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# IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF *IPOMOEA* SPECIES COLLECTED FROM EASTERN INDIA USING INTER SIMPLE SEQUENCE REPEAT MARKERS

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## ABSTRACT

*Ipomoea* genus is widely distributed in the tropical and subtropical regions of the world. The exploration of genetic diversity is pre-requisite for genome organization in the wild species and the related domesticated ones. Inter-simple Sequence Repeat (ISSR) markers was used to assess the identification of 21 important *Ipomoea* species and determination of the genetic relationships among these species. Out of 20 ISSR primers tested, twelve primers produced 218 detectable fragments, of which 207 (94.9 %) were polymorphic across the species. Each of the twelve primers produced fingerprint profile unique to each of the species studied, and thus could be solely used for their identification. Forty four unique bands specific to fifteen species were detected. These may be converted into species-specific probes for identification purposes. Genetic relationships among these species were evaluated by generating a similarity matrix based on the Dice coefficient and the Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendogram. The results showed a clear cut separation of the 21 *Ipomoea* species and were in broad agreement with the morphology. Both molecular and morphological markers will be useful for preservation of the *Ipomoea* germplasm collected from Eastern part of India.

## KEY WORDS

Inter Simple Sequence Repeat (ISSR), *Ipomoea* spp., Phylogeny, Molecular documentation, Fingerprinting

## INTRODUCTION

*Ipomoea* genus is constitute one of the largest genera of family Convolvulaceae with about 600 species of annual, perennial herbaceous and shrubs primarily occurring in subtropical and tropical regions world. Among the *Ipomoea* species, *Ipomoea batatas* (Sweet potato) is an important crop and is cultivated in more than 100 countries due to its significant source of calories, proteins and vitamins (Woolfe, 1992). Asia is the world's largest sweet potato producing region, with china alone producing 90% world production (CIP, 2008). Some

varieties are genetically more homogeneous and therefore more vulnerable to pathogens and adverse environmental conditions. Wild relatives and crop landraces are important gene resources for improving resistance to and increasing genetic heterozygosity and large chromosome number (Veasey et al., 2008). Wild species of *Ipomoea* are an important reservoir of useful genes and may provide a new approach for genetic improvement (Komaki, 2001). However, the taxonomic relationships of *Ipomoea* species and its related wild species have not been fully elucidated. Therefore, it is very essential to understand the extent of genetic diversity and the nature of genetic relationships among *Ipomoea* species and its wild relatives. Apart from morphological, physiological and agronomic traits, the genetic analysis through molecular marker is a pre-requisite to have a deep insight of the genome organization in the wild species and the related domesticated ones.

Several DNA-markers (RAPD, RFLP, SSR and ISSR) have also available to identify the species/ varieties. These markers can be effectively used to answer the phylogenetic relationship among and between *Ipomoea* species (Connolly et al., 1994; Jarret and Austin, 1994; Diaz et al., 1996; Buteler et al., 1999; Dhillon and Ishiki, 1999; Huang and Sun, 2000; Gichuru et al., 2006; Hu et al., 2003; 2004; He et al., 2007; Veasey et al., 2008).

diseases, pests and stresses of modern varieties. The genus is remarkable for the large variation in the habits of its species. Many species are propagated vegetatively. The flowers of sweet potato are self-incompatibility

ISSRs (inter-Simple sequence repeats) are the regions between two microsatellites (also called simple sequence repeats, SSRs).

ISSR overcomes many of the limitations faced by different marker system and has a higher reproducibility. ISSRs can also be easily used as a mapping tool (Sanker and Moore, 2001). Huang and Sun (2000) used ISSR markers system to study the genetic analysis of sweetpotato. As *Ipomoea* species represented by a large number of species/ accessions which are facing genetic erosion. To save these genetic resources, the present investigation is to study the identification and phylogenetic analysis of 21 species of *Ipomoea* collected from India through ISSR markers.

## MATERIALS AND METHODS

### *Plant materials*

Important *Ipomoea* species were collected from different forest areas in Eastern part of India. Identification of the species basing on their morphological characteristics was confirmed in our laboratory and doubtful samples were excluded from the analysis. In total, twenty-one species were taken for this study (Table-1).

