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Congruence Between Morphological and Molecular Approach in Understanding Species Relationship in *Ipomoea* spp.: A Rare Event in Taxonomy

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Abstract: Taxonomic interpretations are based on some evidences. In biosystematics along with conventional morphological parameters, now-a-days molecular markers are extensively used in solving taxonomic disputes. There may be congruence or conflict between these two approaches. In the present study with twelve species of *Ipomoea*, general plant morphological character states as well as RAPD fragment phenotypes were employed to reflect interrelationship and clustering pattern of the species. Species pairs showing significantly greater pairing affinity values in morphological analysis also revealed higher monomorphism in RAPD band profile. Among the species *I. hispida* showed least number of amplified fragments (73) whereas, *I. aquatica* revealed highest number of amplified fragments (213). Dendrograms computed from morphological and RAPD data showed definite clustering pattern of *Ipomoea* species and significantly alike relative closeness. Present study revealed a sharp congruence between morphological and molecular approach.

Key words: *Ipomoea* spp., morphology, RAPD polymorphism, UPGMA method, dendrogram

INTRODUCTION

Ipomoea is the largest genus of the family Convolvulaceae comprising 650 species distributed all over the world (Mabberley, 1997). The genus occurs throughout the tropical and subtropical regions of the globe (Miller *et al*., 2004) and comprises annual and perennial herbs, shrubs and even small trees. Most of the species are twining, climbing plants.

Taxonomic interpretations are based on some evidences. Morphological features are age-old parameters but molecular techniques have earned ample importance to solve the taxonomic disputes. Randomly Amplified polymorphic DNA markers (Williams *et al*., 1990) have been used by several workers to evaluate species relationship, also to maintain clonal cultivars. RAPD markers have been utilized to study inter and intrageneric diversity of genotypes from two Jute species (Ogunkanmi *et al*., 2010), for identification of mutant tomato (Kulkarni and Deshpande, 2010). RAPD molecular marker proved to be superior over morphological approach to characterize large number of landrace rice accessions (Ogunbayo *et al*., 2007). RAPD profile was used to analyse the genetic diversity and distance between clonal cultivars of *Ipomoea batatas* L. (Connolly *et al*., 1994; Zhang *et al*., 1998). Jarret and Austin (1994) proved the utility of RAPD marker for evaluating genetic diversity in sweet potato and for establishing taxonomic as well as evolutionary relationship in *Ipomoea* species. Phylogeny of sweet potato and its wild species was analysed involving morphological variation, crossing ability and RAPD pattern of sweet potato and its closely related species (Katsumi, 2001). Morphometrics and quantitative characteristics of mature cotyledon (Ogunwenmo, 2003) and morphological variations both qualitative and quantitative (Mondal *et al*., 2006) were utilised for identification of *Ipomoea* taxa. Morphological and molecular parameters when used simultaneously to study species relationship or infra-specific diversity analysis showed conflicting interpretation in most of the cases (El-Shazly and El-Mutairi, 2006; Paul *et al*., 2010).

In the present investigation, the morphological character states as well as RAPD fragment phenotype were employed. Primary goal was to study relationship between different species of *Ipomoea*. Secondary aim was to explore congruence if any between the interpretations based on morphological and molecular approach. The later is a rare event in taxonomy especially when different unrelated species are engaged in the study.

MATERIALS AND METHODS

Materials: Twelve species of *Ipomoea* collected from different parts of lower Gangetic plain of West Bengal, India, included nine wild species – *I. hispida* Roem and Schult., *I. hederifolia* L., *I. pes-caprae* (L.) Sweet., *I. fistulosa* Mart ex Choisy., *I. sepiaria* Koen., *I. obscura*

(L.) Ker-Gawl., *I. pes-tigridis* L. *I. chryseides* Ker-Gawl., *I. triloba* (L.) Roth., two horticultural species- *I. quamoclit* L., *I. nil* (L.) Roth. and one semi cultivated species *I. aquatica* Forsk. Seeds of all the species were collected from different locale of West Bengal and were grown in experimental farm of Bose Research Institute, Kolkata, for consecutive three years for morphological and various bimolecular characterizations.

