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Influence of Carbon Dioxide Enrichment on Accumulation of Secondary Metabolites in Plants

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Abstract: Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other primary functions. These chemicals are extremely diverse such as phenolics and terpenoids. Some of these compounds use as medicines or flavorings. Secondary metabolism is linked to primary metabolism. Different species respond differently to elevated CO_2. Elevated CO_2 usually shows an increase in the carbon/nitrogen ratio. High level of CO_2 usually leads to increased rates of net photosynthesis and can alter plant growth and partitioning to secondary metabolites. The concentration of these substances in plants varies among organs, tissues and developmental stage, and is influenced by environmental factors such as temperature, nutrients, soil water, and atmospheric CO_2 concentration. Environmental factors may interact with CO_2 levels and alter the predictions. This review focuses on experimental researches regarding the effects of carbon dioxide on accumulation of secondary metabolites in plants.

Key words: Medicinal Plants, Phenolics, Photosynthesis, Active Substances.

INTRODUCTION

When the earth was in the first stage of evolution, four-and-a half billion years ago, it is believed that the concentration of carbon dioxide in the air was about 1,000,000 ppm. This concentration has been decreased over time. By 500 million years ago, the atmosphere's CO_2 concentration is estimated almost 7500 ppm and by 300 million years ago, it had declined to 370 ppm close to the current CO_2 concentration , after which it rose to about five times where it now stands at 220 million years before present (Berner 1990, 1992, 1993, 1997; Kasting, 1993).

There are several documents that show atmospheric CO_2 increment from about 280 ppm before the industrial revolution to about 360 ppm at present (Baker and Enoch, 1983; Keeling *et al.*, 1995). Doubling of present CO_2 concentrations with rising of global air temperature (1.8-4.0°C) over this century are predicted. The influences of simulated environmental changes, such as temperature, soil water content, nutrient availability, UV-B radiation and CO_2 concentration, on plant growth and productivity have already been studied in arctic (Chaturvedi *et al*., 2009).

Different species respond differently to elevated CO_2 (Bazzaz *et al*., 1995). High levels of CO_2 usually leads to increased rates of net photosynthesis because of increment in activity of Rubisco enzyme and can alter plant growth and accumulation of secondary metabolites (Ghasemzadeh and Jaafar, 2011). The CO_2-stimulation of photosynthesis and the actual biomass responses can mismatch (Korner *et al*., 2007).

The concentration of flavonoids and other phenolics in plants varies among organs, tissues and developmental stage, and is influenced by environmental factors such as temperature, UV and visible radiation, nutrient and water availabilities, and atmospheric CO_2 concentration (Estiarte *et al.*, 1999).

The use of photosynthetic assimilates for carbon (C)-based secondary metabolites such as phenolic compounds depend on genotype and environmental factors. If elevated CO_2 concentration, which increases C supply, or mineral nutrient deficiency, which decreases C demand, makes C available in excess of that needed for growth and maintenance, it could be used to construct C-based secondary metabolites such as flavonoids, proanthocyanidins, lignin and other phenolic compounds. In contrast, sufficient mineral nutrient availability, which promotes optimal growth, might result in lower utilization of photosynthates to produce secondary metabolites (Booker and Maier, 2001).

The synthesis of phenylpropanoids and derived compounds (e.g. condensed tannins) competes directly with the synthesis of proteins, and therefore with plant growth, because of a common precursor, phenylalanine. In contrast, the biosynthesis of terpenoids and of hydrolyzable tannins proceeds presumably without direct competition with protein synthesis. Therefore, accelerated plant growth induced by fertilization may cause a reduction in concentrations of phenylpropanoids but may affect less or not at all the levels of other classes of secondary compounds (Haukioja *et al*., 1998). Several reports indicated the effects of elevated CO_2, known as CO_2 fertilization, on plants (Kimball, 1983; Acock and Allen, 1985; Cure and Acock, 1986; Allen, 1990; Rozema *et al.*, 1993; Allen, 1994; Allen and Amthor, 1995; Idso *et al*., 2000; Ziska *et al*., 2008).

