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3 Effective Role of Growth Stimulators in Mitigating the Adverse Effect of Salinity Stress on Wheat (Triticum astivum L.) at Seedling Stage

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Abstract
Wheat is a crop of global significance and is a staple food of millions of people. Gibberellic acid (GA_3) and ascorbic acid (AsA) are considered as the most effective exogenous protectants which may alleviate the harmful effect of salinity stress. The laboratory experiment was conducted with four replications. Seeds of wheat cultivar GW-496 were soaked in distilled water (control), three concentrations of ascorbic acid (AsA) viz., (50, 100 and 150 mg L^{-1}) and three concentrations of gibberellic acid (GA_3) viz., (150, 200 and 250 mg L^{-1}) solutions for 2 hr. Three concentrations NaCl (50 mM, 75 mM and 100 mM) were added to induce salinity stress, whereas distilled water was provided as control. Root/shoot fresh weight ratio, Root/shoot dry weight ratio, total carbohydrates, total soluble protein and protein profile were measuredon 11th day after germination. It was observed that salinity stress at all three concentrations showed progressive reduction in all measured parameter showever, the reduction was most prominent at severe stress level (100 mMNaCl). Salt stress negatively affected protein synthesis and less number of protein bands/lanes were found under mild and moderate stress condition then severe stress condition. In addition, under NaCl treatment an appearance of a new band of Rm value 0.125 under saline condition. Ascorbicacid@ 100 mg L^{-1} was found efficient in enhancing all measured parameters and also increased the number of bands in protein profile with the appearance of five new protein bands of different Rm values three bands of Rm value 0.116 and two bands of Rm value 0.168 for the treatments under mild and moderate stress condition. For gibberellic acid, the highest concentration (250 mg L^{-1}) proved most efficient in enhancing all given parameters. GA_3@ 250mg L^{-1}also enhanced the number of bands and appearance 11 new protein bands were also observed. Hence, this study proves the role of these bio-stimulators in ameliorating the deleterious effect of salinity stress

Keywords: Stimulators, Mitigating, Salinity Stress, Wheat, Seedling Stage

Introduction
Wheat is a unique gift of nature to the mankind as it provides more than one-quarter of the total world cereal output, which signifies the great importance of wheat in agriculture, trade and nutrition. Wheat has a remarkable adoption to a wide range of environments and its role in world economy is well known. Soil salinity is a global eco-threat to sustainable agriculture and is also increasing over the time. Salinity is one of the most devastating forms of land degradation which severely affects crop growth and production worldwide especially in arid and semiarid regions (Shomeili *et al.,* 2011). The effect of salinity on plant may cause disturbance in plant metabolism (El-Tayeb, 2005). It may also induce biochemical changes such as osmotic stress, oxidative stress and protein denaturation in plants, which lead to cellular adaptive responses and accumulation of compatible organic solutes such as soluble carbohydrates, amino acids, proline, betaines, etc ultimately leading to crop growth reduction(Mohamed *et al.,* 2010). Presoaking treatment of seeds with growth regulators and vitamins could be employed to improve seed germination and subsequent seedling establishment under saline conditions. Pre-sowing seed treatments have been shown to enhance stand establishment in saline areas, Basra *et al.,* (2010).They have shown that prime importance of pre-soaking treatment is to improve physiology and biochemistry of plant in terms of enhancing the seed germination, seedling vigour index, vegetative growth and yield of crop plant under critical level of salinity stress. In terms of biochemical changes under salinity stress condition pre-soaking treatment causes accumulation of ions, increase in sugar content, proline content, organic compounds, antioxidants etc leading to high speed of germination and proper growth Abro *et al*., (2009).

