

# Use of a pooling strategy to identify RAPD and SSR markers associated with maturity, plant height, and grain yield in grain sorghum

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## Abstract

Maturity is a critical trait for better adaptation and productivity of sorghum (*Sorghum bicolor* L. Moench) in stress environments where this crop is usually grown. Despite accrued knowledge of how genotypes with different maturity respond to environments, breeding efficiency is still limited by poor characterization of individual quantitative trait loci (QTL) affecting this trait. We examined DNA marker association with genetic factors of sorghum maturity (days to anthesis), plant height, and grain yield, using genotypes contrasting for maturity: the 10% earliest and 10% latest maturing F5 lines were identified in cross FGxM90812. Parents and progeny were evaluated at one temperate location in the US and six semi-arid tropical locations in Niger. Field data were statistically analyzed considering six alternative groupings of locations. Genetic variability for maturity and plant height was high. Seventy-three random amplified polymorphic DNA (RAPD) and six simple sequence repeat (SSR) markers were used in single marker analyses. Seventeen markers detected maturity loci with stable expression across environments. Most of these markers also identified plant height factors in all environments, but grain yield and maturity were related only when the temperate location was included. It is concluded that the pooling strategy was efficient in accessing genetic components of maturity in grain sorghum. DNA markers may allow an easier selection of specific maturity loci for adaptation to a wide array of growing conditions.

## Introduction

Many agricultural traits of economic importance display continuous variation in populations. Improvement of such traits is difficult since phenotypic evaluation is limited in its ability to identify the individual loci or QTL responsible for complex inheritance. Continuous traits have thus been generally studied through their association with simply inherited entities or

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genetic markers. The best markers are those existing in large numbers, presenting a high level of variation, and having codominant alleles with no phenotypic effect (Falconer and Mackay, 1996, p.359). Morphological markers allow the partitioning of a complex trait into discrete classes but they are rare and often present undesirable phenotypic effects. The discovery in the 1950s of isozymes and gel electrophoresis to identify protein products of different alleles (allozymes) offered a simple method with codominant markers; unfortunately isozymes exist in limited numbers and their detection is not always independent of background or environmental influence (Burow and Blake, 1998). Finally in the early 1980s, it was reported that certain DNA-based variations existed in large numbers, and could be used as Mendelian codominant markers in genetic analysis (Botstein et al., 1980). The best-known DNA markers are restriction fragment length polymorphisms or RFLP (Botstein et al. 1980). These markers are codominant and have found the greatest application in mapping; however their analysis requires the use of radioactive material, is labor-intensive and time-consuming, and limited in the number of allelic variants. Introduction of the polymerase chain reaction or PCR (Mullis and Faloona, 1987) gave a new boost to the use of DNA markers. Of the numerous DNA markers developed from PCR, the most recognized are random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs) also called microsatellites, and amplified fragment length polymorphisms (AFLP). The RAPD assay was first described by two independent groups (Williams et al., 1990; Welsh and McClelland, 1990). RAPD are based on sequence complementarity between template DNA fragment and a short random primer that can be extended by the enzyme *Taq* polymerase. This assay became popular due to high marker polymorphism, lower cost, simplicity and the availability primers on the market; but RAPD markers behave as dominant alleles containing less information than RFLP. Microsatellites are short nucleotides repeated several times throughout the genome of most eukaryotic systems (Hearne et al., 1992). Microsatellites are assayed like RAPD except for the need to design two longer primers based on the flanking sequence of the repeat in each DNA strand. The high degree of SSR polymorphism is due to variation in the number of repeats. SSR are easy to manipulate like RAPD, and are codominant like RFLP. Their isolation is, however, time-consuming and the cost of primer design may be high. The third important type of PCR markers, AFLPs, are based on the selective amplification by random primers, of DNA digested beforehand with restriction enzymes. This assay produces a large number of bands (50-100) which are mostly dominant markers.

DNA markers may potentially explain such genetic issues and phenomena as the number of genes influencing a complex trait, their chromosomal location, the phenotypic effect of each gene, gene dosage, pleiotropy, environmental sensitivity, and epistasis (Paterson et al., 1991). However, their most important application in agriculture so far, has been in mapping to identify QTL. The principles of saturating the genome with markers for QTL mapping, were

demonstrated by Thoday (1961) and effectively applied for the first time by Paterson et al. (1988). Some breeding applications of molecular markers include knowledge of genetic diversity, parental selection, fingerprinting, and control of genetic purity (Dudley, 1993). Marker-assisted selection has been suggested as a method to improve breeding efficiency. Lande and Thompson (1990) suggested that marker information could be integrated with phenotypic data in establishing selection indices. Lee (1995) indicated that marker-assisted selection may improve genetic gain by increasing rate of gain and lowering costs. Marker-assisted selection may be useful by allowing selection in non-target environments (Paterson, 1998; Ribaut et al., 1997), or by improving the effectiveness of early generation testing (Eathington et al., 1997). But the usefulness of marker-assisted selection will ultimately depend on the identification of markers tightly-linked to QTL and accurately-defined phenotypes (Staub et al., 1996).

