Efficient production of flavonoids in *Fagopyrum tataricum* hairy root cultures with yeast polysaccharide elicitation and medium renewal process

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ABSTRACT

Background: Tartary buckwheat (*Fagopyrum tataricum)*, an excellent edible and medicinal crop, has been widely used as a daily diet and traditional medicine for a long time. The major functional components of *Fagopyrum tataricum* have been demonstrated to be flavonoids (i.e. rutin and quercetin), which had notable andioxidant, antidiabetic, hypocholesterolemic and antitumor activities. Hairy root culture is a convenient and efficient plant tissue culture system for large scale production of bioactive metabolites. **Objective:** To enhance the functional flavonoids production in hairy root culture of *F. tataricum*. **Materials and Methods:** The elicitation treatment in combination with medium renewal strategy was applied for efficient promoting flavonoids production in *F. tataricum h*airy root cultures. **Results:** The exogenous yeast polysaccharide (YPS) elicitor notably stimulated the functional metabolites production in *F. tataricum* hairy root cultures, and the stimulation effect was concentration-dependent. Combination with the YPS elicitation (200 mg/L) and medium renewal process, the maximal flavonoids yield was enhanced to 47.13 mg/L, about 3.2-fold in comparison with the control culture of 14.88 mg/L. Moreover, this research also revealed the accumulation of these bioactive metabolites resulted from the stimulation of the phenylpropanoid pathway by YPS treatment. These results indicated that the *F. tataricum* hairy root culture could be an effective system for rutin and quercetin production.

Key words: *Fagopyrum tataricum*, flavonoids, hairy root culture, medium renewal, yeast polysaccharide elicitation

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INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) (Polygonaceae), an excellent edible and medicinal crop mainly grown in the mountainous regions of Southwest China, northern India, Bhutan and Nepal, has been widely used as a daily diet and traditional medicine for a long time.[1,2] Tartary buckwheat along with the buckwheat-based products is attracting many researchers' attention because of its high nutrition value and health benefits. It contains proteins with high biological value and balanced amino acid composition, relatively high resistant starch, dietary fiber, vitamins, minerals, trace elements and various bioactive phytochemicals.[3-5] The major functional components of *F. tataricum* have been demonstrated to be flavonoids such as rutin, quercetin, orientin, vitexin and kaempferol. Lots of researches have revealed that these bioactive compounds had notable antioxidant, hypocholesterolemic, antidiabetic, antimicrobial and antitumor activities, and were beneficial for human health.[6-9]

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Hairy root culture is a convenient and efficient plant tissue culture system for plant science and biotechnology research and development. It has the advantage of rapid growth over normal tissue cultures, genetic stability and hormone-free growth over cell suspension cultures. The hairy root cultures of many plant species have been explored for the production of bioactive metabolites useful as pharmaceuticals, cosmetics and food additives.[10-12] The normal *Fagopyrum esculentum* and *F. tataricum* hairy root cultures have been established as a potential means for the production of rutin, qucertin and other bioactive phenolic compounds.[13,14]

Since the biosynthesis of many secondary metabolites in plants is usually a common defense response of plants to biotic and abiotic stresses, their accumulation can be stimulated by biotic and abiotic elicitors. Therefore,

elicitation, treatment of plant tissue cultures with elicitors, is one of the most effective strategies for enhancing secondary metabolites production in plant tissue cultures.[15,16] The most common and effective elicitors used in previous studies mainly include the components of microbial cells, especially poly- and oligosaccharides (biotic), and heavy metal ions, UV radiation, and hyperosmotic stress (abiotic), and the signaling molecules in plant defense responses such as salicylic acid (SA) and methyl jasmonate (MJ).

Yeast polysaccharide (YPS) has been demonstrated to be an efficient biotic elicitor for stimulating secondary metabolite accumulation in plant cell and tissue culture. Production of many valuable bioactive compounds (*i.e.* artemisinin, azadirachtin, β-amyrin, tanshinones) has been successfully stimulated by YPS elicitors.[17–21] To the best of our knowledge, there were no previous reports about the effects of YPS on functional metabolites accumulation in the hairy root culture of *F. tataricum*. Therefore, we carried out a research program to investigate the effects of YPS elicitation, as well as the medium renewal process on *F. tataricum* hairy root growth and flavonoids production in this study. Moreover, the intracellular phenylalanine ammonia lyase (PAL) activity, as well as the medium pH and conductivity of *F. tataricum* hairy root cultures induced by YPS was also examined.