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The CO_2 fertilization effect may not be clear under conditions where other growth factor is severely limiting, such as low temperature (Long, 1991).

Plants grown in some conditions, where limitations of rooting volume , light, or other factors restrict growth, have not shown a sustained response to elevated CO_2 (Kramer, 1981; Arp, 1991).

Enhanced photosynthetic CO_2 fixation is the first evidence of CO_2 fertilization. Non-structural carbohydrates tend to accumulate in leaves and other plant organs as starch, soluble carbohydrates or polyfructosans, depending on species. In some cases, there may be feedback inhibition of photosynthesis associated with accumulation of non-structural carbohydrates. Researchers and breeders should focus on betterment of photoassimilate utilization for the future (Hall and Allen, 1993).

Secondary metabolism is linked to primary metabolism by the rates at which substrates are diverted from primary pathways and allocated into the secondary biosynthetic routes. Due to these, several environmental factors affecting growth, photosynthesis and other parts of primary metabolism will also affect secondary metabolism (Bryat *et al*., 1983).

This review focuses on experimental researches about effects of carbon dioxide on accumulation of secondary metabolites in plants.

Effects of Co_2:

An experiment showed that concentrations of total soluble phenolics, catechin and proanthocyanidins in needle extracts of loblolly pine (*Pinus taeda* L.) increased about 11% in response to the elevated CO_2 treatments. There were no significant treatment effects on foliar lignin concentrations. The treatments were ambient CO_2 concentration, ambient plus 175 or ambient plus 350 ppm. (Booker and Maier, 2001).

A report regarding the *Podophyllum hexandrum* Royle at an alpine expanse of Garhwal Himalaya by doubling in the atmospheric CO_2 concentration from June to September indicated that Photosynthetic rate was stimulated during the first 30 days thereafter a significant decrease in its rate was recorded. Transpiration rate decreased significantly throughout the CO_2 enrichment and stomatal conductance has shown a significant reduction initially. Total dry matter production significantly increased by 96.40 % after 90 days of CO_2 exposure. Organic Carbon in aboveground and belowground compartment has shown a significant increment whereas in case of total nitrogen results were found non-significant. They concluded that the medicinally used part of this plant (rhizome and root) have shown a significant increment (Chaturvedi *et al*., 2009).

Under controlled well-watered conditions, elevated CO_2 (about triple ambient) increased plant dry weight production in woolly foxglove (*Digitalis lanata*) by 63%, while under water-stressed conditions the CO_2-induced dry weight increase was 83%. In addition, the concentration of digoxin within the plant dry mass was enhanced by 11% under well-watered conditions and by 14% under conditions of water stress (Stuhlfauth *et al.*, 1987).

Another study in central Panama was carried out to evaluate the effects of elevated CO_2 (about twice ambient) on secondary metabolites of young tropical trees. On average, leaf phenolic contents were 48% higher under elevated CO_2. Biomass accumulation was not affected by CO_2, but starch, total non-structural carbohydrates and C/N ratios all increased (Coley *et al*., 2002).

Tropical spider lily (*Hymenocallis littoralis* Jacq. Salisb.) was exposed to elevated levels of atmospheric CO_2 over four growing seasons. This study showed that a 75% increase in ambient CO_2 concentration increased 56% in the bulb biomass. They observed 6% increase in the concentration of a two-constituent (1:1) mixture of 7-deoxynarciclasine and 7-deoxy-*trans*-dihydronarciclasine, 8% increase in pancratistatin, 8% increase in trans-dihydronarciclasine, and 28% increase in narciclasine (Idso *et al.*, 2000).

Another study was conducted with St. Johns' wort. The active substances of this plant are hypericin. pseudohypericin, and hyperforin. One of the problems associated with medicinal plant preparations including St. John's wort is the extreme variability in the phytochemical content, mostly due to environmental variability, and biotic and abiotic contamination during cropping. Plants were grown for 42 days under well watered and fertilized conditions in a greenhouse. The CO_2 concentration was about 360 ppm during the photoperiod, and in computer-controlled environment chambers maintained at a mean photoperiod CO_2 concentration of 1000 ppm. They indicated that the net photosynthetic rates of the plants in the CO_2-enriched chambers were 124% greater than those of the plants growing in ambient air, and that their dry weights were 107% greater. 1000 ppm treatment increased plant concentrations of hypericin and pseudohypericin by just over 100% (Zobayed and Saxena, 2004).

