

The effect of spraying tryptophan amino acid on the physiological characteristics of the periwinkle plant (*Catharanthus roseus* L.) under drought stress

Farshid Yousefi

farshid.yousefi22@gmail.com

Agricultural Sciences and Natural Resources University of Khuzestan

Alireza Abdali  Mashhadi

Agricultural Sciences and Natural Resources University of Khuzestan

Amin Lotfi jalal abadi

Agricultural Sciences and Natural Resources University of Khuzestan

Alireza Shafeinia

Agricultural Sciences and Natural Resources University of Khuzestan

Saied Jalily

Agricultural Sciences and Natural Resources University of Khuzestan

Narges Soltani

Lorestan University

Research Article

Keywords: Catalase, peroxidase, root length, root volume, vincristine, and vinblastine

Posted Date: November 4th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7678921/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on November 27th, 2025. See the published version at <https://doi.org/10.1186/s12870-025-07831-w>.

Abstract

Background

Periwinkle (*Catharanthus roseus* L.) is a significant source of two valuable anticancer alkaloids, vincristine and vinblastine. Amino acids serve as precursors for alkaloid biosynthesis, and environmental stresses are known to induce an increase in the ratio of secondary metabolites in plants. This study was conducted using a completely randomized design with a factorial arrangement and three replications at the Khuzestan Agricultural Sciences and Natural Resources University under controlled greenhouse conditions. The experimental factors included foliar application of the amino acid tryptophan at varying concentrations (control, 50, 100, 150, 200, and 250 ppm) and drought stress levels (100%, 70%, and 40% of field capacity).

Results

Results from the mean comparisons indicated that increasing the concentration of tryptophan up to 250 mg per liter, combined with maintaining soil moisture at 40% of field capacity, exerted a positive and significant effect on biochemical and physiological parameters of both root and aerial organs. These parameters included dry root and plant weight, root volume, photosynthetic pigments, activities of catalase and peroxidase enzymes, total protein content, phenolic compounds, flavonoids, total amino acids, and the concentrations of vinblastine and vincristine alkaloids. Data analysis revealed that all measured traits improved significantly at high concentrations of tryptophan (above 200 ppm). Conversely, severe drought stress (40% field capacity) resulted in a significant reduction in dry plant weight, total protein, and photosynthetic pigments. Nonetheless, these conditions also contributed to an increase in the content of vinblastine and vincristine alkaloids, as well as levels of enzymatic and non-enzymatic antioxidants.

Conclusions

Ultimately, the application of tryptophan at concentrations of 200 and 250 ppm was effective in alleviating the adverse impacts of drought stress by enhancing dry weight, photosynthetic pigments, and antioxidant enzyme activities in the periwinkle plant, culminating in increases in vincristine and vinblastine levels by 230% and 488%, respectively, under severe drought stress (40% field capacity).

Introduction

The plant periwinkle, scientifically designated as (*Catharanthus roseus* L.), is a member of the family Apocynaceae and the order Gentianales [1, 2]. Periwinkle is recognized as a medicinal plant species indigenous to Madagascar [3]. It is cultivated for both medicinal and ornamental purposes in various settings, including parks, gardens, and farms, as well as for enhancing the aesthetic appeal of scenic locations [4]. This plant typically exhibits a height ranging from 40 to 90 centimeters, with a main root length of 20 to 40 centimeters, and its stems may present a pale red or green coloration. The leaves are characterized as simple, glossy, ovate, and arranged oppositely on the stem [5]. Among its notable properties are the reduction of blood pressure, facilitation of wound healing, purification of the blood, treatment of skin disorders such as eczema, alleviation of urinary tract infections, and mitigation of bronchitis inflammation. Furthermore, this plant is known to contain 130 distinct types of indole terpene alkaloids (TIAs). Notably, two dimeric alkaloids, vinblastine and vincristine, possess anti-tumor properties and are employed in the treatment of various cancers [6, 7].

Environmental stresses, particularly drought and salinity, exert a considerable influence on the biosynthesis of bioactive compounds in medicinal plants [8]. Drought stress, recognized as a major abiotic stressor, negatively impacts plant growth and development through morphological, physiological, and molecular changes [9]. In response to drought conditions, plants activate complex defense mechanisms that frequently result in increased production of secondary metabolites, including phenolic compounds, flavonoids, terpenoids, and alkaloids [10]. These metabolites play critical roles in plant stress responses and possess significant pharmaceutical value [11]. Amino acids have emerged as significant agents in enhancing plant stress tolerance [12]. Under drought conditions, the exogenous application of amino acids can compensate for impaired endogenous amino acid metabolism and improve plant resilience [13, 14]. Among amino acids, L-tryptophan (β -3-indolylalanine) is particularly noteworthy due to its dual role as both a protein building block and a precursor for auxin biosynthesis [15]. Foliar application of L-tryptophan has been demonstrated to improve various plant growth parameters, enhance stress tolerance, and potentially influence the production of secondary metabolites [16].

While numerous studies have investigated the bioactive compounds of *C. roseus*, there remains a paucity of information regarding the combined effects of drought stress and tryptophan application on its growth physiology and alkaloid production. Therefore, this study aimed to evaluate the efficacy of tryptophan foliar application in alleviating the effects of drought stress while enhancing the production of valuable alkaloids in *C. roseus*.

Materials and Methods

This experiment was conducted at Khuzestan Agricultural Sciences and Natural Resources University under Greenhouse conditions were implemented with a temperature of $25 \pm 2^\circ\text{C}$, a relative humidity of 60%, and a light/dark cycle of 16 hours of light and 8 hours of darkness. F1 seeds of periwinkle (*Catharanthus roseus* var. Vinca) were obtained from the Zaeem Agricultural Institute and surface sterilized with 1% sodium hypochlorite (for 1 minute) before being sown in seed trays. Once the seedlings reached the four-leaf stage, they were transplanted into pots with dimensions of 20 cm in height and 15 cm in diameter, containing a mixture of light soil, decomposed manure, and sand. The experiment was conducted as a two-factor factorial within a completely randomized design (CRD), incorporating three replications. The factors included: (1) Tryptophan foliar application at concentrations of 0, 50, 100, 150, 200, and 250 ppm. (2) Drought stress levels established at 100%, 70%, and 40% of field capacity (Fig. 1). Tryptophan foliar application was performed twice (at the eight-leaf stage and at the beginning of the flowering stage). Two days after the first foliar application, drought stress treatments were initiated and continued for one month until the start of flowering.

Applying drought stress

Soil moisture was measured gravimetrically to ascertain the optimal timing for irrigation. Irrigation requirements were calculated based on the moisture content and the depth of the root zone. Moisture levels at the field capacity point (19%) and the permanent wilting point (8%) were quantified using a pressure plate device. Additionally, the soil moisture prior to irrigation in the non-stressed treatment was determined to be 13.5% by weight. The net irrigation requirements for the treatments at 100%, 70%, and 40% were found to be 318, 222, and 127 cc, respectively. These values were derived using the following equation [17].

$$V = (\theta_{fc} - \theta_i) \times Z \times A$$

V: Volume of irrigation water (L).

θ_{fc} : Volumetric soil water content at field capacity (cm^3/cm^3) :

θ_i : Initial volumetric soil moisture content before irrigation (cm³/cm³).

Z: Root zone depth (cm).

A: Surface area of the pot (cm²).

Measurement of physiological traits of root and plant performance

After completing the experiment and cutting the aerial parts, the roots of each pot were completely and separately removed from the soil by splitting the pots. After several washes with water, the roots were separated from the soil. To calculate the root volume, the roots of each pot were placed in a graduated cylinder with a specific amount of water, and after the water level rose, the root volume was calculated in cubic centimeters. Root volume was determined using the water displacement method in a graduated cylinder. Dry weights were obtained following oven-drying at 70°C for a duration of 48 hours. The root lengths were measured using a ruler with an accuracy of one millimeter [18]. Root surface area (1), root diameter (2), and root surface density (3) were also calculated using the following formulas [19].

Equation 1, Root surface area = $2 \times (\text{Root length} \times \pi \times \text{Root radius})^{0/5}$

Equation 2, Root diameter = $(4 \times \text{fresh root weight} / (\pi \times \text{root length}))^{0/5}$

Equation 3, root surface density = length of the root $\times \pi \times$ Diameter of the root

Chlorophyll a, b, total and carotenoids

Measurement of greenness and photosynthetic pigments was conducted using the Arnon method before the flowering stage [20]. Chlorophyll and carotenoids were extracted from 1 g of fresh leaf tissue using 80% acetone in conjunction with liquid nitrogen, followed by centrifugation at 6000 rpm for 10 minutes. The absorbance at wavelengths of 663 nm, 645 nm, and 470 nm was measured utilizing a SPEKOL model 2000 Analytikjena spectrometer. The following formulas were used to calculate chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in milligrams per gram of fresh sample weight.

