sweet corn populations such as Stowell´s Evergreen from Ithaca (New York State, USA), Golden Bantam from New York State (USA), and Spanish Gold from Connecticut (USA), have been developed in temperate areas using this strategy (Tracy, 2001). Many studies have shown the potential of flint and dent temperate corn germplasm for improving sweet corn resistance to European corn borer (Joyce and Davis, 1995), resistance to corn earworm (Guo et al., 2001, 2004), stalk and root quality (Treat and Tracy, 1993), yield plus agronomic and quality traits (Tracy, 1990), and for breeding new sweet corn patterns (Davis et al., 1988; Revilla et al., 2000b; Velasco et al., 2002). The maize breeding group of Misión Biológica de Galicia has also used field corn for

improving agronomic performance of sweet corn (Cartea et al., 1996a, b; Malvar et al., 1997a, b; Revilla et al., 1998; Revilla et al., 2000a).

A few investigators have tried to establish heterotic patterns in *su1* sweet corn, either by defining heterotic relationships among sweet corn cultivars (Revilla and Tracy 1997), or by crossing sweet corn cultivars to cultivars representing field corn heterotic patterns (Davis et al., 1988; Revilla et al., 2000b). Heterotic patterns have not been defined for other sweet corn mutants, where variability is narrower than for *su1* germplasm (Revilla et al., 2006b). Revilla and Tracy (1997) investigated the heterotic patterns among six open-pollinated sweet corn cultivars representing the relevant variability within *su1* germplasm. These authors reported two heterotic patterns among sweet corn cultivars: 1) "Country Gentleman" × "Golden Bantam", "Pease Crosby", and "Lindsey Meyer Blue"; and 2) "Stowell´s Evergreen" × "Golden Bantam", "Pease Crosby", and "Lindsey Meyer Blue". Midparent heterosis was 80% for "Country Gentleman" × "Golden Bantam" and 42% for "Stowell´s Evergreen" × "Golden Bantam". Revilla et al., (2000b) reported significant specific heterosis effects for yield and other traits in crosses among sweet and field corn populations.

6- Fitness and viability of the sweet corn mutant *su1*

Mutations represent the raw material for the evolution of species over time. Natural selection depends largely on the fitness of the mutation and its viability. The environment and the genetic background in which the mutation occurs are the two main factors that limit the stability of a mutation and its

propagation (Djemel et al., 2012). Most of the mutations are deleterious, but some of them are viable and economically profitable.

It is important to determine the positive and negative fitness effects of mutations 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). Indeed most mutations affecting fitness and fitness components are harmful (Garcia-Dorado et al., 1998).

Using field corn to broaden the genetic base for breeding programs and to improve the agronomic performance of sweet corn is a simple method as it deals with single recessive alleles but practical results can be disappointing because some undesirable genetic factors of field corn could be incorporated into new sweet corn varieties (Tracy 1990; Revilla et al., 2000a, 2006a, 2010). The success of such introductions depends on the viability of *su1su1* in the genetic background where it is introduced (Revilla et al, 2006a). The gene *su1* is considered lethal or semi-lethal when introduced in most field corn genetic backgrounds (Tracy 1990), as it cannot be maintained except when heterozygous (Tracy 1994), however it survives well enough in other genetic backgrounds.

Martins & Da Silva (1998) studied the variation of gene and genotype frequencies of the mutant *su1* and the *su1su1* individuals, respectively, through five successive generations from crosses *Su1Su1*×*su1su1*; the gene frequency of *su1* was steadily reduced across generations, indicating a directional selection against the allele *su1*. They also found that germination rate might be a factor in the reduction of viability of *su1su1* kernels and other factors, such as viability of plants after emergence and fertility, could decrease or increase the frequency respectively.

Revilla et al. (2000a) studied *su1* viability in crosses between *Su1Su1* and *su1su1* populations and found that *su1* frequency was reduced across recombinations in all crosses. The authors also found that the viability of the *su1* allele depends on the *Su1Su1* genetic background where the allele is inserted and identified the most favorable genetic background for the *su1* allele. Revilla et al. (2000a, 2006a, 2010) and Ordás et al. (2010) also reported that the viability of the *su1* and *sh2* mutants depend on specific sweet × field corn genotype interaction, with genetic background playing a major role in the viability of those mutants.

The opportunity for a mutation to survive in 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 lifespan, depending on genetic background and environmental factors. Furthermore, Yamamoto et al., (2009) confirmed that the size of the genetic effects in wild background was highly correlated with the size of the main effect of mutations, indicating evolutionary potential for enhancing or suppressing effects of single mutations. These studies demonstrate that the chance of mutant viability can only be understood in the light of its genetic and environmental interactions.

Breeding sweet corn for the European Atlantic coast, as for other regions with cold springs and short growing seasons, faces some problems with germination and early development (Ordás et al., 1994; Cartea et al., 1996a, b; Malvar et al., 2007a, b). When the recessive mutant *su1* is segregating in a maize breeding population, the selection against the mutant acts first through viability (Ordás et al., 2010), and then through fertility (mating ability and kernel formation)

(Cisneros-Lopez et al., 2010; Zhang et al., 2011). Germination is the first limiting factor of the viability and the next important factor is early vigor (Martins & Da Silva 1998; Revilla et al., 2000a; Gad & Juvik 2002; Juvik et al., 2003; Revilla et al., 2006a). Tracy (2001) also reported that these two characters are affected by genetic factors in sweet corn, both at planting and during kernel production. The limiting factors for those regions are seedling emergence and early vigor that are normally low for sweet corn particularly at low temperatures (Hotchkiss et al., 1997; Revilla et al., 2003). These authors have evaluated the available sweet corn cultivars for cold tolerance (Hotchkiss et al., 1997) and the heterotic patterns identified among them (Revilla et al., 2003).

Although, traits at early developmental stages, as early vigor, are the most important for *su1* survival, other traits, as kernel yield, kernel moisture, plants height, ear length, and kernel row number, can also play a role in the survival of the mutant (Revilla et al. 2000b).

The viability of kernels homozygous for *su1* is not solely a function of the allele, but it is also controlled by other genes (Djemel et al., 2013). The fitness of *su1* is under genetic control probably due to multiple genes with minor contributions (Djemel et al., 2012). The interaction of genetic background with alleles could have evolutionary implications by increasing or decreasing the probability of mutant fixation.

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 recombinant inbred lines (RIL) populations to create the Nested Association Mapping (NAM) population. Two of the 25 inbreds were *su1* and for the RILs released from both sweet corn lines (IL14h and P39) there was a significant segregation distortion against the *su1* mutant. We analyzed

the two populations at the molecular level and found some indirect evidence of multiple regions having epistatic effects of minor size with the *su1* gen (Djemel et al., 2013). The combined analyses of phenotypic and molecular data would shed new light on the subject.

The currently available information on the different genetic and agronomic variables that control the dynamics of the *su1* mutation remains limited and genes involved in *su1* viability have not been identified yet. Therefore, further research is needed to deepen the theoretical knowledge concerning the genetic regulation of *su1* fitness along the plant growth cycle, identifying genes and investigating the mechanisms involved in the regulation of mutant fitness.

Objectives

II. Objectives

The objective of this work was to identify the genetic mechanisms regulating the fitness of the mutant *su1* in maize. In order to do so, we identified, in two NAM's biparental populations derived from the cross of a *su1* and a *Su1* line:

-The molecular markers associated to vegetative and reproductive traits.

-The relationship between those markers and the *su1* gen.

This could allow a better understanding of the regulatory mechanisms involved in mutant viability and the design of genomic selection programs for improving mutant viability.

Materials and methods

III. Materials and methods

Plant materials

The material consists of RILs released from B73×P39 and B73×IL14H (Table 1). We use molecular characterization data with SNPs published in the Maize Diversity Project (www.panzea.org) for an analysis of QTLs associated with *sugary1* viability in these two populations. The RILs have been multiplied by Dr. Tracy of the University of Wisconsin (USA), who has sent samples of the two populations comprising a total of 392 RILs: 179 from B73×P39 and 213 from B73×IL14h). These inbreds have been multiplied again in 2012 in Pontevedra (Spain) in order to have two seed origins that allow the estimation of environmental effects in the identification of QTLs.

Table 1.Background and endosperm type of the sweet *(su1*) and field (*Su1*) corn inbreds used in this study.

Genotype	Background	Endosperm type
Field corn		
B73	Stiff Stalk Synthetic	*Su1*
Sweet corn		
P39	Golden Bantam	*su1*
IL14h	Stowell's Evergreen	*su1*

Molecular characterization

The NAM genetic map consists of 1478 SNPs, with an average marker density of one marker every 1.3 centiMorgans (cM) (McMullen et al., 2009). As the *Su1* locus was not mapped in the 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. In a previous work with these genotypes, Djemel et al (2013) classified the RILs into field corn or sweet corn types when both flanking markers of *Su1* had the B73 or the alternative allele from the sweet corn inbred line (P39 or IL14h), respectively, excluding all intervals with missing values. In the current work, the inbreds were classified as *Su1* or *su1* based on the observed phenotype, i.e. the *su1* kernels are shriveled and crystalline while the *Su1* have the normal wild appearance.

Growth conditions

The 392 RILs were multiplied and characterized in the field. During the growth period of these RILs in the field, the following data related to the viability of the mutant and wild type plants were collected: seedling emergence, early vigor, chlorophyll content, quantum efficiency of photosystem II (ΦPSII), male and female flowering, plant and ear height, ear length, number of kernel rows per ear, number of leaves, plant appearance, plant color, common rust and 100 kernels weight, as explained below.

All genotypes were evaluated under cold and control conditions in a growth chamber (40 m^3) equipped with VHO (very high-output) fluorescent lamps with a photosynthetic photon flux (PPF) of 228 μmol $m^{-2}s^{-1}$ and the distance between the shelf and the lamps was 0.5 m.

Two separated but simultaneous experiments were carried out for the RILs from B73×P39 and B73×IL14H under cold conditions and two more simultaneous experiments under control conditions. The genotypes evaluated were:

B73×P39: 179 RILs + 2 parental lines + the hybrid B73xP39

B73×IL14H: 213 RILs + 2 parental lines + the hybrid B73xIL14h

For each trial the (RILs + parental lines + hybrids) were evaluated following a Randomized Complete Block Design (RCBD) with 6 repetitions and one kernel per repetition. Entries were evaluated in plastic multi-cell seed trays (each seed tray consists of 104 alveoli of 8 × 13 mm).