Two *Zingiber officinale* varieties (Halia Bentong and Halia Bara) were exposed to different CO_2 concentrations (400 and 800 ppm). High photosynthesis rate (in Halia Bara) and plant biomass (in Halia Bentong) were observed at 800 ppm CO_2. Elevated CO_2 concentration resulted in reduced stomatal conductance and increased water use efficiency. Total flavonoids, total phenolics, total soluble carbohydrates, starch and plant biomass increased significantly in all parts of the ginger varieties under elevated CO_2 (Ghasemzadeh and Jaafar, 2011).

Another work with broccoli (*Brassica oleracea var. italica* Plenck) cv Marathon was carried out in a controlled greenhouse environment with ambient (430-480 ppm) and elevated (685-820 ppm) CO_2 concentrations. Irrigation and fertilization of plants were well done. They indicated that elevated CO_2 concentration increased the fresh weight of the broccoli heads by approximately 7%, while it increased the total glucosinolate concentration of the broccoli inflorescences by 14%, due primarily to identical 37% increases in two methylsulfinylalkyl glucosinolates : glucoiberin and glucoraphanin (Schonhof *et al.*, 2007).

Tobacco and jimson weed plants grown in controlled environment chambers maintained at atmospheric CO_2 concentrations of 294 ppm (reduced), 378 ppm (ambient) or 690 ppm (elevated) and mean air temperatures of 22.1 or 27.1°C. Nicotine of tobacco and atropine or scopolamine of jimson weed was measured. They indicated that at the time of final harvesting, the elevated CO_2 increased the aboveground biomass production of tobacco by approximately 89% at 22.1°C and 53% at 27.1°C, and to have increased that of jimson weed by approximately 23% and 14% at the same respective temperatures. Concentration of nicotine decreased in tobacco, concentration of scopolamine increased in jimson weed and concentration of atropine did not show significant changes in jimson weed (Ziska *et al.*, 2005).

Influence of elevated CO_2 under Mediterranean environment in the greenhouse conditions on growth, concentration and composition of secondary metabolites of *Hypericum perforatum* and *Echinacea purpurea* was investigated. In *Hypericum* plants, leaf dry weight was 33% higher in elevated CO_2. This difference also appeared in allocation of biomass, roots and stems. Flavonoids composition was affected by phonological stage of plants, so after blossom these were increased in elevated CO_2 plants. Plants of *Echinacea* subject to high CO_2 conditions after 7 months had 79%, 339%, 546% and 57% greater dry weight productions of leaves, flowers, stems and roots. Increased CO2 only promoted significant changes in amounts of caftaric acid and total phenols at root level (Save *et al*., 2007).

In a Japanese research with a marine alga, unicellular *Nannochloropsis* sp. under normal (370 ppm) and elevated (3000 and 20000 ppm) atmospheric CO_2 concentrations, eicosapentaenoic acid, a polyunsaturated omega-3 fatty acid, increased when 20000 ppm CO_2 was supplied 12 hours prior to the end of the exponential growth, and the total eicosapentaenoic acid production during 4-day cultivation was about twice that obtained with ambient air (Hoshida *et al*., 2005).

Growth and alkaloid content of wild poppy, (*Papaver setigerum*) investigated in the experimental CO_2 values (300, 400, 500 and 600 ppm) correspond roughly to the concentrations that existed during the middle of the twentieth century, the current concentration, and near and long-term projections for the current century (2050 and 2090), respectively. Elevated carbon dioxide resulted in significant increases in leaf area and above ground biomass. Elevated CO_2 increased the number of capsules, capsule weight and latex production. The amount of all alkaloids morphine, codeine, papaverine and noscapine increased significantly on a per plant basis, with the greatest relative increase occurring with recent increases in atmospheric carbon dioxide (e.g. from 300 to 400 ppm). They concluded that as atmospheric CO_2 continues to increase, significant effects on the production of secondary plant compounds of pharmacological interest could be expected (Ziska *et al*., 2008).

