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SPERMODERM DIVERSITY IN ASHWAGANDHA, *WITHANIA SOMNIFERA* (L.) Dunal

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Seeds morphology of twelve different morphotypes of *Withania somnifera* is studied to elucidate intraspecific variability in spermoderm pattern that may have value as a microtaxonomic parameter for botanical identification of seed mixtures. Four different types of testa have been observed i.e., fossulate, striate, sinuous and muri type in which striate and sinuous are more common. Most of the seeds were characterised by hair like structures or fibrils which surround each cell. Spermoderm pattern in the diploid is quite different in the derived tetraploid, the latter showing enlarged cell size. One way ANOVA depicted significant differences between the genotypes.

Keywords : Ashwagandha, *Withania somnifera*, seed surface architecture, spermoderm, SEM

The *Withania somnifera,* commonly known Ashwagandha an important ancient medicinal plant belonging to family solanaceae. *Withania* species are perennial herbs. The plant is native to India and offers vast diversity in its morphology, growth habit, reproductive features and geographical distribution (Atal and Schwarting 1962). There are twenty six species under this genus of which two species *Withania somnifera* and *Withania cogulans* are found in India (Javanshir 2000, Sharma 2004, Panwar and Tarafdar 2006). The roots have been in use in the Traditional Systems of Medicine (TCM) of Ayurveda and Unani, for its immunomodulatory potential. Leaves have also been found to contain medicinally important withanolides (Lavie *et al.* 1968). The plant has since acquired the status of "Indian ginseng" (Singh and Kumar 1998) in common parlance for its adaptogenic properties.

The plant is usually erect, branched, unarmed, shrubby, reaching upto 50 cm to 1.50 m in height. Leaves are simple, petiolate, elliptic-ovate to broadly ovate, acute, entire, glabrous and up to 8cm long. The fruit (berry) contains numerous seeds that are whitish yellow in color. Each seed is reni-form or almost disc shaped and contain oil which is rich in linolenic acid, oleic acid and palmitic acid (Sattar *et. al* 1988). Seed morphology was considered a useful criterion for determining the intraspecific variability. The aim of this study to illustrate the variability within the species of *Withania somnifera*.

MATERIALS AND METHODS

The Seeds of different varieties/wild types of *Withania somnifera* enlisted in Table 1 were obtained from the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow and other wild sources from different geographic locations were used for the present study. Seeds were washed two to three times thoroughly in distilled water and were left to dry at room temperature. The dry seeds were carefully mounted on SEM stubs by using adhesive tape and uniformly coated with gold/palladium. These samples were fixed to the specimen holder of SEM-JEOL, Japan JSM – 6490LV. The photomicrographs were taken at different magnifications. The terminology of seed coat surface sculpturing used is according to Barthlott (1981) and Sutton (1988). Data were subjected to statistical analysis to work out ANOVA for the comparison of length width ratio of seeds (Figure 1).

OBSERVATIONS

Seeds of the genus *Withania somnifera* are characteristically yellow in colour with a smooth and shiny surface. Each seed is

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Table 1: Comparisons of seed surface characters measured in *Withania somnifera*

S. N.	Variety/Locality or source	Seed coat pattern	Seed parameters		
			Length (mm)	Width (mm)	L:W ratio
1.	Poshita/ CIMAP	Striate, highly invaginated and fibrils were absent	2.05±0.001	1.87±0.002	0.91
2.	Nagori/ CIMAP	Fossulate, highly invaginated and irregular grooves on the surface of the testa.	2.29± 0.01	2.02± 0.001	0.88
3.	Pratap/ CIMAP	Striate, hair like fibrils with thick lateral wall thickening	2.77± 0.02	2.01±0.12	0.75
4.	NMTLI-116/ CIMAP	Striate, hair like fibrils with lateral wall thickning	2.24 ±0.03	2.11± 0.03	0.94
5.	Chetak/ CIMAP	Sinuous, highly lignified and fibrils were lacking	2.17± 0.04	1.83± 0.004	0.84
6.	Pratap/ Farm CIMAP	Fossulate, hair like fibrils with lateral wall thickening	2.10± 0.02	1.62±0.002	0.77
7.	Poshita Tetraploid/ CIMAP	Striate, narrow groves with thick lateral wall	3.01±0.001	3.2± 0.01	1.06
8.	Wild/ Bans village, IMT Haryana	Sinuous, Lumen shallow, fibrils absent	2.25± 0.02	1.96± 0.02	0.87
9.	Wild/ Rajasthan university campus	Sinuous, Lumen invaginated	2.11± 0.01	1.90± 0.001	0.90
10.	Wild/ Rampura Haryana	Muri, network of ridges with thick lateral wall	2.35±0.003	2.06±0.12	0.87
11.	Wild/ Rewari Haryana	Sinuous, fibrils absent	2.10± 0.01	1.82±0.3	0.86
12.	Wild/ Lucknow university campus	Sinuous pattern and hair like fibril present	2.8±0.01	2.03±0.002	0.72