Maize kernels were planted in seedbeds filled with sterilized peat (Gramoflor GmbH & Co.KG, Vechta, Germany) with one kernel per cell. Each kernel was sown in a cell with a surface of 3 × 2.5 cm and 5 cm depth; therefore, average distances were 3 cm between seedlings within a column and 2.5 cm between

seedlings within a row. Experiments were watered after planting and afterwards trials were watered two times per week.

This evaluation system is the most suitable when a large number of genotypes are used because reduce experimental units and thereby control environmental variation associated to stress evaluations. We tested two different origins of kernels (Wisconsin and Pontevedra) evaluated also in separate trials. Therefore, four consecutive runs were made for each combination of origin and growing condition (2 origins × 2 growth conditions).

First, evaluation in two environments (cold and control temperature) of kernels from Wisconsin. The first run, the RILs were evaluated in cold conditions (14 °C / 14 h with light and 10 °C /10 h without light). Then the second run, the RILs were evaluated in control conditions (20 °C / 14 h with light and 15 °C / 10 h without light).

Second, evaluation in two environments (cold and control temperature) of kernels from Pontevedra. The third run, the RILs were evaluated in control conditions (20 °C / 14 h with light and 15 °C / 10 h without light). Then the fourth run, the RILs were evaluated in cold conditions (14 °C / 14 h with light and 10 °C /10 h without light).

In all experiments, fitness data in all plants were recorded the same day (when most of the plants were at the three-leaf stage), except for days to emergence and to formation of the second leaf that were recorded as each plant reached the corresponding stage.

The evaluation of a second origin of kernels will be useful for estimating the stability of the QTL across kernels origin, which is a critical factor in controlled evaluations of kernel emergence and early development.

Measurement of traits

Germination related traits

Days from sowing to emergence

The number of days from sowing to emergence of the coleoptile of each plant.

Proportion of emergence

The proportion of plants germinated out of the six kernels sown (%).

Germination rate

The rate of germination calculate as $100 \times \Sigma Gt/Dt$ where Gt is the number of germinated plants in the day t and Dt is the number of days from sowing to the day t (for the total of 6 replicates).

Days to the second leaf

The number of days from sowing to the complete development of the second leaf of each plant (when the ligule is formed) was counted every two days up to a maximum of 30 days under cold conditions and 15 days under control conditions.

Vigor related traits

Vigor

Using a visual scale from 1= small weak plants with clear color to 9= large strong plants with dark green color. When vigor is determined at three-leaf stage (heterotrophic stage), it is seedling vigor, while in the field early vigor is recorded five weeks after sowing (autotrophic stage).

Chlorophyll (also Photosynthesis related trait)

Relative leaf chlorophyll content measured by using a hand-held Chlorophyll Content Meter, CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA).

Dry weight

The dry weight of each plant was determined after drying the stalk at 60 °C during one week.

Stand

The proportion of plants that are alive at the end of the trial out of the six kernels sown (%).

Photosynthesis related traits

Fluorescence

The maximum quantum efficiency of photosystem II (ΦPSII) was determined on dark-adapted leaves using a hand fluorometer (Opti- Science, Inc., USA).

Chlorophyll fluorescence parameters were measured on the second fully expanded leaf at the third leaf stage (V3) under both cold and control conditions using an OS-30p Chlorophyll Fluorometer.

With this instrument, we reordered the following parameters:

F_0: The basal level of fluorescence is affected by any environmental stress, which causes alterations at the pigment level of PS II. This parameter, along with F_m depends on the instrument used to record the trait. Therefore, we used the same instrument for all measurements.

F_m: The maximal fluorescence decreases after exposure of the leaf to high but not injurious temperatures.

F_v: The difference **F_m - F_0** is denominated variable fluorescence. Normally this value lowered by environmental stresses, which cause damage on the thylakoids. Examples of this kind of stress are heat, freezing and photoinhibition.

F_m/F_0: This ratio depends on the leaf water potential.

F_v/F_m: The quantum yield of the photochemical phase of the photosynthesis. A decrease of this relation is a good indicator for damage of photo inhibition caused by the light when the plants undergo diverse types of environmental stresses, as drought, cold, freezing or salinity. This ratio is used as an indicator of stress induced on the photosynthetic apparatus.

Adult-plant traits in the field

Male and female flowering

Days from sowing to the appearance of anthers or silks, respectively.

Plant height

Distance from the soil to the top of the tassel (cm).

Ear height

Distance between the soil and the insertion of the uppermost ear measured in three plants (cm)

Number of ears

Number of ears per plant.

Ear length

Distance between the bottom and the top of the ear (cm).

Number of kernel rows per ear

Number of rows of kernels in each ear.

100 kernel weight

Dry weight at room temperature of 100 kernels from each ear (g).

Common rust (*Puccinia sorghi*)

As a visible infection of common rust was observed in 2012, we recorded the damage by using a visual scale from 1 = healthy plant to 9 = plant covered by rust.

Number of leaves

The total number of leaves in a sample of three plants per RIL was counted.

Plant appearance

Using a visual scale from 1= small, week and unhealthy plants to 9= large, strong and healthy plants at flowering stage.

Plant color

Using a visual scale from 1= small weak plants with clear color to 9= large strong plants with dark green color.

Statistical analysis

Individual analysis of variance (ANOVA) of each phenotypic trial recorded in the growth chamber under cold and control conditions was performed using Proc Mixed procedure of SAS, version 9.1 (SAS Institute 2009). Repetitions and genotypes were considered random effects. The mean of the RILs were estimated by best linear unbiased predictor (BLUP). Variance components were estimated by restricted maximum likelihood (REML). The heritability was calculated as H = ½ genetic variance / (½ genetic variance + (error variance/6))
6 = number of replications.

To examine the existence of chromosomal regions that influence the *su1* allele, SNPs variability was classified into two main types (sweet corn and field corn) and, within each type, the number of RILs sharing the sweet corn allele in the loci throughout the genome was calculated. Under the null hypothesis of no relationship between a marker and the *su1* allele, the expected proportion of the allele derived from the *su1* line and the allele derived from the wild line should be 1:1 in the sweet corn and in the field maize group of lines. The deviation from the expected number was tested with chi square goodness of fit test (χ^2) ($p < 0.05$).

QTL analysis

QTL analysis was performed with PLABQTL software (Utz and Melchinger, 2003) separately for each RIL population and for data recorded in the field and for data recorded in the growth chamber, and within this, for the experiment under cold conditions and for the experiment under control conditions. A different QTL analysis was performed for each seed origin. A linkage map with a set of 1478 markers was used. A likelihood odds (LOD) threshold was chosen for declaring the putative QTL significant that ensures an experiment wise error rate of $P < 0.30$. The LOD score threshold was obtained by the permutation test method with 1000 permutations (Churchill and Doerge 1994). The proportion of phenotypic variance explained by all QTLs was determined by the adjusted coefficient of determination of regression (R^2adj), fitting a model including all detected QTLs.

Because phenotypic evaluation was made on RIL populations, only the additive effects of the QTL are reported. QTL positions are described as chromosome number followed by the position of the QTL peak in parenthesis.

Results

IV. Results

Phenotypic data

In the analysis of variance of the RIL populations derived from B73×P39 and B73×IL14h for both origins of kernels (Wisconsin and Pontevedra) under both cold and control conditions, the differences among genotypes were highly significant ($P<0.001$) for all traits (Appendix).

In the RILs population B73×P39, F_v/F_m had the highest heritability (0.86 to 0.99) under both cold and control conditions in both origins of kernel (Wisconsin and Pontevedra). Days to emergence and days to the second leaf had the lowest heritability under both cold and control conditions (0.35 to 0.55 and 0.29 to 0.57, respectively). In general, the heritability under control conditions was higher than the heritability under cold conditions. Except for chlorophyll content, F_0, F_m and F_v, the heritability was higher under cold conditions only in the origin kernel of Pontevedra (Tables 1 and 2).

In the RIL population B73×IL14h, the heritability under cold and control conditions was generally lower than the heritability of the other population (B73×P39). The reason for the lower heritability values in the second RIL populations compared to the first one was that the genetic variances were lower for B73×IL14h than for B73×P39 for most traits, while the error variances were lower in the second RIL population than in the first one for half of the cases and higher for the other half. The heritability was from 0.31 to 0.58 under cold conditions. While under control conditions, the heritability varied from 0.35 to

0.65 except for F_0 in the origin kernel of Wisconsin was high (0.76) (Tables 3 and 4).

Table 1.Variance components and heritability of traits recorded in the growth chamber for B73×P39 under cold and control conditions for the kernel origin of Wisconsin

Trait	Genetic variance		Error variance		Heritability	
Environment	Cold	Control	Cold	Control	Cold	Control
Days to emergence	0.26	0.22	1.46	1.03	0.35	0.39
Chlorophyll	3.18	4.13	5.46	4.62	0.64	0.73
Vigor	0.52	-	0.60	-	0.72	-
Days to 2nd leaf	1.7	1.23	12.65	6.62	0.29	0.36
Dry weight	0.002	0.002	0.003	0.001	0.68	0.79
F_0	990.05	680.19	2534.8	1067.5	0.54	0.66
F_m	7063.5	8445.9	6337.7	3703.6	0.77	0.87
F_v/F_m	0.06	0.08	0.03	0.02	0.86	0.92
F_v	1614.1	4989.2	1934.7	1845.8	0.71	0.89
F_m/F_0	0.35	1.92	0.47	0.67	0.69	0.90

Table 2. Variance components and heritability of traits recorded in the growth chamber for B73×P39 under cold and control conditions for the kernel origin of Pontevedra

Trait	Genetic variance		Error variance		Heritability	
Environment	Cold	Control	Cold	Control	Cold	Control
Days to emergence	0.89	0.32	2.23	0.80	0.54	0.55
Chlorophyll	3.95	4.25	3.64	3.44	0.77	0.57
Vigor	0.39	0.50	0.72	0.79	0.62	0.66
Days to 2nd leaf	1.54	1.53	7.04	9.44	0.40	0.57
F_0	1217.8	2205.9	1170.4	3917.1	0.76	0.63
F_m	10087.2	2163.3	3636.9	4217.4	0.89	0.61
F_v/F_m	0.07	0.43	0.02	0.01	0.94	0.99
F_v	4481.00	1897.1	1359.8	1643.4	0.91	0.78
F_m/F_0	0.27	0.96	0.61	0.36	0.57	0.89

