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Research Article

Genetic Diversity Analysis of CIMMYT Bread Wheat (*Triticum aestivum* L.) Lines by SRAP Markers

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Abstract
Genetic diversity is one of the key factors for the improvement of many crop plants including wheat. Many wheat scientists have studied genetic diversity in wheat germplasm using different molecular markers which have provided a powerful approach to analyze genetic relationships among wheat germplasms. In this study, genetic diversity of CIMMYT (International maize and wheat improvement center) bread wheat lines collected from Russia was evaluated using 30 sequence-related amplified polymorphism (SRAP) primer combinations. 686 DNA band was obtained from the 23 primer combinations and approximately 90% of them were found to be polymorphic. Ratio of polymorphic loci, Shannon's diversity index and gene diversity were found 82.61%, 0.39 and 0.26 respectively. The three main clusters were found by using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis method and the average rate of genetic similarity with 0.462. Two main clusters were shown in principal component analysis (PCA) which is consistent with the result of UPGMA. It can be concluded that SRAP markers can be used for wheat genetic diversity studies and have potential linkage mapping, molecular characterizations and marker assisted selection (MAS) breeding.

Key words:
Bread wheat, CIMMYT, genetic diversity, SRAP, *Triticum aestivum*.

Genetic diversity is defined as the amount of genetic variability which is reflected by differences of DNA sequence, biochemical characteristics, physiological properties or morphological characters among individuals of a variety or a population. Plant genetic diversity is changed by evolution and by breeding history during which intensive selection often reduces genetic diversity in the elite germplasm pool (Auvuchanon, 2010). The knowledge of genetic diversity of germplasms is critical for their utilization in the improvement of crops. As a result, it is necessary to investigate the genetic diversity in wheat germplasm to expand genetic variation in wheat breeding.

Triticum aestivum, also known as bread wheat (common wheat) is a cultivated wheat species. Wheat, together with maize (*Zea mays* L.) and rice (*Oryza sativa* L.), is one of the three major food crops in the world. It is grown in a variety of environments, ranging from fully irrigated to high rainfall and drought-prone regions (Dreisigacker *et al.*, 2004). Scientific classification included in the genus *Triticum* is a plant family *Poaceae*. Bread wheat is a segmental hexaploid (6x), which regularly forms 21 pairs of chromosomes (2n = 42) during meiosis. These chromosomes are subdivided into 3 closely related (homoeologous) groups of chromosomes, the A, B, and D genomes and each of these homoeologous groups normally contains 7 pairs of chromosomes (AABBDD). Bread wheat was used in various studies, some of these are germplasm identification (Zhu *et al.*, 2011), gene tagging and mapping (Zhuang et al., 2008), genetic diversity with the molecular marker such as AFLP (Tian *et al.*, 2005) and SSR (Stepien *et al.*, 2007; Wang *et al.*, 2010)., SRAP (Fufa *et al.*, 2005; Dong *et al.*, 2010; Al-Doss *et al.*, 2010).

Molecular markers are useful complements to morphological and physiological characterization of cultivars (Barakat *et al.*, 2010). SRAP is based on two-primer amplification and the primers are 17 or 18 nucleotides long and consist of the following elements (Li and Quiros, 2001). SRAPs amplify several reproducible and polymorphic loci and alleles, and they may amplify functional genes since they are sequence related. As opposed to SSR markers, which tag single multiallelic loci, SRAP markers possess multiloci and multiallelic features, which make them potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting genotypes (Fufa *et al.*, 2005). Compared with the other marker systems, SRAP markers are more reproducible and not complex. SRAP had been applied in various researches such as genetic linkage map construction (Li and Quiros, 2001; Wang et al., 2010), genetic diversity (Dong *et al.*, 2010) and evolutionary study (Budak *et al.*, 2004a; Filiz *et al.*, 2009). The objectives of this study were to assess genetic diversity of 56 inbred CIMMYT wheat lines collected from Russia using SRAP molecular markers.

