



RESEARCH COMMUNICATION

Assessment of variability in *Asystasia gangetica* (L.) T. Anderson from the Western Ghats of Kerala, India

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Abstract

The variability shown by *Asystasia gangetica* (L.) T. Anderson has been thoroughly analyzed by considering gross and micromorphology. The species shows variability in flower color and leaf shape among the accessions collected from different geographical locations. However, the microspore sculpturing was found to be uniform and the seed surface architecture showed variation in one of the accessions as well as *A. gangetica* var. *krishnae*.

Keywords

Intraspecific variability, pollen morphology, spermoderm architecture

Introduction

The genus *Asystasia* (1), belonging to the family Acanthaceae, is represented by more than 70 species, distributed in the Old World (2). The term *Asystasia* means inconsistency, which is related to more or less regular corolla. The inconsistency in corolla is unusual in the family Acanthaceae. As per the latest reports (3), there exist 9 species and 3 varieties in India, of which 6 species are from Kerala. *A. gangetica* is the most common species in the genus. There occurs a variety of flower colors in the species, ranging from white, yellow and purple as well as variously blotched patterns. The tubular flowers have a roughly bilipped corolla, ovate leaves, didynamous yellow-colored stamens and a yellow to green-colored gynoecium.

During the field explorations, the authors collected different accessions of *A. gangetica* showing varied flower colors (Accessions 1-7) and *A. gangetica* var. *krishnae* from various geographical regions. The vegetative and floral analysis of all the color variants showed similar features except for pubescence on stem, shape of leaf and size of flowers in some accessions. A total of 50 individual specimens have been examined for each accession. The vegetative, floral as well as micromorphological features of seed and pollen have been considered for evaluating the variability.

Materials and Methods

The present investigation was carried out in Plant Systematics Laboratory, Department of Botany, University College, Thiruvananthapuram, based on field explorations, examination of herbarium specimens at BSI, CAL, MH, UCBD and TBGT (acronyms after Thiers (4)) and analysis of relevant literature (5-10). Plants from 10 populations of each color variant of *A. gangetica* and the variety *krishnae* were analyzed to elucidate and conclude veg-

etative and floral features. Detailed micromorphology of seeds and pollen were analyzed for comparative studies.

Morphological comparison was made using the traits viz. habit, height of plant, color of stem, leaf shape and size, length of internode. The floral traits included size and color of flowers, number of flowers in the peduncle, sepals, corolla, androecium, gynoecium and fruit. Micromorphology of seed and pollen were studied using SEM (Carl Zeiss EVO 18). Seeds were mounted directly on aluminium stub using double sided adhesive tape and pollen was dusted on the stub. The mounted pollen and seed were sputter coated with gold-palladium. The SEM micrographs were taken for analysis. The micromorphological features of seed and pollen remained more or less uniform.

Taxonomic treatment

Asystasia gangetica (L.) T. Anderson, Enum. Pl, Zeyl.: 235 (1860). (Synonym: *Justicia gangetica* L., Cent. Pl. II : 3 (1756); *Ruellia gangetica* (L.) R. Br., Prodr. Fl. Nov. Holland.: 478 (1810) (6, 11, 12)).

Habitat

The plants grow in waste lands, along forest outskirts and roadsides with 5-20 individuals per population and sometimes in more numbers forming somewhat larger patches.

Phenology

January to June.

Distribution

Asystasia gangetica is known from almost all districts. We could observe the variety *krishnae* from 3 districts

(Thiruvananthapuram, Kollam and Kasaragod). The associated taxa include *Synedrella nodiflora*, *Ipomoea* sp. and *Dicanthelium* sp.

