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EFFICACY OF THE ISOZYME DATA AND EMBRYOLOGICAL CHARACTERS TO SOLVE THE RIDDLE OF JUSTICIA-RUNGIA COMPLEX

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ABSTRACT

Traditionally embryological characters have been used for solving various taxonomic problems regarding the plant systematics. The biomolecules such as the Isozymes has been one of the very important tools for studying various aspects in plant biology.Taxonomic position of several members of Family Acanthaceaeis debated. The complex is *Justicia –Rungia* Complex is highly debatable. *Justicia* is basically a Linnaean Genus which included *Rungia* also. But later *Rungia* was alienated from *Justicia* based on some trivial characters. *Justicia* hasfurther given way to the formation of new genus *Rostellularia.*

In the present investigation Isozymes and embryological data was used to study the *Justicia –Rungia*Complex. The study has revealed a clearer scenario regarding its position. Some of the plants whose isozymes and embryology were studied are *Justiciaprocumbens, Rungiarepens, Haplanthusverticillatus* and *Blepharisrepens.* About nineteen isozymes were studied for the current work but Amylases, NAD diaphoreses, Esterase, Polyphenol oxidases, Ribulosebiphosphate carboxylase and Superoxide dismutase showed better resolution. Thus the embryological and isozyme study using UPGMA clearly indicates a very close similarity between *Justicia procumbens* and *Rungiarepens*.

Keywords*: Acanthaceae, Embryology, Isozyme, Molecular Marker, UPGMA.*

I. INTRODUCTION

Since the advent of plant classification morphological characters have been the backbone of taxonomy. With the changing time the embryological characters have acquired greater significance in plant taxonomy, especially when the external morphological characters are inconclusive and misleading as a result of convergence (Kapil and Bhatnagar, 1980). Embryology has been at the forefront in the systematic placement of the taxon under investigation. However with the advent bio-molecular and genetic techniques it has become complementary for the investigators to us both the old techniques and new techniques in plant systematics. The biomolecules such as the Isozymes has been one of the very important tools for studying various aspects in plant biology (Labhane, 2020). Isozymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. They are encoded by different genetic loci, which usually arise through gene duplication and divergence. The use of molecular tools like RAPD, RFLP, AFLP, etc in plant systematics is further been exploited to draw cladogram and phylogenetic trees.

The family Acanthaceae is extremely heterogeneous and there is not a single morphological character by means of which it would be possible to delimit it from its allies. Thus the family Acanthaceae consists of very closely related taxa, whose placement is doubtful (Bremekamp, 1953). One of the exciting complexes present in family Acanthaceae is Justicia–RungiaComplex. Justiciais a Linnean genus, based on Justici a adhatodaL. was split up by Nees (1832) into Adhatoda, Justicia, Gendarussa and Rostellularia. However, this splitting was not extensively followed and BREMEKAMP (1944) resurrected these genera. AdhatodaMill, JusticiaL. and Rostellularia Reichb classification was again followed by Gunn& al. (1992) and later by Naik(1998) but with the submergence of Rostellulariain Justicia. Due to this the Justiciais sometime identified as Rungiaand some time it is named as Rostellularia. This complex is hence known as Justicia-Rungia complex. The present investigation is aimed at solving this riddle by using Isozymes and embryological/ reproductive data.

METHODS& MATERIALS

The investigation includes four members from family Acanthaceae, namely Blepharisrepens(Vahl) Roth., Haplanthodesverticillata(Roxb.) Majumdar, Justicia procumbens Linnaeus and Rungiarepens(Linnaeus) Nees., which were collected mostly from the plants growing around Nagpur region with only exception, that Haplanthodesverticillata(Roxb.) Majumdar was collected from Sanjay Gandhi National Park (SGNP) Borivali, Mumbai. The plant material were identified with the help of renowned flora namely viz., the Flora of Maharashtra (Singh & al., 2001) and Flora of Marathwada (Naik, 1998). The plant species under investigation were preserved and deposited in the form of herbarium specimen in the Department of Botany, Rashtrasant TukadojiMaharaj Nagpur University, Nagpur with the accession numbers NML/201 – Blepharisrepens, NML/202 – Haplanthodesverticillata, NML/203 – Justiciaprocumbensand NML/204 – Rungiarepens. The planned study the Justicia – RungiaComplex includes the closely related Justicia procumbens and Rungiarepenswhile Blepharisrepens and Haplanthodesverticillataare considered as out groups.

