Mol Breeding (2019) 39: 3
https://doi.org/10.1007/s11032-018-0911-1

Linkage disequilibrium between fitness QTLs and the *sugary1* allele of maize

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Received: 14 February 2018 / Accepted: 12 December 2018 / Published online: 28 December 2018

Abstract Understanding how biological systems evolve across changing conditions has been a crucial focus of research. Mutations change the genetic context in which genes are expressed and yet the mechanisms underlying mutation fitness are still unclear. We use the sweet corn mutant *sugary1* (*su1*) as a model for understanding the genetic regulation of mutant fitness, focusing on the mutant × genotype interaction across diverse environments. In a previous work, we identified quantitative trait loci (QTLs) affecting fitness in a mapping population of recombinant inbred lines (RILs) derived from a cross between field corn (B73) × sweet corn (P39 or IL14h) parents; however, the epistatic effects of these QTLs on *su1* fitness were not investigated. In the present study, we estimated fitness for two seed production environments. Viability of *su1* is under genetic and environmental controls, regulated by multiple genes with minor contributions, and these genes depend on the genotype into which the mutation is introduced and on the environment. Some QTLs were in linkage disequilibrium with the maize gene *Su1* and had epistatic effects on *su1* fitness. These QTLs could be used by sweet corn breeders by combining the most favorable alleles associated with *su1* viability in breeding new genotypes from field × sweet corn crosses. These results also have implications for mutagenesis breeding or genome editing because the epistatic effects of the target genome on the new alleles generated by these techniques could affect the success of the breeding program.

Keywords *Zea mays* L. · Sweet corn · Epistasis · *sugary1*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11032-018-0911-1) contains supplementary material, which is available to authorized users.

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Introduction

Mutations (new alleles) play a key role in selection and evolution, and the fitness of a mutant depends on the genetic background. Therefore, the interaction of genetic background with new alleles determines the probability of allele fixation. Understanding the genetic basis of fitness of new alleles is of interest in terms of increasing the efficiency of incorporation of new alleles, whether natural or created by new technologies into diverse germplasm. In maize, the recessive recurrent mutant

sugary1 (*su1*) is located on the short arm of chromosome 4 (Bin 4.05). This mutation produces high levels of water-soluble polysaccharides and decreases starch levels (Tracy 1994; Tracy et al. 2006). As a defective mutant, the fitness of *su1* is lower than that of the wild allele *Su1*, as shown by the directional natural selection reported against *su1* (Revilla et al. 2006a). In general, the fitness of the su1 mutant depends on genotype × gene interaction and on environmental effects (Revilla et al. 2000, 2006b, 2010; Ordás et al. 2010).

Selection against *su1* is expressed firstly by reduced viability (germination and early vigor) and then by reduced fertility (grain formation) (Martins and Da Silva 1998; Revilla et al. 2000; Tracy 2001; Gad and Juvik 2002; Juvik et al. 2003; Revilla et al. 2006b; Ordás et al. 2010; Cisneros-Lopez et al. 2010; Zhang et al. 2011; Viesselmann et al. 2014). Accordingly, Djemel et al. (2013) concluded that some traits, such as stand, silking date, plant height, and ear length, could have significant effects on mutant viability. Genetic control of *su1* fitness has been studied by Djemel et al. (2012, 2013). These authors also reported that *su1* fitness is under the genetic control of multiple genes with minor contributions, with additive, dominant, and epistatic effects that depend on the genetic background and environmental conditions. Allam et al. (2016) studied quantitative trait loci (QTLs) involved in cold tolerance using two populations of recombinant inbred lines (RILs) involving sweet corn inbred lines developed from B73 × P39 and B73 × IL14h. Large numbers of QTLs related to germination (QTLs in bin 4.05 and in bin 4.07) and early development-traits (QTLs in bin 5.03 and bin 6.07) were detected, with the favorable allele provided by the sweet corn inbred line; in addition, a large linkage block was detected around *su1* locus in both RILs populations. These authors suggested that all these QTLs could contribute to increase the performance of sweet corn inbred lines (Allam et al. 2016). Butler (1977) also concluded that the mutants' fitness value, with both excesses and deficiencies, was probably influenced by their linkage with other genes.

The objective of this work reported here was to investigate the relationship between those single nucleotide polymorphisms (SNPs) and *su1* fitness and the linkage disequilibrium between the *sugary1* mutant and QTLs for mutant fitness. We chose RILs segregating for the *Su1* and *su1* alleles in order to specifically study the epistatic relationship between background alleles and the *Su1* and *su1* alleles. These results could have implications for mutagenesis breeding and genome editing.

Materials and methods

Plant materials and molecular characterization

We used 179 RILs released from B73 × P39 and 213 from B73 × IL14h. B73 is a homozygous wild type (*Su1Su1*), while P39 and Il14h are homozygous for the mutant allele *su1*. The 179 RILs released from B73 × P39 were classified as 144 *Su1Su1* and 35 *su1su1*, based on their phenotype for the *Sugary1* locus; the 213 RILs released from B73 × IL14h were classified as 198 *Su1-Su1* and 15 *su1su1*. The molecular characterization of these RILs was made using 1478 SNPs in the Maize Diversity Project (www.panzea.org), and we used the nested association mapping (NAM) genetic map (McMullen et al. 2009). In order to have two seed production environments, seed of all RILs was produced at Madison, Wisconsin (USA) in 2011 and in Pontevedra (Spain) in 2012. *Su1* is on chromosome 4 between positions 53.7 and 55.2 cM and is flanked by the SNPs PZA01751.2 and PZA00445.22 (www.maizesequence.org; verified 9 March 2012). Based on kernel phenotype, inbreds were classified as *Su1* or *su1*.

