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PHYLOGENETIC RELATIONSHIP OF TEN *IPOMOEA* JACQ. SPECIES BASED ON RAPD ANALYSIS

Vinaya A Rane*, Behnaz B Patel

Department of Botany, R. Ruia College, Matunga, Mumbai 400 019

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For Correspondence:

Vinaya A Rane

Department of Botany,

R. Ruia College, Matunga,

Mumbai 400 019

E-mail:

mhatre_vinaya@yahoo.com

ABSTRACT

Ipomoea is a large and complex genus containing over 500 species of vines and shrubs widely distributed throughout the tropics and subtropics. The phylogeny of 10 species of *Ipomoea* Jacq. representing the three genera were evaluated using Random Amplified Polymorphic DNA (RAPD) markers. The amplification profiles of five primers, which produced amplification for most of the varieties, were used in the final analysis. Based on their scoring matrix the data was subjected to analysis using *MVSP-3.2*. The genetic similarity matrix was calculated using Jaccard's coefficient and the dendrogram was based on Jaccard's similarity Index obtained using UPGMA. Dendrogram showed two major clusters, formed of six species and the remaining four species were connected to the major clusters of species being studied here, with variation in their similarity.

INTRODUCTION

Ipomoea genus is constitute one of the largest genera of family Convolvulaceae with about 500 species of annual, perennial herbaceous and shrubs primarily occurring in subtropical and tropical regions world. The genus is remarkable for the large variation in the habits of its species. Many species are propagated vegetatively. Wild species of *Ipomoea* are an important reservoir of useful genes and may provide a new approach for genetic improvement[1]. 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.

Molecular markers are commonly used by plant biologists to perform a number of tasks, including the genetic fingerprinting of plant varieties, determining similarities among inbred varieties, mapping of plant genomes, and establishing phylogeny among plant species. New techniques for the extraction, purification, and amplification of plant DNA are being developed on a regular basis, enabling researchers to decrease preparation time and obtain readily reproducible results. Plants can now be compared at the molecular level in several ways, via examination of restriction fragments, identification of isoenzymes or products of the polymerase chain reaction (PCR). Numerous molecular markers have been developed and applied for analyses of genetic diversity and relatedness, and all marker systems have their strengths and weaknesses[2,3]. The choice of molecular marker system is determined by the objectives of the study as well as mating system of the studied species, and available financial support.

DNA-based techniques (RFLP, QTL, RAPD, AFLP, SSR, SCAR, VNTR and CAPS) are used to evaluate variation at the DNA sequence level[4].

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[5,6,7,8,9,10,11,12,13,14,15]

RAPD (Random Amplified Polymorphic DNA) is a technique which can provide useful data for the comparison of plant types is the random amplified polymorphic DNA. RAPD polymorphism is caused by a nucleotide substitution within the target site, or an insertion or

deletion of a DNA-fragment within the amplified region. Even in comparisons of inbred strains, the RAPD protocol may be more efficient in the long run relative to other techniques for generating random DNA markers. The RAPD protocol can provide genomic fingerprints that simultaneously scan loci dispersed throughout the genome. RAPD markers have, been widely used in plant genetics and breeding, to estimate genetic diversity, detect phylogenetic relationships among species ,sub-species and cultivars, and create genetic maps[3]. RAPD is low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.

MATERIALS AND METHODS

Ten species of *Ipomoea* were collected from wild habitat, in and around Mumbai, identified and authenticated in consultation with Blatter Herbarium, St. Xavier's College, Mumbai, India. One copy of the herbarium was deposited in the Blatter Herbarium and the other copy is preserved in The Late Yashwantrao Adbal Herbarium, of the Department as voucher specimen (Table 1).

Table 1: The ten species of *Ipomoea* collected and deposited in The Late Yashwantrao Adbal Herbarium, of the Department.

