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APPLICATION OF MOLECULAR MARKERS IN BULK SEGRAGANT ANALYSIS OF YIELD IN MAIZE (*ZEA MAYS* L.) SYNTHETIC POPULATIONS

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Chromosome regions which carry potential QTLs for high grain yield in two synthetic maize populations - B73xMo17 and L1xMo17, were identified by bulk segregant analysis (BSA). Yield was evaluated on F_2 testcross families in field trials using a Nested design. Based on yield data, F_3 families with the corresponding highest and lowest testcross yields were selected for BSA. Genome analysis of F_3 families was carried out with 58 RFLP markers. Allele frequency differences were detected at four RFLP loci n chromosomes 1, 2, 6 and 10 (B73xMo17), i.e. four RFLP loci on chromosomes 1, 2, 6 i 9 (L1xMo17). Only one region, at chromosome 6, was identified in both populations, but with two different RFLP markers. In B73xMo17 it was umc65 and in L1xMo17 umc21 RFLP marker. Bulk segregant analysis was shown to be a quick and informative method for identification of chromosome regions which determine high yield expression in maize, i.e. for identification of RFLP markers closely linked to potential genes involved in expression of the trait.

Key words: maize, grain, yeld, BSA, RFLP, chromosome regions

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INTRODUCTION

Investigations on the genetic basis of quantitative traits have been facilitated by the discovery and applications of molecular markers, which have been used in many plant species to dissect genetic factors underlying quantitative traits such as grain yield. In maize the first pioneer experiments in yield QTL (quantitative trait loci) identification revealed that these QTLs are evenly distributed throughout the genome, but that some chromosome regions contribute more to the phenotypic variability of the trait (STUBER *et al.*, 1992). By comparing identified QTLs for the same cross (B73xMo17) in different experiments, BEAVIS *et al.* (1992) concluded that in one experiment only a subset of yield QTLs can be identified, depending on several factors such as source of the parental lines, type and size of the population analyzed. Environment factors (VELDBOOM and LEE, 1996; AUSTIN and LEE, 1998) and tester choice (KERNS *et al.*, 1999; KRAJA and DUDLEY, 2000) also have a significant impact on the QTL identification, while superior allele detection was shown as the most stable component in these experiments (AUSTIN and LEE, 1996; KRAJA and DUDLEY, 2000).

Various adaptations of conventional QTL analysis have been suggested whereby the size of population to be genotyped is reduced, providing considerable savings in genotyping costs while retaining most of the statistical power for detection of QTLs. MILCHEMORE *et al.* (1991) developed bulk segregant analysis (BSA) where DNA from plants with contrasting expression of a trait is combined into two pools (one high expressing and the other low expressing), thus reducing the number of samples to be genotyped. In this case the frequency of segregating alleles at target QTLs will significantly differ from the expected 1:1 ratio characteristic for most populations. Although there are no many experiments with BSA for QTL detection, this approach was successfully used in drought tolerance component QTL identification in composite populations of maize (QUARRIE *et al.*, 1999).

In the present study grain yield was investigated with the objective to: (i) identify chromosome regions that determine high grain yield, (ii) compare identified chromosome regions in two different crosses (with one parental line in common) grown in the same environmental conditions and (iii) check the validity of BSA approach in QTL detection.

MATERIAL AND METHODS

Field trials

F_2 plants of two crosses, B73xMo17 and L1xMo17, were selfed and also top-crossed to F7R as a tester. Testcrosses were evaluated for grain yield. Field trials with 80 testcross families per cross were performed at two locations in two years, using a Nested design with four sets of 20 families and a standard hybrid.

RFLP analysis

Based on yield data F_3 families with the highest and lowest testcross yields were selected for BSA. Each family was represented with 10-15 plants and 5 to 8 families were pooled into high and low yield bulks. DNA was isolated from

leaves with CTAB buffer. DNA samples were digested with three restriction enzymes - EcoRI, BamHI and HindIII. Restricted fragments were separated on 1% agarose gel, Southern blotted on positively charged nylon membranes and hybridized with 90 RFLP probes labeled with dig-11-dUTP by PCR. Detection was done using Anti-digoxygenin Fab fragments conjugated to alkaline phosphatase and chemiluminiscent substrate CSPD (The Dig System User's Guide for Filter Hybridization, Boehringer Mannheim,1995).

RFLP films were scanned and individual bands (alleles) within each sample were quantified using TotalLab v1.10 program package. The frequencies of the alternative alleles within the bulks were calculated using the pixel area values for each band and expressed as percent of the total pixel area (sum of pixel area values for all bands within a bulk). The RFLP allele frequency difference was taken to be the difference between calculated percents of the total for the same allele in corresponding high and low yield bulks.

RESULTS

Yield data (t/ha) for three locations were analyzed in relation to the standard hybrid yield and expressed as percents of the standard hybrid yield (standard hybrid yield was 100%). Data from one location in one year were rejected because of inappropriate crop density due to poor seed germination rate.

