

Original Research Paper

Palynological investigations in *Jasminum* spp.

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ABSTRACT

The present investigation was carried out at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2017-2019. The study involved nine jasmine genotypes, four falling under the commercially cultivated types and five belonging to underutilized species or 'lesser-known species'. The study was undertaken to investigate and document the palynological parameters of jasmines which could serve as a reliable reference for future jasmine breeding programmes. The palynological investigations were carried out using by Scanning Electron Microscopy (SEM), haemocytometry, acetocarmine test and *in vitro* pollen germination. The pollen morphology analysis indicated wide variation among the species for shape of pollen grain, ranging from tricolpate to prolate; the exine ornamentation was reticulate in all the genotypes. Pollen output was the highest in *J. rigidum* (28,660 pollen/flower) and the lowest in (625 pollen/flower) in *J. sambac* cv. Ramanathapuram Gundumalli. The maximum pollen germination rate and pollen tube length was recorded in *J. rigidum*.

Key words: *Jasminum* spp., Palynology, Pollen morphology and Pollen germination

INTRODUCTION

Jasmine (*Jasminum* sp.) belonging to family Oleaceae is one of the most important and popular traditional flowers of India. It is native to South and Southeast Asia. A large number of species of *Jasminum* are centered around the regions comprising India, China and Malaysia (Nirmala *et al.*, 2017). The area coverage under flower crops in India is 3,07,000 ha with a production of 18,05,000 MT of loose flowers and 7,04,000 MT of cut flowers (2017-18) (www.indiastat.com). In India, Tamil Nadu is the leading producer of jasmine in the country with an annual production of 1,36,901 tonnes from an area of 13,246 ha with a productivity of 11.21 t/ha (Anonymous, 2019). The genus *Jasminum* consists of more than 200 species and is mostly tropical in distribution (Dickey, 1969).

Though there are a large number of species and varieties of jasmine, commercial cultivation is confined to only a very few species. Besides the three commercial jasmine species namely, *J. sambac*, *J. grandiflorum* and *J. auriculatum* which have

attained importance in commercial cultivation (Rimando, 2003; Green and Miller, 2009) and the less exploited, *J. multiflorum* (Syn: *J. pubescens*) which is cultivated to some extent in Karnataka, three lesser known species namely, *J. nitidum*, *J. calophyllum* and *J. flexile* possess economic importance since they produce flowers which are suitable for use as loose flower, besides being ideal garden plants. These three species have the added merit of flowering throughout the year (Ganga *et al.*, 2015), unlike the three popular commercial species namely, *J. sambac*, *J. grandiflorum* and *J. auriculatum* which undergo 'off-season' during the cooler months. The species *J. rigidum* also possesses year-round flowering potential, though the flowers have shorter shelf life.

Since the demand for jasmine flowers is growing day by day owing to its wide range of uses, there arises a pressing need for improving its production and productivity, besides exploring newer strategies to evolve improved genotypes. Flowers of the three commercially important jasmine species are



unavailable in the market during the 'off-season' coinciding with the cooler months (November to March). The above mentioned lesser-known species possess year-round flowering potential but produce flowers with milder fragrance. Interspecific hybridization can enable introgression of desirable genes within the species. Interspecific hybridization in jasmine has been attempted earlier (Anon., 1974; Veluswamy, 1981) but was not successful, the predominant reason being failure of seed germination owing to hybrid unviability and a possible endosperm antagonism in operation (Veluswamy, 1981).

Palynological data are essential pre-requisites of any plant breeding programme. Domez and Isik (2008) and Hanif *et al.* (2013) emphasized the association of palynological aspects with the cytological status of plant species. The present study was undertaken to investigate and document the palynological parameters of commercial and lesser-known species of *Jasminum* which would serve as a reliable reference for future jasmine breeding programmes.

MATERIALS AND METHODS

(i) Plant materials

Plant materials were collected from the jasmine germplasm of the Department of Floriculture and Landscape Architecture, TNAU, Coimbatore located at 11°02' N latitude and 76°57' E longitude at an altitude of 426.72 m above MSL. The weather condition at Coimbatore during the study was moderately warm with hot early summer months during March-May. In open field conditions, the maximum temperature fluctuated between 25°C and 35°C with a mean of 30°C. The minimum temperature ranged between 17°C and 23.5°C with a mean of 20°C. The annual rainfall was 750 mm and relative humidity ranged between 60 and 90 per cent with a mean of 75 per cent.