Tests are performed using methods of single-marker analysis (Sax, 1923; Edwards et al., 1987) or interval mapping (Lander and Botstein, 1989); the approach of interval mapping uses maximum likelihood to identify the most probable QTL position inside a bracket of two markers. When interest is primarily on one trait, alternative and more powerful methods of QTL detection have been devised. Lebowitz et al. (1987) discussed trait-based analysis, whereby different marker frequencies in phenotypic pools are used to test for the presence of a QTL. This is possible because lines with high and low trait values are expected to have plus and minus alleles of important QTL and associated markers. Lander and Botstein (1989) suggested selective genotyping, a method where individuals with extreme phenotypes are sampled for genotyping because they are the most informative. A third method, bulk segregant analysis, was described by Michelmore et al. (1991) as a pooling strategy for single gene traits, also suitable for quantitative traits controlled by a few major genes. Darvasi and Soller (1994) showed that selective genotyping and selective DNA pooling effectively reduced the amount of genotyping without losing much testing power. Wang and Patterson (1994) reviewed the usefulness of the various DNA pooling strategies for QTL identification. They concluded that success was more likely to be achieved for QTL with large effects. The relative advantage of using advanced segregating populations, when the pooling is for traits with low heritability, was also suggested by Soller and Beckmann (1990).

Grain sorghum (*S. bicolor* L. Moench) is the crop of choice in many countries of the Semi-Arid Tropics (SAT). Crop adaptation and yield are largely dependent on an appropriate maturity (Bunting, 1975; Laurie et al., 1995; Bezant et al., 1996). Curtis (1968) noted the strong influence of heading date on yield of Nigerian landraces of sorghum, and emphasized the need to understand the basis of their maturity and adaptation. Dalton (1967), Crook and Cassady (1974), and Plett et al. (1991), have all reported the superior yield of late-maturing

sorghum genotypes compared to early-maturing ones. Using DNA markers to tag maturity genes will lead to a better understanding of how specific factors influence adaptation and productivity. This may speed up selection strategies and contribute to crop improvement in less favorable environments. We used RAPD and SSR markers to assess genetic variation in sorghum advanced progenies. The objective of our study was to identify genomic regions affecting maturity (days to anthesis) of sorghum lines grown at one temperate and six subtropical locations. Markers with significant tests for maturity and plant height and/or grain yield, were examined as evidence of the same genomic regions influencing the traits.

## **Materials and methods**

### *Plant material*

Feterita Gishesh (FG), a sorghum landrace cultivar originating from Sudan and M90812, a breeding line from the Purdue University sorghum improvement program, were crossed at Lafayette, Indiana (Purdue University Agricultural Research Center, PU-ARC), to develop segregating progeny. The two parental lines diverge for maturity. FG is extremely early maturing, and while poor yielding, it provides a guaranteed minimum grain production under erratic rainfall in Sudan. M90812 is an improved line but is very late in maturity. Extreme early and late F3-derived F5 families were obtained following single seed descent methods in a large F2 population of cross FG × M90812.

### *Field experiment*

F5 families and two parents were grown in field trials at one temperate (Lafayette, Indiana) and six semi-arid tropical locations in Niger. Trials were organized as randomized complete block design with two replications. Entries were planted in four-row plots; depending on location, row length varied from 3.84 m to 4.6 m. All data were obtained from the middle two rows. The following traits were measured at all locations:

- days to anthesis: the number of days after planting when 50% of plants have shed pollen half-way down the panicle.
- plant height: average height from the base of plants to the tip of panicles, measured at physiological maturity.
- grain yield: harvested panicles were dried and threshed to obtain grain weight; this was used to calculate yield in kg/ha= {[grain weight(g)/ area harvested(m<sup>2</sup>)] × 10,000 m<sup>2</sup> /1 ha × 1 kg/ 1,000 g }.

### *DNA extraction*

Leaf tissue was collected at PU-ARC from field-grown seedlings of F5 families and parental lines, frozen at  $-70^{\circ}\text{C}$ , lyophilized and ground into fine powder in a Udy Cyclone mill. One gram of ground tissue was mixed in a centrifuge tube with 25 mL of CTAB buffer (2% CTAB, 5M NaCl, 1M Tris pH8.0, 0.5 M EDTA)/2-mercaptoethanol, and incubated for 1 hr. The solution was extracted with chloroform:octanol (24:1) and centrifuged at 3400 rpm for 12 min. The upper layer was transferred to a tube containing microliters of RNase (10mg/mL) and cold isopropanol for DNA precipitation. DNA threads were washed successively in solutions of 76% ethanol/10 mM sodium acetate and 76% ethanol/ammonium acetate and resuspended in TE (10mM Tris pH 8.0, 1mM EDTA). DNA quality was checked on a 0.6 % agarose gel and the concentration read on a TKO 100 minifluorometer.

