ASSOCIATION OF MOLECULAR MARKERS WITH PHENOTYPIC TRAITS OF BREAD WHEAT GENOTYPES

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Genetic diversity is one of the key factors for the improvement of many crop plants including wheat. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity/distance estimates among genotypes are helpful in the selection of parents to be used in subsequent breeding programs (Van Becelaere *et al.*, 2005). Morphological characters, in association with multivariate techniques, have been employed in quantifying genetic similarity in wheat (Zeven and Schavhi, 1989; Van Beuningen and Bush, 1997; Maric *et al.*, 2004; Khaled *et al.*, 2013).

Molecular markers provided excellent tools to estimate the genetic diversity (Sofalian *et al*., 2008). The genomic simple sequence repeats (g-SSRs), Expressed Sequence Tagged, EST-derived SSR (e-SSRs), inter-simple sequence repeats (ISSRs), sequence-tagged site (STS), and sequence-related amplified polymorphism (SRAP) molecular markers were used to evaluate wheat parents and their derived lines (Cui *et al.*, 2014). ISSRs are one of the DNA-based markers that have become widely used in various areas of plant research (Karaca and Izbirak, 2008). The ISSR molecular markers are semi-arbitrary. Single forward primers with 16-18 nucleotide length comprises repetitive units and anchors 2-4 arbitrary nucleotides at the 3' or 5' end. This method did not require the information about genomic sequences and therefore by means of these primers, high level of polymorphism could be realized (Zietkiewicz *et al.*, 1994). This technique has been widely used in studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology (Yang *et al.*, 1996). Moreover, ISSRs markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes (Najaphy *et al.*, 2011).

SRAPs which are based on the amplification of open reading frames (ORFs) developed from genome sequence data of arabidopsis (Li and Quiros, 2001). SRAP targets functional genes and therefore can be efficiently used for purposes including gene tagging, marker-assisted selection (MAS), and genome-wide association (Li and Quiros, 2001; Aneja *et al.*, 2012), Moreover, SRAPs have numerous other advantages such as multilocus and multiallelic features, cost-effectiveness, and a lack of crop specificity. Wang *et al.*

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(2009) reported that SRAP is a molecular marker which could provide high polymorphism and plentiful information. To date, few SRAP markers have been identified in wheat (Li *et al.*, 2007; Aneja *et al.*, 2012; Al-Doss *et al.*, 2010 and 2011; Elshafei *et al.*, 2013; El-Rawy and Youssef, 2014). Limited information's are available on chromosomal locations of SRAP markers (Fufa *et al.*, 2005), their linkage with plant traits and the potential of SRAP markers for genetic diversity studies in wheat. Therefore, these markers were employed to examine their potential for genetic diversity analysis in durum wheat. SRAP has been used successfully for the evaluation of wheat genotypes (Zaefizadeh and Goliev, 2009; El-Rawy and Youssef, 2014). Molecular variation evaluated in their study in combination with phenotypic characters of wheat can be useful in traditional and molecular breeding programs. Using linkage-based association analysis (including QTL interval mapping) in bread wheat, a large number of genes for various traits (quality traits, resistance to biotic and abiotic stresses, etc.) have already been tagged with markers (Gupta *et al.*, 1999; Varshney *et al.*, 2005). The objectives of the present study were to assess the level of genetic diversity and marker-trait associations in 36 bread wheat genotypes using phenotypic, ISSRs and SRAP markers.

MATERIALS AND METHODS

Seed materials and field experiment

Seeds of 36 bread wheat genotypes were classified as: L1 to L34 genotypes obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and Sids-12 (L35) and Egypt-1 (L36) from Egypt. Information on the name/pedigree of the accessions is available elsewhere (Hamam *et al.*, 2015). Wheat genotypes were sown in the field at two dates, 15 November (favorable) and 28 December (heat stress), during winter season of 2012/2013 and 2013/2014. These genotypes were grown in a randomized complete block design with three replications at the experimental farm, Sohag University, Egypt. Each genotype was sown in a plot of 10.5 m^2 area.

Recording of phenotypic data

Data with an average of 15 plants (five plants per replication) of each genotype were recorded on the following 8 phenotypic traits: leaf area (cm^2), days to heading, plant height (cm), biomass (ton/ht.), spike length (cm), number of kernel per spike, 1000-kernel weight (g) and grain yield (ton/ht.).

DNA extraction, ISSRs and SRAP assays

Total Genomic DNA was extracted from young leaf pieces (approximately 1 cm^2) using the BioSprint 96 Workstation (Laboratory of Plant Reproduction and Development (RDP), ENS of Lyon, France) and the DNA Plant Kit (Qiagen), according to the instructions of the supplier. Genomic DNA was diluted 10-fold in water prior to 40 cycles of PCR amplification with the GoTaq Core System mixture (Promega). The Thermal Cycler was programmed as: 1 cycle (an initial denaturing

step) of 5 min at 95°C, 40 cycles of 30 sec at 95°C (denaturation step), 30 sec at 35°C to 58°C (annealing step, optimized for each primer), 1 min 30 sec at 72°C (elongation step) and 5 min at 72°C (final extension), then kept at 20°C. PCR products were visualized by conventional agarose gel electrophoresis (Sambrook *et al.*, 1989). For ISSRs assay, 13 primers produced polymorphic banding patterns among 36 wheat genotypes. For SRAP assay, seven out of ten pair of primers (forward and reverse) revealed genetic polymorphism among genotypes.

