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1. **Manuscript Title**

**5-Aminolevulinic Acid Lessened Growth Suppression in Common Bean (*Phaseolus vulgaris* L.) Exposed to Shallow Water Table**

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**ABSTRACT**

Shallow water table (SWT) is an acute problem in cultivating vegetables at riparian wetland during transitional period from dry to rainy season since it reduces volume of aerobic rhizosphere and limits oxygen availability. Meanwhile, 5-aminolevulinic acid (ALA) is known as one of plant regulators, effective in offset the effects of abiotic stresses. The aim of this study was to evaluate effectiveness of ALA application at different timings before and during continuous 20-day SWT exposure to common bean plants. SWT exposures were set at 5 cm (W-5), 10 cm (W-10), and 15 cm (W-15) below substrate surface. ALA applications were at 4 days before SWT initiation (14 DAP), middle of SWT exposure period (28 DAP), and at end of SWT exposure (38 DAP). Results of this study indicated that SWT exposure affected growth of shoots and roots, leaf water status, proline content, and SPAD value in bean plant. Shallowest water table (SWT-5) caused the most severe effect. Effectiveness of ALA application was varied, depending on time differences between ALA application and initiation of SWT exposure. ALA application did not significantly improve recoverability of bean plants after SWT exposure was terminated.

**KEYWORDS**

Abiotic stress, growth analysis, proline, riparian wetlands, vegetable production.

**INTRODUCTION**

Increasing food production is a global challenge as population continue to increase, especially in densely populated and land limited developing nations. Wetlands have been the last option for agriculture activity due to low soil fertility, risk of heavy metal toxicity to crops, problematic water management, expensive investment in agricultural infrastructure development, limited adaptable crops, and uncertain impacts on the ecosystem. At present, tidal wetlands have been more intensively cultivated than riparian wetlands in Indonesia. Most of riparian wetlands have only been cultivated with one rice crop annually. Since increase in food demand is inevitable in the future; therefore, knowledge and technology for intensifying food production at the riparian wetlands should be well prepared.

The riparian wetlands have two extreme limitations for expanding crop growing season, i.e. flooding and dry period. Local smallholder farmers grow rice at end of flooding period as floodwater has subsided to a depth of less than 15 cm (Lakitan et al., 2019). Based on length of annual flooding period, riparian wetlands are classified as short (<3 months), medium (3-6 months), and long (>6 months) flooding period. Main reason for local farmers to start growing rice as early as possible is to avoid drought condition during reproductive stage at riparian wetlands with short flooding period, or to avoid shallow water table or early flooding at riparian wetlands with long flooding period.

Vegetable cultivation during flooding period has been introduced to local famers and proven to be possible using floating culture systems (Jaya, Lakitan, & Sodikin, 2019). One to three crops of short-life-cycle vegetables can be grown during flooding, depending on length of flooding period. Successful corn cultivation during dry season at short flooding (meaning long dry season) riparian wetlands has also been reported by Bakri, Imanudin, & Bernas, (2015) using dual-function subsurface piping installation for discharging excess water during wet season and maintaining water table at depth of 50 cm below soil surface during dry season. For a continuous year-long cultivation cycle at riparian wetlands, there is transitional period from end of dry to early rainy season to be covered. During this transitional period, occurrence of shallow water table can cause severe yield loss, and at worst case, can cause total failure.

Results of many studies indicated that 5-aminolevulinic acid (ALA), a natural plant growth regulator, was effective in alleviating negative effects of various abiotic stresses in plants (Akram & Ashraf, 2013; Anwar, Yan, Liu, Li, & Yu, 2018) or for enhancing plant resistance to stress (Wu, Liao, Dawuda, Hu, & Yu, 2018). ALA can be applied exogenously.

This research was focused of improving common bean (*Phaseolus vulgaris* L.) survivability under unfavorable shallow water table (SWT) conditions by application of 5-aminolevulinic acid (ALA). At riparian wetlands, the SWT condition occurs soon after floodwater subsided and during early rainy season. Time-wise, these two transitional periods can be grown with short life cycle vegetables, such as common bean. A success in crop cultivation during these transitional periods will complete fundamental knowledge on the full annual cycle of crop cultivation at the riparian wetlands. However, more ‘fine-tuning’ researches are required for increasing reliability and case-specific scenario for intensifying food production at riparian wetlands in Indonesia.

**MATERIALS AND METHODS**

The pot experiment was conducted at an off- campus research facilities at Jakabaring (104o46’43.6” E; 3o01’35.4”S) in Palembang, Indonesia, from August to October 2018. The experiment was done in wet-culture pools with three shallow water table regimes. Bushy common bean (*Phaseolus vulgaris* L.) variety of Ranti was used in this experiment. The size of pots used was 25 cm in diameter and 30 cm in height. All pots were filled with mixed substrate of soil and manure at ratio of 3:2 v/v to 25 cm thickness, but the substrate settled on average at about 20 cm after subsidence during 18 days prior to SWT treatment.

