Korean J. Plant Res. 26(3):389-396(2013)
http://dx.doi.org/10.7732/kjpr.2013.26.3.389

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Comparative Antioxidant Enzyme Activity of Diploid and Tetraploid *Platycodon grandiflorum* by Different Drying Methods

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Abstract - The antioxidant enzyme and DPPH radical scavenging activity with variations in drying methods of diploid and tetraploid in *Platycodon grandiflorum* were determined. Antioxidant enzyme activities were measured as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX). The roots of *Platycodon grandiflorum* were freeze-dried, indoor-dried, hot-air dried, and microwave dried. The root extract of *P. grandiflorum* have shown the highest SOD enzyme activity of 92% in tetraploid of freeze-dried and indoor-dried while diploid of microwave dried showed the lowest SOD enzyme activity of 47.5%. The activity of CAT showed higher values in the root of tetraploid than in the diploid of *P. grandiflorum* in all drying methods. The APX activity showed relatively higher values in the root extract of freeze-dried both the diploid and tetraploid, but the difference in comparison with other extracts was not significant. The POX activities according to drying methods of diploid and tetraploid in *P. grandiflorum* showed relatively high values in freeze-dried and indoor-dried compared with other drying methods, and the POX activity between the diploid and tetraploid was not significant difference in each drying method. The DPPH radical scavenging activity with variation in drying methods of diploid and tetraploid in *P. grandiflorum* was the highest in the freeze-dried, and was higher in tetraploid than diploid in all the concentrations. In conclusion, the root of *P. grandiflorum* had the potent biological activities in both diploid and tetraploid. In particular, the tetraploid root of *P. grandiflorum* showing high antioxidant enzyme activity could be good materials for development of source of functional healthy food.

Key words - Antioxidant enzyme, DPPH radical scavenging activity, Drying method, Diploid, Tetraploid, *Platycodon grandiflorum*

Introduction

Platycodon grandiflorum is a perennial flowering plant belonging to the family Campanulaceae and is grown commercially in East Asia. Roots of *P. grandiflorum* have been used as a traditional oriental medicine and food for bronchial asthma, hepatic fibrosis, bone disorders (Lee *et al.,* 2004; Choi *et al.,* 2009; Jeong *et al.,* 2010; Lee, 1973), hypercholesterolemia and hyperlipidemia (Kim *et al.,* 1995). Roots of *P. grandiflorum* containing triterpenoid saponin, inulin, phytosterin, platycidinin, proteins, lipids, carbohydrates, iron, and fibers are similar to the ginseng roots and are being cultivated for food or medicine material. Recent studies indicate that platycodins are one of the most essential functional components in *P. grandiflorum* in terms of the inhibition of pancreatic lipase (Zhao and Kim, 2004), cholesterol lowering, and antiobesity effects (Zhao *et al.,* 2006). *P. grandiflorum* is well known to affect various pharmacological effects for human health and its consumption is increasing. In order to develop functional products using the physiological functionality of *P. grandiflorum* is needed a mass production of natural materials and the breeding of superior varieties. The creating of giant *P. grandiflorum* by the polyploidy breeding method can maximize its effects.

