



5-Aminolevulinic Acid Lessened Growth Suppression in Snap 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. SWT limits volume of aerobic rhizosphere and reduces oxygen availability. Meanwhile, 5-aminolevulinic acid (ALA) has been known for its effectiveness in offsetting negative effects of abiotic stresses. The aim of this study was to evaluate effectiveness of ALA application at pre- or during continuous 20-day SWT exposure in snap bean. SWT exposures were set at depth of 5 cm (SWT₅), 10 cm (SWT₁₀) and 15 cm (SWT₁₅) below substrate surface. ALA was applied at 4 days before SWT initiation (14 DAP), mid of SWT exposure period (28 DAP), or 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 snap bean plant. Shallowest water table (SWT₅) caused the most severe effects. Effectiveness of ALA application was depended on time difference between ALA application and onset of SWT exposure. ALA application did not significantly improve recovery of snap bean plants after SWT exposure was terminated.

INTRODUCTION

Increasing food production is a global challenge as human population continues to increase, especially in densely populated and land limited developing countries. In most cases, wetland becomes the last option for agriculture activity (Anand & Oinam, 2020) but with some challenges, including low soil fertility, risk of heavy metal toxicity to crops, problematic water management, expensive investment in agricultural infrastructures, limited adaptable crops, and uncertain impacts on the ecosystem (Lakitan et al., 2019; Lebrun et al., 2019; Sulaiman, Sulaeman, & Minasny, 2019). 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 the increase of

food demand is inevitable in near future; therefore, knowledge and technology for intensifying food production at the riparian wetlands should be well established.

The riparian wetlands have two extreme limitations for expanding crop production, i.e. flooding during rainy and extreme drought during dry seasons. Local smallholder farmers grow rice at end of flooding period as floodwater has subsided to the depth less than 15 cm. Based on the duration of annual flooding, riparian wetlands are classified into short (<3 months), medium (3-6 months), and long (>6 months) flooding periods. The main reason for local farmers to plant rice as early as possible is to avoid drought condition during reproductive stage at riparian wetlands if dry period is longer than expected. Early planting is also dedicated to

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avoid shallow water table in case flooding at riparian wetlands arrives sooner.

Vegetable cultivation during flooding period has been introduced to local farmers and successfully applied using floating culture systems (Siaga *et al.*, 2018). One to three fast growing vegetables can be planted during flooding, depending on duration of flooding period. Successful corn cultivation during dry season at riparian wetlands has been reported by Bakri, Imanudin, & Masreah Bernas (2015) using dual-function subsurface piping installation for discharging excess water during wet season and maintaining the depth of water table at 50 cm below soil surface during dry season. For a continuous year round production cycle at riparian wetlands, there is transitional period from the end of dry to early rainy season need to be covered. During this period, the occurrence of shallow water table can cause severe yield loss, or even total crop failure.

Many efforts have been attempted to alleviate growth inhibition due to abiotic stresses. The application of exogenous plant growth regulators have been reported to be able to plant resistance to abiotic stress (Wu, Liao, Dawuda, Hu, & Yu, 2019). 5-aminolevulinic acid (ALA) has been reported to be an effective growth regulator in counteracting the damaging effects of various abiotic stresses in plants (Akram & Ashraf, 2013; Anwar, Yan, Liu, Li, & Yu, 2018). Effective range of ALA concentrations varied among annual plants. Under drought condition, the growth response of canola was positively increased by 0.895 mM ALA application (Akram *et al.*, 2018). Similar findings was also reported by Chen *et al.* (2017) on water melon seedlings under salt stress with ALA concentration of 1.25 mM.

This research was focused on the growth improvement of snap bean (*Phaseolus vulgaris* L.) to offset unfavorable shallow water table (SWT) conditions using ALA application. At riparian wetlands, the SWT condition occurs soon after floodwater subsided and during early rainy season. During these two transitional periods, wetlands can be planted fast growing vegetables, such as snap bean. A success in crop cultivation during these transitional periods will comprehend the fundamental knowledge on annual crop growing cycle at riparian wetlands. However, more studies are still required to increase the reliability and case-specific scenario for intensifying crop production at riparian wetlands in Indonesia.

