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Palynological studies and their implication in the compatibility of tuberose cultivars

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ABSTRACT

Determination of palynological characteristics of commercial cultivars is significant in determining the compatibility and intraspecific relationship, an essential criterion in tuberose breeding. As confirmed through various studies, self-incompatibility and seed sterility in tuberose have greatly hindered conventional breeding methods. However, the regulation of the incompatibility system and the principal factor behind it has yet to be thoroughly investigated. Understanding both male and female gametophytes and their functioning is a prerequisite that needs to be explored in tuberose. Seventeen tuberose genotypes studied showed the same inclination towards viability and germination, determining a positive correlation. Analysis of pollen morphology and ultra-structure using a scanning electron microscope (SEM) revealed marked variations among the genotypes. The distinct pollen ultra-structures revealed would serve as essential classification and character identification features in taxonomy. It also ascertains the pollen function as evidenced by the shrunken pollen of genotypes previously reported as sterile. These findings are vital to fully comprehending tuberose cultivars' breeding systems and reproduction biology.

Keywords*: Agave amica*, Pollen morphology, Pollen viability, Pollen germinability.

INTRODUCTION

Pollen biology includes viability, germination and tube growth defines the success of pollination, determining seed set. Insight knowledge into the pollen biology of a crop is a pre-requisite to incorporate any rational approach to improve crop quality and increase productivity (Abdelgadir *et al*., 1). Performance of pollen in terms of viability and germinating ability has a significance on flower pollinators interaction and ultimately the fruit set. Evaluation of pollen viability assesses the ability of the pollen grain to carry out its function following compatible pollination whereas *in vitro* germination capacity defines the crossability of cultivars (Tedesco *et al*., 18). Knowledge of pollen biology thus contributes to the cultivation, conservation, and genetic improvement of a crop. Pollen morphology studies are of great significance to plant taxonomy and palaeobotany. It establishes pollen characteristics for identification and to determine the interspecific and intraspecific relationships between the species or cultivars (Javady and Arzani, 10).

Tuberose (*Agave amica* (Medik.) Thiede & Govaerts) is a bulbous perennial flowering plant and belongs to the family *Asperagaceae* formerly known as *Agavaceae* (Gutierrez and Garay, 7). It is native to Mexico, commonly grown both as cut and loose flower and also for its essential oil (Bailey, 2). Tuberose is a cross-pollinated crop and improved cultivars have been developed through selection and intraspecific hybridization and to a small extent through induced mutations and interspecific hybridization (Huang *et al*., 9). Genetic variability of the crop is very limited due to self-incompatibility, dichogamy and poor seed setting (Karihaloo, 11). To address and overcome the issue of sterility and incompatibility, it is necessary to examine the viability of pollen, the physiology of its germination and tube growth. There has been no detailed study on pollen morphology of tuberose cultivars, which is fundamental for taxonomic classification and identifies unique features of pollen. Therefore, this study was taken up to assess the pollen biology and morphology of tuberose to elucidate the special pollen characters and understand the intraspecific relationship among the cultivars. This could be further exploited in analyzing the breeding systems governing tuberose such as sterility and incompatibility.

MATERIALS AND METHODS

Seventeen genotypes were used for viability and germination studies *viz*. Arka Shringar, Mexican Single, IIHR-10, IIHR-9, Variegated, Arka Sugandhi, Hyderabad Single, Arka Prajwal, Arka Nirantara,

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IIHR-12, Phule Rajani, GK-T-C4, Calcutta Single, Bidhan Snigda, Bidhan Ujwal, Bidhan Jyoti and IIHR-6. For morphological studies, Arka Prajwal, Arka Shringar, Arka Sugandhi, Arka Nirantara, IIHR-6, IIHR-12, Variegated and Mexican Single were selected. The genotypes are single flower type and maintained at the Division of Floriculture and Medicinal crops, Indian Institute of Horticultural Research (IIHR), Bengaluru located at 12º 58' North latitude and 78º 35' East longitudes, at an altitude of 930 m above MSL in the Eastern Dry Zone (Zone 5) of Karnataka, India.

