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RESEARCH ARTICLE

The Evaluation of Genomic Relationships and Diversity of Wild and Cultivated Wheats Possessing A Genome in Different Ploidy Levels Using SSR Markers

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Abstract: Genomic relationships and diversity of 37 wild and cultivated wheat (*Triticum* sp.) possessing A genome include four *T. urartu* (A^u), thirteen wild einkorn (A^m), four cultivated einkorn (A^m), seven durum wheat (BBA^uA^u), three *T. zhukovskyi* ($A^tA^tA^mA^mGG$) and six common wheat (BBA^uA^uDD) were evaluated by simple sequence repeats (SSR) analysis. Genetic distance was calculated by Nei and Li using UPGMA for construct phylogenetic tree. 24 out of 35 primer pairs amplified and 22 pairs produced polymorphic amplicons (109 alleles). The highest amplified fragments (11 alleles) and polymorphism information content (0.90) was for Xgwm165-4A locus. The highest and the lowest genetic distance within groups for *T. urartu* and *T. zhukovskyi* were 0.86 and 0.55, respectively. The most similarity was between *T. urartu* and wild einkorn species (0.009). The highest dissimilarity observed between cultivated einkorn and common wheat, although *T. urartu* was more close to durum and common wheat than other diploid species.

Keywords: *Triticum*, A genome, genetic relationships, SSR.

INTRODUCTION

The genus *Triticum* includes wild and cultivated species [12]. The cultivated species cytogenetically associated in four groups that summarized in Table 1. There are investigations on the genetic structure of natural wild wheat populations [6] and the genetic mapping of both cultivated and wild wheats [13]. Criteria for estimation of genetic diversity can be different: pedigree records, morphological traits and molecular markers [7]. The knowledge of diversity of wheat genetic resources is critical for their utilization in plant breeding programs. Genus *Triticum* is the desirable source of useful resistance genes of biotic stresses (such as pathogens and insects) and abiotic stresses (such as drought and salt) for common wheat, so we investigate the genetic diversity in wheat resources, to broaden genetic variation in wheat breeding [38]. Therefore, the evaluation of genus *Triticum* and determining genomic relationship between them is necessary for identification of desirable and transferable gene resources. Genetic diversity in wheat was characterized using morphological traits [31, 34] and DNA based markers such as random amplified polymorphic (RAPD) [23], amplified fragment length polymorphism (AFLP) [3], restriction fragment length polymorphism (RFLP) [5] and microsatellites [16, 9, 19]. In the past, morphological traits were used as marker for assessing genetic diversity but these markers are often influenced by environment and therefore are unreliable. However, DNA based markers has enhanced the utilization of biotechnology in crop improvement [21, 36]. PCR based markers have been shown to be powerful

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Table 1. Cytogenetic classification of *Triticum* species [10, 20, 39 and 33].

Ploidy level	Name	Group No.	Species	Comments
Diploid 2n= 2x= 14	Einkorn AA	**Group I**	*T. monococcum* ssp. *aegilopoides* Link. em. Thell.; syn. *T. boeoticum* Boiss.	domesticated form (*T. monococcum* L. ssp. *monococcum*)
		Group II	*T. urartu* Tum.ex. Gandil	existing only as a wild form that restricted to the Fertile Crescent regions
Tetraploid 2n= 4x= 28	Emmer AABB	**Group I**	*T. turgidum* L.	one wild form (*T. turgidum* ssp. *dicoccoides* Korn. ex Asch. & Graebnerem. Thell.) and several cultivated subspecies
	Timopheevii AAGG	**Group II**	*T. timopheevii*	one wild ancestor (*T. timopheevii* ssp. *armeniacum* Jakubz. em. Slageren) and one cultivated form (*T. timopheevii* ssp. *timopheevii*)
Hexaploid 2n= 6x= 42	Common wheat AABBDD	**Group I**	*T. aestivum* L.	a cultivated form (*T. aestivum* L.) with several subspecies
		Group II	*T. zhukovskyi* Menabde & Ericzjan,	often used in breeding programmes.

