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RESEARCH ARTICLE

ROLE OF OSMOPROTECTANTS AND COMPOST APPLICATION IN IMPROVING WATER STRESS TOLERANCE IN SOYBEAN *(Glycine Max L.)*

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ABSTRACT

Water stress is a serious threat to the agricultural production and is likely to further increase. Osmoprotectants, plays an important role in the protects of plants from various environmental stresses. Moreover, Soybean is susceptible to yield loss from water deficit. Therefore, a greenhouse research was conducted aimed at assessing the Impact of exogenous osmoregulators and compost application in alleviating the adverse effects of water stress on soybean. The results indicated that, water Stress significantly reduced nitrogen content which results in the reduction of seed yield. While, Water stress resulted in a significant changes of proline content in leaves. In respect to the tolerance against water stress was observed, the improvement of water stress tolerance resulted from compost with compare mineral application, as well as exogenous osmoprotectants were improved proline and nitrogen content which results in the increase of seed yield. Interestingly, the combined application mineral fertilizer and exogenous osmoprotectants could to be more effective in alleviating the adverse effect of water stress to improve the growth and production of soybean.

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INTRODUCTION

Soybean *(Glycine max L.)* is considered as a miracle crop due to its extraordinary qualities. It contains 40 to 42% good quality protein and 18 to 22% oil, so it is highly desirable in human diet. As the best source of protein, it truly deserves the title "the meat that grows on plant" (Arshad *et al*., 2006). Soybean is considered highly sensitive to drought stress, especially during critical growth stages resulting reduction in yield.Moreover, Water stress as a key abiotic stress limiting for soybean production can cause reduction up to 40% of the yield or even more (Pathan *et al*., 2007). Plants Acclimation to deficit of water is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan *et al*., 2007).

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Various chemicals such as osmoprotectants, growth regulators and stress signaling molecules are being successfully used to induce the tolerance against several biotic and abiotic stresses (Farooq *et al*., 2010). The role of proline probably is not to reduce the osmotic potential, but to protect enzymes against dehydration and salt accumulation (Heidari and Moaveni 2009)**.** Exogenous application of proline is known to induce abiotic stress tolerance in plants (Ashraf and Foolad 2007).Glycine betaine, a member of quaternary ammonium compounds, is an osmolyte that is pre-dominant in higher plants subjected to drought condition (Chaitanya *et al*., 2009). Glycine betaine has been found to act as osmoprotectant and improve the growth and development of plants exposed to a variety of abiotic stresses including drought, temperature and salinity. Previous studies have demonstrated that biochemical, physiological and morphological changes occur in plants in response to water deficit (Basu *et al*., 2010; Chaum *et al*., 2010; Cha-um and Kirdmanee 2010). Application Compost fertilizer derived from agricultural wastes can improve crop tolerance and increase plant growth via providing better soil structure, supply of nutrients and building up antagonistic microorganisms (Tejada *et al*., 2009). Moreover, prolonged chemical fertilizers using alone in intensive cropping systems leads to unfavorable soil fertility, harmful effects on soil physic-chemical and biological properties and undermine sustainable production of crops. Organic matter deficit makes the situation worst for oil seed crops (Mamatha *et al*., 2015). researchers have studied the utilization of available organic materials revealed that straw mulch under water stress led to increased rate of photosynthesis and free proline accumulation (Chaum and Kirdmanee 2009). Keeping in view the importance of this technique the present study was planned to determine whether compost and exogenous applications of osmoprotectants increases drought tolerance of soybean plants, and if such tolerance is associated with changes in physiological aspects and seed yield.

MATERIALS AND METHODS

Plant material and culture conditions

Experiments were conducted in the Greenhouse at the Graduate School of Biosphere Science, Hiroshima University, Japan in 2011- 2012 .The seeds of soybean cultivar Giza 111, was collected from Egypt, (ARC). The seeds were sown into basin from wood (length10 meter ,width 50cm ,height 50cm and depth 350cm) containing a soil mixture of granite regosol soil and perlite (2:1 v/v). The experiment was designed as A Completely Randomized block with arranged a spilt - split plot arrangement with four replications. Each plot was fertilized at a rate of 40 kg N /ha, 12 kg P_2O_5 / ha and 10 kg K_2O_5 / ha using fertilizer mixture and calcium carbonate (300 kg/ha). The treatments could be summarized as follows. Main plots included (a) water stress at different soil moisture levels consisting of (100%, 75%, and 50% of field water holding capacity). Sub plots included (b) Compost application (Mineral (Control), compost (24 t/ha) compost was manufactured using wood poop, chicken poop and palm, Chemical analysis of compost was (N, 91%; phosphorous, 90%, potassium, 50 % and C/N, 24%). (**c**) Sub-sub plots included exogenous proline and glycine betaine at the concentrations of (control, 25 Mm) for each, was applied in two growth stages (V1 and R1). Where, (V1) one fully developed trifoliolate leaf node, (R1) Beginning Bloom (flowering).