Species	Locality	Voucher Specimen
***Ipomoea nil*(Linn.) Roth.**	Yeoor, Thane Dist.	1
***Ipomoea aquatica* Forsk.**	Vikhroli, Mumbai	2
***Ipomoea pes-caprae* (Linn.) R.Br.**	Arnala, Virar, Thane Dist.	3
***Ipomoea carnea* Jacq.**	Alibaug, Raigad Dist.	4
***Ipomoea hederifolia* Linn.**	Yeoor, Thane Dist.	5
***Ipomoea triloba* Linn.**	Yeoor, Thane Dist.	6
***Ipomoea cairica* (Linn.) sweet**	Goregaon, Mumbai	7
***Ipomoea turbinata* Lag.**	Yeoor, Thane Dist.	8
***Ipomoea quamoclit* Linn.**	Yeoor, Thane Dist.	9
***Ipomoea violacea* Linn.**	Airoli, Navi Mumbai	10

The seeds of the different species collected were catalogued separately and stored.

All the species of *Ipomoea* collected here were found to have seed coat dormancy and failed to germinate in water. Acid scarification was performed to break the dormancy. Following scarification and washing the seeds were surface sterilized in 1% sodium hypochloride(v/v). They were washed in sterile distilled water and placed for germination in sterilized cocopeat.

Fresh, healthy, uninfected leaves of the ten species of *Ipomoea* were used for RAPD analysis. The RAPD analysis involved the following steps like genomic DNA extraction followed by RAPD profiling.

Genomic DNA extraction: Genomic DNA was extracted from non-scenescent, healthy leaves using following buffers:

Nuclei extraction buffer: It was prepared by mixing together 0.35M glucose + 0.1M Tris-HCl(pH-8.0) + 0.005M sodium EDTA(pH-8.0) + 2%PVP + 1% Ascorbic acid + 0.2% β-mercaptaethanol.

Nuclei Lysis buffer: It was prepared by mixing together 0.14M Sorbitol + 0.22M, Tris-HCl (pH-8.0) + 0.8M NaCl + 0.22M sodium EDTA (pH-8.0) + 0.8% w/v CTAB + 1%w/v PVP-10, 1% Sarcosyl + 5ug/ml proteinase + 0.1% Ascorbic acid + 0.2% β-mercaptaethanol.

The genomic DNA was isolated from 200mg of the leaf tissue using a protocol given by Chaudry[16]. Leaf tissue was ground to a fine powder with mortar and pestle using liquid nitrogen. 1ml ice cold nuclei extraction buffer was added in the w/v ratio of 1:5 in 1.5ml vial. The resuspended ground powder was kept on ice for 20 min. All the samples were centrifuged at 2700g at 4^{o}C for 20 mins. Then the supernatant poured off taking care that the pellet was saved, which contains the nuclei. 0.8ml of nuclei lysis buffer was added and the pellet was gently resuspended by inverting the tube several times gently. This mixture was incubated at 65^{o}C for 25 min. in a water bath followed by addition of chloroform:isoamyl alcohol (1:1 v/v) and the vial was inverted several times gently to remove proteins. It was the centrifuged at 2700g for 5 minutes at room temperature to separate the aqueous and organic phase. The chloroform : isoamyl alcohol step was repeated to the above supernatant. From this the aqueous layer was used to precipitate the DNA. DNA was precipitated by addition of 0.6 ml of Ice cold Isopropanol to 0.6ml of aqueous layer in another eppendorf tube. This solution was kept in refrigerator till the aggregates of DNA were formed (~ 15 min.). DNA was spooled out using a sterile fine capillary rod, followed by washing three times with 70% Ethanol. The pellet of DNA was air dried to remove all traces of ethanol. DNA was resuspended in 200µl of Tris EDTA buffer (pH- 8.0) in a 1.5ml vial. The DNA extracted was analyzed on 0.8% Agarose gel[17] and DNA quantification was done using Nanodrop (UV-Spectrophotometer).

RAPD profiling of extracted genomic DNA:

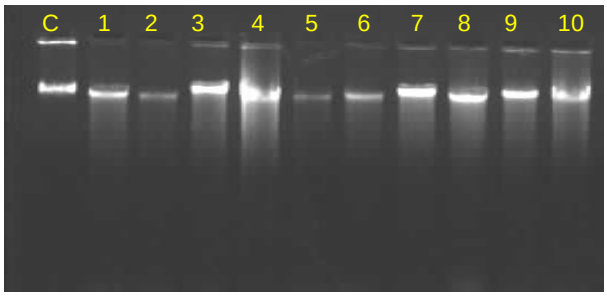
The genomic DNA was diluted accordingly, reaction mixture contains Template-4µl(25ng) 10x Taq assay buffer 1.5µl, MgCl2:0.3µl, Forward Primer: 5µl(1pmoles/µl), Reverse Primer: 5µl(1pmoles/µl) and Taq polymerase: 0.3µl the reaction volume was made up to 15µl with water and amplification was performed in a programmable Thermal Controller (*Eppendorf*

