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SIMULTANEOUS EFFECT ON METABOLIC PROFILING OF TERPENE INDOLE ALKALOID PATHWAY IN LEAVES AND ROOTS OF *CATHARANTHUS ROSEUS* UNDER DROUGHT STRESS

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ABSTRACT

Catharanthus roseus is an important medicinal plant which produces important compounds in roots and leaves through terpene indole alkaloid (TIA) pathway under strict molecular regulation, when subjected to stresses. Working of TIA pathway and alkaloid accumulation requires involvement of tissue and organ-specific compartmentalization. Our knowledge about the effect of drought stress on changes in metabolites accumulation of TIA pathway in roots and leaves is still very less. In present study, various biochemical parameters and metabolite profiling in leaves and roots were investigated simultaneously under drought stress. Different levels of drought stress were supplied to three-month-old C. roseus plants for 2 months and analyzed at 5 and 10 days after treatment (DAT) in field grown conditions. Results showed reduced chlorophyll content in leaves and increased soluble sugar, proline and free amino acid contents in roots and leaves. HPLC analysis revealed increase in tryptophan, tryptamine, serpentine, catharanthine contents in leaves and roots without affecting tabersonine content concomitantly with treatment levels. Contents of secologanin, vindoline, vinblastine and vincristine were found only in leaves with increasing amount. Ajmalicine was found only in roots which decrease under drought stress. This experiment indicated that the drought stress is responsible for increasing contents of important metabolites and precursors of TIA pathway and showed that the pathway work as a whole set of interconnected networks of tissues and organs where metabolites are differentially adjusted for coping with drought stress.

KEY WORDS

Alkaloid, Catharanthus roseus, drought stress, HPLC, Terpene Indole Alkaloid (TIA) Pathway

Introduction:

Plants secondary metabolites are unique sources of new drugs that do not have any fundamental role on life processes but they are produced to cope up and interact between plant and environmental conditions. Among these metabolites, alkaloids are largest class of pharmacologically active basic compounds having low molecular weight and heterocyclic nitrogen. Terpene indole alkaloids (TIAs) form important section of all alkaloids and constitute the largest family of complex natural plant products. These are used as important drugs for malaria, diabetes, cardio-vascular diseases and in several types of cancers. [1]

Catharanthus roseus (L.) G. Don is an important medicinal plant in which biosynthesis of TIAs has been extensively studied. This plant belongs to the family Apocynaceae and harbors more than 100 types of TIAs including commercially important dimeric anti-cancerous alkaloids vinblastine and vincristine. All TIAs in plant are produced by a complex TIA pathway in different compartments of cell under strict transcriptional control. [2] Central precursors for all TIAs in biosynthetic pathway is strictosidine, formed by the

condensation of tryptamine and secologanin of indole pathway and iridoidal pathway respectively by an enzyme strictosidine synthase (STR) in vacuoles of leaves and roots cells. During the biosynthesis of alkaloids, iridoidal pathway is considered to play an important role, where an enzyme geraniol 10 hydroxylase (G10H) initiates first committed step in formation of iridoid monoterpenoid secologanin and is a site of regulatory control. Then, route is diverted towards the biosynthesis of specific monoterpene indole alkaloids (MIAs) in *C. roseus*[1]. An enzyme strictosidine-β-D-glucosidase (SGD) encoded by *Sgd* gene form an intermediate strictosidine-aglycone which gives a specific direction to TIAs biosynthesis and accumulate many important MIAs like ajmalicine and serpentine in roots and tabersonine and catharanthine in epidermal cells of leaf. In late steps, tabersonine converted into vindoline, specifically in leaf by a sequence of six steps. These steps include Aromatic hydroxylation, O-methylation, hydration of the 2,3-double bond, N (1)-methylation, hydroxylation at position 4, and 4-O-acetylation by means of enzymes tabersonine 16-hydroxylase (T16H), O-methyltransferase (OMT), N-methyltransferase (NMT), unknown hyratase, desacetoxyvindoline-4-hydroxylase(D4H), and deacetylvindoline-4-O-acetyltransferase (DAT). Another important anticancerous dimeric compounds vinblastine and vincristine are formed after the coupling of vindoline and catharanthine. [3]

Biosynthesis of all alkaloids and their precursors showed wide differences in their formation in aerial and underground tissues. [4] These differences indicated variations in expression pattern of structural and regulatory genes of TIA pathway in roots as well as in aerial parts. Accumulation of all secondary metabolites is greatly influenced by abiotic stress conditions, as they can induce a wide variety of responses like readjustment of transport systems and metabolic processes in both parts. Abiotic stresses also trigger some initial sensors which finally lead to the induction of stress responsive gene expression and physiological and biochemical changes. Drought stress is one of the most important environmental factors that affect plants growth and productivity. [5] It also affect biosynthesis of secondary metabolites, photosynthesis rate, accumulation of abscisic acid, proline, sugar, free amino acids, synthesis of new proteins and mRNAs. [6]

Large number of studies has been carried out to understand the effect of drought stress on changes occurred in amount of important metabolite in roots or leaves extract. But information regarding the changes in distribution and accumulation of metabolites and their precursors through which they are formed by TIA pathway together at a same time in roots and leaves has not been reported so far. By keeping this view in mind, objective of present study was designed to understand the effect of different levels of drought treatments, simultaneously on certain biochemical aspects in roots and leaves and accumulation of all important TIAs and their precursors at different time intervals. Therefore, present study will help to understand the changes or adjustment occurred in physiological processes and their effect on metabolites biosynthesis by TIA biosynthetic pathway along with their distribution in leaves and roots of *C. roseus* plants under different levels of drought stress condition. In future, results may also help in determining the suitable biotechnological or genetic engineering approach by which content of medicinally important metabolites can be increased.