Equation 4, Chlorophyll a = $(19.3 \times A_{663} - 0.86 \times A_{645}) V/100W$

Equation 5, Chlorophyll b = $(19.3 \times A_{645} - 3.6 \times A_{663}) V/100W$

Relationship 6, Chlorophyll a + b = Chlorophyll a + Chlorophyll b

Equation 7, Carotenoids = $100 (A_{470}) - 3.27 (\text{mg chl. A}) - 104 (\text{mg chl. B})$

V = Volume of filtered solution (upper solution resulting from centrifugation). A = light absorption at wavelengths of 663, 645 and 470 nm. W = wet weight of the sample in grams

Measurement of total phenol, flavonoid, and free amino acid content in leaves

The total phenol content was measured using the Folin-Ciocalteu colorimetric method, with gallic acid as the standard. The extract was obtained using 1 gram of dry leaf weight in 96% pure ethanol. To 0.1 milliliters of plant extract or standard solutions (concentrations of 0-100 mg ml⁻¹ gallic acid in distilled water), 2.8 milliliters of distilled

water and 0.1 milliliters of diluted Folin-Ciocalteu reagent (1:10 v/v) were added. After 5 minutes, 2 milliliters of 7.5% sodium carbonate solution were added to the mixture and kept at room temperature for 90 minutes. The absorbance of the samples was then determined at a wavelength of 760 nanometers. Finally, the total phenol content was calculated based on the standard curve in milligrams of gallic acid per gram of dry weight [21].

The total flavonoid content was quantified using the aluminum chloride colorimetric method, with quercetin serving as the standard reference. A volume of 0.5 mL of the extract solution, prepared in a manner identical to that used for the total phenol assay of leaves, was combined with 150 μ L of 95% methanol, 100 μ L of 10% aluminum chloride, 100 μ L of 1 M potassium acetate, and 280 μ L of distilled water. The samples were maintained at room temperature for 30 minutes, after which the absorbance of the resulting mixture was measured at a wavelength of 415 nm. A standard curve was generated using quercetin, and the results were expressed in milligrams of quercetin per gram of dry leaf. By substituting the absorbance value of the extract into the linear equation derived from the standard curve, the total flavonoid content in the leaves was calculated, with the data ultimately expressed as milligrams of quercetin per gram of leaf [22].

To quantify total free amino acids, 0.2 grams of dried leaf tissue were ground with 5 milliliters of 95% ethanol in a porcelain mortar. The resulting extract was subsequently transferred to a microtube and subjected to centrifugation at 18,000 rpm for 20 minutes. Following centrifugation, the supernatant was carefully separated using a sampler and transferred to a separate container. Subsequently, 100 microliters of the extract were aliquoted into a new microtube, to which 100 microliters of distilled water and 1 milliliter of ninhydrin solution were added. The microtubes were incubated in a water bath at 95°C for a duration of 4 to 7 minutes, after which 2 milliliters of 5% alcohol were incorporated into the solution. The solution was then allowed to cool, and the absorbance of the samples was measured at a wavelength of 570 nanometers. A standard solution was prepared using glycine and acetic acid, and the total free amino acid content was calculated in milligrams per gram of dry tissue weight [23].

Measurement of catalase enzymes, peroxidase, and total protein

Extract preparation

First, 0.2 grams of the plant sample, 0.02 grams of PVP, and 2 milliliters of 10 mM potassium phosphate buffer with a pH of 7 were mixed together in a porcelain mortar. The mixture was thoroughly ground using the mortar pestle until the juice was extracted, resulting in a homogeneous and uniform extract. The homogenized mixture was then transferred to a microtube and centrifuged at 15,000 rpm for 25 minutes at 4°C. The clear phase was separated and stored in a freezer at -80°C [24]. All steps were conducted on ice.

The measurement of peroxidase enzyme activity was conducted by adding 10 microliters of enzyme extract to 2 milliliters of a solution (100 mM potassium phosphate buffer with pH = 7, double-distilled water, 70 mM hydrogen peroxide dissolved in 100 mM potassium phosphate, and 10 mM guaiacol in double-distilled water). The peroxidase enzyme activity was then monitored for 120 seconds at a wavelength of 470 nm using a spectrophotometer [25]. The correct trend of the peroxidase enzyme curve at a wavelength of 470 nm follows an upward trajectory. For catalase enzyme measurement, 2.5 milliliters of sodium phosphate buffer (25 mM concentration, pH = 6.8), 0.5 milliliters of H₂O₂ (10 mM concentration), and 100 microliters of enzyme extract were mixed. After homogenizing the solution, the reading was taken at a wavelength of 240 nm [26]. The decomposition of H₂O₂ due to catalase enzyme activity was reflected by a decrease in absorbance at 240 nm, and the value was expressed per microgram of protein in the enzyme extract.

To measure the protein content, 5 milliliters of Bradford solution and 290 microliters of extraction buffer were mixed together, followed by the addition of 10 microliters of the prepared extracts. The resulting solution was thoroughly mixed using a vortex and then placed for absorbance measurement at a wavelength of 590 nanometers (Bradford, 1976). The protein content was used to assess enzyme activity based on protein concentration. To prepare the Bradford solution, 0.1 grams of Coomassie Brilliant Blue G-250 was added to 50 milliliters of 96% ethanol and mixed using a magnetic stirrer. Then, distilled and double-sterilized water was added to bring the volume to 800 milliliters. Subsequently, 100 milliliters of 85% phosphoric acid was added, and finally, the volume was adjusted to 1000 milliliters with distilled and double-sterilized water. The resulting solution was filtered using filter paper [27].

Measurement of Vinblastine and Vincristine Alkaloids

Leaf samples were collected from both the 3-day and 7-day treatments to quantify the levels of vinblastine and vincristine alkaloids. For sample preparation, a methanol:water (80:20) mixture was employed as the extraction solvent, in accordance with established procedures [28, 29]. The leaf samples were air-dried in the shade. Subsequently, 1 ml of n-hexane was added to 0.5 g of powdered leaf tissue. After the evaporation of the n-hexane, the extracting solvent was introduced to the samples in three sequential stages of 24 hours, with the extracts from all three stages being combined. Following the evaporation of the methanol in the extraction solvent, 10 ml of distilled water was added to the sample, and the pH was adjusted to 3.5 using hydrochloric acid. The samples underwent three rounds of washing with chloroform, with the aqueous phase containing the alkaloids collected each time. The pH of the collected aqueous phase was then elevated to 8.5. Chloroform was added, and the sample was stirred, allowing for the chloroform phase to be collected three times. Finally, the collected chloroform phase was dried, and the resulting dried samples were dissolved in methanol for subsequent High-Performance Liquid Chromatography (HPLC) analysis.

Quantification of Vinblastine and Vincristine Alkaloids by (HPLC)

High-performance liquid chromatography (HPLC) was conducted utilizing a Eurospher II 100-5 C18 column equipped with a precolumn (250 × 4.6 mm) and a UV detector (model K-2600) integrated with a KNAUER WellChrom model HPLC system, operating at a wavelength of 254 nm. The mobile phase comprised a mixture of acetonitrile and sodium dihydrogen phosphate (0.1 M), augmented with 0.5% acetic acid, in a ratio of 21:79 (pH = 3.5), with a flow rate set at 1 mL/min [30]. Subsequently, data analysis was performed using SAS software (version 9.4), which included analysis of variance and mean comparison conducted via the least significant difference method (Duncan's test).

Statistical analysis

Statistical analysis of the data was performed using SAS software (version 9.4), charting was done using Excel software, and mean comparison was conducted with Duncan's test at a 5% error probability level.

Results

Based on the results presented in (Table 1) regarding root volume and dry weight, it was determined that the interaction effect of drought stress and the amino acid tryptophan was significant at a 5% error probability level, while it was not significant for traits such as length, surface area, diameter, and root surface density. The analysis of variance results showed that the main effects of drought stress and the amino acid tryptophan were significant at a 1% error probability level for all traits except root diameter.

Table 1

Results of variance analysis on the effect of drought stress treatment and the amino acid tryptophan on the root indices of the periwinkle plant.

