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CROPS AND SOILS RESEARCH PAPER

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

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SUMMARY

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

INTRODUCTION

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

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

The allele *su1* can be lethal or near lethal when it is introgressed into some field maize genetic backgrounds, being maintained only in heterozygotes, but it survives well enough in other genetic backgrounds. Sweet corn lines homozygous for this allele often have nearly 1·00 germination (Tracy 1990).

The gene and genotype frequencies of *su1* and *su1 su1* individuals, respectively, were monitored throughout five successive generations of *Su1* × *su1* by Martins

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

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

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

MATERIALS AND METHODS

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

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

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

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

All crosses to P39 were successively selfed in 2006, 2007, 2008, 2009 and 2010 in Pontevedra, Spain (42°24′, 8′38′N, 20 m asl), a location in the northwest of Spain where annual rainfall is in the range of 1600–1700 mm. Around 100 plants from each of the 12 entries were self-pollinated by hand, harvested and conserved in bulk. From each bulk, a sample of 150 kernels was sown in blocks 3 m long, spaced 0·8 m apart; plants within the row were 0·20 m apart. The self-pollination generations were repeated up to 5 years.

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

$$q = [(N \times 2) + C \times (1500 - N)]/3000$$

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

Linear and quadratic regressions of the gene frequency on the number of selfed generations were computed for each cross. The coefficients of regression (b) were tested for homogeneity. For each selfed generation, the expected number of *su1* kernels was calculated from the number of the *su1* kernels in the previous generation and was compared with the observed number using the χ^2 test. In order to estimate fitness, the selective value (s) of *su1* was calculated as 1 minus the proportion of the contribution of offspring to the next generation ($1-s$) (Falconer 1981). In order to estimate the effects of the proportion of unfavourable genotypes on the fitness of *su1*, A619 × A632 and EP42 × A661, one unfavourable and one favourable parent were defined within each cross, concerning the effects on the fitness of *su1* when this mutant is introduced through backcrosses. From these crosses the respective F_1, F_2, BC_1 and BC_2 were developed, which carried different proportions of the genome of the unfavourable parent: $P_1=1$, $P_2=0$, $F_1=0{\cdot}50$, $F_2=0{\cdot}50$, $BC_1=0{\cdot}75$ and $BC_2=0{\cdot}25$. Regression analyses of the selective value (s) along the successive generations on the proportion of unfavourable genotypes were carried out for each design and across designs.

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

The variance of the generation means (s_i^2) are not the same; this heterogeneity among variances was adjusted in the analyses by weighting the means differently; these weights being the reciprocals of the squared standard errors (Mather & Jinks 1971). In addition, weight was taken into account when solving for m, a, d, aa, ad and dd. For the six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) the solution is obtained in the form of a matrix by the SAS (SAS Institute 2005) statistical package using PROC IML as follows:

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

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

RESULTS

In both designs, A661 × EP42 (Table 2) and A619 × A632 (Table 3), the reduction of *su1* frequency across self-pollination generations was less important in the first generation. In the first design, the frequencies in all generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) decreased linearly and showed a continuous and pronounced fall from 0·534 to 0·270 and from 0·491 to 0·161 in A661 and EP42, respectively (Fig. 1*a*). The same tendency was observed for the second design, i.e. there was a reduction of *su1* frequency from 0·466 to 0·051 in A619 and 0·504 to 0·171 in A632 (Fig. 1*b*). The χ^2 test for comparison between observed and expected number of *su1* kernels revealed significant differences for almost all crosses across self-pollination generations (data not shown).

For both designs, the coefficients of regression were all negative and significantly different from zero. The coefficients of determination were above 0·90, except for EP42 and the segregating population F_2 involving A619 and A632. The quadratic regression was not significant in any case (data not shown). The reduction on frequency of *su1* for EP42 fluctuated more across years than for A661. However, the behaviour of A619 and A632 concerning *su1* fitness was more stable across years.

The coefficients of regression for each generation in the first design were not homogeneous; seed viability of *su1* mutant was higher for A661 than that for the backcross to EP42 with $b=-0{\cdot}065$ and $b=-0{\cdot}097$, respectively. Regression coefficients for EP42, F_1, F_2 and the backcross of A661 were not heterogeneous; however, the backcross of A661 had the most unfavourable coefficient ($b=-0{\cdot}086$) for *su1* seed viability. In addition, the coefficients of regression for F_1 ($b=-0{\cdot}078$) and F_2 ($b=-0{\cdot}075$) were higher than the coefficients for both parents.