Materials and Methods:

(i) Plant material and treatments:

Catharanthus roseus (L.) G. Don. cv Nirmal seeds were procured from Central Institute of Medicinal and Aromatic Plants, Lucknow and sterilized in 0.2% $HgCl_2$ solution for 10 min followed by washing and incubation at 37ºC for 48 h before sowing. Two months old plantlets were transferred into pots filled with soil having clay and pertile in the ratio of 3:1. The pots were placed under natural light during the growth season with an average day/night temperature of 22/16 ± 2 °C. Experiment was conducted on three-month-old plants in triplicate (three plants per pot), where different levels of drought treatments were given to the plants as Control, 1-week regime (irrigation every week), 2 weeks regime (irrigation every 2 weeks) and 3 weeks regime (irrigation every 3 weeks) for each set up to two months. And then leaves and roots samples were collected on day 5 and 10 days after treatment (DAT) and analyzed for estimation of contents of chlorophyll, soluble sugar, proline and free amino acid and tryptophan, tryptamine, secologanin, strictosidine, ajmalicine, catharanthine, vindoline, tabersonine, vinblastine and vincristine.

(ii) Biochemical Analysis:

Estimation of Chlorophyll contents:

Chlorophyll estimation was performed by using DMSO (dimethyl sulfoxide) method. [7] Chopped Fresh leaves material was added in 5 ml DMSO in vials and placed in oven at 65 °C for 1 h for complete leaching of pigments. The volume of DMSO was made up to 10 ml and then chlorophyll content was measured by using UV-Vis spectrophotometer (Lambda BIO 20, Perkin Elmer, Germany) at 645 and 663 nm. Chlorophyll *a*, Chlorophyll *b,* and total chlorophyll contents were calculated by Arnon's (1949) equations[8], and expressed in mg g^{-1} FW.

Determination of Soluble sugar:

Soluble sugar content was estimated by using anthrone sulfuric acid reagent in aqueous solution.[9] Fresh leaf and roots tissue powder (100 mg) was hydrolyzed by boiling and then filtered extracts were mixed in 4.5 ml of anthrone reagent (0.2 g anthrone, 8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml sulfuric acid). The mixtures were boiled and then allowed to cool rapidly. Absorbance of the solutions were measured for total carbohydrate content at 630 nm against blank in a UV-Vis spectrophotometer (Lambda BIO 20, Perkin Elmer, Germany) and expressed as mg g^{-1} FW. The standard curve was prepared by using glucose as a standard.

Determination of Proline content:

Ninhydrin method was used for proline estimation.[10] Fresh leaf and root samples (1 g) were homogenized in 10 ml of aqueous 3% sulfosalicylic acid for over-night. The Homogenates were centrifuged and the supernatants (2 ml) were mixed with equal volume of freshly prepared acid ninhydrin and glacial acetic acid solution. Mixtures were incubated at 100 °C (1 h) and then the reaction was terminated by adding toluene (4 ml). The fraction of chromophore-containing toluene was aspirated and observed at 520 nm wavelength by using UV-Vis spectrophotometer (Lambda BIO 20, Perkin Elmer, Germany). L- proline was used as standard for preparing standard curve the content was measured in nmol g^{-1} FW.

Determination of Free amino acid content:

Free amino acid content was determined by using ninhydrin method[11] and standard curve was prepared from glycine. Washed fresh leaves and roots (100 mg) were grounded and dipped overnight in ethanol and then centrifuged at 5500 rpm. Alcohol was evaporated by placing supernatants at 100°C for 1 h in water bath. The pellets were dissolved in 10 ml of 0.5 M citrate buffer (pH 5.6) and then 0.5 ml aliquot was mixed in 55% glycerol and 1.0% ninhydrin solution. Mixtures are boiled till the appearance of blue color and then volume was set up to 6 ml. Absorbance was recorded at 570 nm wavelength on UV-Vis spectrophotometer (Lambda BIO 20, Perkin Elmer, Germany) and expressed in µmol g^{-1} FW.

(iii) Terpene indole alkaloid (TIA) contents analysis by HPLC:

Alkaloid extraction:

Alkaloid extraction was done by using dried leaf and root samples of *C. roseus* after certain minor changes in protocol of Singh and others (2000).[12] Briefly, 5.0 g leaf and root powders were extracted in methanol (30 ml x 3) for 12 h and concentrated to 10 ml after filtering by syringe filter (0.22 µm, Millipore, Ireland). Extracts were acidified with 10 ml 3% HCl and then washed with 30 ml hexane. The aqueous Phases were separated and basified with ammonia to the pH 8.5 and extracted thrice with 30 ml chloroform, followed by washing and drying over sodium sulfate. They were allowed to concentrate and residues re-dissolved in 5 ml methanol for use.

