

## **A pedigree marker-assisted selection (PMAS) strategy for improvement of Tajan-derived wheat lines in Iran**

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### **ABSTRACT**

*Pedigree-based selection method combined with marker-assisted selection (MAS) provides a suitable resource for deriving elite lines with more favorable characters. Using field experiments and molecular markers we evaluated diversity of wheat advanced F<sub>5</sub> and F<sub>6</sub> lines derived from cross Tajan/PBW299//MILAN/SHA7 aimed to select elite line(s) with favorable characters [resistance to yellow rust (YR) and fusarium head blight (FHB)]. Selection for several important traits, including resistance to yellow rust, fusarium head blight and general Tajan plant type was conducted during 5 and 6 generations. Genetic diversity of F<sub>5</sub> and F<sub>6</sub> lines was studied using 32 and 47 polymorphic loci produced by long AP-PCR primers. Results showed that there was a great diversity within and between studied advanced lines. Average gene diversity across polymorphic loci for the two generations was 29% and 28.8%, respectively. Genome content of Tajan cultivar in F<sub>5</sub> lines ranged between 35% to 76.5% (with average of 57.5%). Five percent of F<sub>5</sub> lines harbored 77% of Tajan genome. However, 62.8% to 90.2% (in average 75.8%) of F<sub>6</sub> genome was inherited from Tajan. Five percent of F<sub>6</sub> lines harbored ~90% of Tajan genome. These results indicate that advancing progenies from F<sub>5</sub> to F<sub>6</sub> increased the contribution of genome content of Tajan cultivar due to selection pressure in favor of Tajan plant type. Phenotypic evaluations in combination with MAS helped us to identify and select a few F<sub>6</sub> elite lines resistant to YR and FHB with Tajan plant type. Our results markedly show that selection made by breeder has diverse effects on genetic structure of plant material, particularly in favor of fixating genetic background of superior parent.*

**Key words:** Wheat, Diversity, Marker-assisted selection (MAS), Pedigree.

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### **INTRODUCTION**

Bread wheat (*Triticum aestivum*) plays a vital role in food security at global level. Many studies conducted by scientists and field breeding employed by farmers are intensively aimed to develop more productive cultivars with desirable characters being targeted. These characters include

adaptation to extreme environmental conditions and resistance to pathogens causing heavy losses in production [14].

Molecular marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. In the context of MAS, DNA-based markers can be effectively utilized for tracing favorable allele(s) (dominant or recessive) across generations and identifying the most suitable individual(s) among the segregating progeny, based on allelic composition across a part or the entire genome. Pedigree MAS [11] is especially relevant for crops such as wheat, where pedigrees of elite germplasm are known. Fingerprinting elite wheat materials must be conducted in a set of lines actively used in the breeding programme, and in elite materials to be subsequently released. The data may be combined with the phenotypic data collected during different selection cycles to identify favorable alleles for trait(s) of interest, so that if an elite line contains alleles for yield performance in a target environment, their frequency should be higher than the expected random frequency in offspring derived from this elite parental line. This shift in allelic frequency reflects phenotypic selection by breeders and may be identified by comparing fingerprinting data of parents and their offspring. Once the favorable alleles are identified, DNA markers closely linked to the target genomic regions can be used to accelerate fixation of favorable alleles in the next selection step [5].

Assessment of crop germplasm diversity phenotypically is usually devoid of the resolving power needed to identify an individual genotype. Identification based on morphological characters is time consuming and requires extensive field trials and evaluation [4]. In addition, morphological differences may be epigenetic- or genetic-based characters [8, 9, 17]. Molecular markers due to their advantages against morphological and biochemical markers such as their plentifully, independence of tissue or environmental effects, diversity identification and selection in the earlier stages of plant development, can be a useful complement to morphological and physiological characterization of plants [3]. Among the various molecular markers available, RAPD analysis is a simple, rapid, and effective method for detecting polymorphism in wheat [18]. Arbitrary primed polymerase chain reaction (AP-PCR) is a special case of RAPD, wherein discrete amplification patterns are generated by employing single primers of 10–50 bases in length in PCR of genomic DNA. The final products are structurally similar to RAPD products. Recently, it has been simplified by separating the fragments on agarose gels and using ethidium bromide staining for visualization [7]. Saini *et al.* [13] compared AP-PCR using long primers with RAPD and showed that AP-PCR yields more polymorphism per primer than RAPD in mung bean. The use of molecular marker analysis (e. g. AP-PCR) studying genetic diversity and marker-assisted selection in wheat lines advanced by pedigree method was less reported. The objective of this work was to analyze genetic diversity within and between wheat advanced lines and to evaluate the fixation rate of genetic background of superior parent Tajan within F<sub>5</sub> and F<sub>6</sub> lines advanced by pedigree method.

