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Use of image based pollen morphology of cultivated cotton species (*Gossypium* sp) for the establishment of distinctiveness

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Abstract : In this study, the image-based pollen morphology of the four species of cotton was examined to know the variations among cotton cultivars within a species and between species. To explore the variation among cultivars and species, the pollen morphological traits like pollen area, perimeter, radius, length, width and height and filament length were subjected to descriptive statistics and cluster analysis. Among the four cultivated species, G. *barbadense* recorded higher values for all the pollen parameters; however, higher values for filament length were noted in G. *hirsutum*. Cluster analysis formed three clusters which were Cluster I for G. *barbadense*, cluster II for G. *hirsutum* and cluster III was occupied by diploids (G. *arboreum* and G. *herbaceum*).

Keywords: Cluster analysis, diploids, variation, Image analysis, pollen image, tetraploids

Cotton is the "King of Fibres" cultivated across the continent for its fibre. The cotton fibres are economically important, has been used to spun, woven and dyed since prehistoric times. Botanically four economically important cotton species are cultivated; *viz.*, *Gossypium arboreum* (tree cotton), *G. barbadense* (American pima cotton), *G. herbaceum* (levant cotton) and *G. hirsutum* (American upland cotton) (Brubaker, 2002).

These species have two different origins. Gossypium herbaceum and *G. arboreum* are Old World cottons that originated from the African-Arabian gene pool and they are both diploid species. *Gossypium hirsutum* and *G. barbadense* are New World species and are indigenous to Mesoamerica and South America and they are polyploid (Saad, 1960).

Cotton pollen grains are large, spherical, echinate, and porate having a ring of pores. Each cotton flower produces an average of 30,000 to 40,000 pollen grains, representing a mass of 19 to 26 mg of fresh pollen (Vaissiere and Vinson, 1994). The morphological characteristics of pollen grains are very much useful for the study of plant taxonomy because pollen traits are strongly influenced by the selective forces involved in the reproductive processes, including pollination, dispersal, and germination (Erdtman, 1952; Moore *et al.*, 1991; Stuessy, 1990). At the same time, characters subject to strong selection can mislead if they reflect convergent evolution – similar evolutionary responses by unrelated taxa to similar environmental conditions. Thus, the use of pollen morphology as a taxonomic character is challenging and pollen characteristics must be considered in concert with other characteristics in evolutionary reconstructions.

Earlier research conducted elsewhere in this context with examination of pollen grains of the Malvaceae have shown a key that can differentiates the four economically important species of *Gossypium* (Jones and McCurry, 2012). For the establishment of Distinctiveness, Uniformity and stability of a cotton variety on the basis of twenty-two essential characteristics which were defined in the National test guidelines published by the Protection of Plant Varieties and Farmers Rights Authority. However, there is a raising demand for incorporation of newer stable characteristics in the list of essential characteristics as to provide border space for a genotype to establish its distinctiveness by itself and thereby get protected under PPV and FR Act, 2001. Keeping this in mind the present attempt has been made to explore the range of variations that exists in pollen characteristics among the tetraploids and diploids and to find out the feasibility of using these characteristics for the classification of cotton genotypes.

MATERIALS AND METHODS

Pollen grains from 20 extant cotton varieties, five each from the cultivated species that were grown during winter 2016-2017 at Central institute for Cotton Research, Coimbatore under DUS trial formed the material for the present study. The varieties included were Deviraj, G. Cot.12, G. Cot.10, LRA 5166 and Pratima (*G. hirsutum*); NCB 578-1, Sujatha, Suvin, SWCH 5017BGII and TCB 209 (*G. barbadense*); AAH1, AKA7, Aravinda, PA255 and Phule anmol (*G. arboreum*); G. Cot.23, DDHC11, Jayadhar, K11 and Ragavendra (*G. herbaceum*). During fifty per cent flowering anthers were collected in butter cover and used for measurement of filament length, pollen grain area, and perimeter of pollen, radius, length, width and pollen grain height under stereo zoom microscope (RADICAL-RSMr-10) with 80X magnification using IS capture software and expressed in micrometer. Ten measurements were taken for all the pollen traits with pollen grains taken from randomly collected flowers in each variety. The observed data were subjected to descriptive statistics and cluster analysis using Past software and the results are presented below.

