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ABSTRACT: $Musa \times calicutensis$ K. P. Smisha & M. Sabu *hybrid nov*. (Musaceae), a new manually crossed interspecific hybrid of two wild parent plants *Musa haekkinenii* (female) and *Musa beccarii* (male) is described and illustrated. A comparison of characters with a key to the new hybrid M. \times calicutensis with its parents and related variety are provided.

KEY WORDS: Hybrid, India, Interspecific, Musa beccarii, Musa haekkinenii.

INTRODUCTION

Musaceae are the most primitive family of the order Zingiberales with species diversity centered in Old World tropics mainly in Africa and from South and South East Asia to northern Australia (ca.75 spp.). The family comprises of three genera; Musa L., Ensete Bruce ex Horan., Musella (Franch.) H.W.Li. Musa is the largest genus of the family occurs in tropical Asia (ca. 65 spp.) from South India to Eastern Himalayas to northern Australia including Sri Lanka and in Africa. Ensete is native to Africa and Asia (ca.9 spp. and 1 var.), distributed discontinuously in Asia, sub-Saharan Africa and Madagascar (Simmonds, 1960). Musella is a monotypic genus and has narrow distribution in South-West China. Bananas (Musa spp.) form the fourthmost important food crop (Christelova et al., 2011, Davey et al., 2013, de Jusseu et al., 2013, Novak et al., 2014). Present edible bananas which produce fruits without seeds are the hybrids derived from natural hybridization of two wild species M. acuminata Colla and M. balbisiana Colla (Simmonds and Shepherd, 1955). Many studies reported the occurrence of naturally hybridizing wild Musa species (Simmonds, 1962). To explore the potential value of ornamental bananas for landscaping application several hybridization experiments were carried out by different workers. Recently a new hybrid, Musa × georgiana Rich. H. Wallace, was reported with commercial breeding relevance and ornamental potential (Wallace and Hakkinen, 2009).

As a part of Indian Musaceae revisionary project, much of *Musa* species were collected and conserved at Calicut University Botanical Garden (CUBG). Collections of several exotic *Musa* species were also maintained in the garden because of their potential ornamental value. Among them recently published *Musa* species, *M. haekkinenii* N.S. Ly & Haev. and *M. beccarii* N.W. Simmonds got much attention because of their highly attractive inflorescence and special floral architecture. As a part of our studies on reproductive biology of Musaceae and with a purpose of breeding evaluation and potential ornamental value, intra and intersectional hybridization had been done. This paper focuses on a new hybrid, resulted from artificial breeding of *M. haekkinenii* (female parent) and *M. beccarii* (male parent) and resulted a new hybrid M. × calicutensis which shared vegetative and floral characters of both parents and expressed some new distinct characters also.

MATERIALS AND METHODS

The present study was conducted at Calicut University Botanical Garden (11°25'45"N, 75°45'50"E) during the period of 2013-2016. Stigma receptivity of M. haekkinenii (female parent) and pollen viability of M. beccarii (male parent) were assessed at different time intervals from anthesis to flower closing using MTT test (Dafni et al., 2005) and TTC test by Shivanna and Rangaswamy (1992) respectively. Manual cross pollination was done in a period of maximum stigma receptivity and pollen viability which coincides. The self-compatability was assessed by a method suggested by Dafni et al. (2005). The seed set and seedling germination was observed for 3-4 months. The phenological events of new interspecific hybrid were observed with 10 plants of F1 hybrid. Morphometric analysis of vegetative and floral characters was done with a scale and LEICA M80 stereomicroscopes. Colour comparison of new hybrid with that of parents was referred by colour code of Kornerup and Wanscher (1978). Descriptions of a new hybrid and parent plants are given using INIBAP Musa descriptor list (IPGRI-INIBAP/CIRAD, 1996). Photographs were taken with Sony HX400V digital camera. Voucher specimens were deposited at Calicut University Herbarium (CALI) and herbarium of Botanical Survey of India, Southern Regional Centre, Coimbatore (MH).



