Quantification of critical factors affecting fitness of the sugary1 mutant in maize

Djemel A¹, Ordás B¹, Khelifi L², Ordás A¹, Revilla P^{1*}

¹Misión Biológica de Galicia (CSIC). Apartado 28. Pontevedra, E-36080.

Spain

²École Nationale Supérieure Agronomique, Avenue Pasteur, Hassan Badi,

El Harrach-Alger 16000. Algérie

* Corresponding author: previlla@mbg.csic.es

Abstract

Fitness of *sugary1* (*su1*) is affected by some critical traits that depend on the genotypes and environments while their effects have not been quantified with convincing statistical methods. The objective of this work was to identify and quantify the critical factors of *su1* fitness with different genotypes and environments. We used two pairs of field corn inbreds that differentially affected *su1* viability to develop F₁, F₂, BC₁, and BC₂. After selfing, *Su1* and *su1* kernels were evaluated under controlled environmental conditions and in field trials. Multiple regressions showed that dry weight of juvenile plant was affected by early vigor (plant size, color and health) and emergence in cold conditions; ear weight by plant appearance, number of plants and chlorophyll content; number of ears by plant appearance, number of plants, chlorophyll content and female flowering; and kernel weight by ear weight, number of plants, row number and ear length. The main critical factors for *su1* fitness were early vigor and emergence under cold conditions at initial stages, while several adult traits were related with final fitness.

Key words: *Zea mays* – maize - mutant – fitness — *sugary1* – emergence – early vigor – cold tolerance

Introduction

Sweet corn (*Zea mays* L.) mutant *sugary1* (*su1*) has limited fitness when compared to the wild type allele *Su1* and behaves as a lethal or semi lethal allele when it is introgressed into some field corn germplasm (Tracy 1990a); besides, the frequency of *su1* plants decreases during selection in a breeding program (Martins and Da Silva 1998). The fitness of *su1* depends on specific sweet corn $\times$ field corn genotype interactions and genetic background plays a major role in the fitness of this mutant (Revilla et al. 2000, 2006, 2010). The fitness of *su1* is under genetic control and depends on many genes with small effects on a variety of viability-related traits throughout the genome (Djemel et al. 2012, 2013a).

Selection against the mutant *su1* when it is segregating in a maize breeding population, acts first through viability (germination and early vigor) (Ordás et al. 2010) and afterwards through fertility (mating ability and grain formation) (Cisneros-López et al. 2010, Zhang et al. 2011). The viability of *su1* plants has been investigated mainly at the first stages of plant growth, being germination the first limiting factor followed by early vigor (Martins and Da Silva 1998, Revilla et al. 2000, Gad and Juvik 2002, Juvik et al. 2003, Revilla et al. 2006). Revilla et al. (2010) compared in different maize genetic backgrounds the performance of plants carrying *su1* in order to choose the most appropriate field corn varieties for improving the agronomic performance of sweet corn. In that study, early vigor was the most limiting factor for *su1* plant development. Tracy (2001) also reported that early plant stages are affected by genetic factors in sweet corn, both at planting and during seed production. Finally, Djemel et al. (2013b) studied

the genetics causes of the differences in the fitness of *su1* when different field corn genotypes were used as recurrent parents. The authors concluded that depending on specific sweet $\times$ field corn interaction, seedling vigor and particularly seedling chlorophyll content (CCM) were the most critical traits in determining *su1* viability. However, the magnitude of the diverse traits on *su1* fitness has not been quantified with a convincing statistical method. Therefore, the objective of this work was to identify and quantify the critical factors of *su1* fitness for different genotypes and environments.