The study involved nine genotypes of jasmine belonging to eight *Jasminum* species. The commercial species category included four genotypes namely, *J. sambac* cv. Ramanathapuram Gundumalli, *J. auriculatum* cv. CO.1 Mullai, *J. grandiflorum* cv. CO.1 Pitchi and *J. grandiflorum* (White). The lesser known (underutilized) species category included five genotypes namely, *J. calophyllum*, *J. flexile*, *J. multiflorum* (Pink flowered type), *J. nitidum* and *J. rigidum*.

(ii) Methods adopted

The methods adopted to study the palynological aspects of the above listed *Jasminum* species are briefly discussed below.

Pollen morphology

For assessing the morphological characteristics of pollen, flower buds at mature bud stage were involved. In the laboratory, anthers were isolated from the flower buds in petri-dishes and were maintained for 24-48 hours at room temperature (24°C) to facilitate release of pollen. Then the petri-dishes with pollen were transferred to a desiccator, where they were kept until analysis. Preparation of pollen for analysis was performed by mounting two-layer transparent tape on the object carrier on the microscope and applying pollen with a brush. The prepared samples were observed under a Scanning Electron Microscope (SEM) at a magnification of 2000X (whole grain) to 15,000X (exine pattern). The pollen size was measured and the range was recorded for a sample of 30 pollen grains from each of the nine genotypes of jasmine.

Pollen output

Pollen production per flower was estimated using Haemocytometer as suggested by Sathiamoorthy (1973). Three samples of anthers from each *Jasminum* species were collected just prior to dehiscence. The anthers were crushed with a small glass rod in a vial containing 2.5 ml of distilled water and a drop of teepol for obtaining a good suspension of pollen grains in water. The contents were thoroughly shaken and two drops of it were pipetted out and placed on each of the two counting chambers of a Spencer bright line Haemocytometer. The number of pollen grains in each of the eight "corner squares" was recorded. This was repeated five times for each sample and was designated as sub samples. The average number of pollen grains per square multiplied by 2500 gave the quantity of pollens per anther.

Pollen viability

The pollen viability and fertility were assessed by the following two methods.

(a) Acetocarmine test

Freshly dehisced pollen grains were collected from each of the *Jasminum* species in sterilized petri-

dishes. The pollen grains were dusted on the cavity slide and a drop of Acetocarmine stain was placed on the pollen grains. Deeply stained, normal and plump pollen grains were considered viable while shriveled, deformed and weakly stained pollen grains were considered as sterile ones. Pollen viability was assessed for three days *viz.*, first day, second day and third day of anther dehiscence and expressed in percentage.

(b) *In vitro* pollen germination

Pollen from freshly dehisced anthers were collected and tested for germination on the day of anther dehiscence. Pollen was dusted in cavity slide in which 10 per cent of sucrose and 10 ppm of boric acid solution were dropped over gently with a pointed dropper and pollen grains were mixed thoroughly in the solution with the help of a needle. The cavity slides were kept in petri dishes over small glass rods containing moist filter paper at the bottom and closed properly. These were then incubated at ambient room temperature (25°C) for optimum germination. The slides were examined under microscope after one hour, two hours and three hours. Germinated and non-germinated pollen

grains were counted separately in several random fields containing a total of 100 pollen grains and the length of the pollen tube was measured in micrometer (μm).

RESULTS AND DISCUSSION

Pollen morphology

The details of pollen morphology of the nine genotypes of jasmine are furnished in Table 1. Scanning Electron Microscope (SEM) images of the *Jasminum* genotypes indicated wide variations in shape of pollen among the genotypes.

Pollen size

The pollen size ranged between 36.2 μm and 57.9 μm in *J. sambac* cv. Ramanathapuram Gundumalli, 33.0 μm and 42.9 μm in *J. auriculatum* cv. CO. 1 Mullai, 32.32 μm and 41.22 μm in *J. grandiflorum* cv. CO. 1 Pitchi, 33.0 μm and 46.2 μm in *J. grandiflorum* (White), 53.5 μm and 68.4 μm in *J. calophyllum*, 30.2 μm and 53.2 μm in *J. flexile*, 30.5 μm and 53.5 μm in *J. multiflorum* (Pink), 33.0 μm and 52.8 μm in *J. nitidum* and 39.6 μm and 49.5 μm in *J. rigidum* (Table 1).

