Turkish Journal of Biology

Volume 34 | Number 1 Article 11

1-1-2010

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MOHD IDREES

M. NAEEM

M. MASROOR A. KHAN

Turk J Biol
34 (2010) 81-88

doi:10.3906/biy-0808-11

The superiority of cv 'rosea' over cv 'alba' of periwinkle (*Catharanthus roseus* L.) in alkaloid production and other physiological attributes

Mohd IDREES, M. NAEEM*, M. Masroor A. KHAN

Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh - 202 002, INDIA

Received: 19.08.2008

Abstract: A pot experiment was conducted during the winter season of 2006, on 2 cvs. of periwinkle (*Catharanthus roseus* L.), namely 'rosea' (Pink) and 'alba' (White), to investigate their performance in terms of growth, yield, and alkaloid production, as well as other physiological and biochemical attributes. The plants were harvested at 60, 90, and 150 days after planting (DAP) and the yield was recorded at 210 DAP. The results showed that 'rosea' was superior to 'alba' in plant height, total and individual leaf area, and fresh and dry weights at 90 DAP. Net photosynthetic rate, stomatal conductance, carbonic anhydrase activity and nitrate reductase activity were also higher in 'rosea' than in 'alba' at 60 and 90 DAP. Likewise, nitrogen content in leaf was also higher in 'rosea'. At harvest, 'rosea' registered higher number of pods, number of seeds, and seed-yield per plant than did 'alba'. Furthermore, alkaloid content was found to be maximum in leaves at 90 DAP and in roots at 60, 90, and 150 DAP in 'rosea' compared to 'alba'. The contents of alkaloids, viz., vincristine and vinblastine, were also found to be maximum at 90 DAP in both cultivars. Thus, 'rosea' excelled over 'alba' in the overall performance of the plant.

Key words: Carbonic anhydrase activity, nitrate reductase activity, vincristine and vinblastine contents, cultivar 'rosea' and 'alba', *Catharanthus roseus* L.

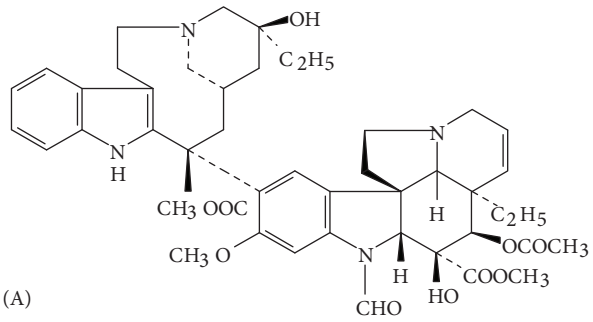
Cezayir menekşesi cv 'rosea'sının cv 'alba'sına alkoloid üretim ve diğer fizyolojik özellikler bakımından üstünlüğü

Özet: Kabuklanma deneyi 2006 yılının kış sezonu boyunca Cezayir menekşesinin "rosea" (pembe) ve "alba" (beyaz) olarak isimlendirilen iki cvs.'nin büyüme, verim ve alkaloid üretim performansı yanı sıra diğer fizyolojik ve biyokimyasal özelliklerine göre tespiti gerçekleştirilmiştir. Ekimden 60, 90 ve 150 gün sonra bitkiler hasat edilmiş ve ekimden 210 gün sonraki verimlilik kaydedilmiştir. Sonuçlar cv. 'rosea'nın bitki boy uzunluğu, toplam ve bireysel yaprak alanı, ekimden 90 gün sonraki taze ve kuru ağırlık bakımından cv. 'alba'dan üstün olduğunu göstermiştir. Ekimden 60 ve 90 gün sonrasında total fotosentetik oran, stoma iletkenlik oranı, karbonik anhidraz ve nitrat redüktaz aktivitesi 'rosea'da 'alba'dan daha yüksektir. Aynı şekilde 'rosea'da yapraktaki nitrojen içeriği daha yüksektir. Hasat zamanında 'rosea'da kabuklanma sayısı, tohum sayısıve tohum verimliliği "alba"dan daha yüksektir. Ayrıca, 'alba' ile kıyaslandığında 'rosea'da ekimden 90 gün sonraki yapraklarda ve ekimden 60, 90 ve 150 gün sonraki köklerde alkaloid içeriği daha fazladır. Alkoloidler, viz., vincristine ve vinblastine içeriği her iki yetiştirmede ekimden 90 gün sonrasında daha fazladır. Böylece, bitkilerin üstün performans özellikleri bakımından 'rosea', 'alba' üzerine üstündür.

