Crop Breeding and Applied Biotechnology 11: 64-69, 2011
Brazilian Society of Plant Breeding. Printed in Brazil

Genetic variability in populations of sweet corn, common corn and teosinte

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Received 17 May 2010

Accepted 15 September 2010

ABSTRACT - *The maize* (Zea mays L. ssp. mays) *has several related species, called teosinte, which are distributed in various subspecies of Zea and other genera. Among the different types of corn, sweet corn shows a great potential for human food. This type was originated from mutations, which increased the amount of polysaccharide in the endosperm. In Brazil there are populations of sweet corn, common maize and teosinte, however, little is known about their genetic variability. Hence, the aim of this present paper was to analyze the genetic variability in two populations of sweet corn (BR 400 and BR 402), two common corn (Pampa and Suwan) and teosinte, using microsatellite markers. The results showed a low intra-population genetic variability in populations of maize, and high variability for the population of teosinte, suggesting that the maize populations may have limitations in future cycles of breeding.*

Key words: *Breeding, SSR markers, genetic resources*.

INTRODUCTION

The Maydeae tribe contains several genera, including the genus *Zea*, which presents a high economic importance including cultivated species of corn and teosinte. This genus has several species of teosinte, such as *Z. diploperennis* (Iltis, Doebley and Guzman), *Z. perennis* (Hitda), *Z. luxurians* (Durieu and Aschersonia) and *Zea mays*, which includes the subspecies maize (*Z. mays* L. ssp. *mays*) and teosintes *Z. mays* L. ssp. *parviglumis* (Iltis and Doebley), *Z. mays* L. ssp. *Huehuetenangensis*, and *Z. mays* L. ssp. *mexican* (Schrader) (Iltis and Doebley 1980, Molina and Narang 1987). Teosinte has been considered a genetic source for the improvement of agronomic characteristics of maize (Reeves 1950, Cohen and Galinat 1984, Lubberstedt et al. 1998). Spontaneous hybrids have occurred between these species, allowing the transfer of genes between them (Ellstrand et al. 2007).

Among various types of corn, sweet corn has the greatest potential for use as human food. Sweet corn is originated through mutation and it is characterized by having at least one of the eight mutant genes. The main genes are: *Shrunken*-2 (sh2) on chromosome 3, *Brittle* (bt) and *Amylose Extender* (ae) on chromosome 5, Sugary Enhancer (if), Sugary (su) and "Brittle-2" (BT2) on chromosome 4; "Dull" (du) on chromosome 10, and Waxy (wx) on chromosome 9 (Tracy et al. 2006).

Some cultivars open pollination has been done in Brazil by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) through mass selection of germoplasm introduced from Hawaii (Super sweet, sweet of Cuba). The population Suwa (BR-105) is originally from Thailand and derived from recurrent selection S1, where there was

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intense selection. The population Pampa (BR 5202) is a variety developed for the South of Brazil. These varieties are important genetic resources for the breeding of maize in Brazil and knowledge of the genetic viability is very important for genetic gain in breeding programs.

Several methods in molecular genetics have been developed in order to characterize genotypes, from which the DNA-based markers are the most commonly used. Among the major markers, the microsatellites or SSR (simple sequence repeats) have advantages, such as: frequently found in the genome; present in many eukaryotic organisms; generally have a high level of polymorphism and are co-dominants (Ek et al. 2005, Xu and Crouch 2008, Agarwal et al. 2008). Theses molecular markers have been used to estimate variability in maize (Bered et al. 2005, Silva et al. 2009). Hence, the aim of this present paper was to analyze the genetic variability in populations of sweet corn, common corn and teosinte.

MATERIAL AND METHODS

The genotypes were cultivars of open pollination of southern Brazil, and the teosinte represents a wild population from Mexico, also cultivated in southern Brazil. All accesses were obtained from Embrapa Clima Temperado, Pelotas, Brazil and from Embrapa Milho e Sorgo, Sete Lagoas, Brazil.

For molecular study, 10 primers (Table 1) from the maize database were used (http://www.maizegdb.org/ssr.php). The primers were analyzed in two populations of sweet corn (BR400-bt and BR402-su), in two of common corn (Suwan and Pampa) and in one population of teosinte. Each population was represented by 17 to 20 individuals and the genomic DNA extractions were carried out by the CTAB method (Saghai-Maroof et al. 1984), concentrations were estimated using a spectrophotometer (GENESYSTM 2).

PCR reactions were carried out in a 25 mL volume, containing 60 ng genomic DNA, buffer 10X (Gibco BRL), 1.5 mM $MgCl_2$ (Gibco BRL), 0.2 mM dNTP (Gibco BRL), 1 U of Taq-DNA polymerase (Gibco BRL), 30 pmol each forward and reverse of primers. The thermal cycling condition amplifications were performed in a thermocycler (PTC-100, MJ Research, Inc.), with 18 cycles of 94 ºC for 1 min, followed by a decrease of 1 ºC in every 2 each (64 ºC a 55 ºC), and 72 °C for 1 min. Additional 30 cycles at 94 °C for 1 min, 55 ºC for 1 min and 72 ° C for 1 min. Products of the PCR were separated by electrophoreses on 3.2 % agarose gel using a 100pb ladder as a size standard and stained with ethidium bromide.

