DOI : 10.5958/0974-0112.2016.00054.2

Indian J. Hort. 73(2), June 2016: 238-244

Genetic diversity and relationship study of single and double petal tuberose (*Polianthes tuberosa* L.) cultivars based on RAPD and ISSR markers

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ABSTRACT

Tuberose (*Polianthes tuberosa* L.) is a commercial ornamental bulbous crop with highly fragrant flowers. In the present study, genetic diversity among 12 tuberose cultivars were studied using randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Altogether 86 amplicons were amplified with 16 selected RAPD primers out of which, 70 (81.39%) were polymorphic with an average of 4.37 amplicons per primer. Similarly, nine ISSR primers amplified a total of 40 scorable amplicons with sizes ranging between 100-3000 bp. Out of 40 ISSR amplicons, 37 (92.50%) were polymorphic, with an average of 4.1 amplicons per primer. RAPD markers based analysis grouped 12 cultivars into two clusters, whereas ISSR markers based analysis grouped 12 cultivars in three clusters. Analysis of molecular variance (AMOVA) study showed only 5% of variation between single and double petal population, whereas, greater variation (95%) was observed only among the cultivars of each petal type. Principal Coordinate Analysis (PCoA) showed the percentage of variation explained by the first 3 axes was 51.44%.

Key words: Genetic diversity, RAPD, ISSR, tuberose.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.), is a member of family Agavaceae and native to Mexico. In India, the tuberose occupies a prime position among the commercial ornamental bulbous crops because of its highly fragrant flowers, which are used in different forms such as, in making of bouquets or in manufacture of perfume from its essential oil (Benschop, 1; Sadhu and Bose, 15). Tuberose essential oil is reported to have different biological activities such as antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing (Nidiry and Babu, 9).Tuberose plants have a considerable capacity of metal absorption and its accumulation, a requisite for remediation of soil contaminated with different levels of chromium (Ramana *et al.*, 12). Characterization of accessions of tuberose provides unique opportunity for their identification, conservation, management and its subsequent utilization in crop improvement programmes. Molecular approaches represent a potential tool for effective characterization of germplasm (Ferguson *et al*., 3; Figliuolo and Perrino, 4; Maestri *et al*., 7), which circumvents the limitations associated with morphological and biochemical characterization (Caetano-Anollecs, 2). Majd *et al*. (8) reported variation in tuberose collections using Randomly Amplified Polymorphic DNA (RAPD) markers and grouped seven populations into two major groups. Sarkar *et al*. (17) analyzed twenty tuberose genotypes comprising both single and double petal types collected from different parts of India using RAPD markers. Kameswari *et al*. (6) studied the genetic relationship of seven tuberose genotypes by ISSR (Inter simple sequence repeats) molecular markers.

Characterization of tuberose cultivars through molecular markers is much desired due to prevailing confusion in ascribing unique identity to genotypes existing in different geographical region of India as they are loosely referred as single and/or double petal cultivars. It is expected that all single petal genotypes might have evolved from single petal parent and accordingly, all double petal genotypes from its respective double petal parent. At present only few studies are recorded with regard to diversity and genetic relationship in tuberose using DNA markers. The present study has been undertaken with the objective of an overall assessment of genetic diversity and genetic relationship among the twelve cultivars of tuberose (six each of single and double petaled respectively) using RAPD and ISSR markers.

MATERIALS AND METHODS

Tuberose cultivars (single and double petal types) were collected from the Directorate of Floricultural Research, Pune, India and were maintained at IARI Farm, New Delhi. These cultivars were characterized for 10 qualitative characters as shown in Table 1.