Results & Discussion

The accessions collected (Fig. 1, 2, Table 2) have been subjected to taxonomic analysis. Leaf shape ranged from ovate to orbicular (Accession 1-6), roughly cordate and ovate to lanceolate leaf in 2 accessions (Accession 7 and 8 respectively). The size of the leaf ranges from 3-6 x 2.3-4.6 cm in all accessions. Length of the inflorescence showed a range from 5-15 cm (Accession 1-6) and 12-25 cm (Accession 7 and 8). The flower color of accessions varying from yellow (Accession 1), white (Accession 2), pale purple (Accession 3), corolla white with violet blotch on lower middle lobe (Accession 4), corolla yellow with dark purple blotch on lower middle lobe (Accession 5) corolla light lavender with dark purple blotch lower middle lobe (Accession 6) purple (Accession 7), corolla white with violet blotch on lower middle lobe (Accession 8). Maximum number of flowers per inflorescence was observed for *A. gangetica* with purple flower (Accession 7), minimum number of flowers per inflorescence was observed for *A. gangetica* having pale purple flower and *A. gangetica* having yellow corolla with dark purple blotch on lower middle lobe (Accession 3 & 5) and all other accession show intermediate range. The yellow colored flowers range in size from 3-4.9 x 1.2 -3 cm (Accession 1). Accession 2-7 show a size ranges from 4-5 x 1.2-4 cm. *A. gangetica* var. *krishnae* (Accession 8) have small sized flowers (1-1.5 x 1.8-2 cm). Androecium, gynoecium and bract of the collected accessions of *A. gangetica* don't show marked vari-

