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Induction in the antioxidative systems and lipid peroxidation in suspension culture roots of *Panax ginseng* induced by oxygen in bioreactors

Mohammad Babar Ali [a], Nguyen Trung Thanh [a], Kee-Won Yu [a], Eun-Joo Hahn [a], Kee-Yoeup Paek [a,*], Hyung Lae Lee [b]

[a] *Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Gaeshin Dong, Cheong-ju 361-763, Republic of Korea*
[b] *Department of Urology, College of Medicine, Chungbuk National University, Cheong-ju 361–763, Republic of Korea*

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Abstract

Roots of mountain ginseng (*Panax ginseng*) were exposed to various levels of oxygen (O_2) (30, 40 and 50%) for 15, 30 and 45 days in 5 L (working volume 4 L) airlift bioreactors. Ginsenoside accumulation and dry weight was enhanced up to 40% O_2; but thereafter declined ginsenoside and dry weight of the roots by increasing level of O_2. Gradual increase in H_2O_2 content and lipoxygenase activity (LOX), resulting in cellular damage and oxidative stress as indicated by increased malondialdehyde (MDA) content after 30 and 45 days at all O_2 levels was shown. Increased levels of O_2 (above ambient) resulted in increases in non-protein thiol (NP-SH) and cysteine content. Higher activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), catalase (CAT), guaiacol peroxidase (G-POD), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione *S* transferase (GST) activities indicated that antioxidant enzymes played an important role in protecting the roots from O_2 up to 45 days, except at 50% O_2 where GR, GST and GPx decreased compared to the control. However, after 45 days, SOD activity decreased significantly compared to the control in the O_2-treated roots. This reflects the sensitivity of enzymes to O_2 toxicity. In stress related experiment, roots showed increased synthesis of ginsenosides when 25 and 50 μM H_2O_2 was applied. However, higher dose and increasing treatment inhibited ginsenoside synthesis. The results indicate that plant roots could grow and protect themselves from O_2 stress by coordinated induction of various antioxidant enzymes and metabolite contents. These results suggest that O_2 supplementation is useful for ginsenoside accumulation using 5-L bioreactors.
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Keywords: Antioxidant enzymes; Ginsenoside; Growth; Lipid peroxidation; Oxygen; *P. ginseng*

1. Introduction

Panax ginseng is an important medicinal plant cultivated largely in Korea. The mature roots of these plants are used in many traditional medical formulations to treat different diseases [1]. Native ginseng is a slow growing plant taking 5–7 years between seed planting and mature root harvesting, during which time much cares is needed since its growth is susceptible to many environmental factors such as soil, shade, climate, pathogens and pests. Nowadays, wild ginseng has become extremely scarce and the ginseng supply depends almost exclusively on field cultivation, which is a time-consuming and labor-intensive process. The demand for the plant in the international market, makes bioreactor technology is a useful tool for production of root biomass on a large scale. Therefore, suspension culture of ginseng roots in bioreactors is viewed as a primary alternative method for large-scale production and recently our laboratory has developed a protocol for the in vitro culturing of *P. ginseng* [2]. This has enabled the study of physiological changes during growth of *P. ginseng* in bioreactor under stress.

* Corresponding author. Tel.: +82 43 266 3227; fax: +82 43 275 7467.
E-mail address: paekky@chungbuk.ac.kr (K.-Y. Paek).

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Ginsenosides are secondary metabolites of plant cells, whose accumulation is usually stimulated in response to challenges by biotic and abiotic stresses. Among various stresses applied to plant tissue cultures, O_2 stress is most relevant to plant growth in the natural environment, which may arise during water deficits. Although the O_2 level is generally considered as an important factor for plant tissue cultures, it has been given much less attention than any other culture conditions such as nutrients, light and temperature for secondary metabolite production. Little is known about the relationship between O_2 and ginsenoside production in tissue-cultured roots. While stimulating the secondary metabolite production of plant cells, the O_2 also caused significant growth inhibition, especially when it was applied to the culture at the early growth stage and was toxic at high levels. In the natural environment, only 1–2% of O_2 undergoes univalent reduction, producing the superoxide radical ($O_2^{\bullet -}$) and derivatives, which induce the antioxidative defense in plant cells. Increased levels of O_2 are toxic for most living systems generating active oxygen species (AOS) [3], which are harmful to the cell. These are superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$). The plant cells contain antioxidant defenses that remove the AOS. The damaging potential of superoxide is reduced by SOD, which catalyzes its dismutation to O_2 and H_2O_2. H_2O_2 is subsequently detoxified in O_2 and H_2O by catalase and peroxidases such as glutathione peroxidase or ascorbate glutathione cycle enzymes [4–6].