## MATERIAL AND METHODS

### Plant Material

The plant material used in this study were the two sister line populations of advanced F<sub>5</sub> plants each containing 20 individuals, and two sister line populations of advanced F<sub>6</sub> plants each containing 10 individuals, derived from Tajan/PBW299/MILLAN/SHA7 cross, which had passed five and six generations of selection for several important traits such as resistance to fusarium head blight (FHB) and yellow rust (YR), and general Tajan plant type. They were obtained from Cereal Research Institute, Gorgan, Iran and were sown in 2009 and 2010.

### Sampling and DNA Extraction

Genomic DNA was isolated from young leaves of plants from each sister line population using CTAB method [12] with some modifications [2]. DNA was checked for the quality and quantity by electrophoresis on 1% agarose gels after staining with ethidium bromide.

### AP-PCR marker analysis

A total of 10 AP-PCR primers were used for PCR amplification of DNA from advanced lines as well as from Falat cultivar. PCR was performed using a PeqSatr Thermocycler. The PCR reaction mixture contained 5  $\mu$ l dH<sub>2</sub>O, 5  $\mu$ l PCR Master Mix (Cinnagen, Iran), 0.5  $\mu$ l primer and 1  $\mu$ l template DNA in a 0.2 ml tube. PCR amplification was performed with a hot start of 94°C (5 min) followed by 35 cycles of denaturation at 94°C for 35 sec, annealing at 55°C for 30 sec., extension at 72°C for 1 min. final extension was carried out at 72°C for 7 min. Amplified DNA fragments were separated by 2.5% agarose gel electrophoresis in 1x TBE buffer. The DNA bands for each primer determined based on their relative migration to molecular weight size. They were visualized by staining with ethidium bromide and were photographed under UV light using the gel documentation system (BioRad, USA).

### Band scoring and data analysis

AP-PCR bands were scored as present (1) or absent (0). Data analysis was performed using PopGen32 software. Several indices of population genetics, such as number of polymorphic loci, observed number of alleles ( $n_o$ ), effective number of alleles ( $n_e$ ), Nei's gene diversity ( $h$ ), Shannon's information index ( $I$ ) were calculated. Similarity matrix was computed based on Nie's unbiased measures of genetic identity and genetic distance [10] and used to construct dendrogram by unweighted pair group methods with arithmetic average (UPGMA) [16].

## RESULTS AND DISCUSSION

Ten AP-PCR primers showed multi-locus pattern (Figure 1), so they produced 2 to 14 bands, from which 2 to 13 were polymorphic. In average, 26 (79%) and 47 (90%) polymorphic markers were detected in F<sub>5</sub> and F<sub>6</sub> generations, respectively, which is relatively higher than the use of RAPD markers tested by Abd-El-Haleem *et al.* [1] in durum wheat.

In the case of first sister line in F<sub>5</sub> generation, number of observed alleles ranged between 1-2, with the average of 1.67. The average number of effective alleles was 1.40, and ranged between 1-1.99. Total number of detected loci was fifty, 32 of them were polymorphic. Percentage of polymorphic loci was 62.7%. Shannon information index for polymorphic loci ranged from 0.191 to 0.692, with average of 0.35. Nie's gene diversity ranged from 0.091 to 0.499, with average of 0.23 (Table 1).

For the second sister line in F<sub>5</sub> generation, number of observed alleles ranged between 1-2, with the average of 1.95. The average of number of effective alleles was 1.60. Total number of detected loci was twenty, 19 of them were polymorphic. Percentage of polymorphic loci was 95%. Shannon information index for polymorphic loci ranged from 0.191 to 0.692, with average of 0.52. Nie's gene diversity ranged from 0.091 to 0.499, with average of 0.35 (Table 1). Altogether, F<sub>5</sub> generation showed 0.28 gene diversity.