The embryological investigations were carried out using standard protocol (Johansen, 1945). Twenty seven (27) characters were recorded from the taxa whose reproductive and embryological studies are investigated. Nineteen (19) isozymes were studied in order to ascertain the bio-molecular characterization of the taxa whose taxonomic position is disputed. Standard isozyme protocols are followed (Wendel and Weeden, 1989; Sadasivan and Manickam. 1996; Soltis&Soltis, 1989).

The cluster analysis was performed for the nineteen isozyme system and twenty seven reproductive and embryological characters by unweighted pair group method using arithmetic averages (UPGMA) (Sneath & Sokal, 1973). The dendrogram was generated with the SAHN subroutine of NTSYS-PC to show similarity coefficient between the taxa using embryological characters and isozymes data (Rohlf, 1993).

II. RESULT & DISCUSSION

Isozyme analysis

In the present investigation, nineteen enzyme system were examined viz. Acetyl esterase (AEST – E.C.3.1.1.6), Acid phosphatase (APH – E.C. 3.1.3.2), Alcohol dehydrogenase (ADH – E.C. 1.1.1.1), Alkaline phosphatase (ALP – E.C.3.1.3.1), Amylase (AMY – 3.2.1.1), Catalase (CAT –E.C.1.11.1.6), Esterase (EST – E.C.3.1.1.1), Glucose phosphate isomerise (G6PDH – E.C.1.1.1.49), Glutamate dehydrogenase (GDH – E.C.1.4.1.2), Malate dehydrogenase-NAD dependent (MDH – E.C.1.1.1.37), Malate dehydrogenase-NADP dependent (MDH – E.C.1.1.1.40), NAD- diaphorase (DIA – E.C.1.6.2.2), Peroxidase (PRX – E.C.1.11.1.7), Phosphoenol pyruvate carboxylase (PEPcase – E.C.4.1.1.38), Polyphenol oxidase (PPO – E.C.1.10.3.2), Ribulosebiphosphate carboxylase (RBC – E.C.4.1.1.39), Succinic dehydrogenase (SDH – E.C.1.3.99.1), Superoxide dismutase (SOD – E.C.1.15.1.1) and Xanthine dehydrogenase (XDH – E.C.1.1.1.204) (Table-1).

Nineteen enzyme system described earlier resolved eighty four putative alleles with sufficient clarity and constancy. The highest number of alleles (bands or electromorphs) perceived was eleven in Succinic dehydrogenase (Table-1).

Table-1 Allele scoring and frequency in the taxa investigated

Name of Enzyme	Allele	Blepharis	Haplanthodes	Justicia	Rungia	% of shared loci(P)
Acetyl Esterase	1	1.000	0.000	1.000	1.000	75.00
	2	1.000	0.000	1.000	1.000	75.00
	3	0.000	1.000	0.000	0.000	25.00
	4	1.000	0.000	0.000	0.000	25.00
Allele Frequency		0.750	0.250	0.500	0.500	
Acid Phosphatase	1	0.000	0.000	1.000	0.000	25.00

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Name of Enzyme	Allele	Blepharis	Haplanthodes	Justicia	Rungia	% of shared loci(P)
	2	0.000	1.000	1.000	1.000	75.00
	3	0.000	1.000	0.000	0.000	25.00
	4	1.000	1.000	1.000	1.000	100.00
	5	0.000	1.000	1.000	1.000	75.00
Allele Frequency		0.200	0.800	0.800	0.600	
Alcohol Dehydrogenase	1	0.000	0.000	1.000	1.000	50.00
	2	1.000	0.000	1.000	1.000	75.00
Allele Frequency		0.500	0.000	1.000	1.000	
Alkaline Phosphatase	1	0.000	0.000	1.000	1.000	50.00
	2	0.000	0.000	1.000	1.000	50.00
	3	1.000	0.000	0.000	0.000	25.00
Allele Frequency		0.333	0.000	0.667	0.667	
Amylase	1	1.000	0.000	0.000	0.000	25.00
	2	0.000	1.000	1.000	0.000	50.00
	3	0.000	0.000	1.000	0.000	25.00
	4	0.000	0.000	0.000	1.000	25.00
	5	1.000	0.000	0.000	0.000	25.00
	6	1.000	0.000	1.000	1.000	75.00
Allele Frequency		0.500	0.167	0.500	0.333	
Catalase	1	1.000	1.000	1.000	1.000	100.00
Allele Frequency		1.000	1.000	1.000	1.000	
Esterase	1	0.000	0.000	1.000	1.000	50.00
	2	0.000	1.000	1.000	1.000	75.00
	3	1.000	1.000	1.000	1.000	100.00
	4	1.000	1.000	1.000	1.000	100.00
	5	1.000	1.000	1.000	1.000	100.00
	6	1.000	1.000	1.000	1.000	100.00
	7	1.000	1.000	1.000	0.000	75.00
	8	1.000	1.000	0.000	1.000	75.00
Allele Frequency		0.750	0.875	0.875	0.875	
Glucose 6 P dehydrogenase	1	1.000	0.000	1.000	1.000	75.00
	2	0.000	0.000	1.000	1.000	50.00
	3	1.000	0.000	1.000	1.000	75.00

