UDC 575.633.11
https://doi.org/10.2298/GENSR1801131S
Original scientific paper

GENETIC DIVERSITY OF WHEAT WILD RELATIVES USING SSR MARKERS

Marzeih SALEHI[1], Ahmad ARZANI[1*], Majid TALEBI[2], and Asad ROKHZADI[3]

[1]Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan-8415683111, Iran
[2]Department of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan-8415683111, Iran
[3]Agronomy Department, Islamic Azad University-Sanandaj Branch, Sanandaj, Iran.

Salehi M., A. Arzani, M. Talebi, and A. Rokhzadi (2018): *Genetic diversity of wheat wild relatives using SSR markers*.- Genetika, Vol 50, No.1, 131-141.

Wild relatives of wheat are potential sources of valuable genetic materials for wheat improvement. Knowledge of the genetic diversity of wild relative species of wheat is crucial for their conservation and utilization. The objective of the current study was to investigate the genetic diversity of inter and intra species of *Triticum monococcum* ssp. *aegilopoides* (AA), *Aegilops tauschii* (DD) and *Aegilops cylindrica* (CCDD) originating from northern and western Iran. Thirty microsatellite (SSR) markers belonging to A, B, C and D genomes were used for analysis and 20 found to be polymorphic within and between species. The SSR markers generated a total number of 180 alleles with an average of 9 alleles per locus in 21 genotypes. The genetic diversity for all loci ranged from 0.74-0.90 with an average of 0.83. The highest genetic diversity was estimated for *Xgwm186* and *Xgwm205* which the latter could amplify in the A, D and CD genomes of *T. monococcum*, *Ae. tauschii* and *Ae. cylindrica*, respectively. In addition, the number of bands generated by *Xgwm*205 along with other four markers in *Ae. cylindrica* (CD) was two-fold than that of *Ae. tauschii* (D). Polymorphic information content ranged from 0.7-0.89 with an average of 0.82. The dendrogram obtained from the neighbor-joining method divided the genotypes of the three species into three distinctive groups. It can be concluded that SSR markers can be useful not only in differentiating wild species of wheat possessing A, D and C genomes, but also in assessing the genetic variation of genotypes within these species.

Keywords: Aegilops spp., *Triticum monococcum*, SSR markers, wild species

Corresponding author: Ahmad Arzani, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan-8415683111 Iran, Tel. +98 31 33913453, Fax. +98 31 33912254, E-mail: a_arzani@cc.iut.ac.ir

INTRODUCTION

Wild relatives of cultivated wheat are potential sources of valuable genetic materials for wheat improvement. Because biodiversity is fundamental to the breeder efforts, management of genetic resources via exploration, collection, characterization, evaluation and conservation needs to be reinforced. While limited genetic variability has been found within primary gene pool of bread wheat germplasm, additional genetic variation requires being exploited (GUPTA, 2016). The genetic diversity of wild species belonging to primary and secondary gene pools of wheat is important in employing in wheat breeding program, particularly for tolerance to biotic and abiotic stresses (ARZANI and ASHRAF, 2016). In most cases, the domestic gene pools lack the genetic diversity to enable plant breeders to select for the required abiotic stress tolerance (REDDEN, 2015; ARZANI and ASHRAF, 2016). Many agronomically important characteristics, including resistance to biotic and abiotic stresses, have been transferred from the relative wild species to wheat. The genes from the homologous genome in wild relatives can be easily introgressed into the cultivated wheat through the recombination of the homologous chromosomes (ARZANI and ASHRAF, 2016). The role of introducing genes from the wild relatives into crop cultivars of major importance to global food security has been emphasized (REDDEN, 2015; BROZYNSKA *et al*., 2015).

