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Use of SSR Markers for Characterizing Cultivated Durum Wheat and Its Naturally Occurring Hybrids with Wild Wheat

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ABSTRACT

Eleven SSR primers were used to analyze 56 accessions of durum wheat consisting of 29 wheat-like accessions, 12 black glumes white awned, 12 white glumes black awned accessions and three local landraces in order to assess natural hybridization of cultivated wheat with its wild progenitor *Triticum turgidum* ssp. *dicoccoides* and to search for evidence of the introgression of wild relatives genes into durum wheat. A total of 738 bands were scored across the accessions. Of the 24 genomic loci totally assayed, 20 (83%) were found to be polymorphic and the remaining four monomorphic. The white glumes black awned accessions were completely separated in one cluster, whereas the black glumes white awned were grouped with wheat-like accessions in the main and its sub-clusters using UPGMA analysis. A total of 560 markers were scored for both wheat and black glumes white awned accessions in which 70% of genomic loci showed to be polymorphic. This high occurrence of polymorphism was most likely a consequence of introgressive hybridization involving emmer wheat. The present study demonstrated the efficiency of SSR technology for polymorphism detection and introgression assessment in wheat, thereby offering wide scope of applications in marker aided breeding programs of wheat.

Keywords*:* Durum Wheat, *T. dicoccoides*, Natural Hybridization, SSR Technique, Gene Flow.

1. INTRODUCTION

Wheat wild relatives and landraces harbor a high level of genetic diversity for important economical traits needed in breeding programs. Gene introgression is one of the factors that play a significant role in affecting genetic diversity of natural populations of crop species. *Triticum turgidum* ssp. *dicoccoides* (genomic constitution AABB) is distributed all over the Fertile Crescent in the Middle East, but its center of distribution is in the Upper Jordan Valley and its surroundings. It grows mainly on *basaltic* and *terra rossa* soil types and often grows together with wild barley and wild oats (Nevo and Beiles, 1989).

Introgression has been defined as "infiltration of genes of one species into another through repeated back crossing of hybrids to the parental species" (Ladizinsky, 1978; IPGRI, 2000). Introgression of desirable traits into crop cultivars as a part of breeding program is known. The extent and significance of natural introgression or farmer-assisted introgressions are uncertain and studies

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on the level of farmer knowledge and their activity in intercrossing between two related taxa is very much limited (Jarvis and Hodgkin, 1999). Transfer of genes from landraces to wild relatives may exhibit greater fitness in the wild populations or even in the new hybrids (Darmency and Vigouroux, 2000).

There is an increasing concern over the loss of germplasm diversity in the gene pools of cultivated crops in areas of crop domestication where the diversity is concentrated (Darmency and Vigouroux, 2000). The exchange of genes have been observed in both directions: from wild to cultivated and from cultivated to wild populations. Crosses sometimes take place between forms of different ploidy levels in the wheat and they may result in substantial increases in variation (Harlan, 1992). Smith (2000) indicated that crops will hybridize with wild relatives and that gene transfer between species does occur. Wild emmer of the Near East do come into contact with cultivated wheat sufficiently to cross occasionally. The extent of natural out-crossing has been reported to range from 0-4% (Briggs et al., 1999), and cross-pollination is influenced by both plant and environmental factors.

Introgression has played a role in structuring the genetic diversity of species (Wendel *et al.*, 1989; Rieseberg, 1997) in the origin of new adaptations (Rieseberg, 1991), in the transfer of adaptation between species (Heiser, 1973; Rieseberg *et al.*, 2003), in the formation of new ecotypes (Levin, 1967; Abbott, 1992; Rieseberg, 1997) or species (Arnold *et al.*, 1991; Soltis and Soltis, 1999), and in the evolution of invasiveness (Anttila *et al.*, 1998; Ellstrand and Schierenbeck, 2000). *T. dicoccoides* (2n = 4x = 28; genome AABB), the progenitor of most cultivated wheat, is crossable with both durum wheat (*T.durum*) (2n = 4x = 28; genome AABB) and common wheat (*T.aestivum*) (2n = 6x = 42; genome AABBDD). Its chromosomes are fully homologous to those of durum wheat and to chromosomes of the A and B genomes of common wheat. Thus, simple plant breeding procedures enable an efficient transfer of desirable alleles from every 'wild' chromosome into its 'cultivated' homologue. Wild emmer carries many agronomically important traits that can be exploited for wheat improvement (Feldman and Millet, 1995), including resistance to different wheat diseases (Nevo, 1995; Nachit *et al.,* 1995) and grain quality (Maali *et al.,* 1998).

Jordan is located within the center of origin of wheat diversity (Hawkes, 1993). Populations of wild emmer wheat from Jordan revealed high within population genetic diversity (Valkoun *et al*., 1994). Natural hybrids found in farmer's fields were products of spontaneous crosses between durum wheat landraces and wild emmer growing together in the same field (Jaradat, 1993). Natural hybridization between wild and domesticated plants is a wide spread phenomenon especially in the center of genetic diversity. New combination of genes resulting from hybridization and introgression between crop cultivars and wild relatives have been important in the evolution of domesticated crop species and may continue to be a factor in increasing the genetic diversity of modern crops (IPGRI, 2000).

Introgression occurred in the northern region of Jordan valley where wild emmer was reported to be extremely abundant, giving rise to wild varieties 'races' of wild emmer (Blumler, 1998). It was reported that only limited gene flow could occur in wheat, so that introgression would seem to be less likely than in other crop progenitors, which are common in cultivated fields (Goldenberg, 1989). Swarms hybrid was identified in regions surrounding Northern Jordan valley, on the edge of formerly cultivated fields, indicating high opportunities for introgression. Furthermore, domesticated wheat had opportunities to come in contact with wild plants

especially in non-tilled field margins. Based on similarity in seed size and other morphological characters between the wild and domesticated races, it was suggested that a large-seeded race of wild emmer wheat (*T. dicoccoides*) from the mentioned region was likely the progenitor of cultivated emmer and its successors, durum (Blumler, 1998).

Approach to identify introgression could be based on morphological or molecular comparisons (Blumler, 1998). The occurrence of hybrid swarms characterized by having indehiscent spikes within the wild populations of *T. dicoccoides* suggested the most recent introgression (Blumler and Byrne, 1991). Thus, the outcome of introgression would be the high variability within wild emmer wheat populations (Harlan, 1992) which could be estimated via molecular approaches (Blumler, 1998; IPGRI, 2000). Owuar *et al*. (1999) indicated that gene flow could occur freely between wild barley population as indicated by the higher genetic diversity detected by RAPD markers. RAPD markers were also used to provide evidence of introgression occurrence in wheat (Tzion *et al*., 1997). More recently, Maccaferri *et al*. (2007a, b) carried out a comparative analysis of SSR and AFLP markers, and phenotypic data to reconstruct genetic relationships among durum wheat accessions.