RESULTS AND DISCUSSION

The data thus generated were subjected to analysis of variation that revealed significant differences among the genotypes for filament length, pollen grain area, and perimeter of pollen, radius, length, width and pollen grain height. The mean, standard deviation, maximum, minimum and range of values for four species of cotton are presented in Table 1 and 2. The polymorphism was observed among the species of cotton for filament length which ranges from 60-242 µm long. Among the four species, *G. hirsutum* had the longest filament length followed by *G. barbadense*, *G. herbaceum* and *G. arboreum*.

The area of pollen grain ranged from 19 to 48 µm2. Pollen perimeter ranged from 17 to 24 µm, radius of pollen grain 2 to 3 µm, length of pollen grain 4 to 7 µm, width of pollen 4 to 7 µm and pollen grain height 1 to 3 µm. Among the species *G. barbadense* expressed the largest area, perimeter, radius, length, width and height followed by *G. hirsutum*, *G. arboreum* and *G. herbaceum*. Similar observations were reported from the earlier study of Saad (1960) and Jones and McCurry (2012) and they have reported that *G. barbadense* and *G. hirsutum* had the largest pollen grains while *G. herbaceum* and *G. arboreum* had the smallest. This indicated that the genome size had a correlation with pollen size. Since, *G. barbadense* and *G. hirsutum* belong to tetraploids and *G. herbaceum* and *G. arboreum* diploids. Saad (1960) also reported that the difference in the size of the pollen grains among the different species of *Gossypium* can be due to the differences in chromosome number.

Frequency distribution

The frequency distribution of morphological data of pollen characters shows that across the four species of cotton, anther filament length had shown the highest variation. The anther filament length was distributed in normal pattern among genotypes of tetraploids, where as reduced length was noted among the genotypes of diploid cotton. The pollen area measured was larger and normally distributed in *G. barbadense* when compared to *G. arboreum* and *G. herbaceum*. The mean data of pollen width and length fall on the similar pattern of pollen area. Such level of distribution may help to find out the amount of variation that exists among cotton species (Fig. 1 and 2).

Morphological observation of colouration of filament length and pollen colour was also

Table 1. Descriptive statistics of pollen morphology of different varieties of cotton species