tools for studying genetic diversity and discriminating wheat cultivars [8, 29 and 35]. Simple sequence repeats (SSRs) or microsatellites are PCR-based markers with high level of polymorphism that permits to discriminate among cultivars and even among closely related wheat breeding lines [28]. Moreover, SSR is widely used in linkage mapping, QTL mapping, marker-assisted selection and phylogenetic survey. In fact, SSRs are co-dominant, locus-specific and generally have high polymorphic information content. Microsatellite loci are also multiallelic, thus suggesting their relative superiority in detecting DNA polymorphism [22, 27].

In the current study, the genomic relationships and diversity analysis of cultivated and wild wheats with different ploidy levels were evaluated by microsatellite markers.

MATERIALS AND METHODS

Plant materials

Thirty seven *Triticum* accessions were used in this study which including thirteen wild einkorn, four *T.urartu*, seven durum wheat and six common wheat collected from different eco-geographical regions of western and north-western of Iran. Moreover, three *T. zhukovskyi* and four domesticated einkorn obtained from Triticarte P/L - Australia (Table 2).

Molecular analysis

DNA extraction was performed from two weeks-old fresh leaves using CTAB method [24] with minor modification (2% CTAB, 4M NaCl, 100mM Tris-HCl, 0.5M EDTA. Na, 0.20% β-mercaptoethanol). Thirty-five primer pairs described by Röder *et al.* [30] were used in the current study (Table 3). The selected primers covered all chromosomes i.e. each chromosome was covered by five pair of primers. Each 25-µl PCR reaction included 100 ng DNA, 0.5 µM of each primer, 0.2 mM dNTPs, and 0.75U *Taq* DNA polymerase. The PCR conditions were an initial denaturation step of 4 min at 94°C and then 35 cycles as follows: 30 s at 94°C, 30 s at 60 or 65°C then 30 s at 72°C. After 35 cycles a final extension of 10 min at 72°C was done. PCR products were run on 1.5% agarose gel in TBE (1x) buffer for 1.5h. Ethidium bromide was used as staining dye for detection of amplified fragments.

Statistical analysis

Detected bands were scored according to their presence or absence at samples. Genetic distances were calculated by Nei and Li [25] using UPGMA method with DARwin program v. 5.0.146. MEGA v. 3.1 program was used for constructing phylogenetic tree.

Polymorphism information content values were calculated for each primer and chromosome by Anderson *et al.* [2] (Eq. 1).

$$\text{Eq.1)} \qquad PIC = 1 - \sum_{i=1}^{n} Pi$$

(where Pi is the frequency of the ith allele)

Table 2. Description of wild and cultivated wheat accessions used in this study.