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The polyploidy breeding method in plants is a way to increase radically the emergence of new useful traits and the quantity by polyploidy obtained through quantitative doubling of the genome, which is a set of chromosomes. Polyploids, although frequently encounter low seed setting rates or complete sterility (Lewis, 1980), usually show larger organ size and superior cold tolerance (Kato and Birchler, 2006). For medicinal plants, polyploidy may increase the amounts of secondary metabolites (Thao *et al.,* 2003) which functional compounds are accumulated in the vegetative parts such as purple coneflower (Gao *et al.,* 1996; Nilanthi *et al.,* 2009). So polyploidy breeding is an effective approach of germplasm development for medicinal plants. Methods using colchinine for polyploidy induction are common for a wide range of plant species (Luckett, 1989; Ishizaka and Uematsu, 1994; Pinheiro *et al.,* 2000; Petersen *et al.,* 2003; liu *et al.,* 2007). Some reports were presented that the tetraploid induction of *P. grandiflorum* by colchicine treatment have also been available for breeding (Kim *et al.,* 2003; Wang *et al.,* 2006; Wu *et al.,* 2011). Recently, plant and plant-derived products are treated a part of the healthcare system by applying the bioactive phytochemicals. Antioxidant compounds in food play an important role as a health-protecting factor. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to eliminate free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Plant have antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) against ROS (reactive oxygen species) (Zhou *et al.,* 2005). The production of activated oxygen species occurs when plants are subjected to stress conditions (Dionisio-Sese and Tobita, 1998). Both enzymatic and nonenzymatic antioxidant systems are present in plants. Superoxide radicals are detoxified by SOD and hydrogen peroxide is destroyed by CAT and different kinds of peroxidases (Kang and Saltveit, 2002). A major hydrogen peroxide-detoxifying system in plant is the ascorbateglutathione cycle that includes APX and glutathione reductase (GR) (Asada, 1994). It is now widely accepted that reactive oxygen species (ROS) are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure (Moftah and Michel, 1987; Kandpal *et al.,* 1981), and needs to be scavenged for maintenance of normal growth. Ascorbate peroxidase, catalase and peroxidase, together with low-molecular mass scavengers such as ascorbate, glutathione and proline, act as the main defense against ROS produced in various parts of plant cells (Apel and Hirt. 2004). The induction of ROS-scavenging enzymes, such as SOD, POXs and CAT, is the most common mechanism for detoxifying ROS synthesized during stress responses (Wojtaszek, 1997; Mittler, 2002). The objective of this study was conducted to obtain tetraploid to have higher contents of pharmaceutical constituents as well as higher yield in *P. grandiflorum* by colchicine treatment, and their antioxidant activity and the antioxidative defence system enzymes SOD, CAT, APX and POD were compared with diploid.

Materials and Methods

Plant material

The diploid plant of *Platycodon grandiflorum* was grown in Geumsan county, Chungcheong province and purchased from local market. Tetraploid plants were provided by the cooperative research laboratory of this study, Chungbuk National University. Root samples were freeze-dried, indoor-dried, hot-air dried, and microwave dried and then ground. Each sample powder was stored at -20℃ for experiments.

Induction of tetraploid

Tetraploid mutants were induced in a similar method to the procedure described by Kim *et al.* (2003). The colchicine treatment on seedlings were performed at the time of cotyledon emergence. After the treatment, seedlings were washed 3~4 times with distilled water and planted on 12cm pots containing of equal volume of perlite and coarse sand. Survival rate and chromosome numbers were counted 30 days after transplanting. After 4 months, measurement of stomates and morphological characters were made. To induce polyploids in adult plants, growing points were covered with cotton balls and sprayed with concentration of 0.05% colchicines solution 3 times a day for 3 days. The ploidy level

of *Platycodon grandiflorum* was estimated chromosome counting of root tips from obtained mutants by morphological characteristics.

Assay of antioxidant enzyme

SOD activity

The superoxide dismutase (SOD) activity was measured using SOD assay Kit-WST purchased from Sigma-Aldrich (Sigma-Aldrich Co., Japan). This assay is based on the colorimetric assay for the measurement of total antioxidant capacity of crude aqueous fractions. The 60 μL of sample solution (sample and blank2) or doubledistilled water (blank1 and blank3) was mixed with 600 μL of WST working solution. For Blank2 and Blank3, 60 μL of dilution buffer was added. Then, 60 μL of enzyme working solution was added to each sample and blank1. The plate was incubated at 37℃ for 20 min, and the OD (Optical density) was determined at 450 nm using a spectrophotometer (Biochrom Co., England). SOD activity (inhibition rate percent) was calculated using the following equation:

$$\text{SOD activity} = \{[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})] / (A_{blank\ 1} - A_{blank3})\} \times 100.$$

CAT activity

Catalase (CAT) activity was assayed by the method of Mishra et al. (1993). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 11 mM H_2O_2, and the crude enzyme extract. The reaction was initiated by addition of H_2O_2 to the mixture, and enzyme activity was determined by monitoring the decline in absorbance at 240 nm (ε=36 M^{-1} cm^{-1}), because of H_2O_2 consumption.

APX activity

Ascorbate peroxidase (APX) activity was determined by monitoring the decline of absorbance at 290 nm as ascorbate (ε=2.8 mM^{-1} cm^{-1}) was oxidized, by the method of Chen and Asada (1989). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 0.2 mM H_2O_2.