Data analysis and dendrograms constructed

The DNA banding patterns generated by ISSRs and SRAP were analyzed by computer program Gene Profiler (version 4.03). The presence (1) or absence (0) of each band was recorded for each genotype for all studied primers. To measure the informativeness of the ISSR and SRAP techniques in differentiating among 36 wheat genotypes, the polymorphic information content (PIC) was calculated according to the formula of Ghislain *et al*. (1999), as PIC= 1- $[(p)^2 + (q)^2]$, where *p* is the frequency of allele band present and *q* is frequency of allele band absent across the tested genotypes. The marker index (MI) was also calculated for each ISSR and RAPD primer as MI= PIC x $\eta\beta$, where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphism (Powell *et al*., 1996). Analysis of variance (ANOVA) was conducted using the 0-1 data. The association analysis was conducted using simple linear regression. For this, data on individual phenotypic trait were regressed on whole 0-1 binary marker data for each individual marker using Excel programme. The coefficient of determination $(R)^2$ was calculated as $R^2 = 1 - (SSE / SST)$, where SSE is the sum of squares of error and SST is the total sum of squares. Genetic similarity estimates for ISSRs and SRAP markers were determined using Jaccard's coefficient (Jaccard, 1908). Dendrograms were generated with the unweighted pair group method with arithmetic mean algorithm (UPGMA) using the computational package MVSP version 3.1. A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the matrices using the Mantel test (Mantel, 1967) method. Finally, the correlation between each distance pair was calculated using NTSYS-pc version 2.2 (Rolhf, 2000).

RESULTS AND DISCUSSION

Phenotypic data

For each of the eight phenotypic traits included in the present study, data on mean and analysis of variance are available with another study published elsewhere (Hamam *et al*., 2015).

Level of polymorphism based on ISSR

In the present study, thirteen out of 25 primers revealed different degrees of percentage of polymorphism (%P) among

genotypes. A total of 61 amplified bands were polymorphic. The %P ranged from 20 (UBC-876 primer) to 100 (UBC-834 primer) with an average of 64.89% (Fig. 1 and Table 1). The number of polymorphic bands ranged from 1 (UBC-876) to 12 (UBC-845) with an average of approximately 5 bands per primer. The bands size ranged from 250 bp to 3.9 kb generated by UBC-815 and UBC-845 primers, respectively. The %P using ISSR in the previous studies was varied. In this regard, Nagaoka and Ogihara (1997) obtained %P of 53.6% between common wheat, while Carvalho *et al.* (2009) documented a high %P (98.5%) using 18 ISSR primers in 99 wheat accessions. Also, Emel (2010) reported a %P of 76.07% among 11 triticale cultivars. On the contrary, Tok *et al.* (2011) showed very low of %P (17.59%).

The ISSRs primers used in this study were composed of di-, tetra- and penta-nucleotide repeat sequences. In this study, the using of di-nucleotide repeat primers showed the highest levels of polymorphism, the UBC-845 and UBC-834 and belonged to (CT) and (AG) repeats primer groups produced 92.31 and 100% polymorphism, respectively (Table 1). It is clear that the highest polymorphism level was obtained in the case of di-nucleotide repeat primers. These results are in agreement with those obtained by Najaphy *et al.* (2011) who reported that the ISSR primers with di-nucleotide motifs (GA)n, (CT)n and (AG)n produced a high level of polymorphism. Unlikely, Song *et al.* (2002) and Sofalian *et al.* (2008) proposed that the polymorphism rates were higher when the motifs comprise three to five nucleotides of microsatellite primers in wheat. In this direction, Sofalian *et al.* (2008) and Emel (2010) obtained a level of polymorphism of 78.6% and 76.07%, respectively using the same primer sequences for wheat cultivars. In agreement with the result obtained by Najaphya *et al.* (2011) our results showed that the primer (UBC-876) belong to tetra-nucleotide repeat sequences (GATA) produced lower level of polymorphism of 20% (Table 1).

Level of polymorphism based on SRAP

Ten pairs of SRAP primers were screened across the 36 wheat genotypes, and seven pairs of them were polymorphic. A total of 55 bands were amplified, of which 40 bands (72.72%) were polymorphic (Fig. 2 and Table 2). The number of bands varied from 4 (ME-7/ EM-3 primer combination) to 12 (ME-1/EM-5 primer combination). The %P ranged between 20 and 100% with an average of 72.72% (Table 2). The mean number of bands and polymorphic bands were 7.86 and 5.71 per primer, respectively. The ME-1/EM-2 and EM-7/EM-5 primer combinations showed higher levels of polymorphism of 77.78% and 100%, respectively. Similarly, Mansing (2010) documented the average of %P (68.73%) using the same forward primers with different reverse primers; they have comparable levels of polymorphism of 71.43% and 100%, respectively. On the other hand, Al-Doss *et al*. (2011) reported lower level of polymorphism than our (35.0%),

among 6 durum wheat genotypes. Also, Zaefizadeh and Goliev (2009) found 56.73% polymorphism in 40 *Triticum durum* genotypes.

PIC and MI analysis

The Polymorphism information content (PIC) values for the 13 ISSRs primers varied from 0.04 to 0.28 with an average of 0.15. The lowest and highest PIC indices were recorded for UBC-876 and UBC-834, respectively (Table 1). Similarly, The PIC values for SRAP primers varied from 0.04 to 0.20 with an average of 0.16. The lowest and highest PIC indices were recorded for primer combinations (ME-2/EM-3) and (ME-7/EM-5 and ME-7/EM-6), respectively (Table 2). The PIC index has been used extensively in many genetic diversity studies (Tatikonda *et al.*, 2009; Talebi *et al.*, 2010; Thudi *et al.*, 2010). Moreover, the PIC value of markers indicates the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation (Peng and Lapitan, 2005). In the present study, the average PIC values of ISSR and SRAP primers are lower than those of a previous study, 0.93 detected for SRAP and 0.90 for ISSRs which observed among 6 wheat genotypes (Tok *et al*., 2011). On the other hand, Najaphy *et al.* (2011) showed moderate values of PIC for the ISSR primers that could be attributed to the diverse nature of the wheat accessions and/or highly informative ISSR markers.

