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Genetic Relationship amongSome Wheat Genotypes Using Ten ISSR Markers

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Abstract: *Ten of ISSRs (inter- Simple Sequence Repeats) markers were used for revealing genetic relationship, genetic diversity and DNA fingerprint of ten bread wheat (Triticumaestivum L.) genotypes. Primers varied among them in giving unique DNA fingerprint hence primers HB12, UBC811andUBC852 gave unique fingerprint for all ten studied wheat genotypes, others varied between 2-6 fingerprint genotypes while primer 17899A gave no unique fingerprint. High genetic distance was 0.661 while low genetic distance was 0.131.Cluster analysis (Phylogenetic tree) grouped studied genotypes in to two main groups ,the first small one included only one genotype while the other large main group included the rest genotypes which further divided in to two sub groups .ISSRs represent good marker for revealing genetic diversity and genotypes fingerprinting. The results could help plant breeder in breeding programs.*

Key words*:Bread heat, ISSR markers, Geneticdiversity, Dendrogram,cluster analysis*

I. Introduction

Wheat is an annual plant belongs to the grass family Poaceae (Graminae) (Pathak and Shrivastav, 2015), which contributes more calories and proteins to the world diet than any other cereal crops (Shewry, 2007).The first step in crop improvement including wheat is complete molecular characterization of germplasm. Our Knowledge about genetic relationships and genetic diversity among breeding lines could help in strategies used for crop improvement (Abbas *et al*., 2008). Genetic diversity of plants determines their potential for improvement and their use for breeding, which eventually enhanced food production. (Khodadadi*et al*., 2011). Characterization of genotypes is accomplished by different markers including; morphological markers (phenotypic markers) which depend on the experience of the breeder to correlate a phenotypic trait with a trait of interest (Wettstein-Knowles, 1992), they are easy to detect and measure, and their relevance to germplasm users and breeders (Lombard *et al*., 2001),but there is complex genetic control of many morphological traits and they can be influenced by environmental conditions (Lombard *et al*., 2001).Biochemical markers are reliable in genetic studies and breeding of plant species because their stability in expression independently of surrounding environmental factors, ability to assess co-dominance, high reproducibility , ease of use and low costs (Kumar *et al*., 2009),but enzyme locus describes small and not randomly part of genome and very affected by methods of extraction, plants growth stages and plant tissues (Mondini*et al*., 2009) , in addition they are relatively low in their distribution and their low level of polymorphism (Krieger and Ross, 2002).

DNA markers defined as a sequence of DNA or a gene, which is situated on a chromosome (Collard *et al*., 2005andSchulmann, 2007) by which it could detect differences between individuals by showing polymorphism (Collard *et al*., 2005). DNA markers are consider very efficient in selection of plant material, not affected by the environment, their segregation as single genes. DNA is extracted easily from plant materials (Ovesna*etal*., 2002).DNA markers usefully used in identification of genetic materiales, selection of parents, detection of the progeny, characterization of the varieties for protection of both, consumers and breeders rights. (Ovesna, *etal*., 2002). Among DNA markers, inter simple sequence repeats (ISSR) markers need small quantities of DNA and not require radioactive labels, it is simple and faste.ISSRwas a powerful, reproducible, simple, rapid, and inexpensive tool to estimate genetic variation and identifying differences among closely related cultivars in many species. (Solimana*et al*.,2014) ,because breeders use common breeding material of other breeding institutes or share common parents/lines, this lead to similar parents in breeding programs which result in lack of genetic variation . (Iqbal*et al*., 1997, Rehman*et al*., 2002).It is very important to study germplasm genetic composition of modern-day cultivars and compare them with their related species and ancestors. This will provides information's about their phylogenetic relationship and produce a chance to find a new useful genes, because accessions possess distinct DNA profiles is always contain a large number of novel alleles (Messmer*et al*., 1992),so this study was conducted to evaluate wheat germplasm variation and fingerprinting studied genotypes.

