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Effects of Genistein on Nodulation, Nitrogen Fixation and Physiological Attributes of Soybean under Salt Stress

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Abstract

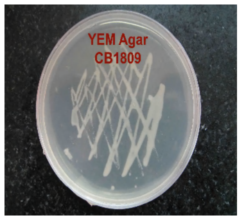
Legumes are highly important food, feed and bio-fuel crops, that can enter into a symbiotic relationship with specific soil bacteria called rhizobia. This interaction results in conversion of atmospheric nitrogen into useable forms of nitrogen, thus reducing/replacing fertiliser demand. Isoflavones, such as genistein in soybean play important roles in plant-microbe interactions, c.f., the legume root nodule symbiosis. Therefore, we evaluated the role of exogenous genistein in the *Bradyrhizobium* inoculation medium on nodulation, nitrogen fixation and some physiological attributes of soybean grown under salt stress. There was a significant difference between presence or absence genistein on nodule number and nitrogenase activity. Genistein increased nodule number and nitrogenase activity in each level of salinity compared with control. Moreover, genistein enhanced maximum photochemical efficiency, photosynthetic rate, stomatal conductance and transpiration rate while catalase, superoxide dismutase and peroxidase activity in leaves and roots were not affected by genistein but salt stress induced large and significant changes on both photosynthetic parameters and antioxidant enzyme activity. The former was suppressed and latter was amplified. There was significant decrease in nodule number and nitrogenase activity because of salt stress. According to these results it can be deduced that, genistein application into rhizobia inoculant improves plant growth through improved nodulation and nitrogen fixation in both normal and salt stress conditions.

Introduction

Soybean (*Glycine max* L.) is the most important legume crop in the world. An integrate interaction between the soil bacterium *Bradyrhizobium japonicum* and its plant host results in the formation of nitrogen fixing root nodules. The symbiosis benefits both partners as the prokaryotic partner receives carbohydrate and the symbiotic bacteria provide the plant with nitrogenous compounds. In general, legume plants exude into their rhizosphere complex cocktails of sugars, flavones or isoflavones, which are perceived as *nod*-gene inducers in 'Rhizobium' bacteria. Specifically for soybean, the isoflavones genistein released by plant roots induce the common nodulation genes of the bacterium. Genistein is the major signal components of soybean root extracts. Reported studies showed that pre-incubation of *B. japonicum* with genistein increased nodule number and nitrogen fixation. In addition, several studies have documented the use of flavonoid inducer molecules as a tool in enhancing nodulation and nitrogen fixation. All stages of the soybean nitrogen fixation symbiosis are inhibited by suboptimal conditions such as drought, temperature, acidity and salinity. In legumes, salinity can limit affects microbial activity and plant growth, because both hyper-ionic and hyper-osmotic stress effects, depress symbiotic performance. Salt stress limits plant productivity in legumes through diminished photosynthetic efficiency, carbon metabolism, leaf-chlorophyll content as well as nitrogen fixation. The objective of this study was to evaluate soybean responses to salinity stress and genistein pre-treated *B. japonicum* inocula in terms of nodulation, nitrogen fixation and selected physiological and biochemical changes.

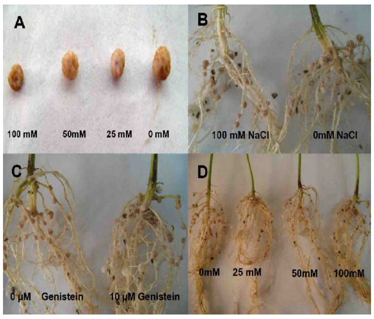
Material and Methods

Soybean seeds (cultivar: L17) were sown into plastic pots filled up with autoclaved perlite and vermiculite. The pots were placed into a growth cabinet (L/D=16/8 h, T=28/25C), and watered with full strength of Broughton and Dilworth solution (B&D). Bradyrhizobium japonicum (CB1809) was cultured in liquid YMB culture, after 24 h genistein was added into YEB to reach final concentration of 10 µM. The five days old plants were inoculated with a liquid YMB and watered with B&D solution containing 8 mM KNO_3 and 0, 25, 50 and 100 mM NaCl. Four weeks after inoculation, maximum photochemical efficiency of PSII (Fv/Fm), photosynthetic rate, stomatal conductance and transpiration rate were measured. Number of nodules and nitrogenase activity (GC method) was assayed. Fresh leaves were frozen in liquid N_2 for antioxidant enzyme activity assay.