Experimental design

The 392 RILs were multiplied in the field in Madison, Wisconsin (USA) (43.09° N, 89.43° W), a location characterized by a continental climate, in 2011, and in Pontevedra (Spain) (42.43° N, 8.64° W), a location with humid Mediterranean climate in the northwest of Spain, in 2012, along with both original hybrids (B73 × P39 and B73 × IL14h, respectively) and the three inbred parents (Allam et al. 2016). Genotypes were evaluated under cold (10 °C/14 °C, dark [10 h] and light [14 h] conditions, respectively) and control (20 °C/25 °C, dark [10 h] and light [14 h] conditions, respectively) conditions in a growth chamber (40 m^3) equipped with fluorescent lamps (photosynthetic photon flux 228 μmol m^{-2} s^{-1}). RILs, parental lines, and hybrids were evaluated using a randomized complete block design with six repetitions and one kernel per repetition. Maize kernels were planted in seedbeds filled with sterilized peat (Gramoflor GmbH & Co.KG, Vechta,

Germany). Experiments were watered after planting and thereafter trials were watered two times per week.

In the growth chamber we evaluated:

- Days to seedling emergence
- Emergence index [$100 \times \Sigma Gt/Dt$ where Gt is the number of emerged seedlings on day t and Dt is the number of days from sowing to day t (for the total of six replicates)]
- Days to second leaf: days from sowing to emergence of the ligule of the second leaf of each plant
- Early vigor rated at the end of the heterotrophic stage (visual rating (1–9), where 1 indicates small plants with light color and 9 indicates large plants with dark color)
- Chlorophyll content (relative leaf chlorophyll content, measured using the hand-held CCM-2000 Chlorophyll Content Meter; Opti-Sciences, Tyngsboro, MA, USA)
- The fluorescence-related parameters F_0 (initial fluorescence), F_m (maximum fluorescence), F_v (variable fluorescence = $F_m - F_0$), and F_v/F_m were determined on leaves maintained under dark conditions by using a plastic forceps for at least 20 min and a hand fluorometer (Opti-Sciences)
- Stand (% of surviving plants at the end of the trial from the emerged seedlings)
- Dry weight (dry weight of each plant (in grams) after drying at 80 °C for 1 week) of juvenile plants harvested when most of the plants were at the three-leaf stage.

In all experiments, fitness data (proportion of emergence, early vigor, chlorophyll content, and fluorescent-related parameters) of all plots were recorded on the same day (when most of the plants were at the three-leaf stage). For days to emergence and to formation of the second leaf, data were recorded as each plant reached the corresponding stage. The evaluation of two seed production environments is useful for estimating the stability of QTLs across production environments, a critical factor in evaluations of seedling emergence and development.

Segregation and linkage disequilibrium analyses

Allam et al. (2016) identified QTLs associated with germination and early growth related traits in all chromosomes for these RILs grown under cold and control conditions. The RILs were classified into two main types (*su1su1* or *Su1Su1*) based on the observed kernel phenotype. At each locus, the allele coming from the field corn parent (B73) was denoted '*A*" and the allele coming from the sweet corn parent (P39 or IL14h) was denoted '*C*".

In the RILs with the *Su1Su1* genotype, we would expect the same number of RILs with the *A* and with the *C* allele for each marker if the marker was not linked to the locus *Su1*; and this is also true for the *su1su1* RILs. This expectation assumed that the selection did not act during the development of the RILs and, therefore, the A allele had the same possibility of fixation as the *C* allele for all markers. We tested for deviation (segregation distortion) from the expected allele frequencies (*A* vs. *C*) under no selection (1:1) within each type of lines (*Su1Su1* and *su1su1*) with a chi-square goodness-of-fit test ($P < 0.05$) for each marker.

Epistasis for fitness

For each trait, an analysis of variance (ANOVA) was performed for each SNP with significant segregation distortion with the *Sugary1* locus. Sums of squares were partitioned into sources of variation attributable to the gene sugary (*Su1* or *su1*), the marker alleles (*A* or *C*), sugary × marker interaction, and error. A significant interaction at a particular marker implies that *AA Su1Su1–AA su1su1* is significantly different from *CC Su1Su1–CC su1su1*; that is, the effect of the *su1* allele depends on the specific marker analyzed (epistatic effect). When the sugary × marker interaction was significant, the means of *Su1Su1AA*, *su1su1AA*, *Su1Su1CC*, and *su1su1CC* was compared by a *t* test. The PROC GLM (SAS Institute Inc. 2009) was used for the analyses of variance.

Results

Segregation distortion analysis

In both populations, maximum distortion was found on chromosome 4, both for marker S_48241786 ($\chi^2 = 90.35$) located in 54 cM for B73 × P39, and marker S_13572749 ($\chi^2 = 20.88$) located in 58 cM for B73 × IL14h. A large linkage block was observed on chromosome 4 for both RIL populations. Of the SNPs located on chromosome 4, 51% (B73 × P39) and 28% (B73 × IL14h) exhibited significant deviation from expected

Mendelian distributions. The length of the linkage group on chromosome 4 was larger for P39 (from 29 to 93 cM) than for IL14h (from 34 to 76 cM).

To check for the presence of chromosomal regions that interact with *su1*, we examined the degree and direction of bias in allelic frequencies for these SNPs on chromosome 4 and found that the allele frequencies of these SNPs did not fit the expected 1:1 ratio. The distortion ratio bias was highly directional. All distorted frequencies had an excess of *Su1* SNPs and a deficit of *su1* homozygous SNPs.

The SNPs showing distorted segregation were unevenly distributed over chromosomes and were located in several regions along chromosomes that varied from 2 to 32 cM in size. For B73 × P39, all SNPs were skewed towards the *A* allele (coming from B73) in *Su1* RILs and towards the *C* allele (coming from P39) in *su1* RILs (Electronic Supplementary Material [ESM] Fig. 1). However, in B73 × IL14h RILs, the SNP located on chromosome 3 was skewed towards the *A* allele in *su1* RILs, and three SNPs located on chromosome 5 were skewed towards the *C* allele in *Su1* RILs (ESM Fig. 2).