The Marker index (MI) values ranged from 0.04 to 2.40 for UBC-876 and UBC-845, respectively with an average of 0.79 for ISSRs assay. MI values for SRAP markers were between 0.04 and 2.20 for the combinations ME-2/EM-3 and ME-7/EM-5, respectively with an average of 1.03. So, SRAP were more efficient than the ISSRs markers, where the SRAP technique exhibited higher average (1.03) of marker index compared to ISSR one (0.79). The results of MI values were smaller than those reported by Najaphy *et al.* (2011) (from 0.41 to 3.36).

Single marker analysis

The present study involved a set of 36 genotypes, which constitute important and diverse genotypes of bread wheat, exhibiting moderate to high genetic variability for the 8 phenotypic traits examined during the present work (Hamam *et al*., 2015). Using simple linear regression method, a total of 101 polymorphic molecular markers (ISSR = 61; SRAP = 40) were identified, only 8 of which showed significant association with 3 of 8 tested traits. In analysis, one ISSRs marker was identified for days to heading trait, followed by five markers each for spike length trait (Table 3). Finally, only one SRAP marker was identified for number of kernel/spike trait (Table 3). The ISSRs markers; UBC-808_{780bp} and (UBC-808_{700bp}, UBC-811_{870bp}, UBC-819_{980bp}, UBC-845_{1035bp}, UBC-880_{1650bp}), were regarded as candidate markers, linked to the days to heading and spike length per plant genes (Fig. 1). The associated markers each explained a maximum regression of 10.74 (days to heading) to 11.60% (spike

length) of the total available variation for individual associated traits (Table 3). The SRAP marker ME-7/EM-6_{420bp} (Fig. 2C) was regarded as candidate marker which linked to number of kernel/spike. Significant regression (258.93*, p=0.047) was observed on it, of 40 SRAP markers (Table 3). Roy *et al*. (2006) showed the associated markers each explained a maximum of 8.12 and 29.38% for tiller numbers and florets per spike traits analyzing a total of 99 and 133 polymorphic SSR and AFLP bands, respectively, in bread wheat. Markers identified during the present study need to be subjected to validation and/or functional analysis of respective traits, which is beyond the scope of the present work. However, we believe that at least one of the markers identified would be validated and used for marker-assisted selection.

Cluster analysis

A cluster analysis realized using Jaccard's coefficient (Jaccard, 1908) for the ISSRs and SRAP markers, revealed similarity coefficient values ranged from 0.51 to 1.00 (Table 4, below diagonal) with an average of 0.76, and from 0.44 to 0.96 (Table 4, above diagonal) with an average of 0.70, respectively. Similar to our findings, Abou-Deif *et al*. (2013) obtained levels of genetic similarity ranged from 0.47 to 0.94, with an average of 0.71 among 20 wheat varieties using ISSRs markers. The UPGMA cluster analysis based on the ISSR marker separated the studied genotypes into two significantly different clusters (Fig. 3). The first cluster was with genotypes "L5, L6, L7 and L8" at 1.00 similarity coefficient, which branched at similarity coefficient (0.57) with the second cluster, which divided into eight sub-clusters. The sub-clusters "a, b, c and d" contains genotypes "L14, L31, L12 and L10", respectively. Genotypes "L35, L27 and L26" formed sub-cluster "e", while genotypes "L20 and L16" were gathered in the sub-cluster "f". The sub-cluster "g" was the biggest which includes 22 genotypes (Fig. 3). Genotypes "L36 and L1" are belonged to sub-cluster "h".

Based on SRAP markers the dendrogram gathered the genotypes into two clusters, which separated at 0.58 similarity coefficients. The first cluster contains genotypes "L28, L30, L29 and L14" (Fig. 4). The cluster II subdivided into nine sub-clusters: "a", "b", "c", "f", and "g" with genotypes "L9", "L19", "L27", "L31" and "L21", respectively. Genotypes "L33, L34 and L12" and "L32, L26 and L5" grouped together belonged to sub-clusters "d" and "e", respectively. Sub-cluster "h" was the biggest with 20 genotypes (Fig. 4). On the sub-cluster "i", genotype "L1" was placed alone. Interestingly, the genotypes "L28, L29 and L30", that grouped together based on ISSRs analysis, formed one cluster based on the SRAP analysis. It is clear that those genotypes "L28, L29 and L30" were developed from the same parents as well as genotypes "L5, L6, L7 and L8".

Correlation analysis

In order to compare the extent of agreement among both molecular markers, a distance matrix was constructed for each assay and compared, using the Mantel test. Comparison of ISSRs and SRAP matrices showed a positive and highly significant correlation (r = 0.63**, p=0.000). Ahmadi and Zar, (2011) reported that the cophenetic correlation value for the dendrogram based on their RAPD and ISSRs data was high (r = 0.81) studying 26 bread wheat cultivars.

