The Pharma Innovation Journal 2023; 12(2): 1925-1930

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 1925-1930

www.thepharmajournal.com
Received: 19-12-2022
Accepted: 24-01-2023

VA Mane-Deshmukh
Department of Plant Biotechnology, MGM IBT, Aurangabad, Maharashtra, India

BD Ghodake
Department of Plant Biotechnology, College of Agricultural Biotechnology Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

PS Kharpude
Department of Plant Biotechnology, MGM IBT, Aurangabad, Maharashtra, India

AM Patil
Department of Plant Biotechnology, MGM IBT, Aurangabad, Maharashtra, India

Corresponding Author:
VA Mane-Deshmukh
Department of Plant Biotechnology, MGM IBT, Aurangabad, Maharashtra, India

Morphological and molecular characterization of onion (*Allium cepa* L.) genotypes using RAPD markers

VA Mane-Deshmukh, BD Ghodake, PS Kharpude and AM Patil

DOI: https://doi.org/10.22271/tpi.2023.v12.i2w.18684

Abstract
The present study carried out to with a view to characterize and validate the RAPD marker's genetic diversity at morphological and molecular levels in thirty cultivars of onion (*Allium cepa* L.). This work aimed to determine their important traits, DNA polymorphism and decipher their genetic variation. The agro-morphological characters such as germination percentage, plant height, leaf colour, leaf waxiness, leaf foliage attitude, bulk colure and bulk shape of thirty onion genotypes were studied. The genetic diversity assessed using four RAPD markers (OPB-09, OPA-16, OPB-16, and OPA-09). It revealed significant genotypic differences in the onion genotypes. Significant genetic diversity was observed and revealed by UPGMA analysis. The RAPD analysis yielded 98.21% average polymorphism (genetic diversity) with 901 amplicons. The OPA-16, OPB-16, and OPA-9 showed 100% polymorphism and only OPB-9 produced 92.85% polymorphism. The RAPD primers discriminated thirty cultivars into two major clusters by the method of UPGMA. The major cluster (cluster-A) contains 28 genotypes and another was a minor cluster (cluster-B) which contains only two genotypes (1403 and 1432/33). The cultivars exhibited statistically significant variability, which gives an opportunity to use them for varietal development through breeding programs. The key findings of the current study would be an insight for breeding strategies in onion cultivars.

Keywords: RAPD, Genetic diversity, *Allium cepa* L., onion genotypes, UPGMA

Introduction
Onion (*Allium cepa* L.) belongs to *Amaryllidaceae* (*Alliaceae*) family and the genus *Allium* (Fritsch and Friesen, 2002). It is an old-world crop and domesticated in Iran and Pakistan i.e. Central Asia. The green leaves, immature and mature bulbs are eaten raw or used in vegetable preparations and consumed as a vegetable or condiment. It is used to enhance the flavour of different recipes. Onion has many medicinal values and is used for the preparation of various Homoeopathic, Unani and Ayurvedic medicines. The nutritive value of an onion varies from genotype to genotype, small size onion is more nutritive than big size. Onion ranks medium in caloric, low in protein and very low in vitamins.

The advantage of onion diversity has not been fully exploited but with the liberalization of policies, multinational companies have entered into hybrid onion development (Khar *et al.*, 2011) [9]. The use of molecular markers for diversity analysis, varietal identification, colour and quality improvement, male sterility analysis and genome sequencing in *Alliums*. Accurate morphological descriptions of cultivars have proved reliable and provided the basis of assurance to farmers and breeders that they are being offered specific genotypes or classes of products to a certain minimum standard of quality and purity.

Many tools are available to study relationships among cultivars including various types of molecular markers, however, morphological characterization is the first step in description and classification. It is commonly known that morphological data can be of dubious taxonomic reliability because of environmental interaction and the largely unknown mechanisms of genetic control of these traits (Comstock and Moll, 1963; Camussi *et al.*, 1983) [3, 2].

Characterization and grouping based on phenotype are influenced by environmental variations; molecular markers are preferred because of polymorphic nature, co-dominance, selective neutral behaviour, easy and fast assay, high reproducibility and easy exchange of data between laboratories (Joshi *et al.*, 1999) [17]. Morphological traits have many limitations including low polymorphism, low heritability, and late expression and may be controlled by epistatic and pleiotropic gene effects (Cramer and Havey, 1999) [4].