Morphological study: For plant morphological study 64 characters with character states including habit and habitat were taken into consideration. Measurements were taken from 10 readings for each character state. Few prominent character shared by all taxa were also considered for analysis.

RAPD analysis: Plant DNA was extracted from young seedlings by CTAB method (Rogers and Bendich, 1988). The DNA concentration in each sample was adjusted to 25 ng μL^{-1} for PCR.

PCR amplifications were performed according to the method of Williams *et al.* (1990) using DNA amplification kit from GENEI Bangalore, India, a set of 12 oligonucleotide primer, OPA 01–OPA 12 (Table 1) from Operon technologies, Inc, Alameda, Calif.) and Perkin Elmer Cetus thermal cycler. Temperature profile of each cycle was 40 sec. for denaturation at 94°C, 1 min. for Annealing at 35°C and 90 sec for extension at 72°C. Reaction continued for 40 cycles followed by 7.5 min. Hold at 72°C to ensure that extension reactions completed. PCR reaction mixture of 25 μL comprised 1X buffer, 0.2 mM of dATP, dCTP, dTTP, dGTP, 2 mM $MgCl_2$, 0.2 μL of primer, 100 ng of template DNA and U of Taq DNA Polymerase. PCR reactions were started 'hot' i.e., template DNA in 20 μL reaction mixture without Taq Polymerase was denatured first at 94°C for 5 min. followed by addition of 1 U of Taq polymerase at 72°C. The amplification products were electrophoresed with ϕ X 174 Hae III digested DNA (GENEI) as DNA size marker.

Statistical analysis: In case of morphological analysis on the basis of presence or absence of a particular character state in all the species, a similarity matrix was prepared giving each character a definite number. Pairing Affinity (PA) values between different combinations of species pair were calculated as follows:

$$\text{pA values} = \frac{\text{Character states common to species a and B}}{\text{Total number of character states in species A and B}} \times 100$$

In case of RAPD fragment polymorphism analysis pairing affinity values were calculated as follows:

Table 1: Random Oligonucleotide primers used in RAPD fragment polymorphism analysis

Primers	Primer sequence (5'-3')
OPA 01	5'-CCGGCCCTTC-3'
OPA 02	5'-TGCCGAGCTG-3'
OPA 03	5'-AGTCAGCCAC-3'
OPA 04	5'-AATCGGGCTG-3'
OPA 05	5'-AGGGGTCTTG-3'
OPA 06	5'-GGTCCCTGAC-3'
OPA 07	5'-GAAACGGGTG-3'
OPA 08	5'-GTGACGTAGG-3'
OPA 09	5'-GGGTAACGCC-3'
OPA 10	5'-GTGATCGCAG-3'
OPA 11	5'-CAATCGCCGT-3'
OPA 12	5'-TCGGCGATGA-3'

$$\text{pA values} = \frac{\text{Number of bands common to species A and B}}{\text{Total number of RAPD bands in species A and B}} \times 100$$

Using pairing affinity values, Dendrograms on morphology and RAPD fragment polymorphism were computed following Unweighted Pair Group Method with Average (UPGMA) method.

RESULTS

Morphological analysis: Most of the species were annual excepting perennial species like- *I. fistulosa*, *I. aquatica* and *I. pes-caprae*. Species were mostly terrestrial twining herbs excepting *I. fistulosa*, a terrestrial erect shrub., *I. aquatica* a semi-aquatic prostrate herb and *I. pes-caprae,* a terrestrial prostrate herb. Flower colour ranged from white to purple. Purple colour appeared to be the most prevalent colour with exception in *I. chryseides* (yellow), *I. nil* (blue).

The morphological features of uniform appearance in all the species were- simple exstipulate leaves; sepals 5, polysepalous, persistent; petals 5 gamopetalous, petals with 5 prominent bands; stamens 5, epipetalous, filaments unequal with hairy outgrowth at base; fruit valved, dehiscent capsule.

