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ASSESSMENT OF GENETIC DIVERSITY AMONG SOME IMPORTANT WILD SPECIES OF NON – TUBEROUS *SOLANUM* USING RAPD AND ISSR MARKERS

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ABSTRACT

Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers were used for assessing genetic diversity and species relationships among eight species of *Solanum*. Both random and SSR primers revealed 90.9 and 96.1 % polymorphism, respectively. ISSR markers were found to be more efficient than the RAPD assay. The marker index and polymorphic information content varied from 40.8 - 47.0, 40.9- 49.0 and 0.41 -0.49, 0.43-0.49 respectively for both the RAPD and ISSR markers. Mean value of number of observed alleles, number of effective alleles, Nei's gene diversity and Shannon's index were 1.95-1.99, 1.47-1.75, 0.26-0.37 and 0.45-0.43 respectively for both the markers. Both UPGMA and PCA analyses of the data revealed clear groupings among the species. Genetically distinct genotypes identified by using RAPD and ISSR markers could be potential sources of germplasm for conservation and further improvement of the genus *Solanum*.

Keywords: **Genetic diversity; *Solanum*; North – East India; RAPD; ISSR**

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INTRODUCTION

The family *Solanaceae* comprises of many species that are well known for food and medicine. Species like *Solanum melongena* L.*, S. tuberosum* L., *Lycopersicum esculentum* L. and *Physalis* spp.*etc*. are good sources of minerals and vitamins[1]. Plants belonging to this family are important sources of almost 300 different kinds of alkaloids[2]. Solanine, scopolamine, atropine and hyoscamine are the key alkaloids of solanaceous plants[3]. North-east India being a biodiversity hotspot is a natural home for the genus *Solanum* and the members of this genus are found to grow wild throughout the region. In the present study, eight species of *Solanum* of this region were selected. These were *Solanum ferox* L., *S. indicum* L., *S. nigrum* L., *S. torvum* Swartz., *S. spirale* Roxb., *S. xanthocarpum* Schrad. & Wendl., *S. sisymbrifolium* Lam. and *S. macranthum* Dunal, and were used for studying the genetic diversity and phylogenetic relationships prevailing within them.

The analysis of genotypes derived from different geographical areas is important to study genetic diversity. The conservations and use of this native genetic material will ensure that it does not disappear, but enjoys a projection for the future with new uses. Moreover, any kind of plant improvement program requires a basic knowledge of the morphology and genetic nature of the plant. In this context, new advances in molecular biology with the use of more sensitive molecular markers able to detect changes in the genotype of the individuals, greatly contributed to the generation of information on this important aspect. Random amplified polymorphic DNA (RAPD) markers and inter - simple sequence repeats **(**ISSR**)** markers are two molecular approaches that have been used to detect variation among plants. Each method has been used extensively to identify and determine relationships of the species and cultivar levels[4,5,6]. The usefulness of the random amplified polymorphic DNA (RAPD) technique to investigate genetic diversity between different plant groups has been demonstrated in several studies[7,8, 9,10,11,12]. RAPD technique is technically simple, less expensive and has been used to estimate the genetic variability in a wide range of plant species[13,14,15,16,17]. Inter-simple sequence repeat (ISSR) markers have been revealed to be useful as novel DNA markers in studies on purposing crop improvement, such as genomic fingerprinting, phylogenetic analysis, gene tagging *etc*., in a wide range of crop plant species since they are highly informative, universal, quick, easy to apply and highly reproducible[18,19,20,21,22]. Although ISSR markers have similar benefits to RAPD markers, which are one of the representative low cost DNA markers, ISSR markers are more reliable ,reproducible and highly polymorphic DNA markers than RAPD ones because of comparatively longer length of the primers and hence higher annealing temperature[23,24]. In order to assess genetic diversity as well as to discriminate *Solanum* species, morphological and biochemical (isozymes and chromatography) approaches were used[25,26,27]. But reports are lacking in genetic diversity studies on *Solanum* members from the rich flora of Northeast India. Again, due to the lack of immediate known utilities for some of the members of this group has led to their neglect and subsequent genetic erosion in this region. Little informations are available on genetic diversity studies on certain commercially important members of *Solanaceae* [28,29,30]. No information regarding a phylogenetic study by using PCR based RAPD and ISSR are available for *Solanum* species of North East India. A systematic study is, thus, essential in this genus in order to examine the existing genetic diversity among the species and also to gain knowledge on the impact of disturbance on genetic variability of this species. Hence the objective of the present study was to assess the diversity at the DNA level using RAPD and ISSR techniques as molecular genetic fingerprints.

MATERIALS AND METHODS

Plant material

Eight different species of *Solanum* L. *viz*. *S. ferox* L., *S. indicum* L., *S. nigrum* L., *S. torvum* Swartz., *S. spirale* Roxb., *S.*

xanthocarpum Schrad. & Wendl., *S.sisymbrifolium* Lam. and *S. macranthum* Dunal were collected from different parts of North- East India (Table 1). Fresh, healthy green leaves of these plants were used for DNA isolation and molecular analysis.