Genetic diversity can be quantified indirectly, by the estimation of genetic distance using pedigree information, or directly, using molecular markers which compare DNA-sequence variation among genotypes.

In wheat, DNA markers represent an exciting new tool for studying genetic relationships between species, populations and cultivars. For this purpose different marker types have been used with varying results. Recently, microsatellites (Röder *et al*., 1998) also referred to as sequence tagged microsatellite sites (STMSs) or simple sequence repeats (SSRs), have found increasing application because of their known advantages over other systems. Wheat microsatellites have been successfully used for the construction of genetic linkage maps of wheat (Röder *et al*., 1998; Nachit *et al*., 2001; Elouafi and Nachit, 2004), for detection of genetic diversity (Fahima *et, al*. 1998; Li *et al*., 2000a, b, c) and for mapping of agronomical important genes (Korzun *et al*., 1998; Chague *et al*., 1999; Peng *et al*., 1999; 2000a, b, c).

The aim of the present work is to assess natural hybridization of cultivated wheat with its wild progenitor *Triticum turgidum* ssp. *dicoccoides* and to search for evidence of the introgression of wild relative genes into durum wheat.

2. MATERIALS AND METHODS

Plant Material

The genetic material used consisted of plants collected during May 2004 by Drs. Duwayri, Ajlouni, and Amri from a population found in a farmer field in Tafila region, located 100 m east of the cement factory in Kadissya (Farmer field coordinates N: 30 40 809; E: 35 38 559; Altitude: 1581 m). The population consisted of a mixture of plants of durum wheat, *Triticum urartu*, *Triticum dicoccoides*, and large number of plants apparently issued from natural hybridization. The accessions were collected and conserved *ex situ* at the genebanks of the International Center for Agricultural Research in the Dry Areas (ICARDA) and of the National Center for Agricultural Research and Technology Transfer (NCARTT). The plants issued from interspecific crosses were spotted using morphological characters such as non-shattering, and large seed size compared to the cultivated wheat and the wild *Triticum* species. Part of these accessions were grown at the greenhouse of the campus at the University of Jordan and their morphological and agronomic characterization was conducted in the field at the campus

during 2004-05 season for confirmation of the interspecific nature of the plants. The accessions were subdivided into three groups based on spikes characteristics: a group resembling to cultivated wheat (WHLIKE group,) a group with black awns white glumes (WHGLU group) and a group with white awns black glumes (BLGLU group). Hourani durum wheat landrace is also introduced as reference standard for comparison.

Isolation of Genomic DNA

Genomic DNA was extracted from the leaves of 2-weeks-old seedlings grown in the greenhouse, using a Promega Wizard genomic DNA purification plant kit according to instructions provided by the manufacturer (http://www.promega.com). A 40 mg of leaf tissue was processed by freezing with liquid nitrogen and grinded into a fine powder using screwdriver in 1.5 ml microcentrifuge tube. A total of 600 µl of nuclei lysis solution was added to the fine powder and mixed to wet the tissue. The mixture was incubated at 65^{o}C in the water bath for 15 min. After that 3 µl of RNase was added to the cell lysate and incubated at 37^{o}C for 15 min. A 200 µl of protein precipitation solution was added and the samples centrifuged for 3 min at 13,000Xg and the supernatant containing the DNA was carefully removed from the tubes and transferred to a cleaned 1.5 ml microcentrifuge tubes contained 600 µl of room temperature isopropanol. The solution then was centrifuged at 13,000Xg for 1 min at room temperature. The supernatant was carefully decanted leaving the DNA pellet adhered at the bottom of the tube. A 600 µl of room temperature 70% ethanol was added to the tubes containing DNA pellet to wash the DNA and the pellets were air dried and the DNA rehydrated using TE buffer (Tris- EDTA) solution and then was stored at -20^{o}C. The DNA quality and concentration was determined using a spectrophotometer reading.

Microsatellite Markers and PCR Analysis

Twenty nine primer pairs of wheat microsatellite with different chromosomal locations were chosen for analysis. Microsatellites used in this study were described by Röder *et al*. (1995, 1998). A pair was chosen for each arm (S and L) of chromosome (1-7) of both genomes (A and B) and only one pair for genome D (Li *et al., 2*003). Primer sequences and loci are shown in Table 1.

PCR was performed in 10-µl reactions containing 20 ng of template DNA, 0.2 µM of each primer, 100 µM of each dNTP, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 and 1 unit of Taq DNA polymerase. Amplification was carried out in a PTC-200 thermal cycler (MJ Research, USA) as follows: 5 min at 94°C, followed by 35 cycles of 45 sec. at 94°C, 45 sec. at 55°C, 60°C or 65°C (depending on the annealing temperature of the primers used), 1 min at 72°C, and 7 min at 72°C for final extension. Amplified products were electrophoresed in 3.0% metaphore agarose gels run at 100 volts for 1:30 hours and the banding patterns were visualized using ethidium bromide staining.100 base pair DNA ladder was loaded with the amplified PCR products. All the chemicals used are supplied from Promega (Washington, USA. http://www.promega.com).

Marker Data Analysis

Microsatellite markers were scored as present (1) or absent (0) over all DNA samples of both wheat and emmer accessions. Genetic similarity estimates of Jaccard (1974) between accessions were calculated in all possible pair-wise comparisons. The ordination analysis was performed according to the unweighted pair-group arithmetic average method (UPGMA) clustering algorithm (Sneath and Sokal, 1973), and the dendrogram of all accessions were constructed from the symmetrical genetic similarity matrix using the SPSS program.

Table (1): Description of wheat microsatellite primer sets and their loci.