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For molecular analysis, DNA was extracted from the leaf tissues using CTAB (Cetyltrimethyl ammonium bromide) method of Saghai-Maroof *et al*. (16) with minor modification. To remove excess phenolics present in the sample 2% PVP was added. Eighty RAPD and 47 ISSR primers for polymorphism among tuberose genotypes. Finally, 16 RAPD and 9 ISSR primers, which produced consistent results, were used for the generation of markers profiles. PCR amplification was optimized for RAPD and ISSR with reaction volume of 25 µl containing 1x PCR Buffer, 2.5 Mm of $MgCl_2$, 0.2 mM of each dNTPs (dATP, dCTP, dTTP and dGTP), 1U *Taq* polymerase (Fermentas, USA) and 30 ng DNA. Amplification was carried out in a thermo-cycler (G-STORM, UK) with following thermal cycle conditions. For RAPD the amplification cycles included: Initial denaturation at 94°C for 2 min. followed by 40 cycles of denaturation (94°C) for 1 min., annealing at 37°C for 1 min., extension at 72°C for 2 min. followed by final extension at 72°C for 10 min. For ISSR the amplification cycles were as follow: Initial denaturation at 94°C for 5 min. followed by 35 cycle of denaturation (94°C) for 30 sec, annealing at (42°C) for 1 min., extension at 72°C followed by final extension at 72°C for 5 min. The amplified products were separated by agarose gel electrophoresis (1.2%) and gel was stained with ethidium bromide. DNA ladder (100 bp; Fermentas, USA) was used as size standard. Gel pictures were taken under UV light using gel documentation system (Alpha Imager®, USA).

Amplified products were scored across the lane with respect to their molecular size. Presence of band was scored as (1) and absence of band scored as (0). Reproducible amplified fragments of RAPD and ISSR (*i.e.* bands present in two replications for each sample) were scored manually. Weak bands with negligible intensity and smeared bands were excluded from the final data analysis. Genetic similarity between two genotypes I and J was estimated by the formula Genetic similarity (GS) = 2Nij/ (Ni+Nj), where Nij is the number of band present in genotype i and genotype j, Ni (resp. J). A cluster analysis was done by UPGMA method with NTSYS 2.02 software (Rohlf, 13). The ability of a primer or technique to distinguish between large numbers of genotypes, *i.e*. Resolving Power (Rp) of RAPD and ISSR primers were determined as described by Prevost and Wilkinson (11). To measure reliability of the resulting genotypic groups, the original matrix was bootstrapped 1000 times by employing Winboot in order to group the accessions into discrete clusters. Bootstrap values obtained with Winboot were placed on the nodes of each cluster on the dendrogram generated by NTSYSpc 2.1. Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA) were performed using software GenAlEx V6.5 (Peakall and Smouse, 10).

RESULTS AND DISCUSSION

Twelve different tuberose cultivars were subjected to 10 different qualitative characters analysis (Table 1). Out of 12 cultivars, six were of single petal type, whereas, remaining were double petal type. In general, the qualitative characters recorded in the present study clearly showed diversity among cultivars at morphological level.

Initially, 80 RAPD primers were screened in order to identify consistent and reproducible markers and only 16 RAPD primers were identified consistent and

Table 1. Qualitative characters of 12 tuberose cultivars.

Cultivar	No. of rows of petals	Foliage attitude	Intensity of green leaf	Anthocyanin colour at base	Density of leaves	Floret colour	Petal shape	Floret tube shape	Bud colour	Flower spike curvature
Hyderabad Single	Single	Semi-erect	Medium	Absent	Medium	WG155A	Subobtuse	Less curve	Pinkish	Absent
Shringar	Single	Semi-erect	Light	Absent	Medium	WG155B	Subobtuse	Less curve	Pinkish	Absent
Mexican Single	Single	Erect	Medium	Present	Dense	WG155B	Acute	Less curve	Greenish	Absent
STR 505	Double	Drooping	Light	Present	Dense	WG155A	Acute	Straight	Pinkish	Absent
Pearl Double	Double	Erect	Medium	Present	Medium	WG155A	Acute	Straight	Pinkish	Absent
Suvasini	Double	Semi-erect	Light	Present	Dense	WG155A	Acute	Straight	Pinkish	Absent
Calcutta Double	Double	Semi-erect	Medium	Present	Dense	WG155A	Acute	Straight	Pinkish	Absent
Vaibhav	Double	Drooping	Light	Present	Medium	WG155A	Acute	Straight	Greenish	Absent
Sikkim Selection	Single	Erect	Medium	Present	Sparse	WG155A	Obtuse	Less curve	Pinkish	Present
GKTC4	Single	Drooping	Medium	Absent	Medium	WG155A	Subobtuse	Less curve	Greenish	Absent
Arka Nirantara	Single	Semi-erect	Dark	Present	Dense	WG155B	Subobtuse	More curve	Pinkish	Present
Swarn Rekha	Double	Drooping	Dark	Present	Sparse	WG155C	Acute	Straight	Pinkish	Absent