DNA isolation

Genomic DNA was extracted from 0.5 g of young fresh leaves using CTAB (Hexadyltrimethyl - ammonium bromide) method[31]. The purity of genomic DNA was evaluated by measuring absorbance (A260 nm/A280 nm ratio) with a Double Beam UV spectrophotometer. The size, purity and integrity of DNA isolated were determined by agarose gel (0.8%) electrophoresis stained with ethidium bromide and using ladder DNA as a size standard.

Primer used in PCRs

For RAPD, 15 deca- mer primers from Operon technologies (Alameda, California, USA) were initially screened for their repeatable amplification with eight different species of *Solanum* L. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved polymorphic amplified products within the species of *Solanum* L. To ensure reproducibility, the primers generating weak products were discarded. Characters of selected primer are given in Table 2. For ISSRs, fourteen primers were utilized following their ability to detect distinct, clearly resolved polymorphic amplified products (Table 2). These oligonucleotides were obtained from UBC primer set 100/9 (University of British Columbia).

RAPD amplification

The reaction mixture of 20 µl contained 50 ng/µl of template DNA, 1 x assay buffer (100 mM Tris sulfonic acid, pH 8.8, 15 mM MgCl2, 500 mM KCl and 0.1 % gelatin), 0.2 mM each dNTPs (B'L Genei, India), 5 pM of each primer and 0.5 U of Taq polymerase (B'L Genei, India). The reaction was performed in 0.2 ml microfuge tubes (Dialabs). PCR amplification was carried out in a Mini Thermal Cycler (Make: Applied Biosystems 9700). Thermal cycling conditions were as follows: pre-denaturing step of 5 min at 94 ºC, followed by 35 cycles each of 45 sec at 94 ºC, annealing for 1 min at 32 ºC, extension for 1 min at 72 ºC and followed by one final extension cycle of 5 min at 72 ºC. The amplification products were electrophoresed in 2 % agarose gels in 0.5 x TBE (10 x stock contained 0.8 M Tris, 0.8 M boric acid, 0.5 M EDTA). The gels were photographed using gel documentation and image analysis (Make: Gel Doc. 2000, Bio Red).

ISSR amplification

ISSR amplification reactions were carried out in 20-µl volume containing 50 ng template DNA, 0.5 U Taq DNA polymerase, 10 mM dNTP, 10 µM primer in 1× reaction buffer that contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, and 0.01% gelatin. PCR amplification was carried out in a Mini Thermal Cycler (Applied Biosystems 9700). Amplification conditions were one cycle at 94°C for 5 min., and 36 cycles each with 94°C for 30 sec., 42°C for 30 sec., and 72°C for 1 min. Amplified products were loaded on 2% agarose gel and separated in 0.5× TBE buffer at 75 V and documented using a gel documentation and an image analysis system (Make: Gel Doc. 2000, Bio Red).

Data scoring and analysis

Duplicate samples from each individual were tested and only well resolved and reproducible bands amplified in both cases were considered for scoring and analysis of the data. The numbers of polymorphic and monomorphic amplification products were determined for each primer in eight *Solanum* species. Scoring was done as 1 for the presence and 0 for the absence across the eight *Solanum* genotypes. Polymorphic information content (PIC) was estimated by using the formula PIC = 1- $\sum Pi^2$,where *Pi* is the frequency of *i*th allele at a given locus[32]. Marker index was calculated by using standard formula [33]. Genetic diversity, the number of observed allele (n_a), mean number of effective alleles (n_e), Nei's gene diversity index (h) and the Shannon index (I) were calculated using the POPGENE software. The similarity matrix was computed that estimated all pair wise difference similarity matrices in the amplification product, using sequential, hierarchical clustering option of the SPSS version 11.0 software package (Information

Technology Science Centre, Lingnam University, 2002). Level of similarity among species was established as percentage of polymorphic bands and a matrix of genetic similarity compiled by using the Dice's coefficient[34]. The program also generated a dendrogram which grouped the species on the basis of Nei Genetic distance using an unweighted pair group method with arithmetic average (UPGMA) cluster analysis[35,36]. Finally, a principal coordinate analysis was performed to determine the similarities between the species under investigation using the Euclidean distances obtained from the standardized values with the help of NTSYS-pc-2.2 software.

RESULTS

Genomic DNA isolation

The genomic DNA of eight species of *Solanum viz*. *S. torvum* Swartz., *S. indicum* L., *S. ferox* L., *S. nigrum* L., *S. xanthocarpum* Schrad. & Wendl., *S. spirale* Roxb., *S. sisymbrifolium* Lam. and *S*. *macranthum* Dunal was extracted. The genomic DNA extracted were pure and intact bands of each sample was obtained when run in 0.8 % agarose gel (Fig 1). The size of the genomic DNA was varied in the range of 60 – 700 ng/µl.

