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SSR MARKERS IN CHARACTERISATION OF SWEET CORN INBRED LINES

Jelena SRDIĆ, Ana NIKOLIĆ and Zorica PAJIĆ

Maize Research Institute, „Zemun Polje“ - Belgrade, Serbia

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Sweet corn differs from field corn in many important traits. So its breeding although includes some standard procedures demand application of techniques that are important for determining special traits, all because of the specificity of its usage. Application of molecular markers becomes almost a necessity for the breeding of sweet corn, especially because this is the type of maize in which still no definitive heterotic patterns have been determined. So getting to know genetic divergence of the sweet corn inbred lines is of great importance for its breeding.

In this paper we analyzed genetic similarity of six sweet corn inbreds based on SSR markers. 40 SSR primers were used in DNA amplification. Results were compared and correlated with the data on specific combining ability, obtained by the diallel analysis.

The results of SCA were in concurrence with genetic similarity. Values of rank correlation coefficient were negative, indicating that more

Corresponding author: Jelena Srdić, Maize Research Institute, Slobodana Bajića 1, 11080 Zemun – Belgrade, Serbia, phone: #381 11/3756-704, e-mail: jsrdic@mrizp.co.yu

similar inbred lines had smaller estaimates of SCA, and lines that were less simliar had higher estimates of SCA. Rank correlation coefficient between SCA and GS according to Dice coefficient was between -0,16 and -0,57*.

Key word: genetic similarity, SSR, sweet corn, SCA.

INTRODUCTION

Genetic diversity in maize plays a key role in planning breeding programs (REIF *et al.,* 2003). Knowledge of germplasm diversity and of relationships among breeding material has significant impact on improvement of crop plants and is useful in planning crosses for hybrid and line development and assigning lines to heterotis groups (HALLAUER *et al*., 1988).

In corn (*Zea mays* L.), sweet corn has not benefited from yield gains due to genetic improvement as field corn has. Possible reasons include the narrowness of the genetic base of sweet corn, the lack of heterotic groups, and the greater effort devoted to improving yield in field corn (TRACY, 1990). Also sweet corn breeding has been directed toward product quality and appearance as well as yield and agronomic performance (TRACY, 1994). Historical date indicate that present sweet corn germplasm originates from only couple self-pollinate varieties such as Golden Bantam, Country Gentleman и Stowell's Evergreen (GERDES and TRACY, 1994), that resulted in a very narrow genetic variability of sweet corn today.

Application und utilization of heterotic patterns in standard grain quality maize had significant influence on improvement of breeding programs in yield increase, making more efficient testing of hybrids and increasing the probability of identifying hybrids with desirable traits TRACY (1990). Various researches could not, up to now, identify such models among sweet corn inbreeds, and one of the main causes is the narrow genetic variability of this type of maize. As a result, there are no clearly defined heterotic groups in sweet corn germplasm like those in field maize (GOODMAN, 1985).

The development of molecular markers provides a tool for assessing the genetic diversity at the DNA level in plant species (MELCIHNGER and GUMBER, 1988). In particular, SSR markers show potential for large-scale DNA fingerprinting of maize genotypes due to the high level of polymorphism detected (SMITH *et al.,* 1997), their analyses by automated systems (SHARON *et al*., 1997), and their high accuracy and repeatability (HECKENBERGER *et al*., 2002).

AMORIM *et al.* (2003), detected genetic variability by the methods of RAPD and SSR markers applied on 13 sweet corn genotypes. While the SSR markers detected significantly larger variability among genotypes, produced clusters showed better agreement of RAPD markers with pedigree data than those produced by SSR markers.

MATERIAL AND METHODS

In this study we analyzed 6 sweet corn inbred lines selected in Maize Research Institute. Their origin was from introduced varieties from Mexico, Iran, populations of sweet corn made in Maize Research Institute Zemun Polje, and F_2 population of hybrid Jubilee. Those six lines were crossed into 15 crosses in a diallel without reciprocal combinations. The experimental design was randomized complete block design with three replications in three treatments. The treatments were: 1. no irrigation, 2. with irrigation and 3. late sowing ANOVA of combining ability for yield was evaluated after GRIFFING (1956).