S.O.V	D.F	Mean squares					
		Root Length	Root Volume	Dry Weight	Root Surface Area	Root Diameter	Root Surface Density
Drought	2	172.6**	11.04**	0.23**	83.6**	0.047**	26.37**
Tryptophan	5	69.9**	3.71**	0.028**	168.3**	0.00013 ^{ns}	67.6**
Drought × Tryptophan	10	1.18 ^{ns}	0.97**	0.0022**	16.9*	0.00006 ^{ns}	1.99*
Error	36	1.41	0.3	0./00068	7.73	0.00009	0.78
C.V (%)	-	4.35	16	4.72	8.22	2.99	3.27
** and * indicate significance at $p < 0.01$ and $p < 0.05$, respectively; ^{ns} : non-significant							

Root Volume and Dry Weight

The results indicate that root volume and dry weight exhibited a positive correlation with increasing concentrations of the amino acid tryptophan. Specifically, an application of 250 milligrams per liter of tryptophan at a 100% field capacity (FC) moisture level resulted in the highest recorded root volume of 6.35 cm (Fig. 2a). Concurrently, this concentration at the same moisture level yielded the maximum root dry weight of 0.801 grams (Fig. 2b). Conversely, a reduction in tryptophan concentration to below 50 milligrams per liter (control), accompanied by an increase in drought stress at a 40% field capacity level, led to a decline in both root volume and dry weight. Notably, the lowest recorded root volume was 2.333 cm (Fig. 2a), and the minimum root dry weight averaged 0.39 grams (Fig. 2b).

Root Surface Area and Root Surface Density

The findings from mean comparisons indicated that increased drought stress resulted in a reduction of both root surface area and root surface density in the Provanesh plant. Conversely, under conditions of no stress, elevated concentrations of the amino acid tryptophan led to enhancements in root surface area and root surface density. Specifically, the application of 250 mg per liter of tryptophan at 100% field capacity moisture level (control) yielded the highest measurements for root surface area and root surface density, recorded at 47.9 cm² and 5.33 g/cm³, respectively. These values represent increases of 63% for root surface area and 30% for root surface density compared to the control. It was also noted that when tryptophan concentrations were below 50 mg per liter (control) and combined with increasing drought stress at 40% field capacity, both root surface area and root surface density experienced a decline. The lowest recorded averages were 28.2 cm² for root surface area and 1.23 g/cm³ for root surface density, which corresponded to approximately a 4% decrease in root surface area and about a 1% decrease in root surface density compared to the control (Fig. 2c and d).

Root Length and Diameter

The results of the comparisons indicated that an increase in drought stress levels resulted in a corresponding increase in the root length of the Parvansh plant. The maximum root length recorded was 28.30 cm at 40% of field capacity, while the minimum root length was 24.1 cm at 100% of field capacity (control) (Fig. 2e). Similarly, the largest root length was observed at a concentration of 250 mg/L of the amino acid tryptophan (14.32 cm), whereas the minimum

root length was noted at 0 mg/L (12.24 cm) (Fig. 2f). Additionally, the minimum root diameter measured was 0.27 cm at 40% of field capacity, and the maximum root diameter was 0.37 cm at 100% of field capacity (control) (Fig. 2g).

Table 2

Results of the analysis of variance on the effects of drought stress treatment and the amino acid tryptophan on the physiological and morphological indices of the periwinkle plant.

S.O.V	D.F	Mean squares				
		Phenol	Flavonoids	Total Amino Acids	Fresh Weight	Dry Weight
Drought	2	261.3**	3.61**	56.1**	4909**	374**
Tryptophan	5	46.6**	3.85**	20.4**	1044**	59.4**
Drought × Tryptophan	10	7.88*	0.26*	3.1**	45.8*	9.74**
Error	36	3.61	0.114	0.542	13.6	1.96
C.V (%)	-	9.32	14.4	4.72	2.87	13.1
** and * indicate significance at $p < 0.01$ and $p < 0.05$, respectively; ^{ns} : non-significant						

Based on the results presented in (Table 2) regarding phenol, flavonoids, and the fresh and dry weight of the plants, it was determined that the interaction effect of drought stress with the amino acid tryptophan was significant at the 5% error level, and the total amino acid and dry weight were significant at the 1% level, while the traits of length and number of pods were not significant. The results of the variance analysis indicated that the simple effect of drought stress and the amino acid tryptophan was significant for all traits except for root diameter at the 1% error level.

Evaluation of total phenol, flavonoid, and free amino acid content in leaves

The results of the analysis of variance table indicated that the effects of drought stress and foliar application of tryptophan amino acid on the concentration of phenol and flavonoid were significant at the five percent probability level (Table 2). Drought stress and foliar application of tryptophan increased the phenol and flavonoid content in the plant. The mean comparison results showed that the highest amount of phenol (17.30 mg of gallic acid per gram of dry leaf weight) and flavonoid (3.77 mg of quercetin per gram of dry leaf weight) were obtained from a concentration of 250 mg per liter under severe drought stress (40 percent of field capacity), which represented increases of 15.7 and 14.6 percent compared to the control for phenol and flavonoid, respectively. Additionally, the lowest amount of phenol (15.57 mg of gallic acid per gram of dry leaf weight) and flavonoid (1.008 mg of quercetin per gram of dry leaf weight) were obtained from a concentration of zero and without drought stress (100 percent of field capacity) (Fig. 3a and b). Furthermore, drought stress combined with foliar application of tryptophan increased the total amino acid content in the plant. The highest total amino acid content (28.91 mg per gram of dry leaf) was found in the foliar application of tryptophan at a concentration of 250 mg per liter under 40 percent drought stress, while the lowest amount (14.57 mg per gram of dry leaf) was recorded at a concentration of zero without drought stress (100 percent of field capacity) (Fig. 3c).

fresh and dry weight of the plant

Based on the results of the analysis of variance, the simple effects of drought stress and tryptophan on all morphological traits (fresh and dry weight of the plant) in the plant were significant at the one percent level (Table 2). The mean comparison results indicated that the highest fresh and dry weight of the plant were 74.03 and 20.33 grams,

respectively, from a concentration of 250 mg per liter under non-drought stress (100 percent of field capacity), while the lowest were 14.81 and 3.37 grams, respectively, from a concentration of zero tryptophan under severe drought stress (40 percent) (Fig. 3d and e).

Evaluation of Chlorophyll a, b, total and carotenoids

The results of the analysis of variance indicated that the interaction between drought stress and the amino acid tryptophan was significant for all traits. Specifically, it was significant at the 5% probability level for chlorophyll a, total chlorophyll, carotenoids, and total protein, while it was significant at the 1% probability level for chlorophyll b, peroxidase enzyme, and catalase (Table 3). The comparison of treatment means showed that the highest levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were obtained with averages of 1.83, 1.88, 3.71, and 0.93 mg per gram, respectively, from a concentration of 250 mg per liter of tryptophan without drought stress (100% field capacity). The concentration of these metabolites decreased under drought stress, with the lowest concentrations for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids being 1, 0.86, 1.87, and 0.1 mg per gram, respectively, from a concentration of zero under severe drought stress (40% field capacity) (Fig. 4d, e, f and g).

Evaluation of Total Protein, Peroxidase Enzyme Activity, and Catalase Activity

The trend of changes in antioxidant enzyme activity showed that the foliar application of the amino acid tryptophan on the Periwinkle plant increased the activity of catalase and peroxidase enzymes, as well as the leaf protein content. The highest protein content was 32.36 mg per gram from the treatment of 250 mg per liter of tryptophan without drought stress (100% field capacity), while the lowest protein content was 16.33 mg per gram from the zero concentration of tryptophan under severe drought stress (40% field capacity) (Fig. 4a). It was also observed that with the increase in tryptophan concentration, the activity of peroxidase and catalase enzymes showed an increasing trend; however, there was no significant difference in peroxidase enzyme activity among the tryptophan levels under severe drought stress. At high levels of tryptophan (200 and 250 mg per liter) under severe drought stress (40% field capacity), the highest peroxidase enzyme activity reached 0.15 standard units per milligram of protein. The lowest activity of this enzyme was obtained from the zero concentration without drought stress, measuring 0.02 standard units per milligram of protein (Fig. 4b). The application of tryptophan at high concentrations also improved catalase enzyme activity. At a concentration of 250 mg per liter of tryptophan under severe drought stress (40% field capacity), the catalase activity was 0.17 standard units per milligram of protein, which compared to the zero concentration without drought stress, increased the enzyme activity by 9.29 times (Fig. 4c).

Table 3

Results of the analysis of variance on the effects of drought stress treatment and the amino acid tryptophan on the physiological and morphological indices of the Periwinkle plant.