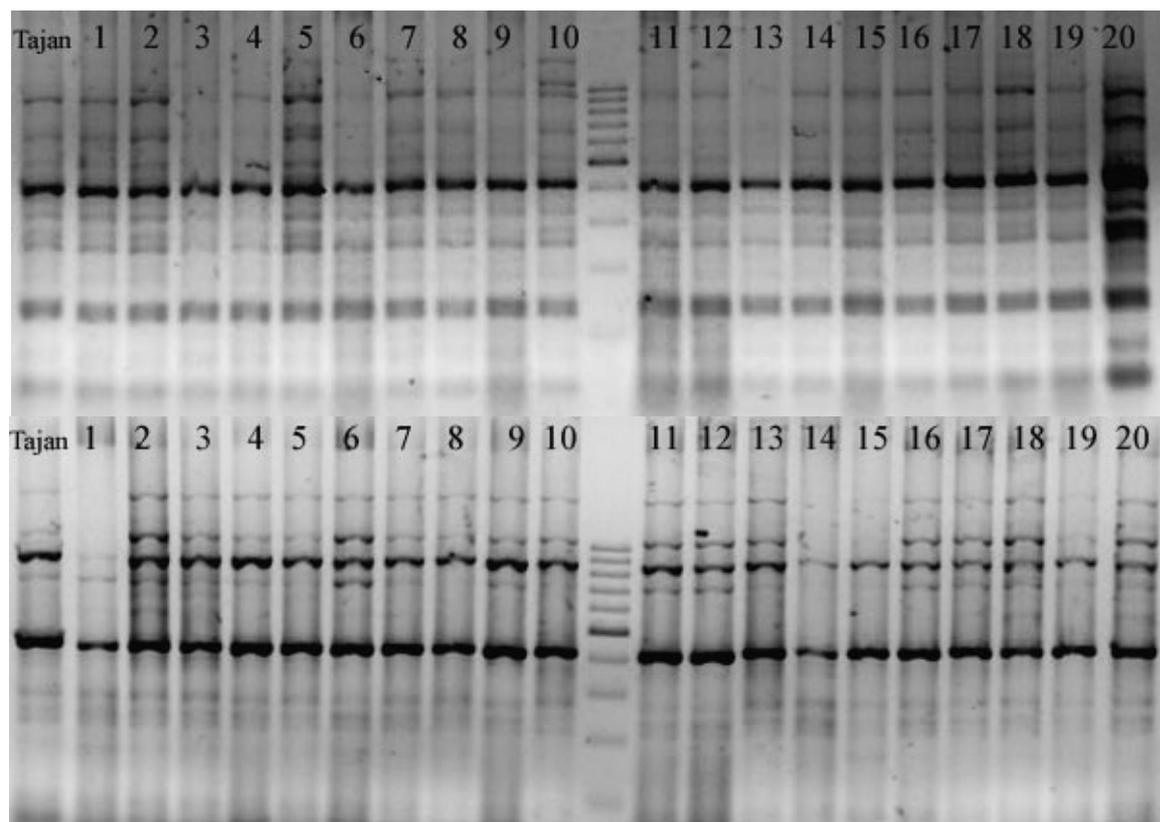


Figure 1. A sample of banding pattern produced by two AP-PCR primers on Tajan cv. and 20 advanced F<sub>6</sub> lines.

Table 1. Genetic diversity parameters for two sister lines in F<sub>5</sub> generation (Pop<sub>1</sub> and Pop<sub>2</sub>) and one population in F<sub>6</sub> generation (Pop<sub>3</sub>) each consisted of 20 individuals.

	No. samples	n <sub>o</sub>	n <sub>e</sub>	No. loci	No. polymorphic loci	Polymorphic loci (%)	Shannon information index	Nie's gene diversity	Contribution of Tajan genome (%)
Pop <sub>1</sub>	20	1.67	1.40	50	32	62.7	0.348	0.232	68.3
Pop <sub>2</sub>	20	1.95	1.60	20	19	95.0	0.517	0.348	46.8
Mean	20	1.81	1.50	35	25.5	78.8	0.433	0.290	57.5
sd <sub>1</sub>	-	0.476	0.377	-	-	-	0.199	0.282	-
sd <sub>2</sub>	-	0.224	0.320	-	-	-	0.230	0.182	-
Pop <sub>3</sub>	20	1.92	1.47	52	47	90.4	0.442	0.288	75.8
sd <sub>3</sub>	-	0.272	0.310	-	-	-	0.203	0.153	-

n<sub>o</sub> and n<sub>e</sub>: observed and effective number of alleles, respectively; sd<sub>1</sub>, sd<sub>2</sub> and sd<sub>3</sub>: standard deviations of Pop<sub>1</sub>, Pop<sub>2</sub> and Pop<sub>3</sub>, respectively.

