**Genotypic effects on field performance of maize plants carrying the
allele *sugary1***

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Abstract

Some mutants reduce fitness when incorporated to breeding populations, particularly the allele *sugary1* (*su1*) in maize (*Zea mays* L.). The objective of this work was to compare in different maize genetic backgrounds the performance of plants carrying *su1*. The crosses among three *su1* populations and three *Su1* synthetics were random mated three times. The plants *Su1* and *su1* were evaluated separately. The *su1* plants had poorer performance than the *Su1* plants, particularly at earlier stages of development, and early vigor seemed to be the most limiting factor for *su1* plant development. The differences in performance between *su1* and *Su1* plants decreased along plant growth and were not significant for most adult traits. Specific crosses between field corn from sweet corn populations had different effects on performance of *su1* plants. These results suggest that the use of defective mutants in plant breeding should take into account the genotypic effects on mutant fitness.

Key words: *Zea mays*, maize, *sugary1*, viability.

Introduction

Sweet corn is an important crop worldwide, but the viability of a commercially important mutant matters for many other crops, besides maize, where mutants are also used for releasing new varieties. Indeed, when plant breeders find a valuable new mutant allele, such as *su1*, they introduce the mutant allele into wild type varieties with irregular success because some mutant alleles reduce plant viability. Sweet corn breeders frequently use field corn to improve sweet corn agronomic performance (Butrón et al., 2008, Tracy, 2000), and have to deal with the reduced viability of *su1* plants within some field corn genetic backgrounds (Tracy 1990, 2000). Actually, *su1* is lethal or semi-lethal for some specific sweet × field corn crosses (Revilla et al., 2000a, 2006, Tracy 2000) and the frequency of *su1* plants is reduced during selection programs (Martins and Da Silva, 1998).

Sweet corn was primarily defined for being homozygous for the allele *sugary1* (*su1*). The mutant *su1* has arisen through spontaneous mutation and crosses to field corn in a variety of field corn backgrounds (Tracy et al., 2006). Indeed, *su1* is stable in those backgrounds, but it is instable under stress (Revilla et al., 2005) and could not be maintained when crossed to other field corn genotypes (Tracy, 2000).

Little is known about the causes of the different survival rates of *su1* when different field corn genotypes are used as recipients, except that the deleterious effects of *su1* are mainly related to the amount of carbohydrates available in the kernel (Tracy and Chang, 2007); therefore, different field corn recipients carrying *su1* could accumulate variable amounts of

carbohydrates, which should be critical for *su1* plant growth during the growing cycle.

The viability of *su1* plants has been investigated mainly at germination; for example Revilla et al. (2000a, 2006) studied the effects of germination on *su1* frequencies in crosses among field corn and sweet corn genotypes. Revilla et al. (2000a) concluded that the main cause of reduction in *su1* frequency was the poor germination of *su1* kernels compared to *Su1* and that *su1* plants were more viable when Corn Belt Dent germplasm was crossed to the sweet corn population Stowell's Evergreen than when Spanish germplasm was crossed to the sweet corn populations. Revilla et al (2000b) reported significant specific heterosis effects for yield and other traits in crosses among these sweet and field corn populations. Specific heterosis for yield and plant height was positive for crosses sweet × field corn, and negative for crosses sweet × sweet and field × field corn. These authors concluded that there is some genetic affinity between Golden Bantam and field corn from northern Spain and between Stowell's Evergreen and field corn from southern Spain.

Although differences between sweet and field corn are perceptible at the adult plant stage, particularly for ear traits, no report has been published on the performance of *su1* vs *Su1* at the adult plant stage, different genotypes being used as field corn recipients and sweet corn donors. The objective of this work was to compare in different genetic backgrounds the performance of *su1* plants in order to choose the most appropriate field corn varieties for improving the agronomic performance of sweet corn.

Materials and methods

The three sweet corn (*su1*) populations used were Golden Bantam (NY) (PI 255976) (GB) from the North Central Regional Plant Introduction Station of Iowa, and Country Gentleman (CG) and Stowell's Evergreen (SE) from Cornell University. These populations represent the largest variability among modern sweet corn inbreds, and Golden Bantam is the source of most commercial inbreds (Tracy, 2000). The three field corn synthetics used were EPS10 from the US Corn Belt, EPS13 from northern Spain, and EPS14 from southern Spain (Ordás 1991, Vales et al. 2001). EPS10 is a synthetic made by crossing four Corn Belt Dent populations with medium flowering time and dent yellow kernels; EPS13 is a breeding population obtained from a synthetic of four northern Spanish varieties with early flowering time and flint yellow kernels; and EPS14 is another breeding population derived from a synthetic of four southeastern Spanish varieties with medium flowering time and semi-flint yellow kernels. All six varieties are adapted to temperate areas.