Nowadays many tools are available to study relationships among cultivars including various types of molecular markers, powerful PCR-based techniques have also emerged which is very

fast. A molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored. There are different marker systems available for crop plants such as random amplified polymorphic DNA (RAPD), (Semagn *et al.*, 2006) [13]. For diversity analysis, the RAPD marker is widely used by horticulturist's simplicity and no need for prior sequence information. The knowledge of genetic diversity helps in the efficient management of germplasm and the selection of parents for crossing. The integrity of inbred lines was studied using RAPD (Bradeen & Havey, 1995) [1].

The molecular markers have offered evaluation and characterization of genetic diversity within and between species. However few marker systems *viz*. SSR; (Fischer and Bachmann, 2000) [6] and RAPD; are simple, economical, quick, and easily automated (Jones *et al.*, 1997) [8]. Therefore, the present study focused on genetic diversity using the RAPD marker approach. Considering this background information and research gaps, it is proposed to undertake the present study to characterize the morphological and molecular diversity in onion genotypes.

Materials and Methods

Plant sample

The thirty onion genotypes (Table 1) were selected for morphological and molecular characterization.

RAPD markers

The total 10 RAPD primers (OPA-16, OPD-1, OPB-16, OPC-4, OPA-9, OPC-10, OPB-9, OPB-17, and OPA-8) were preselected, custom oligo-synthesized and dissolved in a suitable volume of sterile distilled water to get desired concentration of 10 picomole/µl of primer.

Morphological character study

Thirty onion genotypes (Table 1) were grown in pots and used for the analysis of morphological characters such as germination percentage calculated after 8 days of sowing; plant height measured after 15 days of sowing and leaf colour, leaf waxiness, leaf foliage attitude, bulk colure and bulk shape observed after 2 months.

Isolation and quantification of DNA

Genomic DNA of 30 onion genotypes was extracted from the leaves following the cetyltrimethyl ammonium bromide (CTAB) method with some modifications as described by Doyle and Doyle, 1990. The 2% CTAB extraction buffer was prepared by using components (2% CTAB, 5M NaCl, 1M Tris, 0.5M EDTA) and adjusting to 100ml with distilled water and added 40 µl β-mercaptoethanol then buffer prewarmed at 65ºC. DNA quantity was assessed by using Nanodrop (Thermoscientific-Nanodrop1000, USA) at the absorbance ratio of 260 and 280 nm providing a value of 1.8 which determines pure DNA preparation. The concentration of DNA in the sample was calculated using the given formula:

Concentration of DNA = A260× 50 µg× dilution factor
Purity of the DNA = A260: A280 ratio = A260 /A280.

Agarose gel electrophoresis

The 0.8% Agarose gel was prepared by dissolving 0.8g agarose powder in 1X TAE buffer of 100ml and warmed in a microwave oven. Then 10mg/ml ethidium bromide was added to it after cooling down to 50 °C. The gel was poured into a gel casting tray, in which the comb was inserted and kept for 1hr. After solidification, the comb was gently removed. 5µl DNA was mixed with 1µl to 6X gel loading dye and loaded on the gel. The electrophoresis was carried out at 100 volts for 1hr using 1X TAE buffer.

Screening with primers linked to genetic diversity

Out of the total preselected 10 RAPD primers were custom oligo-synthesized and dissolved in a suitable volume of sterile distilled water to get desired concentration of 10 picomole/µl of primer. The amplification reaction mixture was prepared in sterile 0.2 ml PCR tubes, containing the following components. The total volume of each reaction mixture was 20-25 µl.

The 96-well thermal cycler was used to perform the amplification (Applied Biosystem). 25 ng of gDNA, 1X reaction buffer with 1.5 mM MgCl2, 10 pM primer, 0.2 mM dNTPs, 1 unit of Taq polymerase, and nuclease-free water were used to prepare the final volume of 25 µl PCR reaction. Initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 40-42°C for 1 min, extension at 72°C for 1 min, and final extension at 72 °C for 10 minutes. The PCR products were separated by electrophoresis on a 0.8% agarose gel for 80-90 minutes at 100 volts. As a size marker, a 1kb DNA ladder (Rovalab) was used. The gel image was captured using a gel documentation system (Bio-rad-XR+ System).

Statistical analysis of RAPD Data

In order to score the banding patterns gel was checked under Gel Documentation System, under UV-transilluminator. The bands were designated on the basis of their molecular size. 250bp-25kb DNA ladder for PCR product loaded simultaneously with primer products in the gel was used to estimate the molecular size. The presence of each band was scored as '1' and its absence as '0'. The data were analysed using NTSYS pc version 2.02 and constructed dendrogram.

