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Foliar application of 5-aminolevulinic acid for offsetting unfavorable effects of shallow water table on growth and yield in snap bean (*Phaseolus vulgaris*)

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Abstract

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Shallow water table (SWT) limits volume of aerobic substrate for roots to grow in most terrestrial plants, including snap bean (*Phaseolus vulgaris*). Aminolevulinic acid (ALA) has been reported to increase plant tolerance to various abiotic stresses. Aim of this study was to evaluate effectiveness of foliar applied ALA in alleviating negative effects of SWT exposure in snap bean plants. In this study, each seed of snap bean was directly sown in 25 cm diameter pot filled with soil-manure mix. Foliar applications of ALA at rates of 0, 0.15, 0.30, and 0.45 mM were applied at 14 days after sowing. Three days after ALA application, the plants were exposed to SWT for 20 consecutive days in controllable experimental pools; therefore, water table can be set and maintained at 10 cm below substrate surface. Results of this study indicated that SWT exposure decreased SPAD value, root length, and pod fresh weight. These decreases cannot be counterbalanced by ALA applications at rates up to 0.45 mM. However, application of ALA at rate of 0.30 mM or higher was able to compensate for potential reduction in number of harvested pods. Leaf water status, SPAD value, and proline content were not significantly affected by applications of ALA at rate up to 0.45 mM. After recovery period, root biomass increased despite root elongation was restricted during SWT exposure. Relative leaf expansion rate (RLER) at early leaf development was sensitive to SWT exposure. In general, rate of ALA application up to 0.45 mM was too low for overcoming negative impact of shallow water table in snap bean plants.

Keywords: abiotic stress; partial saturation; *Phaseolus vulgaris*; riparian wetland; root growth; stress recovery
Abbreviations: ALA – 5-aminolevulinic acid; DAP – day after planting; RLER – relative leaf expansion rate; RLWC – relative leaf water content; RWR – root weight ratio; SLFW – specific leaf fresh weight; SLWC – specific leaf water content; SRR – shoot/root ratio; SWR – shoot weight ratio; SWT – shallow water table; TLA – total leaf area

Introduction

Although challenges in intensifying food production at riparian wetland have been well identified; yet creating effective solution for smallholder farmers to increase food production at this sub-optimal land is still far from being settled. The hardest problem to solve was predicting occurrence of flooding and managing the floodwater to avoid cultivated crops from direct exposure to SWT, water logging, or partial to full submersion.

SWT could reduce growth and yield of sensitive crops. This undesirable condition is more likely to be encountered during transitional period from dry to rainy season and *vice versa* at the riparian wetland. Only few crops have inherent adaptation to this undesirable condition. However, there are some plant growth regulators that could increase tolerability of crops to abiotic stresses. One of the growth regulators is 5-aminolevulinic acid, abbreviated as ALA (Akram & Ashraf, 2013; Zhang et al., 2015; An et al., 2016; Aksakal et al., 2017).

Snap bean plant has been identified as a non-suitable crop for wetlands with risks of SWT or flooding condition. It was reported that SWT decreased growth and yield in snap bean plant (Pociecha, 2013). Our previous study (Lakitan et al., 2018) indicated that snap bean was tolerant to SWT at depth of 15 cm below soil surface or deeper; however, SWT at depth of 10 cm or shallower caused serious damages to snap bean.

Objective of this research was to evaluate effectiveness of foliar application of ALA at rate up to 0.45 mM in preventing growth suppression and yield reduction in snap bean due to SWT exposure, either by altering morphological characteristics and/or physiological processes.

Materials and Methods

This experiment was carried out at outdoor research facilities at Jakabaring, Palembang, Indonesia, from November 2017 to February 2018, during rainy season. Average diurnal air temperature was 29°C and relative humidity was higher than 80%.

Biomaterial and chemical used

Semi-determinate bushy snap bean variety of PV-072 was used in this experiment. Size of pots used was 25 cm in diameter and 30 cm in height. The pots were filled with mixed substrate of soil and manure with ratio 3:2 (v/v). The substrate was treated with mixed of selected isolates of *Streptomyces* sp., *Trichoderma* sp., and *Geobacillus* sp. for controlling soil-borne phytopathogens and for enhancing decomposition of organic matters. NPK fertilizer at rate of 5.8 gram per pot was also added to the mix. An aqueous solution of ALA was used for increasing tolerability of snap bean to SWT exposure for 20 consecutive days.