Materials and Methods

Experimental details

Seeds of wheat cultivar GW-496 were used for the study. Salinity stress conditions were induced by using three concentrations of NaCl (50 mM, 75 mM and 100 mM). For the treatment of growth regulators three concentration of Ascorbic Acid (AsA) *viz*., (50, 100 and 150 mg L^{-1}) and Gibberellic Acid (GA_3) *viz*., (150, 200 and 250 mg L^{-1}) were selected. Seeds were soaked in distilled water (control), ascorbic Acid (AsA) and gibberellic Acid (GA_3) solutions for 2 hrs. Four replications of 25 seeds from each sample were treated with 2.5 g L^{-1}thiram for about 2 min. These seeds were then spread for germination on 12 cm diameter Petri Dishes on What man No.1 filter paper. The sufficient volume of (10 ml from 1st to 5th day and 20 ml from 6th to 11th day) of NaCl solution was added to induce salinity stress, whereas distilled water was provided to the seeds grown under control conditions.

The physiological parameters i.e. root /shoot fresh weight ratio and Root /shoot dry weight ratio was recorded at 11th day after germination. As per formula

$$\text{Root/shoot fresh or dry weight ratio} = \frac{\text{Root fresh or dry weight}}{\text{Shoot fresh or dry weight}}$$

The biochemical parameters i.e. carbohydrate content, protein content and protein profiling were recorded at 11th day after germination. The extraction and estimation of carbohydrate content was done as per the method suggested by Dreywood (1946) and Bruynet. al.,(1968), with some modifications, the protein content was determined by the proceduregiven byLowry *et al*., (1951).The protein profiling was done by method suggested by Walker (2002) through native poly-acrylamide gel electrophoresis which is based on theprinciple that the protein complex moves towards the anode and the separation is based on the size of protein molecules.

Statistical analysis of the data analysis was performed using the software "DSAASTAT" statistical software (Version 1.101). Mean separations were performed by Duncan's Multiple Range Test (DMRT) at 5% level.

Result and Discussion

Root/Shoot Fresh Weight ratio: Salinity (NaCl) stress had an overall adverse effect on root to shoot fresh weight ratio as the same decreased progressively with increasing salinity levels. Khavarinegad *et al*., (2014) and James *et al*., (2006) also observed similar results in lentil and *Sarcobatusvermiculatus* respectively. Remarkable increase in root to shoot fresh ratio was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. It was found that seeds pretreated with AsA @ 50 mg L^{-1} showed best increment under mild and severe stress conditions. Under moderate stress level treatment with AsA at its highest concentration (150 mg L^{-1}) was more effective. With respect to the treatment of wheat seeds with GA_3 under mild and moderate stress condition, the maximum increment was shown by its treatment at highest concentration (250 mg L^{-1}). Seeds treatment with mild concentration of GA_3 (200 mg L^{-1}) performed well under severe stress condition. Similar report was reported by Khavarinegad *et al.,* (2014) who showed that pre treatment of lentil seeds with GA_3 increased the root to shoot fresh weight ratio under saline condition.

Root/Shoot dry weight ratio: Progressive increase in salinity stress had an adverse effect on root to shoot dry weight ratio. There was 8% increase in the present parameter at mild stress level whereas, at moderate and sever stress levels decrease of 21% and 24% respectively were recorded. Similar result was proposed by Ologundudu *et al.,* (2014) who reported that increasing salinity levels reduced root shoot dry weight ratio in rice plant. Increase in root to shoot dry weight ratio was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. With respect to the treatment effect of AsA under both mild and moderate stress condition, the maximum increment was found by its concentration of 100 mg L^{-1}. But Under severe stress condition, the highest concentration of AsA (150 mg L^{-1}) was found more effective. The positive effect of exogenous supply of ascorbic acid on root/shoot dry weight ratio in chick pea under salinity was reported by Beltagi (2008). Further, 200 mg L^{-1} $GA_3$was found best in reducing the adverse effect of salt stress under mild stress condition. However, under moderate stress, GA_3 @ 150 mg $L^{-1}$was more effective and under severe stress condition maximum increment was shown by treatment with GA_3 @ 250 mg L^{-1}. The result is in agreement with Thakare *et al.,* (2011) who reported that GA_3 treatment was useful to increase root/shoot dry weight ratio and vigour index in wheat.

