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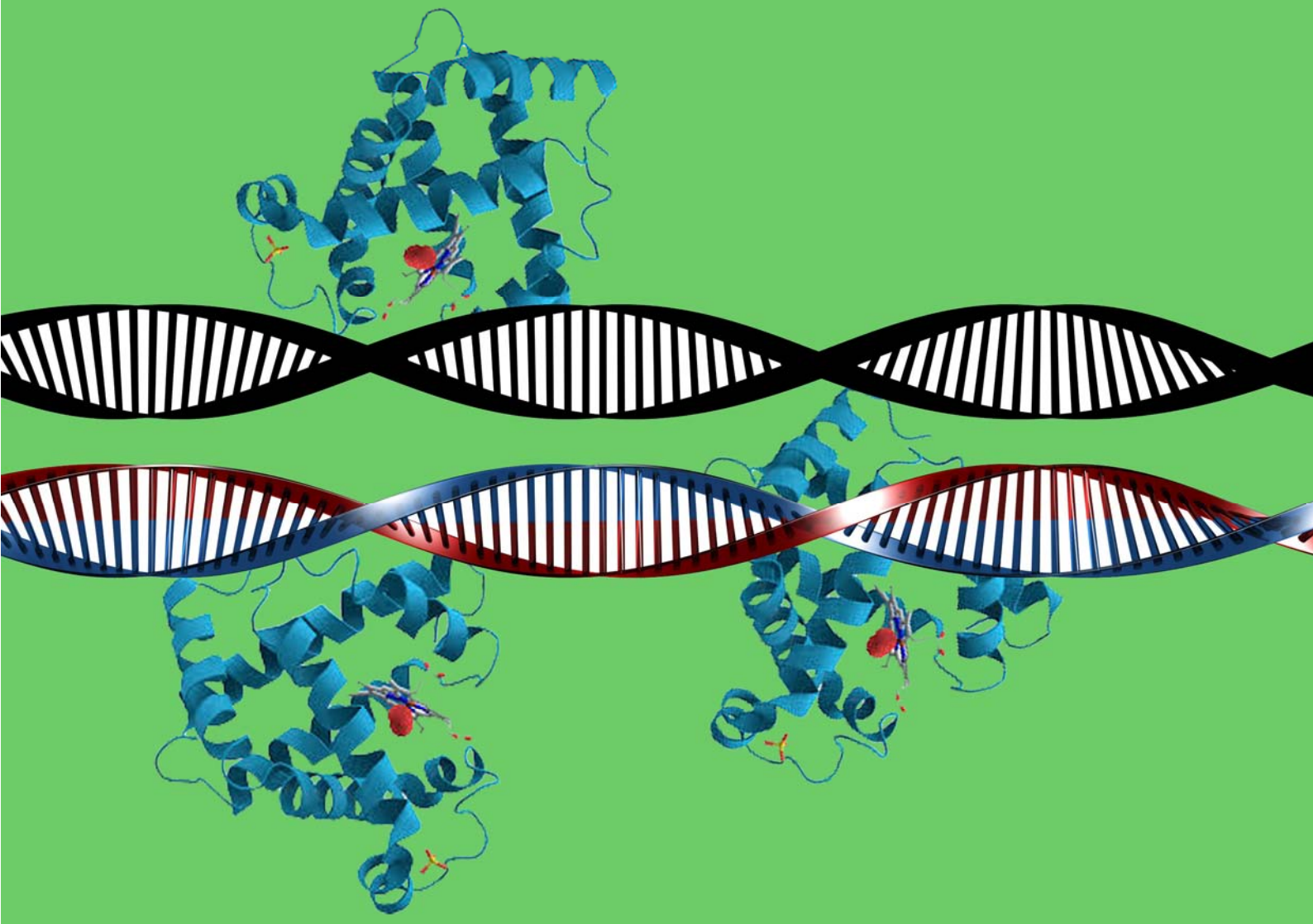

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Genomics and Applied Biology, 2014, Vol. 5, No. 1

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Genomics and Applied Biology, 2014, Vol. 5, No. 2

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Effect of Sodium Chloride on Photosynthetic Pigments and Soluble Protein Content of Green Gram Cultivars (Co6 And Co8)

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Genomics and Applied Biology, 2014, Vol. 5, No. 4

