

Assessing Vinblastine and Proline Contents in *Catharanthus roseus* Cultivated on Inceptisol under Water Stress

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ABSTRACT. *Catharanthus roseus*, formerly known as ornamental plant, has now been explored for its potentials as medicinal herb. One of the secondary metabolites produced, namely vinblastine, has been widely used to treat some human cancers. In addition, as the relatively high tolerance to drought, the plant species could produce proline, which plays important role in maintaining cellular osmotic pressure in an adverse condition. This study aims to assess the profiles of vinblastine and proline in *C. roseus* cultivated on inceptisol under water stress in combination with the applications of Zwavelzure Ammonium (ZA) fertilizer and rice husk biochar (RHB). A Split Plot Design was employed involving three treatments, namely water stress as the main plot and the combination of ZA fertilizer and RHB as the subplot. Three levels of the respective treatment were applied, namely 0, 2 and 4 days without watering; 0, 1.19 and 2.38 g/polybag of ZA fertilizer; and 0, 100 and 200 g/polybag of RHB. The results showed that the highest vinblastine content of 2,516.46 µg/g was obtained in the combination of 4 days without watering, 1.19 g/polybag ZA fertilizer and 200 g/polybag RHB. Meanwhile, the highest proline content of 31.62 µmol/g was found in the applications of 4 days without watering, 2.38 g/polybag ZA fertilizer and 0 g/polybag RHB. The supporting agronomic parameters revealed the best responses in the applications of daily watering, 2.38 g/polybag ZA fertilizer and 200 g/polybag RHB. It can be concluded that the production of vinblastine increases with the higher levels of water stress, ZA fertilizer and RHB, while that of proline increases with only the higher water stress and RHB. ZA fertilizer and RHB applications are shown to increase soil fertility, while daily watering optimizes the growth of *C. roseus*.

Key words: *Catharanthus roseus*, inceptisol, proline, vinblastine, water stress

INTRODUCTION

Madagascar periwinkle [*Catharanthus roseus* (L.) G. Don], previously known as ornamental plant species, has nowadays been widely explored for its potentials as medicinal herbs. More than 130 secondary metabolites produced have been used to treat several non-infectious diseases, such as diabetes, hypertension, asthma, constipation, menstruation problem, and even various human cancers (Mendonce et al., 2025). One of dimeric alkaloids extracted from *C. roseus*, namely vinblastine, is known for its anticancer properties causing high demand in the pharmaceutical industries (Vu et al., 2022). The substance, which is mainly used to treat lymph node cancer and leukemia, can be found abundantly in the stems and leaves (Dhyani et al., 2022). Vinblastine was reported very active in an in vitro condition and showed antitumor effects similar to vincristine, another secondary metabolite, in overcoming medulloblastoma. Hence, it can be considered to substitute vincristine for medulloblastoma treatment in the future, since vincristine has side effect in terms of peripheral nerve damage, particularly in patients with congenital neuropathy (Nobre et al., 2019).

The capacity of *C. roseus* in producing vinblastine was reported to be increased by the applications of precursors (L-phenylalanine and L-tyrosine) and elicitors (chitosan and methyl jasmonate) in hair root clones produced by *Agrobacterium rhizogenes*. Precursors were found more efficient than elicitors in increasing vinblastine synthesis in hair roots of *C. roseus* (Vu et al., 2022). Meanwhile, the applications of methyl jasmonate and ethylene on two cultivars of *C. roseus* showed different effects in inducing synthesis and accumulation of vinblastine, in either roots or shoots. This indicates the necessity of cultivar selection for alkaloids, especially vinblastine (Fraser et al., 2020; Mall et al., 2021). Vinblastine contents in *C. roseus* seedlings increased by 60% after the application of potassium (K⁺) in form of KNO₃, while that in form of K₂SO₄ can increase vinblastine of only 30% (Sahi et al., 2022). The increment of vinblastine contents in *C. roseus* roots can be stimulated by using probiotic microbes *Pseudomonas fluorescens* and *Aspergillus brasiliensis* (Ahmadzadeh et al., 2022).

