RESEARCH ARTICLE **(Open Access)**

# Study of Genetic Polymorphism in Potato Genotypes Using Molecular-Genetic Method

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**Abstract**

The ISSR markers employed in this study have significantly contributed to identifying the genetic polymorphism among potato genotypes. The investigation revealed considerable variation in genetic polymorphism and the degree of genetic similarity across the tested accessions, depending on the primers used. The average polymorphism percentage for the collection of 17 potato accessions was 63.3%. In the dendrogram constructed through cluster analysis, which illustrates genetic relatedness based on ISSR data, all studied genotypes were categorized into four clusters. Notably, genotypes from the same geographical origin were consistently grouped within the same cluster. This study has established the effectiveness of utilizing the UBC 857, UBC 823, and UBC 810 primers for exploring the genetic diversity of potatoes.

**Keywords:** potato, ISSR, genetic diversity, PCoA, cluster analysis

## 1. Introduction

Potatoes stand as a valuable food product and play a crucial role as an industrial and fodder crop. It is called the "second bread" among the people. Potatoes consist of starch, sugar, proteins, and various nutrients, including vitamin C. Potatoes are unmatched as a source of starch when compared to other agricultural crops. Originating in South America, wild species of potatoes can still be found in their native land. The cultivation of potatoes began approximately 9-7 thousand years ago in modern-day Bolivia. Potatoes appeared in Europe in the second half of the 16th century and were initially regarded as ornamental and, curiously, as a poisonous plant. Later, the French agronomist Antoine-Auguste Parmentier (1737-1813) ultimately demonstrated that potatoes possess excellent taste and nutritional qualities. Since then, potatoes began to spread to the provinces of France and subsequently to other countries. Potatoes were introduced to Azerbaijan at the end of the 8th century during the rule of the Qajar dynasty, which controlled the territory comprising modern Iran, Azerbaijan, and part of Central Asia. The first potato was brought to the region by the Russian army, using Azerbaijan as a base during the war with Persia. In Azerbaijan, potatoes are cultivated on 60-70 thousand hectares. Potato cultivation in Azerbaijan is concentrated in the western regions, including Gadabay, Tovuz, Shamkir, partly in Dashkesan, Goygol, and also in Gusar.

As mentioned earlier, potatoes hold strategic importance for food security. Therefore, the study of this agricultural crop is crucial for the country's economy. The success of selection and its future prospects depend on various factors, with the following being particularly decisive: the genetic resources of breeding, its source material, means, techniques, and methods of selection, including biotechnological approaches, and methods of genetic analysis and assessment of genetic diversity in breeding material [24]. To effectively utilize and conserve genetic resources, it is essential to comprehensively investigate biodiversity in plant genetic resource centers and gene banks. This involves identifying variability between accessions and their fingerprinting.

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(Accepted for publication 25.03.2024)
***ISSN**: 2218-2020,* 

The accumulation of new knowledge paved the way for the establishment of a novel scientific discipline – marker-assisted selection [11]. Molecular-genetic methods present extensive possibilities for identifying breeding material [7]. Markers created based on DNA polymorphism hold considerable potential, as they can theoretically be applied to mark any region of the genome. Currently, methods for assessing genetic diversity, using molecular markers, such as Inter Simple Sequence Repeats (ISSR) markers, are widely employed [9]. To create ISSR markers, primers are utilized, which are complementary to microsatellite repeats (typically 4-12 repeat units) and carry a sequence of two to four arbitrary nucleotides at one end (referred to as the "anchor"). These primers enable the amplification of DNA fragments situated between two closely located microsatellite sequences, resulting in the amplification of a multitude of fragments [6]. ISSR markers also exhibit a dominant type of inheritance and do not necessitate prior knowledge of the nucleotide sequence of the DNA under study. The method demonstrates good reproducibility and is effectively employed for identifying interspecific and intraspecific genetic variability, as well as distinguishing species, populations, lines, and, in some cases, for individual fingerprinting [14]. ISSR markers can also be applied for genome mapping and tagging economically valuable traits.

