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Assessment of pollen viability and *in vitro* pollen germination in Nerium cultivars (*Nerium oleander* L.)

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Abstract

Pollen viability and *in vitro* pollen germination was examined in single and double types of Nerium cultivars. Maximum pollen viability per cent was recorded in the cultivar UHSBN-6 (97.90%) followed by the cultivar UHSBN-14 (95.68%) and least per cent was recorded in the cultivar UHSBN-24 (89.58) when methyl blue was used as a dying agent. Potassium iodide showed the maximum per cent pollen viability in the cultivar UHSBN-12 (95.66%). Further, maximum *in vitro* pollen germination was recorded in the cultivar UHSBN-19 (68.69%) in 15% sucrose with 100 ppm boric acid which was on par with the cultivar UHSBN-17 and UHSBN-29 (67.18 and 66.90% respectively). The cultivar UHSBN-12 recorded maximum *in vitro* pollen germination (77.12%) under Brew baker and Kwack medium which was on par with cultivars UHSBN-19 and UHSBN-30 (74.80 and 74.72% respectively). The results obtained from the present investigation can aid in planning crosses and establishment of Nerium hybridization programs using compatible cultivars.

Keywords: Pollen viability, *in vitro*, *Nerium oleander*, pollen germination

Introduction

Nerium oleander L. is one of the evergreen shrub belongs to the family Apocynaceae. The family which is commonly recognized as dogbane family includes about 424 genera distributed in tropical and subtropical regions (Endress and Bruyns, 2000). Nerium distributed in Mediterranean region and subtropical Asia, is indigenous to India and Pakistan. It has been distributed in the Himalayas from Nepal westwards to Kashmir upto 1950m extending to Baluchistan, Afghanistan and found throughout India (Kiran and Prasad, 2014) [14]. Most of the South Indian states are cultivating different kinds of commercial loose flowers and are used for religious and pooja purpose. Cultivation of nerium is highest in Tamil Nadu with an area of 1408 hectares with the production of 33,780 tonnes with the maximum productivity of 24 t/ha, among other loose flowers. Commercial cultivation of nerium has also been found in Karnataka, Kerala and Andhra Pradesh.

Nerium is an evergreen ornamental shrub of immense significance which is used in urban and peri urban landscaping due to its spectacular flowering which can have different colorations as the variety and its resistance to long drought periods (Albornoz *et al.*, 2014) [1]. The incontrovertible aesthetical worth in hand with tolerance to drought, brackish winds and air pollution makes the nerium, an useful plant in all types of urban arrangements, gardens, parks and motorway median floorings widely (Pagen, 1988) [21]. Several cultivars have become very popular as cultivated shrubs because of their fragrant showy blooms and in spite of the poisonous quality of the sap. It is an urbanite plant widely used for ornamental purposes in streets, gardens, hospitals and also used as versatile loose flower and religious offerings. Nerium is also known for its medicinal uses *viz.*, antibacterial (Derwich *et al.*, 2010) [6] antimicrobial, anti-inflammatory, anti-nociceptive (Erdemoglu *et al.*, 2008) [8] and antitumor activities (Manna *et al.*, 2000) [19]. The nerium is also economically important for the pharmaceutical industry because it produces bioactive secondary metabolites, mainly cardenolides, flavonoids and terpenes (Fu *et al.*, 2005) [10]. So to meet the demand of the landscape industry and domestic flower markets, there is a need to carry out a systematic

research on floral biology of nerium flowers. For genetic improvement and domestication of a species, the need to understanding biology of species and crossability pattern is a pre-requisite. One of the important factor for flower fertilization is pollen viability and germination, therefore pollen performance may have a significant role in pollination. Information available on pollen biology of nerium is very limited and the aim of this study was to determine the pollen viability and *in vitro* pollen germination in single and double cultivars. The present study on pollen viability and *in vitro* pollen germination will give an idea about pollen quality and viability in nerium cultivars, which will be helpful in nerium reproduction and hybridization studies.

Material and Methods

Study site and plant materials

The present investigation was carried out at Department of Floriculture and Landscape Architecture, College of Horticulture, University of Horticultural Sciences, Bagalkot during the year 2018-2020. Thirty cultivars of nerium were collected from Tamil Nadu Agricultural University, Coimbatore and different parts of Karnataka were used as experimental material. The list of cultivars and place of collection were listed in the table 1.