MATERIALS AND METHODS

The pot experiment was conducted from August to October 2018 at an off-campus research facilities at Jakabaring (104°46'43.6"E; 3°01'35.4"S) in Palembang, South Sumatera, Indonesia. The experiment was carried out in wet-culture pools with three shallow water table regimes, arranged based on the Strip Plot Design. Bushy snap bean (*Phaseolus vulgaris* L.) cv. Ranti was used in this experiment. The dimension of pot was 25 cm in diameter and 30 cm high. All pots were filled with mixed substrate of soil and manure at ratio of 3:2 v/v up to 25 cm of the pot height. Prior to SWT treatment, the substrate was settled to about 20 cm due to subsidence.

Two weeks before seed sowing, the substrate was sterilized using mixed biosolution of *Streptomyces sp.*, *Trichoderma sp.* and *Geobacillus sp* (STG). The seeds were soaked into water for two hours. Granular NPK fertilizer was applied three times, i.e. at planting time, 15 days and 30 days after planting at rate of 5.8 g per pot.

SWT condition was set up by placing the pots into three experimental pools filled with different water depths, i.e. 15, 10 and 5 cm. The different water depths created different water table positions at 5 cm (W_{-5}), 10 cm (W_{-10}), and 15 cm (W_{-15}), below the substrate surface, respectively. SWT condition was exposed to the snap bean plants for 20 consecutive days, starting from 18 to 38 DAP. Each SWT position was constantly maintained by opening designated valves for free flowing of excess water in the pool.

Referring to ALA concentration used by Akram *et al.* (2018) at 0.895 mM and Chen *et al.* (2017) at 1.25 mM, this study used aqueous solution of ALA at the concentration of 1.0 mM for increasing tolerability of snap bean to shallow water table condition. ALA was only applied once for all treatments but at different application times, i.e., four days prior to SWT exposure at 14 DAP (T_{14}), midpoint during SWT exposure period at 28 DAP (T_{28}), and at 38 DAP or at the termination of SWT exposure (T_{38}). ALA solution was sprayed to adaxial and abaxial leaf surfaces of each plant until both sides of the leaves were fully and evenly wet, following the procedures of Zhang, Miao, & Wang (2015). This foliar application was done at 6.30-7.30 am. Each plant was isolated during ALA foliar application.

Proline content in fresh leaves was analyzed based on modified Bates' protocol. This procedure

was recently reviewed by Kalsoom, Bennett, & Boyce (2016). Proline was determined following the ninhydrine method and using L-proline as a standard. Leaf proline contents were measured at 12 and 18 days after initiation of SWT treatment and after 4 days of recovery. Chlorophyll concentration index was measured using Konica-Minolta Chlorophyll Meter SPAD-502Plus. SPAD values were recorded on the same days as proline measurements.

Leaf area (LA) was estimated using state the estimation of the regression model. Specific 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). Dry weight (DW) was measured after the leaves were exposed to 70°C for 48 h and turgid weight (TW) was measured after rehydration for about 3 h until a constant weight was reached. RLWC was measured at 12 and 18 days of treatment (DoT) and after 4 days of recovery (DoR). SLA, TLA, LAR and biomass data such as root length, root and shoot fresh weight were collected after 20 days of SWT exposure was terminated (58 DAP).

