MS.ID-IJPBS-1264
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (1): 120-125
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Genetic Diversity Analysis in Five Medicinal Plants of Solanaceae Family Using RAPD Markers

***Ravindra Singh, Mahendra Pal Singh and **Sadhana Chaurasia**
***Head, Department of Biological Sciences, **Head, Department of Energy and Environment.**
Faculty of Science and Environment, M.G.C.G. University, Chitrakoot, Satna, 485334 (MP), India.

Received: 14 Oct 2021 / Accepted: 4 Nov 2021/ Published online: 01 Jan 2022
***Corresponding Author Email:** chauhanmp1986@gmail.com

Abstract
An understanding of the extent, composition and patterns of genetic differences is helpful for estimating possible genetic diversity loss and assessing genetic variability and its prospective use in improvement programs. This study assessed patterns of genetic diversity and relationships in five medicinal plants of Solanaceae family using RAPD markers. In this analysis, 8 primers were tested, and 6 of them showed polymorphic amplification. From 6 primers (OPA1, OPA2, OPA6, OPB5, OPB7 and OPB9), a total of 32 bands were generated, in which all were polymorphic with 100% polymorphism. The number of RAPD bands generated per primer ranged from 2 (OPB 5) to 10 (OPA1) with an average of 5.34. The DNA fragments with different molecular weights were amplified and band size ranged from 72 to 3601 bp. The similarity coefficient, which was determined using the presence and absence of bands, ranged from 0.36 to 0.72. Similarity index reveals the maximum similarity between *Solanum xanthocarpum* with *Datura stramonium* and *Solanum nigrum* with *Withania somnifera* (similarity indices 0.72 and 0.70 respectively). While distantly related species were *Withania somnifera* and *Capsicum annuum* with similarity coefficient value of 0.36. The present findings conclude that RAPD technique is a promising method for assessing genetic variability in some medicinal plants of Solanaceae family.

Keywords
Genetic diversity, RAPD, Solanaceae, Dendrogram, UPGMA, Polymorphism

*****

INTRODUCTION
The Solanaceae also known as “nightshade family” is a diverse group of angiospermic plants that includes a number of important agricultural plants, ornamentals, vegetables and many medicinal plants [1]. The name Solanaceae originates from the word Solanum, which means “nightshade” [2]. The family Solanaceae consisted of 3000-4000 species divided into more than 90 genera [3]. Plants/ species of the family Solanaceae are annuals, biennials, perennials and are usually herbs, though some of its species grow as shrubs or small trees [2]. The family’s species can be found in all temperate and tropical habitats but by far the greatest biodiversity of the family is found in the western hemisphere [4].

Genetic diversity is an important feature in crop breeding programs and the analysis of genetic variability within and among breeding materials is a

primary concern for scientists/ plant breeders [5]. Genetic diversity studies can identify alleles that might affect an organism's ability to survive in its natural habitats or that may enable it to survive in more diverse habitats [6]. It is necessary to catalogue the genetic level diversity within the germplasm in order to develop desired characteristics [7]. Knowledge of genetic diversity provides a base for establishing disease free plants. Morphological markers were also used to study genetic variations in crop species [8, 9]. However, estimating genetic diversity based on morphological characteristics has the disadvantages of being limited in number and are influenced by the environment [10]. In recent years, DNA based markers are being increasingly used to solve taxonomic and evolutionary problems in plant species [11]. Moreover, molecular markers are extremely important and their application in diversity analysis has demonstrated advantages over other phenotype-based markers [12].

The recently developed DNA based markers such as SSR [13], RFLP [14], RAPD [15, 16, 17] and AFLP [18] are becoming more popular. Among these molecular marker techniques, RAPD technique became popular and attractive due to its reliability, simplicity, relative ease to perform, low cost and no need of DNA sequence information [19]. RAPD is a valuable tool for the studies of genetic variations [20], linkage maps [22], gene tagging [23] and phylogeny and systematic [21]. There is a scarcity of information on the genetic diversity of selected medicinal plants of Solanaceae family in Chitrakoot region, Satna (M.P.). As a result, the current study was undertaken to evaluate the genetic variations and evolutionary relationships within/between the selected medicinal plants of Solanaceae family by using RAPD markers.

