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Studies on pollen viability in *Jasminum* spp.

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ABSTRACT

In vitro studies on pollen viability of six jasmine species, viz. *Jasminum sambac*, *J. auriculatum, J. grandiflorum*, *J. multiflorum*, *J. flexile* and *J. malabaricum*, was conducted at ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru during 2016-17. Results revealed that species *Jasminum malabaricum* recorded highest pollen viability (96.57%) as well as pollen germination (91.58%) followed by *J. flexile* (84.92% and 81.86%, respectively) and *J. auriculatum* (71.32% and 71.08%, respectively) under *in vitro* condition. The species *J. sambac* significantly recorded lowest pollen viability (34.27%) and germination (34.09%). Significantly highest normal shaped pollen grains were observed in *J. auriculatum* (96.92%) which was at par with *J. malabaricum* (96.89%) and *J. grandiflorum* (93.63%). Modified BK germination medium containing 100 ppm H_3BO_3 + 300 ppm $Ca(No_3)_2$ + 200 ppm $MgSO_4$) with 10% sucrose + 15% PEG 4000 solution was found optimal for highest *in vitro* germination in *J. malabaricum* (91.58%). However, maximum length of pollen tube was recorded in *J. flexile* (1170.68 µm) followed by *J. malabaricum* (951.11 µm). The viable pollen diameter of different species ranged from 38.25 µm (*J. auriculatum*) to 46.53 µm (*J. malabaricum*). Pollen grains collected during evening (05:00 pm to 06:00 pm) at the time of anthesis showed best results. Pollen tubes revealed abnormalities such as bulging and coiling of tips in *J. sambac* and *J. grandiflorum*. This might be one of the reasons for no fruit set when *J. sambac* used as pollen parent. From this *in vitro* pollen germination study, species *J. malabaricum*, *J. flexile* and *J. auriculatum* were identified as most suitable male parents compared to other species.

Key words: *In vitro*, Jasminum, Pollen viability, Pollen germination

Jasmine (*Jasminum* spp.) belonging to the family Oleaceae, order Oleales, with basic chromosome number x=13 (Taylor 1945, Krishnaswamy and Raman 1948), is a climbing, trailing and erect flowering shrub. Flowers are white, yellow or rarely reddish, sometimes solitary, more often in cymose clusters of three to many, usually fragrant, 2 loculed with 1 to 4 erect ovules (Arumugam, 2002). The main beauty and uniqueness of jasmine is its fragrance, which cannot be imitated by any known synthetic aromatic chemical and has a unique status in the perfume world. The most unique type of fragrance is derived from *Jasminum grandiflorum* which is highly appreciated all over the world. The oils are used in the manufacture of high grade perfumes, cosmetics, soaps, confectionery and perfumes.

Pollen viability is the ability of a pollen grain to germinate and develop as a pollen tube (Gerard 1932, Prajapati and Jain 2011). Identification of pollen parents based on the viability decides the extent of success in fruit and seed set.

Dadlani *et al.* (1988) reported that lack of seed setting in *Jasminum* was due to the absence of anthers and/or pollen, or pollen sterility, non-viability or failure to germinate depending upon the genotype involved.

In vitro study is important to understand the physiology and biochemistry of pollen germination. It is possible to germinate pollen grains of a number of species using simple nutrient medium and to achieve a reasonable length of pollen tube growth. Pollen grains are the sexual reproductive unit and the carrier of male genetic material in higher plants, play a vital role in breeding programme and assists successful fruit-set. High fruit set generally depends on viable pollen grains. Pollen fertility and viability have a great importance in hybridization programme. *In vitro* pollen germination is considered as the best indicator of pollen viability (Shivanna *et al*. 1991). Therefore, the present

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investigation was undertaken to examine the pollen viability and germination of six species of jasmine, viz. *Jasminum sambac*, *Jasminum auriculatum*, *Jasminum grandiflorum*, *Jasminum multiflorum*, *Jasminum flexile* and *Jasminum malabaricum*.

MATERIALS AND METHODS

The present study was carried out in the Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru during 2016 to 2017. The experimental material consisted of six species of jasmine, viz. *Jasminum sambac* cv. Ramanathapuram Gundumalli, *Jasminum auriculatum* cv. Parimullai, *Jasminum grandiflorum* cv. Co 1 (Pitchi), *Jasminum multiflorum*+, *Jasminum flexile* and *Jasminum malabaricum*. Following observations were recorded to study pollen viability and germination as per the procedure given below.

The pollen viability was evaluated by different methods such as staining techniques, pollen shape and size, *in vitro* pollen germination test, or analyzing final seed set.