Chromatographic conditions and solvent systems for metabolites quantification:

Analysis of tryptophan, tryptamine, secologanin, strictosidine, ajmalicine, serpentine, catharanthine, tabersonine, vindoline, vinblastine and vincristine of TIA pathway in roots and leaves extracts were done on HPLC system (Waters, Milford, MA, USA) equipped with a 600E system controller, a 996-photodiode array detector and 2707 autosampler (Waters). The system was controlled by Empower 2 software (Europa Science, Ltd., Cambridge, UK) for monitoring and analysis of results. A C_{18} RP-column (Millipore, 250×4.6 mm, particle size 5 µm) was used for chromatographic separation. Column temperature was set at 35 °C. Injection volume was taken 10 µL. Two different mobile phases were used for the quantification of all the alkaloids. Analysis of serpentine, catharanthine, tabersonine, vindoline, vinblastine and vincristine was done by method of Bhadra and others (1993)[13] after slight modifications. Mobile phase consisted of 25:75 (V/V) mixtures of 5 mM di-ammonium hydrogen orthophosphate and methanol (pH 7.0) at flow rate of 1 ml/min. Method of Tikomirroff and Joelicour (2002)[14]

was used for quantification of tryptophan, tryptamine, secologanin, strictosidine and ajmalicine. In this method, acetonitrile and 100 mM H_3PO_4 mixture (pH 2.0) were used as mobile phase in ratio of 5:85 at 1.2 ml/min flow rate. Standard curve was used for identification and quantification of compounds.

Stock solutions of standards were prepared by mixing 1 mg of each standard in 1 ml of methanol separately. Calibration curve were plotted by running them on HPLC system in their respective solvent systems and found linear in the range from 2 to 64 µg/ml.

(iii) Statistical analysis:

All statistical analyses were carried out by using SPSS v.16.0 statistical software for Windows and obtained data were subjected to one-way analysis of variance (ANOVA). Means were compared by least significant differences (LSD) test at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$ levels. Significant differences at levels of significance are represented by stars (*). Data are given as means values ± standard deviation.

RESULTS AND DISCUSSION:

Drought is one of the most common environmental stress inhibiting structural and functional activities of plants. Capacity and sensitivity for tolerating drought stress in plants are depending on genetic structure as well as intensity and duration of stress.[15] Under drought conditions, plants developed mechanism for their survival and to protect themselves from dehydration process. These are characterized by changes in biochemical, physiological, morphological and other metabolic processes and mainly include osmotic adjustment and changes in elastic properties of tissues.[16]

(i) Biochemical analysis

Chlorophyll content:

Photosynthetic activities of plants are directly related to chlorophyll contents. Chlorophyll pigments reduced in *C. roseus* leaves concomitantly with increasing drought levels (Table I). It decreased significantly in 3 weeks regime drought level at 10 DAT in both samplings. Chlorophyll *b* content reduced from 0.22 ± 1.2 (control) to 0.18 ± 0.85 mg g^{-1} FW in 3 weeks regime drought treatment at 5 DAT. At 10 DAT it decreased up to 38 % in 1 and 2 weeks regime and 53% in 3 weeks regime drought treatments. At 5 DAT, no significant effect on total chlorophyll content was observed in all the levels of drought but at 10 DAT it was reduced by 33% in 3 weeks regime treatment level. Chlorophyll contents are reduced under drought stress due to stomatal closure and metabolic impairment.[17] Similar results were also previously reported in *C. roseus*[18], cotton[19] and sunflower plants[20].

Table I: Drought-induced changes in the chlorophyll contents (mg g^{-1} FW) in *C. roseus*

Drought level	Chlorophyll *a* (Mean ± SD)		Chlorophyll *b* (Mean ± SD)		Total Chlorophyll (Mean ± SD)	
	5 DAT	10 DAT	5 DAT	10 DAT	5 DAT	10 DAT
Control	0.94 ± 0.08	0.95 ± 0.02	0.22 ± 0.02	0.26 ± 0.00	1.17 ± 0.09	1.11 ± 0.02
1 W reg	1.14 ± 0.08	0.77 ± 0.04	0.27 ± 0.01	0.16 ± 0.02*	1.39 ± 0.1	0.93 ± 0.02
2 W reg	0.81 ± 0.00	0.71 ± 0.01	0.18 ± 0.00*	0.16 ± 0.01*	0.99 ± 0.00	0.88 ± 0.01
3 W reg	0.88 ± 0.09	0.63 ± 0.05*	0.18 ± 0.01*	0.12 ± 0.00**	1.04 ± 0.09	0.75 ± 0.06*

Values are means (M) of three replicates ± standard deviation. For a given data, statistically significant of differences compared to the value of control plants was conducted. * Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$ and *** Significant at $P \leq 0.001$.

Soluble sugar content:

Irrespective of drought treatment, soluble sugar content in leaves is higher than that of roots. Under drought, it increased with respect to drought level and number of days of treatments. In leaf soluble sugar content was not increased significantly at 5 DAT but at 10 DAT it increased significantly in 2 weeks regime and 3 weeks regime drought treatments. In roots, similar pattern of increasing sugar content was noticed in both the samplings with significant increase at 10 DAT (Fig. I a). It may increase for stabilizing cellular membranes and maintaining cell turgor pressure as it functions as

osmoprotectant under water resistant conditions. Increased soluble sugar contents were also observed in two South Australian bread wheat cultivars[21] and in *Populus euphratica* in vitro[22].