Material and Method

Plant material**:** A total of 56 CIMMYT inbred wheat lines from crosses made during 1891 to 1997 were chosen from Russia for this study (Table 1). Seeds were stratified at 4 ^{0}C for 7–10 days in the dark between moist filter papers in Petri plates. After cold treatment, they were put

under light at room temperature. After germination they were transferred to a peat–soil mixture in small pots. After the seedlings were established, they were transplanted into 15 cm diameter plastic pots containing a mixture of 35% peat, 32% vermiculite, 9% soil, and 24% sand (v/v) and grown under a 16 h light : 8 h dark photoperiod in a greenhouse (Filiz *et al.*, 2009). For basal fertilization, the growth medium was treated with 200 mg/kg N, 100 mg/kg P, 50 mg/kg K, and 20 mg/kg S.

DNA Extraction: Genomic DNA was extracted from greenhouse-grown fresh leaf materials of each bread wheat genotypes, using the CTAB method by Chen and Ronald (1999). The PCR reaction mixtures (25 ml total volume) consisted of 10 mM Tris-HCl, pH 8.8 at 25^0C, 50 mM KCl, 2.0 mM MgCl2, nucleotides dATP, dTTP, dCTP, and dGTP (200 mM each), 0.2 mM primer, 50 ng template DNA, and 1 units/mlof Taq DNA polymerase (Fermentas). Amplifications were carried out in programmed for 35 cycles of 1 min at 94^0C, 1 min at 47^0C, 1 min at 72^0C, and ending with 7 min at 72^0C. Quality of DNA was evaluated by electrophoresis on 2% agarose gel and the DNA was stored at -20 ^{0}C.

SRAP Analysis**:** In SRAP analysis, 30 primer combinations tested and 23 primer combinations were used which are polymorphic (Table 2.). The PCR reaction mixtures (25 ml total volume) consisted of 10 mM Tris-HCl, pH 8.8 at 25^0C, 50 mM KCl, 2.0 mM MgCl2, nucleotides dATP, dTTP, dCTP, and dGTP (200 mM each), 0.2 mM primer, 30 ng template DNA, and 1.5 units/mlof Taq DNA polymerase (Fermentas). Amplifications were carried out in a MJ Research PTC-100 thermocycler programmed for 32 cycles of 1 min at 94^0C, 1 min at 47^0C, 1 min at 72^0C, and ending with 5 min at 72^0C. PCR products were separated by electrophoresis using 2% agarose gel in 1× Tris/Borate/EDTA (TBE) buffer (89-mM Tris, 89-mM boric acid, and 2-mM EDTA) at 90 V for 1 hour and stained using ethidium bromide.

Data Analysis**:** Amplified fragments were scored for the presence (1) and absence (0) for the homologous bands and the matrix of SRAPs data was assembled. The data were analyzed using Popgene version 1.31 (Yeh *et al.*, 1997) and MVSP 3.2 (Multi Variate Statistical Package). The following parameters were estimated: the percentange polymorphic loci (P), Shannon's information index, Nei's gene diversity (He) by using Popgene 1.31 version. A Principal Component Analysis (PCA) was performed based on the variance covariance matrix calculated from marker data using MVSP 3.2. A dendrogram was constructed based on Jaccard's similarity coefficients using the unweighted pair group method with arithmetic average (UPGMA) with MVSP 3.2.

Results and Discussion

Evaluation of genetic diversity and relationships among various accessions is fundamental importance for plant breeding programs. Molecular markers have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity. Advances in our understanding of polymorphisms found in eukaryotic genomes and improved methods for studying genetic markers should facilitate genetic linkage mapping and other applications (Edward and Caskey, 1991). In the present study, SRAP marker systems was first time applied to assess the level of genetic diversity of 56 inbred wheat lines from Russia and total of 686 fragments were amplified with 23 primer combinations, 620 of which were polymorphic (90%), while 66 were monomorphic (10%) (Fig. 1). The number of average polymorphic band is 27 and percentage of polymorphic loci (P), Shannon's information index and Nei's (1973) gene diversity (He) were found 82.61%, 0.39 and 0.26, respectively.