As a plant species originated from Madagascar, which is a tropical region, *C. roseus* grows adaptively in Indonesia. This plant species can grow well in a relatively wide range of altitudes, namely from 100 to

800 m above sea level and is sufficiently tolerant to drought conditions. *C. roseus* exhibits both morphological and physiological adaptations as the mechanism of survival in stress environments, one of which is by producing proline. The formation of this amino acid is a typical adjustment in *C. roseus* growing under water deficit which causes osmotic stress. Many enzymatic activities and biological macromolecule synthesis will be disturbed in the absence of proline formation (Zomorodi et al., 2022). Proline is involved in the cell osmotic regulation by lowering water potential (Hassanvand et al., 2019). As well, it plays important role in plant growth and development, such as triggering seed germination and supporting stomata to minimize water lost (Seleiman et al., 2021).

Dry lands, commonly characterized by shortages in nitrogen, phosphor and potassium, are found in many parts of Indonesia. Inceptisol is an example of soil type newly developing in dry lands thus having low physical, chemical and biological fertilities. This soil type is characterized by the presence of cambic horizon, which is formed through the processes of physical alteration and chemical transformation during pedogenesis. The horizon has a thickness of more than 15 cm with a dominant soil texture of sand to sandy clay. However, small amount of clay still exists, since the soil has not been experiencing further development (Soil Survey Staff, 2022). To optimize inceptisol in dry lands, some improvements on soil quality should be performed, some of which are by the applications of inorganic fertilizers such as Zwavelzure Ammonium (ZA) and soil ameliorants like rice husk biochar (RHB).

With respect to the production of vinblastine and proline in *C. roseus*, only studies involving two variables, such as water stress and ZA fertilizer or water stress and RHB, have been reported so far, while none regarding the simultaneous effects of watering intensity, ZA fertilizer and RHB was available. Based on the background, the objective of our study is to assess the profiles of vinblastine and proline in *C. roseus* cultivated on inceptisol under water stress in combination with the applications of ZA fertilizer and RHB. This will provide scientific support on the cultivation of *C. roseus* in effort of obtaining more efficient production of vinblastine in dry lands.

EXPERIMENTAL SECTION

Research Methodology

The study was carried out experimentally in the greenhouse of the Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Central Java, from March to October 2023. A Split Plot Design was employed in which the main plot was watering intensity and the subplot consisted of two factors, namely ZA fertilizer and RHB. Three levels of watering intensity were used, namely 0 (I0), 2 (I1) and 4 (I2) days without watering respectively. The watering

intensity was performed to fill the field capacity. As well, three levels of ZA fertilizer dosages, namely 0 (N0), 1.19 (N1) and 2.38 g/polybag (N2) and three levels of RHB dosages, namely 0 (B0), 100 (B1), and 200 g/polybag (B2) were applied. All combinations of treatments (ZA fertilizer and RHB) were randomized within the respective main plot (watering intensity) and each was subjected to three replications giving rise to a total of 81 experimental units. Only one plant individual was used in the respective experimental unit (polybag).

Preparation of Growing Media

Inceptisol collected from Karangwangkal, Banyumas, Central Java, was used as growing media for *C. roseus*. The soil pH is relatively low due to water leaching of base cations. In addition, the soil is suffering from drought condition during dry season because of the low water adsorption related to the high contents of sand and dust. The soil was then composited with 2 mm RHB corresponding to the specified dosage. After mixing, the soil was left for approximately 2 weeks enabling RHB to react with the soil. After incubation, the soil was applied with ZA fertilizer according to the respective level of dosage. Along with ZA application, TSP and KCl of 1.66 g/polybag and 2.04 g/polybag respectively were provided as basic fertilizers two days before planting. Then, the media were ready for planting *C. roseus*.

Parameters of Study

The main parameter of study included secondary metabolites contents of *C. roseus*, namely vinblastine and proline, while the supporting parameters were soil and agronomical parameters. The vinblastine contents were measured following Fukuyama et al. (2017), while prolines were determined after Zomorodi et al. (2022). The measurement of vinblastine contents was preceded by grinding sample of 100 g dry leaf using mortar. Then, methanol was added to the sample to obtain extract suspension, which was re-dissolved in Na₂HPO₄ and acetonitrile to produce clear extract. This was subsequently analyzed in High Performance Liquid Chromatography (HPLC) at wavelength of 205 nm. The vinblastine content measured must be included in the series of standard solution. Meanwhile, the measurement of proline contents was preceded by grinding fresh leaf using mortar. The refined sample was then dissolved in 3% sulfosalicylate and toluene. Subsequently, the sample extract was filtered to produce clear extract, which was read for proline content using uv-vis spectrophotometer at wavelength of 520 nm. The proline content measured must be in the range of standard solution series. Soil parameters consisted of total nitrogen, organic carbon and soil pH. Total nitrogen contents were measured using the Kjeldahl method (Ezeabara et al., 2015), while organic carbon contents were determined using the Walkley and Black method (Jha et al., 2014). Soil pH was determined using the electrometric method (Popoola

et al., 2023). Agronomic parameters comprised plant height, total leaf area, root dry weight and shoot dry weight. Plant height was observed weekly until the seventh week after planting. Total leaf area was measured at harvesting using gravimetric method (Chaudhary et al., 2012). Root and shoot dry weights were determined by weighing biomass after being oven-baked for approximately 3 days at 70 – 80°C.