The number of alleles per locus, frequency of alleles of the populations, and polymorphism information content values (PIC) were calculated using the equation:

$PIC = 1 - \Sigma pu^2$, where pu^2 is the frequency of the *u*

Table 1. Primers of microsatellites, sizes and number of alleles per locus and polymorphism (PIC) in four populations of maize and teosinte

Primer	Chromosome	Locus	Nr . of alleles	Size of alleles (pb)	PIC
umc1064	1	Ferredox3	3	140-165	0.64
umc1185	2	Oleosin1	3	115-140	0.48
umc1622	2	Cytokinin response regulator1	3	40-90	0.32
phi029	3	Triose phosphate isomerase44	3	144-165	0.55
phi021	4	Alcohol dehydrogenase2	6	85-114	0.76
nc004	4	Alcohol dehydrogenase2	4	150-180	0.68
nc012	6	Pyruvate, orthophosphate dikinase1	3	110-130	0.39
umc1545	7	Heat shock protein3	3	48-80	0.43
umc1202	8	Ribosome inactivating protein1	3	140-161	0.45
phi032	9	sucrose synthase1	2	215-244	0.26
Mean			3.3		0.50

allele. The genetic distances were calculated using the coefficient Nei 1972, which expresses the differences in allele frequencies among populations. The distance values which were utilized to build dendograms, obtained through the UPGMA procedure, using the NTSYS 2.1 software (Rohlf 2000). The analysis of molecular variance was generated using the Arlequin 2.01 software (Schneider et al. 2000), using the size of each haplotype in pairs of bases.

RESULTS AND DISCUSSION

In 10 loci analyzed, a mean 3.3 alleles were detected, and a number ranging from two in phi032 locus to six in the phi021 locus (Table 1). Ten alleles were specific to the populations of sweet corn and common corn and the frequency of null data was less than 10%. The sizes of the alleles found ranged from 40 to 244 bp (Table 1) and the PIC ranged from 0.26 (phi032) to 0.76 (phi021). A mean 2.44 polymorphic alleles per locus was observed (Table 2) and the percentage of polymorphic loci ranged from 50 to 80 % in the populations. For the genetic diversity, the populations of maize showed lower values in relation to teosinte (Table 2).

The cluster analysis using the coefficient Nei 1972 indicated that the populations of sweet corn (BR400 and BR402) are more related; the common corn population was located in an intermediate position in relation to teosinte (Figure 1).

The genetic structure of the populations obtained by analysis of molecular variance (AMOVA) showed that 56.62% of the allelic variations were observed among populations, only 11.5% of the variation was between individuals within each population, and 32.82% of the variation showed within individuals (Table 3).

The analysis of genetic diversity within populations of common corn and sweet corn showed low diversity (0.22 to 0.33), compared to the main populations of CIMMYT (International Maize and Wheat Improvement Center), which were analyzed by SSR and showed values of 0.45 to 0.61 (Reif et al. 2004). Similar results were found by Yao et al. (2007), with variable rates ranging from 0.59 to 0.81 in accesses of the Chinese corn, also analyzed with SSR.

Many studies have shown that the genetic diversity in maize has been reduced during the process of domestication (Doebley et al. 1984), and during the cycles of selection in breeding, setting some alleles in specific populations (Labate et al. 1997, Hinz et al. 2005, Vigouroux et al. 2005). The data in this study showed low genetic variability in the populations, suggesting that use for breeding shows limitations in genetic gain, especially in recurrent selections. The recurrent selection in corn has decreased the genetic variability after cycles of selection (Labate et al. 1997). Hinz et al. (2005) analyzed the variability of SSR in two large populations of corn (Iowa Corn Borer Synthetic and Iowa Stiff Stalk Synthetic), showing that after 15 cycles of recurrent selection, the inter-population genetic variability decreases significantly, leaving the greatest portion of the variability within and between individuals within each population (78.32%).