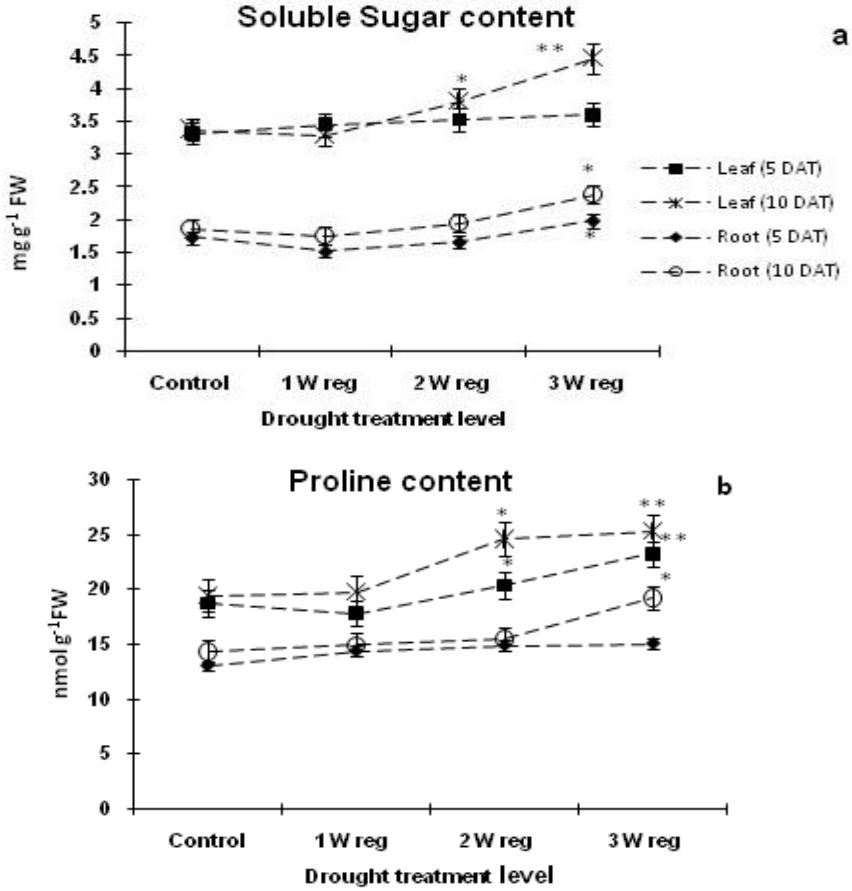
Proline content:

Under drought stress, proline works as one of the compatible osmolytes in plants. In this study, proline content of leaves is comparatively higher than roots of *C. roseus* plant and increased concomitantly with drought levels and number of days of drought treatment. Proline content increased minimally up to the level of 2 weeks regime drought treatment in leaves and roots at 5 and 10 DAT. In 3 weeks regime drought treatment level, proline content increased up to 24.2% and 29.8% in leaves and 15.42% and 32.6% in roots at 5 DAT and 10 DAT respectively (Fig. I b). Increased proline content was also reported in alfalfa[23] and *Zingiber officinale*[24]. The possible cause for increased proline content is the formation of large number of carbon and nitrogen resources in synthesis of osmoregulants in leaves for maintaining cell turgor pressure.

Free amino acid content:

Free amino acids are served as precursors for polyamines which increased significantly in leaves and minimally in roots under drought stress with respect to increasing treatment levels. In 3 weeks regime drought treatment content increased by 64.4% in leaves and 14.38% in roots at 5 DAT and by 92.1% in leaves and 35.88% in roots at 10 DAT (Fig. I c). Under drought stress, biosynthesis of free amino acids increased because they involved in oxidative defense response to high range of water deficit condition. Similar results were also observed by Elfeky and others (2007)[25] and Osman and others (2007)[26] in *C. roseus* shoots under drought stress.

Graph 1: Effect of drought stress on: a. Soluble sugar content, b. Proline content and c. Free amino acid content



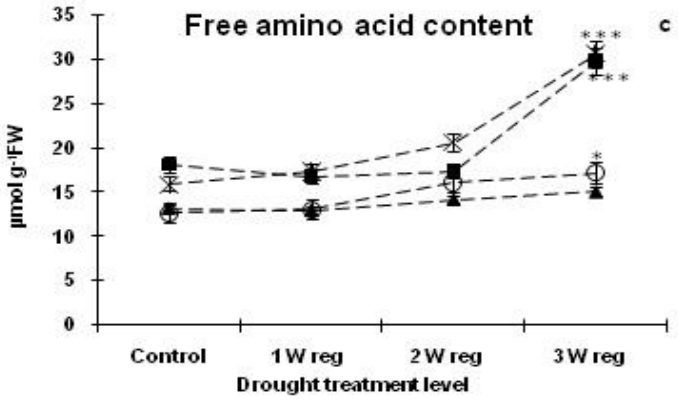


Fig. I Drought induced effect on **a.** Soluble sugar content, **b.** Proline content and **c.** Free amino acid content in ⊸ Leaf at 5 DAT, ✳ Leaf at 10 DAT, ⊸ Roots at 5 DAT and ⊖ Roots at 10 DAT. Values are means (M) of three replicates ±standard deviation (SD). For a given data, statistically significant of differences compared to the value of control plants was conducted. * Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$ and *** Significant at $P \leq 0.001$.

(ii) Important TIAs and precursors analysis:

Water deficit conditions are also responsible for developing changes in biosynthesis of secondary metabolites as they play an important role in defense mechanism of plants. In this study, roots and leaves of C. *roseus* were investigated for products of three late branches (serpentine, vindoline and catharanthine) of TIA pathway i.e monomeric ajmalicine and serpentine and dimeric vincristine and vinblastine. The results of this study not only revealed interesting changes in contents of metabolites present in roots and leaves but also proved evidence for genetically regulated compartmentation of TIAs among *C. roseus* under field grown conditions.

