

Betaine-rich Nano fertilizer improves growth parameters of *Zea mays* var. *saccharata* and *Arabidopsis thaliana* under salt stress

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Abstract

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Salinity or salt stress contributed to major problem in agriculture world. More than 50% of agriculture and irrigated land are affected by salt. This condition limits the available nutrients in the soil, induces physiological disorder and antioxidant dysfunction in plant, and hence influences plant growth and productivity. The relation of betaine and Nano fertilizer remained unknown. The present study aims to determine the effects of betaine-rich Nano fertilizer on growth parameters of sweet corn (*Z. mays* var. *saccharata*) and *A. thaliana* plants under salt stress. Plants were grown with a proper agronomic practices in a controlled plant growth room. Different salinity levels (S0:0 dS/m, S1:2 dS/m, S2:4 dS/m, S3:6 dS/m and S4:8 dS/m), two different concentrations of betaine (B0:0 mM and B1: 50 mM) and with or without the application of Nano fertilizer (N0: without Nano fertilizer and N1: with Nano fertilizer) were arranged as Randomized Complete Block Design with three replicates. Data for growth parameters (plant height, leaf number, root and leaf length and root:leaf length ratio) were recorded. In sweet corn plant, betaine-rich Nano fertilizer significantly increased plant height, number of leaves, root length, leaf length and root:leaf length ratio. In *A. thaliana*, betaine-rich Nano fertilizer showed significant results on plant height, root and leaf length and root:leaf length ratio, but insignificant effect on number of leaves. As a conclusion, this study suggests that application of betaine-rich Nano fertilizer on plant under salinity condition improves the plant growth and development through reducing detrimental consequences of salt stress.

Keywords: betaine-rich; nano fertilizer; salt stress; *Zea mays*; var. *saccharata*; *Arabidopsis thaliana*; growth parameters
Abbreviations: Analysis of Variance (ANOVA), Reactive Oxygen Species (ROS), Sodium Chloride (NaCl)

Introduction

Salinity refers to the soil that contains excessive soluble salts like sodium, calcium, magnesium, sulphate and chloride (Fairhurst et al., 2007; Butcher et al., 2016). Salinity also known as salt stress is an abiotic constraint, contributed a major problem in agriculture world (Habib et al., 2016; Tekeli & Kale, 2017). According to Hossain (2016), 1128 million hectares (Mha) of lands worldwide is estimated salt affected. More than 20% and 50% of agriculture and irrigated land are saline area (Parihar et al., 2015; Habib et al.,

2016; Machado & Serralheiro, 2017). The increase of salt concentration in soil influenced water extraction by plants. This increased reactive oxygen species (ROS) in plant cell, lead the stomata to close, next reduce the loss of water. In a long term, this condition decreased plant water availability, created the osmotic stress which can cause damage to the plant cell and reduce plant water uptake (Sheldon, 2017; Liang et al., 2018). Hence, salt stress restricted plant growth and development, reduced plant productivity and increased the demand of food (Santos et al., 2015; Volkov, 2015; Machado & Serralheiro, 2017).

Betaine is the trimethyl derivative of glycine, an amphoteric quaternary amine, acts as organic osmolytes to withstand with different extreme environmental conditions such as drought, salinity, heavy metals, UV radiation, heat and cold (Khalifa et al., 2016). Some of researchers used the term of glycine betaine to differentiate it from other betaine derivatives like proline betaine and alanine betaine. Organic osmolytes are compatible solute, biologically inert and accumulate at high concentration in cytoplasm without giving harmful effects to overall cellular functions (Zaman et al., 2015). In addition, betaine as osmotic protectant is plethora in chloroplast to maintain photosynthetic efficiency by giving protection to thylakoid membrane. They act in two ways; first acting as nontoxic cytoplasmic osmolyte to raise osmotic pressure, second, to stabilize enzymes and membranes against damage by salt stress (Zeng et al., 2016; Tian et al., 2017; Yu et al., 2017).