**Table**-1

***Morphological characteristics of 21 species of Ipomoea .***

| S.NO | Name of the species | Morphological Characters |
|---|---|---|
| 1. | *Ipomoea quamoclit* | Twinning herbs with simple cordate or lobed or rarely pinnate leaves and usually showy funnel shaped or sub salver shaped ( with weakly funnel shaped tube ) flowers in axillary long peduncle umbellate cymes |
| 2. | *Ipomoea pestigridis* | A slender twinner, hairy or hirsute all over with spreading hairs and deeply palmate 5-9 lobed leaves 2-5" diameter with lanceolate or elliptic acute or acuminate lobes contracted at the base. Flowers 1-1.25" in long peduncle heads with persistent long bracts. |
| 3. | *Ipomoea pescaprae* | A prostate and far creeping herb from a large perennial elongate root. Stems glabrous .Leaves thick, 1.5- 2.5" long and often broader than long, emarginated to deeply 2 lobed at the apex, cuneate or straight at the base where are 2 large coloured glands. Flowers bright rose – purple darker in the throat, 1-2 rarely 3 on peduncles about as long as the leaves. |
| 4. | *Ipomoea wightii* | Climber, spreading hairy. Leaves ovate, 5-11 x 5.5-9cm, deeply 3-lobed, white cottony tomentose beneath; petiole 3-7.5cm long. Flowers purple or pink-purple, in long peduncle; bracts oblong-lanceolate, ciliate. Sepals sub equal, densely ciliate. Corolla funnel-shaped, 2.5-3.5cm long. Capsule globose, 6-7mm long, pubescent. Seeds ovoid. Having wide distribution in the hills of Africa, Sri Lanka and Ganjam in Orissa. |
| 5. | *Ipomoea carnea* | A large straggling shrub with very milky juice ,ovate cordate acuminate leaves ,larger 6" by 4" to 8.5" by 6" ,nearly glabrous & large convolvulus like flowers 3" long & broad. |
| 6. | *Ipomoea emarginata* | A small herb with creeping ,not twinning stems ,rooting at the nodes and small , usually reniform , sometimes ovate cordate leaves and small yellow flowers .5-.75" |
| 7. | *Ipomoea tridentata* | A herb , not twinning , with numerous prostate stems 6-12" long from a woody rootstocks and sessile or subsessile oblong oblanceolate or sub quadrate , often hastate or lyrate leaves .25-.75", rarely upto 1.25" long , usually broadly truncate or emarginated at the tip which has 2-3 teeth , several spinulose teeth , 1-3 hastate base . Flowers pale yellow, 1-3 on a slender peduncle, 5-1" long. |
| 8. | *Ipomoea eriocarpa* | A slender very hairy twinner with narrowly lanceolate to deltoid or ovate acuminate cordate based leaves 2-3" long , or upper smaller .Flowers very small pink or purple , campanulate or urceolate in sessile or subsessile capitates axillary cymes about .5-.75". |
| 9. | *Ipomoea cryseoides* | A slender nearly glabrous twinner with angled stems, deeply cordate ovate acuminate leaves about 1.5"-1.2", shallowly 3 lobed and repand toothed. Stems hairy at the nodes |
| 10. | *Ipomoea aquatica* | An aquatic herb with the stems creeping on mud and floating, with lanceolate to elliptic to oblong or ovate hastate or cordate leaves 1.5-6" long and long slender petioles. Flowers 1-2" long and 1.5" diameters, white or pale purple with a darker purple eye, solitary or in 2-3 flowered. Long floating stout and fistular stems often .3" diameter with milky |

