

## Evidence of a new *Musa* species - *M. swarnaphalya* in India and its confirmation through morpho-molecular characterization

S. Uma\*, M.S. Saraswathi and P. Durai

National Research Centre for Banana, Thogamalai Road, Thayanur Post,  
Tiruchirapalli 620 102, Tamil Nadu

### ABSTRACT

A new species, *Musa swarnaphalya*, Uma, Saraswathi and Durai, has been identified from Sessa village of Balukpong district in southern Arunachal Pradesh. It is characterised by its unique greenish-yellow colour male bud. Diploid status of this species was proven using flow cytometry and its identity as a new species was assessed through morpho-taxonomy and confirmed through RAPD and IRAP markers. *M. swarnaphalya* grouped with *M. itinerans* and *M. nagensium* in a distinct cluster confirming its stand within the section *Eumusa*. Freedom from pests and diseases in its place of distribution suggests its possible utility as a resistant gene source in future breeding programmes.

**Key words:** Banana, *Musa swarnaphalya*, new species.

### INTRODUCTION

*Musa* belonging to the family Musaceae encompasses a broad spectrum of variability. Based on several morphogenetic traits, the Musaceae members are classified into three genera, *Musa*, *Ensete* and *Musella* (Hakkinen and Sharock, 5). *Musa* connotes a large number of ornamental and commercial bananas under five sections *Eumusa* (n = 11), *Rhodochlamys* (n = 11), *Callimusa* (n = 10), *Australimusa* (n = 10) and *Incertae sedis* (n = 7, 9). South and South East Asia are considered as the major centres of banana origin apart from Papua New Guinea and Africa. India is one of the hot spots for *Musa* origin and diversification mainly for two sections *Eumusa* and *Rhodochlamys*. Earlier survey and reports mention the occurrence of several *Eumusa* species like *M. itinerans*, *M. nagensium*, *M. cheesmani*, *M. sikkimensis*, *M. ochracea*, *M. siamea*, *M. flaviflora* etc. (Simmonds and Shepherd, 12; Simmonds, 11) apart from *M. acuminata* and *M. balbisiana*, the major progenitors of cultivated bananas. A number of *Rhodochlamys* members like *M. ornata*, *M. rosacea*, *M. rubra*, *M. aurantiaca*, *M. velutina* etc., are also reported (Sundararaj and Balasubramanyam, 13; Uma *et al.*, 15). The explorations were conducted by NRCB in the last two decades to fill the information void prevailed from 1960's to 1990's which revealed disappearance of more than four *Musa* species from the regions reported earlier by Simmonds (11). Simultaneously, three new species were identified of which this paper describes *M. swarnaphalya* collected from Sessa village in West Kameng district of Arunachal Pradesh. The name *M.*

*swarnaphalya* has been derived from Sanskrit words, *swarna* meaning golden yellow and *phalya* meaning bud.

### MATERIALS AND METHODS

The explorations were conducted during 2001-2004 covering Kilelong, Chakoo, Rupa of West Kameng District, Seppa of East Kameng, Itanagar, Potin, Sekhe and Ziro of Lower Subansiri, Daporijo, Nioku of Upper Subansiri, Along and Basar of West Siang, Dosing, Yemsing and Pasighat of East Siang, Roing of Lower Dibang Valley and Tezu of Lohit districts (Fig. 1). During these explorations, three new species were identified of which *M. swarnaphalya* is discussed in this paper. The new species was identified as a population endemic to Sessa village near Tippy of Balukpong town. This was characterized *in-situ* for 73 traits using *Musa* descriptor (IPGRI-INIBAP/CIRAD, 6). Since, this species had unique yellowish-green male bud it was compared with other *Eumusa* members having yellow and green yellow male bud using the pictorial dictionary of *Musalogue* (Arnaud and Horry, 1; Daniells *et al.*, 3), pictorial dictionary of *Musa* species and *MusaDoc* from 1999 to 2006.

