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EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* ON PHOTOSYNTHETIC ACTIVITY AND GROWTH RESPONSE IN *ACACIA AURICULIFORMIS* SEEDLINGS UNDER ELEVATED CO_2

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Effects of different levels of elevated carbon dioxide (CO_2) on the activity of microbial symbionts (arbuscular mycorrhizal (AM) fungi and *Rhizobium* sp.) in *Acacia auriculiformis* seedlings were studied to understand the relationship between *A. auriculiformis, Rhizobium* sp. and CO_2. Seedlings of *A. auriculiformis* were inoculated with AM fungi and *Rhizobium* sp. and placed in open top chambers fitted with elevated CO_2 supplying facilities. These seedlings were maintained in the open top chambers for 180 days under different levels of elevated CO_2 (ambient control, 600 and 900 ppm). After 180 days, nodule numbers, seedling growth and photosynthesis of *A. auriculiformis* seedlings inoculated with microbial symbionts significantly improved under 600 and 900 ppm of CO_2. The net photosynthetic rate was higher in 900 ppm CO_2 than 600 ppm and ambient control due to inoculation of AM fungi and *Rhizobium* sp. The uninoculated seedlings showed very poor performance under 900 ppm elevated CO_2. This study showed that the microbial symbionts can improve growth and photosynthesis of *A. auriculiformis* seedlings under the elevated CO_2 level conditions (600 and 900 ppm).

Keywords: Nodule numbers, photosynthetic rate, microbial symbionts, photosynthesis

INTRODUCTION

Microbial processes have significant contribution in mitigating global warming. According to the International Panel on Climate Change (IPCC) report (IPCC 2007), global warming is occurring at unprecedented rates and increase in concentrations of anthropogenic greenhouse gases will result in further climate change. Emission of greenhouse gases can be managed with suitable scientific approaches. Soil microorganisms play a big role in the production and consumption of greenhouse gases, including carbon dioxide (CO_2). Soil microbes slow down the global warming as they utilise CO_2 for their survival. However, understanding the role of soil microbes in global warming can help to determine the actual effect of association with plants.

Studies have been conducted on the response of arbuscular mycorrhizal (AM) fungi colonisation to elevated CO_2. The host plant *Bouteloua gracilis* responded to elevated CO_2 with enhanced photosynthesis (Monz et al. 1994). Increased carbon availability from *B. gracilis* may play a significant role in stimulating mycorrhizal colonisation in this species. In another study, mycorrhizal abundance increased 47% under CO_2 enrichment (Treseder 2004).

Investigations have been widely conducted on open top chambers for periods from several weeks to months in several tree species. *Pinus ponderosa* seedlings in open top chamber conditions inoculated with mycorrhizal fungi *Thelephora terrestris* under CO_2 concentration of 175 ppm and 350 ppm showed increased nitrogen levels (Tingey et al. 1995). *Populus euramericana* seedlings showed increased biomass when inoculated with AM fungi under CO_2 concentration of 350 ppm in open top chamber conditions (Lussenhop et al. 1998). Inoculation of AM fungi, *Glomus mosseae,* with *Plantago lanceolata* increased up to twofold biomass under 400 ppm concentration of CO_2 in open top chambers (Staddon et al. 1999). In this study, AM fungi and *Rhizobium* sp. were used to improve the photosynthetic activity and growth of *Acacia auriculiformis* seedlings under elevated CO_2 conditions. *Acacia auriculiformis*, indigenous

to Australia, is being used for afforestation in India. The wood is used for furniture, tools and paper.

MATERIALS AND METHODS

Collection of root nodules and soil

Each 100 g of rhizosphere soil was collected from mature 7-year-old *A. auriculiformis* trees. Root nodules of *A. auriculiformis* were also collected from the same tree and were transported in an ice box and stored at -4 °C. Following Jackson (1973), the physical-chemical analyses showed that average pH of the soil was 5.9 ± 0.2, electrical conductivity 0.19 ± 0.01, nitrogen 20.2 ± 0.5 mg kg^{-1}, phosphorus 14.2 ± 1.1 mg kg^{-1} and potassium 31.5 ± 1.3 mg kg^{-1}.

Isolation and culture of AM fungi

AM fungal spores were isolated from the collected rhizosphere soil samples following the method by Gerdemann and Nicolson (1963). Using the most probable number method (Porter 1979), it was found that the soil samples contained 5.2 ± 0.32 infective propagules (spores, hyphae). The isolated AM fungal spores were identified according to Schenck and Pérez (1990). The identified AM fungal species found in the rhizosphere soils were *Glomus fasciculatum*, *G. geosporum* and *G. aggregatum*. These AM fungi were multiplied in sterile media (Alfisol and sand) with *Zea mays* under greenhouse conditions (relative humidity 65% and temperature 22 °C) for 4 months in pot cultures.