RAPD amplification

The genetic diversity among eight different *Solanum* species was evaluated by 10 selected primers which yielded species specific DNA profiles. The total number of DNA bands amplified with eight species of *Solanum* as well as the number of polymorphic bands along with percentage of polymorphic bands (POL%), polymorphic information content (PIC) and marker Index (MI) is presented in Table 3. A total of 110 mappable RAPD markers were generated by 12 primers. Out of these, 100 were polymorphic (90.9 %).The number of amplification products obtained per primer in the range of 6 to 17 with the primer OPA – 01 producing the minimum number (6) and OPA – 02 producing the maximum number (17) of bands. The POL % ranged from 83.3 per cent to 100.0 per cent. The maximum POL % (100%) was found to be observed in case of primers OPA - 02 and OPA - 05. The PIC value ranged from 0.41 to 0.49. Marker index (MI) value was found to be ranged from 40.8 to 47.0. Primer OPA - 05 showed maximum value of MI *i.e*. 47.0 (Table 3). The RAPD profile screened by OPA 01 for eight different *Solanum* species is shown in Fig 2 A.

Genetic diversity analysis with RAPD marker

Relatively higher level of polymorphism and genetic diversity among eight *Solanum* species were revealed by RAPD markers in this study. The mean values of n_a, n_e ,h and I was found to be estimated as 1.95, 1.47, 0.26 and 0.45 respectively (Table 4).The minimum similarity (0.372) was found between *Solanum xanthocarpum* Schrad. & Wendl. and *Solanum spirale* Roxb and in between *Solanum xanthocarpum* Schrad. & Wendl. and *Solanum indicum* L. Maximum level of similarity indices (0.829) was found between *S*. *macranthum* Dunal and *S. sisymbrifolium* Lam. The genetic similarity value ranged from 0.372 to 0.829 suggesting a wide genetic base within the *Solanum* genotypes used in the present investigation. As indicated by the Fig 2B, the dendrogram based on the similarity matrices of RAPD – PCR banding patterns clearly distinguished the examined *Solanum* species into two main cluster. The first cluster was again divided into two sub clusters consisted of 3 species *viz*. *Solanum torvum* Swartz. , *S. ferox* L. and *S. spirale* L. The second cluster was sub divided into two clusters. One of this sub cluster consisted of 2 species *viz*. *Solanum nigrum* L. and *S. indicum* L. and the other consisted of 3 species *viz*. *Solanum sisymbrifolium* Lam., *S*. *macranthum* Dunal and *S. xanthocarpum* Schrad. & Wendl. Association among eight genotypes was also resolved by PCA (Fig 2C). The overall grouping pattern of PCA corresponded well with the clustering pattern of the dendrogram.

ISSR amplification

The banding patterns of ISSR fragments using ten specific primers with eight different species of *Solanum* revealed a total of 154 bands, of which 148 (96.1 %) were polymorphic. The total number of bands, number of polymorphic bands, percentage of polymorphic bands (Pol %), polymorphic

information content (PIC) and marker index (MI) obtained per primer are shown in Table 3. The reproducibility of the appearance of ISSR banding patterns was confirmed by at least three times of repetition analyses. The maximum POL % (100%) was found to be observed in case of all primers except UBC-840 and UBC-826 (90.9 %). The PIC value was found to be ranged from 0.43 to 0.49. The primer UBC 812 revealed maximum PIC value (0.49).The marker index value was varied from 40.9 to 49.0. The minimum MI value was obtained in case of primer UBC – 840 and maximum MI value was shown by the primer UBC 812. The amplification products obtained by the primer UBC 814 are exhibited in Fig 3A, which exemplified the typical ISSR banding patterns observed.

Genetic Diversity analysis with ISSR marker

The average value of the number of observed alleles (n_a), number of effective alleles (n_e), Nei's gene diversity index (h) and Shannon's index (I) were found to be obtained as 1.99, 1.75, 0.37 and 0.41 ± 0.19 respectively (Table 4).The ISSR data revealed that the genetic similarity indices ranged from 0.304 to 0.652. The nearest relationship was detected between *S. macranthum* Dunal and *Solanum indicum* L. On the other hand, the farest relationship of similarity matrices was detected between *Solanum spirale* Roxb. and *Solanum ferox* L. The dendrogram based on the ISSR similarity matrices revealed that all the species were grouped into two main clusters (Fig 3B). Within each main cluster, two distinct sub clusters could be visualized. The first main cluster included 5 species *viz*. *Solanum ferox* L., *S. xanthocarpum* Schrad. & Wendl., *S. torvum* Swartz., *S. sisymbrifolium* Lam. and *S. nigrum* L. The another main cluster was consisted of 3 species viz. *Solanum spirale* Roxb., *S. indicum* L. and *S. macranthum* Dunal. A close association was observed between *Solanum indicum* L. and *S. macranthum* Dunal. Principal component analysis was performed to determine similarities between the genotypes under study (Fig 3C). In agreement with dendrogram, PCA also showed the separation of eight genotypes into two distinct groups.