Table 1. Pollen morphology, pollen size (μm) and pollen output in *Jasminum* species

Sl. No.	<i>Jasminum</i> genotype	Pollen shape	Morphological characters		Pollen size range (μm)	Pollen output
			Exine ornamentation	Aperture		
1.	<i>J. sambac</i> cv. Ramanathapuram Gundumalli	Tricolpate	Reticulate	Poorly defined	36.2-57.9	625
2.	<i>J. auriculatum</i> cv. CO.1 Mullai	Late obovatus	Reticulate	Sunken	33.0-42.9	14,175
3.	<i>J. grandiflorum</i> cv. CO.1 Pitchi	Prolate	Reticulate, granular	Prominent	32.32-41.22	17,920
4.	<i>J. grandiflorum</i> (White)	Spheroidal	Coarsely Reticulate	Poorly defined	33.0-46.2	23,816
5.	<i>J. calophyllum</i>	Obtuse-angular	Reticulate	Prominent	53.5-68.4	8,769
6.	<i>J. flexile</i>	Circular	Reticulate and smooth	Poorly defined	30.2-53.2	13,778
7.	<i>J. multiflorum</i> (Pink)	Prolate	Distinctly reticulate	Poorly defined	30.5-53.5	17,002
8.	<i>J. nitidum</i>	Circular	Coarse	Sunken	33.0-52.8	21,056
9.	<i>J. rigidum</i>	Prolate	Reticulate, conspicuous furrows	Prominent	39.6-49.5	28,660

Pollen output

Pollen output (average number of pollen produced/flower) for the *Jasminum* species ranged between 625 and 28,660. The pollen output was 28,660 in *J. rigidum* which was the highest among the nine genotypes and 625 in *J. sambac* cv. Ramanathapuram Gundumalli which was the least among the genotypes. The pollen output was 14,175 in *J. auriculatum* cv. CO.1 Mullai, 17,920 in *J. grandiflorum* cv. CO.1 Pitchi, 23,816 *J. grandiflorum* cv. White, 8,769 in *J. calophyllum*, 13,778 in *J. flexile*, 17,002 in *J. multiflorum* (Pink) and 21,056 in *J. nitidum* and (Table 1).

Makde (1982) opined that in *Jasminum* species, a majority of pollen grains were characterized by large scale vacuolation and scanty cytoplasm resulting in degeneration of pollen grains prior to dehiscence, leading to low pollen output.

Pollen viability

Data on pollen viability are furnished in Tables 2 and 3 and pictorial details are furnished in Fig. 1

to 3. The percentage of fertile pollen in *J. sambac* cv. Ramanathapuram Gundumalli was 5.67 per cent on the day of dehiscence and it gradually decreased to 0 per cent on the third day. The percentage of fertile pollen in *J. auriculatum* cv. CO.1 Mullai was 94.87 per cent on the day of dehiscence and it gradually decreased to 43.33 per cent on the third day. The percentage of viable pollen in *J. grandiflorum* cv. CO.1 Pitchi was 77.17 per cent on the day of dehiscence and gradually decreased to 18.67 per cent on the third day. The percentage of viable pollen in *J. grandiflorum* cv. White was 95.50 per cent on the day of dehiscence and it gradually decreased to 18.67 per cent on the third day. (Table 2).

In *J. flexile*, the percentage of viable pollen was 90.67 per cent on the day of dehiscence and it gradually decreased to 22.33 per cent on the third day. The percentage of viable pollen in *J. calophyllum* was 77.17 per cent on the day of dehiscence and it gradually decreased to 18.67 per

