

MORPHOLOGICAL, ANATOMICAL, CYTOLOGICAL AND PALYNOLOGICAL CHARACTERIZATION OF TWO CULTIVARS OF *ABELMOSCHUS MOSCHATUS* (L.) MEDIK (MALVACEAE)

Dubey Kumari Priyanka^{1*}, Datta K. Animesh¹, Mandal Aninda¹, Saha Aditi² and Sengupta Sonali³

¹Department of Botany, Cytogenetics and Plant Breeding Section, Kalyani University, Kalyani, West Bengal, India

²Department of Botany, Narasinha Dutt College, Howrah, India

³P.G. Department of Botany, Hoogly Mohsin College, Hoogly, India

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ABSTRACT

Seeds of 2 cultivars (cultivar I: seed moisture content 3.5%, 100 seed weight 1.94 gm \pm 0.03, seed size 3.47 mm \pm 0.08 \times 3.20 mm \pm 0.06; cultivar II: seed moisture content 1.5%, 100 seed weight 1.25 gm \pm 0.01, seed size 2.92 mm \pm 0.07 \times 3.33 mm \pm 0.03) of *Abelmoschus moschatus* (L.) Medik (Family: Malvaceae) were grown in the Experimental plots of University of Kalyani (West Bengal plain; 22°99' N, 88°45' E, elevation 48 feet above sea level, sandy loamy soil, organic carbon 0.76%, soil p^H 6.85) during the rainfed seasons of 2009 and 2010 (July to December) and morphological (taxonomical details), anatomical (transverse sections of ovary, stem and root), stomatal, cytological (meiotic chromosome behavior) and pollen attributes (fertility, viability and acetolysis studies) were assessed. Results indicated marked differences between the cultivars and based on observations cultivar I is recommended as tall, branched whereas cultivar II as dwarf, unbranched types and the cultivars may further be explored for pharmacological research.

KEYWORDS: *Abelmoschus moschatus*, cultivars, characterization, tall branched type, dwarf unbranched type.

***Corresponding Author**

Dubey Kumari Priyanka, Department of Botany, Cytogenetics and Plant Breeding Section

University of Kalyani, Kalyani – 741235, West Bengal, India Email: dattaanimesh@gmail.com

INTRODUCTION

Abelmoschus moschatus (L.) Medik syn. *Hibiscus abelmoschus* L. of the family Malvaceae, popularly known as ambrette (common names- Filipino: ambrette, kastuli; French: ketmie musquee; Indonesian: kasturi; Malay: kapas hutan; Thai: som-chaba; Vietnamese: b[us]p, v[af]ng- Widodo¹; English name- Musk-mallow), is native to India² and is cultivated in the tropical regions of Asia, Africa and South America for its seeds possessing a characteristic musky odour^{3,4}. The essential oil obtained from ambrette seeds (present in outer layer of seed coat- Nee *et al.*⁵) finds application both in flavor and fragrance formulations⁶ notwithstanding the significance of the species (roots/leaves rarely/seeds) in traditional system of medicine^{7,8}. Maheshwari and Kumar⁹ reported antimicrobial activity of *A. moschatus* leaf extracts. Further, ambrette seed oil is reported to be edible and therefore the species is under nontraditional oil seed crop of economic value under rainfed conditions^{10,11} and may be considered as an alternative

crop in comparison to the traditional oil crops. However, its commercial cultivation is restricted in Java, India (mainly in the Deccan and Carnatic), Madagascar and in parts of Central and South America on a small scale¹. Therefore, attention must be paid to keep the species under cultivation for sustainable use following propagation in regions conducive for their growth including West Bengal plains thereby providing wider base for its exploitation (value added product) both in National and International markets. The present communication describes morphological, anatomical, cytological and pollen attributes of 2 cultivars of *A. moschatus* with an objective to characterize the germplasms and to provide information to breeders and geneticists so that the crop may further be improved (higher seed yield/seed oil content) through efficient breeding and maximizing trade.