Figure 1

Ethidium bromide stained agarose gel showing genomic DNA of eight species of Solanum. M = Marker. Species abbreviations are as follows: S 01= S. torvum, S 02 = S. nigrum S03 = S. ferox, S 04 = S. indicum, S 05 = S. sisymbrifolium , S 06 = S. spirale, S 07 = S. macranthum , S 08 = S.xanthocarpum.

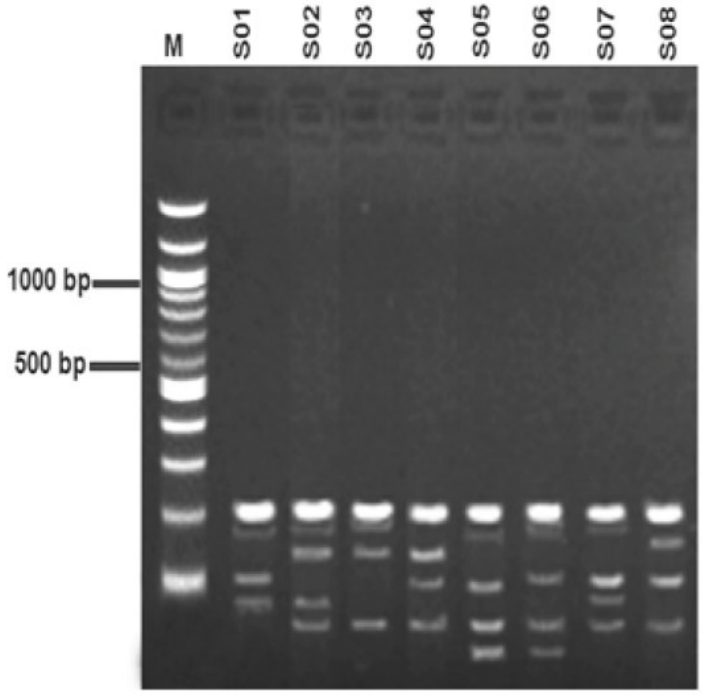

Figure 2A

Polymorphism on the band profiles of genotypes of Solanum revealed by the PCR Amplification generated by OPA=01 resolved on 2.0 % gel. M = Marker. Species abbreviations are as follows: S 01= S. torvum, S 02 = S. nigrum S 03 = S. ferox, S 04 = S. indicum, S 05 = S. sisymbrifolium , S 06 = S. spirale, S 07 = S. macranthum , S 08 = S.xanthocarpum.