Aegilops species are closely related to the genus *Triticum* and therefore, serve as an important reservoir of agronomically valuable traits, in particular for resistance to biotic and abiotic stresses. *Ae. tauschii,* as a progenitor of the D-genome of common wheat, is a potential donor of genetic variability for the improvement of common wheat (KHALIGHI *et al.*, 2008; NAGHAVI *et al*., 2009; SCHNEIDER *et al*., 2008). Jointed grass (*Ae. cylindrica* Host; 2n=4x=28; genome CCDD) is an allotetraploid of the *Triticeae* tribe that is formed via amphidiploidization of a hybrid or hybrids between *Ae. tauschii* Coss. (2n=2x=14; DD) and *Ae. markgrafii* (Greuter) Hammer (2n=2x=14; CC). *Ae. cylindrica* is known as a source of useful traits such as salinity and cold tolerance (FAROOQ *et al*., 1996; KIANI *et al*., 2015; ARZANI and ASHRAF, 2016). *T. monococcum* (2n=2x=14; AA) is considered as the origin of genome A in the wheat and is also known as a source of stem rust resistance genes (ROUSE and JIN, 2011).

Molecular markers are a promising tool for evaluating genetic diversity among plant materials, including RFLP, AFLP, RAPD and SSR (TEKLU *et al*., 2007). SSR markers are tandem repeat motifs composed of one to six nucleotides, which are ubiquitous, abundant, codominant and highly polymorphic (TEKLU *et al*., 2007). The SSR primers have the potential to be used for amplifying orthologous sequences in phylogenetically closely related species of the wheat (KUMAR, *et al*., 2016). LEONOVA *et al.* (2009) used SSR markers to assess the polymorphism between the accessions belonging to *Aegilops cylindrica* (DDCC) and *Aegilops triuncialis* (UUCC) and found 21 SSR markers revealing polymorphism between the accessions of these species.

GHORBANI *et al.* (2015) reported a higher intraspecific variability in meiotic attributes obtained for the studied species of *Ae. cylindrica* than *T. monococcum* ssp. *aegilopoides*. They accordingly hypothesized that *Ae. cylindrica* may be considered as being a recently originated amphidiploid. However, there is little information available on the genetic variation in these species at molecular level. Therefore, the objective of this study was to assess the inter and intra specific genetic diversity of *T. monococcum* ssp. *aegilopoides* (AA), *Ae. tauschii* (DD) and *Ae. cylindrica* (CCDD) species, originating in Iranian habitats differing climatic conditions, with SSR molecular markers.

MATERIALS AND METHODS

Plant materials

In this study, 21 genotypes including eight genotypes of *T. monococcum* ssp. *aegilopoides*, eight genotypes of *Ae. cylindrica* and five genotypes of *Ae. tauschii* collected from northern and western Iran were used. Details of collection sites are given in Table 1. Individual plants were taken from each collection site, the collected seeds of each plant were grown in a separate row and thus seed increase for each pure line (from now on termed "genotype") was conducted in 2011-2012 growing season under field conditions. A geographical representative of *Ae. cylindrica* genotypes has already been studied for salinity tolerance (KIANI *et al*., 2015) was used in this study.

Table 1. The studied genotypes belong to Aegilops cylindrica (Ac), Triticum monococcum subsp. aegilopoides (Tm) and Aegilops tauschii (At) species with their locations and geographical coordinates

Genotypes*	Location	Longitude	Latitude	Altitude
Ac1, Tm1	Km 55, Kermanshah-Ravansaer Rd	46º 42' 022" E	35º 39' 089' N	1397m
Ac2, Tm2	Km 44, Kermanshah-Ravansaer Rd	46º 46 602" E	34º 33' 457" N	1335 m
Ac3, Tm3	Paghaleh village, Kamyaran	46º 53' 475" E	34º 43' 336" N	1408 m
Ac4, Tm4	Km 55, Kermanshah-Ravansaer Rd	46º 42' 022" E	34º 39' 089" N	1397 m
Ac5, Tm5	Km 55, Kermanshah-Ravansaer Rd	46º 42' 022" E	34º 39' 089" N	1397 m
Ac6, Tm6	Km 44, Kermanshah-Ravansaer Rd	46º 46' 602" E	34º 33' 457" N	1335 m
Ac7, Tm7	Km 44, Kermanshah-Ravansaer Rd	46º 46' 602 E	34º 33' 457" N	1335 m
Ac8, Tm8	Gelkanvillage, Sanandaj-Marivan Rd	46º 55' 268" E	35º 24' 33" N	1675m
At1	Babolsar, Caspian Sea shore	---	---	---
At2	Ramsar, Caspian Sea shore	---	---	---
At3	Fereidoon'kenar, Caspian Sea shore	---	---	---
At4	Noushahr, Caspian Sea shore	---	---	---
At5	Farahabad, Caspian Sea shore	---	---	---