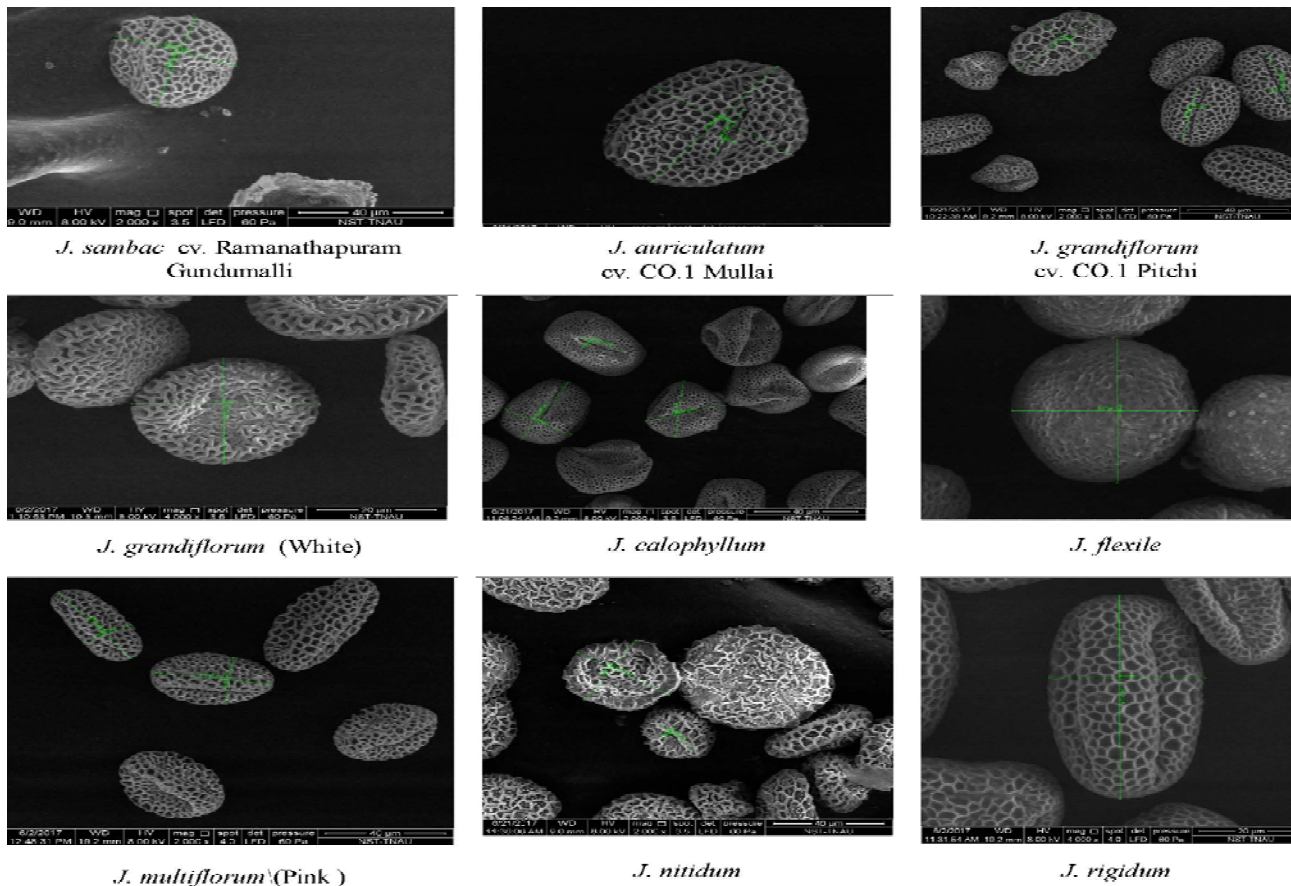


Fig. 1. Pollen morphology *Jasminum* species visualized under SEM



cent on the third day. The percentage of viable pollen in *J. multiflorum* (Pink) was 54.67 per cent on the day of dehiscence and gradually decreased to 17.33 per cent on the third day. The percentage of fertile pollen in *J. nitidum* was 53.33 per cent on the day of dehiscence and it gradually decreased to 18.33 per cent on the third day. The percentage of fertile pollen in *J. rigidum* was 95.33 per cent on the day of dehiscence and it gradually decreased to 27.67 per cent on the third day (Table 3).

Pollen germination rate

The mean germination percentage in *Jasminum* species ranged between 0 per cent and 94.34 per cent, with the highest in *J. rigidum*. No germination was observed in *J. sambac* cv. Ramanathapuram Gundumalli. The mean germination percentage was 42.56 per cent in *J. auriculatum* cv. CO.1 Mullai, 25.3 % in *J. grandiflorum* cv. CO. 1 Pitchi, 59.9 % in *J. grandiflorum* cv. White, 31.72 % in *J. flexile*,

24.0 % in *J. calophyllum*, 1.39 % in *J. multiflorum* (Pink) and 8.86 % in *J. nitidum* (Table 4).