Pollen viability tests

The pollen viability and fertility were studied by acetocarmine method as suggested by (Sathiamoorthy, 1973 and Ranchana *et al.*, 2015) [23]. The pollen viability and fertility were studied by using three different stains *viz.*, Methyl blue, Potassium iodide and Acetocarmine. The freshly dehisced pollen grains were collected from flowers of all cultivars in sterilized petri dish. The pollen grains were dusted on the cavity slide followed by pouring a drop of stain on the slides. Deeply stained, normal and plumped pollen grains were considered as viable while, shrivelled, deformed and weakly stained pollen grains were recorded as non-viable ones (Figure 1). Pollen fertility was expressed in percentage (%).

In vitro pollen germination

Effect of sucrose in combination with boric acid on *in vitro* pollen germination and *in vitro* pollen germination in Brewbaker and Kwack medium were studied (Brewbaker and Kwack, 1963 and Ranchana *et al.*, 2015) [23]. Brewbaker and Kwack medium contains Sucrose 15% W/V, Boric acid 100 ppm, Calcium Nitrate 200 ppm, Magnesium Sulphate 200 ppm and Potassium Nitrate 100 ppm. To study the *in vitro* pollen germination, newly opened flowers were collected in the morning hours (6 AM - 8.00 AM) and transferred to polythene bag. The fresh pollen grains samples were sown on several grooved slides containing solution of 15% sucrose combinations with 100 ppm boric acid and Brewbaker and Kwack medium. Further, the slides were then kept in petri dishes lined with moist filter paper and examined under trinocular compound photo microscope (Model BM-3200T) attached with IS Capture (ISC) software. A pollen grain was considered as germinated if pollen tube length becomes double the diameter of the normal pollen grains (Figure 1). The experiments were replicated thrice in a completely randomized design (CRD) in each treatment. The data were analyzed computing ANOVA and critical difference at 0.5% probability.

Result and Discussion

Significant variations were found with pollen viability for Methyl blue and Potassium iodide stains in the nerium

cultivars (Table 2). Maximum pollen viability per cent was recorded in the cultivar UHSBN-6 (97.90%) followed by the cultivar UHSBN-14 (95.68%) and least per cent was recorded in the cultivar UHSBN-24 (89.58) when methyl blue was used as dying agent. Potassium iodide showed the maximum per cent pollen viability in the cultivar UHSBN-12 (95.66%) which was on par with the cultivars UHSBN-15, UHSBN-14 and UHSBN-3 (94.98, 94.38 and 94.28% respectively). While, least pollen viability was recorded in the cultivar UHSBN-25 (88.53%) followed by UHSBN-24 (89.06%). Aceto carmine dye was observed to be non significant results for pollen viability. High pollen viability was observed in the present investigation was in conformity with the observation made by Usman *et al.* (2016) [30] in *Rauvolfia serpentine*. Kulloli and Shreekala (2009) [18] in *Rauvolfia micrantha* observed that nearly 82% pollen grains were viable after 6 hours of anthesis and gradually reduced on successive days after anthesis. Lopes and Machado (1999) studied the pollen viability in *Rauvolfia grandiflora* and reported that pollen viability was 97.4 per cent. Similar observations were made in *Gloriosa rothschildiana* by Anandhi (2013) and Kuligowska *et al* (2016) [17] in *Hibiscus* species section Muenchhusia. Pollen viability is an ability of a pollen grain to germinate and develop as a pollen tube (Prajapati and Jain, 2011; Sarika and Mary-Varkey, 2012) [24]. Growth of the pollen tube can be taken as the measure of pollen viability since the non-viable pollen could not be grown in to a pollen tube. Good pod set cannot be achieved unless pollen is viable with high germination percentage. Significant variations were observed for *in vitro* pollen germination among the selected nerium cultivars (Table 3). In the present experiment, maximum *in vitro* pollen germination was recorded in the cultivar UHSBN-19 (68.69%) in 15% sucrose with 100 ppm boric acid which was on par with the cultivar UHSBN-17 and UHSBN-29 (67.18 and 66.90% respectively). While, the least pollen germination was recorded in the cultivar UHSBN-16 (60.33%) followed by the cultivar UHSBN-15 (61.20%) in the same media combination. Mondal and Ghanta (2009) in *Lowsonia inermis* made similar observation that sucrose in combination with 100 ppm boric acid gave 92% pollen germination. Sucrose in combination with boric acid promoted pollen germination because boron makes a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionised sugar molecules (Gauch *et al.*, 1955 and Sindhu *et al.*, 1986). The pronounced effect of sucrose and boric acid on pollen germination might be reflected with the views of Johri and Vasil (1961) [13] and Shivanna and Johri (1985) [26] that externally supplied sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism. Boron takes part in pollen germination and style tube formation, therefore has a vital function in fertilization of flowering crops. Similar observation on *in vitro* pollen germination was reported by Deng *et al.* (2017) [5] in Single Petal and Double Petal type of jasmine and revealed that, highest *in vitro* pollen germination rates for jasmine types are 10.1% and 20.3%, respectively. Korkmaz and Guneri (2019) [15, 16] reported in pomegranate the highest pollen germination rate at 10 mg/ L boron application were 62.17%.

