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Comparison of Genetic and Morphological Distance with Heterosis with RAPD Markers in Hybrids of Barley

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ABSTRACT

In the present study, we surveyed the genetic divergence among 12 genotypes of barley using DNA markers. Some fragments generated by seven primers of a 10-mer arbitrary sequence were used to study their potential power in different parents with different characteristics and to predict the yield performance of the hybrid produced from parental materials. Many traits such as biomass weight, plant height, peduncle and spike length, peduncle extrusion, yield per plant and its component such as number of seeds per spike and 100-seeds weight and other morphological characters were measured. Experimental results showed that the 12 barley genotypes were divided into several groups. Although the genetic distance based on RAPD markers has not been significantly correlated with hybrid performance and heterosis in all of the traits, the genetic distance was used to predict hybrid performance with mixed results. It appears to be impossible to predict the hybrid performance from the genetic distance itself. Comparisons of genetic and morphological distance were studied with RAPD and morphometric approaches. Experimental results showed that there was not significant correlation between them.

Key Words: Barley; RAPD markers; Heterosis; Genetic distance

INTRODUCTION

Identification of combinations with strong yield heterosis is the most important step in developing crop hybrids. Generally, parents with a higher general combining ability and long genetic distance can produce a hybrid with better yield performance (Shamsuddin, 1985; Cox & Murphy, 1990; He, 1991; Boppenmaier *et al.,* 1993; Diers *et al.,* 1996). But the identification of combining ability based on morphological characters is costly and time-consuming and may be influenced by environment factors. Therefore, some scientists have been trying to predict yield heterosis on the molecular level. In contrast, molecular markers are not directly influenced by environmental effects or epistatic interactions and can provide large numbers of loci. Several methods such as isozyme analysis or restriction fragment length polymorphisms (RFLPs) have been used to investigate the genetic relationships between and within different species (Liu & Furnier, 1993; Nocelli *et al.,* 1999). Methods that detect variation at the level of the DNA sequence have proved to be an extremely effective tool for distinguishing between closely related genotypes (Hartl & Seefelder, 1998) and the variety of these are now available. One of these methods, randomly amplified polymorphic DNA (RAPDs), has been widely employed because of its simplicity and ability to detect genetic variation among very closely related genotypes in a number of genera such as Brassica (Jain *et al.,* 1994). Furthermore, RAPDs have been widely used to study the population genetic structure, genetic diversity and relationships, and phylogenetic relationships (Baker *et al.,* 1999). At present, other molecular markers such as amplified fragment length polymorphisms (AFLPs) or simple sequence repeats (SSRs) are being used because they tend to detect higher levels of polymorphism (Powell *et al.,* 1996; Perera *et al.,* 1999). However, latter two methods also have some disadvantages compared to RAPDs, such as the need for radioactive labeling or previous sequence information.

The aims of the present study were: (i) to evaluate the genetic diversity, (ii) investigate the relationship between hybrids performance and genetic diversity based on RAPD markers in barley, and (3) to compare the molecular and morphological methods based on RAPD markers.

MATERIALS AND METHODS

Plant materials. The hybrids population was developed by crossing of 6 accessions of Iranian barley with 6 accession of Japanese barley that were selected to study genetic diversity following RAPD assay (Table I).

Field experiment. 13 traits of the parents and their hybrids were measured in the field such as biomass weight, plant height, peduncle, length of awns, spike length, number of fertile tillers, number of sterile tillers, yield per plant and its component such as number of seeds per spike and 100-seeds weight and other morphological characters.

DNA extraction. DNA was extracted using Dellaporta method (Dellaporta *et al.,* 1983) with some modifications. Leaves were ground to fine powder in the presence of liquid nitrogen then 400 µL of extraction buffer (100 mM Tris pH 7, 50 mM EDTA pH 8, 250 mM Nacl, 5% SDS) was added and mixed gently. The contents were transferred to sterile tubes and placed in 65°Ċ for 30 min. 200 µL Sodium Acetate (5 M) was added and mixed by inversion and kept for 10 min on 0°Ċ. 500 µL of Chloroform: iso-amyl alcohol, prepared in a ratio of 24:1, was added to the tubes and they were centrifuged for 15 min at 5,000 r.p.m and room temperature. The aqueous phase was transferred to a clean tube, and one volume of isopropanol (2-propanol) was added and mixed well by inversion so that nucleic acids

were precipitated. Samples were centrifuged for 15 min at 5,000 r.p.m and room temperature. The supernatant was poured off and DNA pellets were washed using 70% ethanol and dried at room temperature. DNA was assessed by spectrophotometer.

