Genetic variation between Spanish and American versions of sweet corn inbred lines

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Abstract

Conservation of maize inbred lines in different stations causes variability among strains. The objective of this research was to determine agronomic and molecular differences in American sweet corn inbreds maintained in Spain. American and Spanish strains of five sweet corn inbred lines were characterized by using 34 RAPD primers that produced 168 consistent bands. Strains of four of these inbreds were crossed in a diallel design, and hybrids were evaluated in four environments in northwestern Spain. The RAPD characterization showed no differences between strains for two inbreds, while divergence between strains was largest for the inbred I5125. Most primers did not reveal any variability between pairs of strains, while some primers produced variations at high rates. Differences in agronomic performance among Spanish and American strains were most important for P51, followed by P39, while strains were not significantly different for I453 or I5125. Molecular differences between strains were not related to agronomic performance. Residual heterozygosity or outcrossing cannot explain these results. Lack of adaptation could have caused stress-induced mutagenesis. Natural selection could have eliminated unfavorable selective mutations, but neutral mutations can be found at the molecular level and favorable mutations could have been selected at the agronomic level.

Key words: Zea mays - variability - RAPD - germplasm conservation.

Variability among strains of maize inbreds has been observed since the beginning of the inbred-hybrid system (Jones 1945) until the development of molecular markers (Tracy et al. 2000). Jones (1945) found several degenerative single-gene changes in five inbreds that produced large heterosis in crosses to their respective wild types, but the mutant lines did not reduce the yield of crosses to unrelated lines. Based on previous works and their own experiments, Tracy et al. (2000) concluded that most observed changes involve several loci, that changes are produced at different rates depending on the genotype, and that the causes of such changes are unclear.

Fleming et al. (1964) reported divergence in conservation of maize inbred lines in different locations, and suggested that genetic changes were due to residual heterozygosity or mutation. Russell and Vega (1973) evaluated several maize inbred lines maintained at different stations for ten years and found significant differences for several quantitative traits among some inbreds, though most changes had no practical importance. These authors reported that genetic changes were independent and occurred continuously. Bogenschutz and Russell (1986) concluded that the method used to maintain the inbreds induces genetic variation. Gethi et al. (2002) have shown that there was small but significant variation among different strains of important inbred lines. According to these authors, such variation could affect germplasm conservation and several steps of genetic studies.

Genetic changes occur during conservation (Murata 1991), and multiplication of germplasm can be produced through selection, errors, and

outcrosses (Parzies et al. 2000, Kameswara Rao and Jackson 1996). Revilla et al (2004) reported variability during conservation of maize inbred lines and suggested natural selection for viability and vigour within inbred lines during storage. Therefore, germplasm conservation and multiplication may have a major impact on genetic composition and structure of gene pools.

There are no published reports on the variability among inbred strains maintained at environments were they were poorly adapted. Sweet corn is poorly adapted to northwestern Spain (Ordás et al. 1994). The objective of this research was to determine agronomic and molecular differences in American sweet corn inbreds maintained in Spain.

Materials and Methods

Plant material: American (US) and Spanish (SP) strains of the five inbred lines I453, I5125, P39, P51, and C13 of sweet corn, Zea mays L., were characterized by using 34 Random Amplified Polymorphic DNA (RAPD) primers that yielded 168 loci. The US seed was provided by the North Central Regional Plant Introduction Station (Ames, Iowa) in 1997. These inbred lines had been released in the USA more than thirty years before they were introduced into Spain and had been self-pollinated enough times to be considered completely homozygous. The University of Minnesota provided the SP seed of I453 and P51 in 1976, and of C13 in 1978, and the seed of I5125 and P39 was provided in 1976 by Crookham Company. The SP strains have been multiplied in northwestern Spain 10 times, except for P51 than was multiplied 7 times. Conservation was carried out by the standard procedure consisting on self-pollinating the original seed and choosing three representative ears from the descendants of one ear. This process is repeated every time the inbred line has to be multiplied. Therefore, the seed available today in Spain descends from one unique US seed.

Genomic DNA isolation and amplifications: Maize kernels were germinated at 25 ºC for a week and one of the first leaves was collected and frozen immediately in liquid nitrogen. Genomic DNA was extracted from individual leaves of each inbred line according to Liu & Whittier (1994) with

