MOLECULAR GENETIC IDENTIFICATION OF SOME WHEAT GENOTYPES

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Wheat represents one of the major sources of food all over the world and the most important cereal crop in Egypt. Wheat proteins quality is generally recognized as the most important factor affecting bread making. Although there is a strong and direct relationship between total flour protein content and loaf volume, the slope of this relationship depends on the inherent quality of different wheat cultivars (Jerry *et al.,* 2003).

In Egypt, improvements of wheat yield and grain quality, modification of its plants architecture and increasing its tolerance to drought and lodging and resistance to insects and pathogens are being done properly. Many wheat cultivars have been produced. The ability to discriminate between such cultivars is a fundamental to the operation of the modern seed industry and seed trade as the basis of modern crop production.

The biochemical genetic fingerprinting can be considered as a good tool for characterization and genetic evaluation of the conserved material (Cardy and Beversdorf, 1984). Therefore, biochemical genetic fingerprinting would satisfy both adequacy and accuracy for the characterization of the conserved material. Furthermore, electrophoretic method is considered a rapid and accurate test to identify and characterize species. It is now possible to determine a fingerprint for each species to distinguish its identity and its properties by the use of appropriate and techniques. Cultivar identification and the techniques to asses cultivar purity are essential for commercial seed production and crop certification.

Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used technique in protein studies. It is considered a low-cost, reproducible, and rapid method for quantifying, comparing and characterizing of proteins.

Isozymes electrophoresis has been successfully applied to many organisms from bacteria to numerous animal and plant species since 1960s (May, 1992). The studies have encompassed various fields (e.g., physiology, biochemistry, genetics and breeding) and purposes (population structure, mating system, hybridization, polyploidy and systematic)

Murphy *et al.* (1990).

El-Ghubashy (2009) used the electrophoretic methods of storage grain proteins to identify ten wheat genotypes of storage grain proteins and water soluble protein. He reported that some genotypes contained specific bands which could be used to identify and characterize them among others.

The aim of the present investigation was to identify ten wheat genotypes using electrophoretic methods of isozymes and soluble and insoluble proteins.

MATERIALS AND METHODS

Materials

Grain samples of ten wheat (*Triticum aestivum* L.) genotypes were used in this study: {Line 772, Line 1198, Line 1827, Line 2174, Sakha 69 (Sa.69), Beni-Swaif 1(Ben.1), Beni-Swaif 3 (Ben.3), Sids 1 (Si.1), Gemiaza 9 (Gem.9), Sakha 61 (Sa.61)}. Samples were obtained from the Wheat Crops Research Department (WCRD), Field Crops Research Institute and the National Gene Bank (NGB), Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

This trial was carried out at the laboratories of the departments of Seed Technology Department, Field Crops Research Institute, Agricultural research center (ARC) and Agricultural Biochemistry Department, Faculty of Agriculture, Al-Azhar University.

Methods of Analysis

1-Isozymes electrophoresis

Native polyacrylamide gel electrophoresis (Native-PAGE) technique was used to charactarize the isozymes profiles of wheat genotypes i.e. esterase (EST), peroxidase (Prx) and glutamate oxaloacetate transaminase (GOT). Isozymes fractionation was performed on vertical slab (19.8cm x26.8cm x.02cm) using the gel electrophoresis apparatus according to Jonathon and Wendel (1990) and Graham *et al.* (1964) as shown in Table (1).

Qualitative and quantitative determination of isozymes bands

The Rf values and approximate molecular weights were used to determine the positions of the protein bands. The gels were densitometrically scanned using color flatbed scanner (Epson GT 8000) connected with computer and printer. The software used was Scan Peak.

2-SDS- protein electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique was used to characterize the different genotypes by their protein fingerprint such as water soluble protein (albumin and globulins) and non-water soluble proteins (gliadins and glutenins). Protein profiling was carried out according to Laemmli (1970) as modified by Studier (1973).

2-2- Calculation and documentation of protein:

Calculation and documentation of the protein on gel electrophoresis depend on the staining, measure of the distance of migration of the proteins and the tracking dye.

Analyzer gel images were analyzed depending on Unweighted Pair Group with Arithmetic Mean (UPGMA) method using diversity database software provided by BioRad manufacturer.

2-3- Data analysis

The similarity matrices were done using gel works advanced software UVP-England Program. The relationships among varieties as revealed by dendrograms were done using SPSS Windows (version 10) Program.

