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CHITOSAN AMELIORATES GROWTH AND BIOCHEMICAL ATTRIBUTES IN MUNGBEAN VARIETIES UNDER SALINE CONDITION

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ABSTRACT

The pot experiment was conducted to evaluate the effect of chitosan on the morphological, biochemical parameters of four Mungbean varieties (BARI Mung3, BARI Mung6, BINA Mung5 and BINA Mung8) under salinity condition. Each pot having eight kilograms of soil was prepared to grow three plants of each variety. The experiment comprised with four different conditions in triplicates viz. control, saline (40 mM NaCl, 25 days after sowing- DAS), saline plus chitosan (25 ppm chitosan, 30DAS on saline condition) and chitosan (25 ppm chitosan on control condition). Seed collections followed by data analysis were done. Proline content was measured accordingly. Salinity caused reduction in all growth and yield contributing attributes compared to control groups of all four varieties. Proline accumulation was enhanced due to saline condition, and this accumulation was not enhanced by application of chitosan. However, application of chitosan played as an outstanding stimulating role in all morphological parameters like number of flowers plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1} and thousand seeds weight under salinity stress.

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INTRODUCTION

Abiotic stresses severely reduce the productivity of almost all pulse crops including mungbean (Gao *et al*., 2007). As reported earlier, compare to the most of the known pulse crops, mungbean is relatively more sensitive to salt stress. Mungbean is generally known as a salt sensitive crop (Chakrabarti and Mukherji, 2003). Thus, it is expected that its metabolic processes are severely affected by salt stress. However, the stress-induced adverse effects may vary at various growth stages. It is observed that the adverse effect on grain yield is prominent at the reproductive stage than that of other stages (Thomas *et al*., 2004). Thus its growth retards with increase in saline regimes. Photosynthetic capacity is reduced in mungbean under saline regimes (Mortant-Manceau *et al*., 2004). The decrease in photosynthetic rate is ascribed to the reduction of stomata conductivity and consequently, inhibition in CO_2 availability for carboxylation (Koyro, 2006). Since the adverse effect of salinity causes remarkable loss in yield and quality of crops different techniques like salt resistant variety development, modulation of intercultural operation or application of some bio-stimulators is continuously being practiced by researchers. Application of chitosan (as a biostimulator) could be one of the approaches to decrease the adverse effect of abiotic stress on crop plants. Chitosan is a cationic polysaccharide produced by alkaline N-deacetylation of chitin. Beneficial roles of chitosan in enhancing tolerance of plants to biotic and abiotic stresses and its relevance to agriculture have been described (Farouk et al., 2011; Farouk and Amany, 2012). Antioxidant activity of chitosan has also been suggested (Park et al., 2004). Chitosan modulates the plant response to several abiotic stresses including salt and water stresses (Ruan and Xue 2002, Dzung et al. 2011). Recently, chitosan enhanced plant growth and development have been reported by some authors (Chibu *et al*. 2002, Mondal *et al*. 2012).

MATERIALS AND METHODS

Experimental site and period

The experiment was conducted at the laboratory of the department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh during the period from January to June, 2015.

Materials

Seeds of four Mungbean varieties were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur and Bangladesh Institute of Nuclear Agriculture (BINA),
BAU, Mymensingh. The collected seeds were stored in a refrigerator (at -18^0C) till use for experimental purposes.

Treatments

The experiment was comprised with four individual groups for different treatments as:
T_0- control condition was maintained by growing plants under natural environment only applying normal water and normal doses of fertilizers.
T_1- 40mM saline condition was induced by applying 20g salt pot^{-1} at 25 DAS.
T_2- saline+chitosan condition was induced by applying chitosan (25 ppm chitosan solution pot^{-1}) in the pot containing salt after one week of salt application.
T_3- chitosan condition was maintained by applying chitosan (25 ppm chitosan solution pot^{-1}) at 30 DAS in control condition.