The substrate was treated with STG solution, containing selected isolates of *Streptomyces sp*., *Trichoderma sp*., and *Geobacillus sp*., at two weeks before seed sowing. Prior to sowing, seeds were soaked in water for two hours. Granular NPK fertilizer was applied three times, i.e. at time of planting, 15 days after planting (DAP), and 30 days after planting at rate of 5.8 gram per pot.

SWT condition was set up by placing pots containing substrate mix of 20 cm depth after subsidence into three experimental pools filled with different depths of water, i.e. 15 cm, 10 cm, and 5 cm. The depth differential created different position of water table at 5 cm (W-5), 10 cm (W-10), and 15 cm (W-15) below substrate surface, respectively. SWT condition was exposed to the common bean plants for 20 consecutive days, starting from18 DAP to 38 DAP. Each SWT position was constantly maintained by opening designated valves for free flowing of excess water in the pool.

An aqueous solution of ALA at 1.0 mM was used for increasing tolerability of common bean to shallow water table condition. ALA was applied at four days prior to SWT exposure or at 14 DAP (T14), at midpoint during the SWT exposure period or at 28 DAP (T28), and at time of SWT exposure was terminated or at 38 DAP (T38). ALA solution was sprayed to upper and lower leaf surfaces of each plant until both sides of the leaves were fully and evenly wet (Zhang, Miao, & Wang, 2015). This foliar application was done at 6.30-7.30 a.m. Each plant was singly isolated during ALA foliar application.

Severity effects of SWT exposure was evaluated using the pairwise comparison procedure, by comparing plants exposed to each SWT condition but without ALA treatment, i.e. W-5-ALA, W-10-ALA, and W-15-ALA with control plants without SWT exposure and ALA treatment. Effectiveness of ALA application was evaluated within each SWT exposure treatment.

Proline content in fresh leaves was analyzed based on modified Bates’ protocol. This procedure was recently reviewed by Kalsoom et al. (2016). Chlorophyll concentration index was measured using Konica-Minolta Chlorophyll Meter SPAD-502Plus. Leaf proline contents and SPAD value were measured at 30 DAP, 36 DAP and 42 DAP.

Basic agronomical traits were measured directly and treated as primary data. Meanwhile, leaf area (LA) was estimated using reliable regression model. Spesific leaf area (SLA), total leaf area (TLA), leaf area ratio (LAR), and relative leaf water content (RLWC) were calculated based on measured primary growth parameters (Meihana et al., 2017; Lakitan, Kadir, Wijaya, & Susilawati, 2018). Most of agronomic traits were collected at 30 DAP, 36 DAP, and 42 DAP, except for biomass data which were collected after 20 days of SWT exposure was terminated, i.e. 58 DAP.

Data were analyzed using analysis of variance (ANOVA) based on the Strip Plot Design. The significant differences amongst levels of SWT exposure and time of ALA applications were determined using the Least Significant Difference (LSD) test at p < 0.05; meanwhile, comparison between paired designated population were evaluated using the T-test at level 0.05.

**RESULTS AND DISCUSSION**

**Growth Suppression due to SWT Exposure**

Roots are directly affected organ during occurrence of stress related to substrate water status (Yu et al., 2018). This research confirmed the argument, as root length and fresh weight were affected significantly by SWT exposure (Table 1). Root extension was significantly limited and root fresh weight was significantly reduced if water table was at the shallowest position, i.e. at 5 cm below substrate surface (SWT-5). Root fresh weight was cut to less than half if water table was at 5 cm compared to at 15 cm below substrate surface. This indicated that common bean plant was sensitive to SWT condition. Root growth suppression eventually also affected growth of aerial organs.

Water table is considered as transitional thin layer between anaerobic and aerobic zone within the growing substrate. Oxygen availability below water table is very low and oxygen transport within water saturated substrate is also very slow (Phukan, Mishra, &Shukla, 2016). Therefore, this hypoxic condition seriously disturbed aerobic metabolism in roots of sensitive plants (António et al., 2016), such as common bean. However, root regrowth after SWT exposure was terminated had been previously observed (Lakitan, Kadir, Wijaya, & Susilawati, 2018).