Nowadays, the usage of fertilizers and unsafe pesticides has led to environmental pollution such as eutrophication, groundwater pollution, air pollution and soil quality. For instance, excess fertilizer mixed with soil will leach out to the lake or absorb by the soil during rainy season (Chhipa, 2017; Chen et al., 2018). Therefore, the existence of Nano technology developed fertilizer in Nano size and shape (Rai et al., 2012) which provides site specific for the active ingredients to be released gradually to the plants and soils. The smaller the size, the larger the surface area next increase the opportunity of interaction between fertilizer and soil. Hence, the absorption of nutrients by plant and soil increase efficiently and the loss of fertilizer can be controlled. Then, the agriculture production can be sustained (Chhipa, 2017; Ma & Tyro, 2017; El-Ramady et al., 2018).

Sweet corn or *Zea mays* L. var. *saccharata* is a monocot, belongs to the genus *Zea*, in the grass family, Poaceae (Fahrurrozi et al., 2016). It stores more sugar than field corn due to naturally occurring genetic mutation. Currently, it becomes most important crop in the world for cereals production and provides raw materials for industry (Butcher et al., 2016; Jiang et al., 2017; Siyuan et al., 2018). People consumed sweet corn habitually due to its nutritional values like minerals, phytochemicals properties and dietary fibre, which can lower the risk of cardiovascular disease, diabetes, obesity, anti HIV and improve digestion system (Rouf, 2016; Xiang et al., 2017; Siyuan et al., 2018). According to Farooq et al. (2015) and Shtereva et al. (2015), sweet corn is known to be a moderately salt sensitive plant. Paradoxically, Mahajan and Tuteja (2005) stated that, it is categorized as salt sensitive plant which also known as glycohytes.

Arabidopsis thaliana (L.) Heynh. is a model plant in the research world, belongs to the genus *Arabidopsis* in the family Brassicaceae (Durvasula et al., 2017). *A. thaliana* was the first used for genetics research in the mid of 1940's. Then, in the mid of 1980's, this plant was being used for plant physiology and biochemistry studies. *Arabidopsis* have rapid life cycle, each plant produces many seeds in eight weeks. Many mutant seed lines of *Arabidopsis* exist, allow researchers or geneticist to study the effects of absent or additional genes. Moreover, researchers use *A. thaliana* to find the genetic sequences of interest due to completed sequences of small genome and it can be easily transformed by *Agrobacterium tumefaciens* to insert new genes to the plant (Cotter, 2005).

There are several reports on role of betaine in reducing salinity stress and the effects of Nano fertilizer on growth and crop production of *Punica granatum* cv. 'Ardestani' (Davaranpanah et al., 2016), *Oryza sativa* (Hoang et al., 2016) and *Prunus dulcis* var. *amara* (Badran & Savin, 2018). However, the effect of both betaine and Nano fertilizer in overcome salinity stress remains to be examined. In this study, we showed the effects of betaine-rich Nano fertilizer on growth of *Z. mays* var. *saccharata* and *A. thaliana* plant under saline condition.

Materials and Methods

Plant materials and treatments

Five levels of salinity [S0; 0 dS/m, S1; 2 dS/m, S2; 4 dS/m, S3; 6 dS/m and S4; 8 dS/m], two different concentrations of betaine [B0; 0 mM (without betaine) and B1; 50 mM (with betaine)] and with (N1; 3 mL/L) and without Nano fertilizer (N0) were used based on the treatments combination. The combination gave a total of 20 treatments (Table 1). Then, the treatments were arranged as Randomized Complete Block Design (RCBD) with three replications (Table 2). Salt (Bendosen, Malaysia), betaine (Sigma-Aldrich, Finland), inorganic fertilizer (Vitagrow, Malaysia) and Nano fertilizer (New Suryamin, India) were applied two times after sowing.