### *RAPD assay*

RAPD genotyping was done for parental inbreds and F5 families. Reactions were prepared following standard protocols (Welsh and Mcleland, 1990; Williams et al., 1992), and amplified using slightly-modified cycling conditions (Williams et al., 1992; Wittwer and Garling, 1991). Amplifications were performed in 25  $\mu\text{L}$  volume containing 50 mM KCl, 10 mM Tris (pH 8.8 at  $25^{\circ}\text{C}$ ), 1.5 mM  $\text{MgCl}_2$ , 0.1% Triton X-100, 0.1 mM dNTPs, approximately 0.2 mM primer, 1 unit *Taq* DNA polymerase, and 25 ng of sorghum template DNA. Reaction mixtures were overlaid with one drop of mineral oil. Amplification was carried out in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA) programmed for 45 cycles at the fastest possible ramp time between temperatures. Each cycle was made of 20 seconds denaturation at  $94^{\circ}\text{C}$ , 40 seconds annealing at  $37^{\circ}\text{C}$ , and 1 min extension at  $72^{\circ}\text{C}$ . Following PCR, RAPD products were analyzed by electrophoresis in 1.6% agarose gels run in TBE (Tris/Boric acid/EDTA) buffer. Gels were stained with ethidium bromide for viewing under UV light.

A set of 400 RAPD primers obtained from Operon Technologies Inc (Alameda, Ca) were used. Only primers showing polymorphic bands in the screening of parental inbreds were evaluated on the 24 individual progeny or RIL. Marker scoring was done as 0 (FG allele) or 1 (M90812 allele). RAPD loci were named by Operon kit number, eg OPB15; if more than one band was scored for a particular primer, small case alphabetical letters were used to distinguish between markers, e.g., OPU6a, OPU6b.

### *SSR assay*

SSR genotyping was done for parental inbreds and F5 families. Twenty-four sorghum SSR primers were produced on a Oligo 1000M DNA synthesizer (Beckman, Fullerton, CA), in the Department of Agronomy, Purdue University, based on sequences described by Brown et al. (1996). Amplification reactions were performed in 25  $\mu$ L volumes containing 50 ng sorghum genomic DNA, 10 pmol of each primer, 1.25 mM MgCl<sub>2</sub>, 1.25 mM each dNTP, 10X PCR buffer (50 mM KCl, 10 mM Tris, 0.1% Triton X-100), and 1 unit of *Taq* DNA polymerase. Reaction mixtures were overlaid with one drop of mineral oil. Amplification was carried out in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA). Amplification conditions were as follows, modified from those published by Brown et al. (1996): initial denaturation at 94°C for 2 min; six cycles where the annealing temperature was reduced by one degree every two cycles (denaturation: 94°C, 30 s; annealing: 62°C-60°C, 60 s, extension: 73°C, 60 s); 15 cycles of 94°C-30 s, 59°C-60 s, 73°C-60 s; and 25 cycles of 94°C-30 s, 57°C-60 s, 73°C-60 s. Following PCR, SSR products were analyzed by electrophoresis in 3 % Methaphor<sup>TM</sup> (FMC BioProducts) agarose gels run at 44 volts overnight in TBE (Tris/Boric acid/EDTA) buffer. Gels were stained with ethidium bromide for viewing under UV light. SSR loci were designated by their id as given in Brown et al. (1996). The alleles were scored as 0 (FG) or 1 (M90812).

### *Statistical analysis*

Analyses of field data were performed for all locations combined as a random factor (Analysis 1), and for five different partitions of locations into fixed or random effects: temperate vs. semi-arid tropical latitudes (Analysis 2), all semi-arid tropical locations (Analysis 3), irrigated vs. rainfed locations (Analysis 4), irrigated locations only (Analysis 5), and rainfed locations only (Analysis 6). The six analyses are summarized in Table 1. Analyses of marker data followed Table 1 with treatments partitioned into marker genotypes (fixed) and entries (random) nested within each marker.

The MIXED procedure in SAS (SAS, 1996) was used for all tests. For fixed effects, this procedure provided type III significance levels and comparisons of adjusted means. SAS MIXED was used because of its greater flexibility compared to SAS GLM, providing correct standard errors of means, and a correct test of significance when more than one mean squares are used in the test denominator. But MIXED does not provide an F test for random effects. Significance of random effects (location by group, location by marker) was determined by the

likelihood ratio test, which is based on  $\chi^2$  with one degree of freedom (Steel et al., 1997, p. 412).