Locus	Location	Left Primer	Right Primer	Repeat	[1]An. Temp.	[2]Opata (bp)	[3]Synth. (bp)
gwm18	*1BS*	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	(CA)17GA(TA)4	50	188	182
gwm60	*7AS*	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG	(CA)30	60	190	224
gwm95	*2AS*	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	(AC)16	60	128	116
gwm99	*1AL*	AAGATGGACGTATGCATCACA	GCCATATTTGATGACGCATA	(CA)21	60	117	120
gwm120	*2BL*	GATCCACCTTCCTCTCTCTC	GATTATACTGGTGCCGAAAC	(CT)11(CA)18	60	162	174
gwm124	*1BL*	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	(CT)27(GT)18imp	60	190	197
gwm136	*1AS*	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC	(CT)58	60	278	321
gwm162	*3AL*	AGTGGATCGACAAGGCTCTG	AGAAGAAGCAAAGCCTTCCC	(CA)14AA(CA)4	60	202	208
gwm169	*6AL*	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	(GA)23	60	220	193
gwm186	*5AL*	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	(GA)26	60	132	106
gwm369	*3AS*	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	(CT)11(T)2(CT)21	60	184	—
gwm219	*6BL*	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	(GA)35imp	60	184	153
gwm251	*4BL*	CAACTGGTTGCTACACAAGCA	GGGATGTCTGTTCCATCTTAG	(CA)28	55	110	109
gwm294	*2AL*	GGATTGGAGTTAAGAGAGAACCG	GCAGAGTGATCAATGCCAGA	(GA)9TA(GA)15	55	96	102
gwm332	*7AL*	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	(GA)36	60	290	211
gwm340	*3BL*	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	(GA)26	60	159	—
gwm361	*6BS*	GTAACTTGTTGCCAAAGGGG	ACAAAGTGGCAAAAGGAGACA	(GA)20imp	60	125	123
gwm368	*4BS*	CCATTTCACCTAATGCCTGC	AATAAAACCATGAGCTCACTTGC	(AT)25	60	259	271
gwm389	*3BS*	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	(CT)14(GT)16	60	117	128
gwm408	*5BL*	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	(CA) 22(TA)(CA)7(TA)9	55	182	148
gwm415	*5AS*	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	(GA)25imp	55	133	131
gwm429	*2BS*	TTGTACATTAAGTTCCCATTA	TTTAAGGACCTACATGACAC	(CT)25	50	211	209
gwm459	*6AS*	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	(GA).28	55	118	126
gwm537	*7BS*	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTCCT	(CA)18(TA)13	60	207	203
*Xgwm*540	5BS	TCT CGC TGT GAA ATC CTA TTT C	AGG CAT GGA TAG AGG GGC	(CT)3(CC)(CT)16	55	133	117
*Xgwm*577	7BL	ATG GCA TAA TTT GGT GAA ATT G	TGT TTC AAG CCC AAC TTC TAT T	(CA)14(TA)6	55	164	155
*Xgwm*601	4AS	ATC GAG GAC GAC ATG AAG GT	TTA AGT TGC TGC CAA TGT TCC	(CT)17	60	152	142
*Xgwm*637	4AL	AAA GAG GTC TGC CGC TAA CA	TAT ACG GTT TTG TGA GGG GG	(CA)18	60	159	157
*Xgwm*428	7DL	CGA GGC AGC GAG GAT TT	TTC TCC ACT AGC CCC GC	(GA)22	60	137	133

1: Annealing temperature, 2: Mexican wheat variety Opata 85 from CIMMYT , 3: International Triticeae Mapping Initiative (ITMI) Opata 85 X W7984.

3. RESULTS AND DISCUSSION

A total of 738 fragments were obtained from the 11 SSR primers across the genotypes screened. Of the 24 genomic loci totally assayed, 20 (83%) were found to be polymorphic and the remaining four monomorphic .An example showing SSR patterns in the wheat populations using primers (*gwm*120, *gwm*124 and *gwm*136) is presented in Figure 1. The genetic relatedness among accessions was estimated by computing a similarity matrix based on all reliable bands detected and on all possible pair-wise comparisons between accessions. The application of the SPSS program allowed us the construction of dendrograms and the visualization of accession clusters and sub-clusters on the basis of Jaccard's genetic similarity coefficient (Table 2).

The mean similarity indices ranged from 0.28 to 1.00 between samples. All samples showed an average similarity index of 0.63 (Table 2), which means that the accessions share on average 63% of their marker alleles at the investigated SSR loci. This wide range of similarity estimates indicated a high polymorphism at the DNA level among the accessions and, therefore, a large amount of genetic variation among homologous DNA sequences.

The results based on the dendrogram (Figure 2) showed that the samples were clearly separated into three main groups: white glumes black awned, black glumes white awned, and classified wheat-like accessions. The results showed also that there is a clear separation for the white glumes black awned accessions (Figure 2), whereas the black glumes accessions were clustered with the wheat-like accessions (Figure 3).