The pollen viability (%) of six species was estimated by using Alexander staining solution (Alexander 1969). Freshly dehisced pollen grains were taken on a slide with one to two drops of Alexander stain on which cover slip was placed and kept for two to three minutes for proper and uniform staining. The pollen grains which were round and deep purplish pink colour stained were recorded as viable. The shriveled and green pollen grains recorded as sterile/ non-viable. The average viable pollen grains was estimated and expressed in percentage.

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen}}{\text{Total number of pollen}} \times 100$$

Round shape pollen grains were recorded as normal shaped pollen grains in all six species. Ten randomly selected pollen grains were used to measure the pollen diameter in each replication. The images were obtained with a photomicroscope and the cell sens standard software attached to the microscope.

$$\text{Normal shaped pollen grains (\%)} = \frac{\text{No. of round shaped pollen}}{\text{Total number of pollen}} \times 100$$

Pollen germination of six *Jasminum* species was tested by using hanging drop technique under *in vitro* (Stanley and Linskens 1974) and expressed in percentage.

Pollen germination was estimated by using Modified Brewbaker and Kwack's (1963) medium which was prepared by using following protocol. The base medium constituted of sucrose (10%) supplemented with boric acid (100 ppm), calcium nitrate (300 ppm) and magnesium sulphate (200 ppm). The concentrations were altered based on the response of pollen germination percentage (%).

In hanging drop technique (Stanley and Linskens 1974), small drop of medium was placed on cover glass and dusted with pollen. Cover glass was slowly and gently tilted to correctly fix on to the cavity of slide. Cavity slides then kept in Petri dishes lined with moist filter paper and examined under an Olympus-BX43 microscope at low magnification (10X) at one, two, three, four and twenty four hours time intervals to know the germination percentage and pollen tube length. The pollen grain was considered as germinated when its pollen tube length becomes equal to or larger than the pollen diameter (Chagas *et al.* 2010). The pollen tube growth was observed and images were captured with a photomicroscope.

RESULTS AND DISCUSSION

Pollen viability in six species of jasmine varied significantly (Table 1). The significantly maximum pollen viability was recorded in *Jasminum malabaricum* (96.57%) followed by *Jasminum flexile* (84.92%) and *Jasminum auriculatum* (71.32%), while the minimum percentage of pollen viability was recorded in *Jasminum sambac* (34.27%). Fertile pollen along with viable pollen favours better fruit set and consequently an acceptable yield (Sezaiercisli 2007).

On the perusal of the data presented in Table 2 revealed significant variation in pollen size and shape among six species of jasmine. The viable pollen diameter in the six *Jasminum* species ranged from 38.25 µm (*J. auriculatum*) to 46.53 µm (*J. malabaricum*). The highest viable pollen diameter was recorded in *Jasminum malabaricum* (46.53 µm) which was at par with *Jasminum multiflorum* (45.20 µm) and *Jasminum flexile* (44.77 µm). Diameter of viable pollen reduced when pollen viability decreased as a result of shrinkage.

Table 1 Number of viable/non-viable pollen and pollen viability (%) in six *Jasminum* species

Species	Number of viable pollen grains	Number of non-viable pollen	Total number of pollen	Viability (%)
J. sambac	24.50	47.00	71.50	34.27 (35.82)
J. auriculatum	335.75	135.00	470.75	71.32 (57.99)
J. grandiflorum	315.00	407.00	722.00	43.63 (41.27)
J. malabaricum	295.25	10.50	305.75	96.57 (79.44)
J. multiflorum	172.75	183.25	356.00	48.53 (44.26)
J. flexile	375.75	66.75	442.50	84.92 (67.12)
SEm±	24.01	19.02	40.41	0.99
CD (P=0.05)	71.36	56.52	120.07	2.94

Figures in parenthesis are the *arc sine* transformed means

Table 2 Number and per cent of normal shaped, pollen grains and viable pollen diameter of six *Jasminum* species

Species	Total number of pollen grains	Number of normal shaped pollen grains	Normal shaped pollen grains (%)	Viable-pollen diameter (µm)
J. sambac	71.50	50.50	70.63 (57.23)	42.75
J. auriculatum	470.75	456.25	96.93 (79.90)	38.25
J. grandiflorum	722.00	676.00	93.63 (75.47)	43.91
J. malabaricum	305.75	296.25	96.89 (79.96)	46.53
J. multiflorum	356.00	287.25	80.69 (63.35)	45.20
J. flexile	442.50	405.75	91.70 (73.24)	44.77
SEm±	40.41	38.54	1.46	0.64
CD (P=0.05)	120.07	114.51	4.35	1.91

Figures in parenthesis are the *arc sine* transformed means.