Species	Genome	Location	Longitude	Latitude	Height
Common wheat-1	BBA^uA^uDD	CV Chamran	-	-	-
Common wheat-2	BBA^uA^uDD	CV Darab	-	-	-
Common wheat-3	BBA^uA^uDD	CV Shirvan	-	-	-
Common wheat-4	BBA^uA^uDD	CV Karmishe	-	-	-
Common wheat-5	BBA^uA^uDD	CV Sardary	-	-	-
Common wheat-6	BBA^uA^uDD	CV Azar	-	-	-
***T. zhukovskyi*-1**	$A^tA^tA^mA^mGG$	Triticarte P/L (Australia)	-	-	-
***T. zhukovskyi*-2**	$A^tA^tA^mA^mGG$	Triticarte P/L (Australia)	-	-	-
***T. zhukovskyi*-3**	$A^tA^tA^mA^mGG$	Triticarte P/L (Australia)	-	-	-
Durum wheat-1	BBA^uA^u	Shush	48.244	32.194	63
Durum wheat-2	BBA^uA^u	Kermanshah	47.065	34.314	1389
Durum wheat-3	BBA^uA^u	Behbahan	50.245	30.598	326
Durum wheat-4	BBA^uA^u	Kermanshah	47.065	34.314	1389
Durum wheat-5	BBA^uA^u	Khoramabad	48.361	33.477	1457
Durum wheat-6	BBA^uA^u	Shush	48.244	32.194	63
Durum wheat-7	BBA^uA^u	Bistoun	46.436	34.385	1671
Wild einkorn-1	A^mA^m	Qorveh	47.804	35.157	1925
Wild einkorn-2	A^mA^m	Kermanshah	47.065	34.314	1389
Wild einkorn-3	A^mA^m	Ahar	47.087	38.47	1327
Wild einkorn-4	A^mA^m	Khoramabad	48.361	33.477	1457
Wild einkorn-5	A^mA^m	Huband	46.292	38.08	1395
Wild einkorn-6	A^mA^m	Marivan	46.175	35.522	1286
Wild einkorn-7	A^mA^m	Kermanshah	47.065	34.314	1389
Wild einkorn-8	A^mA^m	Taleqan	52.233	33.267	1979
Wild einkorn-9	A^mA^m	Islamabad	46.528	34.111	1326
Wild einkorn-10	A^mA^m	Naqan	50.742	31.926	1874
Wild einkorn-11	A^mA^m	Sepiddasht	51.181	32.133	2154
Wild einkorn-12	A^mA^m	Junqan	50.691	32.151	2039
Wild einkorn-13	A^mA^m	Khoramabad	48.361	33.477	1457
***T. urartu*-1**	A^uA^u	Marivan	46.175	35.522	1286
***T. urartu*-2**	A^uA^u	Shahrekord	50.864	32.326	2061
***T. urartu*-3**	A^uA^u	Naqan	50.742	31.926	1874
***T. urartu*-4**	A^uA^u	Kermanshah	47.065	34.314	1389
Einkorn-1	A^mA^m	Triticarte P/L (Australia)	-	-	-
Einkorn-2	A^mA^m	Triticarte P/L (Australia)	-	-	-
Einkorn-3	A^mA^m	Triticarte P/L (Australia)	-	-	-
Einkorn-4	A^mA^m	Triticarte P/L (Australia)	-	-	-

RESULTS

Twenty-two out of 24 SSR markers showed polymorphism and 2 markers produced monomorphic. From 22 SSR markers, 109 alleles were detected. The number of allele per locus ranged from two to eleven with an average of 4.95 alleles per locus. The maximum number of alleles was observed with *Xgwm*165 (chromosome 4A) and *Xgwm427* (chromosome 6A), and minimum number of alleles were observed with *Xgwm*357 (chromosome 1A) and *Xgwm*570 (on chromosome 6A). The highest value of PIC (0.90) was recorded for *Xgwm*165 on chromosome 4A). The highest average of PIC value was for chromosome 5A (Table 4).

Cluster analysis based on UPGMA method using the Jacard genetic dissimilarity coefficient revealed that 37 genotypes were clustered into three main groups (Fig. 1). Group I consist of seven durum wheat, two *T. urartu*, six wild einkorn, and six common wheats, group II consist of two *T. urartu*, and seven wild einkorn, and group III consist of four einkorn and three *T. zhukovskyi* and were common in A^mA^m genomes. Group I divided to three sub-group contained diploid, tetraploid and hexaploid species that indicating closely relationship and similar evolutionary pattern. Group Ia included accessions with genome BBA^uA^u group Ib included accessions with BBA^uA^uDD and group Ic included accessions with genome A^bA^b and A^uA^u.

Table 3. Chromosomal location, Locus and Primers (Forward and reverse).