Research protocols

Hydro-priming was applied to snap bean seeds prior to sowing. Two seeds were planted in each pot, but only one seedling was kept at 7 days after sowing. Tip of primary stem was pinched off after the third trifoliate leaf had fully developed, i.e. for eliminating apical dominance and inducing development of lateral branches. NPK fertilizer was applied three times, i.e. prior to seed sowing, at 15 days and 30 days after sowing.

Foliar application of ALA was done at 6.00-7.30 am on the 14th day after sowing. ALA was sprayed to the whole plant canopy, until both sides of the leaves were entirely wet, as practiced by Zhang et al. (2015). Each plant was isolated during application of ALA. Treatments consisted of (1) control plants, without ALA application and did not exposed to SWT; (2) exposed to SWT but without ALA application (A_0); (3) exposed to SWT and applied with ALA at 0.15 mM (A_{15}); (4) exposed to SWT and applied with ALA at 0.30 mM (A_{30}); and (5) exposed to SWT and applied with ALA at 0.45 mM (A_{45}). Each treatment replicated 3 times and each replication consisted of 5 plants.

SWT treatment was commenced at 18 and terminated at 38 DAP. After termination of SWT treatment, the plants were allowed to recover. The SWT treatment was done by filling the experimental pool (4 m long × 2 m wide × 0.5 m deep) with water to the depth of 15 cm. Since height of the substrate within pots was 25 cm; therefore, water table position was set at 10 cm below surface of growing substrate. This shallow water table position was constantly maintained during treatment by controlling height of water surface within the pool. Average water electrical conductivity was 0.21 mS.

Leaf proline content was analyzed based on modified Bates protocol (Bates et al., 1973). In our procedure, filtrate was homogenized using toluene 0.5% and temperature for heating the mixture was 95°C. Leaf proline contents were measured at 17, 31, and 44 DAP. SPAD value was measured using (Konica-Minolta Chlorophyll Meter SPAD-502Plus) at 17, 25, 31, 35, and 44 DAP.

Data collection

Agronomic traits and growth analysis were directly measured, estimated using reliable regression model, or calculated based on directly measured primary data. Non-destructive leaf area was estimated according to Lakitan et al. (2017). Relative leaf expansion rate (RLER), relative leaf water content (RLWC), specific leaf fresh weight (SLFW), specific leaf water content (SLWC), and total leaf area (TLA) were calculated based on related primary growth parameters (Meihana et al., 2017; Widuri et al., 2017; Lakitan et al., 2018).

RLER was daily calculated based on non-destructive measurements on the same leaves, repeated at three different periods as the plants grow. Measurements were started with young unfolded leaves with midrib length less than 2 cm; then, daily measured until each sampled leaf had reached its full size. Daily leaf dimension measurements for RLER calculation were done at three different periods, i.e. during pre-to-early, middle, and late-to-recovery from SWT exposure, each initiated at 14, 23, and 34 DAP, respectively. RLER was daily calculated, started on the next day after initiation of leaf measurements.

RLWC, SLFW and SLWC were measured at 17, 31 and 44 DAP. Meanwhile, during vegetative growth stage, fresh weight and dry weight were partitioned into stem, root, and leaf. Cumulative number and fresh weight of harvested pods were collected during 16 days of harvesting period, started at 39 DAP and terminated at 54 DAP. Since snap bean used in this study is intended for consumption as vegetable; then, the pods were harvested at marketable size while they were young and easy to snap.

Data analysis

Since substrate used, pot size, water used (circulate within a single experimental pool), and microclimate were uniform; therefore, collected data were analyzed based on the Completely Randomized Design (CRD). Analysis of variance was applied for testing significant effect of treatments. Mean comparisons were further tested using the Least Significant Difference (LSD) test at $p \leq 0.05$.

