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Research Article

Marker based assessment of genetic diversity in *aethiopicum* and *melongena* species of genus *Solanum*

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Abstract
The genetic diversity among 14 genotypes of *Solanum melongena* and *Solanum aethiopicum* was assessed using 40 polymorphic markers of SSR, ISSR and RAPD. Twelve RAPD markers with polymorphism percentage of 63.0 to 100.0 amplified 7.75 loci per marker with a total of 93 polymorphic bands. Sixteen polymorphic ISSR markers revealed 73 polymorphic loci with 4.56 polymorphic loci per marker and polymorphism percentage of 43.0 to 100.0 whereas 12 SSR markers amplified 43 polymorphic alleles with average polymorphic alleles per marker of 3.58. Forty markers of RAPD, ISSR and SSR detected a total of 57 unique/novel alleles with *aethiopicum* whereas 19 unique/novel alleles were generated in different genotypes of *melongena*. Each marker system individually as well as altogether exhibited least similarity coefficients of 0.24 to 0.29 between *aethiopicum* and *melongena* species of *Solanum* whereas higher order of similarity was observed within *melongena* group. Dendrograms generated using marker data of RAPD, ISSR, SSR and RAPD+ISSR+SSR separated *aethiopicum* and *melongena* in two divergent groups. We did not observe exactly similar pattern of grouping of different genotypes of *melongena* group in more homogenous cluster when different marker systems were compared. The Mantel test between the two Jaccard's similarity matrices indicated high correspondence between RAPD and ISSR, RAPD and SSR, and ISSR and SSR based similarity matrices. Distribution of genotypes from different regions did not show clear clustering pattern when three marker systems were compared. Three dimensional plots of genotypes based on principal component analysis indicate close correspondence with UPGMA clustering approach.

Key words
Eggplant, melongena, *aethiopicum*, markers, diversity

Introduction

Eggplant (*Solanum melongena* L.) is one of the important vegetable crops of Asia, Europe, Africa and America of genus *Solanum* and subgenus, *Leptostemonum*, which includes many wild relatives as well as other cultivable species, such as Gboma eggplant (*Solanum macrocarpon* L.) and the Scarlet eggplant (*Solanum aethiopicum* L.), mostly grown in Africa for their fruits and leaves (Tümbilen *et al.*, 2011). A large number of land races, wild/weedy species are found in India as well as Indo-china regions in the *Solanum melongena* complex, in which *S. insanum* has been regarded as the progenitor of cultivated eggplant (Lester and Hasan 1991). *S. melongena* has been an object of intensive research mainly in the countries namely Western Europe, Turkey, India, China, Japan where eggplant cultivated intensively. In some African countries, cultivars of *S. aethiopicum* are bred, but on a small scale, due to economic, sociological, and political situation in this region (Sekara *et al.*, 2007). Hebert (1985) showed high level of resistance to bacterial wilt is available in the *S. aethiopicum* Aculeatum group. Efforts to introgress gene in eggplant from *S. aethiopicum* and wild species has been limited due to sexual incompatibilities. However, Ano *et al.*, (1991) successfully introgressesd resistance to bacterial wilt (*Pseudomonas solanacearum*) in cultivated variety of *S. melongena* through hybridization from *S. aethiopicum* indicating possibility to channelized species diversity in the improvement of eggplant.

Success of hybrid as well OPV development programme is based on the availability of potential diverse germplasm. The degree of heterotic effect of F_1 population is correlated with genetic divergence of parental lines, as parents are more divergent, the heterosis is higher and vice- versa (Prasad and Singh 1986). Moll *et al.*, (1965) however observed that heterosis increases with increased divergence within a restricted range, but may not hold true over the entire range of divergence encountered in the species. Cress (1966) demonstrated that GD is necessary for significant heterosis but not sufficient to guarantee it. Genetically dissimilar parents may produce poor hybrids in the presence of linkage and epistasis. Similarly, multiple allelism can also give the same result even in the absence of linkage and epistasis. Falconer (1981) has defined heterosis as the product of directional dominance and square of differences in gene frequency in parents. Therefore, it is apparent that the presence of both dominance and initial differences in gene frequency in parental lines causes heterosis in F_1. Heterosis will be the greatest, as pointed out by Falconer (1981), when one allele is in homozygous

state in one parent and the other allele in the other parent. Further, significance of genetic diversity seems to be obvious if objective of a breeding programme is to develop a variety through identification of transgressive segregants in segregating generation of a biparental or multiparental cross. The diversity at species level has great significance especially in introgressing biotic and abiotic stress tolerance in cultivated eggplant. Thus, characterization and grouping of germplasm seems to essential step in exploitation of allelic diversity from diverse sources in hybrid or varietal development programme.

There are many approaches used to quantify the diversity at intra as well as interspecies level, however, molecular markers are considered to have enormous potential to explore genetic diversity by detecting polymorphisms at DNA level. They are useful tools for breeding, genotype identification, and the determination of genome organization and evolution in plants. Among the marker systems, random amplified polymorphic DNAs (RAPDs) and Inter simple sequence repeats (ISSR) are polymerase chain reaction (PCR) based and considered to be the simplest marker system. Analysis of ISSR involves the PCR amplification of regions between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (di-, tri-, tetra-, or pentanucleotides) containing primers anchored at the 3′or 5′end by two to four arbitrary, often degenerate nucleotides (Zietkiewicz *et al.,* 1994). The primers used in our analysis were anchored at 3′end to ensure that perfect annealing of the primer occurs at the 3′end of the microsatellite motif, thus obviating internal priming and smear formation. The anchor also allows only a subset of the targeted inter-repeat regions to be amplified, thereby reducing the high number of PCR products expected from the priming of dinucleotide inter-repeat region (Zietkiewicz *et al.,* 1994). ISSR markers have been utilized for the analysis of repeat motifs in eggplant and other species (Isshiki *et al.,* 2008; Ali *et al.,* 2011; Shilpha *et al.,* 2013). RAPD markers, on the other hand, provide a rapid, inexpensive and effective system for studying plant genetic relationships. Since 1990, the RAPD technique has been extensively used in plant systematic studies, especially in the identification of germplasm resources and the measurement of variation to establish evolutionary relationships within or between species, subspecies or populations and genomes. RAPD markers have been used successfully in determination of genetic diversity in eggplant and other species (Nunome *et al.,* 2001; Ali *et al.,* 2011; Shilpha *et al.,* 2013).