Data Analysis

Data on both main and supporting parameters were analyzed using ANOVA. When significant difference among treatments was observed, further analysis was carried out employing the Duncan Multiple Range Test (DMRT) with a confidence level of 5%. The data analysis was implemented employing SPSS software.

RESULTS AND DISCUSSION

During study, the greenhouse had an average temperature of around $22.8 \pm 3.80^\circ\text{C}$. Meanwhile, the average air humidity was $75.4 \pm 11.26\%$. Thus, in general the environmental conditions can be categorized as quite cool and slightly humid supporting optimum growth of *C. roseus*.

Vinblastine and Proline Contents

Vinblastine and proline contents of *C. roseus* under the respective combination of treatments applied are depicted in **Figure 1**. It is shown that vinblastine contents are highly affected by watering intensity in that higher water stress results in the higher vinblastine contents regardless the dosages of ZA fertilizer and RHB. The treatments of 4 days without watering (I2) result in the highest vinblastine contents. It is also revealed that I2N1B2 has the highest vinblastine content, namely $2,516.46 \mu\text{g/g}$, in comparison to any other combinations of treatments (**Figure 1A**). However, this is not significantly different from that of I2N2B2 resulting in vinblastine of $2,285.25 \mu\text{g/g}$. The lowest vinblastine content, namely $642.1 \mu\text{g/g}$, was observed in control (I0N0B0).

Vinblastine content, produced by *C. roseus*, is influenced by total soil nitrogen content, since the structure consists of complex amine groups (Kadhim et al., 2020). The nitrogen content will increase the production of primary metabolites through photosynthesis in plants. On the other hands, synthesis of secondary metabolites occurs when plants are suffering from stress. Vinblastine is made from the precursor vindoline in the biosynthesis pathway of tryptophan, where this amino acid is synthesized in the presence of drought stress (Siddiqui et al., 2023). This corresponds to Hashemabadi et al., (2018) that extreme drought stress can stimulate the production of secondary metabolites as the conversion of primary metabolite synthesis in form of protein.

RHB application serves as maintaining nitrogen availability in soil to avoid lost due to volatilization and leaching. Nitrogen in form of ammonium and nitrate applied on soil will be adsorbed by RHB, so that nitrogen content in soil remains sufficient for simulating plant to perform metabolism properly. High soil nitrogen content will affect plant nutrient uptake and secondary metabolite production (Deng et al., 2022). The treatment I2 (4 days without watering) has highly significant effects both on vinblastine and proline contents. In the exposure to drought condition, plant cells of *C. roseus* will be subjected to inflammation. This will be quickly relieved by means of vinblastine production when primary metabolites are produced in a large amount (Hassan et al., 2021).

Similar to vinblastine, proline content is also highly influenced by water intensity. It is shown in **Figure 1B** that the treatment I2N2B0 results in the highest proline content, namely $31.62 \mu\text{mol/g}$. Meanwhile, the lowest proline content is found in control (I0N0B0) showing proline content of only $15.31 \mu\text{mol/g}$. Water intensity has highly significant effect on the proline content of *C. roseus*. Watering of once in 4 days (I2) exhibits the highest value for proline content in comparison to watering of once in 2 days (I1) and daily watering (I0).

