Journal of Medicinal Plants Research Vol. 6(38), pp. 5128-5134, 3 October, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.120
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effects of oligosaccharide elicitors from endophyitc *Fusarium oxysporum* Dzf17 on diosgenin accumulation in *Dioscorea zingiberensis* seedling cultures

Peiqin Li, Jingfeng Lou, Yan Mou, Weibo Sun, Tijiang Shan and Ligang Zhou*

College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China.

Accepted 26 April, 2012

The effects of the oligosaccharides prepared from endophytic fungus *Fusarium oxysporum* Dzf17 on diosgenin accumulation in *Dioscorea zingiberensis* seedling cultures were investigated. Among three oligosaccharide fractions (DP2-5, DP5-8, and DP8-12), DP8-12 at concentration of 20 mg/L resulted in the highest diosgenin content and yield of the seedlings with the values individually as 1.28 mg/g dw and 18.05 mg/L, which were 4.76-and 5.65-fold higher, respectively compared to the control. Among three oligosaccharide monomers (DP4, DP7, and DP10) separately derived from fractions DP2-5, DP5-8, and DP8-12, DP10 showed the most significant enhancement on diosgenin accumulation. When the seedlings were treated with DP10 at 6 mg/L and harvested on day 36 of culture, both diosgenin content and yield increased to 1.85 mg/g dw and 27.69 mg/L, which were 7.24-and 9.33-fold higher respectively compared to the control. The results indicated that addition of the oligosaccharides from endophytic fungus *F. oxysporum* Dzf17 as elicitors could significantly enhance diosgenin production in *D. zingiberensis* seedling cultures.

Key words: *Dioscorea zingiberensis*, endophytic fungus, *Fusarium oxysporum* Dzf17, seedlings, polysaccharide, oligosaccharide, diosgenin.

INTRODUCTION

Dioscorea zingiberensis C. H. Wright (Dioscoreaceae) is a traditional Chinese medicinal herb, which is indigenous to the south of China (Chen et al., 2003). It has been considered as the preferred species to produce diosgenin (Zhang et al., 2006; Zhu et al., 2010), which is an important precursor of semi-synthetic steroids, such as corticosteroids, sex hormones, and other steroidal drugs in pharmaceutical industry (Fernandes et al., 2003; Li and Ni, 2011). However, overexploitation of natural *D. zingiberensis* has resulted in a dramatic decrease of this plant resource and a sharp shortage of the raw materials. The tissue and cell culture of *D. zingiberensis* has been regarded as an alternative means for efficient and controllable production of diosgenin. In addition, increasing diosgenin content and yield in field cultivation of *D. zingiberensis* should be another aspect (Dicosmo and Misawa, 1995; Zhou and Wu, 2006).

Compared with the growth of whole plants in the natural environment or on agricultural farms, growth of plant tissue cultures has the advantages of well-controlled and reproducible conditions without the limitations of natural factors such as geographical location and climatic variation (Kieran et al., 1997; Zhou and Wu, 2006). However, the cultured plant cells, tissues or organs usually produce low amounts of desired secondary metabolites when compared with the natural intact plants. Therefore, the great efforts have been made to search strategies for improvement of secondary metabolite production in plant tissue cultures, such as cultivation of

*Corresponding author. E-mail: lgzhou@cau.edu.cn.

Abbreviations: WPS, Water-extracted mycelial polysaccharide; **TO,** total oligosaccharide; **DP,** degree of polymerization; **DP2-5,** fraction composed of oligosaccharides with DPs from 2 to 5; **DP5-8,** fraction composed of oligosaccharides with DPs from 5 to 8; **DP8-12,** fraction composed of oligosaccharides with DPs from 8 to 12; **DP4,** oligosaccharide with DP as four; **DP7,** oligosaccharide with DP as seven; **DP10,** oligosaccharide with DP as ten; **dw,** dry weight; **Dzf17,** endophytic fungus *Fusarium oxysporum* Dzf17.

specific plant organ cultures, selection of cell lines with high productivity, optimization of medium and culture conditions, application of genetic engineering and biotransformation, employment of immobilization and permeabilization for cell cultures, and enhancement of secondary metabolite production by using elicitors (Dicosmo and Misawa, 1995). Among the manipulative techniques available to promote the productivity of plant secondary metabolites in plant tissue culture system, elicitation has been regarded as the most likely one to obviously increase yield of the metabolites (Wiktorowska et al., 2010).

Elicitation refers to the induction of secondary metabolite production by the molecules or treatments called elicitors (Zhong, 2002). According to their origins, elicitors are generally classified as biotic or abiotic. Fungi as biotic elicitors have been widely studied to enhance the production of plant secondary metabolites in plant culture systems, such as tanshinone production in *Salvia miltiorrhiza* hairy root cultures (Ge and Wu, 2005), betalain production in *Beta vulgaris* hairy root cultures (Savitha et al., 2006), oleandrin production in *Nerium oleander* cell cultures (Ibrahim et al., 2007), anthraquinone derivatives production in *Rubia tinctorum* cell cultures (Orban et al., 2008), and bilobalide and ginkgolide production in *Ginkgo biloba* cell cultures (Kang et al., 2009).