reproducible. A total of 86 amplicons were amplified with 16 selected RAPD primers (5.37 amplicons per primer), of which 70 (81.39%) were polymorphic (Table 2) with an average of 4.37 amplicons polymorphic per primer. The number of amplicons produced per primer ranged between 3 (SBSB-17) to 8 (SBSD-13). Majd *et al*. (8) used 14 RAPD primers to analyze seven populations of tuberose (single and double petal types) and observed polymorphism with RAPD primers was 58.8%. The resolving power (Rp) of the 16 RAPD primers used in the present study ranged from 3.52 (SBSD-19) to 11.49 (SBSB-10) with an average of 6.75 per primer. The primers with the high Rp values were more informative as they were able to distinguish greater number of tuberose genotypes. However, in contrast to present study, the resolving power value reported by Sarkar *et al*. (17) for the RAPD primers was though lower.

From our initial analyses with 47 ISSR primers, nine primers were found informative for subsequent molecular characterization. These nine ISSR primers amplified 40 scorable amplicons (4.44 amplicons per primer). The number of amplicons produced per primer ranged between 2 (UBC-856) to 8 (UBC-834).

Table 2. RAPD primers used for characterization of tuberose cultivars with total number of bands, total polymorphic bands, per cent polymorphism and resolving power.

Primer	TNB	TPB	P%	Rp
SBSB-05	5	5	100	5.82
SBSB-10	7	5	71	11.49
SBSB-11	6	5	83	8.3
SBSB-17	3	1	33	5.82
SBSB-18	6	6	100	5.04
SBSC-04	4	1	25	7.12
SBSC-09	7	7	100	5.62
SBSC-11	6	6	100	5.66
SBSC-12	4	4	100	3.82
SBSC-14	4	4	100	4.68
SBSC-15	6	5	83	4.5
SBSD-13	8	6	75	7.3
SBSD-16	6	6	100	7.78
SBSD-18	6	2	33	5.04
SBSD-19	4	4	100	3.52
SBSD-20	4	4	100	6.16
Total	86	70		
Average	5.37	4.37	81.39	6.75

TNB = Total No. of bands; NPB = No. of polymorphic bands; P% = Polymorphism percentage; Rp = Resolving power

Out of 40 amplicons produced, 37 were polymorphic (92.50%) with an average of 4.1 amplicons per primer (Table 3). Kameswari *et al*. (6) used six ISSR primers to characterize seven tuberose genotypes and reported 85.48% polymorphism, which supports our finding of having higher polymorphism with ISSR marker system. The Rp values ranged from 1.66 (UBC-856) to 6.28 (UBC-834) with average value of 3.62. The other primers with high Rp values were UBC-835 (4.48), UBC-827 (4.28) and UBC-836 (3.66).

Jaccard's similarity coefficient from our analyses, the range of genetic similarity among cultivars varied from 20.8% (Hyderabad Single and GKTC4) to 91% (Suvasini and Pearl Double). Such a range in terms of genetic similarity suggested that the tuberose cultivars were genetically diverse. Jaccard's similarity coefficient based UPGMA analysis grouped all twelve cultivars into two major clusters (Fig. 1). Calcutta Double and Hyderabad Single were grouped in cluster 1, whereas, 7 cultivars were grouped in cluster 2 and cultivars GKTC4, Vaibhav and Arka Nirantra remained ungrouped. Earlier, Sarkar *et al*. (17) used 17 RAPD primers to characterize 20 tuberose genotypes and UPGMA based cluster analysis grouped all genotypes into two major clusters. The bootstrap values showed that cultivar GKTC was distinct with 100% reliability whereas, Arka Nirantra and Vaibhav had 75 and 61% bootstrap values, respectively. The bootstrap value in above analyses varied from 98 to 16% among the clusters (Fig. 1).