For analysis of field and marker data for the five remaining analyses of partitioning locations, the models were modified from those above to take into account relevant fixed and/or random effects.

Associations between markers were evaluated by arranging marker genotypes in  $2 \times 2$  contingency tables for a  $\chi^2$  test (Steel et al., 1997). This analysis was performed by the FREQ procedure in SAS.

## **Results**

### *Field data*

The results of different analyses of variance for fixed effects are presented in Table 2. For anthesis there were highly significant differences among groups (early progeny, late progeny, parent 1, parent 2). For plant height, there were no significant differences among groups in the irrigated trials, and when latitudes were contrasted, differences were significant at the 5% level; otherwise, there were highly significant differences in all other analyses. For grain yield, no analysis showed significant differences. These results suggest that genetic variation for anthesis and plant height but not for grain yield was expressed by the genotypes tested. Two important environmental factors were represented by latitudes (Analysis 2) and water status (irrigation or rainfall; Analysis 4). Results in Table 2 show they did not contribute significantly to total variation. Random effects (results not shown) were however significant in all analyses. Thus, although there was considerable genetic variation for anthesis and plant height in this germplasm, unidentified environmental factors also explained some of the phenotypic variation.

Results of means comparison (contrasts) are shown in Table 2 for the first analysis where all locations were combined as a random factor; results of the five other analyses were practically similar. It appears that the group of early progeny was significantly earlier maturing (55 days to anthesis) and shorter (163 cm) than the group of late progeny (81 days, 227 cm) but there was no significant difference among the two groups for grain yield. These differences paralleled those in the parents although the height difference in the latter was not significant.

### *Marker data*

Of the 79 RAPD and SSR markers analyzed, 27 were associated with at least one trait in one analysis (Table 3). Figures 1-3 show, respectively, the segregation of RAPD markers OPU6,

OPK14, and SSR marker Sb4-72, in parents and selected progeny. Seventeen markers were associated with maturity in all six analyses, except for marker OPR16c which was not present in Analysis 2. This result indicates that maturity factors with stable expression in various environmental conditions were detected by the markers. Figure 4 shows the localization of a putative QTL for maturity near the SSR marker Sb4-72. A total of 19 markers were linked to plant height factors, twelve of them in all analyses. Thus plant height factors with stable expression were also detected in this cross. Nine markers were associated with grain yield in a variable number of analyses. This suggests that genetic factors for grain yield were more environmental specific. In the list of markers associated with the three traits, twelve markers that detected maturity factors in all environments also detected height factors in most environments. On the other hand, only two of the maturity markers were associated with grain yield factors and only in a few environments. This may indicate that in cross FG × M90812, the same or closely-linked genetic factors influenced maturity and plant height but not maturity and grain yield.

The 27 important markers were submitted to a test of independence (Steel et al., 1997). Results are shown in Table 4. There appears no definite grouping based on the significance of marker-marker association. However, markers that had a strong association with maturity and height factors, also tend to be linked to each other, especially RAPD markers OPK14, OPT14, OPU6, OPR15b, OPZ19, and SSR markers Sb4-7 and Sb4-72 (linked with probability  $p=0.001$ ). These markers may have identified a single genomic region with pleiotropic effects on maturity and height, or different but very tightly-linked factors for the two traits. For grain yield, OPM15a and OPAA17b were the most strongly associated to each other ( $p=0.001$ ) indicating a probable separate genomic area that influences this trait.

## **Discussion**

The six different statistical analyses used with F5 maturity extremes, empirically suggest that environmental stresses increased from the model including a temperate location to those including only Niger locations, and inside Niger as water status became unpredictable (from irrigated to rainfed conditions). The number of markers associated with maturity factors was practically the same for different environments based on the number of locations included. This showed that genomic segments with stable effects on maturity were identified, which makes them potentially useful in selection. There were significant differences for plant height, and at least half of the markers for this trait were identical to those identified for maturity. For grain yield, there was no genetic variability. Under stress conditions that prevailed at most of the locations of this study, genetic variability for yield was probably difficult to separate from strong environmental effects. Observations of low genetic variability for grain yield under

stress are well documented (Blum, 1988; Zavala-Garcia et al., 1992). Nevertheless, a few markers were associated with grain yield factors except under irrigated growing conditions of Niger. The results of the present study suggest the importance of genetic backgrounds not only in producing maturity phenotypes, but also in how this may affect agronomic traits like plant height and grain yield.

