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Phylogeny of *Musa paradisiaca*, *Ravenala madagascariensis* and *Heliconia rostrata* based on Morphological, Biochemical, Amino Acid Sequences of rbcL Protein and matK DNA Sequences

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ABSTRACT: *Musa paradisiaca* L., *Ravenala madagascariensis* Sonn. and *Heliconia rostrata* Ruiz & Pav. are three morphologically similar genera retaining confusion regarding their systematic position and phylogenetic relationship. These genera had been placed by various taxonomists in different systems of classification under different families. Therefore, the present study was undertaken to infer the phylogenetic relationship between these genera and their respective families. To analyze the intergeneric and interfamilial phylogenetic relationship; the morphological and biochemical analyses were carried out and the data were elucidated for phylogenetic trees by using Mesquite 2.75 (http://mesquiteproject.org). However, the phylogenetic trees so obtained failed to show any close relationship. Other analyses were carried out by using MEGA 5.0 (www.megasoftware.net/) where amino acid sequence of rbcL protein and matK DNA sequences were retrieved from NCBI (www.ncbi.nlm.nih.gov/), aligned by clustalW software and had been analyzed for maximum parsimony, maximum likelihood and neighbour joining. The phylogenetic trees so obtained clearly showed that *M. paradisiaca, H. rostrata* and *R. madagascariensis* are three distinct genera, belonging to different families. This supports the Cronquist's modern system of classification and also justifies the placement of these three plants under three different families in APG III system of classification. Thus, *M. paradisiaca, R. madagascariensis* and *H. rostrata* must be recognized under three different families as they constitute a common clade known as Commelinids on the basis of morphological, biochemical and molecular evidences.

Key words: Ash analysis, Protein content, Angiospermic phylogeny, Commelinids

I. INTRODUCTION

Biological chemistry has been rapidly evolving and has wide applications and often used in studying taxonomy and phylogenetic relationship when coupled with molecular data. In general, species are recognized on the basis of morphological species, biological species, the phylogenetic species concept or a combination of all these (Watanabe *et al.*, 2011). But these criteria are not enough and thus, create a dispute over the proper systematic position of the species or genus. One such type of debate going on is the taxonomic position of the monocot genera and species viz. *Musa paradisiaca, Ravenala madagascariensis* and *Heliconia rostrata* and also the relationship among their respective families. *Musa paradisiaca, Ravenala madagasacariensis* and *Heliconia rostrata* bears striking morphological resemblances. Morphologically they are almost similar and thus create a dilemma for their placement among the families. Despite the data available, uncertainties remain as to placement of these plant species and also infer their phylogenetic relationships. However, different systematic botanists have placed these three genera under different families and orders. According to Bentham and Hooker's Natural system of classification (1883), the genus, *Musa* was included under the family Musaceae, while the genus, *Ravenala* and *Heliconia* were placed in Cannaceae/ Marantaceae (Unspecified) (Singh *et al.*, 2006). Engler and Prantl (1899) had treated *Musa* and *Heliconia* under a single family Musaceae and the genus, *Ravenala* under Marantaceae. In John Hutchinson's phylogenetic system of classification (1934), *Musa* and *Heliconia* were placed under Musaceae and *Ravenala* under Marantaceae respectively. While on the other hand, Takhtajan (1969-1980) included *Musa* under Musaceae while the genus *Ravenala* and *Heliconia* under Marantaceae. According to Cronquist's Modern system of classification (1919), *Musa* belongs to Musaceae, while *Ravenala* to Strelitziaceae and *Heliconia* in a new family Heliconiaceae. Hereby, it is evident that various taxonomists have placed these three genera in different groups. Although, in all systems of classification, *Musa* have been placed under Musaceae, yet there is a controversy regarding the systematic position and phylogenetic relationships among these three genera. Thus, the present study was undertaken with a view to solve this perplexity by using biochemical studies along with bioinformatics tools.

From the beginning of molecular systematic to the present day, the most popular phylogenetic markers utilized in plants are various regions in the chloroplast genome (Logacheva *et al.*, 2007). The sequence of the larger sub unit of the enzyme ribulose bisphosphate carboxylase gene (rbcL) was one of the first molecular markers (Duvall, 1993). Many works based on this have appeared till now and many conclusions of these authors were later confirmed (Noud *et al*., 2002). The set of chloroplast genes for phylogenetic analysis at high taxonomic level was subsequently supplemented with genes such as matK (Logacheva *et al.*, 2007). The matK gene codes for the enzyme maturase which is involved in group II intron RNA splicing process. Thus, in this study, the rbcL protein and matK gene sequences were analyzed to infer phylogenetic trees using three methods viz. Neighbour joining, Maximum likelihood and Maximum parsimony.