Genetic similarity was determined by the technique of SSR markers. Genomic DNA was isolated from leaves by Mini CTAB method (WILLIAMS *et al*., 1993). The set of 40 primers was used for DNA amplification. The amplified bands were scored based on 1/0 (presence/absence) system. Genetic similarity among all possible pairs of inbred lines were estimated from SSR data according to Dice coefficient (DICE, 1945). Cluster analysis was carried out on the matrix of genetic distances by the UPGMA method, and the dendrogram was constructed with NTSYS-pc software (ROHLF, 2000). PCA was constructed by the GGE biplot program, and the results are given in 2D diagram form.

Correlations between GD and SCA, based on RAPD markers were calculated by Spearman's rank correlation coefficient (ZAR, 1999).

RESULTS

Results of the ANOVA revealed significant effects of GCA and SCA for ear yield. However, higher value of SCA pointed out that dominant gene action had the crucial effect on the inheritance of this trait. The ratio of GCA/SCA which was <1, confirmed that non-additive gene action had more influence on the inheritance of ear yield of sweet corn (Table 1).

Table 1. ANOVA of combining ability for ear yield

Sources of Var.	D.F.	Mean Square		
		Location 1	Location 2	Location3
GCA	5	1,83*	1,63	3,20**
SCA	15	12,66**	13,33**	7,70**
Error	40	0,70	0,67	0,33
GCA/SCA		0,14	0,12	0,42

*,**- significant at the 0,05 and 0,01 probability level

Three out of 15 hybrid combinations had significant and highly significant estimates of SCA in all three treatments. Hybrid combination ZPLsu5 x ZPLsu3 had two highly significant estimates, and one significant and was ranked 2nd and 3rd among all hybrid combinations. Also hybrid combinations ZPLsu2 x ZPLsu1 and ZPLsu5 x ZPLsu2 were highly ranked and also with significant and highly significant estimates of SCA in three treatments (Tab. 2).

The lowest estimates among all hybrids had ZPLsu5 x ZPLsu4, which was always ranked 15th, and with all three negative estimate of SCA (Table 2).

Table 2. SCA for six sweet corn inbred lines

Hybrid combinations	Treatment1		Treatment 2		Treatment 3	
	SCA	Rank	SCA	Rank	SCA	Rank
ZPLsu 2 x ZPLsu 1	2,26*	4	3,30**	1	2,05*	3
ZPLsu 3 x ZPLsu 1	1,10	11	-0,78	13	0,04	14
ZPLsu 4 x ZPLsu 1	2,08*	6	2,65*	6	1,37	9
ZPLsu 5 x ZPLsu 1	1,84	7	3,02**	2	1,59*	6
ZPLsu 6 x ZPLsu 1	1,48	10	0,67	12	0,43	13
ZPLsu 3 x ZPLsu 2	2,79*	3	1,93	10	1,91*	4
ZPLsu 4 x ZPLsu 2	1,77	9	2,79*	4	3,24**	1
ZPLsu 5 x ZPLsu 2	4,51**	1	2,58*	8	1,6*	5
ZPLsu 6 x ZPLsu 2	1,84	8	2,22*	9	1,58*	7
ZPLsu 4 x ZPLsu 3	2,18*	5	1,88	11	1,4*	8
ZPLsu 5 x ZPLsu 3	3,13**	2	2,8*	3	2,91**	2
ZPLsu 6 x ZPLsu 3	0,86	12	2,61*	7	1,05	10
ZPLsu 5 x ZPLsu 4	-1,93	15	-1,04	15	-0,51	15
ZPLsu 6 x ZPLsu 4	0,73	14	2,67*	5	1,01	11
ZPLsu 6 x ZPLsu 5	0,76	13	-1,04	14	0,75	12
SE	1,02		1,00		0,70	

*,**- significant at the 0,05 and 0,01 probability level respectively

Results of the polymorphism of SSR markers in this study showed that each of the analyzed genotype had specific profile. From the 40 primers applied in this study 84 bands were scored. The number of bands per primer was from 1 to 4. 32 primers were polymorphic, while 8 gave monomorfic picture.

The genetic similarity calculated for 15 combinations of 6 sweet corn inbred lines, based on SSR markers ranged from 0,381 in the combination

ZPLsu 6 x ZPLsu 2, to 0,744 between ZPLsu 6 and ZPLsu 5. The average value was 0,533 (Table 3).