POX activity

Peroxidase (POX) activity was determined specifically with guaiacol at 470 nm (ε=26.6 mM^{-1} cm^{-1}), following the method of Egley et al. (1983). The reaction mixture contained 40 mM potassium phosphate buffer (pH 6.9), 1.5 mM guaiacol, and 6.5 mM H_2O_2 in 1 ml with crude enzyme extract. Control assays in which the enzyme extracts or substrates were replaced by buffer were performed.

Assay of DPPH radical scavenging rate

100 μL of various concentrations (100, 250, 500, 1000, 2500, 5000 and 10000 mg L^{-1}) of extracts of diploid and tetraploid in *Platycodon grandiflorum* were added to 900 μL of 100% methanol containing 100 μM DPPH, and the reaction mixture was shaken for 5 min in the slight vortex. Leaving room temperature for 30 min under darkness, the absorbance of DPPH was determined by spectrophotometer at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation: Scavenging effect on DPPH radical (%) = [(A-B)/A] × 100, Where A is the absorbance at 517 nm without pigment compositions and B is the change in absorbance at 517 nm with pigment compositions incubation (Brand-Williams *et al*., 1995).

Data analysis

The statistical analysis was performed using the procedures of the Statistical Analysis System. ANOVA procedure followed by Duncan Multiple Range Test was used to determine the significant difference at $p < 0.05$, 0.001 highly significant.

Results and Discussion

Antioxidant enzyme activity

This study shows that the plants induced polyploidy mutants can be achieved the activities of SOD, CAT, APX, and POX. The comparative results of the antioxidant enzyme activity in different drying methods of diploid and tetraploid in *P. grandiflorum* plants are shown in figure 1~4. The root extract of *P. grandiflorum* had the highest SOD enzyme activity of 92% in tetraploid of freeze-dried and indoor-dried while diploid of microwave dried showed the lowest SOD enzyme activity of 47.5% (Figure 1). The activity of CAT showed higher values in the root of tetraploid than in the

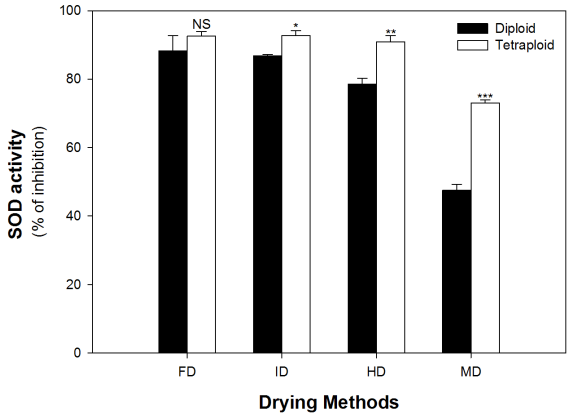

Fig. 1. SOD activities according to drying methods of diploid and tetraploid in *Platycodon grandiflorum*. Asterisk indicates a significant difference between diploid and tetraploid at *p<0.05, **p<0.01, ***p<0.001 level. The bars represent the standard error. NS: not significant. FD: freeze dried, ID: indoor dried, HD: hot air dried, MD: microwave dried.

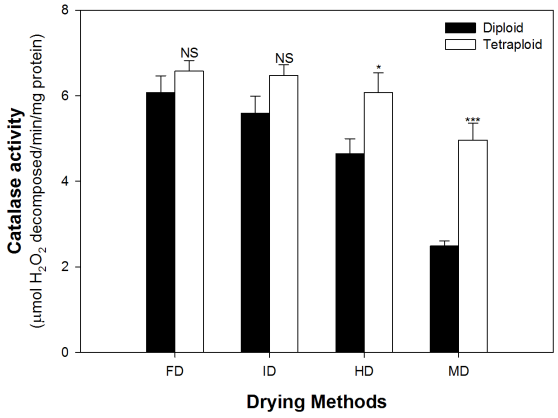

Fig. 2. CAT activities according to drying methods of diploid and tetraploid in *Platycodon grandiflorum*. Asterisk indicates a significant difference between diploid and tetraploid at *p<0.05, **p<0.01, ***p<0.001 level. The bars represent the standard error. NS: not significant. FD: freeze dried, ID: indoor dried, HD: hot air dried, MD: microwave dried.

diploid of *P. grandiflorum* in all drying methods (Figure 2). The APX activity showed relatively higher values in the root extract of freeze-dried both the diploid and tetraploid, but the difference in comparison with other extracts was not significant (Figure 3). The POX activities according to drying methods of diploid and tetraploid in *P. grandiflorum* showed relatively high values in freeze-dried and indoor-dried compared with other drying methods, and the POX activity

