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Original scientific paper

GENETIC DIVERSITY OF CHICKPEA GENOTYPES AS REVEALED BY ISSR AND RAPD MARKERS

Saida HASANOVA[1], Zeynal AKPAROV[1], Alamdar MAMMADOV[2], Litfer AMİROV[1], Sevda BABAYEVA[1], Jale NASİBOVA[1], Zumrud MUKHTAROVA[1], Kamila SHİKHALİYEVA[1], Vusala İZZATULLAYEVA[1], Mehraj ABBASOV[1,3]

[1]Genetic Resources Institute of National Academy of Sciences of Azerbaijan, Baku, Azerbaijan
[2]Institute of Molecular Biology and Biotechnologies of ANAS, Baku, Azerbaijan
[3]Baku State University, Baku, Azerbaijan

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Genetic diversity of 62 chickpea accessions was studied using 8 ISSR and 11 RAPD primers. In the study RAPD primers detected more polymorphism (98%) than the ISSR primers (80%). Genetic diversity index was high (0.73 for ISSR and 0.85 for RAPD) for each of these marker systems. Cluster analysis performed from both separate and combined data of RAPD and ISSR markers using SPSS software package. Jaccard's similarity coefficient for 62 chickpea genotypes was 0.65. Cluster analyses based on combined data generated a dendrogram that separated genotypes into 11 clusters. Four clusters contained only one genotype showing the genetic uniqueness of these accessions. The studied chickpea collection has been proved to constitute a rich source of biodiversity as revealed by RAPD and ISSR markers. Crossing between distantly related genotypes is expected to yield more vigorous plants constituting much of the different traits contained in the two parental lines.

Keywords: chickpea, dendrogram, genetic diversity, ISSR, RAPD

INTRODUCTION

The genus *Cicer* includes a total of 43 species which involves 9 annual species containing cultured chickpea, 33 perennial species and 1 unclassified specie (SETHY *et al*., 2006a). Among these groups, the only variety which has economic importance is *Cicer arietinum* L. It is a

Corresponding author: Mehraj Abbasov, Genetic Resources Institute of National Academy of Sciences of Azerbaijan, Azadliq Ave 155, AZ1106, Baku, Azerbaijan, e-mail: mehraj_genetic@yahoo.com

cultivated chickpea and has a genome of 750 Mbp in size. *C. arietinum* is *a* self pollinated diploid legume with a basic chromosome number of 8 (SETHY *et al.*, 2006b). Genetic polymorphism is extremely low in cultured chickpeas so that developing new varieties is difficult through classical breeding methods. In case of that, molecular markers have been used in breeding programs in recent years (WINTER *et al*., 1999).

Molecular markers reflect with more accuracy the genotypes, independently from the environmental effects and therefore are used in identifying cultivar, registration of new varieties, classification of crop species and in studying phylogenesis and genetic diversity, thereby improving the efficiency of chickpea breeding programs in cultivar development. These markers are divided into 2 groups: specific (SSR, SNP, CAPS etc.) and non-specific (RAPD, ISSR, AFLP, SRAP etc.) (FERNANDEZ *et al*., 2002). Different marker types were used to study genetic diversity in different crops, as well as in chickpea (AHMAD, 1999; CHOWDHURY *et al*., 2002; SUDUPAK *et al*., 2002; ALIYEV *et al*., 2007; BABAYEVA *et al*., 2009; HAJIYEV *et al*., 2015). Among different marker systems RAPD and ISSR are often chosen to perform genetic diversity studies considering their advantages compared to other DNA markers. These methods are widely applicable because they are rapid, inexpensive, simple, do not require prior knowledge of DNA sequence, and require very little starting DNA template (ESSELMAN *et al*., 1999). RAPD markers are the amplification products of anonymous DNA sequence using single, short and arbitrary oligonucleotide primers, while ISSR markers consider the amplification of DNA segment between two identical microsatellite repeat regions oriented in opposite direction. RAPD and ISSR have already been used for cultivar identification and taxonomic relationship studies in chickpeas. IRUELA *et al*. (2002) showed that RAPD markers successfully identified genetic variation in Cicer. RAO *et al*. (2007) reported that the ISSR data in cultivated chickpea together with pedigree data and morphological traits can help the selection of good parental material in chickpea breeding programs.