The viability of pollen was determined by the stainability of the pollens to Alexandrin (is it Alexander or Alexandrin) stain. Mature pollen grains were collected in the morning hours on five floral stages (matured bud, 1st day, 2nd day, 3rd day and 4th day of anthesis), dusted on a glass slide with 1 to 2 drops of Alexandrin stain and kept for 2-3 minutes for uniform staining. Slides were observed under Olympus microscope (Model-DP22, Tokyo) equipped with camera. The dark red stained and round pollen were recorded as viable whereas the unstained or lightly stained and shriveled pollen grains recorded as non- viable. Pollen germination estimation was done by hanging drop technique (Stanley and Linskens, 17) using modified "Brewbaker and Kwack's media" (Brewbaker and Kwack, 3) on five floral stages (matured bud, 1st day, 2nd day, 3rd day and 4th day of anthesis). The modified germination media consisted of 1 ml each of the following components such as 15% Sucrose, 100 ppm H_3BO_3, 150 ppm Ca (NO_3), 100 ppm $MgSO_4$ and 50 ppm KNO_3. The pollens were dusted on a small drop of the prepared media on a cover slip and fixed with a cavity glass slide which was slowly tilted and carefully upturned. The cavity slides were kept in incubator for 1 hour at room temperature and the pollen grains germinated were observed and germination (%) was estimated.

Morphological characteristics of eight genotypes belonging to highly fertile, medium fertile and sterile groups were studied using Hitachi tabletop scanning electron microscope (Model- TM3030 Plus). Freshly dehisced mature pollen grains collected on the 1st day of anthesis in the morning hours were dusted with camel hair brush to stub which was gold sputter coated using an adhesive tape and fed into the vacuum chamber of the system. Pollen micrographs were captured using TM3030 Plus SEM software. Different pollen morphological characters were observed and recorded.

The data collected were processed for analysing following SAS procedures (SAS, 15). Significant difference between the means recorded were determined by the least significant difference (LSD) method at 5%.

RESULTS AND DISCUSSION

Pollen viability test using Alexandrin stain showed variability on the five different stages of flower (Fig. 1). The mean pollen viability was observed highest (51.41%) in the pollen grains collected on 1st day of anthesis (Table 1) and was observed to decline on the subsequent days. Among the 17 genotypes, Arka Nirantara recorded highest pollen viability on the matured bud and first day of anthesis (100%). No viable pollens were recorded for IIHR-12 on all the five stages under study. Stainability is an important measure to determine its viability or sterility of the pollen grains. The difference in the stainability of the pollen grains was observed to be cultivar dependent, determining the specific ability to undergo successful pollination. Variations in viability of tuberose pollen had also been previously reported (Seetharamu, 16). The result showed highest mean viability of the genotypes on the 1st day and slowly decreased on the days following anthesis thus indicating the optimum stage for pollination. The marked variation in viability of the different genotypes including low or no pollen viability reveals the contribution by the specific genetic makeup as well as environmental conditions.

The highest mean pollen germination was noticed on the 1st day of anthesis and declined on the following days (Table 2). Arka Prajwal showed low pollen germination (2.92%) and IIHR-9, IIHR-12 and Bidhan Snigda has shown no pollen germination distinctly on all the flower stage. The genotypes IIHR-6, Mexican Single, Arka Sugandhi and Calcutta Single recorded pollen germination till the 4th day of

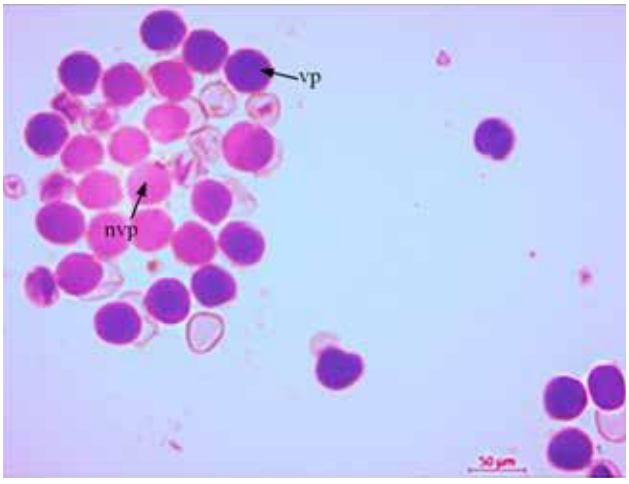

Fig. 1. Viable pollens (vp) and non-viable pollens (nvp) measured by stainability using Alexandrin stain. Scale Bar: 50 µm.