Leaf has been recognized as a sensitive organ to abiotic stress (Clauw et al., 2015). Therefore, this study was focused on leaf response to SWT exposure. Total leaf area per plant (TLA), specific leaf area (SLA), and leaf area ratio (LAR) were significantly and negatively affected by SWT exposure (Table 2). TLA decreased more than three folds in bean plant exposed to water table at 5 cm compared to at 15 cm below substrate surface. The trends were similar for SLA and LAR even magnitude of decreases were more moderate. Decrease in TLA was not only associated with smaller leaves developed during SWT exposure but also due to early senescence of older leaves. Lower SLA is an indication of smaller but thicker leaf; while LAR was associated with photosynthetic efficiency at the whole plant level.

These results justified that disturbance in root functions and metabolism also significantly affected growth of aerial organs, especially leaf. Lower TLA was mainly associated with limited water uptake by roots. Lower cellular water content directly diminished internal hydraulic pressure, starting at individual cell, tissue, and cumulatively affected leaf of the SWT-treated plant. Low SLA was exhibited by appearance of thicker but smaller leaf. Furthermore, plant with low LAR was visually characterized by less number and/or smaller leaves. In this case, fewer leaves were predominantly due to early senescence of old leaves (Sade, del Mar Rubio-Wilhelmi, Umnajkitikorn, & Blumwald, 2017) and some of the leaves had fallen off during exposure to SWT.

Decline in leaf area and other aerial growth parameters were also found in field bean (Pochiecha, 2013), gooseberry (Aldana, Garcia & Fischer, 2014), and white jabon (Sudrajat, Siregar, Khumaida, Siregar & Mansur, 2015) under hypoxic rhizosphere condition. The decrease in plant growth and development was induced by drought stress associated with reduction in plant water status. Furthermore, the stress reduced shoot elongation and leaf expansion followed by decrese in photosynthetic activities (Ntukamazina et al., 2017), mainly due to stomatal closure, photosynthetic enzymes activity, reduced chlorophyll content, and smaller leaf area (Aldana, Garcia & Fischer, 2014).

**Effects of SWT on Leaf Water Status, Proline Content, and SPAD Value**

Relative leaf water content (RLWC) is widely used to indicate plant water status, since it expresses the relative amount of water in plant tissues (An, Qi, & Wang, 2016). Deeper water table below the substrate surface increased volume of aerobic rhizosphere, i.e. more roots subjected to oxygen available substrate, hence less disruption to root metabolism. As a result, the substrate condition was more suitable for root growth; therefore, increased water and nutrient uptakes and their transport to leaves (Lakitan, Kadir, Wijaya, & Susilawati, 2018).

This study disclosed that RLWC significantly increased in bean plants during the first 12 days of SWT treatments (SWT-5 and SWT-10). After 18 days of the SWT treatment, however, RLWC in plant treated with shallowest water table (SWT-5) was significantly decrease (Table 3). This finding implied that function of roots of bean plant on water uptake were not negatively affected and/or the leaves functioned well on preserving water during the first 12 days of the SWT treatment, presumably by reducing transpiration rate via stomatal closure mechanism. Common bean plant exhibited ability to recover within 4 days after termination of SWT exposure (Table 3).

Sufficient oxygen availability in soil could increase ability of root systems to uptake and transport water and minerals to aerial plant parts (Restrevo-Diaz, Melgar & Lombardini, 2010). Stomatal closure was a usual phenomena in many species under hypoxia condition (Biswas & Kalra, 2018) . Stomatal closure increased water saving in plants (Aldana, Garcia & Fischer, 2014; Aydogan & Turhan, 2015). Nevertheless, prolong hypoxia condition in the rhizospher could decrease root ability to absorb water due to metabolism switch from aerobic to anaerobic respiration (Aldana, Garcia & Fischer, 2014). This causes a reduction in ATP production resulting in reduced available energy for normal plant metabolic processes in root (Pradhan & Mohanty, 2013).

Proline content in plant leaf has been widely used as an indicator for abiotic stress whereas proline accumulation detected in most of plants under abiotic stresses (Hayat et al., 2012; Yaish, 2015; Barunawati, Maghfoer, Kendarini, & Aini, 2016), including under excessive soil water content or oxygen deficiency, both associated with SWT condition. In this study, during the first 12 days of the SWT treatments, there was no significant difference amongst plant exposed to water table at 15 cm to 5 cm below substrate surface. The differences were detected at 18 DoT and at 4 DoR (Table 4). Significant differences in proline content at 4 DoR indicated that the bean plant used in this study had not fully recovered despite the RLWC indicated differently (Table 3).

SPAD values of the bean plant exposed to SWT-5 were consistently and significantly lower than those of plant exposed to SWT-15 at all of measurement during and after SWT treatment (Table 4). These consistent differences were similar between primary and secondary growth parameters shown in Table1 and 2. Since SPAD value has been proven as reliable estimation of leaf chlorophyll content; therefore, it can be expected that shallower water table position decreased leaf chlorophyll content and reduced photosynthetic capacity of bean plants. Furthermore, reduction in photosynthetic rates inhibited growth of multiple organs, both their weight and, most cases, also their dimension, i.e. leaf area.