Locus	Primer sequences	Loc[a]	Locus	Primer sequences	Loc[a]
Xgwm164-1A	ttgtaaacaaatcgcatgcg acatttctcccccatcgtc	C	**Xgwm165-4A**	cttttctttcagattgcgcc tgcagtggtcagatgtttcc	S
Xgwm99-1A	gccatatttgatgacgcata aagatggacgtatgcatcaca	L	**Xgwm397-4A**	ctgcactctcggtataccagc tgtcatggattatttggtcgg	L
Xgwm33-1A	cactgcacacctaactacctgc ggagtcacacttgtttgtgca	S	**Xgwm410-5A**	cgagaccttgagggtctaga gcttgagaccggcacagt	L
Xgwm357-1A	aggctgcagctcttcttcag tatggtcaaagttggacctcg	L	**Xgwm595-5A**	gccacgcttggacaagatat gcatagcatcgcatatgcat	L
Xgwm497-1A	ccgaaagttgggtgatatac gtagtgaagacaagggcatt	L	**Xgwm126-5A**	gttgagttgatgcgggagg cacacgctccaccatgac	L
Xgwm10-2A	tggtcgtaccaaagtatacgg cgcaccatctgtatcattctg	L	**Xgwm156-5A**	caatgcaggccctcctaac ccaaccgtgctattagtcattc	L
Xgwm312-2A	acatgcatgcctacctaatgg atcgcatgatgcacgtagag	L	**Xgwm186-5A**	cgcctctagcgagagctatg gcagagcctggttcaaaaag	L
Xgwm372-2A	gaaggacgacattccacctg aatagagccctgggactggg	S	**Xgwm334-6A**	aacatgtgtttttagctatc aatttcaaaaaggagagaga	S
Xgwm249-2A	ctgccatttttctggatctacc caaatggatcgagaaaggga	L	**Xgwm427-6A**	agtgtgttcatttgacagtt aaacttagaactgtaatttcaga	L
Xgwm95-2A	aatgcaaagtgaaaaacccg gatcaaacacacacccctcc	L	**Xgwm169-6A**	gtgctctgctctaagtgtggg accactgcagagaacacatacg	L
Xgwm2-3A	cattctcaaatgatcgaaca ctgcaagcctgtgatcaact	C	**Xgwm459-6A**	agcttctctgaccaacttctcg atggagtggtcacactttgaa	S
Xgwm480-3A	ccgaattgtccgccatag tgctgctacttgtacagaggac	L	**Xgwm570-6A**	atgggtagctgagagccaaa tcgcctttacagtcggc	L
Xgwm369-3A	accgtgggtgttgtgagc ctgcaggccatgatgatg	S	**Xgwm130-7A**	ctcctctttatatcgcgtccc agctctgcttcacgaggaag	L
Xgwm32-3A	tgcttggtcttgagcatcac tatgccgaatttgtggacaa	C	**Xgwm260-7A**	cgcagctacaggaggcc gccccttgcacaatc	S
Xgwm162-3A	agaagaagcaaagccttccc agtggatcgacaaggctctg	L	**Xgwm282-7A**	tctcattcacacacaacactagc ttggccgtgtaaggcag	C
Xgwm4-4A	cactgtctgtatcactctgct gctgatgcatataatgctgt	S	**Xgwm164-7A**	agtgctggaaagagtagtgaagc agccagcaagtcaccaaaac	L
Xgwm610-4A	aatggccaaaggttatgaagg ctgccttctccatggtttgt	L	**Xgwm99-7A**	ttgtaaacaaatcgcatgcg ttcctcactgtaagggcgtt	S
Xgwm637-4A	tatacggttttgtgaggggg aaagaggtctgccgctaaca	L			

[a] C: centromere, L: long arm, S: short arm

The analysis of average genetic distance within groups showed that the highest and lowest genetic distance was within *T. urartu* and *T. zhukovskyi*, respectively (Table.5). The highest average genetic distance was between common wheat and einkorn and the lowest was between *T. urartu* and wild einkorn. Diploid species, wild einkorn and *T. urartu* collected from West and North-West of Iran were more closely related than einkorn species. Moreover, average genetic distance between common wheat and *T. urartu* was lowest, which illustrate genome A^uA^u in *T. urartu* is more similar to genome common wheat than other species. Principle coordinate analysis (PCoA), clustered genotypes into three groups (Fig. 2). The first axis indicated 33.36% of the total variation.