Isolation and culture of *Rhizobium*

The collected root nodules from *A. auriculiformis* trees were surface sterilised with 30% H_2O_2 and kept at room temperature for 10–20 min. Under aseptic conditions, the nodules were rinsed in sterile distilled water and 0.3 g of root nodule was ground manually in a sterile mortar and pestle. The nodule solution was centrifuged at 1000 rpm for 20 min and the supernatant was filtered through Whatman No. 1 filter paper. The suspension was then spread on Yeast Extract Mannitol Agar Medium (YEMA) (Graham 1969) plates and incubated at 25 °C for 15 days. Congo Red solution (1%) was also added into the medium. The pH of the medium was 6.8. After 15 days of incubation, *Rhizobium* sp. which appeared as slimy white colonies was transferred into YEMA broth for upscaling of the inoculum. Broth cultures of *Rhizobium* sp. in 250 mL conical flasks were incubated at 32 °C in an orbital shaker at 1000 rpm for 20 min. The cultures were then homogenised in the centrifuge at 1000 rpm for 10 min. Mass multiplied *Rhizobium* sp. culture was stored under refrigerated conditions (4 °C).

Propagation of *A. auriculiformis* seedlings

The seeds of *A. auriculiformis* were collected from mature trees and soaked in water at 60 °C for 30 min. Seeds treated with hot water were sown in mother bed containing pure sand for germination. After 7 days, germinated seedlings were transplanted to polythene bags (width 14 cm × height 27 cm) containing sterile red soil and sand (1:1). The seedlings were maintained under shade house for 1 month.

Inoculation of AM fungi and *Rhizobium* sp.

Pot cultures of *Z. mays* containing 20 g chlamydospores of all three AM fungi (*G. fasciculatum*, *G. geosporum* and *G. aggregatum*) were placed 5 cm below the soil surface in each polythene bag. Inoculation of cultured *Rhizobium* sp. was achieved by applying 10 mL of rhizoidal suspension in the root zone of *A. auriculiformis* seedlings raised in each bag of the respective rhizoidal treatment. These microbial symbionts were inoculated into the 1-month-old *A. auriculiformis* seedlings of uniform size (30 cm height and 1 cm collar diameter) individually and in combinations. An uninoculated control treatment was also maintained among them. There were four treatments in total: (1) control (20 g sterile soil + 10 mL YEMA broth without *Rhizobium* sp.), (2) AM fungi (20 g), (3) *Rhizobium* sp. (10 mL) and (4) AM fungi (20 g) + *Rhizobium* sp. (10 mL) with 10 replicates of each treatment containing 5 seedlings per treatment, totalling 200 seedlings, in a randomised block design. These seedlings were placed in open top chambers and maintained for 180 days. The open top chamber is a cubical structure with dimensions 3 m × 3 m × 3 m and fabricated with galvanised iron pipe frame and covered with polyvinyl chloride sheet. The upper part of the chamber was uncovered to maintain atmospheric conditions. The Supervisory Control and Data Acquisition (SCADA) system was used to control CO_2 supply.

Seedlings were watered daily but no fertilisers were added. Three open top chambers were used for this study, i.e. with capacity of 600-ppm CO_2 supply day^{-1}, 900-ppm CO_2 supply day^{-1} and an ambient CO_2 controlled chamber. The CO_2 levels were supplied using CO_2 cylinder in the chambers for the entire study period and monitored using SCADA. Ambient CO_2 chamber showed 380 ± 1.1 ppm of CO_2. Average temperature in the chambers was 36.8 ± 1.00 °C and the relative humidity was 65 ± 1.2%. Mean annual rainfall recorded in Coimbatore, India during the period of study was 796.8 mm.

After 180 days inoculation, *A. auriculiformis* seedlings were harvested with their entire root system intact. Parameters measured were root length, shoot length, collar diameter, number of root nodules, and shoot and root biomass of each seedling. The shoot and root biomass were determined after drying in oven at 50 °C for 48 hours.

Net photosynthetic rate

At the end of the study period, light saturated photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) was measured on 15-day-old *A. auriculiformis* leaves from the top of the stem using photosynthetic meter. Leaf chamber of the photosynthetic meter was set at 380 ppm CO_2, temperature 24 °C and saturating photosynthetic rate, 1500 µmol m^{-2} s^{-1}. All *A. auriculiformis* seedlings with or without inoculation of microbial symbionts placed in open top chambers were measured for determination of net photosynthetic rates under ambient, 600 and 900 ppm CO_2 conditions.

Statistical analyses

Each measured variable in the open top chamber experiments and photosynthetic data were statistically analysed using Duncan's multiple range test (SPSS version 17).