MATERIALS AND METHODS

Seeds of cultivar I (cv. I) (moisture content 3.5%, 100 seed weight 1.94 gm \pm 0.03, seed size 3.47 mm \pm 0.08 \times

3.20 mm \pm 0.06) and cultivar II (cv. II) (moisture content 1.5%, 100 seed weight 1.25 gm \pm 0.01, seed size 2.92 mm \pm 0.07 \times 3.33 mm \pm 0.03) of *Abelmoschus moschatus* (L.) Medik were collected from Medicinal Plant Garden, Narendrapur, Ramkrishna Mission, Govt. of West Bengal and sown in the Experimental plots of University of Kalyani (West Bengal plain; 22°99' N, 88°45' E, elevation 48 feet above sea level, sandy loamy soil, organic carbon 0.76%, soil p^H 6.85- Bhattacharya and Datta¹²) during the rainfed seasons of 2009 and 2010 (sowing 1st week of July and harvesting late November to early December; temperature 25.5°C to 39°C max., 18.4°C to 24.2°C min., relative humidity 82.2% to 95.2% max., 48.8% to 76.7% min., rainfall 1.08 mm to 15.2 mm, Agrometeorological data was obtained from Bidhan Chandra Krishi ViswaVidyalaya, Mohanpur, Nadia, West Bengal; line sowing, seeds were sown 25 cm apart and between lines 35 cm distance were maintained).

The mature plants of cv. I and II were examined and the study includes detailed description of every part of the specimen with Olympus binocular dissecting microscope under 10X. Measurements of the leaves, floral parts and seeds were made. Color of different parts of the specimens was laid down with reference to RAL CLASSIC COLOUR CHART, UK. Chlorophyll (mg/gm of leaf tissue) content was assessed (identically matured leaves were taken) as per Arnon¹³. Viability of seeds was tested following tetrazolium method¹⁴. Voucher specimens of the cultivars were deposited in Herbarium, Botany Department, Kalyani University.

Anatomical Studies

Transverse hand sections of the stem (56.0 to 61.0 cm from base) and root (10.0 cm below ground level) were made in both cultivars from fully matured plants (at fruit ripening stage; 120-130 days from sowing) were doubled stained using 1.0% safranin dissolved in 50% alcohol and 1.0% light green dissolved in 90% alcohol¹⁵.

Study of Stomatal Parameters

For the study of stomata, quick fix image impression (leaf imprints from 3 plants of each cultivar were scored, and leaves near apex at bud initiation stage were assessed) techniques was adopted¹⁶. Stomatal frequency and its distribution (upper and lower leaf surfaces along with near mid vein -NMV and away mid vein- AMV) were recorded (light microscope observation-10X \times 40X). Size (μ m) of the stomata and aperture were measured. Considering that the shape of stomata to be elliptical its area was calculated as per Ghosh *et al.*¹⁷ using the formula [$\pi/4(L \times B)$], where L and W are length and breadth.

Meiotic Analysis

Flower buds of suitable sizes from 3 randomly selected plants of each cultivar were fixed (6:30 to 7:00 am) in Carnoy's solution (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid) and three changes were given in the fixative at an interval of 24 hours. The fixed buds were preserved in 70% alcohol and stored in a refrigerator. Anthers were squashed in 2% propinocarmine solution and meiotic data pooled over the plants were recorded at metaphase I (MI) and anaphase I (AI). Photomicrographs were taken from temporary squash preparations.

Pollen Attributes

Fertility and viability

Pollen grains from matured anthers were stained in 2% propinocarmine solution and fully stained pollen grains were considered fertile¹⁸. Pollen grains viability (Lugol's - detects the presence of starch, viable pollen turns black-Bengtsson¹⁹; aniline blue in lactophenol- detects the presence of callose on pollen wall, viable pollen turns blue- Bengtsson¹⁹) were also assessed in the cultivars.

Acetolysis studies

Acetolysis technique was adopted as per Erdtman²⁰ to study the shape, size and ornamentation of the pollen grains in both cultivars.