personal master cycler) for an initial denaturation of 4 min. at 94°C , followed by 35 cycles of denaturation at 94°C for 1min., annealing at 38°C for 1min., and extension for 2 min. at 72°C. A final extension for7 min. at 72°C was included after the last cycle. The products of amplification were cooled at 4°C. A negative control containing sterile water was included in each reaction set instead of templates. Amplified products along with DNA molecular weight markers were separated in a horizontal gel electrophoresis unit using 1.2 % agarose gel[17] stained with ethidium bromide and visualized on a UV transilluminator and the observations were recorded on Gel-Doc. The number of monomorphic bands, number of polymorphic bands, and intensity of bands were recorded. Primers, which on amplification produced the maximum number of bands, were used to amplify the DNA.

Dendrogram construction: The amplification profile using five primers were recorded. The RAPD bands were represented as '1' (present) and '0' (absent). Of the twenty primers tried, only five primers produced polymorphism. They were OPA-16 OPA-09 OPB-10 OPC-19 OPE-06. The amplification profiles of these primers, which produced amplification for most of the varieties, were used in the final analysis. Based on their scoring matrix the data was subjected to analysis using *MVSP-3.2* version RAPD analysis software. The genetic similarity matrix was calculated using Jaccard's co efficient and the dendrogram was based on Jaccard's similarity Index obtained using UPGMA.

RESULTS

Genomic DNA was extracted from all the 10 *Ipomoea* species under study. This extracted DNA was confirmed to be a genomic DNA through Agarose Gel Electrophoresis, wherein even the control DNA was run along with other samples. All the 10 lanes showed a single band each the like control DNA lane, confirming pure genomic DNA preparation; as shown in Fig.1. Quantification of extracted genomic DNA is given in Table: 2



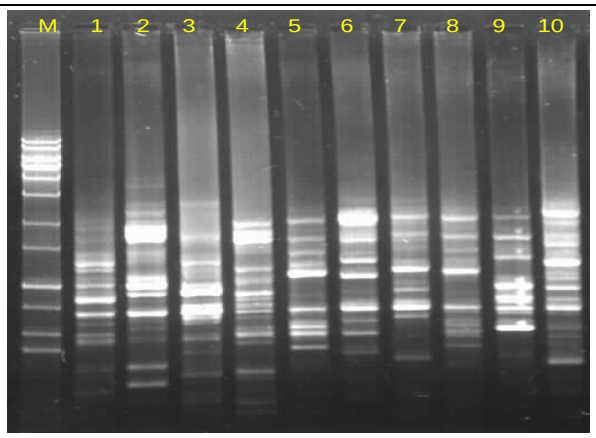
C:Control DNA
Lane1-10:Genomic DNA of plant samples from 1-10

Fig. 1: 0.8% Agarose Gel Electrophoresis of Genomic DNA

Table: 2 DNA Quantification using Nanodrop Spectrophotometer

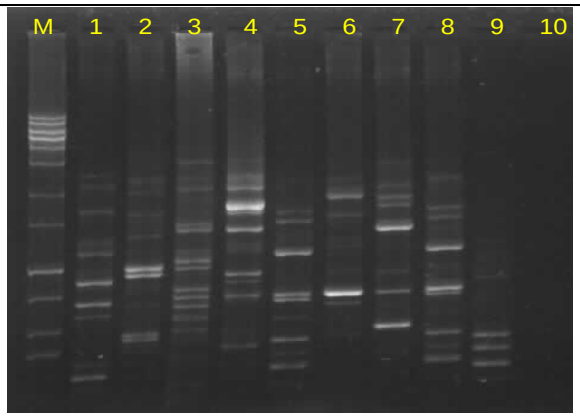
Sample	ng/ µl	260/280	constant
I. nil	252.37	1.93	50
I. aquatica	167.89	1.85	50
I. pes-caprae	222.97	1.89	50
I. carnea	203.81	1.85	50
I. hederifolia	178.67	1.91	50
I. triloba	233.30	1.87	50
I. cairica	189.47	1.99	50
I. turbinata	349.87	1.83	50
I. quamoclit	157.41	1.83	50
I. violacea	233.47	1.89	50