II. Materials and methods

DNA extraction and PCR amplification:ten of wheat genotypes with diverse characterization and pedigree were used for DNA extraction **(table.1)**. Fresh young leaves at age of two weeks were used for DNA extraction using The Genomic DNA Mini Kit (Geneaid Biotech. Ltd; Taiwan Company). PCR reaction mixture was prepared as follows: 6µl template DNA and 3 µl of primer (10 pmole/µl), were added to each PCR Pre Mix tube and sterilized deionized distilled water was added to to the final volume of 20 µl.Ten ISSR markers were examined for fingerprinting genotypes as listed in **table .2**. Amplification was performed in thermocycler programmed according to annealing temperatures produced in **table .3.** The amplified DNA product were separated by electrophoresis on 2 % agarose gels stained with ethidium bromide (5 µl of ethidium bromide solution) electrophoresis runs for 3 hr at 70V and then visualized under UV light and photographs.Presence of a product was identified as (1) and absence was identified as (0). Data were scored for all genotypes, their amplification product and primers. The data then entered into NTSYS-PC (Numerical Taxonomy and multivariate Analysis System), Version 1.8 (Applied Biostatistics) program [19]. A Dendrogram was constructed based on genetic distance: genetic distance (GD) =1- genetic similarity (GS) using the Unweighted Pair-Group Method with Arithmetical Average (UPGMA).

Table (1) Genotypes characterization.**Table (2)** Names and sequences of ISSRs primers .

No.	Name	Pedigree
1	Furat	Foreign genetic structures 5H in F2 (second generation)
2	Baghdad	Australian strain ×Aras
3	Hashimia	A-901Y-902D-OY/LD*6/FB6628-F30069BR12*3/3BR14)
4	Buhuth22	The Ministry of Science and Technology/Iraq
5	Latifia	Australian strain×MexicanArasvareity
6	Dijla	American genetic structures 655in their F2 generation
7	Abaa 99	(Ures/Bow"s"/3/Jup/Biy"s"/urse)
8	Rasheed	Radiation of pure strain of maxi pack with Gama ray (10Krad)
9	Faris	The Ministry of Science and Technology/Iraq
10	Iraq	Radiation of pure strain of maxi pack with Gama ray

ISSRs Primers	Sequence 5' ⟶ 3'
844A	5' CTC TCT CTC TCT CTC TGC 3'
844B	5' CTC TCT CTC TCT CTA 3'
17889A	5' CAC ACA CAC ACA AC 3'
HB12	5' CAG CAGCAG GC 3'
UBC814	5'CTCTCTCTCTCTCTCTCTA3'
UBC-811	5'GAGAGAGAGAGAGAGAAC3'
UBC-881	5'GGGTGGGGTGGGGTG3'
UBC 852	5' GATAGATAGACAGACA 3'
HBS10	5' GAG AGA GAG AGA CC 3'
17899A	5' CAC ACA CAC ACA AG 3'

Table (3): ISSRs primers with their PCR amplification programs.

ISSRs annealing temperature	Step	Temperature	Time
UBC-852 (49 C°) UBC881 (52 C°) UBC814 (53 C°) 844B (37 C°) UBC811 ,844A (48 C°) HB12,HBS10 (48 C°) 17889A,17899A (48 C°)	Initial denaturation	94C°	5 min
	No. of Cycles = 30 Cycles		
	Denaturation	94C°	1min
	Annealing	Variable	1min
	Extension	72C°	2min
	Final extension	72C°	7min
Sofalian*et al* .,2008 and Abou-Deif*et al* ., 2013 with slight modification			

