ISSN-1991-3036 (Online) & ISSN-2075-1621 (Optical)
Int. J. Sustain. Crop Prod. 5(3):29-34 (August 2010)

STUDY ON DIFFERENTIATION OF WHEAT VARIETIES THROUGH MORPHOLOGICAL AND MOLECULAR APPROACHES

S. TASNUVA[1], M. HASANUZZAMAN[1], G. FARUQ[2], F. SHARMEEN[1] AND M. ARIFUZZAMAN[1]

[1]Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science & Technology University, Dinajpur-5200, Bangladesh; [2]Senior Scientific Officer, Wheat Research Centre, Dinajpur, Bangladesh.

Corresponding author & address: M. Arifuzzaman, E-mail: arif_gpb@yahoo.com
Accepted for publication on 15 July 2010

ABSTRACT

Tasnuva S, Hasanuzzaman M, Faruq G, Sharmeen F, Arifuzzaman M (2010) Study on differentiation of wheat varieties through morphological and molecular approaches. *Int. J. Sustain. Crop Prod.* 5(3), 29-34.

Genetic variation within species has been assessed by many methods and from several perspectives. In present study, both RAPD markers and morphology were successfully used to differentiate four cultivars of wheat named Bijoy, Prodip, Sourav and Shatabdi. Four distinct morphological characters were identified to differentiate four varieties. In lower glume beak length, at physiological maturity stage, distinctness was observed. In case of Bijoy variety, it was almost rudiment (1-2mm), but in Prodip, its length was the highest (15-18mm), medium length (12-15mm) was observed in Shatabdi and Sourav showed small (8-l0mm) glume beak length. In Zadok growth stage 25-29, a distinct difference was observed in four wheat varieties. Growth habit of Sourav was erect, Shatabdi semi-erect and Prodip intermediate. Heading days at Zadok growth stage were 68 for Sourav, 71 for Shatabdi, 63 for Bijoy and 60 for Prodip. Culm glaucosity was strong in Sourav, weak in Shatabdi and Prodip and medium in Bijoy. Two primers (OPA-02 and OPB-O1) yielded comparatively higher number of amplification products among the eleven primers with high intensity, minimal smearing and good resolutions with clear bands. All of them (100%) were considered as polymorphic and no monomorphic band was found. The primer OPA-02 produced 16 bands and the other primer OPB-O1 generated 17 bands respectively. The result of RAPD marker was more distinct than the morphological marker for the differentiation of wheat varieties. Phenotypic characters were influenced by the environment but in case of molecular marker the reproducibility of RAPD markers were the same in the same reaction conditions.

Key words: *genetic variation, primer, RAPD marker*

INTRODUCTION

Wheat (*Triticum aestivum*) is the world's most widely cultivated food crop. It is used in various forms by more than one thousand million human beings in the world. Wheat is the second most important cereal crop after rice in Bangladesh. In Bangladesh wheat covered in 400 thousand ha area, yielded 1.85 m ton/ha (BBS, 2008). Several morphological traits were used to differentiate the varieties. Incase of four popular wheat varieties named Sourav, Bijoy, Shatabdi, Prodip, some important phenotypic traits are used to find out the differentiation of those varieties. But the morphological traits are, however, often influenced by environmental conditions (Jasienski *et al.* 1997; Kercher and Sytsma, 2000).

Molecular markers are the molecules that could be used to trace a desired gene(s) in test genotypes. Infact, a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evolution of morphological traits (Stuber *et al.* 1987). However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single gene and they are not affected by the environment. Recently the concept of marker-aided selection had provided an advantage of molecular marker based approaches for crop improvement as compared to selection based solely on phenotype (Tansksley *et al.* 1989; Paterson *et al.* 1991).