RAPD analysis using 5 primers gave a distinct pattern of polymorphic DNA bands as shown in Fig. 2, 3, 4, 5 and 6. RAPD profile of 5 primers used here, showed 106 scorable bands. Out of these 99 polymorphic bands were observed. Primer OPA-09, OPB-10 produced highest number of fragments among the primers used with an average of 24 fragments while OPC-19 produced lowest number of fragments (17) as given in Table 3. Percent polymorphism per primer is given in Table 4.



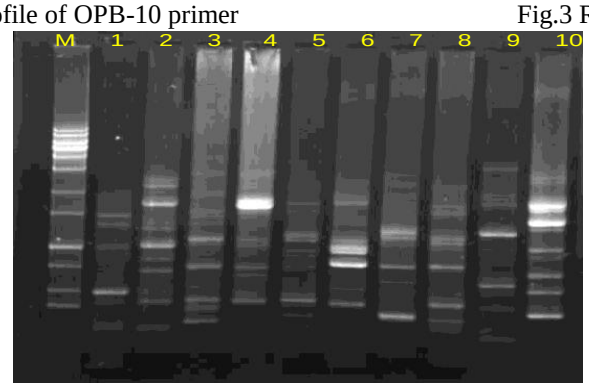
M-high range DNA ruler
Lane1-10 –RAPD profile for OPB-10 primer for plant 1-10

Fig. 2 RAPD profile of OPB-10 primer



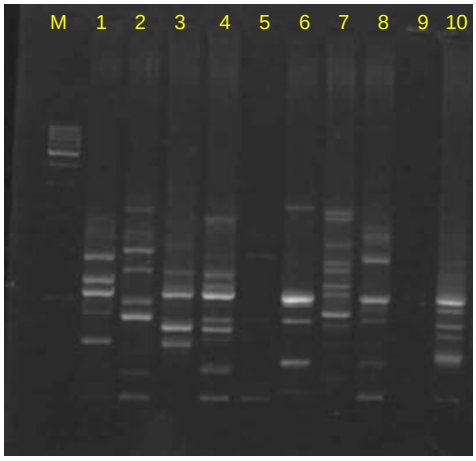
M-high range DNA ruler
Lane1-10 –RAPD profile for OPA09 primer for plant 1-10

Fig.3 RAPD profile for OPA09 primer



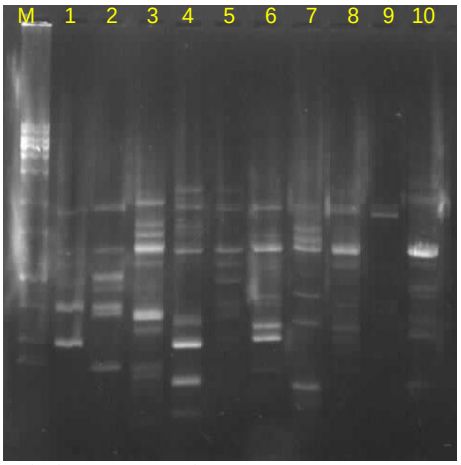
M-high range DNA ruler
Lane1-10 –RAPD profile for OPA16 primer for plant 1-10

Fig. 4 RAPD profile for OPA 16 primer



M-high range DNA ruler
Lane1-10 –RAPD profile for OPC-19 primer for plant 1-10

Fig. 5 RAPD profile of for OPA 19 primer



M-high range DNA ruler
Lane1-10 –RAPD profile for OPE-06 primer for plant 1-10

Fig. 6 RAPD profile for OPA 06 primer

Table 3 : Percentage of polymorphism

Primer Name	No. of amplicons	No. of polymorphic amplicons	% of polymorphism
OPA-09	24	23	95.3%
OPA-16	22	21	95.4%
OPB-10	24	22	91.6%
OPC-19	17	15	88.2%
OPE-06	19	18	94.7%