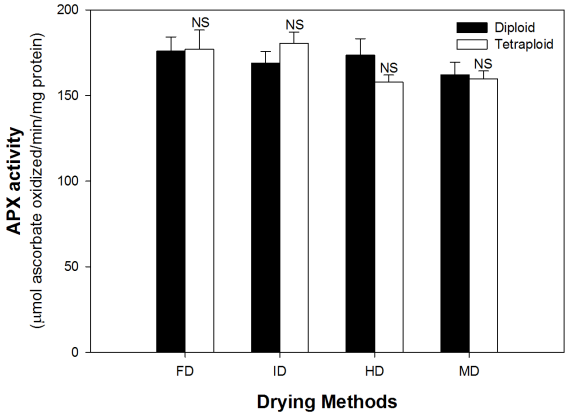

Fig. 3. APX activities according to drying methods of diploid and tetraploid in *Platycodon grandiflorum.* The bars represent the standard error. NS: not significant. FD: freeze-dried, ID: indoor-dried, HD: hot-air dried, MD: microwave dried.

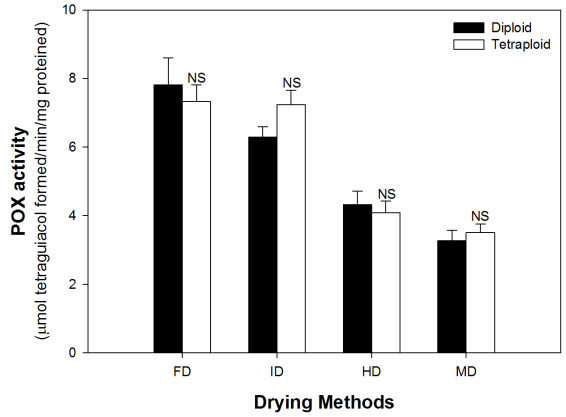

Fig. 4. POX activities according to drying methods of diploid and tetraploid in *Platycodon grandiflorum.* The bars represent the standard error. NS: not significant. FD: freeze-dried, ID: indoor dried, HD: hot-air dried, MD: microwave dried.

between the diploid and tetraploid was not significant difference in each drying method (Figure 4). Significant roles of POX have been suggested in plant development processes (Gaspar *et al.,* 1985), which was involved in scavenging of H_2O_2 produced in chloroplasts (Asish and Anath, 2005). The antioxidant enzyme activities differ significantly in different plants. The SOD is one of the enzymes, in vivo, to catalyze the reaction that converts the harmful reduced oxygen formed in cell due to rancidity into hydrogen peroxide; is generated in most aerobic or anaerobic biological organisms; is switched to water and oxygen by the CAT and APX, and

loses then its toxicity. In other words, the SOD and APX enzymes of the cellular antioxidative system involved in H_2O_2 metabolism; SOD, which catalyses the disproportionation of O_2^- radicals into H_2O_2 and molecular oxygen, and APX, an enzyme that scavenges H_2O_2 (Bonnet *et al.,* 2000). Typically, the APX plays the most important scavenger role in the cytoplasm and chloroplasts of plants, and ascorbic acid is used as a reduction substrate (Wheeler *et al.,* 1998). APX activity, which is important component of the antioxidant system, plays a key role in eliminating H_2O_2 molecules and in the modulation of its steady-state levels in various plant subcellular compartments (Najami *et al.,* 2008). The CAT is also an antioxidant enzyme that protects cells by dispatching of in vivo harmful oxygen and is a typical enzyme that acts to decompose and scavenge the H_2O_2 together with APX. In this study, we are considered that relatively high antioxidant enzyme activity in the tetraploid of *P. grandiflorum* is influenced by providing some stress on plant under forced polyploidy induction. The antioxidant enzymes, indicating a high activity to remove harmful free radicals, have the effect of prevention and inhibition of various diseases and aging. In the tetraploid of *P. grandiflorum*, we can also expect to see these benefits for the next variety of natural foods and cosmetics where the need to apply functional substances may also be required. That is, with this study, in a variety of resource plants, we can sure to take advantage of their higher value as valuable materials of healthy functional foods, as they showed higher antioxidant enzyme activity. In conclusion, we have showed that the root of *P. grandiflorum* had the potent biological activities in both diploid and tetraploid. In particular, the tetraploid root of *P. grandiflorum* showing high antioxidant enzyme activity could be good materials for development of source of functional healthy food.

