# GENETIC RELATIONSHIPS AMONG WHEAT VARIETIES BASED ON ISSR MARKERS

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## ABSTRACT

DNA based molecular markers allow precise, objective and rapid cultivar identification and discrimination. In the present study, the genetic variability and relationships among 65 hexaploid (*Triticum aestivum* L.) and 8 tetraploid (*Triticum durum* L.) wheat varieties were evaluated using five inter simple sequence repeats (ISSR) primers. The used primers generated 63 DNA fragments with an average of 12.6 bands per primer. Polymorphism percentage ranged from 53.33 % (ISSR-844) to 78.57 % (HB12) with an average of 64.95 %. The 308bp (HB12) DNA fragment was unique marker for durum wheat varieties while the 855bp (ISSR-814) fragment was unique maker for bread wheat genotypes. Low PIC values were found indicating the wide differences between the p and q alleles tested by the ISSR markers. The ISSR primers (HB12 and ISSR-844) possessed high RP values and therefore seem to be the most informative primers for distinguishing wheat varieties. The dendrogram indicated that the ISSR markers succeeded in distinguishing the tested varieties in relation to their ploidy level and location, which the tetraploid varieties were put together in one group as well as the hexaploid varieties.

**Keywords:** Wheat, PCR, ISSR, Molecular markers, genetic variability, dendrogram

## INTRODUCTION

Wheat is one of the leading cereals in the world and the most important human food crop. Genetic diversity among wheat genotypes is useful for genetic development of new varieties. The maintenance of variation is an important goal in breeding programs and the assessment of genetic diversity is the first step for efficient management of genetic resources. Genetic variation could be evaluated by several methods, among which DNA markers are more efficient and reliable. Inter-simple-sequence-repeat (ISSR) marker system is one of the best choices to detect the genetic variation and polymorphism in higher plants (Zietkiewicz *et al.*, 1994; Nagaoka and Ogihara, 1997).

The ISSR molecular markers are semi-arbitrary in which the 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). Najaphy *et al*., (2012) reported that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic variability among wheat genotypes. Several studies revealed that ISSR markers could be efficiently used to evaluate genetic variation and establishing genetic relationship in wheat genotypes (Sofalian *et al.*, 2009 and El - Assal and Gaber, 2012).

Chowdhury *et al.*, (2008) used ISSR markers for fingerprinting in a set of 27 Indian bread wheat varieties and found that the cluster analysis based on molecular data is in agreement with their known origin. Abou-Deif *et al.* (2013) analyzed the genomic DNA of 20 wheat genotypes using ISSR markers. The dendrogram succeeded in distinguishing most of the 20 varieties in relation to their genetic background and geographical origin. Recently, Zamanianfard *et al*. (2015) evaluated the molecular diversity of 25 durum wheat genotypes using 11 ISSR primers. The average of polymorphism information content index (PIC) was 0.31, indicating the efficiency of the markers in discrimination of the populations. They found that the cluster analysis based on UPGMA algorithm and Dice similarity coefficient classified the 25 genotypes into four separated groups. The present investigation aimed to study the efficiency of ISSR markers to measure the relationships between 8 durum and 65 bread wheat genotypes.

## MATERIALS AND METHODS

**Materials:** A total of 73 genotypes including 65 hexaploid (*Triticum aestivum* L.) and 8 tetraploid

(*Triticum durum* L) wheat varieties were used in this study (Table 1).

**A. DNA extraction**

Genomic DNA was extracted from young leaves of 2-weeks-old seedlings, bulked from 5 different plants per genotype following the CTAB procedure described by Murray and Thompson (1980) with some modifications. The quantity and quality of genomic DNA was tested by the spectrophotometer and agarose gel electrophoresis.

**B. PCR amplification and Electrophoresis**

Five ISSR primers (Table 2), obtained from (metabion international AG), were used to amplify the DNA. The ISSR-PCR method was carried out, according to Nagaoka and Ogihara (1997). Amplification reactions were carried out in 25µL volumes, containing (11.0 µl $dH_2O$, 3 µl of 10x buffer, 3.0 µl of dNTPs (2.5mM ) 4 µL of Mg Cl2 (25 m M), 3.0 µL primer (2.5 µ L ) ,0.3 µl of Taq polymerase( 5U/ µL) and 2.0 µL of genomic DNA (50 ng/ µL). Amplification was performed in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K.). Programmed for an initial denaturation at 94°C 5 min, 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 40°C and 2 min extension at 72°C followed by final extension for 10 min at 72°C. The amplified products were separated on 1.5% agarose gel in TBE buffer. The DNA bands were visualized by staining the gels with ethidium bromide and photographed under UV light using gel documentation system.

**C. Band scoring and data analysis**

Banding pattern of the ISSR markers in the individuals were scored as presence (1) and absence (0) of the band. The pairwise comparisons between the tested genotypes were used to calculate the coefficient of genetic similarity matrix (Gs) according to Nei and Li (1979). The similarity matrix was subjected to cluster analysis using un weighted pair group method with arithmetic means (UPGMA) clustering procedure and a dendrogram was generated using the software package MVSP (Multi-Variate Statistical Package).