The three *su1* populations were crossed to the three field corn populations in 1995 in Pontevedra, Spain. Population crosses were made in both directions in 5 pairs of 15-plant rows using each plant once as male or as female. We obtained about 50 ears from each cross, which means that approximately 50 individuals from each population were sampled. The nine sweet $\times$ field corn crosses were recombined three times in 1996 (R1), 1997 (R2), and 1998 (R3). For each recombination, plants from ten 15-plant rows were randomly crossed, using each plant once, as female or male. We

obtained about 50 ears from each recombination, thus around 100 plants were involved. Twenty seven populations were obtained from the nine field × sweet corn crosses recombined once, twice and three times. The 50 ears from each population were dried and shelled in bulk, yielding above 10000 kernels per recombination, which were stored in a cold chamber at 4 °C and 50% relative humidity. From each of the 27 populations, 1000 kernels were randomly sampled and *su1* and *Su1* kernels were separated for further evaluation in the field. Therefore, 54 genotypes were produced, consisting of 27 *su1* genotypes (the *sugary1* grains coming from each of the three recombinations made in each cross between sweet and field corn populations) and 27 *Su1* genotypes (the *Sugary1* grains coming from each of the three recombinations made in each cross between sweet and field corn populations).

The field arrangement for each field trial was a split-plot design with two replications; the alleles (*su1* and *Su1*) were assigned to the main plots and to subplots the 27 genotypes, including the six original populations. For most traits, xenia or border effects are not important at all. For yield, the separation of *su1* and *Su1* in blocks minimized the xenia effect, and the fact of having heterozygous plants in the *Su1* block should not have biased yield comparisons because the pollen was freely moving across neighbour plants and the frequency of *su1* kernels was below 0.14 averaged over crosses, according to our previous calculations (Revilla et al. 2000).

Each two-row plot consisted of 17 hills per row with two kernels per hill. Rows were spaced 0.80 m apart, and hills were spaced at 0.21 m. When

the plants had four to five leaves, the plots were thinned to one plant per hill, for a final plant density of approximately 60 000 plants ha⁻¹. Evaluations were located in Pontevedra (20 m above sea level) in Northwestern Spain. Pontevedra has a humid climate with an annual rainfall of about 1600 mm. Trials were sown in 28 May 1999, 23 May 2000, and 16 May 2002 and harvested during the first half of November, at dry-grain stage.

Traits measured on the whole plot were emergence (%), early vigor (rated at the five leaf stage on a visual scale from 1=poor to 9=excellent), pollen date (number of days from planting to 50% of plants producing pollen), silk date (number of days from planting to 50% of plants exhibiting silks), plant height (cm from the soil to the top of the tassel), ear height (cm from the soil to the main ear node), adult plant performance (rated at the full development stage on a visual scale from 1=small canopy and pale color to 9=large canopy and dark green), stand (percentage of plants that reached maturity), ears per plant, yield (kg ha⁻¹ at 140 g kg⁻¹ moisture content), kernel moisture (g kg⁻¹ of moisture content), root lodging (% of plants leaning more than 30 °), stalk lodging (% of stems broken below the main ear), ear length (cm), ear diameter (mm), kernel length (mm), and kernel row number.

Analyses of variance included environments, replications, genes (*Su1* vs. *su1*), recombinations, field corn populations, sweet corn populations and the appropriate interactions, as sources of variation. Environments, replications and their interactions were considered as random effects, and recombination, gene, field corn and sweet corn populations, and their

appropriate interactions as fixed effects. All analyses were performed with the procedure GLM of SAS (2000).

Results

Genes (*Su1* vs. *su1*) differed significantly only for early vigor (Table 1). Differences among field or sweet corn genotypes were significant for more traits than differences among recombinations, and only for adult plant appearance differences within each source of variation were significant. Interactions were seldom significant.

There were no significant differences for any source of variation for seven traits, including emergence. Differences among field x sweet corn crosses were significant for one quarter of the traits, and were largely more important than any other effect, including recombinations and genes (Table 1).