MATERIAL AND METHODS

Plant Material

Five selected medicinal plants (i.e. *Solanum nigrum*, *Solanum xanthocarpum*, *Capsicum annuum*, *Withania somnifera* and *Datura stramonium*) of Solanaceae family were used for this investigation and leaf samples pooled from all selected species were collected into labeled bags and stored in liquid nitrogen for further use.

Genomic DNA isolation

The CTAB method [24] was used to isolate genomic DNA from young leaves of selected medicinal plants, which was then purified and quantified by Nanodrop spectrophotometer.

Selection of the primer

Initially, eight decamer primers namely OPA1, OPA2, OPA3, OPA6, OPB5, OPB6, OPB7 and OPB9 were selected and screened by PCR for their ability to produce polymorphic patterns in selected medicinal plants and 6 primers (OPA1, OPA2, OPA6, OPB5, OPB7 and OPB9) which gave reproducible and distinct polymorphic amplified bands were selected finally for RAPD analysis.

Table 1. List of RAPD primers used for DNA amplification

S. No.	Primer Name	Sequence (5`to 3`)	S. No.	Primer Name	Sequence (5`to 3`)
1	OPA1	CAGGCCCTTC	5	OPB 5	TGCGCCCTTC
2	OPA2	TGCCGAGCTG	6	OPB 6	TGCTCTGCCC
3	OPA3	AGTCAGCCAC	7	OPB 7	GGTGACGCAG
4	OPA6	GGTCCCTGAC	8	OPB 9	TGGGGGACTC

DNA amplification

Polymerase chain reaction was carried out based on the protocol of [15] with some modifications. Amplification was carried out in 20µl reaction mixture containing 2 µl PCR buffer (10X), 2µl dNTPs (0.5 mM), 2µl primer, 2µl MgCl2 (2.5mM), 0.4µl Taq polymerase, 9.6µl sterile water and 2 µl of template DNA (52ng/µl). The amplification was performed with a template program consisting of the initial denaturation at 95% for 2 minutes, followed by 40 cycles of denaturation at 95% for 1 minute, annealing for 1 minute at 34°C and extension at 72°C for 2 minutes. Final extension was performed at 72°C for 5 minutes.

Agarose gel electrophoresis

Amplified DNA was then separated on 1.2% agarose gel containing 10 µl/100 ml ethidium bromides. After completing the process of PCR amplification reaction, 2 µl of 6 X loading dye (bromophenol) was added to each PCR tubes. 1.2% agarose gel in 1X TAE buffer was prepared and the contents of the PCR tube loaded into the gel. Electrophoresis was carried out on 50 V for 120 minutes. When the loading dye touched the gel front, the amplified DNA was viewed through the transilluminator, and the images were taken using gel documentation system.

Data analysis

All amplified bands were scored as (1) for its presence and (0) for its absence. Jaccard's coefficient

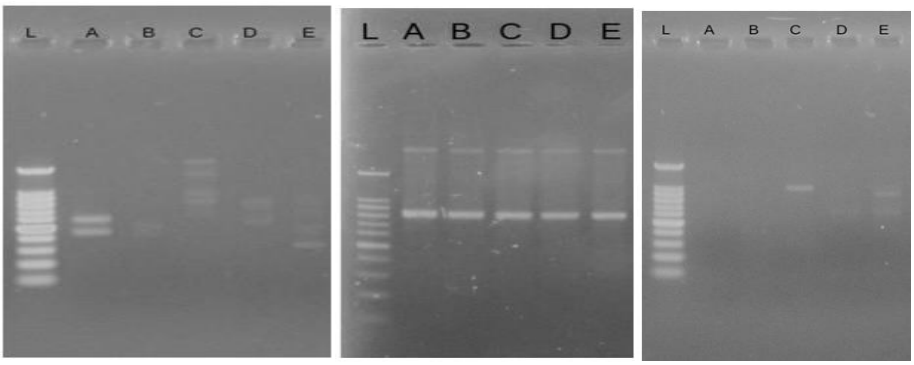
has been used to generate the similarity matrix among the five medicinal plants of Solanaceae family. The similarity coefficient was then used to generate Dendrogram by using UPGMA (unweighted pair group method of arithmetic means) through the programme NTSYSpc software [25].