The dendrogam was constructed by the Unweighted Pair-Group Method (UPGMA) based on Jaccard's genetic similarity coefficient of the 56 bread wheat lines (Fig. 2). The dendrogam showed that 56 samples were classified into three major clusters and the genetic similarity coefficient among bread wheat genotypes ranged from 0.05 to 0.75, with a mean of 0.46. UPGMA cluster analysis demonstrated clear genetic relationships among 56 bread wheat lines, while there is a weak correlation between wheat pedigree and cluster.

Principal component analysis (PCA) was performed based on the 0/1 matrix using the Multi-Variate Statistical Package (MVSP 3.2) to better understand relationships among them and there are two main groups based on PCA analysis (Fig. 3). The PCA revealed similar groupings as the UPGMA analysis and there is a weak correlation between wheat pedigree and cluster. The genotype Karagandinskaya 70 was observed to be alone in one cluster, while the other genotypes included in two major groups.

In present study, genetic diversity level of wheat genotypes is higher than earlier genetic diversity studies using different marker systems with bread wheat such as SSR (Achtar *et al.*, 2010), RAPD (Bibi *et al.*, 2009) and AFLP (Altintas *et al.*, 2008). SRAP markers mainly targets exons which are expected to be evenly distributed along all chromosomes with GC-rich regions and introns with AT-rich regions (Li and Quiros, 2001). Large and complex wheat genome taken into consideration, many intron and exon regions may

have influenced the number of excess polymorphic bands.

Budak *et al.*, (2004b) compared the four marker systems in buffalo grass and found the values of the average discriminating power as: SRAP, SSR, ISSR and RAPD. 34 SRAP primer combinations were tested and these gave a total of 263 bands, and 249 of these were observed polymorphic (95%), and genetic similarities among all individuals ranged from 0.25 to 0.95, with a mean similarity of 0.62. In another study, Budak et al. (2004c) reported that 19 SRAP primer combinations demonstrated high level polymorphism with 21 turfgrass species and 19 primer combinations with a range of 2 to 4 reproducible bands per primers. Twenty-three SRAP markers were used and gave 468 amplified fragments (including 60 monomorphic fragments) with an average genetic diversity of 0.418 and range of 0.10–0.90 with red winter wheat cultivars (*T. aestivum*) and the diversity estimates for SRAP markers ranged from 0.11 to 0.677 with an average value of 0.357 (Fufa *et al.*, 2005).

Zaefizadeh and Goliev (2009) used 12 SRAP combinations primers to determine genetic diversity of *Triticum durum* which included 38 landraces and two cultivars, of which 56.73% was polymorphic for all 40 genotypes. In similar study, Dong *et al.* (2010) showed that genetic diversity and population structure of 15 wild emmer wheat (*Triticum dicoccoides*) populations from Israel were detected using 30 sequence-related amplified polymorphism primer pairs. 244 fragments out of 438 were polymorphic and the proportion of polymorphic loci (P), the genetic diversity (He), and Shannon's information index were 0.557, 0.198, and 0.295, respectively. The numbers of polymorphic bands per primer were found with a mean of 8.1. 19 SRAP primers were used and gave 128 amplified fragments (including 35% polymorphic) among the six wheat durum genotypes exposed to heat stress with mean 6.7 band per primer, the size of fragment ranged from 100 to 1300 bp (Al-Doss *et al.*, 2010). These findings imply that SRAP markers are useful and efficient to estimate genetic diversity level in wheat genotypes.

In plants, the distribution pattern of genetic variation can be influenced by various life-history traits, particularly breeding system (Li and Ge, 2006). Our study revealed that the genetic diversity of *T. aestivum* was relatively high in terms of the three genetic diversity measurements (viz., percentage of polymorphic loci (P), Shannon's information index and Nei's (1973) gene diversity (He) were found 82.61%, 0.39 and 0.26 respectively. It could be explained that we used historical *T. aestivum* inbred lines which are collected long time period from Russia (between 1891-1997) and its genetic background might more differentiated than other plant genomes because of large genome size of wheat (16.000 Mb), which is approximately 8-fold larger than that of maize and 40-fold larger than that of rice. The A, B, and D genomes of common wheat have undergone dynamic evolution since they came together to form hexaploid wheat (Gill *et al.*, 2004).