For molecular characterization, the protocol described by Williams *et al.* (17) was followed for RAPD analysis. Thirty three random primers (Operon Technologies Inc. USA) were used for the study of which nine were considered for the genetic analysis. Likewise, Inter Retro-transposon Amplified Polymorphic (IRAP) markers (Kalendar *et al.*, 7), which are suitable for Polymerase Chain Reaction (PCR) amplification of DNA lying between retro-elements were exploited in the present study. Three primer combinations namely

\*\*Corresponding author's E-mail: umabinit@yahoo.co.in.

Sukkula + LTR 6149, Nikita + LTR 6150 and Sukkula + LTR 6150 were tested for DNA amplification as per the protocol of Teo *et al.* (14).

The amplified products from both IRAP and RAPD were subjected to electrophoresis in 2 and 1.5% agarose gels respectively using 1x TAE buffer. The gels were stained with ethidium bromide. Analysis of the similarity matrix within the NTSYS program (Rohlf, 8) using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) was used to produce the dendrogram and to determine the values of genetic distance.

The protocol of Dolezel (4) was adopted with nuclei of chicken red blood cells (CRBC) as internal reference standard. The fluorescence detection was carried out using the flow cytometer whereby relative fluorescence intensities were translated into histograms corresponding to the relative DNA content, an indicative of ploidy status.

## RESULTS AND DISCUSSION

Arunachal Pradesh, the largest state of seven sister states of the North-Eastern India and located in the eastern Himalayan ranges, is situated between latitude of 26°-30' to 29°-28'-West and longitude of 91°-35' to 97°-24'-East. The new species was identified near the village Sessa between a latitude of 27°-48' 36"-North and a longitude of 92°-26' 38"-East, near Tippy on the way to Bomdila from Balukpong in the state of Arunachal Pradesh. *M. swarnaphalya* has a narrow distribution in the moist deciduous forest of this particular area located at an elevation of 1,000 m above MSL. Location has a mean maximum temperature of 32°C and minimum temperature of 8°C with an annual rainfall of 900-1,200 mm distributed from May to October. *M. swarnaphalya* grows in ally with *M. nagensium*,

*M. itinerans*, *M. sikkimensis* and the members of the section Rhodochlamys (*M. rosaceae* and *M. aurantiaca*) in its vicinity. The morphological uniqueness of *Musa swarnaphalya* is discussed in view of the other related species prevailing in Arunachal Pradesh.

Availability of wide *Musa* diversity in North Eastern India, Western Ghats and southern India was revealed by earlier collection missions and literatures reviewed by Uma *et al.* (15). They made valuable collections and assembled them for studies at Botanical Garden, Kolkata made earliest taxonomic reference. This mainly included the seeded types collected from Chittagong forest of Eastern India and Madras provinces in the south. Schumann (10) mentioned 42 species in his monograph including *Eumusa*, *Rhodochlamys* and *Physocaulis* (now referred as *Ensete*). Wild non-stoloniferous banana from Assam, belonging to *Physocaulis* and two other ornamental bananas with a description of bright coloured bracts and erect bunches, which later were identified as *M. velutina*, and *M. ornata*, respectively. Simmonds and Shepherd (12) reported the occurrence of *M. cheesmani*, *M. flaviflora* in Assam, *M. sikkimensis* in Manipur, Assam, and Sikkim. Reports were also made on inter- and intra-sectional hybrids which have been collected and described (Uma *et al.*, 15).