Analysis of genetic identity of individuals in 2 F<sub>5</sub> populations using unbiased measure of genetic identity and genetic distance [10] showed that contribution of Tajan cultivar genome in F<sub>5</sub> individuals was variable, ranging from 62.8% to 76.5% (with average of 68.3%) for first sister line, and from 35% to 65% (with average of 46.8%) for second sister line. Five percent of F<sub>5</sub> lines harbored 77% and 65% of Tajan genome, respectively in the case of first and second sister lines (Figure 2). Average calculated genetic identity of the two sister lines obviously deviates from expected value (68.3% vs. 50% for first sister line and 46.8% vs. 50% for second one). These results indicate that selection effect was considerable, but in opposite directions (positive in the case of first sister line and negative in the case of second one). This finding shows the importance of making selections based on molecular markers to obtain lines with desirable traits

from donor parent along with maintaining general plant type of commercial recipient parent; Thus, our results again confirms the preference of MAS for crop improvement [15].

In  $F_6$  generation, average number of observed alleles was 1.92 (sd=0.272). The average of number of effective alleles was 1.47 (sd=0.31). Total number of detected loci was 52 and 47 of them (90.4%) were polymorphic. Shannon information index for polymorphic loci ranged from 0.198 to 0.692, with average of 0.442 (sd=0.153). Nie's gene diversity ranged from 0.095 to 0.499, with average of 0.288 (sd=203) (Table 1).

Analysis of genetic identity of individuals in  $F_6$  population showed that contribution of Tajan cultivar genome in  $F_6$  individuals was variable, ranging from 62.8% to 90.2%, and averaged to 75.8%. Five percent of  $F_6$  lines harbored ~90% of Tajan genome (Figure 3) which obviously deviates from expected value (0.50), indicating that in this population selection effect in favor of Tajan genome was considerable. Therefore, breeders must use molecular markers to select those plants with much contribution of Tajan genome (e. g a plant with 90.2% similarity; Figure 3) and carrying desirable donor traits such as yellow rust and FHB resistances.