Another possible explanation could be that local ecogeographic factors and evolutionary forces might affect genome compositions such as gene flow, pollen dispersion etc. Also, wheat lines used in the present study are derived from CIMMYT material. CIMMYT's wheat breeding program aimed at increasing genetic diversity on a large scale by taking into account the need for biological diversification, environmental sustainability and geographic adaptation of the germplasm as an important breeding goal (Rajaram and Van Ginkel, 2001). Our genotypes may have been subjected to national or local breeding programs and genome compositions were affected these breeding programs and indicate the presence of great genetic variability among bread wheat lines. In conclusion, the level of polymorphic markers generated with polymorphic 23 SRAP primer combinations in this study proved to be sufficient to estimate genetic diversity and phylogenetic relationships. SRAP marker system is simple and efficient system and was applicable for the molecular characterization and the investigation of phylogenic relationships in bread wheat and has potential for marker-aided selection, linkage mapping, and evolutionary studies and breeding purposes.

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Table 1. Pedigrees of the 56 CIMMYT bread wheat lines

Name	Country	Collected Date	Pedigree
Noe	Russia	1891	Volga region variety
Smena	Russia	1919	Local (Siberia) variety
Lutescens 956	Russia	1919	Local (Siberia) variety
Irtyshanka 10	Russia	1964	Skala/Saratovskaya 36
Omskaya 12	Russia	1970	Lade(Norway)/FKN–25 (USA)
Tulunskaya 12	Russia	1970	Biryusinka / Bezostaya 1
Selenga	Russia	1978	Buryatscaya 34 / Buryatscaya 79
Rosinka	Russia	1979	Mutant Sibakovskaya
Altayskaya 92	Russia	1981	Novosibirskaya 67 / Lutescens 4029
Omskaya 26	Russia	1986	Novosibirskaya 22 / W.W.16151
Shernyava 13	Russia	1985	OmSHI 6 / ANK 17 // OmSHI 6
Strada Sibiri	Russia	1986	Rang/ Hibryd 21// Irtishanka 10
Pamyaty Azyeva	Russia	1987	Saratovskaya 29 / Lutescens 99–80–1
Omskaya 32	Russia	1989	1989 Lutescens 162–84–1 / Chris (USA)
Kazanskaya	Russia	-----	Omsraya 20 / Lutescens 204–80-//Lutescens
Yubileynaya	Russia	1992	86–6
Omskaya 36	Russia	1994	Lutescens 150–86–10 / Runar
Omskaya 21	Russia	1986	Self-hybrid
Omskaya 34	Russia	1993	Omskaya 21 / Lutescens 4979c
Tarskya 6	Russia	1996	Lutescens 89–87–29 / Narskaya 5
Tarskya 7	Russia	1993	Lutescens 89/87–29 / Omskaya 26
Boevshanka	Russia	1994	Lutescens 70–94 / Lutescens 73–94
Omskaya 27	Russia	1983	Omskaya 17 // Atlas 66 / Lutescens
Marquis	Russia	1892	Hard Red Calkytta / Red Five
Albidum 3700	Russia	1925	Local (Siberia) variety
Saratovskaya 29	Russia	1938	Lutescens 91 / Sarroza // Lutescens
Sybakovskaya 3	Russia	1965	Bezostaya 1 / Saratovskaya 29
Sibiryashka 4	Russia	1966	Sibiryashka 2 / Saratovskaya 29
Omskaya 17	Russia	1972	Lutescens 1138–166 / Red River 68
Tselinnaya 26	Russia	----	Shortandinskaya 25 /FKN 25
Omskaya 19	Russia	1973	Lutescens 1138–70 / Lutescens
Dias 2	Russia	1973	Novosibirskaya 67 / Rang
Omskaya 20	Russia	1980	Irtishanka 10 // Graecum 114

Table 1. Contd..