The high number of markers associated with maturity is expected since inbred parents differed for this trait (Lebowitz et al., 1987; Wang and Paterson, 1994). The fact that most of these markers were the same or linked to each other suggests that a major locus (QTL) for maturity was detected by the pooling strategy, and this QTL also affected plant height, through pleiotropy or tight linkage (Edwards et al., 1987; Veldboom et al., 1994; Austin and Lee, 1996). Linkage between maturity and plant height loci has been reported in sorghum (Quinby, 1967). On the other hand, there was very little relationship between maturity and grain yield at the molecular level in cross FG × M90812. The two markers (OPT2b and OPX20) simultaneously associated with the two traits identified a genomic region separate from the major maturity locus. In addition, markers most tightly-linked to factors of grain yield (OPM15a, OPAA17b) were not associated with maturity. In this cross major maturity factors may not influence grain yield, probably in relation with the low yielding potential of early maturing parent FG. These results show that grain yield may be determined by factors separate from those factors with the greatest influence on maturity.

The pooling of progeny based on maturity phenotype was useful for identification of important genomic regions. Selective genotyping is however more likely to find QTL with large effects (Wang and Paterson, 1994), therefore other genomic segments influencing maturity were likely to go undetected in this study, and their effects on plant height and grain yield cannot be addressed.

There was evidence for unexplained environmental influence, other than was due to different latitudes or water treatments. Maturity is influenced by a score of environmental factors, but usually the most recognized are daylength and temperature. Flower (1995) noted that spatial and temporal climatic variability was high even between neighboring locations in the sorghum growing area of northern Nigeria. Empirical observations during the present study support that information. Drought, planting dates, and temperature seemed to all have differential effects on the sorghum lines, at locations that are supposed to be uniform for daylength and temperature.

We conclude that DNA markers were efficient in accessing genetic components of maturity in grain sorghum. Maturity loci with stable expression across contrasting environments, including

various water regimes, were detected. DNA markers may allow an easier selection of specific maturity loci for adaptation to a wide array of growing conditions.

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**Table 1.** Sources of variation and degrees of freedom in analyses of field data for F5 maturity extremes of sorghum cross FG × M90812.

Source	df	Analyses <sup>a</sup>								
		An1	An2	An3	An4	An5	An6			
Locations	6	√								
Temperate vs. semi-arid tropical	1		√							
Among semi-arid tropical	5		√	√						
Irrigated vs. rainfed	1				√					
Between irrigated	1					√				
Among rainfed	3						√			
Reps/locations	7	√	√	√	6	√	√	2	√	4
Treatments	25	√	√	√	√	√	√	√	√	√
Among groups (early, late, P1, P2)	3	√	√	√	√	√	√	√	√	√
Entries/early	11	√	√	√	√	√	√	√	√	√
Entries/late	11	√	√	√	√	√	√	√	√	√
Location × treatments	150	√	√	√	125	√	√	25	√	75
Location × among groups (early, late, P1, P2)	18	√								
(Temp vs. SAT) × among groups	3		√							
Among SAT loc × among groups	15		√	√						
Irrig vs.rainfed × among groups	3					√				
Between irrig × among groups	3						√			
Among rainfed vs. among groups	9								√	

**Table 1.** Continued.

Source	df	Analyses <sup>a</sup>					
		An1	An2	An3	An4	An5	An6
Locations × entries/early	66	√					
(Temp vs. SAT) × entries/early	11		√				
Among SAT loc × entries/early	55		√	√			
Irrig vs.rainfed × entries/early	11				√		
Between irrig × entries/early	11					√	
Among rainfed vs. entries/early	33						√
Locations × entries/late	66	√					
(Temp vs. SAT) × entries/late	11		√				
Among SAT loc × entries/late	55		√	√			
Irrig vs.rainfed × entries/late	11				√		
Between irrig × entries/late	11					√	
Among rainfed vs. entries/late	33						√
Error	175	√	√	√ 150	√	√ 50	√ 100
Total	363	√	√	√ 311	√	√ 103	√

<sup>a</sup> Analyses are for the following environmental settings: An1= all seven locations, no partitioning; An2= locations partitioned into temperate vs. semi-arid tropical latitudes; An3= six semi-arid tropical locations, no partitioning; An4=semi-arid tropical locations partitioned into irrigated vs. rainfed treatments; An5= between two semi-arid tropical irrigated locations; An6= among four semi-arid tropical rainfed locations.

**Table 2.** Analysis of variance for sorghum traits in F5 maturity extremes of cross FG × M90812 averaged across diverse locations (Analyses 1-6), and genotypic means in Analysis 1.