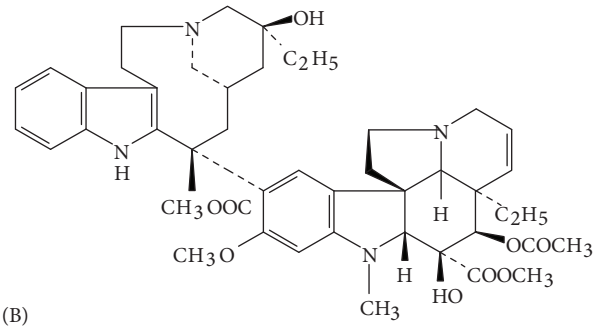
Anahtar sözcükler: Karbonik anhidraz aktivitesi, nitrat reduktaz aktivitesi, vincristine ve vinblastine içeriği, 'rosea' and 'alba' yetiştirme, *Catharanthus roseus* L.

Introduction

Catharanthus roseus L., known as the Madagascar periwinkle, is an important source of indole alkaloids, which are present in all plant parts. The plant is used for the treatment of diabetes, fever, malaria, throat infections, and chest complaints, for the regulation of menstrual cycles, and as a euphoriant (1). The physiologically important and antineoplastic alkaloids, vincristine and vinblastine (Figure 1), are mainly present in the leaves and antihypertensive alkaloids are found in roots such as ajmalicine, serpentine, and reserpine (2). Vincristine and vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia (3,4). These *Catharanthus* alkaloids are also used for the treatment of both malignant and non-malignant diseases and in platelet and platelet associated disorders. There are 3 important cultivars of *Catharanthus roseus* L., which are distinguishable on the basis of their flower colors (1), and 2 of them, namely 'rosea' (Pink) and 'alba' (White), are commonly found in Aligarh. The cultivar 'ocellata' is rarely found in this region.



(A)



(B)

Figure 1. Structure formulae of vincristine (a) and vinblastine (b) in *Catharanthus roseus* L. (Courtesy of Synder, 1996).

Keeping in mind the medicinal importance of periwinkle in the modern as well as traditional systems of medicine, the aim of the present study was to investigate the performance of 2 common cultivars of periwinkle on the basis of growth, physiological and biochemical, yield and quality parameters, and alkaloid production under the agro-climatic conditions of Aligarh in pot culture.

Materials and methods

The seedlings of the cultivars of periwinkle were obtained from Wood Row Nursery, Aligarh. Healthy seedlings of uniform size were selected for transplanting and one seedling per pot was maintained in the net house of the Department of Botany, Aligarh Muslim University, Aligarh. Prior to transplanting, 5 kg of a homogeneous mixture of soil and organic manure in the ratio of 4:1 was placed in 25-cm earthen pots. The soil was maintained at proper moisture to ensure the optimum growth of the plants. The experiment was conducted according to a simple randomized block design with 4 replications. The plants were kept free from weeds and irrigated as and when required.