Data were analyzed using analysis of variance (ANOVA). The mean comparisons among the treatments were tested using LSD ($p < 0.05$). The comparison between control and plants under SWT treatments (for evaluating severity of SWT treatments), and between the plants with and without ALA treatments under SWT exposure (for testing effectiveness of ALA treatments) were evaluated by T-test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Growth Suppression due to SWT Exposure

Roots are the directly affected organ during the occurrence of stress related to substrate water

status (Koevoets, Venema, Elzenga, & Testerink, 2016). Our research findings accordingly confirmed, as root length and fresh weight were affected significantly by SWT exposure (Table 1). Root growth was restricted and root fresh weight was reduced when the water table was at the shallowest position (SWT_{.5}). In SWT_{.5}, the value of root fresh weight was less than a half from SWT_{.15}. The root growth restriction under SWT indicated that snap bean was a sensitive plant to excessive water stress. 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 (Perata, 2020). This hypoxic condition seriously disturbed aerobic metabolism within the root of sensitive plants (Antônio *et al.*, 2016), like snap bean. However, after termination of SWT exposure, the plants were able to regenerate their root growth.

The leaf has been recognized as a sensitive organ to abiotic stress (Widuri *et al.*, 2017). Therefore, this study was also focused on leaf response to SWT exposure. Total leaf area per plant (TLA), specific leaf area (SLA), and leaf area ratio (LAR) were significantly affected by SWT exposures (Table 2). TLA decreased more than three folds when the plant was exposed to SWT_{.5} compared to SWT_{.15}. The trends were similar for SLA and LAR even the magnitude of decreases was more moderate. Decrease in TLA was not only associated with smaller developed leaves during SWT exposure but also due to early senescence of older leaves. Lower SLA is an indication of smaller and thicker leaf; while LAR was associated with photosynthetic efficiency at the whole plant level.

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
LSD 0.05	5.60	9.01	17.64

Remarks: ^z) Means followed by the same letter within each column are not significantly different based on the LSD test at $p \leq 0.05$

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

Treatment	TLA (cm ²)	SLA (cm ² /g)	LAR (cm ² .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
LSD 0.05	289.94	61.17	-

Remarks: ^z) Means followed by the same letter within each column are not significantly different based on the LSD test at $p \leq 0.05$

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 (Rosas *et al.*, 2019; Zhou *et al.*, 2020). 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, 2018) and some of the leaves had fallen off during exposure to SWT.

Reduction in leaf area and other aerial growth parameters were also found in field bean (Pociecha, 2013) and white jaboron (Sudrajat, Siregar, Khumaida, Siregar, & Mansur, 2015) under hypoxic condition in rhizosphere. The decreases in plant growth were induced by water stress associated with reduction in plant water status. The stress reduced shoot elongation and leaf expansion and photosynthetic activities (Ntukamazina *et al.*, 2017), and these, mainly due to stomatal closure, diminished photosynthetic enzymes activity, lower chlorophyll content, and smaller leaf area (Lawson & Matthews, 2020).

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

Relative leaf water content (RLWC) was 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. These mean the more roots were subjected to available oxygen within the 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.

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 on SWT₋₅ treated plants was significantly decrease (Table 3). These findings implied that the functions of roots on water uptake and leaves on preserving water were not negatively affected during the first 12 days SWT treatments. These predicted to have relation with physiological adjustment through reducing transpiration rate via stomatal closure (Lawson & Matthews, 2020). Snap bean plant exhibited ability to recover within 4 days after termination of SWT exposure (Table 3). The absent of hypoxia stress within the substrates could increase ability of the root system to uptake and transport water and minerals to upper plant parts (Liu *et al.*, 2020). Stomatal closure was a usual phenomena in many species under hypoxia condition (Biswas & Kalra, 2018). Stomatal closure increased water saving in plants (Lawson & Matthews, 2020). Nevertheless, prolong hypoxia condition in the rhizosphere could decrease root ability to absorb water due to the switch of metabolic pathway from aerobic to anaerobic respiration. This causes a reduction in ATP production resulting in reduced available energy to support plant metabolic processes in root (Pradhan & Mohanty, 2013).

