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EFFECTS OF BETAINE-RICH NANO FERTILIZER ON IMPROVING PHYSIOLOGY OF *Zea mays* var. *saccharata* AND *Arabidopsis thaliana* UNDER SALT STRESS

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ABSTRACT

Salinity is the second major obstacle after drought conditions that hinder the high productivity of plants. Many factors contribute to the salinity problem, consequently affects the plant physiology, growth as well as production. Therefore, this research aims to determine the effects of betaine-rich Nano fertilizer on physiological parameters of sweet corn (*Z. mays* var. *saccharata*) and *A. thaliana* under salt stress. In this study, plants were grown in a controlled plant growth room. Five salinity levels (S0:0 dS/m, S1:2 dS/m, S2:4 dS/m, S3:6 dS/m, and S4:8 dS/m), two concentrations of betaine (B0:0 mM and B1: 50 mM) and the application of Nano fertilizer (N0: without Nano fertilizer and N1: with Nano fertilizer) were tested in Randomized Complete Block Design (RCBD) with three replicates. Data for chlorophyll content, chlorophyll fluorescence (F_V/F_M), and leaf relative water content (RWC) were recorded. The results were analyzed using a two-way analysis of variance (ANOVA) followed by the Duncan posthoc test. Sweet corn and *A. thaliana* under salt stress showed a significant increase in chlorophyll content and leaf RWC, but not in F_V/F_M. Therefore, it is anticipated that betaine-rich Nano fertilizer could reduce salt stress by improving the physiological parameters in sweet corn as well as in *A. thaliana*.

Key words: Betaine-rich Nano fertilizer, salt stress, *Z. mays* var. *saccharata*, *A. thaliana*, physiology

INTRODUCTION

Saline soil refers to the soil that contains excessive soluble salts like sodium, calcium, magnesium, sulphate, and chloride (Butcher *et al*., 2016). More than 20% and 50% of agriculture and irrigated land are saline areas, respectively (Habib *et al*., 2016). Saline soil influences water extraction by plants. This condition increases reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide ion (O_2-), hydroxyl radical (OH), and hydroxyl ion (OH^-) in the plant cell, leads to the closure of stomata and reduces the water loss. In a long term, this condition decreases plant water availability, creates osmotic stress which can cause damage to the plant cell (Liang *et al*., 2018). Hence, salt stress restricted plant growth and productivity subsequently increased the demand for food (Machado & Serralheiro, 2017).

Betaine is the trimethyl derivative of glycine, an amphoteric quartery amine, that acts as organic osmolytes to withstand different extreme environmental conditions such as UV radiation, heavy metals, drought, salinity, heat, and cold (Day & Kempson, 2016). Some of the researchers used the term glycine betaine to differentiate it from other betaine derivatives like proline betaine and alanine betaine (Tian *et al*., 2017). Betaine acts in two ways; first acting as a nontoxic cytoplasmic osmolyte to raise osmotic pressure, second, to stabilizes enzymes and membranes against damage by salt stress (Tian *et al*., 2017).

Nowadays, the application of fertilizers and unsafe pesticides has led to environmental pollution such as eutrophication, groundwater pollution, air pollution, and poor soil quality (Chen *et al*., 2018). Therefore, the existence of Nanotechnology has

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developed fertilizers in Nano size and shape which provide site-specific for the active ingredients to be released gradually to the plants and soils (Chhipa *et al*., 2017; León-Silva *et al*., 2018). As a result, the loss of fertilizer can be controlled and the absorption of nutrients by plant and soil can be increased efficiently (El-Ramady *et al*., 2018). Then, agriculture production can be sustained.

Zea mays L. var. *saccharata* or sweet corn stores more sugar than field corn due to naturally occurring genetic mutation. Currently, it becomes the most important crop in the world for cereals production and provides raw materials for industry (Siyuan *et al*., 2018). People consumed sweet corn habitually due to their nutritional values like minerals, phytochemicals properties, and dietary fiber (Siyuan *et al*., 2018). *Arabidopsis thaliana* (L.) Heynh. is a model plant used in scientific researches such as for plant physiology and biochemistry studies. Both sweet corn and *A. thaliana* are categorized as a salt-sensitive plant which is known as glycophytes (Joshi *et al*., 2015).