RESULTS

Growth and biomass under elevated CO_2 conditions

The response of *A. auriculiformis* seedlings to elevated CO_2 conditions at 600 ppm showed improved growth and biomass compared with ambient CO_2 conditions inoculated with AM fungi and *Rhizobium* sp. (individually and combined) (Table 1). The number of root nodules after combined inoculation with AM fungi and *Rhizobium* significantly increased in the 600 ppm elevated CO_2 conditions. However, under 900 ppm elevated CO_2 conditions, growth and biomass were lower than 600 ppm elevated CO_2 conditions. The nodule number was similar with that of ambient CO_2 conditions.

At the end of the 180 days, *A. auriculiformis* seedlings inoculated with AM fungi and *Rhizobium* sp. showed improved growth and biomass under 900 ppm elevated CO_2 conditions than control seedlings. The shoot biomass (3.01 g $plant^{-1}$) and root biomass (2.59 g $plant^{-1}$) and number of nodules (16.8 $plant^{-1}$) were significantly ($p = 0.05$) increased in *A. auriculiformis* seedlings inoculated with AM fungi + *Rhizobium* sp. under 900 ppm elevated CO_2 conditions (Table 1). Nodule number was higher under elevated CO_2 conditions due to inoculation of *Rhizobium* sp. than ambient CO_2 conditions. Uninoculated control plants grown under 900 ppm elevated CO_2 had poor growth and biomass than ambient and 600 ppm elevated CO_2 conditions. AM fungi and *Rhizobium* sp. inoculations significantly increased collar diameter of seedlings under 600 and 900 ppm elevated CO_2 conditions. Under ambient CO_2 conditions, seedlings inoculated with AM fungi + *Rhizobium* sp. showed significantly higher growth and biomass and number of nodules than control plants (Table 1).

Overall results showed that seedlings inoculated with AM fungi and *Rhizobium* sp. had improved growth and biomass under elevated CO_2 conditions, whereas the uninoculated control plants had poor performance under elevated CO_2 conditions particularly under 900 ppm.

Net photosynthetic activity under elevated CO_2 conditions

Acacia auriculiformis seedlings showed increased photosynthetic rates in 600 and 900 ppm elevated CO_2 in the presence of AM fungi + *Rhizobium* sp. (Figure 1). In this treatment, net photosynthetic rate was 8.48 µmol m^{-2} s^{-1} under 900 ppm elevated CO_2 condition and 6.1 µmol m^{-2} s^{-1} under

Table 1 Response of *Acacia auriculiformis* seedlings inoculated with AM fungi and *Rhizobium* sp. to elevated CO_2 after 180 days

CO_2 treatment	Inoculation treatment*	Shoot length (cm)	Root length (cm)	Collar diameter (cm)	No. of nodules /seedlings	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
Ambient control	AM	40.2 b	23.5 a	2.2 a	0	2.04 a	1.88 a	3.92 b
	R	44.2 b	26.4 b	2.5 a	22.4 b	2.21 a	1.32 a	3.53 b
	AMR	52.6 c	28.3 b	3.4 b	17.2 a	3.06 b	2.46 b	5.52 c
	Control	36.3 a	18.6 a	1.8 a	0	1.85 a	0.54 a	2.39 a
CO_2- 600 ppm	AM	52.1 b	25.6 a	2.8 a	0	2.8 a	2.1 a	4.9 b
	R	54.6 b	28.3 ab	2.9 a	25.7 a	3.4 b	2.8 b	6.2 c
	AMR	59.3 c	30.8 b	3.2 b	28.4 b	4.2 b	3.1 c	7.3 d
	Control	40.1 a	24.6 a	2.2 a	0	2.1 a	0.95 a	3.05 a
CO_2- 900 ppm	AM	35.6 a	18.4 a	2.3 a	0	1.85 a	1.62 a	3.47 a
	R	44.3 b	22.2 b	1.9 a	14.3 a	2.05 a	1.22 a	3.27 a
	AMR	46.2 b	24.4 b	2.6 b	16.8 b	3.01 b	2.59 b	5.6 b
	Control	39.3 a	19.3 a	1.92 a	0	2.03 a	1.58 a	3.61 a

Values are means of 10 replicates; means followed by same letter in the same column are significantly not different at 5% level of DMRT ($p < 0.05$); *AM = arbuscular mycorrhizal fungi, R = *Rhiozbium* sp. and AMR = AM fungi + *Rhizobium* sp.

