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Nitrogen availability modulates CO_2-induced responses of *Catharanthus roseus*: Biomass allocation, carbohydrates and alkaloids profile

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ABSTRACT

The effects of elevated CO_2 (560 ± 25 ppm) and N supply (no supplemental N: 0 kg N ha^{-1}, recommended N: 50 kg N ha^{-1}, double recommended N: 100 kg N ha^{-1}) on biomass allocation and alkaloids production in periwinkle (*Catharanthus roseus* L.) were examined using open top chambers. Elevated CO_2 significantly stimulated total biomass of the plant grown in moderate and high N condition. Further, change in pattern of biomass partitioning was also observed under different CO_2 and N treatments and plant allocated its maximum biomass toward stem under elevated CO_2 at all N treatments. Leaf biomass showed its highest proportion with high N, under elevated CO_2. Elevated CO_2 rather significantly increased starch, organic carbon, while it decreased soluble sugar in higher N supply. Plants grown with low level of N showed significant reduction in total foliar nitrogen due to CO_2 enrichment. Under elevated CO_2, total alkaloids, vinblastine and vincristine were highest at moderate N; however, catharanthine content was increased linearly with increasing supply of N. On the other hand, vindoline content was reduced under elevated CO_2 with high N. Principal component analysis and factor rotation were conducted to understand the cumulative response of all the variables to elevated CO_2 and N availability. The differential response of the test plant under varying CO_2 and N levels concluded that C-allocation toward biomass and metabolites synthesis under future projected level of CO_2 will be greatly modified by soil nitrogen availability. Furthermore, moderate N supply would be more beneficial for medicinal value of the test plant.

1. Introduction

Global atmospheric CO_2 concentration has linearly increased from 280 ppm since preindustrial time to current level 398 ppm (Mishra et al., 2013) due to excessive combustion of fossil fuel and deforestation. Moreover, Intergovernmental Panel on Climatic Change (IPCC) has predicted a further ongoing increase in atmospheric CO_2 upto 550 ppm by 2050 (IPCC, 2007). A recent report of IPCC stated 40% increase in CO_2 in the current atmosphere compared to 1750 (IPCC, 2013). Since CO_2 is utilized as a substrate in photosynthesis; it directly affects growth and metabolism of the plants. The earlier studies have confirmed that elevated CO_2 increased light-saturated carbon uptake, C assimilation, growth and above-ground biomass production, while decreased stomatal conductance (Ainsworth and Long, 2005) and photorespiration rate (Long et al., 2004).

Since nitrogen (N) is another limiting resource required during growth and development of the plants, as is recognized as most abundant part of photosynthetic apparatus. Nitrogen concentration usually decreased under elevated CO_2 because of increased carbohydrate synthesis and growth (Taub and Wang, 2010; Duval et al., 2012). Therefore, it is assumed that a continuous shift in nitrogen status may complicate the sustainability of plant response to elevated CO_2 (Aranjuelo et al., 2005; McCarthy et al., 2010). Pleijel and Uddling (2012) have reported that N economy of plants could be negatively affected under CO_2 enrichment. Bloom et al. (2010) reported that rising CO_2 reduces N uptake in leaves because of reduced rate of transpiration. Previous studies suggested that elevated CO_2– induced down regulation of photosynthesis was more pronounced under limited N because of poor sink capacity (Ainsworth et al., 2004; Rogers and Ainsworth, 2006). Hence, supply of additional N improves photosynthesis, growth and biomass production by improving sink strength under elevated CO_2 (Sanz-Sáez et al., 2010). Increased availability of carbon and N further

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modify the tissue chemistry of leaves (Koike et al., 2006) in terms of primary as well as secondary metabolites.

Plants are the source of vast diversity of metabolites. Carbohydrates, product of photosynthesis are classified as primary metabolites, as they play important role in growth and development of plants. Secondary metabolites have been acknowledged for their important role in providing protection against pathogen invasion and herbivores in plants and disease causing microbes in human beings (Ziska et al., 2005). Selected plants and their products are used as therapeutic medicines since ancient times (Newman et al., 2003). At present, about 80% of world population depends on medicinal plants for of their primary health care despite of abundance and advancement of synthetic medicines (World Health Organization, 2002), hence increasing the demand of herbal remedies.

Any type of change in abiotic resources such as water and nutrient availability may affect carbon uptake and its pattern of allocation, and hence production of secondary compounds in plants. Though the medicinal plants are used widely, only few studies evaluated the effects of elevated atmospheric CO_2 on medicinal plants. Increase of alkaloids contents in wild poppy (Ziska et al., 2008), and increase in content of scopolamine and decrease of nicotine in jimson weed under elevated CO_2 have been reported (Ziska et al., 2005). Idso et al. (2000) have found that elevated CO_2 enhanced the content of secondary compounds synthesized in water lily bulb. Oliveira et al. (2010) have demonstrated higher production of inulin, an anti-cancerous agent in *Vernonia herbacea* due to increased carbon assimilation under elevated CO_2. Synthesis of total alkaloids was increased in *Catharanthus roseus* (Gholamhosseinpour et al., 2011) and in *Ilex vomitoria* (Palumbo et al., 2007) with increasing N availability. Total alkaloids and caffeine content increased by 5–10 times in *I. vomitoria* grown in nitrate (250 mg N wk^{-1}) fertilized soil compared to without N fertilization.