Total carbohydrate: Salt stress adversely affected the carbohydrate content, a drastic reduction in the same was found with increase in salinity levels. NaCl, at severe and moderate stress levels showed huge reduction in the given parameter to the tune of (73%) and (69%) respectively, whereas, comparatively less reduction of (31%) was observed under mild stress level. Rahdari and Hoseini (2015) also recorded reduction in total carbohydrate in wheat plant by salt stress. The decrease in carbohydrate content may be attributed to the decreased chlorophyll content and chlorophyll stability index under stress condition. Ascorbic acid and gibberellic were effective in counteracting the adverse effect of salinity stress through increase in carbohydrate content. Carbohydrate content was found maximum with the application of AsA @ 100 mg L^{-1} under all three stress condition. The study is in agreement with Farahat*et al.,* (2013) who found that the combined treatment of ascorbic acid (100 ppm and 200 ppm) with salinity level gave significantly increased total carbohydrates % content as compared with control plants. Several reports also indicated that the beneficial effects of additional antioxidants on plant survival under different salt stress are associated with the partial inhibition of ROS formation and protecting macromolecules (carbohydrate, protein, enzymes, DNA etc) from oxidative damage thereby maintaining their level in the plant. In case of seeds treated with GA_3 under all three stress conditions its application at highest concentration (250 mg L^{-1}) was best in increasing carbohydrate content. Similar report was given by Shaddad *et al.,* (2013) that application of different levels of gibberellic acid improved carbohydrate concentration in wheat under salinity stress. This might be due to the fact that GA_3 enhanced the synthesis of carbohydrate through better chlorophyll content and reduced oxidative damage of carbohydrate through better antioxidant system thereby, imparting stress tolerance.

Total soluble protein content: Irregular reduction in protein content was observed with increasing salinity levels. Salt stress at its severe level showed drastic reduction of (67%) in the given parameter but at moderate concentration a minimum reduction of (54%) of in protein content was observed and at

the mild stress level 64% reduction in protein content was observed. Similar result was reported by Rahdari and Hoseini (2015) in wheat, Anjali and Aruna (2013) in spinach, Ebrahimian and Bybordi (2012) in rice, and Tammam *et al.*, (2008) in wheat. The reason of such decrease might be due to oxidative damage of protein by ROS produced under salinity stress condition. A remarkable increase in protein content was found after pretreatment of wheat seeds with ascorbic acid and gibberellic acid under saline condition. It was found that seeds pretreated with AsA @ 100 mg L^{-1} was the best treatment under mild as well as severe stress levels while, under moderate stress level, treatment with highest concentration of AsA (150 mg L^{-1}) was more effective in ameliorating the effect of salinity stress. In favor of the present result Bassuony *et al.,* (2008) reported that vitamin C treatments on stressed *Zeamays* plants resulted in de novo synthesis of new proteins and the increased accumulation of certain existing proteins may be involved in increasing the tolerance of maize plant. Similar result of enhancement in protein content was reported by El- Hameda and Hanan (2015) in sweet pepper. AsA also play crucial role in protecting protein from denaturation due to oxidative damage and dehydration during salt stress by activating antioxidant system and enhancing production of osmolytes. Among the GA_3 treated seeds under all three stress conditions seeds applied with GA_3 @ 250 mg $L^{-1}$was best in reducing the adverse effect of salt stress. Sakhabutdinova *et al.,* (2010) reported similar result in Wheat. Gibberellic acid play important role in reducing the proline and free amino acid accumulation thus enhanced protein synthesis.