Fungi, used as elicitors to promote production of plant secondary metabolites, usually refer to pathogenic or non-pathogenic fungi. Plant endophytic fungi, belonging to non-pathogenic fungi, show symbiotic relationships with their host plants and induce no visual symptoms to host plants (Suryanarayanan et al., 2009). Endophytic fungi have attracted increasing attention because they could not only protect plants against diseases and ward off insect pests, but also increase the fitness of plants by enhancing their tolerance to abiotic stresses, such as drought and heavy metal stresses (Backman and Sikora, 2008; Ganley et al., 2008; Kane, 2011). Moreover, endophytic fungi have been considered as important and novel potential sources of natural bioactive compounds (Schulz et al., 2002; Zhou et al., 2010; Zhao et al., 2011).

Plant endophytic fungi have been investigated as the elicitors to enhance plant secondary metabolite production. Typical examples included paclitaxel (taxol) enhancement in *Taxus chinensis* cell cultures induced by the endophytic fungus *Aspergillus niger* (Wang et al., 2001a), artemisinin production in *Artemisia annua* hairy root cultures elicited by the endophytic *Colletotrichum* sp. (Wang et al., 2001b), paclitaxel formation in *Taxus cuspidata* cell cultures treated by the endophtic *Fusarium mairei* (Li and Tao, 2009), and alkaloid production in *Catharanthus roseus* cell cultures induced by its endophytic *Fusarium oxysporum* F9 (Tang et al., 2011). Endophytic fungus *F. oxysporum* Dzf17 was isolated from the healthy rhizomes of D*. zingiberensis*, and the effects of its mycelia and filtrate have been investigated to enhance diosgenin production in *D. zingiberensis* cell cultures (Zhang et al., 2010). Moreover, polysaccharides and oligosaccharides were prepared from this fungus, and their enhanced effects on diosgenin production in *D. zingiberensis* cell cultures were also observed (Zhang et al., 2009; Li et al., 2011a, b). Diosgenin content in seedling cultures of *D. zingiberensis* was examined to be higher compared to cell cultures (Yin et al., 2011). In this work, effects of total oligosaccharide, its fractions (DP2-5, DP5-8, and DP8-12), and oligosaccharide monomers (DP4, DP7, and DP10) on growth and diosgenin production of *D. zingiberensis* seedlings were studied. The aim of this investigation is to seek appropriate oligosaccharide elicitors from endophytic fungus *F. oxysporum* Dzf17 applying in *D. zingiberensis* seedling cultures to increase diosgenin yield.

MATERIALS AND METHODS

Seedling culture of *D. zingiberensis*

The seedlings of *D. zingiberensis* C. H. Wright were initially obtained by callus redifferentiation on Murashige and Skoog (MS) medium supplemented with 6-benzyladenine (5.0 mg/L), kinetin (2.0 mg/L), sucrose (30 g/L), and agar (8 g/L) at 25°C under 12 h daily illumination of approximately 2000 lux provided by cool fluorescent tubes. The pH medium was adjusted to 5.8 before autoclaving. Subculture of the seedlings was conducted on MS medium supplemented with sucrose (30 g/L) and agar (8 g/L) at 25°C at an interval of 30 days under 12 h daily illumination of approximately 2000 lux (Yin et al., 2011). The seedlings subcultured for 5 generations were taken as the plant materials. Each 125 ml Erlenmeyer flask was filled with 50 ml of MS solid medium, and three seedlings (about 1.0 g fresh weight) were inoculated in each flask.

Preparation of the oligosaccharides

Endophytic fungus *F. oxysporum* Dzf17 was cultured in a 1000 ml Erlenmeryer flask containing 300 ml of liquid medium consisting of glucose (50 g/L), peptone (13 g/L), NaCl (0.6 g/L), K_2HPO_4 (0.6 g/L), and $MgSO_4.7H_2O$ (0.2 g/L). All flasks were maintained at 25°C on a rotary shaker at 150 rpm for 14 days. A total of 150 L of fermentation broth was obtained and centrifuged at 7,741 x*g* for 20 min. The mycelia were collected and washed twice with deionized water, and then lyophilized. About 600 g of mycelia in dry weight (dw) was obtained. Water-extracted mycelia polysaccharides (WPS), total oligosaccharide (TO), oligosaccharide fractions (DP2-5, DP5-8, and DP8-12), as well as oligosaccharide monomers (DP4, DP7, and DP10) were prepared according to Li et al. (2011a, b). All the oligosaccharide elicitors from endophytic fungus *F. oxysporum* Dzf17 (total oligosaccharide, oligosaccharide fractions, and monomers) were independently dissolved in distilled water as the concentrate stock solution, filter-sterilized through a membrane (pore size, 0.45 µm) and stored at 4°C.

