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GROWTH AND NITROGEN DYNAMICS OF *GLYCINE MAX* INOCULATED WITH *BRADYRHIZOBIUM JAPONICUM* AND EXPOSED TO ELEVATED ATMOSPHERIC CARBON DIOXIDE

AFSHAN REHMAN, NEELOFER HAMID AND FAIZA JAWAID

Department of Botany, University of Karachi,
Karachi-75270, Pakistan

Abstract

Seeds of *Glycine max* (soybean) were inoculated with N-fixing bacterium *Bradyrhizobium japonicum* and grown in growth chamber to investigate interactive effects of atmospheric CO_2 and plants Nitrogen status on root and shoot length and biomass, nodule formation and Nitrogen concentration. Plants were grown with CO_2 at 3500 and 1000ppm with or without *Bradyrhizobium japonicum* inoculation. Root and shoot length and dry mass of *Glycine max* increased significantly with CO_2 enrichment provided with *Bradyrhizobium japonicum* as compared to deficient Nitrogen fixing bacterium. While ambient and enriched CO_2 levels resulted in increased Nitrogen concentration of *Glycine max* shoot and root which is inoculated with N-fixing bacterium. Nodule formation was also enhanced in plants supplied with *Bradyrhizobium japonicum* as compared to plants which is *Bradyrhizobium japonicum* deficient at both CO_2 concentrations.

Introduction

Glycine max (L) Merr., is economically significant bean in the world. Many countries of Asia have grown soybean mostly for their seeds (Desai, 2004). *Glycine max* is resourceful crop with many uses and they can be used in hundreds of human foods like oil, meal, flour, animal feeds and industrial products (Kirakosyan & Kaufman, 2009). The global atmospheric CO_2 concentration has enhanced from a pre-industrial value of about 280–368 ppm in 2001, and could more than double by the end of this century (Anon., 2001). Increasing atmospheric CO_2 concentration stimulates plant growth in a number of plant species (Ainsworth & Long 2005). Numerous experiments showed that high atmospheric carbon dioxide concentration leads to increase in photosynthetic rate and whole-plant growth in many plants species (Finzi *et al*., 2001). It has been suggested that plant aboveground carbon assimilation may be restricted by nutrients, predominantly nitrogen (Luo *et al*., 2004). Recently some studies showed that as CO_2 enrichment enhances soil C: N, decomposing microorganisms require more N which resulted in reduced net N mineralization, this result in competition for mineral N between plants and microbes, and can lead to a decrease in plant production (Jun-Qiang *et al*., 2008). Thus, the relations between C and N might influence the terrestrial ecosystem response to elevated atmospheric CO_2 concentration which are huge in consequence to atmosphere-biosphere interactions, and thus to global climate (Gai-ping, 2006). In the present study we investigated the role of N-fixation on growth parameters, nitrogen concentration and nodule formation of *Glycine max* at ambient and elevated levels of carbon dioxide which is inoculated with *Bradyrhizobium japonicum*.

Materials and Methods

Experiments were conducted on soybean (*Glycine max*). The seeds were obtained from the Market. Healthy seeds were surface sterilized with 0.1% Mercuric chloride solution for 5 minutes followed by rinsing with distilled water. Seeds were sown in 15cm diameter plastic pots containing 1500gm of soil contaning N-fixing bacterium *Bradyrhizobium japonicum* and soil with out N-fixing bacterium. Half-strength Hoagland's solution was used to irrigate all the plants throughout experimental period (Hoagland & Arnon, 1950). Two weeks old seedlings were exposed to two different ambient (350ppm) and elevated (1000ppm) CO_2 in controlled environment chamber for 40 days. Air is drawn into chamber by cylinder which were obtained from "The National Gas Limited Pakistan" and blown through the chamber. Chambers were maintained at ambient (350ppm) and elevated (1000ppm) CO_2 levels at 30°C temperature. Plants were fumigated for 1 hour per day for 40 days duration, inside chamber light was delivered by fluorescent and luminescent lamps, which adjusted at top of the canopy. CO_2 gas was released at the air inlet of the elevated CO_2 chamber and the gas emission rate was regulated in proportion to the ventilation rate. Mean CO_2 concentration that was recorded at 30 minutes intervals through the experiment was 350ppm in ambient and 1000ppm in elevated level. After fumigation the pots were kept in natural environmental condition in the net house for growth. There were there replicates for each treatment. After 10, 20, 30 and 40 days interval three plants were randomly harvested from each stand and separated into leaves, stem, root and nodule. Root and nodules were washed with gentle stream of water to ensure least possible damage to the root of plants. Shoot and root length were carefully calculated and observations were recorded in cm. Dry mass (gm) was determined after drying in an oven at 70˚C for 48 hours. Nitrogen was determined using method described by Kjeldhal (1883).