II. MATERIALS AND METHODS

A. Collection of samples and assigning character matrices

The plant specimens of *Musa paradisiaca*, *Ravenala madagascariensis*, *Heliconia rostrata* (RFRI/JRT/MP-01,RFRI/JRT/RM-02,RFRI/JRT/HR-03 respectively) were collected from Jorhat district, Assam, India and their morphology was studied. However, these specimens were also confirmed by taking the help of herbarium specimens of Herbarium of Assam Agriculture University, Assam. Morphology of *Commelina communis* were also analyzed as a representative of Commelinaceae, as it is the distantly related family of the entire three genera (Hayashi *et al*., 1956; Penny and Bowling, 1974). The qualitative and quantitative morphological characters were analyzed and character matrices of qualitative and quantitative morphological characters were prepared by assigning numeric value 1 for advanced character and 0 for primitive character as per literature review (Dowell, 2008).

B. Qualitative biochemical studies

To carry out the biochemical analysis, the fruiting body each of *Musa paradisiaca*, *Ravenala madagascariensis* and *Heliconia rostrata* were collected freshly, and the water extract was used for further analysis. The presence of glucose was detected using Fehling test and Benedict test. The presence of non reducing sugars was detected using the Fehling test and Benedict test for non reducing sugars. To detect protein, Xanthoproteic test and Buiret test was done. Starch was detected using KI. Amino acids were detected using Paper chromatography. To study the quality of the biochemicals, qualitative plant ash analysis was done (Akpabio *et al*., 2012; Behera and Raina, 2011; Indrayan *et al*., 2005; Mommin and Kadam, 2011) The method was adopted from AOAC **(**http:// www.aoac.org/, accessed on September, 2009).

C. Quantitative biochemical analysis

The plant specimens from all the three species were collected and the total fresh weight was taken. The samples were fresh dried in hot air oven at 60^{o}C and the total dry matter was calculated.

The relative water content was measured by using the following formula.

$$\theta = \frac{m_{\mathrm{wet}} - m_{\mathrm{dry}}}{\rho_w \cdot V_b}$$

Where :

m_{wet} and m_{dry} are the masses of the sample before and after drying in the oven; ρ_w is the density of water; and V_b is the volume of the sample before drying the sample.

Only 0.1g of dried and powdered sample in weight was used to estimate total nitrogen content by using Micro- Kjedahl's method (Rangana, 1986). Phosphorus was estimated by using the method given by King (1932). It was estimated from the ash solution by using the formula given by Ward and Johnson (1962). Calcium was determined by Flame Photometric method using Calcium chloride as standard (Rangana, 1986). Protein was estimated by Lowry's method (1951) using BSA as standard. Carbohydrate was determined calorimetrically by using the method given by Somogyi (1952). The total sugar content was estimated using the method given by Gupta, (2003). While the starch content was estimated using the method given by Ibrahim *et al.*, (2010); Vermani *et al.*, (2010).

D. Phylogenetic analyses

The phylogenetic analyses were done by using two softwares viz. Mesquite (Mesquite: a modular system for evolutionary analysis, version 2.75, http://mesquiteproject. org) and MEGA, version 5.00 (www.megasoftware.net,). These two softwares are widely used in evolutionary biology to solve phylogenetic disputes using various methods such as Maximum likelihood, Maximum parsimony and Neighbour joining method. For analysis using Mesquite the morphological and biochemical data were used. A character matrix was made scoring the characters as 0 and 1 where 0 represents the primitive state and 1 as the derived state.

Then, it was computed and the results were used for further analysis under MEGA (Molecular evolutionary genetics analysis). Phylogenetic relationships using MEGA 5.0 were inferred by using rbcL protein sequences and matK gene sequences of 4 species viz. *Commulina communis*, *Musa paradisiaca*, *Ravenala madagascariensis* and *Heliconia rostrata*. The amino acid sequences were obtained from the NCBI site (http://www. ncbi. nlm. nih. gov/entrez/query. fcgi?db=protein) and alignment was build. Likewise, nucleotide sequences of matK gene were also obtained from the website (http://www. ncbi. nlm. nih. gov/entrez/query. fcgi?db=nucleotide) and a new alignment were build. Both the amino acid sequences and nucleotide sequences were aligned using ClustalW and then the phylogenetic relationships were computed using Maximum parsimony, Maximum likelihood and neighbor joining method. The result obtained were then compared and analyzed.