Indole pathway contents:

In indole pathway, tryptophan is decarboxylysed by tryptophan decarboxylase (TDC) enzyme to form tryptamine. Tryptophan content is increased significantly at 5 DAT in leaves and roots at high drought levels. It increased by 24.13% in leaves and 23.52% in roots in 2 weeks regime and 48.27% in leaves and 41.17% in roots in 3 weeks regime drought levels respectively. At 10 DAT, tryptophan content is increased in dose dependent manner minimally in leaves and significantly in roots (Fig. II a). Tryptamine is an important precursor for biosynthesis of strictosidine, it is present in higher amount than tryptophan in leaves and roots irrespective of treatment. Tryptamine content in leaves was not changed significantly under all drought Treatment levels at 5 DAT and 10 DAT. In contrast, content increased significantly with increasing drought levels and number of days of treatment in roots. It is increased by 45.23% and 59.52% in 2 weeks and 3 weeks regime drought treatment levels respectively at 5 DAT and at 10 DAT it increased by 53.12% in 2 weeks regime and 81.25% in 3 weeks regime drought treatment (Fig. II b). This study demonstrated increased tryptophan content significantly in early days of stress. While, amount of tryptamine in roots increased significantly in both samplings. These changes occurred mostly due to the pleiotropic response and declined rate of protein synthesis under drought stress.[27] In evidence of these results, increased tryptophan content in significant amount was observed in wheat[28] and hybrid poplar[29] under various drought stress conditions. On the other hand, tryptamine content under drought stress in plants are not much studied so far but it is found to be increased significantly in barley leaves when irradiated by UV-light and inoculated by powdery mildew fungus.[27]

Secologanin is a final product of iridoidal pathway which played a significant role in biosynthesis of many important TIAs. It is present in high amount in leaves and in minimal amount in roots and increased in leaves and roots with respect to treatment level significantly at 5 DAT. In 3 weeks regime drought treatment level secologanin content increased by 67.5% and 45.1% in

leaves and 38.7% and 27.5% in roots at 5 DAT and 10 DAT respectively (Fig. II C). Under drought stress content of secologanin significantly changes in early days. In relation to this finding, increased secologanin content along with many other TIAs were observed in phytoplasma infected *C. roseus* leaves[30] and under increased glucose level in cell suspension culture[31].

TIAs content after coupling of indole and iridoidal pathway products:

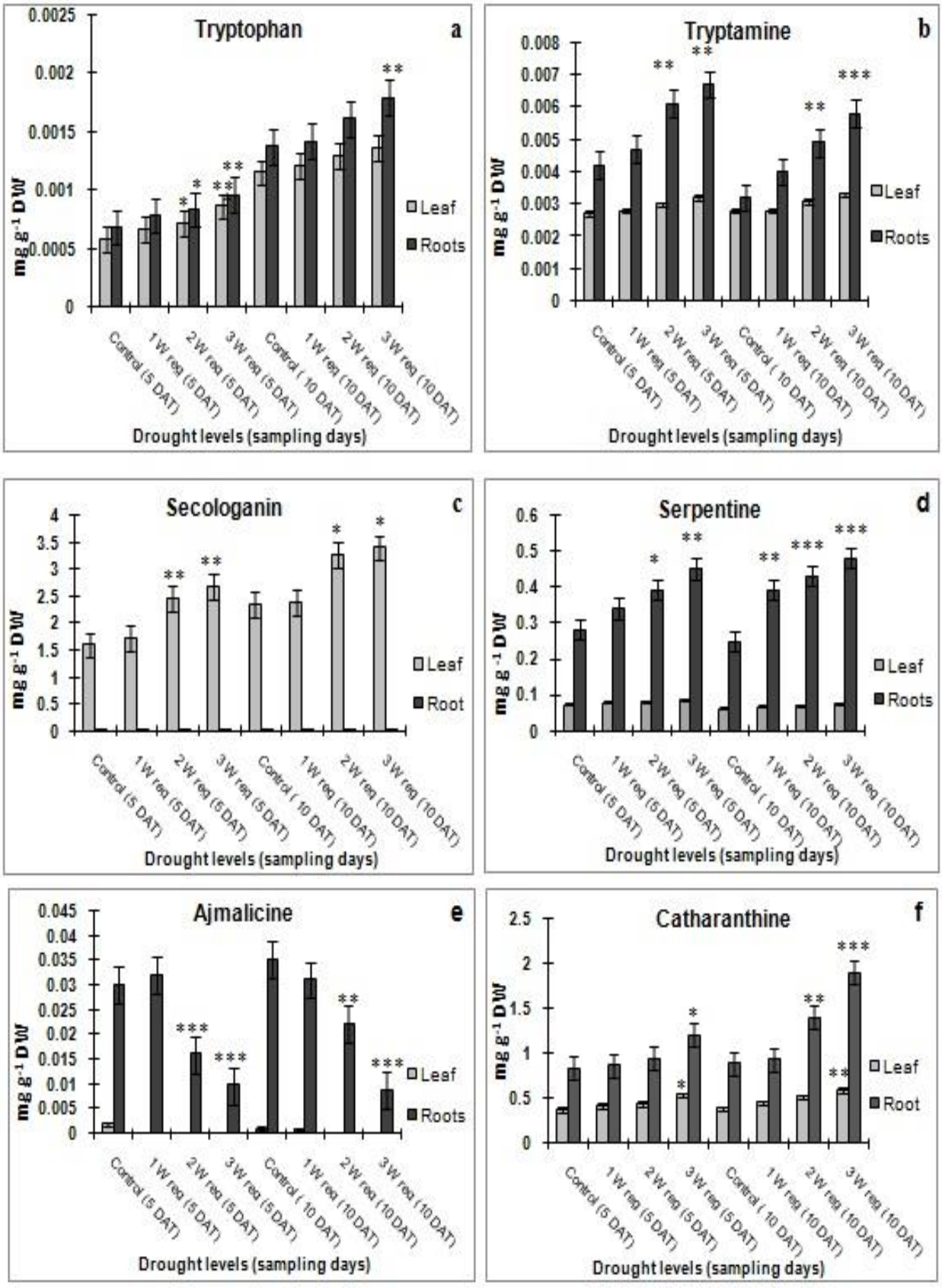
Coupling of tryptamine and secologanin form an intermediate and central precursor strictosidine, which further diverted towards the formation of many important TIAs and intermediates like serpentine, ajmalicine, catharanthine and tabersonine. Ajmalicine and serpentine have important medicinal properties for cardio vascular disorder and both are produced in roots in higher quantity. In the present study, ajmalicine content was absent in both controlled conditions as well as under drought treatments. In roots, it decreased concomitantly with increasing drought level. After 3 weeks regime drought treatment it decreased by 67% at 5 DAT and 75% at 10 DAT. In contrast to this result increased ajmalicine content was reported by Jaleel and others (2008)[18] under drought treatment in *C. roseus* roots. In this study, decreased ajmalicine content could be due to its rapid oxidation by peroxidase enzyme under drought stress, which results in formation of serpentine in large amount. On the other hand, serpentine content increased significantly in roots which increased by 60.7% at 5 DAT and 92% at 10 DAT in 3 weeks regime drought treatments. In contrast, leaves contain less amount of serpentine which also increased with increasing drought treatment levels and number of days of treatment. It increased by 17.56% at 5 DAT and 39.4% at 10 DAT in samples treated with 3 weeks regime drought treatment (Fig. II d). Increased serpentine content were also observed by Binder and others (2009)[32] in cell culture suspension under UV light treatment.