To evaluate the efficiency of selected primers for investigation of genetic diversity, the polymorphism information content (PIC) was calculated as: [PIC = 1- Σ $fi^2$] where, *fi* is the frequency of the $i^{th}$ allele (Weir 1990). The Resolving power (Rp) of each primer was calculated according to Prevost and Wilkinson (1999) as: Rp = ΣIb where, Ib = band informativeness, Ib = 1- [2×(0.5-p) ] and p = total number of bands present.

## RESULTS AND DISCUSSION

Inter-simple sequence repeats (ISSR) using primers based on di-, tetra- or penta-nucleotide repeats have now become a routine among the researchers (Zietkiewicz *et al.,* 1994). Since ISSR markers is simple procedure, low-cost, good stability and high reproducibility, highly polymorphic, highly informative and quick, it has been successfully used in genetic mapping (Najaphy *et al.,* 2012 and Sadigova *et al.,* 2014).

The five ISSR primers generated a total of 63 bands with an average of 12.6 bands per primer. The size of bands ranged from 170 bp (ISSR-844) to 972 bp (HB13) (Figs. 1&2 and Table 3). Primer ISSR-844 amplified the maximum of 15 bands, while the minimum of 10 bands were amplified by the primer HB15. The genotypes (G29) displayed the higher number of DNA fragments (49 bands) followed by G28 (48 bands), G7, G18 and G57 (46 bands, each), while G55 revealed the least number of amplified bands (37 bands) (Table 3). These variation in number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome (Kernodle *et al.,* 1993).

The maximum number of polymorphic bands (11) was obtained from primer HB12 while minimum number (6 bands) was obtained by HB15 with an average of 8.2 per primer. Polymorphism percentage ranged from 53.33 % (ISSR-844) to 78.57 % (HB12) with an average of 64.95 % (Table 2). A significant positive correlation (0.61, $p<0.01$) was observed between the total number of bands and the number of polymorphic bands amplified by five ISSR primers.

The high level of polymorphism among the wheat genotypes indicating the high efficiency of ISSR markers to reveal genetic diversity in the case of these genotypes. These results are in agreement with those obtained by Mehdiabadi *et al.* (2015)

who found high percentage of ISSR polymorphism among 16 advanced lines of durum wheat. Najaphy *et al.* (2012) reported that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes.