The degree of distortion varied among linkage groups. In addition to chromosome 4, we identified three regions containing multiple distorted SNPs for B73 × P39 (Table 1). These regions were on three different linkage groups, and most were defined by SNPs with an excess of *Su1* genotypes. Two SNPs were located on the same genomic regions on chromosome 2 (116–136 cM), one SNP was located on chromosome 3 (33–45 cM), and one SNP was located on chromosome 5 (16–47 cM). Nine distorted SNPs were detected for B73 × IL14h (ESM Fig. 2) in six genomic regions: region 116–118 cM on chromosome 3; region 61–81 cM on chromosome 7; regions 120–124 cM; regions 67–70 and 98–99 cM on chromosome 5; and two very close regions on chromosome 6 (14–22 and 24–41 cM).

Epistasis

Among the B73 × P39 RILs produced in Wisconsin, those RILs with allele *C* at the S_22,390,507 marker and the *su1* allele had higher chlorophyll content than RILs with allele *C* and the *Su1* allele. In contrast, those RILs with allele *A* at the same marker and allele *su1* had lower chlorophyll content than RILs with allele *A* and allele *Su1*. Thus, there was a favorable epistatic effect (1.02 ± 0.48) (Table 2). The same tendency was observed with seeds produced in Pontevedra with $e = 1.08 \pm 0.44$ (Table 3).

Table 1 Single-nucleotide polymorphisms showing segregation distortion in the recombinant inbred lines from B73 × P39 and B73 × IL14h for C the allele (coming from either maize parent P39 or IL14h) versus the A allele (from B73) outside chromosome 4

Marker	Bin	Genetic position (cM)	Physical position (cp)	χ^2	RIL population
S_ 221,805,316	2.08	131	221,805,316	4.733*	B73 × P39
S_ 22,390,507	2.09	134	223,905,071	4.255*	B73 × P39
S_ 11,980,490	3.03	37	11,980,490	7.329**	B73 × P39
S_ 10,706,932	5.02	33	10,706,932	5.125*	B73 × P39
S_ 2,102,534	3.08	118	210,253,465	4.571*	B73 × IL14h
S_ 8,615,162	5.04	68	86,151,625	4.709*	B73 × IL14h
S_ 1,338,249	5.04	70	133,824,940	5.380*	B73 × IL14h
S_1912530	5.05	99	19,125,302	4.212*	B73 × IL14h
S_ 8,971,453	6.02	15	89,714,530	5.451*	B73 × IL14h
S_ 1,066,322	6.04	30	106,632,270	7.60**	B73 × IL14h
S_ 1,099,445	6.04	32	109,944,535	9.679**	B73 × IL14h
S_ 1,124,691	6.04	37	112,469,120	8.489*	B73 × IL14h
S_ 1,282,382	7.03	62	128,238,230	5.191*	B73 × IL14h

RIL, Recombinant inbred line

Physical (base position on chromosomes) and genetic (centiMorgan [cM] values) position are given for each marker. Molecular characterization data with single-nucleotide polymorphisms (SNPs) is published in the Maize Diversity Project (www.panzea.org)

*, ** Significant at $P<0.05$ and 0.01, respectively

Table 2 Means of six traits and standard error for the single-nucleotide polymorphisms that showed segregation distortion for alleles *A* (from B73) and *C* (from P39) and the *Su1* and *su1* alleles in the recombinant inbred line population B73 × P39 of Wisconsin origin and under cold conditions

Marker (bin)	Trait	Mean ± standard error				
		AA Su1Su1	*AA su1su1*	*CC Su1Su1*	*CC su1su1*	Difference[a]
S_221,805,316 (2.08)	Days to emergence	7.31 ± 0.06	7.18 ± 0.07	8.1 ± 0.23	8.06 ± 0.15	0.09 ± 0.29
	Chlorophyll	3.74 ± 0.16	3.43 ± 0.19	4.04 ± 0.28	4.47 ± 0.3	0.74 ± 0.48
	Vigor	3.67 ± 0.06	3.54 ± 0.07	3.67 ± 0.13	3.5 ± 0.1	−0.04 ± 0.19
	Days to second leaf	22.52 ± 0.22	21.84 ± 0.24	23.19 ± 0.56	22.89 ± 0.38	0.38 ± 0.75
	Dry weight	0.12 ± 0.004	0.11 ± 0.004	0.11 ± 0.01	0.09 ± 0.005	−0.01 ± 0.01
	F_v/F_m[b]	0.42 ± 0.02	0.38 ± 0.02	0.53 ± 0.03	0.47 ± 0.03	−0.02 ± 0.05
S_22,390,507 (2.09)	Days to emergence	7.3 ± 0.06	7.24 ± 0.07	8.16 ± 0.2	8.01 ± 0.16	−0.09 ± 0.27
	Chlorophyll	3.83 ± 0.16	3.3 ± 0.19	4.02 ± 0.26	4.51 ± 0.32	1.02 ± 0.48*
	Vigor	3.68 ± 0.06	3.48 ± 0.07	3.48 ± 0.13	3.61 ± 0.1	0.33 ± 0.19
	Days to second leaf	22.45 ± 0.22	22.11 ± 0.26	23.47 ± 0.53	22.67 ± 0.39	−0.46 ± 0.74
	Dry weight	0.12 ± 0.004	0.11 ± 0.004	0.1 ± 0.01	0.09 ± 0.01	0 ± 0.02
	F_v/F_m	0.43 ± 0.02	0.38 ± 0.02	0.51 ± 0.03	0.48 ± 0.03	0.02 ± 0.05
S_11,980,490 (3.03)	Days to emergence	7.33 ± 0.06	7.18 ± 0.07	8.2 ± 0.23	8.02 ± 0.15	−0.03 ± 0.29
	Chlorophyll	3.1 ± 0.14	4.17 ± 0.2	3.9 ± 0.3	4.11 ± 0.26	−0.86 ± 0.47
	Vigor	3.49 ± 0.06	3.72 ± 0.06	3.71 ± 0.16	3.45 ± 0.09	−0.49 ± 0.19*
	Days to second leaf	22.47 ± 0.22	22.09 ± 0.25	24.07 ± 0.56	22.83 ± 0.4	−0.86 ± 0.76
	Dry weight	0.11 ± 0.004	0.13 ± 0.005	0.11 ± 0.01	0.09 ± 0.005	−0.04 ± 0.01*
	F_v/F_m	0.36 ± 0.02	0.46 ± 0.02	0.52 ± 0.04	0.46 ± 0.03	−0.16 ± 0.06*
S_10,706,932 (5.02)	Days to emergence	7.21 ± 0.06	7.31 ± 0.07	7.72 ± 0.23	8.23 ± 0.14	0.41 ± 0.28
	Chlorophyll	3.51 ± 0.16	3.77 ± 0.18	4.48 ± 0.32	4.26 ± 0.27	−0.48 ± 0.48
	Vigor	3.57 ± 0.06	3.69 ± 0.06	3.95 ± 0.11	3.47 ± 0.09	−0.6 ± 0.17*
	Days to second leaf	22.55 ± 0.24	22.01 ± 0.23	22.89 ± 0.52	23.08 ± 0.38	0.73 ± 0.72
	Dry weight	0.12 ± 0.004	0.12 ± 0.004	0.11 ± 0.01	0.09 ± 0.01	−0.02 ± 0.02
	F_v/F_m	0.39 ± 0.02	0.44 ± 0.02	0.51 ± 0.03	0.5 ± 0.02	−0.06 ± 0.05