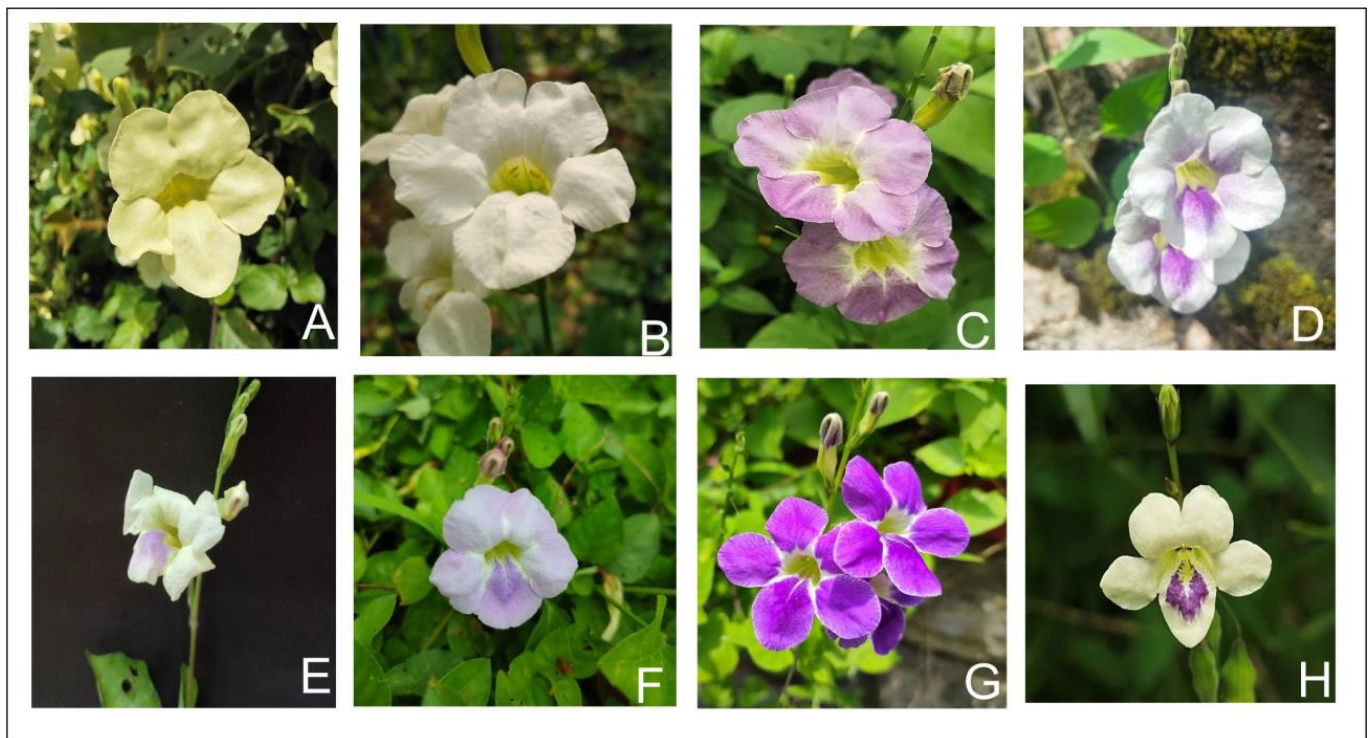


Fig 1. Flower color variation shown by different accessions *Asystasia gangetica*. **A.** Accessions 1 (*A. gangetica*), **B.** Accession 2, **C.** Accession 3, **D.** Accession 4, **E.** Accession 5, **F.** Accession 6, **G.** Accession 7, **H.** Accession 8 (*A. gangetica* var. *krishnae*).