Previous reports have demonstrated the role of betaine in reducing salt stress and the effects of Nano fertilizer on the physiology of plants such as *Ocimum basilicum* L. (Alipour *et al.*, 2016), *Allium cepa* L. (Rady *et al*., 2018), and *Brassica napus* L. (Hezaveh *et al*., 2019). However, the effect of both betaine and Nano fertilizer to overcome salt stress remains to be examined. In this study, we showed the effects of betaine-rich Nano fertilizer on the physiology of *Z. mays* var. *saccharata* and *A. thaliana* under saline conditions and the interactions between salinity and betaine-rich Nano fertilizer on the plant physiology.

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; 0 mL/L) were used based on the treatments combination (B0N0, B1N0, B0N1 and B1N1). Then, the treatments were arranged as a Randomized Complete Block Design (RCBD) with three replications. The betaine-rich Nano fertilizer consists of salt (NaCl) (Bendosen, Malaysia), betaine (Sigma-Aldrich, Finland), inorganic fertilizer (Vitagrow, Malaysia), and Nano fertilizer (New Suryamin, India). The treatments were applied two times after sowing.

The seed of sweet corn (Narm Tao, Thailand) and *A. thaliana* (Ohio State University, Columbus) were used in this study. The growing media of sweet corn consisted of sandy soils and peat moss in ratio 3:1. For *A. thaliana,* the growing media consisted of vermiculite 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 araflat (6 cm in diameter) for *A. thaliana*. 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 araflat were labeled according to the RCBD. After that, seeds were sown followed by the application of treatments. For sweet corn, betaine and Nano fertilizer were poured onto the soil, while for *A. thaliana*, betaine and Nano fertilizer were applied by the foliar method. The methods of application were different due to the different apparatus used to grow the plants. Plants were grown in a controlled growth room under a photoperiod of 16 hr of light and eight hr of the dark cycle, temperature (22 ± 2°C), and relative humidity (60 ± 10%). The second treatment was applied in the second week and third week for sweet corn and *A. thaliana*, respectively. Data for physiological parameters were recorded started from 17 days after sowing for sweet corn and 27 days after sowing for *A. thaliana*.

Measurement of chlorophyll content and chlorophyll fluorescence (F_V/F_M)

Chlorophyll content was measured by using SPAD-502plus portable chlorophyll meter (Konica Minolta, Japan) according to Rady *et al*. (2018). JUNIORPAM chlorophyll fluorometer (Walz, Germany) was used to record chlorophyll fluorescence [F_V/F_M (maximal quantum efficiency of PS II)].

Determination of leaf relative water content (RWC)

Fresh leaf was detached from the plant then immediately weighed by using analytical balance to determine the fresh weight (FW). Then, the leaf was soaked in a Petri dish containing distilled water for 24 hr to determine the turgid weight (TW). Before weigh, excess water was removed by using tissue paper. Next, the leaf was kept in a universal oven (Memmert) at 60°C for 24 hr then weighed to get dry weight (DW). The leaf RWC was calculated based on the formula (Syuhada & Jahan, 2016):

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 23. Means were separated by Duncan post-hoc test and the differences were considered as statistically significant when $p<0.05$.

RESULTS

Effects of betaine-rich Nano fertilizer on chlorophyll content of sweet corn and *A. thaliana*

SPAD-502plus portable chlorophyll meter (Konica Minolta, Japan) was used to measure chlorophyll content of sweet corn and *A. thaliana* under different salinity levels (0, 2, 4, 6 and 8 dS/m) and treatments [without betaine and Nano fertilizer (B0N0), with a single application of betaine (B1N0), with a single application of Nano fertilizer (B0N1) and a combination of betaine-rich Nano fertilizer (B1N1)] and a control (B0N0) at 0 dS/m. A statistically significant interaction between salinity levels and betaine-rich Nano fertilizer on chlorophyll content of sweet corn and *A. thaliana* ($p<0.0005$) was found.

Figure 1A proved that the chlorophyll content of sweet corn treated with B0N0 was significantly declined under saline conditions. In contrast, B1N1 was significantly increased under saline conditions. B1N1 at 0 dS/m (41.27 nm) and 8 dS/m (41.07 nm) were significantly increased compared to the control (39.57 nm). However, B1N1 at 2 dS/m (40.13 nm) showed no significant difference, while B1N1 at 4 dS/m (37.37 nm) and 6 dS/m (37.87 nm) were significantly decreased compared to the control (39.57 nm).

Chlorophyll content of *A. thaliana* treated with B1N1 was significantly elevated compared to B0N0 (Figure 1B). Moreover, B1N1 at 0 dS/m (28.63 nm), 2 dS/m (26.53 nm), 4 dS/m (26.10 nm), 6 dS/m (28.37 nm) and 8 dS/m (24.87 nm) were significantly higher from the control (21.53 nm). B1N1 at 0 dS/m was significantly increased from B1N1 at 2, 4, and 8 dS/m, nonetheless, it was comparable to B1N1 at 6 dS/m.