su1 (*sugary1*), Recessive recurrent mutant; *SU1 sugary1* wild-type allele; *A* allele coming from the field corn parent (B73); *C* allele coming from the sweet corn parent (P39 or IL14h)'

[a] Difference was calculated as (*AASu1Su1-AAsu1su1*) − (*CCSu1Su1-CCsu1su1*)

[b] F_v, Variable fluorescence; F_m, maximum fluorescence

* Significant at $P<0.05$

Epistatic effects between allelic combinations for B73 × P39 RILs were significant for five of 24 cases for the seed from Wisconsin and for six of 20 cases for seed produced at Pontevedra (Table 4). The proportion of significant epistasis was greater in the B73 × IL14h RILs than for B73 × P39 RILs (Table 5).

For B73 × IL14h, all markers exhibited significant epistasis with *sugary1* for at least one trait. With the exception of S_1,282,382 (Wisconsin), all significant epistasis effects were favorable for vigor-related traits (chlorophyll content, seedling vigor, dry weight, and the quantum efficiency of photosystem II (F_v/F_m) and negative for germination-related traits (days to emergence and days to second leaf).

For all SNPs, the *C* alleles (from IL14h) showed a positive epistatic effect with the *su1* locus, increasing the fitness for all traits, except for the S_1,282,382 as the *A* allele (from B73) was more favorable for dry weight and F_v/Fm. For SNPs S_8,615,162 and S_1,338,249, RILs combining the *A* allele plus *Su1* were more fit than expected for all traits for seed from Wisconsin. However, RILs combining the *A* allele plus *Su1* performed worse when they were produced in Pontevedra.

Table 3 Means of six traits and standard error for the single-nucleotide polymorphisms that showed segregation distortion for alleles *A* (from B73) and *C* (from P39) and the *Su1* and *su1* alleles in the recombinant inbred line population B73 × P39 of Pontevedra origin and under cold conditions

Marker	Trait	Mean ± standard error				
		AA Su1Su1	*AA su1su1*	*CC Su1Su1*	*CC su1su1*	Difference
S_221,805,316 (2.08)	Days to emergence	9.16 ± 0.09	9.19 ± 0.1	10.98 ± 0.23	10.74 ± 0.14	−0.27 ± 0.3
	Days to second leaf	29.76 ± 0.17	29.9 ± 0.2	29.81 ± 0.34	31.06 ± 0.19	1.11 ± 0.47*
	Vigor	3.69 ± 0.06	3.5 ± 0.06	3.64 ± 0.11	3.3 ± 0.09	−0.15 ± 0.17
	Chlorophyll	4.17 ± 0.16	4.09 ± 0.18	4.26 ± 0.26	4.37 ± 0.25	0.19 ± 0.43
	F_v/F_m	0.49 ± 0.02	0.46 ± 0.02	0.61 ± 0.02	0.52 ± 0.02	−0.06 ± 0.04
S_22,390,507 (2.09)	Days to emergence	9.15 ± 0.09	9.21 ± 0.1	11 ± 0.21	10.71 ± 0.15	−0.35 ± 0.29
	Days to second leaf	29.77 ± 0.17	29.85 ± 0.2	30.07 ± 0.28	30.89 ± 0.22	0.74 ± 0.44
	Vigor	3.69 ± 0.06	3.45 ± 0.06	3.37 ± 0.11	3.45 ± 0.1	0.32 ± 0.17
	Chlorophyll	4.19 ± 0.16	4.03 ± 0.19	3.78 ± 0.25	4.7 ± 0.26	1.08 ± 0.44*
	F_v/F_m	0.49 ± 0.02	0.45 ± 0.02	0.56 ± 0.03	0.55 ± 0.02	0.03 ± 0.05
S_11,980,490 (3.03)	Days to emergence	9.29 ± 0.09	9.05 ± 0.09	10.83 ± 0.21	10.82 ± 0.15	0.23 ± 0.29
	Days to second leaf	30.02 ± 0.16	29.56 ± 0.2	30.7 ± 0.36	30.59 ± 0.22	0.35 ± 0.49
	Vigor	3.46 ± 0.06	3.8 ± 0.06	3.52 ± 0.12	3.34 ± 0.09	−0.52 ± 0.17*
	Chlorophyll	3.64 ± 0.14	4.72 ± 0.19	3.7 ± 0.29	4.55 ± 0.25	−0.23 ± 0.45
	F_v/F_m	0.43 ± 0.02	0.52 ± 0.02	0.56 ± 0.03	0.54 ± 0.02	−0.11 ± 0.05*
S_10,706,932 (5.02)	Days to emergence	9.09 ± 0.09	9.28 ± 0.1	10.56 ± 0.2	10.92 ± 0.15	0.17 ± 0.28
	Days to second leaf	29.71 ± 0.19	29.73 ± 0.18	31.03 ± 0.35	30.48 ± 0.19	−0.57 ± 0.48
	Vigor	3.71 ± 0.06	3.5 ± 0.06	3.53 ± 0.14	3.39 ± 0.08	0.07 ± 0.18
	Chlorophyll	4.01 ± 0.15	4.31 ± 0.18	4.81 ± 0.27	4.18 ± 0.23	−0.93 ± 0.43*
	F_v/F_m	0.47 ± 0.02	0.51 ± 0.02	0.6 ± 0.02	0.55 ± 0.02	−0.09 ± 0.04*