Three samplings were done: at the vegetative stage (60 DAP, i.e. days after transplanting), flowering stage (90 DAP), and fruiting stage (150 DAP). Before transplanting, uniform basal doses of nitrogen, phosphorus, and potassium were given at the rate of 15, 25, and 25 kg per ha, respectively, in the form of urea, potassium dihydrogen orthophosphate, and muriate of potash. Yield parameters were recorded at the time of harvest (210 DAP). At all growth stages, the plants from each pot were taken out carefully with intact roots, and washed with tap water to wipe off adhering foreign particles. The height of the plants was measured with the help of a meter scale. The total area of the leaf was measured using transparent graph paper. Leaf-area index was determined according to Watson (5). The moisture of the plant roots were removed with blotting sheets and the fresh weight was recorded. Then the plants were dried in a hot air oven at 80 °C for 24 h to record dry weight. The total chlorophyll in the fresh leaves was estimated by the method of Lichtenthaler (6). Chlorophyll content was measured in 80% acetone extract using a spectrophotometer (Spectronic 20D, Milton Roy,

USA). The total carotenoid content was estimated by the method of MacLachlan and Zalik (7). The net photosynthetic rate in the leaves was measured using an IRGA (Infrared Gas Analyzer), Li-Cor 6200 Portable Photosynthesis System (Lincoln, Nebraska, USA). The activity of nitrate reductase in fresh leaves was determined by the method of Jaworski (8). The carbonic anhydrase activity in fresh leaves was analyzed using the method of Dwivedi and Randhawa (9). The nitrogen and phosphorus contents in the leaves were analyzed in leaf powder by following the procedure of Lindner (10) and Fiske and Subba Row (11), respectively. The potassium content in the leaves was analyzed by flame-photometry (12). The concentrations of vincristine and vinblastine in fresh leaves were estimated spectrophotometrically by the method of Nagaraja et al. (13). The absorbance of each sample was compared with that of the calibration curve using standard vincristine and vinblastine (Cipla Ltd., Mumbai, India) and was expressed as a percentage on a fresh weight basis.

Total alkaloid content in the dried leaves and root powder was estimated by the method of Afaq et al. (14). The experimental data were statistically analyzed using the 'analysis of variance' techniques according to Gomez and Gomez (15). In applying the 'F' test, the error due to replicates was also determined. When the 'F' value was found to be significant at the 5% level of probability, the least significant difference (LSD) was calculated.

Results

The mean data and the least significant difference (LSD) ($P \leq 0.05$) of the selected growth, physiological and biochemical and yield and quality attributes of 2 cultivars of periwinkle are presented in Figures 2A-D, 3A-D, and 4A-B and the Table. The results revealed that 'rosea' was significantly different from 'alba' and showed superior response over 'alba' at 90 DAP. *Cv*. 'rosea' exhibited 12.1%, 6.1%, 2.61%, 7.24%, and 4.19% higher values for plant height, area per leaf, leaf area per plant, and fresh and dry weights per plant, respectively at 90 DAP (Figure 2A-C). The value for total chlorophyll content was not significantly different amongst the cvs. at all the stages (Figure 2D). The data in Figure 2D reveal that 'rosea' had 9.38% and 36.2% higher total carotenoid content than 'alba' at 60 and 150 DAP and it also had greater net photosynthetic rate and stomatal conductance by 23.0% and 16.8%, and 15.9%, and 20.5%, respectively, at 60 and 90 DAP than 'alba' (Figure 3A). The activities of nitrate reductase and carbonic anhydrase and N content in the leaves were also higher in 'rosea' by 7.4% and 34.3%, and 18.6% and 12.7% and 0.94% and 0.54% at 90 and 150 DAP than they were in 'alba' (Figure 3B). However, the phosphorus and potassium contents were not significantly different in both cultivars at 60, 90, and 120 DAP (Figure 3C). Total alkaloid in the dried leaves of 'alba' was much higher than that of 'rosea' and was significant only at 90 DAP (Figure 4A).

Table. Yield parameters (number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight, and seed-yield of 2 cultivars of *Catharanthus roseus* L. studied at 210 days after planting i.e. DAP (means of 4 replicates).