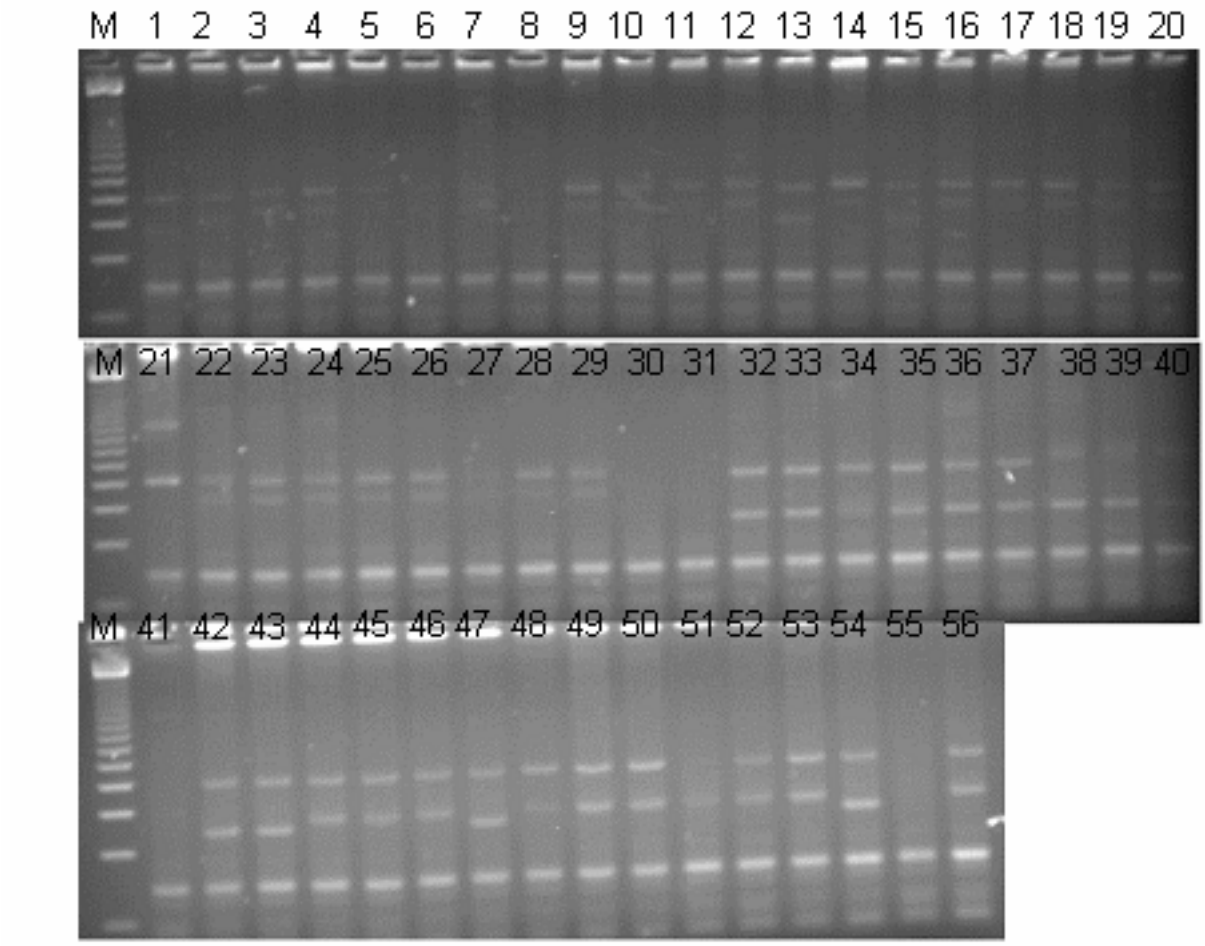

Fig. 1. SSR patterns in the wheat populations using primers (*gwm*120, *gmw*124 and *gwm*136). From left to right, lanes are: M, correspond to 100 bp DNA ladder; 1, Hourni,; 2, Jubiha; 3-12 and 14-32, wheat like genotypes (WHLIKE group); 13, Hourani; 33-44 white awns black glumes (BLGLU group); 45-65, black awns white glumes (WHGLU group).

The overall mean diversity index was chosen as cutoff value and this enabled the definition of eight clusters. The largest cluster was composed of 26 accessions most of which belonging to the wheat-like group, including also eight out of the twelve black awned glumes accessions. The white glumes black awned accessions were grouped in the second cluster and none of the accessions from other wheat groups were grouped in this main cluster. The third main cluster grouped the remaining wheat-like accessions and included the Hourani landrace. The other smaller clusters encompass the rest of accessions from black glumes and wheat-like accessions and in one of the clusters one accession of white glumes group was observed. The genetic relatedness among the two *T. diccocoidess* groups was also studied (data not shown) clearly separated both groups into two main clusters ranged in similarity from 0.5 to 1.0 (Table 2).

Table (2): Jaccard's coffecient of similarity for the wheat accessions used in the study.

	HOURANI	WHLIKE1	WHLIKE2	WHLIKE3	WHLIKE4	WHLIKE5	WHLIKE6	WHLIKE7	WHLIKE8	WHLIKE9	WHLIKE10	WHLIKE11	WHLIKE12	WHLIKE13	WHLIKE14	WHLIKE15	WHLIKE16	WHLIKE17	WHLIKE18	WHLIKE19	WHLIKE20	WHLIKE21	WHLIKE22	WHLIKE23	WHLIKE24	WHLIKE25	WHLIKE26	WHLIKE27	WHLIKE28	WHLIKE29	BLGLU1	BLGLU2	BLGLU3	BLGLU4	BLGLU5	BLGLU6	BLGLU7	BLGLU8	BLGLU9	BLGLU10	BLGLU11	BLGLU12	WHGLU1	WHGLU2	WHGLU3	WHGLU4	WHGLU5	WHGLU6	WHGLU7	WHGLU8	WHGLU9	WHGLU10	WHGLU11	WHGLU12
WHLIKE1	64																																																					
WHLIKE2	73	91																																																						
WHLIKE3	64	82	75																																																			
WHLIKE4	60	64	58	80																																																		
WHLIKE5	64	67	62	82	80																																																	
WHLIKE6	55	58	54	73	89	90																																																
WHLIKE7	47	71	67	71	57	71	64																																															
WHLIKE8	47	71	67	71	57	71	64	100																																														
WHLIKE9	47	71	67	71	57	71	64	100	100																																													
WHLIKE10	47	71	67	71	57	71	64	100	100	100																																												
WHLIKE11	50	77	71	77	62	64	57	93	93	93	93																																											
WHLIKE12	62	64	71	77	62	64	57	89	89	89	89	73																																										
WHLIKE13	50	77	71	77	62	64	57	93	93	93	93	100	73																																									
WHLIKE14	60	64	58	50	45	64	55	57	57	57	57	50	40	50																																							
WHLIKE15	58	62	57	50	46	62	54	56	56	56	56	50	41	50	73																																						
WHLIKE16	39	59	56	59	47	59	53	82	82	82	82	76	58	76	47	65																																				
WHLIKE17	44	67	63	67	53	56	50	81	81	81	81	87	65	87	44	63	88																																				
WHLIKE18	47	60	56	60	47	60	53	65	65	65	65	59	50	59	47	67	72	71																																				
WHLIKE19	44	67	63	67	53	67	60	81	81	81	81	75	56	75	53	73	88	88	81																																			
WHLIKE20	47	60	56	60	57	71	64	65	65	65	65	59	50	59	57	79	82	71	65	81																																		
WHLIKE21	39	59	56	59	47	59	53	82	82	82	82	76	58	76	47	65	100	88	72	88	82																																
WHLIKE22	44	67	63	56	44	56	50	71	71	71	71	65	47	65	53	73	88	76	81	88	81	88																																
WHLIKE23	54	57	53	47	43	57	50	53	53	53	53	47	39	47	67	92	71	59	63	69	86	71	80																															
WHLIKE24	38	50	47	60	57	60	64	75	75	75	75	69	59	69	38	56	82	81	75	81	65	82	71	53																													
WHLIKE25	39	59	56	59	47	59	53	82	82	82	82	76	58	76	47	65	100	88	72	88	82	100	88	71	82																											
WHLIKE26	41	63	59	63	50	63	56	88	88	88	88	81	61	81	50	69	94	94	76	94	76	94	82	65	88	94																									
WHLIKE27	43	57	53	69	67	57	62	63	63	63	63	67	56	67	33	53	71	80	73	80	63	71	69	50	86	71	75																							
WHLIKE28	60	64	58	80	100	80	89	57	57	57	57	62	62	62	45	46	47	53	47	53	57	47	44	43	57	47	50	67																							
WHLIKE29	46	50	47	62	58	75	67	67	67	67	67	60	60	60	46	47	56	53	47	53	56	56	44	44	56	56	59	44	58																						
BLGLU1	39	50	47	59	47	59	53	72	72	72	72	67	67	67	39	56	79	78	72	78	63	79	68	53	82	79	83	71	47	65																					
BLGLU2	41	53	50	63	50	63	56	76	76	76	76	71	61	71	41	59	83	82	76	82	67	83	72	56	88	83	88	75	50	69	94																			
BLGLU3	47	60	56	71	57	71	64	87	87	87	87	80	69	80	47	47	72	71	65	71	56	72	61	44	75	72	76	63	57	79	82	88																		
BLGLU4	44	56	53	67	53	67	60	71	71	71	71	65	65	65	44	63	78	76	71	76	71	78	67	59	81	78	82	69	53	73	88	94	81																
BLGLU5	44	56	53	67	53	67	60	71	71	71	71	65	65	65	44	63	78	76	71	76	71	78	67	59	81	78	82	69	53	73	88	94	81	100															
BLGLU6	31	44	41	53	50	53	57	69	69	69	69	63	53	63	31	33	58	56	50	56	42	58	47	32	69	58	61	56	50	71	67	71	80	65	65														
BLGLU7	46	40	38	40	46	50	54	39	39	39	39	33	33	33	46	69	47	44	56	53	56	47	53	64	56	47	50	53	46	47	56	59	47	63	50														
BLGLU8	35	47	44	56	53	47	50	61	61	61	61	65	56	65	28	44	68	76	61	67	53	68	58	42	81	68	72	80	53	53	78	82	71	76	76	75	63												
BLGLU9	55	46	43	46	55	46	50	35	35	35	35	38	38	38	42	67	44	50	64	50	53	44	50	62	53	44	47	62	55	33	44	47	35	50	50	29	67	50											
BLGLU10	54	69	64	69	54	57	50	63	63	63	63	67	56	67	43	53	61	69	73	69	53	61	69	50	63	61	65	71	54	53	71	75	63	69	69	56	53	69	50										
BLGLU11	50	64	60	64	50	53	47	59	59	59	59	63	44	63	50	71	67	75	59	75	80	75	59	67	75	56	69	67	71	79	50	50	76	81	69	75	75	53	60	75	57	92							
BLGLU12	50	53	50	53	50	53	47	59	59	59	59	63	44	63	50	71	67	75	59	75	69	67	65	67	59	67	71	67	50	41	58	61	50	56	56	37	50	56	57	56	63							
WHGLU1	47	50	47	50	47	50	44	65	65	65	69	50	69	47	56	63	71	47	61	56	63	53	53	56	63	67	53	47	47	55	58	56	53	53	42	39	53	44	44	50	80							
WHGLU2	44	67	63	67	53	56	50	71	71	71	75	75	75	44	53	68	76	53	67	61	68	58	50	61	68	72	59	53	53	68	63	61	67	67	47	37	58	41	50	56	56	71						
WHGLU3	41	53	50	63	50	53	47	67	67	71	71	71	33	42	65	72	58	63	50	65	55	40	67	65	68	65	50	59	53	53	68	63	61	67	67	47	37	42	72	39	65	71	53	67	82			
WHGLU4	41	63	59	63	50	53	47	76	76	76	76	81	61	81	41	50	74	82	58	72	58	74	63	47	67	74	78	65	50	50	65	68	67	63	63	53	35	63	39	56	61	71	88	82	78			
WHGLU5	41	63	59	63	50	53	47	76	76	76	76	81	61	81	41	50	74	82	58	72	58	74	63	47	67	74	78	65	50	50	65	68	67	63	63	53	35	63	39	56	61	71	88	82	78	100		
WHGLU6	41	63	59	63	50	53	47	76	76	76	76	81	61	81	41	50	74	82	58	72	58	74	63	47	67	74	78	65	50	50	65	68	67	63	63	53	35	63	39	56	61	71	88	82	78	100	100	
WHGLU7	41	63	59	63	50	53	47	67	67	71	71	71	41	50	65	72	50	63	58	65	55	47	58	65	68	56	50	50	50	65	60	58	63	63	45	35	55	39	47	53	61	76	94	78	88	88	88	
WHGLU8	41	63	59	63	50	53	47	76	76	76	76	81	71	81	41	50	74	82	58	72	58	74	63	47	67	74	78	65	50	50	74	68	67	63	53	35	63	39	56	61	61	76	94	88	88	88	88	88
WHGLU9	41	63	59	63	50	53	47	76	76	76	76	81	71	81	41	50	74	82	58	72	58	74	63	47	67	74	78	65	50	50	74	68	67	63	63	53	35	63	39	56	61	61	76	94	88	88	88	88	100
WHGLU10	50	53	50	64	62	64	57	59	59	59	59	63	53	63	40	50	58	65	59	65	59	58	56	47	59	58	61	67	62	60	67	71	69	65	65	53	50	65	47	67	73	63	69	65	81	71	71	71	71
WHGLU11	38	50	47	60	57	50	53	65	65	65	65	69	69	69	29	39	63	71	56	61	47	63	53	37	75	63	67	73	57	47	72	67	65	61	61	59	39	71	44	53	59	50	65	81	88	76	76	76	88	88	69
WHGLU12	64	67	62	54	50	54	46	50	50	50	50	53	44	53	64	75	50	56	50	56	60	50	56	69	41	50	53	47	50	40	42	44	41	47	47	28	50	39	58	47	53	64	71	67	53	63	63	63	63	63	64	50