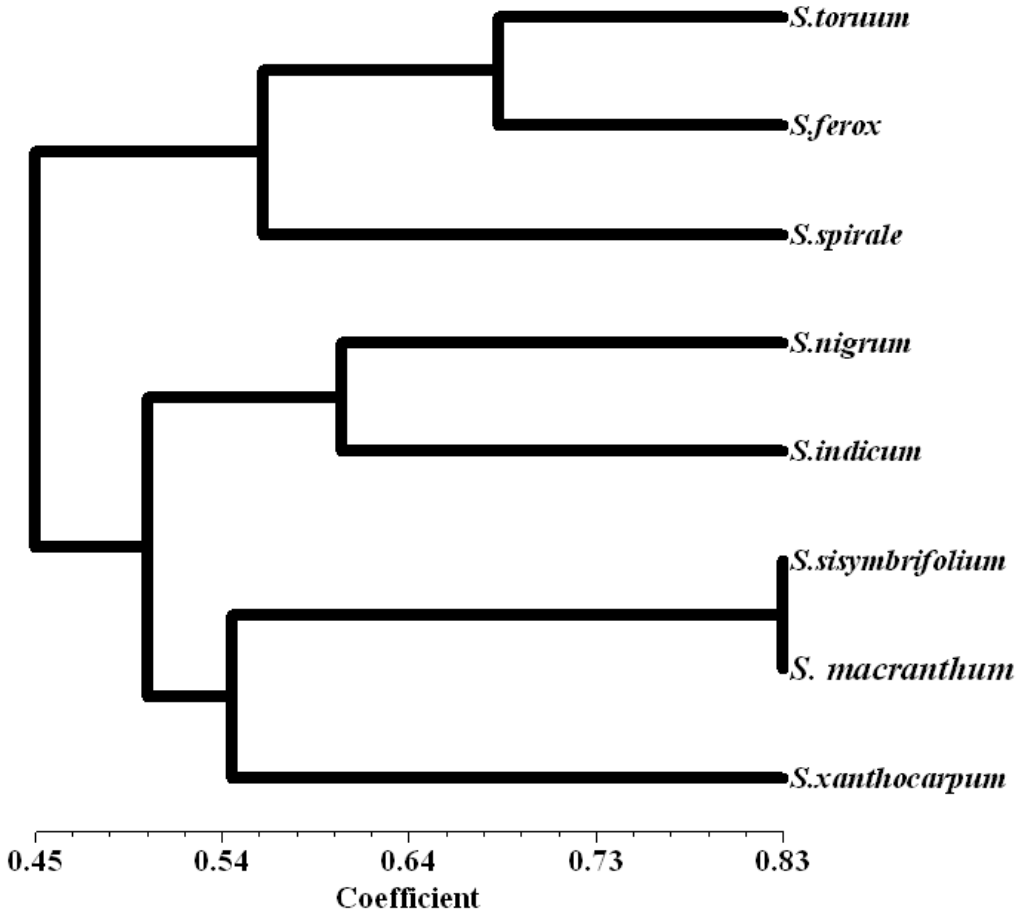

Figure 2B

UPGMA dendrogram based on RAPD data for the studied population of Solanum

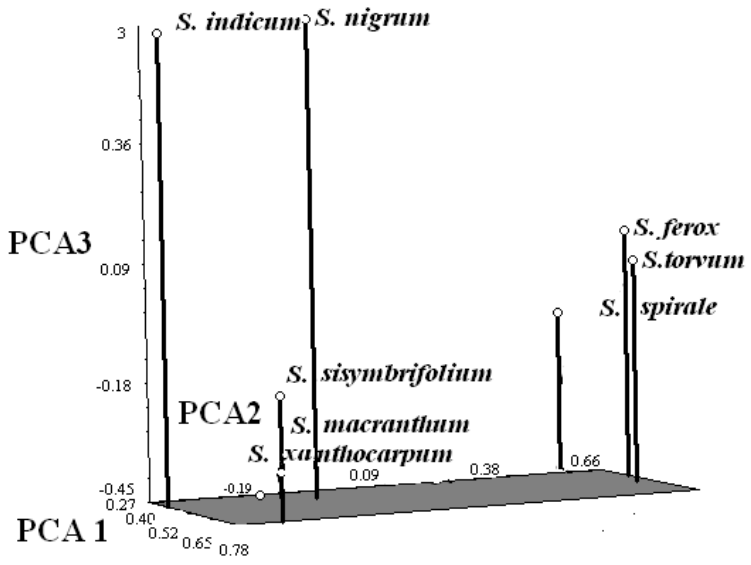

Figure 2C

Three-dimensional plot of principal component analysis (PCA) using RAPD analysis

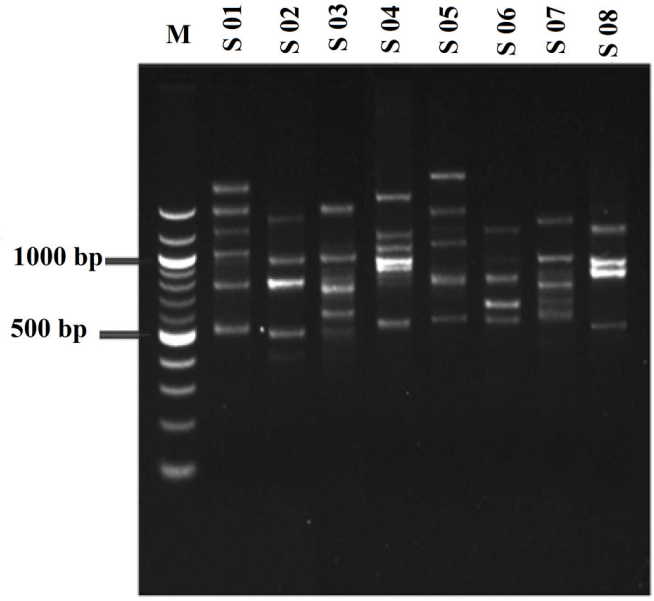

Figure 3A

ISSR amplicon profiles detected in Solanum accessions using primers UBC 814. Species abbreviations are as follows; M = Marker. Species abbreviation are as follows: S 01= S. torvum, S 02 = S. nigrum ,S 03 = S. ferox, S 04 = S. indicum, S 05 = S. sisymbrifolium , S 06 = S. spirale, S 07 = S. macranthum , S 08 = S.xanthocarpum.