Elicitation treatment

Total oligosaccharide (TO) and oligosaccharide fractions (DP2-5, DP5-8, and DP8-12) were separately added to MS medium with the

final concentrations of 5, 10, 20, 40, and 80 mg/L carbohydrate equivalent. Purified oligosaccharide monomers (DP4, DP7, and DP10) were employed to treat the seedlings at concentrations of 2, 4, 6, 8, and 10 mg/L carbohydrate equivalent. The inoculated quantity was three seedlings (about 1.0 g fresh weight) in each flask. The seedlings were harvested after 32 days culture, which was determined according to the dynamics of growth and diosgenin yield of *D. zingiberensis* seedling cultures (Yin et al., 2011). Finally, the optimal oligosaccharide elicitor DP10 was chosen for further investigation. The addition concentration (4, 6, and 8 mg/L) and harvest time (day 30, 33, 36, and 39) for DP10 were studied to realize the maximum enhancement of diosgenin accumulation in the seedling cultures.

Determination of biomass and diosgenin analysis

The seedlings were harvested from the Erlenmeyer flasks and washed with distilled water to remove residual medium, and then lyophilized to a constant weight. Diosgenin extraction and determination were carried out as previously described with some modifications (Zhang et al., 2009; Zhu et al., 2010; Yin et al., 2011). Briefly, 100 mg of powdered dry cultured seedlings was added into a tube with 20 ml of 95% ethanol, and then subjected to ultrasonic treatment for 1 h. After that, 20 ml of 1 mol/L sulfuric acid was added to each tube, and hydrolyzed at 121°C for 2 h. The hydrolyte was extracted for three times with petroleum ether. The combined petroleum ether solution was washed twice with 1 mol/L NaOH, and then twice with distilled water. After dehydration with anhydrous sodium sulfate, the petroleum ether solution was concentrated to dryness under vacuum on a rotary evaporator. The extract was dissolved in acetonitrile, and then filtered through a filter (pore size, 0.22 μm) before analysis.

A high performance liquid chromatography (HPLC) system (Shimadzu, Japan), which consists of two LC-20AT solvent delivery units, an SIL-20A autosampler, an SPD-M20A photodiode array detector, and CBM-20Alite system controller, was employed. A reversed-phase Agilent TC-C_{18} column (250 × 4.6 mm i.d., particle size 5 μm) was used for chromatography by using acetonitrile-water (90:10, v/v) as the mobile phase at a flow rate of 1 ml/min at 30°C, and an LC solution multi-PDA workstation was employed to acquire and process chromatographic data. The injection volume was 20 μl. Changes in absorbance at 203 nm were recorded. The peak area was calibrated to diosgenin content with a chemical standard (Sigma). Diosgenin content in the medium was negligible and not determined.

Statistical analysis

All the experiments were carried out three times. Each treatment was performed in triplicate, and the results were represented by their mean values and standard deviations. The data were submitted to analysis of variance to detect significant differences by PROC ANOVA of SAS version 8.2.

RESULTS AND DISCUSSION

Effects of total oligosaccharide and its fractions DP2-5, DP5-8, and DP8-12

Effects of total oligosaccharide (TO) and its fractions (DP2-5, DP5-8, and DP8-12) on growth, diosgenin content, and diosgenin yield of *D. zingiberensis* seedlings were presented in Table 1. The optimal concentration to promote seedling growth was 20 or 40 mg/L. The highest dry weight (14.58 g dw/L) was observed by using oligosaccharide fraction DP5-8 at 20 mg/L, which was 1.22-fold of control. Similarly, the highest dry weight (14.15 g dw/L) was observed when the seedlings were treated with DP8-12 at 20 mg/L, which was 1.17-fold of control.

These oligosaccharide elicitors resulted in diverse influence on diosgenin content. The maximum diosgenin content (0.82 mg/g dw) was obtained in the seedlings treated with TO at 40 mg/L, which was 3.05-fold of control. The maximum values of diosgenin content generated by DP2-5 and DP5-8 were separately 0.37 mg/g dw and 0.40 mg/g dw, which were respectively 1.37 and 1.49-fold as compared with that of control. Among all the elicitation treatments, oligosaccharide fraction DP8-12 exhibited an excellent effect on enhancement of diosgenin content. The highest diosgenin content (1.28 mg/g dw) was observed when the seedlings were treated with DP8-12 at 20 mg/L, which was 4.76-fold of control and higher than those of TO, DP2-5, and DP5-8.