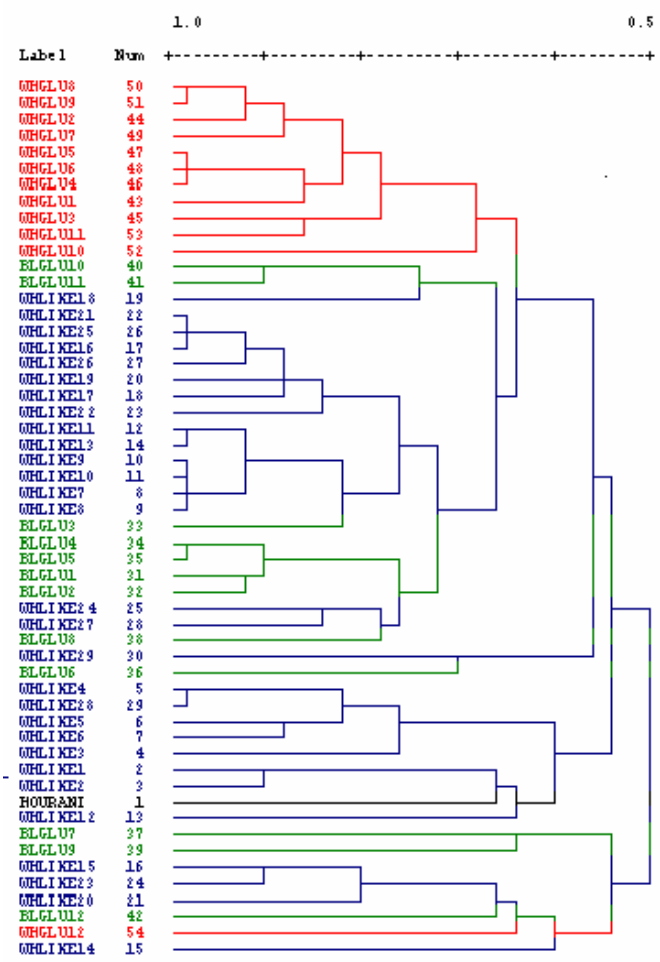

Fig. 2. UPGMA dendrogram of the genetic similarity of the studied accessions based on Jaccard coefficient.