RESULTS AND DISCUSSION

Isozymes electrophoresis

Three different isozymes systems; peroxides (Prx) esterase (Est) and glutamate oxaloacetae transamiase (GOT) were used for the identification of ten wheat genotypes under study based on polyacrylamide gel electrophoresis profiles.

1- . Peroxidase (Prx) isozymes

The peroxidases electrophoretic patterns of different wheat genotypes under study are presented in Table (2). The number of peroxidase bands differed according to the wheat genotypes. The genotypes; L.772, L.1198, L.1827, L.2174, Sa.69, Ben.1, Ben.3, Si.1, Gem.9 and Sa.61 contained 4, 2, 2, 3, 3, 4, 4, 4, 2 and 4 bands, respectively. These results indicated that the various wheat genotypes gave different peroxidases bands. According to the number of peroxidases bands, it was possible to characterize genotypes as follows; the wheat genotypes Sa.69 and L.2174 contained only three bands to distinguish between these varieties depend on R_F values of such three bands which were 0.057, 0.115 and 0.207 for Sa.69 and of 0.06, 0.112 and 0.201 for L.2174, respectively. The Gem.9, L.1198 and L.1827 genotypes contained two bands. Their Rf values for each of the first and second bands were 0.057, 0.115, 0.06, 0.115 and 0.06, 0.112, respectively.

The results in Table (2) showed that each of the genotypes; Ben.1, Ben.3, Si.1, Sa.61 and L.772 contained four bands. The peroxidases produced similar number of bands but the location and Rf values of the bands were different. Hence, these values can be adequately used for the identification among wheat genotypes.

2- Esterases (Est) isozymes

Data in Table (3) showed isozymes patterns of esterases (Est) for the ten wheat genotypes under study. The numbers of the obtained bands were 6, 5, 5, 4, 6, 4, 5, 3, 4 and 5 for Gem.9, Ben.1, Ben.3, Si.1, Sa.61, Sa.69, L.772, L.1198, L.1827 and L.2174 genotypes, respectively. In order to distinguish between such wheat genotypes, the following results and information could be considered:

a- The L.1198 genotype contained three bands; i.e. Rf 0.258, 0.327, and 0.396 which represents unique feature of this studied genotype.

b- Each of the Gem.9 and Sa.69 genotypes exhibited six bands. The Rf of these bands were 0.185, 0.258, 0.327, 0.396, 0.512, 0.658 and 0.123, 0.262, 0.327, 0.396, 0.600, 0.665, respectively. These findings led to characterize these genotypes depending on their Rf values.

c- On the other hand, Ben.1, Ben.3, L.772 and L.2174 genotypes contained five bands not can distinguish between them depending on the difference Rf value because each of the Ben.1, Ben.3 and L.772, L.2174 genotypes contained the same Rf values.

3- Glotamate oxaloacetate transaminase (GOT) isozymes

The results in Table (4) represent the glotamate oxaloacetate transaminases (GOT) electrophoretic patterns for different wheat genotypes under study. All these genotypes showed two bands with approximately the same Rf values. Therefore, it is not possible to characterize between genotypes relying on GOT bands.

The results presented in this study for the genotypes isozymes electrophoresis are along the same line with many authors who studied different isozymes for wheat genotypes identification (Abdel-Tawab *et al.,* 1993; Ebrahim, 1999; Salinas *et al.,* 2006).

SDS-PAGE of water soluble proteins

The polyacrylamide gel electrophoresis of water-soluble proteins of different wheat genotypes are demonstrated in Tables (5 and 6). In total, thirty three polymorphic bands were detected with molecular weights ranged from 97 to 9.7 KD. They are distributed along the gel in a range of Rf: 0.2-0.9. There is a clear variation in the number of bands among genotypes which ranged from 9 in genotypes L 1827, L 2174, Sa.69 and Sa.61 to 10 bands in genotype L 772, L 1198, Ben.1, Ben 3, Si.1 and Gem.9. The results indicated distinct differences in SDS protein banding patterns between the various studied genotypes. Some genotypes have specific bands such as genotype L 772 contained SDS-protein bands with molecular weights of 9.796 and 97.091 KD at mobility Rf of 0.94 and 0.20, respectively. Genotype L 1198 contained protein bands with molecular weights of 21.630 and 40.813 KD at Rf of 0.85 and 0.44. Genotype L 1827 was characterized by protein of molecular weight of 26.782KD at Rf of 0.66. Genotype L 2174 had protein bands with molecular weight of 15.699 KD at Rf 0.88. Ben.1 contained protein bands of molecular weights of 22.739, 23.765, 31.033, 39.990 and 96.671 KD at Rf 0.76, 0.70, 0.65, 0.54 and 0.22, respectively. Ben.3 was characterized by protein band of molecular weight of 23.141 KD at Rf 0.75. Si.1 contained protein bands with molecular weights of 22.413, 23.776, 31.106, 40.715, 52.672 and 80.493 KD at Rf 0.78, 0.70, 0.56, 0.45, 0.36, and 0.30,