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Table 3. Variance components and heritability of traits recorded in the growth chamber for B73×IL14h under cold and control conditions for the kernel origin of Wisconsin

Trait	Genetic variance		Error variance		Heritability	
Environment	**Cold**	**Control**	**Cold**	**Control**	**Cold**	**Control**
Days to emergence	0.35	0.31	1.21	1.15	0.46	0.45
Chlorophyll	2.93	2.36	12.79	7.05	0.41	0.50
Vigor	0.20	-	0.76	-	0.45	-
Days to 2nd leaf	2.04	1.34	10.06	7.53	0.38	0.35
Dry weight	0.001	0.001	0.003	0.002	0.54	0.46
F_0	981.55	541.64	3952.2	512.36	0.44	0.76
F_m	1194.90	529.61	7953.4	1637.90	0.31	0.49
F_v/F_m	0.01	0.004	0.02	0.01	0.44	0.55
F_v	506.01	375.79	2782.30	892.91	0.35	0.56
F_m/F_0	0.12	0.23	0.43	0.37	0.45	0.65

Table 4. Variance components and heritability of traits recorded in the growth chamber for B73×IL14h under cold and control conditions for the kernel origin of Pontevedra

Trait	Genetic variance		Error variance		Heritability	
Environment	**Cold**	**Control**	**Cold**	**Control**	**Cold**	**Control**
Days to emergence	0.65	0.37	1.82	0.76	0.52	0.59
Chlorophyll	1.08	1.32	3.02	2.81	0.52	0.59
Vigor	0.13	0.27	0.81	0.94	0.32	0.47
Days to 2nd leaf	0.98	1.82	5.72	10.14	0.34	0.35
F_0	889.32	15.94	3177.7	64.18	0.46	0.43
F_m	2591.8	388.31	6754	1464.5	0.54	0.44
F_v/F_m	0.01	0.001	0.02	0.01	0.54	0.32
F_v	1161.5	307.07	2523	1302.7	0.58	0.41
F_m/F_0	0.10	0.06	0.29	0.33	0.51	0.37

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Segregation distortion against *su1*

In both RIL populations developed from crosses between B73 and two sweet corn inbreds (B73×P39 and B73×IL14h), a significant segregation distortion was identified for the B73 (*Su1Su1*) : alternative allele (P39 or IL14h) (*su1su1*) (Table 5).

Table 5. Phenotypic segregation distortion of *Su1* : *su1* kernels (P<0.05) in two RIL populations obtained from crosses between the maize inbred line B73 and two diverse lines (P39 and IL14h)

RIL population	Total number of RILs	Number of observed homozygotes		Expected homozygotes per genotype	χ^2
		Su1Su1	*su1su1*		
B73×P39	179	144	35	89.5	66.38**
B73×IL14h	213	198	15	106.5	157.22**

We used the Chi square goodness of fit test (χ^2) ($p < 0.05$) to examine the existence of chromosomal regions that exhibited segregation distortion against the *su1* allele for the RILs developed from B73×P39 and B73×IL14h (Tables 6 and 7).

Table 6. SNP markers showing segregation distortion in the RILs from B73xP39 and B73xIL14h for P39 and IL14h parental alleles vs. B73 parental allele outside the chromosome 4 (origin Wisconsin)

B73xP39			B73xIL14h		
Bin	Marker	$\chi 2$ value	Bin	Marker	$\chi 2$ value
2.09	S_ 2239050	4.255 *	1.08	S_ 2352785	5.605 *
3.03	S_ 1198049	7.329 **	3.08	S_ 2102534	4.571 *
			5.04	S_ 1338249	5.380 *
			6.05	S_ 1304305	4.931 *
			6.02	S_ 8971453	5.451 *
			7.03	S_ 1282382	5.191 *
			8.08	S_ 1723514	4.281 *

Table 7. SNP markers showing segregation distortion in the RILs from B73xP39 and B73xIL14h for P39 and IL14h parental alleles vs. B73 parental allele outside the chromosome 4 (origin Pontevedra)

B73xP39			B73xIL14h		
Bin	Marker	$\chi 2$ value	Bin	Marker	$\chi 2$ value
2.08	S_ 2218053	4.733 *	1.10	S_ 2813894	4.368 *
5.02	S_ 1070693	5.125 *	5.04	S_ 8615162	4.709 *
			6.04	S_ 1099445	9.679 **
			6.04	S_ 1124691	8.489 *
			6.04	S_ 1066322	7.60 **
			7.03	S_ 1282382	4.416 *

For (B73×P39), all the SNPs located on chromosome 4 and three other markers, located on chromosome 2 in the interval position 125-139 (bins 2.08 and 2.09) and bins 3.03 and 5.02, showed deviations from the expected frequencies for the B73 : alternative allele in the *sugary* RILs. However, all the other markers displayed the expected Mendelian distribution of B73 and the alternative allele.

For (B73×IL14h), the SNPs located on chromosome 4 (except those located at bin 4.02 (31 cM), 4.08 (108 cM), (109 cM), 4.10 (134 cM), 4.10 (136 cM)) and other ten

markers showed deviation from the expected frequencies. They were located in bins 1.08, 1.10, 3.08, 5.04, 6.02, 6.05, 7.03, and 8.08 and two very close markers in bin 6.04. They showed deviations from the expected frequencies for the B73 or alternative allele in the *su1* RIL. However, all the other markers in all chromosomes displayed the expected Mendelian distribution of B73 and the alternative allele in the other chromosomes.

QTLs related to *su1* viability

Field study

Several QTLs were detected in both RIL populations (B73×P39 and B73×IL14h) for all traits related to the viability of the mutant in the field. The QTL analysis for the RILs from B73×P39 showed 27 QTLs (Table 8) and showed 24 QTLs for the RILs from B73×IL14h (Table 9). The QTLs were distributed in all chromosomes, except chromosome 10 for both RIL populations.

QTLs for the RILs from B73×P39 in the field

For plant color and number of rows per ear, one QTL was detected on chromosome 5, located in the interval position 81-84 cM (Table 8). This QTL can be considered a major QTL because it explained a large proportion of the phenotypic variance (26.5% for number of kernel rows per ear). An increase in both of these traits was due to the allelic contribution of the field corn parent (B73) as demonstrated by the negative value of the additive effect.

Also on chromosome 5 in the interval position 92-96 cM, another important QTL was detected for kernel weight and number of ears. This QTL explained a high proportion of the phenotypic variance (21.2% for kernel weight), had high

LOD score and had very high additive effect for 100 kernels weight. An increase in these traits was due to the allelic contribution of the field corn parent (B73).

Table 8. Summary of QTLs detected in the RIL population derived from B73×P39 evaluated in the field.

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to emergence	3.05	72-75	4.81	S_156934272	12.8	-1.093
	6.06	70-73	4.91	S_154623250	13	-0.779
Vigor	4.10	140-143	6.09	S_239252139	16.5	-0.443
No. of leaves	5.06	105-107	4.87	S_197693076	13	0.358
	5.08	139-142	5.21	S_213123489	14.1	-0.191
	6.05	61-64	7.57	S_149209155	19.4	-0.255
Chlorophyll	5.07	131-133	5.15	S_210734720	13.8	-3.957
	7.04	104-106	4.88	S_165005627	13	8.353
Plant appearance	8.05	65-68	5.48	S_130408047	14.5	-0.343
Plant color	3.09	152-154	5.50	S_227136056	15	-0.163
	5.04	81-84	4.74	S_169975064	12.7	-0.365
	7.03	78-81	5.76	S_146239395	15.1	0.159
Common rust	4.08	107-110	4.83	S_194193960	12.8	0.457
Ear height	3.06	92-95	4.76	S_179309748	12.6	-5.8
Plant height	5.00	1-4	6.03	S_1195364	17	-7.711
	5.03	57-59	4.86	S_31890893	13	-6.753
	8.05	65-68	7.02	S_130408047	18.2	-7.944
	9.07	98-101	5.16	S_148048875	13.8	-7.305
Number of ears	1.11	181-183	6.94	S_285458111	18	-3.134
	3.03	42-44	5.65	S_12675841	15	2.669
	5.05	93-96	4.70	S_185394531	12.7	-1.216
Ear length	6.05	44-47	5.96	S_121770980	15.7	-0.509
Number of kernel rows per ear	2.04	69-71	6.14	S_40706738	16.1	-1.855
	4.08	100-103	6.31	S_185684080	16.5	-0.778
	5.04	81-83	10.70	S_169975064	26.5	-1.230
	6.06	69-72	4.80	S_154100976	12.8	0.967
100 Kernel weight	5.05	92-94	8.42	S_183693045	21.2	-53.188

For plant appearance and plant height, one QTL was detected on chromosome 8 located in the interval position 65-68 cM. This QTL explained a high proportion of the phenotypic variance (18.2%) and the favorable allele was inherited from the field corn parent in these traits.

For days from sowing to emergence and number of kernel rows per ear, one QTL was detected on chromosome 6 and located in the interval position 69-73 cM. An increase in days to emergence was due to the allelic contribution of the field corn parent (B73). By contrast, an increase in number of kernel rows was due to the allelic contribution of the sweet corn parent (P39).

QTLs for the RILs from B73×IL14h in the field

For plant height and ear height, one QTL was detected on chromosome 8 located in the interval position 66-69 cM (Table 9). This QTL can be considered a major QTL because it explained a high proportion of the phenotypic variance 43.3% and 35.2% for ear and plant height, respectively. An increase in both traits was due to the allelic contribution of the field corn parent (B73).

On chromosome 3, one QTL was detected in the interval position 15-18 cM. This QTL explained a high proportion of the phenotypic variance (20%) and affected number of kernel rows per ear and 100 kernels weight. An increase in both traits was due to the allelic contribution of the field corn parent (B73).

For common rust (*Puccinia sorghi*), one QTL was detected on chromosome 5 located in the interval position 100-103. This QTL explained a high proportion of the phenotypic variance (22.3%) and had a high LOD score. Close to this QTL (at 104-106 cM) we found another QTL for plant height. An increase in common

rust was due to the allelic contribution of the sweet corn parent (P39); therefore, the allele from B73 provided more resistance than the alternative allele. Moreover, an increase in plant height was due to the allelic contribution of the field corn parent (B73).