Table 2 : Quantitative parameters of seed

S. N.	Varieties/Locality	Seed size in µm	No. of Seeds / berry	Dry seed weight (gm)	Striation Lumen size (L x W) in µm	Thickening of the lateral wall of the testa (µm)
1.	Poshita/ CIMAP	3.83±0.12	28.54±1.982	0.0182	10750±1232.06	22.5±2.51
2.	Nagori/ CIMAP	4.60.12±0.3	24.36±1.042	0.0125	13125±3095.67	12.5±0.97
3.	Pratap/ CIMAP	5.81±1.2	20.54±1.00	0.0168	10000±863.84	17±0.90
4.	NMTLI-116/ CIMAP	4.72±0.13	20±1.04	0.017	15156.24±806.84	33.75±1.92
5.	Chetak/ CIMAP	3.9±1.3	23.27±1.04	0.066	24875±3525.15	5.5±0.33
6.	Pratap/ Farm CIMAP	3.4±1.8	30.27±1.16	0.0105	6875±626.70	32.5±2.05
7.	Poshita Tetraploid/ CIMAP	9.6±2.1	18.63±1.49	0.0228	10262.5±1960.02	76.25±1.25
8.	Wild/ Bans village, IMT Haryana	4.41±1.6	22.09±0.58	0.0148	6000±468.97	16.25±1.92
9.	Wild/ Rajasthan university campus	4.009±0.23	21.9±0.93	0.0128	9687.5±897.97	31.25±2.09
10.	Wild/ Rampura Haryana	4.84±0.9	23.45±0.65	0.018	12187.4±767.48	16.75±1.45
11.	Wild/ Rewari Haryana	3.82±1.4	27.81±1.36	0.0168	7250±614.03	22.5±0.83
12.	Wild/ Lucknow university campus	5.68±0.2	22.9±0.82	0.0162	8625±460.52	22.5±2.68

Table 3 **:** ANOVA of the quantitative parameters of seed

Character	significant
Lumen size vs. thickening of lateral wall	***
Lumen size vs. seeds per fruit	***
Lumen size vs. seed size	***
Lumen size vs. dry weight of seed	***
Thickening of lateral wall vs. seeds per fruit	ns
Thickening of lateral wall vs. seed size	ns
Thickening of lateral wall vs. dry weight of seed	ns
Seeds per fruit vs. seed size	ns
Seeds per fruit vs. dry weight of seed	ns
Seed size vs. dry weight of seed	ns