| | | |
|---|---|---|
| | | juice. |
| 11. | *Ipomoea carica* | A slender twiner, hairy or hirsute all over with spreading hairs and palmate with 5 distinct lobed leaves 2-5" diameter with lanceolate or elliptic acute or acuminate lobes contracted at the base. |
| 12. | *Ipomoea hederaceae* | A hairy twiner with retrosely hirsute stems ,ovate cordate 3- lobed leaves 3-5" long & handsome bright blue or rose-colored flower , 1.5- 2" long with very long sepals .Leaves thinly hairy , lobes broadly ovate or oblong very acuminate. |
| 13. | *Ipomoea sepiaria* | A slender, rather variable, twiner glabrous or pubescent with ovate cordate or hastate or sub- 3- lobed acute accuminate or shortly palmately 5-7 nerved. Flowers delicate purple or white with a purple eye, 1.5-2" long. Branches sometimes pilose. |
| 14. | *Ipomoea pilosa* | A medium sized. Very hairy twinner with spreading somewhat swollen –based hairs on stems, petioles and cymes. Leaves 2-6" ovate cordate with sometimes also 2 lateral triangular lobes, white cottony tomentose beneath and with hairs on the nerves hairy above. Flowers about 1" long (including the lobes) purple in few flowered. |
| 15. | *Ipomoea nil* | Hairy twiner; stems hirsute. Leaves ovate, 3-lobed, 5.5-12.5 x 5.5-10cm, base cordate; lobes acuminate; petiole 3-10cm. Flowers bluish purple or pinkish purple, 3.7-5.5cm long, sub umbellate on 1-5 flowered; peduncles 2.5-7.5cm long with reflexed hairs; bracts linear. Sepals long lanceolate pubescent and hirsute, especially at the base. Corolla funnel-shaped. Anthers not twisted. Capsule subglobose, 7.5mm long, glabrous. Frequent in open places and along streams of Bhubaneshwar, Harisankar, Bolangir and Similipal in Orissa. It native in North America. |
| 16. | *Ipomoea batatas* "Acc.No-1" | A prostate plant with milky juice and triangular or ovate usually sharply and irregularly lobed leaves 2-4" long with more or less cordate base and long stout petioles 2-8". Flowers purple 2" long and broad 2-several on long axillary peduncles with short pedicels and very small bracts at the base of the pedicels. Stems glabrous or hairy. |
| 17. | *Ipomoea batatas* "Blackie" | A prostate plant with 5-6 irregularly lobed leaves 2-4" long with more or less cordate base and long stout petioles 2-8" and purple colour. Leaves purplish brown in colour with five distinct purple to black color veins. Stems glabrous or hairy |
| 18. | *Ipomoea batatas* "Acc.No-2" | A prostate plant with milky juice and triangular or ovate usually sharply and irregularly lobed leaves 2-4" long with more or less cordate base and long stout petioles 2-8". Leaves lemon yellow in colour. |
| 19. | *Ipomoea alba* | A large ornamental climber introduced from tropical America into India and now cultivated throughout the warmer parts. Stems glabrous; leaves ovate-acuminate; flowers large, sweet scented, night blooming, white sometimes with greenish bands, in few flowered cymes; capsules ovoid-globose with 4, polished yellow seeds. |
| 20. | *Ipomoea obscura* | A slender twinner with hairy stems, sub orbicular or ovate deeply cordate leaves about 1-2.5" and small flowers, about 0.75" diameter, yellow or white in colour .Leaves with broad and narrow basal sinus, shortly acuminate or apex rounded, hairy beneath. Capsule much exceeding the sepals, ovoid 0.3-0.45", with 4 brown velvety seeds. |
| 21. | *Ipomoea umbellata* | Stems long herbaceous scrambling and sometimes rooting at the nodes , usually not twining ,slightly angular , young pubescent .Leaves 1.5-2.5" oblong or elliptic or lanceolate with broadly cordate or hastate base ,upper sometimes linear and hastate, glabrous or petiole short pubescent or hairy on the upper side . Flowers white or cream on axillary 2-several flowered, peduncles .25-1.5" long, pubescent at base, bracts subulate less than .1". |

## DNA PREPARATION

DNA was isolated using CTAB method following the protocol of Doyle and Doyle (1990), with minor modification. 1.0 - 1.5 g of young non-scenence leaves were ground in liquid nitrogen. Then they were incubated in CTAB buffer (3% w/v CTAB, 100mM Tris-HCl, 20mM EDTA, 1.4M NaCl, 2% v/v β-mercaptoethanol, 2% w/v polyvinyl pyrrolidine, pH 8.0) for 2 hrs at 65 $^{o}$C. The homogenate was then extracted with an equal volume mixture of chloroform: isoamylalcohol (24:1) and centrifuged at 9000 rpm for 10 min. The upper aqueous layer was recovered and precipitated with prechilled isopropanol. The pallet was suspended with Tris-EDTA buffer (pH 8.0). The crude DNA was treated with RNase and incubated for 30 min at 37 $^{o}$C and again extracted with 1 volume phenol and subsequently with chloroform: isoamylalcohol at the ratio of 24:1. The supernatant were collected and precipitated with 3 M sodium acetate and prechilled ethanol. The DNA palate was washed with 70% ethanol, dried and re-suspended in Tris-EDTA buffer. The high molecular weight DNA was checked for quality and quantity electrophoretically using 0.8% agarose gel against a known amount of λ-DNA taken as standard.