Species/Variety	Filament length (μm)					Area(μm^2)					Perimeter (μm)					Length(μm)					Width(μm)				
G. hirsutum	**Mean**	Min.	Max.	Range	SD	**Mean**	Min.	Max.	Range	SD	**Mean**	Min.	Max.	Range	SD	**Mean**	Min.	Max.	Range	SD	**Mean**	Min.	Max.	Range	SD
Deviraj	**151.6**	107	195	88	26.02	**31**	29	33	4	1.63	**18.90**	18	20	2	0.53	**6**	6	6	0	0.00	**5**	5	5	0	0.00
G.Cot. 12	**187**	134	242	108	33.94	**32.5**	30	37	7	2.12	**18.60**	17	20	3	0.57	**5.9**	5	6	1	0.32	**5.2**	5	6	1	0.42
G.cot.10	**160.4**	116	198	82	23.51	**29.9**	27	33	6	1.66	**17.50**	17	18	1	0.74	**5.8**	5	6	1	0.42	**5.5**	5	6	1	0.53
LRA 5166	**190.6**	165	214	49	15.84	**28.4**	24	35	11	2.95	**17.50**	17	18	1	1.18	**5.3**	5	6	1	0.48	**5.2**	5	6	1	0.42
Pratima	**167.6**	120	194	74	21.27	**32.1**	30	34	4	1.60	**17.50**	17	18	1	0.42	6	6	6	0	0.00	**5.7**	5	6	1	0.48
Mean	**171.44**	**128.4**	**208.6**	**80.2**	**24.12**	**30.78**	**28**	**34.4**	**6.4**	**1.99**	**18**	**17.2**	**18.8**	**1.6**	**0.68**	**5.8**	**5.4**	**6**	**0.6**	**0.40**	**5.32**	**5**	**5.8**	**0.8**	**0.54**
G. barbadense																									
NCB 578-1	**185.2**	150	229	79	26.82	**40.6**	34	48	14	3.60	**17.50**	17	18	1	1.15	**6.7**	6	7	1	0.48	**6.2**	6	7	1	0.42
Sujatha	**100.3**	86	124	38	11.64	**35.6**	30	38	8	2.59	**17.80**	15	19	4	0.70	**6**	6	6	0	0.00	**5.8**	5	6	1	0.42
Suvin	**74.4**	60	94	34	11.52	**40**	33	43	10	3.13	**18.40**	17	20	3	0.99	**6.8**	6	7	1	0.42	**6.1**	6	7	1	0.32
SWCH 5017BGII	**165.5**	149	199	50	16.39	**35.2**	30	38	8	2.57	**18.20**	18	19	1	0.67	**6**	6	6	0	0.00	**5.6**	5	6	1	0.52
TCB 209	**88.7**	67	104	37	12.75	**36.9**	30	41	11	3.14	**18.60**	18	19	1	0.94	**6.3**	6	7	1	0.48	**6**	6	6	0	0.00
Mean	**122.82**	**102.4**	**150**	**47.6**	**15.82**	**37.66**	**31.4**	**41.6**	**10.2**	**3.0**	**18.1**	**17**	**19**	**2**	**0.89**	**6.36**	**6**	**6.6**	**0.6**	**0.27**	**5.94**	**5.6**	**6.4**	**0.8**	**0.34**
G. arboreum																									
AAH1	**84.9**	64	117	53	17.63	**29.1**	26	32	6	1.52	**19.50**	19	20	1	0.57	**5.9**	5	6	1	0.32	**5.2**	5	6	1	0.42
AKA7	**116.9**	94	134	40	14.20	**28.5**	25	32	7	2.12	**19.90**	19	21	2	0.97	**5.5**	5	6	1	0.53	**5.1**	5	6	1	0.32
Aravinda	**98.4**	68	127	59	20	**25.5**	24	28	4	1.51	**19.10**	18	20	2	0.53	**5**	5	5	0	0.00	**4.8**	4	5	1	0.42
PA 255	**103.3**	82	128	46	13.17	**25.5**	23	28	5	1.78	**18.50**	17	21	4	0.53	**5**	5	5	0	0.00	**5**	5	5	0	0.00
Phule anmol	**100.9**	71	125	54	15.56	**25.4**	24	26	2	0.70	**19.80**	19	20	1	0.53	**5**	5	5	0	0.00	**5**	5	5	0	0.00
Mean	**100.88**	**75.8**	**126.2**	**50.4**	**16.11**	**26.8**	**24.4**	**29.2**	**4.8**	**1.53**	**19.36**	**18.4**	**20.4**	**2**	**0.63**	**5.28**	**5**	**5.4**	**0.4**	**0.17**	**5.02**	**4.8**	**5.4**	**0.6**	**0.23**
G. herbaceum																									
DDHC 11	**111**	92	129	37	11.85	**25.4**	23	28	5	1.35	**22.00**	20	24	4	0.53	**5.1**	5	6	1	0.32	**4.9**	4	5	1	0.32
G.Cot. 23	**108**	86	123	37	10.39	**26.4**	19	29	10	2.91	**20.60**	19	21	2	1.14	**5.1**	4	6	2	0.57	**4.9**	4	5	1	0.32
Jayadhar	**100**	78	135	57	17.98	**28.4**	25	31	6	1.71	**21.90**	20	23	3	0.84	**5.5**	5	6	1	0.53	**5.1**	5	6	1	0.32
K11	**143.7**	118	173	55	16.89	**27.7**	26	29	3	0.95	**20.70**	19	21	2	0.42	**5.4**	5	6	1	0.52	**5.2**	5	6	1	0.42
Ragavendra	**116.4**	84	153	69	19.52	**28.7**	27	30	3	0.95	**21.00**	19	22	3	0.52	**5.4**	5	6	1	0.52	**5**	5	5	0	0.00
Mean	**115.82**	**91.6**	**142.6**	**51**	**15.33**	**27.32**	**24**	**29.4**	**5.4**	**1.57**	**21.24**	**19.4**	**22.2**	**2.8**	**0.69**	**5.3**	**4.8**	**6**	**1.2**	**0.49**	**5.02**	**4.6**	**5.4**	**0.8**	**0.28**

Table 2. Descriptive statistics of pollen morphology of different species of cotton