Early vigor was lowest for the sweet corn populations Country Gentleman (3.11) and Stowell's Evergreen (3.22) followed by Golden Bantam (4.44), while the synthetic from northern Spain EPS13 (6.22) and from the Corn Belt Dent EPS10 (5.89) had the highest vigor, followed by the southern Spanish synthetic EPS14 (5.22). As recombinations progressed, early vigor decreased from 5.54 for the first recombination (averaged over genes and crosses), to 5.17 for the second and 4.84 for the third recombination. The reduction of early vigor after each recombination was similar, around 6.6 % from the first to the second, and from the second to the third recombination. Concerning the effects of genes, the *Su1* plants were more vigorous than the *su1* plants for any population or cross (Table 2). Averaged over crosses, the most vigorous field corn population was EPS13 (5.48), while differences among EPS14 (5.11) and EPS10 (4.96)

were not significant (Table 2). Golden Bantam (5.32) was the most vigorous sweet corn population averaged over crosses, not differing from Stowells' Evergreen (5.15). EPS13 produced the most vigorous crosses with Golden Bantam (5.5), Stowells' Evergreen (5.5) and Country Gentleman (5.43), though EPS14 $\times$ Golden Bantam (5.44) did not differ significantly from them. Among the sweet corn populations, the *Su1* plants with highest vigor came from crosses involving Golden Bantam (6.12) or Stowells' Evergreen (6.05), while the *su1* plants with largest vigor derived from crosses involving Golden Bantam (4.52) or Country Gentleman (4.43). Among the field corn populations, the *Su1* plants were more vigorous when the parent was EPS13 (6.35) or EPS14 (5.95) than when the parent was EPS10 (5.59), while *su1* plants from these three field corn populations did not differ for vigor (from 4.27 to 4.60).

Silking dates of populations per se varied from 71 days for EPS10 and EPS13 to 80 days for Country Gentleman. The recombinations of crosses involving EPS10 delayed silking from 72 days for the first recombination to 73 for the third, though the second and third recombinations were not significantly different, but the recombination $\times$ field corn population had not significant effects for any other population. Crosses involving Golden Bantam (71 days) produced silks earlier than those of Stowell's Evergreen (74), which did not differ from crosses with Country Gentleman (73). The gene $\times$ sweet corn population interaction was manifested on earlier *Su1* and *su1* plants when Golden Bantam was involved

but; when Stowell's Evergreen was the parent, *Su1* were earlier than *su1* plants.

Adult plant performance was highest for the field corn synthetics EPS14 (6.44) and EPS10 (6.44), followed by EPS13 (6.00) and lowest for the sweet corn population Golden Bantam (2.56), being intermediate for Country Gentleman (4.22) and Stowell's Evergreen (5.44). The crosses involving EPS14 (6.10) had better adult plant performance than for EPS13 (5.85), and crosses with EPS10 (5.85) were intermediate. Among the sweet corn populations, Stowell's Evergreen (6.35) had the best adult plant performance. Grain moisture was lowest for crosses involving Golden Bantam (303 g kg⁻¹) than for any other sweet corn genotype. The *Su1* grains derived from either field corn genotype had less moisture than the *su1* grains.

Yield was lowest for the sweet corn populations Golden Bantam (1618 kg/ha) and Country Gentleman (1754) and highest for the field corn population EPS10 (3398). However, differences were not significant for yield within any source of variation. Nevertheless, the yield components ear diameter, kernel length and row number revealed significant differences. The crosses involving EPS10 (4.4 cm) produced wider ears than those of EPS13 (4.3) or EPS14 (4.2), and the recombination × gene × field × sweet corn population was significant for ear diameter. Kernels were longest when *su1* plants derived from crosses involving Stowell's Evergreen (0.91 cm) or Country Gentleman (0.91), or when the *Su1* plants came from Stowell's Evergreen (0.90). When the sweet corn population was Country Gentleman,

the *Su1* grains were significantly shorter than the *su1* grains. Finally, EPS10 produced more kernel rows (14.6) than EPS13 (13.3) and EPS14 (12.9). The field $\times$ sweet corn combination producing more kernel rows was EPS10 $\times$ Country Gentleman and, and the combination of Country Gentleman with any field corn population produced more rows than the combination of any other sweet corn genotype.