**Table 1:** Pedigree of tetraploid and hexaploid wheat genotypes used in the study.

| Code | Name | Pedigree | Code | Name | Pedigree | Code | Name | Pedigree |
|---|---|---|---|---|---|---|---|---|
| G1 | SOHAG 1 | GDOVZ469/JO//61.130/LDS | G26 | Nour | selected early maturing inbred line (F14) derived from a cross between Shenap*Sakha69 | G51 | ICR-35 | ICARDA |
| G2 | SOHAG 3 | MEXICALI/MAGHREBI 72//51792/DURUM#6 | G27 | 1x15 | Advanced breeding line derived from inter population-interenvironmental cross between early segregates selected in two contrasting enviroments | G52 | ICR-39 | ICARDA |
| G3 | BANI SEWEF 1 | JO"S"/AA"S"/FG"S" | G28 | Line 6 | Advanced long spike, short statured inbred line derived from a cross between two landraces collected from dry areas in Upper Egypt (Omara, 1994) | G53 | ICR-DH18 | ICARDA |
| G4 | BANI SUEF 5 | DIPPER-2/ BUCHEN-3 | G29 | L.S.15 (Long spike 15) | An advanced long-spike inbred line (F14) derived from a cross among landraces collected from stress areas in Upper Egypt (Omara,1994) | G54 | ICR-49 | ICARDA |
| G5 | SVEVO | Cimmyt's Line / Zenit | G30 | SIDS 1 | HD2173/PAVON"S"//1158.57/MAYA 74 "S" | G55 | HD-38 | ICARDA |
| G6 | Ciccico | APPULO/VALNOVA(F6)//VALFORTE/PATRIZIO(F5) | G31 | SIDS 4 | MAYA"S"/MON"S"//CMH74A.592/3/GIZA 157*2 | G56 | Madden | Gamenya//Gabo*3/Khapstein |
| G7 | WK-12 | The landraces were originally collected from farmers' fields near Dandara Temple at Qena Governorate in 1993 (Omara, 1994) and were grown since then every year in order to ascertain the stability of the black glume character. | G32 | SIDS 12 | BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.63014*SX | G57 | ALTAISKAYA 50 | Unknown |
| G8 | LOCAL SBW | unknown | G33 | Lerma Rojo 64 | ((Yaqui 50*(Norin 10*Brevor))*Lerma 52)*(Lerma Rojo)2 | G58 | HONG MANG MAI | OROFEN/DIZIUZHAO |
| G9 | GIZA 157 | GIZA155//PIT62/LR64/3/TZPP/KNOTT | G34 | TOBARI-66 | TEZANOS-PINTOS-PRECOZ/SONORA-64-A[114][144][145][39][1765]; | G59 | SAHEL 1 | NS732/PIMA//VEE#5 |
| G10 | GIZA 160 | CHENAB70/GIZA155 | G35 | SALAMBO | PATO//CC/INIA | G60 | Ejaseed-3 | Unknown |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 80 | | | | |
| G11 | GIZA 163 | F61.70/BON//CNO67/7C | G36 | BACANORA T 88 | JUP/BJY//URES | G61 | CONDOR | WW15*2/3/PJ/GB56//TZPP/NAI60 |
| G12 | GIZA 164 | KVZ/BUHO//KAL/BB | G37 | Sonora 64 | YAKTANA-54//NORIN-10/BREVOR/3/2*YAQUI-54 | G62 | MIRONOVCHANKA | WEIHENSTEPHEN M./MIRONOV-YA 808 |
| G13 | GIZA 165 | CIANO F 67/MARISFUNDIN//MONCHO | G38 | DEBEIRA | HD2160/5/TOB/CNO67//BB/3/NAI60*2//TT/SN64/4/HD1954 | G63 | NASMA | BT1149/BT2511 or BT1149//Florence/Aurore C |
| G14 | GIZA 168 | MIL/BUC//SERI | G39 | EL NIELAIN | S948.A1/7*SANTA ELENA | G64 | GOUMRIA-15 | ICW94-0029-0L-6AP-3AP-2AP-0APS-0AP |
| G15 | SAKHA 8 | CNO67//SN64/KLRE/3/8156 | G40 | MEXIPAK65 | PENJAMO62/GABO55 | G65 | Arrehane | L222 (KLDN) |
| G16 | SAKHA 69 | INIA/RL4220//7C/3/YR | G41 | PAVON F 76 | VCM//CNO/7C/3/KAL/BB | G66 | Saada | BUTTE//BUTTE/ARTHUR71 |
| G17 | SAKHA 92 | NAPO//INIA/WREN | G42 | KBG-01 | 300-SM-501-M/HAR-1709 | G67 | Marchouch | KAL/CNO//2*8156/3/BT908 |
| G18 | SAKHA 93 | SAKHA 92/TR 810328 | G43 | CHAM 4 | FLK/HORK | G68 | HUBARA-5 | Unknown |
| G19 | SAKHA 94 | OPATA/RAYON/3/JUP/BJY//URES | G44 | CHAM 6 | W3918A/JUP | G69 | BerKum | Unknown |
| G20 | GEMMIZA 7 | CMH74.630/5X//SERI82/3/AGENT | G45 | GHAM-8 | ICARDA | G70 | Canada-462 | Unknown |
| G21 | GEMMIZA 9 | ALD'S'/HUAC'S'//CMH74.630/5X | G46 | Reyne 28 | ICARDA ( CIMMYT breeding line) | G71 | Canada-515 | Unknown |
| G22 | Gemmieza 10 | Maya 74 | G47 | Aguilal | SAIS*2/KS85241-14 | G72 | GURAB-2 | ICARDA |
| G23 | Shandaweel1 | Site//Mo/4/Nac/Th.Ac./3*Pvn/3/Mirlo/Buc | G48 | ATTILA | ND/VG9144//KAL/BB/3/YACO/4/VEE#5 | G73 | GK BENCE | ATR/SAVA//LIB |
| G24 | Misr 1 | OASIS/SKAUZ//4*BCN/3/2*PASTOR | G49 | ICR-DH | ICARDA | | | |
| G25 | US3-2 (LIRASA 92 ) | KVZ/TRM//PTM/ANA | G50 | ICR-33 | ICARDA | | | |

.**Table 2.** The codes and sequence of ISSR primers used for amplification with the number of Total bands (TB), polymorphic bands (PB), percentage of polymorphism (PP), polymorphism information content (PIC) and resolving power (RP) for each primer.

| Primer code | Primer sequence | TB | PB | PP | PIC | RP |
|---|---|---|---|---|---|---|
| **HB12** | 5'-GTGGTGGTGGC -3' | 14 | 11 | 78.57 | 0.10 | 6.25 |
| **HB13** | 5'-CTCTCTCTCTCTCTCTAG -3' | 11 | 7 | 63.64 | 0.07 | 5.02 |
| **844** | 5'-GAGGAGGAGGC -3' | 15 | 8 | 53.33 | 0.15 | 5.79 |
| **814** | 5'-CTCTCTCTCTCTCTCTTG -3' | 13 | 9 | 69.23 | 0.18 | 5.01 |
| **HB15** | 5'-CACCACCACGC-3' | 10 | 6 | 60.00 | 0.05 | 4.71 |
| **Average** | | 12.6 | 8.2 | 64.95 | 0.11 | 5.36 |

It is generally reported that polymorphism between cultivars can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming site too distant to support amplification and insertions or deletions that change the size of the amplified product (Powell *et al.,* 1996). Polymorphism also considered as a useful selection tool in monitoring alien genome introgression in wheat breeding programs

**Primers differentiating bread and durum wheat genotypes:**

One DNA fragment at molecular size (308bp) generated by HB12 primer was present only in durum wheat genotypes, whereas absent in bread wheat genotypes. In contrast, the DNA fragment 855bp generated by ISSR-814 primer was present