Percentage based pairing affinity values for different combination of species pair of *Ipomoea* were calculated from the character state distribution pattern. The values ranged from 45.3% (*I. fistulosa* and *I. nil*) to 81.25% (*I. obscura* and *I. triloba*) (Table 2). Significantly higher level of pairing affinity values (70% or above) were shown by species pair like *I. quamoclit* and *I. hederifolia* (70.68%), *I. quamoclit* and *I. chryseides* (71.87%), *I. quamoclit* and *I. triloba* (73.43%), *I. hederifolia* and *I. obscura* (73.43%). *I. hederifolia* and *I. triloba* (71.87%), *I. pes-caprae* and *I. aquatic* (73.43%), *I. aquatic* and *I. sepiaria* (75%) *I. sepiaria* and *I. obscura* (70.31%), lastly *I. sepiaria* and *I. triloba* (75%).

Dendrogram computed from pairing affinity values revealed three major clusters or groups at specific

Table 2: Pairing affinity values between different combinations of species pairs of *Ipomoea* on morphological features

	A	B	C	D	E	F	G	H	I	J	K	L
A	100											
B	59.37	100										
C	60.93	70.68	100									
D	51.56	56.25	54.68	100								
E	51.56	50.50	53.12	68.75	100							
F	57.81	57.81	57.81	73.43	68.75	100						
G	62.50	64.06	67.18	64.06	64.06	75.00	100					
H	57.81	62.50	65.62	51.56	45.31	57.81	64.06	100				
I	62.50	65.62	73.43	64.06	56.25	67.18	70.31	67.18	100			
J	60.93	71.87	71.87	51.56	56.25	54.68	60.93	54.68	67.18	100		
K	65.62	57.81	54.68	53.18	51.56	54.68	64.06	60.93	60.93	62.50	100	
L	60.93	73.43	71.87	67.18	60.93	67.18	75.00	65.62	81.25	68.75	62.50	100

Species of *Ipomoea*, A: *I. hispida*, B: *I. quamoclit*, C: *I. hederifolia*, D: *I. pes-caprae*, E: *I. fistulosa*, F: *I. aquatica*, G: *I. sepiaria*, H: *I. nil*, I: *I. obscura*, J: *I. chryseides*, K: *I. pes-tigridis*, L: *I. triloba*

Table 3: RAPD fragments amplified in different *Ipomoea* spp.

	Primers												
Species of *Ipomoea*	OPA 01	OPA 02	OPA 03	OPA 04	OPA 05	OPA 06	OPA 07	OPA 08	OPA 09	OPA 10	OPA 11	OPA 12	Total No. of RAPDs amplified
A	0	4	14	9	2	0	0	16	16	11	0	1	73
B	16	20	17	18	17	19	12	11	16	12	6	6	170
C	20	16	17	18	20	20	16	13	12	9	9	3	173
D	16	18	13	0	17	9	11	13	13	14	11	4	139
E	10	19	13	17	4	0	0	1	10	0	9	2	85
F	23	18	17	18	24	19	17	13	18	19	14	13	213
G	12	13	13	17	0	5	8	9	17	6	4	2	106
H	19	20	16	18	22	3	16	12	16	13	18	10	183
I	17	21	18	20	21	16	21	8	19	16	20	8	205
J	20	19	16	18	23	8	12	16	13	18	13	14	190
K	21	17	13	21	19	18	14	11	15	18	10	4	181
L	16	20	17	17	18	13	11	11	14	15	13	0	165
Total RAPDs amplified	190	205	184	191	187	130	138	134	179	151	127	67	1883

Species of *Ipomoea*, A: *I. hispida*, B: *I. quamoclit*, C: *I. hederifolia*, D: *I. pes-caprae*, E: *I. fistulosa*, F: *I. aquatica*, G: *I. sepiaria*, H: *I. nil*, I: *I. obscura*, J: *I. chryseides*, K: *I. pes-tigridis*, L: *I. triloba*

similarity level or similarity index. Group-A comprised *I. fistulosa*, *I. pes-caprae* and *I. aquatica*, Group- B included *I. sepiaria*, *I. obscura* and *I. triloba* and Group-C comprised *I. nil*, *I. quamoclit*, *I. hederifolia*, *I. chryseides* and *I. pes-tigridis* and I. *hispida* remained isolated from other members (Fig. 1).