Discussion

The performance of *su1* plants differed from that of *Su1* plants mainly at the first stages of development. Once germination has been accounted for (Revilla et al., 2000a, 2006) the next limiting factor is early vigor. Genotypic effects are of paramount importance (Martins et al., 1998; Revilla et al. 2000a, b, 2006). The presumable effect on viability of loci linked to *su1* is negligible since the effect of recombinations is minor.

Although short growing cycles increase agronomic performance of maize in a region with short growing seasons, the small differences detected for earliness here should have a small contribution to the differences between *Su1* and *su1* plants. Adult plant performance decreased after each recombination, probably due to the reduction on heterosis caused by successive recombinations. Moisture is usually higher in *su1* than in *Su1* grains because of the osmotic pressure produced by the mono and oligosaccharides stored in the *su1* endosperm (Revilla et al. 2000b). Among the *su1* grains, those coming from EPS13 (318 g/kg) had a lower moisture than those coming from EPS10 (334) or EPS14 (340). Low moisture at harvest is a clear advantage for seed production in an area with short growing seasons; therefore, the performance of sweet corn would be favored when *su1* plants are obtained from crosses with field corn from northern Spain compared to southern Spain or the Corn Belt Dent synthetic.

The importance of the differences among sweet corn populations and among field corn populations and few sweet x field corn interactions were significant, therefore differences among field and sweet corn genetic

backgrounds were quite stable. Revilla et al (2000a) concluded that differences in emergence explained most of the differences on *sugary1* viability among genetic backgrounds. The subsequent trait, chronologically, is early vigor, for which a large number of sources of variation were significant. These results are expected if the key factor affecting performance is the amount of kernel reserves, which are genotypically determined (Tracy and Chang, 2007). However, previous reports have shown significant field $\times$ sweet corn population interactions (Revilla et al., 2006). Such discrepancy can be explained by the genotypes involved in each experiment, because in the present work we use populations while in the previous work we crossed inbred lines which implies inbreeding depression per se and high heterosis in crosses to other lines.

As the growing cycle continues, successive traits are less important for the performance of *su1* plants. The lack of significant differences for stand, ears per plant and yield were particularly important because suggests that *su1* had not significant effects on fitness at large. Contrarily, in a previous publication, Revilla et al. (2000b) found significant differences among genotypes for some of fitness-related traits, namely grain yield, grain moisture, plant height, ear length and kernel row number, and the genotype $\times$ environment interaction was also significant for all those traits. In the previous study, the field corn population that produced *su1* plants with best yield components was the Corn Belt Dent synthetic, and the preferred sweet corn population was Country Gentleman.

In order to release sweet corn genotypes with high performance, the base material should be the sweet and the field corn populations producing *su1* plants with better performance, particularly at earlier stages of the growing cycle (Butrón et al., 2008). The present results show that early vigor was the key trait for sweet corn performance. Revilla et al (2000a) found that the decrease of *su1* frequency was larger in the second recombination than in the third, and that reduction strongly depended on the field and sweet corn genetic background. Our present data suggest that lower early vigor of *su1*, compared to *Su1*, could partially explain the reduction in *su1* frequency after each recombination. Martins and Da Silva (1998) found that the frequency of *su1* genotypes decreased during the growing cycle due to a reduction in viability during the vegetative phase. However, these authors focused their research on the reduction on *su1* frequency during the reproductive phase. The reduced early vigor of *su1* plants compared to *Su1* could be one of the causes in such reduction due to competence with weeds and susceptibility to biotic and abiotic stress. Differences among field and sweet corn genotypes on *su1* and *Su1* vigor suggest that it is possible to improve vigor of *su1* plants by selecting appropriate genotypes, as shown by Butrón et al. (2008).

The improvement of *su1* viability would gain from the use of field corn from the north of Spain or sweet corn related to Golden Bantam, because these genotypes would provide high vigor and short growing cycles. Field corn from the south of Spain and sweet corn related to Stowell's Evergreen increased adult plant performance, while field corn from Corn

Belt and sweet corn related to Country Gentleman would improve the yield components.

As a general conclusion, these results suggest that the use of defective mutants in plant breeding should take into account the donor x receptor interaction on the viability of the mutant and on the performance of the plants carrying the mutant. Furthermore, since the genotype affects the fitness of mutants, further research is needed to identify genes involved on mutant fitness.