The data for growth parameters, nitrogen concentration and nodule formation of *Glycine max* inoculated with *Bradyrhizobium japonicum* and exposed to elevated level of CO_2 was analyzed using the "COSTAT" statistical program by two-way analysis of variance (ANOVA) to compare the means of different treatment. "SIGMA PLOT" program was used for graphical representation of data.

Results and Discussion

Nitrogen concentration of shoot and root of *Glycine max* was increased at both CO_2 concentrations (350 and 1000ppm) when inoculated with *Bradyrhizobium japonicum* as compared to non-inoculated *Glycine max* (Fig. 1). Results obtained were statistically significant $^*P<0.05$. Nitrogen is the fourth most common element in plants composition and it is decisive for sustaining photosynthesis and plants production (Ozolincius *et al*., 2007). Elevated atmospheric CO_2 can stimulate photosynthesis and plant production (Anisworth & Long, 2005). Zak *et al*., (2000) showed that increased carbon inputs under elevated CO_2 stimulated the growth of soil microbial biomass, thereby increasing rates of N mineralization. On the other hand Daiz *et al*., (1993) found that increased Carbon inputs under elevated CO_2 stimulated competition between the soil microbial biomass and plants for soil N, leading to decline in soil N availability. Nitrifying enzyme activity in *P. sylvestriformis* under elevated CO_2 would be advantageous to N uptake of plant, also may result in N leaching enhancement and render N lost from ecosystem (Jun-Qiang *et al*., 2008). Elevated CO_2 could also stimulate plant N uptake and soil biological N fixation, further stimulate mineralization of ammonium for microbial nitrification (Hu *et al*., 2001).

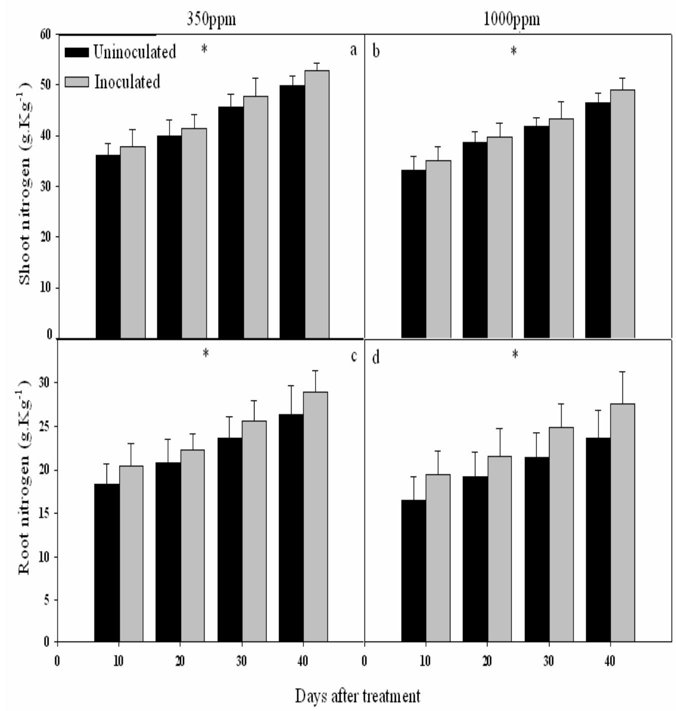

Fig. 1. Nitrogen amount of shoot (a, b) and root (c, d) of uninoculated and inoculated *Glycine max* at ambient (350ppm) and elevated (1000ppm) carbon dioxide. Significant results are denoted by asterists (*$p<0.05$).