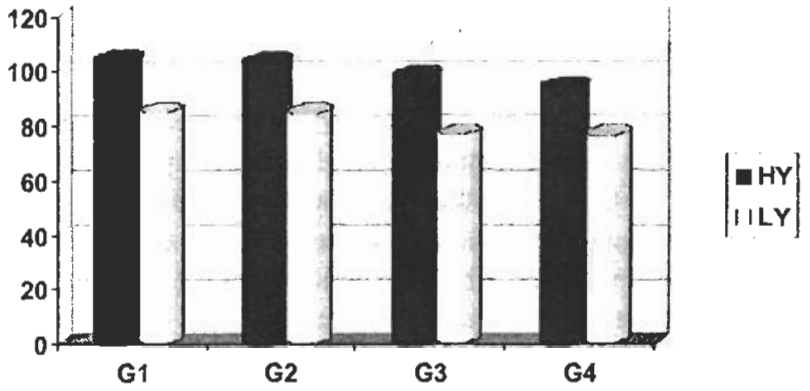
Variance analysis of the yield in each cross revealed that families in some sets did not show significant differences (data not shown). As BSA is based on marker analysis of genotypes with the most extreme phenotype expressions, difference in yield between high and low yield bulks had to be sufficient for detection of RFLP frequency differences. To fulfill this demand two criteria for making bulks, i.e. two types of bulked samples were made for each cross.

The first criterion was based on choosing families that had the most stable yield at each of the analyzed locations. In the cross B73xMo17, five (6.25%) families had higher yield than the standard hybrid on all locations. In the cross L1xMo17, five (6.25%) families had yield higher than the standard hybrid at least at one location. At the other locations their yield was over 90% of the standard. These families were used for high yield (HY) bulks - G1HY for B73xMo17 and G3HY for L1xMo17 crosses. Low yield (LY) bulks also consisted of five families each, thus making the number of families in all four bulks the same. These families showed the least yield variation at different locations. The bulks were designated G1LY for B73xMo17 and G3LY for L1xMo17.

The second criterion was based on the choice of the best (highest yield) two and the worst (lowest yield) two families from each of the four sets. Bulks formed in this way consisted of eight (10%) families each. These bulks were designated G2HY and G2LY for B73xMo17, i.e. G4HY and G4LY for L1xMo17. Mean yield differences between bulks ranged from 17.9% to 21.4% (Graph 1).

These differences in yield were sufficient to enable allele frequency differences between high and low yield bulks to be detected.

	G1	G2	G3	G4
HY	104%	103.2 %	98.3 %	94.4 %
LY	84.9%	84.4%	76.9 %	76.5 %
HY-LY	19.1%	18.8%	21.4%	17.9%



Graph. 1 - Testcross family mean yields (% of the standard) for G1 and G2 bulks (B73xMo17) and G3 and G4 bulks (L1xMo17)

In both crosses some of the families met both sets of criteria and thus were present in both types of bulks. In the cross B73xMo17 three high yield families were present in both G1HY and G2HY, while one family was present in G1LY and G2LY bulks. In the cross L1xMo17 two families were common to G3HY and G4HY, i.e. three families for G3LY and G4LY bulks.

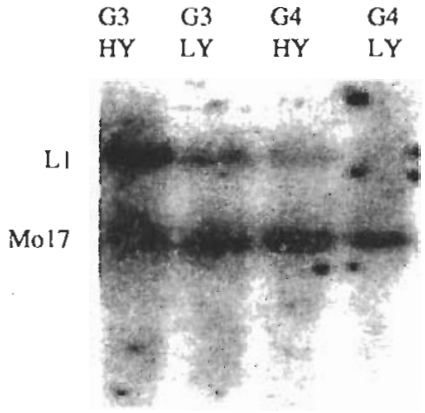
Table 1. - Identified chromosome regions in the B73xMo17 and L1xMo17 crosses

Cross	Probe	Ch.	Bin	Enzyme	Bulk type	Allele frequency difference	Superior allele
B73xMo17	bnl6.32	1	1.12	EcoRI	G1	11 %	Mo17
				HindIII	G1	48 %	Mo17
	bnl8.45	2	2.01	EcoRI	G1	20 %	B73
					G2	9 %	B73
	umc65	6	6.04	EcoRI	G1	6%	B73
					G2	8%	B73
	umc44	9	10.06	EcoRI	G1	30%	Mo17
					G2	20%	Mo17
L1xMo17	umc67	1	1.06	EcoRI	G3	20 %	L1
					G4	30%	L1
	umc34	2	2.04	BamHI	G3	30%	Mo17
					G4	20%	Mo17
	umc21	6	6.05	EcoRI	G3	50%	Mo17
					G4	16%	Mo17
	csu147	9	9.04	EcoRI	G3	59%	L1
				HindIII	G3	54%	L1

Marker analysis was done by a chemiluminiscent RFLP method with 90 probes from UMC (University of Missouri-Columbia) core set. Sixteen *E. coli* clones did not grow on the LB medium, thirteen probes did not amplify during

PCR reaction and nine amplified probes were the wrong size. Together with 52 UMC probes RFLP analysis was done with another six CSU (California State University) probes. These 58 RFLP probes detected 174 enzyme/probe combinations. The best coverage was on chromosomes 1, 6 and 10, while chromosomes 5 and 8 were covered with only one polymorphic marker each. The number of monomorphic loci was 21 for B73xMo17 and 20 for L1xMo17 crosses.