Table 1. Pollen viability of tuberose genotypes.

Genotype	Pollen Viability (%)				
	Matured bud	1st day of anthesis	2nd day of anthesis	3rd day of anthesis	4th day of anthesis
Arka Shringar	20.78	81.67	17.42	21.11	9.82
Mexican Single	61.25	15.48	30.95	9.76	8.13
IIHR-9	27.50	0.00	0.00	0.00	0.00
IIHR-10	26.67	30.95	18.33	10.00	6.33
Variegated	83.75	82.50	25.00	32.39	20.00
Arka Sugandhi	74.29	66.36	56.25	22.50	38.75
Hyderabad Single	36.67	41.67	33.33	29.17	7.85
Arka Prajwal	14.58	26.14	0.00	0.00	0.00
Arka Nirantara	100.00	100.00	41.67	32.22	9.72
IIHR-12	0.00	0.00	0.00	0.00	0.00
Phule Rajani	15.48	52.78	22.88	8.01	7.18
GK-T-C4	13.94	95.00	21.11	26.67	4.06
Calcutta Single	36.67	83.75	16.25	19.40	4.15
Bidhan Snigda	0.00	0.00	0.00	0.00	0.00
Bidhan Ujwal	48.33	36.67	17.71	22.50	0.00
Bidhan Jyoti	43.18	63.33	26.79	5.16	3.61
IIHR-6	45.43	97.75	32.05	19.09	20.32
Mean	38.15	51.41	21.55	15.18	8.23
$SE_{(m)}\pm$	6.92	8.64	3.94	2.86	2.44
CD (p=0.05)	10.51	10.28	9.81	11.74	3.92

Table 2. Pollen germination of tuberose genotypes.

Genotype	Pollen germination (%)				
	Matured bud	1st day of anthesis	2nd day of anthesis	3rd day of anthesis	4th day of anthesis
Arka Shringar	65.45	82.59	17.40	0.00	0.00
Mexican Single	32.79	29.81	16.01	2.34	1.57
IIHR-9	0.00	0.00	0.00	0.00	0.00
IIHR-10	11.70	13.94	0.00	0.00	0.00
Variegated	0.00	25.16	3.88	0.00	0.00
Arka Sugandhi	70.37	45.81	6.86	10.65	6.57
Hyderabad Single	29.12	27.71	12.41	0.00	0.00
Arka Prajwal	25.29	2.92	0.00	0.00	0.00
Arka Nirantara	32.66	46.67	13.94	0.00	0.00
IIHR-12	0.00	0.00	0.00	0.00	0.00
Phule Rajani	57.33	40.97	0.00	0.00	0.00
GK-T-C4	24.87	59.52	0.00	0.00	0.00
Calcutta Single	31.48	49.40	28.71	2.28	4.51
Bidhan Snigda	0.00	0.00	0.00	0.00	0.00
Bidhan Ujwal	37.40	85.86	14.84	0.54	0.00
Bidhan Jyoti	70.38	29.97	8.85	1.52	0.00
IIHR-6	65.32	69.46	18.59	11.65	3.96
Mean	34.08	35.51	8.32	1.70	0.98
$SE_{(m)}\pm$	5.88	7.07	2.15	0.88	0.49
CD (p=0.05)	10.82	14.14	4.81	1.43	0.51

anthesis. Length of the pollen tubes also showed maximum on the 1st day of anthesis with mean tube length of 107.19 µm after 1 hour of incubation. The genotype IIHR-6 recorded the maximum length with 144.15 µm (Table 3). The germination per cent of all the genotypes on the five stages show the same trend as the viability and reduced as the stage advances, which can be correlated as highly viable pollens shows high germination percentage and vice versa (Dey *et al*., 5). The low or inability of certain genotypes to germinate as observed is due to inherent genetic character, defect in microsporogenesis which leads to low viability and loss of vigour of the pollen grains. Low viability and germination of Arka Prajwal pollen had also been previously reported (Ranchana *et al*., 14; Hemanta, 8). The low germination per cent of these genotypes may also be governed by the microspore development and cytological aberrations.