Result and Discussion

A) Agro-Morphological Traits Analysis

The phenotypic or morphological characters were taken into consideration and morphological data were recorded for various quantitative traits (Table 3). The agro-morphological characters of thirty onion genotypes were studied and have been described as follows:

Germination Percent (%)

The Germination Percent (%) of thirty onion genotypes (Table 3) were measured in percent; the genotypes such as 653, 619, 683,1657, 1496, 1190, 619, and 690 have the highest germination Percent i.e. 100%, while 1782, 1146, 1403, 1606, 1631, 1603,1633, 1631, and 1432/33 has the lowest per cent range of 40%.

Leaf colour

The leaf colour was observed as green, dark green, yellowish green, and light green. The mostly observed leaf colour was dark green which was found in sixteen genotypes. Light green and yellowish green were found in 10 and 3 respectively. The green leaf colour was observed only in the 1752 genotype.

Leaf waxiness

The leaf waxiness was observed in 21 onion genotypes; Leaf

waxiness was absent in nine genotypes such as 678, 1784, 1403, 1657, 1496, 1631, 1603, 1190, and 690.

Leaf foliage attitude

The leaf foliage attitude was observed as erect and horizontal. The erect type leaf foliage attitude was found in 19 genotypes and 11 genotypes showed horizontal leaf foliage attitude.

Bulk colour

The bulk colour such as dark red, brown, pink, red, white, and yellow was observed. The red bulk colour of onion was found in nine genotypes; brown, white, and bulk colour were found in eight, and seven genotypes respectively. The pink, yellow and dark red bulk colours were least observed. The dark red bulk colour of the onion was observed only in the 1496 genotype.

Bulk shape

The flat, globe, flat globe, and thick globe bulk shape of the onion were observed (Table 3). The flat bulk shape was found in fifteen genotypes.

Similarly, Sudha *et al*., 2019 [15] observed the germination percentage, root length, seedling length and plumule length contributing most to the separation among the nine cultivars of onion (*Allium cepa* L.).

B) Molecular Genetic Diversity Analysis

Isolation and quantification of genomic DNA

Genomic DNA was isolated by the CTAB extraction method (Doyle and Doyle, 1990). The isolated DNA was less pure; so some modifications were followed

i) Increased the β-mercaptoethanol conc.,
ii) Cancelled the PCI treatment
iii) Cancelled 70% ethanol washing of pellet and
iv) 3µl of RNase used to remove the RNA impurities
v) Reduced the centrifugation time after C: I treatment.

The isolated DNA was checked on 0.8% Agarose gel electrophoresis and used further for RAPD analysis. The quantity and purity of DNA were checked by using both O.D. 260 nm and 280 nm wavelengths on Nano Drop.

Screening of RAPD markers

A set of 10 pre-selected RAPD primers belonging to the Operon© series (Table 2) were used for screening to amplify DNA from thirty onion genotypes to characterize and assess their genetic diversity. Among the 10 primers, four primers showed good amplification and were revealed to be highly polymorphic; while the remaining (six) were sub-optimal, monomorphic, non-distinct and/or non-consistent. The primers showing polymorphic fingerprints are narrated in Table 2 with their polymorphism parameters. These four informative RAPD primers were used for final scoring and further analysis.

Genetic diversity analysis by using RAPD markers

The RAPD analysis produced distinguishable 716 amplification products or scorable bands (fragments). The RAPD markers, OPB-09 produced a maximum number of bands (251) followed by OPA-16 (242), OPB-16 (182), and OPA-09 (168) (Table 2). Out of the total bands produced 92.85 were polymorphic bands in the data set that were sufficient to discriminate among all 30 cultivars (table 2). These bands can be successfully used as genetic markers for the identification of these cultivars. The primers OPB-09, OPA-16, OPB-16 and OPA-09 were found to be the most effective in generating unique bands. The highest number of bands (14) were recorded with primer OPB-09 and while the least number of bands (09) were observed by OPA-09. The OPB-16 and OPA-16 showed 12 and 10 bands respectively. While, Sudha *et al*., 2019 [15] studied and revealed that among 18 RAPDs 10 primers displayed specificity and produced a total of 52 amplified polymorphic fragments.