***= significant ($p \leq 0.001$), ns = non significant

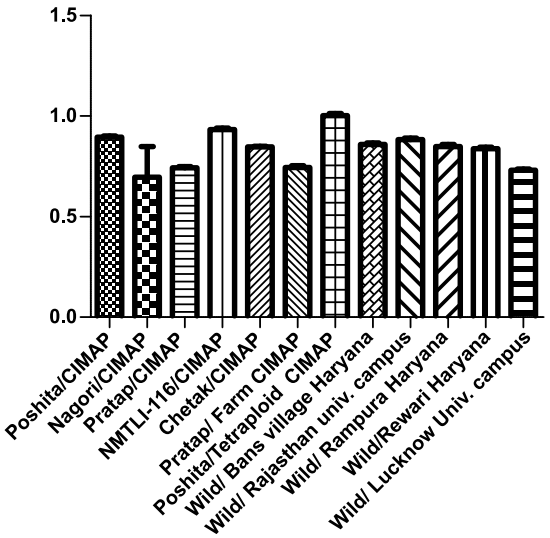

Figure 1 : L:W ratio of Seeds of different morphotypes

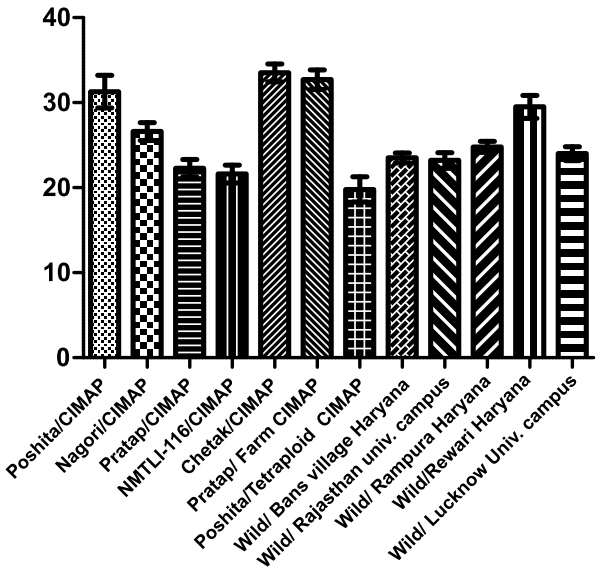

Figure 2 : No. of seeds / berry

reni-form or almost disc shaped, that are formed inside the berry. It is observed that *Withania somnifera* seeds exhibit a wide range of seed coat surface pattern variation constituting striate, fossulate, sinuous and muri type (Plate 1 and 2). Further details, including ANOVA are given in Tables 1, 2 and 3.

DISCUSSION

SEM studies of the seed coat patterns have been known to exhibit existence of genetic diversity at various level of taxonomic hierarchy (Linskens *et al.* 1977, Carolin 1980, Rejdali 1990). Gopinathan and Babu (1985) reported that Seed coat pattern could resolve the classificatory problems, establish evolutionary relationship and help elucidate adaptive significance of seed coat and could also serve as genetic markers.

In the twelve different morphotypes of *Withania somnifera* investigated here the seed coat surface is not uniform, but evince marked intra-specific differences, although there were no differences in seed shape. In diploid and tetraploid there is recognizable differences having thickness of lateral wall of the testa. SEM studies of the seeds of various morphotypes