III. Results and Discussion

Using Bio drop apparatus the concentration of isolated DNA was 73.91µg/ml with purity 1.9,ISSRsprofile results showed variation among studied genotypes through presence of monomorphic, polymorphic and unique bands. Primers HB12, UBC811 and UBC852 gave unique fingerprint for each genotype while primer 17899A failed to give unique fingerprint, other primers ranged (2-5) in their fingerprinted genotypes.**Table 4**. results show that these three primers gave the higher value for polymorphism, HB12, UBC811 and UBC852 (64.7, 68.7 and 52.6) respectively. Polymorphism increased with increasing number of polymorphic bands (Hunter and Gaston, 1988 and Graham and McNichol, 1995), and increases chance of producing unique fingerprint. Primer which produces high polymorphic bands can be further used as polymorphic marker which will prove promising in identification and genetic purity testing of crops (Pal and Singh, 2013).Product of PCR amplification fragment shown in figures (1-10).Results in table (5) show that the higher molecular size and lower molecular size was 1717 bp, 138bp respectively in primer UBC852. Size of amplified fragments related to primer sequence annealed with DNA template. (Mahpara*et al*., 2012).Insertions and deletions could change the size of the amplified product (Powell *et al*., 1996;Fadoul*et al*.,2013) .The higher number of main bands was 19 in

UBC852 while the lower was six bands in primer 844B.The higher number of amplified bans was 142 bands in primer 17899A while lower number of amplified bans was 45 bands in primer 844B**.**Variation in number of main and amplified bands are mainly due to primer structure and that some primers recognize a high number of annealing site, which is more useful than primers recognizing lower number of annealing sites. In this case the number of amplified bands will be higher, thus giving a better chance for detecting DNA polymorphisms among individuals (Williams *et al*., 1990;Tahir, 2014).

Table (4) Wheat genotypes fingerprinting (DNA profile) using ten ISSRs primers and their sequences.

No.	Primers	Sequence (5'-3')	Varieties fingerprinting	No. of varieties fingerprint
1	844A	5' CTC TCT CTC TCT CTC TGC 3'	5,6,8	3
2	844B	5' CTC TCT CTC TCT CTA 3'	1,5	2
3	17889A	5' CAC ACA CAC ACA AC 3'	1,2,3,5,7,8	6
4	17899A	5' CAC ACA CAC ACA AG 3'	-	-
5	HB12	5' CAGCAGCAG GC 3'	1,2,3,4,5,6,7,8,9,10	10
6	HBS10	5' GAG AGA GAG AGA CC 3'	3,5,6	3
7	UBC811	5'GAGAGAGAGAGAGAGAAC3'	1,2,3,4,5,6,7,8,9,10	10
8	UBC814	5'CTCTCTCTCTCTCTCTCTA3'	1,5,8,9,10	5
9	UBC852	5' GATAGATAGACAGACA 3'	1,2,3,4,5,6,7,8,9,10	10
10	UBC881	5'GGGTGGGGTGGGGTG3'	2,3,8,6	4