[a] Difference was calculated as (*AASu1Su1-AAsu1su1*) − (*CCSu1Su1-CCsu1su1*)

* Significant at $P<0.05$

Two genomic regions were detected on chromosome 6, namely, interval 14–22 cM and 24–41 cM. The four sweet corn SNPs (S_8,971,453, S_1,066,322, S_1,099,445, and S_1,124,691) were favored by the *su1* allele, and the *su1* RILs were more fit than expected when the allele was from IL14h for these four markers. These SNPs were found in significant linkage disequilibrium. For vigor-related traits, the RILs combining the *A* allele and *Su1* were more fit than expected for the SNPs S_1,066,322, S_1,099,445, and S_1,124,691; however, they were worse than expected for germination-related traits.

Significant linkage disequilibrium also was found for S_1,282,382, with a chi-square value of 5.191 ($P < 0.05$). Negative epistatic effects were observed for the quantum efficiency of photosystem II (ΦPSII) for the seeds of Wisconsin. However, a positive epistatic effect was detected for seeds of Pontevedra.

Discussion

Our results indicate that there are specific SNPs that affect the viability of *su1* in two RILs populations. We have also detected linkage disequilibrium between some SNPs and the *Sugary1* locus and epistasis for fitness, based on the methods of Felsenstein (1965) and Otto and Feldman (1997). These results are in agreement with those from previous studies indicating that there are specific genes associated with *su1* viability (Revilla et al. 2006a; Djemel et al. 2012, 2013), as well as for other defective mutants, such as *sh2* (Ordás et al. 2010). Our results also support those reported previously indicating that some of these viability-related genes have been detected in several genetic backgrounds, while others are background specific (Revilla et al. 2000, 2006a, 2010; Ordás et al. 2010). Although the authors of earlier reports concluded that there were several genes

Table 4 Means of six traits and standard error for the single-nucleotide polymorphisms that showed segregation distortion for alleles *A* (from B73) and *C* (from IL14h) and the *Su1* and *su1* alleles in the recombinant inbred line population B73 × IL14h of Wisconsin origina and under cold conditions

Marker (bin)	Trait	Mean ± standard error				
		AA Su1Su1	*AA su1su1*	*CC Su1Su1*	*CC su1su1*	Difference
S_2,102,534 (3.08)	Days to emergence	7.76 ± 0.06	7.7 ± 0.07	7.97 ± 0.21	7.67 ± 0.26	−0.24 ± 0.35
	Chlorophyll	6 ± 0.19	6.12 ± 0.23	5.75 ± 0.58	6.16 ± 1.13	0.29 ± 1.3
	Vigor	4.33 ± 0.05	4.37 ± 0.05	4.11 ± 0.17	4.18 ± 0.26	0.03 ± 0.32
	Days to second leaf	23.41 ± 0.19	23.69 ± 0.22	23.43 ± 0.44	23.7 ± 0.67	−0.01 ± 0.85
	Dry weight	0.11 ± 0.003	0.12 ± 0.004	0.12 ± 0.01	0.11 ± 0.02	−0.02 ± 0.02
	F_v/F_m	0.48 ± 0.009	0.47 ± 0.01	0.5 ± 0.03	0.49 ± 0.08	0 ± 0.09
S_8,615,162 (5.04)	Days to emergence	7.66 ± 0.06	7.82 ± 0.07	11.25 ± 0.75	7.72 ± 0.15	−3.69 ± 0.77*
	Chlorophyll	6.45 ± 0.26	5.77 ± 0.18	4.57 ± 2.13	5.88 ± 0.54	1.99 ± 2.22
	Vigor	4.36 ± 0.06	4.32 ± 0.05	3.67 ± 0.33	4.14 ± 0.15	0.51 ± 0.37
	Days to second leaf	23.4 ± 0.23	23.72 ± 0.19	–	23.42 ± 0.38	–
	Dry weight	0.12 ± 0.004	0.11 ± 0.003	0.04 ± 0.01	0.12 ± 0.01	0.09 ± 0.02*
	F_v/F_m	0.49 ± 0.01	0.46 ± 0.01	0.36 ± 0.11	0.51 ± 0.03	0.18 ± 0.11
S_1,338,249 (5.04)	Days to emergence	7.68 ± 0.07	7.82 ± 0.06	11.25 ± 0.75	7.72 ± 0.15	−3.67 ± 0.77*
	Chlorophyll	6.49 ± 0.26	5.79 ± 0.18	4.57 ± 2.13	5.88 ± 0.54	2.01 ± 2.22
	Vigor	4.35 ± 0.06	4.33 ± 0.05	3.67 ± 0.33	4.14 ± 0.15	0.49 ± 0.37
	Days to second leaf	23.46 ± 0.23	23.68 ± 0.19	–	23.42 ± 0.38	–
	Dry weight	0.12 ± 0.004	0.11 ± 0.003	0.04 ± 0.01	0.12 ± 0.01	0.09 ± 0.02*
	F_v/F_m	0.48 ± 0.01	0.47 ± 0.01	0.36 ± 0.11	0.51 ± 0.03	0.16 ± 0.11
S_1912530 (5.05)	Days to emergence	7.71 ± 0.07	7.76 ± 0.06	8.6 ± 0.79	7.8 ± 0.18	−0.85 ± 0.82
	Chlorophyll	6.36 ± 0.23	5.78 ± 0.18	6.3 ± 2.36	5.61 ± 0.54	−0.11 ± 2.44
	Vigor	4.36 ± 0.05	4.32 ± 0.05	3.67 ± 0.24	4.13 ± 0.17	0.5 ± 0.3
	Days to second leaf	23.98 ± 0.22	23.19 ± 0.18	22.5 ± 1.02	23.64 ± 0.44	1.93 ± 1.15
	Dry weight	0.11 ± 0.003	0.11 ± 0.003	0.06 ± 0.01	0.12 ± 0.01	0.06 ± 0.01*
	F_v/F_m	0.48 ± 0.009	0.47 ± 0.01	0.38 ± 0.05	0.53 ± 0.03	0.16 ± 0.06*
S_8,971,453 (6.02)	Days to emergence	7.7 ± 0.06	7.79 ± 0.07	10.38 ± 0.56	7.67 ± 0.16	−2.81 ± 0.59*
	Chlorophyll	6.22 ± 0.21	5.69 ± 0.18	5.1 ± 0.96	5.85 ± 0.64	1.28 ± 1.19
	Vigor	4.39 ± 0.05	4.25 ± 0.05	3.13 ± 0.4	4.11 ± 0.15	1.12 ± 0.43*
	Days to second leaf	23.8 ± 0.19	23.28 ± 0.22	27.67 ± 0.67	23.49 ± 0.39	−3.66 ± 0.83*
	Dry weight	0.11 ± 0.003	0.11 ± 0.003	0.04 ± 0.01	0.11 ± 0.01	0.07 ± 0.01*
	F_v/F_m	0.48 ± 0.01	0.45 ± 0.01	0.4 ± 0.05	0.49 ± 0.03	0.12 ± 0.06*
S_1,066,322 (6.04)	Days to emergence	7.76 ± 0.06	7.66 ± 0.06	9.58 ± 0.54	7.58 ± 0.15	−1.09 ± 0.57
	Chlorophyll	6.25 ± 0.21	5.9 ± 0.2	4.39 ± 0.79	6.1 ± 0.6	2.06 ± 1.03*
	Vigor	4.37 ± 0.04	4.31 ± 0.06	3.25 ± 0.37	4.3 ± 0.15	1.11 ± 0.41*
	Days to second leaf	23.99 ± 0.18	23.02 ± 0.22	27.2 ± 1.2	23.12 ± 0.36	−3.11 ± 1.28*
	Dry weight	0.11 ± 0.003	0.11 ± 0.004	0.05 ± 0.01	0.13 ± 0.01	0.08 ± 0.02*
	F_v/F_m	0.48 ± 0.01	0.46 ± 0.01	0.41 ± 0.05	0.52 ± 0.03	0.13 ± 0.06*
S_1,099,445 (6.04)	Days to emergence	7.8 ± 0.07	7.6 ± 0.06	9.63 ± 0.78	7.59 ± 0.16	−1.84 ± 0.8*
	Chlorophyll	6.27 ± 0.22	5.93 ± 0.21	3.65 ± 1.13	6 ± 0.6	2.69 ± 1.32*
	Vigor	4.37 ± 0.05	4.3 ± 0.06	3.57 ± 0.48	4.27 ± 0.15	0.77 ± 0.51
	Days to second leaf	24.04 ± 0.19	22.98 ± 0.22	26.67 ± 2.03	23.16 ± 0.37	−2.45 ± 2.08
	Dry weight	0.11 ± 0.003	0.11 ± 0.004	0.05 ± 0.01	0.13 ± 0.01	0.08 ± 0.02*