Analysis	Fixed effect	Trait		
		Anthesis (days)	Plant height (cm)	Grain yield (log <sub>10</sub> kg/ha)
1	Groups	***	**	ns
2	Groups	***	*	ns
	Latitudes	ns	ns	ns
3	Groups	***	***	ns
4	Groups	***	**	ns
	Water status	ns	ns	ns
5	Groups	**	ns	ns
6	Groups	***	**	ns
Means (Analysis 1)				
	Early progeny group (E)	54.8	163bc	3.26abc
	Late progeny group (L)	80.7e	227e	3.14adef
	P1=FG	47.0	119bf	3.24bdef
	P2=M90812	85.6e	169cef	3.14cef

<sup>a</sup> Test of fixed effects and contrast between means are from the Mixed procedure of SAS; ns=nonsignificant; \*,\*\*, \*\*\*, significant at p=0.05, 0.01, and 0.001, respectively. For contrasts, means with the same letter are not significantly different at the 5% level of probability; the following letters are used in mean separation for six contrasts: E vs. L (a), E vs. P1 (b), E vs. P2 (c), L vs. P1 (d), L vs. P2 (e), P1 vs. P2 (f).

**Table 3.** RAPD and SSR markers associations with sorghum traits in F<sub>5</sub> maturity extremes of cross FG × M90812 in analyses 1-6.

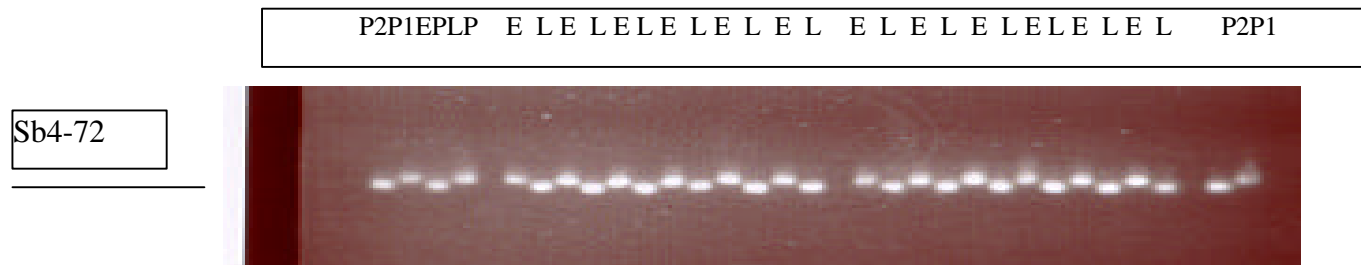
Marker name	Trait <sup>a</sup>																	
	Anthesis						Plant height						Grain yield					
	An1	An2	An3	An4	An5	An6	An1	An2	An3	An4	An5	An6	An1	An2	An3	An4	An5	An6
OPK14	***	***	***	***	***	***	*	*	**	*	*	**						
OPT14	***	***	***	***	***	***	*	*	**	*	*	**						
OPU6	***	***	***	***	***	***	*	*	**	*	*	**						
Sb4-7	***	***	***	***	**	**	**	**	**	**	*	**						
Sb4-72	***	***	***	***	**	**	**	**	**	**	*	**						
OPR15b	***	***	***	***	***	***	*	*	**	*	*	**						
OPZ19	***	***	***	***	***	***	*	*	**	*	*	**						
OPT17d	**	***	*	**	**	**	***	**	**	**	**	***						
OPV14	**	*	*	**	**	**	*	*	*	*		*						
OPT2b	**	**	*	*	*	**	*		*	*		*	*	*				
OPT17b	**	*	*	*	**	*	**	**	**	**	*	**						
OPR16c	*		*	*	*	*	*	*	*	*		*						
OPH9	*	*	*	*	*	*												
Sb6-84	*	*	*	*	*	*												
OPR16a	*	*	*	*	*	*						*						
OPV15a	*	*	*	*	*	*												
OPX20	*	*	*	*	*	*							*					
OPL3a							***	***	***	***	***	***	*		*	*		
OPAB4							*	*	*	*	*	*						
OPZ20							*	*	*	*		*						
OPT17a								*				*						
OPX9b												*		**				
OPP4												*		*				
OPR2														*				
OPT11														*				
OPM15a													**	*	**	*		**
OPAA17b													**	*	**	*		**

<sup>a</sup> An1= all seven locations, no partitioning; An2= locations partitioned into temperate vs. semi-arid tropical latitudes; An3= six semi-arid tropical locations, no partitioning; An4=semi-arid tropical locations partitioned into irrigated vs. rainfed treatments; An5= between two semi-arid irrigated locations; An6= among four semi-arid tropical rainfed locations