Attributes	DAP	Rosea (Pink)	Alba (White)	CD at 5%
Number of pods per plant	210	96	92	2.24
Number of seeds per pod	210	20	20	NS
Number of seeds per plant	210	1920	1840	64.3
100-seed weight (g)	210	1.09	1.07	NS
Seed-yield per plant (g)	210	20.92	19.68	0.58

NS = Non-significant

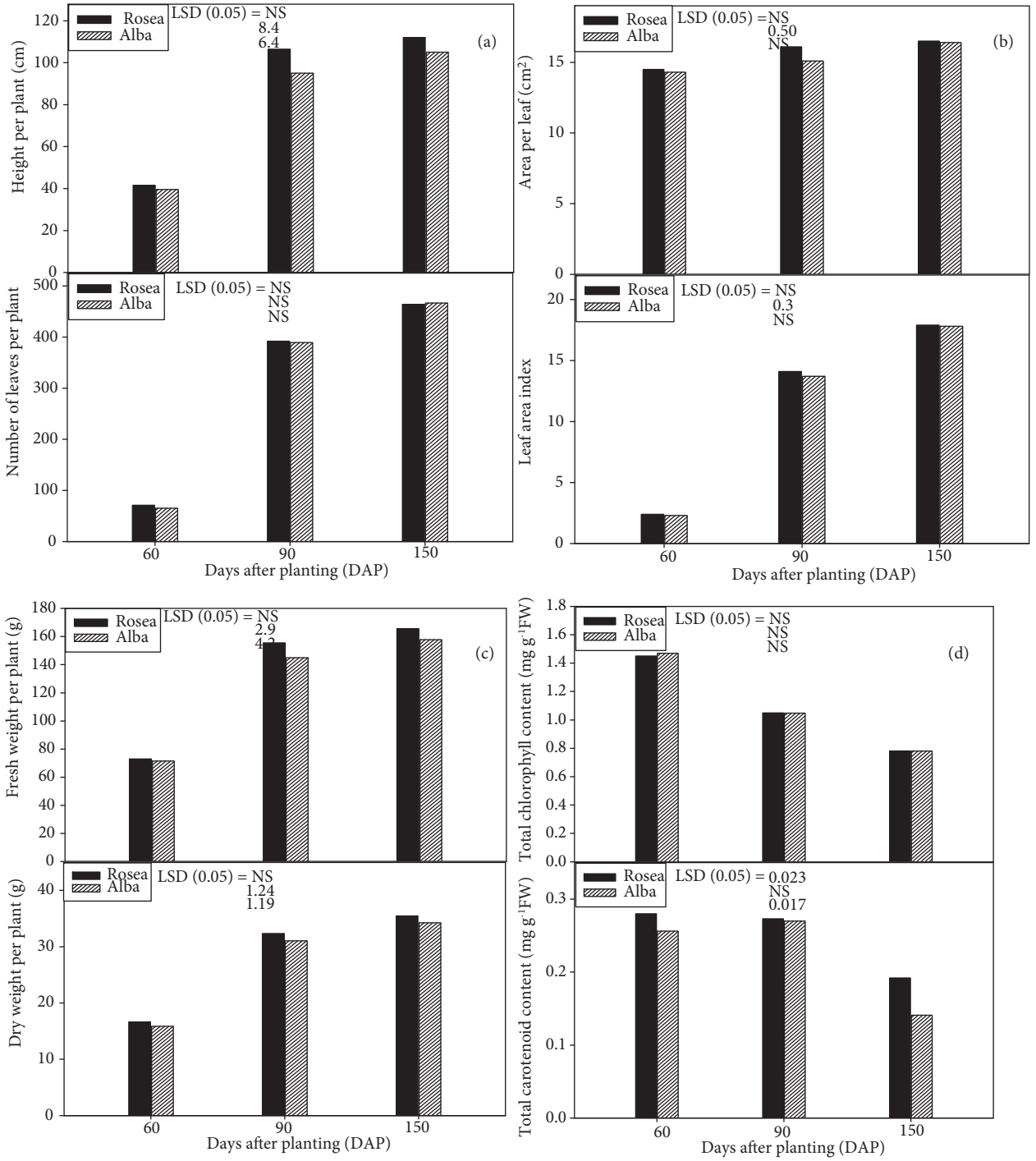


Figure 2 A-D. Plant height, number of leaves, area per leaf, leaf-area index, fresh weight and dry weight per plant, and total chlorophyll and carotenoid content of 2 cultivars of C*atharanthus roseus* L. studied at 60, 90 ,and 150 days after planting, i.e. DAP (means of 4 replicates).