Genomics and Applied Biology 2014, Vol. 5, No. 4, 1-5
http://gab.biopublisher.ca

Research Report **Open Access**

Effect of Sodium Chloride on Photosynthetic Pigments and Soluble Protein Content of Green Gram Cultivars (Co6 And Co8)

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Abstract Investigations were undertaken to study the impact of salt stress (NaCl) in concentrations on seed germination and seedling growth of Green gram (CO5, CO6). Seed germination percentage, seedling growth characters, physiological and bio-chemical parameters were estimated at 10 days after sowing in Petridish. The stress was imposed during sowing time with different concentrations viz., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ppm. The increased total chlorophyll content was noticed in control (distilled water) treated seeds whereas; very less reduction was observed in T_2 to T_5 treatments in the range of 10.5 percent over the T_{11}. The highest reduction percent of 23% were observed in T_{10}, T_{11}, and T_{12} treated seeds. Soluble protein content was significantly reduced due to NaCl treatments. However among the treatments, T_1-T_5 showed very less reduction 11.5 percent than the other treatments, whereas T_6-T_{11} recorded highest reduction of 21~24 percent over control.

Keywords Salt stress; Seed germination; Photosynthetic pigments; Soluble protein; Green gram

1 Introduction

Green gram is the richest protein source of human diet and livestock in poor areas. Apart from that, they are used as green manures and green fodder to animals. Mainly they are used for fixing atmospheric nitrogen to improve the physical and chemical properties of soil. Among the legumes, Green gram was considered as the most important traditional crops of India. Salinity – an abiotic stress is an ever increasing problem that seriously affects crop production in various parts of the world, especially in areas where are irrigated with water containing salts. Salt stress is one of major factors in constraining crop adjustment substances, soluble sugar content, proline production.

About 23% of the world's cultivated lands are saline and 37% is sodic (Khan and Duke, 2001). Salinity affects 7% of the world's land area of about 930 million hectares. Salinity reduces the yield of pulses by more than 50% (Bray, 2000). Soils can be saline due to geo-historical processes or they can be man-made. The water and salt balance, just like in oceans and seas determine the formation of salty soils, where more salt comes in than goes out. Here, the incoming water from the land brings salts that remain because there is no outlet and the evaporation water does not contain salts. Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells. As a result of stomatal closure, photosynthesis declines and photo inhibition and oxidative stress occur. Chlorophyll is the principal agent responsible for photosynthesis and, under adverse conditions, chlorophyll level is a good indicator of photosynthetic activity (XinWen et al., 2008). The deleterious effect of salinity is increased osmotic pressure which restricts the absorption of water into the seeds (Tester and Davenport, 2003). It is also toxic to the embryo and seedlings. Enzyme called α – amylase which is essential for seed germination is inhibited due to salt stress. Starch to

sugar conversion occurs during germination is also affected by salinity. It also delays the synthesis of nucleic acids and RNAase. As regard to the chlorophyll content of the salinized plant, it is apparent that the chlorophyll content was reduced with increasing salinity. When salinity has affected the warning signs were sick or dying trees and declining vegetation. As salinity impacts on any remaining native vegetation and the wildlife that depends on it for survival, the loss of biodiversity escalates. Salinity also reduces the productivity of crops and the sustainability of agriculture. Based on the above constraints, we are taken the objective of Screening of Green gram varieties for NaCl stress tolerance through physiological analysis.

2 Result and Discussion

2.1 Photosynthetic pigments

Photosynthetic pigments are composed of chlorophylls a, b and total, and the main functions are reception and storage of light energy by inductive resonance through antenna complexes, and consequent electron transport carried out by the photosystem II (Taiz and Zeiger, 2002). Salt stress has been stated to reason an inhibition of growth and development, lessening in photosynthesis, respiration and protein synthesis in sensitive species (Boyer, 1982).

2.2 Chlorophyll a (mg/g)

The result on Chlorophyll 'a' was significantly differed in all the treatments. Among the treatments, T_1 showed highest Chlorophyll 'a' content in green gram both CO6 and CO8 (3.35 and 1.86), which was followed by T_2, T_3, T_4, T_5 and T_6. The lowest Chlorophyll 'a' content was recorded in T_{10} and T_{11} treatments (Table 1).