RESULTS AND DISCUSSION

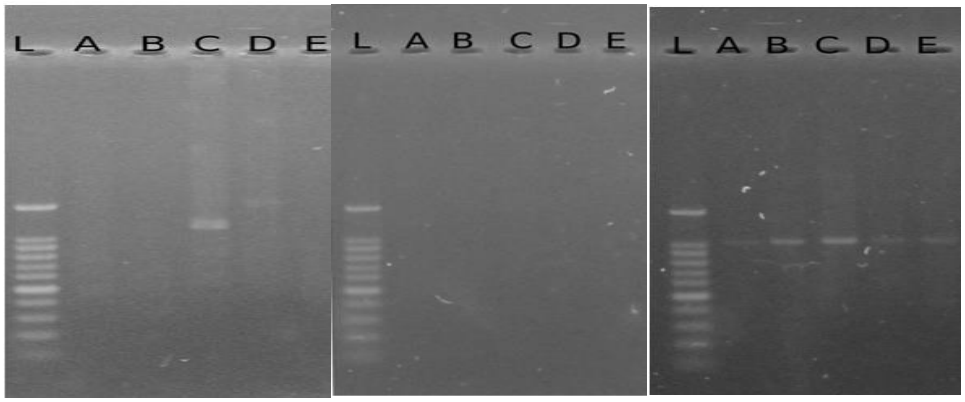
Identification and characterization of plants/species diversity is an essential prerequisite for formulating strategies for plant improvement and conservation of genetic resources. Molecular markers correspond to a powerful and rapid tool for characterizing genetic level variations within the targeted species [26]. Several researchers have demonstrated that the RAPD-PCR method can be applied to assess discriminating differences at intra and inter population level in a wide range of organisms including plant species [27, 28, 29, and 30]. RAPD markers were utilized during this work to study the genetic diversity and interactions within/between medicinal plants of Solanaceae family. A total of 8 RAPD primers (OPA1, OPA2, OPA3, OPA6, OPB5, OPB6, OPB7 and OPB9) were initially screened for this amplification, with 6 (OPA1, OPA2, OPA6, OPB5, OPB7 and OPB9) of them producing a clear and reproducible banding pattern. The pattern of amplified bands among selected medicinal plants generated with the primers OPA1, OPA2, OPA6, OPB5, OPB7 and OPB9 are shown in figure 1. In total 32 amplified bands were obtained of which all bands were polymorphic and showed 100% polymorphism (Table 2). In this study, we found the maximum polymorphism, which is the most significant feature of any marker for identifying a crop species or variety. Similar to present findings [31] reported 100% polymorphism within the genus Solanum by using 11 RAPD markers. [32] used 20 RAPD primers and observed 100% polymorphism in Capsicum genotypes. [33] also worked on *Withania somnifera* and recorded 100% polymorphism by using 10 RAPD primers. The number of RAPD bands were obtained in the range from 2 (OPB5 primer) to 10 (OPA1 primer) with a mean value of 5.3 bands / primer. The present findings are in agreement with [34] who obtained the number of bands in the range of 2 to 9 with an overall mean of 5 bands / primer in different tomato genotypes using 10 RAPD markers. In addition, [35] also found 5.1 bands / primer using 20 RAPD markers across 21 tomato genotypes. The range of generated products was from 72 bp to 3601 bp. These variations related to primer sequence annealed with DNA template [36]. Similar to present findings [37] obtained band size after amplification by RAPD varied in the range of 174 bp to 3603 bp. [38] also obtained the size of amplified bands in the range 100-3500 bp among mango varieties.

Table 2. Amplified DNA bands and polymorphism generated among five medicinal plants of Solanaceae family using 6 RAPD markers

S. No.	Name of primer	Nucleotide Sequences	Band size (bp)	Total no. of bands scored	No. of polymorphic bands	No. of monomor-phic bands	% of polymorphism
1	OPA1	CAGGCCCTTC	216-966	**10**	**10**	00	100
2	OPA2	TGCCGAGCTG	244-2115	05	05	00	100
3	OPA6	GTCCCTGAC	**72-3601**	08	08	00	100
4	OPB5	TGCGCCCTTC	729-1189	**02**	**02**	00	100
5	OPB7	GGTGACGCAG	479-1315	04	04	00	100
6	OPB9	TGGGGGACTC	1016-1078	03	03	00	100
Total				32	32	00	100



(A) **(B)** **(C)**



(D) (E) (F)

Fig. 1. RAPD profiles of selected medicinal plants of Solanaceae family generated by primers OPA 1(A), OPA 2 (B), OPA 6 (C), OPB 5 (D), OPB 7 (E) and OPB 9 (F); L = Marker, A = *Datura stramonium*, B = *Solanum nigrum*, C = *Capsicum annuum*, D = *Withania somnifera*, and E = *Solanum xanthocarpum*.

