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# $CO_2$-induced total phenolics in suspension cultures of *Panax ginseng* C. A. Mayer roots: role of antioxidants and enzymes

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**Abstract**

The effects of different concentrations of $CO_2$ (1%, 2.5% and 5%) on the antioxidant capacity, total phenols, flavonoids, protein content and phenol biosynthetic enzymes in roots of *Panax ginseng* were studied in bioreactor (working volume 4 l) after 15, 30 and 45 days. $CO_2$ induced accumulation of total phenolics in a concentration and duration dependent manner. Total phenols, flavonoids and 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity increased 60%, 30% and 20% at 2.5% $CO_2$ after 45 days compared to control in *P. ginseng* roots which indicated that phenolics compounds played an important role in protecting the plants from $CO_2$. Hypothesizing that increasing the phenolic compounds in roots of *P. ginseng* may increase its nutritional functionality; we investigated whether pentose phosphate pathway (PPP), shikimate/phenylpropanoid pathway enzymes have a role in phenolics mobilization in *P. ginseng* roots. Fresh weight (FW), dry weight (DW) and growth ratio was increased at 1% and 2.5% $CO_2$ only after 45 days, however, unaffected after 15 and 30 days. Results also indicated that high $CO_2$ progressively stimulated the activities of glucose 6 phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25), phenylalanine ammonia lyase (PAL, E.C. 4.3.1.5), cinnamyl alcohol dehydrogenase (CAD, E.C. 1.1.1.195), caffeic acid (CA) peroxidase and chlorogenic acid (CGA) peroxidase after 15, 30 and 45 days. Increased $CO_2$ levels resulted in increases in accumulation of total protein (45%), non-protein thiol (NP-SH) (30%) and cysteine contents (52%) after 45 days compared to control and increased activities of $\beta$-glucosidase (GS, E.C. 3.2.1.21) and polyphenol oxidase (PPO, E.C. 1.10.3.2) in *P. ginseng* roots indicated that they played an important role in protecting the plants from $CO_2$. These results strongly suggest that high concentration of $CO_2$ delivered to ginseng root suspension cultures induced the accumulation of total phenolics possessing high antioxidant properties probably useful for human health. Therefore, roots of *P. ginseng* are considered as a good source of phenolics compounds with high antioxidants capacity and can be produced on a large scale.


*Keywords:* Antioxidant capacity; Cysteine content; Flavonoids; $CO_2$; Glucose 6 phosphate dehydrogenase; *Panax ginseng*; Phenols; Phenylalanine ammonia lyase

## 1. Introduction

The responses of plants to high $CO_2$ levels vary considerably among cultivars and species, and include both undesirable and beneficial physiological and biochemical changes including carbohydrate metabolism, growth and yield of agricultural crop plants [2]. These changes in $CO_2$ will not only influence physiological changes but also have major effects on the primary and secondary metabolism of plants, such as phenolics, usually increase under elevated $CO_2$ [38]. Phenolics compounds are plant carbon-based secondary metabolites and protected plant tissues from oxidative damage, wounding and pathogen infections [10]. Plants produce a variety of secondary metabolites that play important biological roles in their environmental adaptation and are primarily synthesized through the pentose phosphate pathway (PPP), shikimate and phenylpropanoid pathways [8,9,37]. Phenolics are also involved in strengthening the plant cell walls during growth by polymerization into lignins. Some of the key

*Abbreviations:* CA, caffeic acid peroxidase; CAD, cinnamyl alcohol dehydrogenase; CBN-1, Chungbuk National University line-1; CGA, chlorogenic acid peroxidase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; G6PDH, glucose 6 phosphate dehydrogenase; $\beta$;-GS, $\beta$;-glucosidase; PAL, phenylalanine ammonia lyase; PPO, polyphenol oxidase; SKDH, shikimate dehydrogenase.

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enzymes catalyzing the biosynthesis of polyphenols include glucose 6 phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), phenylalanine ammonia lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD). However, PAL is the entry enzyme in the phenylpropanoid biosynthesis pathway and its presence has been demonstrated in all higher plants [9]. Studies with several different species of plants have shown that the activity of PAL increases by the biotic and abiotic factors, such as low temperature [3] and fungal infection [25]. Peroxidases are a group of enzymes involved in phenolics metabolism as well. They are directly involved in the biosynthesis of lignin from cinnamyl alcohols [29]. Besides, glucosidases and polyphenol oxidase (PPO) are widely distributed in the plant and play an important role for oxygen scavenging and defense mechanism against stress [14,45]. Non-enzymatic antioxidant such as non-protein thiol and cysteine contents are good scavenger of reactive oxygen species (ROS) and participates in a wide range of cellular functions [35].