Table 9. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated in the field.

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to emergence	4.08	95-98	5.07	S_181794523	14.4	0.871
Vigor	3.05	66-69	4.96	S_140089114	14.1	0.301
	4.07	89-92	6.06	S_177665741	17	0.346
Chlorophyll	8.03	54-56	4.86	S_91993060	14.2	-5.905
Common rust	1.09	155-158	5.49	S_257892756	15.5	0.453
	5.05	100-103	8.15	S_191253032	22.3	0.588
Ear height	1.06	97-100	4.90	S_175914446	14	-3.783
	3.04	56-59	6.80	S_27109475	18.8	-4.560
	5.06	108-111	5.53	S_201993308	15.7	-4.127
	7.03	63-66	4.89	S_129598959	13.9	-3.819
	8.05	67-69	18.49	S_132879659	43.3	-8.684
	9.03	49-52	6.33	S_26041692	17.8	4.929
	9.06	77-79	6.04	S_138230491	16.9	-4.592
Plant height	5.06	104-106	7.12	S_196414978	19.8	-8.015
	8.05	66-69	14.11	S_130408047	35.2	-11.656
Ear length	6.07	100-103	4.91	S_164891527	15.1	-0.450
	7.01	28-31	7.11	S_6886392	19.7	-0.673
	7.05	115-117	7.71	S_169496703	21.5	-0.653
	9.05	68-70	7.94	S_127013600	21.9	1.460
Number of kernels rows per ear	3.01	15-18	7.05	S_3804316	19.8	-1.254
100 Kernel weight	1.11	185-188	6.34	S_288374247	17.9	-50.554
	2.08	127-130	4.81	S_217444922	13.8	-27.861
	3.01	15-18	6.32	S_3611564	18.2	-33.469
	9.07	111-113	6.62	S_151974142	18.6	33.831

Growth chamber (Origin Wisconsin)

QTLs associated to emergence and early growth-related traits were identified in all chromosomes in the two RIL populations (B73×P39 and B73×IL14H) grown under cold and control conditions, but the location, LOD and weight of the QTLs were not homogeneously distributed throughout the genome (Tables 10 to 13). In B73×P39 the number of QTLs was lower than in B73×IL14h, particularly under control conditions. The number of QTLs was higher under cold than under control conditions. The RILs from B73×P39 had QTLs in six chromosomes and the highest number of QTLs was found in chromosome 4, while the RILs from B73×IL14h had QTLs in all chromosomes and the highest numbers were in chromosome 5.

The LOD score of QTLs varied from 15.94 to 4.81 for B73×P39 under cold conditions and from 9.94 to 2.41 under control conditions (Tables 10 and 11). For B73×IL14h the LOD varied from 18.03 to 4.65 under cold conditions and from 17.54 to 4.61 under control conditions (Tables 12 and 13). The significance of QTLs was usually higher under cold conditions, particularly for B73×P39. The proportion of phenotypic variance explained by the QTLs varied from 38.9 to 13.9 under cold conditions and from 28.2 to 7.4 under control conditions for B73×P39; and from 39.9 to 12.4 under cold conditions and from 43.2 to 12.2 under control conditions for B73×IL14h. The proportion of explained phenotypic variance was usually higher under cold than under control conditions for B73×P39 but not for B73×IL14h. In B73×P39, the favorable alleles of the QTLs were more often provided by the mutant parent (P39) than by the wild type parent (B73), particularly under control conditions. Contrarily, for B73×IL14h, both parents provided equally favorable alleles under cold conditions and the wild type parent provided more favorable alleles under control conditions.

QTLs for the RILs from B73×P39 in the growth chamber

The QTL analysis of the RILs from B73×P39 showed 24 QTLs detected under cold conditions (Table 10). Most of these QTLs were located on chromosome 4 (10 QTLs) and chromosome 2 (8 QTLs), while the remaining QTLs were located on chromosomes 1, 3, 6 and 9. Under control conditions, 13 QTLs were detected, and most of them were located on chromosome 4 (8 QTLs) and the other QTLs were on chromosome 1, 7 and 10 (Table 11).

For the three vigor-related traits (early vigor, leaf chlorophyll content and plant dry weight) and for the fluorescence parameters (F_m, F_v, F_v/F_m and F_m/F_0), several common QTLs were detected. Some of these QTLs were located on chromosome 4 in the interval position 86 and 93 cM. This genomic region can be considered a major QTL because it explained a high proportion of the phenotypic variance for almost all the traits. This QTL was detected for these traits under both, cold and control conditions, except for chlorophyll content only under control conditions. The proportion of explained phenotypic variance by these QTLs was higher under cold conditions than under control conditions. For this QTL, an increase in all these traits was due to the allelic contribution of the sweet corn parent (P39), as demonstrated by the positive value of the additive effect for all these traits.

On chromosome 4, another QTL was detected in the interval position 55 to 60 cM. This QTL was detected also under both cold and control conditions for dry weight, germination rate and days from sowing to emergence. An increase in dry weight and germination rate was due to the allelic contribution of the field corn parent (B73) as demonstrated by the negative value of the additive effect for these traits. Moreover, an increase in days from sowing to emergence was due to the allelic contribution of the sweet corn parent (P39).

For dry weight, vigor and F_m, the same QTL was detected on chromosome 2 in the interval 136-139 cM. This is an important QTL because it explained a high proportion of the phenotypic variance (24.8% for dry weight) and had a high LOD score (9.02 for dry weight). This QTL was detected only under cold conditions, and was associated with *su1*, as indicated by the χ^2 test of independence. For this QTL, an increase in these traits was due to the allelic contribution of the field corn parent (B73).

Another important QTL was detected on chromosome 2 in the interval 157-160 cM affecting dry weight, vigor and F_m. This QTL explained high proportion of the phenotypic variance (19.6% for dry weight) and had high LOD score (6.74 for dry weight). This QTL was detected only under cold conditions, indicating that this QTL was specific to low temperature conditions. The favorable allele was provided by the sweet corn parent P39.

One QTL, in the chromosome 3 in the interval position 41-44 cM, was found for F_0 that explained 15.4%of the phenotypic variance. The allele for increased F_0 was inherited from the sweet corn parent (P39). This QTL is associated with *su1* (χ^2= 7.329**). This QTL was detected only under cold conditions, indicating that this QTL was specific to low temperature conditions.

On chromosome 9, one QTL was detected in interval position 57-60 cM for dry weight and F_m. This QTL explained 14.6% and 13.9% of the phenotypic variance, respectively. For this QTL, an increase in these traits was due to the allelic contribution of the field corn parent (B73). This QTL was also specific for low temperature conditions.

For days from sowing to emergence, two QTLs explained 32% of the phenotypic variance. These QTLs were located on chromosome 3, 73-76 cM and

chromosome 4, 39-42 cM. For these two QTLs, an increase in days to emergence was due to the allelic contribution of the field corn parent (B73), as demonstrated by the negative value of the additive effect.

Table 10 Summary of QTLs detected in the RIL population derived from B73×P39 evaluated under cold conditions for the origin kernel of Wisconsin

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to emergence	3.05	73-76	5.92	S_1593070	16.7	-0.695
	4.03	39-42	5.36	S_1469022	15.3	-0.151
Clorophyll	4.07	89-92	7.53	S_1776657	20.8	1.098
Dry weight	1.09	163-166	6.12	S_2677977	17.2	-0.018
	2.06	85-88	6.12	S_1732505	17.2	0.024
	2.09	136-139	9.02	S_2239050	24.8	-0.016
	2.10	157-160	6.74	S_2360258	19.6	0.014
	4.05	55-57	11.63	S_3986241	30.2	-0.016
	4.07	89-92	15.94	S_1784973	38.9	0.018
	6.01	8-11	4.99	S_6036264	14.8	-0.014
	9.04	57-60	5.06	S_1053195	14.6	-0.01
Seedling vigour	2.06	84-87	5.13	S_1682776	14.7	0.199
	2.09	136-138	7.37	S_2239050	20.7	-0.272
	2.10	157-159	5.35	S_2360258	15.9	0.226
	4.07	89-91	15.36	S_1776657	37.8	0.341
F_0	3.03	41-44	5.41	S_1198049	15.4	8.643
F_m	2.09	136-138	4.81	S_2239050	14.1	-26.406
	2.10	157-159	5.26	S_2360258	15.7	27.535
	4.07	89-92	9.25	S_1776657	24.9	30.364
	9.04	57-60	4.83	S_1027578	13.9	-21.8
F_v/F_m	4.07	89-92	8.03	S_1776657	22	0.109
F_v	4.07	89-92	5.43	S_1776657	15.5	12.963
F_m/F_0	4.07	89-92	5.06	S_1776657	14.5	0.187
Germination rate	4.05	57-60	5.78	S_6582037	16.4	-0.047

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Table 11 Summary of QTLs detected in the RIL population derived from B73×P39 evaluated under control conditions for the origin kernel of Wisconsin						
Trait	**Bin**	**Interval position**	**LOD score**	**Marker**	**R^2 %**	**Additive effect**
Days to emergence	4.05	55-57	9.94	S_3986241	28.2	0.347
Clorophyll	4.08	95-97	2.82	S_1817945	9	0.599
Dry weight	1.08	137-147	2.41	S_2396982	7.4	-0.01
	4.07	86-92	3.57	S_1776657	10.8	0.015
F_0	10.07	90-112	2.47	S_1462238	8.2	-6.476
F_m	4.07	87-92	3.24	S_1776657	10.2	28.867
F_v/F_m	4.07	90-92	5.08	S_1784973	15.2	0.116
F_v	4.07	90-93	4.51	S_1784973	13	26.491
F_m/F_0	4.07	90-93	4.25	S_1784973	12.9	0.506
Days to 2nd leaf	7.03	66-69	3.26	S_1327112	11.9	0.269
	10.03	44-47	3.63	S_8366842	12.7	0.249
Germination rate	4.05	55-60	4.32	S_6582037	13.3	-0.052
Stand	7.02	57-60	3.07	S_1203508	9.9	-0.068

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

QTLs for the RILs from B73×IL14h in the growth chamber

The QTL analysis of the RILs from B73×IL14h showed 46 QTLs under cold conditions (Table 12). The QTLs were distributed in all chromosomes except chromosome 9. The chromosome 5 had high number of QTLs (12 QTLs). Under control conditions, 42 QTLs were detected, most of them were located also on chromosome 5 (8 QTLs) and the others were distributed throughout all chromosomes (Table 13).