The coefficients of regression in the second design were not heterogeneous; therefore, the frequency of the *su1* mutant was similarly reduced across the five

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

Generations	A661	EP42	F_1	F_2	BC_1	BC_2
1	0·534	0·491	0·458	0·474	0·458	0·503
2	0·454	0·353	0·423	0·354	0·319	0·420
3	0·368	0·370	0·256	0·237	0·250	0·284
4	0·328	0·308	0·253	0·234	0·170	0·176
5	0·270	0·161	0·150	0·155	0·099	0·137
b	−0·065±0·004	−0·07±0·015	−0·08±0·011	−0·08±0·012	−0·09±0·007	−0·10±0·009
R^2	0·97	0·83	0·91	0·90	0·97	0·96
	($P<0·001$)	($P=0·019$)	($P<0·01$)	($P \leqslant 0·01$)	($P<0·001$)	($P<0·001$)
s	0·130±0·0071	0·24±0·118	0·27±0·098	0·29±0·095	0·40±0·087	0·29±0·071

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

Generations	A619	A632	F_1	F_2	BC_1	BC_2
1	0·466	0·504	0·476	0·453	0·479	0·483
2	0·365	0·452	0·466	0·416	0·423	0·397
3	0·190	0·380	0·310	0·240	0·310	0·250
4	0·106	0·188	0·277	0·176	0·261	0·159
5	0·051	0·171	0·218	0·158	0·131	0·149
b	−0·109±0·011	−0·09±0·014	−0·07±0·011	−0·08±0·014	−0·09±0·007	−0·09±0·013
R^2	0·95	0·91	0·90	0·88	0·97	0·91
	($P<0·01$)	($P \leqslant 0·01$)	($P<0·01$)	($P=0·011$)	($P<0·001$)	($P \leqslant 0·01$)
s	0·519±0·113	0·21±0·104	0·19±0·071	0·29±0·081	0·28±0·083	0·29±0·096

self-pollination generations. All genotypes have negative selection against *su1* and the highest reduction was for A619 ($b=-0·109$). The comparison between the four inbred lines employed in the present work show that the parent least favourable for *su1* seed viability was A619 and the best was A661.

The selective value s of *su1* is also used as an estimator of seed viability and differed between parents and derived populations. The selection against *su1* occurs in the same direction in both designs (Tables 2 and 3). The coefficient of selection s varied from 0·130 to 0·397 in the first design and from 0·193 to 0·519 in the second design.

The selection effect against *su1* depended on the field maize genotype. EP42 ($s=0·235$) had a larger negative selection effect against *su1* than A661 ($s=0·130$), but differences were not significant. However, the selection effects in F_1, F_2, BC_1 and BC_2 were above the values in the parents. For the second design, A619 ($s=0·519$) was worse than A632 ($s=0·213$).

Mean generation analysis was used to determine the genetics effects and their type of action on *su1* fitness using the coefficients of regression as parameters of seed viability. As shown above, the differences among generations were significant only in the first design (A661 × EP42) and, for this reason, only the genetic parameters were estimated for this cross. The mean generation analysis has shown that the additive model explained adequately the variation observed ($\chi^2_{(4)}=6·75$, with $P=0·149$) with $m=-0·08\pm0·004$ and $a=0·013\pm0·005$.

The coefficient of selection against the mutant showed a tendency to increase along the selfed generations. The combined-over-designs regression analysis and both individual analyses for each design showed that the coefficient of selection of the fifth generation of inbreeding ($s5$) was consistently significant. In addition, coefficients of selection were significantly affected by the contribution of unfavourable genotypes, particularly in the second self-pollination

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

Analysis	Parameter	b	Adjusted R^2
Combined	$s1$	0·17 ± 0·068 (P = 0·032)	0·32
	$s2$	0·24 ± 0·118 (P = 0·069)	0·22
	$s5$	0·53 ± 0·106 (P < 0·001)	0·68
EP42 × A661	$s2$	0·33 ± 0·151 (P = 0·095)	0·43
	$s5$	0·37 ± 0·060 (P < 0·001)	0·88
A619 × A632	$s5$	0·69 ± 0·174 (P = 0·016)	0·75
	s (averaged)	0·24 ± 0·110 (P = 0·094)	0·43

