Estimation Of Genetic Diversity Based On Randomly Amplified Polymorphic Dna (Rapd) In Landraces Of Hexaploid Wheat Collected From Northern Areas Of Pakistan

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Abstract: Common wheat (*Triticum aestivum* L.) is a hexaploid species and is the most important cereal crop of the world. It's annual production worldwide is approximately 700 million metric tons. In Pakistan annual production of wheat is 23 million metric tons. To improve wheat production in mountainous northern area of Pakistan, DNA based research on landraces is very important. Present research is the first documented attempt to utilize recently developed DNA technology for wheat improvement in the area. Seeds of 16 landraces of wheat were obtained from Plant Genetic Resource Institute (PGRI) of National Agricultural Research Centre (NARC), Pakistan. Ten Randomly Amplified Polymorphic DNA primer were used to amplify the DNA isolated from 16 landraces. A total of 73 DNA fragments of various sizes (ranging from 100 to 1300 bp) were amplified. DNA fragments were scored as present and/or absent. Bivariate (1-0) data matrix was constructed and Genetic Diversities (GD) among all the possible combinations was calculated. Genetic diversity estimated among 16 landraces ranged from 0.3 to 0.8. Bivariate data was also used to construct dendrogram. Sixteen genotypes were clustered in 4 groups. Present research indicated that sufficient level of genetic diversity exists among landraces of hexaploid wheat collected from northern areas of Pakistan. It is recommended that the landraces showing high level of genetic diversity (PAK 0016479 and 019353) should be used in future breeding programs designed to improve quality and quantity of wheat production in the area.

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Key words: *Triticum aestivum* L., Genetic diversity, landraces, Northern areas Pakistan, RAPD

Introduction

Common / bread wheat (*Triticum aestivum* L.) is the most important cereal crop of the world. Area under wheat cultivation all over the world is 220 million hectares with a total production of 674.9 million metric tons (FAO, 2012). Pakistan ranks at 7th position in the world for wheat production. Annual production of wheat in Pakistan is 23.5 million metric tons which comes from an area of 8.9 million hectares. Average yield of wheat in Pakistan is 2.6 tons per hectare. In the Northern mountainous area of Pakistan, wheat is cultivated on an area of 17090 hectares with a total production of 36835 tons giving an average yield of 2.1 tons/ha (Anonymous, 2013).

Wheat yield in developed countries like USA, Australia, Canada may be up to approximately 6 tons per hectare while in Pakistan it is only 2.6 tons per hectare. In the Northern areas of Pakistan, average wheat yield is 2.1 tons per hectare which is even less than national average of Pakistan. One of the reasons of low grain yield of hexaploid wheat in northern areas is lack of improved varieties. Like any other crop, improvement in what varieties is possible through Breeding. For successful breeding programs, it is pre requisite to know about the existing Genetic Diversity (GD) present in landraces. To estimate genetic diversity, previously morphological, cytological and/or biochemical markers were commonly used. But these markers are generally less in number and in some cases are influenced by environment so are not considered very suitable for wheat breeding programs. Recently DNA based markers have been developed which are unlimited in number and are not influenced by environment and hence are considered better marker system to estimate genetic diversity (Paterson et al., 1991; Khan, 2010; Mago et al., 2011). Because of economic importance of wheat, a number genetic studies have been conducted worldwide in wheat to study genetic diversity using DNA based markers. These studies include Colomba and Gregorini (2011), Al-Fares and Abu-Qaoud (2012), Naghavi et al., (2009) and Gashaw et al., (2007) etc. They used RAPD merkers and found Genetic diversity in wheat ranging from 0 to 0100%. Banks *et al*., (1995) studied detailed structure of wheat chromosomes using DNA based markers Chao *et al*., (1989) published first genetic map of group 7 chromosome of wheat using RFLPs markers. PCR markers have been found useful in mapping important wheat genes including genes controlling grain protein content (Helguera *et al*.,

2000, 2003, 2005). Autrique *et al*., (1995) tagged rust resistant genes in hexaploid wheat using RAPD markers. Zeb et al., (2009) estimated genetic diversity in 10 hexaploid wheat varieties commonly grown in Pakistan using Simple Sequence Repeat (SSR) primers. They reported that average genetic distances ranged from 16 to 67%. Ahmed et al., (2010) studied Genetic diversity in 32 advanced wheat breeding lines of Pakistan using Randomly Amplified Polymorphic DNA and reported 90 % genetic diversity in their material.

Considering importance of germplasm obtained from high mountain area (present study area) during present research these DNA based markers were used for the first time to estimate Genetic Diversity among landraces of common wheat collected from Northern area of Pakistan.