As a result of several experimental studies, ISSR methods have been demonstrated as a rapid and relatively reliable means to identify genetic differences in various crops at the molecular level. For instance, in a study on the genetic diversity of the endangered *Dalbergia oliveri*, Phong [18] utilized 27 ISSR primers, revealing polymorphism rates of up to 75%. In a study by Muthusamy et al. [13] investigating the effectiveness of ISSR markers in assessing the genetic variability of rice bean (*Vigna umbellata* (Thunb.)), the utilized ISSR primers identified polymorphic loci by 61.79%. Additionally, the calculated PIC value during the study was 0.203, confirming the effectiveness of these markers in assessing genetic polymorphism. ISSR markers were also employed by Mandal and colleagues [12] to evaluate the genetic diversity of *Corchorus* spp. (Tiliaceae), leading to the identification of the most effective primers. Touil and his colleagues [26] conducted a study utilizing ISSR markers to investigate the genetic polymorphism of 80 barley samples from Tunisia. The examined primers, characterized by a high level of polymorphism, are recommended as a valuable tool for assessing the genetic diversity of barley genotypes. Similarly, the works of Pharmawati and Yana [17] reflected the identification of genetic relatedness among *Grevillea* genotypes (*Proteaceae*) using 12 ISSR primers, revealing a remarkable polymorphism level of 99.51%. ISSR markers have been widely employed for assessing genetic diversity, determining phylogenetic relationships, and identifying various crops, such as wheat [10] and legumes [1, 2, 3].

In summary, the studies above affirm the significance and effectiveness of ISSR markers in evaluating genetic diversity and identifying genetic relatedness and differences among a wide range of crops. ISSR markers are also extensively utilized in the study of the genetic diversity of potatoes. For instance, Onamu et al. [15] investigated 35 potato accessions from Mexico, Europe, and the USA, using 19 Random Amplified Polymorphic DNA (RAPD) and 5 ISSR primers to identify genetic diversity and relationships among genotypes. The research revealed polymorphism rates of 81.45% and 82.98% for RAPD and ISSR primers, respectively, with ISSR markers proving more effective than RAPD markers. In another study, Pechenkina et al. [16] explored 12 Russian origin potato varieties to determine genetic diversity using the ISSR method of DNA polymorphism analysis. From 20 ISSR primers, four were identified as the most effective for the studied *S. tuberosum* varieties, including two dinucleotide and two trinucleotide primers. The authors recommended these markers for the examination of *S. tuberosum* cultivars. Given the insights from the above mentioned studies, the aim of this research is to investigate the genetic diversity of potato genotypes using ISSR markers.

## 2. Material and Methods

The research work was carried out on 17 potato accessions taken from government organizations and farms from different regions of Azerbaijan (Table 1). Extraction of nuclear DNA from genotypes was carried out according to the CTAB protocol [22]. PCR reactions for three ISSR primers were conducted in a 20 µl reaction volume, comprising 2 µl of 10x PCR buffer, 2 µl of a dNTP mixture (5 mM), 1.5 µl of MgCl2 (50 mM), 2 µl of each primer (15 pmol/µl), 0.1 µl of Taq-polymerase enzyme (5 U/µl), and 2 µl of the extracted DNA (50 ng/µl). The PCR program included an initial denaturation at 94°C for 5 minutes, followed

by 35 cycles of denaturation at 94°C for 1 minute, annealing for 45 seconds (Table 2), and elongation for 5 minutes at 72°C. The final elongation step was performed at 72°C for 10 minutes. Amplification products were subjected to analysis in a 2% agarose gel in 1x TBE buffer at 90 V. After staining with ethidium bromide, the products were visualized under UV light using the BioRad gel documentation system. Band sizes were determined using Photo-Capt version 12.4, referencing the standard 100 bp ladder.

**Table 1.** List of potato accessions used in the study.

| № | Accession | № | Accession |
|---|---|---|---|
| 1 | SF2 | 10 | SF18 |
| 2 | SF4 | 11 | SF21 |
| 3 | SF7 | 12 | Razara |
| 4 | Anna karenina | 13 | Arizona |
| 5 | SF9 | 14 | SF29 |
| 6 | SF12 | 15 | SF30 |
| 7 | SF13 | 16 | SF31 |
| 8 | SF14 | 17 | SF33 |
| 9 | SF15 | | |

ISSR bands were represented in a binary data matrix. DarWin 6.0 software was used for the generation of an unweighted NJ tree and Principal Coordinate Analysis. Several genetic parametrs, such as genetic diversity index (GDI) [28], polymorphism information content (PIC) [23], effective multiplex ratio (EMR), marker index (MI) [19], resolution power (RP), and mean resolution power (MRP) [20] were computed based on molecular data.