The present study aimed to assess the usefulness of non-specific RAPD and ISSR markers in studying genetic diversity and relationship among chickpea accessions and varieties.

MATERIALS AND METHODS

A total of 62 chickpea genotypes of diverse origin were used in the study (table 1). The accessions named as Flip were previously obtained from ICARDA genebank. The names of the rest local genotypes coincide with their collection region (i.e., Lenkeran 1 was collected from Lenkeran, Ordubad 39 was collected from Ordubad region of Azerbaijan etc.). Narmin and Sultan are local improved varieties. All plants were grown in field condition at GRI of ANAS.

Genomic DNA was extracted from leaf tissue (2g) according to modified CTAB method by Rogers (ROGERS *et al*., 1985). The quality and quantity of extracted DNA was determined by spectrophotometer. The DNA concentration was adjusted to 20 ng μl^{-1} to use for PCR.

Eight ISSR primers out of 15 were used for diversity analysis in chickpea. PCR was carried out in 25 µl reaction volume containing 2.5 µl 10 X PCR buffer, 2 µl dNTP (5 mM), 2 µl primer (10 µM), 1.5 µl $MgCl_2$ (50 mM), 0.2 µl Tag polymerase and 20 ng extracted DNA. PCR condition was as following: initial denaturation at 94°C for 2 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 50-55°C for 45 seconds, extension at 72°C for 1 min and final extension at the same temperature for 7 min.

Eleven RAPD primers out of 30 that produce scorable bands were used to study diversity. PCR mix content was as in ISSR analysis. Amplification was performed in T100 thermal cycler (BioRad) in following conditions: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 35-37°C for 45 seconds, extension at 72°C for 1 min and final extension at 72°C for 5 min.

PCR products were analyzed using 1.8% agarose gel, stained with etidium bromide and documented using BIO-RAD gel documentation system. A 100 bp ladder was used as a molecular size standard.

Fragments amplified by ISSR and RAPD primers were scored for presence or absence and coded as (1) or (0) respectively. WEIR's genetic diversity index (GDI) was calculated based on binary matrix (1990). Cluster analysis was performed using the unweighted pair-group method with arithmetical average (UPGMA) and dendrograms were constructed on the basis Jaccard's genetic similarity index (1908) using SPSS (2003) software package.

Table 1. Name of the chickpea genotypes used in the study

№	Genotype	№	Genotype	№	Genotype
1	Lenkaran1	22	Ordubad 39	43	Flip 00-19
2	Lenkaran 2	23	Ordubad 41	44	Flip 97-32
3	Flip 03-48	24	Gusar 43	45	Sultan
4	Jalilabad 50	25	Gusar 44	46	TH 1- 04
5	Flip 04-4	26	Flip 97-24	47	Flip 03-34
6	Narmin	27	Flip 03-22	48	Flip 03-17
7	Aghstafa 42	28	Baku 30	49	Flip 04-38
8	Aghstafa 35	29	Jalilabad11	50	Flip 03-36
9	Iran 48	30	Aghdash 18	51	Flip 03-71
10	Jalilabad55	31	Flip 06-18	52	Flip 04-35
11	Sabirabad59	32	Shamakhi 25	53	Flip 03-22
12	Ordubad 47	33	Yardimli 27	54	Flip 06-28c
13	Yardimli 28	34	Masalli 30	55	Flip 03-77
14	Flip 06-8	35	Masalli 51	56	Flip 03-27
15	Flip 06-33	36	Bilasuvar 58	57	Flip 04-16
16	Flip 06-61	37	Lerik 33	58	Flip 06-7
17	Absheron 34	38	Aghstafa 36	59	Flip 06-89
18	Flip 06-33	39	Absheron 35	60	Flip 32-79
19	Flip 06-161	40	Flip 22-04	61	Flip 05-19
20	Flip 05-169	41	Flip 23-04	62	Flip 06-144
21	Ordubad 39	42	Flip 02-88		

RESULTS AND DISCUSSION

ISSR analysis

A total of 15 ISSR primers were screened for the amplification of clear and unambiguous polymorphic bands on 5 representative genotypes. No amplification was observed with seven primers (UBC 875, UBC 877, UBC 878, UBC 854, UBC 859, UBC 869 and UBC 874), while 8

ISSR primers produced clear, scorable and polymorphic PCR products. These were chosen for further analyses (Figure 1).