Materials and Methods

Plant Materials

Two pairs of field corn (*Su1Su1*) inbreds that differentially affected *su1* viability (Revilla et al. 2006) were used to develop F₁, F₂, BC₁, and BC₂ in order to study the factors affecting the fitness of *sugary1*. Two separate designs were developed. The first design consisted in two unrelated field corn inbreds: A661 (Corn Belt) and EP42 (European Flint), while the second one involved two unrelated field corn inbreds from the same genetic background (Corn Belt): A619 and A632. Crosses (F₁) between each pair of inbred lines were made in 2001 and subsequently self-pollinated and backcrossed, obtaining the F₂, BC₁, and BC₂. The four parents and the derived generations were crossed with the sweet corn (*su1su1*) inbred P39 as donor of the *su1* allele. All crosses to P39 were successively selfed in 2006, 2007, 2008, 2009 and 2010, producing 30 genotypes for each cross, and *su1* and *Su1* kernels were separated. Therefore, 60 genotypes were produced for each design, consisting of 30 *su1* genotypes and 30 *Su1* genotypes. The 120 genotypes were evaluated in controlled growth chamber under cold and warm conditions, and in the field.

Growth chamber trials

The 120 entries were evaluated in plastic multi-cell seed trays in 2011 at Pontevedra (Spain). Cold treatments followed a randomized complete block with three replications. Growth chamber evaluations were carried out for a period of 30 days. Each experimental plot consisted of 4 kernels per row and one kernel per alveolus. Sowing depth was approximately 2 cm. Trays

were watered every week. The conditions were set at 14 h light with a photosynthetic photon flux (PPF) of 228 $\mu\text{mol}/\text{m}^2/\text{s}$ at 14 °C and 10 h darkness at 8 °C for the cold treatment. Under standard conditions, the temperatures were 25 °C and 18 °C in light and dark, respectively. Data were recorded on proportion of emergence, early vigor (an index combining plant size, color and health taken on the five-leaf stage with a visual scale from 1= weak plant to 9= strong plant); leaf chlorophyll content measured at the three-leaf stage using a hand-held Chlorophyll Content Meter, CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA), and dry plant weight (plants were cut and dried for one week at 80 °C) at the end of each experiment.

Field trials

All entries, except those produced in 2010, were also evaluated in field trials in 2010 and 2011 at Pontevedra (42° 24'N, 8' 38'W, altitude 20 m a.s.l.), a location in the northwest of Spain where annual rainfall is in the range of 1600–1700 mm, and in 2010 at Algiers (36 ° 47 ' N, 2 ° 03' E, altitude 32 m a.s.l.), located in the sub humid North of Algeria with 900 mm of annual rainfall. Each experimental plot consisted of two rows with 25 kernels per row and one kernel per hill. The rows were spaced 0.80 m apart, and the hills were spaced 0.12 m apart. The hills were thinned after emergence to obtain a final density of 75 000 plants ha^{-1} . Labor consisted on preparatory work conducted with a cultivator, fertilization, plowing tillage and rotary cultivator. Weeds were controlled with herbicides and mechanically. Irrigation was made only once at flowering time. Data were recorded on

proportion of emergence, early vigor, leaf chlorophyll content measured, plant weight (g) at the five-leaf stage in a sample of five plants (only at Pontevedra), male and female flowering, plant height, plant appearance (taken on the five-leaf stage on a visual scale from 1= weak plant to 9= strong plant), total adult plants per plot, total ear weight per plot, ear appearance (taken on a visual scale from 1= poor plant to 9= excellent), total number of ears per plot, ears per plant, ear length, number of kernel rows, and 100-kernel weight.

Statistical analyses

In order to identify the traits that were significant covariates on fitness, multiple regressions analyses using the stepwise selection method were made. The weight of juvenile plant in the field, in warm conditions and in cold conditions, as well as ear weight, number of ears, and kernel weight in the field were the dependent variables. All other traits recorded before each of those were the independent variables. The analysis was performed using the Proc Glmselect of SAS (SAS Institute Inc 2008) with *P*-value of SLE (entry significant level) and SLS (stay significant level) equals to 0.001. The sources of variance included in the model were environments, treatments (year of selfing, generation, gene, and the respective interactions), and the environments $\times$ treatments interactions

Results

Multiple regression analyses of fitness-related traits revealed that all of them were affected by several traits along the growth cycle of plant development. The proportion of variance explained varied from one third for kernel weight to three quarters for the weight of juvenile plant in the field (Table 1). Genotype, environment, and the genotype $\times$ environment interaction had also significant effects on some fitness-related traits.