Cultivation of ginseng roots as a potential source of a secondary metabolite, which has been used as a medicinal plant since the Greek and Roman time. *Panax ginseng*, is a perennial plants, cultivated in China, South Korea and Japan. The plant is well known for its anti-inflammatory, diuretic and sedative properties and as healing agent because of its saponin (a secondary metabolite) [19]. Besides saponin, *P. ginseng* also accumulates other secondary metabolites (phenolics compounds) and for the growth of the plant 4–6 years is needed for proper accumulation of secondary metabolites. Tissue culture is an important tool of plant biotechnology and one of its potential applications is for the production of valuable plant secondary metabolites. The accumulation of many secondary metabolites in plants is stimulated by various biotic and abiotic stresses (elicitors) that also activate plant defense mechanisms [11,40]. Therefore, development of an efficient root culture system for commercial production of ginseng root requires integrated enhancement strategies. Since $CO_2$ has a relatively low critical pressure (7.38 MPa) and critical temperature (304 K), and increased carbon source for plants shown a range of effects in various biological systems [15]. However, researches on the effects of $CO_2$ in bioreactor on hairy roots are scarce. Sustainable development of medicinally important root culture in this area should reduce labor cost and production of more bioactive compound because of the slow growth of the plant in field condition. Though, this ginseng species has a relatively well-defined metabolic response under different stress and accumulating lot of ginsenoside in response to stress treatments [19,49]. To the best of our knowledge, no information is available concerning the effects of $CO_2$ on enzymes, flavonoids, NP-SH, cysteine content in bioreactor in *P. ginseng* roots. Therefore, we have interested in the studies on phenolics metabolism and the enzymes responsible for the synthesis of phenolics. The objective of this study was to investigate the developmental mobilization of total phenolics and its influence on related metabolite, scavenging activity in tissue-cultured *P. ginseng* roots and their production in large scale. Here, we report the induction of PPP, shikimate and phenylpropanoid pathway enzymes in roots of *P. ginseng* subjected to $CO_2$ stress and their relation to the simultaneous accumulation of phenolics compounds.

## 2. Result

### *2.1. Effect of $CO_2$ on root growth, total phenolics, flavonoids, total protein, DPPH activity, NP-SH and cysteine contents*

The root growth of *P. ginseng* was given in Table 1. The root growth increased after inoculation slowly and the root growth profiles were almost same until the day 15, which was considered a preparatory period of adaptation of roots in the new environment. The fresh weight (FW), dry weight (DW) and growth ratio remained unchanged until day 30 and

Table 1
The effect of $CO_2$ concentration on ginseng roots growth after 15, 30 and 45 days of bioreactor culture

| $CO_2$ concentration (%) | FW ($g^{-1}$) | DW ($g^{-1}$) | Growth ratio (GR) [d] |
|---|---|---|---|
| | After 15 days of culture | | |
| Control | 19.87 [a] ± 0.42 | 2.02 [a] ± 0.12 | 0.96 [a] ± 0.06 |
| 1 | 19.90 [a] ± 0.47 | 2.02 [a] ± 0.10 | 0.95 [a] ± 0.02 |
| 2.5 | 20.52 [a] ± 0.31 | 1.94 [a] ± 0.09 | 0.97 [a] ± 0.02 |
| 5 | 20.71 [a] ± 0.50 | 2.06 [a] ± 0.20 | 0.98 [a] ± 0.02 |
| | After 30 days of culture | | |
| Control | 70.13 [a] ± 3.52 | 6.72 [a] ± 0.28 | 3.26 [a] ± 0.14 |
| 1 | 70.86 [a] ± 3.23 | 6.11 [a] ± 0.30 | 3.55 [a] ± 0.14 |
| 2.5 | 71.95 [a] ± 3.61 | 6.75 [a] ± 0.27 | 3.37 [a] ± 0.13 |
| 5 | 71.15 [a] ± 3.54 | 6.73 [a] ± 0.23 | 3.36 [a] ± 0.11 |
| | After 45 days of culture | | |
| Control | 126.2 [a] ± 4.5 | 10.16 [b] ± 0.24 | 5.08 [b] ± 0.22 |
| 1 | 127.9 [a] ± 3.9 | 13.92 [a] ± 0.41 | 6.96 [a] ± 0.30 |
| 2.5 | 132.5 [a] ± 4.2 | 12.95 [a] ± 0.50 | 6.47 [a] ± 0.28 |
| 5 | 126.9 [a] ± 4.1 | 11.10 [b] ± 0.35 | 5.55 [b] ± 0.28 |

Mean separations within columns by different letters are significantly different according to DMRT at 5% level. [d] Growth ratio is the quotient of the DW after cultivation and the DW of the inoculum.