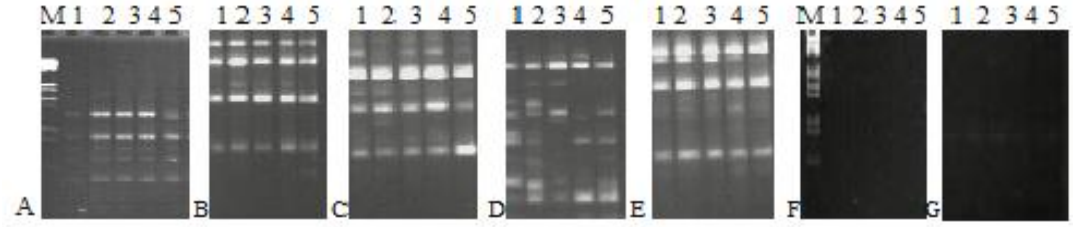

Fig. 1 PCR amplification products in 5 representative genotypes using UBC 873 (A), UBC 864 (B), UBC 880 (C), UBC 810 (D), UBC 809 (E), UBC 854 (F) and UBC 869 (G) primers. M- 100bp DNA ladder.

The eight ISSR primers amplified a total of 41 bands, of which 32 bands (80%) were polymorphic (table 2). The highest number of bands (8) was observed for UBC 810, of which 5 were polymorphic. Primer UBC 880 produced specific bands (630 bp) which were observed only in drought resistant accessions (Flip 00-19, Flip 97-32, Flip 97-81, Masallı 30, Flip 22-04, Shamakhi 25, Aghdash 18, Flip 03-27, Flip 04-38, Flip 97-24) (figure 2). BHAGYAWANT *et al*. (2008) recorded high level of polymorphism for UBC 880. The effectiveness of this primer in distinguishing chickpea genotypes resistant to high temperature was also noted by the authors. So, SCAR (Sequence Characterized Amplified Region) primer can be developed from the ISSR amplicon and used to screen chickpea genotypes for drought resistance.

Table 2. Number of bands, polymorphism and genetic diversity detected by ISSR primers

Primer	Number of bands	Number of polymorphic bands	Percentage of polymorphism	Genetic Diversity Index
UBC-873	4	3	75	0.60
UBC-810	8	5	62.5	0.84
UBC-808	5	4	80	0.84
UBC-112	5	4	80	0.75
UBC-811	6	4	66.7	0.67
UBC-827	5	5	100	0.79
UBC-864	4	4	100	0.74
UBC-809	4	3	75	0.66
Total	41	32		
Mean	5	4	80%	0.73

DODDAMANI *et al.* (2014) provided detailed information on SSRs in chickpea genome. Of the simple SSRs, the most frequently occurring motifs were AT (41%) in di-SSRs, and AAT (13.25%) in tri-SSRs. In the present study primers containing (GA)n (UBC 810, UBC 811, UBC 112, UBC 873) were more polymorphic in comparison to the other primers. RATNAPARKHE *et al*.

(1998) used ISSR markers to determine genetic diversity among chickpea varieties and wild species and noted average level of polymorphism for primers containing (GA)n.

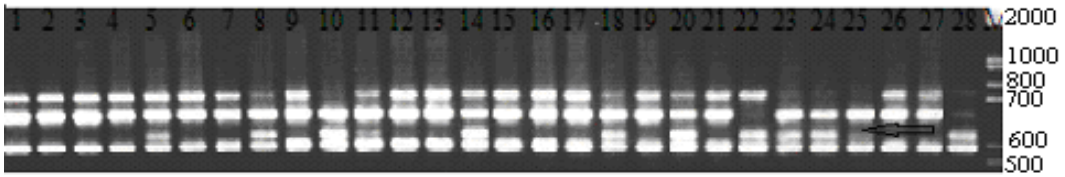

Fig. 2 Specific amplicon synthesized with UBC 880: 5-Flip 00-19, 8-Flip 97-32, 10-Flip 97-81, 14-Masalli 30, 18-Flip 22-04, 20-Shamakhi 25, 22-Aghdash 18, 23-Flip 03-27, Flip 04-38, 28- Flip 97-24. M-100bp DNA ladder.