III. RESULTS AND DISCUSSION

A. Morphological characters observed for analysis

Musa paradisiaca is a perennial gigantic herb, roots are adventitious, stem is an underground rhizome, leaves are large, entire, glabrous, apex obtuse with a distinct midrib and parallel venation, petiolated, with a long and thick petiole, exstipulated ; the inflorescence is a terminal spike covered by red bracts, flowers are sessile, monoecious, unisexual, zygomorphic, epigynous; perianth consist of six tepals, arranged in two whorls of three each, three outer and two inner anterior perianth leaves unite to form a tube like structure, inner perianth leaf is free, petaloid; six stamens present, one of them reduced into staminode, anthers bicelled and basifixed; gynoecium is tricarpellary, syncarpous, ovary inferior, trilocular, axile placentation, style simple, filiform, stigma with three branched lobes.

Ravenala madagascariensis is a medium sized herb, roots rhizomatous, stem is hard and woody, scarred; leaves alternate, distichously arranged, simple, petiolated, stout, blade oblong, base cup shaped, apex rounded, glabrous; inflorescence are an axillary thyrse, bearing circinnate flowers, clusters enclosed in a distichously arranged, large, stiff boat shaped bracts; flowers are bisexual, zygomorphic, trimerous, subtended by bracteoles; sepals free, lanceolate, petals free and lanceolate; ovary inferior, trilocular, stle long, stigma with finger like protuberances.

Heliconia rostrata is a shrub, roots adventitious, stem is underground rhizome; leaves are oblong, entire, glabrous, parallel venation; the peduncle arises in the axils of leaves and bears stiff boat like bracts; perianth consist of six tepals, arranged in two whorls, the outer posterior telas is large and free, the remaining tepals are free and fused to form cymbiform structure, flowers are produced on long drooping panicles and consist of brightly coloured waxy bracts; stamens are six in number while the sixth one is staminode; gynoecium is tricarpellary, syncarpous, inferior, trilocular, stigma capitate. Tables 1 and 2 show the comparative morphological qualitative and quantitative characters among the three species.

Table 1. Qualitative morphological characters.

Sl no.	Characters	*Commelina communis*	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostrata.*
1	Habit	Herb	Herb	Herb	Shrub
2	Growth habit	Erect	Erect	Erect	Erect
3	Stem type	Knotted stem	Pseaudostem	Woody	Scape
4	Branch angles	Acute	Acute	Acute	Acute
5	Leaf shape	Lanceolate	Ovate	Ovate	Ovate
6	Leaf blade	Curved	Curved	Curved	Curved
7	Leaf apex	Sharp	Blunt	Blunt	Blunt
8	Leaf apex habit when exposed to sunlight	Downturned	Downturned	Downturned	Downturned
9	Leaf base shape	Obtuse	Obtuse	Obtuse	Obtuse
10	Leaf base type	Blunt	Blunt	Blunt	Blunt
11	Leaf margin	Entire	Entire	Entire	Entire
12	Leaf angle and pose	Drooping	Drooping	Drooping	Drooping
13	Leaf surface view	Glossy	Glossy	Glossy	Glossy
14	Leaf venation	Parallel	Parallel	Parallel	Parallel
15	Shoot density	Dense	Intermediate	Sparse	Dense
16	Stem colour	Green	Brown	Brown	
17	Petiolar canal	Margin enclosed	Margin enclosed	Margin enclosed	Margin enclosed

18	Peduncle	Glabrous	Glabrous	Glabrous	Glabrous
19	Pedicel	Short	Long	Long	Long
20	Immature leaf color	Green	Green	Green	Green
21	Mature leaf	Green	Green	Greenish orange	Dark
22	Petiole colour	Light green	Light green	Light green	Light green
23	Bract shape	Ovate and pointed	Ovate and broad	Ovate and pointed	Ovate
24	Bract curling	Slightly	Slightly	Absent	-
25	Bract apex	Pointed	Rounded	Rounded	Pointed
26	Bract color	Green	Brownish red	Green	Reddish pink
27	Bract colour fading	Light green	Light green	Yellow	Light
28	Bract scars	Absent	present	Present	Present
29	Free tepal of flowers	Corrugated	Corrugated	Corrugated	Corrugated
30	Flower colour	Blue	yellow	Yellow	Yellow
31	Colour of ovary	Yellow	Yellow	Yellow	Yellow
32	Colour of anther	Yellowish	Yellowish	Brownish	Brownish
33	Gynoecium	Tricarpellary	Tricarpellary	Tricarpellary	Tricarpellary

Table 2. Quantitative morphological characters.