Name	Country	Collected Date	Pedigree
Niva 2	Russia	1982	PS -360/76 / Irtishanka 10
Omskaya 29	Russia	1979	Lutescens 204–80–1 / Lutescens 99
Omskaya 14	Russia	1987	-------
Rosinka 2	Russia	1987	Chemical mutant Tselinnaya 21
Slavyanka Sibiri	Russia	1984	Chemical mutant Lutescens 65
Sonata	Russia	1986	Tselinnaya 20 /Tertsiya
Tuleevskaya	Russia	1989	Olivatseva / Vendel // Lutescens
Omskaya 33	Russia	1992	Lutescens 137–87–39 / Omskaya 28
Svetlanka	Russia	1987	Omskaya 23 / Tselinnaya 26
Milturum 321	Russia	1913	Local (Siberia) redspike variety
Cesium 94	Russia	1923	Caesium 117 / Western Polba
Milturum 553	Russia	1927	Milturum 321 / Citchener (Canada)
Omskaya 9	Russia	1964	Bezostaya 1 / Saratovskaya 29
Omskaya 18	Russia	1977	Omskaya 11 / Geines (WW) (USA)
Karagandinskaya 70	Russia	-----	Lutescens 1594 / Sibiryashka 8
Omskaya 24	Russia	1977	----------
Eritrospermum 59	Russia	1979	ANK 1 / ANK 2 // ANK 3
Tertsiya	Russia	1980	Saratovskaya 36 / I 428010
Omskaya 28	Russia	1985	Lutescens 19 / Hibryd (Canada)
Omskaya 30	Russia	1987	Omskaya 20 / Lutescens 204–80–1
Omskaya 35	Russia	1994	Omskaya 29 / Omskaya 30
Omskaya 37	Russia	1997	Lutescens 61–89–100 / Lutescens
Omskaya 20	Russia	1980	Irtishanka 10 // Graecum 114

Table 2. The sequence of the SRAP primers

Forward primers	Reverse primers
Me1 TGA GTC CAA ACC GGA TA	Em1 GAC TGC GTA CGA ATT AAT
Me2 TGA GTC CAA ACC GGA GC	Em2 GAC TGC GTA CGA ATT TGC
Me3 TGA GTC CAA ACC GGA AT	Em3 GAC TGC GTA CGA ATT GAC
Me4 TGA GTC CAA ACC GGA CC	Em4 GAC TGC GTA CGA ATT TGA
Me5 TGA GTC CAA ACC GGA AG	Em5 GAC TGC GTA CGA ATT AAC
Me6 TGA GTC CAA ACC GGA CA	Em6 GAC TGC GTA CGA ATT GCA
Me7 TGA GTC CAA ACC GGA CG	Em7 GAC TGC GTA CGA ATT CAA
Me8 TGA GTC CAA ACC GGA CT	Em8 GAC TGC GTA CGA ATT CAC
Me9 TGA GTC CAA ACC GGA GG	Em9 GAC TGC GTA CGA ATT CAG
Me10 TGA GTC CAA ACC GGA AA	Em10 GAC TGC GTA CGA ATT CAT
Me11 TGA GTC CAA ACC GGA AC	Em11 GAC TGC GTA CGA ATT CTA
Me13 TGA GTC CAA ACC GGA AG	Em12 GAC TGC GTA CGA ATT CTC
Me12 TGA GTC CAA ACC GGA GA	Em13 GAC TGC GTA CGA ATT CTG
	Em14 GAC TGC GTA CGA ATT CTT
	Em15 GAC TGC GTA CGA ATT GAT
	Em16 GAC TGC GTA CGA ATT GTC