As presented in Table 1, the variation trend of diosgenin yield was mostly dependent on that of diosgenin content. The most significant enhancement on diosgenin yield was generated by DP8-12 at concentration of 20 mg/L, which was 5.65-fold of control (18.05 mg/L). When the seedlings were treated with DP8-12 at 40 mg/L, diosgenin yield was also significant higher than that of control, which was 3.37-fold of control. Elicitor TO also showed a satisfactory effect on enhancement of diosgenin yield. When the seedlings were treated with TO at 40 mg/L, diosgenin yield was increased to 11.32 mg/L, which was 3.54-fold of control. The highest values of diosgenin yield in seedling cultures treated with DP2-5 and DP5-8 were respectively 4.49 mg/L and 5.81 mg/L, which were 1.41- and 1.82-fold of control, respectively, but both were lower than that of DP8-12. It was concluded that the most effective elicitor was oligosaccharide fraction DP 8-12 among the total oligosaccharide and its fractions.

Effects of oligosaccharides DP4, DP7 and DP10

Three purified oligosaccharide monomers were respectively isolated from the oligosaccharide fractions DP2-5, DP5-8, and DP8-12, which were named as DP4, DP7, and DP10, respectively (Li et al., 2011b). The effects of DP4, DP7, and DP10 on growth, diosgenin content, and diosgenin yield of *D. zingiberensis* seedlings were shown in Figure 1. Dry weight of the seedlings did not significantly increase in most of treatments except when the seedlings were elicited by 6 mg/L and 8 mg/L of DP4, 8 mg/L of DP7, and 6 mg/L of DP10. The maximum values of dry weight generated by DP4, DP7, and DP10 were 15.58, 14.49, and 14.43 g dw/L respectively, 1.29-, 1.20-, and 1.20-fold higher compared to control but showed no significant differences among them.

Table 1. Effects of total oligosaccharide and its fractions on growth, diosgenin content, and diosgenin yield of *D. zingiberensis* seedling cultures.

Concentration (mg/L)		Dry weight (g dw/L)	Diosgenin content (mg/g dw)	Diosgenin yield (mg/L)
CK	0	11.93 ± 0.41^{ij}	0.27 ± 0.02^{lm}	3.20 ± 0.16^{kl}
TO	5	12.23 ± 0.30^{hi}	0.31 ± 0.02j^{k}	3.78 ± 0.14^{j}
	10	13.06 ± 0.21^{def}	0.43 ± 0.03^{fg}	5.61 ± 0.34^{f}
	20	13.69 ± 0.18^{bcd}	0.56 ± 0.02^{d}	7.60 ± 0.39^{d}
	40	13.83 ± 0.29^{bc}	0.82 ± 0.02^{b}	11.32 ± 0.39^{b}
	80	10.91 ± 0.21^{l}	0.45 ± 0.05^{f}	4.86 ± 0.40^{g}
DP2-5	5	12.09 ± 0.36^{hij}	0.29 ± 0.02^{kl}	3.50 ± 0.10^{jk}
	10	12.64 ± 0.39^{fgh}	0.34 ± 0.01^{ij}	4.31 ± 0.16^{hi}
	20	12.18 ± 0.39^{hij}	0.37 ± 0.02^{hi}	4.49 ± 0.38^{gh}
	40	11.54 ± 0.38^{jk}	0.24 ± 0.01^{m}	2.81 ± 0.17^{lm}
	80	11.07 ± 0.52^{kl}	0.20 ± 0.02^{n}	2.20 ± 0.16^{n}
DP5-8	5	12.37 ± 0.34^{ghi}	0.30 ± 0.02^{kl}	3.71 ± 0.13^{j}
	10	13.45 ± 0.15^{cde}	0.35 ± 0.01^{i}	4.74 ± 0.22^{gh}
	20	14.58 ± 0.40^{a}	0.40 ± 0.02^{gh}	5.81 ± 0.24^{ef}
	40	13.68 ± 0.34^{bcd}	0.29 ± 0.02^{kl}	3.95 ± 0.21^{ij}
	80	13.09 ± 0.30^{def}	0.20 ± 0.01^{n}	2.65 ± 0.24^{m}
DP8-12	5	12.16 ± 0.34^{hij}	0.38 ± 0.01^{hi}	5.59 ± 0.08^{gh}
	10	12.59 ± 0.21^{fghi}	0.49 ± 0.01^{e}	6.11 ± 0.06^{e}
	20	14.15 ± 0.31^{ab}	1.28 ± 0.02^{a}	18.05 ± 0.44^{a}
	40	13.93 ± 0.63^{bc}	0.77 ± 0.03^{c}	10.76 ± 0.23^{c}
	80	12.96 ± 0.55^{efg}	0.48 ± 0.04^{e}	6.24 ± 0.31^{e}

Each value was expressed as mean ± standard deviation (n = 3). Different letters in each column indicated significant differences at p = 0.05.