Key to the new hybrid and its parents and related variety of *M. beccarii*

1a.	Bracts persistent, seeds barrel-shaped M. haekkinenii
1b.	Bracts deciduous, seeds round-dorsiventrally compressed 2
2a.	Stigma narrowly flat, opaque yellow M. × calicutensis
2b.	Stigma round-globose, cream 3
3a.	Fruits 1-13 cm long, narrowly thickened at the centre
	M. beccarii
3b.	Fruits 7-8 cm long, heavily thickened at the centre
	<i>M. beccarii</i> var. <i>hottana</i>

TAXONOMIC TREATMENT

Musa× calicutensis K.P. Smisha & M. Sabu, hyrid nov. Figs. 1 & 2

Type: India, Kerala, Malappuram District, Thenhipalam, Calicut University Botanical Garden, 11°25'45''N, 75°45'50''E, 09 Dec 2016, *K.P. Smisha* 147907 (holotype: CALI!, isotype: MH!).

Musa \times calicutensis is differs from the female parent M. haekkinenii in having narrowly lanceolate-obovate bract (vs oblong-ovate), bracts adaxially brownish red (vs orange red), and presence of sterile anthers and undeveloped fruits. $M. \times calicutensis$ similar to the male parent M. beccarii by the presence of deciduous bracts but show distinct characters from M. beccarii in presence of lanceolate bract shape (vs obovate), small shoulder (vs larger shoulder), flag leaf shape, absence of horn appendages on compound tepal, sterile anther and undeveloped fruit. Moreover, hybrid M. × calicutensis resembles in bract shape of M. beccarii var. hottana but differs in having bracts adaxially brownish red (vs reddish orange with discoloured stripes), absence of horn appendages on compound tepal, sterile anther and undeveloped fruit (Table 1).

Clump forming, plant slender, herbaceous, suckering freely, 4-5 suckers borne close to the parent plant, 27-30 cm long, oriented vertically. Mature pseudostem slender, 130-140 cm high, 18-20 cm diam. at the base, sap milky. Leaf habit intermediate, petiole 20-25 cm long, green with sparse brown blotches at the base at an age; petiole canal margins incurved, narrow, 0.5-0.7 cm wide, scarious, and clasping pseudostem at the base. Lamina oblong-lanceolate, 90-110×30-35 cm long, obtuse apex, margin corrugated and red, midrib 84-99×0.8-1cm long, grayish green on adaxial surface and pale gravish green on abaxial surface, leaf blade base rounded, adaxial surface dark green and dull, abaxial surface deep green and shiny, insertion point of leaf bases asymmetric on both sides. Inflorescence erect, peduncle 4-5 cm long, 8-12 cm in diam., puberulent, yellowish cream which turn light green at maturity. Flag leaf with colourful bract like base and leafy apex persistent, 80-90 cm long; leaf lamina 30-50×5-5.5 cm long, lamina dark green and dull at adaxial surface, deep green and shiny at abaxial surface; mid rib gravish green on adaxial surface and pale grayish green on

abaxial surface; bract 25-30×5-5.5 cm long, orange red adaxial surface and brownish red abaxial surface. Sterile bracts lanceolate, 25-26×5-6 cm long, adaxial surface dull, brownish red with yellow tinge at base, abaxial surface shiny, brownish red with yellow tinge at base, apex acute, greenish, base grayish orange, shouldered, deciduous. Female bracts lanceolate, 19.5-20×6-7 cm long, adaxial surface dull, brownish red, abaxial surface shiny, brownish red, apex acute to obtuse, green tinted with yellow, base grayish orange, shouldered, margin not revolute, lifting one bract at a time, just open on first day, open and reflexed on second day, deciduous on third day, imbricate. Basal flowers female, borne in 3-4 female hands, flowers 3 per bract in a single row, 8-11 cm long. Compound tepal 4.5-5×1.5-2 cm long, lower half deep yellow and upper half olive green, ribbed at adaxial and abaxial surfaces, apex 5-lobed, rounded, without horn like appendages, two lobes larger and exserted, 0.1×0.2 cm long, middle and lateral lobes are curved backward, 0.1×0.2 cm long. Free tepal $4.5 - 5 \times 1.3 - 1.5$ cm long, ovate, as long as the style, closely appressed to the stigma, translucent, opaque vellow, margin entire, apex corrugated with short acumen, adaxial surface smooth, abaxial surface ribbed. Staminodes 5, lanceolate, creamy yellow, 1.5-1.6×0.1-0.2 cm long. Style straight, 3×0.2 cm long, pale yellow with olive green tinge at apex. Stigma terete, 1×0.5 cm long. Ovary straight, 4-6.5 cm long, lemon yellow, waxy, 3-locular. Male bracts lanceolate, 10-14×4-5 cm long, bract lifting one at a time, open on first day, open and reflexed on second day, deciduous on third day, adaxial surface dull, brownish red, abaxial surface shiny, brownish red, apex intermediate, green tinted with yellow, base gravish orange, not shouldered, margin not revolute. Male flowers borne in 3-4 in male hands, flowers 5-6 cm long; compound tepal 4.5-5.5×1.1-1.3 cm long, lower half deep yellow and upper half olive green, ribbed at adaxial and abaxial surfaces, apex 5-lobed, rounded, without horn like appendages, two lateral lobes larger and exserted, 0.2×0.1 cm long, 3 central lobes are curved backward, 0.1×0.1 cm long; free tepal 3.7- $4.5 \times 1.0 - 1.3$ cm long, ovate, $3/4^{\text{th}}$ of compound tepal, translucent, opaque yellow, margin entire, apex corrugated with short acumen, adaxial surface smooth, abaxial surface ribbed. Stamens 5, 3.5-4.0 cm long; anther 1.8-2×0.05-0.1 cm long, grayish yellow; filament 1.6-2.5×0.1-0.2 cm long, light yellow. Rudimentary style straight, 3.5-4×0.1-0.2 cm long, pale yellow with olive green tinge at apex. Rudimentary stigma terete, yellowish orange, narrowly oblong, 0.4-0.6×0.3-0.4 cm long. Ovary rudimentary, oblong, 4-7 cm long and lemon yellow, waxy. Undeveloped fruit bunch lax, 3-4 hands, 3 fruits per hand, borne in a row, fingers oriented obliquely respected to axis, mature undeveloped fruit narrowly