2.3 Chlorophyll b (mg/g)

The result on Chlorophyll 'b' was significantly differed in all the treatments. Among the treatments, T_1 and T_2 showed highest Chlorophyll 'b' content in green gram both CO6 and CO8 (0.78 and 0.32), which was followed by T_3, T_4, T_5 and T_6. The lowest Chlorophyll 'b' content was recorded in T10 and T11 treatments (Table 1).

Table 1 Effect of salt stress (NaCl) on photosynthetic pigments of green gram (CO6 and CO8)

Treatments	Chlorophyll 'a' (mg/g)		Chlorophyll 'b' (mg/g)		Total Chlorophyll (mg/g)	
	CO6	CO8	CO6	CO8	CO6	CO8
T_1	3.35	1.89	0.78	0.32	4.41	2.95
T_2	3.30	1.84	0.78	0.32	4.36	2.90
T_3	3.08	1.62	0.77	0.31	4.13	2.67
T_4	3.06	1.60	0.76	0.30	4.10	2.64
T_5	3.03	1.57	0.74	0.28	4.05	2.59
T_6	3.00	1.54	0.73	0.27	4.01	2.55
T_7	2.98	1.52	0.67	0.21	3.93	2.47
T_8	2.92	1.46	0.66	0.20	3.86	2.40
T_9	2.85	1.39	0.66	0.20	3.79	2.33
T_{10}	2.80	1.34	0.62	0.16	3.70	2.24
T_{11}	2.90	1.44	0.65	0.19	3.83	2.37
Mean	3.0	1.6	0.7	0.2	4.0	2.6
SE.d	0.160	0.160	0.034	0.018	0.160	0.160
CD (0.05)	0.333	0.333	0.070	0.039	0.333	0.333

2.4 Total Chlorophyll (mg/g)

The result on total chlorophyll content was significantly differed in all the treatments. Among the treatments, T_1 showed highest total chlorophyll content in green gram both CO6 and CO8 (4.41 and 2.95), which was followed by T_2, T_3, T_4 and T_5. The lowest total chlorophyll content was recorded in T_9, T_{11} and T_{10} treatments (Table 1).

There was also significantly maintained in the total chlorophyll content of the seedlings in the T_1~T_5 treatments as 12.6%, with lesser reduction over the other treatments. The highest reduction of 25.3 percent was noticed in T_6-T_{12} treated seedlings. According to Lapina and Popov (1970), saline conditions lead to disruption of the fine structure of chlorophyll and instability of the pigment protein

complex which may also be the cause of the reduced chlorophyll content.

2.5 Carotenoids (mg/g)

The result on Carotenoid content was significantly differed in all the treatments. Among the treatments, T_1 showed highest Carotenoid content in green gram both CO6 and CO8 (2.11 and 1.71), which was followed by T_2, T_3, T_4 and T_5. The lowest Carotenoid content was recorded in T_9, T_{10} and T_{11} treatments (Table 2). The carotenoid pigments in the leaf tissues of extreme salinity stress were degraded by 60% and 72% respectively in sugarcane. Reduction in water use efficiency in this crop under salinity level had a direct impact on photosynthetic pigment degradation, leading to reduce water oxidation in photosystem II (Shao et al., 2008). Total chlorophyll content decreased when plants are subjected to severe drought (50% of field capacity), these could be a result of a reaction centre or a photosystem II modification (Blum et al., 1989).

2.6 Soluble protein (mg/g)

The soluble protein content of the leaf, being a measure of RuBP carboxylase activity was considered as an index for photosynthetic efficiency. There were reports that RuBP-case enzyme forms nearly 80 per cent of the soluble proteins in leaves of many plants (Joseph et al., 1981). The result on soluble protein content was significantly differed in all the treatments. Among the treatments, T1 showed highest Soluble protein content in green gram both CO6 and CO8 (4.19 and 9.16), which was followed by T_3, T_4 and T_5. The lowest Soluble protein content was recorded in T_7, T_8 and T_{11} treatments (Table 2). Martignone et al. (1987) observed that in soybean soluble protein content was the first nitrogenous compound affected under stress conditions, which at severity got denatured and lost the activity. It was further explained that soluble protein, world's most abundant protein containing the enzyme RUBISCO, is involved in CO_2 assimilation; therefore, the reduction in soluble protein might have a direct adverse effect on photosynthesis.