Brewbaker and quack medium is a complex medium consisting of sucrose (15% w/v), Boric acid (100 mg/l), Ca (NO₃) (200 mg/l), MgSO₄ (200 mg/l) and KNO₃ (100 mg/l). It was widely used in the preparation of pollen culture medium. It contains all the basic requirements for successful pollen germination. Apocynacean pollen grains gives positive

response to the *in vitro* test in presence of Brewbaker's medium (Chatterjee *et al.*, 2014) [4]. The cultivar UHSBN-12 recorded maximum *in vitro* pollen germination (77.12%) under Brew baker and Kwack medium which was on par with cultivar UHSBN-19 and UHSBN-30 (74.80 and 74.72% respectively). The lowest pollen germination under Brewbaker and Kwack medium was recorded in the cultivar UHSBN-17 (70.22%) followed by in the cultivar UHSBN-28 (70.70%). The obtained results are in close conformity with earlier findings of Kulloli and Shreekala (2009) [18] and they revealed that, best pollen germination (82%) was achieved in Brewbaker and quack medium containing sucrose after 2 hours of incubation. Despite of sucrose, Brewbakers medium contains other salts such as Boron, Calcium, Magnesium and

Potash, the combined effect of sucrose and salts played significant role in pollen germination. In the present investigation, the pollen viability was less under *in vitro* pollen germination when comparing with staining methods among the nerium cultivars. This was in close conformity with findings of Du *et al.* (2017) in peony cultivars and reported that differential results obtained using different pollen viability assays confirmed that the *in vitro* germination test showed lower viability compared to I2-KI staining method. The present study suggests that, irrespective of nerium cultivars, pollen viability and quality of the pollen grains was good, suggesting that all studied cultivars can be used as male parent in hybridization programme.

Table 1: List of cultivars used for pollen viability and *in vitro* pollen germination studies

Cultivars	Flower colour	Type	Place of collection	Cultivars code
3	White	Single	TNAU, Coimbatore	UHSBN-3
6	Red with yellow center	Single	TNAU, Coimbatore	UHSBN-4
8	Light pink	Single	TNAU, Coimbatore	UHSBN-8
12	Pink	Single	TNAU, Coimbatore	UHSBN-9
14	Pink with yellow centre	Single	TNAU, Coimbatore	UHSBN-14
15	Ivory yellow	Single	TNAU, Coimbatore	UHSBN-15
16	White	Double	TNAU, Coimbatore	UHSBN-16
17	Pink	Double	TNAU, Coimbatore	UHSBN-17
19	Ivory yellow	Single	TNAU, Coimbatore	UHSBN-19
24	Yellow	Double	Bangalore, Karnataka	UHSBN-24
25	Red	Double	Ilkal, Karnataka	UHSBN-25
26	Pinkish orange	Single	Ilkal, Karnataka	UHSBN-26
28	Salmon pink	Single	Bangalore, Karnataka	UHSBN-28
29	Pink	Single	Ilkal, Karnataka	UHSBN-29
30	Dark red	Single	Bagalkot, Karnataka	UHSBN-30

Table 2: Pollen viability in in single and double nerium (*Nerium oleander* L.) cultivars

Treatment	Pollen viability (%)		
	Methyl Blue	Potassium iodide	Aceto Carmine
UHSBN-3	94.30	94.28	93.54
UHSBN-6	97.90	91.36	92.60
UHSBN-8	93.73	92.34	93.50
UHSBN -12	92.31	95.66	93.12
UHSBN-14	95.68	94.38	92.63
UHSBN-15	92.87	94.98	92.82
UHSBN-16	90.42	91.37	90.90
UHSBN-17	93.24	90.33	88.77
UHSBN-19	91.67	92.97	91.48
UHSBN-24	89.58	89.06	89.06
UHSBN-25	90.22	88.53	91.01
UHSBN-26	91.56	92.44	92.61
UHSBN-28	92.93	91.56	92.23
UHSBN-29	93.02	92.03	93.94
UHSBN-30	94.79	93.41	92.53
S.E.m	1.16	0.99	1.33
C.D.(p=0.05%)	3.36	2.87	NS