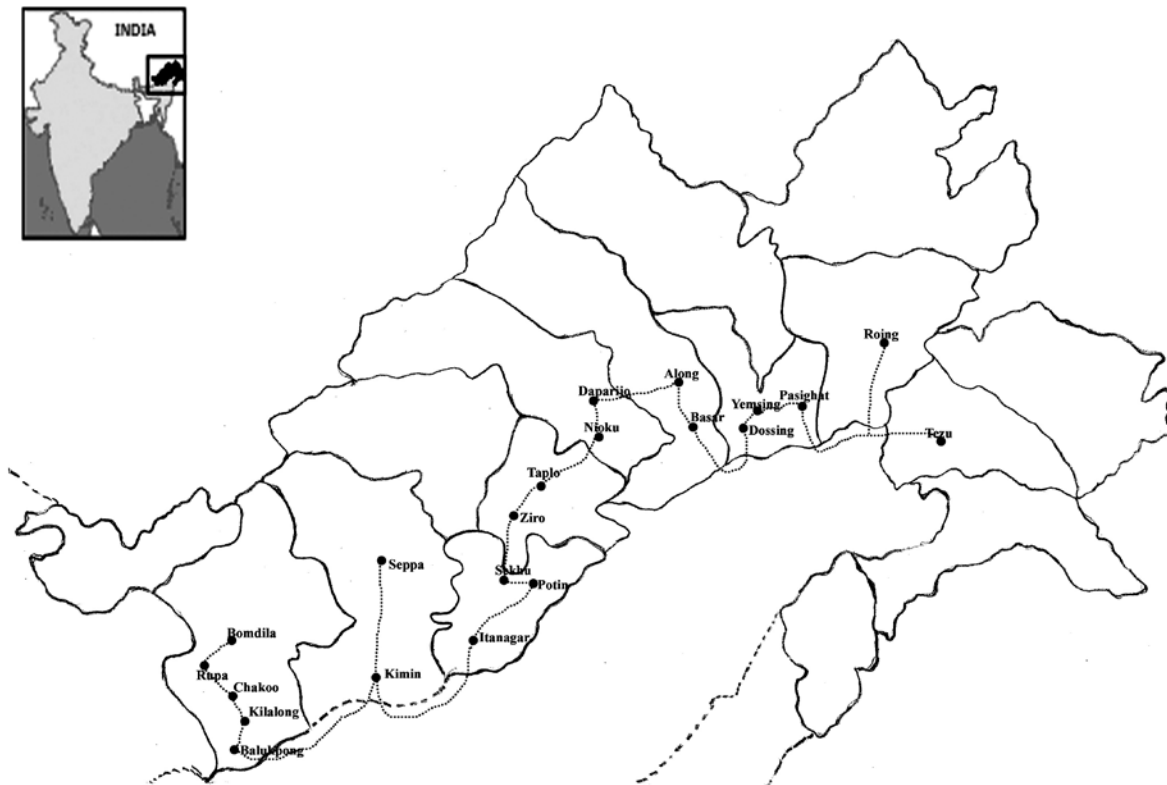
The *M. swarnaphalya* is characterized by robust stature of 8.0 to 8.5 m in height with maroon coloured Pseudostem (Fig. 2a). Leaves are dark green, 150-170 cm long having round laminar bases. Intensely wax coated petioles (50-60 cm) are tightly clasped on the pseudostem. Petiolar canal is closed and the margins are erect, sometimes overlapped. Peduncle is short, 15-20 cm long, glabrous, green, slightly angular in position. Male axis is pendulous, barren and bract scars are not predominant.

**Table 1.** Comparative evaluation of traits of *Eumusa* members with greenish-yellow male bud.

Character	<i>M. acuminata</i> ssp. <i>banksii</i>	<i>M. acuminata</i> ssp. <i>errans</i>	<i>M. basjoo</i>	<i>M. swarnaphalya</i>
Plant height (m)	3-4	4-5	2-2.5	8-9
Pseudostem colour	Green	Green	Green	Maroon
Rhizome	Corm	Corm	Elongated	Corm
Bract imbrication	Convolute	Convolute	Convolute	Imbricate
Bract lifting	1-2	1-2	2-4	2-4
Basal flowers	Hermaphrodite	Hermaphrodite	Female	Female
Pedicle length	Short	Short	Sessile	Long
No. of fruits / hand	15-20	18-20	12-14	6-8
Distribution	Papua New Guinea	Philippines	Philippines	India

**Table 2.** Taxonomic key for the identification of *Musa* species and subspecies with green and green-yellow coloured male buds.

A. Green coloured pseudostem with the range of 3 – 7 m height	<i>M. ac. ssp. banksii</i> , <i>M. ac. ssp. errans</i> , <i>M. schizocarpa</i> and <i>M. basjoo</i>
1. Basal hermaphrodite flowers	<i>M. ac. ssp. banksii</i> <i>M. ac. ssp. errans</i> <i>M. schizocarpa</i>
i). Indehiscent fruit	<i>M. ac. ssp. banksii</i> <i>M. ac. ssp. errans</i>
a) No. of hands per bunch ranging from 8-10	<i>M. ac. ssp. banksii</i>
c) No. of hands per bunch ranging from 15-20	<i>M. ac. ssp. errans</i>
ii). Dehiscent fruit	<i>M. schizocarpa</i>
2. Basal female flowers	<i>M. basjoo</i>
B. Maroon coloured long pseudostem with more than 8 m height	<i>M. swarnaphalya</i>



**Fig. 1.** Exploration route in Arunachal Pradesh and specific distribution of *M. swarnaphalya*.



Fig. 2a. Natural habitat of *M. swarnaphalya*.



