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Parashuram M
Department of Floriculture and Landscaping, Horticulture College and Research Institute, TNAU Coimbatore, Tamil Nadu, India

KR Rajadurai
Assistant Professor, Department of Floriculture and Landscaping, Horticulture College and Research Institute, TNAU Coimbatore, Tamil Nadu, India

S Haripriya
Assistant Professor Hort. Department of Nano Science and Technology, Horticulture College and Research Institute, TNAU Coimbatore, Tamil Nadu, India

Correspondence
Parashuram M
Department of Floriculture and Landscaping, Horticulture College and Research Institute, TNAU Coimbatore, Tamil Nadu, India

Evaluation of nerium cultivars for physiological and biochemical parameters under Coimbatore conditions Tamil Nadu India (*Nerium oleander* L.)

Parashuram M, KR Rajadurai and S Haripriya

Abstract
The present investigation was under taken to study the physiological and biochemical parameters of nerium cultivars (Nerium *oleander* L.) such as leaf area, chlorophyll and proline content, observation on trichromes and stomata at Department of Floriculture and Landscaping, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore in the year 2016-2017. Among the parameters studied highest leaf was recorded in the Pink cultivar followed by Red cultivar and the least in Yellow cultivar. Chlorophyll content is the major factor which influences flower yield. The highest chlorophyll content was recorded in Pink cultivar and the lowest chlorophyll content recorded in Yellow cultivar. Proline content is one of the important amino acid which will give resistance to abiotic stresses. Among the cultivars highest proline content was recorded in Yellow cultivar followed by White cultivar while least proline content was recorded in Pink cultivar. Stomatal observations of nerium cultivar was done on both upper and lower surface of the leaf there was no much differences was observed among the cultivars. In all the cultivars of nerium there is no stomata was observed on upper surface of the leaf. The stomatal crypt was observed on lower surface of the leaf in all nerium cultivars. Around the stomatal crypt, many number of stomata were distributed. In lower surface of the leaves trachoma's were found densely in all the cultivars. Stomata were found at the base of the trichromes on lower surface of the leaves. These preliminary data will be useful in order to examine the physiological and biochemical studies in nerium in future days.

Keywords: *Nerium oleander* (L), proline, chlorophyll, stomatal crypt, trichrome

Introduction
Nerium (*Nerium oleander* L.) is an evergreen shrub or small tree in the dogbane family Apocynaceae. The Apocynaceae is a diverse and species-rich family in the order Gentianales. The family has a widespread distribution throughout tropical and temperate regions. *Nerium oleander* L. is widely cultivated that no precise region of origin has been identified, though South West Asia has been suggested. It is either native or naturalized to a broad area from Mauritania, Morocco, and Portugal eastward through the Mediterranean region and the Sahara to the Arabian peninsula, Southern Asia, and as Far East as Yunnan in Southern parts of China. It typically occurs around dry stream beds. In India nerium distributed throughout the country but commercial cultivation takes place in Tamil Nadu only. Nerium grows to 2–6 m tall, with erect stems that spread outward as they mature. Leaves are 10 to 22 cm. long, narrow, acute and have a prominent mid rib, are "leathery" in texture and usually arise in groups of three from the stem. The plant produces terminal flower heads, usually pink or white. Nerium is an ornamental plant used in the urban landscaping due to its spectacular flowering which can have different colorations as the variety and its resistance to long drought periods. In Tamil Nadu, nerium is used for various purposes like ornamental plants in garden, religious purpose and garlands. Now days in India neruim cultivation gaining more importance due to its wide utility but no systematic research has been carried out. Hence keeping all these in view, the research has been undertaken to assess some physiochemical parameters of nerium cultivars which will help for further physiological and biochemical studies in relation to drought tolerance studies in *Nerium oleander* (L.)