Allele frequency differences were detected at four RFLP loci in each cross. RFLP markers potentially linked to genes for grain yield in the cross B73xMo17 were detected on chromosomes 1, 2, 6 and 10. Bnl6.32 (chromosome 1) was detected only in the G1 type of bulk, while the other three RFLP markers were detected in both G1 and G2 types of bulks. In the cross L1xMo17 allele frequency differences between high and low yield bulks were detected on chromosomes 1, 2, 6 and 9. Csu147 was detected only in G3 type of bulk, while the other markers were detected in both types of bulks. Data for these loci are presented in Table 1. An illustration of RFLP allele frequency differences is given in Picture 1.



Bulk	L1 allele frequency	Mo17 allele frequency	Allele frequency difference
G3HY	66.6%	37.4%	20%
G3LY	42.8%	57.2%	
G4HY	40.3%	59.7%	30%
G4LY	8.9%	91.1%	

Picture 1. - An illustration of RFLP allele frequency differences between HY and LY bulks from L1xMo17 cross revealed by umc67 (bin 1.06) probe in combination with EcoRI enzyme

DISCUSSION

In both populations four chromosomes regions potentially involved in high yield expression were identified. This number would most probably be higher if the coverage of the genome was complete and if the population size used for phenotypic evaluation was larger. Only one common region, at chromosome 6, was identified, but with two different markers - umc65 (bin 6.04) in B73xMo17 and umc21 (bin 6.05) in L1xMo17. Umc21 also identified in several different experiments (AJMONE -MARSAN *et al.*, 1995; AUSTIN and LEE., 1996; KERNS *et al.*, 1999) suggesting the importance of this chromosome region in maize high yield expression. The superior allele on umc65 was from B73 and on umc21 from Mo17 parental lines. This could be explained as detection of different genes on the same chromosome regions or as the result of different epistatic interactions in two different genetic backgrounds. The identified chromosome regions, i.e. RFLP

markers, potentially involved in grain yield expression, were also compared to yield QTLs from the Maize Genetics and Genomics Database - MaizeGDB (www.maize.org/qtl.php). Seven RFLP markers detected in this work (all except umc67) were located at the same chromosome regions in which yield QTLs were reported previously.

Genotypic sampling can largely explain the low rate of congruency between QTL detected in different samples of the same cross (MIHALJEVIĆ *et al.*, 2004). However, for most identified RFLP loci in our work allele frequency differences were detected in both types of bulks. This relatively high congruency could be expected because one to three F_3 families were present in both G1 and G3, i.e. G2 and G4 bulks, meaning that comparison between different types of bulks does not strictly refer to independently selected F_3 families.

The results obtained in this work indicate, by comparison with literature and MaizeGDB data, that BSA can be used as a quick and informative method for identification of chromosomal regions which carry genes for grain yield in maize, i.e. for identification of potential RFLP markers closely linked to genes involved in the expression of the trait. Although BSA approach is adequate only for detecting QTLs with significant contribution to phenotypic variability of the quantitative traits (WANG and PATERSON, 1994), it has been successfully used as a complementary method to map-based QTL analysis in several experiments (QUARRIE et al., 1999; NAVABI *et al.*, 2005). Confirmed RFLP markers (proved as statistically significant in individual plant analysis) could be used in indirect selection for improving maize high yield.

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PRIMENA MOLEKULARNIH MARKERA U ANALIZI PRINOSA GRUPNIH UZORAKA SINTETIČKIH POPULACIJA KUKURUZA (*ZEA MAYS* L.)

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I z v o d

Identifikacija hromozomskih regiona potencijalnih nosioca QTL uključenih u ekspresiju visokog prinosa zrna kukuruza kod dve sintetičke populacije - B73xMo17 i L1xMo17, urađena je analizom grupnih uzoraka. Prinos je određen na familijama test-ukrštanja F_2 biljaka u poljskim ogledima, po Nested dizajnu. Na osnovu podataka o prinosu odabrane su F_3 familije sa najvećim i najmanjim prinosom odgovarajućih familija test-ukrštanja. Analiza genoma visoko i nisko prinosnih grupnih uzoraka F_3 familija je urađena pomoću 58 RFLP markera. Na osnovu razlika u frekvenciji alela identifikovana su četiri RFLP lokusa na hromozomima 1, 2, 6 i 10 (B73xMo17), odnosno četiri RFLP lokusa na hromozomima 1, 2, 6 i 9 (L1xMo17). Samo je jedan region, na hromozomu 6, identifikovan u oba ukrštanja, ali sa dva različita markera - umc65 u B73xMo17 i umc21 u L1xMo17. Analiza grupnih uzoraka se pokazala kao brza i informativna metoda u identifikaciji hromozomskih regiona koji determinišu ispoljavanje visokog prinosa kod kukuruza, tj. u identifikaciji RFLP markera blisko vezanih za potencijalne gene uključene u ekspresiju ove osobine.

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