Protein Profile: Salt (NaCl) stress negatively affected the protein synthesis and hence band formation. The more number of protein bands/lane were found under the mild and moderate stress condition but number of protein bands/lane were dramatically reduced at severe stress condition. In addition NaCl showed an appearance of a new band of Rm value 0.125 under saline condition for the treatment 75 mM NaCl. The result is in agreement with Mohamed *et al*., (2010) in potato and Nagesh and Devaraj (2008) in french bean who reported that NaCl showed induction in the synthesis of new polypeptides under salinity stress and protein banding pattern and showed an entirely different sets of proteins in salt stressed seedlings. Similarly, Kong *et al*., (2005) while working with rice seedlings under salinity stress identified two protein bands (22 and 31 kDa) whose expression specifically increased under salt stress. Pretreatment of wheat seeds with ascorbic acid and gibberellic acid considerably increased the number of bands on gel under saline condition. For AsA treatment the highest number of bands under mild stress condition was found with the applications of AsA @ 100 and 200 mg L^{-1}. Under moderate condition maximum number of bands was found with the treatment of AsA @ 100 and 150 mg L^{-1}. While under severe stress condition all concentrations of AsA showed equal number of bands. It was found that there was an increase in appearance of new bands of different Rm values after pretreatment of wheat seeds with ascorbic acid under saline condition and also showed both qualitative and quantitative changes in the banding patterns of proteins in the wheat seedlings. Among the AsA treated samples five new bands of different Rm values were reappeared in response to the added ascorbic acid treatment which was completely disappeared in response to NaCl. The details of those eleven bands are as follows, three bands of Rm value 0.116 for treatments viz., NaCl 75 mM + AsA 50 mg L^{-1}, NaCl 75 mM + AsA 100 mg L^{-1} and NaCl 75 mM + AsA 150 mg L^{-1} and two bands of Rm value 0.168 for treatments viz., NaCl 50 mM + AsA 100 mg L^{-1} and NaCl 50 mM + AsA 150 mg L^{-1}. Similar result was shown by Mohsen *et al.,* (2013) in faba bean, Beltagi (2008) in chickpea and Ekmekçi and Karaman (2012) in *Silybummarianum* who reported that presoaking of seeds in ascorbic acid (50 ppm) under salinity stress resulted in change in protein pattern and increase in new protein bands formation. Hence application of ascorbic acid induced the synthesis and increased the intensity of the original protein bands and caused the appearance of additional new bands. Such increase could lead to tolerance mechanisms of treated plants towards salinity stress. In case of GA_3 treated seeds the highest number of bands was found with the application of GA_3 @ 200 and 250 mg L^{-1} under mild stress condition. Under moderate stress condition application of GA_3 @ 150 mg L^{-1} showed maximum number of bands. While under severe stress condition all concentrations of AsA showed equal number of bands. Taking in view the appearance of new bands for GA_3 treated samples it was found that eleven new bands of different Rm values were found in response to the added gibberellic acid treatments which completely disappeared in response to NaCl stress. The details of those eleven bands are as follows, one band of Rm value 0.116 for treatments viz., NaCl 75 Mm + GA_3 150 mg L^{-1}. Two bands of Rm value 0.125 viz., NaCl 50 mM + GA_3 200 mg L^{-1} and NaCl 50 mM + GA_3 250 mg L^{-1}.One band of Rm value 0.133 for treatments viz., NaCl 50 mM + $GA_3$150 mg L^{-1}. Three bands of Rm value 0.176 for treatments viz., NaCl 50 mM + GA_3 150 mg L^{-1}, NaCl 50 mM + GA_3 200 mg L^{-1} and NaCl 50 mM + GA_3 250 mg L^{-1}.Two bands of Rm value 0.215 for treatments viz., NaCl 50 mM + GA_3 200 mg L^{-1} and NaCl 50 mM + GA_3 250 mg L^{-1} and two bands of Rm value 0.476 for treatments viz., NaCl 50 mM + GA_3 200 mg L^{-1} and NaCl 50 mM + GA_3 250 mg L^{-1}. The result is in agreement with Abdel-Hamid, A. M., & Mohamed (2014) who reported that gibberellic acid pretreatment of barley induced the formation of unique newly protein that showed clear differences in the number of bands, their molecular weights and the intensity of bands. The results indicated the synergistic interaction between salinity stress, ascorbic acid and gibberellic acid under salt stress resulting in enhanced protein induction and repression in synthesis of new proteins for the inducing salt resistance in wheat seedlings.