DNA isolation and PCR amplification

DNA was isolated from the fresh leaf tissues of plants according to CTAB protocol (MURRAY and THOMPSON 1980) with minor modifications. Thirty SSR markers have already been reported to be highly polymorphic in A, B, C and D genomes, were used for analysis (GOLABADI *et al*., 2011; LEONOVA *et al*., 2009). DNA was quantified electrophoretically using lambda standard DNA on 0.7% agarose gel. The PCR reaction was performed in a 15 µL volume containing 2 µL of DNA template (~50 ng), 1.5 µl of 10X PCR buffer, 0.7 µl of MgCl2, 0.3 µl of dNTPs, 4 pmol of each primer and 2.5 unit of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania). The PCR amplification was performed using a thermal cycler (BioRad model My Cycler™ thermal Cycler) under conditions (5 min of initial denaturation at 94°C was followed by 35 cycles of 1 min at 94°C, 1 min at the respective annealing temperature of 51-59°C (Table 2), 2 min at 72°C and the final extension step of 72°C for 10 min). The PCR products of

selective amplification were electrophoresed on 12–16% non-denaturing polyacrylamide gels. A 100-bp DNA ladder was used to estimate the size of DNA fragments. Gels were stained using the silver staining protocol for visual detection (BASSAM *et al*. 1991).

Data analysis

The genotypes used in this study were homozygous, because individual plants were taken at the collection sites, the seeds of each plant were grown in a separate row and seed increase for each pure line was conducted in 2011-2012 growing season under field conditions. The data was obtained by scoring based on allele size differences and imported into a binary data matrix for further analysis. The number of alleles, the observed number of genotypes, gene diversity and polymorphic information content (PIC) at each SSR locus were estimated using Power Marker version 3.25 (LIU and MUSE, 2005). Based on SSR data, cluster analysis of the genotypes was conducted using neighbor-joining method. Principal coordinate analysis (PCoA) was also conducted to identify the genetic relationships among genotypes using NTSYS-PC (ROHLF, 2002). Analysis of molecular variance (AMOVA) was performed using Arlequin version 3.1 (EXCOFFIER *et al*., 1992) to determine molecular variation within and among species.

RESULTS

PCR amplification and genetic diversity

Twenty out of thirty used SSR markers designed for wheat were highly polymorphic with sharp amplification bands in 21 genotypes belonging to the three wild species. Figure 1 shows an example of a polyacrylamide gel profile generated using *Xgwm165* primer pairs. The total number of detected alleles was 180 with an average 9 alleles per locus. The number of alleles per locus ranged from 5 for the *Xgwm131* to 13 for the *Xgwm186*. The *Xgwm186* marker was able to discriminate between A and CD genomes by producing specific DNA band for each of them. On the other hand, SSR markers *Xgwm*131, *Xgwm*132, *Xgwm*205 and *Xgwm*497 could amplify A genome of *T. monococcum* ssp. *aegilopoides*, D genome of *Ae. tauschii* and C and D genomes of *Ae. cylindrica*. In addition, the number of bands generated in *Ae. cylindrica* (CD) genotypes was two-fold than that of *Ae. tauschii* (D) genotypes. The major allele frequency ranged from 0.14 for *Xgwm205* to 0.39 for *Xgwm497* with an average of 0.27 (Table 2).