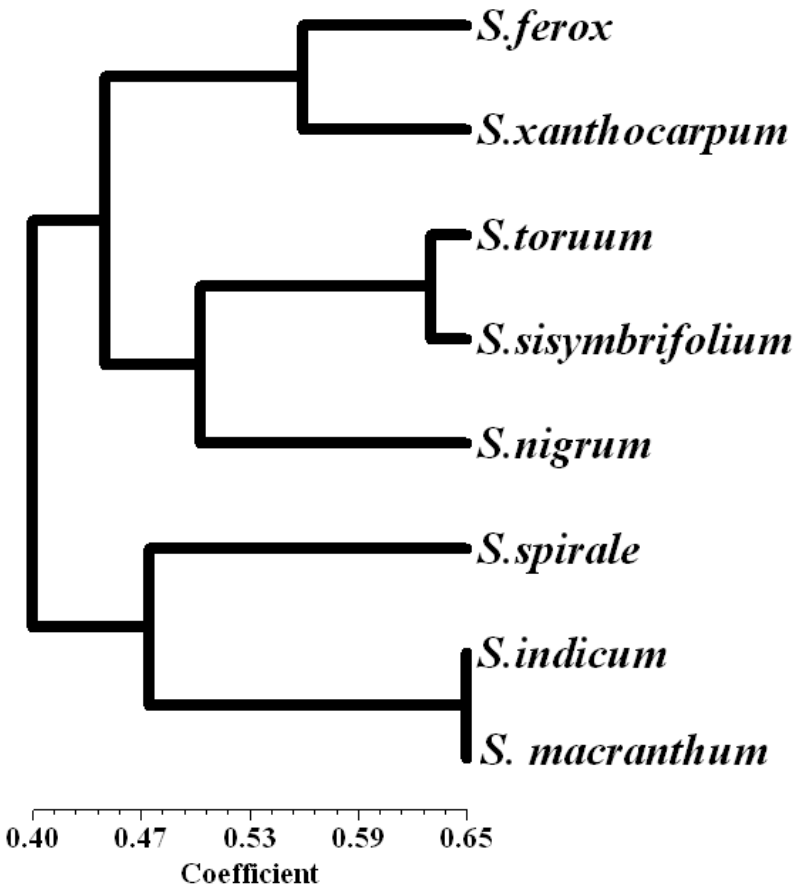

Figure 3B
Dendrograms generated using UPGMA showing relationships between Solanum genotypes using ISSR

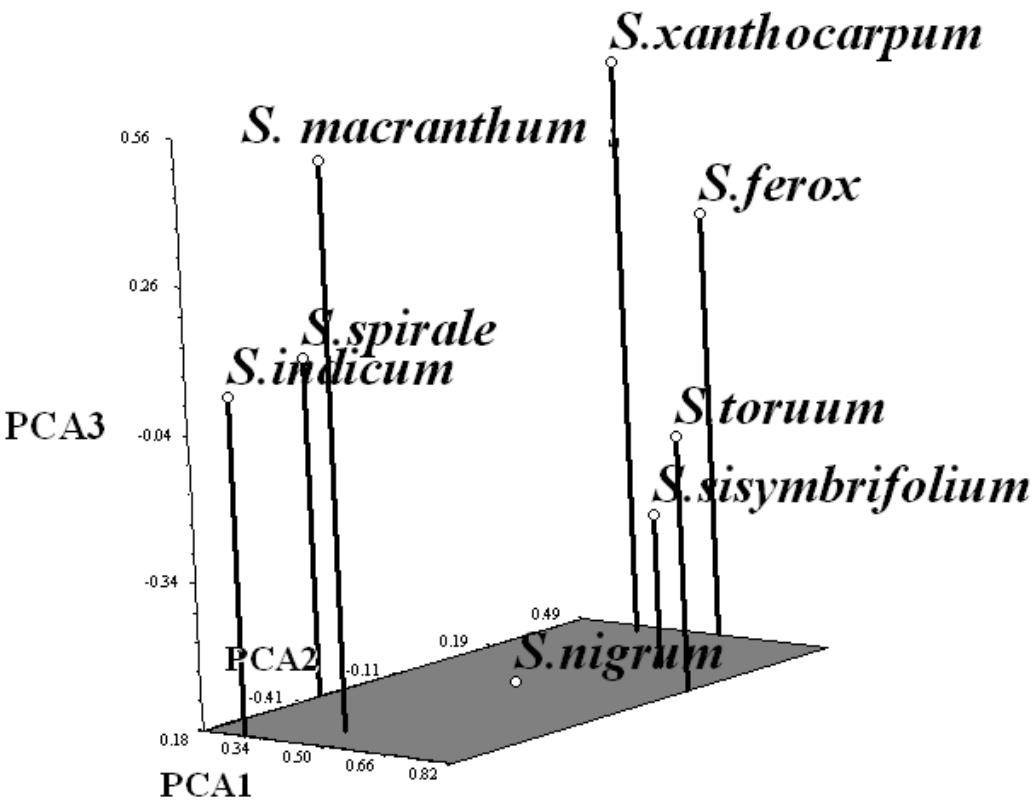

Figure 3C
Three-dimensional plot of principal component analysis (PCA) using ISSR analysis