Sl no.	Characters	*Commelina communis*	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostrata*
1	Length of leaf	7-10 cm	50-250 cm	120-150 cm	50-100 cm
2	Length of mature leaf petiole	1-2 cm	10-30 cm	20-30 cm	5-10 cm
3	Bract length	1-2 cm	30-45 cm	50-70 cm	10-15 cm
4	Bract width	2-3 cm	5-10 cm	10-30 cm	3-5 cm
5	Length of flower	1 cm	10-15 cm	15-20 cm	5-10 cm
6	Width of flowers	1.5 cm	1.7 cm	5-10 cm	5-7 cm
7	Length of stamen	.25 cm	1-2 cm	15 cm	5-6 cm
8	Length of anthers	2 mm	2mm	10 cm	3-4 cm
9	Length of style and stigma	1 cm	6.4 cm	10 cm	7 cm
10	Length of ovary	.25 cm	6-9 cm	15 cm	5-6 cm

The following tables 3 and 4 show the character matrices of qualitative and quantitative morphological characters.

Table 3. Character matrix of qualitative morphological characters.

Sl no.	Characters	*Commelina communis*	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostrata*
1	Habit	0	1	1	0
2	Growth habit	0	0	0	0
3	Stem type	0	0	1	0
4	Branch angles	0	0	0	0
5	Leaf shape	0	0	0	0
6	Leaf blade	0	0	0	0
7	Leaf apex	0	1	1	1
8	Leaf apex habit (when exposed to sunlight)	0	0	0	0
9	Leaf base shape	0	0	0	0
10	Leaf base type	0	0	0	0
11	Leaf margin	0	0	0	0
12	Leaf angle and pose	0	0	0	0
13	Leaf surface view	0	0	0	0
14	Leaf venation	0	0	0	0
15	Shoot density	0	1	1	0
16	Stem colour	0	1	1	0
17	Petiolar canal	0	0	0	0

18	Peduncle	0	0	0	0
19	Pedicel	0	1	1	1
20	Immature leaf color	0	0	0	0
21	Mature leaf	0	0	1	1
22	Petiole colour	0	0	0	0
23	Bract shape	0	1	1	0
24	Bract curling	0	0	1	1
25	Bract apex	0	1	1	0
26	Bract color	0	0	1	1
27	Bract colour fading	0	1	1	1
28	Bract scars	0	1	1	1
29	Free tepal of flowers	0	0	0	0
30	Flower colour	0	1	1	1
31	Colour of ovary	0	0	0	0
32	Colour of anther	0	0	1	1
33	Gynoecium	0	0	0	0

Table 4. Character matrix of quantitative morphological characters.

Sl no.	Characters	*Commelina communis*	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostratra*
1	Length of leaf	0	1	1	1
2	Length of mature leaf petiole	0	1	1	1
3	Bract length	0	1	1	1
4	Bract width	0	1	1	0
5	Length of flower	0	1	1	1
6	Width of flowers	0	1	1	1
7	Length of stamen	0	0	1	1
8	Length of anthers	0	0	1	1
9	Length of style and stigma	0	1	1	1
10	Length of ovary	0	1	1	1

B. Biochemical parameters observed for analysis

From Table 5, the qualitative ash analysis, it was found that the chemicals such as calcium, magnesium, iron, phosphorus, chlorine, sulphur were present. Likewise, the qualitative biochemical analysis also showed the presence of nitrogen, protein, carbohydrate, sugar and starch. Table 2 shows the comparative value of the various biochemical analysis among the three plant species.

From Table 6, it is evident that, the relative water content is highest in *Ravenala madagascariensis*, followed by *Musa paradisiaca* and then *Heliconia rostrata.* However, comparatively, the overall water content is much higher than any other genera. The dry matter content was found to be highest in *Ravenala madagascariensis*, then *Musa paradisiaca* and the least content was found in *Heliconia rostrata.* The nitrogen content was almost equal between *Musa paradisiaca* and *Ravenala madagascariensis* but *Heliconia rostrata* had less content. Likewise, phosphorus content was found to be even between *Ravenala madagascariensis* and *Heliconia rostrata*, but, *Musa paradisiaca* showed comparatively less phosphorus content. The calcium, magnesium, protein, carbohydrate, sugar and starch content were almost equal in all the three plant species.

Table 5. Character matrix of qualitative biochemical analysis.