Fig. 2b. *M. swarnaphalya* in shooting.

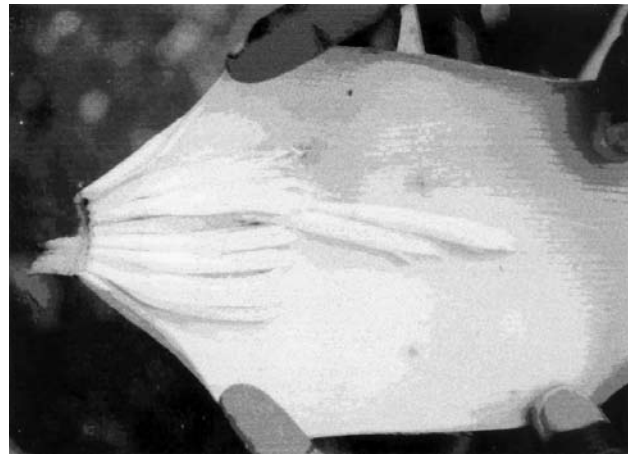


Fig. 2c. Male flowers and bract inner surface.

Male bud is lanceolate in shape, greenish yellow in colour (Fig. 2b) and heavily wax coated, deeply imbricate, outer face of the bract is greenish yellow and inner face is pale orange yellow in colour. Bract tip is blunt as in case of *M. balbisiana* and tinted with purplish brown on its outer side. Bracts open 1-2 at a time and do not revolute. Male flowers are yellow, 3.5-4.0 cm long, compound tepal and lobes are yellow (Fig. 2c). Free tepal is rectangular in shape, 1.6-1.8 cm long with smooth margins; its apex is also smooth and pointed.

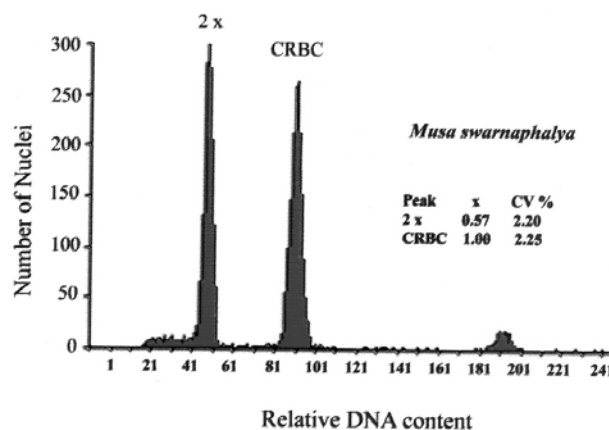


Fig 2d. Nuclear DNA content of *M. swarnaphalya*.

Ovary is straight 1.2-1.3 cm long, yellow in colour, style is straight and translucent white and stigma is orange yellow. Fruits are ashy green (before maturity), biseriate, 3.0-3.5 cm long, pedicel is unusually long with 1.2-1.5 cm and 6-8 fruits per hand. Pollen is fertile and germinability is more than 80 per cent.

Comparison of morphological data with Musalogue and MusaDoc led to identification of accessions with yellow and green-yellow male bud. Of these seven were screened out, since they did

not belong to the section Eumusa (they were Fei hybrids of *M. acuminata* × *M. textilis* of the section Australimusa). This resulted in only 31 accessions of which, 14 either belonged to plantain or cooking group of AA diploid and 17 accessions were various clones of *Musa acuminata* ssp. *banksii*. Although, *M. schizocarpa* and *M. schizocarpa* × *acuminata* hybrids also exhibit green-yellow male bud, they were not considered since *M. schizocarpa* is clearly distinguishable due to dehiscent nature of the fruits. Hence, *M. swarnaphalya* was compared with three nearest species *M. acuminata* ssp. *banksii*, *M. acuminata* ssp. *errans* and *M. basjoo* for their morphological features and the details are provided in Table 1 and Fig. 3.