Plant sampling and physiological Measurements: during the vegetative stage (V6) (Six fully developed trifoliolate leaf nodes Plants in each plot were sampled and separated into the leaves, stems and roots. The fresh samples were kept frozen in liquid nitrogen, and then freeze-dried for Nitrogen determination by the Kjeldahl method and Proline determination, was determined spectrophotometrically following the ninhydrin method described by Bates *et al* (1973), using L-proline as standard and then determined by spectrophotometer (U-3310, Hitachi, Ltd. Tokyo, Japa.

Seed Yield plant: At maturity a random sample of ten plants per replication were taken in order to determine Seed Yield plant (gm).

Statistical analysis: All data collected for both seasons were subjected to analysis of variance according to Gomez and Gomez (1984).and treatment means were compared using Duncun Multiple Range Test (Duncan, 1955).

RESULT AND DISCUSSION

Proline content (μg mg^{-1} DW)

proline accumulation in plant under water stress can reduce water loss of plants. The proline contents increased with the application of osmoprptectants and compost application. It was reported that soybean plants cope with drought stress by accumulating some osmolytes, including proline (van Heerden and Krüger 2002). (Fig.1) showed clearly that proline contents increased water-stressed plants. In this regard, water stress treatment caused an increase in proline as compared with control plants. In the meantime, exogenous osmoprotectants treatment caused further increases in proline compound.Thus, Proline concentration content increased with water stress plus osmoprotectants treatment compared with those values obtained under water stress treatment in the absence of osmoprotectants. It is well known that proline is considered compatible and osmoregulator solutes these compounds accumulate in many plant species under a broad range of stress conditions, such as water shortage, salinity, extreme temperatures, and high light intensity (Abbaspour 2012; Abu-Muriefah 2015).

In our study, proline content in the leaves of soybean plants grown under water stress increased compared to the unstressed control plants. Moreover, supplied osmoprotectants promoted this effect and increased proline (Fig.1). The combined application mineral fertilizer and exogenous osmoprotectants was found to be more effective in alleviating the adverse effect of water stress to improve the accumulation of Proline. (Fig.1). The application of Proline and mineral fertilizer together was found to be more potential in mitigating the adverse effect of water stress to improve the accumulation of Proline. Proline accumulation is considered as an early response to drought stress (Ramanjulu and Sudhakar 2000). Proline accumulation is positively correlated with drought tolerance (Reddy *et al*., 2004). This result agrees with Verbruggen and Hermans (2008) who have been reported Proline accumulation is believed to play adaptive roles in plant stress tolerance. It was clear that,proline contents increased with the application of compost application under water stress with compare mineral with compare mineral as shown in (Fig.1).While, the efficiency of the combined mineral fertilizer with osprotectants-treated plants was better in alleviating the adverse effect of water stress as shown in (Fig. 1).The results revealed that plants grown in soil under organic manure contained more proline content may be due to organic compost enhanced the availability of certain elements and their supply to the plant during growth period (Saleh*et al*., 2003). proline accumulation is one of the key features of protein metabolism under drought stress, and also the first response of plants exposed to water-deficit stress in order to reduce injury to cells (Ashraf and Foolad 2007).

Nitrogen (mg N g^{-1} DW)

The results show that decreasing the soil moisture caused marked significantly decreased in nitrogen contents in shoots under water stress with compare control (Fig.2). The decrease was due decrease in photosynthesis and protein synthesis are not provided; therefore, protein synthesis is dramatically reduced or even stopped (Smiciklas *et al*., 1992).