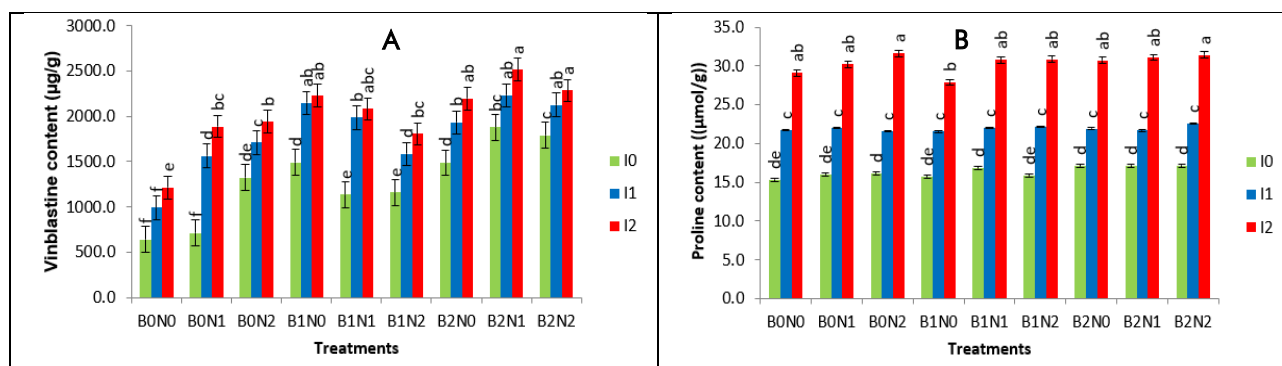


Figure 1. Secondary metabolite contents of *Catharanthus roseus* (A = vinblastine; B = proline) N0 = No nitrogen, N1 = 1.19 g of nitrogen/polybag, N2 = 2.38 g of nitrogen/polybag; B0 = no RHB, B1 = 100 g/polybag RHB, B2 = 200 g/polybag RHB; I0 = everyday watering, I1 = once in two-day watering, I2 = once in four-day watering

Proline is used as an indicator of stress, because this amino acid is produced in a large quantity in the presence of stress on plants. Less water in plant cultivation will reduce agronomical values, but at the same time, it will increase the production of secondary metabolites and amino acids such as proline (Alhverdzadeh & Danaee, 2023). Proline is synthesized as physiological response of plant for survival under drought stress. Providing field capacity of 50% greatly increases proline production in plants. Nevertheless, water deficit will reduce plant quality, stem strength, light interception and carbon assimilation (Patel et al., 2022). Impairs in the hydration status of plants undergoing water stress is the occurrence of oxidative damage characterized by reduced chlorophyll content, increased membrane degradation and lipid peroxidation (Zomorodi et al., 2022).

C. roseus belongs to plant species of drought tolerant thus having sufficiently high proline content. The higher proline content in a plant species, the more

tolerant the species to drought stress (Dullah et al., 2019). Proline functions as nitrogen storage in plants, osmoregulator and protector for particular enzymes. Plant cells of high proline contents are presumably more tolerant to drought conditions (Ghosh et al., 2022). The application of ZA shows significant effect on proline production, because ZA can increase accumulated protein resulting from photosynthesis. Proteins in unstressed plants are synthesized in forms of many other essential amino acids required for plant growth. When drought stress occurs, then the remaining nitrogen reserves will be just synthesized into proline (Meena et al., 2019).

Soil Parameters

The ANOVA of treatments shows significant effects on soil parameters. The applications of ZA fertilizer and RHB have significant effects on total soil N content, organic carbon content and soil pH. Further analysis of DMRT at confidence level of 0.05 results in data presented in **Table 1**.

Table 1. Soil parameters under treatments of water intensity, ZA fertilizer and RHB applications

No.	Treatment	Total soil N (%)	Soil organic carbon (%)	Soil pH
1	I0N0B0	0.236 ef	2.91 f	5.42 cd
2	I0N0B1	0.230 ef	3.35 d	5.54 c
3	I0N0B2	0.237 ef	4.42 a	5.82 a
4	I0N1B0	0.524 cd	2.98 f	5.39 e
5	I0N1B1	0.583 c	3.32 d	5.55 c
6	I0N1B2	0.608 bc	4.32 b	5.65 b
7	I0N2B0	0.674 b	2.95 f	5.25 f
8	I0N2B1	0.792 ab	3.29 d	5.45d
9	I0N2B2	0.885 a	4.22 c	5.6 bc
10	I1N0B0	0.210 f	2.95 f	5.41 cd
11	I1N0B1	0.213 f	3.2 e	5.51 c
12	I1N0B2	0.296 e	4.39 a	5.77 a
13	I1N1B0	0.535 cd	2.93 f	5.33 e
14	I1N1B1	0.562 c	3.3 d	5.54 c
15	I1N1B2	0.674 b	4.31 b	5.61 bc
16	I1N2B0	0.707 ab	2.9 f	5.23 f
17	I1N2B1	0.705 ab	3.21 e	5.44 cd
18	I1N2B2	0.795 ab	4.24 bc	5.58 bc
19	I2N0B0	0.283 e	2.92 f	5.4 cd
20	I2N0B1	0.290 e	3.18 e	5.49 c
21	I2N0B2	0.341 d	4.4 a	5.71 ab
22	I2N1B0	0.529 cd	2.91 f	5.3 d
23	I2N1B1	0.571 c	3.19 e	5.51 c
24	I2N1B2	0.672 b	4.29 bc	5.58 ab
25	I2N2B0	0.690 b	2.88 f	5.2 f
26	I2N2B1	0.709 ab	3.21 e	5.41 cd
27	I2N2B2	0.809 ab	4.21 c	5.56 bc