SUMMARY

Phenotypic traits and molecular markers analyses are very important tools for the estimation of genetic variability among genotypes. In 36 bread wheat genotypes, genetic variability and marker-trait associations were studied for 8 agronomic traits using a set of 101 DNA-based molecular markers (61 ISSR and 40 SRAP polymorphic markers). The results of ISSR and SRAP analyses showed similar range of the percentage of polymorphism (%P) (20 to 100%), as well as the average of polymorphic information content (PIC) which was about 0.15 and 0.16, respectively. Results showed that di-nucleotide repeat primers represented the highest levels of polymorphism, i.e. UBC-845 and UBC-834 belonged to (CT) and (AG) repeats produced %P of 92.31 and 100%, respectively. While, UBC-876 belonged tetra-nucleotide repeat (GATA) produced lower level of polymorphism (20%). Results of marker index (MI) showed that SRAP were more efficient than the ISSRs markers, where the SRAP technique exhibited higher average (1.03) of marker index compared to ISSR one (0.79). Single-marker analysis (SMA) indicated that one ISSR, six ISSRs and one SRAP markers linked to the days to heading, spike length and number of kernel per spike traits, respectively. The cluster analysis based on ISSRs and SRAP data revealed similarity coefficient values ranged from 0.51 to 1.00 with an average of 0.76, and from 0.44 to 0.96, with an average of 0.70, respectively. The genotypes "L28, L29 and L30" grouped together based on ISSR and SRAP markers analyses. Similarity matrices generated by ISSRs and SRAP showed a positive and highly significant correlation (r = 0.63**, p=0.000).

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Table (1): Primers used for ISSR analysis, total number of fragments detected by each pair of primers, %P, PIC and fragments sizes for 36 wheat genotypes.

Primer name	Primer Sequence (5'---------3')	Amplified bands		%P	PIC	MI	Fragments size (bp)	
		Bands number	Polymorphic bands				Larger	Smaller
UBC-808	$(AG)_8C$	11	5	45.45	0.12	0.60	1250	645
UBC-811	$(GA)_8AC$	6	4	66.67	0.21	0.84	1300	615
UBC-812	$(GA)_2GG(AG)_4AA$	7	4	57.14	0.08	0.32	1500	500
UBC-815	$(TC)_8A$	5	2	40.00	0.05	0.10	950	250
UBC-819	$(GT)_8A$	9	7	77.78	0.17	1.19	1600	745
UBC-834	$(AG)_8YT$	6	6	100.00	0.28	1.68	900	365
UBC-840	$(GA)_8TT$	6	3	50.00	0.18	0.54	1150	770
UBC-845	$(CT)_8TT$	13	12	92.31	0.20	2.40	3900	800
UBC-846	$(CA)_8RT$	5	4	80.00	0.24	0.96	930	645
UBC-849	$(GT)_8YA$	8	4	50.00	0.09	0.36	1400	750
UBC-876	$(GATA)_2(GACA)_2$	5	1	20.00	0.04	0.04	2000	1000
UBC-880	$(TC)_8AA$	6	5	83.33	0.17	0.85	1650	720
UBC-881	$(GGGTG)_3$	7	4	57.14	0.10	0.40	1500	615
Total		94	61					
Mean		7.23	4.69	64.89	0.15	0.79		

%P, Percentage of polymorphism; PIC, polymorphism information content; MI, Marker index and bp, base pair.

Table (2): Primers used for SRAP analysis, total number of fragments detected by each pair of primers, %P, PIC and fragments sizes for 36 wheat genotypes.

Primer name	Primer Sequence (5'---------3')	Primers combinations	Bands number	Polymorphic bands	%P	PIC	MI	Fragments size (bp)	
								Larger	Smallest
ME-1(F)	TGAGTCCAAACCGGATA	ME-1/ EM-2	9	7	77.78	0.17	1.19	3250	200
ME-2(F)	TGAGTCCAAACCGGAGC	ME-1/ EM-5	12	8	66.67	0.18	1.44	2200	170
ME-7(F)	TGAGTCCAAACCGGACG	ME-2/ EM-3	5	1	20.00	0.04	0.04	1900	90
EM-2(R)	GACTGCGTACGAATTTGA	ME-2/ EM-5	6	4	66.67	0.16	0.64	1950	410
EM-3(R)	GACTGCGTACGAATTGAC	ME-7/ EM-3	4	3	75.00	0.16	0.48	1000	140
EM-5(R)	GACTGCGTACGAATTAAC	ME-7/ EM-5	11	11	100.00	0.20	2.20	1700	130
EM-6(R)	GACTGCGTACGAATTGCA	ME-7/ EM-6	8	6	75.00	0.20	1.20	1200	250
Total			55	40					
Mean			7.86	5.71	72.72	0.16	1.03		

%P, Percentage of polymorphism; PIC, polymorphism information content; MI, Marker index and bp, base pair.

Table (3): Details analyses of variances (ANOVA) involving simple linear regression (R^2) for traits using 61 ISSR and 40 SRAP polymorphic bands.

Marker	Trait	SV	df	SS	MS	R^2	P-value
UBC-808$_{780bp}$	Days to heading	Genotypes	1	19.00	19.00*	10.74	0.050
		Error	34	157.85	4.64		
		Total	35	176.85			
UBC-808$_{700bp}$	Spike length	Genotypes	1	7.45	7.45*	11.60	0.042
		Error	34	56.74	1.67		
		Total	35	64.19			
UBC-811$_{870bp}$	Spike length	Genotypes	1	7.13	7.13*	11.10	0.047
		Error	34	57.06	1.68		
		Total	35	64.19			
UBC-819$_{980bp}$	Spike length	Genotypes	1	7.16	7.16*	11.11	0.047
		Error	34	57.03	1.68		
		Total	35	64.19			
UBC-845$_{1035bp}$	Spike length	Genotypes	1	7.45	7.45*	11.60	0.042
		Error	34	56.74	1.67		
		Total	35	64.19			
UBC-880$_{1650bp}$	Spike length	Genotypes	1	7.13	7.13*	11.11	0.046
		Error	34	57.06	1.68		
		Total	35	64.19			
ME-7/EM-6$_{420bp}$	Number of kernel/spike	Genotypes	1	258.93	258.93*	11.11	0.047
		Error	34	2071.80	60.93		
		Total	35	2330.73			

*Significant at 5%. SV, source of variation; df, degrees of freedom; SS, sum of squares and MS, mean of squares.