Jaccard`s similarity coefficient value ranged 10-70% based on ISSR data. Maximum similarity was observed between Sikkim Selection and STR550

Table 3. ISSR primers used for characterization of tuberose cultivars with total No. of bands, total polymorphic bands, per cent polymorphism and resolving power.

Primer	TNB	TPB	P%	Rp
UBC-827	5	4	80	4.28
UBC-825	4	4	100	3.14
UBC-834	8	8	100	6.28
UBC-826	4	3	75	3.34
UBC-836	5	5	100	3.66
UBC-855	4	3	75	3.34
UBC-841	3	3	100	2.46
UBC-835	5	5	100	4.48
UBC-856	2	2	100	1.66
Total	40	37		
Average	4.44	4.1	92.50	3.62

TNB = Total No. of bands; NPB = No. of polymorphic bands; P% = Polymorphism percentage; Rp = Resolving power

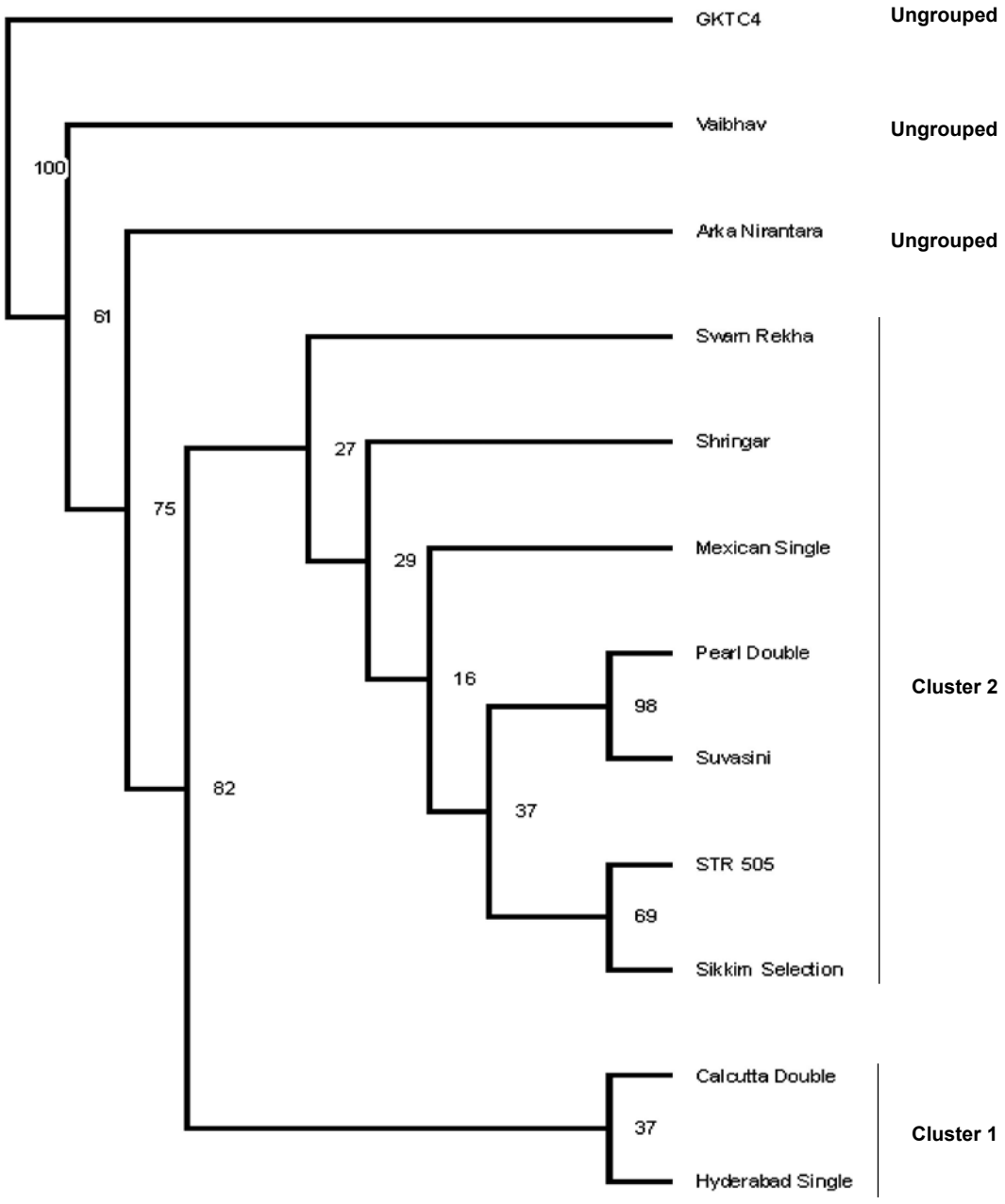

Fig. 1. Phylogenetic tree of twelve tuberose cultivars based on RAPD data.

(70%), whereas, least similarity was observed between Arka Nirantara with both GKTC4 and Calcutta Double (10%). UPGMA analysis based on Jaccard's similarity coefficient grouped all twelve cultivars in three major clusters (Fig. 2). In cluster 3, five cultivars were grouped together and except GKTC4, all other cultivars (Calcutta Double, Vaibhav, Pearl Double and Suvasisni) were double petal type. The bootstrap value of 100% for cluster 1 indicated its high reliability and thus remained separated from cluster 2 and cluster 3; whereas bootstrap value of 3% between cluster 2 and cluster 3 suggested loose grouping and such clustering might change with addition of more number of markers (Fig. 2).

It is assumed that single type and double petal tuberose might have evolved from different genotypes and both maintain their separate identity. To test this hypothesis, single and double petal cultivars in the present study were considered as two independent populations and each population were having six