The weight of juvenile plant (DWJ) for both *Su1* and *su1* grains in the field significantly depended only on early vigor with different values for *Su1* (cumulated $R^2 = 0.65$) and *su1* (cumulated $R^2 = 0.56$) (Table 1). Also, location had significant effects on DWJ for both *Su1* and *su1*, while replication and genotype $\times$ environment interaction had significant effects on DWJ for *Su1* only. Early vigor was the only factor affecting DWJ in controlled warm conditions with higher impact for *su1* ($R^2 = 0.41$) than for *Su1* ($R^2 = 0.36$), while in cold conditions, the main factors were early vigor and emergence (cumulated $R^2 = 0.64$ for *su1* and 0.77 for *Su1*). However, emergence had a negative effect on DWJ ($b = -0.029$ for *su1* and -0.038 for *Su1*) while early vigor had always a positive effect.

The factors affecting ear weight in the field were plant appearance, total number of adult plants and chlorophyll content for both *Su1* (cumulated $R^2 = 0.71$) and *su1* (cumulated $R^2 = 0.70$), all with positive effects on ear weight. The order of these factors was the same for the mutant (*su1*) and the wild type (*Su1*) allele with small differences of magnitude. Also, genotype and repetition had significant effects on ear weight of *su1* entries.

Total number of ears (TE) was affected by total adult plants both for *Su1* and *su1*, but the other factors affecting TE were different for the mutant and the wild type allele. TE of *Su1* plants was affected by genotype, the weight of juvenile plants, and plant appearance, while for *su1* plants the others significant factors were chlorophyll content and silking. Nevertheless, the cumulated R^2 explained by these factors was similar for both *Su1* and *su1* (0.63 and 0.65, respectively).

Kernel weight (KW) of *Su1* and *su1* plants was affected by two common factors: ear weight and kernel row number, and by one single factor for each allele: number of plants for *su1* and repetition for *Su1*. The cumulated R^2 of KW was low in both *Su1* (0.36) and *su1* (0.38).

In general, the cumulated coefficient of regression for each trait varied from $R^2 = 0.36$ for kernel weight to 0.74 for dry weight of juvenile plant. Moreover, the factors affecting the weight of juvenile plant were not the same in the field than in the growth chamber under cold or warm conditions, except for early vigor that was always a significant factor for this fitness related trait.

Discussion

Fitness-related traits of *sugary1* were affected by plant development traits at diverse proportions, as well as by genotype, environment, and the genotype $\times$ environment. Variability for biomass at early stages of development (weight of juvenile plant) was mostly explained by early vigor (plant size, color and health) for both the mutant *su1* and the wild type plants in the field and under controlled conditions. Emergence was a limiting factor of early biomass production (estimated as individual plant weight) but only under cold conditions. Since no other traits were considered as independent variables explaining the variability of the weight of juvenile plant, we can conclude that early development traits were tightly interdependent. This is consistent with previous results showing that the main limiting factors for sweet corn viability were emergence and early vigor (Cartea et al. 1996a, b, Malvar et al. 2007a, b), which are the weaker aspects of *su1* viability when facing natural selection (Ordás et al. 2010). However, there was some controversy concerning the importance of germination *vs.* early vigor as the main limiting factor of *su1* fitness, as germination has been identified as the first limiting factor and early vigor as the second one in some studies (Martins and Da Silva 1998, Revilla et al. 2000, Gad and Juvik 2002, Juvik et al. 2003, Revilla et al. 2006), while early vigor was the main limiting factor in other studies (Revilla et al. 2010). The current results suggest that early vigor is more important than germination as limiting factor of *su1* fitness.

Fitness was measured as ear weight, total number of ears, and kernel weight. The main significant factors affecting these fitness traits were quite

consistent. Indeed, all three fitness-related traits were affected by total adult plants because the effect was positive for ear weight and number of ears of both mutant and wild type plants and negative for kernel weight of *su1*. Plant appearance had also positive effects on ear weight of both *su1* and *Su1* and on number of ears of *Su1*. Chlorophyll content had positive effects on ear weight both *su1* and *Su1* and on number of ears of *su1*. 100- kernel weight behaved slightly different because it was affected by some singular traits but the total effect was small. Accordingly, several authors have shown that mating ability and grain formation were also secondary limitations of *su1* fitness (Cisneros-López et al. 2010, Zhang et al. 2011).