M. beccarii Characters M. × calicutensis M. haekkinenii M. beccarii var. hottana 130-140 Plant height (cm) 250-300 150 - 20080 Watery Milky Watery Waterv Sap watery/milky Suckers 4–5 8-13 6 - 82 Margins recurved Petiole canal Margins erect Margins overlapping Margins recurved and winged Narrowly lanceolate Flag leaf type Narrowly lanceolate bract Broad bract and lamina. Unknown and broad lamina, persistent bract, broad lamina, persistent persistent Sterile bract type Lanceolate. deciduous Lanceolate persistent Obovate. deciduous Unknown Female bud shape Lanceolate-oblanceolate oblanceolate oblanceolate Unknown Female bract type Lanceolate, deciduous Lanceolate, persistent Lanceolate, deciduous Ovoid, deciduous Female bract colour Adaxial surface dull and Adaxial surface dull Adaxial surface dull and Unknown upper/lower surface brownish red abaxial and orange red and orange red and abaxial surface shiny and brownish abaxial surface shinv surface orange red tinted with brownish red red and orange red Flowers per bract 3–4 1–4 2–4 2–3 Grayish green Pale grayish green Unknown Bract apex colour Green tinted with yellow Grayish orange Bract base colour Orange Orange red Unknown Deep yellow Ovary colour Lemon yellow Light green Light green Stigma shape Narrowly flat Round to Globose Round to Globose Flat Male bud shape I anceolate oblanceolate oblanceolate Ovoid Male bract type Lanceolate, deciduous Lanceolate, persistent Obovate, deciduous Ovoid, deciduous Male bract colour Adaxial surface dull, Adaxial surface dull Adaxial surface dull and Adaxial surface orange red and abaxial surface upper/lower surface brownish red and abaxial and orange red and orange red and abaxial surface shiny, brownish red abaxial surface shiny surface orange red tinted red with discoloured and orange red with brownish red stripes Bract apex colour Green tinted with yellow Gravish green Pale grayish green Yellow Bract base colour Unknown Grayish orange Orange red Orange red Flowers per bract 3-4 3-4 4 2 Lower half yellow and Male compound tepal Lower half deep yellow and Lower half reddish Grayish yellow colour upper half olive green vellow and apex deep apex light green green Appendage Absent Absent Present Present Free tepal shape/colour Obovate, opaque vellow Obovate, lower half Narrowly obovate, pale Oblong reddish yellow and grayish yellow apex deep green Anther type, colour Sterile, grayish yellow Fertile, pale grayish Fertile, pale greenish Fertile white white Rudimentary ovary Deep yellow Lemon yellow Pastel pale green Unknown colour Number of Undeveloped fruits 3-4, Fruits 3-4, straight Fruits 4-5, narrowly Fruits 2-3, heavily fruits/undeveloped fruits thickened at the centre thickened at the centre Straight per hand & shape Fruit colour when mature Olive yellow-grayish green Gravish yellow Pale grayish green Light green-creamy yellow Seed type Absent Barrel-shaped, warty Round-dorsiventrally Cylindrical, obpyriform, flattened, warty wartv Reference Hakkinen et al. 2005