Wheat Research Center of Bangladesh Agricultural Research Institute has been released 24 varieties until June 2005. During approval of a variety, National Seed Board. Bangladesh is frequently demanding some marker characters of each variety and sometimes it is not possible to release a good variety due to lack of physical or morphological marker. It is really difficult for the wheat breeders to develop physical marker with other good agronomic characters and they think to do backcrosses to solve these problems, which is also time consuming and sometimes did not find any positive results. DNA finger printing might be a good solution to overcome this problem. RAPD (Random Amplified Polymorphic DNA) markers are frequently using for DNA finger printing since 1995. The whole protocols might be used for the future developed wheat lines, so the complication of identifying characters of new lines will be eliminated.

Therefore, the present investigation was undertaken on marker characterization and to identify distinct characters of four popular wheat varieties named Bijoy, Prodip, Sourav and Shatabdi.

MATERIALS AND METHODS

Four hexaploid wheat varieties named Bijoy, Prodip, Sourav and Shatabdi were collected from Wheat Research Centre (WRC) of Bangladesh Agricultural Research Institute (BARI) were used in the study.

Methods of Distinctness, Uniformity and Stability (DUS) Test for morphological characterization

A plot containing 500 normal wheat plants were grown at commercial seed rate and spacing in research block I of wheat research center. There was another set either 15 days interval in planting or grown in research block II of Wheat Research center in December 15, 2007. This year can be considered as test period as UPOV suggested. UPOV published guidelines and instructions were followed to conduct DUS test.

DNA extraction for Molecular characterization

A plant DNA extraction Kit (Gene i ^{TM}) which was designed as quick single tube method for DNA extraction only 10 to 15 mm long tender wheat plant tissue (10 days aged wheat seedling). Phenol extractions did not involve with this protocol, so the extracted DNA were directly used for PCR amplification.

Conformation of DNA

Five µl 2X loading dye was mixed with 3 µl DNA samples. Then these DNA samples (8 µl) were loaded in the 0.8% agarose gel on the gel tank. The electrophoresis machine was run for 1.0 h at 120 V. After electrophoresis the gel was stained by ethidium bromide for atleast 15 min and the bands were visualized under UV light Gel Doc system (Biometra, Japan). Two methods were applied for checking DNA concentration more precisely viz. λ(lamda) DNA (concentration marker) and spectrophotometer method. Spectrophotometer was used to quantify DNA more precisely.

Application of RAPD markers by Polymerase chain reaction (PCR)

A target DNA sequence is exponentially amplified with the help of arbitrary primers, a thermo stable DNA polymerase, deoxynucleotide triphosphates, magnesium chloride and reaction buffer. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and elongation step. Eleven primers of random sequence (Operon Technologies, Inc., Alameda, California- USA) were screened on a sub sample of two randomly chosen individuals from four different varieties to evaluate their suitability for amplification of their DNA sequences, which could be scored accurately. The details of primers are given in table 1. A final subset of two primers (OPB-O1 and OPA-02) exhibiting good quality banding patterns, and sufficient variability were selected for analysis.

Table 1. Parameter of the random primers used in the present study for Screening

Primer code	Sequence (5'-3)	GC content (%)
OPB-O 1 *	GTTTCGCTCC	60
62AB l OC2	GGACCCAACC	70
63AB l 0A3	GTCGCCGTCA	70
64AB l OG4	TCTGGTGAGG	60
66AB l OG6	ACCTGAACGG	60
68AB l 0A8	GTGTGCCCCA	70
OPA-02*	TGCCGAGCTG	70
70AB l OC 10	GGTCTACACC	60
71 AB l OG 11	AGCGCCATTG	60
72AB l OG 12	AGGGCGTAAG	60
73AB10T13	CTGGGGACTT	60

