

## EMBRYOLOGICAL CHARACTERS TO STUDY THE *JUSTICIA-RUNGIA* COMPLEX (ACANTHACEAE)

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**Abstract:** Family Acanthaceae is characterized by very diverse plants whose taxonomic position is debated. Therefore, study of various macroscopic and microscopic characters had been used for studying the relatedness of the various taxa. Embryological characters are considered as one of the most stable characters. *Justicia-Rungia* Complex is one of the intriguing complexes present in family Acanthaceae. *Justicia* is basically a Linnaean Genus which included *Rungia* also. But later *Rungia* was separated from *Justicia* by Nees. Another offshoot of *Justicia* has been the formation of new genus *Rostellularia* which is also the creation of Nees. Due to this the *Rungia* is sometime confused as *Justicia* and some time it is considered as *Rostellularia*. In the present investigation embryological data is used to study the *Justicia-Rungia* Complex. The plants studied in present investigation are *Justicia procumbens*, *Rungia repens*, *Haplanthus verticillata* and *Blepharis repens*. The embryological study using UPGMA clearly indicates a very close similarity between *Justicia procumbens* and *Rungia repens*.

**Keywords:** Embryology, Acanthaceae, *Justicia-Rungia* Complex, UPGMA

### Introduction

DANIEL (2009) considered the family Acanthaceae as a large pan-tropical family of about 229 genera and 3450 species. BREMEKAMP (1953) considered the family Acanthaceae to be highly heterogeneous and found that there is no single character which can be sufficient enough to classify. Therefore, study of various macroscopic and microscopic characters had been used for studying the relatedness of the various taxa. Embryological characters are considered as one of the most stable characters, since they are less prone to mutations. JOHANSEN (1945), CAVE (1953), KAPIL (1962), AREKAL (1963), JOHRI (1963, 1991), DAVIS (1966), BHANDARI (1971), PHILIPSON (1974), FALSER (1975), RAGHVAN (1997), BHOJWANI & BHATNAGAR (1999), IFRIM (2011) all of them effectively used reproductive and embryological characters for studying systematic placement of the taxon. DAHLGREN (1991) has also stressed the use of embryological characters as a next step towards the natural system of classification of the dicot plants. Family Acanthaceae have been reviewed by several authors from embryological point of view, in order to solve various taxonomic problems (MAURITZON, 1934; CRETE, 1951; MAHESHWARI & NEGI, 1955; JOHRI & SINGH, 1959; PHATAK & AMBEGAOKAR, 1963; MOHANRAM & WADHI, 1965; DAVIS, 1966; MAHESHWARI, 1963; LABHANE & DONGARWAR, 2011; LABHANE, 2011). *Justicia – Rungia* Complex is one of the interesting complexes present in family Acanthaceae, which needs to be studied from embryological stand point. In the present paper the

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reproductive and embryological characters are taken into consideration to study their relative affinities.

### Materials and methods

The materials selected for the present investigation are *Blepharis repens* (Vahl) Roth., *Haplanthodes verticillata* (Roxb.) Majumdar, *Justicia procumbens* Linnaeus and *Rungia repens* (Linnaeus) Nees. All the taxa selected in the present investigation were collected mostly from the plants growing around Nagpur region, which is located in the central India, in the state of Maharashtra, with only exception, that *Haplanthodes verticillata* (Roxb.) Majumdar was collected from Sanjay Gandhi National Park (SGNP) Borivali, Mumbai. Nagpur falls within the tropical to sub-tropical region of central India, with temperature varying from 10-30 °C during rainy and winter season to 40-45 °C during summer. *Haplanthodes verticillata* was collected from SGNP, Mumbai belongs to North-western parts of Maharashtra, which shows more or less moderate climate throughout the year, with average temperature is 20-30 °C. The plant material were identified with the help of standard flora namely viz., the Flora of Maharashtra [SINGH & al. 2001], Flora of Marathwada [NAIK, 1998], Flora of Nagpur [UGEMUGE, 1986] and Flora of British India [HOOKER, 1885]. The taxa under investigation were preserved in the form of herbarium specimen and deposited in the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur with the accession numbers NML/201 – *Blepharis repens*, NML/202 – *Haplanthodes verticillata*, NML/203 – *Justicia procumbens* and NML/204 – *Rungia repens*.