Table 4 (continued)

Marker (bin)	Trait	Mean ± standard error				
		AA Su1Su1	*AA su1su1*	*CC Su1Su1*	*CC su1su1*	Difference
	F_v/F_m	0.48 ± 0.01	0.47 ± 0.01	0.39 ± 0.07	0.51 ± 0.03	0.13 ± 0.08
S_1,124,691 (6.04)	Days to emergence	7.82 ± 0.07	7.62 ± 0.05	9.63 ± 0.78	7.7 ± 0.16	−1.73 ± 0.8*
	Chlorophyll	6.09 ± 0.2	6.05 ± 0.21	3.65 ± 1.13	6.06 ± 0.56	2.45 ± 1.29*
	Vigor	4.37 ± 0.05	4.32 ± 0.05	3.57 ± 0.48	4.18 ± 0.15	0.66 ± 0.51
	Days to second leaf	24.07 ± 0.19	23.22 ± 0.2	26.67 ± 2.03	23.3 ± 0.37	−2.52 ± 2.08
	Dry weight	0.11 ± 0.003	0.11 ± 0.003	0.05 ± 0.01	0.12 ± 0.01	0.07 ± 0.01*
	F_v/F_m	0.47 ± 0.01	0.48 ± 0.01	0.39 ± 0.07	0.51 ± 0.03	0.11 ± 0.08
S_1,282,382 (7.03)	Days to emergence	7.78 ± 0.06	7.69 ± 0.07	7.75 ± 0.34	7.96 ± 0.21	0.3 ± 0.41
	Chlorophyll	6.01 ± 0.19	6.23 ± 0.23	6.09 ± 0.57	5.72 ± 0.68	−0.59 ± 0.94
	Vigor	4.3 ± 0.05	4.4 ± 0.05	4.29 ± 0.35	4.06 ± 0.16	−0.33 ± 0.39
	Days to second leaf	23.48 ± 0.19	23.69 ± 0.21	24 ± 0.93	23.31 ± 0.4	−0.9 ± 1.05
	Dry weight	0.11 ± 0.003	0.11 ± 0.003	0.16 ± 0.03	0.1 ± 0.01	−0.06 ± 0.03*
	F_v/F_m	0.48 ± 0.01	0.47 ± 0.01	0.59 ± 0.03	0.47 ± 0.03	−0.11 ± 0.04*

[a] Difference was calculated as (*AASu1Su1-AAsu1su1*) − (*CCSu1Su1-CCsu1su1*)

* Significant at $P<0.05$

with minor effects throughout the genome (Djemel et al. 2013), they did not identify specific genes associated with *su1*-viability.