Seed of sweet corn (Narm Tao, Thailand) and *A. thaliana* (Ohio State University, Columbus) were obtained and used in this study. The growing media of *Z. mays* var. *saccharata* consisted of sandy soils (UniSZA, Besut Campus) and peat moss (Free Peat, Holland) with ratio 3:1 meanwhile the growing media of *A. thaliana* consisted of vermiculite (Vermicula, Malaysia) and peat moss in ratio 3:1. The growing media were mixed well before being filled into the individual pot (11 cm in diameter) for sweet corn and arafat (6 cm in diameter) for *A. thaliana*. Seeds

Table 1. Combination of the treatments used in this study

	B0N0	B1N0	B0N1	B1N1
S0	T1	T2	T3	T4
S1	T5	T6	T7	T8
S2	T9	T10	T11	T12
S3	T13	T14	T15	T16
S4	T17	T18	T19	T20

Table 2. The Randomized Complete Block Design (RCBD)

Treatment/Block	Block 1	Block 2	Block 3
T1	S0B0N0	S0B0N0	S0B0N0
T2	S0B1N0	S0B1N0	S0B1N0
T3	S0B0N1	S0B0N1	S0B0N1
T4	S0B1N1	S0B1N1	S0B1N1
T5	S1B0N0	S1B0N0	S1B0N0
T6	S1B1N0	S1B1N0	S1B1N0
T7	S1B0N1	S1B0N1	S1B0N1
T8	S1B1N1	S1B1N1	S1B1N1
T9	S2B0N0	S2B0N0	S2B0N0
T10	S2B1N0	S2B1N0	S2B1N0
T11	S2B0N1	S2B0N1	S2B0N1
T12	S2B1N1	S2B1N1	S2B1N1
T13	S3B0N0	S3B0N0	S3B0N0
T14	S3B1N0	S3B1N0	S3B1N0
T15	S3B0N1	S3B0N1	S3B0N1
T16	S3B1N1	S3B1N1	S3B1N1
T17	S4B0N0	S4B0N0	S4B0N0
T18	S4B1N0	S4B1N0	S4B1N0
T19	S4B0N1	S4B0N1	S4B0N1
T20	S4B1N1	S4B1N1	S4B1N1

were soaked in distilled water for half an hour to enhance the germination while Nano fertilizer (3 mL/L), inorganic fertilizer (5 mL/L for sweet corn and 3 mL/L for *A. thaliana*), NaCl [2 dS/m (675 mg/L), 4 dS/m (1350 mg/L), 6 dS/m (2025 mg/L) and 8 dS/m (2700 mg/L)] and betaine [stock solution (500 mM), working solution (50 Mm)] were prepared in solution form using distilled water. Pots and arafat were labelled according to the RCBD (Table 2). After that, seeds were sowed followed by the application of treatments. Betaine and Nano fertilizer were poured to the soil for sweet corn meanwhile for *A. thaliana*, betaine

and Nano fertilizer were applied by foliar method. The methods of application were different due to the different apparatus used to grow the plants. In few days, the seeds were slowly germinating. Lights were turned on after seven days for sweet corn whereas for *A. thaliana*, lights were turned on after transferred to controlled plant growth room. Plants were grown in controlled growth room under 16 hours of light and eight hours of dark cycle, temperature (22 ± 2) and relative humidity (60 ± 10). Cultural practices were handled according to the previous experiment. Weeds were controlled by removing manually using hands. Second treatment was applied in week 2 and week 3 for sweet corn and *A. thaliana*, respectively. Data for growth parameters were recorded started from week 3 and week 4 for sweet corn and *A. thaliana*, respectively.

Measurement and Determination of Plant Growth Parameters

Measurement of Plant Height

The height of the sweet corn plant (17 days after sowing) was measured from the soil surface to the longest leaf emerged from the whorl by straightening the plant to its fullest length while *A. thaliana* (27 days after sowing) was measured started from the media surface along the stem up to flowers by using ruler (Arshad & Ranamukhaarachchi, 2012).

Measurement of Number of Leaves

The leaf of sweet corn (17 days after sowing) with visible leaf collars was counted. Ironically, the leaf number of *A. thaliana* plant (27 days after sowing) was counted from the rosette part of the plant (Pal et al., 2015).