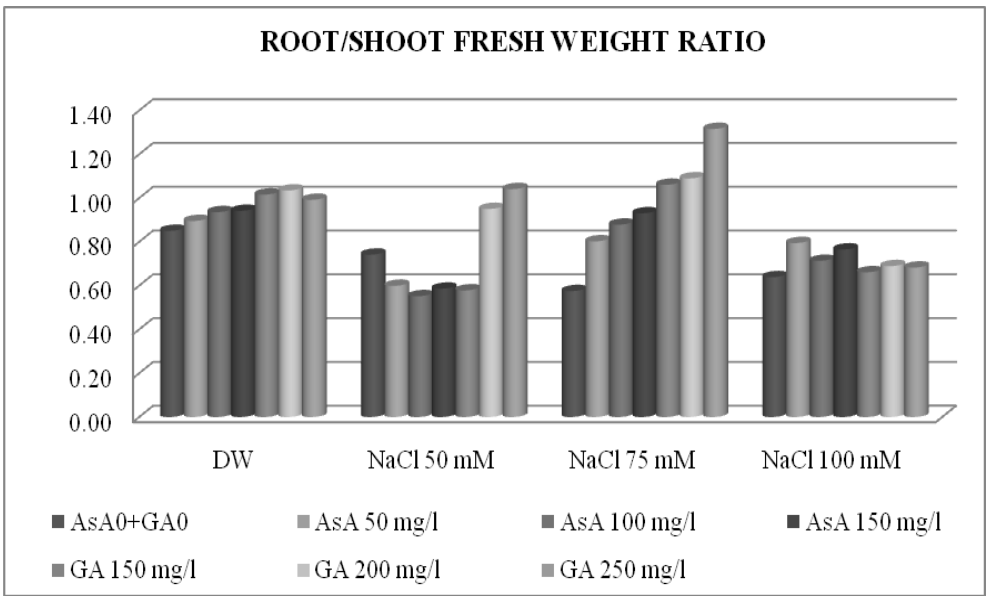

Fig 1: Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on root/shoot fresh weight ratio at 11th DAG of wheat.

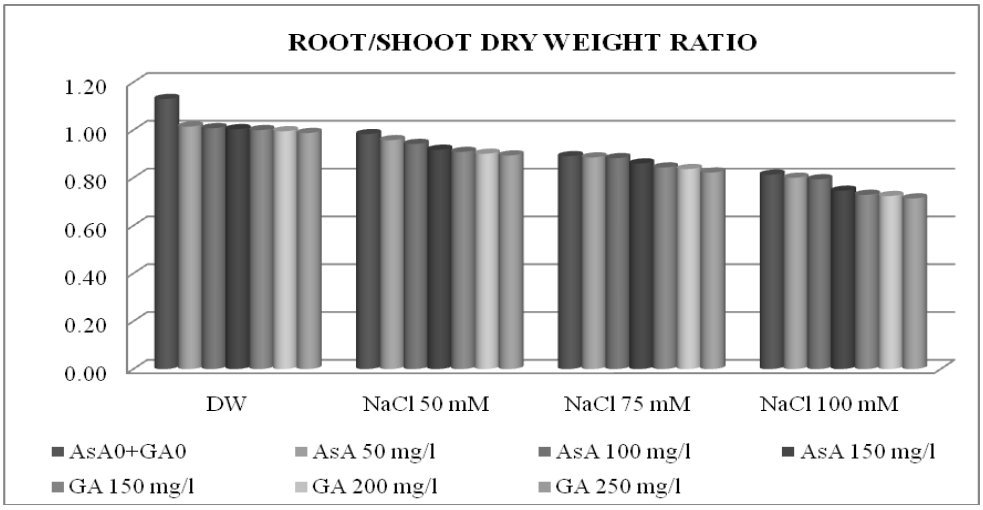

Fig 2: Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on root/shoot dry weight ratio at 11th DAG of wheat.