RAPD polymorphism analysis: In RAPD fragment polymorphism analysis out of 12 primers used only four viz., OPA 02, 03, 08 and 09 revealed amplification for all the species. Total number of amplified fragments were 1877 in all the species from 12 primers. Only primer OPA-08 showed one monomorphic band (i.e., band having similar migrating pattern present in all the species (Fig. 2D). Other primers produced amplified fragments not universally present i.e., were polymorphic in respect of twelve species. Amplified products obtained with different primers are given in Table 3. Among the species investigated *I. hispida* showed least number of amplified fragments (73) whereas *I. aquatica* revealed highest number of amplified fragments (213). Primer OPA-02 (Fig. 2B) produced highest number of amplified products (205) whereas primer OPA -12 produced least number of amplified fragments (63) among the species (Table 3).

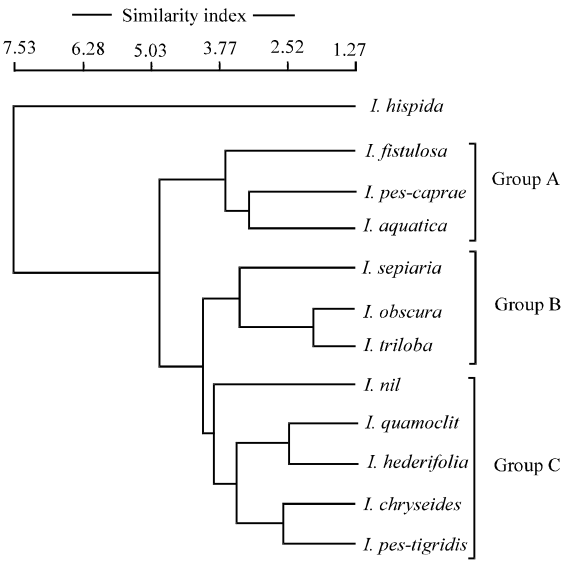

Fig. 1: Dendrogram computed from morphological features of *Ipomoea* spp. Showing cluster pattern

RAPD fragment profile of a species was treated as fragment phenotype and each band was considered as

Table 4: Pairing affinity values between different combinations of species pairs of *Ipomoea* on RAPD fragment profile

	A	B	C	D	E	F	G	H	I	J	K	L
A	100											
B	50.24	100										
C	44.82	56.93	100									
D	39.28	43.47	51.20	100								
E	29.07	45.90	31.93	48.33	100							
F	41.86	53.33	42.03	51.35	51.51	100						
G	41.90	44.44	40.65	45.16	42.85	57.35	100					
H	36.36	44.29	36.24	50.00	43.28	58.02	49.27	100				
I	35.55	43.58	43.13	51.94	43.27	51.80	45.07	64.28	100			
J	36.50	64.23	45.83	37.24	39.70	40.76	24.85	54.08	52.76	100		
K	34.69	38.23	39.09	35.48	35.59	34.24	34.42	43.24	46.05	52.00	100	
L	32.81	40.00	43.66	44.75	39.37	45.75	39.09	35.66	67.08	50.00	51.06	100

Species of *Ipomoea*, A: *I. hispida*, B: *I. quamoclit*, C: *I. hederifolia*, D: *I. pes-caprae*, E: *I. fistulosa*, F: *I. aquatica*, G: *I. sepiaria*, H: *I. nil*, I: *I. obscura*, J: *I. chryseides*, K: *I. pes-tigridis*, L: *I. triloba*