Some of the QTLs we identified for *su1* viability were in linkage disequilibrium with the *Su1* locus, supporting previous results which suggested that there could be genes involved in the viability of *su1* (Allam et al. 2016). In this previous publication, we studied QTLs involved in emergence and seedling vigor traits associated with *su1* fitness using two populations of RILs developed from B73 × P39 and B73 × IL14h, respectively. The QTLs detected depended on seed origin and evaluation conditions.

In both RIL populations used in this study, the highest distortion was observed on chromosome 4. Among 152 codominant loci, 78 (51%; P39) and 43 (28%; IL14h) significantly deviated from Mendelian segregation. The *Su1* locus is on chromosome 4, bin 4.05 (James et al. 1995). Galinat (1978) proposed a linkage block on chromosome 4 that included *Su1*, referring to this block as the chromosome 4 complex. The SNPs close to the gene that causes segregation distortion tend to display distorted ratios (Zamir and Tadmor 1986). This region was found to be under higher selection pressure with a reduced recombination rate (Lu et al. 2002; McMullen et al. 2009).

In the present study, several SNPs located outside the chromosome 4 block in both RIL populations showed non-random distribution of the allelic frequency of the B73 or alternate alleles in both the *su1* and the *Su1* RILs type. The distorted SNPs were concentrated in particular regions of the linkage map, and these regions were largely unidirectional in bias (most of them show an excess of *Su1* homozygous genotypes). Segregation distortion was detected on chromosomes 2, 3, and 5 for the B73 × P39 population, and on chromosomes 3, 5, 6, and 7 for the B73 × IL14h population. Djemel et al. (2013) also reported that SNPs located in bin 2.08, 3.03 and 5.04 were associated with the viability of the *su1* allele and positively associated with the number of sugary kernels.

We hypothesize that the reason a SNP is in linkage disequilibrium with *Su1* is that a particular parent provides an allele that increases the fitness of the mutant *su1* (epistatic interaction). S_22,390,507, the allele derived from the sweet corn line P39, showed a favorable epistatic effect with the *su1* allele, increasing the chlorophyll content from RILs derived from both production locations. Allam et al. (2016) reported that this marker was associated with vigor-related traits and was detected in both seed origins but only under cold conditions. 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) that appeared to be specific to low temperature. For both S_1,338,249 and S_8,615,162 located in bin 5.04, sweet

Table 5 Means of six traits and standard error for the single-nucleotide polymorphisms that showed segregation distortion for alleles *A* (from B73) and *C* (from IL14h) and the *Su1* and *su1* alleles in the recombinant inbred line population B73 × IL14h of Pontevedra origina and under cold conditions