S.O.V	D.F	Mean squares						
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids	Total Protein	Peroxidase	Catalase
Drought	2	1.56**	1.41**	6.07**	2.16**	276**	0.05**	0.037**
Tryptophan	5	0.1**	0.2**	0.58**	0.03**	74.2**	0.003**	0.004**
Drought × Tryptophan	10	0.016*	0.008**	0.033*	0.003*	16.6*	0.0012**	0.0009**
Error	36	0.006	0.001	0.011	0.001	55.01	0.0001	0.0001
C.V (%)	-	5.2	2.8	3.58	6.75	9.28	14.2	15.4

** and * indicate significance at $p < 0.01$ and $p < 0.05$, respectively; ^{ns}: non-significant

Evaluation of Vinblastine and Vincristine Alkaloid Content

Analysis of variance results indicated that the effects of drought stress and the foliar application of the amino acid tryptophan on the concentrations of vincristine and vinblastine in the periwinkle plant were significant (Table 4). Both drought stress and the foliar application of tryptophan resulted in an increase in the content of vincristine and vinblastine in the periwinkle plant. Mean comparison analyses revealed that the highest concentrations of vincristine and vinblastine, measured at 0.87 mg and 0.69 mg per gram of dry leaf weight, respectively, were achieved at a concentration of 250 mg per liter under severe drought stress (40% field capacity). These amounts represent increases of 230% for vincristine and 488% for vinblastine when compared to the control treatment. Furthermore, the lowest concentrations of vincristine and vinblastine, measured at 0.3 mg and 0.29 mg per gram of dry leaf weight, respectively, were obtained from the control treatment, which featured zero tryptophan concentration and no drought stress (100% field capacity) (Fig. 5a and b).

Table 4

Analysis of variance results for the effect of drought stress treatment and tryptophan amino acid at different times based on the physiological indices of the periwinkle plant.

S.O.V	D.F	Mean squares	
		Vincristine	Vinblastine
Drought	1	0.05**	0.2**
Tryptophan	1	0.58**	0.05**
Drought × Tryptophan	1	0.003**	0.016**
Error	8	0.00024	0.0003
C.V (%)	-	4.18	5.5

** and * indicate significance at $p < 0.01$ and $p < 0.05$, respectively; ^{ns}: non-significant

Discussion

The results of the above experiment showed that drought stress increased the root length of the Persian plant. In response to the negative effects of drought stress, plants enhance their root systems. The increase in root length in plants growing under drought stress conditions is considered a desirable trait due to better absorption of moisture and nutrients from the soil, which is significantly important for the survival of plants [31]. The application of spermidine and putrescine improves root length by affecting the root apical meristem due to their role in controlling root cell division and the formation of primary and lateral roots [31, 32]. Under drought stress conditions, water and nutrient absorption increases in plants through the enhancement of root volume, surface area, and diameter [33]. With the increase in root length and area, it is natural for the root volume to also increase [34]. In fact, the increase in root volume is considered a desirable trait in assessing drought resistance, and genotypes with higher root volume have a greater ability to absorb water and nutrients, leading to increased production of aerial organs [35, 36]. The increase in root density can largely be attributed to the transfer of photosynthetically produced materials towards the roots for greater water absorption under drought stress conditions [37]. In this experiment, the application of tryptophan amino acid at high concentrations significantly increased all studied traits of the plant, except for root diameter. Drought stress significantly affects the growth and metabolism of plants, particularly on amino acid concentrations and protein synthesis. Studies have shown that drought generally increases the levels of free amino acids in plants, with a 5.9-fold increase observed in *Brassica napus* [38]. Tryptophan, a crucial amino acid, has been demonstrated to mitigate the effects of drought stress when applied externally to *Zea mays* [39]. A metabolomic analysis of various wheat cultivars revealed that drought-sensitive varieties exhibited increased levels of amino acids, including tryptophan [40]. In *Brassica oleracea*, the application of amino acid mixtures has been shown to alleviate the impacts of drought stress on plant growth and nutritional quality [41]. Drought stress substantially affects plant growth and performance, whereby the external application of amino acids, particularly tryptophan, can help ameliorate these adverse effects. Research indicates that foliar application of L-tryptophan enhances drought tolerance in corn and wheat through improvements in relative water content, leaf membrane stability, and chlorophyll concentration [39, 42]. Furthermore, tryptophan is integral to auxin biosynthesis, stimulates photosynthetic activity, and contributes to the yield and quality of agricultural crops [43]. Additionally, drought stress has been found to elicit varying responses in phenolic compounds within *Brassica napus*, resulting in an increase in total phenol content under heightened drought conditions, while flavonoid content was elevated at lower drought levels [44]. Phenolic compounds and flavonoids are essential non-enzymatic antioxidants that play a pivotal role in mitigating the effects of abiotic stresses, particularly drought stress in plants. These compounds are synthesized via the phenylpropanoid pathway, which becomes activated under stress conditions, leading to an upsurge in phenolic production that aids in neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation [45]. The findings from the research conducted on the medicinal plant Parvaneh under drought stress conditions (40%, 80%, and 100% field capacity) indicate that chlorophyll content was significantly influenced by the primary effects of drought stress. Specifically, drought stress at 40% field capacity resulted in a 40% reduction in total chlorophyll compared to the control treatment at 100% field capacity. Furthermore, additional experiments assessed the activities of antioxidant enzymes in response to the drought stress treatments. Notably, at 100% field capacity, the lowest enzyme activity was recorded in comparison to the other treatments. In most instances, an increase in drought stress was associated with an enhancement in enzyme activity and the antioxidant capacity of the plant, thereby improving the plant's ability to neutralize free radicals. This observation is consistent with the results of the present study [46–48]. Conversely, foliar spraying consistently enhanced enzyme activity and the plant's antioxidant capacity, thereby improving its ability to neutralize free radicals, in accordance with the findings of this investigation. Peroxidases play a crucial role in detoxifying hydrogen peroxide during the dehydration of various substrates. Specifically, the peroxidase enzyme facilitates the decomposition of hydrogen peroxide through the oxidation of hydrogen-donating substrates such as phenolic compounds, syringaldazine, guaiacol, and ascorbate [49]. Additionally, superoxide hydrogen is neutralized to water and oxygen by the action of the peroxidase enzyme or is transformed into the more reactive hydroxyl radical [50]. The

metabolism of antioxidants constitutes a fundamental defensive strategy that plants have developed to mitigate the damage inflicted by reactive oxygen species (ROS). The neutralization of ROS depends on a detoxification mechanism facilitated by an integrated system of reduced non-enzymatic molecules alongside enzymatic antioxidants, including catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) [51]. Additionally, water deficiency intensifies the production of reactive oxygen species, such as H₂O₂ and O₂, which can lead to chloroplast degradation, lipid peroxidation, and diminished chlorophyll content [52, 53]. Drought stress in medicinal plants results in elevated concentrations of ROS and malondialdehyde (MDA), which may have toxic effects [54]. Furthermore, the augmented activation of the chlorophyll-degrading enzyme chlorophyllase induces instability in protein complexes and subsequent degradation of chlorophyll, culminating in a reduction of chlorophyll levels [55]. Various studies have documented a decline in the concentrations of chlorophyll a and b, as well as a decrease in the chlorophyll a to b ratio in thyme leaves experiencing drought stress [56–58]. Elevated levels of drought stress correspond with a reduction in the content of chlorophyll a, chlorophyll b, and carotenoids compared to control conditions [59]. Similarly, photosynthetic pigments, including chlorophyll a and b, total chlorophyll, and carotenoids in *Melissa officinalis*, alongside chlorophyll a and b, carotenoids, and total pigments in *Mentha arvensis* L. and *Mentha pulegium* L., exhibited reductions under water deficit conditions [55]. The carotenoid content in *Thymus daenensis* and the chlorophyll to carotenoid ratio in *Thymus kotschyanus* were also diminished under drought stress conditions [57, 60, 61].

The application of drought stress and the amino acid tryptophan significantly enhanced the production of vincristine and vinblastine in the Periwinkle plant (*Catharanthus roseus*). Under conditions of severe drought stress (40% field capacity) and with a tryptophan concentration of 250 mg per liter, the levels of vincristine and vinblastine in the dried leaves increased by 230% and 488%, respectively, compared to the control treatment. This finding suggests that environmental stressors, particularly drought, can stimulate the production of secondary metabolites in medicinal plants. This research aligns with several other studies indicating that drought stress and plant growth regulators can influence the production of valuable alkaloids, such as vincristine and vinblastine. Specifically, it has been reported that drought stress can elevate the total alkaloid content by up to 187%, with vincristine and vinblastine levels increasing by 175% and 171%, respectively, in comparison to control plants [62]. Additionally, tryptophan serves as a precursor in alkaloid biosynthesis and may enhance their production under stress conditions [63]. Foliar application of indole-3-acetic acid (IAA) at a concentration of 150–200 ppm has been shown to significantly increase the levels of vincristine [64]. Drought stress induces structural and anatomical alterations in plants and contributes to the accumulation of secondary metabolites such as alkaloids, including vincristine and vinblastine [65]. Research indicates that moderate drought stress can enhance both the quality and quantity of secondary metabolites by specifically elevating alkaloid concentrations [65]. Drought stress administered at 75 percent of field capacity, in conjunction with foliar application of proline (300 mg per liter), resulted in increased production of vinblastine and vincristine in both leaves and roots [66]. Conversely, in vitro drought stress induced by polyethylene glycol (PEG) did not significantly affect the production of vinblastine and vincristine in callus cultures; however, it may influence terpenoid production and the differentiation of latex-producing cells [67]. Moreover, it appears that the combination of tryptophan and drought stress synergistically enhances alkaloid levels, as evidenced by the significant increases observed in various studies [63, 68].