Results

Exposure to SWT treatment for 20 days in snap bean plants opened opportunity to evaluate differences in RLER during period of pre-to-early SWT exposure, in the middle of SWT exposure period, and during late-to-recovery from SWT exposure. The obvious difference among stages was observed on first three days after young leaves were unfolded. At the pre-to-early SWT period, RLER on the first day was around 2 cm^2cm^{-2}, dropped to slightly above 1 cm^2cm^{-2} during the middle of SWT exposure, and further decline to about 0.5 cm^2cm^{-2} at the late-to-recovery period (Figure 1). At later leaf development, RLERs were almost similar in all three periods of SWT exposure. Within each SWT period, RLER was not significantly affected by ALA application at rates up to 0.45 mM. Also, there was no RLER difference between plants exposed to SWT but not treated with ALA (A_0) and plants not treated with both SWT and ALA (Control).

RLWC, SLFW, and SLWC were not affected by ALA application at rates up to 0.45 mM (Table 1). However, RLWC and SLFW increased (4.3 and 1.8 percent, respectively) while SLWC decreased (2.5 percent) during middle of SWT exposure. During the late-to-recovery period, all of RLWC, SLFW, and SLWC were dropped to levels 15.1, 9.6, and 19.3 percent lower than that during pre-to-early period, respectively.

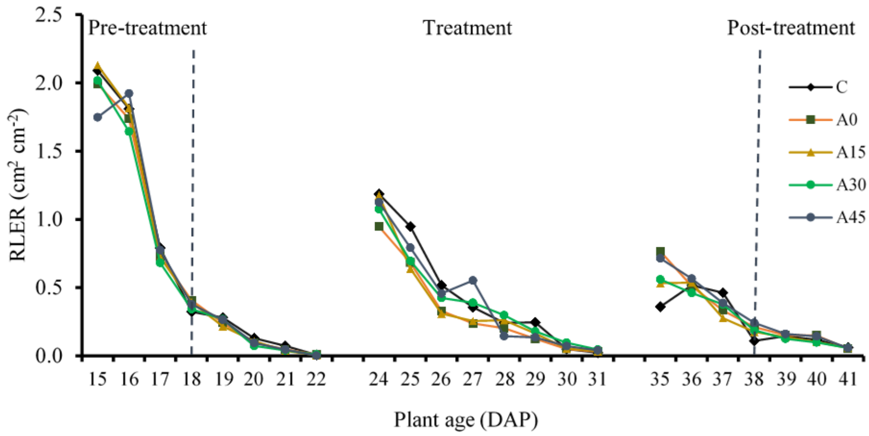

Fig. 1. Relative leaf expansion rate at pre-, during, and post-treatments of shallow water table in snap bean (*Phaseolus vulgaris*) plant

Table 1. Effect of ALA application rates on relative leaf water content, specific leaf fresh weight, and specific leaf water content measured at different periods of shallow water table exposure in snap bean (*Phaseolus vulgaris*) plants

ALA application rate (mM)	Shallow water table treatment		
	Pre-to-early period (17 DAP)	Mid-SWT period (31 DAP)	late-to-recovery period (44 DAP)
	Relative leaf water content (%)		
Control	85.35±2.17	88.24±2.21	75.04±8.84
0.00	83.41±5.96	85.59±3.06	73.20±3.16
0.15	80.30±7.64	83.69±9.16	70.11±5.48
0.30	85.59±0.50	89.07±3.36	66.96±0.70
0.45	84.33±2.95	89.88±0.80	72.77±7.18
F-value	0.64^{ns}	0.91^{ns}	0.87^{ns}
	Specific leaf fresh weight (mg cm^{-2})		
Control	25.20±2.20	23.39±1.97	21.17±2.58
0.00	22.12±1.28	22.76±0.89	19.15±0.30
0.15	23.62±5.88	22.40±0.48	19.38±4.47
0.30	20.62±5.15	22.71±1.52	20.68±1.56
0.45	21.35±0.16	21.26±0.27	19.81±2.41
F-value	0.75^{ns}	1.27^{ns}	0.32^{ns}
	Specific leaf water content (mg cm^{-2})		
Control	23.10±2.29	21.43±1.95	17.26±2.57
0.00	20.30±0.67	19.90±0.82	15.82±0.49
0.15	21.71±5.28	19.99±0.36	15.91±4.02
0.30	19.22±5.10	20.07±1.35	17.05±1.22
0.45	19.50±0.41	18.79±0.38	16.38±2.27
F-value	0.22^{ns}	2.02^{ns}	0.66^{ns}