Cotton species	Parameters	Anther filament length µm	Area µm2	Perimeter µm	Radius µm	Length µm	Width µm	Height µm
G. *hirsutum*	**Mean**	**171.44**	**30.78**	**19.36**	**2.92**	**5.80**	**5.36**	**1.52**
	Range	39.00	4.10	1.40	0.30	0.70	0.50	0.70
	Minimum	151.60	28.40	18.50	2.70	5.30	5.20	1.20
	Maximum	190.60	32.50	19.90	3.00	6.00	5.70	1.90
G. *barbadense*	**Mean**	**122.82**	**37.66**	**21.24**	**2.98**	**6.36**	**5.94**	**1.68**
	Range	110.80	5.40	1.40	0.10	0.80	0.60	0.60
	Minimum	74.40	35.20	20.60	2.90	6.00	5.60	1.30
	Maximum	185.20	40.60	22.00	3.00	6.80	6.20	1.90
G. *arboreum*	**Mean**	**100.88**	**26.80**	**18.00**	**2.36**	**5.28**	**5.02**	**1.26**
	Range	32.00	3.70	1.40	0.90	0.90	0.40	0.20
	Minimum	84.90	25.40	17.50	2.00	5.00	4.80	1.20
	Maximum	116.90	29.10	18.90	2.90	5.90	5.20	1.40
G. *herbaceum*	**Mean**	**115.82**	**27.32**	**18.10**	**2.56**	**5.30**	**4.98**	**1.30**
	Range	43.70	3.30	1.10	0.80	0.40	0.20	0.40
	Minimum	100.00	25.40	17.50	2.10	5.10	4.90	1.10
	Maximum	143.70	28.70	18.60	2.90	5.50	5.10	1.50

done. Among the 20 varieties, all tetraploid varieties had expressed absent for filament colour, however, in dipoids baring AAH1, DDHC 11, G.Cot. 23 others have filament colour. Similarly, the pollen colour of *G. barbadense*, *G. arboreum* and *G. herbaceum* are yellow in colour where as the varieties in *G. hirsutum* have expressed for cream colour except LRA 5166, which is yellow in colour (Fig.3 and 4).

Cluster analysis

The cluster analysis based on Ward's linkage method the dendrogram is presented in (Fig.5) using the past software. The dendrogram showed three distinct clusters based on pollen morphology. The cluster III was the largest comprising of 10 genotypes grouped into two subclusters cluster IIIa and IIIb consists of five genotypes each.

The cluster I is contributed mainly by *G. barbadense* which includes five varieties namely NCB 578-1, Sujatha, Suvin, SWCH 5017BGII and TCB 209; Likewise cluster II is occupied by *G. hirsutum* which consists of Deviraj, G. Cot.12, G.Cot.10, LRA 166 and Pratima; the cluster III is composed of diploid species which includes *G. arboreum* and *G. herbaceum i.e.*, AAH1, AKA7, Aravinda, PA255 and Phule anmol (*G. arboreum*); G.Cot. 23, DDHC11, Jayadhar, K11 and Ragavendra (*G. herbaceum*). The cluster III forms two subclusters which do not form distinct clusters for *G. arboreum* and *G. herbaceum* species.

CONCLUSION

The present findings will facilitate to look forward the range of variations that exists for pollen characteristics among the tetraploid and diploid cotton. Considerable level of variations for pollen morphology among the species and among varieties with in a species was observed. Utilising the variations in parameters like filament length, area of pollen grain, perimeter, radius, length, width and height of pollen grain, different states of a characteristic may be defined and these states will add a new characteristic in the list of existing characters of either tetraploid or diploid cotton. Addition of such new characteristic may increase the probability of establishing distinctiveness in a new candidate cotton variety while under DUS test. In this study the anther filament length was found to encompass greater variation in tetraploids than diploids. The cluster analysis also adjudged the