Only 2 (UBC 827 and UBC 864) out of 8 primers showed 100% polymorphism (table 2). The highest Genetic Diversity Index was recorded for UBC 810 and UBC 808, while primer UBC 873 had the lowest GDI. On the average genetic diversity index observed in studied chickpea collection was 0.73.

RAPD analysis

A total of 30 RAPD primers were checked in 5 genotypes to produce clear and polymorphic bands. Among them 11 primers that showed polymorphism were used for further analyses.

The total number of markers observed in 62 chickpea genotypes was 77, of which 76 were polymorphic (table 3). The polymorphism rate ranged from 80 to 100%. Seven primers showed 100% polymorphism. AHMAD *et al*. (1999) reported the high polymorphism level for chickpea species detected by RAPD markers.

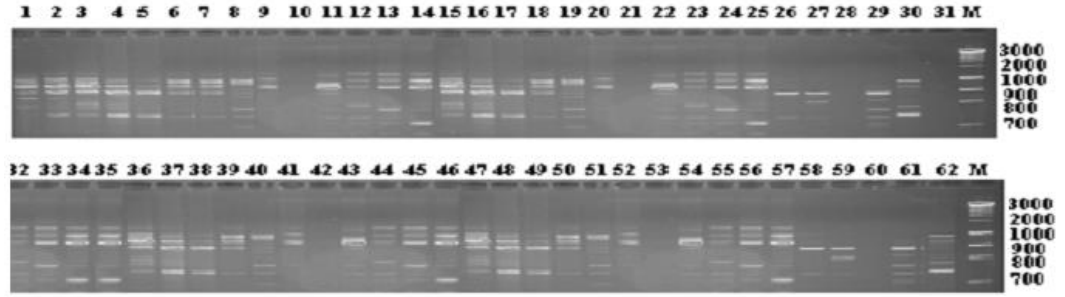

Fig. 3 Electrophoretic profile of PCR products using OPA 19 RAPD primer in 62 chickpea genotypes. M-100bp DNA ladder.

High number of polymorphic bands was observed in OPA 19, OPG 12 and OPF 03 primers, while the least number was noted for primer OPD 4. On the average, each primer amplified 7 bands. Among used primers the highest and lowest genetic diversity indices were 0.97 (OPS 09 and OPF 03) and 0.63 (OPG 14) respectively. The mean GD value was 0.85. This confirms the existence of higher genetic diversity among studied chickpea accessions. The findings were in accordance with SUDUPAK *et al*. (2002) who observed 95 bands with 7 RAPD primers, of which 92 were polymorphic.

Table 3. Number of bands, polymorphism and genetic diversity detected by RAPD primers

Primer	Number of total bands	Number of polymorphic bands	Percentage of polymorphism	Genetic Diversity Index
OPA 19	12	12	100.0	0.66
OPD 02	8	8	100.0	0.91
OPD 11	8	8	100.0	0.93
OPS 09	4	4	100.0	0.97
OPD 4	5	4	80.0	0.94
OPG 12	10	8	80.0	0.75
OPG 14	3	3	100.0	0.63
OPD 10	5	5	100.0	0.72
OPF 03	10	10	100.0	0.97
OPC 16	7	6	80.0	0.85
OPG 4	4	4	75.0	0.90
Total	77	76	98.7	-
Mean	7.0	6.9	98.6	0.85