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Name of Enzyme	Allele	Blepharis	Haplanthodes	Justicia	Rungia	% of shared loci(P)
Allele Frequency		0.670	0.000	1.000	1.000	
Glutamate Dehydrogenase	1	1.000	1.000	1.000	1.000	100.00
Allele Frequency		1.000	1.000	1.000	1.000	
Malate Dehydrogenase (NAD)	1	0.000	1.000	1.000	1.000	75.00
	2	0.000	0.000	0.000	1.000	25.00
	3	1.000	1.000	1.000	1.000	100.00
Allele Frequency		0.333	0.667	0.667	1.000	
Malate Dehydrogenase(NADP)	1	1.000	1.000	1.000	1.000	100.00
Allele Frequency		1.000	1.000	1.000	1.000	
NAD-Diaphorase	1	1.000	0.000	0.000	1.000	50.00
	2	0.000	1.000	0.000	0.000	25.00
	3	0.000	1.000	1.000	1.000	75.00
	4	0.000	0.000	1.000	0.000	25.00
	5	1.000	0.000	0.000	1.000	50.00
	6	1.000	0.000	0.000	0.000	25.00
	7	1.000	1.000	1.000	1.000	100.00
Allele Frequency		0.571	0.429	0.429	0.571	
Peroxidase	1	0.000	0.000	1.000	1.000	50.00
	2	0.000	0.000	1.000	1.000	50.00
	3	0.000	0.000	1.000	1.000	50.00
	4	0.000	1.000	1.000	1.000	75.00
	5	1.000	1.000	1.000	1.000	100.00
	6	1.000	1.000	1.000	1.000	100.00
	7	0.000	0.000	0.000	1.000	25.00
	8	0.000	0.000	0.000	0.000	0.00
Allele Frequency		0.250	0.375	0.750	0.875	
PEP Carboxylase	1	1.000	1.000	1.000	1.000	100.00
Allele Frequency		1.000	1.000	1.000	1.000	
Polyphenol Oxidases	1	1.000	0.000	1.000	1.000	75.00
	2	0.000	0.000	1.000	1.000	50.00
	3	0.000	0.000	0.000	1.000	25.00

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Name of Enzyme	Allele	Blepharis	Haplanthodes	Justicia	Rungia	% of shared loci(P)
	4	1.000	0.000	0.000	0.000	25.00
	5	1.000	0.000	1.000	0.000	50.00
	6	0.000	0.000	1.000	1.000	50.00
	7	1.000	1.000	1.000	1.000	100.00
Allele Frequency		0.571	0.143	0.714	0.714	
Rubisco	1	1.000	1.000	1.000	1.000	100.00
	2	1.000	0.000	1.000	1.000	75.00
	3	0.000	1.000	0.000	0.000	25.00
	4	1.000	0.000	0.000	0.000	25.00
	5	0.000	0.000	1.000	1.000	50.00
	6	0.000	0.000	1.000	1.000	50.00
	7	0.000	0.000	1.000	1.000	50.00
Allele Frequency		0.429	0.286	0.714	0.714	
Succinic Dehydrogenase	1	1.000	0.000	1.000	1.000	75.00
	2	0.000	1.000	1.000	0.000	50.00
	3	1.000	0.000	1.000	1.000	75.00
	4	0.000	0.000	1.000	1.000	50.00
	5	0.000	1.000	0.000	0.000	25.00
	6	1.000	0.000	1.000	0.000	50.00
	7	1.000	0.000	0.000	1.000	50.00
	8	1.000	0.000	0.000	0.000	25.00
	9	0.000	0.000	1.000	1.000	50.00
	10	0.000	0.000	1.000	1.000	50.00
	11	0.000	0.000	1.000	1.000	50.00
Allele Frequency		0.455	0.182	0.727	0.636	
Superoxide dismutase	1	0.000	0.000	0.000	1.000	25.00
	2	1.000	0.000	1.000	0.000	50.00
	3	0.000	0.000	0.000	1.000	25.00
	4	0.000	1.000	1.000	0.000	50.00
	5	1.000	1.000	0.000	0.000	50.00
Allele Frequency		0.400	0.400	0.400	0.400	
Xanthine Dehydrogenase	1	1.000	1.000	1.000	1.000	100.00
Allele Frequency		1.000	1.000	1.000	1.000	
Total Allele Frequency		0.531	0.413	0.716	0.717	