**Role of ALA in Overcoming Stress due to SWT Exposure**

Root length, root fresh weight and shoot fresh weight in common bean were severely decreased under shallower water table conditions (SWT-5 and SWT-10) in plants were not treated with ALA. Meanwhile, application of ALA at 4 days prior to SWT treatment (at 14 DAP) had effectively overcome negative effect of SWT at depth of -5 cm (SWT-5) and -10 cm (SWT-10) as indicated by significantly longer roots in ALA treated plants. However, application of ALA at latter stages (T28 and T38) were not as effective. Effectiveness of ALA application at pre-SWT exposure was also observed in root and shoot fresh weights (Table 5).

Shallow of water table has been well recognized as limiting factor to plant growth and development (Aldana, Garcia, & Fischer, 2014) since it created partially hypoxic condition within rhizosphere. In favorable condition, common bean was responsive to fertilizer application (Santosa, Maghfoer, & Tarno, 2017) but bean plant was not as responsive under abiotic stress, especially during SWT exposure. Roots are directly exposed to oxygen deficient under shallow water table condition. Damage to roots directly reduces water potential and turgor in cells of above ground organs. As a result, cell enlargement is halted, leading to growth inhibition. In this study, reductions of roots and shoot in common bean plant exposed to SWT was overcome by ALA application at 4 days before SWT treatment (Table 5). Suppression of plant growth under abiotic stress was successfully prevented or overcome by ALA applications under salt stress (Anjum et al., 2016), hypoxia (An, Qi & Wang, 2016), and low temperature (Anwar, Yan, Liu, Li & Yu, 2018).

Improvement of roots and shoot growth under SWT stress by ALA application is associated with enhancement in water and nutrient uptake and transport. Plants exposed to hypoxia shift their metabolism from oxidative phosphorylation to anaerobic fermentation to maintain ATP production. Anaerobic fermentation is activated by ALA without resulting root injury (An, Qi & Wang, 2016). Meanwhile, significantly increase in roots and shoot growth after ALA application at 38 DAP associated with reoxygenation of rhizosphere as SWT treatment was terminated. Availability of oxygen in soil supports aerobic respiration and enhances root activity on water and nutrient uptakes. ALA played role on some key physiological processes in plant under abiotic stresses (Akram, Ashraf, & Al-Qurainy, 2012).

 Total leaf area (TLA), specific leaf area (SLA) and leaf area ratio (LAR) decreased significantly in common bean plant exposed to SWT at depth of -5 cm (SWT-5) and -10 cm (SWT-10), compared with control plant (Table 6). Under SWT-10 condition, ALA application at 14 DAP improved TLA, SLA and LAR significantly, i.e. 100.9%, 109.7% and 83.6%, respectively. Similarly, ALA applied at 38 DAP significantly increased TLA (69.3%), SLA (91.5%) and LAR (60.3%) in plant exposed to SWT-10.

TLA, SLA and LAR were higher in bean plant pre-treated with ALA at 14 DAP prior to SWT exposure. Akram & Ashraf (2013) reported that ALA treatment as a foliar spray exhibited considerable inﬂuence during early vegetative growth stage. ALA alleviated harmful effects of abiotic stresses by protecting chlorophyll and the photosynthetic apparatus (Anwar, Yan, Liu, Li, & Yu, 2018) via osmoregulation and inhibitory effects on membrane lipid peroxidation (Ye, Chen, Tao, Wang, &Xu, 2016). Exogenous application of ALA enhanced some key physiological and biochemical processes in plants such as photosynthesis and nutrient uptake (Freije, Saleh, Islam & Manai, 2018). These enhanced processes promoted plant growth and development (Wu, Liao, Dawuda, Hu, & Yu, 2018).

ALA application at end of SWT treatment (38 DAP) enhanced bean plants recovery, as indicated by significant increase in TLA, SLA and LAR during the first four days after SWT treatment was terminated. Significantly higher TLA, SLA, and LAR in ALA treated compared to non-treated bean plants indicated that ALA had contributed in enhancing recovery from SWT exposure.

No significant different in relative leaf water content in common bean plant under SWT condition compared to control plant, except after 18 days SWT exposure at -5 cm below substrate surface (Table 7). ALA application at pre-SWT exposure (14 DAP) at -10 cm depth (SWT-10) measured at 12 DoT exhibited significantly higher RLWC in ALA-treated bean plant. Similarly, Higher RLWC also observed in bean plants exposed to SWT-5 and treated with ALA at 28 DAP. However, in most cases, there was no significant different in RLWC among plants exposed to SWT at each depth, treated with ALA at different time of applications, and measured at 12 DoT, 18 DoT, and 4 DoR (Table 7).