The significant effects on fitness of genotypes, environment, and the genotype $\times$ environment interaction have been also confirmed in previous studies (Revilla et al. 2000, 2006, 2010). However, the current results show limited effects of genotypes on total number of ears of *Su1* plants. There were no significant effects of location, though some significant but small effects of repetitions on the weight of juvenile plants and kernel weight of *Su1* plants. And the genotype $\times$ environment interaction was only significant for dry weight of juvenile *Su1* plants.

Therefore, the importance of the effects affecting *sugary1* fitness varied for each trait, but also depended on genotype, environment, and their interaction. The main critical factor for *su1* fitness was early vigor, followed by emergence under cold conditions at the initial stages, while several adult traits were related with final fitness, particularly number of plants that produced grain. Breeding programs intending the production of sweet corn genotypes with better agronomic performance should focus in early vigor

and emergence, at initial stages, and plant appearance, total adult plants, and chlorophyll content at later stages.

Acknowledgments

Research was supported by the Spanish Plan for Research and Development (project code AGL2007-64218/AGR and AGL2010-22254) and the Diputación Provincial de Pontevedra. A Djemel acknowledges his fellowship from the Spanish Council for Scientific Research (CSIC).

References

- Cartea, M.E., R.A. Malvar, P. Revilla, and A. Ordás, 1996a: Identification of field corn populations to improve sweet corn for Atlantic European conditions. *Crop Sci.* **36**, 1506-1512.
- Cartea, M.E., R.A. Malvar, P. Revilla, and A. Ordás, 1996b: Improvement of early vigor and adaptation of sweet corn to the European Atlantic coast with open-Pollinated Field Corn Populations. *Maydica*, **41**, 119-125.
- Cisneros-López, M.E., L.E. Mendoza-Onofre, H.A. Zavaleta-Mancera, V.A. Gonzalez-Hernandez, G. Mora-Aguilera, L. Cordova-Tellez, and M. Hernandez-Martinez, 2010: Pollen-pistil interaction, pistil histology and seed production in A × B grain sorghum crosses under chilling field temperatures. *J. Agric. Sci.* **148**, 73-82.
- Djemel, A., B. Ordás, L. Khelifi, A. Ordás, and P. Revilla, 2012a: Genetic effects on fitness of the mutant sugary1 in wild-type maize. *J. Agric. Sci.* **150**, 603–609.
- Djemel, A., M.C. Romay, P. Revilla, L. Khelifi, A. Ordás, and B. Ordás, 2013a: Genomic regions affecting fitness of the sweetcorn mutant sugary1. *J. Agric. Sci.* **151**, 396-406.
- Djemel, A., P. Revilla, L. Khelifi, A. Ordás, and B. Ordás. 2013a: Influence of the maize *Sugary1* locus on genetics of economically important traits. *Euphytica*, **191**, 279–290
- Djemel, A., M.C. Romay, P. Revilla, L. Khelifi, A. Ordás, and B. Ordás, 2013b: Genomic regions affecting fitness of the sweetcorn mutant sugary1. *J. Agric. Sci.* **151**, 396-406

- Juvik, J.A., G.Y. Gad, H. Tae-Ho, Y. Tadmor, F. Azanza, W.F. Tracy, A. Barzur, and T.R. Rocheford, 2003: QTL influencing kernel chemical composition and seedling stand establishment in sweet corn with the *shrunk2* and *sugary enhancer1* endosperm mutations. J. Amer. Soc. Hort. Sci. **128**, 864-875.
- Gad, G.Y., and J.A. Juvik, 2002: Enhancement of seedling emergence in sweet corn by marker-assisted backcrossing of beneficial QTL. Crop Sci. **42**, 96-104.
- Malvar, R.A., M.E. Cartea, P. Revilla, and A. Ordás, 1997a: Identification of field corn inbreds adapted to Europe to improve agronomic performance of sweet corn hybrids. Crop Sci. **37**, 1134-1141.
- Malvar, R.A., P. Revilla, M.E. Cartea, and A. Ordás, 1997b: Field corn inbreds to improve sweet corn hybrids for early vigor and adaptation to European conditions. Maydica, **42**, 247-255.
- Martins, M.E.Q.P., and W.J. Da Silva, 1998: Genic and genotypic frequencies of endosperm mutants in maize populations under natural selection. J. Hered. **89**, 516-524.
- Ordás, B., V.M. Rodríguez, M.C. Romay, R.A. Malvar, A. Ordás, and P. Revilla, 2010: Adaptation of super-sweet maize to cold conditions: mutant x genotype interaction. J. Agric. Sci. **148**, 401-405.
- Revilla, P., R.A. Malvar, M.C. Abuín, B. Ordás, P. Soengas, and A. Ordás, 2000: Genetic background effect on germination of *su1* maize and viability of the *su1* allele. Maydica, **45**, 109-111.