DISCUSSION

The knowledge about genetic structure and relationship of wild relatives of crop plants can be used to obtain information about population divergence that is important for conservation of genetic diversity in exploration of natural genetic resources and germplasm resources management for crop plants improvement.

Table 4. PIC value, number of alleles and means of number allele per locus revealed by SSR markers.

Locus (chromosome)	PIC value	Number of alleles
Xgwm164-1A	0.62	3
Xgwm99-1A	0.23	2
Xgwm497-1A	0.64	4
Mean	0.49	3
Xgwm10-2A	0.54	5
Xgwm312-2A	0.45	2
Xgwm372-2A	0.45	2
Xgwm249-2A	0.63	2
Xgwm95-2A	0.61	3
Xgwm2-3A	0.88	9
Mean	0.53	4.6
Xgwm369-3A	0.63	3
Xgwm32-3A	0.75	6
Xgwm162-3A	0.61	4
Mean	0.72	4.33
Xgwm4-4A	0.77	5
Xgwm165-4A	0.90	11
Xgwm397-4A	0.78	6
Mean	0.81	7.33
Xgwm126-5A	0.78	7
Xgwm156-5A	0.82	7
Mean	0.80	7
Xgwm334-6A	0.77	5
Xgwm427-6A	0.86	11
Xgwm169-6A	0.63	3
Mean	0.75	7.67
Xgwm130-7A	0.80	5
Xgwm99-7A	0.46	2
Mean	0.63	3.5
Total mean	0.68	4.95

Table 5. Average genetic distance within and between Triticum accessions.

Species	Within species	Between species				
		Common wheat	Wild Einkorn	*T. urartu*	Durum wheat	*T. zhukovskyi*
Common wheat	0.680	-				
Wild Einkorn	0.826	0.116	-			
T. urartu	0.864	0.108	0.009	-		
Durumwheat	0.738	0.146	0.114	0.085	-	
T. zhukovskyi	0.545	0.304	0.218	0.139	0.267	-
Einkorn	0.568	0.326	0.22	0.161	0.278	0.253

Genetic diversity of wheat from West and North-West of Iran are reflected by average distance within these populations. The highest genetic diversity within-group were in *T. urartu* and wild einkorn species, but distance between them was lowest than other groups. Moreover, *T. urartu* and wild einkorn accessions were clustered together in group II and Ic (Fig.1), that shown SSR fragments investigated in this study have conserved during evolution. Genetic distance among *T. urartu*, durum wheat and common wheat was lower than genetic distance between wild einkorn and einkorn. This result is in agreement with previous reports described by Dvorak et al. [10] and Brandolini et al. [4] that recognized *T. urartu* are the donor of A^u genome to the polyploid wheats. On the other hand, Brandolini et al. [4] investigated A genome of wheat by RFLP markers, observed that all of einkorn species were clustered together and were separated from *T. urartu* species. Cultivated Durum wheat or landrace from Iran has a higher genetic diversity than the common wheat landraces. One reason might be the different domestication history of durum and common wheat. Wild form of tetraploid wheats has exchanged genes with emmer wheats at early stages of domestication and with durum wheat recently, that caused to a high level of polymorphism in durum wheat landraces [15].