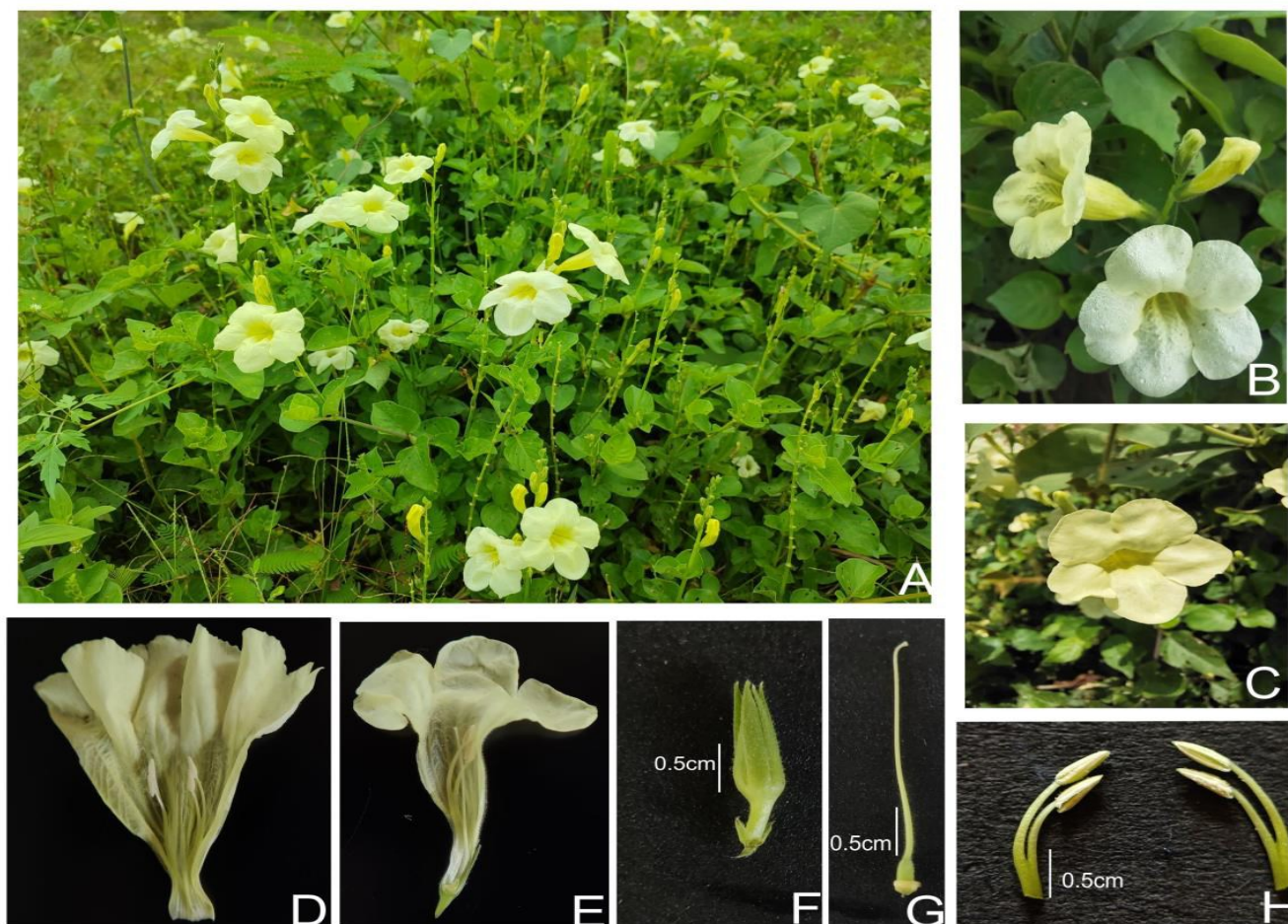


Fig 2. Habit and floral features of *Asystasia gangetica*. **A.** Habit, **B.** Inflorescence, **C.** Flower, **D.** Corolla split opened, **E.** L.S of flower, **F.** Calyx, **G.** Gynoecium, **H.** Stamens.

ation with respect to color, pubescence and size - longer stamen 0.8 -1.3 cm long; shorter stamen 0.5 - 1.2 cm long and length of gynoecium ranges from 1-2.7 cm long, but *A. gangetica* var. *krishnae* have vertical black - blue bands on anther and bract is violet to purple in color. The size of the fruit ranges from 2.5 - 3.1 cm long and the seeds ranges from 0.3-0.4 cm across (Table 1).

Pollen grains are prolate, tricolporate with radial symmetry and reticulate to perforate surface in all accessions of *A. gangetica* and *A. gangetica* var. *krishnae*. Thus, the palynological traits are more or less consistent. The seed surface architecture shows a uniform pattern having raised patches with tubercles all over the surface in most of the accessions except for Accession 7 and *A. gangetica* var. *krishnae*. The seed surface of Accession 7 has eruptive patches of irregularly varying sizes having highly convoluted surface without tubercles. *A. gangetica* var. *krishnae* (Accessions 8) have an entirely different surface architecture with narrowly ovate -polygonal shaped cells in a compact reticulate pattern having sparsely distributed tubercles (Fig. 3).

Reports are on the factors behind the maintenance of flower color variations within populations (13). Flower color is traditionally considered as an adaptation to pollinator visual perception. Non pollinator agents of selection are likely to play a role in populations exhibiting either discrete or continuous color variations.

Generally, presence or absence of anthocyanins affects the coloration of the floral display and this is related to flavonoid biosynthetic pathway. In *Clematis patens*, the anthocyanins for flower color variations have been identified as delphinidin and cyanidin glycosides (14). Reports are on the detection of anthocyanin and 4 flavonols from the petals of 3 species of *Hibiscus* (15). Further, CHS- D is suggested as the CHS gene solely responsible for anthocyanin production in the floral limbs (16, 17). The biochemical pathways behind the flower color phenotypes in *Ipomoea purpurea* has been traced from the molecular and genetic levels and suggested the possibility to associate discrete floral phenotypes with individual genes (16). In *Limonium wrightii*, intraspecific variation of flower color and its distribution has been evaluated (18). Allopatric geographical distribution has been recorded for the frequently observed pink and yellow flower color morphs. Further, the observed orange flower color morphs has been suggested as the hybridization product of pink and yellow flower color morphs. The flower color variations in other plant taxa has also become the subject of research (19). Recently, the flower color variations in *Impatiens balsamina* has reported usage as alternative ecofriendly natural acid- base indicators (20).