Several QTLs were detected on chromosome 5; within these QTLs, one QTL was located in the interval position 63-69 cM affected leaf chlorophyll content and fluorescence parameters (F_0, F_v/F_m, F_v, F_m/F_0) (Tables 12 and 13). This QTL can be considered a major QTL because it explained a high proportion of the

phenotypic variance for all these traits. This QTL was detected for these traits under both, cold and control conditions with similar proportion of explained variance in both conditions. For this QTL, an increase in leaf chlorophyll content, F_v/F_m, F_v and F_m/F_0 was due to the allelic contribution of the sweet corn parent (IL14h), as demonstrated by the positive value of the additive effect for all these traits. By contrast, the increase in F_0 was due to the allelic contribution of the field corn parent (B73). This QTL was very close to one QTL detected for F_v/F_m under control conditions located in the interval 71-73 cM. This QTL was associated with *su1,* as the segregation of this QTL and *su1* was not independent (χ^2= 5.38*).

Table 12. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under cold conditions for the origin kernel of Wisconsin

Trait	Bin	Interval position	LOD score	Marker	R^2 %	Additive effect
Days to emergence	2.01	4-6	8.88	S_1915161	23.2	0.962
	3.05	77-80	5.39	S_1624254	14	0.167
	4.10	135-137	5.32	S_2379092	14.1	0.175
Colorphyll	5.03	66-69	5.57	S_7663466	14.6	0.422
Dry weight	2.00	1-3	4.7	S_1094394	13.1	-0.007
	3.03	38-40	6.77	S_1046345	17.4	0.009
	4.05	60-63	5.22	S_1357274	13.8	-0.009
	4.10	138-141	8.86	S_2386386	22.8	-0.01
	5.03	53-55	9.53	S_2280782	23.6	0.041
	5.08	139-142	5.69	S_2131234	15.1	-0.009
Seedling vigor	4.10	138-141	5.42	S_2386386	14.6	-0.101
	10.06	79-82	5.63	S_1420470	14.8	-0.155
Days to 2nd leaf	1.08	138-141	8.03	S_2296092	20.2	-0.394
F_0	2.01	7-10	5.69	S_2654015	15.3	7.462
	2.02	20-22	5.43	S_6245916	14.1	-7.221
	4.10	133-136	6.01	S_2373952	15.7	5.919
	5.02	34-37	5.13	S_1070693	13.6	6.551
	6.07	101-104	13.16	S_1651952	31.5	-8.949
	7.01	39-41	8.52	S_1328301	21.4	13.627
	8.03	50-52	11.46	S_4617108	27.7	-14.964

Table 12. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under cold conditions for the origin kernel of Wisconsin (Continue)						
F_m	3.03	40-43	4.88	S_1147481	12.9	-7.526
	4.08	108-111	7.94	S_1970728	20	-9.901
	4.10	133-135	7.79	S_2373952	19.9	7.656
	5.02	34-37	6.63	S_1070693	17.2	8.845
	5.03	47-50	5.67	S_1632069	14.8	-7.674
	6.03	23-26	5.44	S_9787388	14.2	7.19
	7.02	58-61	6.01	S_1223503	15.5	6.166
	8.01	5-8	5.15	S_3031491	14.4	5.782
F_v/F_m	2.00	1-4	4.69	S_1094394	13.1	-0.019
	5.03	65-67	17.31	S_6911963	38.7	0.033
	5.08	139-142	4.65	S_2129031	12.5	-0.016
	6.07	96-99	4.91	S_1642091	13.2	0.015
F_v	2.04	69-72	5.83	S_4070673	15.1	-5.296
	5.03	65-67	9.42	S_6911963	23.4	6.264
	6.03	23-26	5.09	S_9787388	13.4	5.357
F_m/F_0	2.00	1-4	4.85	S_1094394	13.5	-0.082
	3.07	110-113	5.66	S_2013903	14.7	-0.071
	5.03	65-67	18.03	S_6911963	39.9	0.139
	5.08	139-142	6.45	S_2129031	16.9	-0.077
	6.06	79-82	4.8	S_1588237	12.6	0.062
	7.04	111-114	6.27	S_1677781	16.1	-0.142
Stand	3.05	72-75	7.25	S_1569342	18.5	-0.08
	5.03	52-55	4.67	S_2204537	12.4	0.071
Germination rate	2.02	20-23	6.61	S_6245916	16.9	0.054
	3.08	122-124	5.08	S_2102534	13.3	0.12
	8.08	122-125	4.94	S_1723514	13	-0.043

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

An important QTL was detected only under control conditions on chromosome 6 located in the interval position 88 to 92 cM (Table 13). This QTL affected germination rate and fluorescence parameters (F_m, F_0 and F_m/F_0). It explained very high proportion of the phenotypic variance (39.5% for F_0) and had a high

LOD score (17.54).

For germination rate, one QTL was detected on chromosome 3 and was located in the interval position 122-124 cM. This QTL was associated with *su1* and specific to low growth temperature. For this QTL, an increase in germination rate was due to the allelic contribution of the sweet corn parent (IL14h). Another important QTL was detected on chromosome 3 in the interval position 79 to 82 cM for days to the second leaf. It explained 25% of the phenotypic variance and the favorable allele was provided by the sweet corn parent.

Table 13 Summary of QTLs detected in the RIL population derived from B73×Il14h evaluated under control conditions for the origin kernel of Wisconsin

Trait	Bin	Interval position	LOD score	Marker	R^2 %	Additive effect
Colorphyll	5.03	63-66	6.55	S_6000276	16.9	0.401
Dry weight	1.06	97-100	4.77	S_1759144	12.6	-0.008
	4.02	29-32	8.5	S_1064600	21.6	0.008
	4.05	60-63	6.48	S_1357274	16.8	-0.007
	7.03	62-65	5.06	S_1282382	13.3	-0.007
	9.03	52-55	5.76	S_3489543	14.9	-0.006
F_0	5.02	39-42	4.93	S_1302019	13	-9.371
	5.03	65-67	11.83	S_6911963	28.4	-7.585
	6.07	90-92	17.54	S_1624052	39.5	-8.81
	8.03	49-52	7.96	S_3085022	20.2	-9.635
	9.07	101-103	5.68	S_1493105	14.9	-9.407
F_m	1.02	30-33	6.49	S_1387227	16.7	-6.253
	1.07	121-124	6.43	S_2073734	16.5	-4.868
	1.11	185-188	5.89	S_2883742	15.3	-4.86
	2.07	106-108	9.29	S_1966416	23	-6.197
	3.09	153-156	4.87	S_2277584	13.3	-4.143
	4.08	107-110	5.65	S_1941939	14.7	-4.646
	4.10	135-137	9.45	S_2379092	23.7	6.019
	6.02	15-18	5.47	S_8971453	14.5	4.395
F_m	6.05	48-50	5.49	S_1304305	14.3	-4.524
	6.07	90-92	12	S_1624052	29.1	-6.929
	10.04	48-50	7.65	S_1080936	19.3	-7.335

Table 13 Summary of QTLs detected in the RIL population derived from B73×Il14h evaluated under control conditions for the origin kernel of Wisconsin						
F_v/F_m	5.02	39-42	4.96	S_1302019	13.1	0.025
	5.04	71-73	6.66	S_1338249	17.1	0.035
	6.07	94-97	6.68	S_1635134	17.4	0.012
	10.05	63-65	8.52	S_1347662	21.4	-0.014
F_v	1.07	128-130	5.61	S_2154688	14.6	-6.058
	2.01	12-14	6.06	S_3995442	16	4.204
	5.03	66-69	11.91	S_7313274	28.6	6.332
	8.01	17-19	4.91	S_7078007	13.3	9.201
	10.04	49-52	4.98	S_1123883	13	-3.704
F_m/F_0	2.02	21-24	6.09	S_6642611	15.7	0.15
	5.03	66-68	20	S_7313274	43.2	0.223
	6.07	90-92	11.31	S_1624052	27.6	0.148
Days to 2nd leaf	1.08	141-144	5.87	S_2352785	15.2	-0.259
	3.05	79-82	10.24	S_1664679	25	0.354
Germination	8.08	123-126	4.61	S_1723514	12.2	-0.055
Stand	1.02	39-42	6.55	S_2158043	16.8	0.066
	3.04	43-45	5.12	S_1345197	13.5	0.061
Germination rate	4.03	42-45	8	S_1782282	20.3	-0.123
	5.03	52-54	5.47	S_2204537	14.3	0.101
	6.07	88-90	5.7	S_1619971	15	-0.09

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

On chromosome 8, one QTL was detected in the interval position 49-52 cM. It explained very high proportion of the phenotypic variance and had very high LOD score. This QTL was detected under both cold and control condition for F_0 and an increase in this trait was due the allelic contribution of the field corn parent (B73).

Growth chamber (Origin Pontevedra)

QTLs associated to emergence and early growth-related traits were identified in all chromosomes in the two RIL populations (B73×P39 and B73×IL14H) grown under cold and control conditions, but the location, LOD and weight of the

QTLs were not homogeneously distributed (Tables 14 to 17). In B73×P39 the number of QTLs was lower than in B73×IL14H, particularly under control conditions. The number of QTLs was higher under cold than under control conditions. The distribution of QTLs across chromosomes depended on the genotype more than on the environmental conditions.

The LOD score of QTLs varied from 10.06 to 2.27 for B73×P39 under cold conditions and from 7.93 to 2.28 under control conditions. For B73×IL14h the LOD varied from 9.29 to 2.27 under cold conditions and from 5.56 to 2.27 under control conditions (Tables 14 to 17). The significance of QTLs was usually higher under cold conditions, particularly for B73×P39. The proportion of phenotypic variance explained by the QTLs varied from 30.2 to 8.2 under cold conditions and from 24.5 to 7.8 under control conditions for B73×P39; and from 27.7 to 7.2 under cold conditions and from 18.9 to 7.4 under control conditions for B73×IL14h. The proportion of explained phenotypic variance was in general higher under cold than under control conditions for B73×P39 and B73×IL14h. In B73×P39, the favorable alleles of the QTLs were more often provided by the mutant parent (P39) than by the wild type parent (B73), particularly under control conditions. In B73×IL14h, also the favorable alleles of the QTLs were more often provided by the mutant parent (IL14h) under cold conditions. However, both parents provided equally favorable alleles under control conditions.