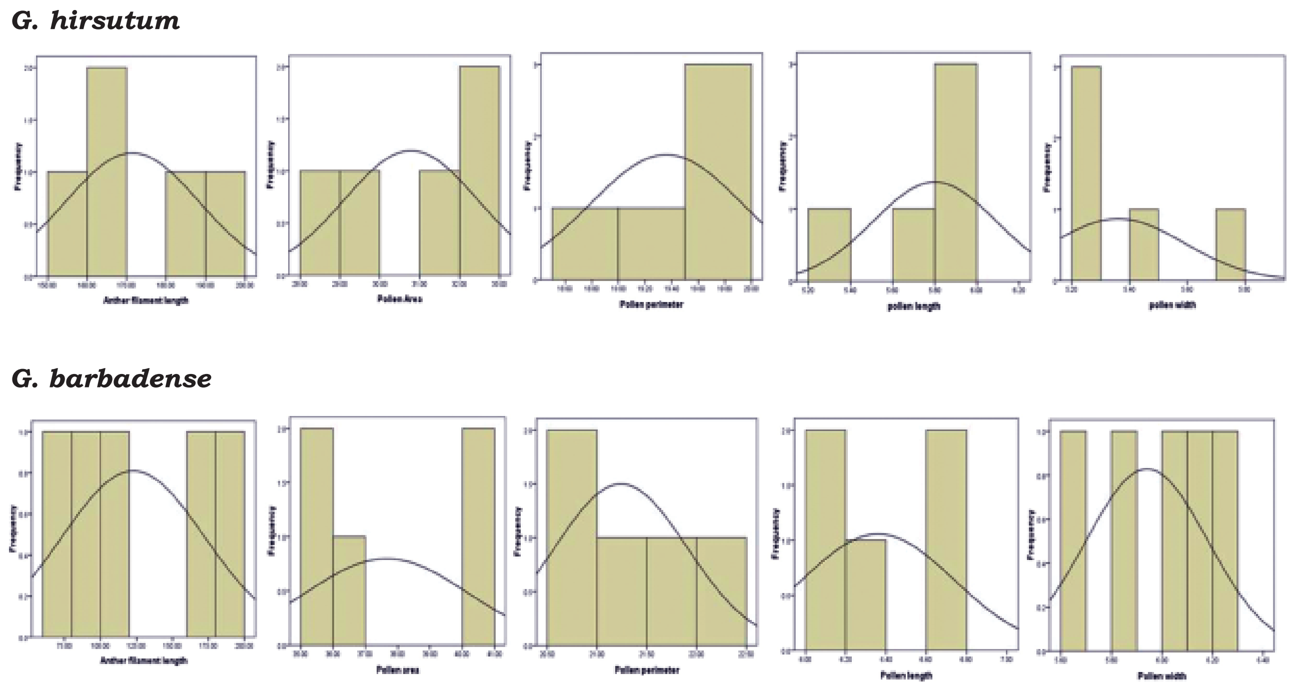
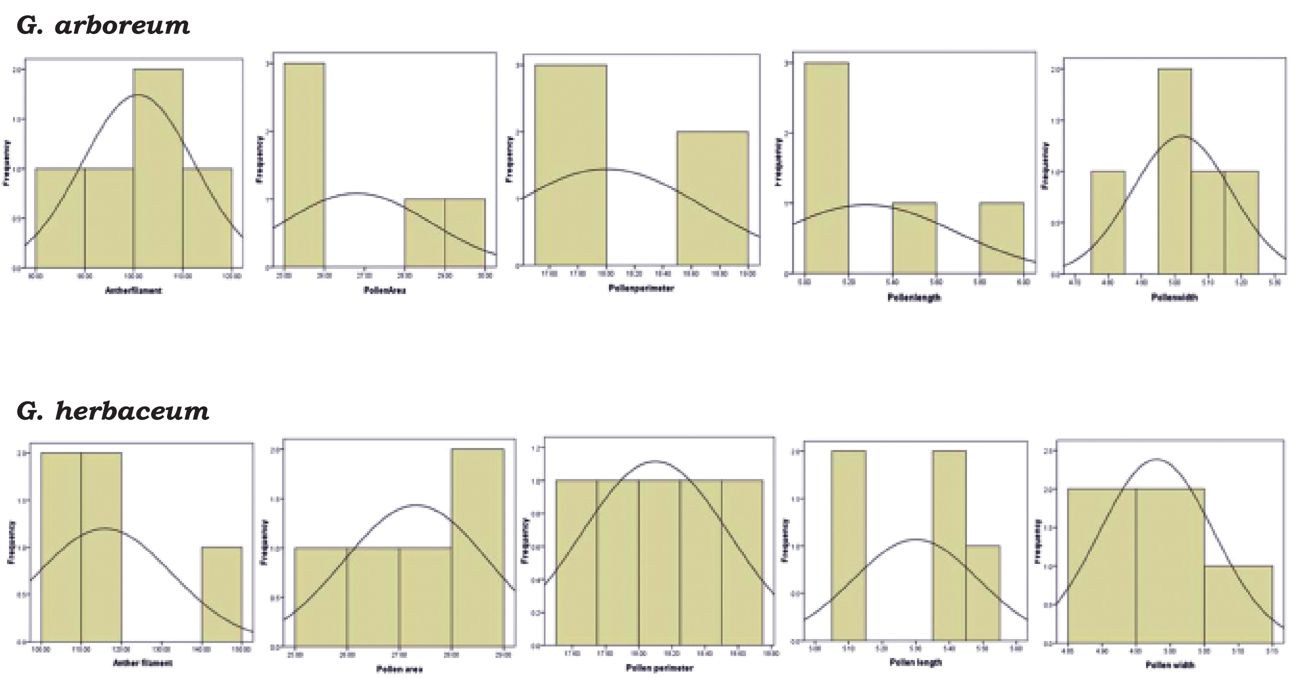