The genetic identity among wheat like accessions and the landrace Hourani also showed high genetic diversity (Figure 4) where the similarity index ranged from 0.5 to 1.0 (Table 2) and two main clusters have been formed where the landrace Hourani grouped in the first main cluster with eight accessions whereas the rest were grouped in the second clusters. The similarity index among wheat like and black glumes accessions ranged from 0.33 to 1.0 indicating a wide range of diversity among these accessions.

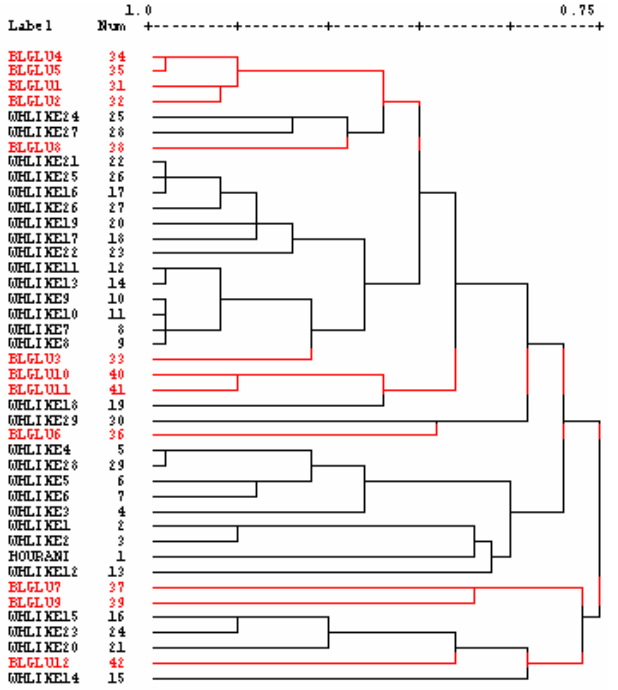

Fig. 3. UPGMA dendrogram of the genetic similarity of the wheat like and black glumes white awned populations based on Jaccard coefficient.

Introgression of genetic variability in wheat from distantly or closely related species often has been specifically aimed at the introduction of simply inherited traits, such as insect and diseases resistance (Friebe *et al*., 1996; Braun et al., 1998, Nachit *et al.*, 1995; Maali *et al.*, 1998) for which narrow or nil genetic variability exists in wheat. The polymorphism identified in these accessions was most likely attributable to occasional introgressive hybridization involving wild emmer. The co-existence of wheat landraces together with wild emmer might have offered the possibility for natural hybridization resulting in spontaneous interspecific hybrids. Our results support the occurrence of natural hybridization between wheat landrace and wild emmer.

Fig. 4. UPGMA dendrogram of the genetic similarity of the wheat like accessions and the landrace Hourani genotype based on Jaccard coefficient.

Exploitation of available variability for improvement of any crop depends on the possibility and ability to introgress desirable genetic traits from wild relatives to cultivated varieties. This strategy is largely facilitated by precise monitoring of alien gene introgression at molecular level (Prakash *et al*., 2002).

The present study demonstrated the efficiency of SSR technology for polymorphism detection and analysis of introgression in wheat, there by offering wide scope of application in marker aided breeding programs of wheat.

In conclusion, our results further demonstrate that the polymorphism identified in these genotypes was therefore the consequences of introgressive hybridizations involving wild emmer and microsatellite analysis can be useful for distinguishing wheat landraces sharing a high degree of genetic similarity, and so for determining the extent of genetic diversity among them. This information is crucial not only for the management of genetic diversity but also for the identification and preservation of core collections of wheat.

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REFERENCES

Abbott, R.J. 1992. Plant Invasions, Interspecific hybridization and the Evolution of New Plant Taxa. ***Trends Ecol.Evol***. 7:401-405.

Anttila, C.K., C.C. Daehler, N.E. Rank and D.R. Strong. 1998. Greater Male Fitness of a Rare Invader (*Spartina alterniflora*, Poaceae) Threatens a Common Native (*Spartina foliosa*) with Hybridization. ***Am. J. Bot.*** 85:1597–1601.

Arnold, M.L., C.M. Buckner and I. Robinson. 1991. Pollen-mediated Introgression and Hybrid Speciation in Louisiana Irises. ***Proc. Natl. Acad. Sci***. USA 88:1398–1402.

Blumler, M.A. 1998. Introgression of Durum into Wild Emmer and the Agricultural Origin Question, 252-268. ***The Harlan Symposium***. Damania A,B., Valkoun, J., Willcox and Qualset.(eds). 10-15 May.1997. ICARDA. Allepo,Syria.

Blumler, M.A. and Byrne.1991. The Ecological Genetics of Domestication and Origin of Agriculture. ***Curr. Anthropol.*** 32:23-54.

Braun, H.J., T.S. Payne, A.I. Morgounov, M. van Ginkel, S. Hegde and S.G Rajaram. 1998. The Challenge: One Billion Tons of Wheat by 2020. 33–40. In A.E. Slinkard (ed.) ***Proc. 9th Int. Wheat Genet. Symp***., Vol. 1, Saskatoon, SK, Canada. Univ. Ext. Press, Saskatoon, SK, Canada.

Briggs, K.G., O.K. Kiplagat and Johnson-Flanagan, A.M.

1999. Floret Sterility and Out-crossing in Spring Wheat Cultivars. ***Can. J. Plant***. Sci.79:321–328.

Chagué, V., Fahima, T., Dahan, A., Sun, G.L., Korol, A. B., Ronin, Y.I., Grama, A., Röder, M.S. and Nevo, E. 1999. Isolation of Microsatellite and RAPD Markers Flanking the Yr15 Gene of Wheat Using NILs and Bulked Segregant Analysis. ***Genome*** 42:1050–1056.

Darmency, H. and Vigouroux, Y. 2000. Gene Flow from Cultivated Crops to Weedy Relatives. ***Third International Weed Science Congress.*** 6-11 June, 2000. Brazil.

Ellstrand, N.C. and K.A. Schierenbeck. 2000. Hybridization as a Stimulus for the Evolution of Invasiveness in Plant. ***Proc. Natl Acad. Sci.*** USA. 97:7043-7050.

Elouafi, I. and Nachit, M.M. 2004. A Genetic Linkage Map of Triticum Durum x Triticum Dicoccoides Population Based on SSRs and AFLP Markers and QTL Analysis for Milling Characters. ***Theor. Appl. Genet***. 108:401–413.

Fahima, T.V., M.S. Röder, A.Grama and E.Nevo. 1998. Microsatellite DNA Polymorphism Divergence in *Triticum dicoccoides* Accessions Highly Resistant to Yellow Rust. ***Theor.Appl.Genet.*** 96: 176-195.