slight modifications. Amplifications were conducted with 10-mer primers from Operon DNA Technologies Inc (Alameda, California, USA). From an initial screening of 80 decamer primers (kits A, B, C, and D) we selected 34 decamer oligonucleotides that showed consistent banding patterns. For the RAPD reactions 25 ng of genomic DNA were used as template in a final volume of 25 µl containing 1 × reaction buffer (20 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1mM DTT, 50% glycerol, 0.5% Tween 20 and 0.5% Nonidet P40), 3.0 mM Mg Cl_2, 200 µM of each dNTP (Ecogen), 30 ng of each primer and 1 U Taq polymerase (ECOGEN, Barcelona, Spain). Amplifications were performed in a PTC-100 Thermal Cycler (MJ Research, Watertown, Massachusetts, USA) under the following conditions: DNA denaturation was done at 95 ºC for 5 min followed by a 45 cycle amplifications (95 ºC, 1 min; 35 ºC, 1 min.; 72 ºC, 2 min) and a final extension step at 70ºC for 7 min. RAPD products (20 µl) were separated by gel electrophoresis in 1.5% agarose gels in TAE buffer. The polymorphic fragments were named by the primer code (OP Operon), the kit letter and its approximate size in base pairs. Only bands that gave a reproducible score in the duplicated experiment were included in the final analysis.

Agronomic analysis: The US and SP strains of I453, I5125, P39, and P51 were crossed in a diallel design in 1997. The hybrids were planted in Pontevedra (northwestern Spain) (20 m above sea level) in experiments arranged as

randomized complete block designs with two replications per environment. This location has a humid climate with annual rainfall about 1600 mm. Planting dates were the 20 of May and the 17 of June in 1998, and the 19th of May and the 15th of June in 1999. Plant density was approximately 60 000 plants ha^{-1}. Trials were harvested when the ear was dry (end of October or November) in order to measure yield as seed production.

Lack of adaptation of maize from USA to European conditions is the main selective force that might alter the genetic constitution of germplasm resources; therefore, we measured the main adaptive-related traits, namely early vigor and dry grain yield. Early vigor is a key adaptive trait for the introduction of maize from the USA in northern and western Europe, where springs are too wet and cool for maize germination. The estimation of early vigor takes into account size, color, and canopy development approximately one month after planting, using a scale from 1 (weak plants) to 9 (vigorous plants), where 5 represents the average plant development of the trial. Dry grain yield is the main adaptive trait because summarizes the total fitness of the plant. Dry grain yield was recorded in kg ha^{-1} at 140 g kg^{-1} grain moisture.

The analysis of variance was made considering hybrids and inbred strains as fixed effect, and any other source of variation as random effect. The analysis was made for each environment separately and combined over environments. A separate analysis of variance was performed for each hybrid, considering strain combinations as fixed effects. For each hybrid, means were compared among the four possible strain combinations, Spanish × Spanish,

Spanish × USA, USA × Spanish and USA × USA. All analyses were performed using the SAS program (SAS, 2000).

Results

RAPD analysis

The inbreds P51 and C13 did not show differences between US and SP strains for the 168 bands obtained from 34 RAPD primers, while the other three inbreds had some variability between strains (Table 1). The inbred I5125 showed the largest variability, 8% of the bands being different between the SP and the US. The strains of I453 differed for 2% of the bands, and the strains of P39 for 1%.

Only eight of the 34 random primers showed differences between strains and, from them, OPC11 differed between strains for the three inbreds and OPC4 for two of the inbreds. Besides, for most of the primers, none of the bands varied between strains, while for some primers, as OPC2, OPC4, OPC8, and OPC11, most of the bands differed between strains.

Field trials

Environments and hybrids and the genotype × environment interaction were significantly for vigor and yield (data not shown). When the analyses of variance were made for each hybrid, differences among strain combinations were always significant, while the genotype × environment interaction was significant only for early vigor of I5125×P51.

Strain combinations SP×SP were more vigorous than US×SP for the hybrids I453×I5125 and I5125×P39, and than the combination SP×US and US×US for the hybrid P39×P51 (Table 2). For the hybrid I453×P39, the most vigorous combination was SP×US, and for I5125×P51, the combination US×SP was more vigorous than SP×US. These results suggest that Spanish strains had experienced some improvement of early vigor while maintained in the wet and cool springs of northwestern Spanish springs.

Concerning yield, SP×US combinations were superior to SP×SP for I453×P39 and to US×SP for I5125×P39, and US×SP yielded more than SP×SP for I5125×P51 (Table 2). The strain combination SP×SP had higher yield than SP×US and US×US for P39×P51. Therefore, although differences among strain combinations were not as significant for yield as they were for early vigor, heterosis in crosses between Spanish and USA strains was larger than in crosses SP × SP or US × US, when available.

Discussion

Variation in SP strains from the original US strain involves both the appearance and disappearance of RAPDs bands (Table 1), therefore, variability has aroused by several mechanisms. Some of the sequences polymerized from these primers varied between strains more than others. Variability at the molecular level was not distributed uniformly among the inbreds or randomly along the genome, suggesting that whatever the cause of variation might be, some genotypes and genomic regions are more affected than others.