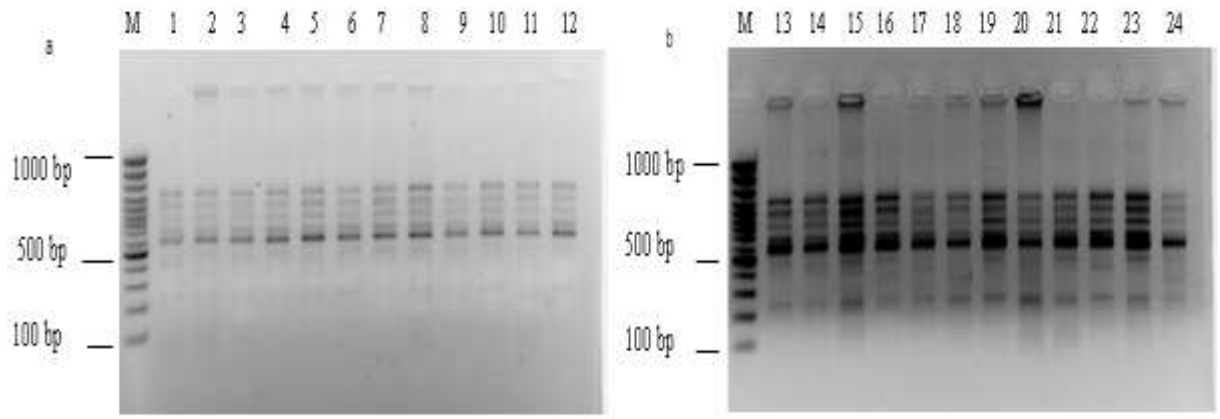

Fig. 1. SRAP amplified result of *T. aestivum* by primer M5E15. The DNA samples were fractionated in 2% agarose gel stained with ethidium bromide. a) Lanes: 1 'Noe', 2 'Smena', 3 'Lutescens 956', 4 'Irtyshanka 10', 5 'Omskaya 12', 6 'Tulunskaya 12', 7 'Selenga', 8 'Rosinka', 9 'Altayskaya 92', 10 'Omskaya 26', 11 'Shernyava 13', 12 'Strada Sibiri' b) Lanes: 13 'Pamyaty Azyeva', 14 'Omskaya 32', 15 'Kazanskaya', 16 'Yubileynaya', 17 'Omskaya 36', 18 'Omskaya 21', 19 'Omskaya 34', 20 'Tarskya 6', 21 'Tarskya 7', 22 'Boevshanka', 23 'Omskaya 27', 24 'Marquis'.