Fig. 1. Frequency distribution of pollen morphological traits among tetraploid cotton species



Contd.., Fig. 1. Frequency distribution of pollen morphological traits among diploid cotton species

variation by forming separate cluster for each species indicating the usefulness of characterisation of cotton genotypes based on pollen morphology and establishment of distinctiveness among them.

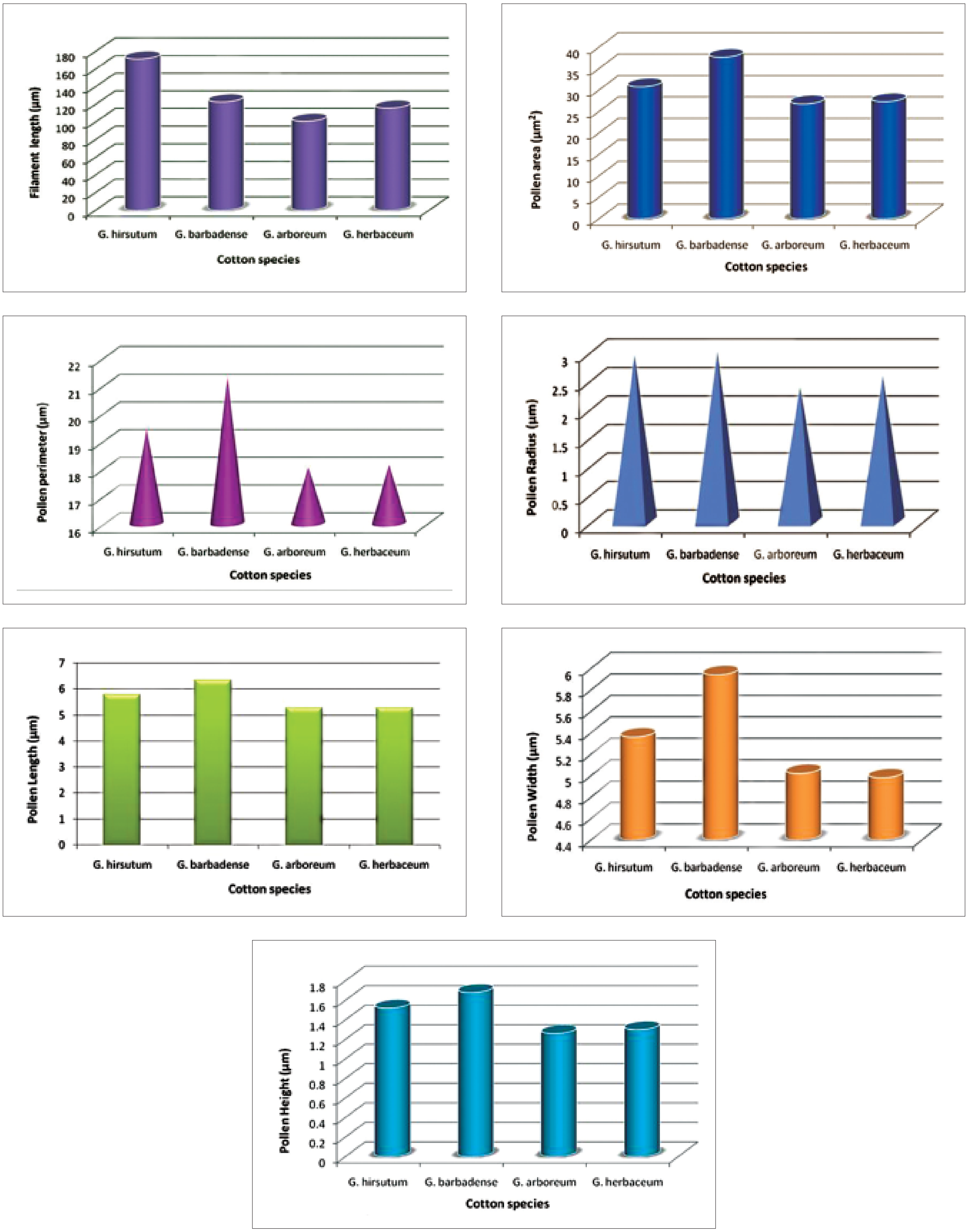


Fig. 2. Variations in pollen morphological traits among cotton species

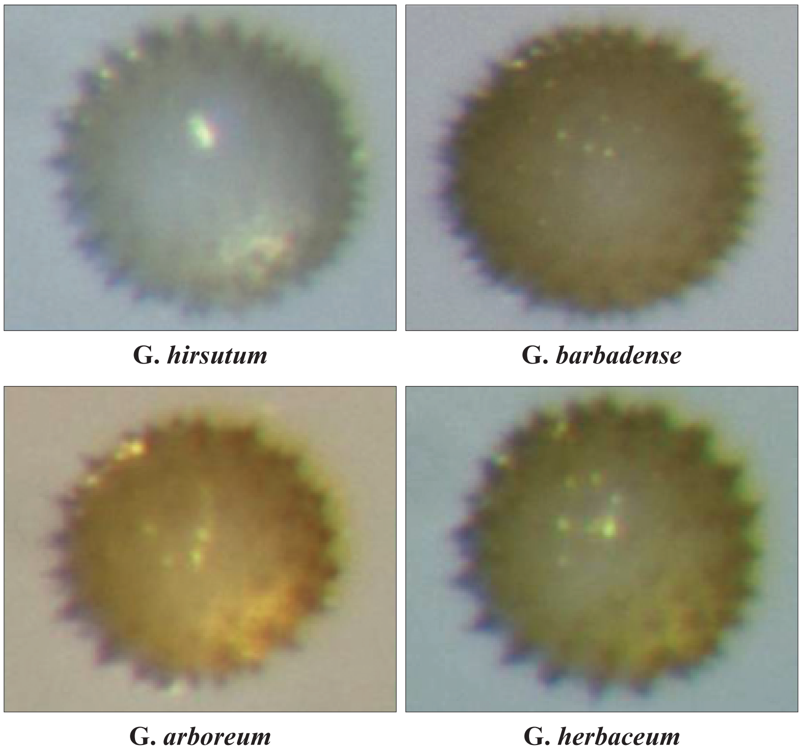


Fig. 3. Variation in morphology of the pollen length of cotton species