Figure 1B presented the effects of DP4, DP7, and DP10 on diosgenin content of the seedlings. The highest diosgenin content was 0.35 mg/g dw when the seedlings were treated with DP4 at 4 mg/L, which was 1.29-fold of control. For DP7, only when its concentration was increased up to 8 mg/L, the diosgenin content reached to the maximum (0.37 mg/g dw), which was 1.28-fold of control and showed no significant difference with that of DP4. However, DP10 resulted in an evident enhancement on diosgenin content at all designed concentrations. When the seedlings were treated with DP10 at 6 mg/L, diosgenin content was dramatically increased to the highest (1.64 mg/g dw) which was 6.16-fold of control.

The effects of DP4, DP7, and DP10 on diosgenin yield were shown in Figure 1C, in which DP10 exhibited the most obvious enhancement on diosgenin yield at concentration range from 2 to 10 mg/L. Especially, when the seedlings were treated with DP10 at 6 mg/L, diosgenin yield was increased up to the highest (23.71 mg/L) which was 7.39-fold of control. However, DP4 and DP7 generated slight enhancement even inhibitory effects on diosgenin yield at the test range of concentrations. The maximum values of diosgenin yield treated with DP4 and DP7 were respectively 4.76 mg/L and 5.34 mg/L, which were just 1.48- and 1.66-fold of that of control respectively. Hence, DP10 was considered as the most effective oligosaccharide elicitor to promote diosgenin accumulation in *D. zingiberensis* seedlings.

Optimization of harvest time and elicitation concentration for DP10

As oligosaccharide DP10 exhibited an excellent promoting effect on diosgenin accumulation in *D. zingiberensis* seedlings (Figures 1B and 1C), the harvest time and elicitation concentration for DP10 were further studied. Three concentrations (4, 6, and 8 mg/L) and four harvest days (30, 33, 36, and 39 days) were chosen to conduct the combination experiments, aiming to determine the optimal elicitation conditions of DP10.

As presented in Figure 2A, dry weight of the seedlings in all these elicitation treatments was higher than that of

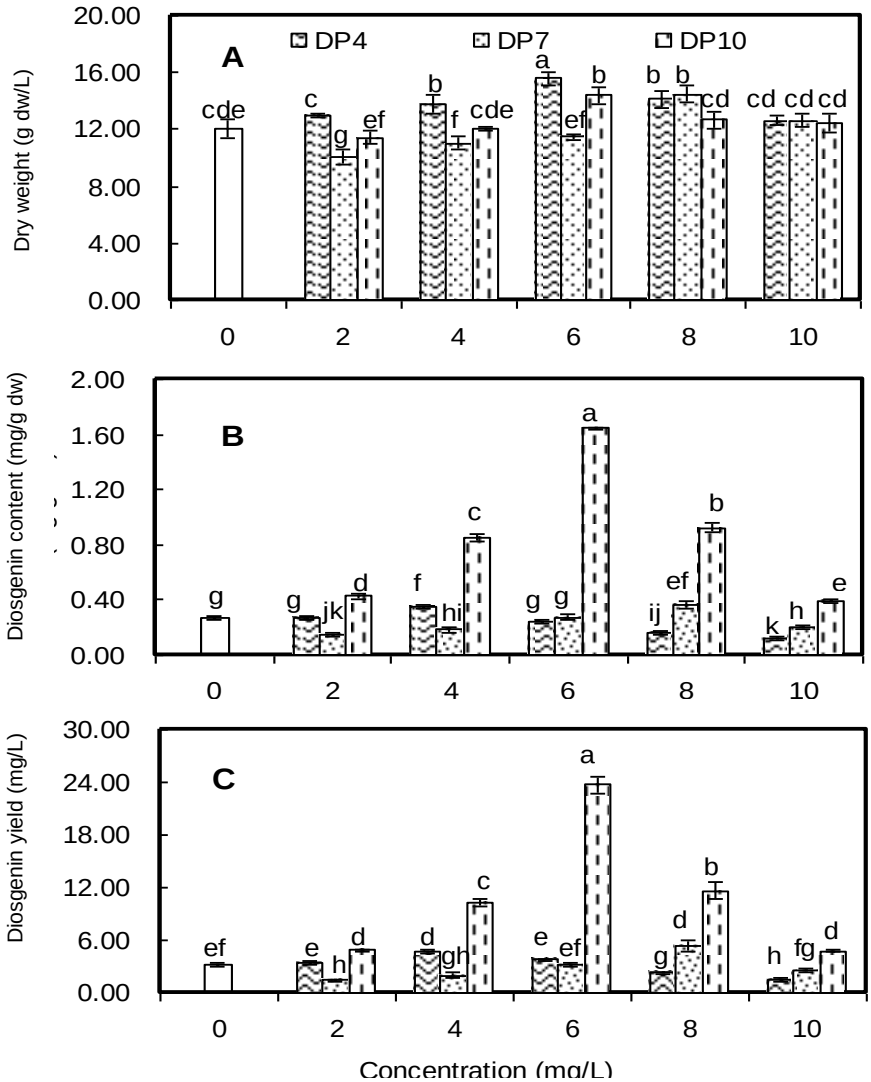

Figure 1. Effects of oligosaccharides DP4, DP8 and DP10 on growth (A), diosgenin content (B) and diosgenin yield (C) of *D. zingiberensis* seedlings. Each value was expressed as mean ± standard deviation (n = 3). Different letters indicated significant differences among the treatments at p = 0.05 level.