Measurement of Root Length, Leaf Length and Determination of Root:Leaf Length Ratio

Root and leaf length were measured by using ruler. Plant was uprooted carefully then cleaned to remove planting media. The length of the root was measured started from base of the stem until the end of the root (Teh et al., 2016). Sweet corn leaf (17 days after sowing) with visible leaf collar was measured from stem till the end of leaf tip. Conversely, leaf length of *A. thaliana* (27 days after sowing) was taken from the end of the petiole until the end of the leaf tip. The ratio of root and leaf length was determined according to Matt (2005) with modifications by dividing length of root with the length of leaf.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Duncan post-hoc test was employed using Statistical Package for Social Science (SPSS) version 23. A value of $p < 0.05$ was considered as statistically significant.

Results

Effects of betaine-rich Nano fertilizer on plant height of sweet corn and *A. thaliana*

Plant height was measured by using ruler to examine whether the application of betaine-rich Nano fertilizer can improve plant height of sweet corn and *A. thaliana* under salinity. Figure 1 showed the mean values of plant height affected by different salinity levels (0, 2, 4, 6 and 8 dS/m) and treatments [without betaine and Nano fertilizer (B0N0), with single application of betaine (B1N0), with single application of Nano fertilizer (B0N1) and a combination of betaine-rich Nano fertilizer (B1N1)] and a control (B0N0) at 0 dS/m. Figure 1 (A) demonstrated plant height of sweet corn was suppressed under saline conditions. Conversely, B1N1 under different salinity levels (0, 2, 4, 6 and 8 dS/m) significantly influenced the plant height of sweet corn compared to the control. B1N1 at 4 dS/m showed the highest plant height (20.26 cm), but the plant height started to decrease from 6 dS/m (13.50 cm) to 8 dS/m (11.43 cm).

Figure 1 (B) revealed that the plant height of *A. thaliana* treated with B1N1 at 0, 2, 4, 6, 8 dS/m were significantly higher than B0N0 at 0 dS/m. Instead of that, B1N1 was fluctuated at 0, 2, 4, 6 and 8 dS/m. B1N1 at 6 dS/m (23.63 cm) was significantly increased than B1N1 at 0 dS/m (13.93 cm), 4 dS/m (18.73 cm) and 8 dS/m (21.33 cm), nonetheless, it was comparable with B1N1 at 2 dS/m.

Effects of betaine-rich Nano fertilizer on number of leaves of sweet corn and *A. thaliana*

According to Figure 2 (A), there were no significant improvement between B1N1 under salinity levels 0, 2, 4 and 6 dS/m (3.33) compared to the control (B0N0 under 0 dS/m). Only B1N1 under 8 dS/m (3.67) significantly increase com-

pared to the control (2.67). In *A. thaliana* (Figure 2 B), B1N1 displayed no significant improvement between salinity levels 0 dS/m (8.00), 2 dS/m (8.00), 4 dS/m (7.33), 6 dS/m (7.33) and 8 dS/m (8.00), but B1N1 at 8 dS/m was significantly higher than B0N0 at 8 dS/m. In addition, B0N1 at 8 dS/m was also significantly higher than B0N0 at 8 dS/m.

Effects of betaine-rich Nano fertilizer on root length of sweet corn and *A. thaliana*

In this study, root length of sweet corn was recorded to justify the effects of different treatments on plants grown in different salinity levels. Figure 3 (A) and (B) displayed mean values of root length of sweet corn and *A. thaliana* influenced by different treatments and salinity levels. Root length of sweet corn treated with B0N0 was fluctuated under different salinity levels meanwhile B1N1 linearly increased with the increase of salinity levels. Moreover, B1N1 under all salinity levels (0, 2, 4, 6 and 8 dS/m) were markedly higher than control (17.20 cm). This proved that betaine-rich Nano fertilizer increased root length of sweet corn plant under salt stress, helped plants to explore and absorb nutrients from various part of the soil. This result might relate with the plant height of sweet corn. B1N1 under 0 dS/m (22.37 cm) was the lowest followed by B1N1 under 2 dS/m (25.54 cm), B1N1 under 4 dS/m (27.90 cm), B1N1 under 6 dS/m (29.35 cm) and B1N1 under 8 dS/m (31.50 cm).