RESULTS AND DISCUSSION

Taxonomical Description

Cultivar I

Annual, branched undershrub (**Fig. 1**), branches 6 to 8 (angle of divergence to main axis- range 60° to 80°) from 7.0 to 12.0 cm from base; branches extended to the length of 145.0 to 155.0 cm but shorter than main shoot, main shoot 165.60 cm \pm 1.55 in length; terrestrial; stem terete, solid, woody (hard), hispid hairy throughout, fallen out at maturity; purple green to light purple green, more purplish surrounding the nodal region; leaves alternate, palmately 3 to 5 lobed (**Fig. 3a**), 15.5 cm \pm 0.74 (14.9 cm to 16.5 cm) long and 23.1 cm \pm 0.56 (21.5 cm to 23.3 cm) width (area: 205.8 cm² \pm 7.08); central lobe longer than laterals; each lobe oblong, acuminate at apex, serrated at margin, lobed, unicostate with 3 to 5 pairs of alternate as well as opposite pinnate secondaries terminated to the teeth; reticulate, herbaceous, hispid hairy, greenish above, dull green below, purple on the major veins, basal part of lamina and distal part of petiole; petiolate; petioles slender, 22.0 to 25.0 cm, hispid hairy, light purple above and greenish beneath; stipulate; stipule free lateral, lanceolate to ovate, acuminate at apex, entire to slightly dentate at margin, hairy along margin, size 0.4 to 0.5 cm; inflorescence cymose, axillary without peduncle and bracts; flower solitary, (length: 3.54 cm \pm 0.21, 3.4 to 3.8 cm; diameter:

4.34 cm \pm 0.17, 4.2 to 4.5 cm), complete, bisexual, actinomorphic, hypogynous, pentamerous, color light yellow (Jonquil BS4- 053, lower part crimson/cherry BS1- 025 – **Fig. 4a**), pedicillate; pedicels slender, terete, 32.0 mm, hispid hairy as well as glandular hairy throughout, light purple above greenish beneath, tumid at the distal part below the epicalyx; bracteolate; epicalyx 4, free, slightly dimorphic, size 0.7 to 0.9 cm in length, breadth 0.5 to 0.6 cm, acute to obtuse at apex, deciduous; calyx tubular, gamosepalous, 2.5 cm long, split at one side almost up to the base; surface glandular hairy throughout, light green, sepals 5 often shows as 2 lobes, triangular about 5.0 mm long; corolla polypetalous, petals 5 (length: 3.8 cm to 4.0 cm; breadth: at the widest part- 2.5 cm to 2.9 cm), twisted to left, each one obliquely spatulate or obovate, light yellow, pinkish at maturity, basal part 1.0 cm long, crimson color (BS1-025) with oblique upper edge; stamen numerous; filaments forming column, monadelphous; column 1.8 cm long attached to the swollen base of petals, anthers throughout the column, 1 celled, reniform, cream yellow; carpels 5, syncarpous; ovary obconical to dome shaped, densely hairy, 5.0 mm diameter, greenish; style 1, slender, 2.5 cm long, white mucilaginous, glabrous; stigma 5, spatulate, swollen, hispid hairy; ovary 5 chambered, many ovules in axile placentation (**Fig. 5**); fruits oblong conical (length: 3.74 cm \pm 0.15, 3.5 to 4.0 cm; width: 1.68 cm \pm 0.16, 1.5 to 1.9 cm), capsule, strongly 5 ribbed and 5 sub-ribbed in between the locules (**Fig. 4a**); dehiscent longitudinal, basipetal; many seeded (33.73 \pm 1.55/fruit); seeds rounded (**Fig. 7a**) with many verrucose interrupted lines (color- Congo brown BS3-038), glabrous; hilum end depressed and notched; seed yield/plant 8.89 gm \pm 2.08, viability 20.0 to 25.0%.