Effects of betaine-rich Nano fertilizer on chlorophyll fluorescence (F_V/F_M) of sweet corn and *A. thaliana*

No significant interaction between salinity levels and betaine-rich Nano fertilizer was observed on F_V/F_M of sweet corn ($p=0.984$) and *A. thaliana* ($p=0.997$). The F_V/F_M of sweet corn treated with B0N0 started to decrease from 2 dS/m to 8 dS/m under saline conditions, whereas B1N1 has fluctuated under saline conditions (Figure 2A). At all salinity levels, no significant difference was found between B1N1 and control, but B1N1 at 0 dS/m (0.81), 2 dS/m (0.82), 4 dS/m (0.72) and 6 dS/m (0.80) were escalated from the control (0.71). However, B1N1 at 8 dS/m (0.71) was found to be similar to the control (0.71).

Figure 2B showed that F_V/F_M of *A. thaliana* treated with B1N1 at 0 dS/m (0.77), 2 dS/m (0.77), 6 dS/m (0.78) and 8 dS/m (0.75) were higher from the control (0.72) but the differences were not significant. Only B1N1 at 4 dS/m (0.65) was significantly lower from the control.

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

The interaction between salinity levels and betaine Nano fertilizer on leaf RWC was statistically significant in *A. thaliana* ($p<0.0005$), but not in sweet corn ($p=0.082$). The leaf RWC of sweet corn treated with B1N1 at 0 dS/m (96.33%), 2 dS/m (96.87%), 4 dS/m (95.71%), 6 dS/m (96.70%) and 8

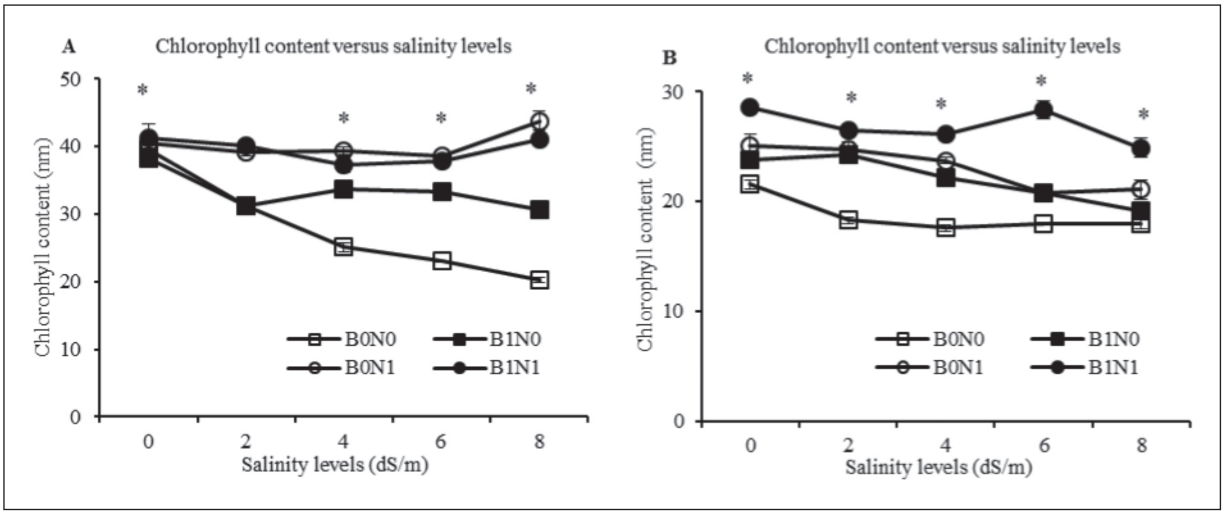

Fig. 1. Effects of different treatments of betaine-rich Nano fertilizer on chlorophyll content of sweet corn (A) and *A. thaliana* (B) under different salinity levels.

B0 = no betaine, N0 = no Nano fertilizer, B1 = with betaine and N1 = with Nano fertilizer. Single asterisk * indicated B1N1 was significantly different from the control (B0N0) at 0 dS/m ($p<0.05$).