Height of this new species is 8-9 m which distinctly varies from other species, likewise, the colour of the pseudostem also differs by having maroon colour. Male bud shows deep imbrications; while in other species, it is convolute in nature. Basal flowers of *M. acuminata* ssp. *banksii* and *M. acuminata* ssp. *errans* are hermaphrodite, while *M. basjoo* and *M. swarnaphalya* have female flowers. Pedicel is unusually long (2 cm) while in others either it is short or sessile. Number of fruits per hand is drastically low (6-8) but *Musa acuminata* ssp. *errans* has more than 20 fruits per hand. This being a quantitative trait, not expected to be influenced by agroclimatic and edaphic factors.

Of the three known species with green yellow male bud, *M. swarnaphalya* is very distinct with respect to

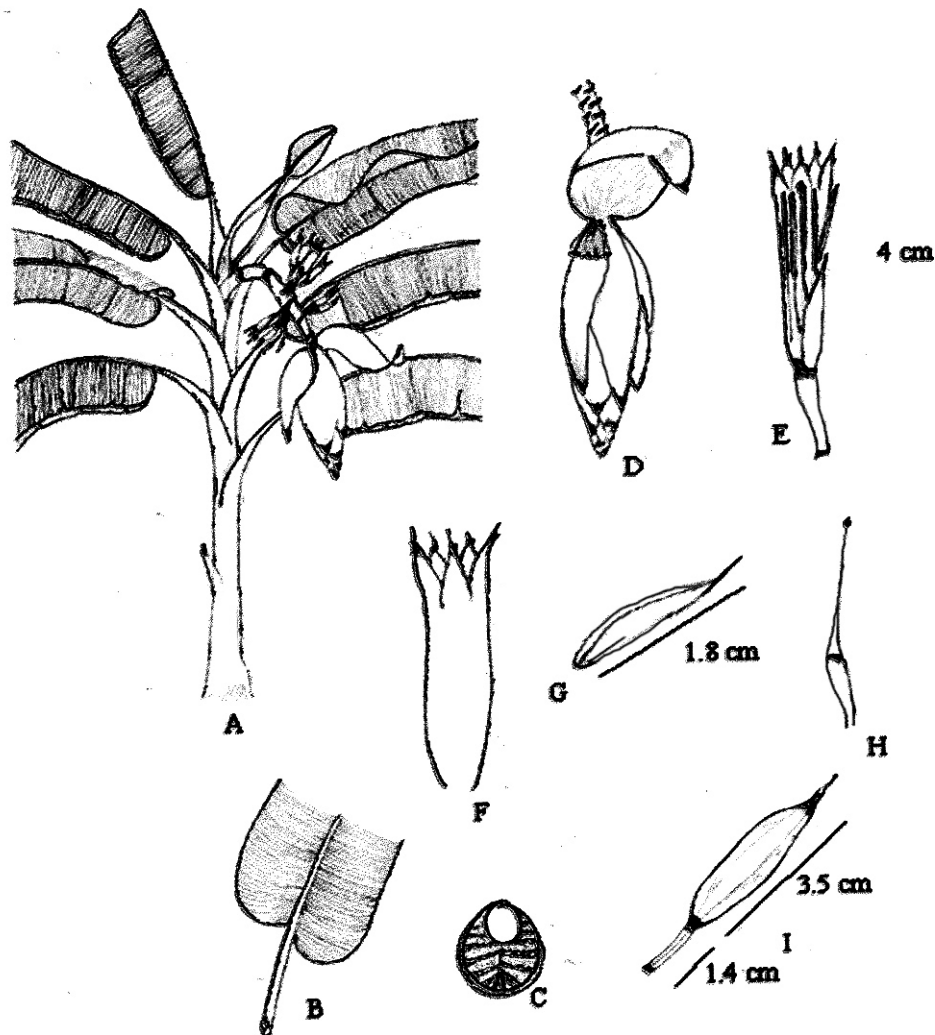
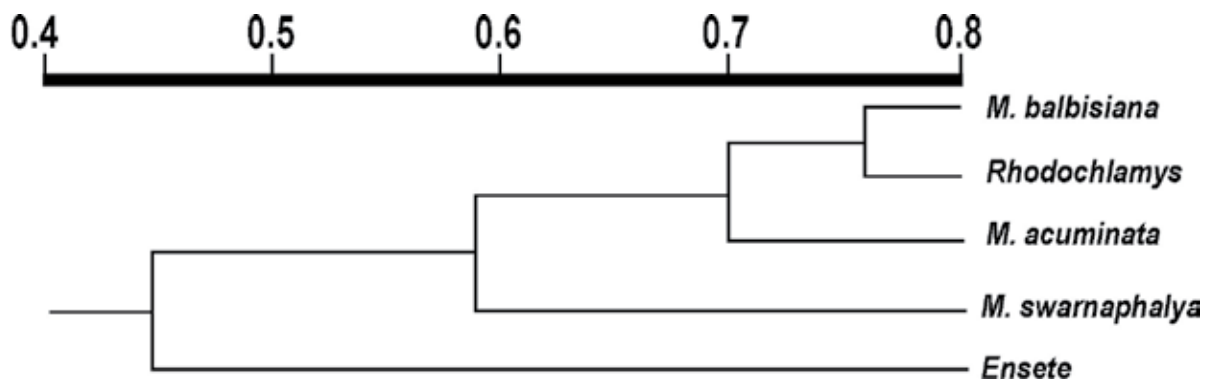