**Table 2.** The ISSR primers used in the study.

| № | Primer | Sequence (5'-3') | Annealing temperature |
|---|---|---|---|
| 1 | UBC 857 | ACACACACACACACACYG | 51.3 °C |
| 2 | UBC 823 | TCTCTCTCTCTCTCTCC | 45 °C |
| 3 | UBC 810 | GAGAGAGAGAGAGAGAT | 41 °C |

## 3. Results and Discussion

This study utilized ISSR markers to assess the genetic diversity among potato genotypes. The results revealed significant variability within the potato genotypes, providing valuable insights into their genetic makeup. For the genetic assessment of potato accessions, PCR was conducted using three ISSR markers, resulting in the synthesis of 19 fragments, of which 14 exhibited polymorphism (Table 3). UBC 810, UBC 823, and UBC 857 generated 8, 5, and 6 total bands, while the number of polymorphic bands for each primer was 5, 3, and 4, respectively. Torabi-Giglou et al. [25] successfully amplified 40 polymorphic bands across 45 potato genotypes, employing 5 ISSR primers. Discrepancies in the number of amplified bands may be attributed to variations in primer characteristics, as well as the diverse ploidy levels present in the material, encompassing three different ploidy levels. Various statistical parameters were calculated based on the obtained results from the primers. In the current investigation, elevated levels of polymorphism were observed in potato genotypes through the utilization of ISSR markers. The rates of polymorphism for the primers were notably high, ranging from 60% to 67%, with an average polymorphism rate of 63.3%. The primer UBC 857 demonstrated the highest level of polymorphism (fig. 1). The observed polymorphism percentages indicate a substantial level of genetic diversity among the examined potato genotypes.

Previous research has shown the promising potential of ISSR markers in distinguishing potato cultivars, as demonstrated by studies conducted by Prevost and Wilkinson [20] and Bornet et al. [4].

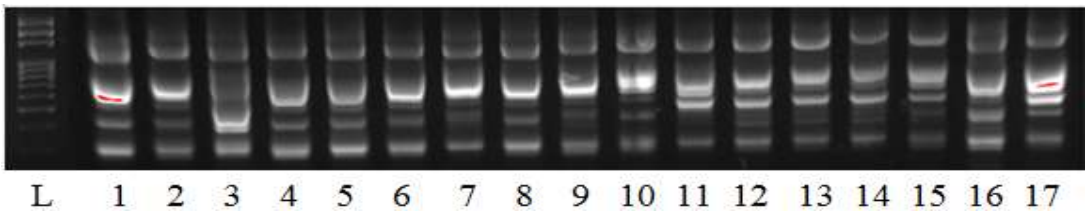


**Figure 1.** Amplification products obtained by UBC 857 for 17 potato accessions.

Utilizing the frequency of occurrence of ISSR profiles in potato accessions, the genetic diversity index was calculated. The average value of the genetic diversity index for the collection was 0.50 units.

**Table 3.** The polymorphism and genetic diversity parameters in potato genotypes based on ISSR analysis

| Primer | Number of total fragments | Number of polymorphic fragments | Polymorphism, % | Genetic diversity index | Polymorphic Information Content | EMR | MRP | Rp | MI |
|---|---|---|---|---|---|---|---|---|---|
| UBC 810 | 8 | 5 | 63 | 0.55 | 0.40 | 3.25 | 0.07 | 2.96 | 1.25 |
| UBC 823 | 5 | 3 | 60 | 0.32 | 0.29 | 3 | 0.25 | 1.35 | 0.88 |
| UBC 857 | 6 | 4 | 67 | 0.61 | 0.37 | 2.67 | 0.10 | 2.42 | 0.99 |
| Total | 19 | 12 | | | | | | | |
| Average | 6.3 | 4 | 63.3 | 0.49 | 0.35 | 2.97 | 0.14 | 2.24 | 1.04 |

Y= C, T

The PIC value serves as a crucial indicator, reflecting the marker's quality and its efficacy in discerning genetic variations in studies. Across the examined 17 potato accessions, the average PIC was 0.36 units. The most informative was the UBC 810 primer, exhibiting the highest PIC value of 0.40. The results obtained in this study surpass those reported by Torabi-Giglou et al. [25], who observed an average PIC value of 0.25 for 45 potato accessions. In addition, the effective multiplexing coefficient, calculated based on the fraction of polymorphic loci, yielded an average value of 2.97 units across all accessions. The UBC 810 primer again demonstrated its prominence, recording the highest EMR value of 3.25 units, showing its exceptional performance in multiplexing genetic analyses.