In the present study, a significant difference in total alkaloid content in roots was clearly observed amongst the cultivars at 60, 90, and 150 DAP. The total alkaloid content in roots (18.10%, 8.67% and 9.86% higher over 'alba') was found at 60, 90, and 150 DAP, respectively (Figure 4A). However, the content

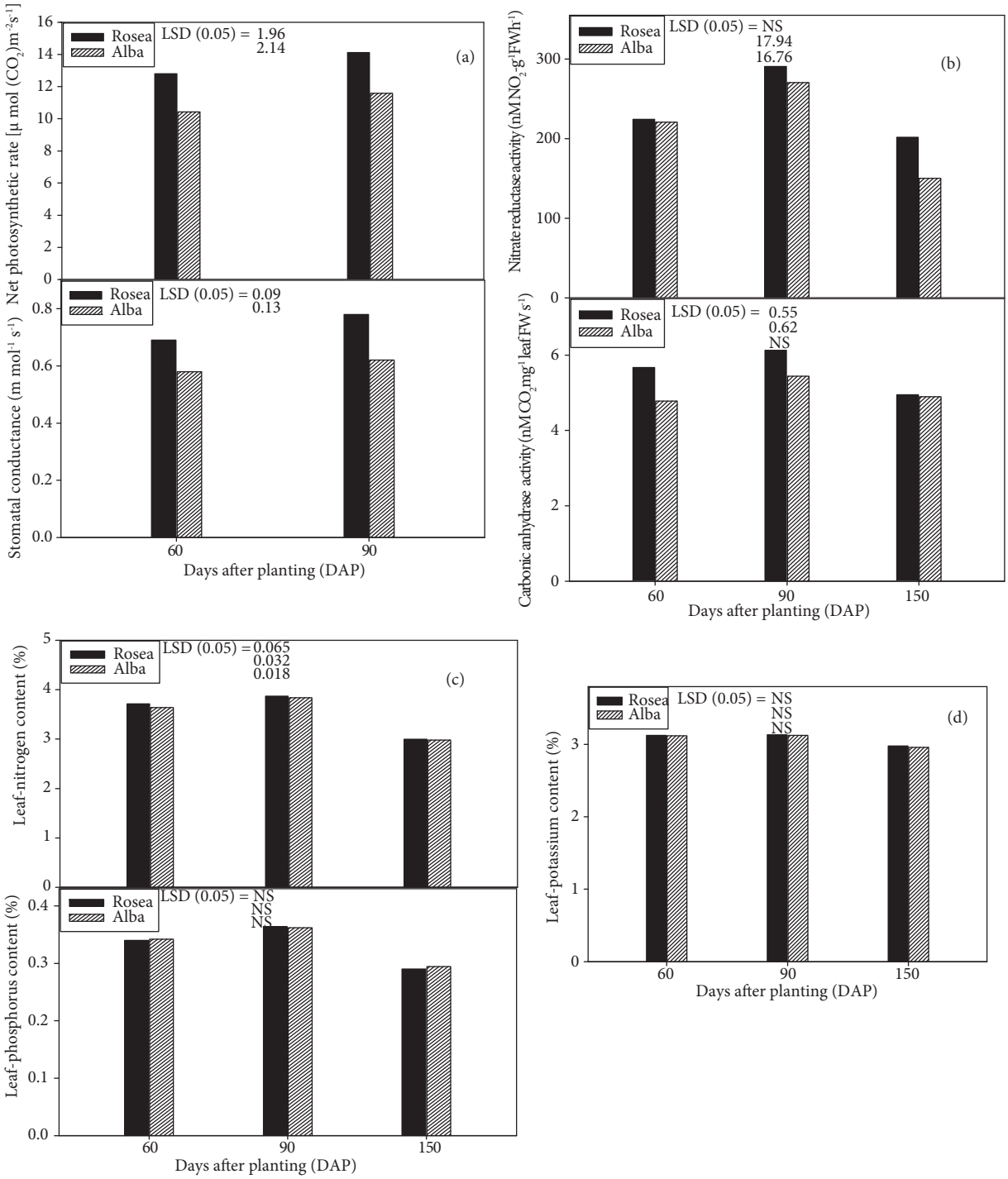


Figure 3A-D. Net photosynthetic rate, stomatal conductance (60 and 90 DAP), nitrate reductase activity, carbonic anhydrase activity, and leaf-nitrogen, -phosphorus and- potassium content of 2 cultivars of *Catharanthus roseus* L. studied at 60, 90, and 150 days after planting, i.e. DAP (means of 4 replicates).