Feldman, M. and Millet, E. 1995. Methodologies of Identification, Allocation and Transfer of Quantitative Genes from Wild Emmer into Cultivated Wheat. ***Proc. VIII Int. Wheat Genet. Symp.***, Beijing 1: 19- 27.

Friebe , B., J. Jiang, W. J. Raupp, R. A. McIntosh and B. S. Gill. 1996. Characterization of Wheat-alien Translocations Conferring Resistance to Diseases and Pests: Current Status. ***Euphytica***, 91(1): 59-87.

Goldenberg, E.M. 1989. Migration Patterns and the Development of Multi-locus Association in Selfing Annual *T. dicoccoides* . ***Evolution***. 43(3):595-606.

Harlan, J.R.1992. Crops and Man. American Asso. of Agron. 2ed ed. Inc. Madison. Wisconsin. U.S.A., 284.

Hawkes, J.G. 1993. International Workshop on Dynamic *in situ* Conservation of Wild Relatives of Major Cultivated Plants: Summary of Final Discussions and Recommendations. ***Israel J. of Botany***. 40:529-536.

Heiser, C.B. 1973. Introgression Re-examined. ***Bot. Rev.*** 39:347–366

International Plant Genetic Resources Institute (IPGRI). 2000. Annual Report, Rome Italy.

Jaccard, A. 1974. The Genetic Structure of Populations, Springer Verlag, Berlin, German Federal Republic.

Jaradat, A.A. 1993.Ecogeographical Races in Natural Populations of *T. dicoccoides*, 397-408. In: Biodiversity and Wheat Improvement. Damania (ed). John Wiley and Sons.

Jarvis, D.I. and Hodgkin.1999. Wild Relatives and Crop Cultivars: Detecting Natural Introgression and Combination in Agroecosystem. ***Molecular Ecology***. 81:S159-S173.

Korzun, V., Röder, M.S., Ganal, M.W., Worland, A.J. and Law, C.N. 1998. Genetic Analysis of the Dwarfing Gene (Rht8) in Wheat. I. Molecular Mapping of Rht8 on the Short Arm of Chromosome 2D of Bread Wheat (*Triticum aestivum* L.). ***Theor. Appl. Genet***. 96:1104–1109.

Ladizinsky, G. 1978. Collection of Wild Cereals in Upper Jordan Valley. ***Economic Botany***. 29:264-267.

Levin, D.A. 1967. Hybridization between Annual Species of Phlox: Population Structure***. Am. J. Bot.*** 54:1122–1130.

Li, Y.C., Fahima, T., Peng, J.H., Röder, M.S., Kirzhner, V., Beiles, A., Korol, A.B. and Nevo, E. 2000a. Edaphic Microsatellite DNA Divergence in Wild Emmer Wheat, *Triticum dicoccoides*, at a Microsite: Tabigha, Israel***. Theor. Appl. Genet.*** 101:1029–1038.

Li. Y.C., Fahima, T., Korol, A.B., Peng, J.H., Röder, M.S., Kirzhner, V., Beiles, A. and Nevo, E. 2000b. Microsatellite Diversity Correlated with Ecological-edaphic and Genetic Factors in Three Microsites of Wild Emmer Wheat in North Israel. ***Mol. Biol. Evol.*** 17:851–862.

Li, Y.C., Röder, M.S., Fahima, T., Kirzhner, V., Beiles, A., Korol, A.B. and Nevo, E. 2000c. Natural Selection Causing Microsatellite Divergence in Wild Emmer Wheat at the Ecologically Variable Microsite at Ammiad, Israel***. Theor. Appl. Genet.*** 100: 985–999.

Li, Y.C., Fahima, T., Röder, M.S., Kirzhner, V., Beiles, A., Korol, A.B. and Nevo, E. 2003. Genetic Effects on Microsatellite Diversity in Wild Emmer Wheat

(*Triticum dicoccoides*) at the Yehudiyya Microsite, Israel**.** ***Heredity*** 90: 150-156.

Maali, A., Duwayri, M. and Nachit, M.M. 1998. Use of *Triticum dicoccoides* to Improve Grain Quality in Durum Wheat. In: Durum Research Network, SEWANA (South Europe, West Asia and North Africa). Eds: M.M. Nachit, M. Baum, E. Porceddu, P. Monneveux and E. Picard. Aleppo, ***ICARDA***. Syria, 319-323.

Maccaferri, M., Stefanelli, S., Rotondo, F., Tuberosa, R. and Sanguineti, M.C. 2007a. Relationships among Durum Wheat Accessions. I. Comparative Analysis of SSR, AFLP and Phenotypic Data. ***Genome*** 50(4):373–384.

Maccaferri, M., Sanguineti, M.C., Xie, C., Smith, J.S. and Tuberosa, R. 2007b. Relationships among Durum Wheat Accessions. II. A Comparison of Molecular and Pedigree Information. ***Genome*** 50(4):385–399.

Nachit, M.M., Asbati, A., Azrak, M., Rbeiz, N. and El-Saleh, A. 1995. Durum Breeding Research to Improve Dryland Productivity in the Mediterranean Region. Cereal Improvement Program, ***ICARDA Annual Report***, 95–96.

Nachit, M. M., Elouafi, I., Pagnotta, M. A., El-Saleh, A., Iacono, E., Labhilili, M., Asbati, A., Azrak, M., Hazzam, H., Benscher, D., Khairallah, M., Ribaut, J.M., Tanzarella, O.A., Porceddu, E. and Sorrells, M.E. 2001.Molecular Linkage Map for an Intraspecific Recombinant Inbred Population of Durum Wheat (*Triticum turgidum* L. var durum). ***Theor. Appl. Gene***. 102: 177-186.

Nevo, E. and A. Beiles.1989. Genetic Diversity of Wild Emmer Wheat in Israel and Turkey: Structure, Evolution, and Application in Breeding. ***Theor. Appl. Genet.*** .77:421-455.

Nevo, E., A. Beiles, Y. Gutterman, N. Storch and Kaplan, D.1984. Genetic Resources of Wild Cereals in Israel and Vicinity. I. Phenotypic Variation within and between Populations of Wild Wheat, *T. dicoccoides*. ***Euphytica***. 33:717-735.

Nevo, E. 1995. Genetic Resources of Wild Emmer, *Triticum dicoccoides*, for Wheat Improvement: News and Views. In: ***Proc. 8th Int. Wheat Genetics Symp.*** Beijing, 79–87.

Owuar, E. D., T. Fahima, A. Beharav, A. Koroland and E. Nevo. 1999. RAPD Divergence Caused by Micro Site Edaphic Selection in Wild Barley. ***Genetica*** 105:177-192.

