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Advances in Applied Science Research, 2010, 1 (3): 153-159



The effects of selection for multiple traits on diversity of advanced wheat lines revealed by molecular markers

Noori A.¹, Ahmadikhah A.^{1*}, Soughi H.², and Dehghan M.²

¹Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran; ²Cereal Research Institute, Gorgan, Iran.

ABSTRACT

It was suggested that selection for multiple desirable traits during generations has meaningful effects on diversity of resultant lines to be released to farmers. However, this idea less was studied at molecular level. We have started investigating effects of selection on diversity of wheat advanced F_5 and F_6 lines derived from Falat//ATTILA/BCN13/MILLAN/SHA7 cross, which passed 5 and 6 generations of selection for several important traits, including resistance to yellow rust, fusarium head blight and general Falat plant type. Genetic diversity of F_5 and F_6 lines was studied using 38 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 two generations was 28% and 53%, ranging 9.1-49.5% and 9.1-49.9%, respectively. In F_5 we identified lines with low identity to Falat as few as 28% and some lines with high identity up to 79%. Average identity to Falat in F_5 was 59.5%. In F_{6} , however, we identified lines with 38-85% identity to Falat, and averaged to 65%. These results indicate that advancing lines from F_5 to F_6 increased their identity to Falat cultivar due to selection pressure in favor of Falat plant type. Our findings obviously show the multidirectional effects of selection made by breeders on genetic diversity of plant material at molecular level.

Key words: Selection, Molecular markers, Wheat, Diversity.

INTRODUCTION

The study of diversity is important in crop breeding programs for the selection of suitable parents to obtain heterotic hybrids as well as for the conservation and management of germplasm [4]. Conventionally, genetic diversity is estimated on the basis of morphological and phenotypic characters. Assessment of crop germplasm diversity phenotypically and morphologically 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 [5]. In addition, morphological differences may be

epigenetic- or genetic-based characters [8, 10, 17]. Estimation by biochemical markers, viz. isozyme analysis, may also be biased as only a minor portion of genome is represented by these markers. Molecular markers due to their advantages against to 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]. In last decade molecular markers such as RFLP, AFLP, RAPD, SCAR and etc., have been used to assess genetic variation at the DNA level [9]. Techniques using isozyme and RFLP are timeconsuming, labor-intensive, reveal low level of polymorphism in wheat owing to its high proportion of reptetive DNA [14]. Among the various techniques available, RAPD analysis is a simple, rapid, and effective method for detecting polymorphism in wheat [18]. Usually arbitrary primers having 10 bases per length are used in RAPD [19]. 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. In the first two cycles, annealing is under non-stringent conditions. The final products are structurally similar to RAPD products. Compared to DAF, this variant of RAPD is not very popular as it involves autoradiography. Recently, however, it has been simplified by separating the fragments on agarose gels and using ethidium bromide staining for visualization [7]. Aba-El-Haleem et al. [1] using RAPD markers obtained up to 70% polymorphism and discriminated 11 durum wheat cultivars in 2 clusters. 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) for detecting polymorphism and study of genetic diversity in wheat advanced lines was less reported. The aims of this study were to detect polymorphism, to analyze genetic diversity within and between wheat advanced lines and to investigate the effect of selection for multiple traits on diversity of the advanced lines.

MATERIALS AND METHODS

Plant Material

The plant material used in this study were the two sister line populations of advanced F_5 plants each containing 20 individuals, and two sister line populations of advanced F_6 plants each containing 10 individuals, derived from Falat//ATTILA/BCN3/MILLAN/SHA7 cross, which had passed five and six generations of selection for several important traits such as fusarium head blight (FHB) resistance, resistance to yellow rust and general Falat 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 μ l dH₂O, 5 μ l PCR Master Mix (Cinnagen, Iran), 0.5 μ l primer and 1 μ 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 extention 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 [11] 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 3 to 9 bands, from which 1 to 9 were polymorphic. Totally, 38 and 47 polymorphic markers were detected in F_5 and F_6 generations, respectively.



Figure 1. A sample of banding pattern produced by an AP-PCR primer named Ctg199 on Falat cv. (Fl) and 20 advanced lines (from 1 to 20) in F_5 generation.

F₅ generation

For the first sister line in F_5 generation, number of observed alleles ranged between 1-2, with the average of 1.73. The average number of effective alleles was 1.35, and ranged between 1-1.98. Total number of detected loci was fifty two, 38 of them were polymorphic. Percentage of polymorphic loci was 73%. Shannon information index for polymorphic loci ranged from 0.191 to 0.692, with average of 0.34. Nie's gene diversity ranged from 0.091 to 0.495, with average of 0.22 (Table 1).