Proline content in plant leaf has been widely used as an indicator for abiotic stress. Proline accumulation was detected in most of plants under abiotic stresses (Barunawati, Maghfoer, Kendarini, & Aini, 2016; Hayat *et al.*, 2012; Yaish, 2015), including under excessive soil water content or oxygen deficiency. The proline content during the first 12 days of the SWT was significantly increase

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on the plants exposed to SWT_{-15} , but negligible of those under SWT_{-10} and SWT_{-5} . Enhance in proline accumulation in plants under shallower water table was due to stress condition experienced by plant. The differences in proline content among the SWT treated plants were detected also at 18 DoT and at 4 DoR (Table 4). Significant differences in proline content at 4 DoR indicated that snap bean plant used in this study had not fully recovered even 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} in every

observation period during and after SWT treatments (Table 4). These consistent differences were similar between primary and secondary growth parameters presented in Table 1 and 2. Since SPAD value has been proven as reliable estimation of leaf chlorophyll content (Donnelly, Yu, Rehberg, Meyer, & Young, 2020); therefore, it can be expected that shallower water table position decreased leaf chlorophyll content and reduced photosynthetic capacity of snap 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.

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	18 DoT	4 DoR
	<i>Relative Leaf Water Content (%)</i>		
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
LSD 0.05	1.38	4.39	-

Remarks: ^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 $p \leq 0.05$

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	18 DoT	4 DoR
	<i>Proline Content ($\mu\text{mol.g}^{-1}$)</i>		
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 b	0.187±0.015 b	0.316±0.118 a
LSD 0.05	0.012	0.006	0.001
	<i>SPAD value</i>		
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
LSD	0.52	0.79	1.61

Remarks: ^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 $p \leq 0.05$

Role of ALA in Overcoming Stress due to SWT Exposure

Without ALA application, root length, root fresh weight and shoot fresh weight in snap bean were severely decreased under shallower water table conditions (SWT₋₅ and SWT₋₁₀). Meanwhile, application of ALA at 4 days prior to SWT treatment (at 14 DAP) had effectively counteract the negative effects on the plants treated by SWT₋₅ and SWT₋₁₀ as indicated by longer roots in ALA treated plants. However, application of ALA at later stages (T₂₈ and T₃₈) were not 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 recognized as one limiting factor to plant growth and development (Lawson & Matthews, 2020) since it created

partially hypoxic condition within rhizosphere. In favorable condition, snap bean was responsive to fertilizer application (Santosa, Maghfoer, & Tarno, 2017) but bean plant was less responsive under SWT exposure. 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 (Rodriguez-Dominguez & Brodribb, 2020). In this study, the root and shoot growth inhibitions of SWT treated bean plants was reduced by ALA application at 4 days before SWT treatment. (Table 5). The successful ALA application on reducing the negative effects of abiotic stress was also reported on the plants under salt stress (Anjum *et al.*, 2016), hypoxia (An, Qi, & Wang, 2016), and low temperature regime (Anwar, Yan, Liu, Li, & Yu, 2018).

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	Without ALA	With ALA			Control vs Exposure to SWT without ALA	Exposure to SWT With vs Without ALA application ^z		
		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)
<i>Root Length (cm)</i>								
SWT ₋₅	25.2	42.8	25.8	32.7	-40.6 ^y *	17.7 *	0.7 ns	7.5 ns
SWT ₋₁₀	35.9	50.7	46.1	49.1	-29.8 *	14.8 *	10.2 ns	13.2 ns
SWT ₋₁₅	61.5	59.0	49.7	60.7	-4.3 ns	-2.5 ns	-11.8 ns	-0.8 ns
Control	65.7							
<i>Root Fresh Weight (g)</i>								
SWT ₋₅	25.8	54.8	33.1	46.6	-23.1 *	29.0 *	7.3 ns	20.8 ns
SWT ₋₁₀	23.5	65.2	27.6	56.2	-25.4 *	41.7 *	4.1 ns	32.7 *
SWT ₋₁₅	84.2	109.3	85.7	91.9	35.2 *	25.2 *	1.6 ns	7.7 ns
Control	48.9							
<i>Shoot Fresh Weight (g)</i>								
SWT ₋₅	72.6	79.7	52.5	80.8	-61.8 *	7.1 ns	-20.1 ns	8.1 ns
SWT ₋₁₀	108.7	138.7	110.7	115.6	-25.7 *	30.0 *	2.0 ns	6.9 ns
SWT ₋₁₅	132.9	158.6	152.3	160.2	-1.6 ns	25.7 *	19.5 ns	27.3 *
Control	134.5							

