Alleviation of Adverse Effects of Salt Stress on Soybean (*Glycine max.* L.) by Using Osmoprotectants and Organic Nutrients

Ayman El Sabagh, Sobhy Sorour, Abd Elhamid Omar, Adel Ragab, Mohammad Sohidul Islam, Celaleddin Barutçular, Akihiro Ueda, Hirofumi Saneoka

***Abstract*—**Salinity is one of the major factors limiting crop production in an arid environment. Despite its global importance soybean production suffer the problems of salinity stress causing damages at plant development. So it is implacable to either search for salinity enhancement of soybean plants. Therefore, in the current study we try to clarify the mechanism that might be involved in the ameliorating effects of osmo-protectants such as proline and glycine betaine as well as, compost application on soybean plants grown under salinity stress. The experiment was conducted under greenhouse conditions at the Graduate School of Biosphere Science Laboratory of Hiroshima University, Japan in 2011. The experiment was designed as a spilt-split plot based on randomized complete block design with four replications. The treatments could be summarized as follows; (i) salinity concentrations (0 and 15 mM), (ii) compost treatments (0 and 24 t ha^{-1}) and (iii) the exogenous, proline and glycine betaine concentrations (0 mM and 25 mM) for each. Results indicated that salinity stress induced reduction in growth and physiological aspects (dry weight per plant, chlorophyll content, N and K^+ content) of soybean plant compared with those of the unstressed plants. On the other hand, salinity stress led to increases in the electrolyte leakage ratio, Na and proline contents. Special attention was paid to, the tolerance against salt stress was observed, the improvement of salt tolerance resulted from proline, glycine betaine and compost were accompanied with improved K^+, and proline accumulation. While, significantly decreased electrolyte leakage ratio and Na^+ content. These results clearly demonstrate that harmful effect of salinity could reduce on growth aspects of soybean. Consequently, exogenous osmoprotectants combine with compost will effectively solve seasonal salinity stress problem and are a good strategy to increase salinity resistance of soybean in the drylands.

***Keywords*—**Compost, glycine betaine, growth, proline, salinity tolerance, soybean.

I. Introduction

SOYBEAN seed production may be limited by environmental stresses such as soil salinity. Growth, development and yield of soybean are the result of genetic potential interacting with environment [1]. Minimizing environmental stress will be optimized the seed yield [2].

Salinity has become more and more important to the scientific and political agenda. Over 6% of the world's total land area and 20% of the irrigated land are salt-affected [3]. Salinity problems are particularly relevant for arid and semiarid areas like an Egypt. Approximately 33% of the cultivated land and most extension agricultural land in Egypt are already salinized [4].

Proline and glycine betaine are known to serve as compatible osmolytes, protectants of macromolecules and also as scavengers of reactive oxygen species (ROS) under stressful conditions [5]. During osmotic adjustment, many plants accumulate proline in response to salt stress widely believed to function as a protector against salt damage [6]. Glycine betaine is one of several such compatible solutes that has an osmoprotective function and is known to improve salt stress tolerance in most crop plants [7].

The productivity of irrigated crops with saline water or crops grown under saline stress can be enhanced by using compost as an amendment reported by [8]. The addition of composted material in soil is responsible for enhancing soil microbial activities [9]. Therefore, the objectives of this work were to study the effect of salinity stress on physiological traits in soybean, and to examine whether alleviation of salt stress-induced adverse effects by the exogenous of proline and glycine betaine accompanied with compost application.

II. The Study Area

The experiment was conducted under greenhouse conditions at Plant Nutritional Physiology, Graduate School of Biosphere Science Laboratory of Hiroshima University, Japan in 2011.