Because of co-dominant inheritance, abundance, wide genome coverage and multi-allelic nature, simple sequence repeats (SSRs) or microsatellites have now become the marker of choice (Sarikamiş *et al.,* 2010; Bisen *et al.,* 2015; Kumawat *et al.,* 2015). A number of SSR markers have been identified in Solanaceae (Yi *et al.,* 2006; Bindler *et al.,* 2007), but the numbers are less in eggplant. The development of SSR markers derived from SSR-enriched genomic library of eggplant has been reported by Nunome *et al.,* (2003, 2009). SSR markers have been used in determination of genetic diversity in eggplant (Nunome *et al.,* 2003; Stagel *et al.,* 2008; Nunome *et al.,* 2009).

Deployment of more than one marker system together in genotyping seems to be more precise and reliable in terms of coverage of genomic regions and determination of genetic diversity among the germplasm when compared with analysis using single marker system. Different markers have different specificity to the genomic regions and detect loci from different regions of the genome. The probability to cover more genomic regions using different markers increases with types and number of markers. Thus, errors or problems occurring with the use of a certain marker system alone could be minimized when markers are used in combination (Saker *et al.,* 2005; Leal *et al.,* 2010; Pakseresht *et al.,* 2013). Different combinations of markers system have been used in diversity analysis in many plant species (Li *et al.,* 2013; Shilpha *et al.,* 2013; Nascimento *et al.,* 2013; Wu *et al.,* 2014) including eggplants where RAPD and AFLP (Nunome *et al.,* 2001), RAPD and SSR (Demir et al 2010: Ali *et al.,* 2011) and SSR and morphological markers (Cericola et al 2013) have been used successfully. Therefore, the objective of this study was to use RAPD, ISSR and SSR markers in combination for a detail analysis of genetic diversity 13 genotypes of *S. melongena* and with one genotype of *S. aethiopicum*.

Materials and method

Plant materials: The materials used in the investigation comprised of one accession of *Solanum aethiopicum* and 13 accessions of *Solanum melongena* have been depicted in Table 1 along with salient features and geographical locations. Each accession was sown in pots and leaf sample from 2-3 week old seedlings were collected for genomic DNA isolation.

Genomic DNA Extraction and quantification: Freshly collected leaf sample of each accession was ground to fine powder in mortar and pestle using liquid nitrogen and immediately it was processed using the Plant Genomic DNA isolation kit (HiMedia Laboratories Pvt. Ltd, India). The kit was especially designed for plant genomic DNA isolation and based on Cetyl Trimethyl Ammonium Bromide (CTAB) method. The RNA content in genomic DNA isolated using the kit was removed using RNase A. Quantity and quality of the genomic DNA was determined using dual

beam spectrophotometer (Systronics, India) and agarose gel electrophoresis (Sambrook and Russel 2001).

DNA Markers: Polymerase chain reaction (PCR) based markers namely RAPD, ISSR and SSR were used to quantify the genetic diversity of the *aethiopicum* and *melongena* species. In total 46 markers comprised of 12 RAPDs, 20 ISSRs and 14 SSRs were used in the investigation.

PCR Amplification: PCR amplification was performed in a volume of 25 µl. Each 25µl reaction mixture consisted of 100 ng of DNA, 200 µM dNTPs, 1U of Taq polymerase, 1X Taq buffer and 1.2 µM primer in case of RAPD and ISSR whereas 0.6 µM each of forward and reverse primers were used in case of SSR marker. Amplication reaction was performed with initial denaturation at 94 ^{0}C for 5 min followed by 35 cycles of denaturation at 94^{0}C for 45 second (RAPD and ISSR) and for 30 second (SSR), annealing of primers for 1 min at 38 ^{0}C (RAPD), 45 ^{0}C (ISSR), 45-60 ^{0}C (SSR), and polymerization for 2 min at 72 ^{0}C (RAPD and ISSR) and for 1 min at 72 ^{0}C (SSR). One cycle of final extension was maintained for 5 min at 72 ^{0}C. Amplification reaction was performed in dual block DNA engine (BioRad, USA). PCR amplified DNA fragments were resolved using 1.2% agarose gel electrophoresis for RAPD and ISSR markers whereas 3% agarose gel was used to resolve the fragments generated using SSR primers. The gels were stained with ethidium bromide (0.5mg/ml) and documented using Gel Documentation System (Alpha Imager EC).

Scoring of gel and analysis of data: Each genotype was scored as presence (1) or absence (0) of respective allele against each primer. Same procedure was used for recording observation separately with all the three markers system. Pair wise Jaccard's similarity coefficients were calculated to determine the similarity of *S. melongena* and *S. aethiopicum* genotypes. Unweighted pair group method with arithmetic average (UPGMA) based dendrogram was generated to determine marker based genetic relationship amongst the 14 genotypes. The data of each marker were analysed separately as well as in combination using the NTSYS-pc software (Rohlf, 2000). Statistical stability of the branches in the cluster was estimated by bootstrap analysis using the Winboot software program (Yap and Nelson 1996). Correlation between Jaccard's similarity matrices of pair wise marker systems was determined by means of the Mantel matrix correspondence test (Mantel 1967). This test yields a product moment correlation (r) that is one measure of the relatedness between the two matrices. Three dimensional plots of 14 genotypes were obtained using principal component analysis.

Result and discussion

In the present investigation, 46 markers comprised of 12 RAPD markers, 20 ISSR markers and 14 SSR markers were used to characterize 13 genotypes of *S. melongena* and one genotype of *S. aethiopicum* and further to quantify the molecular diversity among these genotypes.