There is a dearth of information on interactive effects of elevated CO_2 and nitrogen supply on medicinal plants. Therefore, the present study was conducted to evaluate the effects of elevated atmospheric CO_2 and varying soil nitrogen availability on growth, carbohydrates pool, total alkaloids and active ingredients (catharanthine, vindoline, vincristine and vinblastine) of a medicinal plant, *C. roseus* (L.) G Don. *C. roseus* is one of the most valuable medicinal plants, producing more than 130 monoterpenoid indole alkaloids (Zhu et al., 2014). These alkaloids are condensation products of a nitrogen-containing indole derived from tryptamine and a monoterpenoid component derived from secologanin (Fig. 1).

Some of the known compounds widely used in medicine include vinblastine and vincristine, the powerful antitumor drugs (Ziegler and Facchini, 2008). Ajmalicine and serpentine, the other important compounds in this plant are used in heart related problem and to improve the blood circulation in the brain (Schmeller and Wink, 1998). These compounds are very costly in market because of the fact that they present in very small amounts and extraction is carried out along other compounds of similar physical nature (Loyola-Vargas et al., 2007). This plant also grows in wild and often suffers from nitrogen limitation, which not only affects the growth, but also the alkaloids yield.

2. Material and methods

2.1. Experimental site

A field experiment was conducted during April 2011 to August 2011 in Botanical garden of Banaras Hindu University, Varanasi, Uttar Pradesh (25°18′N latitude, 82°01′E longitude, and 76.19 m above sea level) situated in eastern Gangetic plains of India, using open top chambers (OTCs). Different meteorological parameters were collected periodically from a weather observatory near the experimental site. Daily maximum temperature varied from 32 to 40 °C, while minimum temperature varied from 20 to 25 °C. The mean relative humidity varied from 52.1 to 89.3% and total rain fall was 763 mm during the experimental period.

Experiment was performed in OTCs of 1.9 m height and 2.05 m diameter. OTCs were constructed following the design of Kumari et al. (2013). Each chamber was attached with a high speed blower for the continuous supply of air at the rate of three changes per minute. Elevated CO_2 was supplied to OTCs via CO_2 cylinders connected with blowers and elevated level was monitored through solenoid valve attached to CO_2 analyzer.

2.2. Experimental design

The experiment was designed as a split plot with CO_2 as the main plot and nitrogen as sub plots. There were two CO_2 treatments i.e., ambient 375 ppm (AC) and elevated 560 ppm (EC). The elevated dose of CO_2 was decided on the basis of predicted concentration of atmospheric CO_2 i.e., 535–983 ppm by IPCC, 2007 and we have selected the lower side of the projection. Three nitrogen concentrations were used for each CO_2 treatment i.e., without supplemental nitrogen (NF0), recommended dose of nitrogen (NF1) and double recommended dose of nitrogen (NF2). Recommended and double recommended nitrogen doses were 50 and 100 kg ha^{-1}, respectively. Nitrogen was supplied to the soil in form of urea.

2.3. Raising of the test plants

Periwinkle (*C. roseus* (L.) G Don.), a perennial plant of family Apocyanaceae was selected as test plant. The field was prepared by ploughing up to 20 cm depth. Eighteen open top chambers (OTCs) were installed at the experimental site, three replicate chambers for each treatment. Soil from each experimental plot was collected for measurement of N content before transplantation of plantlets. Then the deficit amount of N was added to the soil to attain NF1 and NF2 concentrations in two splits (Table 1). One third dose of nitrogen was given as basal dressing before transplantation and two third dose was given as top dressing 30 days after transplantation (DAT). Seeds of periwinkle were sown in a plot ($2.5 \times 2.5\ m^2$) and plantlets of 15 days were transplanted in rows in open top chambers at 20 cm distance. There were 15 plants in each chamber. Similar soil moisture at field capacity was maintained in each chamber through drip irrigation.

Measurements of microclimatic parameters (temperature, humidity and light) were done within and outside OTCs during the experimental period. It was observed that temperature and relative humidity were higher by 0.1–0.3 °C and 2–6%, respectively within the chambers compared to outside. Light intensity was 4–5% less inside the chambers than outside.

2.4. CO_2 monitoring

CO_2 monitoring was done inside and outside the OTCs using CO_2 analyzer (LI-820, LI-COR Biosciences, Lincoln, USA) and variations in diurnal concentration of CO_2 were recorded. The mean concentration of ambient CO_2 was recorded 375 ± 30 ppm. The elevated CO_2 dose was maintained at 560 ± 25 ppm.

2.5. Plant sampling and analyses

2.5.1. Biomass measurement

For biomass measurement, three plants were randomly selected from each replicate chamber of different treatments from the beginning of the experiment. Plants were harvested at 120 days