Cluster analysis

Cluster analysis performed from both separate and combined data of RAPD and ISSR markers using SPSS software package. Dendrogram separated genotypes into 8 and 12 clusters based on ISSR and RAPD markers, respectively (data not shown). So, in our study based on the results of electrophoretic analysis in agarose gel RAPD markers revealed more diversity in compare with ISSR markers. CHOWDHURY *et al*. (2002) used 22 RAPDs and 22 ISSRs to study 19 chickpea genotypes and indicated low level of diversity detected by ISSR. Both marker systems showed the same tendency in the grouping of accessions. So, both RAPD and ISSR analysis grouped accessions Flip 32-79, Flip 05-19, Flip 06-144 in the same clusters. On the other hand, varieties Lankaran 1, Yardimly 28, Aghstafa 35, Masalli 52 were placed in different clusters as revealed by both RAPD and ISSR markers. The similarity between two marker systems on revealing genetic relationship among genotypes was also noted in the number of literature (FERNANDEZ *et al*., 2002; TANYOLAC *et al*., 2003; IZZATULLAYEVA *et al*., 2014).

Combined ISSR and RAPD analysis

Cluster analyses based on combined data generated a dendrogram that separated genotypes into 11 clusters (figure 4). Four clusters contained only one genotype. Cluster I that included 39 accessions was further divided into 2 subclusters with 20 and 19 genotypes respectively. Eighteen accessions in the first subcluster were introduced from ICARDA. Flip 00-19, Flip 97-32, Sultan and TH 1-04 varieties, which were estimated as highly perspective by MIRZEYEV *et al*. (2010), were also located in this group. Other perspective varieties such as Narmin, Aghstafa 42, Lankaran 2 and Jalilabad 50 which were distinguished for plant height and productivity were grouped in cluster V.

Some relationship was observed between genetic structure of genotypes and geographic location. So, varieties Ordubad 41 and Ordubad 39, Gusar 43 and Gusar 44, Flip 32-79, Flip 05-19, Flip 06-144, Flip 06-89 fall into the same clusters.

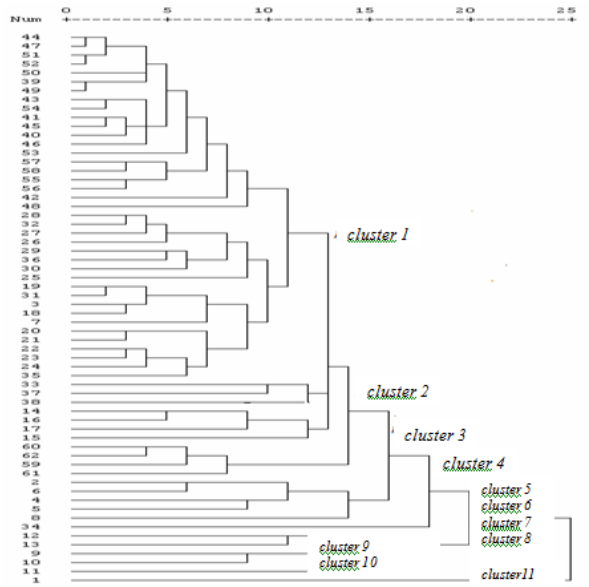

Fig. 4 A dendrogram showing genetic similarity among chickpea genotypes based on combined ISSR + RAPD data

Jaccard's similarity coefficients were calculated for 62 chickpea genotypes. The mean similarity index was 0.65. Flip 03-71 and Flip 04-35, Sultan and TH 1-04, Flip 97-32 and Flip 03-34, Flip 22-04 and Flip 04-38, Flip 00-19 and Flip 06-28, Flip 02-88 and Sultan, Flip 03-48 and Flip 06-33, Flip 06-18 and Flip 06-161, Flip 05-169 and Ordubad 39 showed the highest similarity index, while the lowest similarity index (0.27) was noted between Lankaran 1 and Gusar 43; Lankaran 1 and Ordubad 41; and Yardimli 28 and Flip 97-32.

Hybridization between any distantly related genotypes with best agronomic traits is expected to yield more heterotic and vigorous plants constituting much of the different traits contained in the two parental lines. Using genetic diversity and similarity parameters simultaneously for improvement programs would be the best approach (FIKIRU *et al*., 2007).