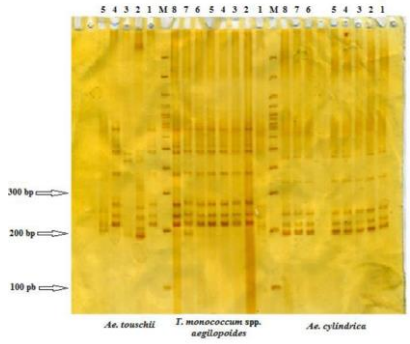

Figure 1. Polyacrylamide gel profile in three wheat wild specie using *Xgwm165* primer. Numbers represent the genotypic numbers listed in Table 1 and M is a lane of 100 bp molecular ladder.

Genetic diversity ranged from 0.74-0.90 with an average of 0.83. The highest genetic diversity was estimated for *Xgwm186* and *Xgwm205* and the lowest for *Xgwm131*. All primers showed polymorphic lines that ranged from 0.7 for *Xgwm131* to 0.89 for *Xgwm186* and *Xgwm205* with an average value computed for all loci of 0.82 (Table 2).

The matrix of genetic distance was formed using the neighbor-joining method. Genetic distance coefficients between genotypes ranged from 0.32 to 1, indicating a high genetic diversity among genotypes studied.

Table 2. Summary of microsatellite allele data revealed by 20 polymorphic microsatellite loci in the studied genotypes

Primer	Major allele frequency	Number of allele	Number of genotype	Gene diversity	PIC	annealing temperature
Xgwm 3	0.31	6	6	0.78	0.75	57.5
Xgwm 60	0.25	9	9	0.85	0.84	58
Xgwm71.2	0.24	9	9	0.85	0.83	59
Xgwm131	0.38	5	5	0.74	0.70	59
Xgwm132	0.32	10	10	0.84	0.83	53
Xgwm135	0.25	7	7	0.82	0.80	58
Xgwm148	0.19	10	10	0.88	0.87	57
Xgwm160	0.33	7	7	0.80	0.78	57
Xgwm165	0.24	9	9	0.84	0.83	58
Xgwm186	0.19	13	13	0.90	0.89	59
Xgwm190	0.19	9	9	0.86	0.85	59
Xgwm191	0.30	10	10	0.85	0.84	59
Xgwm205	0.14	12	12	0.90	0.89	59
Xgwm219	0.19	10	10	0.88	0.87	59
Xgwm299	0.33	8	8	0.78	0.76	51
Xgwm332	0.24	9	9	0.85	0.84	59.5
Xgwm497	0.39	8	8	0.77	0.74	59
Xgwm499	0.35	8	8	0.80	0.78	58
Xgwm518	0.22	10	10	0.87	0.86	53
Xgwm604	0.26	11	11	0.86	0.85	51
Mean	0.27	9	9	0.84	0.82	57.15

Principal coordinate analysis (PCoA) and cluster analysis

The PCoA was used to visualize the genetic relationships among genotypes. The first three components accounted for 25.71% of the total variation, implying that the used markers possessed a suitable dispersion of markers in the genome. Since the first components do not explain much of the total variation, the analysis of genetic relationships among genotypes should be based on cluster analysis.

The wild wheat genotypes belonging to the three species were clearly discriminated based on the dendrogram generated by cluster analysis of SSR markers (Figure 2). The *T. monococcum* spp. *aegilopoides* and *Ae. tauschii* seemed to be more closely related than *Ae. cylindrica*.

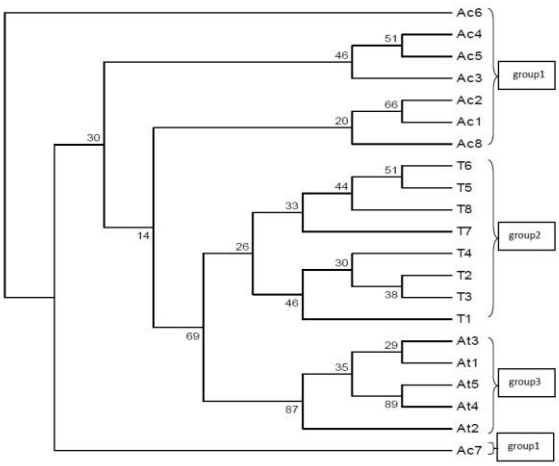

Figure 2. Neighbor-joining dendrogram of 21 genotypes belonging to three wild-relative species of wheat based on SSR markers. More details on the origin of the genotypes can be found in Table 1.