RAPD marker based genotyping: Twelve RAPD markers amplified a total of 114 reproducible loci. The loci amplified by each marker varied from minimum of 4 with random marker AC-11 to maximum of 17 with OPA-01 with an average of 9.5 loci per marker (Table 2). Of the 114 bands, 21 were found to be monomorphic whereas 93 bands were polymorphic. The average polymorphic bands were recorded to be 7.75 per primer. The polymorphism percentage ranged between 63.00 % (OPA-11) to 100 % (OPA-10 and OPA-18). All the RAPD markers except OPA-07 detected 20 unique bands (1.67/marker) in *aethiopicum* whereas only four markers generated 7 unique bands (0.58/marker) in *melongena* genotypes. The marker OPA-01 was identified to be most effective marker in terms of detecting and amplifying unique loci in *aethiopicum*. The pair wise Jaccard's similarity coefficients estimated based on 12 RAPD markers were found to vary from 0.26 (between *S. aethiopicum* and PB-4 of *S. melongena*) to 0.86 (between NDB-1 and PB-70 of *S. melongena*) followed by 0.85 (between WB-1 and PB-67). The UPGMA based dendrogram generated based on RAPD markers divided 14 genotypes into two major groups A and B at 0.33 similarity coefficient (Fig 1a). The first major group consisted of only one genotype of *S. aethiopicum* while second major group included thirteen genotypes of *S. melongena*. PB-4 and KS-331 of group II formed two separate clusters namley cluster I and cluster II, respectively, with similarity coefficient of 0.68 wheraes these two clusters differentiated from other members of this group at similarity coeffcient of ca 0.69. The cluster III grouped six genotypes and differentiated at similarity coefficient of 0.76 with five members of cluster IV. Three dimensional plots of 14 genoypes using RAPD marker data revealed differental distribution of *aethiopicum* from *melongena* (Fig 2a). Distribution of S. *aethiopicum* genotype made clear cut distinction from the distribution pattern of 13 genotypes of S. *melongena*. Three dimensional plots of 14 genotypes gave pattern of grouping similar to those observed with dendrogram based grouping.

ISSR marker based genotyping: Sixteen polymorphic ISSR markers of the 20 initially

screened were used to characterize the genetic diversity among 14 genotypes. Of the total of 96 loci detected with 16 ISSR primers, 73 were noted to be polymorphic and 23 were monomorphic (Table 3). The average number of polymorphic loci detected by each marker was 4.56. The number of amplified loci varied from 2 with primer 8161-059 to a maximum of 11 each with marker 8161-041 and 8161-042. The least polymorphic ISSR marker was 8161-048 (43.00 %) whereas three ISSR markers namely 8161-045, 8161-053 and 8161-059 were identified to be most polymorphic (100 %). The unique loci amplified with 16 ISSR markers were 26 in *aethiopicum* and 6 in *melongena.* The ISSR markers 8161-041 generated maximum of 6 unique bands followed by 8161-042 with 5 unique bands in *aethiopicum.*

ISSR marker based pair wise Jaccard's similarity coefficients was found to vary from 0.267 (between *aethiopicum* and LC-7 of *melongena*) to 0.955 (between BARI and PB-66, BARI and NDB-1 of *S. melongena*) followed by 0.94 (between PB-66 and NDB-1). The UPGMA based dendrogram analysis classified 14 genotypes in to two broad groups designated as Group I and Group II (Fig 1b). Group I consisted of one genotype of S. *aethiopicum* whereas the other group consisted of all the genotypes of *S. melongena*. These two groups were separated to each other at similarity coefficient of ca 0.29. The group II was divided into four clusters. Cluster I consisted of four genotypes and was separated from one member of cluster II at similarity coefficient of ca 0.85. Cluster III formed a separate node and related to cluster II at similarity coefficient of 0.87 and with seven members of IV at similarity coefficient of ca 0.89. The principal component analysis of ISSR marker data and three dimensional depictions of 14 genotypes on plot revealed wide relationship between *S. aethiopicum* genotype and 13 genotypes *S. melongena* (Fig 2b). Within *S. melongena*, the distribution patterns were also noted to be similar to the pattern observed with dendrogram based grouping of the genotypes.

SSR marker based genotyping: Of the 14 SSR markers used initially, 12 were found to be polymorphic which amplified a total of 47 alleles. Out of 47, 43 alleles were noted to be polymorphic (Table 4). Amplified alleles varied in size from 80 bp to 380 bp amongst different genotypes with different markers. The marker EM145 detected one allele in all the genotypes of *melongena* group whereas the same gave null allele in *aethiopicum*. The average number of alleles per marker was 3.92 while in case of polymorphic bands, only 3.58 bands per primer were noted. Eleven unique alleles were noticed with all the SSR markers in case of *aethiopicum* whereas only six unique alleles were amplified in different genotypes of *melongena* (Table 4).

Pair wise Jaccard's similarity coefficients based on 14 SSR markers data revealed minimum similarity of 17.8% between *S. aethiopicum* and PR of *S. melongena* to maximum similarity of 94.1% between PB-71 and NDB-1.The dendrogram generated using UPGMA method on 14 SSR markers data separated *S. aethiopicum* (Group I) from genotypes of *S. melongena* (Group II) at similarity coefficient of 0.24 (Fig 1c). Based on the similarity values, the 13 genotypes of group II were further divided into four clusters. Cluster I consisted of four genotypes and separated from one member of cluster II at similarity coefficient of ca 0.74. Cluster III and IV separated to each other at similarity coefficient of ca 0.82 and together with these two clusters established similarity coefficient of ca 0.76 with cluster II. The SSR data based PCA generated three dimensional plot where *S. aethiopicum* occupied position quite distant from the positions of 13 genotypes of *S. melongena* (Fig 2c). The pattern of three dimensional plots of 14 genotypes was similar to grouping done by SSR marker based dendrogram.

RAPD +ISSR+ SSR marker based genotyping: In order to obtain more accurate genetic estimates, combined analysis was carried out using RAPD, ISSR and SSR markers data. Forty polymorphic markers together with generated 257 alleles with an average of 6.42 alleles per marker. The average percentage of polymorphism across the 40 markers was observed to be 83.29. The pair wise Jaccard's similarity coefficient between the genotypes was estimated using pooled markers data. The *S. aethiopicum* genotype had least similarity with all the genotypes of *S. melongena* where similarity coefficients varied from 0.267 between *S. aethiopicum* and KS-331 of *S. melongena* to 0.319 between *S. aethiopicum* and WB-1 of *S. melongena*. Among the *S. melongena* genotypes, PS and NDB-1 exhibited maximum similarity coefficient (0.881) whereas PB-4 and KS-331 had minimum similarity (0.717).