Marker (bin)	Trait	Mean ± standard error				
		AA Su1Su1	*AA su1su1*	*CC Su1Su1*	*CC su1su1*	Difference
S_2,102,534 (3.08)	Days to emergence	9.25 ± 0.07	9.44 ± 0.09	11.18 ± 0.34	12.25 ± 0.95	0.88 ± 1.01
	Days to second leaf	30.71 ± 0.17	30.79 ± 0.19	31.3 ± 0.79	–	–
	Vigor	3.86 ± 0.05	3.76 ± 0.05	2.97 ± 0.2	3.75 ± 0.25	0.88 ± 0.33*
	Chlorophyll	4.36 ± 0.11	4.08 ± 0.12	3.94 ± 0.39	2.68 ± 0.5	−0.98 ± 0.65
	F_v/F_m	0.48 ± 0.01	0.45 ± 0.01	0.49 ± 0.03	0.43 ± 0.04	−0.03 ± 0.05
S_8,615,162 (5.04)	Days to emergence	9.24 ± 0.08	9.36 ± 0.07	11.6 ± 0.24	11.24 ± 0.37	−0.48 ± 0.46
	Days to second leaf	31.36 ± 0.17	30.28 ± 0.19	–	31.3 ± 0.79	–
	Vigor	3.71 ± 0.06	3.84 ± 0.05	2 ± 0.41	3.19 ± 0.19	1.06 ± 0.46*
	Chlorophyll	3.75 ± 0.12	4.45 ± 0.11	2.3 ± 0.23	3.94 ± 0.37	0.94 ± 0.47*
	F_v/F_m	0.4 ± 0.01	0.51 ± 0.01	0.31 ± 0.05	0.49 ± 0.03	0.07 ± 0.05
S_1,338,249 (5.04)	Days to emergence	9.25 ± 0.08	9.37 ± 0.07	11.6 ± 0.24	11.24 ± 0.37	−0.48 ± 0.46
	Days to second leaf	31.28 ± 0.17	30.29 ± 0.19	–	31.3 ± 0.79	–
	Vigor	3.7 ± 0.05	3.83 ± 0.05	2 ± 0.41	3.19 ± 0.19	1.06 ± 0.46*
	Chlorophyll	3.73 ± 0.12	4.47 ± 0.11	2.3 ± 0.23	3.94 ± 0.37	0.9 ± 0.47
	F_v/F_m	0.4 ± 0.01	0.5 ± 0.01	0.31 ± 0.05	0.49 ± 0.03	0.08 ± 0.05
S_1912530 (5.05)	Days to emergence	9.25 ± 0.08	9.41 ± 0.07	10.67 ± 0.47	11.32 ± 0.44	0.49 ± 0.65
	Days to second leaf	31.03 ± 0.17	30.5 ± 0.18	33 ± 0.003	30.86 ± 1.08	−1.61 ± 1.11
	Vigor	3.76 ± 0.05	3.86 ± 0.05	2.5 ± 0.42	3.19 ± 0.21	0.59 ± 0.48
	Chlorophyll	3.77 ± 0.1	4.54 ± 0.12	3.6 ± 0.68	3.89 ± 0.42	−0.48 ± 0.81
	F_v/F_m	0.44 ± 0.01	0.49 ± 0.01	0.43 ± 0.07	0.49 ± 0.03	0.01 ± 0.07
S_8,971,453 (6.02)	Days to emergence	9.19 ± 0.07	9.47 ± 0.08	12.63 ± 0.75	10.93 ± 0.35	−1.98 ± 0.84*
	Days to second leaf	30.93 ± 0.17	30.54 ± 0.19	–	31.3 ± 0.79	–
	Vigor	3.86 ± 0.05	3.76 ± 0.05	2.25 ± 0.52	3.3 ± 0.2	1.15 ± 0.56*
	Chlorophyll	3.85 ± 0.09	4.5 ± 0.13	2.28 ± 0.35	4.15 ± 0.4	1.22 ± 0.55*
	F_v/F_m	0.46 ± 0.01	0.46 ± 0.01	0.31 ± 0.07	0.53 ± 0.02	0.22 ± 0.08*
S_1,066,322 (6.04)	Days to emergence	9.2 ± 0.07	9.53 ± 0.09	11.38 ± 0.42	11.27 ± 0.4	−0.44 ± 0.59
	Days to second leaf	30.87 ± 0.16	30.7 ± 0.21	–	31 ± 0.82	–
	Vigor	3.83 ± 0.04	3.75 ± 0.06	2.29 ± 0.42	3.25 ± 0.2	1.04 ± 0.47*
	Chlorophyll	3.81 ± 0.1	4.67 ± 0.14	3.7 ± 0.87	3.79 ± 0.39	−0.77 ± 0.96
	F_v/F_m	0.45 ± 0.01	0.48 ± 0.01	0.46 ± 0.09	0.48 ± 0.03	−0.01 ± 0.09
S_1,099,445 (6.04)	Days to emergence	9.19 ± 0.07	9.56 ± 0.09	11.38 ± 0.42	11.27 ± 0.4	−0.48 ± 0.59
	Days to second leaf	30.93 ± 0.16	30.66 ± 0.22	–	31 ± 0.82	–
	Vigor	3.85 ± 0.04	3.78 ± 0.06	2.29 ± 0.42	3.25 ± 0.2	1.03 ± 0.47*
	Chlorophyll	3.82 ± 0.1	4.64 ± 0.14	3.7 ± 0.87	3.79 ± 0.39	−0.73 ± 0.96
	F_v/F_m	0.44 ± 0.01	0.48 ± 0.01	0.46 ± 0.09	0.48 ± 0.03	−0.02 ± 0.09
S_1,124,691 (6.04)	Days to emergence	9.23 ± 0.07	9.48 ± 0.08	11.38 ± 0.42	11.27 ± 0.4	−0.36 ± 0.59
	Days to second leaf	30.86 ± 0.16	30.62 ± 0.19	–	31 ± 0.82	–
	Vigor	3.83 ± 0.05	3.76 ± 0.05	2.29 ± 0.42	3.25 ± 0.2	1.03 ± 0.47*
	Chlorophyll	3.94 ± 0.1	4.34 ± 0.12	3.7 ± 0.87	3.79 ± 0.39	−0.31 ± 0.96
	F_v/F_m	0.45 ± 0.01	0.47 ± 0.01	0.46 ± 0.09	0.48 ± 0.03	0 ± 0.09
S_1,282,382 (7.03)	Days to emergence	9.3 ± 0.07	9.38 ± 0.09	12.83 ± 0.79	11 ± 0.33	−1.91 ± 0.87*
	Days to second leaf	30.67 ± 0.17	30.79 ± 0.19	–	31.11 ± 0.86	–
	Vigor	3.81 ± 0.05	3.85 ± 0.05	3 ± 0.52	3.07 ± 0.2	0.03 ± 0.56
	Chlorophyll	3.96 ± 0.1	4.38 ± 0.13	2.9 ± 0.39	3.99 ± 0.41	0.67 ± 0.59
	F_v/F_m	0.46 ± 0.01	0.46 ± 0.01	0.34 ± 0.04	0.52 ± 0.03	0.18 ± 0.05*

[a] Difference was calculated as (*AASu1Su1-AAsu1su1*) − (*CCSu1Su1-CCsu1su1*)

* Significant at $P<0.05$

corn RILs performed better for all traits (positive epistatic) when the SNP carries the allele provided by the sweet corn parent Il14h. As a result, the RILs that combined the *su1* locus with the allele from Il14h occurred more frequently than predicted, according to the prediction made by Kouyos et al. (2007), who stated that if a combination of two alleles at different loci are more fit than expected, then this combination will be found more frequently than predicted from the allele frequencies (Kouyos et al. 2007). Djemel et al. (2013) reported that the marker umc_1221 located on chromosome 5 (bin 5.04) was associated with early vigor and that the proportion of sugary seeds with roots deviated from the random distribution in the same RIL populations used in this study. Djemel et al. (2013) also reported that this region seems to be a good candidate to contain genes involved in *su1* fitness.

These results have implications for both evolutionary studies and breeding programs. Indeed, mutations have a key role both for breeding and evolution. When a mutant is introduced into a genotype or a gene is modified through mutagenesis or genome editing, the viability of the modified gene depends on its interaction with the rest of the target genome. In this process, the environment and the original genetic background in which the mutation occurs affect the fitness of a mutation and its propagation (Djemel et al. 2013). Natural selection and recombination generate new genotypes, some of which increase the fitness of the new mutation.

In conclusion, specific genes were involved in the viability of the sweet corn mutant *su1* and the effect of these genes depends on the genotype and on the environment. There were SNPs in linkage disequilibrium associated with vegetative and reproductive traits, particularly with germination and early vigor-related traits. Some SNPs had significant epistasis in traits related to fitness of the *su1* mutant. The SNPs identified in this study could be used by sweet corn breeders by combining the most favorable alleles associated with *su1* viability in breeding new genotypes from field × sweet corn crosses. These results also have implications on mutagenesis breeding or genome editing because the epistatic effects of the target genome on the new alleles generated by these techniques could affect the success of the breeding program.

Acknowledgements Molecular data was provided by the Maize Diversity Project (https://www.panzea.org/). Seed was provided by the North Central Regional Plant Introduction Station of the USA.

Funding This work was supported by the Spanish Plan for Research and Development (grant number AGL2016-77628-R); FEDER (grant number AGL2016-77628-R); and the College of Agricultural and Life Sciences (University of Wisconsin-Madison).

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