Based on Figure 3 (B), root length of *A. thaliana* treated with B1N1 were significantly increased compared to B0N0 under saline conditions (0, 2, 4, 6 and 8 dS/m). The reduction of root development might be due to the toxic effects of NaCl and unbalanced nutrients absorption. B0N0 was fluctuated under saline conditions while B1N1 was constantly increased from 0 to 4 dS/m, significantly increased at 6 dS/m then decreased at 8 dS/m.

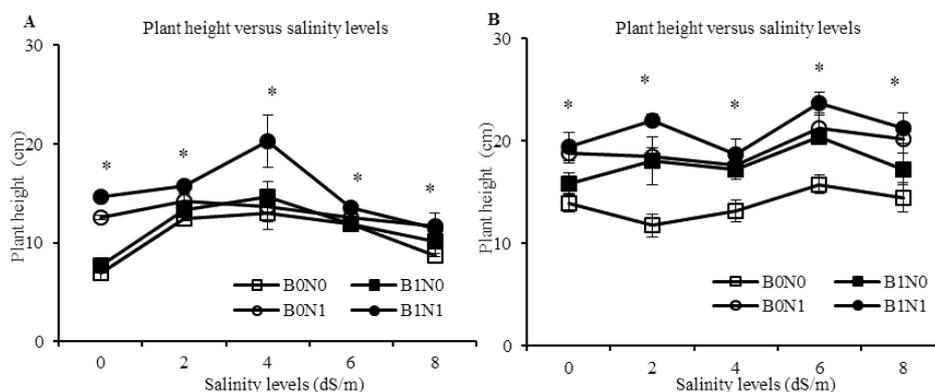


Fig. 1. Effects of different treatments on plant height of sweet corn (A) and *A. thaliana* (B) under different salinity levels
 Note: B0 and N0 show no betaine and Nano fertilizer while B1 and N1 show with betaine and Nano fertilizer. Single asterisk * indicated B1N1 was significantly different than control ($p < 0.05$)

Effects of betaine-rich Nano fertilizer on leaf length of sweet corn and *A. thaliana*

Figure 4 (A), B1N1 in saline conditions (0, 2, 4, 6 and 8 dS/m) were significantly higher than B0N0. B1N1 was increased from 0 to 2, and then slightly decreased from 2 dS/m till 8 dS/m. Besides, B1N1 at 0 dS/m (45.67 cm), 2 dS/m (50.33 cm), 4 dS/m (43.67 cm) and 6 dS/m (41.10 cm) showed significant increment but B1N1 at 8 dS/m (35.30 cm) was comparable to control (34.67 cm).

Based on Figure 4 (B), leaf length of *A. thaliana* treated without betaine and Nano fertilizer (B0N0) was reduced with the increasing levels of salinity. B1N1 at 0 dS/m (3.94 cm) and 8 dS/m (3.27 cm) were significantly higher than control (B0N0 under 0 dS/m) while B1N1 at 2 dS/m (3.73 cm), 4 dS/m (3.43 cm) and 6 dS/m (3.27 cm) were comparable to the control. B1N1 at 0 dS/m was significantly higher

than 4, 6 and 8 dS/m whereas B1N1 at 8 dS/m significantly lower than 2 dS/m. Other than that, B1N1 at 4 dS/m was insignificantly higher than 8 dS/m whereas B1N1 at 6 dS/m was notably higher than 8 dS/m.

Effects of betaine-rich Nano fertilizer on root:leaf length ratio of sweet corn and *A. thaliana*

The effects of various treatments on root:leaf length ratios of sweet corn and *A. thaliana* are displayed in Figure 5 (A) and (B), respectively. Figure 5 (A) showed that B0N0, B1N0, B0N1 and B1N1 were increased from 0 to 8 dS/m. Root:leaf length ratio under B1N1 at 4 dS/m (0.65), 6 dS/m (0.72) and 8 dS/m (0.89) were notably higher than control (0.50) while B1N1 at 0 dS/m (0.51) and 2 dS/m (0.49) were comparable to the control.