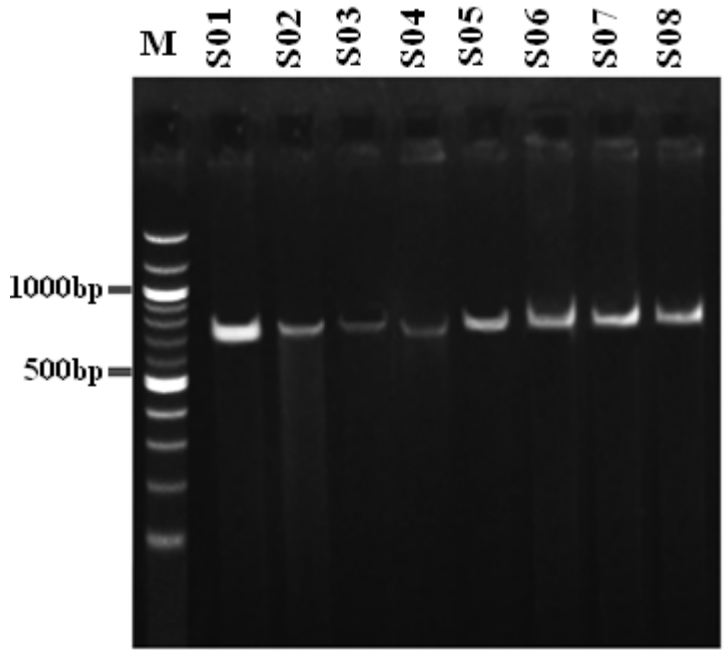

Table 1
Plant materials used in the study

Name of the species	Acc. No.	Locality	Geographical location(Altitude/latitude)
Solanum torvum Swartz.	1 = S 01	Rani, Assam	26°3'43.84872"N and 91°36'3.90852"E
Solanum nigrum L.	2 = S 02	Jalukbari,Guwahati, Assam	26°8'38.8914"N and 90°39'41.3809"E
Solanum ferox L.	3 = S 03	Numbor, Golaghat, Assam	26°30'36"N and 93°85'0"E
Solanum indicum L.	4 = S 04	Jalukbari,Guwahati, Assam	26°8'38.8914"N and 90°39'41.3809"E
Solanum. sisymbrifolium Lam.	5 = S 05	Goalpara, Assam	26°10'0"N and 90°37'0"E
Solanum spirale Roxb.	6 = S 06	West Kameng, Arunachal Pradesh	27°15'38.3004"N and 92°25'12.8172"E
S. macranthum Dunal	7 = S 07	Shillong, Meghalaya	25°34'0"N and 91°53'0"E
Solanum xanthocarpum Schrad.& Wendl	8 = S 08	Jalukbari,Guwahati, Assam	26°8'38.8914"N and 90°39'41.3809"E

Table 2
Characters of Primers used in the study

Marker	Primer code	Sequence (5´ - 3´)	Annealing Temperature(ºC)
RAPD	OPA 01	CAGGCCCTTC	34
	OPA 02	TGCCGAGCTG	34
	OPA 03	AGTCAGCCAC	32
	OPA 04	AATCGGGCTG	32
	OPA 05	AGGGGTCTTG	32
	OPAA 01	AGACGGCTCC	34
	OPAB 01	CCGTCGGTAG	34
	OPAB 14	AAGTGCGACC	32
	OPAH 13	TGAGTCCGCA	32
	OPC 07	GTCCCGACGA	34
	OPAM 20	ACCAACCAGG	32
	OPAO 01	AAGACGACGG	32
	OPAP 10	TGGGTGATCC	32
	OPAJ 19	ACAGTGGCCT	32
	OPX 20	CCCAGCTAGA	32
ISSR	UBC 809	AGAGAGAGAGAGAGAGG	52
	UBC 812	GAGAGAGAGAGAGAGAA	50.9
	UBC 814	CACACACACACACACACAA	55
	UBC 818	CACACACACACACACAG	55
	UBC 824	TCTCTCTCTCTCTCTCG	52
	UBC 826	ACACACACACACACACC	52
	UBC 840	GAGAGAGAGAGAGAGAAT	58
	UBC 844	CTCTCTCTCTCTCTCTAC	54
	UBC 848	CACACACACACACACAAG	50.9
	UBC 857	ACACACACACACACACYG	55
	UBC 864	ATGATGATGATGATGATG	52
	UBC 872	GATAGATAGATAGATA	40
	UBC 878	GGATGGATGGATGGAT	50.5
	HB 13	GAGGAGGAGGC	38
	HB 15	GTGGTGGTGGC	38

Table 3

Degree of polymorphism, percentage of polymorphism (Pol %), polymorphic information content (PIC) and marker index (MI) for RAPD and ISSR primers in the eight genotypes of Solanum

Marker	Primer code	Total No. of bands	Total No. of polymorphic bands	POL%	PIC	MI(Pol% x PIC)
RAPD	OPA 01	6	5	83.3	0.49	40.8
	OPA 02	17	17	100	0.41	41.0
	OPA 03	8	7	87.5	0.49	42.8
	OPA 04	9	8	88.8	0.49	43.5
	OPA 05	11	11	100	0.47	47.0
	OPAA 01	8	7	87.5	0.48	42.0
	OPAB 01	10	9	90.0	0.46	41.4
	OPAB 14	7	6	85.7	0.48	41.1
	OPC 07	8	7	87.5	0.48	42.0
	OPAH 13	9	8	88.8	0.48	42.2
	OPAO 01	9	8	88.8	0.47	41.7
	OPAP 10	8	7	87.5	0.49	42.8
ISSR	UBC 809	9	8	88.8	0.49	42.6
	UBC 812	12	12	100	0.49	49.0
	UBC 814	12	12	100	0.44	44.0
	UBC 818	12	12	100	0.47	47.0
	UBC 824	12	12	100	0.46	46.0
	UBC 826	11	10	90.9	0.48	43.6
	UBC 840	11	10	90.9	0.45	40.9
	UBC 844	11	10	90.9	0.46	41.8
	UBC 848	11	11	100	0.43	43.0
	UBC 857	11	10	90.9	0.47	42.7
	UBC 864	10	10	100	0.47	47.0
	UBC 872	12	12	100	0.46	46.0
	UBC 878	10	9	90	0.48	43.2
	HB 15	10	10	100	0.47	47.0