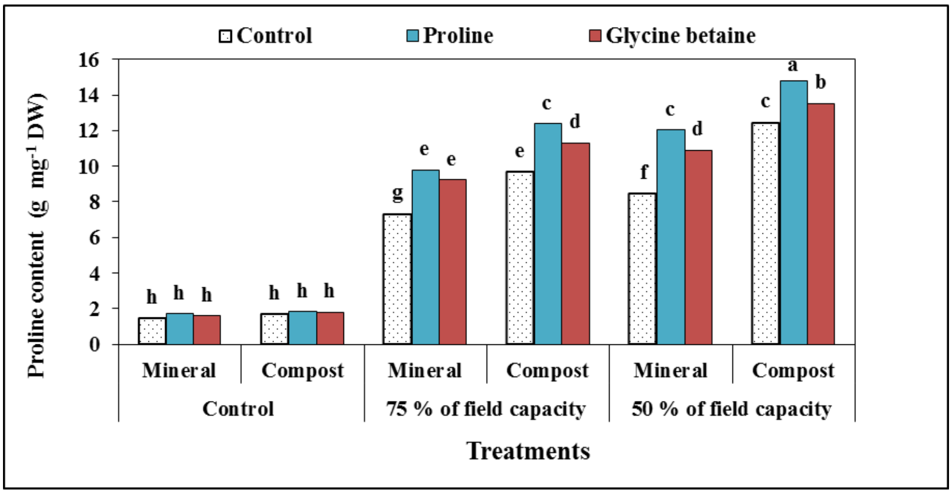

Fig.1: The effects of water stress on proline content (g mg^{-1} DW) in soybean leaves as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test

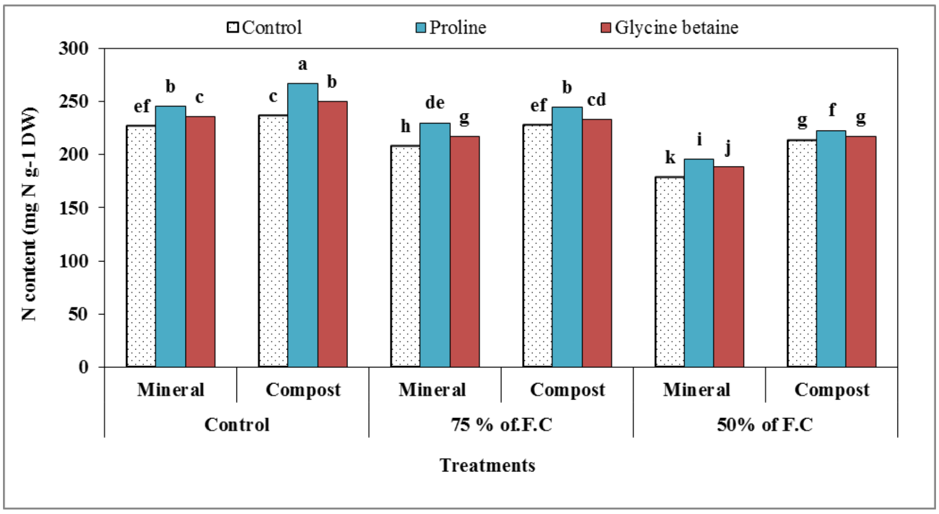

Fig 2. The effects of water stress on N content (mg N g^{-1} DW) in soybean as affected by compost and exogenous proline and glycine betaine. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test. FC; field capacity