*Primer selected for RAPD analysis for all samples of the cultivars

During the experiment, PCR buffer, dNTPs, and primer solutions were thawed from frozen stocks, mixed by vortexing and placed on ice. DNA samples were also thawed out and mixed gently. The primers were pipetted first into 0.2 ml PCR tubes. For each DNA sample being tested, a pre-mix was then prepared including the following order: buffer, dNTPs, DNA template and sterile distilled water. DNA then added to the tubes containing primers. Samples were added and the tubes were then sealed and placed in a thermocycler and the cycling was started immediately. DNA amplification was performed in an oil-free thermal cycler. The reaction mix was preheated at 94°C for 3 minutes follower by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 54°C and elongation or extension at 72°C for 2 minutes. After the last cycle, a final step for 7 minutes at 72°C to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°C. Agarose gel (1.5%) was prepared and poured into platform carefully when the gel solution cooled to 55°C. Let the gel polymerize for at least 30 minutes before removing the combs. After removing the casters, gel with platform was placed at the tank and poured 0.5X TBE buffer into the tank to submerge the gel.

Then the combs were removed cautiously that gel slots were not injured. Then the PCR products were mixed with 5µl of 2X gel loading dye. 15 µl of the mixture was loaded slowly per well on the gel in the gel tank allow them to sink in the bottom of the wells. The tank was covered and all connections were checked. Electrophoresis machine run for 1-1.5 hr at 100 volts. The separation process was monitored by the migration of the dyes in the loading buffer. When the bromophenol blue dye had reached about three-fourth of the gel length, the electrophoresis was stopped. After completion of electrophoresis the gel was soaked in ethidium bromide (l0mg/ml) solution for 15-20 min.

Documentation of the samples

After staining the gel was taken out carefully from the staining tray and placed on high performance ultraviolet light box (UV transilluminator) of gel for checking the DNA bands. The DNA was observed as band and saved the records.

Data Analysis

Since RAPD markers are dominant, we assumed the each band represented the phenotype at a single allelic locus (Williams *et al*. 1990). All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer.

RESULTS AND DISCUSSION

Morphological characterization

Morphological traits are, however, often influenced by environmental conditions (Jasienski 1997; Kercher and Sytsma, 2000), which in turn may influence the estimation of genetic variation and relatedness. Agronomical important traits are valuable for a species in cultivation, and form the basis for the breeder's selection of promising plant material. Other morphological traits are used mainly for identification of genotypes and cultivars, e.g. the UPOV (International Union for the Protection of New Varieties of Plants) guidelines for evaluation of the genetic variation within a species, e.g. leaf shape analysis. In case of wheat morphological characters are usually determined by DUS (Distinctness, Uniformity and Stability) test. DUS parameters are considered in Zadok growth stages (Soller and Beckmann, 1983), where wheat lifecycle is usually considered in 100 days. In the present study, in Zadok growth stage 25-29 a distinct different was observed in four wheat varieties. Sourav habit was erect, Shatabdi was semi-erect and Prodip was intermediate. Heading days were (Zadok growth stage 60) 68 days for Sourav, 71 days was for Shatabdi, 63 days was for Bijoy and 60 for Prodip. Culm Glaucosity was observed strong in Sourav, weak in Shatabdi and Prodip, medium in Bijoy. Lower glume beak length was medium in Sourav and Shatabdi, short in Bijoy and very long in Prodip.

Identifying distinct phenotypic marker

Figure 1. Distinct phenotypic markers (lower glume beak length) of four wheat varieties

Four wheat varieties were raised up in Field I, WRC, BARI, Dinajpur and Field II, WRC, BARI, Dinjpur. No significant differences were observed in lower glume beak length in same variety in the both fields. However, significant differences were observed among the varieties in the two fields. Observed results have been presented in Table2 and Table 3.

Table 2. Differences in lower glume beak length in same varieties in two locations

Location	Beak length (mm)			
	Bijoy	Prodip	Sourav	Shatabdi
Field 1, WRC, BARI, Dinajpur	1.2	15	8	10
Field 1, WRC, BARI, Dinajpur	1.3	16	8.5	10
LSD (0.05)	NS	NS	NS	NS

Table 3. Differences in lower glume beak length in four varieties in different locations

Variety	Lower glume beak length(mm)	
	Field I	Field II
Bijoy	1.2	1.3
Prodip	15	16
Sourav	8.0	8.5
Shatabdi	10	10
% CV	2.5	1.9
LSD(0.05)	4.73	3.08