Pollen tube growth

In the present study, pollen tube growth as measured in terms of the length of pollen tube in the *Jasminum* species ranged between 0 µm and 1917.10 µm. The maximum pollen tube length of 1917.10 µm was recorded in *J. rigidum*. The mean length of pollen tube was 447.81 µm in *J. auriculatum* cv. CO. 1 Mullai, 552.61 µm in *J. grandiflorum* cv. CO. 1 Pitchi, 449.82 µm in *J. grandiflorum* cv. White, 222.15 µm in *J. flexile*, 641.15 µm in *J. calophyllum*, 884.27 µm in *J. nitidum*. Pollen tube was not conspicuous in *J. multiflorum* (Pink).

Pollen viability is an important factor, since the probability of fertilization usually declines when pollen grains with low viability are deposited on the stigma (Wilcock and Neiland, 2002; Zhao *et al.*, 2004; Teng *et al.*, 2012). Lai (1995) and Deng *et al.* (2014) attributed low pollen fertility and Deng *et al.* (2016)

Table 2. Pollen of commercial jasmine genotypes

Age of pollen grains	No. of. pollen grains tested	<i>J. sambac</i> cv. Ramanthapuram Gundumalli		<i>J. auriculatum</i> cv. CO. 1 Mullai		<i>J. grandiflorum</i> cv. CO. 1 Pitchi		<i>J. grandiflorum</i> (White)	
		Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)
1 st day of dehiscence	100	5.67	94.33	94.87	5.13	77.17	22.83	95.50	4.50
2 nd day of dehiscence	100	3	97	78.33	21.67	56.00	44.00	62.67	37.33
3 rd day of dehiscence	100	0	100	43.33	56.66	18.67	81.33	18.67	81.33

Table 3. Pollen viability of underutilized jasmine genotypes

Age of pollen grains	No. of. pollen grains tested	<i>J. nitidum</i>		<i>J. calophyllum</i>		<i>J. multiflorum</i> (Pink)		<i>J. flexile</i>		<i>J. rigidum</i>	
		Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)
1 st day of dehiscence	100	53.33	46.67	83.33	16.67	54.67	45.33	90.67	9.33	95.33	4.67
2 nd day of dehiscence	100	44.33	55.67	75.33	24.67	45.33	54.67	69.33	30.67	78.00	22.00
3 rd day of dehiscence	100	18.33	81.67	25.67	74.33	17.33	82.67	22.33	77.67	27.67	72.33

Table 4: *In vitro* pollen germination in *Jasminum* species (after 3 hr incubation)

Species	Germination %	Length of pollen tube (μm)
<i>J. sambac</i> cv. Ramanathapuram Gundumalli	0.00	-
<i>J. auriculatum</i> cv. CO. 1 Mullai	42.56	447.81
<i>J. grandiflorum</i> cv. CO. 1 Pitchi	25.3	552.61
<i>J. grandiflorum</i> (White)	59.9	449.82
<i>J. flexile</i>	31.72	222.15
<i>J. calophyllum</i>	24.0	641.15
<i>J. multiflorum</i> (Pink)	1.39	-
<i>J. nitidum</i>	8.86	884.27
<i>J. rigidum</i>	94.34	1917.10