ALA application under various abiotic stresses was effective in maintaining RLWC, such as stresses due to high salinity (Akram, Ashraf, & Al-Qurainy, 2012; Liu, Nguyen, Ueda, & Saneoka, 2014; Yang, Chang, Sun, Yu, & Huang, 2014); low temperature (Manafi, Modarressanavy, Aghaalikhani, & Dolatabadian, 2015); and waterlogging condition (An, Qi, & Wang, 2016). ALA maintained cellular hydration (Yang, Chang, Sun, Yu, & Huang, 2014), stabilized root vigor and enhanced their water uptake capacity (An, Qi, & Wang, 2016).

In this study, RLWC maintenance in SWT-exposed plants (Table 7) was at cost of TLA reduction (Table 6) in effort to balance limited water uptake by partially damaged root system (Table 5). Ability to maintain RLWC in bean plants was more as a survival mechanism since it did not significantly follow by new growth and development. There were smaller new leaves developed but, at the same time, there also some older leaves fallen off. Since at time the bean plants was allowed to recover from stressful impact of the 20-day SWT exposure, the plants had shifted into reproductive stages; therefore, development of flowers and pods were severely halted.

**Effects of Timing of SWT Exposure and ALA Application**

Proline, an amino acid, plays an important role in plants. It protected the plants from various biotic and abiotic stresses and also supported plants to recover from stress more rapidly (Hayat et al., 2012). In this study, proline content measured during SWT exposure (12 DoT and 18 DoT) were significantly higher in plant exposed to SWT than in control plant. However, proline content was significantly decreased in the SWT-exposed plants only after 4 days of recovery (Table 8).

Measurement of proline content at 12 DoT disclosed that ALA application at 14 DAP significantly increased and decreased in plant exposed to SWT-5 and, respectively. These results indicated that ALA application at 14 DAP was only effective in reducing stress at SWT-15 but not at shallower water table at SWT-5. Meanwhile, measurement at 12 DoT (2 days after of ALA application at 28 DAP), proline contents were significantly decreased in all SWT exposures. This finding leads to argument that effect of ALA in alleviating stress is only effective in short term. During recovery period, application of ALA at pre- and during SWT exposures did not contribute to stress alleviation in bean plants as indicated by proline content in ALA-treated plants was higher than non-treated plants. Therefore, bean plants recovery is presumably more related to increase of oxygen availability after termination of SWT exposure rather than as effect of ALA application (Table 8). Similarly, Yaish (2015) also reported that proline was not a major factor in the mechanism leading to stress tolerance.

The correlation between proline accumulation and abiotic stress tolerance in plants was not always apparent. Accumulation of proline in the leaves was believed to be a symptom of stress injury rather than a sign of stress tolerance in rice plants (An, Qi, & Wang, 2016). Proline provided protection to plant from stress by cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of proteins/enzymes (Hayat et al., 2012; Manafi, Modarressanavy, Aghaalikhani, & Dolatabadian, 2015). ALA enhanced antioxidant enzymes activities, such as superoxide dismutase, peroxidase, and catalase. The enhancing of antioxidant enzymes activities reduced the detrimental effect of reactive oxygen species (ROS) to plant cells (Manafi, Modarressanavy, Aghaalikhani, & Dolatabadian, 2015; An, Qi, & Wang, 2016).

SWT exposure consistently and significantly reduced SPAD value compared to control plant. Meanwhile, applications of ALA at pre- (14 DAP), during (28 DAP), and end of (38 DAP) SWT exposures were effectively increased SPAD value subsequently measured (Table 9). SPAD value has been used for predicting leaf chlorophyll content. Strong correlation between SPAD value and leaf chlorophyll content has been reported by Zhu, Tremblay & Liang (2012) and Jiang, Johkan, Hohjo, Tsukagoshi, & Maruo (2017).

This finding indicated that exogenous applied ALA stimulated leaf chlorophyll synthesis under SWT condition. ALA is precursor of heme containing photosynthetic pigments. As a metabolic intermediate in higher plants, ALA directly associated with chlorophyll and carotenoids biosynthesis (Akram & Ashraf, 2013; Anjum et al. 2016; An, Qi, & Wang, 2016). Furthermore, ALA contributed in increasing plant growth under abiotic stress (Wu, Liao, Dawuda, Hu, & Yu, 2018).