Figure 5 (B) demonstrated that root:leaf length ratio of *A. thaliana* grown under salt stress (0, 2, 4, 6 and 8 dS/m)

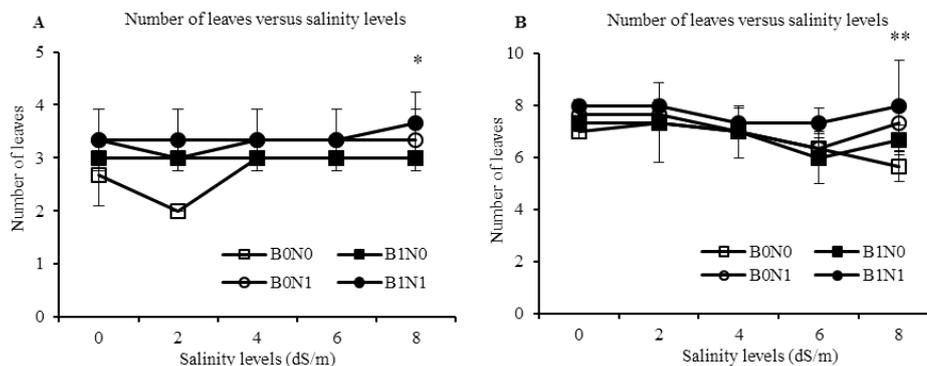


Fig. 2. Effects of different treatments on number of leaves of sweet corn (A) and *A. thaliana* (B) under different salinity levels

Note: B0 and N0 show no betaine and Nano fertilizer while B1 and N1 show with betaine and Nano fertilizer. Single asterisk * indicated B1N1 was significantly different than control ($p < 0.05$). Double asterisks ** indicated B1N1 and B0N1 were significantly different than B0N0 under 8 dS/m ($p < 0.05$)

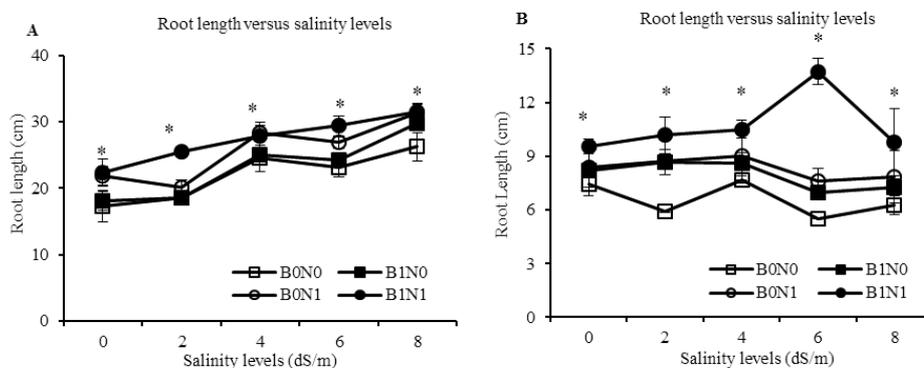


Fig. 3. Effects of different treatments on root length of sweet corn (A) and *A. thaliana* (B) under different salinity levels

Note: B0 and N0 show no betaine and Nano fertilizer while B1 and N1 show with betaine and Nano fertilizer. Single asterisk * indicated B1N1 was significantly different than control ($p < 0.05$)

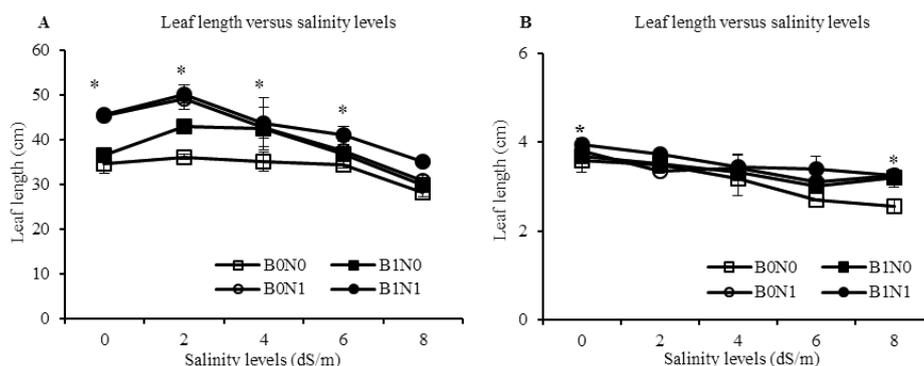


Fig. 4. Effects of different treatments on leaf length of sweet corn (A) and *A. thaliana* (B) under different salinity levels
 Note: B0 and N0 show no betaine and Nano fertilizer while B1 and N1 show with betaine and Nano fertilizer. Single asterisk * indicated B1N1 was significantly different than control ($p < 0.05$)