Table (4): Similarity matrix for 36 wheat genotypes according to Jaccard's coefficient obtained from 94 ISSR fragments (below diagonal) and from 55 SRAP fragments (above diagonal).

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34	L35	L36
L1		0.69	0.68	0.76	0.6	0.67	0.64	0.67	0.54	0.72	0.67	0.61	0.75	0.62	0.63	0.69	0.67	0.65	0.64	0.70	0.58	0.65	0.71	0.63	0.66	0.57	0.64	0.56	0.58	0.60	0.63	0.65	0.59	0.69	0.65	0.74
L2	0.88		0.79	0.89	0.81	0.90	0.80	0.83	0.59	0.83	0.90	0.77	0.75	0.62	0.73	0.74	0.90	0.89	0.74	0.82	0.73	0.81	0.83	0.84	***0.96***	0.77	0.74	0.56	0.58	0.55	0.73	0.75	0.80	0.80	0.81	0.80
L3	0.80	0.91		0.89	0.74	0.83	0.86	0.83	0.58	0.77	0.77	0.70	0.74	0.66	0.67	0.79	0.83	0.82	0.73	0.89	0.77	0.80	0.89	0.77	0.76	0.66	0.73	0.59	0.61	0.54	0.72	0.69	0.68	0.68	0.74	0.79
L4	0.78	0.86	0.94		0.77	0.86	0.82	0.86	0.59	0.86	0.86	0.79	0.83	0.63	0.74	0.82	0.86	0.85	0.76	0.92	0.74	0.83	0.92	0.80	0.85	0.73	0.70	0.56	0.58	0.56	0.82	0.77	0.76	0.76	0.83	0.89
L5	0.83	0.94	0.94	0.88		0.90	0.75	0.78	0.60	0.78	0.84	0.72	0.71	0.68	0.79	0.65	0.84	0.83	0.65	0.71	0.74	0.76	0.83	0.84	0.83	0.84	0.79	0.57	0.64	0.65	0.69	0.71	0.75	0.75	0.76	0.75
L6	0.83	0.94	0.94	0.88	***1.00***		0.83	0.87	0.62	0.87	0.87	0.74	0.73	0.65	0.76	0.72	0.93	0.86	0.72	0.79	0.81	0.84	0.86	0.93	0.86	0.81	0.82	0.58	0.61	0.62	0.71	0.73	0.77	0.77	0.78	0.83
L7	0.83	0.94	0.94	0.88	***1.00***	***1.00***		0.90	0.54	0.72	0.77	0.77	0.75	0.62	0.63	0.80	0.90	0.82	0.80	0.89	0.78	0.81	0.83	0.84	0.77	0.67	0.74	0.60	0.58	0.51	0.73	0.70	0.69	0.74	0.75	0.74
L8	0.83	0.94	0.94	0.88	***1.00***	***1.00***	***1.00***		0.57	0.75	0.81	0.80	0.78	0.60	0.66	0.83	0.93	0.79	0.72	0.86	0.76	0.78	0.86	0.87	0.80	0.70	0.71	0.54	0.56	0.54	0.77	0.73	0.72	0.72	0.73	0.77
L9	0.84	0.92	0.92	0.87	0.92	0.92	0.92	0.92		0.57	0.57	0.56	0.51	0.53	0.63	0.54	0.62	0.59	0.46	0.55	0.58	0.60	0.61	0.58	0.61	0.62	0.64	***0.44***	0.49	0.48	0.58	0.65	0.69	0.59	0.60	0.59
L10	0.79	0.77	0.79	0.79	0.82	0.82	0.82	0.82	0.83		0.81	0.69	0.78	0.60	0.81	0.77	0.81	0.79	0.72	0.79	0.81	0.78	0.80	0.81	0.80	0.75	0.71	0.58	0.56	0.62	0.71	0.68	0.72	0.72	0.78	0.90
L11	0.80	0.85	0.94	0.88	0.91	0.91	0.91	0.91	0.89	0.82		0.74	0.73	0.60	0.71	0.72	0.87	0.86	0.72	0.79	0.71	0.84	0.80	0.81	0.86	0.75	0.71	0.54	0.56	0.54	0.77	0.73	0.77	0.77	0.84	0.77
L12	0.76	0.84	0.87	0.81	0.87	0.87	0.87	0.87	0.82	0.75	0.89		0.67	0.54	0.65	0.71	0.74	0.79	0.71	0.72	0.60	0.72	0.73	0.70	0.79	0.74	0.71	0.57	0.59	0.53	0.70	0.72	0.77	0.83	0.72	0.71
L13	0.78	0.86	0.89	0.89	0.89	0.89	0.89	0.89	0.88	0.86	0.86	0.82		0.58	0.79	0.81	0.78	0.71	0.70	0.83	0.74	0.71	0.83	0.74	0.72	0.68	0.65	0.53	0.54	0.56	0.74	0.71	0.65	0.65	0.76	0.81
L14	0.69	0.64	0.62	0.62	0.64	0.64	0.64	0.64	0.64	0.60	0.61	0.58	0.60		0.61	0.57	0.65	0.68	0.62	0.63	0.61	0.63	0.69	0.61	0.64	0.60	0.62	0.73	0.83	0.77	0.61	0.58	0.62	0.67	0.63	0.57
L15	0.86	0.89	0.84	0.86	0.86	0.86	0.86	0.86	0.85	0.80	0.83	0.79	0.87	0.64		0.68	0.71	0.74	0.63	0.69	0.77	0.69	0.75	0.71	0.75	0.81	0.72	0.55	0.57	0.63	0.67	0.69	0.73	0.68	0.74	0.84
L16	0.81	0.81	0.81	0.79	0.81	0.81	0.81	0.81	0.85	0.77	0.81	0.72	0.77	0.64	0.79		0.77	0.76	0.74	0.89	0.73	0.70	0.77	0.73	0.71	0.62	0.60	0.51	0.53	0.55	0.73	0.65	0.69	0.64	0.75	0.80
L17	0.84	0.87	0.92	0.87	0.87	0.87	0.87	0.87	0.91	0.83	0.92	0.83	0.85	0.63	0.82	0.85		0.86	0.72	0.86	0.81	0.84	0.86	0.93	0.86	0.75	0.77	0.58	0.61	0.58	0.77	0.73	0.77	0.77	0.78	0.77