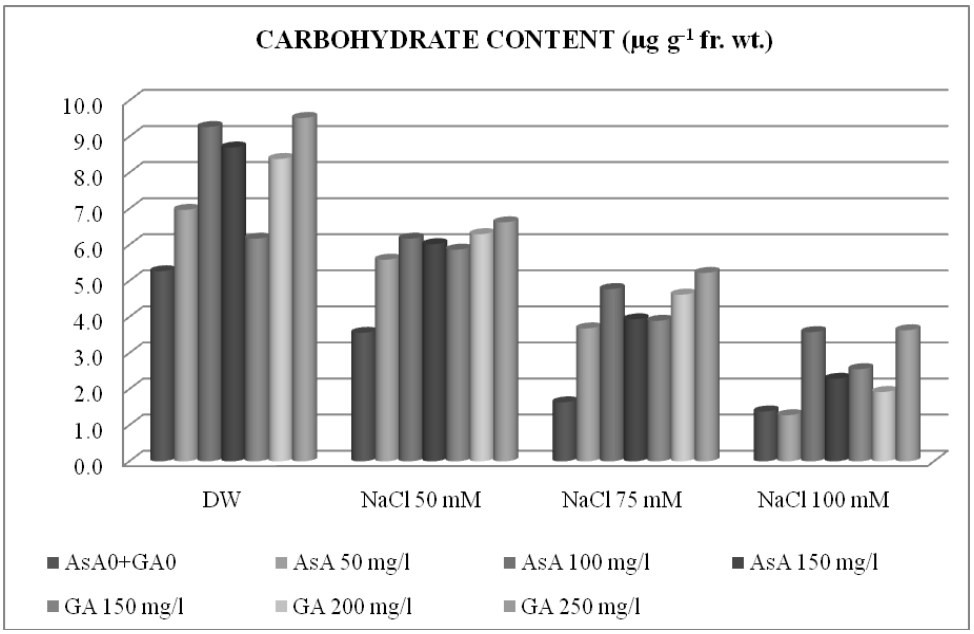

Fig 3: Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on carbohydrate content at 11th DAG of wheat.