QTLs for the RILs from B73×P39 in the growth chamber

The QTL analysis of the RILs from B73×P39 showed 20 QTLs detected under cold conditions (Table 14). Most of these QTLs were located on chromosome 4 (9 QTLs) and the other QTLs on chromosome 1, 2, 3, 9 and 10. While under control conditions, 13 QTLs were detected, and most of them were located on

chromosome 4 (10 QTLs) and the other QTLs were on chromosome 2, 5 and 6 (Table 15).

Table 14 Summary of QTLs detected in the RIL population derived from B73×P39 evaluated under cold conditions (Pontevedra).

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to emergence	2.00	0-2	2.27	S_800641	8.2	-0.198
	4.05	56-59	10.06	S_65820374	30.2	0.462
	4.06	64-71	2.62	S_157612318	9.1	0.289
Chlorophyll	3.04	61-64	3.16	S_67695082	11.5	0.606
	3.06	94-99	2.81	S_181708617	10.4	-0.550
	4.07	87-96	2.63	S_177665741	9.7	0.632
	10.07	111-112	3.65	S_150253470	13.3	-0.617
Vigor	2.08	125-136	3.72	S_221805316	12.2	-0.239
	4.07	87-92	5.57	S_177665741	17.3	0.233
	9.00	0-2	2.76	S_984943	9.9	-0.170
Days to 2nd leaf	1.09	156-166	2.58	S_262609794	9	-0.219
	9.05	70-73	4.09	S_131410821	13.4	0.282
F_0	3.04	60-63	2.52	S_67695082	9.3	9.115
	4.07	86-96	3.18	S_177665741	11.7	10.285
F_m	4.07	90-93	5.40	S_178497329	16.9	41.375
F_v/F_m	4.07	90-92	6.55	S_178497329	19.3	0.125
F_v	4.07	90-93	5.88	S_178497329	18.2	29.624
F_m/F_0	4.07	90-93	2.47	S_178497329	8.2	0.112
Emergence	9.02	36-39	2.87	S_16858233	9.8	-0.056
Germination rate	9.02	26-30	2.72	S_11894246	9.3	-0.042

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

On chromosome 4, two QTLs were detected in the interval position 87-96 cM affecting vigor traits and 47-59 cM affecting germination traits (Table 14). These two QTLs were stable because they were detected before in the origin of Wisconsin. Both of them were found under both cold and control conditions, the same like the other origin and affecting the same traits. These QTLs also explained high proportion of the phenotypic variance. However, the QTL in bin

4.05 explained higher proportion of the phenotypic variance in this origin of kernels.

On chromosome 2, two QTL were detected in the interval positions 0-2 cM and 125-136 cM. These QTLs were detected only under cold conditions. The favorable allele was provided by the field corn parent B73 for days from sowing to emergence and early vigor. Both of these QTLs were detected before in the other kernel origin.

For leaf chlorophyll content and F_0, one QTL was detected on chromosome 3 located in the interval position 60-64 cM. This QTL was specific for low temperature conditions. An increase in both traits was due to the allelic contribution of the sweet corn parent (P39).

Table 15. Summary of QTLs detected in the RIL population derived from B73×P39 evaluated under control conditions (Pontevedra).

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to emergence	4.05	54-56	7.93	S_36356063	24.5	0.251
	5.02	27-44	2.74	S_10706932	9.7	0.136
Days to 2nd leaf	4.04	47-58	2.30	S_30247943	8.6	0.281
	6.02	14-15	3.45	S_88522572	12	-0.331
Vigor	4.07	87-92	4.20	S_177665741	13.6	0.644
Chlorophyll	2.06	82-90	2.28	S_175544792	7.8	0.158
	4.07	87-92	3.41	S_176002564	11.4	0.248
	4.08	103-106	2.47	S_187910369	8.2	0.173
F_0	4.07	90-92	4.46	S_178497329	13	-22.161
F_v/F_m	4.07	89-92	6.35	S_177665741	18.9	0.091
F_v	4.07	89-92	3.40	S_177665741	11.5	12.631
F_m/F_0	4.07	89-92	6.98	S_177665741	20.4	0.453
Germination rate	4.05	56-59	3.61	S_48241786	11.5	-0.079

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

QTLs for the RILs from B73×IL14h in the growth chamber

The QTL analysis of the RILs from B73×IL14h showed 19 QTLs under cold conditions (Table 16). Most of these QTLs were located on chromosome 6 (8 QTLs) and the other QTLs on chromosome 1, 3, and 5. And under control conditions, 18 QTLs were detected, most of them were located also on chromosome 3 (7 QTLs) and the others were distributed throughout all chromosomes except chromosome 4 and 8 (Table 17).

One QTL was detected on chromosome 5 in the interval position 66-72 cM affecting F_v, F_v/F_m and F_m/F_0. An increase in these fluorescence parameters was due to the allelic contribution of the sweet corn parent IL14h. This QTL was detected in the origin kernel of Wisconsin. In both origins, this QTL explained a high proportion of the phenotypic variance. However, this QTL was only detected for these traits under cold conditions in this origin of Pontevedra.

Table 16. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under cold conditions (Pontevedra).

Trait	Bin	Interval position	LOD score	Marker	R^2%	Additive effect
Days to 2nd leaf	3.05	68-73	2.39	S_150351409	7.9	0.145
	5.04	83-86	3.96	S_171780740	12.8	-0.195
Vigor	1.10	171-174	2.67	S_276934002	7.6	-0.065
F_0	6.08	110-111	8.06	S_169398019	23.2	-10.447
F_m	1.11	199-209	2.43	S_296021192	8.1	-9.990
	5.05	98-107	2.27	S_191253032	7.2	9.731
	6.04	28-32	2.93	S_106632270	9.1	17.459
	6.08	110-111	4.69	S_169398019	14.8	-15.035
F_v/F_m	5.04	68-71	9.29	S_81355643	27.7	0.035
	6.05	49-52	2.74	S_132463856	8.6	0.018
	6.07	81-103	2.28	S_162405290	8.1	0.018
F_v	3.07	111-114	2.98	S_202178692	9.7	-7.776
	5.03	66-69	5.34	S_73132746	17.4	10.534
	6.04	32-35	3.49	S_109944535	10.7	7.721
F_m/F_0	5.04	69-72	9.43	S_86151625	26.7	0.125

Table 16. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under cold conditions (Pontevedra).						
F_m/F_0	6.07	97-100	4.28	S_164583618	12.8	0.080
Emergence	1.07	119-129	3.10	S_210788543	9.6	0.072
Germination rate	1.10	174-181	3.14	S_281389433	10.5	-0.049
	6.04	37-40	2.37	S_112469120	7.3	-0.043

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Another important QTL was detected on chromosome 6 located in the interval position 110-111 cM. This QTL was detected only under cold conditions and affected F_m and F_0. This QTL explained high proportion of the phenotypic variance and had high LOD score. For this QTL, an increase in these traits was due to the allelic contribution of the field corn parent (B73).

Under cold and control conditions, one QTL was detected on chromosome 6 in the interval position 81-103 cM. This QTL affected F_0, F_v/F_m and F_m/F_0. An increase in F_v/F_m and F_m/F_0 was due to the allelic contribution of the sweet corn parent. By contrast, an increase in F_0 was due to the allelic contribution of the sweet corn parent (P39). This QTL was detected before for the other origin of kernels Wisconsin only under control conditions. However, it was detected under both cold and control conditions in this origin.

Table 17. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under control conditions (Pontevedra).						
Trait	**Bin**	**Interval position**	**LOD score**	**Marker**	**R^2%**	**Additive effect**
Days to emergence	1.08	131-143	2.59	S_229609244	8.3	-0.131
	5.07	114-116	2.42	S_204759286	7.9	0.186
Days to 2nd leaf	1.08	139-142	3.90	S_232724707	12.9	-0.337
	3.06	83-86	4.32	S_171444374	13.6	0.345
	7.03	62-65	2.47	S_128238230	8.3	0.282
Vigor	2.06	82-85	3.58	S_153170753	10.2	0.131
F_0	6.07	87-90	5.56	S_161793121	18.9	-1.345

Table 17. Summary of QTLs detected in the RIL population derived from B73×IL14h evaluated under control conditions (Pontevedra).						
F_m	3.09	147-153	3.12	S_224619474	10.2	-4.357
	6.01	0-9	2.78	S_28011906	9.3	3.935
F_v	3.09	131-146	2.74	S_217455785	8.1	-3.303
F_v	3.09	146-153	2.64	S_224619474	7.9	-3.295
F_m/F_0	3.03	33-36	3.75	S_8789496	12.1	-0.051
	3.09	131-134	3.69	S_216847626	11.8	-0.047
	9.02	33-36	2.38	S_15123546	7.9	0.041
Emergence	3.09	162-163	2.27	S_232488322	7.4	0.044
Stand	1.08	146-149	2.88	S_245456793	8.3	0.038
Germination rate	1.11	190-193	2.50	S_291718049	9.2	-0.065
	10.07	104-107	2.32	S_148622338	8.5	0.062

F_0: The ground fluorescence of dark adapted leaves, **F_m:** The maximal fluorescence yield, **F_v:** The difference **F_m - F_0**, **F_m/F_0:** This ratio of F_m and F_0 and **F_v/F_m:** The maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Discussion

V. Discussion

The study of the genetic control of *su1* viability is of great importance for using this mutant in breeding programs. Sweet corn breeders frequently use field corn to broaden the genetic base for the breeding programs and to improve the agronomic performance of sweet corn (Tracy 2001, Butrón et al. 2008). To do so they have to deal with the reduced viability of *su1* plants within some field corn genetic backgrounds (Tracy 1990, 2001). The *su1* viability is under genetic and environmental controls with significant additive effects that are probably due to multiple genes with minor contribution (Djemel et al. 2013). Both emergence and seedling vigor are the most critical traits affecting the viability of *su1* plants (Revilla et al. 2000a, 2006a). Tracy (2001) also reported that these traits are affected by genetic factors, both at planting and during seed production. Previous studies with maize have suggested that stand establishment (seedling emergence and growth rate) is under polygenic control with relatively low heritability (McConnell and Garner, 1979; Yousef and Juvik, 2001).