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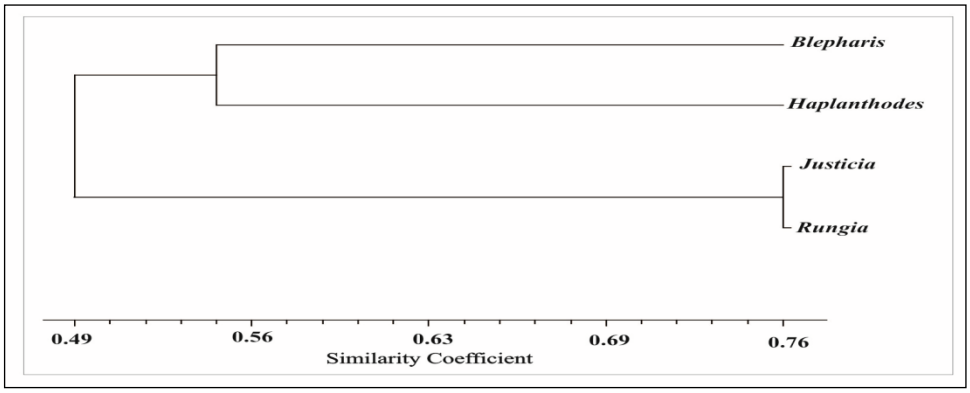

Figure-1.Dendrogram of isozyme profile prepared by unweighted pair group using arithmetic averages (UPGMA)

Krishnamurthy et al. (2004) used the isozyme studies on eight enzyme system to confirm the separation of Citrullusfrom Praecitrullus as two distinct genera, similar study on Luffa and Momordica has led to confirmation as separate genera. Apavatjrut et al. (1999) using isozyme as a molecular marker to identify seven taxa of early flowering groups of Curcuma. Mateu-Andres and Segarra-Moragues (2003) studied eight taxa belonging to six species of Antirrhinum using isozyme belonging to thirteen enzyme system in order to establish taxonomic delimitation and relationship among taxa related to A. graniticumand A. meonanthum. Siddiquee et al. (2010) effectively used eight isozyme patterns to establish relationship among three species of Trichoderma. Sammour et al (2019) worked on Lathyrussativus L

B. repensand H. verticillata were selected as an out groups in order to ascertain that even though they belong to same family Acanthaceae, how much similarity it shows in comparison with the Justicia-Rungia complex. J. procumbens and R. repens represent similar allelic frequency in sixteen out of total nineteen enzymes explored. Its fifty putative alleles out of total eighty four putative alleles resolved shows similarity. B. repensand H. verticillata share similar allelic frequency in eight out of total nineteen enzyme system investigated and it represents about nineteen putative alleles out of total eighty four alleles resolved shows close similarity. The total allelic frequency of Justiciaand Rungia is 0.716 and 0.717 respectively, whereas the allelic frequency of Blepharis and Haplanthodes is 0.531 and 0.413 respectively (Figure-1).

Embryological Characters- The embryological / reproductive twenty seven (27) characters which are selected to assess the coefficient of similarity between the taxa under consideration are mentioned in Table- 2 (Labhane &Dongarwar, 2014). Schnarf (1931) was the first to use embryology in solving taxonomic problems since embryological characters are considered as relatively stable and being less prone to adaptive stress. Dahlgren (1991) has also stressed the use of embryological characters as a next step towards the natural system of classification of the dicot plants. Labhane &Dongarwar (2014) have used the embryological data to establish relationship between the Justicia and the Rungia.