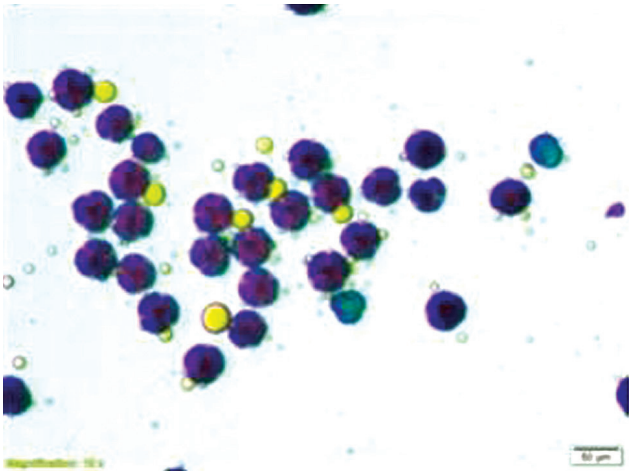

Fig 1 Viable and non-viable pollen grains of *J. malabaricum*

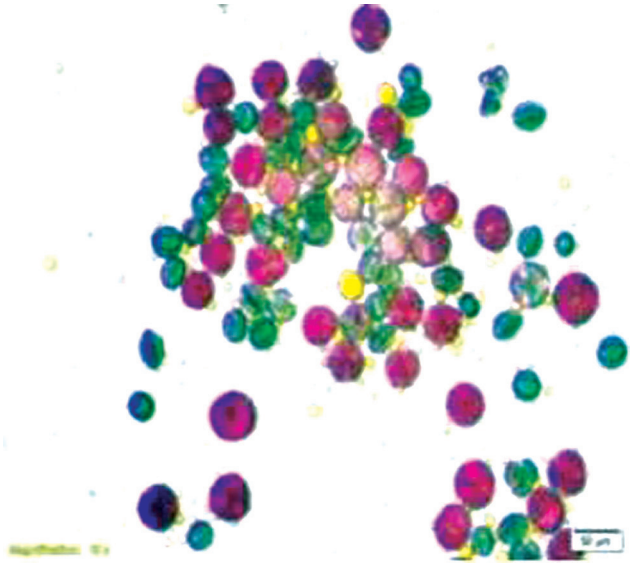

Fig 2 Spherical and triangle shape of pollen grains in *J. multiflorum*

Highest normal shaped pollen grains were recorded in *Jasminum auriculatum* (96.93%) which was at par with *Jasminum malabaricum* (96.89%) and *Jasminum grandiflorum* (93.63%), while minimum normal shaped pollen grains were recorded in *Jasminum sambac* (70.63%).

Pollen germination was conducted to ascertain the viability of pollen grains while selecting pollen parents. *In vitro* pollen germination showed variation in pollen germination of individual species in different media concentration. The ideal pollen germination media for individual species and pollen germination percentage of individual species after one, two, three, four and twenty-four hours of germination are given in Table 3, Table 4 and Fig 3, respectively.

The maximum pollen germination was recorded in *Jasminum malabaricum* (91.58%) followed by *Jasminum flexile* (81.86%) and *Jasminum auriculatum* (71.09%), however, minimum pollen germination was recorded in *Jasminum sambac* (34.08%) (Table 4). Liu Hui-chao *et al*. (2011) recorded highest percentage of pollen germination in *Jasminum nudiflorum* (91.04%). Pollen germination increased with the increase in the time of incubation, while, minimum *in-vitro* germination *per cent* was recorded 1 hr after incubation. Maximum pollen germination was recorded 24 hr after anthesis. The reason for higher pollen germination

Table 3 Ideal pollen germination media for different *Jasminum* species

Species	Sucrose (%)	H_3BO_3 (ppm)	$Ca(NO_3)_2$ (ppm)	$MgSO_4$ (ppm)	PEG4000 (%)	KNO_3 (ppm)
J. sambac	15	100	300	200	10	100
*J. auriculatum*and *J. multiflorum*	15	100	300	200	15	-
J. grandiflorum and *J. flexile*	10	100	300	200	20	-
J. malabaricum	10	100	300	200	15	-

H_3BO_3- Boric acid; $Ca(NO_3)_2$ - calcium nitrate; $MgSO_4$ - magnesium sulphate; PEG 4000 - polyethylene glycol 4000; KNO_3 - potassium nitrate