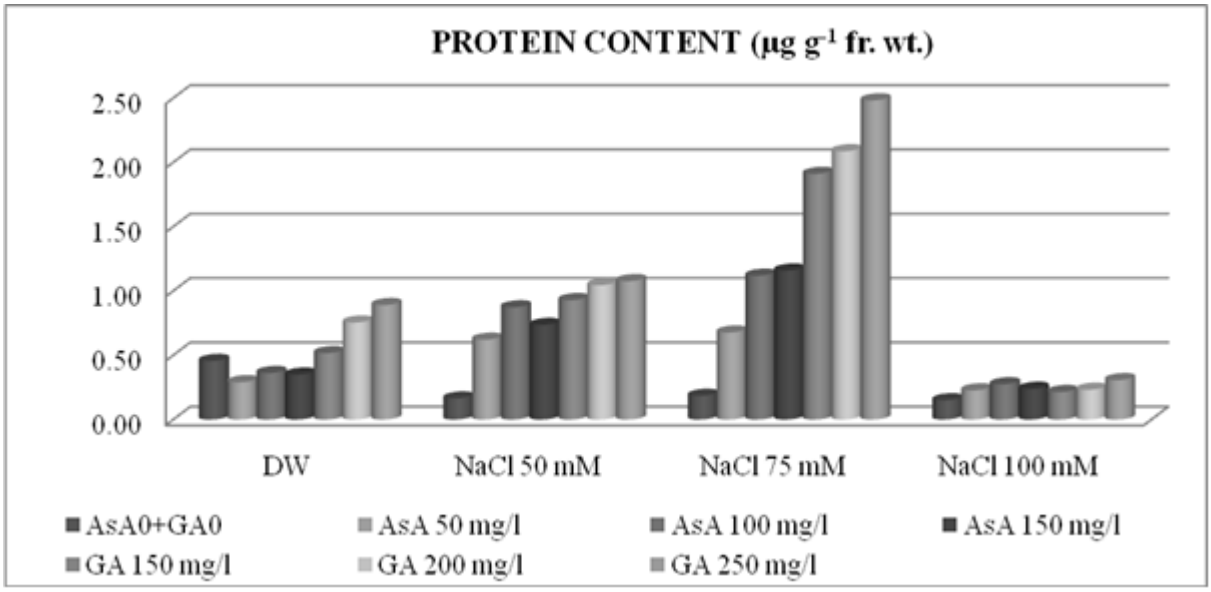

Fig 5: Effect of NaCl induced salinity stress, ascorbic acid (AsA) and gibberellic acid (GA_3) on protein content at 11th DAG of wheat.

Table 1: The Rm values of Proteins obtained *via* Native Page Gel Electrophoresis from control and treated (Salt, Giberellic Acid and Ascorbic acid) wheat seedlings at 11th DAG

Treatments	**Rm values**									
At 11th day	**0.116**	**0.125**	**0.133**	**0.168**	**0.176**	**0.215**	**0.317**	**0.377**	**0.428**	**0.476**
Distilled Water										
Distilled Water (DW)	-	-	-	-	-	-	+	+	+	+
DW + AsA 50 mg L-1	-	-	-	-	-	-	+	+	+	+
DW +AsA 100 mg L-1	-	-	-	+	-	-	+	+	+	+
DW + AsA 150 mg L-1	-	-	-	-	-	-	+	+	+	-
DW + GA3 150 mg L-1	-	-	-	-	-	-	+	+	+	-
DW + GA3 200 mg L-1	-	-	-	-	-	-	+	+	+	-
DW + GA3 250 mg L-1	-	-	-	-	-	-	+	+	+	-
MildStress										
NaCl 50 mM	-	-	-	-	-	-	+	+	+	-
NaCl 50 mM + AsA 50 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 50 mM + AsA 100 mg L-1	-	-	-	+	-	-	+	+	+	-
NaCl 50 mM + AsA 150 mg L-1	-	-	-	+	-	-	+	+	+	-
NaCl 50 mM + GA3 150 mg L-1	-	-	+	-	+	-	+	+	+	-
NaCl 50 mM + GA3 200 mg L-1	-	+	-	-	+	+	+	+	+	+
NaCl 50 mM + GA3 250 mg L-1	-	+	-	-	+	+	+	+	+	+
Moderate Stress										
NaCl 75mM	-	+	-	-	-	-	+	+	+	+
NaCl 75 mM + AsA 50 mg L-1	+	-	-	-	-	-	+	+	+	-
NaCl 75 mM + AsA 100 mg L-1	+	-	-	-	-	-	+	+	+	+
NaCl 75 mM + AsA 150 mg L-1	+	-	-	-	-	-	+	+	+	+
NaCl75mM + GA3 150 mg L-1	+	-	-	-	-	-	+	+	+	-
NaCl 75mM + GA3 200 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 75mM + GA3 250 mg L-1	-	-	-	-	-	-	+	+	+	-
Severe Stress										
NaCl 100 mM	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + AsA 50 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + AsA 100 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + AsA 150 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + GA3 150 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + GA3 200 mg L-1	-	-	-	-	-	-	+	+	+	-
NaCl 100 mM + GA3 250 mg L-1	-	-	-	-	-	-	+	+	+	-
A) Protein bands with moderate intensity (+)								**C)** No protein bands (-)		
B) Protein bands with dark intensity (+)										