Genetic relationship and cluster tree analysis

The RAPD data thus obtained were used to construct similarity coefficient matrix of five medicinal plants of Solanaceae family using NTSYS-pc software. (Table 3). The Jaccard's similarity analysis among five medicinal plants exhibited genetic similarity in the range of 0.36 to 0.72 with a mean value of 0.54 across the five selected medicinal plants of Solanaceae family. The values of similarity coefficient in the present findings are almost similar to the observation of [39] who reported similarity coefficient in the range of 0.30 to 0.77 with an overall mean of 0.535. Maximum similarity was observed between *Solanum xanthocarpum* and *Datura stramonium* (0.72) followed by *Solanum nigrum* and *Withania somnifera* (0.70), *Solanum nigrum* with *Solanum xanthocarpum* (0.60) and *Withania somnifera* with *Solanum xanthocarpum* (0.60). The lowest similarity coefficient was exhibited between *Withania somnifera* and *Capsicum annuum* with 0.36 similarity value.

Table 3. Jaccard's similarity coefficient for five medicinal plants of Solanaceae family based on RAPD markers

	Solanum nigrum	***Withania somnifera***	***Solanum xanthocarpum***	***Datura stramonium***	***Capsicum annuum***
Solanum nigrum	1.00	0.70	0.60	0.57	0.42
Withania somnifera		1.00	0.60	0.51	**0.36**
Solanum xanthocarpum			1.00	**0.72**	0.51
Datura stramonium				1.00	0.42
Capsicum annum					1.00

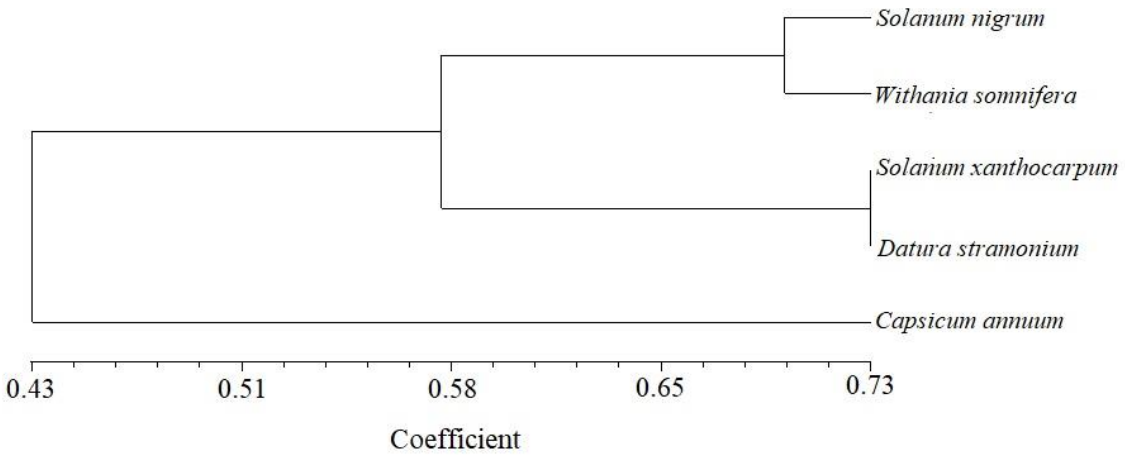


Fig. 2. Dendrogram constructed with UPGMA clustering method of five medicinal plants of Solanaceae family using RAPD primers

The RAPD cluster tree analysis of some medicinal plants of family Solanaceae revealed that they could be mainly grouped into two clusters. Cluster I included four medicinal plants (*Withania somnifera*, *Solanum nigrum*, *Solanum xanthocarpum* and *Datura stramonium*) and it can be again divided into two sub clusters I and II. Sub cluster I included *Solanum nigrum* and *Withania somnifera* while sub cluster II contained *Datura stramonium* and *Solanum xanthocarpum*. Cluster II included only single species *Capsicum annuum* which occupies a distinct place as revealed in the tree.