Table 2 Effect of salt stress (NaCl) on carotenoids and soluble protein content of green gram (CO6 and CO8)

Treatments	Carotenoids (mg/g)		Soluble protein (mg/g)	
	CO6	CO8	CO6	CO8
T_1	2.11	1.71	4.19	9.16
T_2	2.06	1.66	3.87	8.86
T_3	1.84	1.44	4.47	8.56
T_4	1.82	1.42	4.27	7.34
T_5	1.79	1.39	4.01	7.14
T_6	1.76	1.36	5.49	6.87
T_7	1.74	1.34	2.99	6.68
T_8	1.68	1.28	2.55	6.47
T_9	1.61	1.21	5.91	6.36
T_{10}	1.56	1.16	3.71	5.64
T_{11}	1.66	1.26	2.69	6.17
Mean	1.8	1.4	4.0	7.2
SE.d	0.160	0.116	0.418	0.160
CD (0.05)	0.333	0.242	0.867	0.333

3 Materials and Methods

The experiment was carried out at Vanavarayar Institute of Agriculture (TNAU affiliated), Pollachi, Tamil Nadu, India during 2013~2014. The experiment consists of ten treatments with three replications were laid out in completely randomized block design with two cultivars of CO5 and CO6. Seeds of green gram varieties obtained from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, were used for the study and the details of the varietal characters were listed in Table 3. Green gram varieties (Table 3) were screened for tolerance to various levels of sodicity stress, based on germination per cent, seedling growth and vigour index, seeds were allowed to germinate in Petri dishes. The germination medium was prepared following the procedure mentioned below. Petri dishes were sterilized using 0.01% $HgCl_2$ and 70% ethanol and finally washed with distilled

water. Before placing the germination sheet, Petri dishes were cleaned thoroughly with a cotton swab. The surface sterilized (70% ethanol) 15 seeds from each variety were placed in each Petri dish. For imposing sodicity (11 levels as considered as Treatments) stresses, sodium chloride (NaCl) solution at the concentration of T_1: control (without NaCl), T_2:10, T_3:20, T_4:30, T_5:40, T_6:50, T_7:60, T_8:70, T_9:80, T_{10}:90 and T_{11}:100 ppm were prepared. The seeds were allowed to germinate, by sprinkling the salt solution of 10 mL each in alternate days. Distilled water was used for maintaining the control. The pH and EC details of the salt solution used for experiment were given in Table 4.

Table 3 Varietal Details

S.No	Crop	Variety	Duration (days)	Yield (kg/ha)	
				Rainfed	Irrigated
1.	Green gram	CO6	62~67 days	900	1050
2.		CO8	70 days	900	1050

Table 4 pH and EC of the salt solution used for experiment

S.No	(NaCl) Salt solution	pH	EC (dS/m)
1.	0 ppm	7.1	0.42
2.	10 ppm	7.3	2.0
3.	20 ppm	7.5	4.1
4.	30 ppm	7.6	5.3
5.	40 ppm	7.7	6.9
6.	50 ppm	7.8	7.0
7.	60 ppm	8.2	7.6
8.	70 ppm	8.4	8.1
9.	80 ppm	8.7	8.5
10.	90 ppm	9.2	9.00
11.	100 ppm	9.5	10.00

3.1 Observation recorded

The germination percentage, root length, shoot length and Vigour index were measured at 15th DAS and chlorophyll 'a' 'b' and total chlorophyll and carotenoid content was estimated based on the procedure given by Yoshida et al. (1979) and expressed as mg/g fresh weight.

3.2 Photosynthetic pigments

The chlorophyll content was estimated following the method suggested by Yoshida et al. (1971) and expressed as mg/g fresh weight.

3.3 Procedure

Take 250 mg of leaf sample is macerated with 10 mL of 80% acetone using a pestle and mortar and the extract is centrifuged at 3000 rpm for 10 minutes. The supernatant solution is transferred into a 25 mL volumetric flask and made up to 25 mL using 80% acetone. Then the color intensity of the green pigment is read at 480 nm, 510 nm, 645 nm, 663 nm and 652 nm for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content respectively using Spectrophotometer.

3.4 Soluble protein content

Soluble protein content of leaf was estimated as per the method of Lowry et al. (1951) and expressed as mg/g fresh weight.

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