control. Dry weight of the seedlings increased with the prolongation of harvest time, and arrived at the maximum (15.34 g dw/L) on the 36th day at concentration of 4 mg/L, which was 1.32-fold of control. The highest dry weight of the seedlings treated at 6 mg/L was still observed on the 36th day, which was 1.29-fold of control but slightly lower than that of 4 mg/L. For the seedlings treated with DP10 at 8 mg/L, the highest dry weight was obtained on the 39th day, which was 1.17-fold of control. So, the optimal concentration to promote growth of the seedling cultures was 4 mg/L or 6 mg/L.

The optimal harvest time of the seedlings treated with DP10 at 6 mg/L was on the 36th day, on which diosgenin content increased to 1.85 mg/g dw which was 7.24-fold of control and higher than (1.64 mg/g dw) on the 32nd day. For the seedlings treated with DP10 at 4 mg/L or 8 mg/L, the highest values of diosgenin content were separately observed on the 36th and 33rd day, which were respectively 4.74-and 3.19-fold of control.

Diosgenin yields in seedling cultures treated with DP10 at 4, 6 or 8 mg/L and harvested on the 30th, 33rd, 36th, and 39th day were presented in Figure 2C. When the seedlings were treated with DP10 at 6 mg/L, the values of diosgenin yield were higher than those of 4 or 8 mg/L. Diosgenin yield in seedling cultures reached maximum (27.69 mg/L) when treated at 6 mg/L and harvested on the 36th day, which was 9.33-fold higher than the control and far higher (7.29-fold) than that on the 32nd day. For the seedlings treated with DP10 at 4 mg/L or 8 mg/L, the harvest time with the highest diosgenin yield was the 36th