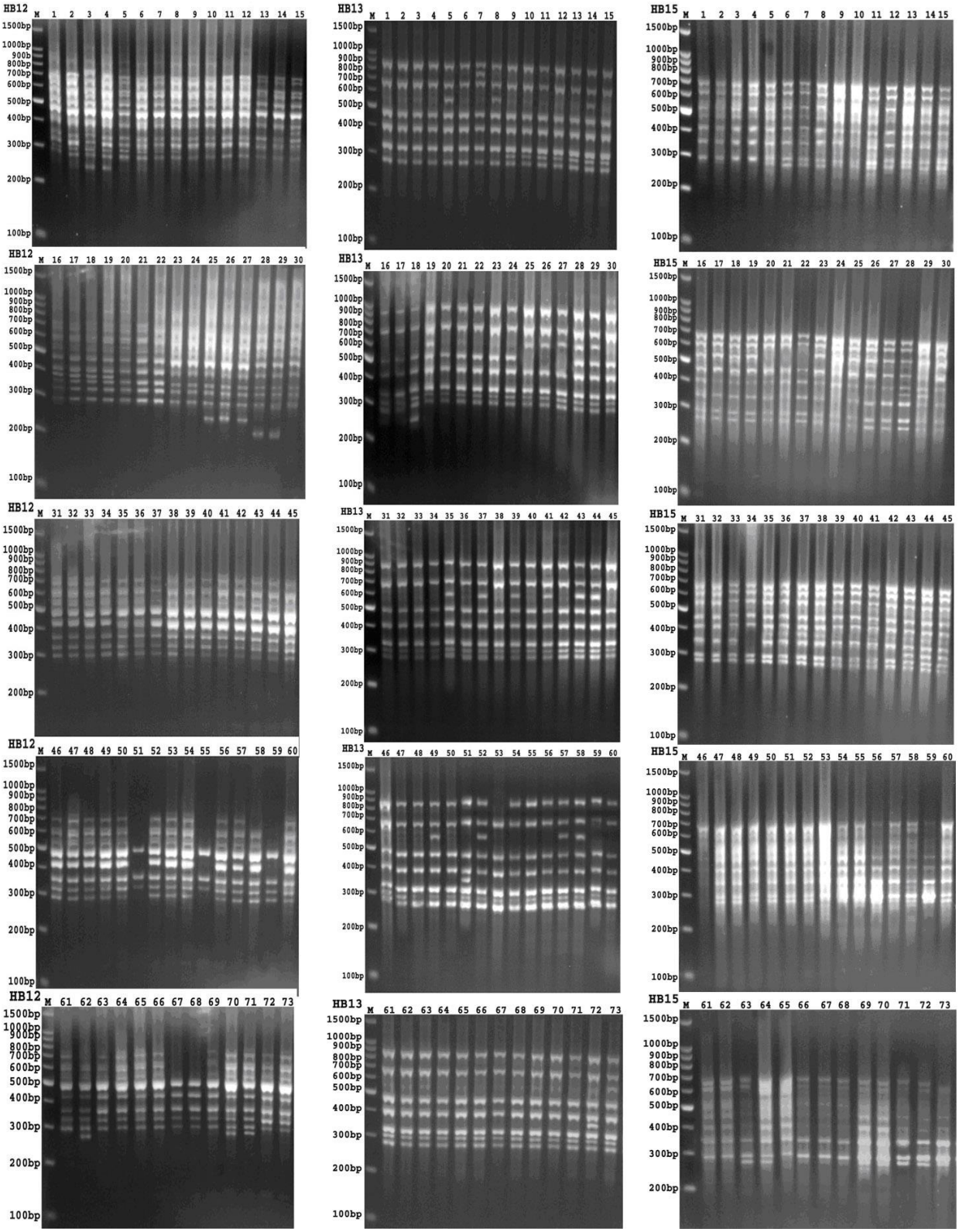


**Fig. 1:** ISSR banding profile of 8 durum (lanes 1 to 8) and 65 bread (lanes 9 to 73) wheat genotypes amplified with the primer HB12, HB13 and HB15. Lane M represented the DNA ladder.