L18	0.83	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.89	0.87	0.82	0.76	0.83	0.61	0.83	0.84	0.87		0.76	0.85	0.74	0.77	0.79	0.80	0.92	0.79	0.75	0.61	0.63	0.56	0.75	0.71	0.82	0.82	0.77	0.76
L19	0.81	0.89	0.86	0.86	0.86	0.86	0.86	0.86	0.94	0.83	0.83	0.77	0.87	0.64	0.87	0.82	0.85	0.89		0.76	0.63	0.70	0.71	0.68	0.71	0.62	0.64	0.60	0.58	0.55	0.63	0.65	0.64	0.69	0.70	0.74
L20	0.84	0.84	0.78	0.81	0.81	0.81	0.81	0.81	0.85	0.83	0.78	0.72	0.82	0.69	0.87	0.85	0.85	0.86	0.90		0.80	0.77	0.85	0.80	0.79	0.68	0.65	0.56	0.58	0.51	0.82	0.71	0.70	0.70	0.77	0.82
L21	0.85	0.94	0.88	0.88	0.91	0.91	0.91	0.91	0.92	0.82	0.85	0.79	0.89	0.64	0.92	0.81	0.87	0.91	0.95	0.89		0.74	0.75	0.82	0.70	0.66	0.72	0.64	0.57	0.55	0.67	0.60	0.68	0.63	0.69	0.73
L22	0.83	0.91	0.88	0.91	0.91	0.91	0.91	0.91	0.89	0.82	0.82	0.79	0.89	0.61	0.89	0.84	0.87	0.91	0.89	0.89	0.94		0.83	0.79	0.77	0.73	0.74	0.66	0.59	0.56	0.74	0.71	0.70	0.75	0.87	0.75
L23	0.83	0.91	0.88	0.88	0.91	0.91	0.91	0.91	0.95	0.84	0.85	0.79	0.89	0.64	0.89	0.84	0.87	0.94	0.95	0.89	0.97	0.94		0.81	0.79	0.74	0.71	0.57	0.65	0.61	0.76	0.77	0.71	0.71	0.83	0.83
L24	0.85	0.91	0.88	0.88	0.91	0.91	0.91	0.91	0.92	0.84	0.88	0.81	0.92	0.64	0.92	0.84	0.89	0.91	0.92	0.89	0.97	0.94	0.97		0.81	0.76	0.77	0.55	0.57	0.63	0.72	0.69	0.73	0.73	0.74	0.78
L25	0.84	0.92	0.87	0.87	0.89	0.89	0.89	0.89	0.91	0.80	0.84	0.78	0.88	0.65	0.91	0.80	0.85	0.89	0.94	0.88	0.98	0.92	0.95	0.95		0.80	0.76	0.57	0.59	0.57	0.76	0.72	0.83	0.83	0.77	0.77
L26	0.79	0.87	0.81	0.76	0.84	0.84	0.84	0.84	0.88	0.72	0.78	0.72	0.77	0.64	0.79	0.77	0.80	0.81	0.85	0.79	0.86	0.81	0.86	0.84	0.88		0.71	0.54	0.61	0.58	0.66	0.84	0.77	0.77	0.73	0.77
L27	0.78	0.84	0.78	0.81	0.81	0.81	0.81	0.81	0.85	0.77	0.78	0.74	0.82	0.64	0.84	0.77	0.79	0.83	0.87	0.84	0.89	0.83	0.89	0.89	0.88	0.88		0.56	0.54	0.56	0.63	0.65	0.74	0.74	0.69	0.69
L28	0.58	0.58	0.56	0.58	0.58	0.58	0.58	0.58	0.59	0.66	0.58	0.61	0.60	0.60	0.63	0.56	0.55	0.60	0.60	0.58	0.60	0.56	0.60	0.60	0.59	0.56	0.63		0.80	0.69	0.54	0.49	0.51	0.65	0.57	0.51
L29	0.58	0.58	0.56	0.54	0.55	0.55	0.55	0.55	0.58	0.58	0.55	0.59	0.55	0.62	0.58	0.52	0.55	0.55	0.55	***0.51***	0.55	***0.51***	0.55	0.55	0.55	0.56	0.58	0.74		0.78	0.56	0.54	0.58	0.68	0.59	0.53
L30	0.60	0.60	0.60	0.60	0.59	0.59	0.59	0.59	0.62	0.64	0.59	0.58	0.64	0.61	0.64	0.55	0.58	0.63	0.64	0.59	0.63	0.59	0.63	0.63	0.63	0.60	0.64	0.76	0.83		0.54	0.49	0.60	0.60	0.61	0.60
L31	0.74	0.79	0.74	0.76	0.76	0.76	0.76	0.76	0.77	0.72	0.73	0.70	0.82	0.60	0.79	0.69	0.75	0.76	0.79	0.77	0.84	0.78	0.81	0.84	0.85	0.77	0.85	0.61	0.63	0.69		0.64	0.68	0.68	0.74	0.73
L32	0.79	0.84	0.82	0.87	0.84	0.84	0.84	0.84	0.85	0.75	0.81	0.78	0.82	0.65	0.85	0.78	0.80	0.84	0.85	0.82	0.90	0.90	0.90	0.90	0.91	0.80	0.85	0.58	0.53	0.59	0.83		0.70	0.70	0.71	0.75
L33	0.78	0.85	0.80	0.83	0.82	0.82	0.82	0.82	0.84	0.76	0.77	0.74	0.81	0.64	0.83	0.76	0.81	0.85	0.86	0.83	0.91	0.88	0.88	0.88	0.92	0.81	0.86	0.58	0.53	0.59	0.84	0.92		0.80	0.75	0.69
L34	0.77	0.85	0.82	0.82	0.82	0.82	0.82	0.82	0.86	0.79	0.79	0.81	0.89	0.61	0.83	0.75	0.81	0.82	0.89	0.83	0.88	0.85	0.88	0.88	0.86	0.78	0.86	0.62	0.57	0.68	0.83	0.81	0.82		0.75	0.69
L35	0.78	0.83	0.78	0.78	0.82	0.82	0.82	0.82	0.78	0.76	0.77	0.76	0.81	0.59	0.83	0.73	0.76	0.80	0.81	0.78	0.85	0.82	0.82	0.85	0.87	0.81	0.86	0.60	0.55	0.63	0.81	0.81	0.82	0.85		0.75
L36	0.88	0.88	0.86	0.80	0.85	0.85	0.85	0.85	0.87	0.77	0.85	0.81	0.81	0.69	0.84	0.81	0.90	0.83	0.84	0.84	0.88	0.83	0.85	0.88	0.87	0.81	0.84	0.56	0.56	0.60	0.79	0.82	0.83	0.82	0.80	