Primer selection and RAPD profiles

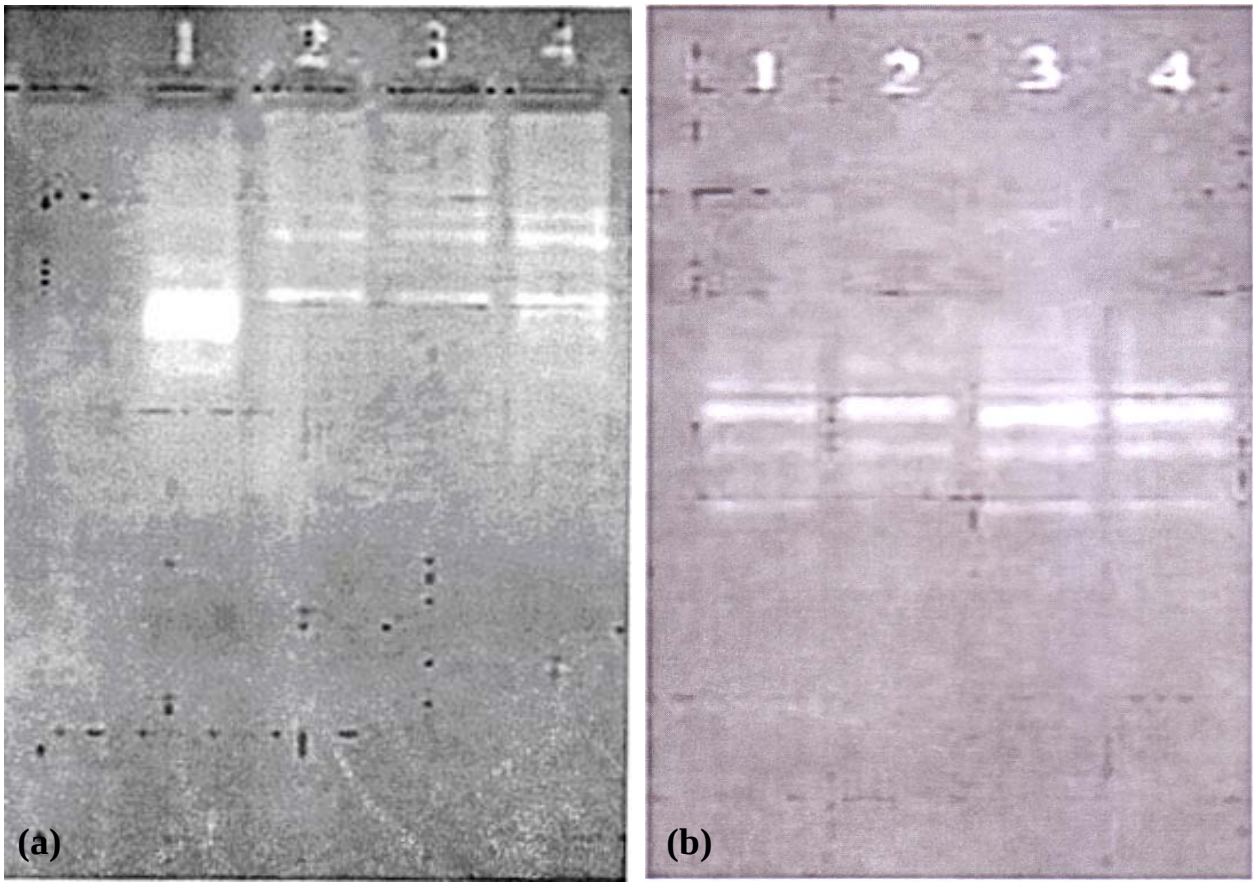
Among the eleven primers initially tested, two primers (OPA-02 and OPB-O1) yielded comparatively higher number of amplification products with high intensity, minimal smearing and good resolutions with clear bands. Two random primers (OPA-02 and OPB-O1) produced RAPDs with varied patterns. The two primers generated 33 distinct bands. All of them (100%) were considered as polymorphic and no monomorphic band was found. The primer OPA-02 produced 16 bands and the other primer OPB-O1 generated 17 bands respectively.