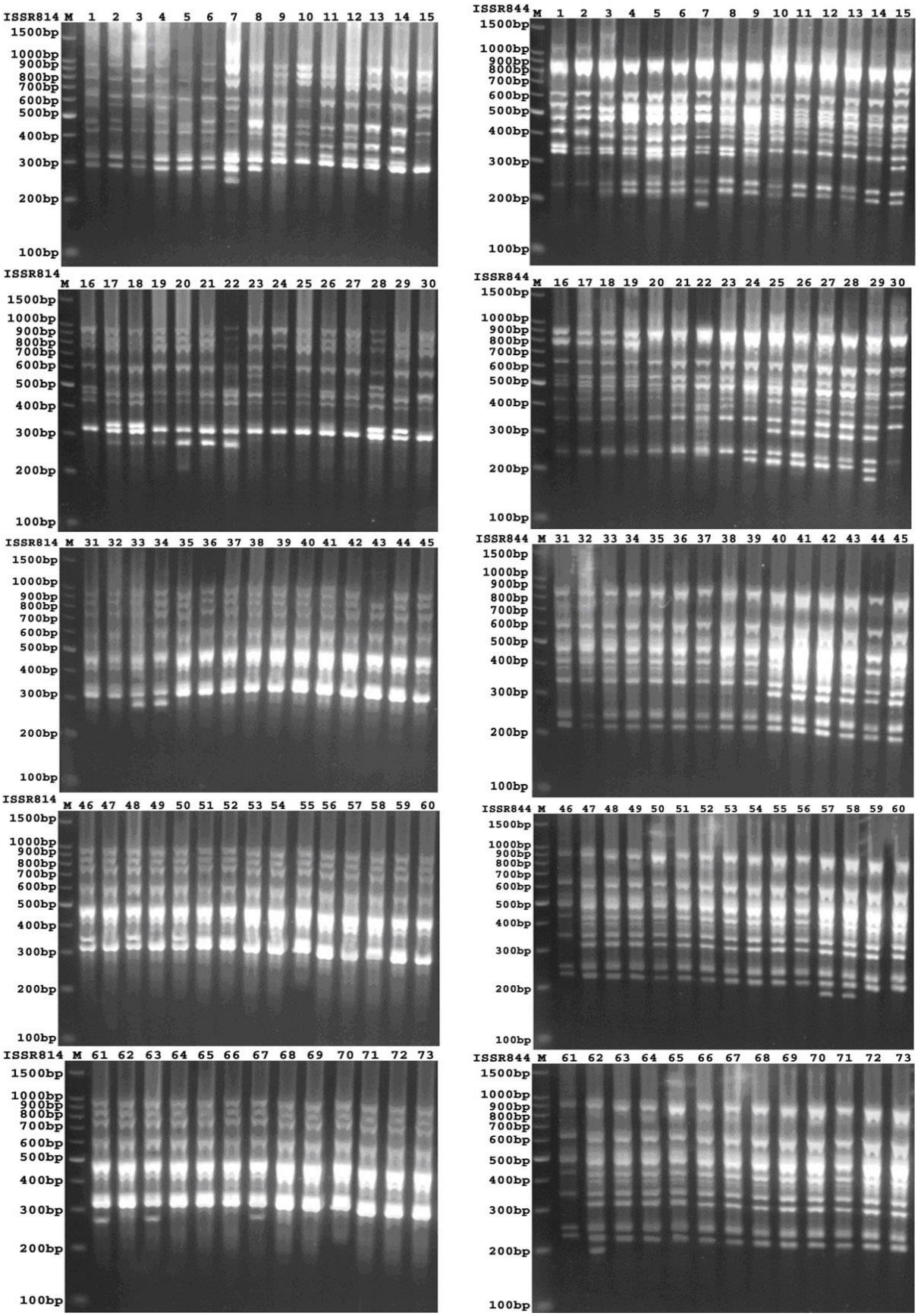


**Fig. 2:** ISSR banding profile of 8 durum (lanes 1 to 8) and 65 bread (lanes 9 to 73) wheat genotypes amplified with the primer ISSR814 and ISSR844. Lane M represented the DNA ladder.