Preparation of pot

Earthen pots were prepared for seed planting of mungbean varieties (BARI Mung3, BARI Mung6, BINA Mung5 and BINA Mung8). For each variety pots were prepared as triplicates. Thus total 48 (12×4) pots were filled with 8kg soil and then 5 seeds were sown at 08 April 2015 in each pot. At 18 DAS thinning (keeping 3 plants in each pot) and fertilization (Urea 4g; muriate of potash 2.5g; & boric acid 1.5g) were performed.

Determination of growth parameters

At 40 DAS, 45 DAS and 50 DAS number of flowers plant^{-1} were counted and at 60 DAS number of pods plant^{-1} were determined. Then numbers of seeds pod^{-1} were counted and thousand seed weight were measured using weighing machine.

Determination of proline

The fresh leaf sample was collected at the booting stage. Proline content of leaves was determined according to the method developed by Bates *et al.* (1973). Fifty milligrams of fresh leaf sample was homogenized in a mortar with pestle using 10ml of 3% sulfosalicylic acid. The homogenate was centrifuged and then filtered through Whatman no. 1 filter paper.

The extraction procedure was repeated and the two portions of the filtered were taken together. Two milliliter of the filtered was pipette into the test tube and 2ml acid ninhydrin and 2ml glacial acetic acid were added to it and the mixture was shaken well. The test tubes were incubated for one hour at 100^{0}C in a hot water bath. After that they were transferred to an ice bath to terminate the reaction. Four milliliter of toluene was added to each of the test tube, and then stirred vigorously for 15-20 seconds. The toluene was separated from the aqueous phase and collected carefully. Absorbance of the collected toluene was measured at 520nm in a UV- spectrophotometer (Shimadzu, UV-1201) against reagent blank. A standard curve was prepared with analytical grade proline and proline contents in sample were calculated by using the standard curve. Each analysis was done in duplicate from fresh leaf sample. Finally, the percentage of proline present in the leaves was expressed as mg/100g fresh leaves.

RESULTS

Yield contributing characters

Number of flowers plant^{-1}

Number of flowers plant^{-1} of all genotypes was significantly (1% level) reduced under salinity stress condition. Number of flowers plant^{-1} varied from 6 to 18 among different treatments. The highest number of flowers plant^{-1} (18) was recorded at chitosan applied condition and the lowest number was (6) recorded at under salinity condition.

Number of pods plant^{-1}

The effect of salt stress on the number of pods plant^{-1} was statistically significant (1% level) for all varieties. Number of pods plant^{-1} was varied from 3 (highest) to 1(lowest) among four varieties under salinity stress. The highest number of pods plant^{-1} (7) was recorded at control condition and the lowest number (1) was recorded at salinity stress.

Number of seeds pod^{-1}

Number of seeds plant^{-1} was varied from 9 to 4 among four varieties under salinity stress. The highest number of seeds plant^{-1} (10) was recorded at control condition and the lowest number (4) was recorded at soil salinity. The highest number (10) of seeds pod^{-1} was observed at both control condition and chitosan applied condition. Chitosan alone enhanced pod number plant^{-1} significantly in all genotypes compared to joint application (salt+chitosan) even more then control (BARI Mung3) conditions.

Thousand seeds weight (g)

A significant variation was found in all genotypes between control condition and salinity. The lowest seed wt. (34gm) and highest seed wt. (35gm) was recorded under saline condition which were significantly (5 to 1% level) less then control condition (Highest 38gm, Lowest 36gm). Thousand seeds weight enhanced significantly at chitosan induced saline condition compared to saline condition. However, there was no significant difference of thousand seed weight either at control or at chitosan induced condition.

Table 1. Number of flowers plant^{-1} under different conditions of four Mungbean Varieties

Variety	Number of flowers plant^{-1}				SE±	CV (%)	LSD	Level Of Sig.
	T_0	T_1	T_2	T_3				
1.BARI Mung3	17^a	10^c	14^b	16^a	0.351	4.26	1.21	**
2.BARI Mung6	16^b	9^c	16^b	18^a	0.415	4.87	1.43	**
3.BINA Mung5	15^b	8^c	15^b	17^a	0.305	3.85	1.06	**
4.BINA Mung8	15^b	6^c	15^b	17^a	0.238	3.11	0.823	**