### *Primer screening*

Twenty synthesized ISSR primers (M/S Bangalore Genei, Bangalore, India) were initially screened using one species of *Ipomoea* (*Ipomoea batatas*) to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species of *Ipomoea*. To ensure reproducibility, the primers generating weak, or complex patterns were discarded. A few well amplified fragments that were not reproducible across 2 replicate of DNA extraction were also discarded from analysis.

### *PCR amplifications and electrophoresis of PCR products*

For ISSR study, the initial optimization of PCR was conducted including concentration of template DNA, primer, $MgCl_2$, number of PCR cycle and annealing temperature. The PCR reaction had a total volume of 25 µl containing 20 ng templates DNA, 100 mM each dNTPs, 20 ng of oligonucleotides synthesized primer (M/S Bangalore Genei, Bangalore, India), 2.5 mM $MgCl_2$, 1x Taq buffer [10 mM Tris-HCl) pH 9.0, 50 mM KCl, 0.01% gelatin] and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, Bangalore, India). DNA amplification was performed in a PTC –100 thermal cycler (M J Research, USA) programmed for a preliminary 5 min denaturation step at 94$^{0}$ C, followed by 40 cycles of denaturation at 94 $^{0}$C for 20 sec., annealing temperature depending on the primer (50 - 56 $^{0}$C) for 30 sec. and extension at 72 $^{0}$C for 45 sec., finally at 72 $^{0}$C for 5 min. Amplification products were separated alongside a low range molecular weight marker (M/S Bangalore Genei, India) on a 2 % (w/v) agarose gel electrophoresis in 1x TAE (Tris Acetate- EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad, California, USA).

### *Analysis of amplification profile*

Clearly defined ISSR bands that behave as dominant markers were scored for the presence (1) or absence (0) for all the species/cultivars and entered into a data matrix. The genetic relationships among the species/cultivar were determined by calculating the Dice coefficient, estimated as $S = 2N_{AB} / N_A + N_B$ .Where $N_{AB}$ is the number of amplified product common to both A and B. $N_A$ and $N_B$ corresponds to number of amplified product in A and B respectively. Diversity pattern were represented in the form of a dendogram that was generated by subjecting the genetic similarity matrix to Unweighted Pair-group Method Arithmetic average (UPGMA) cluster

analysis with software NTSYS-pc, Version 2.0 (Rohlf, 1995).

## RESULTS AND DISCUSSION

The present study illustrates that to provide additional and rapid molecular markers suitable for identification of genetic resources and also to assess phylogenetic relationship among the *Ipomoea* species occurring in Eastern India. Twenty ISSR primers were used to assess the 21 species of *Ipomoea*. Twelve, out of the 20 primers generated clear multiplex banding profiles (good/good with less bands), among which six primers IG-01 (AGGGCTGGAGGAGGGC),IG-02 (AGAGGTGGGCAGGTGG),IG-03 (GAGGGTGGAGGATCT),IG-06 (GACAGATAGACAGATA), IG-10 [$(AC)_8T$] and IG-14 [$(GA)_8T$], produced the best ISSR profiles. However, the rest 8 primers produced either smear with bands or no products at all. Modification on the annealing temperature, primers concentration, $MgCl_2$ and template concentration did not improve their patterns. In addition, the results also showed that the most of the primers based on GA/AG and GT/TG dinucleotides core repeats generated good banding profiles. The amplification of ISSR markers was consistent across two replicate DNA extractions from three samples, over with 98% of scorable fragments reproducible. This was in concordance with previous studies an oilseed rape where the reliability of the protocol was demonstrated between PCRs and DNA extraction (Charters *et al*., 1996). A higher concentration of $MgCl_2$ (2.5mM) gave best results. This may be due to non-specific amplification because of reduced enzyme fidelity (Hopkins and Hilton, 2001). The concentration of $MgCl_2$ affects the specificity and yield of reaction by increasing the stringency of primer annealing or has a direct effect on Taq Polymerase (Saiki, 1989).