Table 1. Comparison of characters of Musa × calicutensis with parents and related variety.

oblong, 6–6.5 cm long, olive yellow, not waxy, straight, bear persistent floral relicts, 3.5–4 cm long.

Etymology: The name of a new hybrid refers to the place, Calicut University Botanical Garden where the interspecific hybridization was done.

DISCUSSION

A new hybrid $Musa \times calicutensis$ is developed here by artificial breeding techniques. The new hybrid is highly relevant for breeding and with potential ornamental value and also to improve the knowledge of parental compatibility in detail. Authors crossed M. *haekkinenii* (female parent) and M. *beccarii* (male parent) with *M. ornata*, *M. siamensis*, *M. laterita*, *M. velutina*, *M. markkuana* and none of them produced fruit. But the cross between *M. haekkinenii* (female parent) and *M. coccinea* (male parent, a close relative of *M. haekkinenii*) resulted in fruit setting and seed production. Reciprocal crosses also showed positive results. But the reciprocal cross between the parents of *M.* × *calicutensis*; *M. beccarii* (male parent) and *M. haekkinenii* (female parent) was a failure without fruit and seed production. The rate of fruit set of both pollination (cross pollination and interspecific hybridization) resulted >30% fruit set by self-incompatibility index method. It has been confirmed that both species as highly self-compatible.



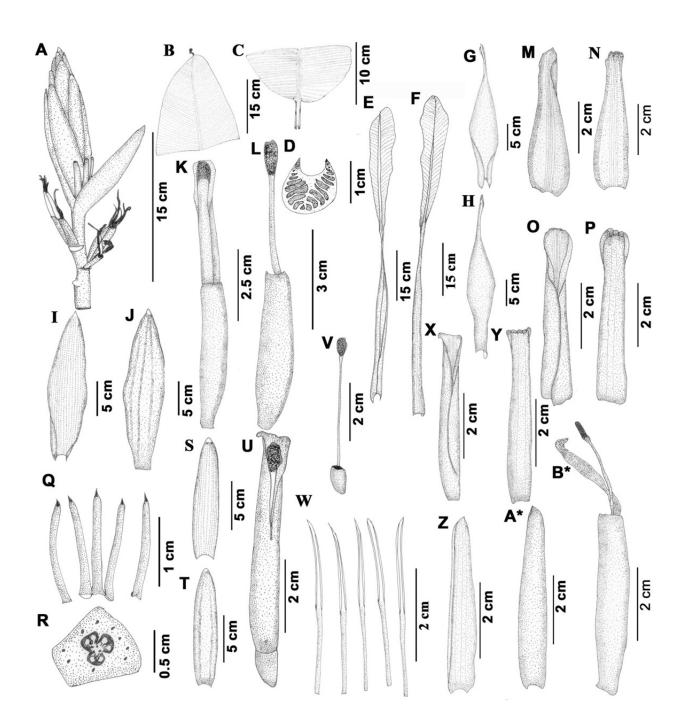


Fig. 1. Illustration of *Musa* × *calicutensis* K. P. Smisha & M. Sabu *hyb*rid *nov*. A: Inflorescence. B: Leaf apex. C: Leaf base. D: Cross-section of petiole. E–F: Flag leaves. G–H: Sterile bracts. I–J: Female bracts. K: Female flower. L: Ovary with style and stigma. M–N: Compound tepals. O–P: Free tepals. Q: Staminodes. R: Cross-section of ovary. S–T: Male bracts. U: Male flower. V: Rudimentary ovary with style and stigma. W: Stamens. X–Y: Compound tepals. Z–A*: Free tepals. B*: Undeveloped fruit.