The morphological study of the pollen of tuberose genotypes conducted using scanning electron microscope (SEM) revealed that tuberose pollen in general ranged from medium (25-50 µm) to large (50-100 µm) size and observed to be monad and heteropolar in nature with radial symmetry (Table

Table 3. Pollen tube length of tuberose genotypes.

Genotype	Length of pollen tube (µm)			
	Matured bud	1st day of anthesis	2nd day of anthesis	3rd day of anthesis
Arka Shringar	77.09	136.84	23.66	18.16
Arka Nirantara	91.98	87.10	60.88	37.95
Arka Sugandhi	46.31	91.81	23.28	0.00
IIHR-6	110.50	144.15	59.91	42.04
Mexican Single	53.96	93.43	36.98	0.00
Variegated	86.12	89.79	46.48	18.84
Mean	77.66	107.19	41.87	29.25
$SE_{(d)}$	15.68	17.24	11.98	5.73
CD (p=0.05)	34.16	37.56	26.11	12.49

Table 4. Characteristic pollen morphology of tuberose genotypes.

Genotype	Pollen units	Orientation of polarity	Symmetry	Pollen size	Shape classes	Equatorial outline	Polar outline	Exine ornamentation	Shape index (P/E ratio classes)
Arka Shringar	Monad	Heteropolar	Radially symmetric	Large	Oblate speroidal	Circular to elliptic	Triangular	Reticulate	Sub-transverse
Arka Prajwal	Monad	Heteropolar	Radially symmetric	Medium	Shrinked	Elliptic	Triangular	Perforate	Semi-transverse
Arka Nirantara	Monad	Heteropolar	Radially symmetric	Large	Sub-oblate	Circular to elliptic	Triangular	Micro-perforate	Semi-transverse
Arka Sugandhi	Monad	Heteropolar	Radially symmetric	Large	Sub-oblate	Circular to elliptic	Triangular	Rugulate	Semi-transverse
IIHR-6	Monad	Heteropolar	Radially symmetric	Large	Sub-oblate	Circular to elliptic	Triangular	Reticulate	Semi-transverse
IIHR-12	Monad	Heteropolar	Radially symmetric	Medium	Shrinked	Circular to elliptic	Triangular	Perforate	Sub-transverse
Mexican Single	Monad	Heteropolar	Radially symmetric	Large	Oblate speroidal	Elliptic	Triangular	Reticulate	Sub-transverse
Variegated	Monad	Heteropolar	Radially symmetric	Large	Sub-oblate	Circular to elliptic	Triangular	Micro-perforate	Semi-transverse

4). Tuberose has tetrasporic anther and pollen grains are produced in four pollen sacs of anther like Bougainvillea (Chang *et al*., 4). The heteropolar nature of the pollen revealed that the distal and proximal faces of the tuberose pollen are not similar. The pollen grains at maturity are dissociated into single pollen grain called monad and are radially symmetric and they have no horizontal plane of symmetry. According to NCP classification (Erdtman, 6), the pollen grains of tuberose were classified as 243, ditreme (200) denoting the number of apertures, equatorial aperture (040) denoting the position of the aperture and colpate (003) denoting the character or type of the aperture. The pollen aperture was identified to be colpate, located in the sexine region of the pollen and elliptic shape of colpate with mean colpi length of 46.89 µm. The NCP classification basically, denotes the features of pollen aperture such as the number and position of the aperture and character or type of the aperture (Erdtman, 6). When the length/breadth ratio of the pollen aperture is more than 2.0, it is termed as colpus, a simple aperture with elliptic shape.