Table 2. Number of pods plant^{-1} under different conditions of four Mungbean Varieties

Variety	Number of pods plant^{-1}				SE±	CV (%)	LSD	Level Of Sig.
	T_0	T_1	T_2	T_3				
1. BARI Mung3	5^a	3^c	4^b	5^a	0.164	6.69	0.568	**
2. BARI Mung6	6^b	1^d	5^c	7^a	0.218	7.96	0.756	**
3.BINA Mung5	6^b	2^d	5^c	7^a	0.228	7.90	0.789	**
4.BINA Mung8	7^a	2^c	6^b	7^a	0.169	5.33	0.585	**

Table 3. Number of seeds pod^{-1} under different conditions of four Mungbean Varieties

Variety	Seeds pod^{-1}				SE±	CV (%)	LSD	Level Of Sig.
	T_0	T_1	T_2	T_3				
1. BARI Mung3	8^{ab}	4^c	7^b	9^a	0.337	8.36	1.17	**
2. BARI Mung6	10^a	6^b	9^a	10^a	0.285	5.65	0.988	**
3. BINA Mung5	10^a	9^b	9^b	9^b	0.173	3.24	0.599	*
4. BINA Mung8	9^a	4^b	9^a	9^a	0.270	6.04	0.935	**

Table 4. Thousand seeds weight under different conditions of four Mungbean Varieties

Variety	Thousand seeds wt. (gm)				SE±	CV (%)	LSD	Level of Sig.
	T_0	T_1	T_2	T_3				
1. BARI Mung3	37^a	34^b	36^a	37^a	0.337	1.63	1.17	**
2. BARI Mung6	37^a	35^b	37^{ab}	38^a	0.578	2.72	2.00	*
3. BINA Mung5	38^a	34^b	35^b	38^a	0.746	3.56	2.58	*
4. BINA Mung8	36^{ab}	34^b	36^a	37^a	0.550	2.67	1.90	*

Proline accumulation

In the present study proline contents in the leaves of mungbean plant at early reproductive stage were presented in (Figure 1). A general trend of increase in proline accumulation was observed in stress plant compared to control one. The maximum (9.06mg/100gm) proline accumulation was found in BINA Mung8 genotype under salinity. Proline content gradually decreased for the application of chitosan with salt and chitosan alone in all genotypes. The lowest (4.66Mg/100gm) proline accumulation was found in BARI Mung6 under chitosan applied condition.