Table 3: *In vitro* pollen germination in single and double Nerium (*Nerium oleander* L.) cultivars

Cultivars	<i>In vitro</i> pollen germination (%)	
	Sucrose (15%) +100ppm Boric acid	Brewbekar and Kwack
UHSBN-3	65.95	74.12
UHSBN-6	66.26	73.19
UHSBN-8	61.74	72.48
UHSBN -12	66.78	77.12
UHSBN-14	63.29	73.74
UHSBN-15	61.20	70.92
UHSBN-16	60.33	72.21
UHSBN-17	67.18	70.22
UHSBN-19	68.69	74.80
UHSBN-24	65.65	73.71

UHSBN-25	64.60	70.90
UHSBN-26	64.96	71.80
UHSBN-28	63.69	70.70
UHSBN-29	66.90	74.17
UHSBN-30	65.95	74.72
S.E.m	0.636	0.843
C.D.(p=0.05%)	1.845	2.44

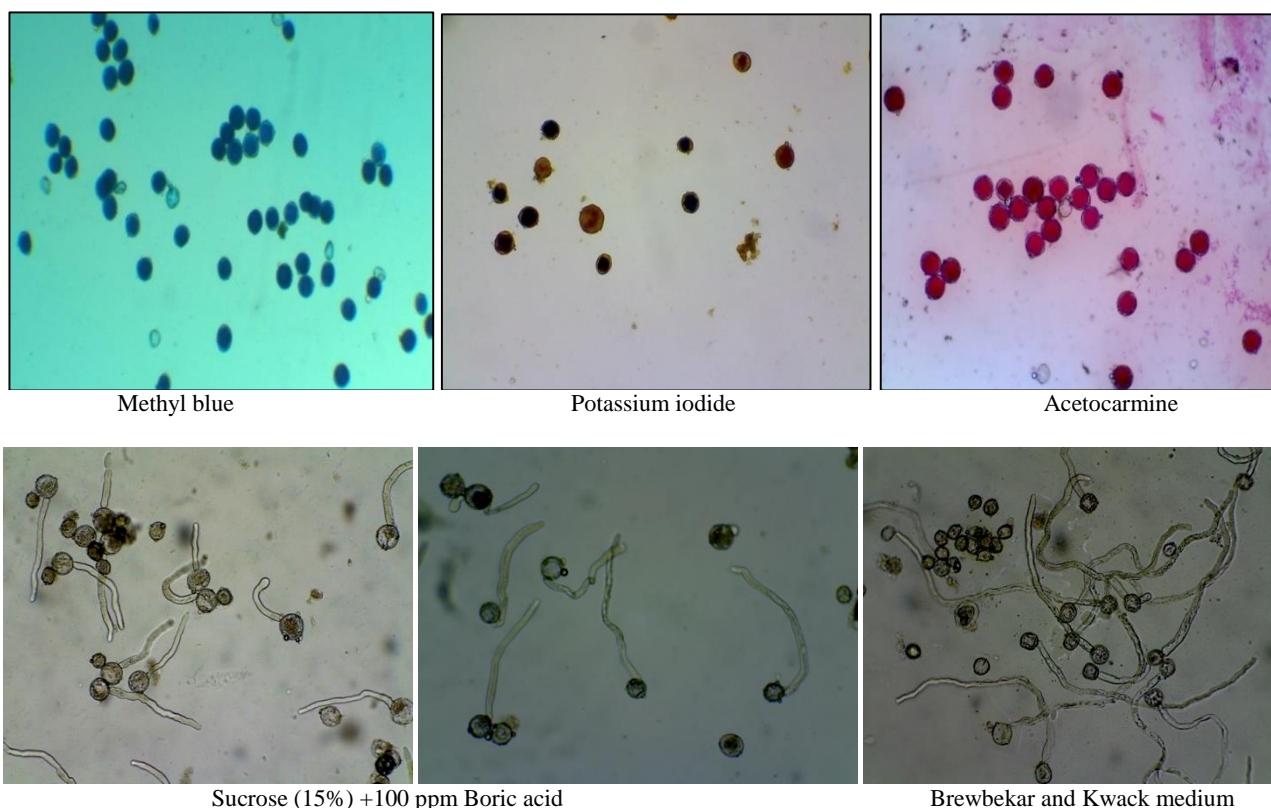


Fig 1: Pollen viability and *in vitro* pollen germination under different media in *Nerium* cultivars

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