Two apertures (di-zonocolpate) were observed on the equatorial zone of the pollen grains and the apertural view of the pollen grains was heterocolpate (Table 5). The pollen grain shapes varied from oblate spheroidal to sub-oblate. Shrunken and shapeless pollens were observed in the genotypes Arka Prajwal and IIHR-12. Mexican Single and Arka Prajwal obtained an elliptic shape whereas the remaining genotypes obtained circular to elliptic shape (Fig. 2). Completely shrunken or distorted pollen grains were noticed on the anthers of Arka Prajwal and IIHR-12 while the elliptic plump pollen grains are observed in Mexican Single (Fig 3). Variations in pollen grains size were observed in the genotype Variegated which displayed pollens of different sizes such as large and small along with shrunken pollen grains (Fig. 4). Reticulate type of exine sculpturing which showed a net like structure comprising of muri and lumina was observed in the genotypes Arka Shingar, IIHR-6 and Mexican Single. The exine surface provided with small perforations or microperforated was observed in Arka Nirantara and Variegated, whereas Arka Prajwal and IIHR-12 reported perforated type of exine with large perforations. Rugulate type of sculpturing was observed in Arka Sugandhi where the ridges run irregularly (Table 4 and Fig. 5). Exine ornamentation or pollen exine pattern serves as a prime factor in pollen classification and identification (Koubouris *et al*., 12). The presence of lumina and pollenkitt are particularly imperative for the survival and evolution of self-incompatible tuberose as in the case of *Bougainvillea* (Chang *et al*., 4).

The mean length of pollen measured in equatorial view was 55.67 µm whereas in polar view, mean length was 47.31 µm (Table 6). Length of the genotype Arka Prajwal and IIHR-12 were significantly

Table 5. Characteristics of pollen grain aperture of tuberose genotypes.

Genotype	Type of aperture	Shape of aperture	No. of apertures	NCP classification	Aperture view
Arka Shringar	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
Arka Prajwal	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
Arka Nirantara	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
Arka Sugandhi	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
IIHR-6	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
IIHR-12	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
Mexican Single	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate
Variegated	Colpate	Elliptic	Di-zonocolpate	243	Heterocolpate

Table 6. Pollen morphological parameters of tuberose genotypes.

Genotype	Length of pollen in equatorial view (µm)	Length of pollen in polar view (µm)	Length of colpi (µm)	P/E ratio
Arka Shringar	56.29	49.42	46.42	0.88
Arka Prajwal	45.37	35.83	47.78	0.79
Arka Nirantara	64.91	51.40	47.00	0.79
Arka Sugandhi	61.29	53.55	48.59	0.87
IIHR-6	60.61	49.86	46.00	0.82
IIHR-12	42.40	38.69	46.57	0.91
Mexican Single	51.92	47.46	40.78	0.91
Variegated	62.60	52.28	51.97	0.84
Mean	55.67	47.31	46.89	0.85
$SE_{(d)}$	3.27	2.45	4.39	0.10
CD (p=0.05)	6.94	5.20	NS	NS

lesser as compared to the other genotypes both in equatorial and polar axis. This is due to the shrinkage of the pollen grains which were reported to be non-viable in nature. Similar findings had also been observed (Pipino *et al.,* 13) in hybrid tea roses where the mean diameter of high fertile genotypes was larger as compared to the mean diameter of the low fertile genotypes. According to the P/E ratio the pollen grains belong to sub-transverse class (0.88-1) and semi-transverse class (0.75-0.88). Variations in the pollen shape depend upon their P/E ratio. The completely shrunken or distorted pollen grains of Arka Prajwal and IIHR-12 could be explained by the non-viability or sterility of the pollens which resulted in low viability and inability to germinate as observed in the same study. This is in conformity with previous study as no fruit set was observed in the cultivar Arka Prajwal (Hemanta *et al*., 8). The amber shape of the pollen are also known to contribute to determining the mode of pollination. The difference in outline view of the pollen grains is evident by the differences