The young buds and flowers in sufficient quantity were collected and fixed in formalin-acetic-alcohol and stored in 70% alcohol. The selected taxa were collected from Nagpur and Mumbai, during the months of August to March 2005-2010, when the plant bears buds, flowers and fruits. Customary methods of dehydration, infiltration and embedding were followed. Sections were cut 8-14 mm thick and stained with Heidenhain's iron-alum-haematoxylin. Light green was used as a counter stain. Twenty seven characters were recorded from the taxa whose reproductive and embryological studies are investigated (Fig. 2).

The cluster analysis was performed for the twenty seven reproductive and embryological characters by unweighted pair group method using arithmetic averages (UPGMA) [SNEATH & SOKAL, 1973]. The reproductive and embryological characters are used to study the *Justicia – Rungia* Complex, the closely related *Justicia procumbens* and *Rungia repens* are taken into consideration. *Blepharis repens* and *Haplanthodes verticillata* are included in the present investigation as out groups. The dendrogram was generated with the SAHN subroutine of NTSYS-PC to show similarity coefficient between the taxa [ROHLF, 1993].

### Results and discussion

Twenty seven reproductive and embryological characters are selected carefully to estimate relative similarities (Tab. 1). The reproductive and embryological characters show close similarity between the two closely related *Justicia* and *Rungia* species (Fig. 2). The cluster analysis using un-weighted pair group arithmetic averages (UPGMA) shows the presence of three distinct clusters amongst the four taxa, when reproductive and embryological characters are taken into consideration (Fig. 1). The *Justicia–Rungia* cluster

shows a similarity coefficient of nearly 97%. The *Justicia–Rungia* cluster shows a similarity coefficient of 55% with the second cluster represented by *Haplanthodes*, which in turn shows a similarity coefficient of 22% with the *Blepharis* cluster. Thus the embryological characters assumes that the genera *Justicia* and *Rungia* are 97% similar to each other, whereas the most distantly placed taxa investigated is *Blepharis* which shows a similarity coefficient of just 22%.

BREMEKAMP (1938, 1944, 1953, 1955) gave a detailed account regarding his view on the subdivision of the family Acanthaceae and concluded in his paper titled ‘The delimitation of the Acanthaceae’ that the family 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. Study of embryology, palynology, chemotaxonomy, anatomy etc had been used for studying the relatedness of the various taxa. *Justicia–Rungia* Complex is one of the exciting complexes present in family *Acanthaceae*. *Justicia* is a Linnean genus, based on *Justicia adhatoda* L. was split up by NEES (1832) into *Adhatoda*, *Justicia*, *Gendarussa* and *Rostellularia*. But this splitting was not widely followed. Later, BREMEKAMP (1944) resurrected these genera. GUNN & al. (1992) accepted *Adhatoda* Mill, *Justicia* L. and *Rostellularia* Reichb. This same course has been followed by NAIK (1998) in his flora of Marathwada with the submergence of *Rostellularia* in *Justicia*. Due to this the *Justicia* is sometime confused as *Rungia* and some time it is considered as *Rostellularia*. Hence, in order to understand the degree of similarity among the most closely related *Justicia procumbens* and *Rungia repens*, the reproductive and embryological characters are used to study the *Justicia–Rungia* Complex. Both *Justicia procumbens* and *Rungia repens* shows close similarity with respect to morphology. Another two taxa namely *Blepharis repens* and *Haplanthodes verticillata* are also included in the present investigation as out groups, since these taxa are morphologically quite distinct from the *Justicia–Rungia* Complex.

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. 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 & BHATNAGAR, 1980]. MAHESHWARI (1950, 1963), and JOHN (1963) on the basis of their extensive work on embryology have provided list of families, genera, species, etc where embryology has either supported earlier classification or has proposed a new systematic position for the taxa concerned. Thus embryological evidences have been used in solving taxonomic problems at almost all levels and have helped to resolve the doubtful systematic position of several taxa. LABHANE & DONGARWAR (2011) based on embryological studies also confirmed the close relationship between the two species of *Justicia* and *Rungia*. However, previous attempts regarding the placement of taxonomic species lacks the statistics or quantitative element to descriptions. Statistical methods allow more rigorous comparisons between different forms and it has great significance in distinct grouping or separation of closely related species, which is used in the present study. The use of embryological characters and its analysis using the UPGMA for ascertaining the taxonomic alignment clearly indicates a very close relationship between the species of *Justicia procumbens* and *Rungia repens*.

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**Tab. 1.** The reproductive / embryological characters selected for taxonomic alignment.