There was interaction between SWT exposure and time of ALA application on proline content measured at 12 DoT, 18 DoT and 4 DoR (Fig. 1). At the midterm of SWT exposure (12 DoT), the highest proline content was observed in bean plant exposed to -5 cm water table (SWT-5) and ALA applied at 14 DAP (Fig. 1A). In this first case, measurement of proline was undertaken at 16 days after ALA application at pre-SWT treatment. At the near end of SWT treatment (18 DoT), the highest proline content was found in bean plant exposed to -10 cm water table (SWT-10) and ALA applied at 28 DAP (Fig. 1B). In this second case, proline was measured at 8 days after ALA application. Meanwhile, at the recovery stage (4 DoR), the highest proline content was oberved in plant exposed to -15 cm water table (SWT-15) and ALA applied at 38 DAP (Fig. 1C). In this third case, proline measurement was done only 4 days after ALA application or at as early as 4 days after SWT exposure was terminated. It is clear from these three cases that bean plant is suffer more during SWT exposure at longer period of time after ALA application but, reversely, it suffered more at earlier stage of recovery period and getting better as time progresses.

**CONCLUSIONS**

SWT exposure affected growth of above ground organs and roots, leaf water status, proline content, and SPAD value in bean plant. Severity of the effects depended on depth of water table with shallowest water table (SWT-5) caused the most severe effect. Common bean used in this study exhibited ability to recover after SWT exposure was terminated. Effectiveness of ALA application on reducing negative effects of SWT exposure was varied, depending on depth of SWT exposure and timing of its application, with regards to duration of the plant has been exposed to SWT before ALA was applied. ALA application did not significantly improve recoverability of bean plants after SWT exposure was terminated. Instead, recoverability was more associated with improvement in soil aeration since there was no significant difference in growth between ALA-treated and non-treated plants during the recovery period.

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Table 1. Effects of shallow water table on root length, root fresh weight, and shoot fresh weight

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Root length (cm) | Root fresh weight (g) | Shoot fresh weight (g) |
| SWT-5 | 33.78±8.55 | c z | 44.82±10.96 | b | 70.99±16.03 | c |
| SWT-10 | 48.62±2.32 | b | 49.69±19.61 | b | 121.67±14.97 | b |
| SWT-15 | 56.44±5.93 | a | 95.65±12.23 | a | 157.05±4.15 | a |

z) Means followed by the same letter within each column are not significantly different based on the LSD test at p < 0.05

Table 2. Effects of shallow water table on leaf characteristics in common bean plant

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | TLA (cm2) | SLA (cm2.g-1) | LAR (cm2.g-1) |
| SWT-5 | 1072.59±343.10  | c z |  230.40±35.96  | c | 60.72±17.72 |
| SWT-10 | 2406.55±756.47  | a | 308.63±30.68  | b | 91.09±15.24 |
| SWT-15 | 3666.26±257.79  | b | 373.03±14.67  | a | 105.91±6.80 |

z) Means followed by the same letter within each column are not significantly different based on the LSD test at p < 0.05

Table 3. Relative leaf water content measured during and after SWT exposure at variable depths

|  |  |
| --- | --- |
| Depth of water table | Time of measurement z |
| 12 DoT | 18DoT | 4 DoR |
| SWT-5 | 82.61±0.33 | a y | 64.62±9.15 | b | 69.14±1.51 |
| SWT-10 | 81.50±1.62 | a | 76.01±1.80 | a | 70.07±2.57 |
| SWT-15 | 77.78±0.83 | b | 78.65±2.01 | a | 69.97±2.66 |

z)DoT = days after treatment initiation; DoR = days of recovery. Duration of SWT treatment was from 18 DAP to 38 DAP

y) Means followed by the same letter within each column are not significantly different based on the LSD test at p < 0.05

Table 4. Proline content and SPAD value measured during and after SWT exposure at variable depths

|  |  |
| --- | --- |
| Depth of water table | Time of measurement z |
| 12 DoT | 18DoT | 4 DoR |
|  | *Proline Content (µmol.g-1)* |
| SWT-5 |  0.295±0.045 a y | 0.170±0.011 | c | 0.256±0.058 | b |
| SWT-10 | 0.306±0.024 a | 0.213±0.019 | a | 0.230±0.017 | c |
| SWT-15 | 0.225±0.018 a | 0.187±0.015 | b | 0.316±0.118 | a |
|  | *SPAD value* |
| SWT-5 | 44.63±1.23 | b | 39.22±0.50 | b | 34.84±1.78 | c |
| SWT-10 | 44.33±1.89 | b | 44.38±1.05 | a | 37.08±0.61 | b |
| SWT-15 | 46.38±0.40 | a | 43.85±0.65 | a | 39.23±0.58 | a |

z)DoT = days after treatment initiation; DoR = days of recovery. Duration of SWT treatment was from 18 DAP to 38 DAP

y) Means followed by the same letter within each column are not significantly different based on the LSD test at p < 0.05

Table 5. Pairwise comparison for evaluating effect of SWT exposure on root length, root fresh weight, and shoot fresh weight; and for comparing between SWT exposed plants treated with or without ALA at different time of application.