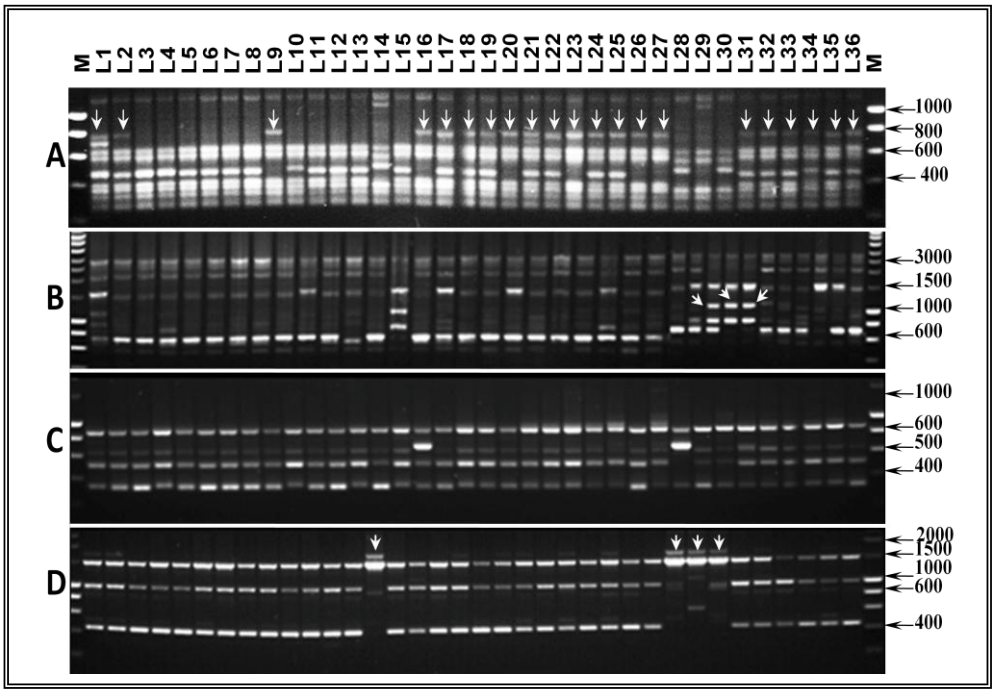

Fig. (1): ISSRs profiles obtained for 36 wheat genotypes amplified with primers: A, UBC-808; B, UBC-845; C, UBC-846 and D, UBC-880; M = 100 bp ladder size marker.