of vincristine and vinblastine was not significantly different amongst 'rosea' and 'alba' (Figure 4B). Cultivar 'rosea' registered higher number of pods, number of seeds, and seed-yield per plant by 4.34%, 4.35%, and 6.30% than that of 'alba' (Table).

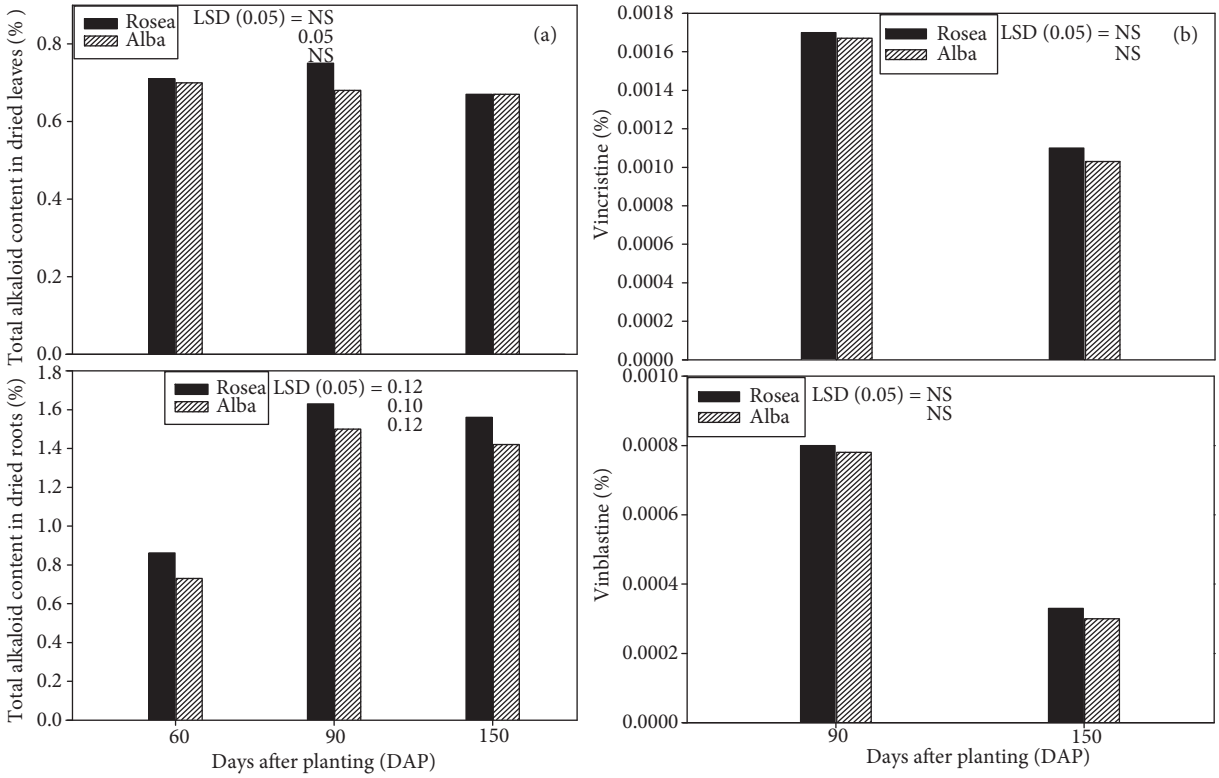


Figure 4A-B. Total alkaloid content in leaves and roots (60, 90, and 150 DAP) and vincristine and vinblastine content of 2 cultivars of *Catharanthus roseus* L. studied at 90 and 150 days after planting, i.e. DAP (means of 4 replicates).