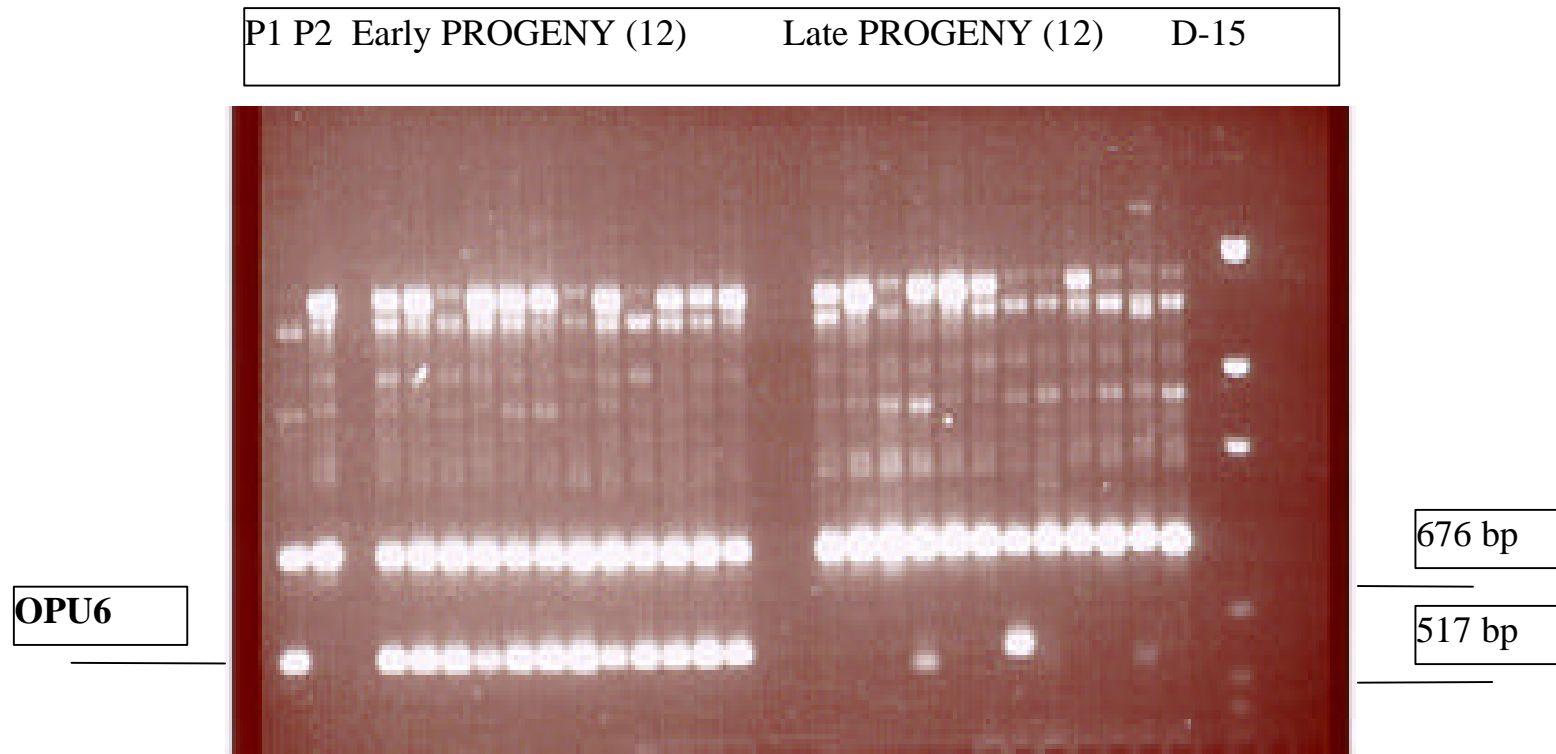
**Table 4.** Association among RAPD and SSR markers linked to sorghum traits in cross FG × M90812 in analyses 1-6.

Marker <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
1. OPK14	x	***	***	***	***	***	***	**	*	*		*	*		*										*	*	*	
2. OPT14		x	***	***	***	***	***	**	*	*		*	*		*										*	*	*	
3. OPU6			x	***	***	***	***	**	*	*		*	*		*										*	*	*	
4. Sb4-7				x	***	***	***	**	*	**	**	*	*	*	*	*	*								*			
5. Sb4-72					x	***	***	**	*	**	**	*	*	*	*	*	*								*			
6. OPR15b						x	***	**	*	**	**	*	*	*	*	*	*								*			
7. OPZ19							x	**	*	**	**	*	*	*	*	*	*								*			
8. OPT17d								x	*	**	***							***	*									
9. OPV14									x	**					***	*												
10. OPT2b										x	*	*																
11. OPT17b											x							***	*					*				
12. OPX20												x																
13. OPH9													x	***														
14. Sb6-84														x														
15. OPR16a															x	*									*	*		
16. OPV15a																x									*	*		
17. OPR16c																	x											
18. OPL3a																		x						**	**			
19. OPAB4																			x				***					
20. OPZ20																				x								
21. OPM15a																					x	***						
22. OPAA17b																						x						
23. OPT17a																							x	*				
24. OPP4																									x	*		
25. OPX9b																										x		
26. OPR2																											x	***
27. OPT11																												x