In conclusion, the present study confirms the suitability and effectiveness of RAPD and ISSR markers in evaluation of genetic diversity and relationship in chickpea collection. The most effective RAPD primers were OPD 02, OPD 11, OPS 09, OPD 4, OPF 03 and OPG 4, whereas UBC 810, UBC 808 and UBC 827 were the best ISSR markers. The studied chickpea collection has been proved to constitute a rich source of biodiversity as revealed by RAPD (0.85) and ISSR (0.73) markers.

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REFERENCES

ALİYEV, R.T., M.A. ABBASOV, A.CH. MAMMADOV (2007): Genetic identification of diploid and tetraploid wheat species with RAPD markers. Turk. J. Biol., *31:* 173-180.

AHMAD, F. (1999): Random Amplified Polymorphic DNA (RAPD) Analysis Reveals Genetic Relationships Among the Annual *Cicer* Species. TAG**,** *98*: 657-663.

BABAYEVA, S., Z. AKPAROV, M. ABBASOV, A. MAMMADOV, M. ZAIFIZADEH, K. STREET (2009): Diversity analysis of Central Asia and Caucasian lentil (*Lens culinaris* Medikus) germplasm using SSR fingerprinting. Gen. Res. Crop Evol., *56***:** 293-298.

BHAGYAWANT S.S. and N. SRIVASTAVA (2008): Genetic fingerprinting of chickpea (*Cicer arietinum* L.) germplasm using ISSR markers and their relationships. Afr. J. Biotech.**,** *7*(24): 4428-4431.

CHOWDHURY, M.A, B. VANDENBERG, T. WARKENTIN (2002): Cultivar Identification and Genetic Relationship Among Selected Breeding Lines and Cultivars in Chickpea (*Cicer arietinum* L.). Euphytica**,** *127*:317–325.

DODDAMANI, D., M.A. KATTA, A.W. KHAN, G. AGARWAL, T.M. SHAH,R.K. VARSHNEY (2014): CicArMiSatDB: the chickpea microsatellite database. BMC Bioinformatics, *15*: 212.

ESSELMAN, E.J., J.Q. LI, D. CRAWFORD, J.L. WINDUSS, A.D. WOLFE (1999): Clonal diversity in the rare Calamagrostis porteri ssp. In sperata (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. Mol. Ecol., *8*: 443–45.

FERNANDEZ, M.E, A.M. FIGUEIRAS, A.M. BENITO (2002): The Use of ISSR and RAPD Markers for Detecting DNA Polymorphism, Genotype Identification and Genetic Diversity among Barley Cultivars with Known Origin. TAG, *104*: 845-851.

FİKİRU, E., K. TESFAYE, E. BEKELE (2007): Genetic diversity and population structure of Ethiopian lentil (*Lens culinaris* Medikus) landraces as revealed by ISSR marker. Afr. J. Biotech., *6*(12): 1460-1468.

HAJIYEV, E.S., Z.I. AKPAROV, R.T. ALIYEV, S.V. SAIDOVA, V.I. IZZATULLAYEVA, S.M. BABAYEVA, M.A. ABBASOV (2015): Genetic polymorphism of durum wheat (*Triticum durum* Desf.) accessions of Azerbaijan. Russ. J. Gen.**,** *51*(9): 1009-1017.

JACCARD, P. (1908): Nouvelles Reserches sur la Distribution Florale. Bull. Soc.Vaud. Sci. Nat.**,** *44:* 223-270.

IRUELA, M., J. RUBIO, J.I. CUBERO, J. GIL, T. MILLÁN (2002): Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. TAG, *104*: 643- 651.

İZZATULLAYEVA, V.İ., Z.İ. AKPAROV, S.M. BABAYEVA, J. OJAGHİ, M.A. ABBASOV (2014): Efficiency of using RAPD and ISSR markers in evaluation of genetic diversity in sugar beet. Turk. J. Biol., *38***:** 429-438.

MİRZEYEV, R.S., L.A. AMİROV, A.A. JAHANGİROV (2010): Study of perspective chickpea accessions. Collection of scientific works of APII, *12***:** 96-98.