Data are presented as mean±standard deviation. The LSD test was not performed since F-value of all treatments was not significant

SPAD values measured at 17, 31, 35, and 44 DAP were significantly affected by exposure to SWT exposure in snap bean plants, with exception at 25 DAP; but there was no significant difference in SPAD value among plants treated with ALA at rates up to 0.45 mM. Meanwhile, leaf proline contents were not affected both by SWT exposure and rate of ALA application (Table 2).

Presence of water saturated layer in growing substrate below water table significantly limited root elongation; as a result, roots of snap bean plants exposed to SWT exposure were significantly shorter than those of controlled plants. Shoot/root ratio (SRR), root weight ratio (RWR), and shoot weight ratio (SWR) were not significantly different if they were calculated based on fresh weight; however, the differences were significant if they were calculated based on dry weight (Table 3). Differences between roots measured based on fresh and dry weights indicated that roots water content under SWT condition was much higher than that of untreated control plants.

Total leaf area (TLA) was consistently lower in SWT treated plants compared to that of control plants; even though, the difference was not statistically significance. Accumulative number of harvested pods in plants exposed to SWT exposure and treated with low (0.15 mM) ALA application rate was significantly lower than that of control plants; whereas, those treated with higher ALA rates (0.30 and 0.45 mM) were statistically comparable to control plants. All SWT treated plants produced lower pod weights than control plants did (Table 4). More significant difference in pod weight than in cumulative number of harvested pods indicated that there was also difference in pod size between SWT treated and untreated plants.

Discussion

Early RLER as indicator of stress due to SWT

The fastest leaf enlargement process in snap bean plants occurred during the first 3 days after young leaf had been unfolded, as indicated by the highest RLER during this 3-day period. The period during leaf development was the most sensitive to abiotic stresses such as SWT exposure (Figure 1). Lakitan et al. (2018) previously found that within 3–4 days after the leaf was unfolded, the leaf had reached about 70–90 percent of its final size in snap bean plnat; however, it took few days longer to reach the final/mature leaf size in chili pepper (Widuri et al., 2017) and tomatoes (Meihana et al., 2017).

The first 3-day RLER decreased by half during middle of SWT exposure period compared with during pre-to-early period. RLER further decreased during late-to-recovery period. RLER at and after 4 days of leaf development did not significantly different among three periods of SWT exposure. This finding leads to argument that RLER during early leaf development is the best time for studying abiotic stress in snap bean plant. It requires further studies to prove if this argument is also valid for other plants and/or exposed to different abiotic stresses.

Effect of foliar applied ALA depends on concentration used

Leaf water status, as indicated by RLWC, SLFW, and SLWC, was not significantly affected by SWT exposure and ALA application (Table 1). Similarly, the SWT exposure and ALA application did not significantly affect leaf proline content. However, SPAD value was affected by SWT exposure but not by ALA application at rate up to 0.45 mM (Table 2).

Leaf water status was not affected albeit root length was

Table 2. Effect of ALA application rates on SPAD value and leaf proline content measured at pre-, during, and post-treatments of shallow water table in snap bean (*Phaseolus vulgaris*) plants

ALA application rate (mM)	Pre-treatment (17 DAP)	Shallow water table period			Post-treatment (44 DAP)
		Early period (25 DAP)	Middle period (31 DAP)	Late period (35 DAP)	
	SPAD Value				
Control	34.87±0.29 a	34.93±0.03	36.38±0.27 a	36.37±1.21 a	36.51±1.60 a
0.00	33.74±0.62 b	34.13±0.38	33.51±1.15 b	31.47±0.71 b	27.06±1.42 b
0.15	33.77±0.32 b	34.31±0.43	34.81±0.79 b	33.39±2.90 b	27.81±1.27 b
0.30	33.67±0.38 b	35.76±1.35	34.60±0.33 b	31.53±0.78 b	27.62±0.88 b
0.45	33.59±0.47 b	34.36±0.92	34.31±1.00 b	32.06±1.35 b	27.66±1.98 b
F-value	4.55*	2.19^{ns}	5.24*	4.95*	22.33*
	Leaf proline content (μmol/g)				
Control	0.222±0.020	–	0.224±0.017	–	0.243±0.022
0.00	0.216±0.003	–	0.239±0.003	–	0.264±0.008
0.15	0.247±0.044	–	0.239±0.017	–	0.293±0.015
0.30	0.213±0.001	–	0.243±0.060	–	0.273±0.032
0.45	0.219±0.005	–	0.225±0.002	–	0.272±0.009
F-value	0.274^{ns}	–	2.285^{ns}	–	1.119^{ns}