Result showed that root and shoot length and dry mass of root and shoot were significantly (**$p<0.01$) increased in inoculated *Glycine max* with *Bradyrhizobium japonicum* at both CO_2 treatments as compared to without inoculated plant. In elevated CO_2 concentration (1000ppm) with *Bradyrhizobium japonicum* inoculation increase was highest as compared to non-inoculated ambient CO_2 (350ppm) treatment (Fig. 2). Plants showed higher growth responses to elevated CO_2 when other resources such as nutrients and water are not limiting (Curtis & Wang 1998). Plants grown from seeds inoculated with N-fixing bacteria *Pseudomonas* genus have been found to have better growth over plants not inoculated with these bacteria (Somova *et al*., 1997). Nitrogen fixing plants would have an advantage in this respect, because N_2-fixation is stimulated by the increasing supply of photosynthate from the host plant at elevated CO_2, consequently it has been hypothesized that plant species that form a symbiotic association with N_2–fixing bacteria exhibit a significant increase in seed production at elevated CO_2 (Ainsworth *et al*., 2008). Elevated CO_2 increased dry mass production more in legumes than in other species (Kimball *et al*., 2002). Present result indicated that number of nodules in Carbon dioxide (350 and 1000ppm) was increased in *Glycine max* inoculated with *Bradyrhizobium japonicum* (Fig. 3) in contrast to non-inoculated both CO_2 treatments. Nitrogen fixation is the microbial processes, which is responsible for the transformation of N into forms that are easily utilized by plant or lost from rhizosphere and their rates could be modified by the rising CO_2 because the processes in the soil are likely to be sensitive to these CO_2-induced changes in soil labile C, soil water content and litter quality (Barnard *et al*., 2005).