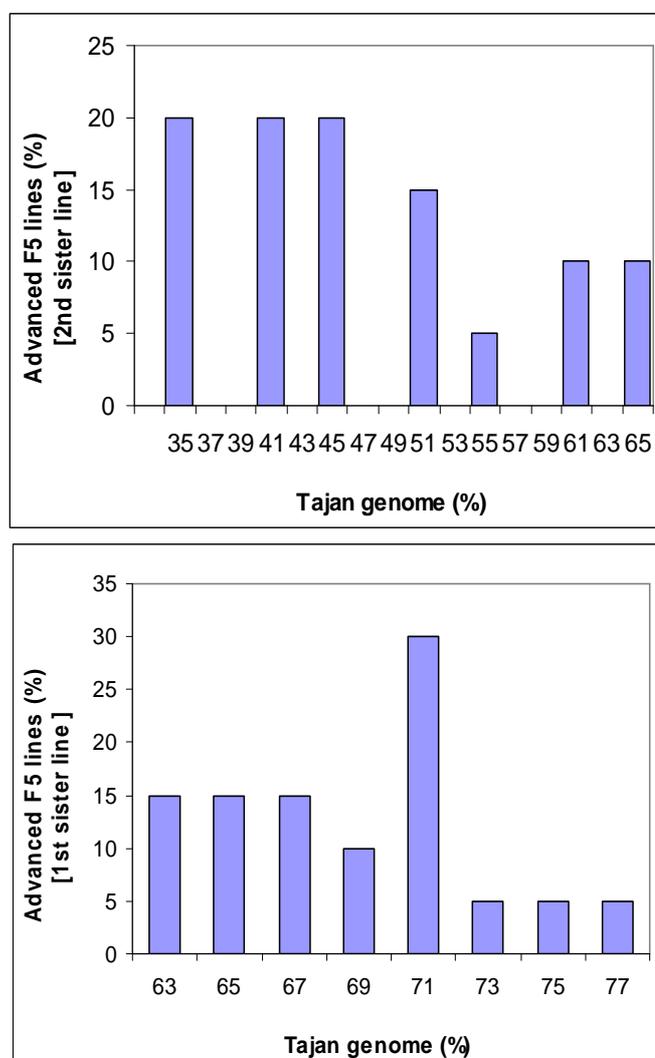
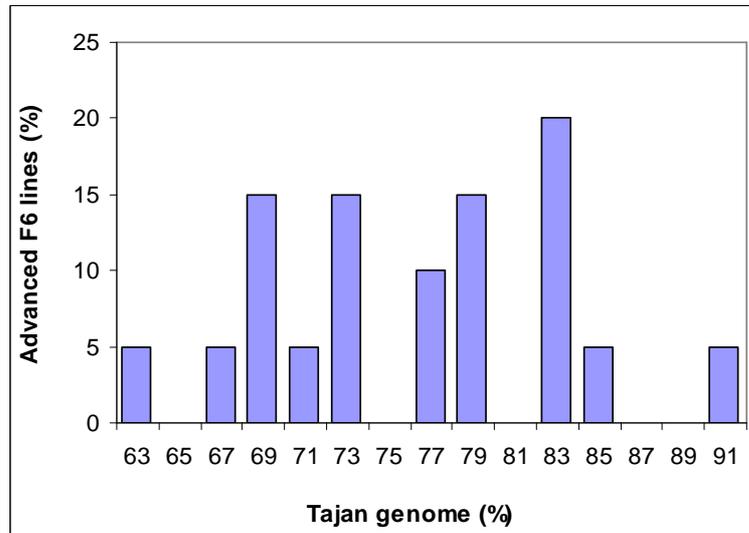


Figure 2. Background marker-assisted selection to obtain contribution of Tajan genome in 2 advanced sister lines of  $F_5$  generation.

Values of contribution of Tajan cultivar are shown on horizontal axis.

As seen in Table 1, average gene diversity in F<sub>5</sub> was calculated equal to 29% and that of F<sub>6</sub> equal to 28.8%. This shows that advancing from F<sub>5</sub> to F<sub>6</sub> based on phenotyping selection did not decrease genetic diversity due to selection pressure in favor of Tajan plant type.



**Figure 3. Background marker-assisted selection to obtain contribution of Tajan genome in advanced lines of F<sub>6</sub> generation.**

*Values of contribution of Tajan cultivar are shown on horizontal axis.*



**Figure 4. Field evaluation of Tajan-derived F<sub>6</sub> lines (left and middle) for yellow rust resistance using natural infection by culturing spreader cultivar (right).**

Genetic identities to Tajan in two F<sub>5</sub> advanced lines were 68.3% and 46.8% (in average 57.5%) and in F<sub>6</sub> was 75.8% (Table 1). Therefore, selection effect in favor of fixation of Tajan genome F<sub>6</sub> is 18.3%. This indicates that phenotypic selection for general Tajan plant type has been very effective. Furthermore, only a few F<sub>6</sub> lines after 6 generations of selection for resistance to yellow rust (see Figure 4) and fusarium head blight showed resistance to these two constraints, indicating that these traits have not been yet fixated and hence, selection must be continued until fixation of these traits.

The results of this work indicate that improving a line of Tajan type with resistances to yellow rust and fusarium head blight will be more promising when using marker-assisted selection (MAS). Gadaleta *et al.* [6] using molecular markers in a set of 28 BC<sub>3</sub>F<sub>7</sub> lines found that contribution of recurrent parent genome ranged between 76 to 99% which did not deviate from expected value.

### CONCLUSION

Our results indicate that advancing plants from F<sub>5</sub> to F<sub>6</sub> increased contribution of Tajan genome due to selection pressure in favor of Tajan plant type. Detection of plants in F<sub>5</sub> and F<sub>6</sub> generations with variable contribution of Tajan genome indicates the possibility of combined selection for obtaining elite lines with favorable characters from donor plants and harboring background of known commercial cultivars. Our results markedly show that selection made by breeder has diverse effects on genetic structure of plant material, particularly in favor of fixating genetic background of superior parent.

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