Results and Discussion

Salinity had negative effects, and pre-incubation of *B. japonicum* with genistein had positive effects on all of the traits except for antioxidant enzyme activity (Table 1). The combined effect of genistein and NaCl was significant on maximum photochemical efficiency of PSII , transpiration rate, photosynthesis rate, nodule number and nitrogenase activity (Table 2). Stomatal conductance, photosynthetic rate, and transpiration rate were reduced by NaCl while pre-incubation with genistein increased these parameters. The highest transpiration and photosynthesis rate were recorded in control plants inoculated with 10 µM genistein while the lowest transpiration and photosynthesis rate were obtained at the highest salt concentration without genistein use.



Here-observed increases of stomatal conductance and transpiration rate with concomitant increased photosynthesis rate can be attributed to more nitrogen fixation and nitrogen availability due to an enhancing effect of genistein on nodulation (Miransari and Smith, 2008). In contrast, genistein treatment alleviated the effects of salt stress and enhanced Fv/Fm. There were significant differences among salinity and genistein levels for this parameter. Salt stress reduced nodule number while inoculated soybean plants with genistein pre-incubated *B. japonicum* cells improved soybean nodulation. Acetylene reduction assays for nitrogenase activity showed that the maximum and the minimum C_2H_4 concentration were determined from the treated plants with pre-incubated *B. japonicum* with 10 µM genistein under no stress condition and treated plants with 100 mM NaCl without genistein application, respectively. It would seem that increase of C_2H_2 reduction can be due to an increase in nodule number as well as nodule weight affected by genistein. Decreased ability of nodules to reduce C_2H_2 under salinity has been well-documented for other legumes (Ferri et al. 2000). The inhibition of acetylene reduction by salt stress may be due to a limitation of oxygen diffusion in nodules or due to toxic effects of Na or Cl accumulation (Serraj, 1998). Activities of antioxidant enzymes in roots and shoots exhibited a similar change. Genistein application had not significant effect on enzyme activity but obvious differences existed due to salinity stress. Specifically, peaks of activities of antioxidant enzymes in roots and shoots appeared at the highest NaCl concentration. It indicated that reactive oxygen species scavenging system was activated efficiently and removed reactive oxygen species.

Table 1: The main effects of salinity on stomatal conductance and antioxidant enzymes activity

Salinity	Stomatal conductance	CAT		POX		SOD	
		Leaf	Root	Leaf	Root	Leaf	Root
0 mM	**0.05a**	104.88c	113.71c	134.33d	152.60d	1.26b	1.04c
25 mM	0.04b	107.73c	124.15c	147.49c	167.62c	1.34b	1.33b
50 mM	0.03c	161.88b	192.56b	165.58b	199.91b	1.37b	1.34b
50 mM	0.02d	**194.27a**	**228.38a**	**185.99a**	**212.30a**	**1.76a**	**1.63a**

Table 2: Interaction of salinity and genistein levels on maximum photochemical efficiency, transpiration rate, photosynthetic rate, nodule number and nitrogenase activity

Genistein	Salinity level (mM)	Fv/Fm	Transpiration rate	Photosynthetic rate	Nodule number	Nitrogenase activity
(0 µM)	**0**	656.33e	1.48bc	14.45d	42.00c	7.47e
	25	644.66f	1.35de	12.52e	36.00d	6.59f
	50	632.00g	1.28e	10.85ef	24.66e	6.22fg
	100	613.00h	0.99f	10.60f	16.66f	5.76g
(10 µM)	**0**	**716.66a**	**1.86a**	**25.50a**	**74.66a**	**18.16a**
	25	687.33b	1.58b	20.13b	68.33b	17.29b
	50	677.33c	1.53bc	18.33bc	47.00c	10.28c
	100	664.66d	1.44cd	16.78c	43.66c	8.10d

Ferri, A., Lluch, C., Ocana, A., 2000. Effect of salt stress on carbon metabolism and bacteroid respiration in root nodules of common bean (*Phaseolus vulgaris* L.). Plant Biology 2, 396-402.

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