Note: I = water intensity; N = ZA fertilizer; B = rice husk biochar; the values in bold are the best one in the respective parameter; numbers followed by the same letter in each column show no significant different at 0.05% level of confidence

It is shown that the application of daily watering in combination with ZA fertilizer of 2.38 g/polybag and RHB of 200 g/polybag (I0N2B2) reveals the highest total soil N content, namely 0.885%. This is significantly different from that obtained in control and some other treatments of daily watering with no nitrogen application (I0N0B0, I0N0B1 and I0N0B2), which results in total soil N content of 0.236%, 0.230% and 0.237% respectively. These are considered very low in comparison to those obtained from ZA applications of 2.38 g/polybag (N2).

The distribution of total soil N is determined by soil depth. The more the soil depth, the lower total soil N content because of the lesser the source of soil nitrogen. Nitrogen availability is influenced by mineralization process, decomposition of organic matters and the existence of nitrogen-fixing microorganisms. The total soil nitrogen content is related to several factors, such as the amount of organic matters, the number of soil microorganisms, land use intensity, vegetation, fertilizing interval and soil drainage (Widyasunu et al., 2023). In addition, RHB application will maintain nitrogen availability in soil, because the cation exchange capacity (CEC) and water retention increase avoiding easy lost of ammonium and nitrate ions from soil (Aslam et al., 2021). RHB application of 15 ton/ha can reduce nitrogen lost due to volatilization up to 40.7% and diminish N₂O emission up to 24% (Selvarajh et al., 2021). Watering and RHB applications can increase

soil humidity thus stimulating microorganisms to decompose soil. The soil decomposition will convert organic compounds into minerals, which are essential for plant growth (Hale et al., 2013).

RHB application of 200 g/polybag with no ZA fertilizer and 4 days without watering (I2N0B2) is found capable of increasing soil organic carbon of 4.4%. Meanwhile, combinations of treatments of the same ZA and RHB dosages with different intervals of watering reveals non-significant different results. ZA application of 2.38 g/polybag with no RHB and 4 days without watering (I2N2B0) shows the lowest soil organic carbon, namely 2.88%. Thus, it can be said that any treatment without RHB application shows lower soil organic carbon in comparison to those with RHB applications.

The addition of organic matters is the key for soil quality improvement, because it can increase soil decomposition due to the role of microorganisms therein. This corresponds to do Carmo et al., (2016) that organic matter application and fertilization can increase plant productivity due to the increasing physical, chemical and biological soil fertilities. This will in turn increase nutrient supply in soil. The application of organic matters can also increase aggregate stability, which serves as plant growth media. The contents of soil organic matters are influenced by several factors, such as parent materials, vegetation of legumes and cogongrass, and humid climate (Paiman & Effendy, 2020).