Dendrogram generated using combined marker data separated *S. aethiopicum* genotype in one group from another group of 13 *S. melongena* genotypes at similarity coefficient of ca 0.30 (Fig 1d). Group II consisted of 13 *S. melongena* genotypes were further divided into four clusters. Cluster I consisted of three genotypes. Cluster II and III exhibited similarity coefficient of ca 0.81 and together with these two clusters established similarity coefficient of 0.77 with cluster I. Two members of clusterIII diverged from seven members of cluster IV at similarity coefficient of ca 0.82. Three dimensional plots of 14 genotypes based on principal component analysis of combined marker data also gave distribution

pattern similar to what we observed with dendrogram based distribution of genotypes (Fig 2d).

The matrices for RAPD, ISSR and SSR markers were also compared using Mantel's test so that correspondence between the matrices generated using different markers could be determined. The correlation between the matrices of cophenetic correlation values for the dendrogram based on RAPD and ISSR, RAPD and SSR, ISSR and SSR data were observed to be high and positive (0.904, 0.87 and 0.97, respectively). All the three markers systems had similar pattern of grouping of 14 genotypes into two major clusters. The combination of three marker systems also grouped 14 genotypes in to two major groups. However, 13 genotypes of *S. melongena* grouped together were further differentiated into different clusters with variable genotypes in each using molecular data of RAPD, ISSR, SSR and combination these markers.

Discussion:The evaluation of germplasm and assigning the values is crucial for breeders to plan effective hybridization programme to produce new cultivars for higher productivity, adaptability and quality parameters. Molecular markers are considered to most effective tools in characterizing and quantifying allelic diversity in a set of germplasm. In brinjal, molecular marker namely ISSR and RAPD (Ali *et al.,* 2011; Shilpha *et al.*, 2013) and SSR (Nunome *et al.*, 2003; Stagel *et al.*, 2008; Nunome *et al.,* 2009) have been used in determining molecular diversity. Many investigators have used combinations of two markers systems in diversity analysis in many plant species (Li *et al.,* 2013; Shilpha *et al.,* 2013; Nascimento *et al.,* 2013; Wu *et al.,* 2014) whereas combination of RAPD and AFLP (Nunome *et al.*,, 2001), RAPD and SSR (Demir et al 2010: Ali *et al.,* 2011) and SSR and morphological markers (Cericola et al 2013) systems have been used successfully in diversity analysis in brinjal. In the present investigation, we used 40 markers of RAPD, ISSR and SSR to analyse diversity between *melongena* and *aethiopicum* species of *Solanum.*

Of the three marker system, 12 RAPD markers produced 112 alleles whereas 16 ISSR and 12 SSR markers 96 and 47 alleles, respectively. The high level of average polymorphism across the three markers was estimated to 83.29% which was lower than the level of polymorphism revealed by SSR (94.0%) but higher than the polymorphism revealed by RAPD (80.42%) markers ISSR markers (75.44%). High level of polymorphisms with different markers may not be surprising because of the geographical evolution of the species in the different agroclimatic zones and homeologous chromosome complement of *S. aethiopicum* and *S. melongena* revealed by moderate bivalent formation coupled with higher percentage of multivalent formation in crossed progenies (Roxas et al 1995).

All the three markers analysis independently as well as in combination discriminated *S. aethiopicum* from 13 genotypes of *S.melongena.* Thus, of the two major groups formed with individual as well as combination of three marker system, group one with one genotype of *S. aethiopicum* was differentiated with second group with 13 genotypes of *S. melongena* by similarity coefficient of 0.33 with RAPD markers, 0.29 with ISSR markers, 0.24 with SSR markers and 0.31 with combination of three marker systems. The marker analysis therefore indicates that *S. aethiopicum* had wide molecular diversity with *S. melongena*. High diversity is further supported by the homoeologous chromosome complement of *S. melongena* and *S. aethiopicum* has revealed by moderate bivalent formation coupled with higher percentage of multivalent in the F1 plus the occurrence of univalent (Roxas et al 1995). Ona (1995), however, noted close proximity between these two species based on diversity analysis among five *Solanum* species.

The separation of S. *aethiopicum* accession from the accessions of *S. melongena* and constituting two divergent groups using RAPD, ISSR and SSR marker data independently and also using combined data together indicate that clustering patterns are quite similar. Significant positive cophenetic correlations between dendrogram based on RAPD and ISSR, RAPD and SSR, ISSR and SSR data further support that clustering of accessions using the three marker systems in the present investigation were quite similar. The Principal Component Analysis using RAPD, ISSR, SSR data independently and also in combination and presentation in the form of three dimensional plots indicates divergent relationship between *S. aethiopicum* and *S. melongena*. Thus, principal component analysis using different markers data also corroborated well with their UPGMA clustering. A close correspondence between the similarity matrices of RAPD, ISSR and SSR noted in the present investigation indicate either of the markers individually or in combination can be effectively used in determination of genetic relationships among the accessions of S. *aethiopicum* and *S. melongena*. High positive cophenetic correlations were also noted earlier by Panwar *et al.,* (2010) in finger millet and by Singh *et al.,* (2014) in mungbean and other *Vigna* species. ISSR and SSR based clustering seems to have more correspondence than the RAPD and ISSR followed by RAPD and SSR. Souframanien and Gopalakrishna (2004) also noted low magnitude of correlation between RAPD and ISSR Jaccard's similarity coefficient value probably because of the difference in resolution of RAPDs

and ISSRs since two-marker techniques target different portions of the genome.

Clustering of 13 genotypes of within *S. melongena* group was not uniform when RAPD, ISSR and SSR derived dendrograms were compared. Loarce *et al.,* (1996) opined that such differences may be attributed to marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars. The difference in clustering pattern may also be attributed because of the distribution as well as target genomic regions of the different markers. In fact, the RAPD and ISSR markers revealed the diversity of the entire genome since they are scattered throughout the genome while specific loci are targeted with the SSR markers (Yang *et al.,* 2010; Gimenes *et al.,* 2007). Fernandez *et al.,* (2002) further stated that putatively similar size of bands originating with random markers in different genotypes may not necessarily be homologous and genetic relationship based on such banding pattern may be erroneous.