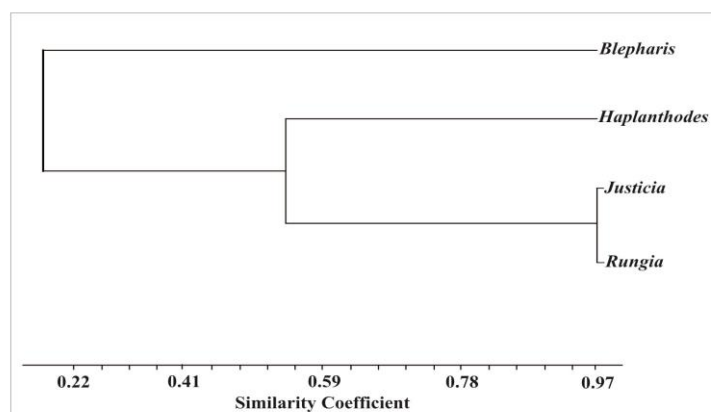
	Characters	<i>Blepharis</i>	<i>Haplenthodes</i>	<i>Justicia</i>	<i>Rungia</i>
1	Number of stamens	4	2	2	2
2	Anther cells <i>equal/unequal</i>	Unequal	Equal	Equal	Equal
3	Anther cell <i>spurred/not spurred</i>	Not spurred	Not spurred	Spurred	Spurred
4	Epidermal cells <i>small/large</i>	Small	Large	Small	Small
5	Stomium <i>pronounced/not pronounced</i>	Not pronounced	Not pronounced	More pronounced	More pronounced
6	Endothecium <i>1/2 layered</i>	1	2	1	1
7	Fibrous thickening <i>present/absent</i>	Absent	Present	Present	Present
8	Tapetal cells <i>2-3 / 2-4 nuclei</i>	2-4 nuclei	2-3 nuclei	2-3 nuclei	2-3 nuclei
9	Microspore tetrad <i>spherical/elongated</i>	Elongated	Spherical	Spherical	Spherical
10	Pollen grain <i>elongated/spherical/triangular</i>	Elongated	Triangular	Spherical	Spherical
11	Anther cells <i>parallel/superimposed</i>	Parallel	Parallel	Superimposed	Superimposed
12	Exine <i>uniform/not uniform</i>	Uniform	Uniform	Not Uniform	Not Uniform
13	Pollens <i>mono/dimorphic</i>	Monomorphic	Monomorphic	Dimorphic	Dimorphic
14	Number of ovules	2 Ovules	6-10 Ovules	4 Ovules	4 Ovules
15	Schizogenous Cavity <i>present/absent</i>	Absent	Present	Present	Present
16	Jaculator <i>long/short</i>	Long and lanceolate	Short and obtuse	Long and acute	Long and acute
17	Both micropylar and chalazal haustorium - <i>present/absent</i>	Absent	Present	Present	Present
18	Micropylar caecum <i>present/absent</i>	Absent	Absent	Present	Present
19	Micropylar haustorium at maturity- <i>present/absent</i>	Absent	Present	Absent	Absent
20	Chalazal haustorium at maturity- <i>present/absent</i>	Absent	Present	Absent	Absent
21	Secondary haustorium <i>present/absent</i>	Absent	Present	Present	Present
22	Endosperm <i>present/absent</i>	Absent	Present	Present	Present
23	Mature embryo <i>straight/curved</i>	Straight	Straight	Curved	Curved
24	Ornamentation on embryo <i>present/absent</i>	Present	Absent	Absent	Absent
25	Seed Coat <i>present/absent</i>	Absent	Present	Present	Present
26	Tubercles on seed <i>present/absent</i>	Absent	Present	Present	Present
27	Seed dispersal by <i>splitting/ degeneration</i>	Degeneration	Splitting	Splitting	Splitting

### Conclusions

The present investigation shows that the two taxon, *Justicia procumbens* L. and *Rungia repens* (L.) Nees appears to be very similar with respect to the development of the embryological and reproductive characters. The genus *Rungia* is segregated from *Justicia* on the basis of minor morphological characters. However, the cluster analysis using UPGMA taking into consideration twenty seven embryological and reproductive characters showed 97% similarity coefficient between the two taxa and hence, the inclusion of species of *Rungia* under *Justicia* is justified. It seems that the two species might have got segregated very recently during the process of evolution leading to the formation of two distinct taxa, however the present study suggest that both the taxa *Justicia procumbens* and *Rungia repens* are reproductively very similar.