Analysis of molecular variance

Analysis of molecular variance revealed a significantly higher within-species genetic variation than that between species, where 76.4% of the computed genetic variation belonged to within species and the remaining 23.6% belonged to between species (Table 3). The AMOVA results revealed that there were significant (P=< 0.001; F_{ST}= 0.23571) genetic differences between the three species. AMOVA results also provided other parameters such as mean gene copy number, number of alleles, expected heterozygosity and genetic diversity. *T. monococcum* spp. *aegilopoides* and *Ae. tauschii* possessed the highest and lowest values of these parameters, respectively.

Table 3. Analysis of molecular variance (AMOVA) in microsatellite data among and within species

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among species	2	51.24	1.51	23.57
Within species	39	191.05	4.90	76.43
Total	41	242.29	6.41	

F_{ST} : 0.23571

DISCUSSION

The genetic diversity of 21 genotypes belonging to three species closely related to wheat (A, D and AD genomes) collected from northern and western Iran was evaluated using 20 polymorphic SSR markers. A total of 180 alleles ranging from 5 to 13 alleles per locus were

amplified (Table 2). Our results, therefore, were in agreement with those of LI *et al*. (2008), who emphasized that SSR was an effective marker system for detecting genetic diversity among wheat and related species genotypes and provided useful information about the phylogenic relationships. WANG *et al*. (2013) screened the genetic diversity of wheat (*Triticum aestivum* L.) and the related species with SSR markers and reported an average of 6.32 alleles per locus with a range of 2 to 13 alleles per locus. ZHANG *et al*. (2006) reported an average of 5.7 alleles per locus among wheat landraces from Oman with a range of 2 to13 alleles per locus. In contrast, some studies have observed higher allelic diversity. BORDBAR *et al*. (2011) used 25 pairs of SSR primers to analyze D-genome species of *Aegilops* and *Triticum* from Iran and detected a total of 500 alleles, with each primer detecting 13 to 30 alleles (average 20.1).

Application of molecular markers to monitor alien chromatin during introgression is a powerful complement to other methods for identifying desirable genes in a successful transfer. Thus, the availability of molecular markers which are specific to the alien chromosome region in wheat background increases the efficiency of identifying translocations and shorter recombinants (GONG *et al*., 2016). The generation of A, C and D genome specific PCR primers by means of microsatellite analysis will be fruitful to trace their chromatin introduced into wheat, as in the case of our study. The *Xgwm186* marker was able to discriminate between A and CD genomes. On the other hand, all other SSR markers located on the A genome in *T. monococcum* spp. *aegilopoides* were able to amplify the D genome in *Aegilops* species with considerable polymorphism. GUYOMARCH *et al*. (2002) showed that SSR markers amplifying products from A and B genomes generated amplification fragments which were mapped on the D genome. In this study, SSR markers *Xgwm*71.2, *Xgwm*131, *Xgwm*132, *Xgwm*205 and *Xgwm*497 could also be amplified in C genome, because the band number produced by these markers in *Ae. cylindrica* (CD) was two-fold than that of produced in *Ae. tauschii* (D). Likewise, LEONOVA *et al*. (2009) demonstrated that a number of *Xgwm* markers in A, B genomes can be used to distinguish the C genome. Common alleles are apparently older, and because of a polymorphism suppressor mutation at an alternative locus could attain an intermediate frequency.

The PIC observed for *Xgwm186* and *Xgwm205* markers was the highest recorded and thus, these markers could be more efficient in genotypic differentiation of closely related species of wheat. The highest genetic diversity was obtained from the *Xgwm186* and *Xgwm205* markers, producing 13 and 12 alleles, respectively (Table 2). TAHERNEZHAD *et al*. (2010) evaluated the genetic diversity of Iranian *Ae. tauschii* using SSR markers and found a high level of polymorphism and genetic diversity. In addition, high level of genetic diversity in *Aegilops tauschii* using SSRs was also reported by SAEIDI *et al*. (2006). In the analysis of 31 *Triticum* and *Aegilops* genotypes using AFLP markers, KHALIGHI *et al*. (2008) reported a total of 414 reliably detectable fragments, of which 387 (93.5%) were polymorphic between two or more accessions. The relationships between allele frequency, genetic diversity and PIC revealed that the markers with higher PIC and genetic diversity possessed less major allele frequency, and therefore, could be preferentially used to aid in choosing appropriate parental genotypes for molecular mapping, or assessing the genetic diversity within a species (ANDERSON *et al*., 1993).