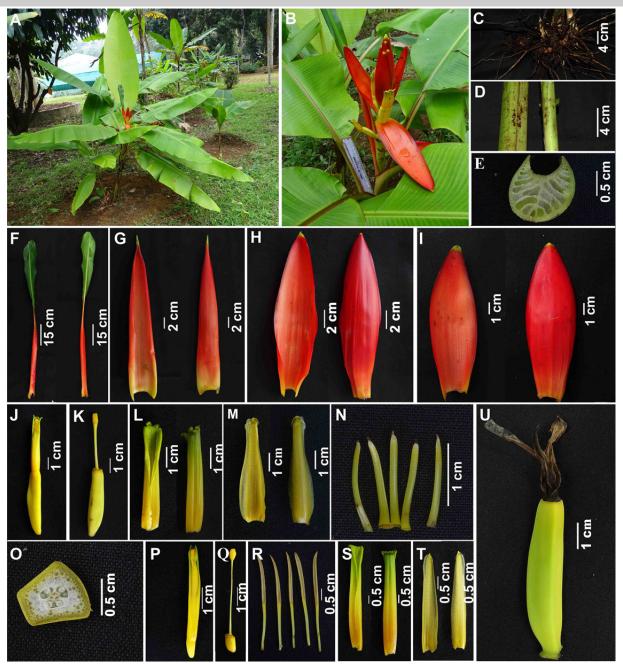


Fig. 2. *Musa* × *calicutensis* K.P. Smisha & M. Sabu *hybrid nov.* A: Habit. B: Inflorescence. C: Corm. D: Pseudostem. E: Cross-section of petiole. F: Flag leaves. G: Sterile bracts. H: Female bracts. I: Male bracts. J: Female flower. K: Ovary with style and stigma. L: Compound tepals. M: Free tepals. N: Staminodes. O: Cross section of ovary. P: Male flower. Q: Rudimentary ovary with style and stigma. R: Stamens. S: Compound tepals. T: Free tepals. U: Undeveloped fruit.

The cross pollination of *M. haekkinenii* expressed average seed number $56.80 \pm 2.15\%$ and seed weight $14.75\pm 00.00\%$. But average seed number and seed weight of interspecific hybridization was $6.83 \pm 1.78\%$ and $0.07 \pm 00.00\%$ respectively. Seed germination test, a reliable method for assessing reproductive success exhibited 87% and 84% germination in cross pollination and interspecific hybridization respectively.

Musa haekkinenii is a native wild banana of

Vietnam and only known under cultivation (Calicut University Botanical Garden (CUBG), Kerala, India and Suriana Botanic Garden, Penang, Malaysia) today. The centre of origin of the species is Northern Vietnam and so far no wild population of species has been reported and existence of the species in wild is still uncertain and recognized as a Data Deficient species according to IUCN Red List categories and criteria (Ly *et al.*, 2012). The plant has a potential ornamental value



because of the presence of highly attractive orange red coloured inflorescence with persistent bracts which last up to 4–7 months. *Musa beccarii*, a wild banana is distributed in Borneo and Indonesia. The species is remarkable for its highly attractive bracts and flowers which adds the ornamental value.

The taxonomic placement of *M. beccarii* is still ambiguous because of the basic chromosome number 2 n = 2 x = 18 and increased distribution of rRNA genomes (Cizkova *et al.*, 2016). Based on morphological characters Hakkinen (2004) placed *M. beccarii* in the section *Callimusa*. But the cytological study did not support *M. beccarii* in *Callimusa-Australimusa* group by Bartos *et al.* (2005). Recently phylogenetic analysis by Li *et al.* (2010) included *M. beccarii* in *Callimusa-Australimusa* clade because of the close relationship of *M. beccarii* with three *Australimusa* species namely *M. maclayi*, *M. peekelii* and *M. textilis*. Under these circumstances, we prefer to present the new hybrid *M. × calicutensis* as an interspecific hybrid without assigning any sectional classification.

The new interspecific hybrid M. × *calicutensis* which express intermediate characters of M. haekkinenii (female) and M. beccarii (male) and exhibits new characters also. The basal unisexual female flowers are fertile with receptive stigma and unisexuality is recognized as a unique adaptation for cross pollination. But the male flowers are sterile without any pollen grain production and recognized as a self-incompatible hybrid. No parthenocarpic fruit development was noticed. However, our new hybrid significantly adds the ornamental value because of the presence of highly attractive bracts and flowers of inflorescence which lasts up to 3-5 months. Currently, many newly explored wild Musa species are used as staple food, medicine and ornamentals. So the interspecific hybridization between wild species has an immense relevance for breeding purposes. We are focused to improve the knowledge on molecular phylogenetic and cytological investigations of new hybrid M. × *calicutensis* and its parent plants to ensure relevant systematic placement and to explore breeding applications.