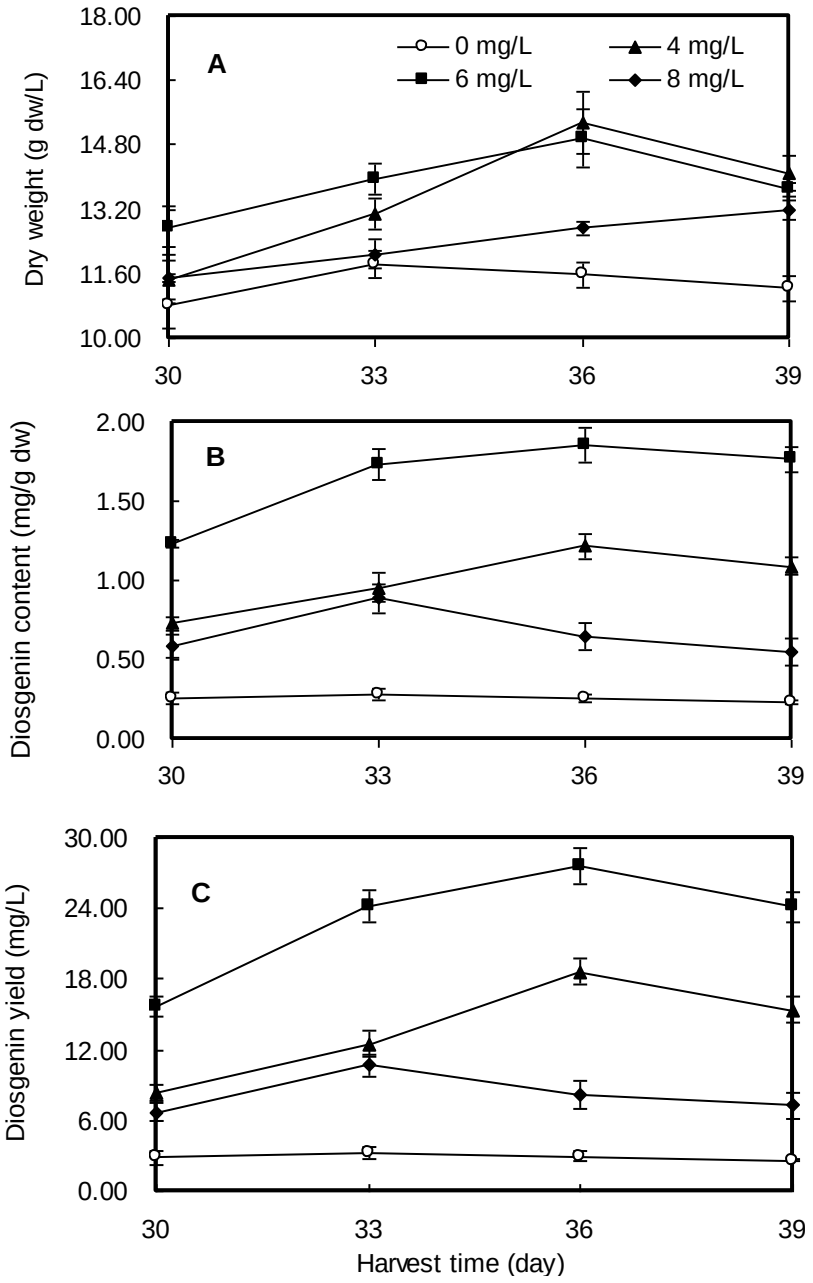

Figure 2. Effects of different concentrations of oligosaccharide DP10 on growth (A), diosgenin content (B), and diosgenin yield (C) of *D. zingiberensis* seedlings with different harvest time. Each value was expressed as mean ± standard deviation (n = 3).

and 33rd days, which were respectively 6.27- and 3.25-fold higher than the control. Therefore, the optimal concentration of DP10 and the optimal harvest time for maximization of diosgenin accumulation in *D. zingiberensis* seedlings were separately determined as at 6 mg/L and on the 36th day.

In conclusion, three oligosaccharide fractions (DP2-5, DP5-8 and DP8-12) were prepared from total oligosaccharide (TO) which was obtained by acid hydrolysis of the water-extracted mycelial polysaccharide (WPS) from endophytic fungus *F. oxysprorum* Dzf17. Three oligosaccharide monomers (DP4, DP7, and DP10) were separately derived from fractions DP2-5, DP5-8, and DP8-12. Among them, DP10 showed the most significant enhancement on diosgenin accumulation. When the seedlings were treated with 6 mg/L of DP10 and harvested on day 36 of culture, both diosgenin content and yield increased to 1.85 mg/g dw and 27.69 mg/L,

which were respectively 7.24- and 9.33-fold higher compared to the control. The results indicated that addition of oligosaccharides from endophytic *F. oxysporum* Dzf17 as elicitors could significantly enhance diosgenin production in *D. zingiberensis* seedling cultures. However, the specific mechanisms of how these oligosaccharides regulate the growth and secondary metabolism are rarely understood. The oligosaccharides prepared from endophytic fungus *F. oxysporum* Dzf17 could be used as elicitors to enhance diosgenin production either in large-scale culture of the seedlings or in field cultivation of *D. zingiberensis* intact plants in the future.

ACKNOWLEDGMENTS

This work was co-financed by the grants from the Hi-Tech R&D Program of China (2011AA10A202), and the National Basic Research Program of China (2010CB126105).

REFERENCES

Backman PA, Sikora RA (2008). Endophytes: an emerging tool for biological control. Biol. Control, 46: 1-3.

Chen Y, Fan J, Luo Z, Fu Y (2003). Rapid clonal propagation of *Dioscorea zingiberensis*. Plant Cell Tiss. Org. Cult., 73: 75-80.

Dicosmo F, Misawa M (1995). Plant cell and tissue culture: alternatives for metabolite production. Biotechnol. Adv., 13: 425-453.

Fernandes P, Cruz A, Angelova B, Pinheiro HM, Cabral JMS (2003). Microbial conversion of steroid compounds: recent developments. Enzyme Microb. Technol., 32: 688-705.

Ganley RJ, Sniezko RA, Newcombe G (2008). Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. For. Ecol. Manag., 255: 2751-2760.

Ge X, Wu J (2005). Tanshinone production and isoprenoid pathways in *Salvia miltiorrhiza* hairy roots induced by Ag^{+} and yeast elicitor. Plant Sci., 168: 487-491.

Ibrahim AK, Khalifa S, Khafagi I, Youssef D, Khan I, Mesbah M (2007). Stimulation of oleandrin production by combined *Agrobacterium tumefaciens* mediated transformation and fungal elicitation in *Nerium oleander* cell cultures. Enzyme Microb. Technol., 41(3): 331-336.

Kane KH (2011). Effects of endophyte infection on drought stress tolerance of *Lolium perenne* accessions from the Mediterranean region. Environ. Exp. Bot., 71: 337-344.

Kang SM, Min JY, Kim YD, Karigar CS, Kim SW, Goo GH, Choi MS (2009). Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. J. Biotechnol., 139: 84-88.

Kieran PM, MacLoughlin PF, Malone DM (1997). Plant cell suspension cultures: some engineering considerations. J. Biotechnol., 59: 39-52.