Cultivar II

Annual undershrub (**Fig. 2**), rare often in a few plant incipient branches (1 to 2) were noted at the onset of flowering; main shoot 109.2 cm \pm 4.14 in length; terrestrial; stem terete, soft, solid with mucilage, hispid hairy, slight purple to reddish; purple shade near to nodes; leaves alternate, palmately lobed, usually 5 lobed (**Fig. 3b**), length 13.4 cm \pm 0.41 (12.6 cm to 14.9 cm), width 19.3 cm \pm 0.69 (17.2 cm to 21.9 cm), area 179.7 cm² \pm 10.07; lobes further irregularly sublobed and serrated; lamina at base hastate to sagittate; herbaceous, 5 veined divergent terminated at apex with prominent secondaries, hispid hairy throughout, green above, dull green beneath, primary veins often purple color; petiolate; petioles slender, 10.0 to 16.0 cm long, hispid, purple above and greenish beneath; stipulate; stipule free lateral, linear about 0.4 to 0.5 cm long, hispid, hairy, green, deciduous; inflorescence cymose with solitary

flower, axillary, without peduncle and bracts; flowers solitary, (length: 4.7 cm \pm 0.17, 4.5 to 5.0 cm; diameter: 6.3 cm \pm 0.17, 6.0 to 6.5 cm), complete, bisexual, actinomorphic, pentamerous, color yellow (luminous yellow- RAL 1026 -**Fig. 4b**), pedicillate; pedicels slender about 7.5 cm long, hairy, greenish with purple at base to about 1 cm long; bracteolate; bracteoles as epicalyx, 9 segments; each segment linear, variable in sizes (6.0 mm to 20.0 mm in length, 1.0 to 3.5 mm in width), acute to acuminate at apex, entire at margin, broadened at base, hairy, green, persistent, united to form a narrow tube; calyx tubular to urceolate, about 3.5 cm long, dentate at top, irregularly 3 to 5 lobed, lobed about 4.0 mm long, tube with distinct nervation, hairy, dull green, deciduous; sepals 5; corolla polypetalous, petals 5 (length: 5.0 cm to 5.2 cm; diameter at the widest part- 3.1 cm to 3.6 cm), spatulate, strongly veined, glabrous, cream yellow, basal part crimson color (BS1-025), 1.0 cm long, twisted to left; stamen numerous; filaments forming column- 2.0 cm long, anthers throughout the column, cream yellow; carpels 5, syncarpous; ovary obpyriform, 6.0 mm long and 6.0 mm across, hairy, green; style 1, slender- 1.6 cm long, whitish, glabrous; stigma 5, clavate to rounded at top, crimson color; ovary 5 chambered, many ovules in axile placentation (**Fig. 6**); fruits capsule, ovoid spindle shaped (length: 6.2 cm \pm 0.32, 5.0 to 7.0 cm; width: 2.54 cm \pm 0.21, 2.5 to 2.6 cm), narrow to both ends, 5 angular with 5 ridges and 5 furrows (**Fig. 4b**), hispid hairy throughout, green, dehiscence; many seeded (24.8 \pm 2.31/fruit); seeds reniform (**Fig. 7b**) with longitudinal verrucose lines (color- Mahogany brown RAL 8016), glabrous; hilum depressed and notched; seed yield/plant 7.76 gm \pm 1.82; viability 20.0%.

Anatomical Studies

Stem anatomy of cv. I (Fig. 9)

Cuticle thin, single layered parenchymatous cells, cells oblong, barrel shaped or rectangular; hypodermis one layered, transversely oblong, thin walled, compact with black contents; outer cortex 8 to 10 cell layered thick, cells rectangular to tetraangular or rounded, compact, thin walled; inner cortex 6 to 8 cell layered thick, parenchymatous, cells transversely oblong to rounded, some cells disintegrate to form mucilage; endodermis indistinct; vascular bundles conjoint, collateral, open forming a rounded ring; cambium 2 to 3 cell layered thick, cells rectangular; phloem zone in 3 patches; outermost towards periphery- 8 to >50 cells in irregular clusters, cells polygonal thick walled; 2nd row of patches of extra phloem fibre, rectangular to tetraangular in outline, 8 to 25 cells in cluster; 3rd row- 4 to >20 cells in cluster, irregular in outline; xylem endarch, covering a larger span, ray cells 1-2 seriate; pith parenchymatous,

compact, polygonal; mucilage cavity present encircled with 6 to 7 peripheral cells.