Table 4. Total scoreable bands and polymorphic bands amplified by two RAPD primers in studied four wheat cultivars

Primer	Sequences (5'-3')	Scored band	Polymorphic band
OPB-O1	GTTTCGCTCC	17	17
OPA-02	TGCCGAGCTG	16	16

The level of polymorphism (100%) indicated the effectiveness of RAPID technique to study substantial amount of polymorphisms among the different varies of *Triticum aestivum*. This proportion of polymorphism higher compared to some previous RAPID analysis in *Triticum aestivum*, e.g. 71% in Maxican wheat (Castgna *et al.* 1997), 80% in Chinese (Sun *et al*. 1998) wheat cultivars. This difference can be attributed to the primers used and genotypes evaluated.

The banding patterns of four wheat cultivars using two different primers are shown in figure 2(a) and 2(b)



Lane 1= Prodip, Lane 2= Bijoy, Lane 3= Shatabdi, Lane 4= Sourav

Figure 2. Random amplified polymorphic DNA (RAPD) patterns found in four wheat genotypes (a) using primer OPA-02 and (b) using primer OPB-O1

The present experiment produced a total of 33 bands by using two primers (OPA-02 produced 16 and OPB-O1 produced 17 bands) and they were polymorphic RAPD markers. This high level of polymorphism detected by the arbitrary primers was almost similar to the previous reports in other RAPD studies on *Triticum aestivum* cultivars, such as 31 scored per Primers in Mexico.

We observed that the morphological characters might be changed depends on environmental factors, such as drought, high nitrogen application (micro environment), prolong winter. As a result some variation was found in our research also lower glume beak lengths in four wheat cultivars. In the case of molecular markers it is expected that the reproducibility of RAPD markers will be the same in the same reaction conditions. But it is needed to do in a developed analytical environment and it is expensive. So it is concluded that besides development of molecular markers, distinct morphological marker identification is also very important to differentiate the wheat varieties. In the present study, it was attempted and developed distinct morphological markers besides development of RAPD markers, which differentiated the four popular wheat varieties Bijoy, Prodip, Shatabdi and Sourav.

REFERENCES

BBS (2008) Statistical Pocket Book of Bangladesh. Statistic Division. Ministry of planning, Government of the People's Republic of Bangladesh, Dhaka. p.203.

Castagna R, Gnocchi S, Perenzin M, Heun M (1997) Genetic variability of the wild diploid wheat *Triticum urartu* revealed by RFLP and RAPD markers. Theor Appl Genet 94, 424-430.

Jasienski M, Ayala FJ, Bazzaz FA (1997) Phenotypic plasticity and similarity of DNA among genotypes of an annual plant. Heredity.78, 176-181.

Kercher SM, Sytsma KJ (2000) Genetic and morphological variation in populations of the rare prairie annual Agalinis skinneuiana (Wood) Britton (Scrophulariaceae). *Natural Areas Journal,* 20, 166-175.

Paterson AH**,** Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments.Genetics 127, 181-197.

Soler M, Beckmann JS (1983) Genetic polymorphism in varietal identification and genetic improvement. *Theoretical and Applied Genetics*. 67(1), 25-33.

Stuber CW, Edwards MD, Wendal JF (1987) Molecuilar marker facilited investigations of quantitative traits loci in maize 2. Factors influencing yield and its component traits. Crop Sci. 27, 639-648.

Sun GL, Diax O, Salomon B, Bothmer R (1998) Microsatellite variation and its comparison with allozyme and RAPD variation in *Elymus fibrosus* (Schrenk) Tzvel. (Poaceae). Hereditas, Lund. 129, 275-282.

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. BioTechnology 7, 257-264.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, Oxford. 18, 6531-6535.

.