Fig. 3. Drawings of *M. swarnaphalya*. Uma, Saraswathi and Durai

A. The whole plant, B. Basal portion of the leaf, C. Cross section of petiole, D. Male bud, E. Male flower, F. Compound tepal, G. Free tepal, H. Pistil, I. Fruit.



**Fig. 4.** Dendrogram showing the relationship of *M. swarnaphalya* with other members of *Musa* and *Ensete* sections in banana.

its stature growing to a height of 8-9 m and robust nature. Maroon colouration of pseudostem is distinct from other basic green coloured pseudostems. Most distinct feature is the deep imbricate nature of male bud like in case of *M. textilis* and greenish-yellow colour of the bract. Pedicel is unusually long as in *M. nagensium* and each hand put forth only 6-8 fingers per hand. Provisional taxonomic key has been developed for the identification of species and sub-species of *Musa* with green and green in yellow male buds and it is provided in Table 2.

Preliminary molecular characterization of *M. swarnaphalya* was carried out using RAPD to confirm its uniqueness using 19 known reference species. They included *M. acuminata* ssp. *burmannica* (AAw), *M. acuminata* ssp. *burmannicoides* (AAw) and Lairawk (AAw), Athiakol (BBw) Attikol (BBw) and Bhimkol (BBcv), *M. nagensium* and *M. itinerans* of the section Eumusa. Three distinct groups were observed with all three wild *acuminata* species grouping into one cluster, all three *M. balbisiana* species (both wild and cultivated) grouping into separate cluster. The species *M. itinerans* and *M. nagensium* grouped with *M. swarnaphalya* into a distinct cluster suggesting its unique species status within Eumusa section.

Molecular characterization using retro-elements (IRAP-Inter-Retrotransposon Amplified Polymorphism) also exhibited similar results with *M. swarnaphalya* sharing 58 per cent similarity with Eumusa members (*M. acuminata* ssp. *burmannica* and *M. acuminata* ssp. *burmannicoides*) (Fig. 4) and was distinct from *Rhodochlamys* and *Ensete* members (Saraswathi, 9; Uma *et al.*, 16). The results of flow cytometry indicated the diploid nature (2x) of *M. swarnaphalya* (Fig. 2d).

*M. swarnaphalya* belongs to the section Eumusa and field observation suggests its complete immunity to leaf spot. Phylogenetic studies of *M. swarnaphalya* with other commercial varieties using advanced marker

systems like SNP's, nuclear protein coding gene loci etc. has shown its paternal lineage for hardy Poovan (AAB) subgroup of bananas (Carreel *et al.*, 2).

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