Table 4: Percent polymorphism per primer

Primers	*I.nil*	*I.aquatica*	*I. pescaprae*	*I.carnea*	*I.hederifolia*	*I.triloba*	*I.cairica*	*I.tubinata*	*I.quamoclit*	*I.violacea*
OPA09	8/9	10/11	14/15	13/14	9/10	9/10	9/10	12/13	6/7	0/1
OPA16	5/6	11/12	9/10	8/9	6/7	8/9	10/11	10/11	8/9	10/11
OPB10	10/12	13/15	11/13	12/14	9/11	10/12	8/10	11/13	7/9	9/11
OPC19	7/7	6/6	9/9	10/10	3/3	7/7	4/4	6/6	1/1	7/7
OPE06	5/6	5/6	11/12	10/11	7/8	8/9	5/6	7/8	6/7	9/10
% Polymorphism	87.5%	90%	91.5%	91.3%	87.1%	89.36%	87.87%	90.1%	84.8%	87.5%

The similarity amongst the ten species of *Ipomoea* was calculated using UPGMA for Jaccard's Similarity Coefficient as given in Table 5. Grouping of *Ipomoea* species into clusters based on RAPD Data is given in Table 6.

Table 5: UPGMA Jaccard's Coefficient Similarity Matrix

Ipomoea species	*I. nil*	*I. aquatica*	*I. pes-caprae*	*I. carnea*	*I. hederifolia*	*I. triloba*	*I. cairica*	*I. turbinata*	*I. quamoclit*	*I. violacea*
I. nil	1									
I. aquatica	0.344	1								
I. pes-caprae	0.282	0.295	1							
I. carnea	0.343	0.282	0.363	1						
I. hederifolia	0.385	0.344	0.319	0.324	1					
I. triloba	0.333	0.286	0.394	0.38	0.333	1				
I. cairica	0.259	0.221	0.333	0.338	0.281	0.306	1			
I. turbinata	0.456	0.348	0.397	0.403	0.456	0.468	0.355	1		
I. quamoclit	0.161	0.25	0.254	0.186	0.275	0.217	0.2	0.288	1	
I. violacea	0.309	0.242	0.247	0.343	0.333	0.27	0.237	0.317	0.275	1

Table 6: Grouping of *Ipomoea* species into clusters based on RAPD Data

Node	Group 1	Group 2	Similarity	Object in group
1	*I. triloba*	*I. turbinata*	0.468	2
2	*I. pes-caprae*	Node 1	0.396	3
3	*I. nil*	*I. hederifolia*	0.385	2
4	Node 2	*I. carnea*	0.382	4
5	Node 3	Node 4	0.356	6
6	Node 5	*I. aquatica*	0.316	7
7	Node 6	*I. cairica*	0.299	8
8	Node 7	*I. violacea*	0.287	9
9	Node 8	*I. quamoclit*	0.234	10

Based on UPGMA Jaccard's Coefficient of Similarity, Dendrogram was prepared, which showed two major clusters, formed of six species and the remaining four species were connected to the major clusters of species being studied here, with variation in their similarity, as shown in Fig. 7