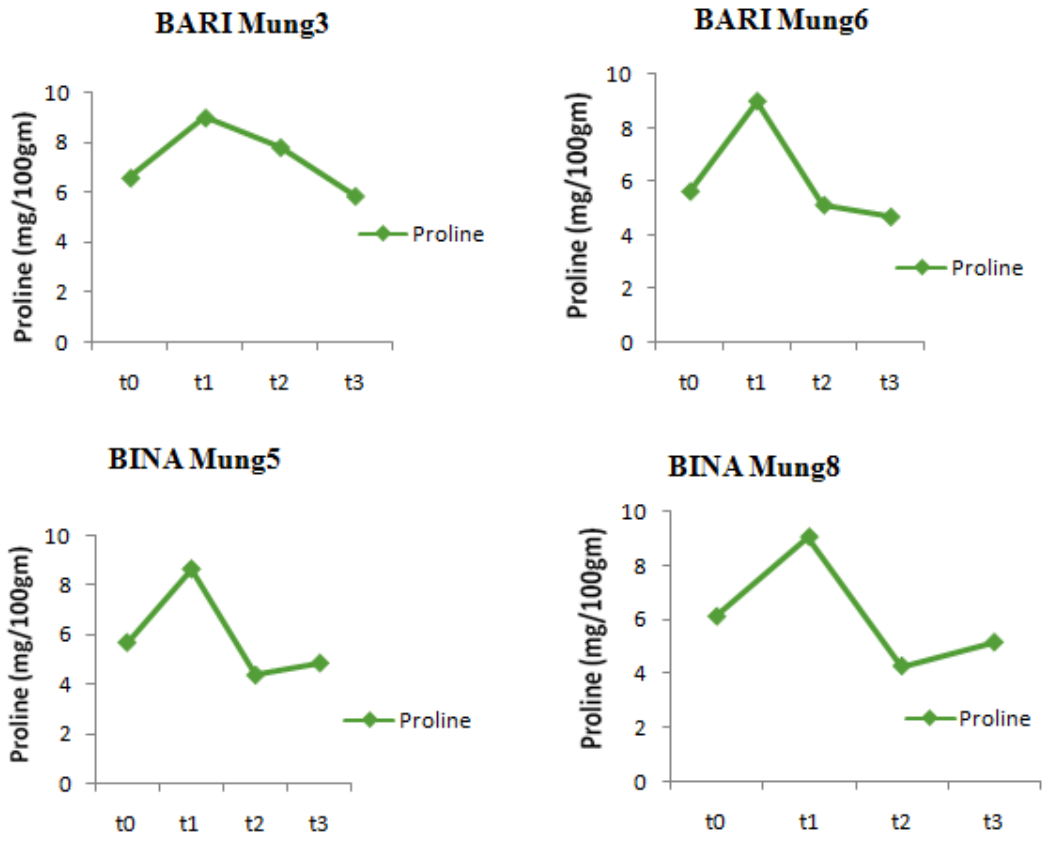

Figure 1. Proline accumulation under different treatments like t_0 = control, t_1 = salinity, t_2 = salinity+chitosan, t_3 = chitosan.

DISCUSSION

It was observed in a study (Khan *et al.,* 2010) that accumulation of toxic ions under salinity stress reduced the water and osmotic potential that further caused disturbances in photosynthetic processes. Thus loss of chlorophyll content caused chlorosis of leaves that later turned into necrosis. These adverse effects finally caused leaf and flower senescence. It is observed that yield might be reduced under salinity conditions due to increasing the rate of flower abscission and pod abortion (Liu *et al.,* 2003). On the other hand, the increase in pod formation due to chitosan application could be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photo assimilates from source to sink tissues. Such stimulating effect of chitosan on seed could be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful reactive oxygen species (ROS) by increasing antioxidants and enzyme activities (Guan *et al.,* 2009). The stimulating effect (1% to 5% level) on thousand seed weight in chitosan induced mungbean varieties was

found at saline condition in this study. This phenomenon could be attained through the increases in plant biomass which may be due to improving photosynthetic machinery (Khan *et al.,* 2002). However, Ghoname *et al.* (2010) also observed that foliar application of chitosan on sweet pepper increased significantly the number of fruits per plant and the mean weight of fruit, as well as quality characteristics of the fruit. Chitosan has been proved to work as a positive factor in enhancing seed weights in bean plants treated with the chitosan (Sheikh and Malki, 2011). A common response to water deficit in plants is the accumulation of osmoprotectants such as proline (Moradshahi *et al.,* 2004).

In this study, saline condition significantly (1% level) increases the proline accumulation in all mungbean varieties. It was reported that proline content in plant during salt stress condition could be increased mainly due to two reasons. Under salt stress condition, increment in proline accumulation often did occur due to onset of adaptive process (Aspinall and Paleg, 1981). Many others reported that increase in proline accumulation might occur from cellular injury as well (*Hanson* and Hitz, 1982) stress under salinity. The superoxide anion scavenging mechanism of chitosan related to its Nelson, structure that has many hydroxyl and amino groups available to react with ROS (Xie *et al.,*1978). Chitosan scavenged the excessive superoxide radical produced due to osmotic stress (Li *et al.,* 2002; Sun *et al.,* 2004).

CONCLUSION

Salinity imposition suppressed the morphological attributes especially vegetation compared to their respective controls. Salinity induces osmotic stress and toxicity which further could interfere with nutrients, signaling processes and related metabolic pathways. However, chitosan application could generate vigorous vegetation like the control. Therefore, it can be concluded from the results that chitosan is an effective bio-stimulator to enhance plant growth, yield and plant tolerance to oxidative stress under salinity stress and could overcome severe stress by scavenging of ROS through induction of enzyme activities.

COMPETING INTEREST

There is no conflict of interest among the authors.

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