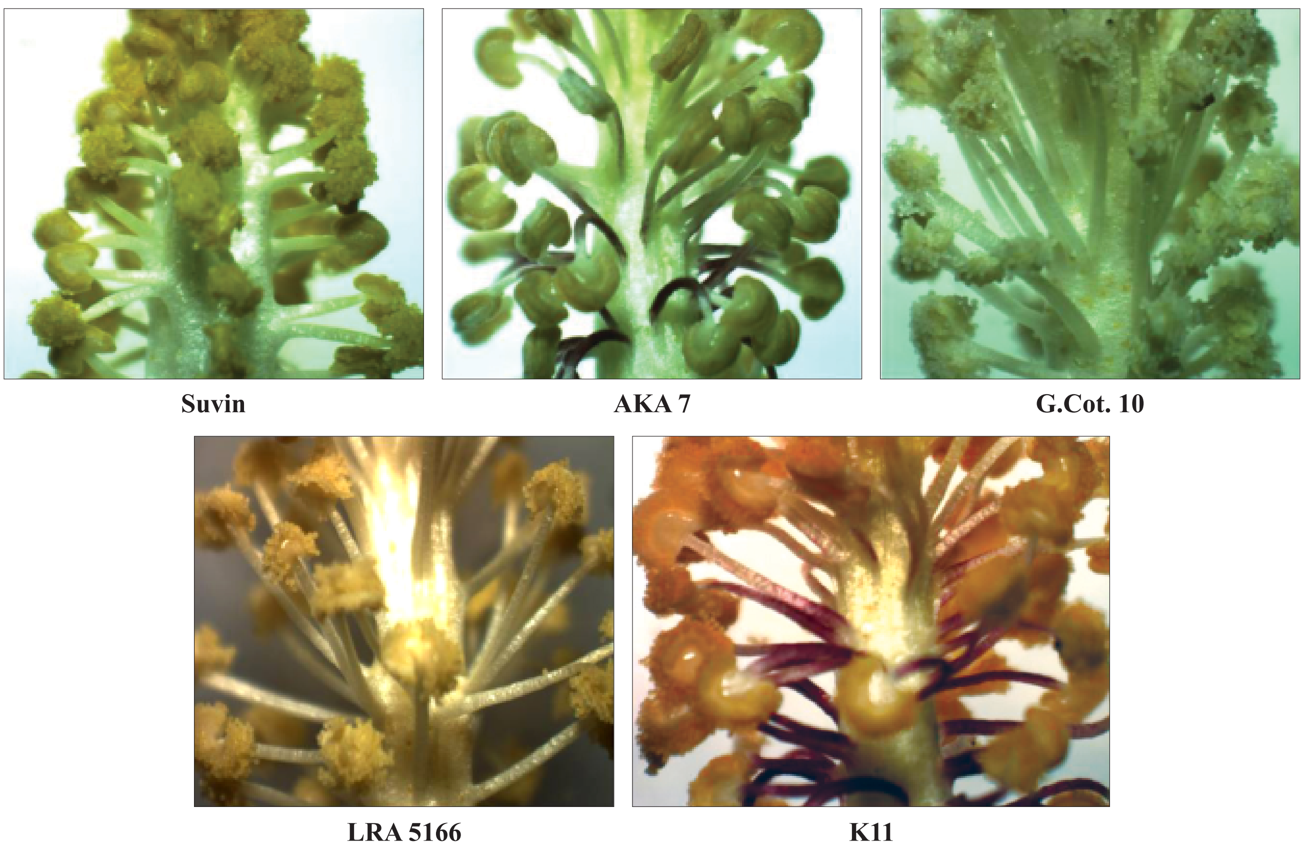


Fig. 4. Variations in length and filament colouration among cotton varieties

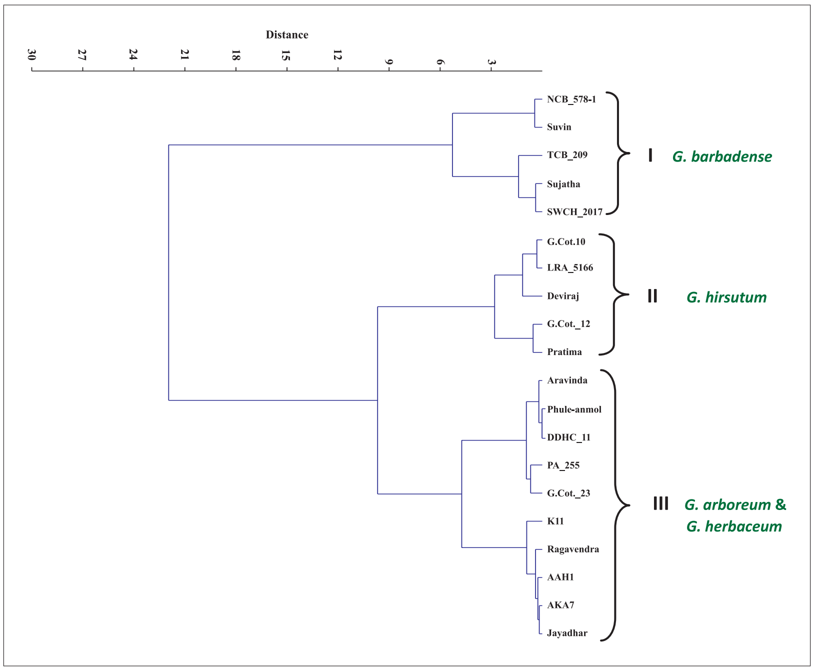


Fig. 5. Ward's linkage method of clustering cotton varieties of different species based on pollen morphology

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