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**Fig. 1.** Dendrogram based on embryological characters prepared by using unweighted pair group using arithmetic averages (UPGMA)

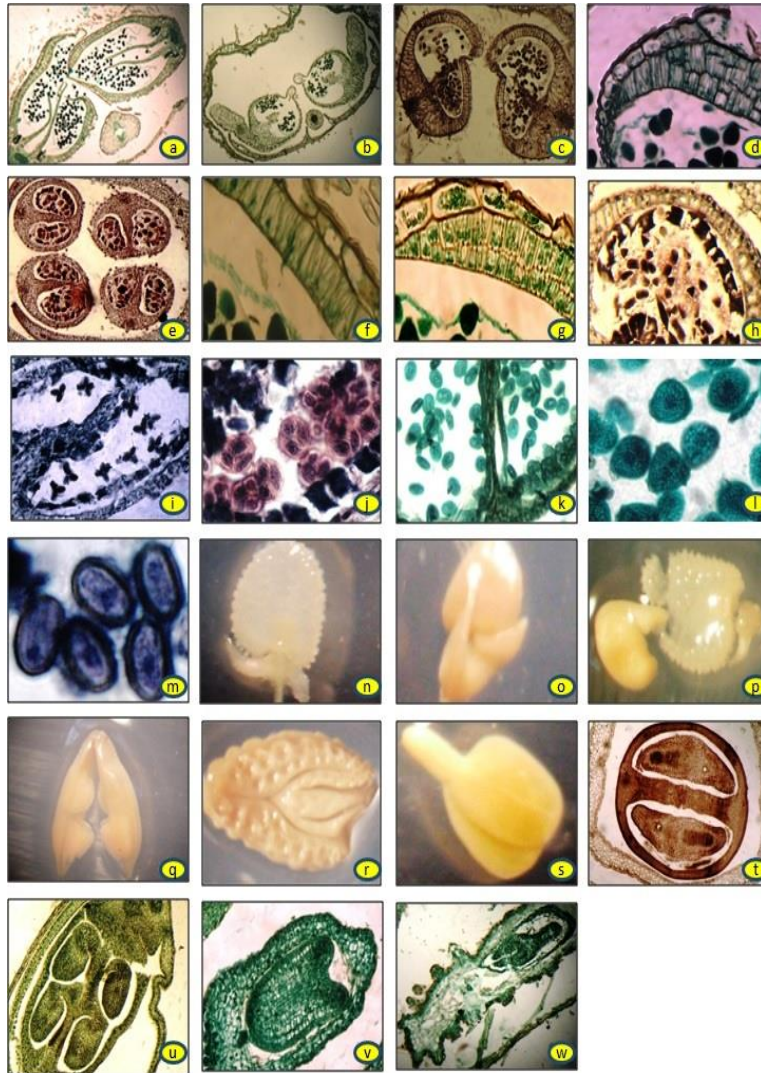
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**Fig. 2.** a- Two anther with equal cells in *H. verticillata*; b- Spurred anther cell and prominent stomium in *J. procumbens*; c- Anther cells unequal and one layered endothecium in *B. repens*; d- Two layered endothecium *H. verticillata*; e- Four stamens without spurred anther cell in *B. repens*; f- Large epidermal cells in *H. verticillata*; g- Two layered fibrous endothecium in *H. verticillata*; h- Small epidermal cells without fibrous endothecium in *B. repens*; i- Elongated microspore tetrad in *B. repens*; j- Spherical microspore tetrad in *J. procumbens*; k- Pollen grains dimorphic and 2-3 nucleate tapetum in *J. procumbens*; l- Triangular pollen grains in *H. verticillata*; m- Monomorphic elongated pollen grains showing uniform thickening in *B. repens*; n- Long and acute jaculator in *J. procumbens*; o- Short and obtuse jaculator in *H. verticillata*; p- Mature embryo curved with seed coat as in *J. procumbens*; q- Four seeds in mature fruit in *J. procumbens*; r- Ornamentation (rumination) present with straight embryo in *B. repens*; s- No rumination with straight embryo in *H. verticillata*; t- Seed coat absent in *B. repens*; u- Four ovules in *J. procumbens*; v- Dicot embryo with endosperm in *H. verticillata*; w- Dicot embryo with endosperm and ornamentation on seed coat in *J. procumbens*.