Root anatomy of cv. I (Fig. 11)

Cork 8 cell layered thick towards periphery, inner cells transversely oblong to rectangular; secondary growth prominent, primary vascular bundle exarch, inconspicuous in the centre; most part of vascular tissue comprises of xylem tissue; phloem fibre patches in rings, usually 6.

Stem anatomy of cv. II (Fig. 10)

Cuticle medianly thick, single layered, cells barrel shaped to rounded with trichomes; hairs hispid, 2 celled, dipped within the mass of dermal cells appearing as tumid structure; hypodermis usually 2 cell layered thick, 3 to 4 cell layer thick below, cells rectangular to tetragonal, medianly thick walled, compact; outer cortex 4 to 5 cell layer thick, cells polygonal to rounded, compact, thin walled; inner cortex 3 to 4 cell layered thick but often disintegrate forming secretory cavities, endodermis distinct below inner cortex; vascular bundles conjoint, collateral, open forming a circular ring; cambium 1 to 2 cell layered thick, rectangular; phloem towards periphery, 10 to 15 cell layered thick with extra phloem fibres in patches of 10 to 25 cells in clusters, cells polygonal, compact, thin walled; xylem endarch single or many layered intermingled with xylem parenchyma; ray cells uniseriate, medianly thick walled; pith parenchymatous, compact, cells polygonal, variable in sizes, thin walled; mucilage cavity present, distinctly encircled with 4 to 6 peripheral cells.

Root anatomy of cv. II (Fig. 12)

Same as cv. I excepting that irregular patches of phloem fibre, 8 to 12 cells.

Stomatal Attributes

Stomata in both the cultivars were amphistomatic, paracytic (rare often anomocytic), isodiametric, polygonal and elongated in nature (Fig. 8). Guard and subsidiary cells of the stomata were with randomly distributed chloroplast and the surface had wavy, continuous striations. However, striations were absent in juvenile leaf impressions. Stomatal frequency (cv. I: upper surface- 29.13 ± 3.4 , AMV 29.17 ± 2.7 , NMV 22.10 ± 1.8 ; lower surface- 50.47 ± 4.2 , AMV 54.0 ± 3.3 , NMV 48.47 ± 2.2 ; cv. II: upper surface- 29.4 ± 1.6 , AMV 29.73 ± 2.1 , NMV 27.7 ± 0.9 ; lower surface- 58.63 ± 3.2 , AMV 48.57 ± 2.8 , NMV 52.53 ± 2.6), stomatal area (cv. I: upper- $537.9 \mu\text{m}^2 \pm 9.62$, lower- $807.5 \mu\text{m}^2 \pm 8.81$; cv. II: upper- $572.2 \mu\text{m}^2 \pm 7.74$, lower- $619.3 \mu\text{m}^2 \pm 7.44$) and pore area (cv. I: upper- $169.1 \mu\text{m}^2 \pm 4.48$, lower- $82.6 \mu\text{m}^2 \pm 3.69$; cv. II: upper- $59.0 \mu\text{m}^2 \pm 5.62$, lower- $39.8 \mu\text{m}^2 \pm 3.21$) were also assessed.

Meiotic Analysis

Meiotic chromosome behavior was nearly normal and same in both cultivars. The chromosome number recorded at MI was $2n=72$ (Figs. 13-14) always with mean association of $35.81 \text{ II} + 0.39 \text{ I}$ per cell (36 meiocytes scored) in cv. I and $35.8 \text{ II} + 0.34 \text{ I}$ per cell (35 PMCs analyzed) in cv. II. The predominant association was studied to be 36 II (cv. I- 86.1%; cv. II- 82.6%). The AI chromosome segregation was always (100.0%) equal (36/36) in the cultivars (cv. I- 27 cells scored; cv. II- 41 cells observed).