Conclusions

Based on the findings of the present study, the application of tryptophan as a foliar treatment on *Catharanthus roseus* plants not only mitigates the adverse effects of drought stress but also exerts a beneficial influence on root tissue development, plant dry weight, enzymatic and non-enzymatic antioxidant levels, photosynthetic pigments, and the biosynthesis of valuable alkaloids. This treatment demonstrates the potential to enhance both agricultural yield and

the concentrations of the alkaloids vincristine and vinblastine. Consequently, the foliar application of tryptophan is recommended as a viable strategy to augment the commercial production of alkaloids in *Catharanthus roseus* and as an effective method to address agricultural challenges associated with drought stress.

Declarations

Acknowledgements

We would like to express our sincere gratitude and appreciation to the esteemed professors and experts at Khuzestan Agricultural Sciences and Natural Resources University, as well as to the staff of the university's Central Laboratory, for their collaboration in the conduct of this research.

Author Contributions

F.Y: Data collection, formal analysis, validation, original draft of the manuscript, funding. A.A: Formal analysis of the biochemical section, funding, and Review and editing of the manuscript. A.L.j: Review and editing of the manuscript. A.SH: Conceptualization, review and editing of the manuscript. S. J: Formal analysis, review and editing of the manuscript. N.S: Review and editing of the manuscript and HPLC analysis.

Funding

provided by Dr. Alireza Abdali Mashhadi, Farshid Yousefi, and Ms. Narges Soltani.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing of interests

The authors declare no competing interests.

Clinical trial

Not applicable.

References

1. Tolambiya P, Mathur S. A Study on Potential Phytopharmaceuticals Assets in *Catharanthus roseus* L.(Alba). International Journal of Life Science Biotechnology and Pharma Resarch 2016;5(1):6.

2. Alam MM, Naeem M, Khan MMA, Uddin M. Vincristine and vinblastine anticancer catharanthus alkaloids: Pharmacological applications and strategies for yield improvement. *Catharanthus roseus*: Springer; 2017. p. 277-307.
3. Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: pharmacognosy and biotechnology. *Current medicinal chemistry*. 2004;11(5):607-28.
4. Adekomi DA. Madagascar periwinkle (*Catharanthus roseus*) enhances kidney and liver functions in Wistar rats. *Eur J Anat*. 2010;14(3):111-9.
5. Shukla AK, Shasany AK, Verma RK, Gupta MM, Mathur AK, Khanuja SP. Influence of cellular differentiation and elicitation on intermediate and late steps of terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*. *Protoplasma*. 2010;242:35-47.
6. Nayak B, Pinto Pereira LM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complementary and Alternative medicine*. 2006;6:1-6.
7. Jaleel CA, Gopi R, Manivannan P, Gomathinayagam M, Murali P, Panneerselvam R. Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. *Pesticide Biochemistry and Physiology*. 2008;90(2):135-9.
8. Azad N, Rezayian M, Hassanpour H, Niknam V, Ebrahimzadeh H. Physiological mechanism of salicylic acid in *Mentha pulegium* L. under salinity and drought stress. *Brazilian Journal of Botany*. 2021;44:359-69.
9. Choudhary S, Zehra A, Mukarram M, Naeem M, Khan MMA, Hakeem KR, et al. An insight into the role of plant growth regulators in stimulating abiotic stress tolerance in some medicinally important plants. *Plant Growth Regulators: Signalling under Stress Conditions*. 2021:75-100.
10. Yeshi K, Crayn D, Ritmeijer E, Wangchuk P. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*. 2022;27(1):313.
11. Pathania D, Sharma M, Kumar S, Thakur P, Torino E, Janas D, et al. Essential oil derived biosynthesis of metallic nano-particles: Implementations above essence. *Sustainable Materials and Technologies*. 2021;30:e00352.
12. Dinkeloo K, Boyd S, Pilot G, editors. Update on amino acid transporter functions and on possible amino acid sensing mechanisms in plants. *Seminars in cell & developmental biology*; 2018: Elsevier.
13. Basha D, El-Aila H. Response of foliar spraying with amino acids and integrated use of nitrogen fertilizer on radish (*Raphanus sativus* L.) plant. *International Journal of ChemTech Research*. 2015;8(11):135-40.
14. Hildebrandt TM, Nesi AN, Araújo WL, Braun H-P. Amino acid catabolism in plants. *Molecular plant*. 2015;8(11):1563-79.
15. Mustafa A, Imran M, Ashraf M, Mahmood K. Perspectives of using l-tryptophan for improving productivity of agricultural crops: A review. *Pedosphere*. 2018;28(1):16-34.
16. Mohamed MF, Thalooth A, Essa R, Gobarah ME. The stimulatory effects of tryptophan and yeast on yield and nutrient status of wheat plants (*Triticum aestivum*) grown in newly reclaimed soil. *Middle East J Agric Res*. 2018;7(1):27-33.
17. Walker WR, editor The effects of geometry and wetted perimeter on surface irrigation infiltration. *World Environmental and Water Resources Congress 2008: Ahupua'a*; 2008.
18. Selahvarzi Y, Tehranifar A, Gazanchian A. Physiomorphological changes under drought stress and rewatering in endemic and exotic turfgrasses. *Journal of Horticultural Sciences and Techniques of Iran*. 2007;9(3):193-204. (In Persian).
19. Hajabbasi MA. Tillage effects on soil compactness and wheat root morphology. *Journal of Agricultural Science and Technology*. 2001;3(1):67-77.

20. Arnon A. Method of extraction of chlorophyll in the plants. *Agronomy journal*. 1967;23(1):112-21.
21. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food chemistry*. 2005;91(3):571-7.
22. Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*. 2002;10(3).
23. Hwang M-N, Ederer GM. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *Journal of Clinical Microbiology*. 1975;1(1):114-5.
24. Ponce A, Del Valle C, Roura S. Natural essential oils as reducing agents of peroxidase activity in leafy vegetables. *LWT-Food Science and Technology*. 2004;37(2):199-204.
25. Chance B, Maehly A. [136] Assay of catalases and peroxidases. 1955;2:775-64.
26. Cakmak I, Horst WJ. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia plantarum*. 1991;83(3):463-8.
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*. 1976;72(1-2):248-54.
28. Singh D, Maithy A, Verma R, Gupta M, Kumar S. Simultaneous determination of Catharanthus alkaloids using reversed phase high performance liquid chromatography. 2000.
29. Siddiqui MJA, Ismail Z, Saidan NH. Simultaneous determination of secondary metabolites from *Vinca rosea* plant extractives by reverse phase high performance liquid chromatography. *Pharmacognosy magazine*. 2011;7(26):92.
30. Gupta M, Singh D, Tripathi A, Pandey R, Verma R, Singh S, et al. Simultaneous determination of vincristine, vinblastine, catharanthine, and vindoline in leaves of *Catharanthus roseus* by high-performance liquid chromatography. *Journal of chromatographic science*. 2005;43(9):450-3.
31. Hossinzadeh SR, Salimi A, Ganjeali A. Effects of methanol on morphological characteristics of chickpea (*Cicer arietinum* L.) under drought stress. *Environmental Stresses in Crop Sciences*. 2012;4(2):139-50.
32. Habba EE, Abdel Aziz N, Sarhan A, Arafa A, Youssefi N. Effect of putrescine and growing media on vegetative growth and chemical constituents of *Populus euramericana* plants. *Journal of Innovations in Pharmaceutical and Biological Sciences*. 2016;3(1):61-73.
33. Ganjali A, Kafi M, Bagheri A, Ahmadi FS. Allometric relationships for root and shoot characteristics of chickpea seedlings (*Cicer arietinum* L.). 2004.
34. Serraj R, Krishnamurthy L, Kashiwagi J, Kumar J, Chandra S, Crouch J. Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Research*. 2004;88(2-3):115-27.
35. Ding H, Zhang Z, Dai L, Song W, Kang T, Ci D. Responses of root morphology of peanut varieties differing in drought tolerance to water-deficient stress. *Acta Ecologica Sinica*. 2013;33(17):5169-76.
36. Li X, Guo Z, Lv Y, Cen X, Ding X, Wu H, et al. Genetic control of the root system in rice under normal and drought stress conditions by genome-wide association study. *PLoS Genetics*. 2017;13(7):e1006889.
37. Leport L, Turner NC, Davies S, Siddique K. Variation in pod production and abortion among chickpea cultivars under terminal drought. *European Journal of Agronomy*. 2006;24(3):236-46.
38. Good AG, Zaplachinski ST. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. *Physiologia plantarum*. 1994;90(1):9-14.
39. Rao SR, Qayyum A, Razzaq A, Ahmad M, Mahmood I, Sher A, et al. ROLE OF FOLIAR APPLICATION OF SALICYLIC ACID AND L-TRYPTOPHAN IN DROUGHT TOLERANCE OF MAIZE. *Journal of Animal and Plant Sciences*. 2012;22:768-72.