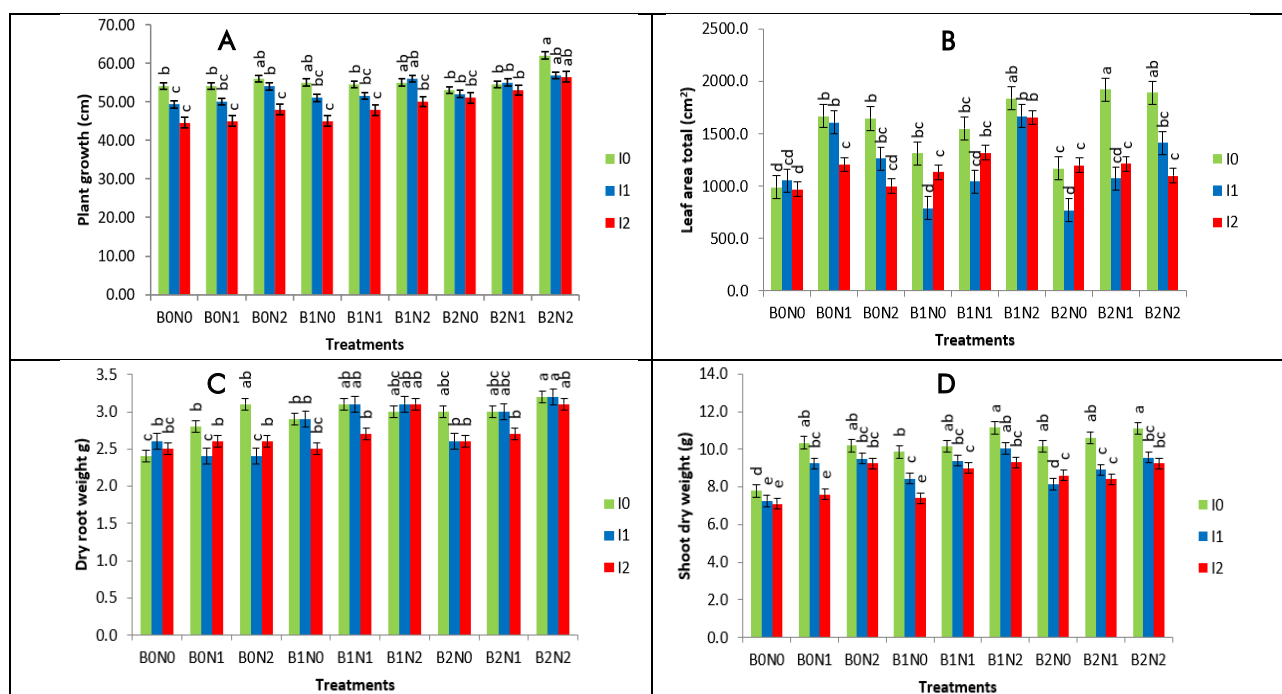


Figure 2. Agronomical parameters of *Catharanthus roseus* under treatments of water intensity, ZA fertilizer and RHB applications (A = plant height; B = total leaf area; C = root dry weight; D = shoot dry weight) N0 = No nitrogen, N1 = 1.19 g of nitrogen/polybag, N2 = 2.38 g of nitrogen/polybag; B0 = no RHB, B1 = 100 g/polybag RHB, B2 = 200 g/polybag RHB; I0 = everyday watering, I1 = once in two-day watering, I2 = once in four-day watering

With respect to soil pH, the combination of treatments I0N0B2 shows the best result as capable of increasing soil pH up to 5.82. Meanwhile, the combination of treatments with no ZA, RHB of 200 g/polybag and 2 days without watering (I1N0B2) shows non-significant different from I0B0N2. On the other hands, ZA application of 2.38 g/polybag with no RHB and 4 days without watering (I2N2B0) shows significant decrease in soil pH and is the lowest among other treatments, namely 5.2. RHB and any other types of biochars serve as ameliorants, which are amphoteric depending on the surrounding soil conditions.

Soil of low pH can be restored by the application of soil ameliorants in form of RHB. Biochars are ameliorants of high organic matter and functional groups contents. These are amphoteric and capable of chelating Al, Fe, Mn and Ca which bind to P in soil. Amine is functional group playing role in increasing soil pH, while carboxyl group serves to reduce soil pH in regulating soil pH (Ghorbani et al., 2019). In addition, the application of ZA in a large quantity will lead to decreasing soil pH. This is because ZA fertilizer contains nitrogen and sulphur, which in binding to water will release many H⁺ ions into the soil (Havlin et al., 2017). Too low soil pH can reduce plant productivity due to plant cell metabolism impairs (do Carmo et al., 2016). Watering can increase soil pH as a result of carbonate ions formation in soil (Widyasunu et al., 2023).

Agronomical Parameters

The ANOVA of watering intensity in combination with ZA and RHB applications reveals significant effects on some agronomical parameters, namely plant height, dry weight of roots and shoots. Further analysis of DMRT at confidence level of 0.05 is shown in **Figure 2**.