Remarks: ^z) T₁₄ = ALA application at 4 days prior to SWT exposure (at 14 DAP), T₂₈ = ALA application at midpoint during the SWT exposure period (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

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Improvement of roots and shoot growth under SWT stress by ALA application is associated with enhancement in water and nutrient uptake and their transport. Plants exposed to hypoxia shift their metabolism pathways from oxidative phosphorylation to anaerobic fermentation to maintain ATP production (Liu *et al.*, 2020). Anaerobic fermentation was activated by ALA without resulting root injury (An, Qi, & Wang, 2016). The significant increase of roots and shoot growths after ALA application at 38 DAP was associated with reoxygenation of rhizosphere after the termination of SWT treatments. Availability of oxygen in soil supports aerobic respiration and enhances root activities 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 snap bean plant exposed to SWT₋₅ and SWT₋₁₀, compared with control plant (Table 6). Under SWT₋₁₀ condition, ALA application at 14 DAP retained TLA, SLA and LAR comparably to non-SWT treated plants at 100.9%, 109.7% and 83.6%, respectively. However, later ALA application at 38 DAP was less effective with the values of TLA (69.3%), SLA (91.5%) and LAR (60.3%) in plant exposed to SWT₋₁₀.

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	Without ALA	With ALA			Control vs Exposure to SWT without ALA	Exposure to SWT With vs Without ALA application ^z		
		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)
<i>Total Leaf Area (dm²)</i>								
SWT ₋₅	7.05	13.74	6.99	11.45	-23.98 ^y *	-0.06 ^{ns}	-0.06 ^{ns}	4.40 ^{ns}
SWT ₋₁₀	11.01	31.20	16.13	24.87	-20.01 *	5.12 *	5.12 ^{ns}	13.86 *
SWT ₋₁₅	25.91	39.33	34.18	36.48	-5.11 ^{ns}	8.27 *	8.27 ^{ns}	10.57 ^{ns}
Control	31.02							
<i>Specific Leaf Area (dm²/g)</i>								
SWT ₋₅	1.87	2.51	1.89	2.51	-1.37 ^{ns}	0.65 ^{ns}	0.02 ^{ns}	0.64 ^{ns}
SWT ₋₁₀	1.62	3.39	2.78	3.09	-1.61 *	1.77 *	1.16 ^{ns}	1.48 *
SWT ₋₁₅	3.39	3.57	3.85	3.77	0.16 ^{ns}	0.18 ^{ns}	0.46 ^{ns}	0.39 ^{ns}
Control	3.23							
<i>Leaf Area Ratio (cm²/g)</i>								
SWT ₋₅	47.1	77.0	41.9	63.3	-71.9 *	29.9 ^{ns}	-5.2 ^{ns}	16.2 ^{ns}
SWT ₋₁₀	45.4	106.9	76.5	89.8	-73.6 *	61.5 *	31.1 ^{ns}	44.4 *
SWT ₋₁₅	86.5	112.3	106.6	98.8	-32.5 ^{ns}	25.8 ^{ns}	20.1 ^{ns}	12.3 ^{ns}
Control	119.0							

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

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TLA, SLA and LAR were higher in pre-treated bean plant with ALA at 14 DAP prior to SWT exposure. Akram & Ashraf (2013) reported that foliar application of ALA gave positive effects 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, 2018) and promoted plant growth and development (Wu, Liao, Dawuda, Hu, & Yu, 2019).

ALA application at end of SWT treatment (38 DAP) contributed to support the regrowth of the plants during recovery period. These phenomena referred to the significant increase of TLA, SLA and LAR during the first four days after SWT treatment was terminated. The higher TLA, SLA, and LAR in ALA treated compared to non-treated plants indicated that ALA had contributed in enhancing

recovery from SWT exposure.