- Revilla, P., R.A. Malvar, V.M. Rodríguez, A. Butrón, B. Ordás, and A. Ordás, 2006: Variation of *sugary1* and *shrunk2* gene frequency in different maize genetic backgrounds. *Plant Breed.* **125**, 478-481.
- Revilla, P., R.A. Malvar, B. Ordás, V.M. Rodríguez, and A. Ordás, 2010: Genotypic effects on field performance of maize plants carrying the allele *sugary1*. *Plant Breeding*, **129**, 92-95.
- SAS Institute Inc., 2008: The SAS system, version 9. SAS Inst., Cary, North Carolina, USA.
- Tracy, W.F., 1990: Potential of field corn germplasm for the improvement of sweet corn. *Crop Sci.* **30**, 1041-1045.
- Tracy, W.F., 2001: Sweet corn. In: Specialty corns. Ed. A.R. Hallauer. Boca Raton, Florida, USA: CRC Press. pp. 155-199.
- Zhang, K., Y. Li, and L. Lian, 2011: Pollen-mediated transgene flow in maize grown in the Huang-Huai-Hai region in China. *J. Agric. Sci.* **149**, 205-216.

Table 1. Multiple regression analyses, using the stepwise procedure, from the analyses of covariance for each trait on dry weight of juvenile plant, ear weight, ear number, and 100-kernel weight from maize genotypes grown at two locations in two years. Only significant coefficients (b) are shown along with their accumulated coefficients of determination (R^2)				
Covariate	<i>Su1</i>		<i>su1</i>	
	b	Cumulative R^2	b	Cumulated R^2
Dry weight of juvenile plant in field conditions (g)				
Location		0.53		0.53
Early vigor (1-9)	33.632±8.321	0.65	24.795±5.772	0.56
Repetition (Location)		0.67		
Genotype x Location		0.74		
Dry weight of juvenile plant in warm conditions (g)				
Early vigor (1-9)	0.134±0.014	0.36	0.155±0.014	0.41
Dry weight of juvenile plant in cold conditions (g)				
Early vigor (1-9)	0.170±0.007	0.75	0.123±0.008	0.61
Emergence (N)	-0.038±0.010	0.77	-0.029±0.008	0.64
Ear weight in field conditions (g)				
Plant appearance (1-9)	0.704±0.050	0.62	0.426±0.064	0.66
Total adult plants (N)	0.068±0.008	0.68	0.051±0.012	0.68
Chlorophyll content (CCM)	0.027±0.005	0.71	0.033±0.007	0.70

Genotype				0.77
Repetition (Location)				0.80
Number of ears in field conditions (N)				
Total adult plants (N)	0.900±0.067	0.41	1.158±0.064	0.50
Chlorophyll content (CCM)			0.342±0.039	0.63
Genotype		0.59		
DWJ (g)	0.012±0.003	0.61		
Plant appearance (1 – 9)	2.031±0.430	0.63		
Silking (days)			-0.460±0.122	0.65
100-K weight in field conditions (g)				
Ear weight (kg)	1.049±0.165	0.20	1.800±0.159	0.30
Total adult plants (N)			-0.129±0.032	0.34
Number of kernels rows (N)	-0.695±0.137	0.29	-0.521±0.132	0.38
Repetition (Location)		0.35		
Ear length (cm)	0.466±0.123	0.36		
¹ b: coefficient of regression. R ² : coefficient of determination.				
***: All coefficients included are significant at P=0.001				