Material and Methods
The study was conducted during 2016-2017 to study the physiological and biochemical parameters of nerium cultivars at Department of Floriculture and Landscaping, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The four cultivars of nerium was collected from different districts of Tamil Nadu. The well-established ground layered plants were used for plating. In each replication five plants were selected in all

the treatments and tagged for recording the observation on physiological and biochemical parameters. The observation were recorded are leaf area, chlorophyll content, proline content, stomatal and trichome observation. Leaf area was measured from base of leaf blade to the tip of the leaf using Bio-vis software and in expressed centimetre square (cm^2).The total chlorophyll content and its fractions were assessed by following the method of Yoshida *et al*., 1976.Proline content in the green leaves was estimated by the method suggested by Bates *et al.,* 1973 [3]. Stomatal observation was done using lactic acid method, during this experiment. Nerium leaves are very thick and leathery and hence the other methods are not suitable to observe the stomata in nerium. For observing the stomata medium mature leaves were collected from each cultivars. The leaves were washed with clean water and then leaves cut in to small bits. Leaf bits were kept in boiling water for 5 to 6 minutes for softening. Then the leaves were kept in 70 % alcohol to remove the chlorophyll for 24 hours. After that the leaf bits were collected and rinse with clean water for 2 to3 times, then to soften the leaf bits boiled with the lactic acid for 4 to 5 minutes. Now leaf sample was ready for the observation. Both upper and lower leaf surface of stomata was observed with light microscope at 100x and 400x magnification and photo was took with help the of scope image 9x software with camera attached. The statistical analysis was done by adopting the standard procedures of Panse and Sukhatme (1985). The critical difference was worked out at five per cent (0.05) probability. Analysis was carried out with AGRES software package and MS Excel® spreadsheet.

Result and Discussion

A perusal of data embodied in table 1 revealed that narium cultivars differed significantly with respect to leaf area. Among the cultivars the highest leaf area of 47.81cm^2 was recorded in the Pink cultivar followed by Red cultivar (38.54 cm^2) and the least in Yellow cultivar (37.81cm^2). Leaf area will directly influences the plant yield by increasing the photosynthetic rate. This is in accordance with observation of (Mackay *et al*., 2005 and Kumar and Haripriya. 2010) [6] in nerium respectively. The similar observation was made by (Mollick *et al.,* 2011) [7] in *Codiaeum variegatum* (L.) cultivars reports that leaf area of cultivars ranges between 30.8 to 209.8 cm^2.

The data on chlorophyll content are presented in table 2. Significant variations were found regarding chlorophyll content among the different nerium cultivars. Among the nerium cultivars highest chlorophyll content (7.76 mg g^{-1}**)** was recorded in Pink cultivar which is on par with White cultivar (7.35 mg g^{-1}) while least chlorophyll content was observed Yellow cultivar (6.57 mg g^{-1}). Chlorophyll content is the major factor which influences flower yield.The highest chlorophyll content was recorded in Pink cultivar and the lowest chlorophyll content recorded in Yellow cultivar. The variation in chlorophyll content of leaf among the varieties might be attributed to genetic constitution. Similar observation in chlorophyll content among the varieties was observed by (Shiragur *et al.,* 2004) [11] in carnation.