BARI, one of the genotypes of *melongena* group included in the study is a popular variety in Bangladesh. Similarly, genotypes WB1, SMB 115 and KS331 and NDB1 are the cultivars belonging to West Bengal, Orissa and Uttar Pradesh provincial state of India, respectively. The remaining of the experimental materials has been developed at Pantnagar which is situated in foothills and *tarai* region of Uttarakhand state. Distribution of genotypes from different regions did not show clear clustering pattern of with different marker systems, which indicated that the association between genetic similarity and geographical distance is less significant. Gene flow intentional or unintentional in the past might be a probable reason for such a distribution pattern based on marker data. Yee *et al.,* (1999) and Li *et al.,* (2013) noted similar observation while analyzing Azuki bean and Ruthenia Medic.

Genotypes possessed most distinct DNA profiles are expected to contain the greatest number of unique/novel alleles. Such genotypes are likely to serve as source to uncover the largest number of unique and potentially agronomic useful alleles (Souframanien and Gopalakrishna 2004). In the present investigation, *S. aethiopicum* exhibited most distinct DNA profile with 20 unique/novel alleles with RAPD, 26 with ISSR and 11 with SSR markers. The novel alleles may be agronomically important however, it requires further investigation to uncover the potential of novel alleles identified with *S. aethiopicum.* Unique/novel alleles identified with different markers in *S. melongena* also prove to be associated with desirable agronomical traits may serve as potential germplasm in improvement programme.

In the present investigation, we, therefore successfully characterize and determine the diversity between *aethiopicum and melongena* species of *Solanum* that may be further utilized in breeding or pre-breeding programme of eggplant.

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References

Ali, Z., Xu, Z.L., Zhang, D.Y., He, X.L., Bahadur, S., Yi, J.X. 2011. Molecular diversity analysis of eggplant (*Solanum melongena*) genetic resources. *Genet. Mol. Res.*, **10:** 1141-1155.

Ano, G., Hebert, Y., Prior, P., Messiaen, C.M. 1991. A new source of resistance to bacterial wilt of eggplants obtained from a cross: *Solanum aethiopicum* L x *Solanum melongena* L. *Agronomy*, **11:** 550-560.

Bindler, G., Van der Hoeven, R., Gunduz, I., Plieske, J. 2007. A microsatellite markers based linkage map of tobacco. *Theor. Appl. Genet.,* **114:** 341-349.

Bisen, A., Khare, D., Nair, P. and Tripathi, N. 2015. SSR analysis of 38 genotypes of soybean (GlycineMax (L.)Merr.) genetic diversity in India. *Physiol Mol Biol Plants*, **21:**109-115.

Cericola, F., Portis, E., Toppino, L., Barchi, L., Acciarri, N. 2013. The Population Structure and Diversity of Eggplant from Asia and the Mediterranean Basin. *PLoS ONE*, **8:** e73702.

Cress, C.E., 1966. Heterosis of the hybrid related to gene frequency difference between two populations. *Genetics*, **53:** 269-274.

Demir, K., Bakir, M., Sarikamis, G., Acunalp, A. 2010. Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. *Genet. Mole. Res.*, **9:** 1568-1576.

Falconer, D.S., 1981. Introduction to Quantitative Genetics. 2nd Edition, Longman, London. 340p.

Fernández, M.E., Figueiras, A.M., Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet.,* **104:** 845-851.

Gimenes, M.A., Hoshino, A.A., Barbosa, A.V., Palmieri, D.A., Lopes, C.R. 2007. Characterization and transferability of microsatellite markers of the cultivated peanut (*Arachis hypogaea*). *BMC Plant Biol.*, **7:** 9.

Guohao, H.E., Ronghua, M., Melanie, N., Guoquing, G., Roy, N.P., Prakash, Channapatna, S. 2003. Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). *BMC Plant Biol.*, **3:** 3.

Hebert, Y. 1985. Comparative resistance of 9 species *Solanum* to bacterial wilt (*Pseudomonas solanacearum*) and nematode *Meloidogyne incognita*. Interest in improving eggplant (*Solanum melongena* L) region tropical moist. *Agronomy*, **5:** 27-32.

Isshiki, S., Iwata, Khan, N.M.R. 2008. ISSR variation in eggplant (*Solanum melongena* L.) and related *Solanum* species. *Sci. Hort.*, **117:** 186-190.

Kumawat, G., Singh, G., Gireesh, C., Shivakumar, M., Arya, M., Agarwal, D.K. and Husain, S.M. 2015. Molecular characterization and genetic diversity analysis of soybean (*Glycine max* (L.) Merr.) germplasm accessions in India. *Physiol Mol Biol Plants*, **21:**101-107.

Leal, A.A., Mangolin, C.A., do Amaral, A.T.J., Goncalves, L.S. 2010. Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. *Genet. Mol. Res.*, **9:** 9-18.

Lester, R.N., Hasan, S.M.Z. 1991. Origin and domestication of the Brinjal Eggplant, *Solanum melongena*, from *S. incanum*, in Africa and Asia. In: Solanaceae III Taxonomy, Chemistry, Evolution (Hawkes J. G. Lester R. N. Nees M. and Estrada, N., eds). Royal Botanic Garden, Kew, 369-387

Li, H., Li, Z., Cai, L., Shi, W., Mi, Fu., Shi, F. 2013. Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* L.) in Inner Mongolia using ISSR and SSR markers. *Genet. Resour. Crop Evol.*, **60:** 1687–1694.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Res.*, **27:** 209–220.

Moll, R.H., Lonquist, J.H., Velez Fortuno, J., Johnson, E.C. 1965. The relationship of heterosis and genetic divergence in maize. *Genetics*, **52:** 139-144.

Nascimento, W.F., Rodrigues, J.F., Koehler, S., Gepys, P., Veasey, E.A. 2013. Spatially structured genetic diversity of the Amerindian yam (*Dioscorea trifida* L.) assessed by SSR and ISSR markers in Southern Brazil. *Genet. Resour. Crop. Evol.*, **60:** 2405–2420.

Nunome, T., Ishiguro, K., Yoshida, T., Hirai, M. 2001. Mapping of fruit shape and color development traits in eggplant (Solanum melongena L.) based on RAPD and AFLP markers. *Breed. Sci.*, **51:** 19-26.

Nunome, T., Negoro, S., Kono, I., Kanamoti, H., Miyatake, K., Yamaguchi, H., Ohyama, A., Fukuoka, H. 2009. Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). *Theor. Appl. Genet.*, **119:** 1143-1153.

Nunome, T., Suwabe, K., Iketani, H., Hirai, M. 2003. Identification and characterization of microsatellites in eggplant. *Plant Breed.*, **122:** 256-262.