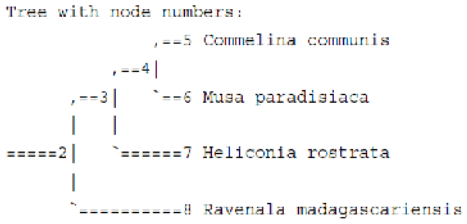
Sl no.	Metals	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostratra*
1	Calcium	✓	✓	✓
2	Magnessium	✓	✓	✓
3	Iron	✓	✓	✓
4	Phosphorus	✓	✓	✓
5	Chlorine	✓	✓	✓
6	Sulphur	✓	✓	✓

Table 6. Quantitative biochemical analysis.

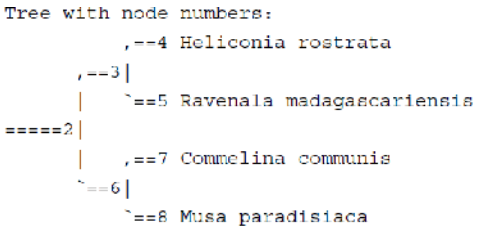
Sl no.	Biochemical aspect	*Musa paradisiaca*	*Ravenala madagascariensis*	*Heliconia rostrata*
1	Relative water content	85.1	88	78.86
2	Dry matter	6.4	7.28	5.02
3	Nitrogen content	2.38	2.49	1.57
4	Phosphorus content	0.24	0.35	0.32
5	Potassium content	5.67	6.06	5.56
6	Calcium content	650.17	632.49	624.35
7	Magnessium content	182.47	180.45	179.84
8	Protein content	1.63	1.65	1.59
9	Carbohydrate content	36.77	35.79	34.65
10	Sugar content	4.1	5.05	5.02
11	Starch content	12.03	11.48	11.49

C. Phylogentic trees obtained after analyses

First of all, phylogenetic analysis was done using mesquite 2.75. For this, based on the above characters, a character matrix was made where 0 is designated as primitive state and 1 as derived state. Table V shows the character matrix prepared after morphological analysis. Table VI shows the character matrix using data obtained from biochemical analysis. When the maximum parsimony tree was computed using only morphological data, 10 different trees were obtained. The trees so obtained in Newick format are as follows: (((*Commelina communis*, *Musa paradisiaca*), *Heliconia rostrata*), *Ravenala madagascariensis*); ((*Heliconia rostrata*, *Ravenala madagascariensis*), (*Commelina communis*, *Musa paradisiaca*)); (*Heliconia rostrata*, ((*Commelina communis*, *Musa paradisiaca*), *Ravenala madagascariensis*)); ((*Musa paradisiaca*, (*Commelina communis*, *Heliconia rostrata*)), *Ravenala madagascariensis*); (((*Commelina communis*, *Heliconia rostrata*), *Ravenala madagascariensis*), *Musa paradisiaca*); ((*Commelina communis*, *Heliconia rostrata*), (*Musa paradisiaca*, *Ravenala madagascariensis*)); ((*Commelina communis*, (*Ravenala madagascariensis*, *Musa paradisiaca*)), *Heliconia rostrata*); ((*Commelina communis*, (*Heliconia rostrata*, *Ravenala madagascariensis*)), *Musa paradisiaca*); *(Commelina communis*, ((*Heliconia rostrata*, *Ravenala madagascariensis*), *Musa paradisiaca*)); ((*Heliconia rostrata*, (*Musa paradisiaca*, *Ravenala madagascariensis*)) ,*Commelina* communis); The following Figure 1 shows the trees obtained after morphological analysis.



Tree 1



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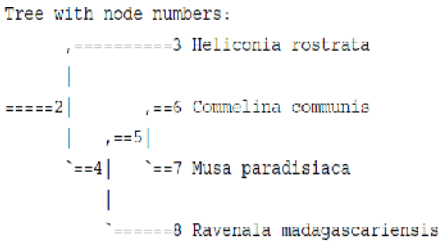

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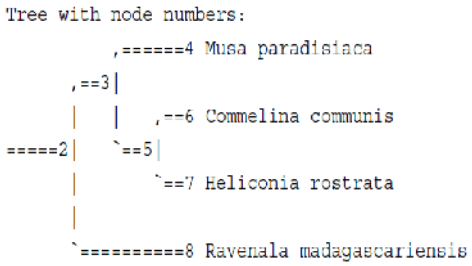

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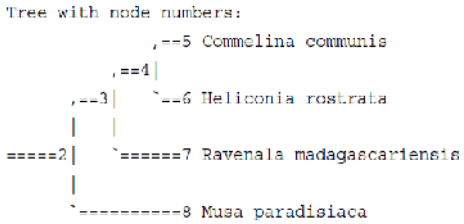