It is shown in **Figure 2** that watering intensity in combination with ZA and RHB applications has significant effects on agronomical parameters of *C. roseus*. The combination of treatment I0N2B2 results in the highest plant height, total leaf area, root dry weight and shoot dry weight, which are 62 cm, 1,923.3 cm², 3.4 g and 11.15 g respectively. When compared to control, the combination of treatment I0NB2 shows highly significant difference. On the other hands, the lowest response was observed in control with watering once in 4 days (I2N0B0). Plant height, total leaf area, root dry weight and shoot dry weight in this treatment are 44 cm, 970 cm², 2.2 g and 7.1 g respectively.

Nitrogen plays important role on the plant growth. This element accelerates meristematic cell division, so that it stimulates the growth of both plant roots and shoots (Wulandari et al., 2023). In addition, higher nitrogen content can increase metabolism activities, photosynthesis and regulates stomata opening (Sampaio et al., 2014).

Organic matter application will increase nutrient uptake, especially nitrogen, so that fertilization effectivity will be increased. RHB application considerably increases the efficiency of nitrogen fertilization as it significantly prevents the lost of ammonium and nitrate ions (Li et al., 2021). ZA fertilization can increase plant height, because in comparison to urea it has better persistence in soil due to its non-hygroscopic properties (Supriyono et al., 2021). Water intensity also influence significantly agronomical parameters. Optimum water availability will increase photosynthesis rate and maintain cell turgor, so that the metabolism processes occur faster. Moreover, water serves also as fertilizer solvent (Hashemabadi et al., 2018).

Broader leaves can capture more light and have more stomata, so that higher photosynthesis rate can be obtained. Photosynthesis activity is influenced by light intensity, stomata number and foliar chlorophyll contents (Huang et al., 2019). Larger size and number of leaves represent optimalization of nutrient uptake giving rise to increasing dry matters of plants (Supriyono et al., 2023). ZA application can stimulate protein synthesis and increase the rate of meristematic cell division at root and meristems (Aslani et al., 2021).

ZA fertilization can increase N availability in soil thus increasing N uptake by roots. The nitrogen absorbed by plants through a large number of roots will increase metabolism activities, formation of proteins composing plant cells and photosynthesis (Acharya et al., 2020). The photosynthates thus produced by plants will be distributed to all parts of plant body, particularly roots that meristematic root cells will remain to divide. The cell division will increase the growth of root hairs, root elongation and root enlargement, so that expanded range of nutrient uptake will be obtained. In addition, the increasing root biomass will result in better plant vigour (Supriyono et al., 2023).

ZA fertilizer will increase the dynamics of nitrogen and sulphur. Deficiency of sulphur can increase nitrogen fertilization efficiency, and vice versa. Watering is an effort to be performed in protecting soil moisture from drying out. Moist soil will increase soil microorganism colonies, so that soil decomposition can occur more rapidly. In addition, soil in dry condition will impair plant metabolisms and reduce soil physical, chemical and biological fertilities (Kolega et al., 2020).

In general, secondary metabolites of vinblastine and proline in *C. roseus* are influenced by both soil and agronomical parameters. The total soil N enhances soil fertility, so that *C. roseus* is capable of growing well. A higher content of nitrogen in plants can lead to the increasing level of tryptophan, so that vinblastine is produced more rapidly when *C. roseus* is grown in sub-optimum conditions (Rosa et al., 2023). Higher organic carbon is used as nitrogen

coating and stimulates soil microorganism activities in soil decomposition. The intensive soil decomposition will provide nitrogen by means of mineralization (Widyasunu et al., 2023). In addition, neutral soil pH of 6 – 7 can provide several essential nutrients for plant growth (Abbas et al., 2017). Better soil fertility will enhance photosynthesis rate, which can in turn lead to better agronomical parameters. On the other hands, agronomical parameters such as plant height, leaf area, root and shoot dry weights are subjected to decrease in the presence of vinblastine and proline synthesis. Photosynthesis rate will be reduced when plants are under adverse environmental conditions. Water stress lowers enzymatic performance in the process of primary metabolite synthesis (Supriyono et al., 2021). Hence, the three variables, namely soil fertility, agronomical attributes and water stress altogether influence the production of vinblastine and proline.

In summary, it can be concluded that the production of vinblastine increases with the higher levels of water stress, ZA fertilizer and RHB, while that of proline increases with only the higher water stress and RHB. ZA fertilizer and RHB applications are shown to increase soil fertility, while daily watering optimizes the growth of *C. roseus*. This provides valuable information for further studies in efforts of increasing the vinblastine and proline contents in *C. roesus*.

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