RAPD amplification. 40 UBC primers[1] were used for PCR amplification and produced reproducible and informative marker patterns. Polymerase chain reaction (PCR) was performed in a volume of 25 µL containing 100 mM of Tris-Hcl pH 8.8, 50 mM Kcl, 0.01% Triton X-100, 1.14 mM $MgCl_2$, 0.175 mM of each dNTP, 0.5 µM primer, 25 ng of genomic DNA and 1 unit of Tag DNA polymeras. DNA amplification was performed in a DNA Thermal cycler (perkin elmer) programmed for an initial denaturation step of 2 min at 94°Ċ, then 45 cycles at 92°Ċ (1 min), 35°Ċ (1 min), 72°Ċ (2 min) for denaturation, primer annealing and primer extension, respectively, and a final primer extension at 72°Ċ for 5 min. Amplifed products were analyzed by electrophoresis in 6% polyachrylamide gels run at 200V in 1X TAE and detected by staining in ethidium bromide (5 ng/mL). The gels were photographed on a digital gel documentation system.

Statistical analysis. RAPD bands were scored as 0 for absent or 1 for presence in each individual. Polymorphic RAPD markers were transformed into a binary matrix. Pair-wise genetic similarities (Sij) between accession i and j were estimated using the similarity coefficient of simple matching by SPSS software. Dendogram was constructed using unweighted pair group method of arithmetic averages (UPGMA), based on distance matrices.

RESULTS

After screening 40 primers for DNA amplification, and presence of polymorphic fragments, 6 primers produced informative banding patterns. Seven primers on 12 accessions generated 379 amplified fragments, and 64 (16.8%) were polymorphic. The polymorphism among primers ranged from 11.76, with primer UBC100 to 24.13 with primer UBC96 (Table II).

The fragments size per primer ranged from 300 to 1500 bp. It is important to note that the number of amplified fragments per primer and fragment strength may depend upon the degree to which the RAPD assay is optimized. Williams *et al.* (1993) found that even minor alteration in reaction conditions can affect banding patterns.

DISCUSSION

The outcome of this research showed that the RAPD marker is a very fast and cheap way for evaluating the genetic diversity and family relationship in a large number of samples. Likewise, according to the similarity coefficient of the genotypes in this research, the existence of such a variation in genotypes was not favorable and they must take action in developing germplasm, collecting samples and restoring the germplasm of genotypes.

According to the cluster analysis of morphological data, the dendrogram (Fig. 2) and comparison of plant groups and their F1, No. 3 genotypes of collection, F1 (4 x 5), Japanese 1, collection 4, Japanese barley, F1 (3 x Japanese barley), Japanese 10, F1 (7 x 50) , collection 7, collection 5 were placed in the first group and genotypes No. 11 Japanese, F1 (10 x 15), collectin 17, F1 (11 x 17), F1 (5 x 1), collection 15 in the second group and genotypes No. 5 and 50 (Japanese) in the third group. It was observed that in 83.3% of samples, the hybrids of parents were at least grouped together with one of the parents, which shows that the hybrid is near to one of the parents. For example, F1 (3 x Japanese barley) and Japanese barley were in one group, which shows that the hybrid plant in comparison between two parents is more like the Japanese parent. Thus, this classification can show us the way of generation and gene

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Table I. Cross between 6 barley parents produced 6 F1 hybrid

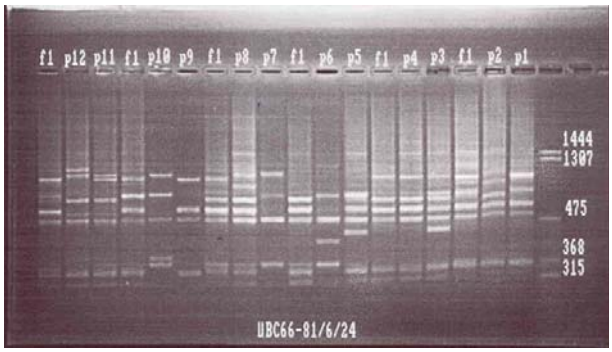
Male parent	Female parent
Japanese 50	number 7 from collection
Japanese 5	number 4 from collection
Japanese	number 3 from collection
Japanese10	number15 from collection
Japanese 11	number 17 from collection
Japanese 1	number 5 from collection