Characters	Blepharis	Haplanthodes	Justicia	Rungia
Number of stamens	4	2	2	2
Anther cells- equal/ unequal	Unequal	Equal	Equal	Equal
Anther cell-spured/not spured	Not spured	Not spured	Spured	Spured
Epidermal cells- small/ large	Small	Large	Small	Small
Stominum- Pronounced /not pronounced	Not pronounced	Not pronounced	More pronounced	More pronounced
Endothecium- 1/ 2 layered	1	2	1	1

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Characters	Blepharis	Haplanthodes	Justicia	Rungia
Fibrous thickening- Present / Absent	Absent	Present	Present	Present
Tapetal cells - 2-3 / 2-4 nuclei	2-4 nuclei	2-3 nuclei	2-3 nuclei	2-3 nuclei
Microspore tetrad- sperical/ elongated	Elongated	Sperical	Sperical	Sperical
Pollen grain- Elongated/ sperical/triangular	Elongated	Triangular	Sperical	Sperical
Anther cells- Parallel/ Superimposed	parallel	parallel	Superimposed	Superimposed
Exine- uniform/ Not uniform	Uniform	Uniform	Not Uniform	Not Uniform
Pollens- Mono/ Dimorphic	Monomorphic	Monomorphic	Dimorphic	Dimorphic
Number of Ovules	2 Ovules	6-10 Ovules	4 Ovules	4 Ovules
Schizogeneous Cavity- Present/ Absent	Absent	Present	Present	Present
Jaculator- Long / short	Long &lanceolate	Short and obtuse	Long and acute	Long and acute
Both micropylar and chalazalhaustorium- Present/ Absent	Absent	Present	Present	Present
Micropylar caecum- Present/ Absent	Absent	Absent	Present	Present
Micropylarhaustorium at maturity- Present/ Absent	Absent	Present	Absent	Absent
Chalazalhaustorium at maturity- Present/ Absent	Absent	Present	Absent	Absent
Secondary haustorium- Present/Absent	Absent	Present	Present	Present
Endosperm- Present/ Absent	Absent	Present	Present	Present
Mature embryo- straight/ curved	Straight	Straight	Curved	Curved
Ornamentation on embryo- Present/Absent	Present	Absent	Absent	Absent
Seed Coat- Present/ Absent	Absent	Present	Present	Present
Tubercles on seed- Present/Absent	Absent	Present	Present	Present
Seed dispersal- Splitting/ degeneration	Degeneration	Spliting	Spliting	Spliting

The cluster analysis was performed for the embryological data from twenty seven (27) embryological/reproductive characters by unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973). The dendrogram was generated with the SAHN subroutine of NTSYS- PC to show similarity coefficient between the genotypes (Rohlf, 1993). The embryological data generated using twenty seven (27) characters shows the presence of three distinct in which the cluster Justiciaandungia shows a similarity coefficient of nearly 97%. Thus, the embryological data explicitly shows close similarity between the Justicia and the Rungia, whereas the Blepharis is more distantly placed to this Justicia-Rungia complex than the Haplanthodes(Figure-2) (Labhane &Dongarwar, 2014).

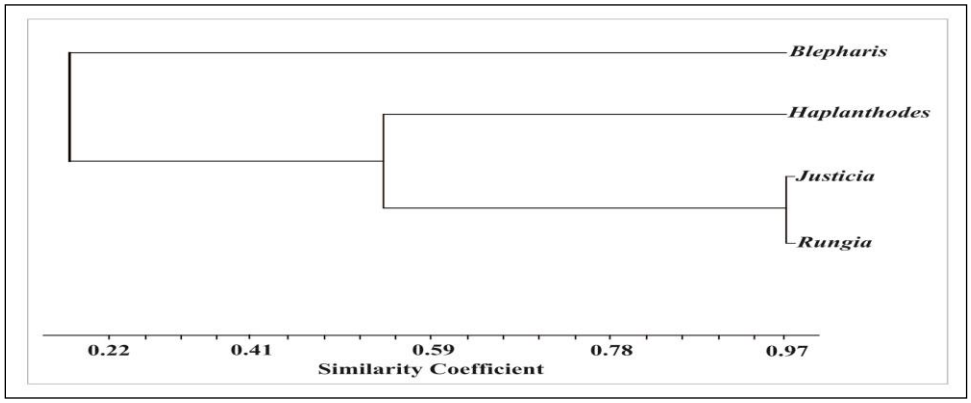

Figure 2. Dendrogram of embryological characters prepared by using unweighted pair group using arithmetic averages (UPGMA)