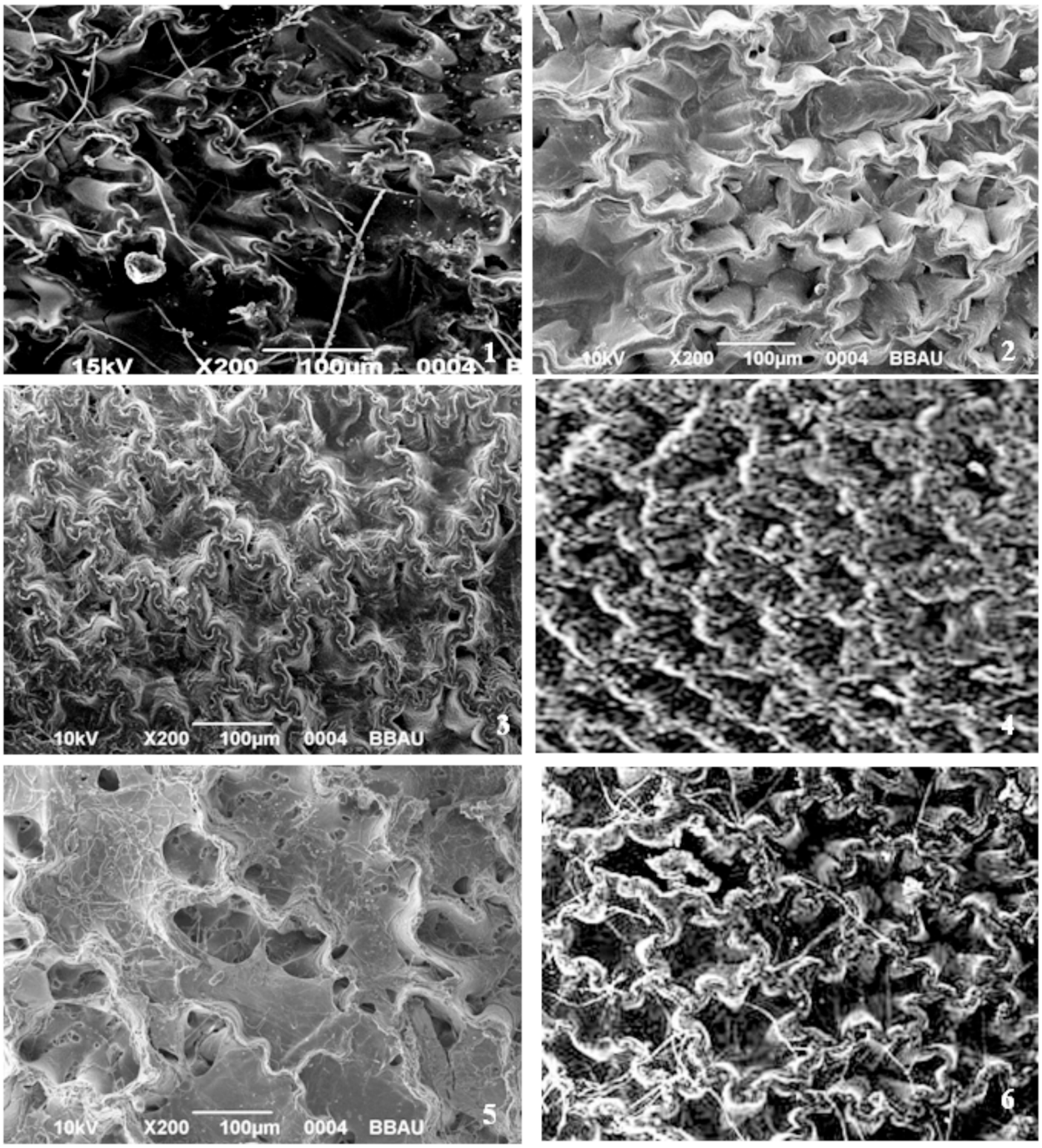

Plate 1. Scanning electron microphotographs of spermoderm patterns of different varieties of Ashwagandha. 1. Poshita, 2. Nagori, 3. Pratap, 4. NMTLI-116 5..Chetak and 6. Pratap Farm. Fig; 1, 3, 4. shows striate type. 2, 6. fossulate type.

indicate that sinuosus and striate type seed coat pattern are more common. Most of the seeds were somewhat similar in size and shape but in some morphotypes the convolutions were more invaginated, as observed in Nagori, Posita and Pratap. In Pratap, Wild/Rajasthan, Wild/Rampura Haryana and NMTLI-116 long, curved, and irregularly spread fibrils were present which were lacking in other morphotypes. The present SEM studies show intraspecific seed coat pattern variation in twelve different types of *Withania somnifera.* Statistical analysis showed significant variation in Nagori vs. Teatraploid (***$p \leq 0.001$); also in Pratap, vs. teatraploid; Pratap vs. wild (Lucknow) (**$p \leq 0.01$) and Nagori vs. NMTLI-116. (*$p \leq 0.05$). Seeds/berry (Fig. 2)