III. Materials and Methods

A. Plant Material and Culture Conditions

The seeds of soybean cultivar (Giza 111) were sown into wood made basin (length 10 meter, width 50 cm, height 50 cm and soil depth 35 cm). The basin was filled with a mixture of soil, perlite and peat moss at the ratio of 4:2:1 (v/v/v). Each basin was fertilized at a rate of 40 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1}, 100 kg K_2O_2 ha^{-1} and calcium carbonate (300 kg ha^{-1}). The experiment was designed as a completely randomized block design with arranged a spilt split plot arrangement; each treatment was replicated four times. The trial materials could

A. E., S. S., A. O., and A. R. are with the Department of Agronomy, Faculty of Agriculture, Kafrelsheikh University, Egypt, (Corresponding author e-mail: aymanelsabagh@gmail.com).

M. S. I. is with Department of Agronomy, Faculty of Agriculture, Hajee Mohammad Danesh Science University, Bangladesh

C. B. is with Department of Field Crops, Faculty of Agriculture, Cukurova University, Turkey, (cebar@cu.edu.tr).

A. U. and H. S. are with Plant Nutritional Physiology, Graduate School of Biosphere Science, Hiroshima University, Japan, (e-mail: akiueda@ hiroshima-u.ac.jp and saneoka@hiroshima-u.ac.jp).

be summarized as follows (i) Main plots included two salinity treatments (control and 15 mMNaCl) salinity were applied in fully developed trifoliolate leaf node (V1). (ii) Sub plots included compost (control, 24 ton ha^{-1}) was mixed with soil and manufactured using wood poop, chicken poop and palm. Chemical analysis of compost was (N 9.1%, phosphorous: 9.0%, potassium: 5.0% and C/N: 24) and (iii) Sub-sub plots included exogenous proline and glycine betaine: (control, 25 mM) one level of proline and glycine betaine was applied in fully developed trifoliolate leaf node (V1).

B. Plant Sampling and Measurements

During the vegetative stage (V6: six fully developed trifoliate leaf stage and near the flowering stages) plants in each plot were sampled after treatments imposition and separated. The fresh samples were kept frozen in liquid nitrogen then freeze-dried and we measured the dry weight. Dry samples were ground into fine powder using a vibrating sample mill (Model TI-100, Heiko Seisakusho Ltd., Tokyo, Japan) for chemical analysis.

1. Electrolyte Leakage Rate (ELR)

It was measured with the method described by [10]. Electrolyte leakage rate can be defined as follows: ELR (%) = EC1/EC2 x 100). Where; EC1: Electrical conductivity of the bathing solution was determined after 24 h; EC2: Electrical conductivity of samples were then autoclaved at 120°C for 20 min and a last conductivity reading.

2. Total N Content

Nitrogen content was determined using a Kjeldahl Nitrogen Digester and Distillator (Kjeldatherm Type TT100 &Vapodest Type 20, Gerhardt, Germany).

3. Determination of Na and K Content

The Na and K content in leaves were measured using a flame photometer (ANA-135; Tokyo Photoelectric, Tokyo, Japan) according to the method of [11]. Dried samples were gently agitated in 1 N HCl overnight, and the content of Na and K ions was estimated from the Na and K standard curves.

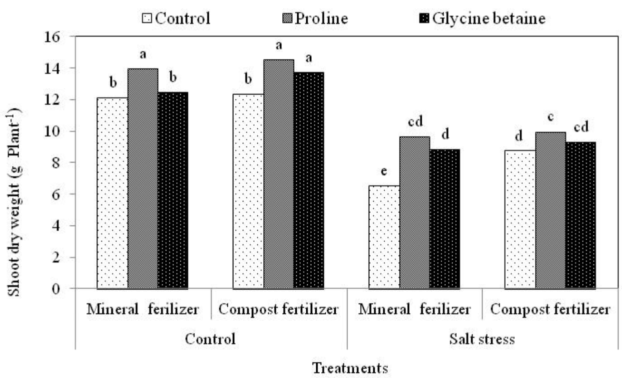

Fig. 1 The effects of salinity on shoot dry weight in soybean as affected by compost application and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

4. Proline Content

It was determined spectrophotometrically following the ninhydrin method described by [12] using l-proline as a standard.