The six selected primers showed highly polymorphic banding profile. Furthermore, each of these primers produced fingerprints profiles unique to each of the species. Therefore, each primer can be used separately to identify these species in the future. ISSR amplification for all samples resulted in multiple band fingerprint profile for the six selected ISSR primers (Figure 1). The average number of scorable fragments per primer was 18, with a range from 14 to 25, while the average number of polymorphic fragments per primer was 18 with a range from 13 to 25. Out of the 218 scorable fragments, 207 were polymorphic revealing 94.95% polymorphism across the 21 species studied (Table-2). Forty four ISSR loci were recorded as germplasm specific as they occurred in fifteen species/accession. These may be developed into species specific probes for identification purposes. In the study primers based on GA/AG and GT/TG dinucleotides core repeats generated good profiles, which seem to indicate that the more frequent microsatellite in *Ipomoea* contains the repeated dineucleotides (AG/GA) n and (GT/TG)n**.** These results are in accordance with the SSR assay. ISSR techniques have high reproducibility, possibly due to the use of longer primers (16 – 25 mers) compared with RAPD primers (Williams et al., 1990) which permits the subsequent use of a higher annealing temperature, leading to high stringency. Rajapakse et al (2004) analysed the phylogenetic relationships of 13 accessions sweetpotato and its wild progenitors by using nucleotide sequence variation of a nuclear encoded beta-amylase gene. They found that *I.ramosissima* and *I.umbraticola* are quite different from other species. Veasey *et al* (2008) indicated that genetic diversity in Brazillian Sweet potato landraces by using microsatellite markers. They found eight SSR loci were assessed among the 78 accessions (58 landraces and 20 putative clones). In present study indicates that there was a distant variation in DNA amplification of 21 species of *Ipomoea* collected from Eastern India. Thematrix calculated for all possible pair wise comparisons between accessions showed that the index value varied from a minimum of 0.33 between *I.wightii* and *I.pestigridis* to a

maximum of 0.66 between *Ipomoea batatas* "Acc no.1" and *I.batatas* "Blackie" (Table 3). All the others ones have displayed different intermediate levels of similarity. Cluster analysis showing a dendrogram using Dice coefficient subjecting to UPGMA method (Figure-2) divided into three major clusters at 0.25 similarity level : A, B and C. Cluster-A and B had only one species and cluster – C having 19 species. Cluster-C is again divided into two subclusters i.e. C1 and C2. Subculster C1 having seven species and C2 having twelve species. C1 again divided into two minor clusters i.e D1 and D2. D1 having three species with 58% similarity. D2 having four species with 60% similarity. *Ipomoea pestigridis* and *Ipomoea eriocarpa* has been grouped with *Ipomoea alba*. Subcluster C2 is divided into four minor clusters i.e. D3, D4, D5 and D6 with 55% similarity with each other. D3, D4, D5 and D6 having three species each. *I.batatas* 'Blackie' and *I.batatas* 'Acc no. 1' are group together with *I.batatas* 'Acc no -2". *Ipomoea carica* and *I.aquatica* have also making one group with *I.carnea*. *I.wighitti* and *I.pescaprae* are making independent group. In the present study, we identified 22 ISSR loci that were specific to fifteen species. The frequency of specific fragments was within the 2% margin of error detected in the repeatability assay. These may be developed into species specific probes useful for identification purposes. More extensive sampling within each of the species would be required to estimate the proportion of fragments that are truly specific, as opposed to those that represent PCR artifacts or reflect sampling variation from heterogeneous templates. Our results indicate that the genetic relationship among the *Ipomoea* species, inferred by ISSR markers, were in accordance with their morphological characters. *Ipomoea batatas* 'Blackie' is morphologically similar to *Ipomoea batatas* "Acc no.1" and "*I. batatas* "Acc no.2 " with irregularly lobed leaves, long with more or less cordate base and long stout petioles. This modification may be due to bud-sport, and most plants have been distributed in Eastern India. The source of new diversity (typically wild relative, landraces) should be sought to enhance the information base (Swanson, 1996). Landraces, which indirectly reflect cultural and environmental diversity, are of interest for future breeding program.

In conclusion, this study indicates that the information for species identification and the presence of species in the natural distribution have been confirmed with ISSR markers. This analysis is quick and reproducible, can generate sufficient polymorphism to identify the i*pomoea* species, although most ISSR alleles are dominant rather than co-dominant. Using some of the co-dominant markers like SSR can further check the findings. Assessment of the genetic diversity and relationships among the species is of interest not only for germplasm conservation but also for breeding program.