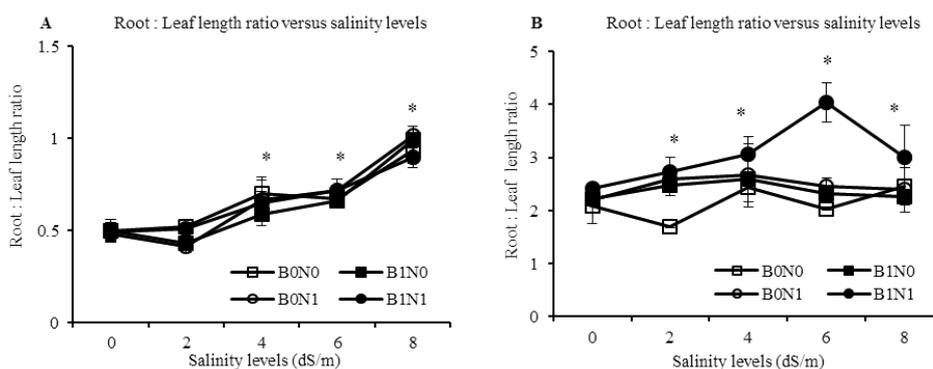


Fig. 5. Effects of different treatments on root: leaf length ratio of sweet corn (A) and *A. thaliana* (B) under different salinity levels

Note: B0 and N0 show no betaine and Nano fertilizer while B1 and N1 show with betaine and Nano fertilizer. Single asterisk * indicated B1N1 was significantly different than control ($p < 0.05$)

treated with B1N1 were significantly improved compared to those treated with B0N0. B1N1 at 2 dS/m (2.73), 4 dS/m (3.07), 6 dS/m (4.04) and 8 dS/m (3.00) were statistically significant compared to the control (2.09). Among B1N1, 6 dS/m showed the highest root:leaf length ratio (4.04) followed by 4 dS/m (3.07), 8 dS/m (3.00), 2 dS/m (2.73) and 0 dS/m (2.43).

Discussion

Betaine is believed to improve the growth parameters of two ornamental shrubs, *Viburnum lucidum* L. and *Callistemon citrinus* (Curtis) Stapf (Cirillo et al., 2016). This result was in agreement with Torabian et al. (2017) who reported that foliar application of Nano fertilizer could improve growth of

Helianthus annuus L. and *Moringa peregrine* (Forssk) (Soliman et al., 2015) under saline conditions. Previous research also found similar result where foliar application of betaine improve growth *Z. mays* 'DK 647F1' grown under salt stress with controlled temperature ($27 \pm 2^\circ\text{C}$) and relative humidity ($65 \pm 10\%$), (Kaya et al., 2013). On the contrary, our study used soil application method on glycophytes, *Z. mays* var. *sacchrata* and foliar application methods on model plant, *A. thaliana* grown in a controlled plant growth room with temperature ($22 \pm 2^\circ\text{C}$) and relative humidity ($60 \pm 10\%$). In this paper, we showed the effects of different application methods of betaine-rich Nano fertilizer in order to improve detrimental consequences of salt stress on plants.

Soil application methods allowed nutrients to enter plants through root hairs, lenticels, mucilage and exudates (Farooq

qui et al., 2016) while foliar application of betaine and Nano fertilizer is a beneficial method as it easily penetrated by the leaves through stomata, hydathodes and trichomes, later transported to all parts of the plant via phloem pathway (Hussain et al., 2018; Sturikova et al., 2018; Kheir et al., 2019).