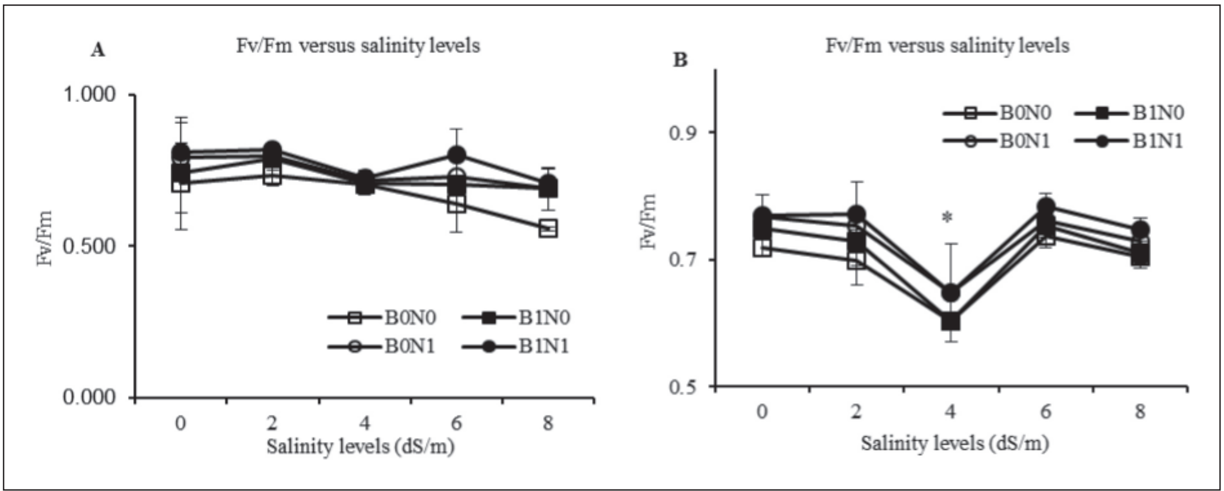

Fig. 2. Effects of different treatments of betaine-rich Nano fertilizer on chlorophyll fluorescence (Fv/Fm) of sweet corn (A) and *A. thaliana* (B) under different salinity levels.

B0 = no betaine, N0 = no Nano fertilizer, B1 = with betaine and N1 = with Nano fertilizer. Single asterisk * indicated B1N1 was significantly different from the control (B0N0) at 0 dS/m ($p<0.05$).

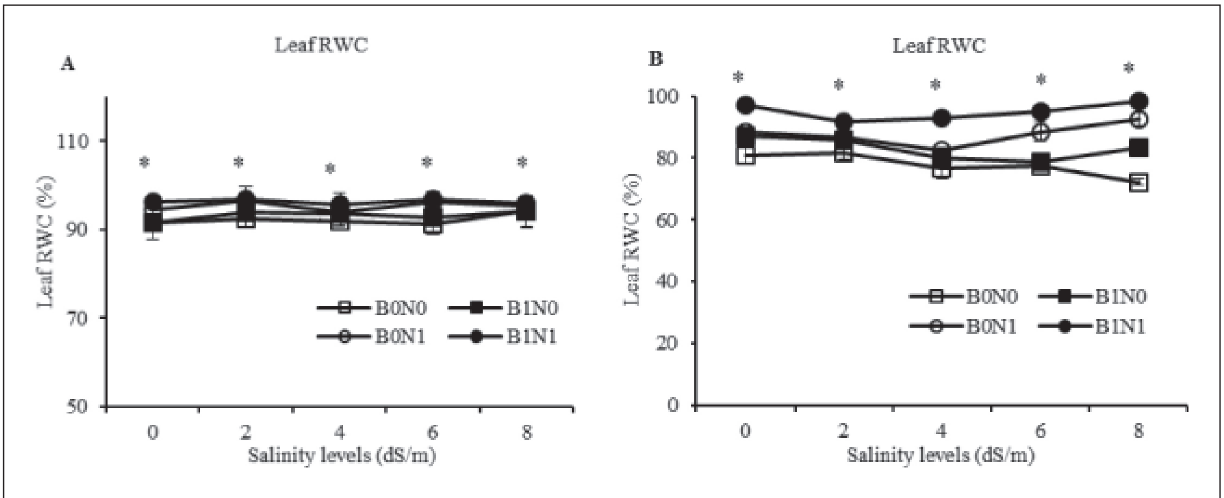

Fig. 3. Effects of different treatments of betaine-rich Nano fertilizer on leaf RWC of sweet corn (A) and *A. thaliana* (B) under different salinity levels.

B0 = no betaine, N0 = no Nano fertilizer, B1 = with betaine and N1 = with Nano fertilizer. Single asterisk * indicated B1N1 was significantly different from the control (B0N0) at 0 dS/m ($p<0.05$).

dS/m (95.90%) were statistically significant compared to the control (91.49%) (Figure 3A).