Peng, J.H., Fahima, T., Röder, M.S., Huang, Q.Y., Dahan, A., Li, Y.C., Grama, A. and Nevo, E. 2000c. High-Density Molecular Map of Chromosome Region Harboring the YrH52 Gene and Its Preliminary Discrimination from Yr15 in Wild Emmer Wheat, *Triticum dicoccoides*. ***Genetica*** 109:199–210.

Peng, J.H, Fahima, T., Röder, M.S., Li, Y.C., Dahan, A., Grama, A., Ronin, Y.I., Korol, A.B. and Nevo, E. 1999. Microsatellite Tagging of the Stripe-rust Resistance Gene YrH52 Derived from Wild Emmer Wheat, *Triticum dicoccoides*, and Suggestive Negative Crossover Interference on Chromosome 1B. ***Theor. Appl. Gene***. 98: 862–872.

Peng, J.H., Fahima, T., Röder, M.S., Li, Y.C., Grama, A. and Nevo, E. 2000a. Microsatellite High-density Mapping of the Stripe Rust Resistance Gene YrH52 Region on Chromosome 1B and Evaluation of Its Marker-assisted Selection in the F2 Generation in Wild Emmer Wheat. ***New Phytol.*** 146:141–154.

Peng, J.H., Korol, A.B., Fahima, T., Röder, M.S., Ronin, Y.I., Li, Y.C. and Nevo, E. 2000b. Molecular Genetic Maps in Wild Emmer Wheat, *Triticum dicoccoides*: Genome-Wide Coverage, Massive Negative Interference and Putative Quasi-linkage. ***Genome Res.*** 10:1509–1531.

Prakash, N. S., Marie-Christine, C., Naveen, S. and Philippe, L. 2002. AFLP Analysis of Introgression in Coffee Cultivars (*Coffea arabica* L.) Derived from a Natural Interspecific Hybrid. ***Euphytica*** 124: 265–271.

Rieseberg, L.H. 1991. Homoploid Reticulate Evolution in Helianthus: Evidence from Ribosomal Genes. ***Am. J. Bot***. 78:1218–1237.

Rieseberg, L.H. 1997. Hybrid Origins of Plant Species. ***Annu. Rev.Ecol. Syst***. 28:359–389.

Rieseberg, L.H., O. Raymond, D.M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J.L. Durphy, A.E.

Schwarzbach, L.A. Donovan and Lexer. 2003. Major Ecological Transitions in Wild Sunflowers Facilitated by Hybridization. ***Science*** 301:1211–1216.

Röder, M.S., J.Plaschke, S. Ukönig, A. Börner, M.E. Sorells, S.D. Tanksley and M.W. Ganal. 1995. Abundance Variability and Chromosomal Location of Microsatellites in Wheat. ***Mol.Gen.Genet***. 246:327-333.

Röder, M.S., K. Wendehake, J. Plaschke, M.H. Tixer, P. Leroy and M.W. Ganal. 1998. A Microsatellite Map of Wheat. ***Genetics*** 149: 2007-2023.

Röder, M.S., V.Korzun, B.S.Gill and M.W.Ganal. 1998. The Physical Mapping of Microsatellite Markers in Wheat. ***Genome*** 41:278-283.

Smith, C. M. 2000. Introgression: Occurrence *vs.* Consequence. In ***3rd Int. Weed Science Congress***. Brazil.

Sneath, P.H.A. and Sokal, R. R. 1973. Numerical Taxonomy. Freeman and Company, San Francisco, CA.

Soltis, D. and P. Soltis. 1999. Polyploidy: Recurrent Formation and Genome Evolution. ***TREE*** 14(9). 348-352.

Tzion F. Sun, V. Chague, A. Korol, A. Grama, Y. Ronin and E. Nevo.1997. Identification of Molecular Markers Linked to the Yr15 Strip Rust Resistance Gene of Wheat Introgressed from Wild Emmer Wheat *T. dicoccoides.* ***Plant and Animal Genome. V. Conference***. Htt://www.Intl. pag.org./5abstracts/p.5c-163-html.

Valkoun, J., O.F. Mamluk and I. Amaizer.1994. Yellow Rust Reaction in Natural Population of Wild Triticum Species in 1993-1994, Genetic Resources Unit. ***ICARDA***, 69-71.

Wendel, J.F., P.D. Olson, J. Mc. and D. Stewart. 1989. Genetic Diversity, Introgression and Independent Domestication of Old World Cultivated Cottons. ***Am. J. Bot***. 76:1795–1806.

استخدام واسمات المايكروساتيلايت لتوصيف الهجن الطبيعية بين القمح القاسي والبري

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ملخـص

تم استخدام احد عشر بادئا وراثيا في دراسة 56 سلالة من مجتمع القمح القاسي المتكون من 29 سلالة مشابهة مظهريا للقمح القاسي، و12 سلالة تملك العصافة السوداء والسفا الابيض، و 12 سلالة تمتلك العصافة البيضاء والسفا الأسود، بالإضافة إلى ثلاث سلالات محلية بهدف تقييم التهجين الطبيعي بين السلالات المحلية من القمح القاسي مع السلالات البرية *dicoccoides* القريبة له والبحث عن امكانية وجود بعض الجينات من القمح البري في السلالات المستزرعة من القمح القاسي .تم الحصول على ما مجموعه 738 حزمة من (DNA) من خلال دراسة السلالات والبادئات الوراثية واستطاعت البادئات المدروسة تمييز 24 موقعا على الكروموسومات وبنسبة تباين 83 %، وكانت هناك اربعة مواقع فقط مشتركة في كل السلالات المدروسة.

باستخدام تحليل المتوسط الحسابي للمجموعات الزوجية غير المزانة UPGMA من برنامج SPSS تجمعت السلالات ذات العصافة البيضاء والسفا الاسود في مجموعة واحدة وانفصلت تماما عن بقية السلالات؛ بينما تداخلت السلالات ذات العصافة السوداء والسفا الابيض مع متشابهات القمح القاسي في المجموعات الرئيسية والثانوية. كان مجموع الحزم من (DNA) في السلالات متشابهة القمح وتلك الممتلكة العصافة السوداء 560 بنسبة تباين وصلت الى 70%، وهذا التباين الذي تم الحصول عليه في هذه المجموعة هو نتيجة التهجين الطبيعي بين متشابهات القمح والسلالات ذات العصافة السوداء. بينت هذه الدراسة كفاءة استخدام الواسمات الوراثية وخصوصا المايكروساتيلايت في تمييز التباين الوراثي وتحليل التهجين الطبيعي في القمح، مما يفتح المجال امام استخدام المعلمات الوراثية في أنظمة تحسين المحاصيل النباتية.

الكلمـات الدالـة: القمح القاسي، القمح البري (*dicoccoides*)، التهجين الطبيعي، تقنية المايكروساتيلايت، تدفق الجينات.

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