In our study, the genetic distance of studied genotypes ranged from 0.32 to 1, which was higher than that in the previous studies. In the analysis of genetic diversity in emmer wheat (*Triticum dicoccon* Schrank) by SSR markers, the genetic distance coefficients ranged from 0.63 to 0.97 with an average of 0.82 (TEKLU *et al*., 2007).

Dendrogram resulted from the neighbor-joining method, in which the tree was divided to three genotypic groups corresponding to *Ae. cylindrica*, *T. monococcum* spp. *aegilopoides* and *Ae. Tauschii* species (Figure 2). This was in agreement with that of a previous work using SSR markers for assessing interspecific variation in *Triticum* and *Aegilops* species. For example, LEONOVA *et al*. (2009) reported that the *Aegilops* accessions were clustered into three groups according to their species classification.

The results of AMOVA, based on SSR markers, revealed a significantly greater within species genetic variation than between species (Table 3). Likewise, a previous study on *Aegilops geniculata* and *Triticum durum* showed a greater within species genetic variation than between species (MAHJOUB *et al*. 2012).

CONCLUSION

Knowledge of the genetic diversity of wild relative species of wheat is crucial for their conservation as well as utilization in wheat breeding. In addition, the availability of molecular markers specific to alien chromosomes or arms enables identifying a chromosome region of the interest in wheat background. Our results suggested that there was a high level of genetic diversity in the *Ae. cylindrica*, *T. monococcum* and *Ae. tauschii* species as revealed by SSR analysis. It can, therefore, be assumed that SSR markers are not only useful in differentiating wild species of wheat (A, C and D genomes), but also in assessing the genetic diversity of genotypes within species. To manage, conserve and exploit wheat genetic resources, the studied species can serve as a readily available source of potentially useful variation for wheat improvement. Furthermore, SSR markers have the potential to enhance the efficiency of discriminations in various wheat alien gene introgressions in order to promote wheat improvement, particularly for tolerance to biotic and abiotic stresses.

ACKNOWLEDGMENT

The authors thank Professor Junhua Peng for his assistance and guidance in collecting the germplasm used in this study.

Received, February 11th, 2017
Accepted November 18h, 2017

REFERENCES

ARZANI, A, A. ASHRAF (2016): Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Critical Reviews in Plant Sciences, *35*:146–89.

ANDERSON, J.A., G.A. CHURCHILL, J.E. AUTRIQUE, S.D. TANKSLEY, M.E. SORRELLS (1993): Optimizing parental selection for genetic linkage maps. Genome, *36*: 181–186.

BASSAM, B.J., G.C. ANOLLES, P.M. GRESSHOFF (1991): Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry *196*: 80–83.

BROZYNSKA, M., A. FURTADO, R.J. HENRY (2015): Genomics of crop wild relatives: expanding the gene pool for crop improvement. Plant Biotechnology Journal, *14*: 1070-1085.

BORDBAR, F., M.R. RAHIMINEJAD, H. SAEIDI, F.R. BLATTNER (2011): Phylogeny and genetic diversity of D-genome species of *Aegilops* and *Triticum* (*Triticeae, Poaceae*) from Iran based on microsatellites, ITS, and trnL-F. Plant Systematics and Evolution, *291*:117-131.

EXCOFFIER, L., P.E. SMOUSE, J.M. QUATTRO (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. Genetics, *131*: 479–491.

FAROOQ, S., T.M. SHAH, M. ASGHAR (1996): Intergeneric hybridization for wheat improvement. Cereal Research Communications, *24*: 155–161.