Pollen Fertility and Viability

Wider range but lower frequency of pollen fertility (cv. I: 21.7% to 50.0%; cv. II: 28.1% to 33.9%) was studied but pollen viability (Figs. 17a and 17b) was relatively higher following aniline blue (cv. I: 36.0% to 37.1%; cv. II: 31.9% to 35.9% - detects the presence of callose) and lugols (cv. I: 74.2% to 82.9%; cv. II: 62.0% to 72.6% - detects the presence of starch) staining.

Pollen Morphology Following Acetolysis Technique

Pollen grains were larger in size in cv. I ($153.7 \pm 2.95 \times 147.6 \pm 2.06 \mu\text{m}^2$) than cv. II ($129.2 \pm 1.99 \times 121.9 \pm 1.94 \mu\text{m}^2$; size variations evident- Fig. 15) and they were prolate-spheroidal, pantoporate and isopolar in nature (Fig. 16). Pores (cv. I: number >30 /pollen grains, pore diameter $7.5 \mu\text{m}$; cv. II: number >25 /grains, diameter $7.0 \mu\text{m}$) large, rounded to oval. Exine thick (cv. I: $5.0 \mu\text{m}$; cv. II: $6.5 \mu\text{m}$), tectum echinate, spines (cv. I: number >60 , base $6.0 \mu\text{m}$ wide, length $30.0 \mu\text{m}$, interspinal distance $35.0 \mu\text{m}$; cv. II: number >50 , base $4.7 \mu\text{m}$ wide, length $22.0 \mu\text{m}$, interspinal distance $20.0 \mu\text{m}$) many with blunt to rounded apex and broadened base. Tectum granulate in between spines.

Masters²¹ reported 2 varieties (*multiformis* and *betulifolius*; based on leaf shape primarily) in *A. moschatus* and subsequently Hochreutiner²² added 2 more varieties in the species (*genuinus* and *rugosus*). Brossom-Walkers²³ recognized 3 subspecies in *A. moschatus*, viz., subsp. *moschatus*, subsp. *biakensis* (Hochr.) Bross. and subsp. *tuberosus* (span.) Bross., of which only subsp. *moschatus* was reported from India and was corroborated by Sivaranjan and Pradeep²⁴ from their taxonomical studies on Malvaceae. Hamon and van Sloten²⁵ suggested that the genus *Abelmoschus* appears as a regular series of polyploids with $x=12$, chromosome number varying from 14 to 97, *A. moschatus* had $n=36$. Thus it is apparent that the cultivars studied were *A. moschatus* var. *moschatus*, and the 2 cultivars (the traits were uniform and the plants bred true) varied widely between themselves in relation to the parameters studied. It would be logical to identify cv. I as *tall branched*