Precursor catharanthine and tabersonine are present in roots in high amount as compared to leaves, where catharanthine induced significantly during late stages of treatment and tabersonine content changed minimally only in early days in drought stress. Irrespective of treatment, contents of catharanthine are present in high amount in roots as compared to leaves and increased up to 44.57% at 5 DAT which reached approximately more than double at 10 DAT in 3 weeks regime drought level (Fig. II e). In leaves, amount of catharanthine is increased by 46% at 5 DAT and 51.2% at 10 DAT in 3 weeks regime drought treatment as compared to their respective controls (Fig. II f). Irrespective of treatment, tabersonine amount found three times more in roots than leaves and did not influenced significantly by drought treatment and number of days of sampling (Fig. II g). In support to given results it was documented that tabersonine and ajmalicine accumulation occurred earlier (within 4 h) and catharanthine later after MeJA elicitation.[33]

TIAs content of Vindoline pathway:

Tabersonine is converted into vindoline by six different enzymatic stages and is only detected in leaves. Formation of vindoline is significantly influenced by light exposure because initially conversion of tabersonine requires an involvement of cytochrome P450- dependent enzyme, tabersonine 16-hydroxylase (T16H).[34] Vindoline, vincristine and vinblastine are important anticancerous metabolites which are only observed in leaves and not detected in roots of *C. roseus* plant. Vindoline is induced under drought stress significantly with increasing number of days of treatment and drought levels. At 5 DAT, its amount increased by 35% and at 10 DAT it increased significantly up to 83.2% in 3 weeks regime respectively (Fig. II h). Increased vindoline content is also observed in response to artemisinic acid elicitation, UV-B treatment in *C. roseus* culture suspension[35] and in *C. roseus* seedlings after salinity stress[26]. Coupling of vindoline and catharanthine form α-3', 4'-anhydrovinblastine, reaction is catalyzed by basic peroxidase-like enzyme anhydrovinblastine synthase (AVLBS). In present study, vinblastine content minimally decreased under drought stress in leaves concomitantly to the drought levels. The content decreased by 26.41% at 5 DAT and 16.6% at 10 DAT in 3 weeks regime drought treated sample (Fig. II i). The amount of anticancerous agent vincristine is increased under drought stress concomitantly with drought level. At 5 DAT it increased significantly by 80% at 5 DAT and approximately up to 2 folds at 10 DAT in 3 weeks regime drought treatment level as compared to control (Fig. II j). Coupling of vindoline and catharanthine form α-3', 4'- anhydrovinblastine, reaction is catalyzed by basic peroxidase-like enzyme anhydrovinblastine synthase (AVLBS). It is finally converted to dimeric products vinblastine and vincristine in leaves.[36]

Graph 2: Changes in contents of various alkaloids in roots and leaves under drought stress.