DNA polymorphism by random amplified polymorphic (RAPD) markers

Out of four RAPD primers, three primers (OPA-16, OPB-16, and OPA-9) showed 100% polymorphism and only OPB-9 produced 92.85% polymorphism. Thus, the overall average of 98.21% polymorphism (genetic diversity) was observed based on amplified polymorphic loci or banding patterns of each genotype i.e. band row-wise polymorphism (Table 2). These bands can be successfully used as genetic markers for the identification of onion cultivars. The primers OPB-09, OPA-16, OPB-16 and OPA-09 produced unique bands and were found to be the most effective. Similarly, Sangeeta *et al*., 2006 estimated genetic diversity among 24 cultivars of short-day onions using 15 RAPD primers which yielded 137 bands, of which 91.24% were polymorphic; Maniruzzaman *et al*., 2010 evaluated ten cultivars of onion, 12 primers revealed scorable (168 bands) polymorphisms between cultivars of *A. cepa.*

Clustering analysis based on RAPD markers linked to genetic diversity

The cluster analysis (Fig.1) based on genetic diversity-related RAPD markers across various linkage groups data revealed a relatively broad genetic background of the onion genotypes. Two distinct and major clusters (A, and B) were found to represent 30 genotypes. The RAPD amplification/analysis data obtained were analyzed and subsequently processed by using NTSYS-pc© (Version 2.02i) developed by (Rohlf, 2000) [11] and generated the dendrogram.

A dendrogram (Fig.1) based on UPGMA cluster analysis of RAPD data showed two clearly distinct groups of 30 genotypes. One is a major cluster (cluster-A) which contains 28 genotypes and a minor cluster (cluster-B) contains only two genotypes (1403 and 1432/33). The 1403 and 1432/33 genotypes were closer to each other than the other 28 genotypes. Cluster-A were again divided into two sub-clusters i.e. cluster-C and cluster-D. In Cluster-D 1782 and 1690 are closer to each other than 1779 and 1446. Cluster-C contains the all remaining genotypes. Cluster-C is again subdivided into cluster-E and cluster-F. Cluster-E contains 1360 and 643 genotypes which were closer to each other. Cluster-F is again subdivided into cluster-FI and one simplicifolious line or out-grouped. Cluster-FI was divided into cluster-G and cluster-H. But one simplicifolious line is produced of 1782 genotype which means 1782 is different from cluster-G and cluster-H. In cluster-H the genotypes 693 and 653; 1778 and 678; 1784 and 1752 were closest to each other. In cluster-G, the genotype 1184 was simplicifolious which means this genotype is distinct from other genotypes of cluster-FI. The genotypes 1631 and 619 were closest or nearly similar to each other (Fig.1).

Table 1: List of Onion genotypes

Sr. No.	Genotype Code	Sr. No.	Genotype Code	Sr. No.	Genotype Code
1	1360	11	1782	21	1779
2	643	12	1606	22	683
3	693	13	1657	23	1784
4	653	14	1496	24	1752
5	616	15	1631	25	1403
6	1778	16	1603	26	1631
7	1782	17	1633	27	619
8	678	18	1658	28	1610
9	1690	19	1190	29	690
10	1146	20	1184	30	1432/33

Table 2: DNA polymorphism by RAPD markers

Sr. No.	Primer code	Primer sequence	Total No. of bands	No. of polymorphic bands	Percent polymorphism
1	OPA-16	AGCCAGCGAA	10	10	100
2	OPB-16	TTTGCCCGGA	12	12	100
3	OPA-9	GGGTAACGCC	9	9	100
4	OPB-9	TGGGGGACTC	14	13	92.85