Material and methods

Northern areas of Pakistan is the most magnificent mountainous area of the world surrounded by mighty mountains viz; Himalaya, Karakoram, Hindukush and Pamir. It occupies an area of more than 72,496 km^2 (27,991 sq mi) with a population of more than 1,800,000. Population density of the area is 25/km^2 (64/sq mi). Highest peaks of the area include K2, Rakaposhi, Masha broom, Gashabroom, Golden peak, Nanga Parbat etc. Latitude of the area is 35.3500° N, 75.9000° E. Samples of landraces of hexaploid wheat were originally collected during early 70s through a joint project of Japan International Cooperation Agency (JAICA) and Pakistan Agricultural Research Council. Seeds of 16 landraces of hexaploid wheat representing most part of northern areas were used during present work (Table 1).

Table 1. Collection No and collection sites of 16 landraces of hexaploid wheat used during present study

S.No	ID No	Collection site	District	Altitude (meter above sea level)
1.	PAK 0016479	Siliharng	Ysin valley, Ghizer	2100
2.	PAK 0017434	Manich	Yasin valley, Ghizer	2435
3	011620	-	Ghizer	2840
4	012288	Singal	Ghizer	1965
5	012289	Damas	Ghizer	2065
6	012291	Yasin	Ghizer	2435
7	012319	Talis	Ghizer	2820
8	018710	Bambureet	Chitral	1940
9	018853	Darosh	Chitral	1330
10	019353	Khybar/Gojal	Hunza	1930
11	012284	Dazar	Diamer	1595
12	011768	-	Astore	2300
13	011489	-	Hunza	1550
14	011483	Palandri	Azad Jamu and Kashir	1450
15	011855	Muzaffarabad Pataka	Azad Janu and Kashmir	1250
16	012104	Muzaffarabad Kahala	Azad Jamu and Kshmir	680

Small scale DNA isolation procedure (miniprep) of Doyle, and Doyle, (1990) was used with some modifications to isolate total genomic DNA from the seeds of hexaploid wheat. The quality and quantity of the DNA was checked on 1% agarose/TBE gel. Five µL DNA from each samples was taken, mixed with 2 µL loading dye (Bromophenol Blue) and loaded in the wells. Gel was then run at 70 volts for one and half hour. Gel was observed under UV light using "UVitech" Gel Documentation System. Ten RAPD primers viz; GL DecamerB-07 (GGTGACGCAG), GL DecamerD-06 (ACCTGAACGG), GL Decamer I-07 (CAGCGACAAG), GL Decamer J-04 (CCGAACACGG), GL Decamer K-13 (GGTTGTACCC), GLC-09 (CTCACCGTCC), GLD-07 (TTGGCACGGG), GL C-03 (GGGGGTCTTT), GLA-06 (GGTCCCTGAC), D-02 (GGACCCAACC) (obtained from Gene Link, Inc, USA) were used to amplify genomic DNA isolated from 16 landraces of hexaploid wheat. PCR reactions were carried out in 25 l volume using standard protocols (Devos and Gale, 1992). Genetic diversities among all the possible comparisons were estimated using formula described by Nei (1978)

GD=1-dxy/dx+dy-dxy

Where GD = Genetic diversity, dxy=number of common DNA fragments in two samples, dx=number of total DNA fragments in sample number 1, dy=number of total DNA fragments in sample number 2. A dendrogram was constructed using computer package "popgene ver 3.2" (Yeh et al., 1999).

Results and discussion

An example of amplification profile using RAPD prmer GLD-06 is presented in Figure 1. Only reliably score able DNA fragments were included in analysis. Over all a total of 73 DNA fragments of various size ranging from 100-1300 base pair (estimated using 100 bp ladder, GeneLink) were amplified in 16 land races of hexploid wheat using 10 RAPD primers. DNA fragments were scored as present (1) and/or absent (0). Bivariate (1-0) data matrix was constructed and Genetic Diversities (GD) among all the possible combinations was calculated. Genetic diversity estimated among 16 landraces ranged from 0.3 to 0.8 (Table 2). Minimum genetic diversity (31.1%) was estimated among accessions 019353 (collected from an altitude of 1930 meter) and 011489 (collected from an altitude of 1550 meter). Both the accessions belong to district Hunza. Maximum Genetic diversity (78.3%) was observed among 019353 (collected from altitude of 1930 meter) and PAK 0016479 (collected from altitude of 2100 meter). These 2 accessions belong to district Hunza and district Ghizer.

Bivariate data was also used to construct dendrogram. Sixteen genotypes were clustered in 4 groups viz; A, B, C and D comprising of 5, 3, 5 and 3 accessions, respectively (Fig. 2). Present research indicated that sufficient level of genetic diversity exist among landraces of hexaploid wheat collected from northern areas of Pakistan. It is recommended that the landraces showing high level of genetic diversity (fir example PAK 0016479 and 019353) should be used in future breeding programs aimed at increasing quality and quantity of wheat production in the area.