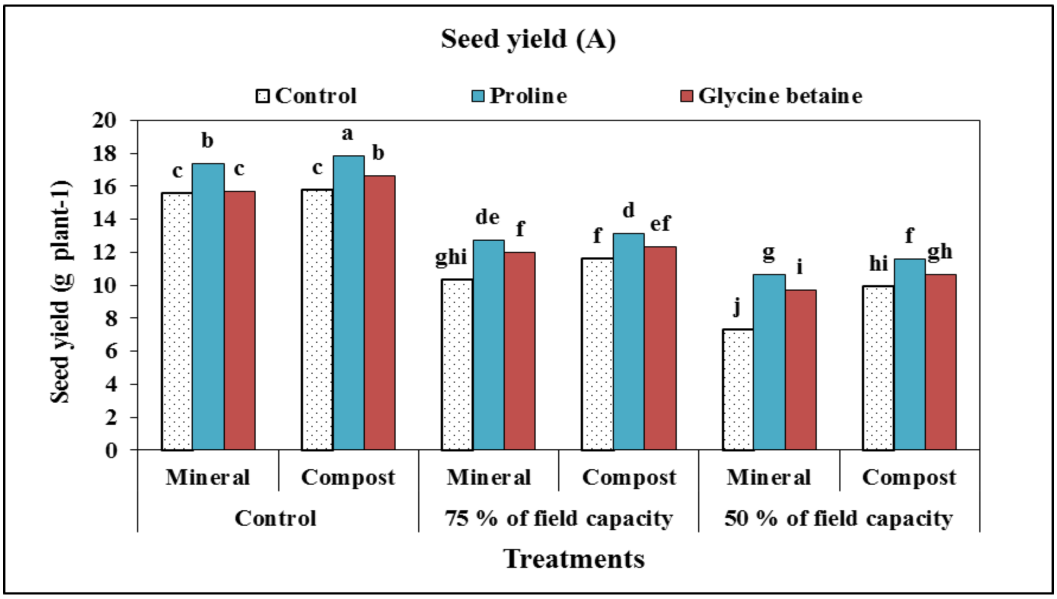

Figure 3 A

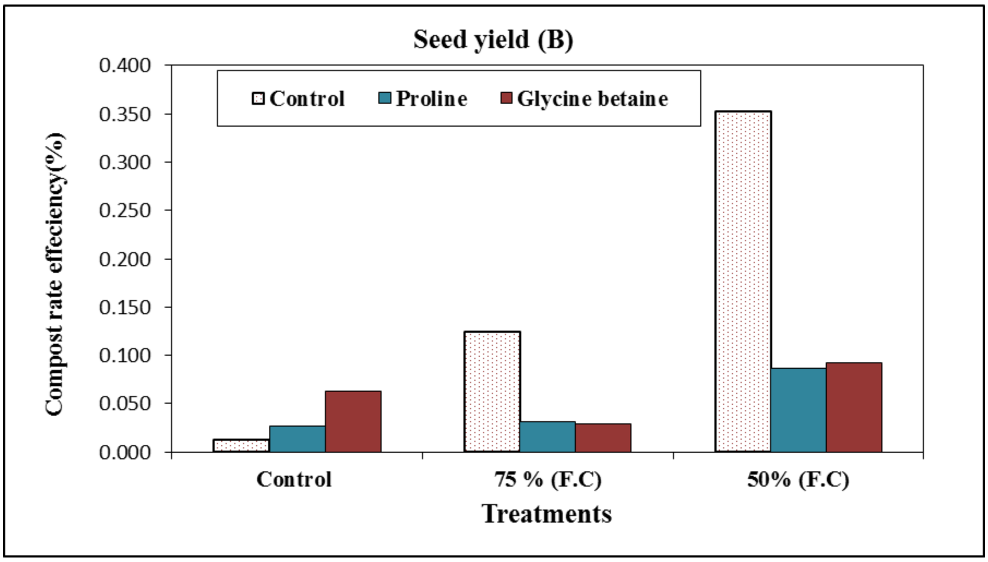

Figure 3 B

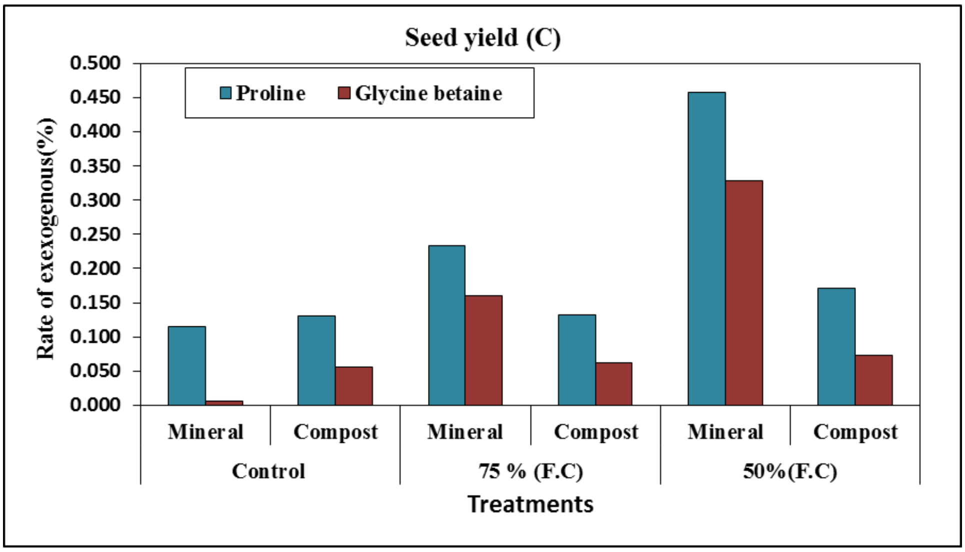

Figure 3 C

Fig. 3 (A, B, C). The effects of water stress on seed yield (g) 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. FC; field capacity