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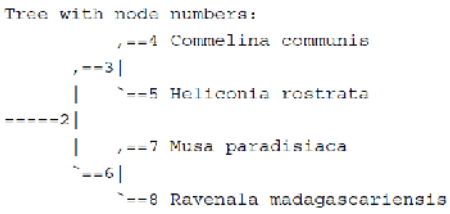

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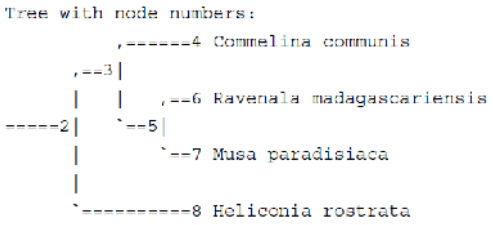
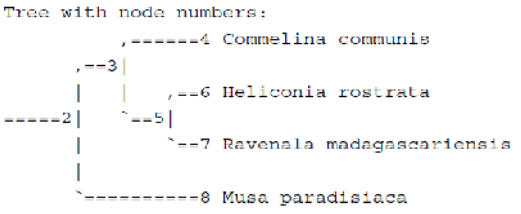
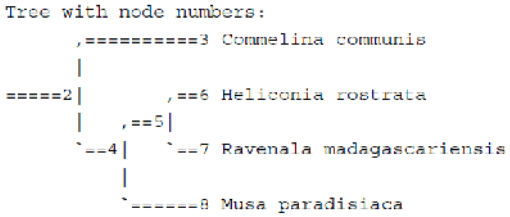


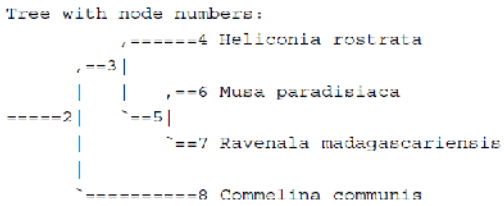
Tree 7



Tree 8



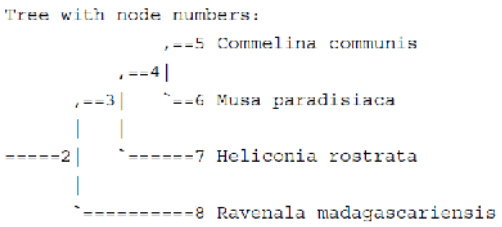
Tree 9



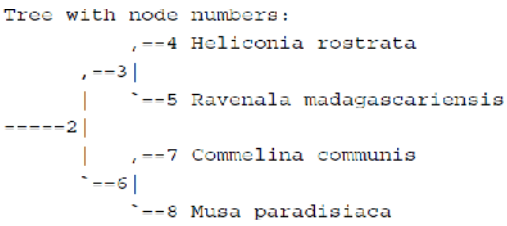
Tree 10

Fig. 1. Tree 1: (((*Commelina communis*, *Musa paradisiaca*), *Heliconia rostrata*), *Ravenala madagascariensis*);
Tree 2: ((*Heliconia rostrata*, *Ravenala madagascariensis*), (*Commelina communis*, *Musa paradisiaca*));
Tree 3: (*Heliconia rostrata*, ((*Commelina communis*, *Musa paradisiaca*), *Ravenala madagascariensis*));
Tree 4: ((*Musa paradisiaca*, (*Commelina communis*, *Heliconia rostrata*)), *Ravenala madagascariensis*);
Tree 5: (((*Commelina communis*, *Heliconia rostrata*), *Ravenala madagascariensis*), *Musa paradisiaca*);
Tree 6: ((*Commelina communis*, *Heliconia rostrata*), (*Musa paradisiaca*, *Ravenala madagascariensis*));
Tree 7: ((*Commelina communis*, (*Ravenala madagascariensis*, *Musa paradisiaca*)), *Heliconia rostrata*);
Tree 8: ((*Commelina communis*, (*Heliconia rostrata*, *Ravenala madagascariensis*)), *Musa paradisiaca*);
Tree 9: (*Commelina communis*, ((*Heliconia rostrata*, *Ravenala madagascariensis*), *Musa paradisiaca*));
Tree 10: ((*Heliconia rostrata*, (*Musa paradisiaca*, *Ravenala madagascariensis*)) ,*Commelina* communis);

The trees obtained after maximum parsimony analysis using both morphological and biochemical data in Newick format are as follows: (((*Commelina communis*, *Heliconia rostrata*), *Musa paradisiaca*), *Ravenala madagascariensis*); ((*Musa paradisiaca*, *Ravenala madagascariensis)*, *(Commelina communis*, *Heliconia rostrata*)); (*Musa paradisiaca*, ((*Commelina communis*, *Heliconia rostrata*), *Ravenala madagascariensis*)); ((*Commelina communis*, (*Musa paradisiaca*, *Ravenala madagascariensis*)), *Heliconia rostrata*); *(Commelina communis*, ((*Musa paradisiaca*, *Ravenala madagascariensis*), *Heliconia rostrata*)); *(Commelina communis*, (*Heliconia rostrata*, (*Musa paradisiaca*, *Ravenala madagascariensis*))). The figure 2 shows the trees obtained using data of morphological as well as biochemical analysis.