**Table 3:** Number of amplified DNA-fragments in seventy-three wheat genotypes investigated with five ISSR primers.

| Genotypes | HB12 | HB13 | 844 | 814 | HB15 | TOTAL | Genotypes | HB12 | HB13 | 844 | 814 | HB15 | TOTAL | Genotypes | HB12 | HB13 | 844 | 814 | HB15 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 11 | 6 | 9 | 8 | 7 | 41 | **26** | 11 | 7 | 11 | 8 | 8 | 45 | **51** | 3 | 8 | 12 | 7 | 8 | 38 |
| **2** | 11 | 6 | 9 | 8 | 7 | 41 | **27** | 11 | 7 | 11 | 7 | 8 | 44 | **52** | 10 | 8 | 12 | 7 | 8 | 45 |
| **3** | 12 | 6 | 10 | 8 | 7 | 43 | **28** | 11 | 8 | 11 | 9 | 9 | 48 | **53** | 10 | 7 | 12 | 7 | 8 | 44 |
| **4** | 12 | 6 | 9 | 8 | 7 | 42 | **29** | 11 | 8 | 12 | 9 | 9 | 49 | **54** | 10 | 7 | 12 | 7 | 8 | 44 |
| **5** | 11 | 7 | 9 | 7 | 8 | 42 | **30** | 10 | 7 | 8 | 7 | 8 | 40 | **55** | 3 | 7 | 12 | 7 | 8 | 37 |
| **6** | 11 | 6 | 9 | 7 | 8 | 41 | **31** | 10 | 7 | 9 | 7 | 8 | 41 | **56** | 10 | 7 | 12 | 7 | 8 | 44 |
| **7** | 11 | 7 | 10 | 10 | 8 | 46 | **32** | 10 | 7 | 9 | 7 | 8 | 41 | **57** | 10 | 8 | 13 | 7 | 8 | 46 |
| **8** | 11 | 8 | 10 | 7 | 8 | 44 | **33** | 10 | 7 | 9 | 8 | 8 | 42 | **58** | 9 | 8 | 13 | 7 | 8 | 45 |
| **9** | 10 | 7 | 9 | 9 | 8 | 43 | **34** | 10 | 7 | 9 | 8 | 8 | 42 | **59** | 4 | 7 | 12 | 7 | 8 | 38 |
| **10** | 10 | 7 | 9 | 8 | 8 | 42 | **35** | 10 | 8 | 9 | 7 | 8 | 42 | **60** | 10 | 7 | 12 | 7 | 8 | 44 |
| **11** | 10 | 7 | 9 | 9 | 8 | 43 | **36** | 10 | 7 | 9 | 7 | 8 | 41 | **61** | 10 | 7 | 12 | 8 | 7 | 44 |
| **12** | 10 | 7 | 9 | 9 | 8 | 43 | **37** | 10 | 8 | 9 | 7 | 8 | 42 | **62** | 10 | 7 | 13 | 7 | 7 | 44 |
| **13** | 10 | 7 | 9 | 9 | 8 | 43 | **38** | 10 | 7 | 9 | 7 | 8 | 41 | **63** | 10 | 7 | 12 | 8 | 8 | 45 |
| **14** | 10 | 8 | 9 | 9 | 8 | 44 | **39** | 10 | 8 | 9 | 7 | 8 | 42 | **64** | 10 | 7 | 12 | 7 | 8 | 44 |
| **15** | 11 | 7 | 11 | 8 | 8 | 45 | **40** | 10 | 7 | 10 | 7 | 8 | 42 | **65** | 10 | 7 | 12 | 7 | 7 | 43 |
| **16** | 11 | 7 | 10 | 8 | 8 | 44 | **41** | 10 | 8 | 10 | 7 | 8 | 43 | **66** | 10 | 7 | 12 | 7 | 7 | 43 |
| **17** | 11 | 7 | 10 | 9 | 8 | 45 | **42** | 10 | 7 | 10 | 7 | 8 | 42 | **67** | 5 | 7 | 12 | 8 | 7 | 39 |
| **18** | 11 | 8 | 10 | 9 | 8 | 46 | **43** | 10 | 8 | 10 | 7 | 9 | 44 | **68** | 5 | 7 | 12 | 7 | 7 | 38 |
| **19** | 11 | 8 | 10 | 8 | 8 | 45 | **44** | 10 | 8 | 11 | 7 | 9 | 45 | **69** | 10 | 7 | 12 | 7 | 8 | 44 |
| **20** | 10 | 7 | 8 | 8 | 7 | 40 | **45** | 10 | 7 | 10 | 7 | 9 | 43 | **70** | 11 | 7 | 12 | 7 | 8 | 45 |
| **21** | 10 | 7 | 9 | 9 | 7 | 42 | **46** | 10 | 7 | 12 | 8 | 7 | 44 | **71** | 11 | 7 | 12 | 7 | 8 | 45 |
| **22** | 10 | 7 | 10 | 9 | 7 | 43 | **47** | 10 | 7 | 12 | 7 | 8 | 44 | **72** | 10 | 8 | 12 | 7 | 8 | 45 |
| **23** | 10 | 7 | 9 | 8 | 8 | 42 | **48** | 10 | 7 | 12 | 8 | 8 | 45 | **73** | 10 | 8 | 12 | 7 | 7 | 44 |
| **24** | 10 | 7 | 9 | 7 | 8 | 41 | **49** | 10 | 8 | 12 | 7 | 8 | 45 | | | | | | | |
| **25** | 11 | 7 | 10 | 7 | 8 | 43 | **50** | 10 | 7 | 12 | 8 | 8 | 45 | | | | | | | |
| **TOTAL** | | | | | | | | | | | | | | | **721** | **524** | **769** | **554** | **578** | **3146** |
| **Average** | | | | | | | | | | | | | | | **9.88** | **7.18** | **10.53** | **7.59** | **7.92** | **43.10** |