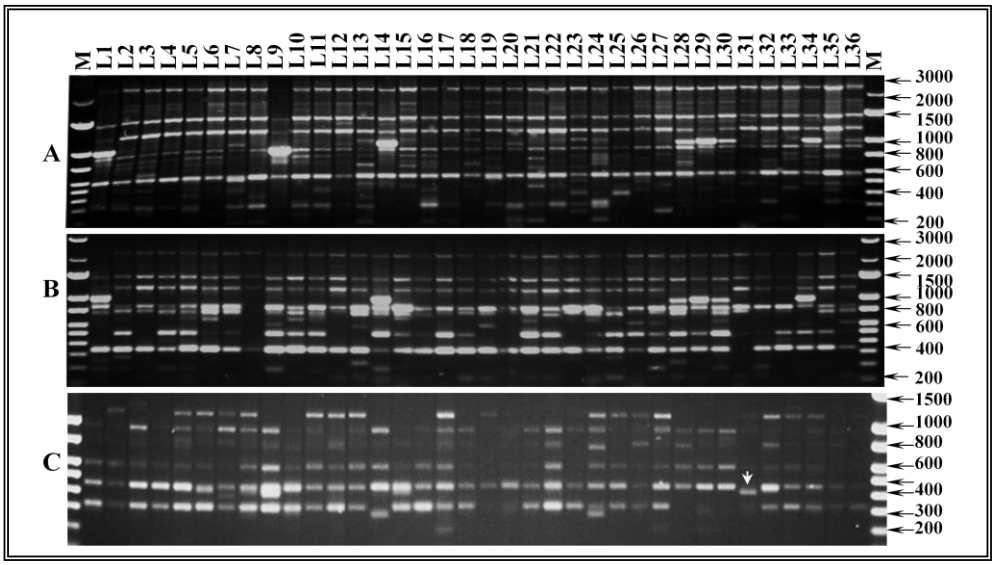

Fig. (2): SRAP profiles obtained for 36 wheat genotypes amplified by primers: A) EM-1F/ME-2R and B) EM-1F/ME-5R and C) ME-7F/EM-6R; M = 100 bp ladder size marker.

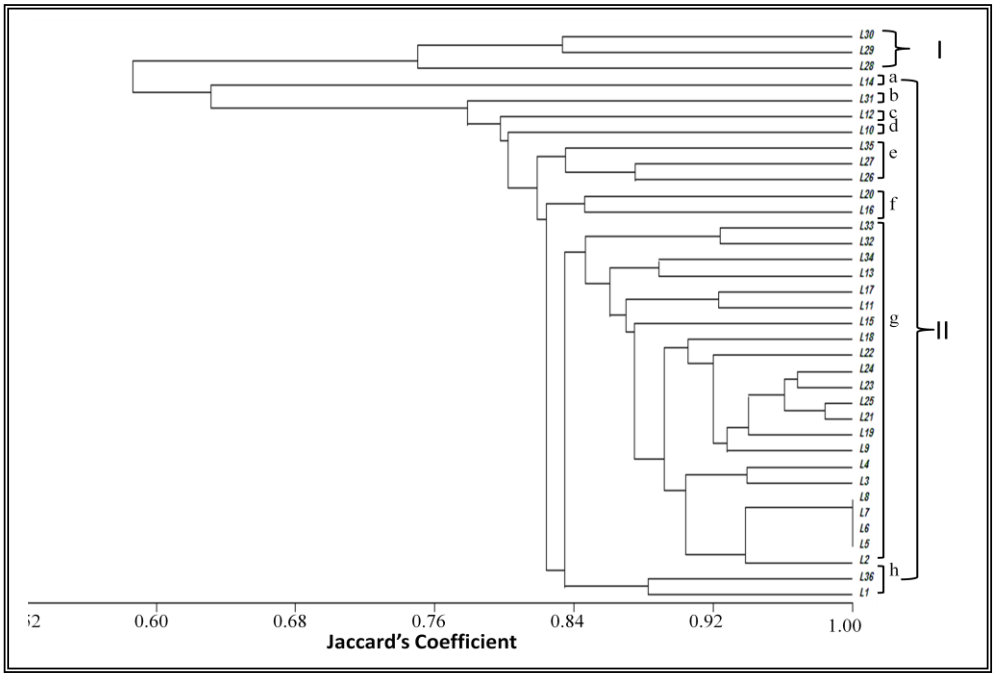

Fig. (3): Dendrogram generated using Jaccard`s coefficient analysis, showing relationships among 36 wheat genotypes, using ISSRs data.

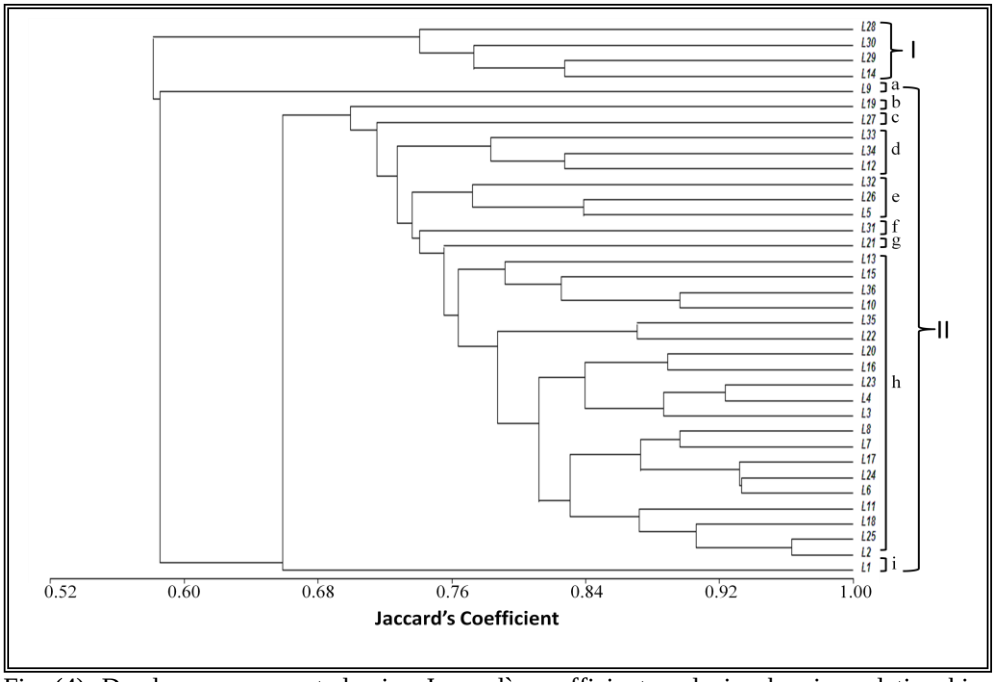

Fig. (4): Dendrogram generated using Jaccard`s coefficient analysis, showing relationships among 36 wheat genotypes, using SRAP data.