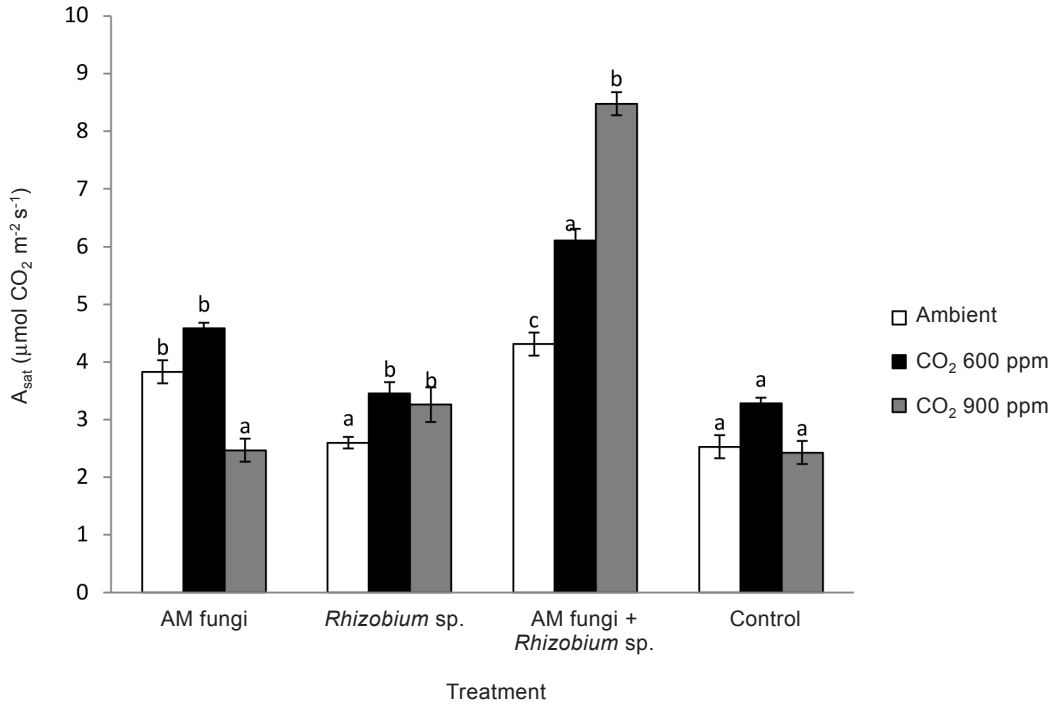

Figure 1 Net photosynthetic rates (A_{sat}) of *Acacia auriculiformis* seedlings under elevated CO_2 conditions

600 ppm elevated CO_2. The net photosynthetic rate for ambient CO_2 was 4.31 µmol m^{-2} s^{-1}. Control plants had poor photosynthetic rates compared with AM fungi- and *Rhizobium* sp.-inoculated seedlings, particularly under 900 ppm elevated CO_2 condition.

DISCUSSION

Soil microorganisms contribute significantly to the production and consumption of CO_2. However, microbial symbionts can contribute to carbon sequestration by increasing nutrient

uptake by plants (Garcia et al. 2011) as shown this study. *Acacia auriculiformis* seedlings rely upon microbial symbionts particularly mycorrhizal fungi and nitrogen-fixing bacteria to acquire nutrients such as phosphorus and nitrogen for growth even under elevated CO_2 conditions. In this present study elevated CO_2 greatly influenced growth and nutrient content in tree crops especially in the presence of microbial symbionts. Increasing levels of elevated CO_2 mitigate stress in plants (AbdElgawad et al. 2015) resulting in growth improvement. In controlled CO_2 chamber, seedlings without microbial inoculations had poor growth due to impact of elevated (natural) CO_2. Seedlings with AM fungi and *Rhizobium* sp., either individually or in combination, had improved growth and biomass. Microbial symbionts scavenged excess CO_2 in the seedlings for microbial survival and increase the growth and biomass through nutrient transfers (Tang et al. 2011, Song et al. 2013, 2015, AbdElgawad et al. 2015). Symbiotic nitrogen fixers *Frankia* and *Rhizobium* promote the growth and biomass of nitrogen-fixing trees under elevated CO_2 conditions (Norby 1987). Increase in the number of root nodules in *A. auriculiformis* increased the nitrogenase activity that led to higher fixation of nitrogen. Elevated CO_2 conditions increased collar diameter compared with ambient CO_2 which is in agreement with Yazaki et al. (2004). Increased photosynthetic rates observed in the present study might have enhanced the rate of nitrogen fixation due to inoculation of *Rhizobium* sp. (Thomas et al. 1991).

CONCLUSIONS

Microbial symbionts play an important role in improvement of plant growth. Under higher atmospheric CO_2 conditions microbial symbionts facilitate tree crops that improve tree growth and survival. This study showed that *A. auriculiformis* benefitted from inoculations using microbial symbionts and had improved growth and biomass. *Acacia auriculiformis* can be used to mitigate increasing CO_2 levels.

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