40. Michaletti A, Naghavi MR, Toorchi M, Zolla L, Rinalducci S. Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. *Scientific Reports*. 2018;8.
41. Haghighi M, Saadat S, Abbey L. Effect of exogenous amino acids application on growth and nutritional value of cabbage under drought stress. *Scientia Horticulturae*. 2020;272:109561.
42. Shabir A, Saqib M, Ahmad M, Latif MT, Bukharia SAH, Ahmad MQ, et al. Enhancing Drought Tolerance of Wheat (*Triticum aestivum* L.) Through Foliar Application of Proline and L-Tryptophan. *Biological Sciences - PJSIR*. 2020.
43. Rezk AI, El-Nwehy SS. Amino Acids and its Role in Plant Nutrition and Crop Production. A review. *Middle East Journal of Applied Sciences*. 2021.
44. Rezayian M, Niknam V, Ebrahimzadeh H. Differential responses of phenolic compounds of *Brassica napus* under drought stress. *Plant Physiology*. 2018;8:2417-25.
45. Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdely C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry*. 2007;45(3-4):244-9.
46. Alhaithloul HA, Soliman MH, Ameta KL, El-Esawi MA, Elkelish A. Changes in ecophysiology, osmolytes, and secondary metabolites of the medicinal plants of *Mentha piperita* and *Catharanthus roseus* subjected to drought and heat stress. *Biomolecules*. 2019;10(1):43.
47. Liu Y, Meng Q, Duan X, Zhang Z, Li D. Effects of PEG-induced drought stress on regulation of indole alkaloid biosynthesis in *Catharanthus roseus*. *Journal of Plant Interactions*. 2017;12(1):87-91.
48. Tan U, Gören HK. Comprehensive evaluation of drought stress on medicinal plants: a meta-analysis. *PeerJ*. 2024;12:e17801.
49. Chandra A, Dubey A. Effect of ploidy levels on the activities of Δ^1 -pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in *Cenchrus* species grown under water stress. *Plant Physiology and Biochemistry*. 2010;48(1):27-34.
50. Wang W-B, Kim Y-H, Lee H-S, Kim K-Y, Deng X-P, Kwak S-S. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant physiology and Biochemistry*. 2009;47(7):570-7.
51. Shi Q, Bao Z, Zhu Z, Ying Q, Qian Q. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. *Plant growth regulation*. 2006;48:127-35.
52. Foyer CH, Lelandais M, Kunert KJ. Photooxidative stress in plants. *Wiley Online Library*; 1994.
53. Tátrai ZA, Sanoubar R, Pluhár Z, Mancarella S, Orsini F, Gianquinto G. Morphological and physiological plant responses to drought stress in *Thymus citriodorus*. *International Journal of Agronomy*. 2016;2016(1):4165750.
54. Li M, Zhang M, Cheng L, Yang L, Han M. Changes in the platycodin content and physiological characteristics during the fruiting stage of *Platycodon grandiflorum* under drought stress. *Sustainability*. 2022;14(10):6285.
55. Omid H, Shams H, Sahandi MS, Rajabian T. Balangu (*Lallemantia* sp.) growth and physiology under field drought conditions affecting plant medicinal content. *Plant Physiology and Biochemistry*. 2018;130:641-6.
56. Abd Elbar OH, Farag RE, Shehata SA. Effect of putrescine application on some growth, biochemical and anatomical characteristics of *Thymus vulgaris* L. under drought stress. *Annals of Agricultural Sciences*. 2019;64(2):129-37.
57. Bahreininejad B, Razmjou J, Mirza M. Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*. 2013.
58. Misra A, Srivastava N. Influence of water stress on Japanese mint. *Journal of Herbs, Spices & Medicinal Plants*. 2000;7(1):51-8.

59. Zamani Z, Amiri H, Ismaili A. Improving drought stress tolerance in fenugreek (*Trigonella foenum-graecum*) by exogenous melatonin. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*. 2020;154(5):643-55.
60. Ashrafi M, Azimi-Moqadam M-R, Moradi P, MohseniFard E, Shekari F, Kompany-Zareh M. Effect of drought stress on metabolite adjustments in drought tolerant and sensitive thyme. *Plant Physiology and Biochemistry*. 2018;132:391-9.
61. Bistgani ZE, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. *The Crop Journal*. 2017;5(5):407-15.
62. Amirjani MR. Effects of drought stress on the alkaloid contents and growth parameters of *Catharanthus roseus*. *J Agric Biol Sci*. 2013;8(11):745-50.
63. Alaakel S, AL-Ammouri Y. Somaclonal Variation in Callus Cultures of Rose Periwinkle, *Catharanthus Roseus* L. Under Induced Salt and Osmotic Stresses. *OBM Genetics*. 2023;7(4):1-14.
64. Muthulakshmi S, Pandiyarajan V. Influence of IAA on the vincristine content of *Catharanthus roseus* (L). G. Don. *Asian J Plant Sci Res*. 2013;3(4):81-7.
65. Shil S, Dewanjee S. Impact of drought stress signals on growth and secondary metabolites (SMs) in medicinal plants. *J Phytopharmacol*. 2022;11(5):371-6.
66. Khudair A, Al-Naseri O, editors. Increase Active Substances in *Catharanthus Roseus* LG Don with Water Tension and Foliar Application of Proline. *IOP Conference Series: Earth and Environmental Science*; 2021: IOP Publishing.
67. Iskandar NN. Vinblastine and Vincristine production on Madagascar Periwinkle (*Catharanthus roseus* (L.) G. Don) callus culture treated with polyethylene glycol. *Makara Journal of Science*. 2016;20(1):2.
68. Khashan KT, Al-Athary MA. Vinblastine and vincristine alkaloids production from callus of *Catharanthus roseus* (L.) G. Don under some abiotic factors. *Al-Kufa University Journal for Biology*. 2016;8(2):9-24.