Elucidating the abiotic stress tolerance in plants will help to develop more resistant crop varieties, improving the production in a climate change scenario. Proline content is one of the important amino acid which will give resistance to abiotic stresses. The data on proline content are presented in table 3 Significant variations were found regarding proline content among the different nerium cultivars. Among the cultivars highest proline content (6.33 mg g^{-1}) was recorded in Yellow cultivar followed by White cultivar (6.12 mg g^{-1}) while least proline content was recorded in Pink cultivar (5.86 mg g^{-1}). Thus Yellow cultivars might have higher abiotic stress tolerance capacity than other types. This findings was accordance with observation made by (Kumar *et al*., 2016) [5] in *Nerium oleander* L. Regarding observations on Stomata the information's were presented in table 3. Stomatal observations of nerium cultivar was done on both upper and lower surface of the leaf there was no much differences was observed among the cultivars. In all the cultivars of nerium there is no stomata was observed on upper surface of the leaf. The stomatal crypt was observed on lower surface of the leaf in nerium cultivars. Around the stomatal crypt, many number of stomata were distributed. In lower surface of the leaves trachoma's were found densely in all the cultivars. Stomata were found at the base of the trichromes on lower surface of the leaves. The sunken type of stomata is present in all the nerium cultivars (Plate 1). Nebelsick *et al*., 2009 [8] studied about the stomatal crypt model in *Banksia ilicifolia* and stated that the in leaves with open or partially closed stomata, crypts reduced transpiration by less than 15% compared with no encrypted, superficially positioned stomata. The similar observation were made by (Strobel and Sundberg., 1983) [12] in nerium and stated that stomatal density in non-succulents like nerium exhibit a relatively high and type of stomata is sunken, which is present in crypt. These findings were in accordance with findings of (Neblsick *et al*., 2008) and (Abdalla *et al*., 2016) in *Bankisia ilicifolia* and *Nerium oleander* L. respectively. Similar observation were made by (Doshi *et al.,* 2012 and Anita *et al.,* 2015) [1, 4] in nerium and stated that the type of stomata in nerium was sunken.

Table 1: Leaf area (cm^2) in nerium (*Nerium oleander* L.) cultivars

Cultivars	Leaf area (cm^2)
Red	38.02
White	38.54
Pink	47.81
Yellow	37.81
Mean	40.55
SEd	0.50
CD (p= 0.05)	1.10

Table 2: Chlorophyll content (mg g^{-1}) in nerium (*Nerium oleande*L.) cultivars

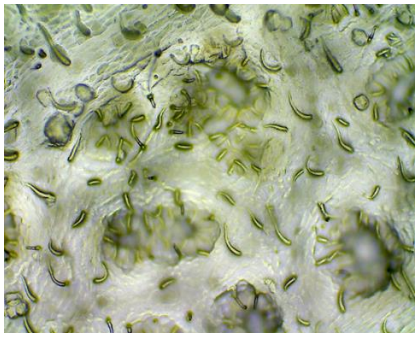
Cultivars	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
Red	3.98	2.794	6.77
White	4.16	3.194	7.35
Pink	4.89	2.872	7.76
Yellow	3.70	2.842	6.57
Mean	4.18	2.93	7.11
SEd	0.09	0.13	0.23
CD (p= 0.05)	0.21	0.30	0.51

Table 3: Proline content (mg g-1) in nerium (*Nerium oleander* L.) cultivars

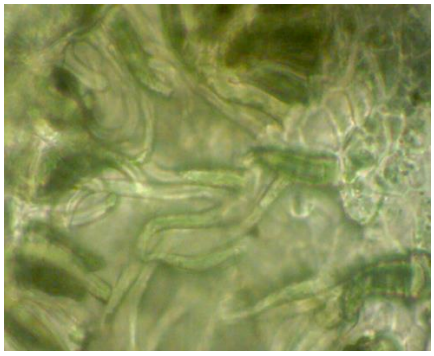
Cultivars	Proline content(mg g-1)
Red	5.38
White	6.12
Pink	5.86
Yellow	6.33
SEd	0.19
CD (p= 0.05)	0.42

Table 4: Stomatal observation in nerium (*Nerium oleander* L.) cultivars

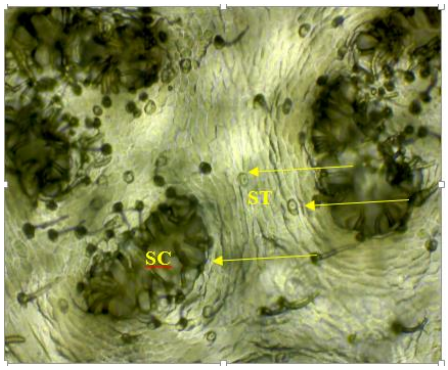
Cultivars	Presence of stomata on upper surface of leaves	Presence of stomata on lower surface ofleaves	Type of stomata
Red	NO	Densely present	Sunken
White	NO	Densely present	Sunken
Pink	NO	Densely present	Sunken
Yellow	NO	Densely present	Sunken