The leaf RWC of *A. thaliana* treated with B1N1 was increased at 4 dS/m (93.07%), 6 dS/m (95.12%) and 8 dS/m (98.38%) although it was dropped from 0 dS/m (96.97%) to 2 dS/m (91.50%). Also, B1N1 under all salinity levels were significantly higher from the control (80.76%) (Figure 3B).

DISCUSSION

The decline of chlorophyll content in plants under saline conditions is a common effect of oxidative stress (Taibi *et al*., 2016). Ion accumulation under saline conditions repressed enzymes chlorophyllase, attributed to the suppression of chlorophyll biosynthesis (Taibi *et al*., 2016). The suppression of chlorophyll biosynthesis caused a decrease in chlorophyll content (Babaei *et al*., 2017) hence the efficiency of photosynthesis is affected (Rady *et al*., 2018). Depletion of F_V/F_M under saline conditions also led to the reduction of chlorophyll pigment (Babaei *et al*., 2017). The F_V/F_M was reduced in saline conditions due to inhibition of electron transport at the acceptor side of PS II (Lucini *et al*., 2015). Destruction of PS II consequently affects carbon dioxide (CO_2) fixation, indirectly halt the activation of Rubisco (Iqbal *et al*., 2015). Hence, the development and growth of cells slowed and inhibited due to low ATP production.

Leaf RWC indicated water status in plants and vital to preserve osmotic adjustment in a plant cell to achieve optimum productivity of the photosynthesis process (Bhuiyan *et al*., 2016). The lowest leaf RWC value in sweet corn and *A. thaliana* were observed at 6 and 8 dS/m, respectively. This relates to the incompletion of cell development due to the decrease of chlorophyll content and fluorescence (F_V/F_M) under saline conditions. Furthermore, saline conditions gave an impact on water and ions absorption in the plant (Babaei *et al*., 2017). The attenuation of water content in the plant created osmotic stress, induces stomatal closure to maintain water balance in the plant (Ghorbani *et al*., 2018).

However, the application of betaine-rich Nano fertilizer through the soil and foliar application methods demonstrated a significant increment in chlorophyll content and leaf RWC under saline conditions compared to the control, meanwhile, chlorophyll fluorescence (F_V/F_M) was comparable to control under saline conditions. In this paper, we showed the effects of betaine-rich Nano fertilizer in reducing the detrimental consequences of salt stress on glycophytes, *Z. mays* var. *sacchrata,* and model plant, *A. thaliana* by using different methods. Soil application methods allowed nutrients to enter plants through root hairs, lenticels, mucilage, and exudates (Farooqui *et al*., 2016) while foliar application easily penetrated by the leaves through stomata, hydathodes, and trichomes, later transported to all parts of the plant via phloem pathway (Sturikova *et al*., 2018).

Betaine and Nano fertilizer alleviated detrimental effects in a salt-stressed plant by discriminating excess salts (Kheir *et al*., 2019) and ROS (Wei *et al*., 2017). The uptake and allocation of K^+ by the plant was improved (Alasvandyari *et al*., 2017; Kheir *et al*., 2019), boosted non-enzymatic antioxidants and enzymatic antioxidant (Torabian *et al*., 2017). Consequently, promoted water holding capacity in the plant cells (Kheir *et al*., 2019), thus, increased turgor pressure and strength of plant cell wall (Yassen *et al*., 2017). The increase of RWC provided better osmotic regulation in the plant, maintained stomatal aperture which increased chlorophyll content and fluorescence (F_V/F_M) (Babaei *et al*., 2017). Moreover, the absorption of nutrients enhanced, therefore, balanced the nutrient loss (El-Ramady *et al*., 2018).

CONCLUSION

Saline conditions suppressed the chlorophyll content, chlorophyll fluorescence (F_V/F_M), and leaf RWC of sweet corn and *A. thaliana*. The present study proved that the application of betaine-rich Nano fertilizer by foliar or soil methods have significantly improved the chlorophyll content and leaf RWC in both sweet corn and *A. thaliana*. It is believed that betaine and Nano fertilizer ameliorates salt-affected damage by elevating endogenous antioxidant defense in plants under saline conditions. Hence, promotes the photosynthetic activity and induces plant cell to retain water. However, the effects of betaine-rich Nano fertilizer in plants may vary depending on the plant species, duration of salt stress, and repetition of treatments. Therefore, a field study is needed to examine the effects of betaine-rich Nano fertilizer on salt-stressed plants in further detail.

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