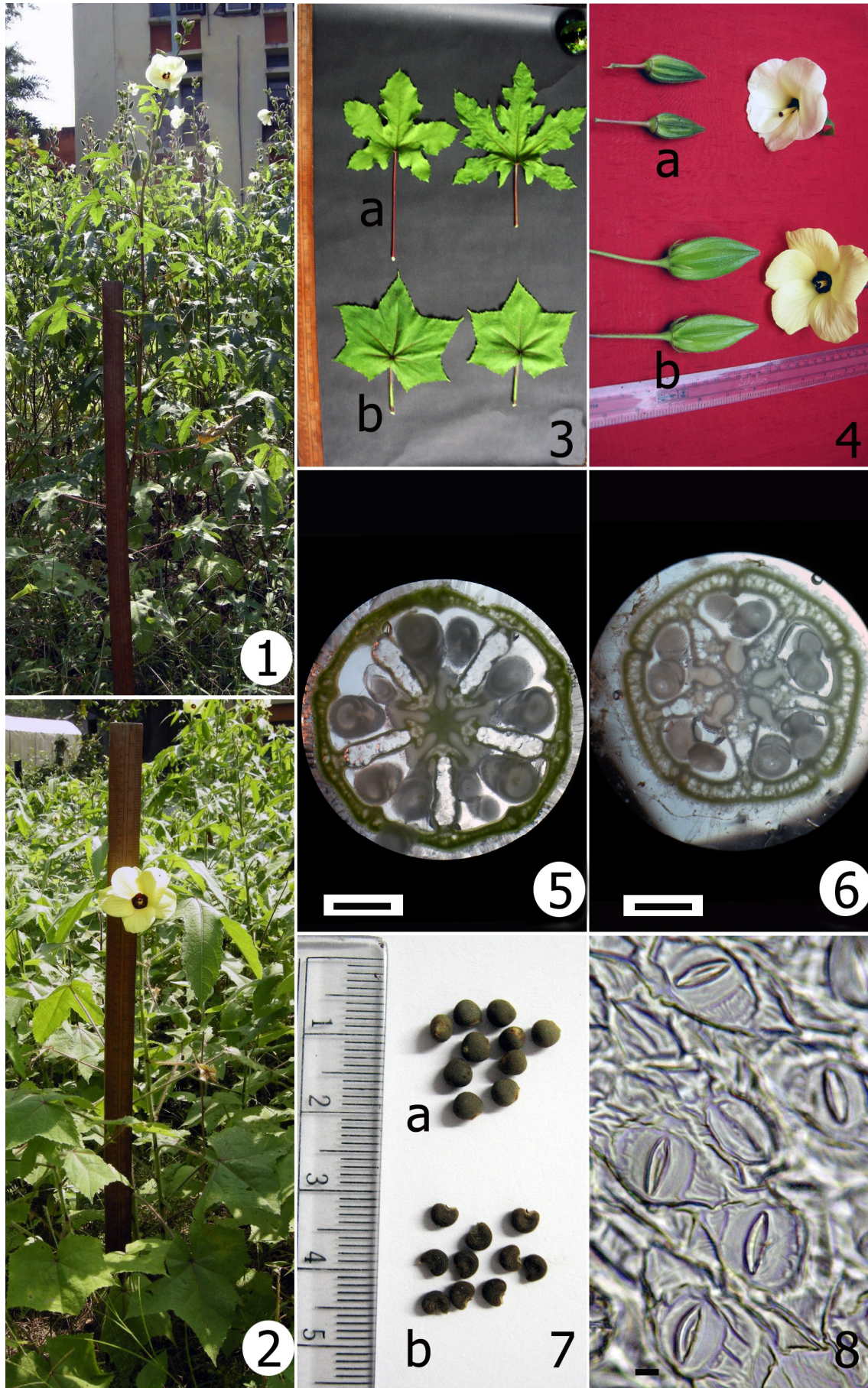
whereas cv. II as *dwarf*, *unbranched* types and these 2 cultivars possibly enrich pharmacological research.