CONCLUSION

Determination of the extent and pattern of genetic variations in plant species has important implications for genetic improvement and genetic resource conservation. It allows plant breeders/researchers to develop improved varieties/cultivars with desirable characteristics. Considering all the findings of present work, it is concluded that RAPD analysis is efficient in revealing usable level of genetic variability within/between the selected medicinal plants of Solanaceae family. The RAPD estimation revealed that *Solanum xanthocarpum* and *Datura stramonium* showed highest value of similarity index (0.72) whereas *Withania somnifera* and *Capsicum annuum* having lowest similarity index (0.36) which means that these species are distantly related. It is evident from the present study that the identified diverse species among the five medicinal plants can be used in future breeding programs for improving the quality characteristics of selected medicinal plants.

REFERENCES

1. Shah V.V., Shah N.D., and Shinde S.S., Solanaceae: Historical aspects. International Journal of Pharmaceutical Research and Bioscience. 1(3): 90-95, (2012).
2. Nakade J.G., Awaley M.G., and Patele C.K., Floristic study of family Solanaceae of Amgaon Region, Gondia, Districts. International Journal of Research in Bioscience, Agriculture and Technology. 8(1): 59-71, (2020).
3. Knapp S., Bohs L., Mee M., Spooner D.M., Solanaceae - a model for linking genomics with biodiversity. Comparative and Functional Genomics. 5: 285-291, (2004).
4. Olmstead R.G., Bohs L., Abdul Migid H., Santiago-Valentin F., Garcia V.F., and Collier S.M., A molecular phylogeny of the Solanaceae. Taxon. 57(4): 1159-1181, (2008).
5. Chakkravarthi B.K., and Naravaneni, SSR marker-based DNA fingerprinting and diversity studies in rice (*Oryza sativa* L.). African Journal of Biotechnology. 5: 685-688, (2006).
6. Khan S., and Shah R.A., Assessment of genetic diversity among Indian Ginseng, *Withania somnifera* (L.) Dunal using RAPD and ISSR markers. Research Biotechnology. 7:1-10, (2016).
7. Islam F.M.A., Beebe S., Munoz M., Tohm J., Redden R.J. and Basford, K.E., Using molecular markers to assess the effect of introgression on quantitative attributes of common bean in the Andean gene pool. Theory of Applied Genetics. 108: 243-252, (2004).
8. Islam A.K., and Shepherd K.W., Alian genetic variation in wheat improvement. In: chromosome engineering in plants, genetics, breeding and evolution (Eds.) P.K. Gupta and T. Tsuchiya. In Anonymous Elsevier Science Publishers, Amsterdam. 312, (1991).
9. Aslam S., Saleem M., Qureshi A.S., Khan A., Islam M., and Khan S.M., Heterosis for the improvement of oil quality in sunflower (*Helianthus annuas* L.). Pakistan Journal of Botany. 42(2): 1003-1008, (2010).
10. Meric S., Balaric S., Martincic J., Bejic I., and Kozumplink V., Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficient of parentage. Plant Breeding. 123(4): 366-369, (2004).
11. Sherif M. Sharawy, Taxonomic relationships of some taxa of sub family Asclepiadoideae (Apocynaceae) as reflected by morphological variations and polymorphism in seed protein and RAPD electrophoretic profile. International Journal of Botany. 9(1): 18-29, (2013).
12. Liu Z., and Furnier G.R., Comparison of Allozyme, RFLP and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied Genetics. 87(1-2): 97-105, (1993).
13. Tautz D., Hyper variability of simple sequence as general sources for polymorphic DNA markers. Nucleic Acids Research. 17(16): 6463-6471, (1989).
14. Beckman J.S., and Soller M., Restriction fragment length polymorphism in plant genetic improvement: methodologies, mapping and costs. Theoretical and Applied genetics. 67: 35-43, (1983).
15. William J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., and Tingey S.V., DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18: 6531-6535, (1990).
16. Welsh J., and McClelland M., Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research. 18: 7213-7218, (1990).
17. Karp A., Kresovich S., Bhat K.V., Ayad W.G., and Hodgkin T., Molecular tools in plant genetic resources conservation: a guide to the technologies; in IPGRI Technical Bulletin. No. 2. International Plant Genetic Resources Institute, Rome, Italy, (1997).
18. Vos P., Hogers R., Bleeker M., Reijans M., Lee T.V.D., Hornes M., Frejters A., Pot J., Peleman J., Kuiper M., and Zabeau M., AFLP- a new technique for DNA fingerprinting. Nucleic Acids Research. 23: 4407-4414, (1991).
19. Khanuja S.P., Shasany A.K., Darokar M.D., and Kumar S., DNA fingerprinting of plant genetic resources.