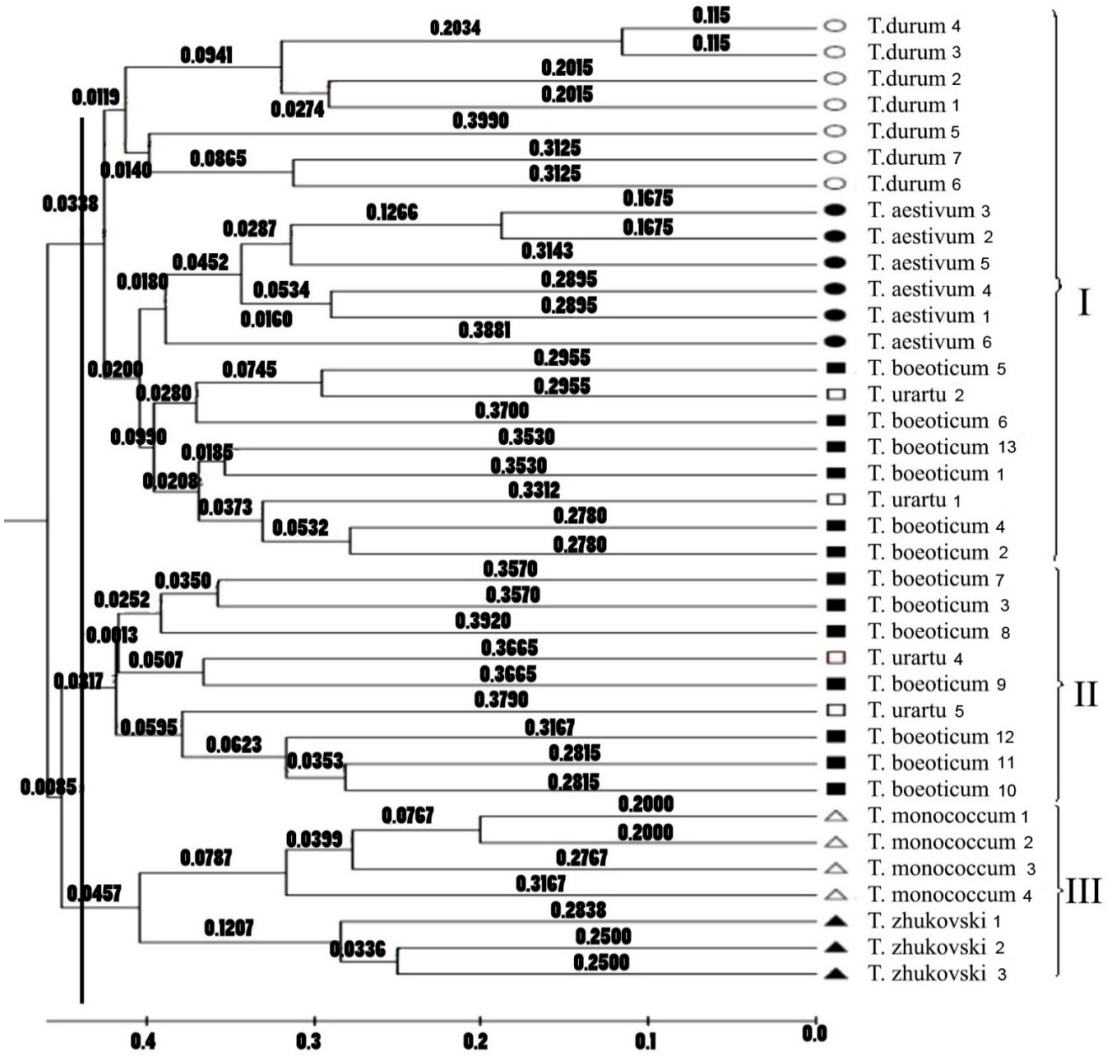

Figure 1. The clustering of 37 accessions of wheat at different ploidy level with 109 alleles from 22 polymorphics loci.

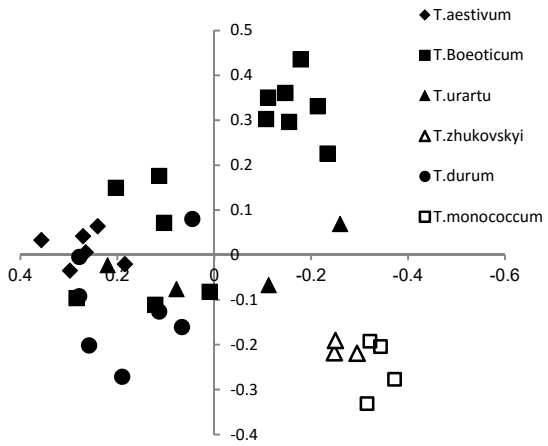

Figure 2. Principle coordinates analysis based on molecular data obtained from Jacard matrix.