Ona, N., dela Vina, A.C., Ramirez, D.A. 1985. Genetic diversity of seed proteins among five *Solanum* species from the Philippines. *Phil. J. Crop Sci.*, **20:** 122-128.

Pakseresht, F., Talebi, R. and Karami, E. 2013**.** Comparative assessment of ISSR, DAMD and SCoT markers for evaluation of genetic diversity and conservation of landrace chickpea (*Cicer arietinum* L.) genotypes collected from north-west of Iran. *Physiol Mol Biol Plants*, **19:** 563–574.

Panwar, P., Nath, M., Yadav, V.K., Kumar, A. 2010. Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn.). *J. Genet.*, **2:** 121-133.

Prasad, S.K., Singh, T.P. 1986. Heterosis in relation to genetic divergence in maize (*Zea mays* L.) *Euphytica*, **35:** 919-924.

Rohlf, F.J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1 Exeter Software, New York, USA

Roxas, D.M.R., Vina, A.C. dela,Ramirez, D.A. 1995 Cytogenetics of *Slanum melongena* L., *Solanum aethiopicum* and their F_1 hybrid. *Philipp. J. Crop Sci.*, **20:** 129-140.

Saker, M.M., Youssef, S.S., Abdallah, N.A., Bashandy, H.S. 2005. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *Afr. J. Biotechnol.*, **4:** 882-890.

Sambrook, J., Russel, D.W., 2001. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sarikamiş, G., Yanmaz, R., Ermis, S., Bakir, M. 2010. Genetic characterization of pea (*Pisum sativum*) germplasm from Turkey using morphological and SSR markers. *Genet. Mol. Res.*, **9:** 591-600.

Sekara, A., Cebula, S., Kunicki, E., 2007. Cultivated eggplants origin, breeding objectives and genetic resources, a review. *Folia Horticulturae Ann.*, **19(**1): 97-114.

Shilpha, J., Silambarasan, T., Pandian, S.K., Ramesh, M. 2013. Assessment of genetic diversity in *Solanum trilobatum* L., an important medicinal plant from South India using RAPD and ISSR markers. Genet. Resour. *Crop. Evol.*, **60:** 807–818.

Singh, A., Dixit, H.K., Jain, N., Singh, D., Yadav, R.N. 2014. Efficiency of SSR, ISSR and RAPD markers in molecular characterization of mungbean and other vigna species. *Indian J. Biotech.*, **13:** 81-88.

Souframanien, J., Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet.*, **109:** 1687–1693.

Stàgel, A., Portis, E., Toppino, L., Rotino, G.L. 2008. Gene-based microsatellite development for mapping and phylogeny studies in eggplant. *BMC Genomics*, **9:** 357.

Tümbilen, Y., Frary, A., Mutlu, S., Anlar, S. 2011. Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses. *Int. Res. J. Biotech.*, **2:** 16-25.

Wu, Z.G., Li, X.X., Lin, X.C., Jiang, W., Tao, Z.M., Mantri, N., Fan, C.Y., Bao, X.Q. 2014. Genetic diversity analysis of yams (*Dioscorea* spp.) cultivated in China using ISSR and

SRAP markers. *Genet. Resour. Crop. Evol.*, **61:** 639–650.

Yang, X.H., Yan, J.B., Shah, T., Warburton, M.L., Li, Q., Li, L., Gao, Y.F., Chai, Y.C., Fu, Z.Y., Zhou, Y., Xu, X.T., Bai, G.H., Meng, Y.J., Zheng, Y.P., Li, J.S. 2010. Genetic analysis and characterization of a new maize association mapping panel for quantitative trait loci dissection. *Theor. Appl. Genet*., **121:** 417–431.

Yap, I.V., Nelson, R.J. 1996. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion Paper Series No. 14. International Rice Research Institute, Manila.

Yee, E., Kidwell, K.K., Sills, G.R., Lumpkin, T.A. 1999. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Sci*., **39:** 268–275.

Yi, G.B., Lee, J.M., Lee, S., Choi, D. 2006. Exploitation of pepper EST-SSRs and an SSR-based linkage map. *Theor. Appl. Genet.,* **114:** 113-130.

Zietkiewicz, E., Rafalfki, A., Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genome*, **20:** 176-183.

Table 1: Salient features of the experimental materials

S. No.	Genotypes	Source	Salient features
1	*Solanum aethiopicum*	Sudan	Erect plant type, round and cluster bearing small green fruits which become scarlet red while ripen, tolerant to phomopsis blight as well as fruit and shoot borer.
2	BARI	Bangladesh	Erect plant type, extra-long fruit having light purple color, tolerant to phomopsis blight.
3	PB-66	Pantnagar (Uttarakhand, India)	Erect plant type having long purple fruit,
4	Pant Rituraj (PR)	Pantnagar (Uttarakhand, India)	Semi erect plant type, round dark purple fruit, suitable to round the year cultivation.
5	WB-1	Koochbihar (W Bengal, India)	Erect growth habit, oblong green fruits, tolerant to phomopsis blight and sturdy stem.
6	PB-67	Pantnagar (Uttarakhand, India)	Semi erect plant type, long green fruited, high yielder.
7	PB-71	Pantnagar (Uttarakhand, India)	Erect plant type having oblong purple fruit.
8	Pant Samrat (PS)	Pantnagar (Uttarakhand, India)	Erect plant type, long purple fruits, cluster bearing, good combiners, tolerant to phomopsis blight.
9	NDB-1	Faizabad (UP, India)	Semi erect plant type, purple and oblong fruits.
10	PB-70	Pantnagar (Uttarakhand, India)	Erect plant type having round green fruits.
11	PB-4	Pantnagar (Uttarakhand, India)	Erect plant type having long purple fruits.
12	SMB-115	Cuttack (Orissa, India)	Erect plant type having small oblong purple fruit, cluster bearing.
13	KS-331	Kalyanpur (UP)	Spreading plant type, long fruit having purple color.
14	LC-7	Pantnagar (Uttarakhand, India)	Erect plant type, oblong, very soft and green fruits with light purple tinge.