Journal of Medicinal and Aromatic Plant Sciences. 20: 348-351, (1998).
20. Zhu H., Zhang H., Mao K., Li H., and Wang A., The genetic diversity of Yunnan local varieties and wild species of tomato. Journal of Yunnan Agricultural University. 19: 373-377, (2004).
21. Millan T., Osuna F., Cobos S., Torres A., and Cukero J., Using RAPDs to study phylogenetic relationships in Rosa. Theoretical and Applied Genetics. 92: 273-277, (1996).
22. Cheung W.Y., Champagne G., Hubert N., and Landry B.S., Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theoretical and Applied Genetics. 4: 569-582, (1997).
23. Tiwari K., Penner G., and Warkentin T., Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene er 1 in pea. Genome. 41: 440-444, (1998).
24. Doyle J.J., and Doyle J.S., (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 19: 11-15, (1987).
25. Rohlf F., NTSYS-pc. Numerical taxonomy and multivariate analysis system version 2.02e. Exeter software New York, (1997).
26. Mir B.A., Koul S., Kumar A., Kaul M.K., Soodan A.S., and Raina S.N., Assessment of characterization of genetic diversity in *Withania somnifera* (L.) Dunal using RAPD and AFLP markers. African Journal of Biotechnology. 10(66): 14746-14756, (2011).
27. Bautista N.S., Solis R., Kamjim O., and Ishii T., RAPD, RFLP and SSLP analysis of phylogenetic relationships between cultivated and wild species of rice. Genes and Genetic systems. 76: 71-79, (2001).
28. Wu Y.J., Chen Y., Wang J., Zhu C.X., and Xu B.L., RAPD analysis of jasmine rice specific genomic structure. Genome. 49: 716-719, (2006).
29. Archak S., and Karihaloo J.L., RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Current Science. 82: 1139-1141, (2002).
30. Malviya N., and Yadav D., RAPD analysis among pigeon pea (*Cajanus cajan* L. Mill sp.) cultivars. Genetic Diversity, Genetic Engineering and Biotechnology Journal. 1-7, (2010).
31. Ahmad S.H.M., and Fadl M.A., RAPD based genetic diversity analysis within the genus Solanum. Egyptian Journal of Botany. 55(2): 175-185, (2015).
32. Dekka S.D., Dadlani M., and Talukdar, A., Morphogenetic characterization, and genetic analysis of Capsicum genotypes. Vegetos. 25(2): 249-256, (2012).
33. Tiwari P., and Shrivastava A., Efficiency of RAPD markers for molecular diversity analysis of *Withania somnifera* (L.) Dunal in Central India. International Journal of Biological Sciences. 3(7): 126-130, (2016).
34. Paul M., and Saha S.R., Assessment of genetic diversity of different tomato genotypes using RAPD markers. Progressive Agriculture. 29(4): 276-283, (2018).
35. Shah M.A., Khan A.I., Awas F.S., Sadaqat H.A., Bahadur S., Rasheed A., and Baloch, F.S., Genetic diversity of some tomato cultivars and breeding lines commonly used in Pakistani breeding program. Turkish Journal of Agricultural Food Science and Technology. 3: 126-132, (2015).
36. Mahpara S., Farooq J., Ali Z., Patrescu-Mag I.V., and Hussain F., Assessment of genetic distance among wheat genotypes through RAPD markers. Advances in Agriculture and Botanics International Journal of the Bioflux Society. 4(1): 3-35, (2012).
37. Al-Ghufaili M.K.F., and Al-Tamini A.J.T., Genetic relationship among ten wheat genotypes using seventeen RAPD markers. Plant Archives. 18(1): 595-600, (2018).
38. Pruthvish R., and Chikkaswamy B.K., (2016). Genetic diversity and relationships among mango varieties using RAPD molecular markers. International Journal of Current Microbiology and Applied Sciences. 5(1): 778-787, (2016).
39. Tidke SH.A., Ramakrishna D., Kiran S., Kosturkova G., and Ravishankar G.A., Analysis of genetic diversity of 12 genotypes of Glycine max by using RAPD marker. International Journal of Current Microbiology and Applied Sciences. 6(7): 656-663, (2017).