5. Statistical Analysis

All data collected for both seasons were subjected to analysis of variance according to [13]. Treatment means were compared using Duncan Multiple Range Test [14]. All statistical analysis performed using analysis of variance technique by “MSTAT-C” computer software package 1990.

IV. Results and Discussion

A. Shoot Dry Weight

The current results had significant differences were observed between salinity treatments with respect to dry weights of shoots (Fig. 1). Hussein et al. [15] reported that a negative relationship was detected between vegetative growth parameters and salinity stress. On the other side, in comparison with control, the treatments of proline application increased dry weights of shoots of soybean under salinity stress, significantly (Fig. 1). The present finding is agreement with earlier reports that the exogenous application of proline alleviates the adverse effect of stress on plant growth [16]. As well as, exogenous application of glycine-betaine significantly increased dry weight under saline conditions (Fig. 1). These findings can be related to some earlier studies in which it has been observed that exogenous application of glycine betaine counteracts the growth inhibition caused by salt stress in different crop plants [17]. In this concern, application of compost caused significant increases in dry weight under investigation (Fig. 1). Previous studies have confirmed that physical, chemical and biological properties in soil in salt affected areas are strongly enhanced upon the application of organic manure, leading to better crop growth and development [18].

B. Leaf Electrolyte Leakage

Leaf electrolyte leakage (LEL) rate is a good strain index as it reflects the degree of plant injury by salt stress. The presence of saline stresses induced a significant increase in electrolyte leakage ratio in leaves of soybean (Fig. 2). Increase in electrolyte leakage has been documented by [19] which reported sodium can increase membrane leakage ratio. Special attention was paid to, the treatments of exogenous proline indicated that maintained a better electrolyte leakage status under salinity conditions. The proposed functions of accumulated proline are osmoregulation and maintenance of membrane [20]. Also, our results showed that exogenous glycine betaine lowered the leaf electrolyte leakage rate in salt-stressed leaves (Fig. 2). This facilitation could be attributed to the glycine betaine-induced antioxidant responses that protect the plant from oxidative damage [21]. Likewise, the application of compost indicated that maintained a better electrolyte leakage status under salinity conditions (Fig. 2). Decrease electrolyte leakage in strawberry grown under wheat straw or black polythene has been reported by [22].

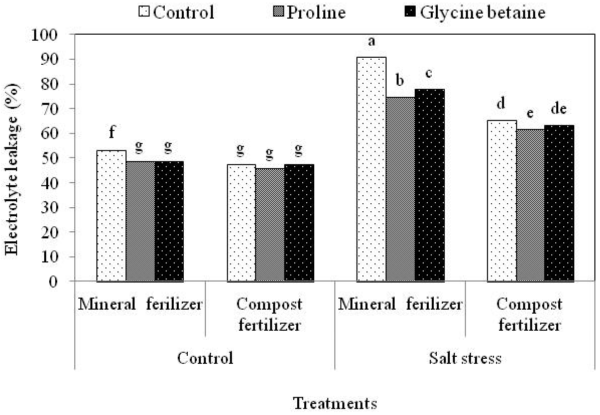

Fig. 2 The effects of salinity on the electrolyte leakage ratio in soybean as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

C. Nitrogen Content

According to the result under control condition the maximum nitrogen content was recorded (Fig. 3). Singh et al. [23] reported that, the decrease in total-N concomitantly with the decrease in soluble-N and protein-N in salinity stressed mungbean plants can be attributed to the effect of salinity on decreasing the biosynthesis of protein and/or the decrease in nitrogen fixation and/or inhibition in nitrate reductase activity. On the contrary, applied proline increased of nitrogen content (Fig. 3). The proposed function of accumulated proline is redulated to osmotic adjustment, protein stability and plant growth [20]. In a similar way, nitrogen contents were significantly affected by exogenous of proline (Fig. 3). Glycine betaine can protect the chloroplasts and their membranes against the deteriorative effects of salinity stress and thus maintaining their integrity [24]. Additionally, the application of organic compost increase nitrogen content of soybean plants compared with control (Fig. 3). These results are in full agreement with those obtained by [25].