Tree 1



Tree 2

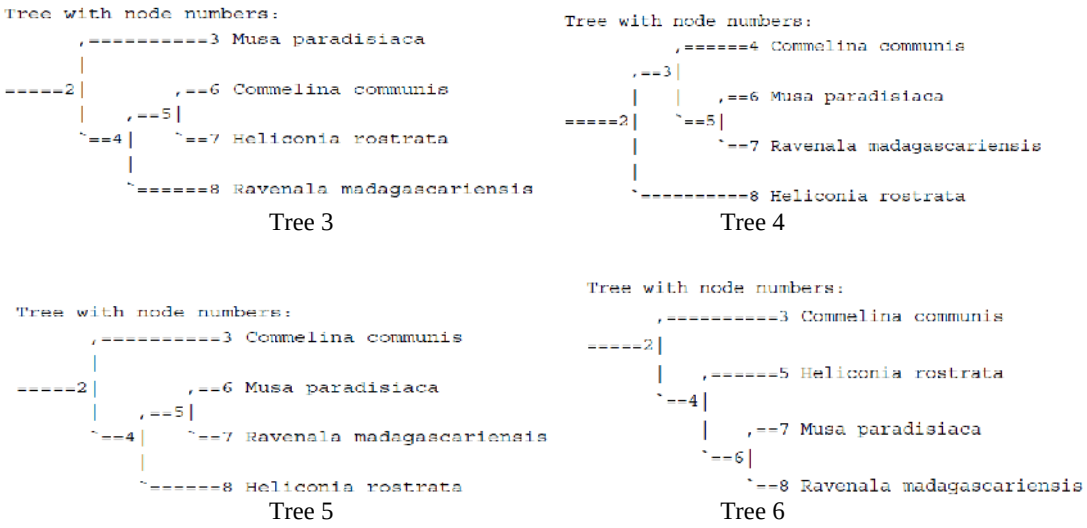


Fig. 2. Tree 1: (((*Commelina communis*, *Heliconia rostrata*), *Musa paradisiaca*), *Ravenala madagascariensis*);
Tree 2: ((*Musa paradisiaca*, *Ravenala madagascariensis*), (*Commelina communis*, *Heliconia rostrata*));
Tree 3: (*Musa paradisiaca*, ((*Commelina communis*, *Heliconia rostrata*), *Ravenala madagascariensis*));
Tree 4: ((*Commelina communis*, (*Musa paradisiaca*, *Ravenala madagascariensis*)), *Heliconia rostrata*);
Tree 5: (*Commelina communis*, ((*Musa paradisiaca*, *Ravenala madagascariensis*), *Heliconia rostrata*));
Tree 6: (*Commelina communis*, (*Heliconia rostrata*, (*Musa paradisiaca*, *Ravenala madagascariensis*))).

When molecular data were used for phylogenetic analysis, using neighbor joining, maximum likelihood and maximum parsimony method based on rbcL protein coding amino acid sequences and matK gene coding nucleotide sequences, all the results obtained showed the same tree. The tree obtained in Newick format are as follows: (*Commelina communis* (*Musa paradisiaca*, (*Heliconia rostrata*, *Ravenala madagascariensis*))). The following figure 3 shows the trees obtained based on molecular data.