DPPH radical scavenging activity

The investigation of the antioxidant activity of natural substances is based on the measuring of the electron donor capacity of DPPH with the ability to inhibit the oxidation by donating electrons in free radicals causing this lipid peroxidation (Boo *et al.,* 2012), that is, free radical are known to be a major factor in biological damages, and DPPH has been used to evaluate the free radical-scavenging activity of natural antioxidants (Yokozawa *et al.,* 1998; Zhu *et al.,* 2001). Active oxygen caused by in vivo metabolism removed by the body's antioxidant system, but excessive free radicals induced stress, causing the lipid peroxidation by combining with unsaturated fatty acids in the cell membrane, and brought intracellular structural and functional damage. The measurement results of free radical scavenging activity at seven different concentrations, 100, 250, 500, 1000, 2500, 5000 and 10000 mg/L are shown in Table 1. The DPPH radical scavenging activity with variation in drying methods of diploid and tetraploid in *P. grandiflorum* was the highest in the freeze-dried, and was higher in tetraploid than diploid in

Table 1. DPPH radical scavenging activities according to drying methods of diploid and tetraploid in *Platycodon gradinflorum*

Plant	Drying methods	DPPH radical scavenging activity, % of control						
		Concentration (mg/L)						
		100	250	500	1000	2500	5000	10000
Diploid	FD	10.0±0.64^{a}	10.5±0.51^{a}	11.3±0.32^{a}	12.1±0.29^{a}	16.0±0.75^{a}	21.4±1.91^{ab}	33.3±1.66^{ab}
	ID	9.8±0.45^{a}	10.1±0.68^{a}	10.9±0.51^{a}	11.2±0.24^{a}	13.3±0.85^{b}	17.1±0.77^{b}	29.5±1.35^{ab}
	HD	9.7±0.18^{a}	10.2±0.24^{a}	10.6±0.62^{a}	11.3±0.10^{a}	13.6±0.37^{ab}	17.2±0.94^{b}	28.2±1.86^{b}
	MD	8.9±0.47^{a}	10.0±0.24^{a}	10.8±0.17^{a}	11.7±0.20^{a}	14.2±0.53^{ab}	18.4±1.13^{ab}	29.6±0.98^{ab}
Tetraploid	FD	10.6±0.88^{a}	10.4±0.72^{a}	10.8±0.98^{a}	12.1±0.26^{a}	16.1±0.60^{a}	22.2±1.04^{a}	35.2±0.87^{a}
	ID	10.4±0.79^{a}	10.5±0.56^{a}	10.9±0.79^{a}	11.1±0.73^{a}	14.9±0.21^{ab}	18.9±1.41^{ab}	31.5±1.21^{ab}
	HD	10.1±0.43^{a}	10.2±0.40^{a}	10.7±0.40^{a}	11.7±0.29^{a}	13.3±0.98^{b}	18.8±1.63^{ab}	32.2±1.86^{ab}
	MD	9.2±0.55^{a}	10.2±0.12^{a}	10.7±0.72^{a}	11.6±0.73^{a}	14.7±1.29^{ab}	19.8±2.11^{ab}	31.6±3.22^{ab}

[z]Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test. FD: freeze-dried, ID: indoor-dried, HD: hot-air dried, MD: microwave dried.

all the concentrations. Overall, the DPPH radical scavenging activity showed that the increase was proportional to the concentration. Cells are oxidized and damaged by the free radical, depending on the growth of cells. It has been reported that saponin components of *P. grandiflorum* have antioxidant capacity to inhibit the oxidation by donating electrons to the free radical due to strong reduction (Fu *et al.,* 2009; Kim *et al.,* 2010; Ryu *et al.,* 2012). In this study, the DPPH radical scavenging activity appeared to concentration dependent, and depending on the polyploid of plants, there were significant differences between tetraploid and diploid. Therefore further study is needed to require about tetraploid characterization to compare functional components between obtained tetraploid and diploid for *P. grandiflorum.* The effective source of the tetraploid plants including *P. grandiflorum* could be employed in all medicinal preparation to combat myriad diseases associated with oxidative stress.

Acknowledgement

This research was supported by High Value-added Food Technology Development Program of IPET, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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(Received 10 June 2013 ; Revised 18 June 2013 ; Accepted 18 June 2013)