D. Sodium Content

Na+ concentration in soybean shoot significantly increased under salt stress as a compared with plants grown in control conditions (Fig. 4). Salt stress commonly stimulate to accumulation of Na+ and a massive K+ efflux from plant tissues [26]. But, application of proline significantly decreased Na+ content in plant undergoing salinity stress (Fig. 4). The lower accumulation of Na^+ in tissues of proline-treated plants, compared to their corresponding salinity treatment, displayed the improved effect of proline on the ability of roots to exclude the salt ions from the xylem sap flowing to the shoot, and thus better growth rates, as suggested by [27]. Also, glycine betaine significantly decreased Na^+ content in plant parts undergoing salinity stress (Fig. 4). This can be involved in osmotic adjustment and turgor maintenance of stressed cells which improve salt tolerance. Glycine betaine is able to decrease the uptake of Na^+ ions in stressed maize plant [28]. Moreover, application of compost significantly decreased Na^+ content in plant parts undergoing saline water irrigation whereas K^+ content showed an increase accordingly in plant parts under saline conditions (Fig. 4). Application of organic manure increased the availability of K^+ content, nitrogen and phosphorus to the whole plant [29].

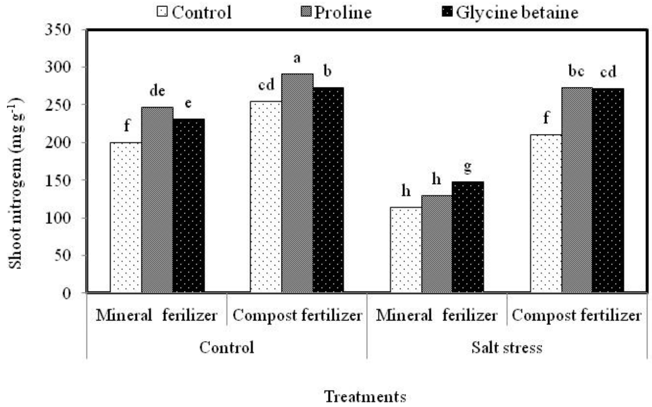

Fig. 3 The effects of salinity on N content (mg N g^{-1} DW) in soybean as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

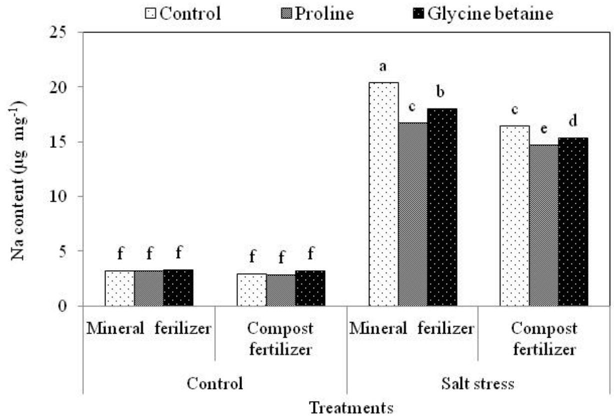

Fig. 4 The effects of salinity on Na content (µg Na mg^{-1} DW) in soybean leaves as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

E. Potassium Content

K^+ concentration showed the highest values in soybean leaves grown in the control and it decreased with salinity stress (Fig. 5). The K content in plant tissues represents the main cation in plant cells, and is an important component of the cell osmotic potential [30]. However, the application of external proline enhanced K uptake in soybean plants (Fig. 5).