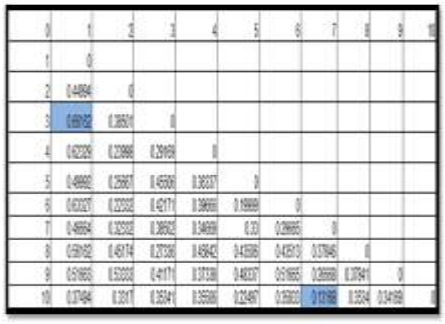
Most of chosen primers in this study were di-nucleotide motif primers, primers of dinucleotide repeat markers revealed maximum amplification which found to be more amenable in wheat and especially 3'-anchored di-nucleotide motif primers which gave clear amplified products, whereas 5'-anchored dinucleotide primers were excluded because they give either poor or no amplification in wheat genotypes as reported (Pujo*et al*, 1999). Monomorphic bands were thirteen in primer 17899A while in primer HB12 were only one band. Al-Judy (2004) reported that genome contains constant identical sequences commonly refer to as conserved sequence. Monomorphic bands are type of these sequences, which reveal that genotypes that belong to one species share some genome sequences and differ in others (Russel, *et al*., 1997; Al-Judy, 2004 and AL-Badeiry, 2013; AL-Tamimi, 2014). The higher polymorphic bands were eleven in HB12 and UBC811 primers whileprimers 844A and 844B gave only two polymorphic bands .High polymorphism observed using primers UBC811, UBC852 and HB12 while primer 17899A gave lowest polymorphism. Studies reported that primers with di-nucleotid GA sequence give low polymorphism(Akkaya*et al*.,1992; Singh and Jaiswal,2016),this was correct for primer HBS10 while primer UBC811 gave high polymorphism ,this may resulted from nucleotide sequence changes by deletion or insertions that may change level of polymorphism by changing primer annealing sites (Powell *et al*., 1996;Fadoul*et al*.,2013), others studies confirmed that GA sequence showed stable amplification and rich polymorphism (Liu *et al*., 2010;Yousefi*et al*.,2015). Polymorphism level increased by using tri- HB12 and tetra- UBC852 nucleotide sequence (Pujo*et al*, 1999).Primers sequences which composed of di- nucleotide sequence give low polymorphism while tetra- and penta-nucleotide repeat sequences gave different high level of polymorphism (Nagoaka and Ogihara, 1997; Sofalian*etal*., 2008).The higher number of unique bands was five in primers HB12 and HBS10 while primer UBC881 gave only one band. Primers 844B, 17899A and UBC814 gave no unique band. the presence of such bands refer to that primer recognized a unique annealing site in genome ,this increase chance of producing a unique cultivar fingerprint (Grewal*et al*.,2007;Vishwanath *et al*., 2010; Fadoul*et al*.,2013;AL-Tamimi,2014) .Primer 17899A gave lowest value for efficiency in contrast to primer HB12 which gave higher value. Discriminatory value in both primers HB12 and UBC811 was the highest while primers 844A and 844B gave the lowestvalue. Both efficiency and discriminatory value of primer concerned with its ability to give uniquefingerprint .(Newton and Graham, 1997; Arif et al., 2010 and AL-Badeiry, 2013; AL-Tamimi,2014),this was clearly observed in primers HB12,UBC811 and UBC852 which were high in their both efficiency and discriminatory value and they gave a unique fingerprint.In table (6) the results showed that the highest genetic distance was observed between Furat and Hashimia genotypes while lowest genetic distance was between Abaa99 and Iraq genotypes. Variation in genetic similarity may be attributed to the genealogy of the cultivars, because some of them have a common parent or not, authors emphasized that there is possibility of forming groups could be linked to the sharing of genetic material from one distant common ancestor; it would explain the genetic similarity, the presence of common ancestors might have influenced the similarity among the cultivars in the present study. (Morale *et al*., 2011).According to dendrogram produced in figure (2) there were two main clusters, the first small cluster included only Furat genotype while the other large cluster included two sub groups; the small one included the genotypes Hashimia, Rasheed and Faris, the other large one included genotypes Baghdad, Buhuth22, Latifia,Dijla,Abaa99andIraq. The evolution leading to adaptation to different agroecological conditions may

mutate the SSR sequences ,which lead to differences in the ISSR amplification pattern result in diverse phyllogenetic relationships Regardless of different geographical locations of the place of release of varieties and different ploidy levels.(Singh and Jaiswal.,2016).

Table (5) Summarized results of ISSRs amplification product include :fragment size range in bp ; No. of : main , amplified ,monomorphic , polymorphic and unique bands ; polymorphism (%) , efficiency and discriminatory value (%) of primers.

No.	Primers	Size in (bp)	main	amplified	mono	poly	unique	Polymorphism (%)	Efficiency	Discriminatory Value (%)
1	844A	160-529	9	74	5	2	2	22.22	0.027	3.846
2	844B	413-1200	6	45	4	2	-	33.33	0.0444	3.846
3	17889A	143-1482	16	118	8	4	4	25	0.0338	7.692
4	17899A	186-1492	16	142	13	3	-	18.75	0.0211	5.769
5	HB12	184-1297	17	66	1	11	5	64.705	0.1666	21.153
6	HBS10	223-1113	15	102	7	3	5	20	0.0294	5.769
7	UBC811	163-606	16	83	3	11	2	68.75	0.132	21.153
8	UBC814	152-866	10	75	6	4	-	40	0.0533	7.692
9	UBC852	138-1717	19	127	6	10	3	52.631	0.0787	19.230
10	UBC881	196-607	11	77	7	3	1	27.27	0.0389	5.769
Total no. of bands			135	909	60	53	22	-	-	-
Average bands per primer			13.5	90.9	6	5.3	2.2	-	-	-
Average per primer %			-	-	-	-	-	37.2656	0.06252	10.1919

Table (6) The genetic distance .