Except after 18 days on SWT-5, there was no significant difference on relative leaf water content between treated and control plants (Table 7). ALA application at pre-SWT treatments (14 DAP) at SWT₋₁₀ induced higher RLWC in ALA-treated bean plant at 12 DoT. Higher RLWC was also observed on ALA-treated SWT₋₅ plants at 28 DAP. In general, however, there was no significant difference in RLWC among ALA treated plants under various SWT treatments 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, Modarres Sanavy, 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).

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	With-out ALA	With ALA			Control vs Exposure to SWT without ALA	Exposure to SWT With vs Without ALA application ^y		
			14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)
<i>Relative leaf water content (%)</i>									
12 DoT	SWT ₋₅	77.3	82.4	82.8	-	-1.3 ns	5.0 ns	5.5 *	-
	SWT ₋₁₀	77.2	82.6	80.3	-	-1.4 ns	5.4 *	3.1 ns	-
	SWT ₋₁₅	77.3	78.4	77.2	-	-1.4 ns	1.1 ns	-0.1 ns	-
	Control	78.6							
18 DoT	SWT ₋₅	63.7	71.1	58.2	-	-16.7 *	7.4 ns	-5.5 ns	-
	SWT ₋₁₀	72.1	74.7	77.3	-	-8.2 ns	2.6 ns	5.1 ns	-
	SWT ₋₁₅	77.5	80.1	77.2	-	-2.8 ns	2.5 ns	-0.3 ns	-
	Control	80.3							
4 DoR	SWT ₋₅	66.8	70.3	67.4	69.7	-5.9 ns	3.5 ns	0.6 ns	2.9 ns
	SWT ₋₁₀	65.4	70.9	72.1	67.2	-7.3 ns	5.6 ns	6.7 ns	1.8 ns
	SWT ₋₁₅	67.9	71.9	66.9	71.1	-4.8 ns	4.0 ns	-1.0 ns	3.2 ns
	Control	72.7							

Remarks: ^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)

In this study, RLWC maintenance in SWT-exposed plants (Table 7) was at cost of TLA reduction (Table 6) to balance the limited water uptake by partially damaged root system (Table 5). Ability to maintain RLWC in bean plants was more as a survival mechanism since the subsequent visible growth was not detected. There were smaller new leaves developed but, at the same time, there also some older leaves fallen off. The plants were allowed to recover after 20 days from SWT exposure, and the plants had shifted into reproductive stages; therefore, development of

flowers and pods were also severely inhibited.

Effects of ALA Application with Reference to Period of SWT Exposure

Proline 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 at 12 and 18 DoT were significantly higher in SWT-treated than control plants. However, proline content significantly decreased in the SWT-exposed plants after 4 days of recovery (Table 8).

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	With-out ALA	With ALA			Control vs Exposure to SWT without ALA	Exposure to SWT With vs Without ALA application ^y		
			14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)
<i>Proline content (μmol.g⁻¹)</i>									
12 DoT	SWT ₋₅	0.29	0.33	0.26	-	0.05 *	0.03 *	-0.03 *	-
	SWT ₋₁₀	0.31	0.32	0.29	-	0.07 *	-0.01 ns	-0.02 ns	-
	SWT ₋₁₅	0.26	0.21	0.24	-	0.02 ns	-0.05 *	-0.03 *	-
	Control	0.25							
18 DoT	SWT ₋₅	0.21	0.18	0.16	-	0.02 *	-0.03 *	-0.05 *	-
	SWT ₋₁₀	0.24	0.20	0.22	-	0.05 *	-0.04 *	-0.02 *	-
	SWT ₋₁₅	0.21	0.17	0.20	-	0.02 *	-0.03 *	-0.01 ns	-
	Control	0.19							
4 DoR	SWT ₋₅	0.18	0.32	0.23	0.22	-0.13 *	0.15 *	0.06 *	0.04 *
	SWT ₋₁₀	0.24	0.25	0.21	0.23	-0.06 *	0.01 ns	-0.03 *	-0.02 *
	SWT ₋₁₅	0.20	0.24	0.26	0.45	-0.10 *	0.04 *	0.06 *	0.25 *
	Control	0.30							