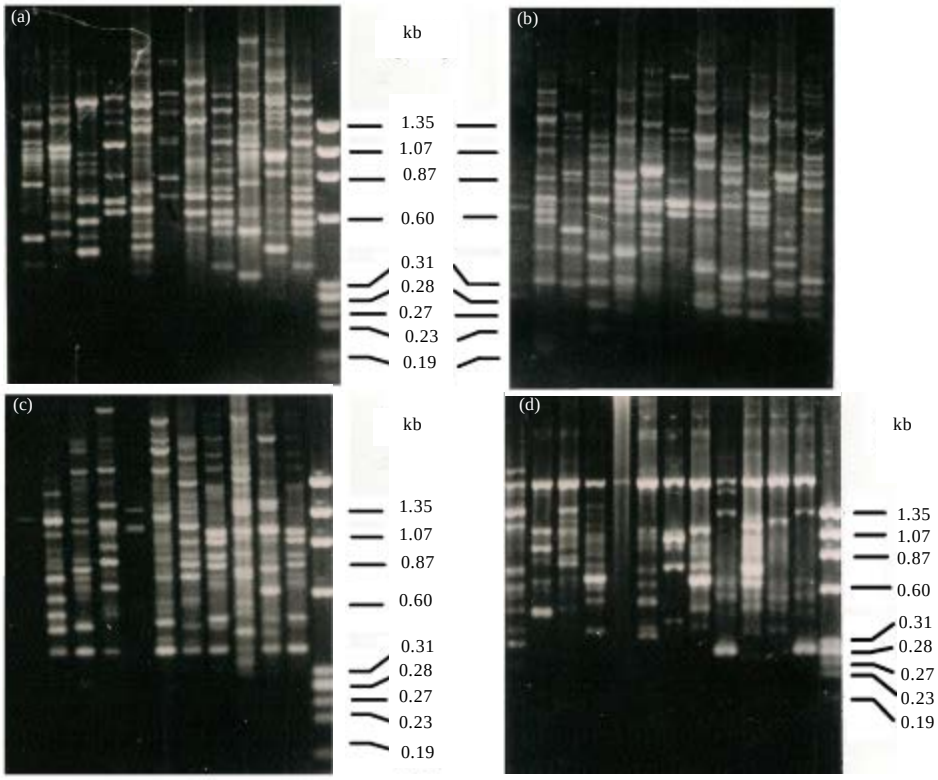

Fig. 2 (a-d): RAPD fragment polymorphism in *Ipomoea* spp., (a) With primer OPA 01, Lane 1-11 1 *I. quamoclit* 2. *I. hederifolia* 3. *I. pes-caprae*, 4. *I. fistulosa* 5. *I. aquatica* 6. *I. sepiaria*, 7. *I. nil* 8. *I. obscura* 9. *I. chryseides*, 10. *I. pes-tigridis*. 11. *I. triloba* M. DNA size marker, (b) With primer OPA 02, Lane 1-12 1. *I. hispida* 2. *I. quamoclit* 3. *I. hederifolia*, 4. *I. pes-caprae*. 5. *I. fistulosa* 6. *I. aquatic,* 7. *I. sepiaria* 8. *I. nil* 9. *I. obscura*, 10. *I. chryseides* 11. *I. pes-tigridis*. 12. *I. triloba*, M. DNA size marker, (c) With primer OPA 05, Lane 1-11 1. *I. hispida* 2. *I. quamoclit* 3. *I. hederifolia,* 4. *I. fistulosa* 5. *I. aquatica* 6. *I. sepiaria*, 7. *I. nil* 8. *I. obscura* 9. *I. chryseides*, 10. *I. pes-tigridis*. 11. *I. triloba* M. DNA size marker, (d) With primer OPA 08, Lane 1-12 1. *I. hispida* 2. *I. quamoclit* 3. *I. hederifolia*, 4. *I. pes-caprae*. 5. *I. fistulosa* 6. *I. aquatic,* 7. *I. sepiaria* 8. *I. nil* 9. *I. obscura*, 10. *I. chryseides* 11. *I. pes-tigridis*. 12. *I. triloba*, M. DNA size marker

potential variable. Pairing affinity values were calculated between different combinations of species pair based on monomorphic band profile (Table 4). Pairing affinity values in one way reflected percentage based

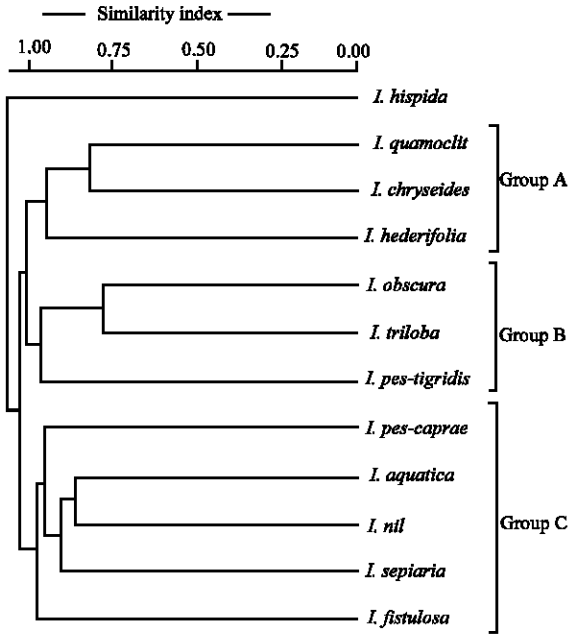

Fig. 3: Dendrogram computed from RAPD fragment polymorphism in *Ipomoea* spp.