Table II. Oligonucleotide primer with their sequences, number of amplified fragment, polymorphic fragments, polymorphism (%) and fragment size range (bp) in barley test array

Primer	Sequences (5' to 3')	Amplified Fragments (N)	Polymorphic fragments (NP)	Polymorphism % (p=NP/NA*100)
UBC96	GGCGGCATGG	58	14	24.13
UBC66	GAGGGCGTGA	34	6	17.64
UBC64	GAGGGCGGGA	56	9	16.07
UBC95	GGGGGGTTGG	69	11	15.94
UBC1	CCTGGGCTTC	56	10	11.85
UBC100	ATCGGGTCCG	68	8	11.76
UBC77	GAGCACCAGG	23	4	17.39
		379	63	16.62

Data analysis: Heterosis (MH) of some traits were determined by the equation MH=(F1-MP)/MP, where MP represents the parent value.

Fig. 1. DNA polymophism detected with UBC66 primer on 12 accessions of parent and 6 F1



From right to left each pair of accessions and their f1, i.e. p1, p2 and their f1 (p1×p2), p3, p4 and their f1(p3×p4), etc.

Fig. 2. The dendrogram of parent's genotypes and their hybrids in morphological data using Dice distance based on UPGMA method

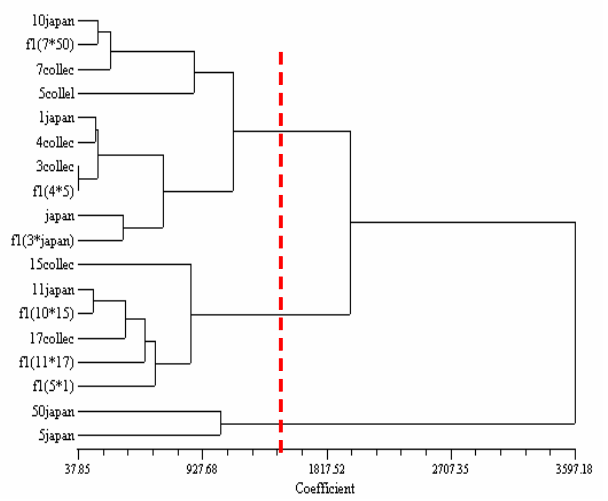
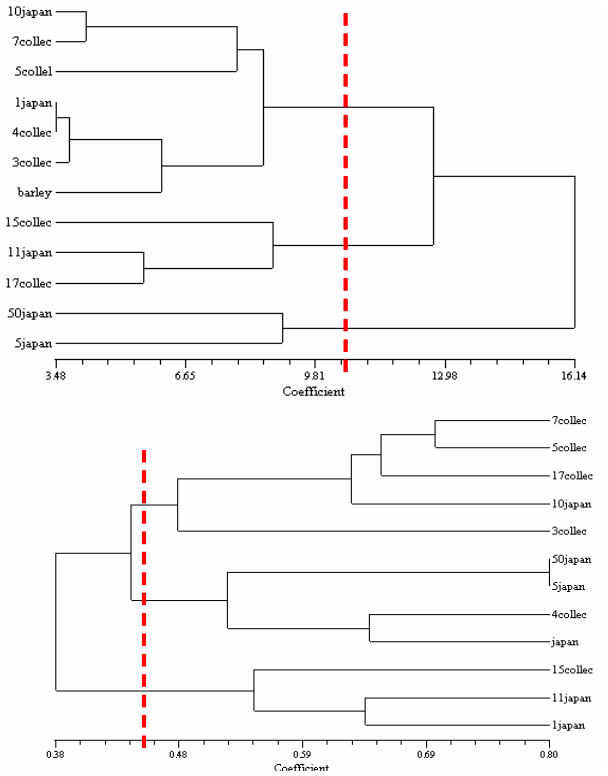


Fig. 3. The dendrogram of 12 genotypes of barley based on UPGMA and on Dice similarity coefficient in RAPD data(A) Jaccard similarity coefficient in morphological data (B).



action, and so we can suggest some theories for the way of generation and gene action.