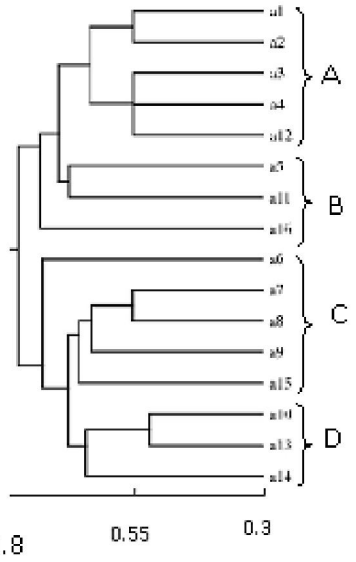
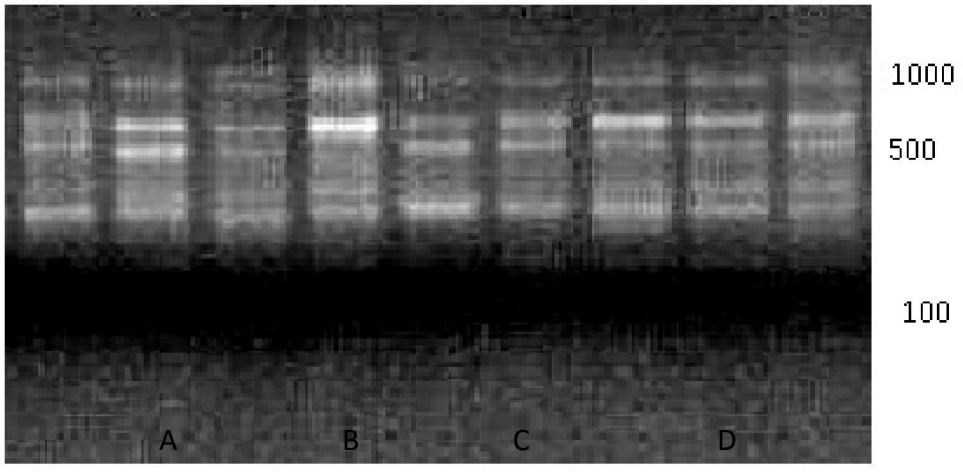


Fig. 2. Dendrogram constructed for 16 landraces of hexplaoid wheat based on data obtained using 10 RAPD primers



A B C D

Fig. 1. PCR amplification profile of fifteen landraces of hexaploid wheat using RAPD primer GLD-06.

Table 2. Estimates of genetic distances among 16 common wheat landraces using ten RAPD primers

1	2	3	4	5	6	7	8	9	10	11	12	13
	14	15	16									
1		0.359	0.440	0.568	0.622	0.696	0.718	0.762	0.622	0.783	0.539	0.508
	0.718	0.672	0.672	0.622								
2			0.440	0.508	0.568	0.648	0.672	0.718	0.568	0.741	0.596	0.440
	0.672	0.622	0.622	0.622								
3				0.359	0.568	0.696	0.622	0.622	0.622	0.648	0.539	0.359
	0.568	0.568	0.568	0.568								
4					0.508	0.696	0.672	0.622	0.622	0.596	0.596	0.359
	0.508	0.508	0.568	0.622								
5						0.596	0.568	0.622	0.508	0.696	0.539	0.568
	0.672	0.672	0.568	0.622								
6							0.539	0.648	0.596	0.508	0.672	0.696
	0.596	0.696	0.596	0.783								
7								0.359	0.440	0.539	0.539	0.622
	0.508	0.622	0.508	0.718								
8									0.508	0.475	0.648	0.568
	0.440	0.508	0.508	0.672								
9										0.539	0.539	0.568
	0.508	0.622	0.508	0.718								
10											0.672	0.596
	0.311	0.539	0.539	0.741								
11												0.539
	0.648	0.648	0.648	0.648								
12												
	0.508	0.440	0.508	0.568								
13												
	0.440	0.440	0.672									
14												
		0.568	0.622									
15												
			0.568									

Present research indicated that high level of genetic diversity exist among landraces of hexaploid wheat which were used to be cultivate in Northern areas of Pakistan before onset of released varieties (approximately in early 70s). It is unfortunate that those landraces have been completely eradicated from the area now. But seeds of those landraces have been preserved in gene bank of Japan International Cooperation Agency JAICA (http://www.jica.go.jp/english/) and Plant Genetic Resource Institute, Islamabad. It is recommended that these landraces (and similar landraces collected from the area during early 70s) should be used in future breeding programs aimed at increasing quality and quantity of wheat production in the area. These landraces may prove better for local agriculture as compared to varieties imported from other areas as these landraces have already been adapted to harsh local environment of the area. It is also important to note that hexaploid wheat germplasm used during present research (obtained from areas surrounded by 3 mighty mountains of Himalaya, Karakorum and Hindukush) has a unique and important value because this germplasm belongs to high altitude (at least more than 1300 meters) and hence these accessions may be value able for genes like cold / frost tolerance.

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