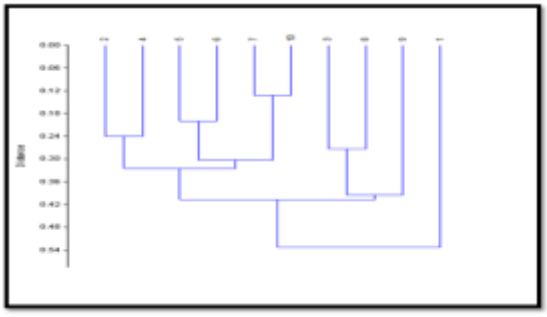
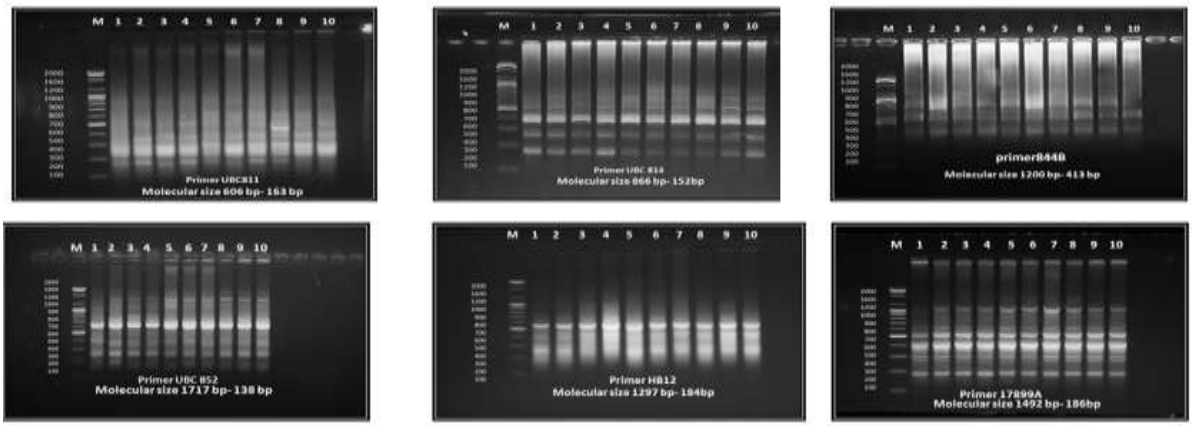


Figure (2) UPGMA dendrogram.

Conclusions:

Low genetic diversity among studied wheat genotypes revealed by ISSR markers,which indicate the narrow genetic base of wheat crop in Iraq, on the basis of results of this study.



Figures (1-10): The electrophoresis profile obtained by ISSR primers, lane M: DNA ladder and lanes 1-10: Wheat genotypes

References

[1]. Abbas, S.J.; Shah,S.R.U.; Rasool,G.andIqbal,A.(2008). Analysis of genetic diversity in Pakistani wheat varieties by using randomly amplified polymorphic DNA (RAPD) primers American-Eurasian Journal of Sustainable Agriculture, 2(1): 29-33.

[2]. Abou-Deif, M. H.; Rashed, M. A.; .Sallam, M. A. A.; Mostafa, E. A. H. and Ramadan,W.A.(2013).Characterization of Twenty Wheat Varieties by ISSR Markers. Middle-East Journal of Scientific Research 15 (2): 168-175.

[3]. Akkaya, M.S.; Bhagwat, A.A. and Cregan, P.B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. Genetics, 132: 1131–9.

[4]. Al–Badeiry , N. A.M.(2013).Molecular and Cytological Studies on Some *Zea mays* Varieties in Iraq .Phd thesis, University of Kufa ,Faculty of Science ,Department of Biology , Iraq.