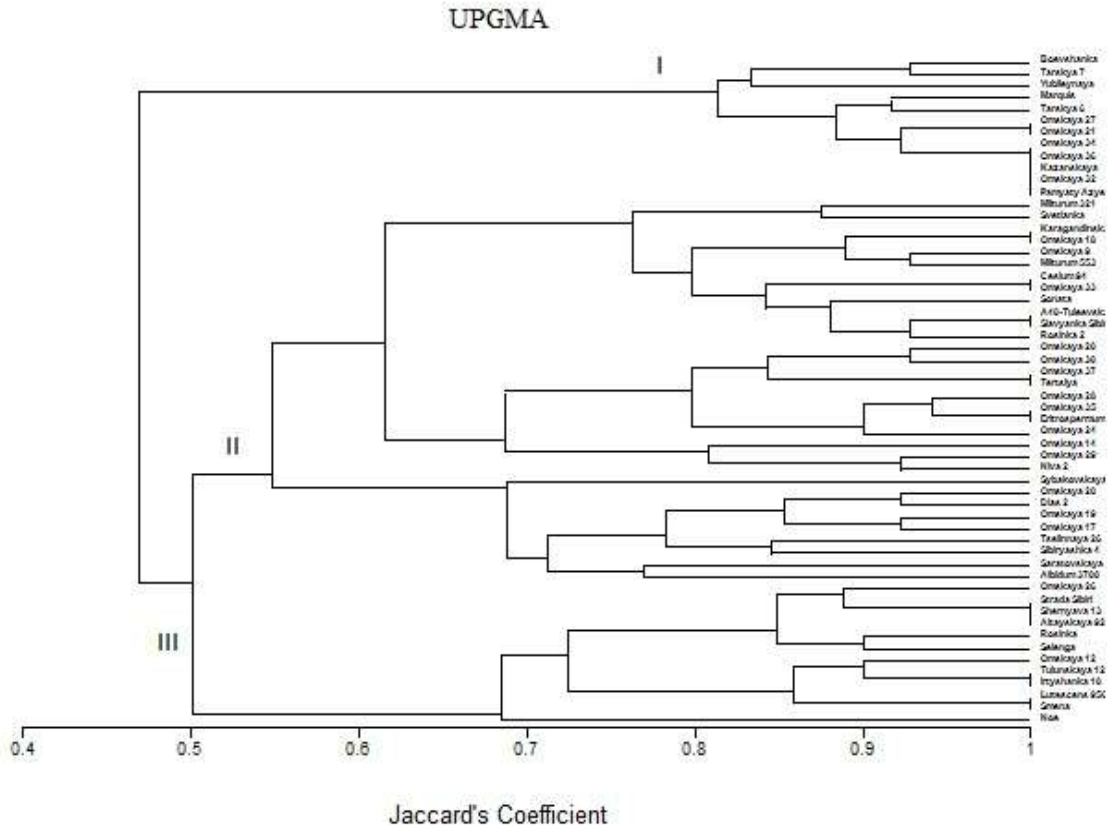

Figure 2. Unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram based on Jaccard's similarity coefficient of 56 inbred wheat genotypes, generated using SRAP markers.

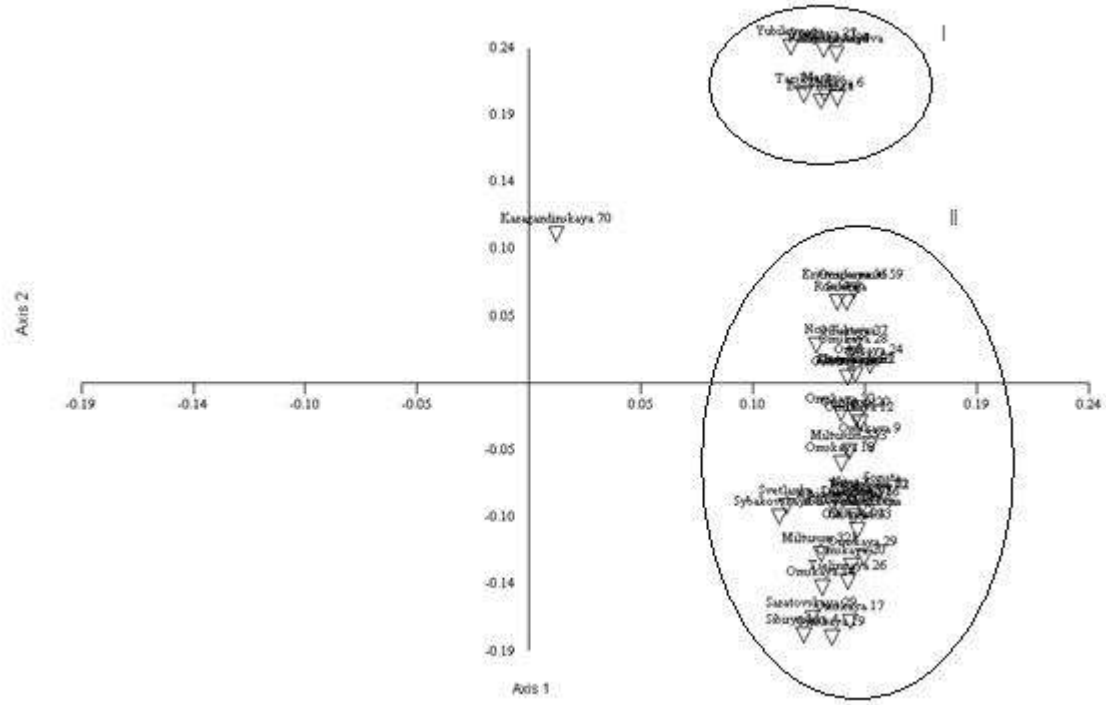

Figure 3. 56 bread wheat lines based on the principal component analysis (PCA)