respectively. Gem.9 contained protein bands with molecular weights of 22.180, 23.247 and 81.900 KD at Rf 0.79, 0.71 and 0.27, respectively. Sa.61 was characterized by protein band of molecular weight of 21.640 KD at Rf 0.85. On the other hand, bands with molecular weights about 26.151 and 50.393 KD at Rf 0.67 and 0.40, were detected in all genotypes except L1827 and Si.1. The band with molecular weight of 31.053 KD at Rf 0.56 was observed in all genotypes except Ben.1 and Si.1. Also, the band with molecular weight of 82.161 KD at Rf 0.25 occurred in all genotypes except Gem.9 and Si 1, and the band with molecular weight of 96.683 KD at Rf 0.21 was present in all genotypes except L772 and Beni.1, while band of 41.248 KD at Rf 0.43 was present in all genotypes except L 1198, Ben.1 and Si.1.

3- SDS-PAGE of water insoluble proteins

The polyacrylamide gel electrophoresis of SDS-water insoluble proteins of different wheat genotypes are shown in Tables (7 and 8). In total, twenty polymorphic bands were detected with molecular weight ranging from 164.762 to 14.032 KD. They are distributed along the gel in a range of Rf: 0.03 to 0.96. There are clear variations in the number of bands between genotypes which ranged from 5 in genotype L 2174 to 8 bands in the rest of the genotypes. The results indicated distinct differences in insoluble protein banding patterns between the various studied genotypes. There was common band that was found in all genotypes which have MW of 49.349 KD at Rf 0.39.

Some genotypes have specific bands which distinguished them from the others. For instance, genotype L772 contained protein bands of molecular weights of 14.262, 16.846 and 31.018 KD at Rf of 0.95, 0.87 and 0.61. Genotype L 1198 contained proteins with molecular weights of 14.403, 89.697 and 122.927 KD at Rf of 0.94, 0.22 and 0.11. Genotype L 1827was characterized by protein bands of molecular weights of 31.398, and 73.164 KD at Rf 0.59 and 0.26. Genotype L 2174 had a band of molecular weight of 17.105 KD at Rf of 0.87. Sa.69 contained a band of molecular weights of 16.488 and 74.014 KD at Rf 0.91 and 0.25. Ben.3 was characterized by protein band of molecular weight of 30.404 KD at Rf of 0.62. On the other hand, bands with molecular weights of 90.069 and 164.762 KD at Rf 0.19 and 0.03 were present in all genotypes except L 1198 and L 2174, respectively. The band with molecular weight of 121.176 KD at Rf of 0.15 was present in all genotypes except L 1198 and L2174. Also, the band at 14.032 KD at Rf 0.96 was appeared in all genotypes except L772 and L 1198. The band at 16.815KD at Rf of 0.88 was present in all genotypes except L772, L2174 and Sa.69 Also, the band at 31.411 KD at Rf of 0.58 was present in all genotypes except L772, L 1827 and Ben.3 while, the band at 73.052 KD at Rf 0.27 was present in all genotypes except L1827 and Sa.69.

Cluster analysis of wheat genotypes was performed based on the results of SDS-PAGE of the combination of water soluble and insoluble proteins using SPSS10 program. The genetic similarity ranged between 100% and 33%. The highest similarity was 100% between Sakha 69 and Beni-Swaif 1, while the lowest similarity was 33% between L.772 and Sids 1 as shown in Table (9).