[5]. Al-Judy, N.J. (2004).Detecting of DNA Fingerprints and Genetic Relationship Analysis in Local and Improved Rice (*Oryza sativa* L.)Varieties in Iraq Using RAPD Markers.Ph.D thesis, College of Science, Baghdad University, pp 166.

[6]. AL-Tamimi, A. J.T. (2014). Genetic Diversity of Some Tomato Genotypes Using RAPD and SSR markers in Iraq .PhD thesis .Faculity of science.University of kufa. p 183.

[7]. Arif, I. A.; Bakir, M. A.; Khan, H. A.; Al-Farhan, A. H.; Al-Homaidan, A. A.; Bahkali, A. H.; Al-Sadoon, M. and Shobrak, M. (2010).Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment.Genet. Mol. Res., 9 (4): 2191-2198.

[8]. Collard, B.C.Y.; Jahufer, M.Z.Z and Pang, E.C.K. (2005).An introduction to markers, quantitative trait loci (QTL) mapping and marker-assissted selection for crop improvement: The basic con-cepts.Euphytica, 142(1-2): 169-196.

[9]. Fadoul, H. E.; El Siddig, M. A.; and El Hussein, A. A. (2013).Assessment of genetic diversity among Sudanese wheat cultivars using RAPD markers. INT J CURR SCI. 6: E 51-57.

[10]. Graham, J. and McNicol, R. J. (1995). An examination of the ability of RAPD markers to determine the relationships within and between Rubus spp. Theo. Appl. Gene., 90: 1128-1132.

[11]. Grewal, A.; Kharb, P.; Malik, R.; Jain, S.and. Jain, R. K. (2007).Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers .indian Journal of Biotechnology , 6, pp 18-23.

[12]. Hunter, P. R. and Gaston, M. A. (1988). Numerical index of discriminatory ability of simpson's index of diversity. J. Clin. Mic., 26:2465-2466.

[13]. Iqbal, M.J., N. Aziz.,N.A. Saeed., Y. Zafar and K.A. Malik. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor, Appl, Genet. 94: 139-144.

[14]. Khodadadi, M.; Hhossein,F. and Miransari,M.(2011).Genetic diversity of wheat (*Triticumaestivum* L.).Genotypes based on cluster and principal componenet analysis for breeding strategies .A.J.C.S.5 (1):17-24.

[15]. Kreiger, M.; Ross, K.G. (2002).Identification of a major gene regulating complex social behavior. Science, 295:328–332.

[16]. Kumar,P.; Gupta,V.K.; Misra,A.K.andPandey,B.K.(2009).Potential of molecular markers in plant biotechnology. Plant Omics Journal.2 (4):141-162.

[17]. Liu, D.; X.He; G.Liu and B.Huang (2010). Genetic diversity and phylogenetic relationship of Tadehagi in southeast China evaluated by intersimple sequence repeat (ISSR). Genet.Resour. Crop Evol. 58: 679-687.

[18]. Lombard, V.; Dubreuil, P.; Dillmann, C. and Baril, C.P. (2001).Genetic distance estimators based on molecular data for plant registration and protection: a review. Acta Horticultura.546:55-63.

[19]. Mahpara, S. Farooq, J. Ali, Z.; Petrescu-Mag, I. V.andHussain, F. (2012).Assessment of genetic distance among wheat genotypes through RAPD markers.Advances in Agriculture &Botanics International Journal of the Bioflux Society. Volume 4 (1):31-35.

[20]. Messmer, M.M.; Melchinger,A.E.; Boppenmaier,J.; Herrmann,R.G. and Brunklaus-Jung,E. (1992). RFLP analysis of early-maturing Eurpeonmaizgermplsm.Theor.Appl.Genet. 83: 1003-1112.

[21]. Mondini,L.; Noorani,A.andPagnotta ,A.(2009). Assessing Plant Genetic Diversity by Molecular Tools. Diversity.1, 19-35.

[22]. Morale,G.F.;Resende, J. T.V.; Faria, M.V.; Andrade, M. C. ; Resende, L.V.; Delatorre, C. A. and Da Silva, P.R..(2011).Genetic similarity among strawberry cultivars assessed by RAPD and ISSR markers. Sci. Agric., 68(6):665-670.