In this study, accessions from different geographical areas fall in the same cluster. For example, the wild accession *T. urartu* and wild einkorn collected from Marivan, Shahrekord, Kermanshah, Khoramabad, Sefid Dasht, Huband, Ahar and Naghan joined together in the phylogenetic tree. This finding support previous investigation of Sasanuma et al that wild wheat from different region fall in same group and phylogenetic relationship among populations does not seem related with their geographical origin [32]. Also Al-Khanjari et al [1] found that all hexaploid landrace accessions originated from the same geographic didn't cluster in the same group. Moreover, because North-West of Iran is one of the origin centers of wheat [14], some wild and

cultivated wheats from different locations have clustered in the same group. Moreover, these accessions from same geographical locations were in the different cluster.

In this study using SSR markers, relationships and genetic diversity of wild and cultivated wheats collected from West and North West of Iran were investigated. The results showed that in west of Iran (East Fertile Crescent) polymorphism exists within wild wheat relatives. Iran not only is one of the main domestication sites of hexaploid and tetraploid wheat [37] but also a main center of wild wheats distribution [18]. Therefore, it is expected that the wild populations of *Triticum* spp. in this region contained high levels of genetic diversity. We could use genetic diversity to detect desirable genes such as protein quality, amino acid content or resistance [26] and transfer these genes to durum and common wheats. Microsatellite markers are useful tools for variety identification and breeding programs.

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ارزیابی روابط و تنوع ژنومی گندم های وحشی و زراعی دارای ژنوم A در سطوح مختلف پلوئیدی با استفاده از نشانگر ریزماهواره

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چکیده

تنوع و روابط ژنتیکی ۳۷ گندم وحشی و زراعی (*Triticum* spp.) دارای ژنوم A شامل چهار *T. urartu* (Au)، سیزده اینکورن وحشی(A^mA^m)، چهار اینکورن زراعی (A^mA^m)، هفت گندم دروم (BBA^uA^u)، سه *T. zhukovskyi* ($A^tA^tA^mA^mGG$) و شش گندم نان (BBA^uA^uDD) بوسیله نشانگر ریزماهواره (SSR) بررسی گردید. فاصله‌های ژنتیکی بوسیله روش نی و لی با استفاده از UPGMA با نرم‌افزار DARwin محاسبه گردید. نرم افزار MEGA برای رسم درخت فیلوژنتیک مورد استفاده قرار گرفت. سی و پنج جفت نشانگر ریزماهواره مورد استفاده قرار گرفت که ۲۴ مورد از آنها تکثیر شد و ۲۲ نشانگر چند شکلی (با ۱۰۹ آلل) از خود نشان دادند. بیشترین قطعات تکثیر شده (۱۱ آلل) و محتوای اطلاعات چندشکلی (۰/۹) برای لوکوس Xgwm165-4A بود. بیشترین و کمترین فاصله ژنتیکی درون گروه‌ها برای *T. urartu* و *T. zhukovskyi* به ترتیب برابر با ۰/۸۶ و ۰/۵۵ بود. بیشترین شباهت بین *T. urartu* و گونه‌های اینکورن وحشی (۰/۰۰۹) مشاهده گردید. بیشترین عدم تشابه بین اینکورن‌های زراعی و گندم نان مشاهده گردید. در میان گونه های دیپلوئید *T. urartu* بیشترین شباهت را به گندم دروم و گندم نان داشت.

کلمات کلیدی: تریتیکوم، ژنوم A، روابط ژنومی، نشانگر ریزماهواره