Both early vigor and yield show that Spanish and US strains are not equivalent and that hybrids between Spanish strains were more vigorous and had less heterosis than crosses between Spanish and US strains. Early vigor is a serious limitation for most US hybrids when grown in Europe (Ordás et al. 1994, Revilla et al. 1999). Therefore, these results show that ex-situ conservation resulted in selection for early vigor and, to a lesser extend, for yield, within these sweet corn inbred lines.

Tracy et al. (2000) detected heterosis among strains of the sweet corn inbred P39. Although we could not calculate heterosis due to lack of US seed and the extremely poor performance of inbreds, divergence among US and Spanish strains seems to more related to selection for early vigor that to heterosis-related effects.

Residual heterozygosity was not the only explanation for the observed variability among P39 strains, as reported by Tracy et al. (2000), and we

completely agree with that conclusion because SP strains are not morphologically different to US strains. These authors considered that the possibility of pollen contamination or outcrossing was unlikely because of a number of reasons, including that the strains are morphologically similar, which is also true in the present experiments.

Possible explanations for the high mutability (8%) of I5125, compared to the other four inbreds, could be the phenomenon known as hyper-mutation (Foster, 2000). Besides, the preferential variation in some sequences could be that some selective agent is causing adaptive mutations (Foster, 2000). Besides hyper-mutation, other models that have been proposed to explain the appearance of adaptive mutations are the direct mutation model (Cairns et al. 1988) and the amplification mutagenesis model (Andersson et al. 1998). Stress induced mutagenesis has been extensively reported in bacteria and yeast, either as a consequence of the stress response of the organism, or as a result of selection (Tenaillon et al. 2004, Hersh et al. 2004). Adaptive mutations could be playing a role in adaptation of maize to marginal or exotic environments, thus disturbing germplasm conservation but facilitating breeding for adaptation.

Agronomic differences between SP and US strains were most important for P51, followed by P39, while strains were not significantly different for I453 or I5125. Differences between strains, as deduced from RAPD data, were not related to agronomic performance. Contrarily, Tracy et al. (2000) concluded that there was a relationship between molecular diversity

and improved performance. Therefore, these data do not support the hypothesis that selection for adaptation within inbred lines has generated molecular variability. However, selection has actually happened, and variability is indispensable for selection to be efficient, therefore, some other sort of variability must be underlying agronomic differences between strains of these inbred lines.

Variation was probably due to lack of adaptation, which might have raised the mutation rate and caused adaptive mutations by several mechanisms, such as transposons. The conservation of inbred lines implies some natural selection that would have eliminated unfavorable selective mutations. Therefore mainly neutral mutation can be found at the molecular level, while agronomic variation reflects the few favorable selective mutations that had been conserved. Besides, other explanations, such as epigenetic variation or gene silencing are possible, but further research is needed to board this issue.

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Headings of Tables

Table 1. Polymorphism for 168 RAPD bands from 34 primers between U.S.A and Spanish strains of five U.S.A. sweet corn inbred lines that are conserved in Spain since 1976.

Table 2. Means for early vigor[1] and yield for crosses among the Spanish (SP) and the U.S.A. (US) strains of four sweet corn inbred lines evaluated in two planting dates and two years.

Table 1

Inbred	Primer	Locus	U.S.A.	Spain
I5125	OPA8	650	-	+
	OPB17	700	+	-
	OPC2	890	-	+
		900	+	-
		950	-	+
		1200	-	+
	OPC4	850	+	-
		1050	+	-
		1100	-	+
	OPC8	1200	-	+
		1250	+	-
	OPC11	1600	+	-
	OPC14	1000	+	-
		1100	+	-
I453	OPC6	700	-	+
	OPC11	1400	-	+
		1600	+	-
P39	OPC4	1100	-	+
	OPC11	1600	+	-

Table 2

Early vigor[1] (1-9)	Strain combination			
Hybrid	SP × SP	SP × US	US × SP	US × US
I453 × I5125	7.1a	6.3ab	6.1b	[3]
I453 × P39	5.0b	6.1a	4.7b	[3]
I453 × P51	6.9a	6.4a	6.3a	[3]
I5125 × P39	6.8a	5.9ab	5.5b	6.0ab
I5125 × P51	5.5ab	4.9b	6.9a	[3]
P39 × P51	7.1a	4.7b	6.8a	4.3b
Yield (kg ha^{-1})	SP × SP	SP × US	US × SP	US × US
I453 × I5125	517a	528a	535a	[3]
I453 × P39	267b	361a	274b	[3]
I453 × P51	416a	440a	405a	[3]
I5125 × P39	400a	466a	284b	413a
I5125 × P51	344b	397ab	524a	[3]
P39 × P51	472a	327b	422ab	340b

[1] Early vigor was estimated by using a scale from 1 (weak plants) to 9 (vigorous plants)

[2] Means followed by the same letter, within the same row do not differ significantly; following Fisher protected LSD (P=0.05)

[3] Due to the poor adaptation of US inbreds, there was not enough seed from these hybrids for trials.