GHORBANI, F., A. ARZANI, M.M. POURSIAHBIDI (2015): Meiotic instability in *Aegilops cylindrica*: a comparison with stable meiosis in *Triticum monococcum* subsp. *aegilopoides*. Caryologia, *68*: 101-108.

GOLABADI, M., A. ARZANI, S.A.M. MIRMOHAMMADI MAIBODY, B.E. SAYED TABATABAEI, S.A. MOHAMMADI (2011): Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. Euphytica, *177*: 207-221.

GONG, W., W. GONG, R. HAN, G. LI, S.K. SEHGAL, H. LI, A. LIU, J. SONG, G. SONG, C. LIU, J. LIU (2014): Chromosome arm-specific markers from *Aegilops searsii* permits targeted introgression. Biologia, *71*: 87-92.

GUPTA, P.K. (2016). Use of alien genetic variation for wheat improvement. In: V.R. Rajpal, S.R. Rao & S.N. Raina (Eds). *Molecular breeding for sustainable crop improvement* (pp. 1-30). Springer International Publishing Switzerland.

GUYOMARCH, H., P. SOURDILLE, G. CHARMET, K.J. EDWARDS, M. BERNARD (2002): Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theoretical and Applied Genetics, *104*: 1164–1172.

KHALIGHI, M., A. ARZANI, M.A. POURSIAHBIDI (2008): Assessment of genetic diversity in *Triticum* spp. and *Aegilops* spp. using AFLP markers. African Journal of Biotechnology, *7*: 552-546.

KIANI, R., A. ARZANI, F. HABIBI (2015): Physiology of salinity tolerance in *Aegilops cylindrica*. Acta Physiologiae Plantarum, *37*: 135. doi 10.1007/s11738-015-1881-0.

KUMAR, S., S.K. VERMA, P. KUNDU, A. AWASTHI, I. SHEIKH, K. SANGWAN, R. PRASAD, H.S. DHALIWAL (2016): Transferability and polymorphism between group 7 chromosome specific simple sequence repeat (SSR) markers of bread wheat and its related non-progenitor *Aegilops* species. Journal of Crop Improvement, *30*: 433-446.

LEONOVA, I.N., M.S. RODER, F. NASYROVA (2009). The application of wheat microsatellite markers for the detection of interspecific variation in tetraploid *Aegilops* species with C and U genomes. Cereal Research Communications, 37: 335–343.

LIU, K., S.V. MUSE (2005): PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics, *21*: 2128–2129.

LI L.J., K.C. YANG, G.T. PAN, T.Z. RONG (2008): Genetic diversity of maize populations developed by two kinds of recurrent selection methods investigated with SSR markers. Agricultural Sciences in China, *7*: 1037-1045.

MAHJOUB, A., K. MGUIS , M. ROUAISSI , R. ABDELLAOUI, N. BEN BRAHIM (2012): RAPD analysis of genetic diversity in natural populations of *Aegilops geniculata* Roth and *Triticum durum* Desf from Tunisia. Agriculture and Biology Journal of North America, *3*: 466–475.

MURRAY, M.G., W.F. THOMPSON (1980): Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, *8*: 4321-4325.

NAGHAVI, M.R., M.J. AGHAEI, A.R. TALEEI, M. OMIDI, J. MOZAFARI, M.E. HASSANI (2009): Genetic diversity of the D-genome in *T. aestivum* and *Aegilops* species using SSR markers. Genetic Resources and Crop Evolution, *56*: 499-506.

REDDEN, R. (2015): Wild relatives for the crop improvement challenges of climate change: the adaptation range of crops. In Redden, R., Yadav, S.S., Maxted, N., Dulloo, M.E., Guarino, L. and Smith, P. (Eds) *Crop wild relatives and climate change* (pp. 61–76). New Jersey, USA: John Wiley & Sons.

ROHLF, F.J. (2002): Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York.

ROUSE, M.N., Y. JIN (2011): Stem rust resistance in A-genome diploid relatives of wheat. Plant Disease, *95*:941-944.