**Figure 1**.

***ISSR banding pattern of 21 species of Ipomoea using synthesis primer IG-02 (A), IG-13 (B) and IG-15 (C). M – low range DNA marker. No. 1 - 21 is the species of Ipomoea as in Table-1.***

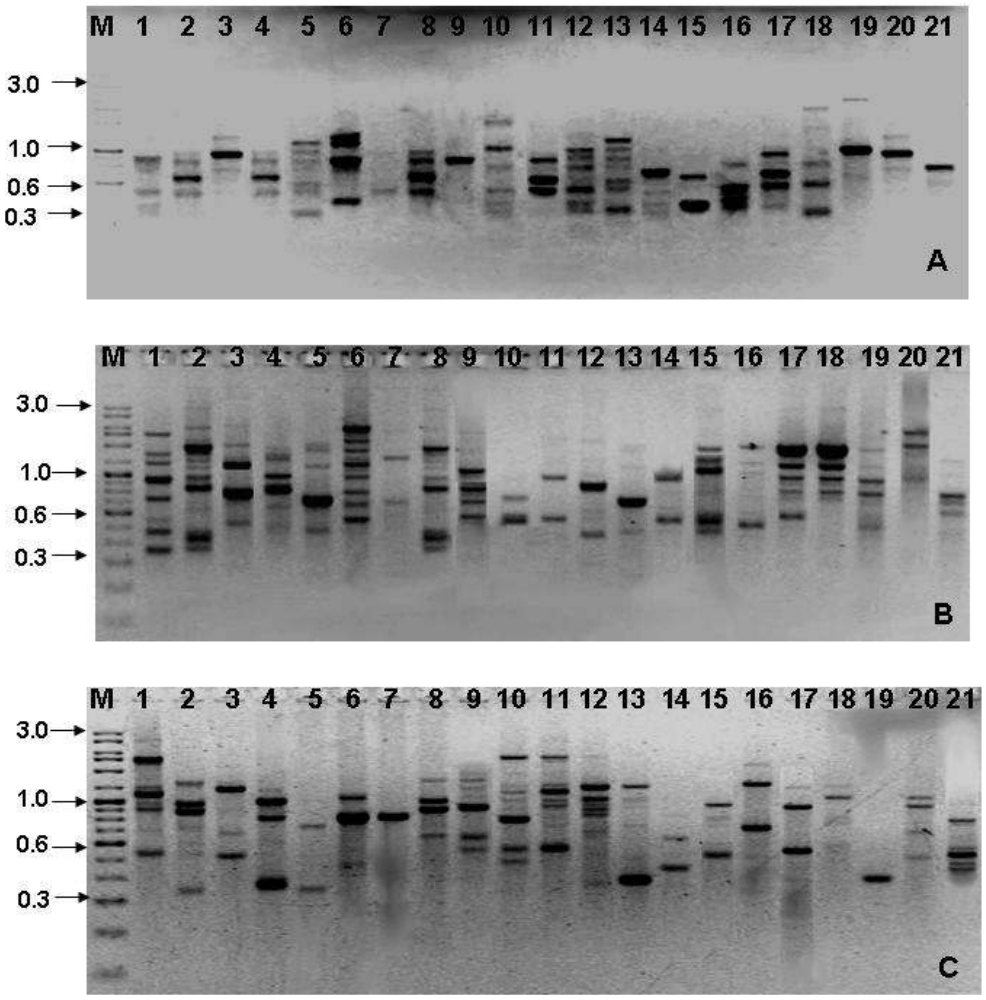


**Figure 1**

**Figure 2**.

***UPGMA dendrogram based on the similarity coefficient, showing the clustering pattern among the 21 species of Ipomoea. F1 - F21 are the 21 species of Ipomoea as in Table-1.***