Discussion

Catharanthus roseus L. as a tropical plant needs a relatively high temperature during the period of growth and development. Two common cultivars of *Catharanthus roseus* L. reported here varied in productivity and quality parameters. Cultivar 'rosea' gave better performance for all growth parameters at 90 DAP than 'alba'. The present study indicates that values for most of the growth parameters in both cultivars were not significant at 60 and 120 DAP. However, fresh and dry plant weights in both cultivars were significant at most of the stages (Figure 2C).

Variation in the biomass production in 2 cultivars may be due to accessions phenology, environment and season (16,17). According to agro-climatic conditions of India, intraspecific variations on many physiological and morphological traits in *Catharanthus roseus* L. have been recorded (18-21). Furthermore, varietal differences among the accessions are greater than the differences between related species or genera (22). It is noteworthy that the variation in the performance of 2 cultivars may be partly due to soil type and fertility. Mineral nutrients, according to the requirements of plants, are one of the most important factors in growth and development. In general, the fertilization of medicinal plants causes an increase in the yield of bioactive compounds. An adequate supply of mineral nutrients (NPK) in the initial stage of plant growth and development plays a pivotal role, since we have supplied uniform basal doses of N, P, and K in the soil. Nitrogen is the main growth and yield determining factor, followed by other macronutrients. It was noted that cultivar 'rosea' occupied a better position in respect of the total carotenoid content at 60 and 150 DAP than 'alba'. The genetic make-up of the plant and temperature range might be responsible for the difference in carotenoid content. Temperature range and light intensity also have an influence on the chlorophyll and carotenoid content of the leaves.

Higher values for net photosynthetic rate, nitrate reductase and carbonic anhydrase activities, and leaf-nitrogen content together with appropriate content of phosphorus and potassium could be responsible for its better growth. Moreover, the positive correlation of leaf-nitrogen content (r = 0.938*), carbonic anhydrase activity (r = 0.992*), and nitrate reductase activity (r = 0.962*) with the alkaloid content of 'rosea' depicts their role in alkaloid production as observed in this study. Hence higher values for fresh and dry weights per plant, number of pods, and number of seeds per plant of 'rosea' contributed to the plant productivity. Seed-yield is a cumulative performance of pod number, seed number per pod, and 100-seed weight**.** Enhancement in yield attributes would ultimately culminate in the seed-yield production of 2 cultivars. De Luca and Laflamme (23), Endt et al. (24), and Datta et al. (25) pointed out that cultivar variations in *Catharanthus roseus* L. on the basis of their morphological traits and in the alkaloid contents are regulated primarily at the level of gene expression.

The differences in the performance of cultivars of periwinkle (*Catharanthus roseus* L.) could be ascribed to the variation in their genetic make-up. Cultivar variation in respect of different parameters was also reported by Virk et al. (26), Singh et al. (27), Mishra et al. (28), Choudhary and Gupta (29), Khan et al. (30), and Naeem et al. (31).

Thus, it may be concluded on the basis of present study that the cultivar 'rosea' of periwinkle is superior to the cultivar 'alba' for growth, physiological and biochemical yield and quality attributes under the agro-climatic conditions of Aligarh, western Uttar Pradesh. Additionally, the best time of harvesting for total alkaloids in the leaves and roots of both cultivars was 90 DAP.

Acknowledgement

The authors are highly grateful to Cipla Ltd., Mumbai, India, for the generous supply of standard Vincristine and Vinblastine samples.

Corresponding author:
M. NAEEM
Plant Physiology Section,
Department of Botany,
Aligarh Muslim University,
Aligarh, 202 002, INDIA
E-mail: naeem_phd@yahoo.co.in

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