The dendrogram (Fig. 1) demonstrated the relationship among the ten wheat genotypes according to the similarity indicies**.** The dendrogram divided the genotypes into two main clusters, where, the first cluster included all the genotypes except Sids 1, which was in a separate cluster**.** The first cluster was divided into two sub clusters; L.1198 was in the first sub-cluster, while all the other genotypes were in the second sub cluster. The obtained results for protein electrophoresis [SDS-PAGE] of soluble and insoluble proteins are in agreement with Ebrahim (1999), Selim (2000), Gianibelli *et al.* (2001), El-Manzalawy (2006), Shuaib *et al.* (2007) and El-Ghubashy (2009).

SUMMARY

This investigation was carried out to identify ten wheat (*Titicum aestivum* L.) genotypes using molecular analysis. The electrophoresis of three isozymes systems; peroxidases (Prx), esterases (Est) and glutamate oxaloacetae transamiase (GOT) and soluble proteins and insoluble proteins of grain were carried out. Isozymes electrophoresis identified wheat genotypes: The number of Prx bands indicated that the genotypes Sa.69 and L.2174 contained only three bands to distinguish between these varieties depend on R_F values. Esterase bands of various genotypes indicated that the L.1198 genotype have three bands only which led to discriminate this variety from the others. GOT bands of all genotyes had two bands. These genotypes contained approximately the same Rf values. Therefore, it was not possible to distinguish between them relying on GOT bands.

The total number of bands for water soluble proteins showed the thirty three polymorphic bands which were detected with molecular weights ranging from 97 to 9 KD, Rf :0.2-0.9. There are clear distinctions in the number of bands between genotypes which range from 9 in genotypes L 1827, L 2174, Sa. 69 and Sa. 61 to 10 bands in genotype L 772, L 1198, Ben.1, Ben 3, Si.1 and Gem.9. Some genotypes have specific bands which distinguished them from others. The total number of bands for insoluble proteins indicated the occurrence of twenty polymorphic bands with molecular weight ranging from 164 to 14 KD, with Rf: 0.03 - 0.9. There is a clear variation in the number of bands between genotypes ranged from 5 in genotype L 2174 to 8 bands in the rest of the genotypes. There was common band that were in all genotypes with MW of 49.349 KD at Rf 0.39, Some genotypes have specific bands which distinguished them among others, for instance, genotype L 772 contained protein bands with molecular weights

14.262, 16.846 and 31.018 KD at mobility 0.95, 0.87 and 0.61. The genetic similarity indicies ranged between 100% and 33%. The highest similarity was 100% between Sakha 69 and Beni-Swaif 1, while the lowest similarity was 33% between L.772 and Sids 1.

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Table (1): The ingredients of the staining solutions.

Enzymes	Compounds and references	Amounts
Prx	A-sodium acetate (1M, pH 4.7) 3, 3, 5, 5 tetramethyl benzidine (TMBZ) B-0.30 % H_2O_2 Graham *et al*. (1964)	50 ml 50 ml 50 ml
EST	Sodium phosphate (100mM, pH 6.0) α -naphthyl acetate Fast blue RR salt Jonathan and Wendel (1990)	50 mg 25 mg 50 mg
GOT	A-Tris (1M, pH 8.5) A-Ketoglutaric acid Aspartic B-pyrodxal-5- phosphate Fast blue BB salt Jonathan and Wendel (1990)	50 ml 50 mg 100 mg 5 mg 150 mg

Table (2): R_f of peroxidase isozymes (Prx) bands in wheat genotypes.

Genotypes	Prx-1	Prx-2	Prx-3	Prx-4
L.772	0.060	0.112	0.170	0.204
L.1198	0.060	0.115	----	----
L.1827	0.060	0.112	----	----
L.2174	0.060	0.112	0.201	----
Sa.69	0.057	0.115	0.207	----
Ben.1	0.057	0.095	0.129	0.172
Ben.3	0.063	0.098	0.129	0.170
Si.1	0.057	0.112	0.170	0.204
Gem.9	0.057	0.115	----	----
Sa.61	0.057	0.095	0.170	0.204

Table (3): R_f of esterase isozymes (EST) bands in wheat genotypes.