Data are presented as mean±standard deviation. Means followed by the same letter within each column of each parameter are not significantly different at LSD level 5%. The LSD test was only performed if F-value of ALA treatment was significant (*)

Table 3. Effect of ALA application rates on root length, shoot-root ratio, root weight ratio, and shoot weight ratio based on fresh and dry weight in snap bean (*Phaseolus vulgaris*) plants after shallow water table exposure

ALA application rate, mM	Root length, cm	Shoot-root ratio	Root weight ratio	Shoot weight ratio
	Fresh weight			
Control	55.2± 9.48 a	7.43±0.53	0.10±0.01	0.76±0.02
0.00	33.3±10.61 b	5.82±1.93	0.15±0.06	0.81±0.04
0.15	35.8± 4.16 b	5.15±1.23	0.16±0.03	0.81±0.03
0.30	33.2±10.25 b	5.97±3.02	0.16±0.08	0.78±0.09
0.45	38.8± 3.28 b	5.11±3.09	0.17±0.10	0.69±0.14
F-value	3.80*	0.55^{ns}	0.57^{ns}	1.12^{ns}
	Dry weight			
Control	–	12.54±4.90 a	0.07±0.022 a	0.80±0.046 a
0.00	–	6.62±1.19 b	0.13±0.019 b	0.85±0.029 a
0.15	–	6.33±0.54 b	0.13±0.008 b	0.83±0.018 a
0.30	–	7.22±0.66 b	0.12±0.008 b	0.83±0.019 a
0.45	–	5.37±0.70 b	0.14±0.024 b	0.75±0.027 b
F-value	–	4.53*	7.67*	5.15*

Data are presented as mean±standard deviation. Means followed by the same letter within each column of each parameter are not significantly different at the LSD level 5%. The LSD test was only pursued if F-value of ALA treatment was significant (*)

significantly shorter due to water-saturated layer at bottom part of growing substrate (Table 3). Roots of snap bean plant did not have ability to grow into hypoxic zone below water table as previously reported (Lakitan et al., 2018). However, the roots immediately regrew after SWT exposure was terminated. Shorter roots with relatively massive branching were able to absorb sufficient water from smaller volume but moist substrate. This is more likely the case for explaining why leaf water status is not affected by SWT exposure.

Many studies reported that proline accumulated during abiotic stress in many plants (Ben Rejeb et al., 2015; Khan et al., 2015; Per et al., 2017). No significant increase or accumulation in leaf proline content may indicated that the snap bean plants in this study have not been experiencing stress condition although they were exposed to 20 consecutive days to SWT exposure, in both with (A_{15}, A_{30}, and A_{45}) or without (A_0) application of ALA.

Table 4. Total leaf area, number of pods, and pod fresh weight per plant in snap bean (*Phaseolus vulgaris*) plant exposed to20 days of shallow water table and treated with different ALA application rates

ALA application rate, mM	Total leaf area, dm^2	Number of pods	Pod fresh weight, g
Control	88.86±11.73	28.67±5.03 a	123.70±28.71 a
0.00	57.01±18.51	17.11±1.68 bc	62.13±20.49 b
0.15	64.44±13.50	13.00±3.28 c	45.68±15.29 b
0.30	71.09±11.60	23.33±1.57 a	74.07±15.91 b
0.45	63.30±15.39	23.22±4.68 ab	54.78±13.73 b
F-value	2.16^{ns}	8.79*	7.35*

Data are presented as mean±standard deviation. Means followed by the same letter within each column are not significantly different at the LSD level 5%. The LSD test was only performed if F-value of ALA treatment was significant (*)

Application of ALA was frequently reported to enhance proline accumulation (Chen et al., 2017; Wang et al., 2018; Xiong et al., 2018). Insignificant effect of ALA application in this study was suspected due to low ALA concentration applied or the plants have not yet experiencing stress. For comparison, Akram et al. (2018) applied ALA at concentration of 0.895 mMin canola (*Brassica napus*) under drought stress condition and Chen et al. (2017) used even higher concentration of ALA at 1.25 mM in watermelon seedlings under salt stress condition.