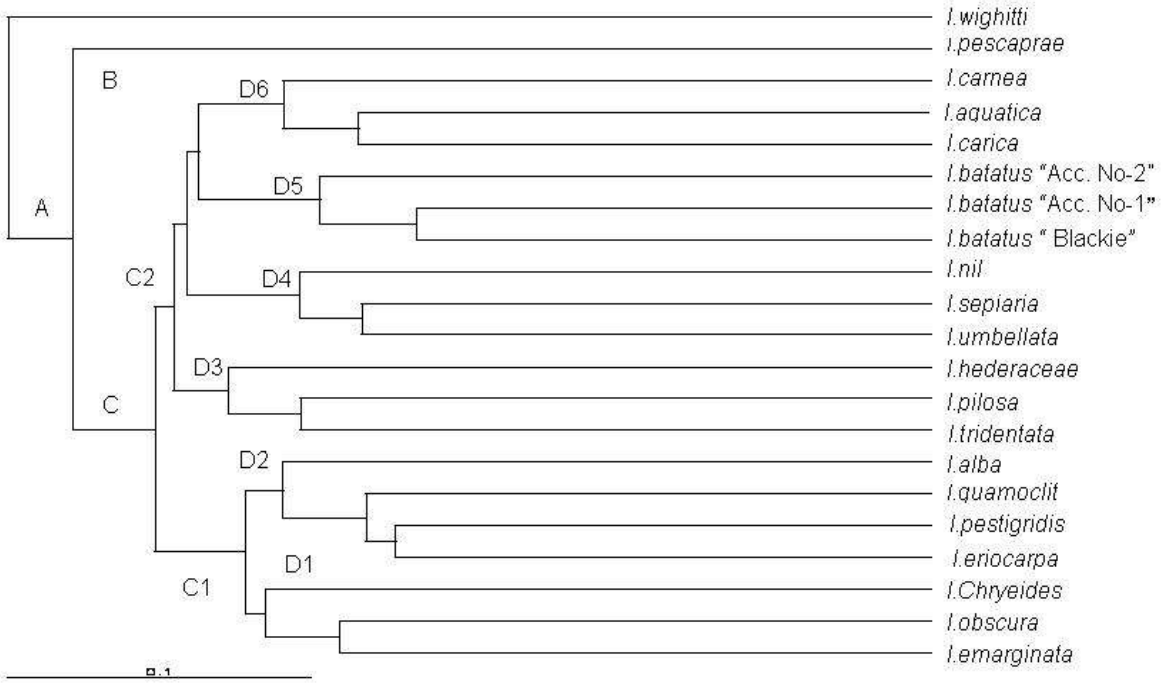


**Figure 2**

**Table-2**

***Twelve ISSR primers used for DNA fingerprinting of 21 species of Ipomoea.***

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| Primer Code | Primer Sequence | Total No. of Bands | No. of Polymorphic bands | Polymorphic Percentage | No. of Unique bands | Bands Range |
|---|---|---|---|---|---|---|
| IG-01 | AGGGCTGGAGGAGGGC | 22 | 21 | 95.45 | 04 | 0.3 - 3.5 |
| IG-02 | AGAGGTGGGCAGGTGG | 20 | 20 | 100.00 | 05 | 0.45 - 1.9 |
| IG-03 | GAGGGTGGAGGATCT | 25 | 25 | 100.00 | 05 | 0.25 - 2.1 |
| IG-06 | GACAGATAGACAGATA | 20 | 19 | 95.00 | 04 | 1.1 - 3.0 |
| IG-09 | $(AG)_8C$ | 06 | 05 | 82.22 | 00 | 0.3 - 2.2 |
| IG-10 | $(AC)_8T$ | 20 | 18 | 90.00 | 04 | 0.5 - 3.0 |
| IG-11 | $(AC)_8G$ | 14 | 14 | 100.00 | 03 | 0.3 - 2.0 |
| IG-13 | $(GA)_8A$ | 19 | 17 | 89.47 | 04 | 0.3 - 2.6 |
| 1G-14 | $(GA)_8T$ | 23 | 21 | 91.30 | 04 | 0.6 - 2.4 |
| IG-15 | $(AT)_8G$ | 18 | 18 | 100.00 | 04 | 0.5 - 3.0 |
| IG-22 | GACACGACAC | 15 | 13 | 86.66 | 03 | 0.6 - 2.1 |
| IG-23 | $(GA)_8C$ | 16 | 15 | 93.75 | 02 | 0.2 - 1.5 |
| TOTAL | ---------- | 218 | 207 | 94.95 | 42 | 0.20 - 3.5 |