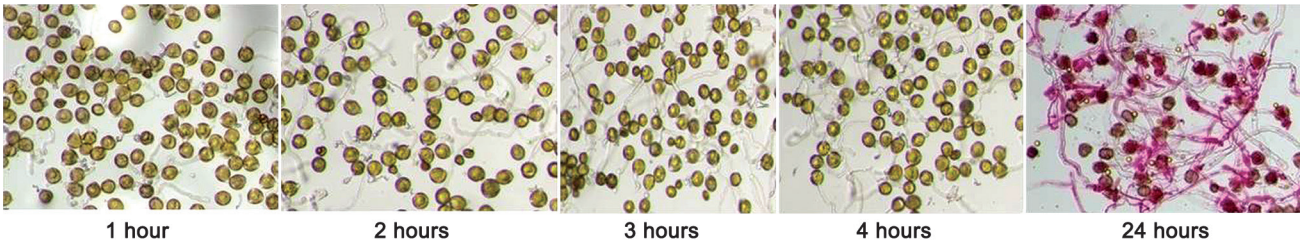

Fig 3 *In vitro* pollen germination in *J. malabaricum* at 1, 2, 3, 4 and 24 hours after incubation.

Table 4 Pollen germination (%) of six *Jasminum* species after 1 hr, 2 hr, 3 hr and 24 hr of inoculation

Species	Number of pollen grains	Pollen grain germination (%)				
		After 1 hr	After 2 hr	After 3 hr	After 4 hr	After 24 hr
J. sambac	69.75	0.79	5.22	9.33	21.99	34.08
J. auriculatum	443.75	15.21	30.55	38.73	46.13	71.09
J. grandiflorum	255.75	0.62	3.90	10.16	21.48	42.99
J. malabaricum	454.75	9.11	20.71	35.10	45.56	91.58
J. multiflorum	302.75	03.05	7.34	24.00	32.55	51.66
J. flexile	354.50	6.81	14.92	29.86	39.89	81.86
SEm±	11.99	1.29	1.20	1.69	1.53	0.90
CD (P=0.05)	35.63	3.84	3.57	5.02	4.56	2.67

with the increase in duration for which the pollen grains were exposed to the pollen germination media could be attributed to adequate amount of nutrient absorption for a longer period.

Raman *et al.* (1970) reported that pollen germination in sucrose solution of different concentrations was generally poor in all the *Jasminum* species and varieties studied. The maximum pollen grains were germinated only when flowers were collected at the time of anthesis. However, Yan-ming *et al*. (2014) reported that pollen for highest viability should be collected at 8.00 - 9.00 am in *J. sambac* Aiton.

The maximum length of pollen tube was recorded in *J. flexile* (1170.68 µm) after 24 hr of germination followed by *J. malabaricum* (951.11µm), *J. auriculatum* (848.15 µm) and *J. multiflorum* (763.30 µm), while minimum pollen tube length was recorded in *J. sambac* (412.17 µm) (Table 5). Increase in length of the pollen tube was observed with increase in duration for which the pollen grains were incubated. The pollen tube length was minimum at 1 hr after incubation. It increased with the increase in incubation time up to 24 hr. The reason for increased pollen tube length with the increase in duration for which the pollen grains were exposed to the germination media might be due to adequate amount of nutrient absorption for a longer period.

Pollen tubes revealed abnormalities like bulging and coiling of tips in *J. sambac* and *J. grandiflorum* which might be the reason for no fruit set when *J. sambac* used as pollen parent and less fruit set in *J. grandiflorum* (male parent). Sun *et al.* (2010) reported that no seed set in the interspecific cross between *Dendranthema grandiflorum* and *D. nankingense*, in that only very few pollen grains germinated on stigmas after pollination and most of them germinated abnormally.

The results indicated that *J. malabaricum* recorded highest percentage of pollen viability followed by *J.*

Table 5 Mean pollen tube length for six *Jasminum* species

Species	Pollen tube length (µm)				
	After 1 hr	After 2 hr	After 3 hr	After 4 hr	After 24 hr
J. sambac	60.03	86.20	115.91	212.56	412.17
J. auriculatum	83.38	104.30	253.56	523.15	848.15
J. grandiflorum	37.78	49.25	151.50	252.81	517.81
J. malabaricum	74.61	235.06	544.34	751.11	951.11
J. multiflorum	87.27	180.94	369.76	538.30	763.30
J. flexile	58.35	220.11	499.04	995.68	1170.68
S.E.m±	6.14	10.83	27.84	26.49	12.10
CD (P=0.05)	18.24	32.19	82.74	78.72	35.96

flexile, and *J. auriculatum*, however, lowest percentage of pollen viability was recorded in *J. sambac*. The species *J. malabaricum*, *J. flexile* and *J. auriculatum* were found to be better pollen parents with better pollen viability as compared to other *Jasminum* species. Quantity of pollen grains obtained from *J. sambac* was very less. This might be the reason for fruit set failure in artificial cross pollination when *J. sambac* was used as a pollen parent.

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