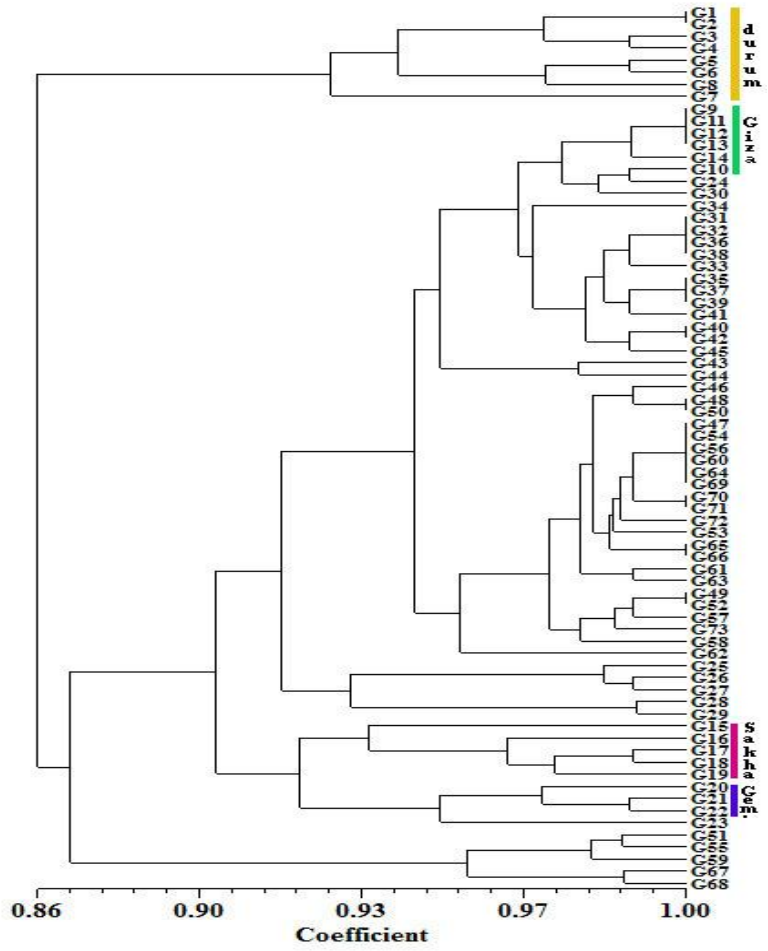


**Fig. (3):** Dendrogram of 73 wheat genotypes developed from ISSR data using UPGMA analysis. The scale is based on Dice coefficients of similarity.

only in bread wheat genotypes, whereas absent in durum wheat genotypes. These fragments generated by HB12 and ISSR-814 could be used as molecular markers to differentiate between bread and durum wheat genotypes. These results were in agreement with Abou-Deif *et al* (2013).

**Genotype Specific Primers:**

The presence of a unique band for a given genotype is referred as positive marker while the absence of a common band served as negative marker. Such bands could be used as DNA markers for genotype identification and discrimination. In this respect, two DNA fragments in G7 [877bp (HB13) and 249bp (ISSR-814)] and one band in G18 [249bp (HB13)] were positive unique markers. While, specific negative markers were recorded for G53 [972bp (HB13)], G43 [954bp (ISSR-814)], G73 [689bp (HB15)] and G34 [340bp (HB15)] (Figs. 1&2). Unique bands have also been observed by Najaphy *et al.* (2012) and Abou-Deif *et al.* (2013).

**Marker performance: -**

The information on the genetic profile of each genotype obtained by the five ISSR primers were used to assess the marker performance through evaluation of three parameters: polymorphic information content (PIC) and resolving power (RP).

**a) Polymorphism information content (PIC)**

The PIC values varied among the five primers from 0.05 in HB15 to 0.18 in ISSR-814 with an average of 0.11(Table 2). Since the maximum value of PIC for dominant markers such as ISSRs is 0.5, the low PIC values indicated the wide differences between the p and q alleles tested by the ISSR markers. Similar results have been reported by Ghobadi *et al.* (2014); Khavarinejad (2014); khaled and Hamam (2015) and Razmjoo *et al.* (2015).

**b) Resolving power (RP)**

The resolving power (RP) is a parameter that indicates the discriminatory potential of the primers chosen. The estimates of RP ranged from 4.71in HB15 to 6.25 in HB12 with an average of 5.36 per primer (Table 2). Prevost and Wilkinson (1999), reported that the RP index provides a moderately accurate estimate of the number of genotypes identified by a primer. Two of the ISSR primers (HB12 and ISSR-844) possessed high RP values (6.25 and 5.79, respectively) and therefore seem to be the most informative primers for distinguishing the genotypes. The resolving power provides information on the ability of a primer to reflect the genetic or taxonomic relationships of a group of genotypes under study.

**Cluster analysis:**

The dendrogram (Fig. 3) grouped the 73 wheat genotypes according to their ploidy level into two main clusters with over all 0.86 genetic similarity (Gs). Cluster 1 including the durum wheat genotypes while cluster 2 including all bread wheat genotypes.

The dendrogram also reflected the differences in genetic diversity within the two ploidy levels and discriminated all genotypes. The durum wheat was divided at 0.93 Gs into two sub-clusters, the first contains G1, G2, G3 and G4 while, the remaining four genotypes were found in the second sub-cluster. The bread wheat genotypes cluster was divided at 0.87Gs into four sub-clusters in which the first contains the genotypes G51, G55, G59, G67 and G68 with common 0.95Gs.

The second sub-cluster contained nine genotypes, five of them (G15, G16, G17, G18 and G19) were produced by Sakha Research Station, and three genotypes (G20, G21 and G22) were collected from Gemmaiza Research Station, while G23 collected from Shandaweel Research Station. Similarly, wheat varieties obtained from Giza Research Station (G9 – G14) were also grouped together in the same sub-cluster. The third sub-cluster included five genotypes (G25, G26, G27, G28 and G29) with 0.93Gs. The fourth sub-cluster comprised all remaining wheat genotypes which represent the genotypes collected from different geographical regions. Powell *et al.* (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured.