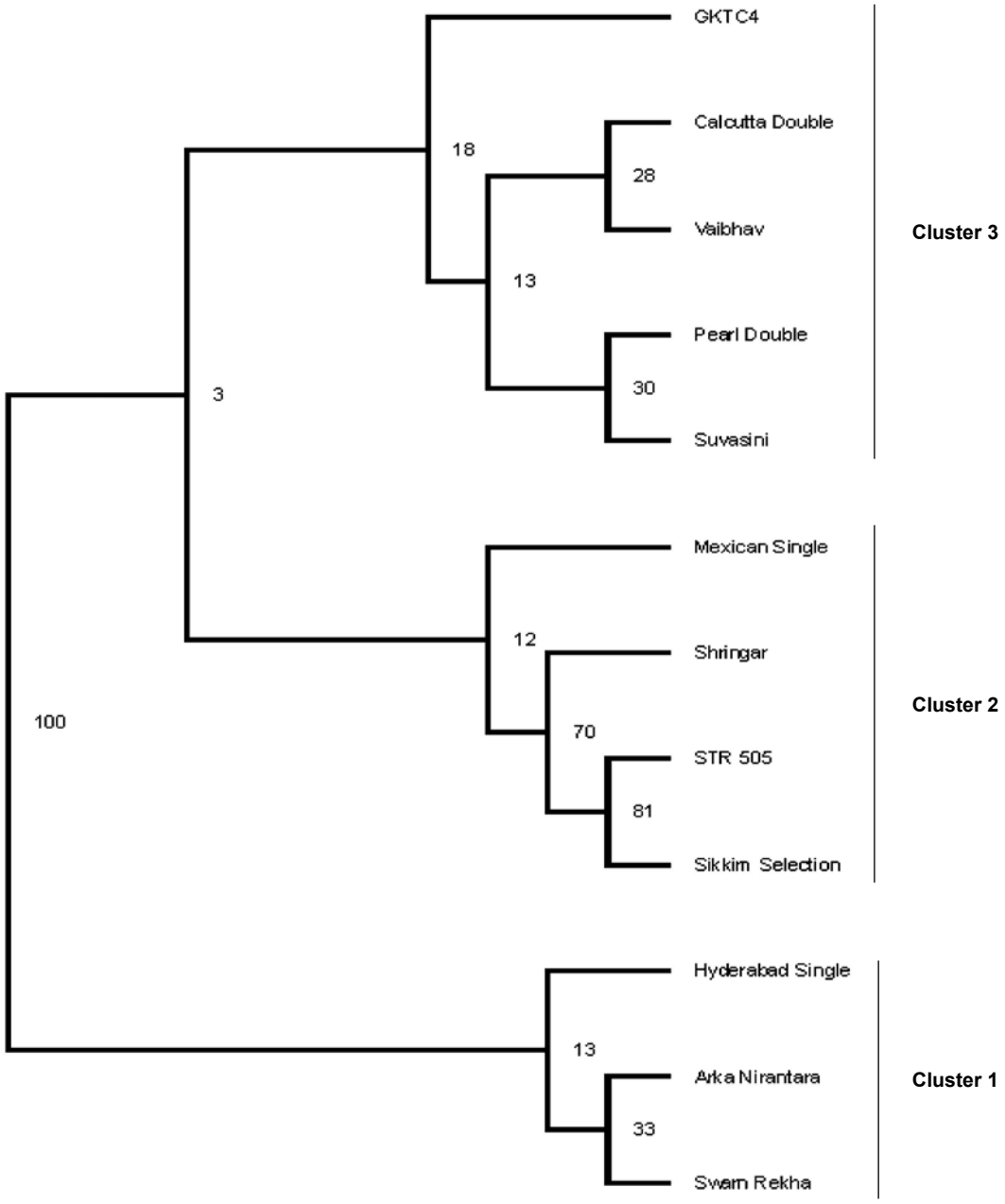

Fig. 2. Phylogenetic tree of twelve tuberose cultivars based on ISSR data.

cultivars. Analysis of molecular variance (AMOVA) analyses for combined RAPD and ISSR data obtained 5% variation between the two populations (Fig. 3), and that indicated less variation between two groups, *i.e*. single and double petal types. We observed maximum variation among cultivars of each population (95%). Similar to our findings, Ganesan *et al*. (5) and Rufai *et al*. (14) also observed significant variation within population of *Moringa oleifera.* The observed partitioning of variation supports UPGMA based clustering and further supports absence of genetic isolation between single and double petal type genotypes.

Principal Coordinate Analysis (PCoA) with combined data of RAPD and ISSR markers demonstrated existence of greater diversity tuberose cultivars. In the above analyses, all cultivars exhibited uniform distribution across the two axes (Fig. 4). The percentage of variation explained by the first three axes was 51.44%. Earlier, Kameswari *et al*. (6) observed