Table 2: Summary of RAPD markers based genotyping of *Solanum* species

Primers code	Sequence (5′-3′)	Number of bands			Unique bands		% Polymorphism
		Total bands	Monomorphic bands	Polymorphic bands	'a'	'm'	
OPA-01	CAGGCCCTTC	17	6	11	6	-	65.00%
OPA-03	AGTCAGCCAC	11	1	10	2	-	91.00%
OPA-04	AATCGGGCTG	6	2	4	1	-	67.00%
OPA-05	AGGGGTCTTG	9	1	8	1	3	89.00%
OPA-07	GAAACGGGTG	6	2	4	-	1	67.00%
OPA-10	GTGATCGCAG	11	0	11	1	2	100.00%
OPA-11	CAATCGCCGT	8	3	5	1	1	63.00%
OPA-14	TCTGTGCTGG	7	2	5	1	-	71.00%
OPA-15	TTCCGAACCC	11	1	10	1	-	91.00%
OPA-18	AGGTGCCGTT	10	0	10	3	-	100.00%
AC-11	CCTGGGTCAG	4	1	3	1	-	75.00%
AC-14	GTCGGTTGTC	14	2	12	2	-	86.00%
	Total	114	21	93	20	7	
	Average	9.5	1.75	7.75	1.67	0.58	80.42

Note: 'a', 'm': for S. aethiopicum and for S. melongena, respectively.

Table 3: Summary of ISSR markers based genotyping of Solanum species

Primers code	Sequence (5′- 3′)	Number of bands			Unique bands		% Polymorphism
		Total bands	Monomorphic bands	Polymorphic bands	'a'	'm'	
8161-041	AGAGAGAGAGAGAGAGC	11	2	9	6	-	82.00%
8161-042	GAGAGAGAGAGAGAGAC	11	1	10	5	-	91.00%
8161-043	GAGAGAGAGAGAGAGAA	10	2	8	2	-	80.00%
8161-044	AGAGAGAGAGAGAGAGTT	7	2	5	-	-	71.00%
8161-045	AGAGAGAGAGAGAGAGCC	6	0	6	2	-	100.00%
8161-046	AGAGAGAGAGAGAGAGTA	6	1	5	2	2	83.00%
8161-047	GAGAGAGAGAGAGAGACC	5	2	3	-	-	60.00%
8161-048	GAGAGAGAGAGAGAGAA	7	4	3	2	-	43.00%
8161-051	GAGAGAGAGAGAGAGAT	4	2	2	1	-	50.00%
8161-052	GAGAGAGAGAGAGAGAC	4	2	2	-	-	50.00%
8161-053	GAGAGAGAGAGAGAGAA	4	0	4	3	1	100.00%
8161-054	GAGAGAGAGAGAGAGACT	4	1	3	1	-	75.00%
8161-055	AGAGAGAGAGAGAGAGTT	6	2	4	2	1	67.00%
8161-056	CTCTCTCTCTCTCTCTGC	5	1	4	-	-	80.00%
8161-058	CACACACACACACACAGT	4	1	3	-	1	75.00%
8161-059	ACACACACACACACACGT	2	0	2	-	1	100.00%
	Total	96	23	73	26	6	
	Average	6.0	1.44	4.56	1.62	0.37	75.44

Note: 'a', 'm': for S. aethiopicum and for S. melongena, respectively.

Table 4: Summary of SSR markers based genotyping of Solanum species

Primers code	Primer Sequence (5′- 3′)	Number of bands			Unique bands		% Polymorphism
		Total bands	Monmorphico bands	Polymorphic Bands	'a'	'm'	
emh11O01	F-GATGTGTCGATGAGATTTTGGTCA R-TAGCTACGTTGGTTTGGTGCTGAA	2	0	2	-	-	100.0
EMB01L13	F-TCAAAAGACTTGAAACCCGATGGT R-GTTTATCAGGTTTTTGATCACCGGACA	2	0	2	-	1	100.0
EMB01H20	F-TCTTGTTCCCAGTCTATCGCTAATCA R-ATCCGAATTTAGTCGGGCTTCAAT	5	0	5	1	-	100.0
emf21C11	F-TGGTTGGAGCCATGATTACTTGAA R-ATGCTACCTATCAAACAGGCGGAA	3	0	3	1	-	100.0
emf21H22	F-CACAAGATGAACAAGACTAAGGAGTGC R-CTTCTTCAACCTGTCTTTAGCCCA	3	0	3	2	-	100.0
EEMS28	F-GACGATGACGACGACGATAA R-TGGACTCACAACTCAGCCAG	6	0	6	1	1	100.0
EEMS48	F-CAATGCAAACAATTATCATTTCG R-TCGATGTTGTTGTCGTCGTT	6	0	6	1	1	100.0
EEMS49	F-TGAAATTGATCAATACCTATAAATTTAG R-GAAAGCCAGGATAGCATTCG	2	0	2	1	1	100.0
EM119	F-CCCCACCCCATTTGTGTTATGTT R-ACCCGAGAGCTATGGAGTGTTCTG	6	2	4	3	1	67.00
EM140	F-CCAAAACAATTTCCAGTGACTGTGC R-GACCAGAATGCCCCTCAAATTAAA	7	1	6	-	1	86.00
EM145	F-CAGTGCTACATAAATTGAGACAAGAGG R-GGAGGTACAACGATTTTCATATGGT	1	0	1	-	-	100.0
EM155	F-CAAAAGATAAAAAGCTGCCGGATG R-CATGCGTGAGTTTTGGAGAGAGAG	4	1	3	1	-	75.00
	Total	47	4	43	11	6	
	Average	3.92	0.33	3.58	0.92	0.5	94.0

Note: 'a', 'm': for S. aethiopicum and for S. melongena, respectively.