Table 3: List of selected 30 Onion genotypes with morphological character study

Sr. No.	Genotype	Germination %	Leaf colour	Plant height (15 days after sowing) (cm)	Leaf waxiness	Leaf foliage attitude	Bulk colour	Bulk shape
1	1360	80	Dark green	4.5	Present	Erect	Brown	Flat
2	643	60	Dark green	6.8	Present	Horizontal	Pink	Thick
3	693	80	light green	5	Present	Erect	Red	Flat
4	653	100	Yellowish green	6.5	Present	Horizontal	Pink	Flat
5	619	100	Dark green	5.8	Present	Erect	Red	Flat globe
6	1778	80	Dark green	5.5	Present	Erect	White	Flat
7	1782	40	Dark green	5.3	Present	Horizontal	White	Flat
8	678	60	light green	6	Absent	Erect	Brown	Globe
9	1690	60	Dark green	3.2	Present	Erect	Pink	Flat
10	1146	40	light green	2.4	Present	Erect	Yellow	Thick globe
11	1779	80	Dark green	5.9	Present	Horizontal	Yellow	Flat
12	683	100	Dark green	5.5	Present	Horizontal	Red	Flat
13	1784	60	light green	7	Absent	Erect	Red	Thick
14	1752	60	Green	5	Present	Erect	White	Flat
15	1403	40	Dark green	5	Absent	Horizontal	Brown	Flate globe
16	1782	40	Yellowish green	5.7	Present	Horizontal	White	Flat
17	1606	40	Dark green	3.2	Present	Erect	Brown	Flat globe
18	1657	100	light green	5.5	Absent	Horizontal	White	Flat
19	1496	100	Dark green	5.5	Absent	Erect	Dark red	Flat
20	1631	40	light green	2.9	Absent	Erect	White	Globe
21	1603	40	Dark green	6.9	Absent	Horizontal	Red	Flat globe
22	1633	40	Light green	0.5	Present	Erect	Brown	Flat
23	1658	60	Light green	4.9	Present	Erect	White	Flat globe
24	1190	100	Light green	3.2	Absent	Erect	Brown	Flat
25	1184	80	Dark green	5.7	Present	Erect	Red	Thick globe
26	1631	40	Yellowish green	2.9	Present	Erect	Brown	Globe
27	619	100	Dark green	5.8	Present	Erect	Red	Flat globe
28	1610	80	light green	3.9	Present	Horizontal	Red	Flat
29	690	100	Dark green	5	Absent	Horizontal	Red	Globe
30	1432/33	40	Dark green	4	Present	Erect	Red	Flat globe

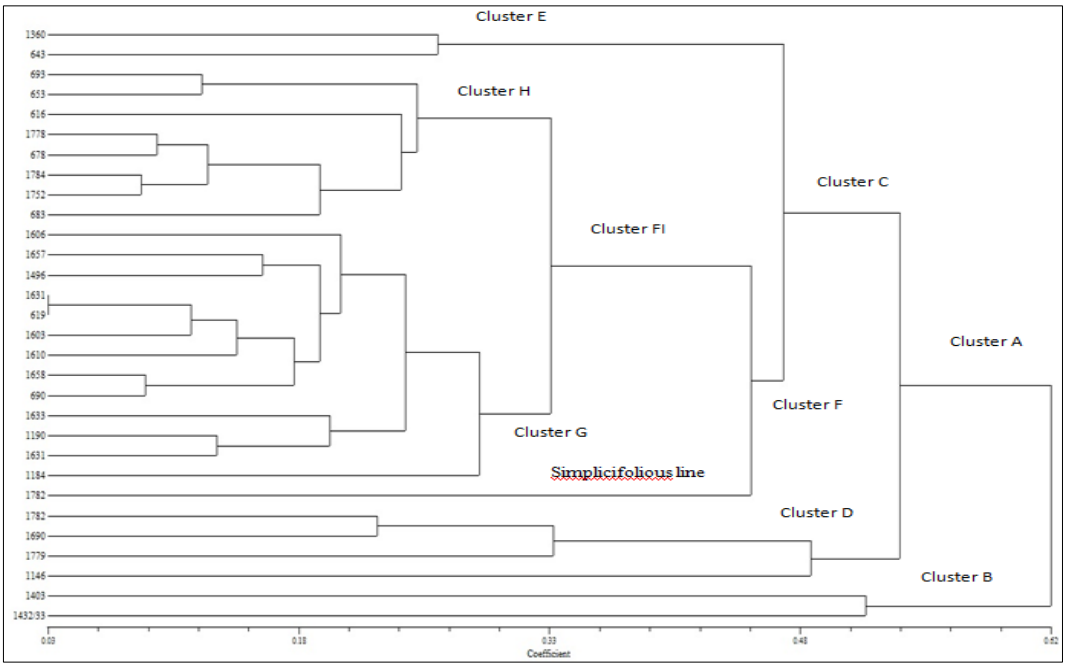

Fig 1: Dendrogram of 30 Onion genotypes revealed by UPGMA cluster analysis based on Jaccard's genetic similarity index estimates derived from RAPD fingerprints (NTSYSv2.02i, Rohlf, 2000) [11]

Summary

In the present investigation, morphological characters such as germination percentage, leaf colour, plant height, leaf waxiness, bulk shape, bulk colour and leaf foliage attitude were studied to differentiate genotypes. Similarly, (Singh *et al*., 2013) [14] concluded that the genotypes evaluated the traits differ clearly from each other and form a very reliable morphological descriptive profile of onion genotypes.