monomorphism in different combinations of species pair, in reverse way the polymorphism percentage. Monomorphism percentage ranged from 24.85 to 67.08%. species pair *I. sepiaria* and *I. chryseides* showed least monomorphism of 24.85% i.e., greatest polymorphism and species pair *I. obscura* and *I. triloba* showed greatest monomorphism of 67.08% i.e., least polymorphism.

Dendrogram computed from monomorphism percentage showed distinct cluster or Groups (Fig. 3). The Dendrogram showed three major groups, Group-A comprised species- *I. quamoclit*, *I. chryseides* and *I. hederifolia*, Group-B comprised species – *I. obscura*, *I. triloba* and *I. pes-tigridis* and Group-C included species *I. pes-caprae*, *I. aquatic*, *I. nil*, *I. sepiaria*, *I. fistulosa*. *I. hispida* remained isolated from other species.

DISCUSSION

In taxonomic research along with the conventional morphological parameters, molecular markers are extensively used in solving taxonomic disputes. Same interpretations may or may not be drawn using both the parameters in respect of a particular taxonomic problem. Most of the previous taxonomic and systematic works on *Ipomoea* focussed the economically important cultivated hexaploid sweet potato (*I. batatas* L.), their origin, evolution and relationship with putative progenitor. *I. trifida* was appeared as a probable progenitor of *I. batatas* by cytogenetical and molecular studies (Srisuwan *et al*., 2006). More or less similar interpretation was drawn previously from combined ISSR and cpDNA dataset (Huang and Sun, 2000). There are large number of weed species interrelationship of which are least investigated.

RAPD phenotypes are inherited in a dominant fashion, therefore do not allow direct estimation of heterozygosity (Tingey and del Tufo, 1993). Prime goal of the present investigation was to group the species according to their relative closeness using morphological character states and RAPD fragment phenotype.

Numerical taxonomic techniques (Harman, 1976; Sneath and Sokal, 1973) have been proved to be a useful means to trace out genetic variability and species relationship (Elisens and Crawford, 1988). It is assumed that there exist a set of common characteristics or variables associated with each unit. The closeness between the two units is formulated in terms of a function and is called the distance.

Cluster pattern in the Dendrogram on morphology revealed clear grouping among the species. The relationship between the species of Group-A (*I. fistulosa, I. pes-caprae* and *I. aquatica*) was supported by erect or prostrate habit, perennial nature, large purple flower and big seeds. Relationship between members of Group-B (*I. sepiaria, I. obscura* and *I. triloba*) and Group-C (*I. nil, I. quamoclit I. hederifolia, I. chryseides* and *I. pes-tigridis*) is evidenced by twining annual habit, ovate-cordate leaves with acute to acuminate tip and small seeds. *I. hispida* is quite separable from other species in having hispid habit, very small white or purplish flower and very small seeds. The evolved members of the family Convolvulaceae are presumed to be twining plants with generally ovate-cordate leaves and showy white or purplish flower (Rendle, 1983), accordingly Group-B and C comprising comparatively evolved members. Large seeds are generally associated with woody perennials that are considered to be primitive (Salisbury, 1942) and accordingly Group-A including comparatively primitive members.

RAPD fragment obtained with different primers showed a high degree of polymorphism. Clustering pattern and relative closeness of the species was significantly alike. Dendrogram on RAPD showed complete isolation of *I. hispida* from other species as in morphological Dendrogram. In both the Dendrograms *I. obscura* and *I. triloba* were in close proximity. *I. quamoclit I. hederifolia* and *I. chryseides* were

clustered in well defined cluster in both the occasions. *Ipomoea* represent one of the few cases where we can find a significant congruence between morphological and molecular approach especially when well defined separate species are considered. The minor deviations and discrepancies might have been minimized or eliminated if large number of RAPD primers were used.

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