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LITERATURE CITED

Bartoš, J., O. Alkhimova, M. Doleželová, E. De Langhe and J. Doležel. 2005. Nuclear genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete* (Musaceae): taxonomic implications. Cytogenet. Genome Res. **109(1-3)**: 50-57.

- Christelová P., M. Valárik, E. Hřibová, E. De Langhe, and J. Doležel 2011. A multi gene sequence based phylogeny of the Musaceae (banana) family. BMC Evol. Biol. 11: 103.
- Čížková, J., E. Hřibová, P. Christelová, I.V. den Houwe, M. Häkkinen, N. Roux, R. Swennen, and J. Doležel. 2015. Molecular and cytogenetic characterization of wild *Musa* species. PLoS ONE **10(8)**: e0134096.
- **Dafni A., P.G. Kevan, and B.C. Husband.** 2005. Practical Pollination Biology. Enviroquest Ltd. Cambridge, Ontario, Canada. pp. 1-590.
- Davey, M.W., R. Gudimella, J.A. Harikrishna, L.W. Sin, N. Khalid, and J. Keulemans. 2013. A draft *Musa* balbisiana genome sequence for molecular sequence in polyploid, inter and intraspecific *Musa* hybrids. BMC Genomics 14(1): 683.
- de Jesus, O.N., S.O. Silva, E.P. Amorim, C.F. Ferreira, J.M.S. Campos, G.G. Silva, and A. Figueira. 2013. Genetic diversity and population structure of *Musa* accessions in *ex situ* conservation. BMC Plant Biology 13(1): 41.
- Häkkinen, M. 2004. Musa voonii, a new species from Northeren Borneo and classification of section Callimusa in Borneo. Acta Phytotax. Geobot. 55: 79-88.
- Häkkinen, M., M. Suleiman, and J. Gisil. 2005. Musa beccarii (Musaceae) varieties in Sabah, Northern Borneo. Acta Phytotax. Geobot. 56: 135-140.
- **IPGRI-INIBAP/CIRAD.** 1996. Description for Bananas (*Musa* spp.). International Plant Genetic Resources Institute, Rome, Italy/ International Network for the Improvement of Banana and Plantain, Montpellier, France/ Centre de Cooperation Internationale en RecherecheAgronomique pour le Developpment, Montpellier, France. pp. 1-58.
- **Kornerup, A. and J.H. Wanscher.** 1978. Methuen handbook of colour, 3rd ed. Methuen, London. pp.1-252.
- Li, L.-F., M. Häkkinen, Y.-M. Yuan, G. Hao and X.-J. Ge. 2010. Molecular phylogeny and systematics of the banana family (Musaceae) inferred from multiple nuclear and chloroplast DNA fragments, with a special reference to the genus *Musa*. Mol. Phylogenet. Evol. 57(1): 1-10.
- Lý, N.S., C.-K. Lê, T.-D. Triệu, A. Haevermans, P.P. Lowry II, and T. Haevermans. 2012. A distinctive new species of wild banana (*Musa*, Musaceae) from northern Vietnam. Phytotaxa 75(1): 33-42.
- Novák, P., E. Hřibová, P. Neumann, A. Koblížková, J. Doležel, and J. Macas. 2014. Genome wide analysis of repeat diversity across the family Musaceae. PLoS ONE 9(6): e98918.
- Shivanna, K.R. and N.S. Rangaswamy. 1992. Pollen Biology: A laboratory Manual. Narosa Publishing House. New Delhi. pp. 1-199.
- Simmonds, N.W. 1960. Notes on banana taxonomy. Kew Bull. 14: 198-212.
- Simmonds, N.W. 1962. The evolution of the bananas. Longmans, London. pp. 1-170.
- Simmonds, N.W. and K. Shepherd. 1955. The taxonomy and origins of the cultivated bananas. J. Linn. Soc. Bot. 55(359): 302-312.
- Wallace, R. and M. Häkkinen. 2009. *Musa* \times *georgiana*, a new intersectional hybrid banana with edible banana breeding relevance and ornamental potential. Nor. J. Bot. **27(3)**: 182-185.