Basic flower color in *A. gangetica* is governed by 3 sets of genes-P (producing purple pigments in the inner epidermis of the limb), B (producing purple in the outer epidermis of the limb and back of the tube) and Y (producing yellow

Table 2. Collection sites

Accession	District	Location	Coordinates	
Accession 1	Kollam	Kulathupuzha	8° 54' N & 77° 3' E	
		Thiruvananthapuram	Attingal	8°41' N & 76°48' E
			Nedumangad	8° 36' N & 77° 0' E
			Maranallur	8° 28' N & 77° 4' E
	Ernakulam	Meenachal	9°37' N & 76° 46' E	
	Thrissur	Athirappally	10°17'N & 76°34'E	
	Kozhikode	Thalassery	11°45'N & 75°29'.E	
		Nadapuram	11°42'N & 75°40'E	
	Alappuzha	Chengannur	9°18.98'N & 76° 37.0'E	
	Idukki	Adimali	10° 0' N & 76° 57' E	
Kottayam	Meenachal	9°37' N & 76° 46' E		
Kasaragod	Mallam	12° 32' N&74° 58' E		
Accession 2	Idukki	Idukki	9°51' N & 76°58' E.	
	Kollam	Punalur	9° 01' N & 76° 55' E	
	Kottayam	Kottayam	9° 35' N & 76° 31' E	
	Ernakulam	Neriamangalam	10°30' N &76°47' E	
Kasaragod	Mallam	12° 32' N&74° 58' E		
	Kasaragod	Neeleswaram	12° 20' N & 75° 6' E	
Thiruvananthapuram	Vamanapuram	8°43' N & 76°54' E		
	Vayyakkal	8°40' N & 76°52' E		
Accession 4	Kasaragod	Cheruvathur	12°19' N & 75°04'E	
	Idukki	Kattapana	9° 45' N & 77° 7' E	
	Kollam	Thenmala	8° 57' N & 77° 3' E	
Kadakkal		8° 49' N & 76°55'E		
Accession 5	Thiruvananthapuram	Karyavattam	8° 34' N & 76° 53' E	
		Neyyar	8°33' N & 77°12.5' E	
Accession 6	Kollam	Pathanapuram	9° 5.45' N & 76° 51.46' E	
		Kottarakara	8° 59.8' N & 76° 46.5' E	
	Pathanamthitta	Pathanamthitta	9° 15' N & 76° 47' E	
Accession 7	Thiruvananthapuram	Kattakkada	8°30' N & 77°04' E	
		Kariavattam	8° 34' N & 76° 53' E	
		Manacaud	8° 28.4' N & 76° 56.8' E	
Accession 8	Kollam	Thenmala	8° 57' N & 77° 3' E	
		Kulathupuzha	8° 54' N & 77° 3' E	
	<i>A.gangetica</i> var. <i>krishnae</i>	Kasaragod	Cheruvathur	12°19' N & 75°04'E
Thiruvananthapuram		Vidyanagar	12° 35' N&75° 0' E	

in the entire limb) which are independent of one another and inherited in a simple Mendelian fashion (21). The genetic variability among the various flower color morphs of *A. gangetica* from Thailand has been analysed using RAPD markers (22) and the study concluded the existence of high level of genetic variability among the selected specimens. The UPGMA dendrogram obtained in the study separated the flower color morphs into various clusters. Apart from these earlier reports on the fundamental genetics and genetic variability, no other studies have been carried out so far regarding the infraspecific color variability in the flowers of this taxon.

Conclusion

Though earlier attempts have been made on the fundamental genetics and preliminary molecular screening of the flower color morphs of *Asystasia gangetica* (L.) T. Anderson, present investigation is the first of its kind regarding the infraspecific variability in terms of qualitative and quantitative data on the vegetative as well as floral traits. The study mainly targets the macro morphological as well as micro morphological features of the various flower color morphs collected from the state of Kerala. Significant variations were not observed except the flower color in the studied collections. However, 2 specimens showed variability with respect to micromorphological traits of spermoderm architecture irrespective of the common palynological features shown by all the specimens. The cytological and molecular studies using reliable markers along with profiling of the floral pigments of the flower color morphs are in progress. These studies are expected to give a better understanding of the color variability exhibited by the flowers of *A. gangetica*.