Phenotypic data

In the present study, individual analysis of variance (ANOVA) of the phenotypic data recorded in the growth chamber for both origins of kernel was performed. All traits showed considerable genetic variability under both cold and control conditions for both origins. Similarly, various researches found significant differences for these traits when investigating the genetic variability for germination and early plant vigor under controlled-environment or field conditions (McConnell and Gardner 1979; Mock and McNeill 1979; Revilla et al.

2000a).

In our study, the heritability for the RIL population B73×P39 was higher than the other RIL populations B73×IL14h. In general, the heritability for photosynthesis related traits (fluorescence parameters) was higher than for the other traits. By contrast, germination-related traits had the lowest heritability. According to Beavis (1998) and Utz et al. (2000), the power, accuracy, and precision of QTL mapping mainly depends on the sample size of the mapping population, the heritability of the trait, and the true number of segregating QTL.

Quantitative trait loci analysis

The different numbers and positions of QTL detected in different populations indicate that early vigor in maize is a complex quantitative trait and depends on the population, the environment and the developmental stage. Many studies have been conducted to map quantitative trait loci (QTL) of important traits in maize (Bernardo, 1999). In the present study, we have identified QTLs in the field and in a growth chamber under cold and control conditions; these analyses are discussed separately.

Field study

Several QTLs were identified for the viability-related traits of the mutant in the field for both RIL populations. In most of the QTLs detected in the field study, the favorable allele was inherited from the field corn parent (B73). Although traits at early developmental stages, as early vigor, are the most important for *su1* survival, other traits, as kernel yield, kernel moisture, plants height, ear

length, and kernel row number, can also play a role in the survival of the mutant (Revilla et al. 2000a).

One QTL was detected in bin 8.05 for both RIL populations. This QTL explained high proportion of the phenotypic variance and affected plant height and plant appearance in both RIL populations and ear height in B73×IL14h. The favorable allele was inherited from the field corn parent. In the same region, several authors have reported the presence of a gene called *vgt1*, involved in flowering time (Chardon et al., 2005). Moreover, Juvik et al., (2003) reported in the same region a major chromosomal region associated with emergence in the *sh2* population across 4 locations. And also this region was the only common region in the genomes of two populations (*shrunken2* and *sugary enhancer1*) containing a QTL that significantly influenced emergence. Also Djemel et al (2013) found a QTL in the same region related to germination of wild type kernels (non-*sugary* kernels with coleoptile).

Two QTLs were detected in the RIL population B73×P39 located in bin 6.05. These QTLs affected number of leaves and ear length. They explained 19.4% and 15.7% of the phenotypic variance, respectively. An increase in both traits was due to the allelic contribution of the field corn parent (B73). This agrees with Djemel et al. (2013) who reported a QTL in the same region that significantly affected ear length and coleoptile development. Also these authors reported that the P39 alleles had negative effects on ear length and germination of wild type kernels (number of non-*sugary* seeds with coleoptile).

Another important QTL was detected in the RIL population B73×P39 that was located in bin 5.04. This QTL explained the highest proportion of the phenotypic variance compared to the other QTLs (26.5% for number of kernel rows per ear). This QTL affected plant color and number of kernels rows per

ear. The favorable allele was inherited from the field corn parent (B73) for both traits. Djemel et al. (2013) reported that this QTL was strongly associated with observed number of *sugary* seeds and also was associated with other important traits, as early vigor and germination of the mutant (proportion of *sugary* seeds with roots). Also these authors reported that this region exhibited a net deviation from the random distribution of the P39 allele in the RIL population B73×P39. Interestingly, this region has been reported as a QTL with major effects on grain yield in other genetic backgrounds (Graham et al, 1997; Schaeffer et al. 2006; McMullen et al. 2009; Schon et al. 2010), so this region seems to be good candidate to contain genes involved in the regulation of maize production and, particularly, in *su1* fitness.

For ear height, one QTL was detected in bin 3.04 for the RIL population B73×IL14h. This QTL explained 18.8% of the phenotypic variance. The favorable allele was inherited from the field corn parent (B73). Djemel et al. (2013) reported in this bin one QTL associated with early vigor, ear weight and seed type.

Growth chamber trials

In our study, two NAM's biparental populations derived from B73×P39 and (B73×IL14H) and two origins of kernels (Wisconsin and Pontevedra) were evaluated in separate trials. The evaluation of a second origin of kernels is useful for estimating the stability of the QTL across kernel origins, which is a critical factor in controlled evaluations of kernel emergence and early development. QTLs associated to seedling stand establishment (seedling emergence and growth rate) were identified in all chromosomes in the two RIL

populations B73×P39 and B73×IL14H grown under cold and control conditions. There were QTLs detected under both cold and control conditions and other QTLs only under cold or control conditions.

One QTL was detected on chromosome 1 in bin 1.08 in both RIL populations B73×P39 and B73×IL14h and was associated with several traits, including dry weight. This QTL was not exclusive for cold tolerance because it was found under both cold and control conditions. It was found in both origins in the RIL population of B73×IL14h. This agrees with Fracheboud et al., (2002), who reported a QTL in this region that was important for the rate of photosynthesis independently of the growth temperature. This QTL showed segregation distortion with the *su1* allele. Therefore, this chromosomal region could contain one or several genes involved in efficiency of photosynthesis and biomass production. Moreover, this gene could be involved in *su1* viability by increasing the ability of the plant to synthetize biomass compensating the unfavorable effect of the defective mutant *su1*.

One QTL on chromosome 2 in the interval position 0-6 cM was detected only under cold conditions for B73×IL14h in the kernel origin of Wisconsin and in B73×P39 in the kernel origin of Pontevedra. This agrees with earlier investigations (Han, 1994), who reported a QTL in this region that was one of the three loci that had shown the largest associated effects for seedling emergence. This also agrees with other reports (Wang, 1997), where the allele from the sweet corn inbred Ia453 linked to this marker locus improved emergence over one cycle of pedigree selection in one sweet corn genetic background across two environments. Studying seedling stand establishment (seedling emergence and growth rate), Juvik et al. (2003) reported also an important QTL in this region in a *sh2* population; this region accounted for 13%

of the total variation in emergence and 21.4% of the variation in kernel starch content (mg/kernel). Therefore, this QTL can be related to emergence of defective kernels such as *su1* or *sh2*.

In the RIL population B73×P39, one QTL located in the interval position 125-139 cM, can be considered a major QTL because it was stable across origins of kernels and explained 25 % of the phenotypic variance for dry weight. This QTL was detected only under cold conditions, indicating that this QTL was specific to low temperature conditions, and also is associated with *su1* because the distribution of this QTL and *su1* were not independent (χ^2= 4.255). Fracheboud et al., (2002) reported, in the same region, a QTL for CO_2 fixation and the quantum yield of electron transport at photosystem II (ΦPSII) which appeared to be specific to low temperature. The authors suggested *ssu2* as a potential candidate gene associated to this QTL (Ribulose bisphosphate carboxylase small unit 2). Also at this locus, an interesting candidate gene is *hcf106* (Maize Genetics and Genomics Database, www.maizegdb.org) (Jompuk et al., 2005). The *hcf106* gene codes for the high chlorophyll fluorescence protein 106, which is a component of the Δph-dependent translocation pathway in the thylakoid membrane (Mori and Cline, 2001).

The QTL on chromosome 3 in bin 3.05 was detected in the RIL populations B73×P39 and B73×IL14h. It was found only under cold conditions, indicating that this QTL was specific to low temperature conditions. This QTL was stable for the RILs of B73×IL14h because it was found in both origins of kernels (Wisconsin and Pontevedra). However, in the RILs of B73×P39 was found only in the origin of Wisconsin. A major QTL for tolerance of photosynthesis to low temperature was reported in the same region by Fracheboud et al. (2002). The authors suggested that the responsible gene(s) at this QTL is involved in the

early development of the chloroplast at low temperature. An investigation of the maize genome database (www.agron.missouri.edu) indicated the presence of an interesting candidate gene for this QTL, *tha1* (thylakoid assembly protein 1). Interestingly, the *tha1* mutation induces a reduction of the efficiency levels of PSII and PSI (Barkan et al., 1995).

In the RIL population B73×P39, two genomic regions were detected under both cold and control conditions across the two origins of kernels. Both of them were located on chromosome 4, in bins 4.05 and 4.07. These genomic regions can be considered major QTLs because they explained very high proportion of the phenotypic variance 30.2 % and 38.9% of dry weight, respectively. Furthermore, they affected multiple traits, indicating pleiotropy or multiple genes in the area. This QTL was not exclusive for cold tolerance for most traits although its effect was more important under cold than under control conditions.

In the RILs from B73×P39, one QTL was detected on chromosome 1 in bin 1.09 (156-166 cM). This QTL was found in both origins of kernels only under cold conditions, indicating that this QTL was associated to cold tolerance. The favorable allele was provided by the field corn parent B73. On chromosome 3 in bin 3.03, one QTL was detected only under cold conditions in both RIL populations but only in the origin of Wisconsin. Interestingly, this QTL was associated with *su1.*

In the RIL population of B73×IL14h, one QTL was detected on chromosome 6 in the interval position 81-104 cM. This QTL was detected under cold and control condition for both origins of kernels. On chromosome 7 in the interval position 62-65 cM, one QTL was detected in the RIL population of B73×IL14h only under control conditions in both origins of kernels. On chromosome 5 in the interval position 63-72 cM, one QTL was detected in the RIL population B73×IL14h

under cold and control conditions in the kernel origin of Wisconsin. However, this QTL was found only under cold conditions in the other origin of kernels (Pontevedra).

The QTLs detected under both cold and control conditions are important for the respective trait independently of growth temperature. On the other side, QTLs detected only under cold conditions, are important for cold tolerance and they are more interesting for the specific objectives of our study, as the viability of *su1* is lower under cold conditions (Ordás et al., 2010).

Segregation distortion against *su1*

In the two populations of RILs involving sweet corn 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). The sweet corn inbred IL14h brought about higher reduction of the *su1* allele when crossed with B73 than the sweet corn inbred P39 when crossed with the same inbred. In the study of Djemel et al. (2012) using the same sweet inbred lines (P39 and IL14h) and non-sweet maize inbred lines (Oh43 and Tx303), reported the same distortion for the *su1* inbred lines. While in the two non-sweet inbreds, the frequency of the alternative allele had a similar frequency as the B73 allele. Therefore, the effect of selection was solely due to the *su1* allele. These results confirm the general observation that the viability of *su1* depends on the genetic interaction sweet × field corn parents (Revilla et al. 2000a, 2006a, 2010; Djemel et al. 2012).