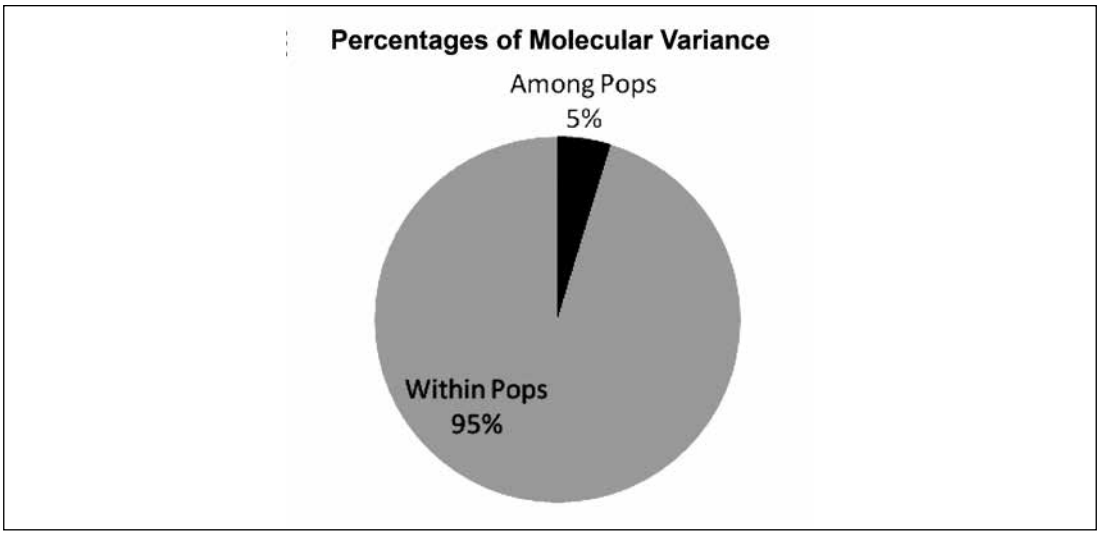

Fig. 3. Analysis of molecular variance (AMOVA) based on pooled RAPD and ISSR data.

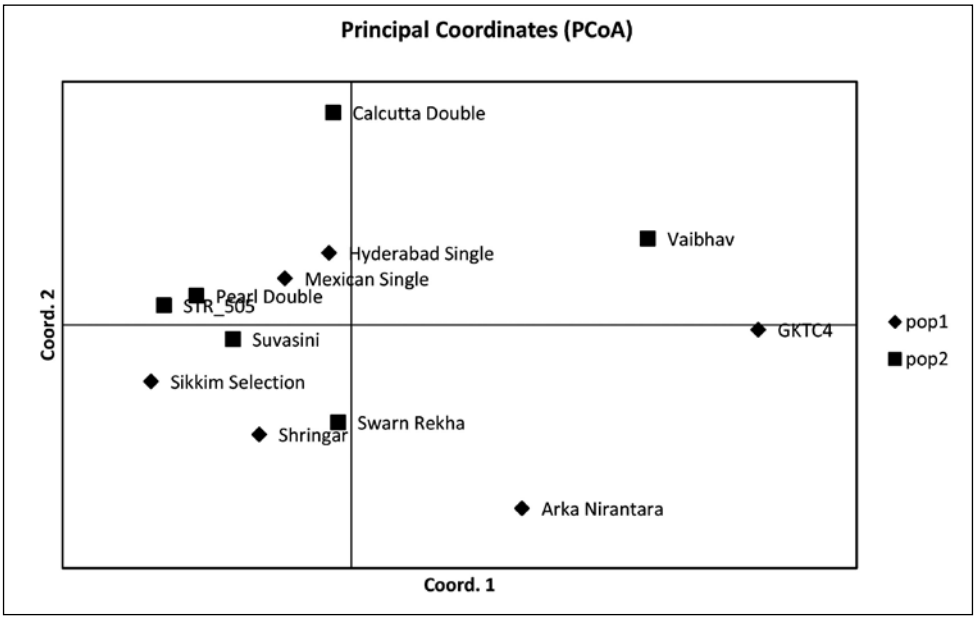

Fig. 4. Principal Coordinate Analysis (PCoA) based on pooled RAPD and ISSR data. Pop1 - Single petals, Pop2 - Double petals.

that the first two principal components accounted for major variations. The first component accounts for 55.76% of all changes and second component accounted for 13.95% of principal changes. The intermixing of cultivars belonging to two different population types across the coordinates, support the UPGMA tree and AMOVA analysis (Fig. 4). Lack of genetic-based clustering of single and double petal types, indicated non-significant diversity between two petal types, however large diversity was observed between the cultivars of both petal types.

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Received : May, 2014; Revised : March, 2016;
Accepted : May, 2016