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Authors contributions

The authors contributed equally to the present work.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

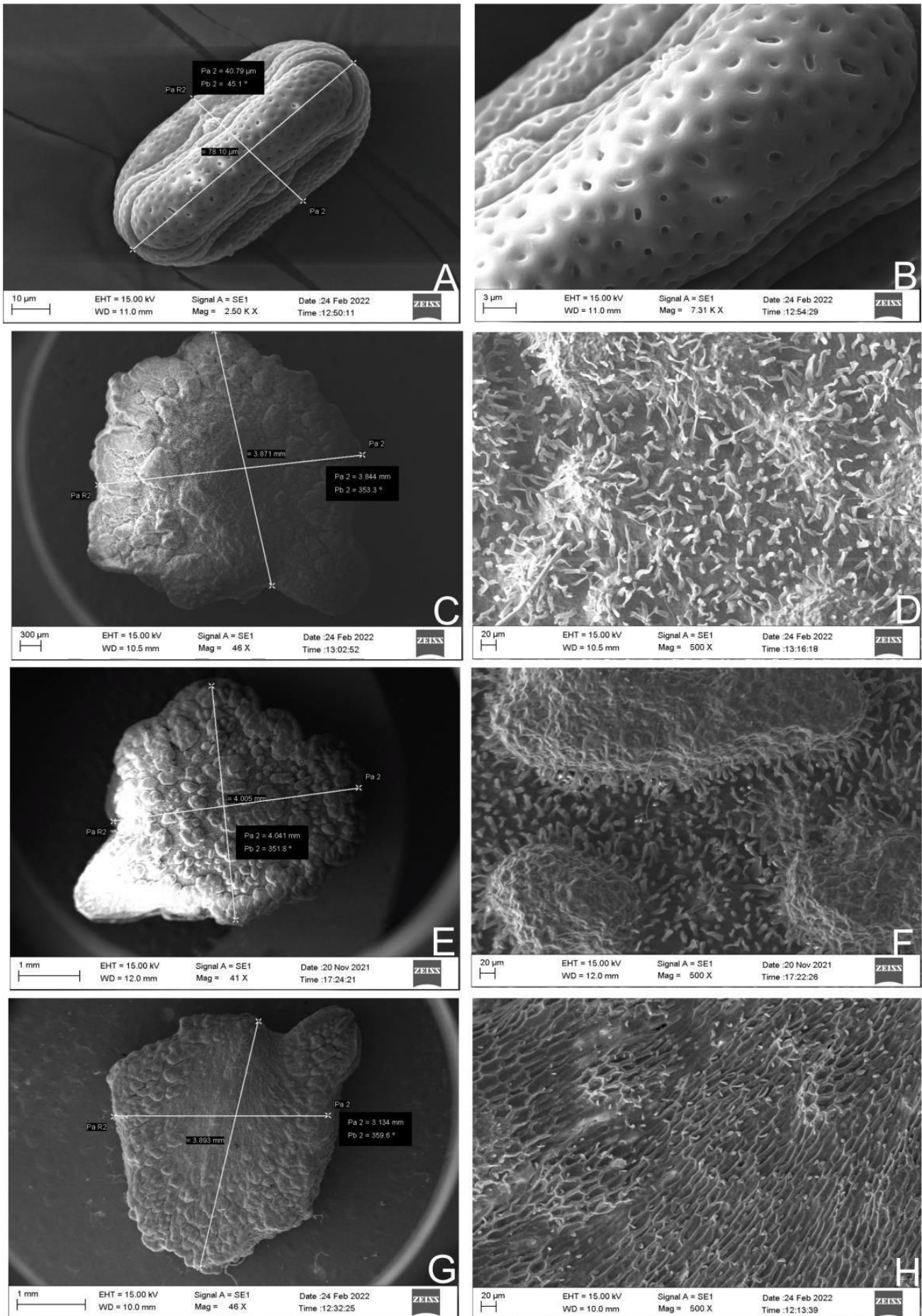


Fig 3. Micromorphological characters of *Asystasia gangetica* as revealed by SEM analysis of pollen and seed. **A.** Pollen grain, **B.** zoomed out view of pollen surface, **C.** Seed of *A. gangetica*, **D.** Seed surface- enlarged view, **E.** Seed (*A. gangetica* accession 7), **F.** Enlarged surface view of the seed of accession 7, **G.** Seed of *A. gangetica* var. *krishnae* (accession 8), **H.** Enlarged.

Table 1. Cluster means for characters in 100 SA induced ricae mutants and their parent

Charac ters	Accession1 (<i>A.gangetic</i>)	Accession2	Accession3	Accession4	Accession5	Accession6	Accession 7	Accession8 (<i>A.gangetica</i> <i>var.krishnae</i>)
Habit	Herb 45-60 cm in height	Herb 45-50 cm in height	Herb 50-55 cm in height	Herb 40-45 cm in height	Herb 45-55 cm In height	Herb 50-60cm in height	Herb 40-50 cm in height	Subscandent herb 40-50cm in height
Flower color	Yellow	White	Pale purple	Corolla white with violet blotch on lower middle lobe	Corolla yellow with darkpurple blotch onlower middle lobe	Corolla light lavender with darkpurple blotch lower middle lobe	Purple	Corolla white withviolet blotch onlower middle lobe
Stem	Quadrangular stem with prominent groove, green reddish brown,occasio nally pubes- cent stem. Hairs at node	Quadrangular stem with prominent groove, red- dish brown at node. Hairs at node	Quadrangular stem with no prominent groove, red- dish brown at nodes Hairs at node	Quadrangular stem with prominent groove, reddish brown- green color. Hairs at node	Quadrangular stem with no prominent groove, reddish brown atnodes Hairs at node	Quadrangular stem with grooves, stem greensparsely pubescent. Hairs at node	Quadrangul ar stem with lesspromi- nent groove, brownish green. Hairs at node	Quadrangular stem with no prominent grooves, red- dish brown at nodes. Hairs at node