Table 3. Genetic similarity of six sweet corn inbred lines by SSR markers

Genotype	ZPLsu 2	ZPLsu 3	ZPLsu 4	ZPL su 5	ZPLsu 6
ZPLsu 1	0,390	0,524	0,409	0,667	0,651
ZPLsu 2		0,439	0,651	0,390	**0,381**
ZPLsu 3			0,545	0,476	0,558
ZPLsu 4				0,545	0,622
ZPLsu 5					**0,744**

Cluster analysis for estimates of genetic similarity of six sweet corn inbreds showed clear grouping of lines into two subclusters. The first subcluster encompassed four inbreds. ZPLsu 5 and ZPLsu 6, that had the highest estimate of GS were most closely attached in this subcluster. This subcluster had also ZPLsu 1, and ZPLsu 3 which was loosely attached to other three lines. The second cluster consisted of lines ZPLsu2 and ZPLsu 4. (Figure 1.)

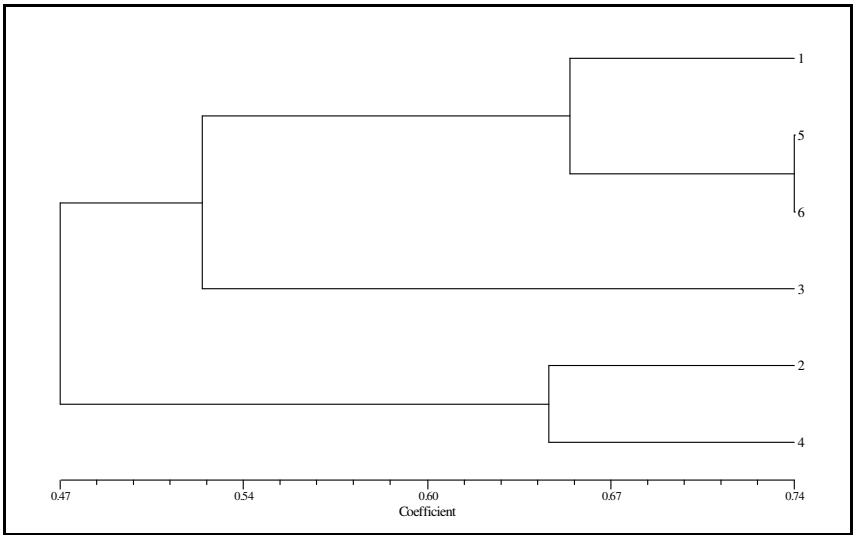

Figure 1. Cluster analysis of genetic similarity for six sweet corn inbred lines

If the grouping of genotypes by CA is taken for assuming the grouping of lines in heterotic groups, this figure correspond with the results of SCA estimates. Hybrid combinations ZPLsu 2 x ZPLsu 1 and ZPLsu 5 x ZPLsu 2 had highest estimates of SCA and in CA were grouped in different subclusters (Figure 1).

The grouping of lines was the same by the PCA as the one based on cluster analysis. Lines ZPLsu 5 and ZPLsu 6 were clossest, as well as lines ZPLsu 4 and ZPLsu 2 on the other side, which was in accordance with CA. The genotype ZPLsu 3 was most distant from all others (Figure 2).