In the other hand, the exogenous osmoprotectants was found to be more effective under water stress to increase the content of nitrogen content (Fig.2). However, the application of Proline and mineral fertilizer together was found to be more potential in mitigating the adverse effect of water stress to increase nitrogen content (Fig 2). This result agrees with Heikal and Shaddad (1982) who reported positive effects of proline in counteracting the injury exerted through its accumulation in the whole plant organs under water stress. Concerning, application of glycine increase nitrogen content of soybean plants under water stress. However, the efficiency of the combined with mineral -treated plants was better in alleviating the adverse effect of water stress to increase nitrogen content (Fig.2). Glycine betaine induces increase in osmotic adjustments for drought tolerance by improving antioxidative defense system including antioxidative enzymes in wheat crop (Wang *et al*., 2010). According to the results, the application of compost increase nitrogen content of soybean plants compared with mineral fertilizer. However, the efficiency of the combined mineral with osprotectants -treated plants was better in alleviating the adverse effect of water stress than compost for increasing nitrogen content (Fig.2). This enhancement of free proline contents could be attributed to compost which blocked soil water evaporation and the effect increased with compost application dynamically (Wesseling *et al* (2009).These results are in agreement with Thakur *et al* (2000) who reported increased photosynthesis with application of compost. (Pongsa-Anutin *et al* (2007) observed that Mulch is retain soil moisture for longer duration providing additional amount of nutrition which help to enhance the rate of photosynthesis as a result, improving plant health. Enhancement of transpiration rate by straw mulch is in accordance with Zhang *et al* (2009) reported that Wheat straw mulch provides the ground with cover to prevent evaporation; the higher evapotranspiration under water limiting conditions improved photosynthesis and ultimately increased crop growth and yield.

Seed Yield/plant

The current results had a negative effect on Seed yield/plant under water stress and these experiments showed that water stress caused a significant reduction in seed yield.(Fig.3A,B,C) Actually, the drought stress initiates a series of biochemical and physiological processes in plants which results in the reduction of crop yield (Shahbaz*et al*., 2011). It was found that Soybean is sensitive to drought stress (van Heerden and Krüger 2002), and a drought-induced decrease in photosynthesis reduces soybean yield (Liu *et al*., 2004).In contrast, supplementary osmpprotectants enhanced seed yield compared to water-stressed plants. Our results demonstrate that exogenous of osmoprotectants compound improved the seed yield under water stress. Moreover, the combined application mineral fertilizer and osmoprotectants was found to be more effective in alleviating the adverse effect of water stress to improve seed yield (Fig.3A,B,C).Several investigators reported that proline plays a regulatory role in activity and function of the enzymes in plant cells and in their participation in development of metabolic responses to environmental factors (Ozturk*et al*., 2002). Likewise, the exogenous of glycine betaine is associated with the increase of seed yield/plant and may help to reduce adverse effects of water stress. However, the combined with mineral application was more effective to improve seed yield under water stress (Fig.3A, B, C).There are many reports demonstrating positive effects of exogenous application of glycine betaine on plant growth and final crop yield under drought stress of soybean(Ashraf and Foolad, 2007). Regarding to Compost caused a positive significant effect on seed yield. While, The efficiency of combined mineral with osprotectants -treated plants was better in alleviating the adverse effect of water stress for enhancing seed yield under stress conditions (Fig.3A,B,C).The improvement in growth with compost application be ascribed to the lowering of daytime soil temperature and increase in moisture supply to the crop (Xue *et al*., 2013). The considerable enhancement in soybean growth was the result of soil water being used for crop growth and yield rather than evaporation of soil water soil profile moisture under compost has important implications in the utilization of water by crop and in soil reactions that control the availability of nutrients and biological nitrogen fixation (Surya *et al*., 2000). Combining deficit irrigation and organic matter can be the key to improve soybean yield under water scarcity, because organic matter improves soil water-holding capacity and increases water and nutrients availability for plant (Wesseling *et al*., 2009). The protection of drought stress in compost -treated plants was associated with longer roots and smaller leaves for absorbing more water and losing less water, which improve stress tolerance in water-stressed plants (Fletcher *et al*., 2010).

Conclusion

We conclude that, application of compost and osmoprotectants alone promoted accumulation of proline and improving the content of nitrogen resulted in an increase in plant seed yield under water-stress. Furthermore, it is evident that, the combined exogenous osmoprotectants and mineral fertilizer together were more effective to alleviate the inhibitory effect of water stress on soybean. Consequently, these treatments play an important role to develop water stress tolerance in soybean and could be the key to reduce the deleterious effects of water stress on soybean growth.

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