The low variability populations of sweet corn may be explained by the fact that they originated from a mass selection of a genotype from Hawaii, and that no mixtures occurred of other breeds of sweet corn, providing a decrease in intra-population genetic variability. On the other hand, the population of teosinte, which is grown in southern Brazil as forage, showed greater genetic variability in relation to maize, reflecting the low selection pressure that this population has been subjected to. Analysis of genetic diversity in natural populations of teosinte (*Z. mays* ssp. *mexicana*) using 93 SSR, showed a high genetic variability (0.85) compared to the one found

Table 2. Genetic variability within populations Suwan, Pampa, BR400, BR402 and teosinte using 10 primers of microsatellites

Populations	Individuals	Polymorphic markers (%)	Total of alleles	Alleles/locus polymorphism	Genetic diversity
Suwan	20	50	11	2.20	0.22
Pampa	17	80	18	2.25	0.32
BR400	20	50	14	2.80	0.33
BR402	20	70	17	2.43	0.31
Teosinte	19	80	20	2.50	0.40
Mean	19.2	66		2.44	0.31

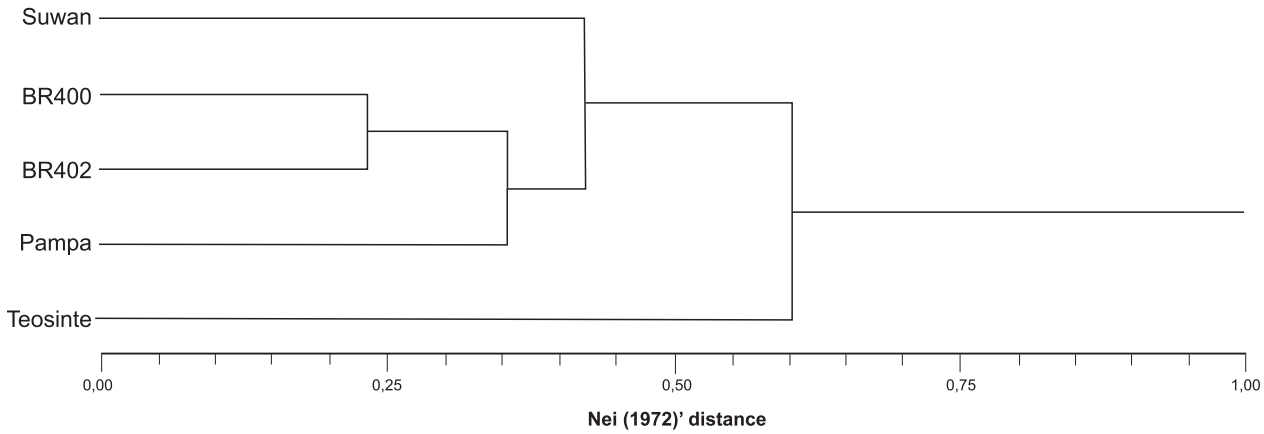

Figure 1. Dendogram of four populations obtained by the matrix of genetic distance, based on the coefficient of Nei (1972).

Table 3. Analysis of molecular variance (AMOVA) of microsatellite data of five populations (Suwan, BR400, BR402, Pampa and Teosinte)

Source of variation	df	SS	Variance component	Variance component (%)
Between population	4	19.456	0.12423	55.62
Between individuals within population	91	11.31	0.02568	11.56
Within population	96	7	0.07292	32.82
Total	191	37.766	0.22214	100

in this study, suggesting that the population of teosinte has considerable genetic diversity (Fukunaga et al. 2005).

The analysis of genetic divergence among populations of maize and teosinte, indicated that the population of sweet corn showed higher genetic divergence in relation to teosinte. Both populations of sweet corn and common corn are "Dent" populations, however, sweet corn is originated from natural mutations, which may have a smaller reduction in variability mainly due to the founder effect, which would result in a lower genetic variability. Data from this study suggest that populations of teosinte showed high genetic variability than the populations of maize and genetic variability in populations of maize were low.

Estimates of genetic variability have been obtained with the most varied types of molecular markers, which reflect the similarity between genotypes from a direct sample of the genome. In breeding, the genetic variation in agronomical and physiological traits are more relevant to the selection. However, estimates of variability with molecular markers have been the strategy adopted by the breeding program, allowing the organization of the germplasm and plan strategies for breeding. In this study, the genetic similarity detected among populations suggests a reduced genetic gain in breeding programs.

Variabilidade genética em populações de milho doce, comum e teosinto

RESUMO - *O milho* (Zea mays L. ssp. mays) *possui diversas espécies relacionadas, chamadas teosinto, que estão distribuídas em várias subespécies de Zea e em diversos outros gêneros. Entre os tipos de milho, o milho doce apresenta um grande potencial para consumo in natura, sendo originário de mutações que aumentam o teor de polissacarídeo no endosperma. No Brasil existem populações de milho doce, comum e teosinto, cujo nível de variabilidade genética não é conhecido. O objetivo desse trabalho foi analisar a variabilidade genética em duas populações de milho doce (BR 400 e BR 402), duas de milho comum (Pampa e Suwan) e uma de teosinto, usando marcadores microssatélites. Os resultados mostraram baixa variabilidade genética intrapopulacional nas populações de milho, e relativamente alta para a população de teosinto, sugindo que essas populações de milho podem apresentar limitações em futuros ciclos de melhoramento genético.*

Palavras-chave: melhoramento genético, marcadores SSR, recursos genéticos.

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