MATERIALS AND METHODS

Hairy root culture

The hairy root culture of *Fagopyrum tataricum* was derived from explants (cultivar Miqiao-01 of *F. tataricum*) infected with a Ri-T-DNA-bearing *Agrobacterium rhizogenes* Ri1601 [Figure 1].[22] Experiments in this study were carried out in shake-flask culture of the hairy roots in 150mL Erlenmeyer flasks placed on an orbital shaker running at 110-120rpm, and at 25°C in the dark. Each flask was filled with 30mL liquid medium and inoculated with 0.3 g fresh hairy roots from three-week-old shake-flask culture. The liquid medium was made of hormone-free MS medium with 30 g/L of sucrose and 0.5 g/L of casein hydrolysate but excluding ammonium nitrate.

Preparation and application of YPS

The yeast extract (YE, Y4250) was purchased from Sigma (St. Louis, MO, USA). The yeast polysaccharide (YPS) was the polysaccharide fraction of YE precipitated by ethanol as described previously.[21] Briefly, YE (20 g) was dissolved in distilled water (100 mL) and then mixed with ethanol (400 mL), and allowed to precipitate for 4 days at 4°C in a refrigerator. The crude polysaccharide fraction was further purified by another round of ethanol precipitation. The final gummy precipitate was dissolved in distilled water (50 mL) and stored at 4°C prior to use. The concentration of YPS was determined by the anthrone test using sucrose as a reference.[23] YPS was applied to the *F. tataricum* hairy root cultures at the following five concentrations (0, 50, 100, 200 and 400 mg/L) on days 10, 15, 20, 25 and 30 of culture, respectively. The hairy root cultures of *F. tataricum* were harvested on day 30 (or on day 33, for YPS addition on day 30) for measurement of their biomass and flavonoids content. After the preliminary experiments, 200 mg/L of YPS was determined to be the most effective elicitation treatment, and it was applied in the next experiments on the time courses of YPS-treated root growth and flavonoids accumulation in *F. tataricum* hairy root cultures. The changes of intracellular PAL activity, medium pH and conductivity in the *F. tataricum* hairy root cultures induced by YPS were also investigated.

Another culture experiment was performed on medium renewal and YPS elicitation treatment. For the single medium renewal process, the spent medium in each culture flask was decanted and replaced with 30 mL fresh MS-N medium. The medium renewal was started on day 25, a few days before the stationary phase, and the cultures were harvested on day 35. In the combined medium renewal and YPS elicitation experiments, the fresh medium was applied to the root cultures on day 25, and the YPS (200 mg/L) was simultaneously added to the cultures for one (on day 25) or two (on days 25 and 30, respectively) times, and the *F. tataricum* hairy roots were harvested on day 35 for measurement.

Measurement of biomass and flavonoid content

The hairy roots were picked out of the culture flasks with a pair of forceps, rinsed thoroughly with distilled water, blotted dry by a paper towel, and then dried at 40-45°C in an oven to attain the constant dry weight (Dw). The dried root samples were ground into powder and then extracted with methanol (20 mg roots/mL) under sonication for 30 min. After removal of the solid, the extract was evaporated to dryness and redissolved in methanol, and applied to high-performance liquid chromatography (HPLC) for the analysis of the rutin and quercetin content. The HPLC system was equipped with two LC-10ATvp pumps and a SPD-M10Avp diode-array detector (Shimadzu, Kyoto, Japan), and using a C_{18} column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA). The separation was performed using a mixture of acetonitrile and distilled water (0.2% H_3PO_4) with a gradient elution: (0-8 min, 20% acetonitrile; 8-13 min, 20-40% acetonitrile; 13-29 min, 40% acetonitrile; 29-30 min, 40-20% acetonitrile; 30-35 min, 20% acetonitrile). The flow rate was set at 1.0 mL/min, and UV detection at 365nm. The temperature of the column was set at 30°C, and the sample injection volume was 20 μL as reported by Zhao *et al.*[24] The rutin and quercetin were detected and quantified with the standards obtained from the Institute for Identification of Pharmaceutical and Biological Products (Beijing, China).