Internodal length	8-16.7 cm	3.5-10.5 cm	5.8-12.6 cm	3-5cm	5.8-12.6 cm	8-17cm	5-7 cm	2-11.5 cm
Leaf shape Tip	Ovate tocor- date Acuminate	Ovate Acuminate	Ovate Acute-acuminate	Ovate Acute	Ovate - orbicular Acuminate	Ovate Acute- acuminate	Cordate Roughly cuspi- date	Ovate -lanceo- late Acuminate Sub cordate
Base	Ovate to round	Ovate	Round-cune- ate	Round	Ovate	Round	Sub cordate - round, Oblique	
Size	3-5.1 x 2.8 - 4.1cm	4.1-6 x 3.2-3.9 cm	3.5-6 x 3.4-4.1cm	3.5-4 x 2.5-3 cm	3.6-4.9 x 3-3.8 cm	4.5-5.6 x 3.8-4.6cm.	3.8-4.2 x 3 - 3.5cm	2-8.6 x 2.3-4.5cm
Petiole	Green 0.7-2.8cm	Green 1.8-3cm	Green 1.5-2.8cm	Green 0.5-2cm	Green 1.5-2cm	Green 1.8-3cm	Green 1.5-2cm	Green 0.2-0.65cm
Vein	5-6 pair	5-6 pair	5-6 pair	5-6 pair	5-6 pair	5-6 pairs	4-6 pair	5-8pair
Inflore scence	One sided raceme, 5-12 flower	One sided raceme,6- 8 flower	One sided raceme, 5-8flower	One sidedra- ceme, 7-9flower	One sided raceme , 5-8 flower Pubescent	One sided raceme, 7-11 flowers,	Raceme, 17-22 flowers, alternate	One sided rceme, 6-12flowers
Peduncle	5-14.5 cm	6-12.9 cm	7-13cm	8-11cm	6.5-12cm	6.5-15cm	14-20 cm	12-25cm
Flower size	3-4.9 x 1.2 -3cm	4.5-5 x 1.2-1.3cm	4.5-4.8 x 3-3.2cm	4.5-5 x 3.5-4cm	4-4.8 x 3 - 3.2cm	4-4.2 x 2.8 - 3cm	4.5-4 x 2.5 - 3	1-1.5 x 1.8-2 cm
Sepal	5,green, pu- bescent, linear -lanceolate, upto 1cm long	5,green, pu- bescent,lanceo- late,up to 0.8 cm long	5,green, densely pu- bescent,linear- lanceolate upto 0.8cm long	5,green,pubece- nt,linear lanceo- late, upto 0.8 cm long	5,green,densely pubecent,linear- lancelate,upto 0.7cm long	5,green, pubes- cent,linear- lanceolate, upto 0.8cm long	5,green, dense- ly pubeulant, linear lanceo- late, upto 0.7cm long	5,green, densely pubescent, linear- lanceo- late 0.3cm