Markers related to the viability of *su1*

In our study, a linkage block was observed in chromosome 4 for both RIL populations. In the RIL population B73×P39 all markers on chromosome 4 showed deviation from the expected frequencies for the B73 or alternative allele in the *su1* RIL. In the RIL population B73×IL14h not all markers on chromosome 4 showed deviations. The markers in bins 4.02, 4.08 and 4.10 displayed the expected Mendelian distribution. The explanation maybe that most of these markers detected on chromosome 4 were located outside the block on chromosome 4. Djemel et al. (2013) reported that the SNPs located on chromosome 4 that showed deviations were located from 39.2 cM to 77.2 cM in B73×IL14h. Galinat (1978) 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. The *Su1* was mapped in 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. Djemel et al. (2013) reported that in both populations B73×P39 and B73×IL14h, the highest deviation from random distribution was observed in chromosome 4. Also these authors reported that all markers on chromosome 4 in both RIL population B73×P39 and B73×IL14h showed deviations from the expected frequencies for the B73 or alternative allele in the *sugary* RIL.

Variation in the fitness value of genes can be caused by a closely linked gene (Butler 1977). Djemel et al. (2013) concluded that there are probably a lot of genes with minor effects affecting the diverse viability-related traits. In order to understand the genetic regulation of the *su1* allele, they examined the variation in the mutation fitness related to the genomic regions associated with *su1*.

In the RIL population B73×P39, three markers located out of chromosome 4 showed deviation from the expected frequencies for the B73 : alternative allele in the *su1* RIL. They were located on chromosome 2 in the interval position 125-139 (bins 2.08 and 2.09), bin 3.03 and bin 5.02. This marker on chromosome 2 was detected in both origins of kernels. While in the RIL population B73×IL14h, 10 markers showed deviation from the expected frequencies. They were located in bins 1.08, 1.10, 3.08, 5.04, 6.02, 6.05, 7.03, 8.08 and two very close markers in bin 6.04. The marker in bins 5.04 and 7.03 were detected in both origins of the kernels.

Previous reports have hypothesized that there are specific genes that are associated to *su1* viability (Revilla et al., 2006a, Djemel et al., 2012, 2013). This is true also for other defective mutants, such as *sh2* (Ordás et al., 2010). Some of these viability-related genes are detected in several genetic backgrounds, while others are background-specific, as previously reported by Revilla et al (2000a, 2006a, 2010) and Ordás et al. (2010). However, the previous reports have not been able to identify specific genes associated with *su1*-viability; actually, they have concluded that there were several genes with minor effects throughout the genome (Djemel et al., 2013). Our results confirm the hypothesis: there are specific genes involved in mutant viability and these genes depend not only on the mutant but also on the genetic background where the mutant is introduced.

These results have implications for both evolutionary studies and breeding programs. Indeed, mutations represent the raw material for evolution, and allow natural selection to generate new genotypes, depending on the fitness of the new mutation that might appear. In this process, the environment and the genetic background in which the mutation occurs limit the stability of a mutation and its propagation (Djemel et al., 2013). On the other side, breeding

sweet corn for stress conditions, particularly cold temperatures at sowing, is strongly limited by the reduced viability of *su1*. Sweet corn breeders frequently search sources of stress tolerance and agronomic performance in field corn genotypes for broadening the genetic base of sweet corn for breeding programs (Revilla et al. 2000b, 2006a, b). The QTLs identified in this study could be used by sweet corn breeders by combining the most favorable alleles associated to *sugary1* viability in breeding new genotypes from field × sweet corn crosses.

Conclusions

VI. Conclusions

The general conclusion is that there are specific genes involved in the viability of the sweet corn mutant *sugary1* and these genes depend on the genotype where the mutant is introduced and on the environment.

The specific conclusions are:

There are QTLs associated with vegetative and reproductive traits, particularly with germination and early vigor-related traits. Some of the QTLs were stable across environments and genotypes while others were specific of a particular genotype or associated solely to cold tolerance.

Some QTLs were in linkage disequilibrium with the maize gene *Sugary1*, suggesting that there could be genes involved in the viability of the mutant *sugary1*.

The QTLs identified in this study could be used by sweet corn breeders by combining the most favorable alleles associated to *sugary1* viability in breeding new genotypes from field × sweet corn crosses.

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Appendix

Appendix

Mean squares for genotypes and error from the analysis of variance of RIL population B73xP39 under both cold and control conditions (origin Pontevedra)				
Trait	Environment	Source of variation	d.f.	MS
Days to emergence	cold	Genotype	174	6.7896***
		Error	721	2.2343
	control	Genotype	173	2.4838***
		Error	744	0.7936
Second leaf	cold	Genotype	165	12.5057***
		Error	414	7.0625
	control	Genotype	172	11.0288***
		Error	686	3.4194
Vigor	cold	Genotype	173	2.6217***
		Error	684	0.7151
	control	Genotype	173	3.3689***
		Error	744	0.7796
Chlorophyll	cold	Genotype	173	22.0464***
		Error	646	3.6196
	control	Genotype	172	30.8558***
		Error	716	9.3917
F_0	cold	Genotype	173	6805.288***
		Error	640	1169.369
	control	Genotype	172	20834.404***
		Error	698	2201.683
F_m	cold	Genotype	173	48980.588***
		Error	640	3629.15
	control	Genotype	172	14851.692***
		Error	698	4201.915
F_v/F_m	cold	Genotype	173	0.3379***
		Error	640	0.0150
	control	Genotype	172	0.2103***
		Error	698	0.0128
F_v	cold	Genotype	173	21360.361***
		Error	640	1356.660
	control	Genotype	172	10551.646***
		Error	698	1634.305
F_m/F_0	cold	Genotype	151	1.6595***
		Error	493	0.5768
	control	Genotype	172	4.8034***
		Error	690	0.3603

Mean squares for genotypes and error from the analysis of variance of RIL population B73xP39 under both cold and control conditions (origin USA)				
Trait	Environment	Source of variation	d.f.	MS
Days to emergence	cold	Genotype	180	2.9337***
		Error	855	1.2631
	control	Genotype	181	2.2809***
		Error	860	0.9594
Second leaf	cold	Genotype	179	20.8989***
		Error	670	11.2566
	control	Genotype	179	12.8775***
		Error	718	5.9976
Vigor	cold	Genotype	180	3.5524***
		Error	846	0.5889
	control	Genotype	-	-
		Error	-	-
Chlorophyll	cold	Genotype	180	21.8979***
		Error	764	5.2430
	control	Genotype	180	25.8855***
		Error	763	4.1856
F_0	cold	Genotype	180	6323.912***
		Error	657	2365.324
	control	Genotype	181	4687.8644***
		Error	802	1058.978
F_m	cold	Genotype	181	42183.869***
		Error	782	5994.15
	control	Genotype	181	48941.164***
		Error	803	3520.39
F_v/F_m	cold	Genotype	181	0.3344***
		Error	783	0.0265
	control	Genotype	181	0.4653***
		Error	802	16.2105
F_v	cold	Genotype	167	7434.387***
		Error	569	1680.118
	control	Genotype	181	28429.225***
		Error	802	1711.911
F_m/F_0	cold	Genotype	167	1.8050***
		Error	569	0.4041
	control	Genotype	181	10.9341***
		Error	801	.6542

Mean squares for genotypes and error from the analysis of variance of RIL population B73xIL14h under both cold and control conditions (origin Pontevedra)				
Trait	Environment	Source of variation	d.f.	MS
Days to emergence	cold	Genotype	174	6.7896***
		Error	721	2.2343
	control	Genotype	204	2.6133***
		Error	885	0.7553
Second leaf	cold	Genotype	165	12.5057***
		Error	414	7.0625
	control	Genotype	204	9.2751***
		Error	802	2.7879
Vigor	cold	Genotype	173	2.6217***
		Error	684	0.7151
	control	Genotype	204	2.4031***
		Error	878	0.9451
Chlorophyll	cold	Genotype	173	22.0464***
		Error	646	3.6196
	control	Genotype	204	19.4124***
		Error	843	10.1611
F_0	cold	Genotype	173	6805.288***
		Error	640	1169.369
	control	Genotype	203	145.8759***
		Error	837	64.2992
F_m	cold	Genotype	173	48980.588***
		Error	640	3629.15
	control	Genotype	203	3449.3138***
		Error	837	1459.666
F_v/F_m	cold	Genotype	173	0.3379***
		Error	640	0.0150
	control	Genotype	203	0.0142***
		Error	837	0.0076
F_v	cold	Genotype	173	21360.36***1
		Error	640	1356.660
	control	Genotype	203	2875.8105***
		Error	837	1296.884
F_m/F_0	cold	Genotype	151	1.6594***
		Error	493	0.5768
	control	Genotype	203	0.6559***
		Error	835	0.3274

Mean squares for genotypes and error from the analysis of variance of RIL population B73xP39 under both cold and control conditions (origin USA)				
Trait	Environment	Source of variation	d.f.	MS
Days to emergence	cold	Genotype	214	3.0159***
		Error	958	1.1485
	control	Genotype	215	2.8310***
		Error	953	1.0848
Second leaf	cold	Genotype	211	19.6041***
		Error	743	9.3697
	control	Genotype	213	13.6439***
		Error	818	6.6532
Vigor	cold	Genotype	214	1.8529***
		Error	945	0.7202
	control	Genotype	-	-
		Error	-	-
Chlorophyll	cold	Genotype	213	28.0400***
		Error	893	12.2733
	control	Genotype	215	19.8202***
		Error	937	6.9194
F_0	cold	Genotype	214	9046.525***
		Error	901	3829.691
	control	Genotype	215	3470.7829***
		Error	928	494.429
F_m	cold	Genotype	214	14218.910***
		Error	901	7758.83
	control	Genotype	215	4466.4688***
		Error	928	1579.357
F_v/F_m	cold	Genotype	214	0.0568***
		Error	901	0.02216
	control	Genotype	215	0.0325***
		Error	928	0.0101
F_v	cold	Genotype	214	5535.432***
		Error	901	2670.555
	control	Genotype	215	2883.8071***
		Error	928	872.783
F_m/F_0	cold	Genotype	214	1.0542***
		Error	901	0.4021
	control	Genotype	215	1.5977***
		Error	928	0.3633