Fig. 2. Pollen stainability in *Jasminum* genotypes

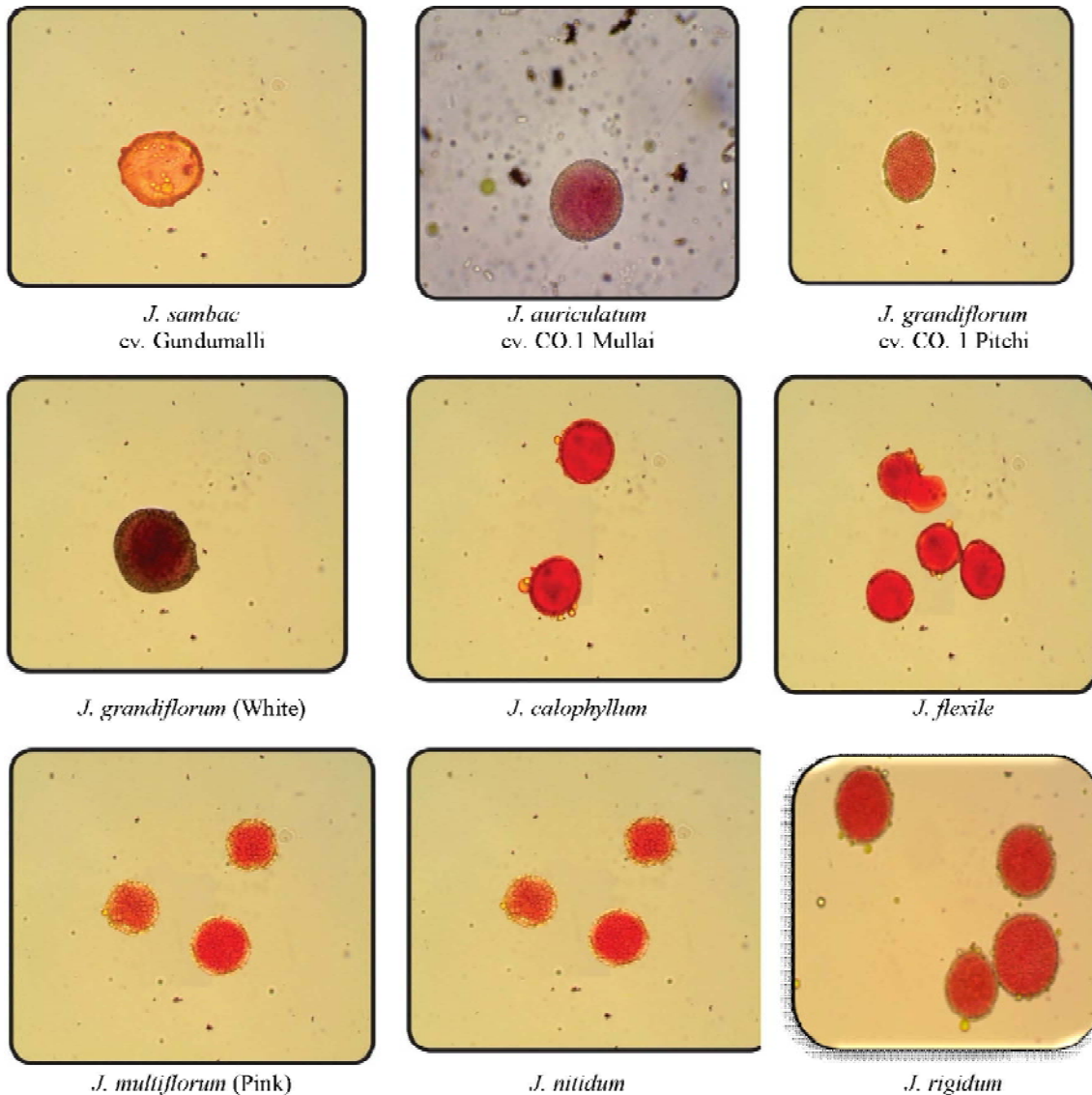
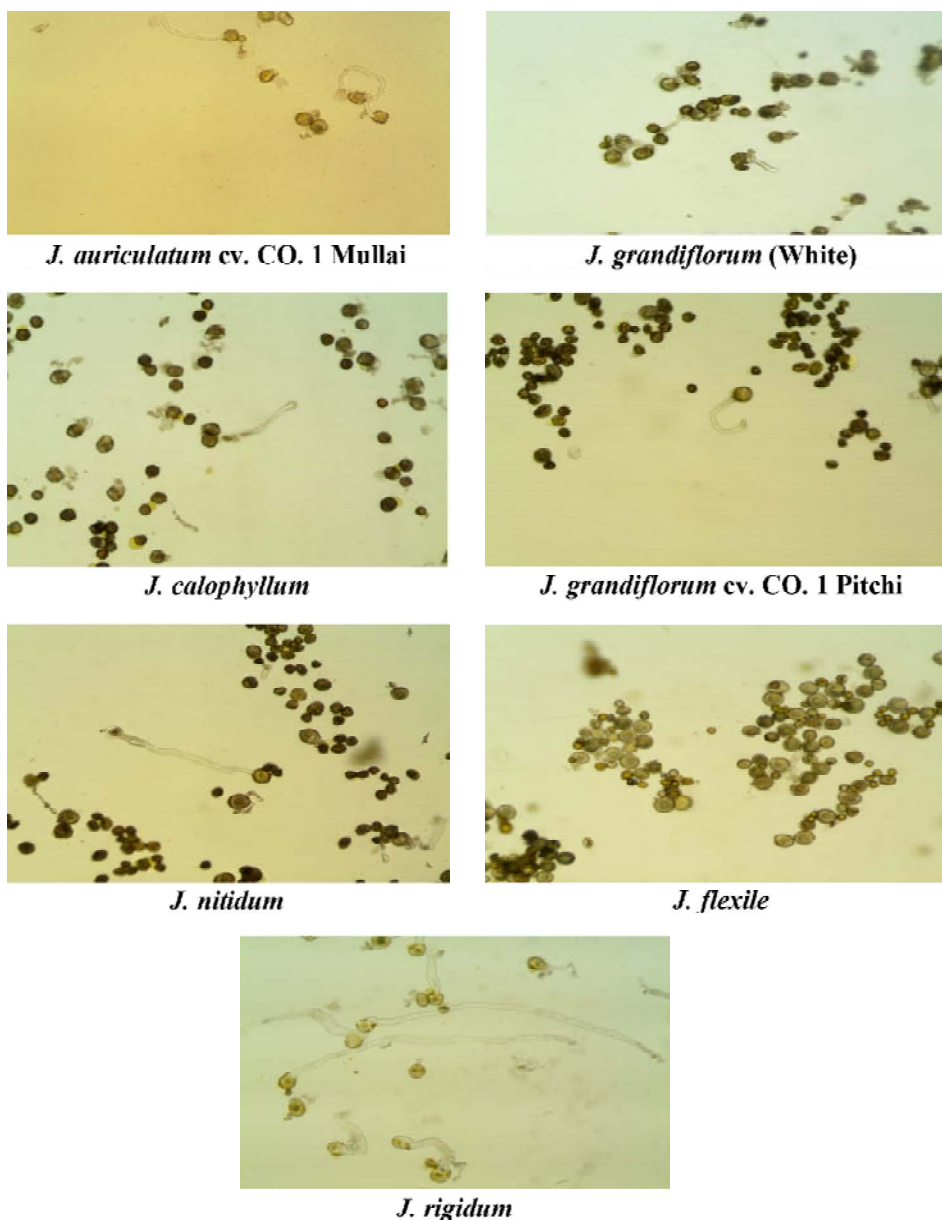