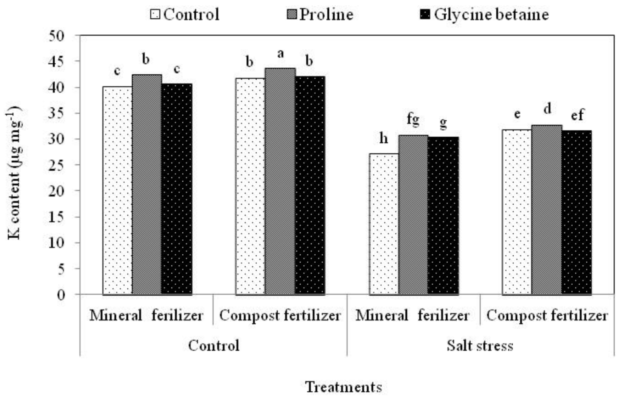

Fig. 5 The effects of salinity on K content (µg K mg^{-1} DW) in soybean leaves as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

The results agree with [31] which showed that exogenous application of proline counteracted the adverse effects of salinity on early seedling growth and enhances the K/Na ratio. Thus, proline caused a reduction in Na^+ ion absorption and toxicity. This could explain the mitigating effects of proline on the growth of soybean plants in saline condition. Similarly, the application of external glycine betaine increased K^+ uptake in soybean plants (Fig. 5). These results indicated that glycine betaine may play a role in maintaining cytosolic K^+ homeostasis by suppressing Na^+-enhanced apoplastic flow to reduce Na^+ uptake [28]. In this concern, the application of compost increases K+ content (Fig. 5). Organic matter (OM) can function as salt ion binding agents who detoxify the toxic ions, particularly Na^+ and Cl^- [32].

F. Proline Content

The results show that salt stress was significantly increased proline content (Fig. 6). The increase due to salinity was also demonstrated by [33]. Moreover, exogenous of proline to plants exposed to salinity stresses generally, provides a stress preventing or recovering effect (Fig. 6). Increased accumulation of proline in stressed plants may be an adaptation to compensate for the energy for growth and survival and thereby help the plant tolerate stress, as reported by [34]. In the same way, glycine betaine treatment, in the present work was shown to increase proline concentrations (Fig. 6). This may be due to the effect of glycine betaine in increasing the proline which can be involved in increasing the ability of soybean to cope with salinity stress [35]. Importantly, exogenous application of proline and glycine betaine in stressed plants further enhanced the endogenous proline content and in every case the effect of proline was higher than glycine betaine (Fig. 6). Moreover, the results revealed that plants grown in the organic compost soil has more proline content than the control (Fig. 6). Organic manure increased proline content under saline conditions which was reflected on producing better growth parameters, photosynthesis apparatus and sugar content. This free amino acid accumulates in plant tissues in response to several types of abiotic stresses as salt, drought or high temperature. The accumulation of proline might be resulted from increasing of protein turnover [36].

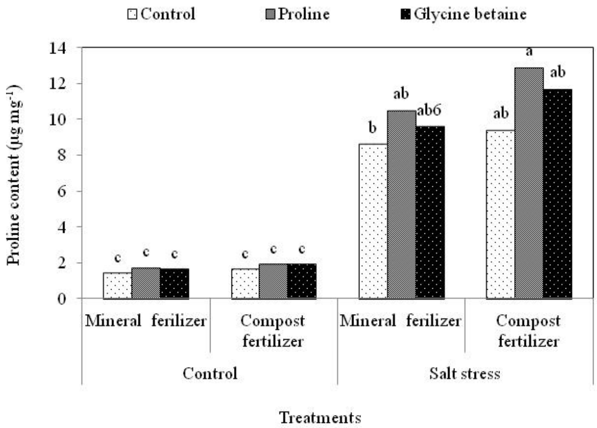

Fig. 6 The effects of salinity on proline content (µg mg^{-1} DW) in soybean leaves as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

V. Conclusion

This study indicated that that exogenous application of proline and glycine betaine combining with compost application alleviated detrimental effects of salt stress and may be a practical approach to improve salt stress tolerance in soybean. Consequently, the research will effectively solve seasonal salinity stress problem and are a good strategy to increase salinity resistance in soybean.

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