Pedicle	Green, 0.2-0.5 cm	Green, 0.3-0.5 cm	Green, 0.7-0.75 cm	Green, 0.7 - 0.8 cm	Green 0.5- 0.7 cm	Green, 0.2-0.5 cm	Green, 0.1-0.2 cm	Green 0.5- 0.1 cm
Bract	Small,hairy, lanceolate,green	Small,hairy, lanceo- late,green	Small,hairy, lanceolate,green	Small, hairy, lanceolate, green	Small,hairy, lanceo- late,green	Small,hairy lanceolate, green	Small,hairy, lanceolate,green	Small,hairy, violet-purple
Stamen	Longer, up- to1.3 shorter upto 1.2 cm; pubescent on antherand filament, pale yellow	Longer, up to 1.2shorter up to 1cm; pubescent on antherand filament, pale yellow	Longer, upto 1.3 shorterupto 1.1cm ; pubescent on anther and fila- ment, pale yel- low	Longer, up to 1.1shorter upto 0.8cm; pubescent on anther and filament, pale yellow	Longer, up to 1.2shorter upto 1 cm; pubescenton anther and filament, pale yellow	Longer,upto 1cm shorter up to 0.8 cm, pubescent on anther and filament, pale yellow	Longer, upto 0.8 shorter upto 0.7 cm; pubescent on anther and filament, pale yellow	Longer, upto 0.8 shorter up to 0.5 cm; pubescent on anther and filament, vertical violet- black bands
Gynoece- ium	Up to 2.7cm long, pale yellow to green, base portion of style and ovary pu- bescent	Up to 2.2 cm long, pale yellow to green, base portion of style and ovary pu- bescent	Up to 2.6cm long, Pale yellow to green, base portion of style and ovary pu- bescent	Up to 2.6 cm long, pale yellow to green, base portion of style and ovary pubes- cent	Up to 2.7 cm long, pale yellow to green, base portion of style and ovary pubes- cent	Up to 2.5cm long, pale yellow to green, base portion of style and ovary has pubescence	Up to 2cm long, pale yellow to green, base portion of style and ovary pubescent	Up to 1cm long, pale yellow to green, base portion of style and ovary pubescent
Fruit	Capsule, up to 3cm long, 2-4 seed, 0.35cm across	Capsule, up to 2.7cm long 2-4 seed, 0.3cm across	Capsule, up to 3cm long 2-4 seed, 0.4cm across	Capsule, up to 2.9cm long 2-4 seed, 0.35cm across	Capsule, up to 3.1cm long 2-4 seed, 0.3cm across	Capsule, up to 2.7cm long 2-4 seed, 0.3cm across	Capsule, up to 2.8cm long, 2-4 seed, 0.35cm across	Capsule, up to 2.5cm long, 2-4 seed, 0.35cm across

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