Figures

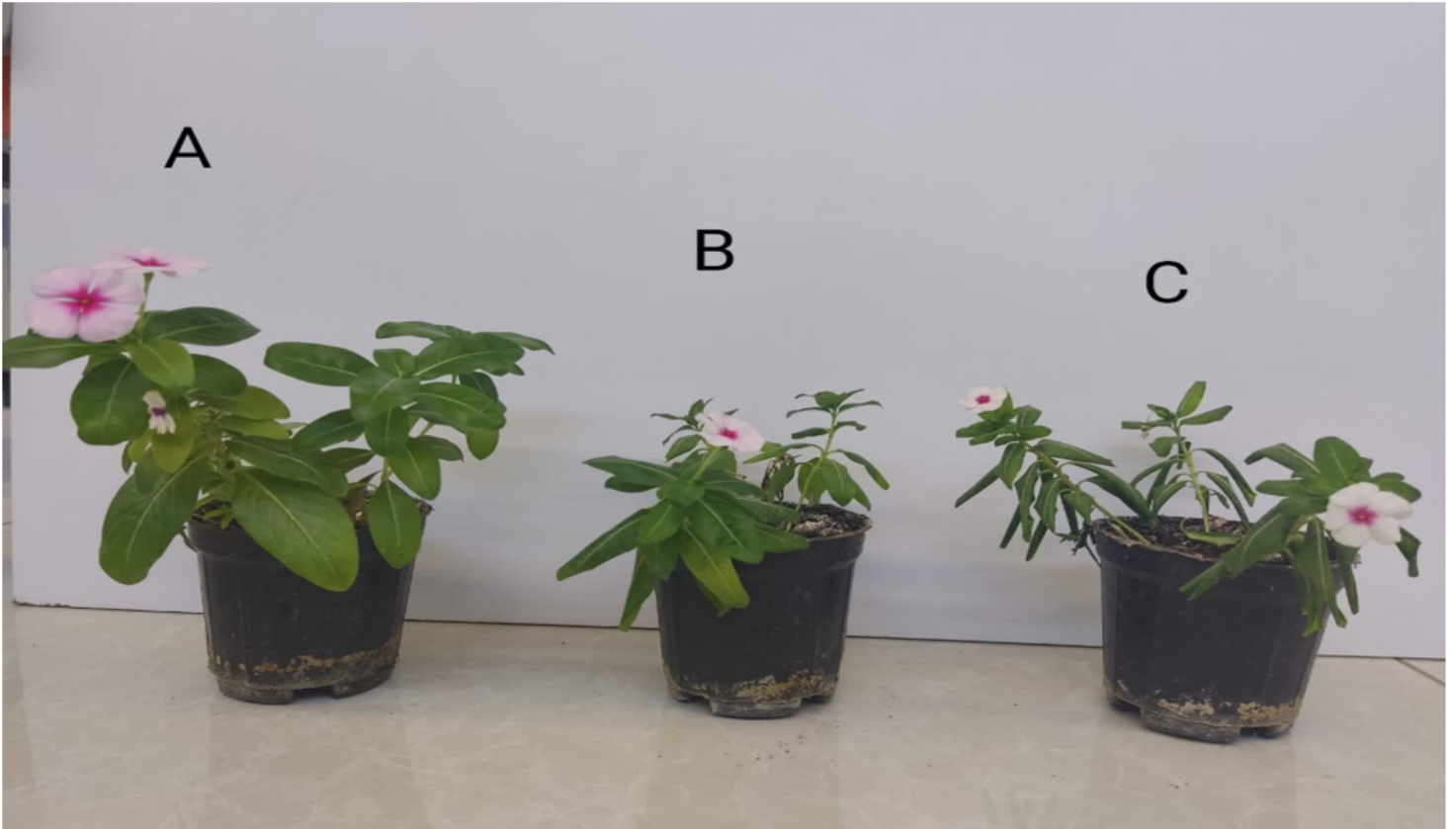


Figure 1

Seedlings of *C. roseus*. **A:** Control seedlings 100%, **B:** Seedlings under drought stress of 70% and **C:** Seedlings under drought stress of 40%.

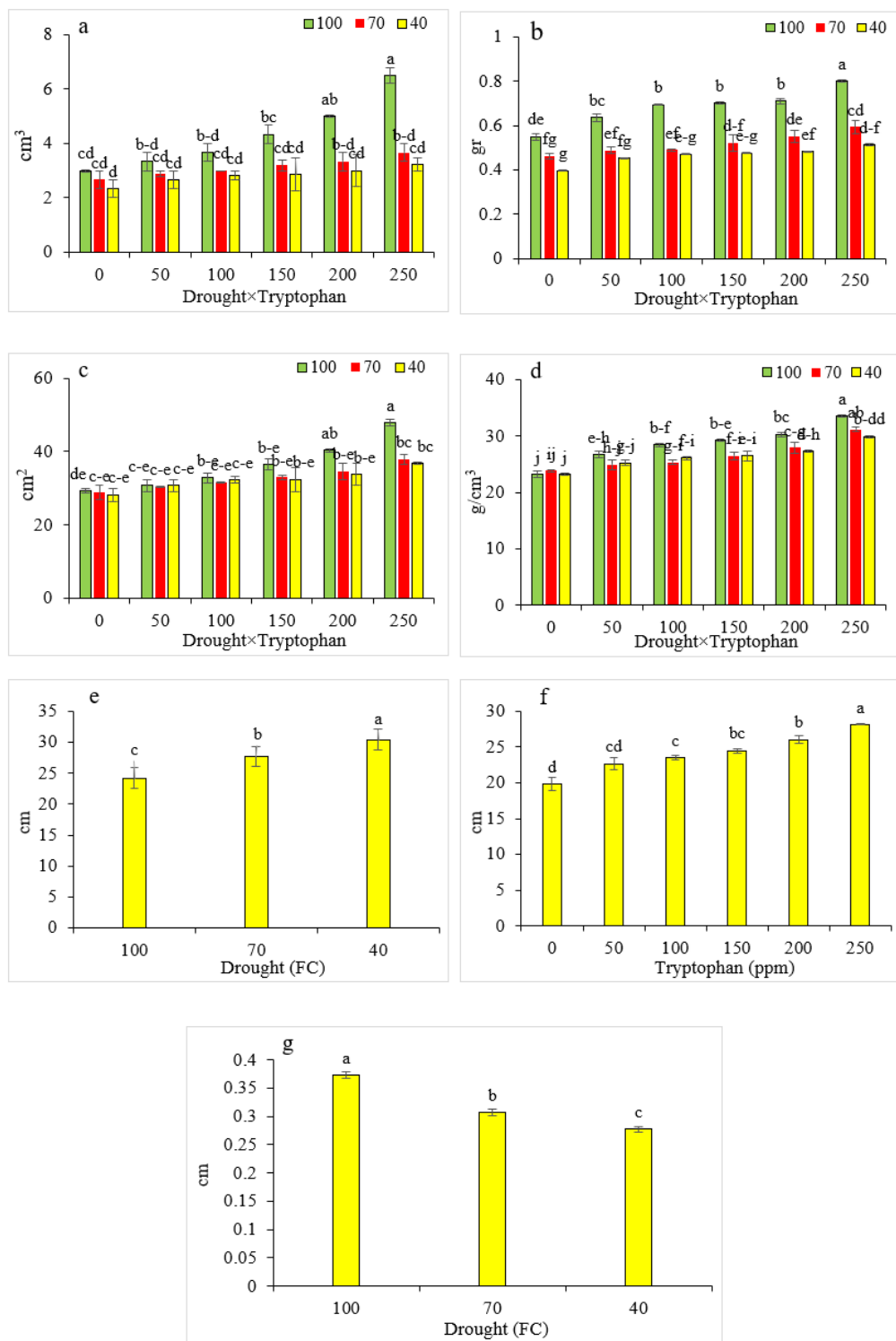


Figure 2

Comparison of the response to drought stress treatment with varying concentrations of the amino acid tryptophan, as measured by root volume (a), root dry weight (b), root area (c), root area density (d), root length (e,f), and root diameter (g). Columns sharing the same letters indicate no statistically significant difference at the 5% error probability level, as determined by Duncan's test.