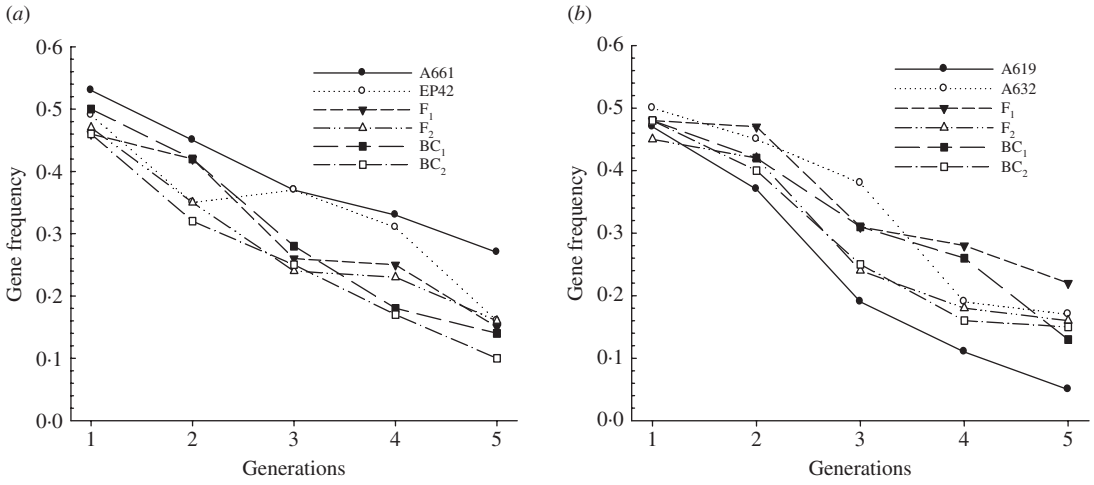

Fig. 1. Change of gene frequency across five selfing generations of crosses between the *su1* inbred P39 and six basic generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from crosses between two pairs of field maize inbred lines: (*a*) first design and (*b*) second design.

generation ($s2$) in the combined analysis and in the first design (Table 4).

DISCUSSION

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

The tendency of *su1* gene frequency reduction occurred in both designs but with different intensities depending on the field maize inbred lines involved, as previously reported by Revilla *et al*. (2006*a*, 2010). Three background types were used in the present study; A619 and A632 were released from Lancaster and Reid (Corn Belt Dent race), while A661 was released from Corn Belt germplasm different from Reid or Lancaster and EP42 comes from a European flint population genetically distinct from the American germplasm pools. Seed viability of *su1* mutant was lower in the Reid and Lancaster inbreds than in the cross involving inbreds with different genetic backgrounds, A661 and EP42. These dynamic aspects of the *su1* gene frequency have been reported by Revilla *et al*. (2006*b*, 2010), who concluded that seed viability of *su1* gene depends on specific *su1* × field maize interactions. Sweet corn breeders are aware of the unfavourable effects of base germplasm on *su1* seed viability (Tracy 1990) and the present results suggest that it could be worthwhile searching for favourable field maize genotypes for sweet corn breeding outside the current sources, for example, in hard endosperm maize (Alonso Ferro *et al*. 2008). However, previous results are not encouraging, at least for European germplasm (Cartea *et al*. 1996*a*, *b*; Malvar *et al*. 1997*a*, *b*; Revilla *et al*. 1998).

A net selection was revealed acting against *su1*, with the fitness of the mutant allele highly dependent of the

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

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

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

In congruence with the additive effects of the genotypes on the fitness of *su1*, it was found that the variation of the proportion of the unfavourable genotype explains a considerable part of the variation observed for the selective value (*s*), particularly in the last generation where the mutant is expected to have the highest exposure as a consequence of homozygosity advance. These results suggest that the fitness of a mutant depends on the genetic background in general rather than on single genes. Magwire *et al*. (2010) used *Drosophila* as a model system to provide an explanation of genetic and environmental factors that affect variation in lifespan and senescence, concluding that variations observed were especially due to genetic background and epistatic effects. The present research also confirms the suggestion of Yamamoto *et al*. (2009) that the variation in the magnitude of the genetics effects among the wild genetic backgrounds could have evolutionary implications. Furthermore, the ability of genotypes for moderating the fitness costs of new genetic variants has been hypothesized by Raymond *et al*. (2011). Altogether, these evidences and the current data suggest that genotypes could modulate the fitness of new mutations.

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

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