SAEIDI, H., M.R. RAHIMINEJAD, S. VALLIAN, J.S. HESLOP-HARRISON (2006): Biodiversity of diploid D-genome *Aegilops tauschii* Coss. in Iran measured using microsatellites. Genetic Resources and Crop Evolution, *53*: 1477–1484.

SCHNEIDER A., I. MOLNAR, M.M. LANG (2008): Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica, *163*: 1-19.

TAHERNEZHAD, Z., M.J. ZAMANI, M. SOLOUKI, M. ZAHRAVI, A.A. IMAMJOMEH, M. JAFARAGHAEI, M.R. BIHAMTA (2010): Genetic diversity of Iranian *Aegilops tauschii* Coss. using microsatellite molecular markers and morphological traits. Molecular Biology Reports, *37*: 3413-3420.

TEKLU, Y., K. HAMMER, M.S. RODER (2007): Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccon* Schrank): Analysis of genetic diversity and differentiation. Genetic Resources and Crop Evolution, *54*: 543-554.

WANG, Y.J., C.Y. WANG, H. ZHANG, Z.N. YUE, X.L. LIU, W.Q. JI (2013): Genetic analysis of wheat (*Triticum aestivum* L.) and related species with SSR markers. Genetic Resources and Crop Evolution, *60*: 1105–1117.

ZHANG, P., S. DREISIGACKER, A. BUERKERT, S. ALKHANJARI, A.E. MELCHINGER, M.L. WARBURTON (2006): Genetic diversity and relationships of wheat landraces from Oman investigated whit SSR markers. Genetic Resources and Crop Evolution, *53*: 1351-1360.

GENETIČKI DIVERZITET DIVLJIH SRODNIKA PŠENICE POMOĆU SSR MARKERA

Marzeih SALEHI[1], Ahmad ARZANI[1*], Majid TALEBI[2], Asad ROKHZADI[3]

[1]Department za poljoprivredu i oplemnejivanje, Poljoprivredni koledž, Isfahan Univerzitet za tehnologiju Isfahan-8415683111, Iran
[2]Department za poljoprivrednu biotehnologiju, Poljoprivredni koledž, Isfahan Univerzitet za tehnologiju, Isfahan-8415683111, Iran
[3]Poljoprivrendi Department, Islamic Azad Univerzitet-Sanandaj Branch, Sanandaj, Iran

Izvod

Divlji srodnici pšenice su potencijalni izvor vrednog genetičkog mateirjala za poboljšanje pšenice. Poznavnaje genetičkog diverziteta divljih srodnika je krucijalno za njihovu konzervaciju i korišćenje. Cilj ispitivanja je genetički diverzitet između i unutar vrsta *Triticum monococcum* ssp. *aegilopoides* (AA), *Aegilops tauschii* (DD) i *Aegilops cylindrica* (CCDD) poreklom iz severnog i zapadnog Irana. Trideset mikrosatelit (SSR) markera koji pripadaju A, B, C i D genomu je korišćeno za analizu i 20 je bilo polimorfno unutar i između vrsta. SSR markeri su dali ukupno 180 alela sa prosekom od 9 alela po lokusu u 21 genotip. Genetički diverzitet za sv elokuse je bio u opsegu od 0.74-0.90 sa prosekom 0.83. Najveći genetički diverzitet je utvrđen za *Xgwm186* i *Xgwm205* koji su mogli da amplifikuju u A, D i CD genomu *T. monococcum*, *Ae. tauschii* i *Ae. cylindrica*. Dodatno, broj traka dobijen sa *Xgwm*205 zajendo sa druga četri markera u *Ae. cylindrica* (CD) je bio dva puta nego *Ae. tauschii* (D). Polimorfni informativni sadržaj je bio u opsegu od 0.7-0.89 sa prosekom 0.82. Dendogram je grupisao genotipove tri vrste u tri različite grupe. Može se zaključiti da SSR marker mogu biti korisni ne smao za razlikovanje divljih srodnika koji poseduju A, D i C genom, već i za određivanje genetičke varijabilnosti genotipova unutar ovih vrsta.

Primljeno 11.II.2017.
Odobreno 18. XI. 2017.