Healthy plantlets of *Artemisia annua* can be produced effectively *in vitro*, using a liquid medium with CO_2 enrichment in photoautotrophic conditions, resulting in plants to survive in *ex vitro* environments, as well as having effective artemisinin accumulation (Supaibulwattana *et al*., 2011).

In vitro culture of mint (*Mentha sp.* L.) and thyme (*Thymus vulgaris* L.) under atmospheres of 5%, 10%, 21%, 32%, or 43% O_2 with either 350 or 10000 ppm CO_2 was investigated. The results indicated that the highest levels of thymol were produced from thyme shoots cultured under 10% and 21% O_2 with 10000 ppm CO_2 and levels were considerably lower in shoots grown under either lower or higher O_2 levels. Higher levels of piperitenone oxide were obtained from mint cultures grown under 21% O_2 with 10000 ppm CO_2 compared to that obtained with lower O_2 levels (Tisserat *et al*., 2002).

In a five years experiment with *Populus nigra* L., elevated CO_2 and nitrogen fertilization, alone or in combination, did not affect lignin concentrations in wood. Soluble phenolics and soluble proteins in wood decreased slightly in response to elevated CO_2. Higher nitrogen supply stimulated formation of carbon base secondary compounds and increased protein concentrations (Luo *et al*., 2008).

A Malaysian medicinal herb *Labisia pumila* Benth. with three varieties alata, pumila and lanceolata was exposed to elevated CO_2 (1200 ppm) combined with four levels of nitrogen fertilization (0, 90, 180 and 270 kg N/ha). No varietal differences were observed, however, as the levels of nitrogen increased from 0 to 270 kg N/ha, the production of total phenolics and total flavonoids decreased in the order leaves>roots>stems (Ibrahim *et al*., 2010). Another experiment with these varieties under three levels of CO_2 enrichment (400, 800 and 1200 ppm) for 15 weeks showed that total flavonoids and total phenolics content, simultaneously, reached their peaks under 1200 ppm exposure, followed by 800 and 400 ppm (Ibrahim and Jaafar, 2011a). The same researchers in another study with the same three levels of CO_2 indicated that glutahione, oxidized gluthathione and antioxidant activities in a descending manner came from the leaf enriched with 1200 ppm CO_2 > leaf 800 ppm CO_2 > leaf 400 ppm CO_2 > stem 1200 ppm CO_2 > stem 800 ppm CO_2 > stem 400 ppm CO_2 > root 1200 ppm CO_2 > root 800 ppm CO_2 > root 400 ppm CO_2 (Ibrahim and Jaafar, 2011b).

Three deciduous tree species were exposed to elevated CO_2, about twice ambient (720 ppm) and temperature. The results revealed that elevated CO_2 generally stimulated increased carbon partitioning to various classes of phenolic compounds, whereas an increase in temperature had the opposite effect. The combined effects of both elevated CO2 and temperature were additive, i.e., canceling one another's individual effects (Veteli *et al*., 2007).

An investigation was conducted with exposure of four carbon dioxide regimes (350, 700, 1050 and 1400 ppm) and three fertilization levels (0, 100 and 500 kg/ha monthly) on seedlings of European white birch (*Betula pendula* Roth). They showed that fertilizer addition reduced the amounts of glucose and fructose while sucrose remained almost unaffected. The sugar content of leaves increased at 700 ppm and 1050 ppm of CO_2 and decreased at the highest CO_2 concentration (1400 ppm). The amounts of proanthocyanidins and flavonoids in leaves decreased with fertilization addition and increased with CO_2 enrichment. The production of simple phenolic glucosides varied according to the fertilization and CO_2 treatments. The triterpenoid content of stems seemed to increase with fertilization and CO_2-addition (Lavola and Julkunen-Tiitto, 1994).

Conclusion:

Elevated CO_2 concentrations can alter secondary metabolites accumulations. On the other hand, several environmental factors such as light, temperature, soil water and nutrients may interact with CO_2 levels and result in non predictable behavior.

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