Salt stress elevated Na^+ and Cl^- in plant cells, suppressed the uptake of K^+ , Ca^{2+} and Mg^{2+} in the plant due to antagonistic effect, then produced extreme ratios like $\text{Na}^+/\text{Ca}^{2+}$, $\text{Ca}^{2+}/\text{Mg}^{2+}$ and $\text{Cl}^-/\text{NO}_3^-$, leading to ionic stress and unbalance nutrient in plants. In addition, salt stress accompanied by oxidative stress (Isayenkov & Maathuis, 2019) due to accumulation of reactive oxygen species (ROS), bring oxidative damage to the plant cells which disrupted large number of enzyme, cellular processes, plant metabolism and physiology processes in plant like photosynthesis process due to retardation synthesis of photosynthetic pigment such as chlorophylls and carotenoids. Rate of photosynthesis decreased and inhibited, thus lower the production of glucose and adenosine triphosphate (ATP) by cellular respiration. The development of cell was restrained, hence limit the cell growth and development of plant height, leaves and root. On the other hand, osmotic stress was created attributable to decrement in water absorption by root and water availability in plants. As a result, it limits the availability of essential nutrients (Ali et al., 2017; Torabian et al., 2017; Hussain et al., 2018). Root: leaf length ratio were markedly increased under saline conditions compared to the control plants, which reflects that leaf growth was adversely affected due to salt stress than the root growth (Kaya et al., 2013; Benjamin et al., 2014; Cirillo et al., 2016). Elevation of root:shoot ratios might be detrimental under salinity as they can build up toxic ions in shoots, leading to nutrient imbalance and decrement of water potential in plant, therefore, restrict plant growth (Xu et al., 2015; Lucini et al., 2015).

Betaine is compatible solute, act as zwitterion, interrelates with hydrophobic and hydrophilic domains of protein complexes and membranes, thus provides stabilization and maintenance of the structural and functional integrity of protein complexes and membranes (Pardo-Domènech et al., 2016; Annunziata et al., 2019). On top of that, betaine alleviates detrimental effects of salt stress on plant by discriminating excess salts like Na^+ and Cl^- (Van Oosten et al., 2017), detracting ROS, to be specific hydrogen peroxide (H_2O_2), superoxide ion (O_2^-), hydroxyl radical (OH) and hydroxyl ion (OH^-), enhanced the uptake of K^+ , boosted non-enzymatic antioxidants like endogenous glycine betaine and enzymatic antioxidant enzymes for instance, catalase (CAT) activity, superoxide dismutase (SOD) activity, peroxidase (POD) activity and ascorbate peroxidase (APX) (Alasvandyari et al.,

2017). Hence, betaine promoted water flow into cells and supported physiological function in plant (Rady et al., 2018).

Nano fertilizer extend the duration of nutrient release to the plant, enhance the absorption of nutrients, for example, increase the accumulation of nitrogen in the leaf to support leaf development, so, balance the nutrient loss due to excess salts (El-Ramady et al., 2018; Hussein & Abou-Baker, 2018). Besides, Nano fertilizer encouraged enzyme activities in plant, as a result, preserved plant cells from undue injury by reactive oxygen species (ROS) in salt stress states (Torabian et al., 2017). Instead of that, Nano fertilizer may help the increment of rigidity, strength and stretchiness of plant cell wall under salinity stress (Yassen et al., 2017). Kheir et al. (2019) further emphasized that, Nano fertilizer improved the uptake and allocation of K^+ by the plant, mitigated the quality of the soil through raising water holding capacity, reduced mineral fixation and soil toxicity.

Conclusions

Salinity reduces plant height, number of leaves, root length, leaf length and root:leaf ratio of *Z. mays* var. *saccharata* and *A. thaliana*. The application of betaine and Nano fertilizer by foliar or soil application method on plants, enhanced antioxidant defense to ameliorate salt induce damage due to osmotic and ionic stress under salinity condition. Thus, promotes plant growth and development in salt stress states. The effects of betaine-rich Nano fertilizer on plant may vary depending on the genus and species, duration of salt stress and repetition of the treatments. More studies are needed to examine the effects of betaine-rich Nano fertilizer on other plants in field conditions.

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