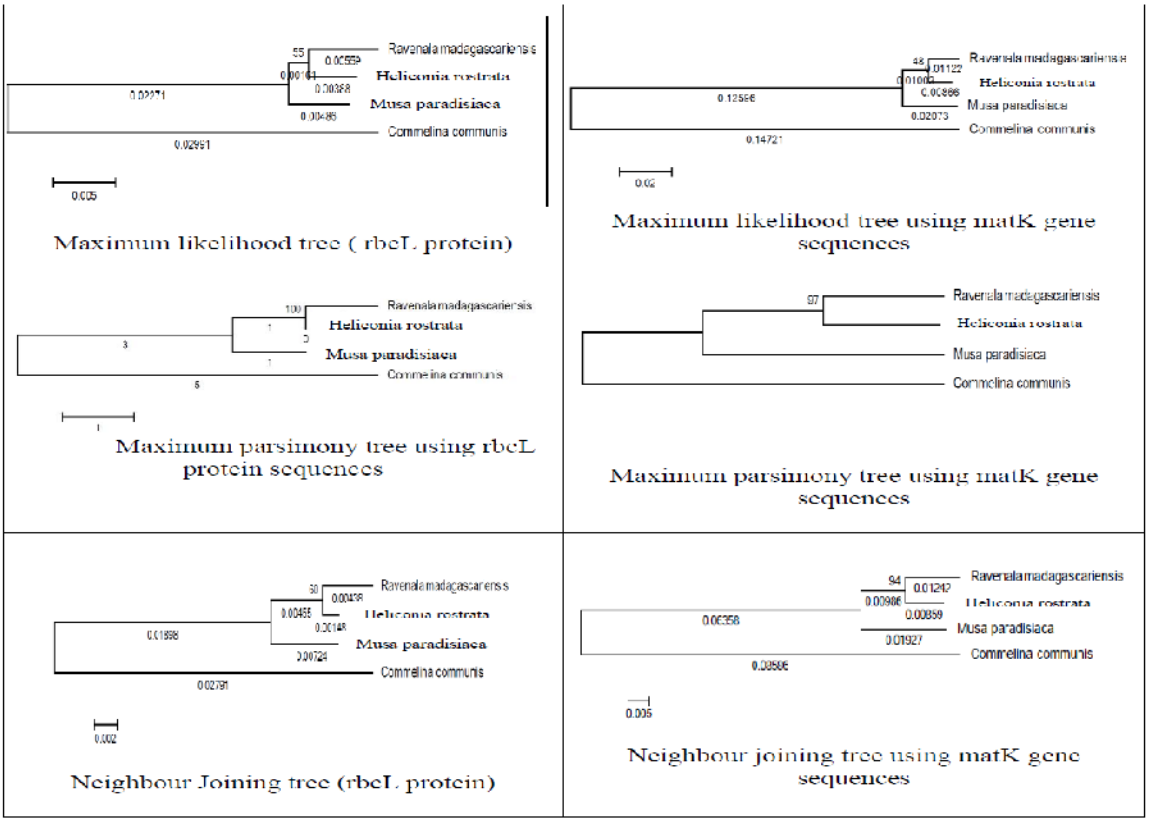


Fig. 3. Phylogenetic trees obtained by using MEGA 5.0.

The maximum parsimony trees showed two sister genera, viz. *Musa* and *Ravenala*; *Heliconia* and *Commelina*. However, the 65 characters used in the analysis though revealed that all the three genera are monophyletic and belongs to a single clade , yet it couldn't specify the phylogenetic relationships of these genera as well as the direction and rate of evolution among them. The phylogenetic relationships were deduced when rbcL protein coding amino acid sequences beside the matK gene coding nucleotide sequences in MEGA 5.0.were used for analysis. The neighbor joining tree, maximum parsimony tree and maximum likelihood tree, all depicted the same results. The trees showed that the genera forms a monophyletic group. It confirmed that *Ravenala* and *Heliconia* are two sister genera as they were supported by 100 % bootstrapping which is a good support. Moreover, as the three genera forms three separate branches, it can be confirmed that they belong to three different families (Futuyma, 2009). Based on the nodes formed, it can be said that the three genera share a common ancestry. Regarding the direction of evolution, based on the branch lengths, it can be said that from *Commelina*, the first genera to be evolved is *Musa* followed by the next two genera (Chase, 2004). The branch lengths too support the view that the rate of evolution of *Ravenala* and *Heliconia* is faster as compared to that of Musa. From the trees obtained, it can also be said that *Ravenala* and *Heliconia* are closely related to each other and both are distantly related to *Musa* (The Angisoperm Phylogeny Group-II, 2003).

CONCLUSION

From this study, it can be concluded that, the three genera are monophyletic and share a common ancestry. The placement of these genera in the commelinid clade is justified. The three genera forms three separate branches which supports its placement under three different families in Cronquist's Modern system of classification and shows the anomalies in their placement in natural system of classification and phylogenetic system of classification. Of the three genera, *Ravenala* and *Heliconia* are sister genera and these two have evolved later than *Musa* but at a faster rate of evolution which is depicted by the short branch lengths. Hence, they are highly evolving. Thus, *M. paradisiaca*, *R. madagascariensis* and *H. rostrata* must be recognized under three different families as they constitute a common clade known as commelinids on the basis of morphological, biochemical and molecular evidences.

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