The Resolving Power exhibited a noteworthy range from 1.35 to 2.96, averaging at 2.24. RP, a parameter crucial for determining the discriminatory potential of chosen primers [27], underscores the efficiency of the selected markers in genetic differentiation. Additionally, the mean resolution power, assessing the discriminatory potential over a substantial material volume [21], varied from 0.07 to 0.25 for the studied potato accessions. The value of the marker index (MI), employed to assess the overall efficiency of the primer-marker system, ranged from 0.88 to 1.25, with an average of 1.04 units.

Information about genetic kinship increases the probability of selecting the best traits, allowing for the choice of different and complementary parental forms [5]. Based on data from 3 ISSR primers, cluster analysis grouped 17 potato genotypes into 4 clusters and demonstrated genetic relatedness among them (fig. 2). The genetic distance index among samples ranged from 0 to 1. Four samples were grouped into Cluster 1 (1, 4, 5, 6), 2 samples into Cluster 2 (2, 8), 8 samples into Cluster 3 (3, 7, 9, 11, 12, 13, 14, 15), and 3 samples (10, 16, 17) were grouped in the Cluster 4.

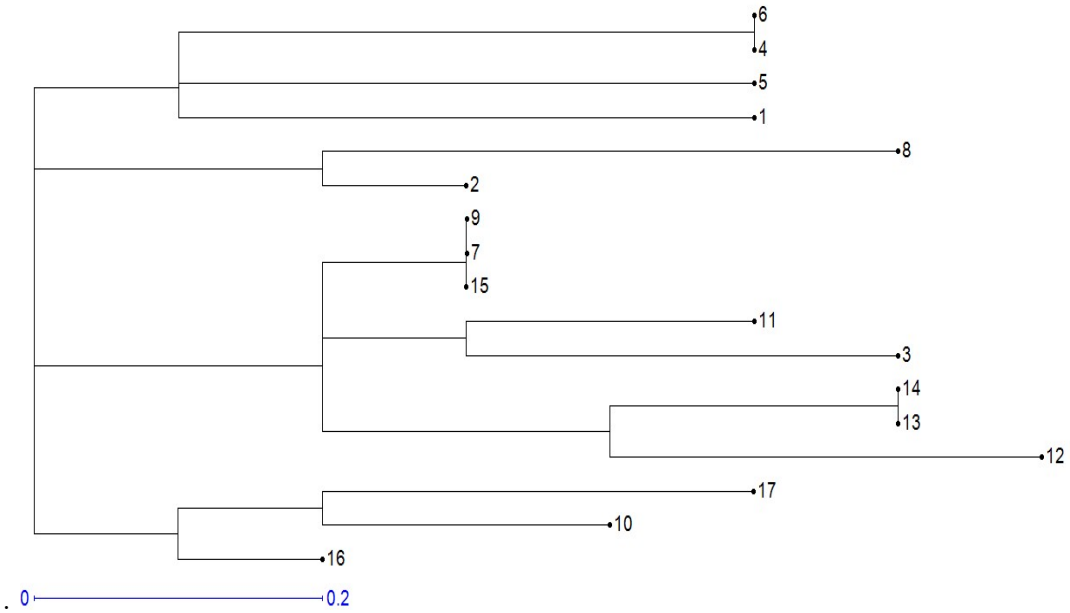


**Figure 2**. Dendogram showing genetic relationship among 17 potato accessions based on ISSR data.

As seen in figure 1, Sample 8 and Sample 12 in Cluster 1, along with Samples 13, 15, and 30 in Cluster 3, as well as Samples 28 and 29, had genetic distance equal to 0 according to the studied ISSR loci. The 4th sample, situated in the 2nd cluster, shares the same group as the 14th sample, but genetically, these samples were distant with GD=0.5.

The research established a correlation between sample clustering and their respective regions of origin. Specifically, potato accessions 8 and 9, clustered in the 1st cluster, were sourced from Gusar, while samples 26, 28, and 29, forming the 3rd cluster, originated from Jalilabad. Samples 31 and 33, grouped into the 4th cluster, were obtained from the Sheki region. Samples from other regions were distributed across different clusters.

Numerous studies have reported the grouping of potato genotypes based on their geographical origins. For instance, Esfahani et al. [8] observed the clustering of European and North American potato genotypes based on their origin. Likewise, in the study of Bornet et al. [4] potato genotypes were differentiated according to their origins in Europe and Argentina.