Remarks: ^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)

ALA application at 14 DAP significantly increased and decreased the proline content in SWT_{-5} and SWT_{-15} plants, respectively, at 12 DoT. These 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} . During recovery period, application of ALA at pre- and during SWT exposures did not contribute to stress alleviation as indicated by higher proline content at ALA-treated than non-treated plants. Therefore, the plants recovery is presumably related with the increase of oxygen availability after SWT treatment rather than the direct effect of ALA (Table 8). Similarly, Yaish (2015) also reported that proline was not the major factor in the mechanism leading to stress tolerance. Overall, ALA application prior to and during SWT exposure effectively declined proline content within the plant compared to those without ALA application. It was related to redirection of glutamic acid pathway in chlorophyll and heme synthesis. Since ALA was the precursor of proline and glutamic acid, so it suppressed proline synthesis and accumulation (Averina, Gritskevich, Vershilovskaya, Usatov, & Yaronskaya, 2010). Enhancing in chlorophyll and heme synthesis by ALA could induce activity of antioxidant enzymes (Xiong, Wang, Tan, Zhang, & Naeem, 2018). The antioxidant enzymes played an important role in declining cell damage caused by Reactive Oxygen Species (ROS) when plants were

experiencing abiotic stresses (Anwar, Yan, Liu, Li, & Yu, 2018), such as under SWT exposure.

The correlation between proline accumulation and abiotic stress tolerance in plants was not always apparent. Accumulation of proline in the leaves has been believed as 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, Modarres Sanavy, 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 (An, Qi, & Wang, 2016; Manafi, Modarres Sanavy, Aghaalikhani, & Dolatabadian, 2015). Thus, the decline in proline content under abiotic stress by ALA application was a symptom of stress tolerance (Liu, Nguyen, Ueda, & Saneoka, 2014).

SWT exposure consistently and significantly reduced SPAD value of SWT treated compared to control plants. The applications of ALA at pre- (14 DAP), during (28 DAP), and end of (38 DAP) SWT exposures were effectively increased SPAD values (Table 9).

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	Without ALA	With ALA			Control vs Exposure to SWT without ALA	Exposure to SWT With vs Without ALA application ^y		
			14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)		14 DAP (T ₁₄)	28 DAP (T ₂₈)	38 DAP (T ₃₈)
<i>SPAD value</i>									
12 DoT	SWT_{-5}	43.1	45.5	43.8	-	-4.8 *	2.4 *	0.7 ns	-
	SWT_{-10}	44.6	45.7	43.0	-	-3.2 *	1.0 ns	-1.6 ns	-
	SWT_{-15}	43.1	46.1	46.7	-	-4.8 *	3.0 *	3.6 *	-
	Control	47.9							
18 DoT	SWT_{-5}	38.4	39.6	38.9	-	-5.8 *	1.2 ns	0.5 ns	-
	SWT_{-10}	41.3	43.3	45.4	-	-2.9 *	2.0 *	4.1 *	-
	SWT_{-15}	39.3	42.7	44.9	-	-4.9 *	3.4 *	5.6 *	-
	Control	44.2							
4 DoR	SWT_{-5}	34.4	36.8	33.4	34.3	-9.0 *	2.4 ns	-1.0 ns	-0.1 ns
	SWT_{-10}	35.8	37.3	36.4	37.6	-7.6 *	1.4 ns	0.6 ns	1.7 ns
	SWT_{-15}	36.1	39.9	38.9	38.8	-7.3 *	3.8 *	2.8 ns	2.7 ns
	Control	43.4							

Remarks: ^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)

Since SPAD value has been used for predicting leaf chlorophyll content (Donnelly, Yu, Rehberg, Meyer, & Young, 2020). These findings indicated that exogenous applied ALA induced chlorophyll synthesis under SWT condition. ALA is the precursor of heme in photosynthetic pigments. As a metabolic intermediate in higher plants, ALA directly associated with chlorophyll and carotenoids biosynthesis (Akram & Ashraf, 2013; An, Qi, & Wang, 2016; Anjum *et al.*, 2016). Thus, ALA contributed in increasing plant growth under abiotic stress (Wu, Liao, Dawuda, Hu, & Yu, 2019).