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1 Table 1 Pertinent mean squares for ten traits showing significant differences from the analyses of variance of *Sugary1(Su1)* and *sugary1*
2 (*su1*) plants obtained from three generations of recombination (R) of crosses among three field corn (*Su1*) and three sweet corn (*su1*) corn
3 genotypes.

4 Source of		Early	Silk	Pollen	Ear	Adult plant	Kernel	Root	Ear	Kernel	Kernel
5 row											
6 Variation	d.f.	Vigor	date	date	height	performance	moisture	lodging	diameter	length	number
7 Gene	1	295.56*	75.85	31.13	516.88	22.69	1689.23	2438.69	11.04	1.48	0.62
8 Recombination (R)	2	19.72*	6.92	1.86	459.23	4.27*	13.08	331.49	8.77	0.36	2.26
9 Field corn genotype (field)	2	11.26*	9.00	10.35	790.18*	12.52*	78.31	505.46	153.22*	9.85	126.30*
10 Sweet corn genotype (sweet)	2	2.57	269.98*	215.06*	3980.91	35.39*	95.92*	296.46	0.49	3.34	161.63
11 Gene × field	2	2.78	6.62	4.90	1.71	1.51	49.07*	349.48	0.10	0.59	2.20
12 Gene × sweet	2	2.66*	21.60*	15.72	414.27	3.13	24.30	64.93	9.03	1.43*	1.77
13 Gene × R	2	0.19	3.52	2.17	68.83	1.34	14.90	37.16	12.04	2.20	1.58
14 R × field	4	0.89	8.77*	3.76	20.71	0.28	4.59	718.80*	4.95	0.27	0.94
15 R × sweet	4	1.80	10.87	5.80	39.83	0.30	9.79	372.15	1.81	0.48	0.31

1	Field × sweet	4	1.20*	18.06	5.95	202.68	2.18	5.79	93.17	3.86	0.36	5.48*
2	Gene × R × field	4	1.20	1.38	1.13	57.47	0.68	27.57	452.16	6.96	0.27	1.01
3	Gene × R × sweet	4	0.52	5.62	4.93	48.15	0.27	10.35	124.27	2.40	0.43	0.89
4	Gene × field × sweet	4	0.97	1.40	2.87	69.18	0.48	22.64	241.42	2.30	0.39	2.72
5	R × field × sweet	8	1.28	6.87	4.08	65.08	0.23	8.77	144.41	1.74	0.18	2.26
6	Gene × R × field × sweet	8	0.98	4.46	4.22	120.09	0.58	8.51	241.11	11.45*	0.85	0.59

7 * Significant at P = 0.05

8

9

1 Table 2. Mean¹ early vigor (1 - 9)² of *Sugary1* (*Su1*) and *sugary1* (*su1*) plants obtained from three generations of recombination (R) of
2 crosses among three field corn (*Su1*) and three sweet corn (*su1*) corn genotypes.

3 Field 4 corn 5 genotype	Sweet corn genotype											
	Golden Bantam			Stowell's Evergreen			Country Gentleman			Mean		
	<i>su1</i>	<i>Su1</i>	Mean	<i>su1</i>	<i>Su1</i>	Mean	<i>su1</i>	<i>Su1</i>	Mean	<i>su1</i>	<i>Su1</i>	Mean
6 EPS10 4.22hi	5.81c-f	5.02b		4.41ghi	5.59ef	5.00b	4.37ghi	5.37f	4.48b	4.33c	5.59b	4.96b
7 EPS13 4.67gh	6.33ab	5.50a		4.41ghi	6.59a	5.50a	4.74g	6.11a-d	5.43a	4.60c	6.35a	5.48a
8 EPS14 4.67gh	6.22abc	5.44a		3.96i	5.96b-e	4.96b	4.19hi	5.67def	4.93b	4.27c	5.95ab	5.11b
9 Mean	4.52c	6.12a	5.32a	4.26d	6.05a	5.15ab	4.43c	5.72b	5.07b	4.40b	5.96a	

10 ¹ Means are compared according to the Fisher's protected LSD (P=0.05). Means followed by the same letter, within each category, are not
11 significantly different. Comparisons are made within each category: means of the field corn genotypes, means of the sweet corn genotypess,
12 means of *su1* and *Su1* for the sweet corn genotypess, means of *su1* and *Su1* for the field corn genotypes, and *su1* and *Su1* for the crosses.

13 ² 1 = weak plants, 5 = average vigour, 9 = highly vigorous

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