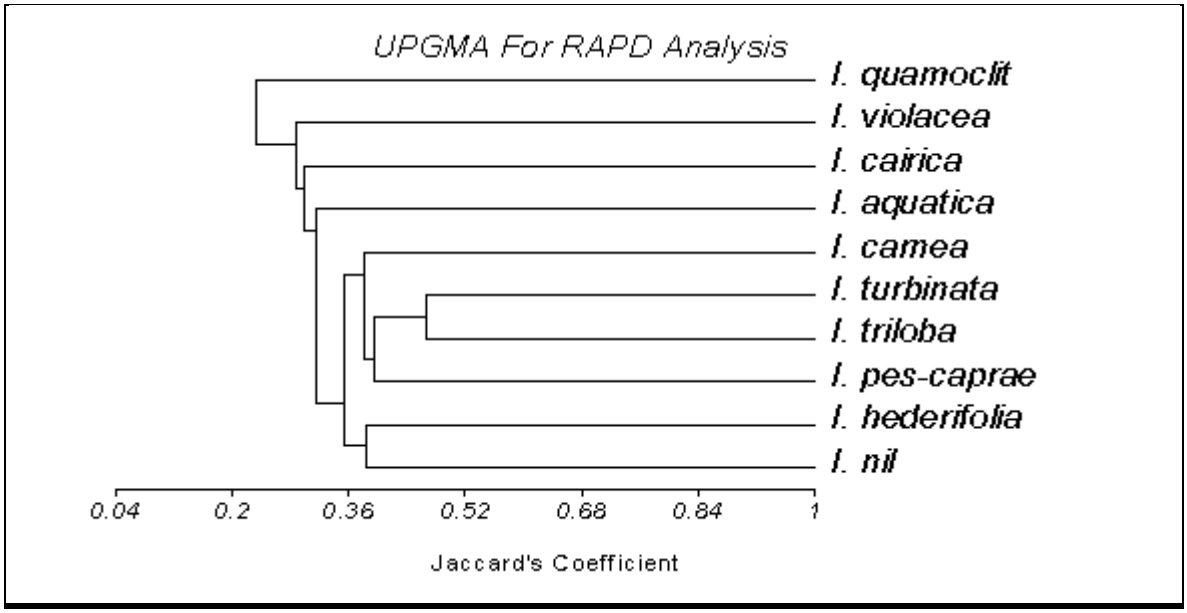


Fig. 7 Dendrogram based on UPGMA Jaccard's Similarity Matrix - RAPD analysis.

DISCUSSION

It was observed that *I. turbinata* has highest amount of gDNA (349.87 ng/µl), followed by *I. nil* (252.37 ng/ µl) and the least amount was observed in *I. quamoclit* (157.41ng/ µl) followed by I. *aquatica* (167.89 ng/ul), amongst the 10 *Ipomoea* species studied.

Among the primers that generated polymorphic amplification products primers OPA16 produced highest percentage of polymorphism (95.4%) followed by OPC-19 produced lowest polymorphism (88%). The proportion of polymorphic bands per total number of bands detected was least for *I. quamoclit* (84.8%) compared to that of *I. pes-caprae* (91.5%) with highest polymorphic bands per total number of bands. The percentage of polymorphic bands

in *I. quamoclit* (84.8%) was the lowest followed by *I. hederifolia* (87.1%), *I. violacea* (87.5%), *I. nil* (87.5%) and *I. cairica* (87.8%). The similarity index was calculated for RAPD polymorphism using UPGMA Jaccard's Coefficient wherein maximum similarity was observed to be between *I. triloba* and *I. turbinata* as 46.8% followed by 45.6% between *I. turbinata* and *I. hederifolia* and also between *I. turbinata* and *I. nil* as shown in Table 3.1.4.; on the other side minimum similarity was found between *I. nil* and *I. quamoclit*, thus they are placed at two extremes of the dendrogram here as seen in Fig. 7

Jaccard's Coefficient of Similarity, Dendrogram showed the first major cluster studied *I. triloba* and *I. turbinata* both of sub-genus *Quamoclit* showed maximum similarity of 46.8% forming Node-1 in the dendrogram, this node is connected to *I. pes-caprae* of sub-genus *Eriospermum* with the similarity index of 39.6%, forming Node-2. At Node-4, *I. triloba*, *I. turbinata* and *I. pes-caprae* get connected to *I. carnea* of sub-genus *Eriospermum* with the similarity of 38.2% as shown in Table 3.1.6. The second major cluster studied here showed 38.5% similarity between *I. nil* of the most primitive section *Pharbitis* and *I. hederifolia* of section *Mina* of the sub-genus *Quamoclit* being connected at Node-3. These two major clusters are connected to each other at Node-5, with the similarity index of 35.6 %. Node-5{[*I.carnea*+ (*I.pes-caprae*+*I.triloba* +*I.turbinata*)]+[*I. nil* +*I. hederifolia*]}, which is the cluster of six species is connected to *I. aquatica* with similarity index of 31.6% forming the next node as Node-6, which is in turn connected to *I. cairica* similarity index of 29.9% forming Node-7, it is then connected to *I. violacea* having similarity index of 28.7% forming the next node as Node-8. Finally all the nine species covered up to Node-8 is connected to *I. quamoclit* with the similarity index of 23.4%.