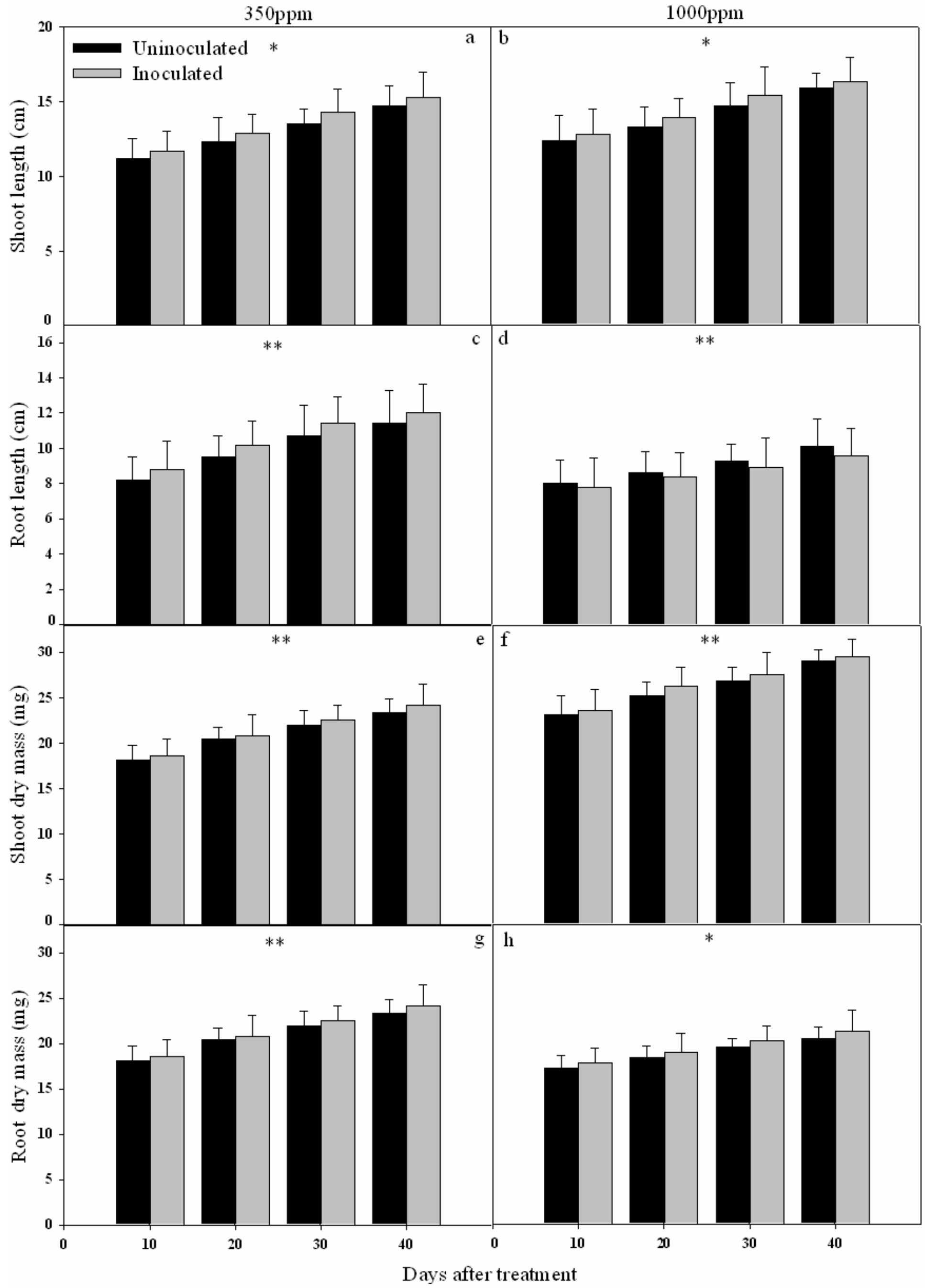

Fig. 2. Shoot length (a, b) and root length (c, d) dry mass of shoot (e, f) and root (g, h) of uninoculated and inoculated *Glycine max* at ambient (350ppm) and elevated (1000ppm) carbon dioxide. Significant results are denoted by asterists (*p<0.05; **p<0.01).

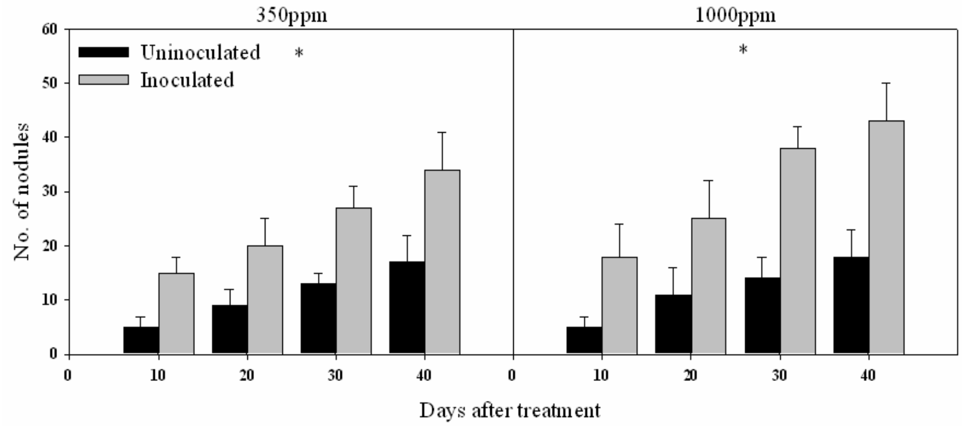

Fig. 3. Number of nodules of uninoculated and inoculated *Glycine max* at ambient (350ppm) and elevated (1000ppm) carbon dioxide. Significant results are denoted by asterists (*p<0.05).

Conclusion

Result of the present study suggested that growth of *Glycine max* increases at ambient and elevated level of CO_2 when inoculated with N-fixing bacterium because Nitrogen fixation not only improves the Nitrogen limitation that was seen during growth of plants in both ambient and elevated CO_2 but able to speed up the growth of plants at elevated CO_2.

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