For the second sister line in F_5 generation, number of observed alleles ranged between 1-2, with the average of 1.96. The average of number of effective alleles was 1.73. Total number of detected loci was twenty five, 24 of them were polymorphic. Percentage of polymorphic loci was 96%. Shannon information index for polymorphic loci ranged from 0.191 to 0.692, with average of 0.34. Nie's gene diversity ranged from 0.091 to 0.499, with average of 0.34 (Table 1). Altogether, F_5 generation showed 0.28 gene diversity.

Genetic identity and genetic distance were calculated for individuals in 2 F₅ populations using unbiased measure of genetic identity and genetic distance [11]. We observed that identity between F₅ individuals and Falat cultivar was variable, ranging from 0.58 to 0.79 (with average of 0.68) for first sister line, and from 0.28 to 0.76 (with average of 0.51) for second sister line (Figure 2). Although average calculated genetic identity of first sister line obviously deviates from expected value (68% vs. 50%), in the case of second sister line well fits to expected value (51% vs. 50%). These results indicate that selection effect on first sister line was more considerable that on second one. This finding shows the importance of making selections based on molecular markers to obtain lines with desirable donor parent traits along with maintaining general plant type of commercial recipient parent and again confirms preference of MAS for crop improvement [15].

	No.	no	n _e	No.	No.	Polymorphic	Shannon	Nie's gene	Identity
	samples			loci	polymorphic	loci (%)	information	diversity	to Falat
	_				loci		index	-	
Pop ₁	20	1.73	1.35	52	38	0.73	0.34	0.22	0.68
Pop ₂	20	1.96	1.62	25	24	0.96	0.50	0.34	0.51
Mean	40	1.85	1.49	38.5	31	0.80	0.42	0.28	0.595
$S.D_1$	-	0.448	0.319	-	-	-	0.249	0.175	-
$S.D_2$	-	0.200	0.388	-	-	-	0.230	0.182	-
Pop3	20	2.0	1.63	47	47	1.00	0.36	0.53	0.65
S.D ₃	_	0.00	0.32	-	-	-	0.143	0.173	_

Table 1. Genetic diversity for 2 sister lines in F₅ generation (Pop1 and Pop2) and one population in F₆ generation (Pop3) each consisted of 20 individuals.

SD₁, SD₂ and SD₃: standard deviations of Pop₁, Pop₂ and Pop₃, respectively.

F₆ generation

For two sister lines in F_6 generation, average number of observed alleles was 2. The average of number of effective alleles was 1.63. Total number of detected loci was 47 and all of them were polymorphic. Shannon information index for polymorphic loci ranged from 0.191 to 0.692, with average of 0.36. Nie's gene diversity ranged from 0.091 to 0.499, with average of 0.53 (Table 1). Genetic identity and genetic distance were again calculated for individuals in F_6 population using unbiased measure of genetic identity and genetic distance. We observed that identity between F_6 individuals and Falat cultivar was variable, ranging from 0.38 to 0.85, and averaged to 0.65 (Figure 3) which obviously deviates from expected value (0.50), indicating that in this population selection effect in favor of Falat genome was considerable. Therefore, breeders must use molecular markers to select those plants with much contribution of Falat genome (e. g a plant with 0.85 value of similarity) and carrying desirable donor traits such as yellow rust and FHB resistances.

As mentioned average gene diversity in F_5 was calculated equal to 0.28 and that of F_6 equal to 0.53. This shows that advancing from F_5 to F_6 based on phenotyping selection not only decreased genetic diversity, but also increased it; a result in opposition to general knowledge of plant breeding, which believes that genetic diversity decreases in final generations of a pedigree-based method.

Genetic identities to Falat in two F_5 advanced lines were calculated 68% and 51% (in average 59.5%) and in F_6 was equal to 65% (Table 1). Therefore, selection effect in favor of Falat cultivar from F_5 to F_6 is 5.5%. This indicates that phenotypic selection for general Falat plant type has been relatively effective.





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Furthermore, F_6 lines after 5 generations of selection for resistance to yellow rust and fusarium head blight showed resistance to these two constraints. These results indicate that improving a line of Falat type with resistance to yellow rust and fusarium head blight using phenotypic foreground and background selections had little success and will be more promising when using marker- assisted selection (MAS). Gadaleta *et al.* [6] using molecular markers in a set of 28 BC₃F₇ lines derived from cross of durum wheat cv. Latino × *Triticum turgidum var. dicoccoides* found that contribution of recurrent parent genome ranged between 76 to 99% and had average of 93% which did not deviate from expected value.





Values of identity to Falat cultivar are shown at right.

COCLUSION

Our results indicate that advancing plants from F_5 to F_6 increased their average identity to Falat due to selection pressure in favor of Falat plant type. Detection of plants in advanced generations with diverse identity to one parent obviously shows the multidirectional effects of selection made by breeders on genetic diversity of plant material at molecular level.

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