The analysis of main coordinates, as observed in the results of Principal Coordinate Analysis (PCoA), aligns with the outcomes of cluster analysis. The first axis explains 34.86% of the variation, the first three axes explain 68.57%, and the first five axes explain 83.6% (fig. 3).

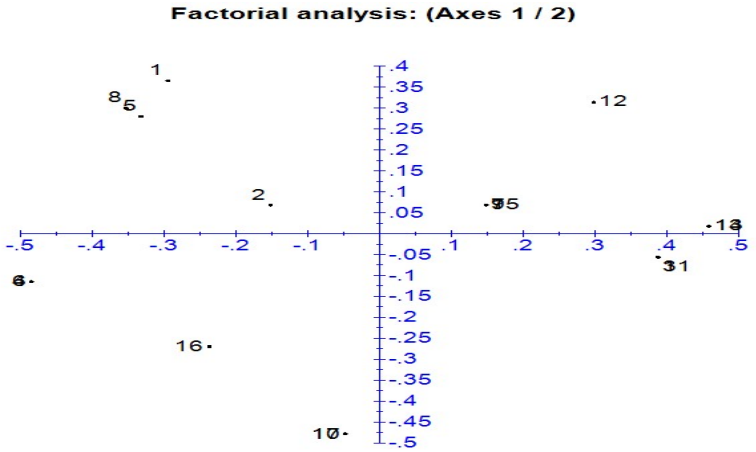


**Figure 3**. Distribution of potato genotypes on a scatter plot according to ISSR data.

## 4. Conclusion

The data presented in the literature on the effectiveness of using ISSR markers to identify genetic polymorphism in various crops is fully confirmed by our studies on potato. The use of three ISSR markers (UBC 857, UBC 823, UBC 810) made it possible to identify genetic polymorphism and the degree of genetic similarity in 17 potato genotypes. The studied genotypes, according to the ISSR data, were grouped into 4 clusters, while a number of genotypes of the same geographical origin were combined into the same cluster. The primers used in the work are effective for the identification of potato genotypes. These findings contribute to the understanding of the genetic landscape of potato cultivars, which is crucial for breeding programs and conservation efforts.

## 5. ACKNOWLEDGMENTS

This work was supported by the Azerbaijan Science Foundation-**Grant № AEF-MCG-2023-1(43)-13/11/3-M-11**

## 6. References

1. Asadova A, Babayeva S, Izzatullayeva V, Akbarova S, Aghazade G, Mirzaliyeva I, Abbasov M: **Molecular characterization of L. sativus L. collection based on ISSR markers.** Genetika 2020, **52**(2): 777-786.
2. Babayeva S. M, Nasibova J. A, Akparov Z. I, Shikhaliyeva K. B, Mammadova A. D, Izzatullayeva V. I, Abbasov M. A: **Application of DNA markers in determination of fusarium resistance and genetic diversity in chickpea**. Legume Research-An International Journal 2018, **41**(4): 537-542.
3. Babayeva S, Akparov Z, Amirov L, Shikhaliyeva K, Hasanova S, Rustamov K, Abbasov M: **Genetic relationship among introduced lentil germplasm using agronomic traits and ISSR markers.** Genetika 2018, **50**(2): 575-590.
4. Bornet B, Goraguer F, Joly G, Branchard M: **Genetic diversity in European and Argentinian cultivated potatoes (Solanum tuberosum subsp. tuberosum) detected by inter-simple sequence repeats (ISSRs).** Genome 2002, **45**(3): 481-484
5. Cabral P.D.S, Souza L.C, Costa G.F. et al.: **Investigation of the genetic diversity ofcommon bean (*Phaseolus vulgaris*.) cultivars using molecular markers.** Genetics and Molecular Research 2018, **17**(4): 1-11.
6. Cekic C, Battey N.H, Wilkinson M. J: **The potencial of ISSR-PCR primer-pair combination for genetic linkage analysis using the seasonal flowering locus in *Fragaria*as a model**. Theor. Appl. Genet. 2001, **103**: 540-564.
7. Dolničar P: **Importance of Potato as a Crop and Practical Approaches to Potato Breeding.** Methods Mol. Biology 2021, **2354**: 3-20.
8. Esfahani S.T, Shiran B, Ghlolamreza B: **AFLP markers for the assessment of genetic diversity in European and North American potato Varieties cultivated in Iran**. Crop Breed. Appl. Biotechnology 2009, **9**: 75-86.
9. Gilberto Codignole Luz, Danuza Kelly Strioto, Claudete Aparecida Mangolin, Maria de Fatima P.S. Machado: **ISSR markers to assess genetic diversity of cultivated populations from artificial selection of *Stevia rebaudiana* (Bert.) Bertoni.** *Breed Sci.* 2020, **70**(4): 508–514.
10. Hajiyev E.S, Akparov Z.I, Aliyev R.T, Babayeva S.M, Izzatullayeva V.I, Abbasov M.A, Mammmadova A.D: **The use of DNA markers for the study of genetic polymorphism of soft wheat** (*T. aestivum* Desf.) genotypes of Azerbaijan. Factors of experimental evolution of organisms, Kyiv 2018: 279-284.
11. Hasan N, Choudhary S, Naaz N, Sharma N, et al.: **Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes.** J. Genet. Eng. Biotechnology 2021, **19**(1): 1-26.
12. Mandal A, Animesh K, Datta S, Gupta S: **Genetic assessment of eight Cor-chorus spp. (*Tiliaceae*) using RAPD and ISSR markers.** The Nucleus 2013, **56**: 23-30.
13. Muthusamy S, Kanagarajan S, Ponusamy S: **Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces.** Elect. J. Biotechnology 2008, **11**( 3): 1-10.
14. Neve G, Meglecz E: **Microsatellite frequencies in different taxa.** Trends Ecol. Evol. 2000, **15** (9): 376-377.
15. Onamu R, Legaria J, Rodríguez J. L, Sahagùn J, et al.: **Molecular characterization of potato**