|  |  |  |
| --- | --- | --- |
| Depth of SWT | Control vs Exposure to SWT | With vs Without ALA application z |
| 14 DAP (T14) | 28 DAP (T28) | 38 DAP (T38) |
| *Root Length (cm)* |
| SWT-5 | -40.6 y | \* | 17.7 | \* | 0.7 | ns | 7.5 | ns |
| SWT-10 | -29.8 | \* | 14.8 | \* | 10.2 | ns | 13.2 | ns |
| SWT-15 | -4.3 | ns | -2.5 | ns | -11.8 | ns | -0.8 | ns |
| *Root Fresh Weight (g)* |
| SWT-5 | -23.1 | \* | 29.0 | \* | 7.3 | ns | 20.8 | ns |
| SWT-10 | -25.4 | \* | 41.7 | \* | 4.1 | ns | 32.7 | \* |
| SWT-15 | 35.2 | \* | 25.2 | \* | 1.6 | ns | 7.7 | ns |
| *Shoot Fresh Weight (g)* |
| SWT-5 | -61.8 | \* | 7.1 | ns | -20.1 | ns | 8.1 | ns |
| SWT-10 | -25.7 | \* | 30.0 | \*. | 2.0 | ns | 6.9 | ns |
| SWT-15 | -1.6 | ns | 25.7 | \* | 19.5 | ns | 27.3 | \* |

z) T14 = ALA application at 4 days prior to SWT exposure (at 14 DAP), T28= ALA application at midpoint during the SWT exposure priod (at 28 DAP) , T38= ALA application at time of SWT exposure was terminated (at 38 DAP).

y) Value of comparison and result of t-Test for paired sample at level 0.05; ns = no significant different, \* = significantly different.

Table 6. Pairwise comparison for evaluating effect of SWT exposure on total leaf area, specific leaf area, and leaf area ratio; and for comparing between SWT exposed plants treated with or without ALA at different time of application.

|  |  |  |
| --- | --- | --- |
| Depth of SWT | Control vs Exposure to SWT | With vs Without ALA application z |
| 14 DAP (T14) | 28 DAP (T28) | 38 DAP (T38) |
| *Total Leaf Area (dm2)* |
| SWT-5 | -23.98 y | \* | 6.69 | ns | -0.06 | ns | 4.40 | ns |
| SWT-10 | -20.01 | \* | 20.18 | \* | 5.12 | ns | 13.86 | \* |
| SWT-15 | -5.11 | ns | 13.42 | \* | 8.27 | ns | 10.57 | ns |
| *Specific Leaf Area (dm2/g)* |
| SWT-5 | -1.37 | ns | 0.65 | ns | 0.02 | ns | 0.64 | ns |
| SWT-10 | -1.61 | \* | 1.77 | \* | 1.16 | ns | 1.48 | \* |
| SWT-15 | 0.16 | ns | 0.18 | ns | 0.46 | ns | 0.39 | ns |
| *Leaf Area Ratio (cm2/g)* |
| SWT-5 | -71.9 | \* | 29.9 | ns | -5.2 | ns | 16.2 | ns |
| SWT-10 | -73.6 | \* | 61.5 | \* | 31.1 | ns | 44.4 | \* |
| SWT-15 | -32.5 | ns | 25.8 | ns | 20.1 | ns | 12.3 | ns |

x) T14 = at 4 days prior to SWT exposure (at 14 DAP), T28= at midpoint during the SWT exposure priod (at 28 DAP), T38= at time of SWT exposure was terminated (at 38 DAP)

y) Value of comparison and result of t-Test for paired sample at level 0.05, ns = no significant different, \* = significantly different

Table 7. Pairwise comparison for evaluating effect of SWT exposure on relative leaf water content and for comparing between SWT exposed plants treated with or without ALA at different time of application.

|  |  |  |  |
| --- | --- | --- | --- |
| Time of measurement z | Depth of SWT | Control vs Exposure to SWT | With vs Without ALA application y |
| 14 DAP (T14) | 28 DAP (T28) | 38 DAP (T38) |
| 12 DoT | SWT-5 | -1.3 | ns | 5.0 | ns | 5.5 | \* | - |
| SWT-10 | -1.4 | ns | 5.4 | \* | 3.1 | ns | - |
| SWT-15 | -1.4 | ns | 1.1 | ns | -0.1 | ns | - |
| 18 DoT | SWT-5 | -16.7 | \* | 7.4 | ns | -5.5 | ns | - |
| SWT-10 | -8.2 | ns | 2.6 | ns | 5.1 | ns | - |
| SWT-15 | -2.8 | ns | 2.5 | ns | -0.3 | ns | - |
| 4 DoR | SWT-5 | -5.9 | ns | 3.5 | ns | 0.6 | ns | 2.9 | ns |
| SWT-10 | -7.3 | ns | 5.6 | ns | 6.7 | ns | 1.8 | ns |
| SWT-15 | -4.8 | ns | 4.0 | ns | -1.0 | ns | 3.2 | ns |

z) Measurements were made at 12 DoT, 18 DoT, and 4 DoR; DoT = days after treatment initiation; DoR = days of recovery. Duration of SWT treatment was from 18 DAP to 38 DAP.