In this experiment after the cluster analysis and drawing the dendrogram of data from the molecular and morphological methods observed (Fig. 3) that: in both the dendrograms, collection genotype No. 3, Japanese 10, collection 7, collection 5, were in one group and the hybrid genotype (No. 5 x Japanese 50) also occupied the same. In molecular and morphological methods, there was no difference in this classification. But in other genotypes, this classification was changed that shows the differences of these genotypes at molecular level and DNA. The most important point is in genotypes No. 5 x 50 Japanese that are exactly like each other from morphological point of view and in molecular methods that the genotypes are studied at DNA level. They are also like each other. In comparison of similarity coefficient of these two genotypes, we understand that the similarities of these genotypes in molecular studies are much more than morphological studies and this is because of the environment effect on phenotype that causes the separation of similar genotypes. After comparison between these two cluster by using the NTSYS software and Mantel test, correlation was r = 0.136 (p[random Z>= observed Z]=0.136) that shows that these two clusters from statistical point of view has no meaningful correlation with each other.

This observation was also recorded by Roldan-Ruiz *et al.* (2001) on ryegrass cultivar. Casiva *et al.* (2002) studied broad bean plant by morphological methods, Isozyme and RAPD and they didn't find a high correlation between morphological and RAPD methods. Szczepaniak *et al.* (2002) in comparison of RFLP primer and morphological method also found this difference. They also observed in their results that the variation from morphological method is obviously more than genetic method that proved in results of previous researches. Kiani *et al.* (2002) found that the cluster analysis and classification of objects based on RAPD has no relationship with morphological and agricultural characters. This means that samples with the same banding pattern in one group may be completely different in morphological and agricultural characters, so that cluster analysis and classification of samples based on RAPD marker may have no correlation with classification of samples based on botanical and morphological characters.

The correlations between heterosis and genetic distance were calculated (Table III). Of all of them only height showed significant correlation with genetic distance based on RAPD markers on 5% level. Length of peduncle also has significant correlation on 10% level and nothing was seen in other characters. It means that in the amount of heterosis with the distance of genotypes there was no special process. Also near and far genotypes have the same pattern for molecular data on the appearance of heterosis.

Zhao *et al.* (1999) has reported that due to complexity of genetical heterosis, it is hard to predict it from genetic distance. Furthermore, Liu *et al.* (1999) studied the relationship between appearance of hybrids and genetic distance based on RAPD marker in barley. They reported that genetic distance based on RAPD markers has no

Table III. Estimating of heterosis of different characters with mean of parents

Genotype	Height	Length of spike	Length of awn	Length of peduncle	Extraction of peduncle	Number of f.stiller
F1(7x50)	0.41	0.43	0.51	0.48	0.89	0.16
F1(4x5)	0.32	0.3008	0.42	0.13	0.43	0.47
F1(3x barley)	-0.032	0.11	-0.089	0.17	0.26	-0.48
F1(10x15)	-0.054	0.064	0.05	0.14	1.15	-0.26
F1(11x17)	0.099	0.032	0	0.2	1.5	0.47
F1(5x1)	0.29	0.14	0.18	0.32	0.34	0.059

Genotype	Number of stiller	biomass	Yield of spike	Number of seed in spike	Total yield	Weight of 100-seed	Harvest index
F1(7x50)	0.16	0.33	0.65	0.33	0.41	0.08	0.2
F1(4x5)	0.47	1.14	-0.84	0.11	0.96	0.14	-0.092
F1(3x barley)	-0.48	-0.37	-0.2	-0.14	-0.58	0.05	-0.32
F1(10x15)	-0.26	-0.42	-0.32	-0.39	-0.43	0.31	-0.009
F1(11x17)	0.47	0.36	-0.013	-0.13	0.54	-0.69	-0.44
F1(5x1)	0.059	-0.06	-0.36	-0.42	-0.34	0.11	-0.28

correlation with hybrid and heterosis performance, therefore, it was concluded that there is no possibility to predict the hybrid performance through genetic distance.

Cerna *et al.* (1997) also studied the relationship between heterosis of yield and heterozygosity of molecular marker in soybean and reported that there is no relationship between heterosis of yield and estimated genetic distance based on RFLP marker, but there is relationship between heterosis for yield and isozyme gene locus. Although there is limitation on allocation of isozyme marker for choosing parents and it's because of the few aysoszyme loci in soybean. Melchinger *et al.* (1990) showed a good possibility to predict the performance of F1 corns based on molecular markers. But this is for the time that studies contain the inside and outside group combinations. Riday *et al.* (2003) compared genetic and morphologic distance with heterosis in medicago and found no relationship between genetic distance and heterosis, despite the matrix morphological distance based on 17 agricultural characters has meaningful correlation with heterosis.