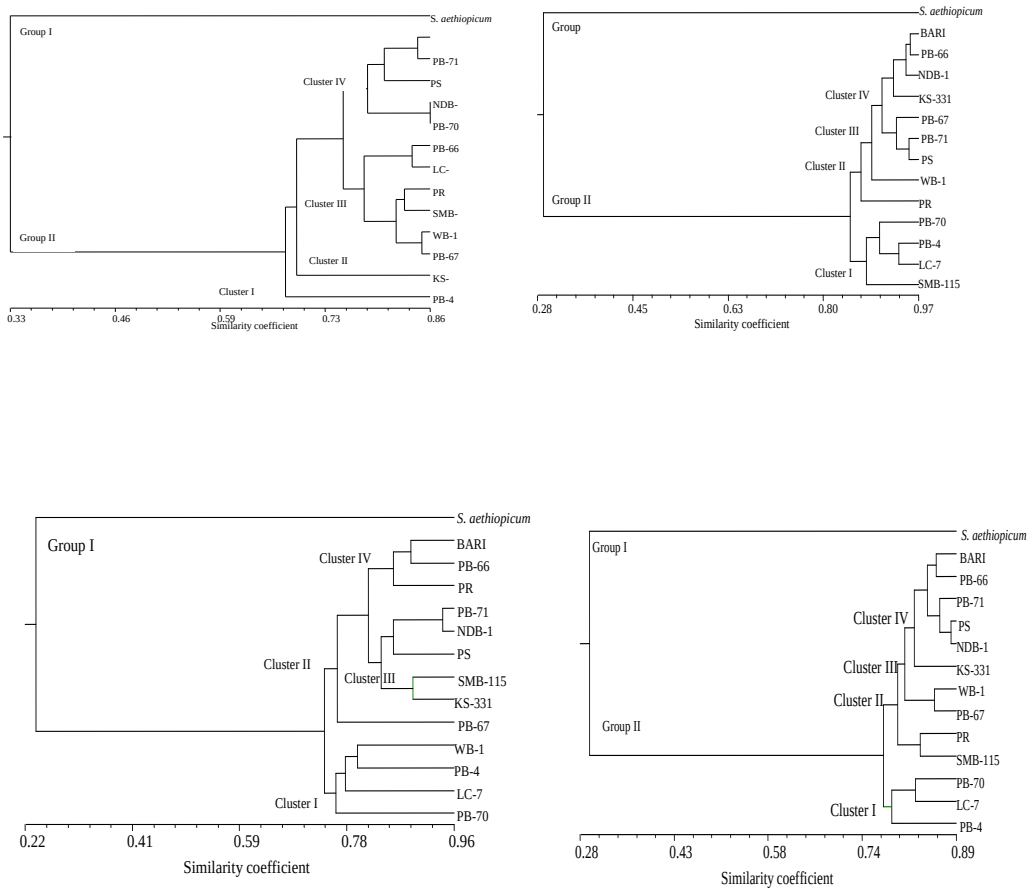

Figure 1: Dendrograms of 14 genotypes of *Solanum* species based on marker data. 1a: RAPD, 1b: ISSR, 1c: SSR, 1d:RAPD+ISSR+SSR

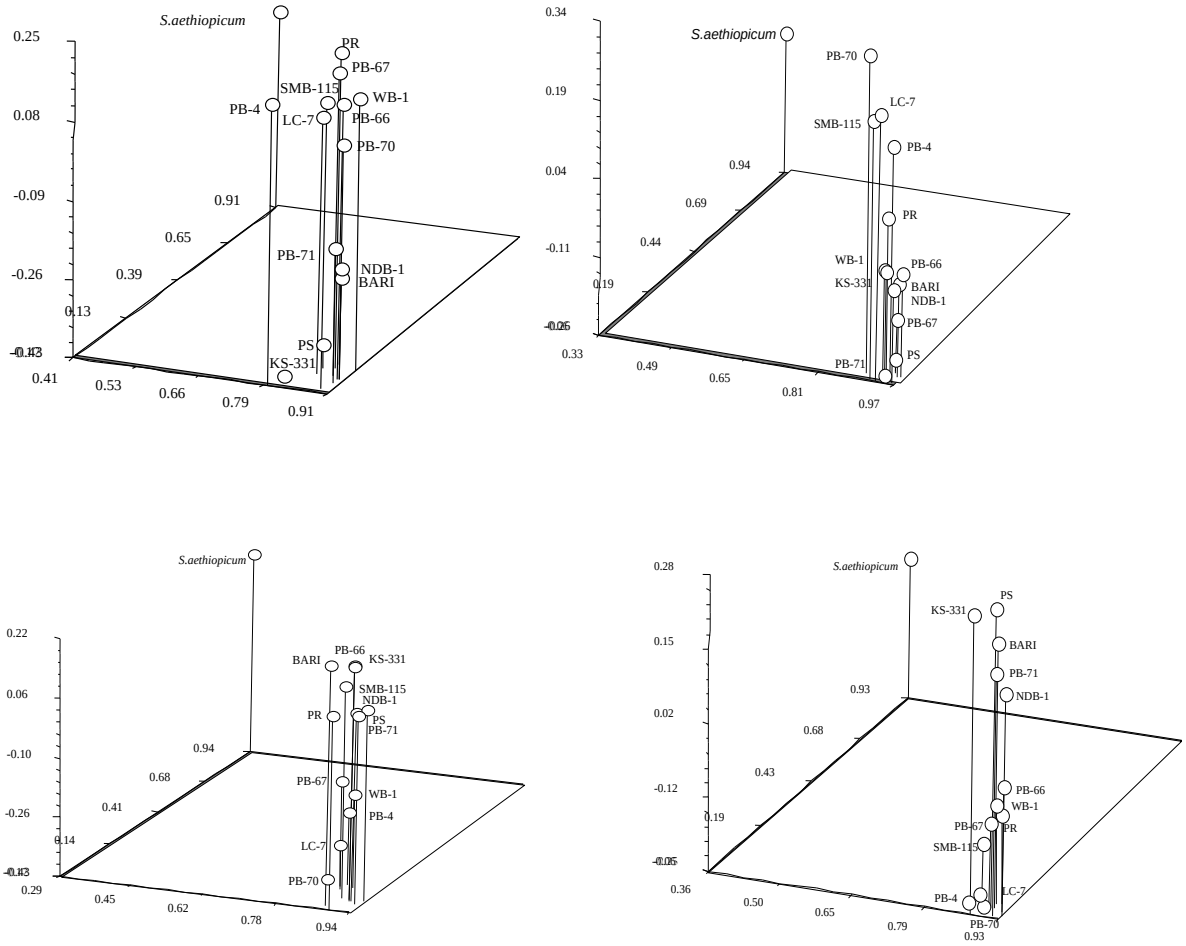

Figure 2: Three-dimensional plots of 14 genotypes of Solanum species using principal component analysis of RAPD, ISSR, SSR and combined data. 2a: RAPD, 2b: ISSR, 2c: SSR, 2d: RAPD+ISSR+SS