Fig. 3. *In vitro* pollen germination in *Jasminum* genotypes



attributed low pollen viability for poor fertilization in jasmine. Deng *et al.* (2016) also reported that among 15% of anthers, tetrads formed pollen mass instead of free microspores, and only one tube grew normally from the tetrad pollen.

The differential pollen viability of the jasmine species recorded in the present study is attributable to the varied ploidy levels and chromosomal forms. In *J. sambac* cv. Ramanathapuram Gundumalli, higher pollen sterility is attributed to its triploid status which leads to meiotic abnormalities. These observations are supported by the earlier reports of Raman (1955) and Deng *et al.* (2017) in *Jasminum* species.

In the various jasmine species studied, pollen germination percentage was low to moderate (Plate 2). This might be associated with the irregular meiosis leading to defective pollen and egg cells, ultimately resulting in sterility. Datta *et al.* (1960) elucidated that structural changes lead to loss of genes as expressed in the suppression of the female reproductive development in *J. grandiflorum*.

CONCLUSION

The inferences from the present study are (i) there is wide variation among the nine genotypes with respect to shape of pollen grain while exine

ornamentation was reticulate in all the genotypes, (ii) *J. auriculatum* cv. CO.1 Mullai, *J. grandiflorum* cv. White, *J. flexile* and *J. rigidum* had high pollen viability. Pollen output (average number of pollen produced/flower) was the highest

in *J. rigidum* (28,660) and the least (625) in *J. sambac* cv. Ramanathapuram Gundumalli; (iii) The highest rate of pollen germination (94.34%) and length of pollen tube (1917.10 μm) was recorded in *J. rigidum*.

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