Combined analysis using both the isozyme and embryological data-

The dendrogram based on combined analysis of the isozyme profile and the embryological characters also shows the presence of three distinct clusters (Fig-4). The most closely related cluster is JusticiaandRungia, which shows a similarity coefficient of 82%. The second cluster is that of Justicia-Rungia and Haplanthodesshowing a similarity coefficient of 49%. Thus the cluster Justicia-Rungia is closely related to Haplanthodes than to Blepharis. The Blepharisis related to the remaining cluster and showing a similarity coefficient of 43% with respect to the other cluster.

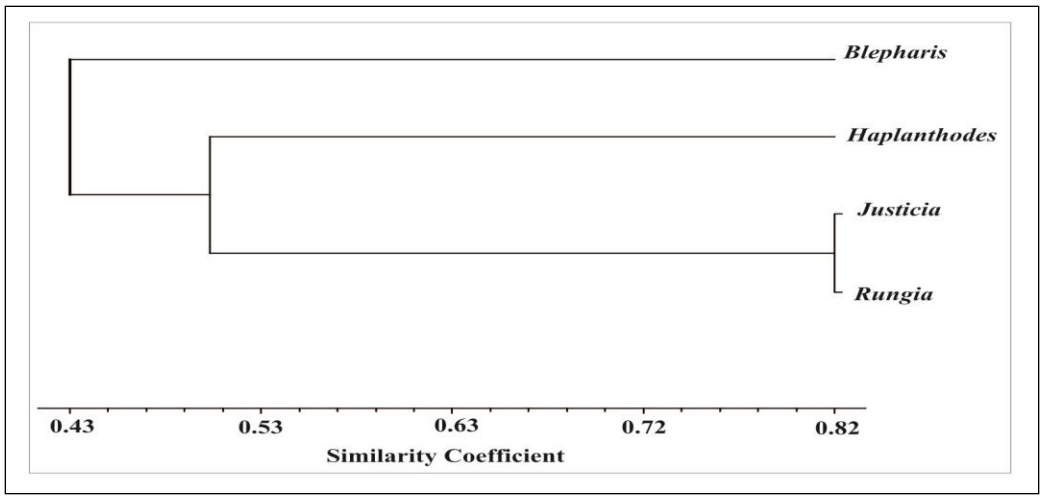

Figure-3. Dendrogram prepared by combined embryological characters and isozyme profile using unweighted pair group using arithmetic averages (UPGMA)

Similar clustering have been worked out for ascertaining the taxonomic affinities by Apavatjrut et al. (1999), Lange and Schifino-Wittmann (2000), Batista and Sosa (2002), Fu and Dane (2003), Mateu-Andres (2004), Gonzalez-Astorgaet al. (2004), Jaaska (2005) and Tripp et al. (2013). Das and Mukherjee (1997) studied twelve species of Ipomoea based on morphological and isozyme data and the dendrogram was prepared to suggest the affinities amongst them. While Labhane &Dongarwar (2014) worked on embryological characters to study the Justicia-Rungia complex.

III. CONCLUSION

Thus the dendrogram generated from data generated from nineteen (19) isozymes suggest a close affinity between both the Justiciaprocumbens and Rungiarepens, whereas the out groups stand apart from the Justicia-Rungia complex. Similarly the dendrogram extracted from the twenty seven (27) embryological characters and reproductive characters also suggest similar result (Labhane &Dongarwar, 2014). When dendrogram is prepared when both the isozyme profile generated from nineteen enzymes system along with twenty seven (27) embryological characters and reproductive characters, the resulting dendrogram again suggest the close association of Justiciaprocumbens and Rungiarepens.

Thus to solve the riddle of Justicia-Rungia complex the above study seems to be very important. The Linnaean genus Justiciarepens was named as Rungiarepens by Nees (1832) based on artificial characters namely, membranous bract- broad or narrow, margin of bract- hyaline or not, separation of the placenta from the valve- elastically or not. Thus separation of Rungiarepens from Justicia seems artificial. But in the light of current investigation based on the embryological and isozyme data, the inclusion of the species Rungiarepens under Justicia seems valid rather than considering as two separate genera.

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