CONCLUSIONS

There was no significant correlation between RAPD marker and hybrid performance. However, an increase in samples reflected a relationship. Therefore, when the structure of samples data is not suitable and there is a large amount of sample, primary screen estimation of the relationship between genetic distance and heterosis is essential.

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REFERENCES

Baker, J.H.A., M. Matthes, G.M. Arnold, K.J. Edwards, I. Ahman, S. Larsson and A. Karp, 1999. Characterisation of genetic diversity in potential biomass willows (Salix spp.) by RAPD and AFLP analyses. *Genome*, 42: 173–83

Casiva, P.V., B.O. Saidman, J.C. Vilards and A.M. Cialdella, 2002. First comparative phenetic studies of Argentinean species of Acacia (Fabaceae), Using morphometric, isozymal, and RAPD approaches. *American J. Bot.,* 89: 843–53

Cerna, F.J., S.R. Cianzio, A. Rafalski, S. Tingey and D. Dyer, 1997, Relationship between seed yield heterosis and molecular marker heterozygosity in soybean. *Theor. Appl. Genet*., 95: 460–7

Cox, T.S. and J.P. Murphy, 1990. The effect of parental divergence on F2 heterosis in winter crosses. *Theor. Appl. Genet.*, 79: 241–50

Diers, B.W., P.B.E. Mcvetty and T.C. Osbom, 1996. Relationship between heterosis and genetic distance based on RFLP markers in oilseed rape (*Brassica napus* L.) *Crop Sci.*, 36: 79–83

Hartl, L. and S. Seefelder, 1998. Diversity of selected hop cultivars detected by fluorescent AFLPs. *Theor. Appl. Genet.*, 96: 112–6

Kiani, P.S., A.A. SH. Booshehri, B.E.S. Tabatabai, B.Y. Samadi and M. Omidi, 2002. Use of RAPD for the study of genetic diversity in *Brassica napus* L.. *Pl. Breed.,* 121: 777–84

Liu, Z. and G.R. Furnier, 1993. Comparison of allozyme, RFLP and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. *Theor. Appl. Genet.*, 86: 97–105

Liu, Z.Q., Y. Pei and Z.T. Pu, 1999. Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat *Triticum aestivum* L. *Pl. Breed.*, 118: 119–23

Melchinger, A.E., M. Lee, K.R. Lamkey and W.L. Woodman, 1990. Genetic diversity for restriction fragment length polymorphisms in relation to genetic effects in maize inbreds. *Crop Sci.*, 30: 1033–40

Nocelli, E., T. Giovannini, M. Bioni and R. Alicco, 1999. RFLP– and RAPD–based genetic relationships of seven diploid species of Avena with the A genome. *Genome*, 42: 950–9

Perera, L., J.R. Russel, J. Provan and W. Powell, 1999. Identification and characterization of microsatellites in coconut (*Coco nucifera* L.) and the analysis of coconut population in Srilanka. *Mol. Breed.*, 8: 344–6

Powell, W., M. Morgante, C. Andre, M. Hanafeg, J. Vogel, S. Tingey and A. Rafalski, 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.,* 2: 225–38

Riday, H., E. Charles Brummer, T. Austin Campbell, D. Luth and P.M. Cazcarro, 2003. Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. *Sativa* and subsp. *Falcata. Euphytica*, 131: 37–45

Roldan–Ruiz, I., F.A. Van Eeuwijk, T.J. Gilliland, P. Pubreuil, C. Dillmann, J. Tallemand, M. Deloose and C.P. Baril, 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theor. Appl. Genet*., 103: 1138–50

Shamsuddin, A.K.M., 1985. Genetic diversity in relation to heterosis and combining ability in spring wheat. *Theor. Appl. Genet.*, 70: 306–8

Szczepaniak, M., E. Cieslak and P.T. Bednarek, 2002. Morphological and AFLP variation of *Elymus reperus* (L.) gould (poaceae). *Cell. Mol. Biol. Letters,* 7: 547–58

Williams, J.C.K., M.K. Hanafey, J.A. Rafalski and S.V. Tingey, 1993. Genetic analysis using random amplified polymorphic DNA marker. *Meth. Enzymol.*, 218: 705–40

Zhao, M.F., X.H. Li, J.B. Yang, C.G. Xe, R.Y. He. D.J. Liu and Q. Zhang, 1999. Relationship between molecular marker heterozygosity and hybrid performance in intra– and inter subspecific crosses of rice. *Pl. Breed.,* 118: 139–44

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