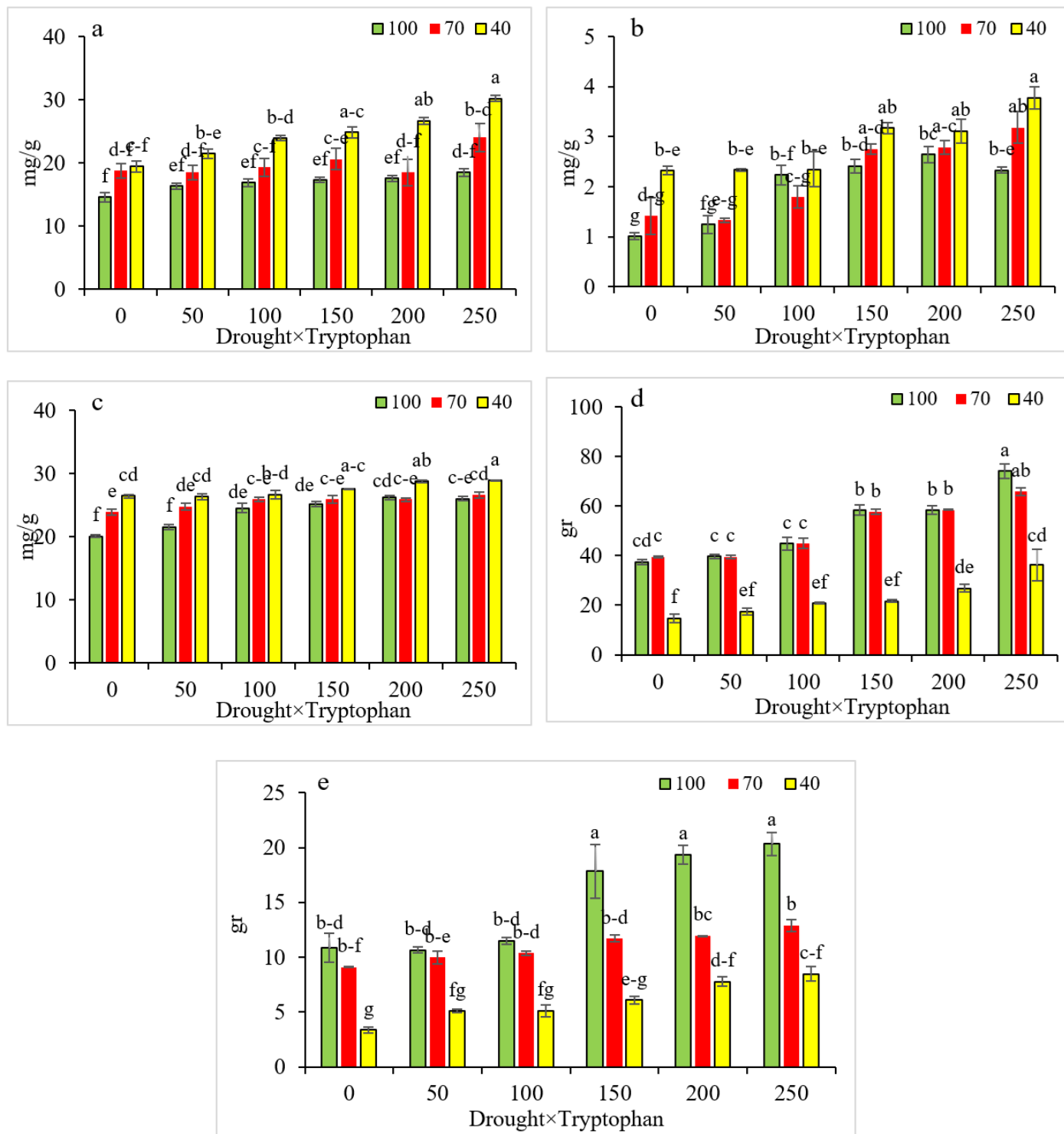


Figure 3

compares the response of drought stress treatment with different concentrations of the amino acid tryptophan in terms of phenol traits (a), flavonoids (b), total amino acids (c), fresh weight (d), and dry weight (e). Columns with similar letters are not statistically significantly different at the 5% error probability level according to Duncan's test.

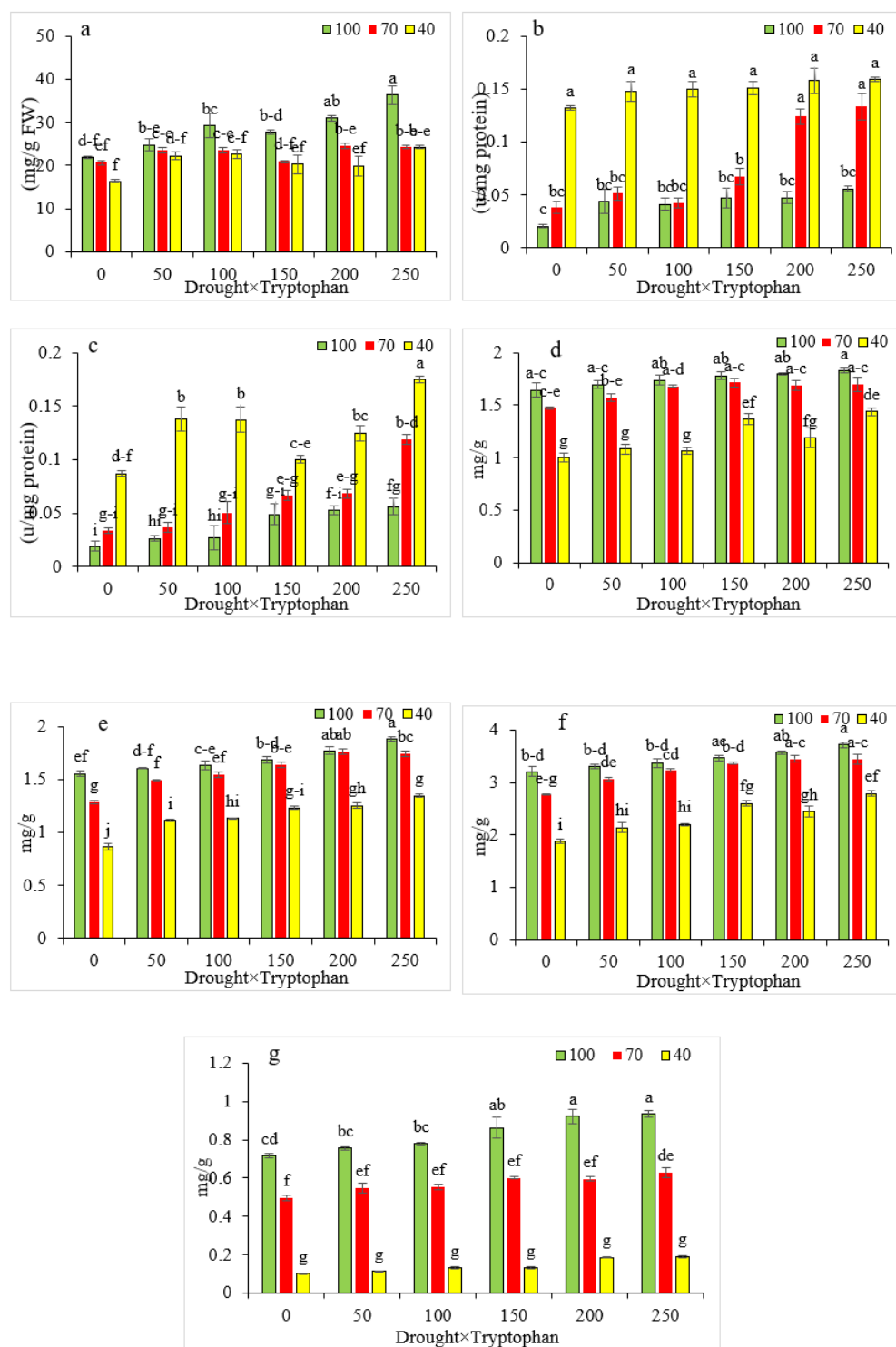


Figure 4

Comparison of the mean response of drought stress treatment with the concentration of the amino acid tryptophan regarding total protein (a), peroxidase enzyme (b), catalase (c), chlorophyll a (d), chlorophyll b (e), total chlorophyll (f), and carotenoid (g). Columns with similar letters are not statistically significantly different at the 5% probability level according to Duncan's test.

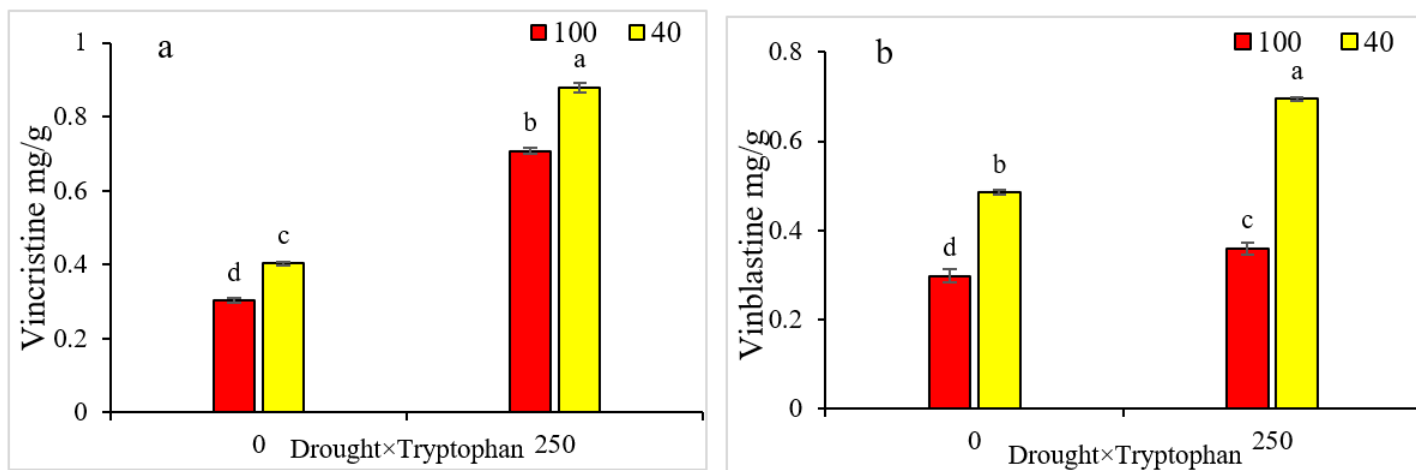


Figure 5

Comparison of the average responses to drought stress treatment with varying concentrations of tryptophan amino acid in relation to vincristine (a) and vinblastine (b) traits. Columns sharing identical letters indicate that there is no statistically significant difference at the 5% error probability level, as determined by Duncan's test.