Table 4

Genetic diversity parameter in eight Solanum species

Parameter	Value	
	RAPD	ISSR
Number of observed alleles (n_a)	1.95 ± 0.32	1.99 ± 0.26
Number of effective alleles (n_e)	1.47 ± 0.45	1.75 ± 0.40
Mean Nei's gene diversity index (h)	0.26 ± 0.17	0.37 ± 0.14
Shannon index (I)	0.45 ± 0.23	0.41 ± 0.19

DISCUSSION

The present study revealed the existence of genetic diversity within the collection of *Solanum* germplasm from different geographical regions of Northeast India. Assessment of genetic variability within a germplasm is of interest in practical applications such as for conservation of genetic resources and for breeding purposes, to predict the ability to combine or to rapidly verify the breeding material. Hence, it is crucial for genetic improvement and elite gene exploitation, such as tolerance genes against abiotic stresses. For breeding, it is necessary to detect polymorphisms among cultivars and

lines. The genus *Solanum* shows diversity in habitat, morpholy and ethnomedicinal use. The knowledge on phylogenetic relationship and genetic diversity among the species of the genus *Solanum* is rather scanty. However, in solanaceous plants, a low frequency of polymorphism among cultivars and intraspecific lines has been reported probably due to its autogamous nature[29,37,,38]. In the present study, the genetic similarity among genotypes was calculated as being between 0.372 and 1, also emphasizing the effectiveness of RAPD markers in detecting polymorphisms. However, the polymorphism obtained by the RAPD markers used produce the greatest number of bands and showed great potential to discriminate polymorphic DNA segments. Prior identification of more polymorphic RAPD markers may provide a better characterization of genotypes. This corroborates with the observations found in other plants like rice bean[39] and barley[40] respectively ISSR molecular markers have been used successfully in germplasm bank characterization especially in the assessment of the differences among species or varieties belonging to the same genus[41,42,43,44,45,46]. In the present study, the ISSR markers were also useful in the characterization of eight different species of *Solanum* generating many polymorphic bands and is in agreement with the findings of earlier works[42]. In this study it was found that the ISSR marker generated more number of polymorphic bands than those by RAPD marker. It clearly showed that ISSR markers are more efficient than RAPD marker in revealing DNA polymorphism among the genotypes. This finding is in agreement with the earlier findings on tomato[47], wheat[48], pea nut[5], *Nothofagus* spp.[49] and *Morus* sp. [50] where higher polymorphism was unrevealed with ISSR marker from closely related species. In fact, the ISSRs have a high capacity to reveal polymorphism and offer great potential to determine intra- and intergenomic diversity as compared to other arbitrary primers like RAPDs[51]. Cluster analysis dendrogram based on both RAPD and ISSR markers clearly distinguished the experimental *Solanum* species. The result is also confirmed by principal component analysis (PCA).This indicates that both the markers have high discrimination ability of the groups of *Solanum* species, therefore, this analysis has been proved as a powerful tool for detecting the phylogenetic relationships among the *Solanum* species. Clustering of genotypes within the group was not similar when RAPD and ISSR derived dendrograms were compared. This differences may be attributed to marker sampling error and/or to 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[52]. The putatively similar bands originating for RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pairs. This situation may lead to wrong results when calculating genetic relationships[40]. Our results indicate the presence of great genetic variability among the wild genotypes of *Solanum* available in North – East India. Thus it is found that the RAPD and ISSR markers are useful in the assessment of *Solanum* diversity, the detection of duplicate sample in germplasm collection, and the selection of a core collection to enhance the efficiency of germplasm management for use in *Solanum* breeding and conservation.

CONCLUSION

The present study of genetic structure is critical for the description of genetic diversity and uncovering evolutionary patterns in the genus *Solanum* of North East India. Evaluation of genetic diversity also has immense significance for *in situ* conservation of important *Solanum* species especially for genetic improvement programmes. Moreover, the results of the present study suggest that the application of molecular fingerprinting provides a rapid and sensitive tool for detecting genetic variations among the different species of the genus as well as other genera of the family *Solanaceae*. Our results confirm that DNA analysis by RAPD and ISSR is an efficient method for the exploration of genetic diversity in *Solanum* populations. The dendrogram reveals the phylogenetic relationships as well as the evolutionary pattern of the selected *Solanum* members.

ISSR markers are found to be more efficient than RAPD marker in revealing DNA polymorphism among the species under study. To our knowledge, this is the first report using RAPD and ISSR markers to assess the genetic variability existing in the germplasm *Solanum* from North - East India.

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