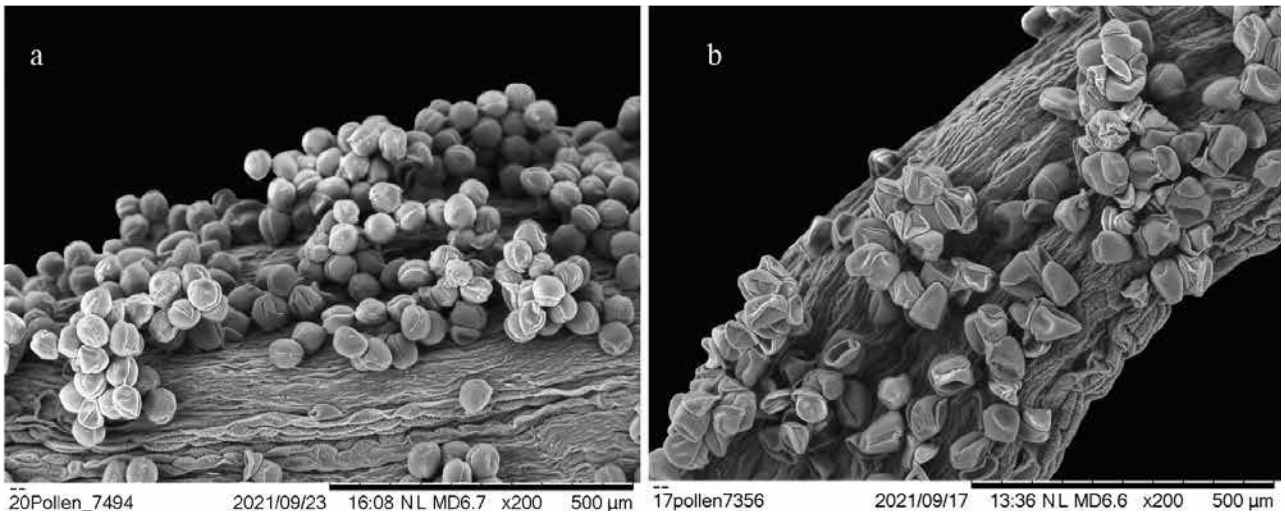

Fig. 2. Variations in pollen shape and structure of different genotypes; a) Arka Shringar b) Mexican Single c) IIHR-6 d) Variegated e) IIHR-12 f) Arka Sugandhi g) Arka Prajwal and h) Arka Nirantara. Scale Bars: 30 µm.

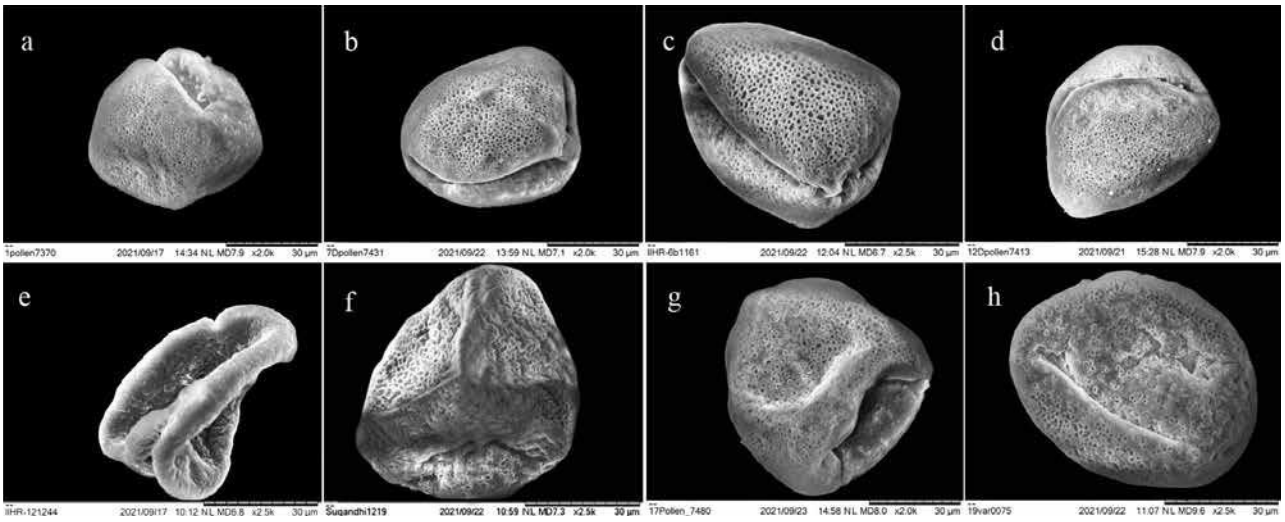

Fig. 3. Distribution of pollen grains on the anther; a) Fertile pollens of Mexican Single b) Non fertile pollens of Arka Prajwal. Scale Bars: 500 µm.