Since the O_2 concentration in air is 20%, in a bioreactor, continuous O_2 exposure would be a useful procedure for disinfection purposes. To improve the developmental capacities of cultured roots in bioreactor, we used higher O_2 that may decrease the concentrations of AOS in roots by improving the production of antioxidant enzymes. In addition, for efficient large-scale production of roots, which contains large amount of saponin (a secondary metabolite), is necessary to determine whether root culture in bioreactor could be applied to large-scale cultivation. However, there have been no reports on the effects of various O_2 levels on growth, lipid peroxidation and antioxidant enzymes in cultured roots. The present study was conducted to evaluate the capacity of tissue-cultured roots to respond to oxidative stress. Activities of antioxidant enzymes in mountain ginseng roots in bioreactor may be helpful in developing a better understanding of tolerance mechanisms to oxygen stress. We hypothesize that increased activities of antioxidant system in tissue-cultured roots would improve their root development.

2. Methods

2.1. Plant material, induction, proliferation of callus and adventitious roots

Four years old fresh ginseng roots (*P. ginseng* C.A. Meyer) were sterilized and cultured as described by Yu et al. [7] with some modifications. Cultures were maintained at 25 ± 2 °C in the dark for 4 weeks for callus induction. Callus was transferred to solid Murashige and Skoog (MS) medium [8] containing 3.0 mg L^{-1} indole butyric acid (IBA), and 3% sucrose to induce adventitious roots. The root line G10 was selected from 15 established adventitious root lines and was proliferated further in 5 L airlift balloon type bubble bioreactor containing 4 L modified MS liquid medium supplemented with 5.0 mg L^{-1} IBA, 2.0 mg L^{-1} naphthalene acetic acid (NAA), 0.5 mg L^{-1} kinetin and 5% sucrose (excluding NH_4NO_3 from the medium) and was named as Chungbuk National University Line 3 (CBN-3). These proliferated roots were used as explants for further experiments.

2.2. Bioreactor culture, supply of oxygen (O_2) and collection of sample

Forty to 45 days old CBN-3 line adventitious roots were cut into small pieces and 5 g FW per liter inoculums were treated with different levels of O_2 (i.e., 30, 40 and 50%) in the airlift balloon type bioreactors containing 4 L 3/4th MS liquid medium supplemented with 7.0 mg L^{-1} IBA, 0.5 mg L^{-1} kinetin and 30 g L^{-1} sucrose. Bioreactor cultures were performed at 25 ± 1 °C in the dark. Ambient room O_2 level was measured using a HEWLET PACKARD HP 6890 series GS system. The percentage of O_2 was obtained in the bioreactors by mixing air with different level of pure O_2 using an EYELA gas control unit F140, Japan. Roots were cultivated after 15, 30 and 45 days separately to avoid infection. Roots were washed under running tap water for 5–7 min and then again with nano pure water. Roots were then blotted dry and weight 1 g in an eppendorf tube and immediately kept under liquid nitrogen and stored at −80 °C until further analysis.

2.3. Dose and time dependence of ginsenoside synthesis by H_2O_2

To evaluate the dose dependence response of ginsenoside synthesis, roots were treated with various concentrations of H_2O_2 (25, 50, 100 and 200 μM) for 1 week. In another series of experiments, roots were treated with 50 μM H_2O_2 and collected after 0, 3, 5, 7 and 9 days of treatment. For this experiments, 45 days old roots were exposed to different concentrations of H_2O_2 containing 500 mL of the same MS medium mentioned above. The cultures were kept in a rotary shaker at 100 rpm in dark at 25 ± 1 °C.

2.4. Growth, extraction and determination of ginsenoside content

The growth of the adventitious roots was measured in terms of dry weights (DW). Roots were placed between the folds of blotting paper to remove excess water. Dry weight was measured after drying the fresh roots in an oven at

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