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(Accepted for publication 25.03.2024)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

**(Solanum tuberosum L.) genotypes using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers.** African Journal of Biotechnology 2016, **15(**22): 1015-1025.

16. Pechenkina V, Zhulanov A, Prishnivskaya Y, Vasilieva Yu, et al.: **Molecular-genetic analysis of national varieties of *Solanum tuberosum* L.** Bulletin of Science and Practice 2018, **4**(3): 11-19.
17. Pharmawati A.C, Yan A, McFarlane B: **Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (*Proteaceae*)**. Australian Systematic Botany 2004, **17**(1): 49-61.
18. Phong D.T, Hien V.T, Thann T.T, Tang D.V**: Comparison of RAPD and ISSR markers for assessment of genetic diversity among endangered rare Dalbergia oliveri (*Fabacea*) genotypes in Vietnam.** Genet. Mol. Res. 2011, **10**(4): 2382-2393.
19. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalsky A: **The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis.** Mol. Breed. 1996, **2**: 225-238.
20. Prevost A, Wilkinson M.J: **A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars.** Theor. Appl. Genet. 1999, **98**: 107-112.
21. Raza A, Farooq U.B, Khan A.W, et al.: **Polymorphic information and genetic diversity in *Brassica* species revealed by RAPD markers.** Biocell Tech Science Press 2020, **44**(4): 769-776.
22. Rogers S.O, Bendich A.J: **Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues.** Plant Mol Bio. 1985, **5**(2): 69-76.
23. Roldan-Ruiz I, Dendauw J, Vanbockstaele E, Depicker A, De Loose M: **AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.).** Mol. Breed. 2000, **6**: 125–134.
24. Salgotra R.K, Chauhan B.S: **Genetic Diversity, Conservation, and Utilization of Plant Genetic Resource.** Genes 2023, **14**(1): 1-20.
25. Torabi-Giglou M, Jaber P, Mohammadi S. A, Nahandi F. Z, Azar A. M, Śliwka, J: **DNA and morphological diversity and relationship analysis of selected cultivated, wild potatoes and some promising hybrids.** Journal of Biodiversity and Environmental Sciences 2015, **6** (2): 175-186.
26. Touil L, Guasmi F, Elfalleh W, Hannachi H, et al. : **The use of ISSR and RAPD markers for genetic diversity among South Tunisian barley.** ISRN Agronomy 2012, 1-10.
27. Verma K. S, Haq Sh, Kachhwaha S, Kothari S.L: **RAPD and ISSR marker assessment of genetic diversity in *Citrulluscolocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress.** Biotechnology 2017, **7**(5): 288
28. Weir B.S: **Genetic data analysis. Methods for discrete population genetic data.**, Sinauer Associates, Suderland 1990, 377