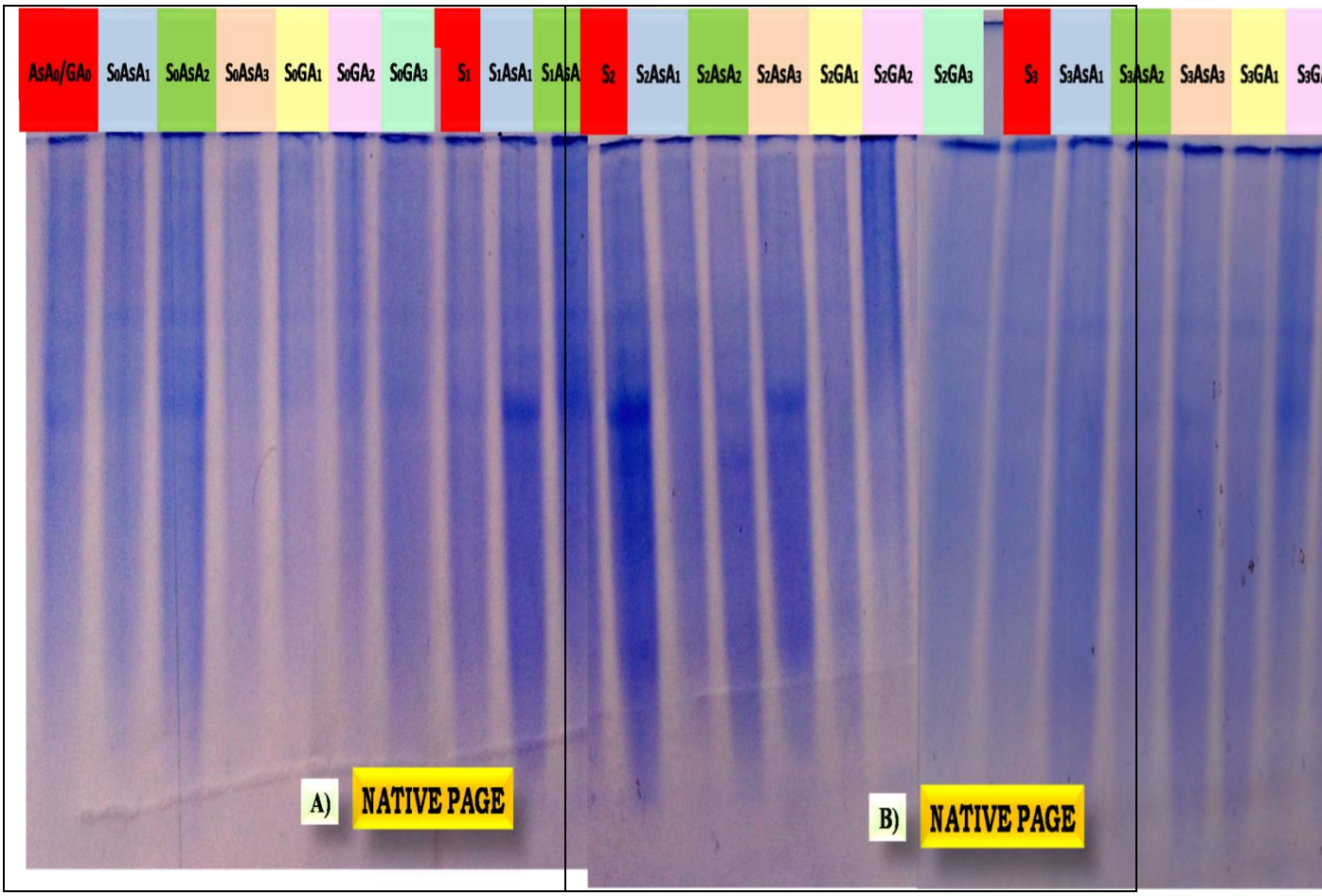

Fig 5: Proteins bands obtained via Native Page Gel Electrophoresis from control and treated (salt, gibberellic acid and ascorbic acid) wheat seedlings at 11 DAG`

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