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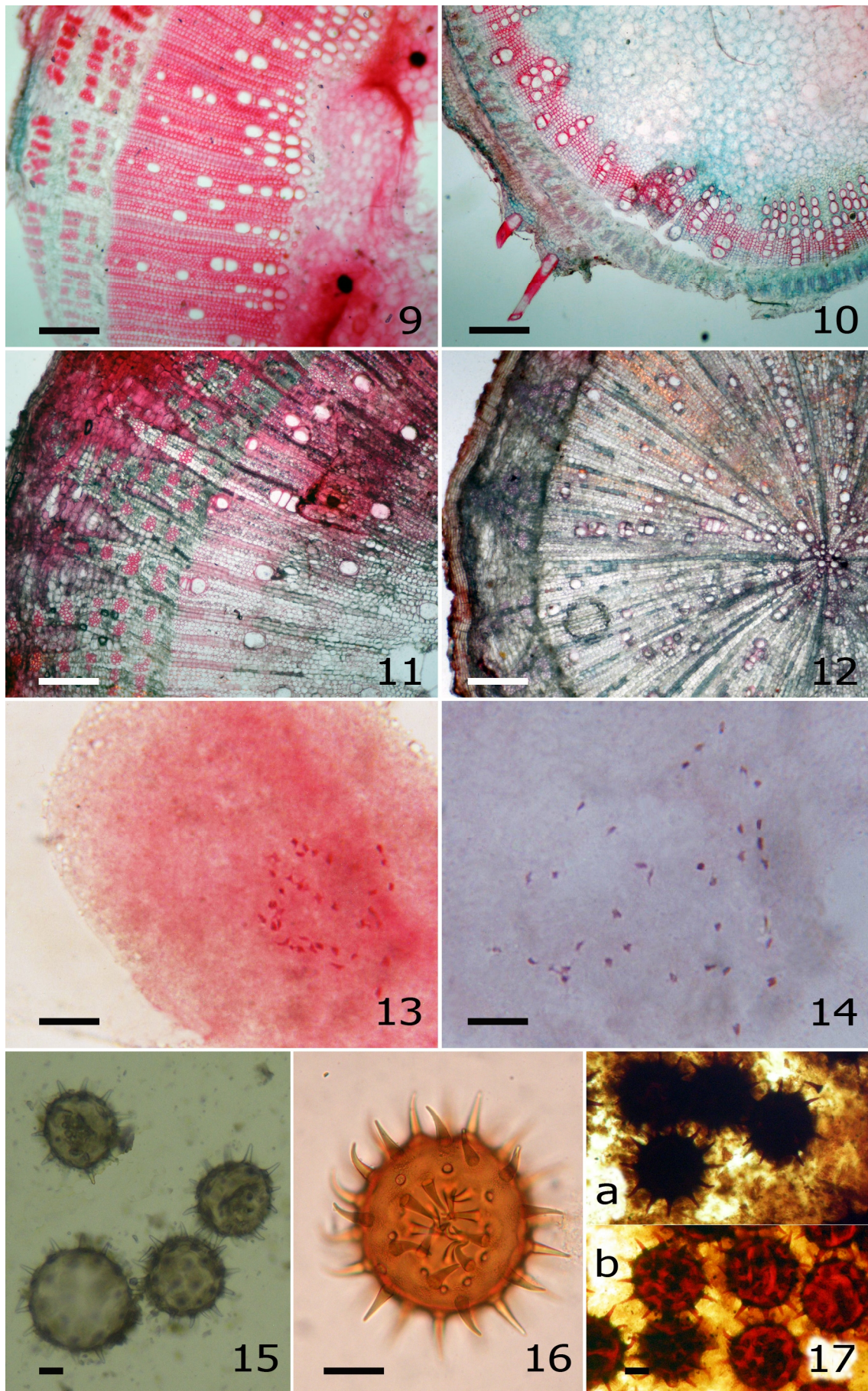
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Figs. 1-8. *A. moschatus*: 1-2. Cultivar I and II at flowering stage respectively. 3. Leaves of cv. I (a) and II (b). 4. Flowers and fruits of cv. I (a) and II (b). 5-6. T.S. of ovary of cv. I (5) and II (6). 7. Seeds (cv. I- a, cv. II- b). 8. Paracytic type of stomata in the cultivars. Scale bar = 2.5 μ m.



Figs. 9-17. *A. moschatus*: 9-10. T.S. of stem (cv. I- 9; cv. II- 10). 11-12. T.S. of root (cv. I- 11; cv. II- 12). 13-14. 36 II ($2n=72$) at MI 15. 15. Pollen grains showing size variations. 16. Acetolysed pollen grains. 17. Viable (a) and nonviable (b) pollen grains after lugol's staining. Scale bar: 9-14 = 2.5 μ m; 15-17 = 50 μ m.