Genotypes	EST-1	EST-2	EST-3	EST-4	EST-5	EST-6
L.772	0.123	0.258	0.327	0.396	0.658	----
L.1198	0.258	0.327	0.396	----	----	----
L.1827	0.258	0.327	0.396	0.600	----	----
L.2174	0.165	0.258	0.327	0.396	0.658	----
Sa.69	0.258	0.327	0.396	0.662	----	----
Ben.1	0.262	0.327	0.400	0.600	0.738	----
Ben.3	0.262	0.327	0.404	0.600	0.738	----
Si.1	0.258	0.327	0.396	0.658	----	----
Gem.9	0.185	0.258	0.327	0.396	0.512	0.658
Sa.61	0.123	0.262	0.327	0.396	0.600	0.665

Table (4): R_f of glotamate oxaloacetate transaminase isozyme (GOT) bands in wheat genotypes.

Genotypes	GOT-1	GOT-2
L.772	0.377	0.455
L.1198	0.368	0.455
L.1827	0.368	0.455
L.2174	0.377	0.455
Sa.69	0.377	0.475
Ben.1	0.377	0.471
Ben.3	0.368	0.447
Si.1	0.363	0.455
Gem.9	0.377	0.455
Sa.61	0.377	0.475

Table (5): Molecular weights (MW) of SDS-PAGE of water soluble proteins of wheat genotypes.

No. bands	MW(KD)	RF	1	2	3	4	5	6	7	8	9	10
1	97.091	0.200	+	-	-	-	-	-	-	-	-	-
2	96.683	0.210	-	+	+	+	+	-	+	+	+	+
3	96.671	0.220	-	-	-	-	-	+	-	-	-	-
4	82.161	0.250	+	+	+	+	+	+	+	-	-	+
5	81.900	0.277	-	-	-	-	-	-	-	-	+	-
6	80.493	0.301	-	-	-	-	-	-	-	+	-	-
7	52.672	0.361	-	-	-	-	-	-	-	+	-	-
8	50.393	0.404	+	+	+	+	+	+	+	-	+	+
9	41.248	0.434	+	-	+	+	+	-	+	-	+	+
10	40.813	0.441	-	+	-	-	-	-	-	-	-	-
11	40.715	0.450	-	-	-	-	-	-	-	+	-	-
12	39.990	0.540	-	-	-	-	-	+	-	-	-	-
13	31.106	0.562	-	-	-	-	-	-	-	+	-	-
14	31.053	0.563	+	+	+	+	+	-	+	-	+	+
15	31.033	0.657	-	-	-	-	-	+	-	-	--	-
16	26.782	0.661	-	-	+	-	-	-	-	-	-	-
17	26.151	0.675	+	+	-	+	+	+	+	+	+	+
18	23.776	0.702	-	-	-	-	-	-	-	+	-	-
19	23.765	0.708	-	-	-	-	-	+	-	-	-	-
20	23.565	0.710	+	+	+	+	+	-	-	-	-	+
21	23.247	0.713	-	-	-	-	-	-	-	-	+	-
22	23.141	0.751	-	-	-	-	-	-	+	-	-	-
23	22.739	0.761	-	-	-	-	-	+	-	-	-	-
24	22.413	0.780	-	-	-		-	-	-	+	-	-
25	22.180	0.791	-	-	-	-	-	-	-	-	+	-
26	21.657	0.821	-	-	-	+	+	+	+	+	+	-
27	21.640	0.850	-	-	-	-	-	-	-	-	-	+
28	21.630	0.850	-	+	-	-	-	-	-	-	-	-
29	18.922	0.869	-	-	-	-	-	-	+	+	+	-
30	15.761	0.879	+	+	+	-	+	+	+	-	-	+
31	15.699	0.880	-	-	-	+	-	-	-	-	-	-
32	11.679	0.920	+	+	+	-	-	-	-	-	-	-
33	9.796	0.943	+	-	-	-	-	-	-	-	-	-

+ = present - = absent

1 = L.772, 2 = L.1198, 3 = L.1827, 4 = L.2174, 5 = Sa.69, 6 = Ben.1, 7 = Ben.3, 8 = Si.1, 9 = Gem.9 and 10 = Sa.61.

Table (6): Total number of bands and the MW of the highest and the lowest bands for the SDS- soluble proteins in wheat genotypes.

Genotypes	High MW (KDa)	Low MW (KDa)	Total bands number
L.772	97.691	9.796	10
L.1198	96.683	11.679	10
L.1827	96.683	11.679	9
L.2174	96.683	15.699	9
Sa.69	96.683	15.761	9
Ben.1	96.671	15.761	10
Ben.3	96.683	15.761	10
Si.1	96.683	18.922	10
Gem.9	96.683	18.922	10
Sa.61	96.683	15.761	9

Table (7): Molecular weights (MW) of SDS- protein PAGE of water insoluble proteins of wheat genotypes.