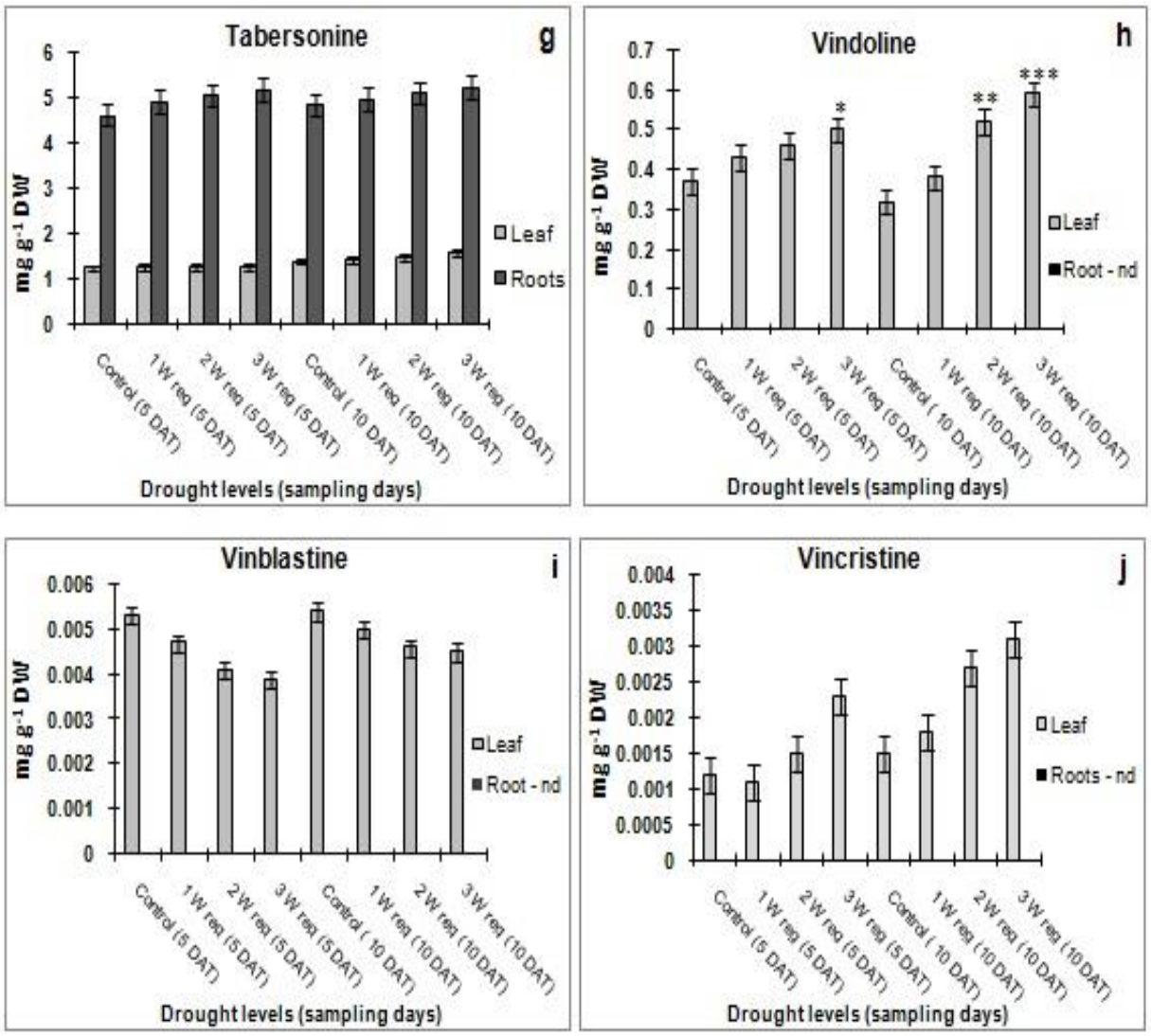


Figure II

Drought induced quantitative changes in the content of **a.** Tryptophan, **b.** Tryptamine, **c.** Secologanin, **d.** Serpentine, **e.** Ajmalicine, **f.** Catharanthine, **g.** Tabersonine, **h.** Vindoline, **i.** Vinblastine and **j.** Vincristine at 5 DAT and 10 DAT (day after treatment) in leaf and in roots. For a given data, statistically significant of differences compared to the value of control plants was conducted. * Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$ and *** Significant at $P \leq 0.001$. In the given data leaf is represented by ▭ and roots are represented by ■.

Conclusion:

Under drought stress, simultaneous quantification of TIA-precursors, intermediates and end products in leaf and roots demonstrated interesting changes in their contents and basic distribution under field grown condition in *C. roseus* plant. Study revealed that the pathway work as a whole set of interconnected network where all metabolites and precursors are differentially adjusted in relation to drought stress. Under drought condition, precursor tryptamine and tabersonine are not affected; on the other hand, ajmalicine and vinblastine contents decreased significantly in root. Tryptophan, serpentine and catharanthine contents increased significantly in roots while vindoline and vincristine increased significantly with respect to drought level and number of days of treatment in leaf sample. This could be occurred due to changes in expression levels of genes and associated enzymes which are responsible for increasing or

decreasing contents of these metabolites. Future research is needed to understand the genomic and proteomic approaches for determining contribution of regulatory networks and effect on genes expression of TIA biosynthetic pathway under drought stress.

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Conflict of Interest:

No conflict of interest to declare.