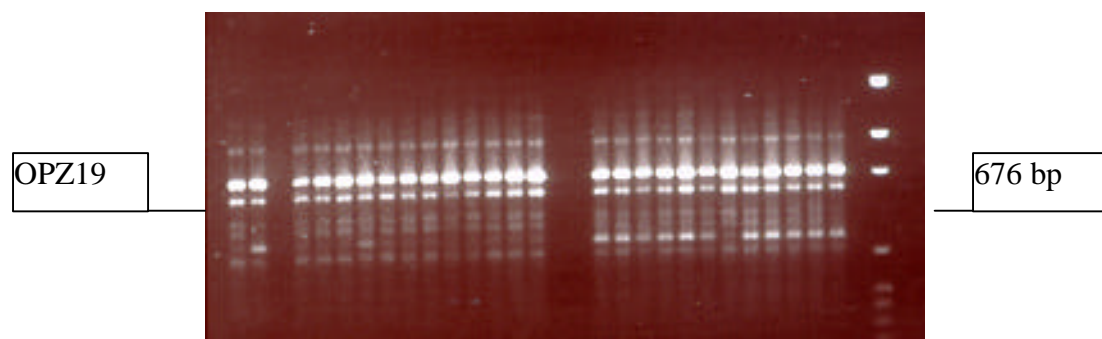
<sup>a</sup> Based on chi-square test of marginal means in 2x2 tables; \*, \*\*, \*\*\*, significant at p=0.05, 0.01, and 0.001, respectively



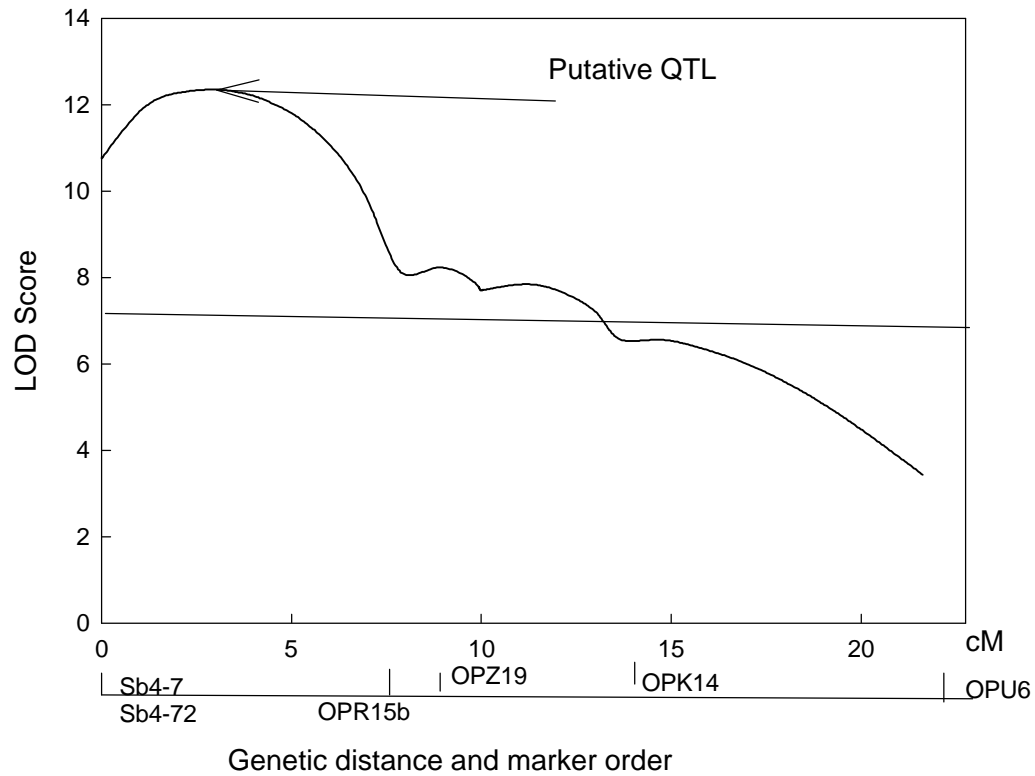
**Figure 1.** The segregation of SSR marker Sb4-72 in maturity extremes of cross FG × M 90812. The SSR Sb4-7 has exactly the same segregation but a product of greater size.



**Figure 2.** The segregation of RAPD marker OPU6 in maturity extremes of cross FG × M 90812.



**Figure 3.** The segregation of RAPD marker OPZ19 in maturity extremes of cross FG  $\times$  M90812. Lanes from l. to r.: FG (marker absent), M90812 (marker present), blank, 12 early lines, blanks, 12 late lines, blank, size marker (D-15)



**Figure 4.** Genetic localization of a putative QTL for maturity in sorghum cross FG × M90812. The QTL was identified in single factor analysis ( $P < 0.001$ ) and positioned using *QTL Cartographer* (LOD threshold 7.27 based on 1,000 permutations).