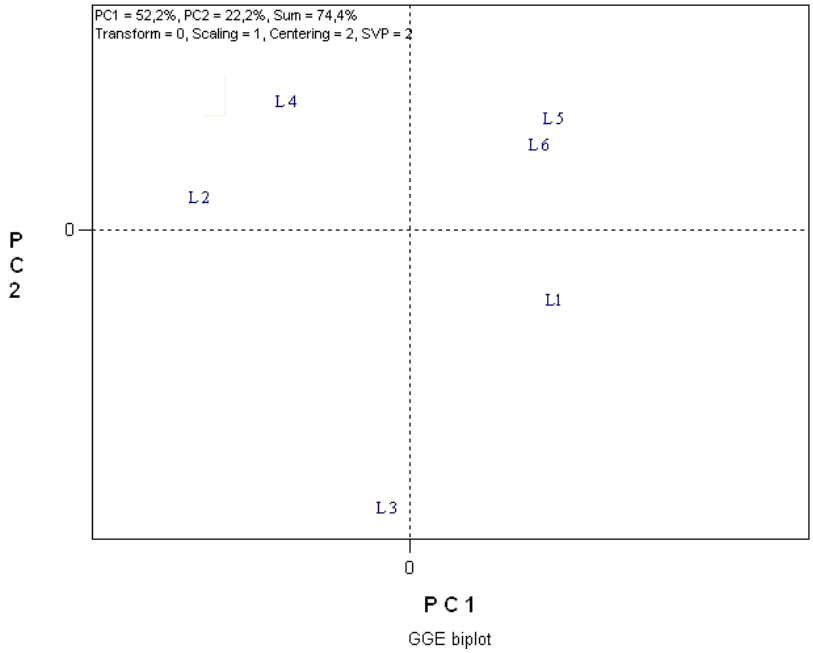

Figure 2. PCA of genetic similarity for six sweet corn inbred lines

The concurrence between the GS and SCA was established by Spearman's rank correlation coefficient. Estimates of correlation between genetic distance and SCA ranged from -0,16 (treatment 2) to -0,57* (treatment 1). Estimates were always negative indicating that genetically more similar genotypes achieved lower esatimates of SCA, and with lower values of GS higher estimates of SCA were calculated. Statistical significance of correlation coeficient indicates that there is good agreement between GS based on SSR markers and SCA of ear yield.

GERDES and TRACY (1994), found significant correlation between RFLP based genetic distance and pedigree relatedness (r= -0.543**), of 43 sweet corn

inbred lines. This suggested that molecular techniques provide an accurate assessment of relationships between sweet corn inbred lines like it is estimated among the lines of standard quality grain maize.

Many reports confirm that applying the methods of molecular markers can be used to establish heterotic groups among maize inbred lines and therefore simplify the choice of parent lines for the production of high-yielding hybrids (LANZA *et al*. 1997; LÜBBERSTEDT *et al*., 2000; REIF *et al.,* 2003).

The narrow genetic base is the reason that no distinctive heterotic groups among sweet corn inbred lines are up to now determined TRACY (1990). Nevertheless the application of molecular markers could provide breeders with essential information about potentially useful hybrid combination and therefore improve effectiveness of sweet corn breeding programs.

CONCLUSION

The results of this study showed that the estimation of the genetic similarity based on SSR markers between six sweet corn inbred lines is in agreement with data of the specific combining ability of their crosses. SSR marker provide a valuable tool for grouping of germplasm and are a good complementation to field trials for identifying groups of genetically similar germplasm. With the results of GS based on SSR markers, field trials can be planned more efficiently. This way the application of SSR markers in breeding of sweet corn would have a great contribution to the efficiency of its breeding, since sweet corn has the narrow genetic base with no determined heterotic patterns.

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SSR MARKERI U KARAKTERIZACIJI SAMOOPLODNIH LINIJA KUKURUZA ŠEĆERCA

Jelena SRDIĆ, Ana NIKOLIĆ i Zorica PAJIĆ

Institut za kukuruz, „Zemun Polje“ - Belgrade, Serbia

I z v o d

Kukuruz šećerca razlikuje se od kukurza standardnog kvaliteta zrna u većem broju značajnih osobina. Zbog toga oplemenjivanje ove kulture pored standardnih procedura obuhvata i neke posebne, a sve zbog specifičnosti njegovog korišćenja. Primena molekularnih markera u oplemenjivanju šećerca postaje neophodnost, naročito zbog toga što kod ovog tipa kukuruza još uvek nisu utvrđene heterotične grupe, pa je upoznavanje genetičke varijabilnosti samooplodnih linija šećerca na molekularnom nivou od velikog značaja za njegovo oplemenjivanje.

U radu analizirana je genetička sličnost 6 samooplodnih linija kukuruza šećerca na osnovu SSR markera. Primenjen je set od 40 prajmera. Dobijeni razultati upoređeni sa podacima specifičnih kombinacionih sposobnosti prinosa klipa, dobijenih dialelnom analizom.

Koeficijent korelacije ranga između GS i PKS bio je negativan i u jednom slučaju statistički značajan, a kretao se od -0,16 and -0,57*. Negativna vrednost ovog koeficijenta ukazuje na to da se sa povećanjem sličnosti genotipova smanjuje vrednost PKS i obrnuto. Statistička značajnost ovog koeficijenta ukazuje na pozdanost primene SSR markera u proceni genetičke sličnosti i planiranju perspektivnih ukrštanja.

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