No. bands	MW(KD)	RF	1	2	3	4	5	6	7	8	9	10
1	164.762	0.03	+	+	+	-	+	+	+	+	+	+
2	122.927	0.11	-	+	-	-	-	-	-	-	-	-
3	121.176	0.15	+	-	+	-	+	+	+	+	+	+
4	90.069	0.19	+	-	+	+	+	+	+	+	+	+
5	89.697	0.22	-	+	-	-	-	-	-	-	-	-
6	74.014	0.25	-	-	-	-	+	-	-	-	-	-
7	73.164	0.26	-	-	+	-	-	-	-	-	-	-
8	73.052	0.27	+	+	-	+	-	+	+	+	+	+
9	49.349	0.39	+	+	+	+	+	+	+	+	+	+
10	31.411	0.58	-	+	-	+	+	+	-	+	+	+
11	31.398	0.59	-	-	+	-	-	-	-	-	-	-
12	31.018	0.61	+	-	-	-	-	-	-	-	-	-
13	30.404	0.62	-	-	-	-	-	-	+	-	-	-
14	17.105	0.87	-	-	-	+	-	-	-	-	-	-
15	16.846	0.87	+	-	-	-	-	-	-	-	-	-
16	16.815	0.88	-	+	+	-	-	+	+	+	+	+
17	16.488	0.91	-	-	-	-	+	-	-	-	-	-
18	14.403	0.94	-	+	-	-	-	-	-	-	-	-
19	14.262	0.95	+	-	-	-	-	-	-	-	-	-
20	14.032	0.96	-	-	+	+	+	+	+	+	+	+

+ = present - = absent

1 = L.772, 2 = L.1198, 3 = L.1827, 4 = L.2174, 5 = Sa.69, 6 = Ben.1, 7 = Ben.3, 8 = Si.1, 9 = Gem.9 and 10 = Sa.61.

Table (8): Total bands and the MW of the highest and the lowest bands for insoluble proteins in wheat genotypes.

Genotypes	High MW (KDa)	Low MW (KDa)	Total bands
L.772	164.762	14.262	8
L.1198	164.762	14.403	8
L.1827	164.762	14.032	8
L.2174	90.069	14.032	5
Sa.69	164.762	14.032	8
Ben.1	164.762	14.032	8
Ben.3	164.762	14.032	8
Si.1	164.762	14.032	8
Gem.9	164.762	14.032	8
Sa.61	164.762	14.032	8

Table (9): Similarity indicies among the ten wheat genotypes based on water soluble and insoluble proteins analysis.

Genotypes	L.772	L.1198	L.1827	L.2174	Sa.69	Ben.1	Ben.3	Si.1	Gem.9
L.1198	.556								
L.1827	.556	.571							
L.2174	.545	.545	.563						
Sa.69	.629	.571	.706	.750					
Ben.1	.629	.571	.706	.750	1.000				
Ben.3	.611	.556	.686	.667	.743	.743			
Si.1	.333	.389	.400	.485	.514	.514	.611		
Gem.9	.500	.500	.571	.667	.686	.686	.778	.667	
Sa.61	.686	.686	.765	.750	.824	.824	.800	.571	.743

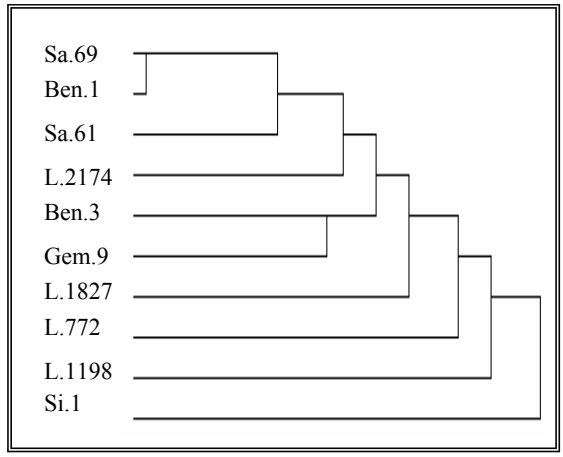

Fig. (1): Dendrogram representing the genetic relationships among the ten wheat genotypes based on SDS-PAGE.