REFERENCES

1. Verpoorte R, van der Heijden R, Moreno PRH. Biosynthesis of terpene indole alkaloids in *Catharanthus roseus* cells. In G.A. Cordell (eds.), The Alkaloids. Academic Press, New York, pp. 1997; 221-299.
2. Van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The *Catharanthus* alkaloids: pharmacognosy and biotechnology. Curr Med Chem. 2004; 11(5): 607–628.
3. De Luca V, Balsevich J, Tyler RT, Eilert U, Panchuk BD, Kurz WGW. Biosynthesis of indole alkaloids. Developmental regulation of the biosynthetic pathway from tabersonine to vindoline in *Catharanthus roseus* cultivar *Little delicate*. J Plant Physiol. 1986; 125: 147–156.
4. Shukla YN, Rani A, Kumar S. Effect of temperature and pH on the extraction of total alkaloids from *Catharanthus roseus* leaves. JMAPS. 1997; 19 (2): 430-431.
5. Boyer JS. Plant productivity and environment. Science. 1982; 218: 443-448.
6. Lichtenthaler HK. Vegetation stress: an introduction to the stress concept in plants. J plant physiol. 1996; 148: 4–14.
7. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot. 1979; 57(12): 1332-1334.
8. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*, Plant physiol. 1949; 24(1): 1-15.
9. Hedge JE, Hofreiter BT. Carbohydrate Chemistry. In R.L. Whistler, J.N. Be Miller (eds.). Academic Press, New York, pp. 1962: 17.
10. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973; 39: 205-207.
11. Lee YP, Takakashi T. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal Biochem. 1966; 14: 71-77.
12. Singh DV, Maithy A, Verma RK, Gupta MM, Kumar S. Simultaneous determination of *Catharanthus* alkaloids using reversed phase high performance liquid chromatography. J Liq Chrom Rel Technol. 2000; 23(4): 601–607.
13. Bhadra R, Vani S, Shanks JV. Production of indole alkaloids by selected hairy root lines of *Catharanthus roseus.* Biotechnol Bioeng. 1993; 41(5): 581-592.
14. Tikhomiroff C, Jolicoeur M. Screening of *Catharanthus roseus* secondary metabolites by high-performance liquid chromatography. J Chromatogr. 2002; 955(1): 87-93.
15. Chaves MM, Flexas J, Pinheiro C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot. 2009; 103: 551–560.
16. Save R, Peñuelas J, Marfá O, Serrano L. Changes in leaf osmotic and elastic properties and canopy structure of strawberries under mild water stress. Hort Sci. 1993; 28(9): 925-927.
17. Lawson T, Oxborough K, Morison JIL, Baker NR. The responses of guard and mesophyll cell photosynthesis to CO2, O2, light and water stress in a range of species are similar. J Exp Bot. 2003; 54(388): 1743–1752.
18. Jaleel CA, Sankar B, Sridharan R, Panneerselvam R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. Turk J Biol. 2008 32(2): 79-83.
19. Massacci A, Nabiev SM, Pietrosanti L, Nematov SK, Chernikova TN, Thor K, Leipner J. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant physiol biochem. 2008; 46(2): 189– 195.
20. Kiani SP, Maury P, Sarrafi A, Grieu P. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. Plant Sci. 2008; 175(4): 565–573.
21. Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. J Exp Bot. 2008; 59(12): 3327-3346.
22. Watanabe S, Kojima K, Ide Y, Sasaki S. Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. Plant Cell Tiss Org. 2000; 63(3): 199–206.
23. Aranjuelo I, Molero G, Erice G, Avice JC, Nogués S. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). J Exp Bot. 2011; 62(1): 111–123.

24. Bhosale KS, Shinde BP. Influence of arbuscular mycorrhizal fungi on proline and chlorophyll content in *Zingiber Officinale* Rosc. grown under water stress. Ind J Fund appl life Sci. 2011; 1: 2231–6345.
25. Elfeky SS, Osman MEH, Hamada SM, Hasan AM. Effect of salinity and drought on growth criteria and biochemical analysis of *Catharanthus roseus* shoot. Int J Bot. 2007; 3(2): 202-207.
26. Osman MEH, Elfeky SS, Kamelia AS, Hasan AM. Response of *Catharanthus roseus* Shoots to Salinity and Drought in Relation to Vincristine Alkaloid Content. Asian J Plant Sci. 2007; 6(8): 1223-1228.
27. Miyagawa H, Hiroshi T, Tsurushima T, Ueno T, Shishiyama L. Accumulation of Tryptamine in Barley Leaves Irradiated with UV Light. Biosci Biotechnol Biochem. 1994; 58(9): 1723-1724.
28. Browne JB, Erwin TA, Juttner J, Schnurbusch T, Langridge P, Bacic A, Roessner U. Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. Mol Plant. 2012; 5(2): 418–429.
29. Barchet GLH, Dauwe R, Guy RD, Schroeder WR, Soolanayanahally RY, Campbell MM, Mansfield SD. Investigating the drought-stress response of hybrid poplar genotypes by metabolite profiling. 2014; Tree physiol doi:10.1093/treephys/tpt080.
30. Choi YH, Tapias EC, Kim HK, Alfons WML, Erkelens C, Jacobus TJ, Berzin J, Zel J, Verpoorte R. Metabolic Discrimination of *Catharanthus roseus* Leaves Infected by Phytoplasma Using ^{1}H -NMR Spectroscopy and Multivariate Data Analysis. Plant Physiol. 2004; 135(4): 2398-2410.
31. Contin A, Van der Heijden R, Lefeber AWM, Verpoorte R. The iridoid glucoside secologanin is derived from the novel triose phosphate/pyruvate pathway in a *Catharanthus roseus* cell culture. FEBS Lett. 1998; 434(4): 413–416.
32. Binder BYK, Christie AM, Jacqueline P, Shanks V, San KY. The effects of UV-B stress on the production of terpenoid indole alkaloids in *Catharanthus roseus* hairy roots. Biotechnol Prog. 2009; 25(3): 861-865.
33. Rischer H, Oresic M, Seppänen-Laakso T, Katajamaa M, Lammertyn F, Ardiles-Diaz W, van Montagu MCE, Inzé D, Oksman- Caldentey KM, Goossens A. Gene-to-metabolite networks for the terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* cells. Proc Natl Acad Sci U S A. 2006; 103(14): 5614–5619.
34. St-Pierre B, De Luca V. A cytochrome P-450 monooxygenase catalyzes the first step in the conversion of tabersonine to vindoline in *Catharanthus roseus.* Plant Physiol. 1995; 109(1): 131-139.
35. He L, Yang LY, Tan R, Zhao S, Hu Z. Enhancement of vindoline production in suspension culture of the *Catharanthus roseus* cell Line C20 by light and methyl, jasmonate elicitation. Anal Sci. 2011; 27(12): 1243-1248.
36. Tang ZH, Guo XR, Yu JH, Yang L, Sun YF, Zu YG. Effects of low light intensity on changes of soluble sugars, alkaloids and phytohormones in *Catharanthus roseus* seedlings. Acta Ecol Sinica. 2007; 27: 4419-4424.

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