There were interactions between SWT exposure and time of ALA application on proline content 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 SWT₋₅ treated plants and ALA applied at 14 DAP (Fig. 1A). In

this first case, measurement of proline was carried out 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 on SWT₋₁₀ treated plants 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 observed in plant exposed SWT₋₅ treatments 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 was clear from these three cases that snap bean plant was 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 retained the growth as time progresses.

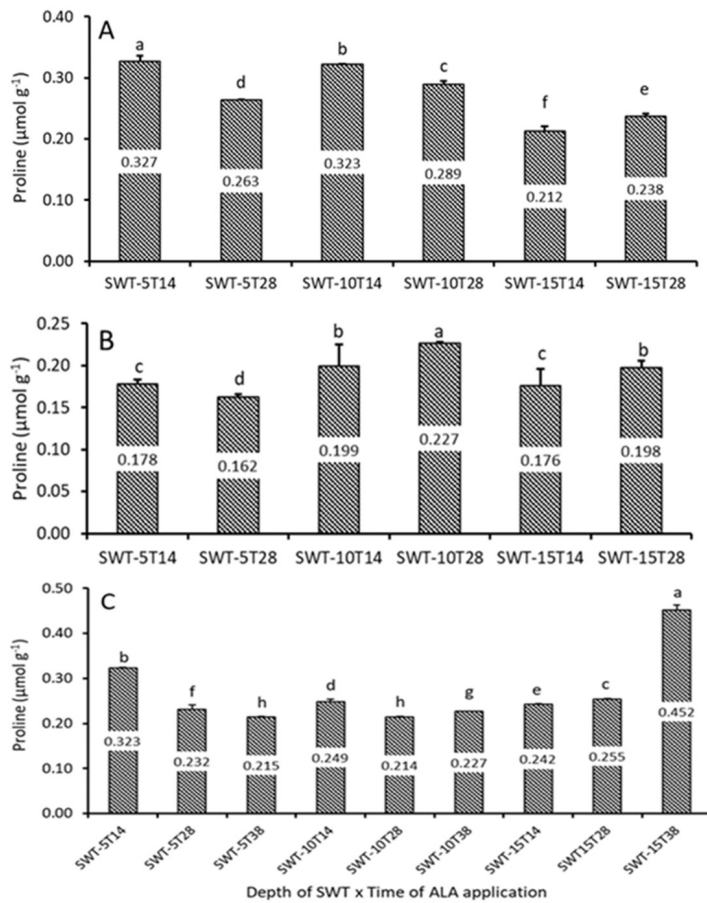


Fig. 1. Proline contents measured after 12 days (A) and 18 days of SWT exposure (B), and after 4 days of recovery (C) in bean plants subjected to different water table regimes of -5 cm (SWT₋₅), -10 cm (SWT₋₁₀) and -15 cm (SWT₋₁₅) and different time of ALA application at 14 DAP (T₁₄), 28 DAP (T₂₈), and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

CONCLUSION

SWT exposure affected root and shoot growths, leaf water status, proline content, and SPAD value in snap bean plant. The shallowest water table (SWT₅) treatment gave the most severe on plant growth. Snap bean used in this study exhibited ability to recover after SWT exposure was terminated. The effectiveness of ALA application in reducing negative effects of SWT exposure was depended on depth of SWT exposure and timing of ALA application. Application of ALA prior to SWT exposure exhibited better results in counterbalancing stress than during or at end of SWT exposure. ALA application gave less effects on plant recovery after SWT exposure.

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