CONCLUSION

As from the dendrogram we can see that *I. triloba* and *I. turbinata* being placed very close to each other, indicating their relative proximity with each other, both belonginfg to sub-genus *Quamoclit.*. Similarly, *I. aquatica* (a hygrophyte, with linear to sagitate leaves and grayish, densely pubescent seeds), *I. cairica* (with palmately lobed leaves and subglobose to ovoid and densely short-tomentose seeds), *I. violacea* (possessing simple cordate leaves and white flowers and ovoid, pubescent with short stiff hairs on seeds) and *I. quamoclit* (with pinnetisect leaves, red flowers and conical, glabrous, tubercled seeds)are distantly separated from each other. The dendrogram outgrouped the psamophyte *I. pes-caprae* of of sub-genus *Eripomoea* from the remaining two species of the sub-genus *I. aquatica* and *I. violacea* though all the three belong to the same section *Eripomoea.*

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REFERENCES

1. Komaki K.(2001) Phylogeny of *Ipomoea* species closely related to sweetpotato and their breeding use. *Bull. Natl. Inst. Crop Sci.* 1: 1-56
2. Schlötterer, C. (2004). The evolution of molecular markers – just a matter of fashion? *Nature reviews/Genetics* 5: 63–69.
3. Weising, K., Nybom, H., Wolff, K. & Kahl., G. (2005). *DNA fingerprinting in plants. Principles, Methods, and Applications*. 2nd edition. CRC Press, Tylor and Francis group. Boca Raton. pp-444.
4. Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., van de Weil, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert R., Volcaert, G., Rueda, R., Linacero, R., Vazgues, A. & Karp, A. (1997). Reproducubility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* 3: 381–390.
5. Connolly, A.G., I.D. Godwin, M. Cooper, and I.H. DeLacy. (1994). Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.) genotypes. *Theor. Appl. Genet*. 88:332–336.
6. Jarret, R.L. and D.F. Austin. (1994). Genetic diversity and systematic relationship in sweetpotato (*Ipomoea batatas* (L.) Lam.) and related species as revealed by RAPD analysis. *Genet. Res. Crop Evol*. 41:165–173.
7. Diaz J, Schmiediche P and Austin DF. (1996). Polygon of crossability between eleven species of *Ipomoea batatus* (Convolvulaceae). *Euphytica,* 88:189-200.
8. Buteler MI, Jarret RL and Labonte DR. (1999). Sequence characterization of microsatellites in diploid polyploid *Ipomoea*. *Theor. Appl.Genet.*, 99:123-132.
9. Dhillon NPS, Ishiki K. (1999). Genomic variation and genetic relationships in *Ipomoea* spp. *Plant Breeding*, 118:161-165.
10. Huang JC, Sun M. (2000). Genetic diversity and relationships of Sweetpotato and its relatives in *Ipomoea* series Batatas (Convolvulaceae) as revealed by intersimple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theor. Appl. Genet.*, 100:1050-1060.
11. Gichuru V,Aritua V, Lubega GW, Edema R, Adipula E, Rubaihayo PR. (2006). A Preliminary analysis diversity among East African sweet potato landraces using morphological and simple sequence repeats markers. *Acta Hortic.*, 703:159-164.
12. Hu J, Nakatani M, Lalusin AG, Kuranouchi T., Fujimura T. (2003). Genetic analysis of sweet potato relatives using inter-simple sequence repeats (ISSRs). *Breeding Science.* 53:297-304.
13. Hu J, Nakatani M, Mizuno K, Fujimura T. (2004). Development and characterization of microsatellite in sweet potato. *Breeding Science*. 54:177-188.
14. He XQ, Liu QC, Ishiki K, Zhai H, Wang YP.(2007). ISSR analysis of genetic diversity and relationships among sweet potato (*Ipomoea batatus*) landraces in China. *Plant GeneticResources Newsletter*, 150: 35-41.
15. Veasey EA, Borges A, Rosa MS, Queiroz- Silva JR, Bressan EA, Peroni N. (2008). Genetic diversity in Brazilian sweet potato (*Ipomoea batatus* (L.) Lam., Solanales, Convolvulaceae) landraces assessed with microsatellite markers. *Genet. Mol. Biol*., 31 (3):725-733.
16. Chaudry, B., Afshan Y. Tayyab ,H. and Riyazuddin S (1999) *Plant molecular biology reporter*. 17: 1-7.
17. Maniatis and Sambrook, F, (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York: pp-1610.