The dendrogram indicated that the ISSR markers succeeded in distinguishing the tested varieties in relation to their ploidy level and location, which the tetraploid varieties were put together in one group as well as the hexaploid varieties. Sofalian *et al.* (2008) also reported that ISSR markers are efficient tools for estimating intra-specific genetic diversity in wheat and these molecular markers could differentiate the local varieties obtained from different locations. Similarly, Carvalho *et al.* (2009) analyzed 48 wheat cultivars by ISSR markers and found that most cultivars belonging to the same botanical variety were clustered in the same main group. Malik *et al.* (2010) reported that the cluster analysis placed the 27 wheat genotypes in six groups in agreement with their known origin.

**REFERENCES**

Abou-Deif, M.H.; M.A. Rashed; M.A.A. Sallam; E.A.H. Mostafa and W.A. Ramadan 2013. Characterization of twenty wheat varieties by ISSR markers. Middle-East Journal of Scientific Research, 15 (2): 168-175.

Carvalho, A.; H. Guedes-pinto and J. Lima-brito. 2009. Genetic diversity among Old Portuguese bread wheat cultivars and botanical varieties evaluated by ITS rDNA PCR-RFLP markers. J Genet., 88: 363-367.

Chowdhury, R.M.V.K., S.J.S. Kundu and R.K. Jain. 2008. Applicability of ISSR markers for genetic diversity evaluation in Indian bread wheat genotypes of known origin. Environ. Ecol., 26: 126-131.

El-Assal, S. and A. Gaber. 2012. Discrimination capacity of RAPD, ISSR and SSR markers and of their effectiveness in establishing genetic relationship and diversity among egyptian and saudi wheat cultivars. American Journal of Applied Sciences, 9(5): 724-735.

Ghobadi, G.; R. Mohammadi; A. Etminan and L. Shooshtari. 2014. Assessment of Genetic Relationships among Durum wheat Genotypes based on Molecular and Morphological Markers. Advances in Environmental Biology, 8(21): 60-65.

Kernodle, S.P.; R.E. Cannon and J.G. Scandalios. 1993. Concentration of primer and template qualitatively affects product in RAPD-PCR. Biotechniques, 1: 362-364.

Khaled, A. G. A. and K. A. Hamam. 2015. Association of molecular markers with phenotypic traits of bread wheat genotypes. Egypt. J. Genet. Cytol., 44:115-130.

Khavarinejad, M.S. 2014. Comparison of Obtained Wheat Genetic Divergence by Molecular and Morphological Analysis Using Cluster. Scientia Agriculturae, 6 (3): 107-113.

Malik, R.; S. Sareen; S. Kundu and J. Shoran. 2010. The use of SSR and ISSR markers for assessing DNA polymorphism and genetic diversity among Indian. bread wheat cultivars. Prog. Agric., 12: 82-89.

Mehdiabadi, S.; R. Mohammadi; A. R. Etminan and L. Shooshtari. 2015. Evaluation of Genetic Diversity in Durum Wheat Advanced Lines. Biological Forum – An International Journal, 7(1): 236-240.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., 8: 4321-4325.

Nagaoka, T. and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and Applied Genetics, 94: 597-602.

Najaphy, A.; R.A. Parchin and E. Farshadfar. 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Australian Journal of Crop Science, 6(2): 326-332.

Nei, M. and W.H. Li. 1979. Mathematical model for studying genetic variations in terms of restriction endonucleases. Proc. Nat. Acad. Sci., 76: 5269-5273.

Powell, W.; M. Morgante; C. Andre; M. Hanafey; J. Vogel; S. Tingey and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Prevost, A. and M.J. Wilkinson. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics, 98: 107—112.

Razmjoo, M.; R. Mohammadi and L. Shooshtari. 2015. Evaluation of genetic diversity in durum wheat genotypes (*Triticum turgidum* var. durum) using ISSR markers. Journal of Biodiversity and Environmental Sciences, 6(1): 522-529.

Sadigova, S.; H. Sadigov; R. Eshghi; S. Salayeva and J. Ojaghi. 2014. Application of RAPD and ISSR markers to analyses molecular relationships in azerbaijan wheat accessions (*Triticum aestivum* L.). Bulgarian Journal of Agricultural Science, 20(1): 87-95.

Sofalian, O.; N. Chaparzadeh and M. Dolati. 2009. Genetic diversity in spring wheat landraces from Northwest of Iran assessed by ISSR markers. Not Bot Horti Agrobo., 37(2): 252-256.

Sofalian, O.; N. Chaparzadeh; A. Javanmard and M.S. Hejazi. 2008. Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. International Journal of Agricultural and Biological Engineering, 10: 466-468.

Weir, B. 1990. Genetic data analysis: Methods for discrete population genetic data. Sinauer Assoc. Sunderland, MA.

Zamanianfard, Z.; A.R. Mohammadi and L. Shooshtari. 2015. Evaluation of Molecular Diversity of durum wheat genotypes using ISSR markers. Biological Forum – An International Journal, 7(1): 214-221.

Zietkiewicz, E.; A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 20: 176-183.