Li H, Ni J (2011). Treatment of wastewater from *Dioscorea zingiberensis* tubers used for producing steroid hormones in a microbial fuel cell. Bioresour. Technol., 102: 2731-2735.

Li P, Mou Y, Shan T, Xu J, Li Y, Lu S, Zhou L (2011a). Effects of polysaccharide elicitors from endophytic *Fusarium oxysporium* Dzf17 on growth and diosgenin production in cell suspension culture of *Dioscorea zingiberensis*. Molecules, 16: 9003-9016.

Li P, Mao Z, Lou J, Li Y, Mou Y, Lu S, Peng Y, Zhou L (2011b). Enhancement of diosgenin production in *Dioscorea zingiberensis* cell cultures by oligosaccharides from its endophytic fungus *Fusarium oxysporum* Dzf17. Molecules, 16: 10631-10644.

Li YC, Tao WY (2009). Effects of paclitaxel-producing fungal endophytes on growth and paclitaxel formation of *Taxus cuspidata* cells. Plant Growth Regul., 58: 97-105.

Orban N, Boldizsar I, Szucs Z, Danos B (2008). Influence of different elicitors on the synthesis of anthraquinone derivatives in *Rubia tinctorum* L. cell suspension cultures. Dyes Pigments, 77: 249-257.

Savitha BC, Thimmaraju R, Bhagyalakshmi N, Ravishankar GA (2006). Different biotic and abiotic elicitors influence betalain production in hairy root cultures of *Beta vulgaris* in shake-flask and bioreactor. Process Biochem., 41: 50-60.

Schulz B, Boyle C, Draeger S, Rommert AK, Krohn K (2002). Endophytic fungi: A source of novel biologically active secondary metabolites. Mycol. Res., 106: 996-1004.

Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS (2009). Fungal endophytes and bioprospecting. Fungal Biol. Rev., 23: 9-19.

Tang Z, Rao L, Peng G, Zhou M, Shi G, Liang Y (2011). Effects of endophytic fungus and its elicitors on cell status and alkaloid synthesis in cell suspension cultures of *Catharanthus roseus*. J. Med. Plants Res., 5: 2192-2200.

Wang C, Wu J, Mei X (2001a). Enhancement of taxol production and excretion in *Taxus chinensis* cell culture by fungal elicitation and medium renewal. Appl. Microbiol. Biotechnol., 55: 404-410.

Wang J, Zhang Z, Tan R (2001b). Stimulation of artemisinin production in *Artemisia annua* hairy roots by the elicitor from the endophytic *Colletotrichum* sp. Biotechnol. Lett., 23: 857-860.

Wiktorowska E, Długosz M, Janiszowska W (2010). Significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis* L. Enzyme Microb. Technol., 46: 14-20.

Yin C, Li P, Li H, Xu L, Zhao J, Shan T, Zhou L (2011). Enhancement of diosgenin production in *Dioscorea zingiberensis* seedling and cell cultures by beauvericin from the endophytic fungus *Fusarium redolens* Dzf2. J. Med. Plants Res., 5: 6550-6554.

Zhang C, Wang Y, Yang Z, Xu M (2006). Chlorine emission and dechlorination in co-firing coal and the residue from hydrochloric acid hydrolysis of *Discorea zingiberensis*. Fuel, 85: 2034-2040.

Zhang R, Li P, Xu L, Chen Y, Sui P, Zhou L, Li J (2009). Enhancement of diosgenin production in *Dioscorea zingiberensis* cell culture by oligosaccharide elicitor from its endophytic fungus *Fusarium oxysporum* Dzf17. Nat. Prod. Commun., 4: 1459-1462.

Zhang R, Li P, Zhao J, Yin C, Zhou L (2010). Endophytic fungi from *Dioscorea zingiberensis* and their effects on the growth and diosgenin production of the host plant cultures. Nat. Prod. Res. Dev., 22: 11-15.

Zhao J, Shan T, Mou Y, Zhou L (2011). Plant-derived bioactive compounds produced by endophytic fungi. Mini-Rev. Med. Chem., 11: 159-168.

Zhong JJ (2002). Plant cell culture for production of paclitaxel and other taxanes. J. Biosci. Bioeng., 94: 591-599.

Zhou L, Wu J (2006). Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. Nat. Prod. Rep., 23: 789-810.

Zhou L, Zhao J, Shan T, Cai X, Peng Y (2010). Spirobisnaphthalenes from fungi and their biological activities. Mini-Rev. Med. Chem., 10: 977-989.

Zhu YL, Huang W, Ni JR, Liu W, Li H (2010). Production of diosgenin from *Dioscorea zingiberensis* tubers through enzymatic saccharification and microbial transformation. Appl. Microbiol. Biotechnol., 85: 1409-1416.