RAO, L.S., P. USHA RANI, P.S. DESHMUKH, P.A. KUMAR, S.K. PANGULURI (2007): RAPD and ISSR fingerprinting in cultivated chickpea (Cicer arietinum L.) and its wild progenitor Cicer reticulatum Ladizinsky. Genet. Resour. Crop. Evol., *54*: 1235-1244.

RATNAPARKHE, M.B., D.K. SANTRA, A. TULLU, F.J. MUEHLBAUER (1998): Inheritance of Inter-Simple Sequence Repeat Polymorphism and Linkage with a Fusarium Wilt Resistance Gene in Chickpea. TAG**,** *96***:** 348-353.

ROGERS, S.O. and A.J. BENDICH (1985): Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol., *5***:** 69-76.

SETHY, N.K., S. CHOUDHARY, B. SHOKEEN, S. BHATIA (2006a): Identification of microsatellite markers from *Cicer reticulatum*: Molecular variation and phylogenetic analysis. TAG**,** *112***:** 347-357.

SETHY, N.K., B. SHOKEEN, K.J. EDWARDS, S. BHATIA (2006b): Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). TAG, *112:* 1416–1428.

SUDUPAK, M.A., M.S. AKKAYA, A. KENCE (2002): Analysis of genetic relationships among perennial and annual Cicer species growing in Turkey using RAPD markers. TAG, *105:* 1220-1228.

TANYOLAC, B. (2003): Inter-Simple Sequnce Repeat (ISSR) and RAPD Variation among Wild Barley (*Hordeum vulgare* subsp. *spontaneum*) Populations from West Turkey. Gen. Res. Crop Evol., *50:* 611-614.

WINTER, P., T. PFAFF, S.M. UDUPA, B. HÜTTEL, P.C. SHARMA, S. SAHI, R. ARREGUIN-ESPINOZA, F. WEIGAND, F.J. MUEHLBAUER, G. KAHL (1999): Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol. Gen. Genetics, *262***:** 90-101.

WEIR, B.S. (1990): Genetic-Data Analysis Methods for Discrete Genetic Data. Sunderland, MA, USA: Sinauer Associates Inc.

GENETIČKI DIVERZITET GENOTIPOVA LEBLEBIJE UTVRĐEN POMOĆU ISSR I RAPD MARKERA

Saida HASANOVA[1], Zeynal AKPAROV[1], Alamdar MAMMADOV[2], Litfer AMİROV[1], Sevda BABAYEVA[1], Jale NASİBOVA[1], Zumrud MUKHTAROVA[1], Kamila SHİKHALİYEVA[1], Vusala İZZATULLAYEVA[1], Mehraj ABBASOV[1,3]

[1] Institut za genetičke resurse Nacionalne akademije nauka Azerbejdžana (ANAS), Baku, Azerbejdžan
[2]Institut za molekularnu biologiju i biotehnologiju ANAS, Baku, Azerbejdžan
[3]Baku državni Univerzitet, Baku, Azerbejdžan

Izvod

U radu je proučavan genetički diverzitet 62 genotipa leblebije pomoću 81 SSR i 11 RAPD prajmera. RAPD prajmeri su detektovali veći polimorfizam (98%) u odnosu na ISSR prajmere (80%). Indeks genetičkog diverziteta bio je visok (0.73 za ISSR i 0.85 za RAPD) za svaki sistem markera. Klaster analiza je urađna za RAPD i ISSR markere, pojedinačno i kombinovano, korišćenjem SPSS softvera. Jaccard-ov koeficijent sličnosti za sve genotipove bio je 0.65. Klaster analizom zasnovanoj na kombinovanim podacima dobijen je dendrogram koji je grupisao genotipove u 11 klastera. Četiri klastera su sadržala samo jedan genotip koje je pokazao genetičku jedinstvenost u odnosu na sve proučavane uzorke. Na osnovu dobijenih rezultata može se zaključiti da ispitivana kolekcija leblebija predstavlja bogat izvor biodiverziteta. Očekuje se da će ukrštanja između udaljenih genotipova dati snažnije biljke, koje će imati bolja svojstva u odnosu na roditeljske linije.

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