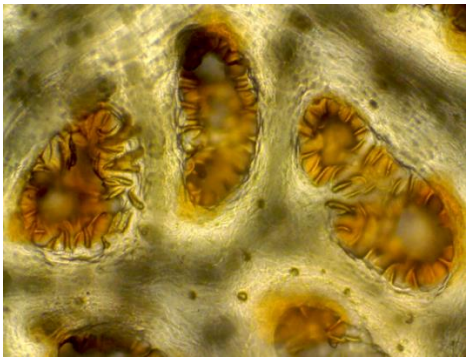
Distribution of trichomes at lower surface of nerium leaves (100X)



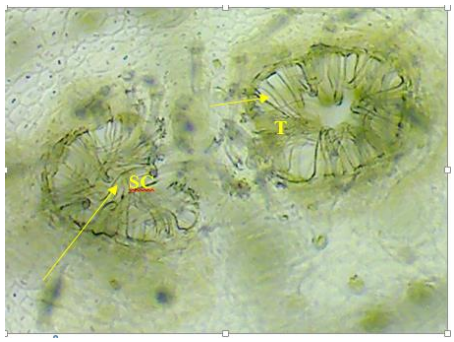
Distribution of trichomes at lower surface of nerium leaves (400 X)



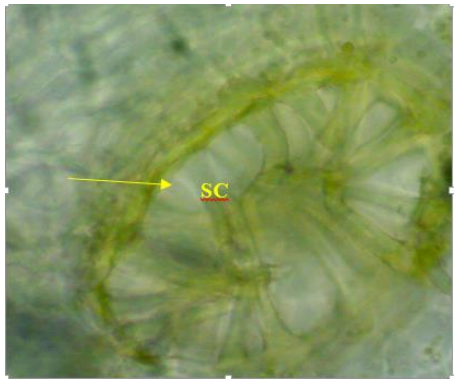
Densely distributed stomatal crypt at lower surface of nerium leaves (100



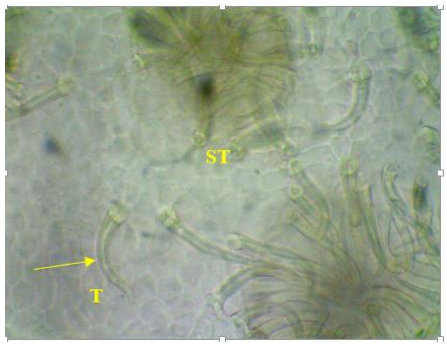
View of stomatal crypt after colouring with acetocarmine stain



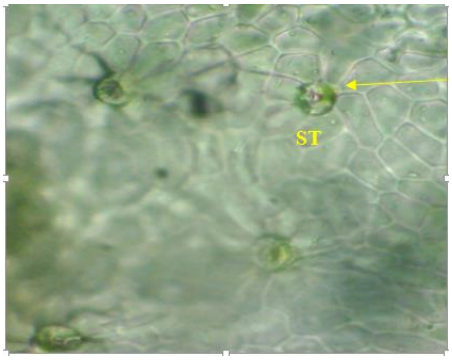
Presence of trichomes around the stomatal crypt (100X)



Presence of trichomes around the stomatal crypt (400X)



Presence of stomata at the base of trichomes (400X)



View of nerium Stomata after removing trichomes (400X)

Fig 1: Observation on trichromes and stomata of nerium cultivars
Note: ST (Stomata) SC (Stomatal crypt) T (Trichome)

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