[23]. Nagoaka, T. and Ogihara, Y. (1997).Applicability of inter- simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet., 94: 597–602.

[24]. Newton, C. R. and Graham, A. (1997).Polymerase Chain Reaction.2nd ed. Bios.Scientific Publishers Ltd., Oxford, U.K.

[25]. Ovesna, J.; Pplakova,K.andLeisova,L.(2002).DNA Analyses and their Applications in Plant Breeding.Czech J. Genet.Plant Breed. 38, 2002 (1): 29–40.

[26]. Pal, D. and Singh, M. (2013).Molecular Profiling and RAPD analysis of Commercial Hybrid Parental Lines in Tomato and Chili. IJIRSET, 2(9): 4288- 4292.

[27]. Pathak and Shrivastav, S. (2015). Biochemical studies on wheat (*Triticumaestivum* L.). Journal of Pharmacognosy and Photochemistry; 4(3): 171-175.

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

[29]. Pujor, S.; Tamhankar, S.A.; Rao, V.S.; Gupta, V.S.; Naik, S. and Ranjekar, P.K. (1999). Arbitrarily primed-PCR based diversity assessment reflects hierarchical groupings of Indian tetraploid wheat genotypes. Theor. Appl. Genet., 99: 868-876.

[30]. Rehman, M., D. Hussain and Y. Zafer.(2002). Estimation of genetic divergence among elite cotton cultivars genotype by DNA fingerprinting technology. Crop Sci., 42: 2137-2144.

[31]. Russell, J.R.; Fuller, J.D.; Macaulay, M.; Hatz, B.; Jahoor, A.; Powell, W. and Waugh, R. (1997).Direct comparison of levels of genetic variation among barley accessions detected by RFLP, AFLPs, SSRs and RAPDs.Theor. Appl. Genet. 95: 714-722.

[32]. Schulmann, A.H. (2007).Molecular markers to assess genetic diversity.Euphytica, 158(3): 313-321.

[33]. Singh, P. and Jaiswal J.P. (2016).Assessing genetic diversity in bread wheat using inter simple sequence repeat (ISSR) markers. Asian Journal ofn bio science.11 (1):141-145.

[34]. Sofalian, O.; Chaparzadeh,N. and Dolati, M.(2008).Genetic diversity in spring wheat landraces from northwest of Iran assessed by ISSR markers.Notul. Bot. Hort. Agric., Cluj-Napoca, 37: 252-256.

[35]. Solimana, M. I. ; Zaghloul, M. S.; Heikal, Y. M. (2014). Genetic variation within and among three Egyptian esembryanthemum species using different genetic markers, Egyptian journal of basic and applied sciences 1, 127-135.

[36]. Tahir, N. A. (2014).Genetic Variability Evaluation A mong Iraqi Rice (*Oryza sativa* L) Varieties using RAPD Markers and Protein Profiling. Jordan Journal of Biological Sciences, 7(1): 13 – 18.

[37]. Vishwanath, K.; Prasanna, K. P. R., Pallvi, H. M.; Rajendra P.; Ramegowda, S. and Devaraju, P. J. (2010). Identification of Tomato (*Lycopersiconesculentum*) Varieties through Total Soluble Seed Proteins Research Journal of Agricultural Sciences, 2(1): 08-12.

[38]. Wettstein-Knowles, P. v (1992).Cloned and mapped genes: Current status. pp. 73-98. In P. R. Shewry, (ed.), Barley: In: Genetics, Biochemistry, Molecular Biology and Bio¬technology. Alden Press, Oxford.

[39]. Williams, J. G.; Kubelik, A. R.; Livak, K. J.; Rafaski, J. A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18 (22):6531-6535.

[40]. Yousefi, V.; Najaphy, A.; Zebarjadi, A. and Safari, H. (2015).Molecular characterization of Thymus species using ISSR markers. Journal of Animal & Plant Sciences, 25(4): 1087-1094.