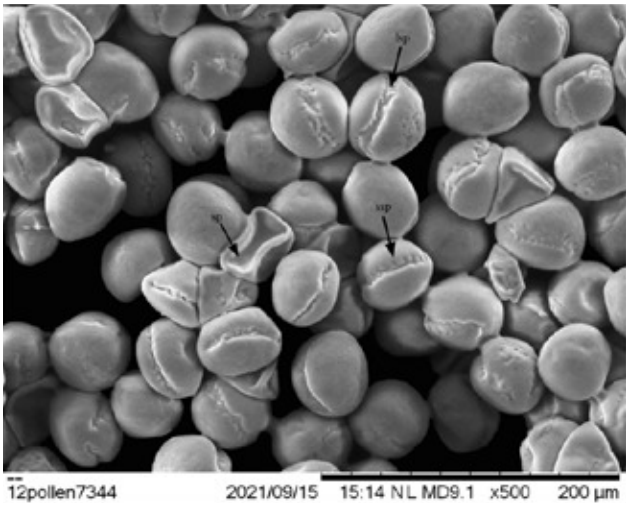

Fig. 4. Pollens of cultivar 'Variegated' displaying fertile pollens of large size (lsp) and small size pollens (ssp). Non-fertile pollens observed as shrunken pollens (sp). Scale Bar: 200 µm.

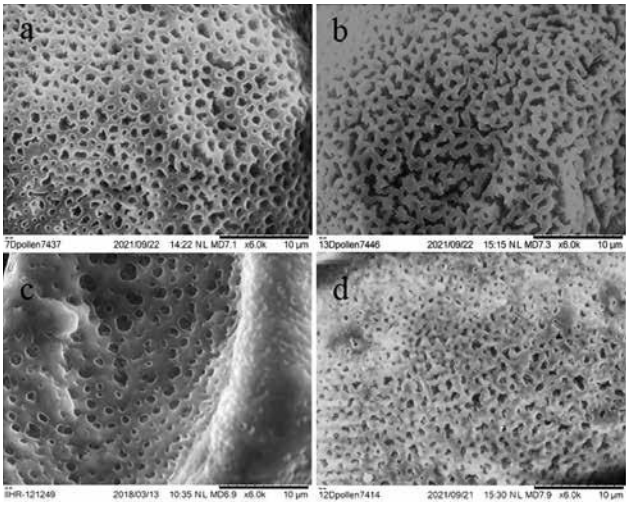

Fig. 5. Exine ornamentation types observed in different genotypes a) Reticulate- Mexican Single b) Regulate- Arka Sugandhi c) Perforate- IIHR-12 d) Microperforate- Variegated. Scale Bars: 10 µm.

observed in the pollen shape of different genotypes.

This investigation concludes that pollen viability and germination are inter-related and varies depending on the genotype. The morphological study reveals unique features of pollen grains and helps to determine the abnormal pollens pertaining to various genotype and also confirms that there is co-relation between pollen biology and morphology as evident from the shrunken and distorted shape of the sterile genotypes, which is observed for the first time and could be significant for further crop improvement studies and breeding for development of novel and improved varieties.

AUTHORS' CONTRIBUTION

Conceptualization of research (TUB); Designing of the experiments (TUB, KSP); Contribution of experimental materials (TUB, SAN, DMV); Execution of field/lab experiments and data collection (RL, KSP); Analysis of data and interpretation (RL, TUB); Preparation of the manuscript (RL, TUB, BSK, MPM, KSP).

DECLARATION

The authors declare that there is no conflict of interest.

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