**Table. 3**

***Similarity coefficient among the 21 species of Ipomoea obtained from ISSR markers. (I1- I 21 is the accessions as indicated in***

| | I 1 | I 2 | I 3 | I 4 | I 5 | I 6 | I 7 | I 8 | I 9 | I 10 | I 11 | I 12 | I 13 | I 14 | I 15 | I 16 | I 17 | I 18 | I 19 | I 20 | I 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I 1 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| I 2 | 0.61 | 1.00 | | | | | | | | | | | | | | | | | | | |
| I 3 | 0.44 | 0.44 | 1.00 | | | | | | | | | | | | | | | | | | |
| I 4 | 0.41 | 0.33 | 0.37 | 1.00 | | | | | | | | | | | | | | | | | |
| I 5 | 0.62 | 0.51 | 0.47 | 0.48 | 1.00 | | | | | | | | | | | | | | | | |
| I 6 | 0.58 | 0.64 | 0.43 | 0.34 | 0.49 | 1.00 | | | | | | | | | | | | | | | |
| I 7 | 0.51 | 0.52 | 0.49 | 0.37 | 0.43 | 0.47 | 1.00 | | | | | | | | | | | | | | |
| I 8 | 0.64 | 0.65 | 0.48 | 0.41 | 0.58 | 0.55 | 0.5 | 1.00 | | | | | | | | | | | | | |
| I 9 | 0.50 | 0.57 | 0.43 | 0.33 | 0.47 | 0.55 | 0.56 | 0.49 | 1.00 | | | | | | | | | | | | |
| I 10 | 0.60 | 0.56 | 0.48 | 0.37 | 0.62 | 0.52 | 0.52 | 0.61 | 0.53 | 1.00 | | | | | | | | | | | |
| I 11 | 0.52 | 0.49 | 0.40 | 0.45 | 0.53 | 0.41 | 0.52 | 0.39 | 0.49 | 0.62 | 1.00 | | | | | | | | | | |
| I 12 | 0.61 | 0.50 | 0.43 | 0.35 | 0.56 | 0.44 | 0.53 | 0.55 | 0.45 | 0.56 | 0.44 | 1.00 | | | | | | | | | |
| I 13 | 0.55 | 0.49 | 0.50 | 0.43 | 0.62 | 0.52 | 0.57 | 0.55 | 0.48 | 0.53 | 0.49 | 0.55 | 1.00 | | | | | | | | |
| I 14 | 0.49 | 0.40 | 0.43 | 0.32 | 0.44 | 0.40 | 0.58 | 0.43 | 0.47 | 0.55 | 0.44 | 0.55 | 0.52 | 1.00 | | | | | | | |
| I 15 | 0.46 | 0.43 | 0.47 | 0.47 | 0.54 | 0.39 | 0.50 | 0.46 | 0.55 | 0.47 | 0.46 | 0.41 | 0.61 | 0.56 | 1.00 | | | | | | |
| I 16 | 0.50 | 0.46 | 0.40 | 0.48 | 0.53 | 0.39 | 0.52 | 0.48 | 0.44 | 0.58 | 0.47 | 0.46 | 0.55 | 0.56 | 0.50 | 1.00 | | | | | |
| I 17 | 0.58 | 0.52 | 0.36 | 0.39 | 0.52 | 0.49 | 0.57 | 0.50 | 0.51 | 0.62 | 0.54 | 0.52 | 0.58 | 0.56 | 0.55 | 0.66 | 1.00 | | | | |
| I 18 | 0.47 | 0.49 | 0.31 | 0.35 | 0.48 | 0.46 | 0.44 | 0.44 | 0.54 | 0.50 | 0.42 | 0.42 | 0.49 | 0.47 | 0.52 | 0.59 | 0.60 | 1.00 | | | |
| I 19 | 0.57 | 0.56 | 0.40 | 0.39 | 0.52 | 0.54 | 0.51 | 0.59 | 0.55 | 0.54 | 0.41 | 0.52 | 0.51 | 0.47 | 0.50 | 0.48 | 0.54 | 0.52 | 1.00 | | |
| I 20 | 0.53 | 0.52 | 0.40 | 0.38 | 0.46 | 0.61 | 0.50 | 0.54 | 0.57 | 0.52 | 0.43 | 0.46 | 0.51 | 0.46 | 0.54 | 0.37 | 0.51 | 0.50 | 0.58 | 1.00 | |
| I 21 | 0.46 | 0.39 | 0.48 | 0.41 | 0.54 | 0.45 | 0.44 | 0.47 | 0.41 | 0.48 | 0.43 | 0.46 | 0.63 | 0.54 | 0.56 | 0.50 | 0.48 | 0.44 | 0.43 | 0.45 | 1.00 |

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