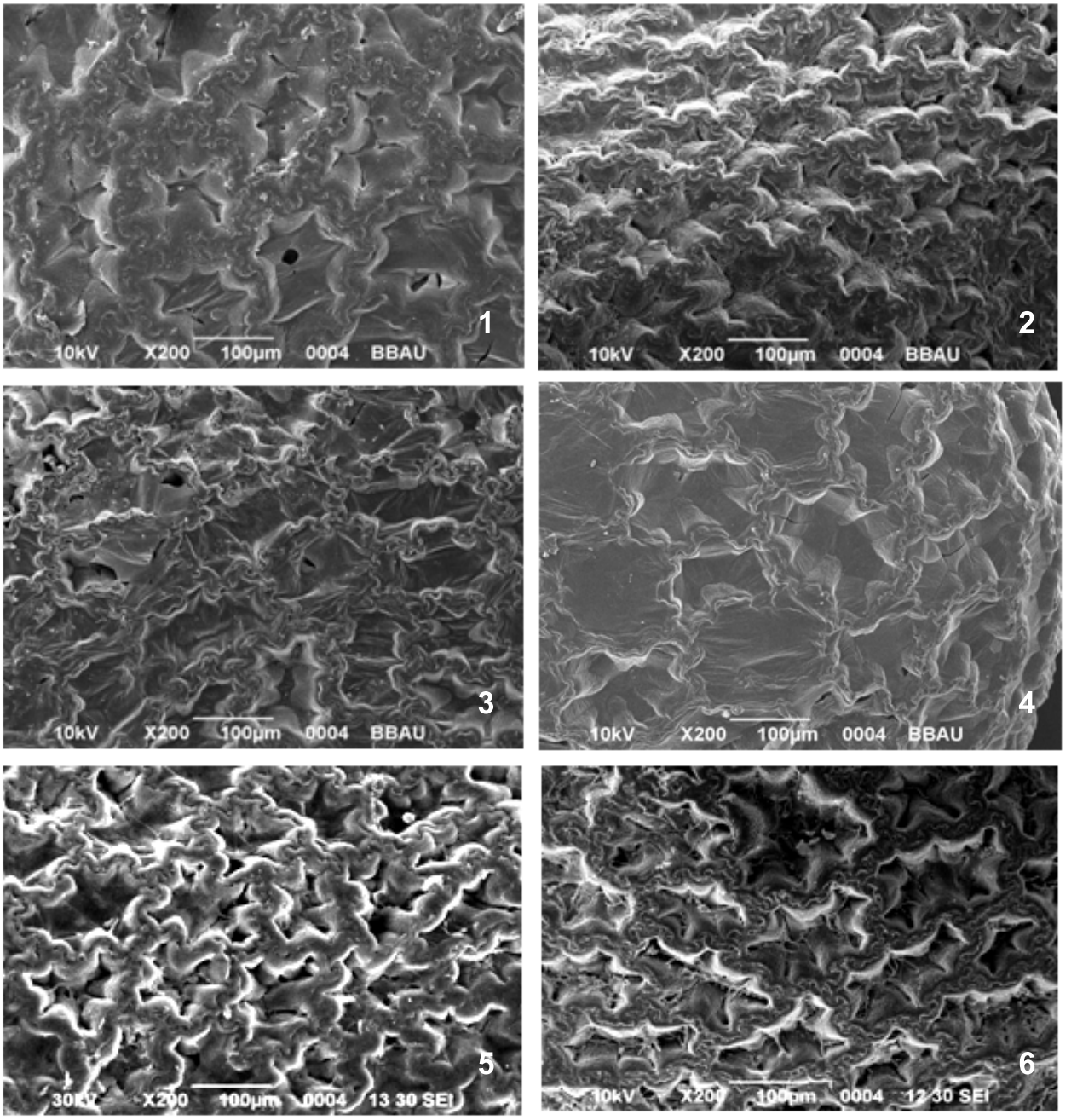

Plate 2. Scanning electron microphotographs of spermoderm patterns of different varieties of Ashwagandha. 1. Tetraploid, 2. Wild/ Bans village, IMT Haryana, 3. Wild Rajasthan university campus, 4. Wild/Rampura Haryana, 5. Wild/Rewari and 6. Wild/Lucknow University campus. Fig; 7. striate type. 8, 9, 11, 12. sinuous type. 10. Muri type.

were found to lowest in tetraploid (18.63±1.49) followed by Pratap CIMAP (20.54±1), NMTLI-116 (20±1.04) and Chetak (23.27±1.04). The thickening of lateral wall of the testa was maximum in tetraploid (76.25± 1.25) followed by NMTLI-116 (33.75±1.92), Pratap (32.05± 2.05) and wild Rajasthan (31.25± 2.09) (Table 2). The lumen size showed a significant results (***p $\leq$ 0.001) with thickening of the radial wall of the testa, seeds/berry, seed size and dry weight of seed (Table 3).

CONCLUSION

Incidence of spermoderm diversity as observed across the genoptyes is a clear indicator that this could be used as an important microtaxonmic parameter for deliniating differences amongst the genotypes. ANOVA of quantitative

parameters showed that the cell size, radial wall thickening of the testa, seed size and seeds per fruit were more significant among morphotypes. The negative correlation obtained between lumen size and radial wall thickening of the testa. The positive correlation obtained for the characters like length:width vs. radial wall thickening of the testa; and dry weight of seed vs. lumen size prove that increase in the dry weight of seed goes with the increase in the lumen size. The highly thickened lignified wall of the testa as a part of its physiological function. High lignin containing seeds provide evidence for more stress tolerance. The differences in seed coat pattern within species are also associated with the growth form on the habit characteristics. Results of the present study reveal that even within the similar species, the testa features of spermoderm greatly varies which can be used as micromorphological markers for identification.

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