y) T14 = at 4 days prior to SWT exposure (at 14 DAP), T28= at midpoint during the SWT exposure period (at 28 DAP), T38= at time of SWT exposure was terminated (at 38 DAP)

Table 8. Pairwise comparison for evaluating effect of SWT exposure on proline content and for comparing between SWT exposed plants treated with or without ALA at different time of application.

|  |  |  |  |
| --- | --- | --- | --- |
| Time of measurement z | Depth of SWT | Control vs Exposure to SWT | With vs Without ALA application y |
| 14 DAP (T14) | 28 DAP (T28) | 38 DAP (T38) |
| 12 DoT | SWT-5 | 0.045 | \* | 0.033 | \* | -0.030 | \* | - |
| SWT-10 | 0.065 | \* | -0.010 | ns | -0.024 | ns | - |
| SWT-15 | 0.015 | ns | -0.051 | \* | -0.026 | \* | - |
| 18 DoT | SWT-5 | 0.018 | \* | -0.031 | \* | 0.047 | \* | - |
| SWT-10 | 0.052 | \* | -0.044 | \* | -0.017 | \* | - |
| SWT-15 | 0.015 | \* | -0.033 | \* | -0.009 | ns | - |
| 4 DoR | SWT-5 | -0.125 | \* | 0.147 | \* | 0.056 | \* | 0.039 | \* |
| SWT-10 | -0.058 | \* | 0.007 | ns | -0.028 | \* | -0.016 | \* |
| SWT-15 | -0.101 | \* | 0.043 | \* | 0.055 | \* | 0.253 | \* |

z) Measurements were made at 12 DoT, 18 DoT, and 4 DoR; DoT = days after treatment initiation; DoR = days of recovery. Duration of SWT treatment was from 18 DAP to 38 DAP.

y) T14 = at 4 days prior to SWT exposure (at 14 DAP), T28= at midpoint during the SWT exposure period (at 28 DAP), T38= at time of SWT exposure was terminated (at 38 DAP)

Table 9. Pairwise comparison for evaluating effect of SWT exposure on SPAD value and for comparing between SWT exposed plants treated with or without ALA at different time of application.

|  |  |  |  |
| --- | --- | --- | --- |
| Time of measurement z | Depth of SWT | Control vs Exposure to SWT | With vs Without ALA application y |
| 14 DAP (T14) | 28 DAP (T28) | 38 DAP (T38) |
| 12 DoT | SWT-5 | -4.8 | \* | 2.4 | \* | 0.7 | ns | - |
| SWT-10 | -3.2 | \* | 1.0 | ns | -1.6 | ns | - |
| SWT-15 | -4.8 | \* | 3.0 | \* | 3.6 | \* | - |
| 18 DoT | SWT-5 | -5.8 | \* | 1.2 | ns | 0.5 | ns | - |
| SWT-10 | -2.9 | \* | 2.0 | \* | 4.1 | \* | - |
| SWT-15 | -4.9 | \* | 3.4 | \* | 5.6 | \* | - |
| 4 DoR | SWT-5 | -9.0 | \* | 2.4 | ns | -1.0 | ns | -0.1 | ns |
| SWT-10 | -7.6 | \* | 1.4 | ns | 0.6 | ns | 1.7 | ns |
| SWT-15 | -7.3 | \* | 3.8 | \* | 2.8 | ns | 2.7 | ns |

z) Measurements were made at 12 DoT, 18 DoT, and 4 DoR; DoT = days after treatment initiation; DoR = days of recovery. Duration of SWT treatment was from 18 DAP to 38 DAP.

y) T14 = at 4 days prior to SWT exposure (at 14 DAP), T28= at midpoint during the SWT exposure period (at 28 DAP), T38= at time of SWT exposure was terminated (at 38 DAP)

|  |
| --- |
|  |
|  |
|  |

Figure 1. Proline contents measured after 12 days (A) and 18 days of SWT exposure (B), and after 4 days of recovery (C) in bean plants subjected to different water table regimes of -5 cm (SWT-5), -10 cm (SWT-10) and -15 cm (SWT-15) and different time of ALA application at 14 DAP (T14), 28 DAP (T28), and 38 DAP (T38).