Among 4 RAPD primers, three primers (OPA-16, OPB-16, and OPA-9) showed 100% polymorphism and only OPB-9 produced 92.85% polymorphism. Thus, the overall average of 98.21% polymorphism (genetic diversity) was recorded in 30 onion genotypes. The dendrogram were constructed on the basis of UPGMA cluster analysis (Fig.1), which showed two clearly distinct groups. One was a major cluster (cluster-A) which contains 28 genotypes and another was a minor cluster (cluster-B) which contains only two genotypes (1403 and 1432/33). The genotypes 1631 and 619 were closest or nearly similar to each other. The 1184 genotype was simplicifolious or out-group, which means this genotype is distinct from other genotypes of cluster-FI. The genotypes 1631 and 619 were closest or nearly similar to each other.

Conclusions

The present investigation validated the use of RAPDs to find new sources of advantageous alleles and could investigate the present divergence with the moderately wide genetic basis of breeding material. The use of landraces in national breeding programmes will benefit from the selection because of the favourable core of markers linked to high genetic diversity. In order to help onion producers and breeders create high-yielding inbreds and hybrids, the molecular information revealed by the current work will be linked with the results of the field evaluation. This study utilized RAPDs for screening out potential onion genotypes. These results would be useful to select onion genotypes based on their genetic distance and/or similarities and morphological characters.

References

1. Bradeen JM, Havey MJ. Randomly amplified polymorphic DNA in bulb onion and its use to assess inbred integrity. Journal of the American Society for Horticultural Science. 1995;120(5):752-758.
2. Camussi A, Spagnoletti Z, Melchiorre P. Numerical taxonomy of Italian maize populations: genetic distances on the basis of heterotic effects. Maydica. 1983;28(4):411-424.
3. Comstock RE, Moll RH. Genotype environment interactions. Statistical genetics and plant breeding (No. REP-1173. CIMMYT.). 1963.
4. Cramer CS, Havey MJ. Morphological, biochemical, and molecular markers in onion. Hort Science. 1999;34(4):590.
5. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13–15.
6. Fischer D, Bachmann K. Onion microsatellites for germplasm analysis and their use in assessing intra-and interspecific relatedness within the subgenus Rhizirideum. Theoretical and Applied Genetics. 2000;101:153-164.
7. Fritsch RM, Friesen N. Evolution, domestication and taxonomy. In Allium crop science: recent advances, 2002. p. 5-30.
8. Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, Van de Wiel C, *et al*. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Molecular breeding. 1997;3:381-390.
9. Khar A, Lawande KE, Negi KS. Microsatellite marker based analysis of genetic diversity in short day tropical Indian onion and cross amplification in related Allium spp. Genetic resources and crop evolution. 2011;58:741-752.
10. Maniruzzaman M, Haque ME, Haque MM, Sayem MA, Al-Amin M. Molecular characterization of onion (*Allium cepa*) using RAPD markers. Bangladesh Journal of

Agricultural Research. 2010;35(2):313-322.
11. Rohlf FJ. Statistical power comparisons among alternative morphometric methods. American Journal of Physical Anthropology. 2000b;111:463–478.
12. Sangeeta Kutty M, Veere Gowda R, Anand L. Analysis of genetic diversity among Indian short-day onion (*Allium cepa* L.) cultivars using RAPD markers. The Journal of Horticultural Science and Biotechnology. 2006;81(4):774-778.
13. Semagn K, Bjornstad A, Ndjiondjop MN. An overview of molecular marker methods for plants. African journal of biotechnology, 2006, 5(25).
14. Singh SR, Ahmed N, Lal S, Ganie SA, Mudasir A, Nusarat J, *et al*. Determination of genetic diversity in onion (*Allium cepa* L) by multivariate analysis under long day conditions. 2013.
15. Sudha GS, Ramesh P, Sekhar AC, Krishna TS, Bramhachari PV, Riazunnisa K. Genetic diversity analysis of selected Onion (*Allium cepa* L.) germplasm using specific RAPD and ISSR polymorphism markers. Biocatalysis and Agricultural Biotechnology. 2019;17:110-118.
16. Wilkie SE, Isaac PG, Slater RJ. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in Allium. Theoretical and Applied Genetics. 1993;86:497-504.
17. Joshi N, Caputo GM, Weitekamp MR, Karchmer AW. Infections in patients with diabetes mellitus. New England Journal of Medicine. 1999 Dec 16;341(25):1906-12.