ALA is one of the key precursors involved in chlorophyll biosynthesis (Kosar et al., 2015). Exogenous foliar application of ALA also reported to enhance photosynthetic enzyme activity, i.e. ribulose-1,5-bisphosphate (RuBP) carboxylase (Wang et al., 2018); increased chlorophyll content, net photosynthetic rate, and above ground biomass (Xiong et al., 2018); increased soluble protein, regulated nitrogen metabolism, and improved plant growth (Chen et al., 2017); and improved seedling growth (Kosar et al., 2015).

SPAD values strongly correlated with leaf nitrogen (Rorie et al., 2011; Vigneau et al., 2011), protein (Nakano et al., 2008; Venkatesh & Basu, 2011), and chlorophyll content (Coste et al., 2010; Jangpromma et al., 2010). Failure to compensate decrease in SPAD value in plants exposed to SWT exposure and application of ALA in this study (Table 2) was other evidence that ALA application at rates up to 0.45 mM was too low.

Increase in root branching during recovery period

Albeit application of ALA had been reported to increase above ground biomass of seedling or mature plant (Kosar et al., 2015; Chen et al., 2017; Xiong et al., 2018); however, in this study, the effect of ALA, specifically at rate up to 0.45 mM, was more pronounced on roots during recovery period after exposure to SWT treatment in snap bean plant, as indicated by increase in RWR and decrease of SRR and SWR (Table 3).

Root length in plants exposed to SWT was consistently shorter regardless of rates of ALA applied (Table 3). Increase in root dry weight with restricted root elongation was achieved by increase of root branching. This finding is consistent with result of our previous study (Lakitan et al., 2018).

ALA contribution on partial yield recovery

ALA at application rates up to 0.45 mM exhibited in significant effect during recovery period after SWT exposure. TLA in plants exposed to SWT exposure was consistently lower than that of control untreated plants. Although statistically TLAs among exposed and control plants were not significantly different (Table 4). It should be bear in mind, however, some of old leaves in SWT treated plants started to senescence earlier during late SWT period and had fallen off at time of measurement.

Total number of harvested pods was not significantly different between control plants and plants exposed to SWT exposure and treated with ALA at rates of 0.30 and 0.45 mM whereas plants untreated or treated with ALA at lower rate of 0.15 mM produced significantly lower number of pods (Table 4). This indicated that exposure to SWT significantly reduced number of pods but application of ALA at rate of 0.30 mM or higher was able to help the plants to prevent flower or pod abortion.

Weight of cumulatively harvested pods decreased significantly in all plants exposed to SWT exposure, including those treated with ALA at rates of 0.30 and 0.45 mM (Table 4). This finding implies that pod size in all plants exposed to SWT was smaller than that of control plants. This smaller pod size may become a concern since it affects market value of this commodity.

Conclusions

RLER at early leaf development was sensitive to SWT exposure. Leaf water status, SPAD value, and proline content were not significantly affected by application of ALA at rate up to 0.45 mM; instead, SPAD value and root length significantly decreased as snap bean plant exposed to SWT. Based on dry weight data, SRR significantly decreased and RWR significantly increased but SWR did not significantly affected by SWT exposure. Increase in root biomass while root elongation was restricted was achieved by increase in root branching. Application of ALA at rate of 0.30 mM or higher was able to compensate for potential reduction in number of harvested pods due to SWT exposure. However, decrease in pod weight and no significant difference in cumulative number of harvested pods indicated that size of pod was smaller in plant treated with ALA at rate of 0.30 mM or higher. In general, rate of ALA application up to 0.45 mM is too low for overcoming negative impact of SWT in snap bean plant.

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