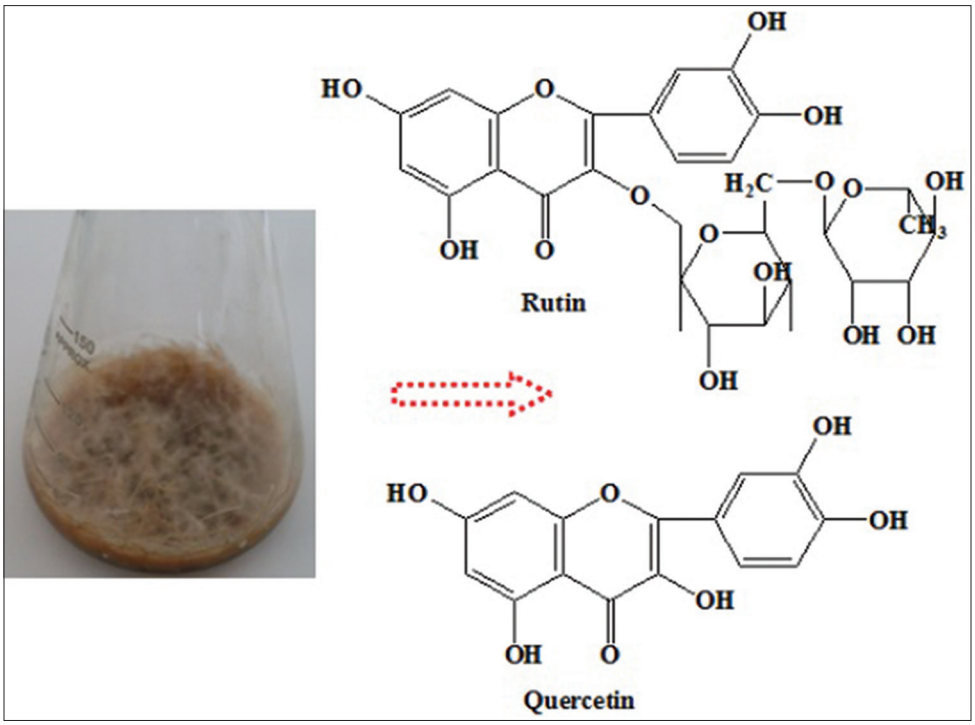

Figure 1: Production of rutin and quercetin in the hairy root culture of *F. tataricum*

Measurement of PAL activity, medium pH, conductivity and sucrose concentration

Phenylalanine ammonia lyase (PAL) was extracted from the *F. tataricum* hairy roots with borate buffer (pH 8.8). The fresh cells were ground in the buffer (0.2 g/mL) for 2 min with a pestle and mortar on ice, and then centrifuged at 8,000 rpm and 4°C for 20 min to obtain a solid-free extract. The PAL activity was determined based on the conversion of L-phenylalanine to cinnamic acid by the method of Wu and Lin.[25] The medium pH and conductivity were measured with the respective electrodes and meters.[21] Sucrose concentration in the liquid medium was determined by the Anthrone test using sucrose as a reference.[23]

Statistical analysis

All treatments were performed in triplicate, and the results were represented by their mean values and the standard deviations (SD). The data were submitted to analysis of variance (one-way ANOVA) to detect significant differences by procedure (PROC) ANOVA of statistics analysis system (SAS) version 8.2. The term significant has been used to denote the differences for which $P \leq 0.05$.

RESULTS AND DISCUSSION

Hairy root growth and flavonoids accumulation of *F. tataricum*

As shown in Figure 2, the time course of *F. tataricum* hairy root growth exhibited a slow growth period in the first 9 days, a rapid linear growth period between days 12-24, and a stationary or declining phase in the later days, reaching the maximum biomass concentration of 9.94 gdw/L around day 27. For the flavonoid accumulation, the total flavonoids (rutin plus quercetin) of hairy roots remained at a very low level from days 1-15, and then increased steadily from days 16-30 to a highest content of 1.50 mg/g [Figure 2a]. The time course of sugar (sucrose) concentration [Figure 2b] was nearly symmetrical to that of hairy root growth, indicating a direct correlation of the hairy root growth to sugar consumption. As the major carbon source, sucrose was essential for the *F. tataricum* hairy root growth, and when it was depleted (around day 27), the hairy root growth stopped and the root biomass concentration began to drop. As seen from Figure 2b, the medium pH showed a notable drop in the first 3 days (due to the consumption of NH_4^+ and release of protons) and a gradual increase after day 6 (due to the consumption of nitrate NO_3^-).[26] These results indicated that day 30 was a suitable time for harvesting *F. tataricum* hairy roots with respect to their high biomass and flavonoids yield.

Effects of YPS on the growth and flavonoids production in *F. tataricum* hairy root cultures

Figure 3 shows the effects of yeast polysaccharide (YPS) on the growth and flavonoids production in hairy root cultures of *F. tataricum*, which were dependent on both YPS dosage and its treatment period. As shown in Figure 3a, compared with the control culture of 9.93 gdw/L, with early treatment, the hairy root biomass decreased by about 2-26% with 50-400 mg/L YPS applied on days 10 and 15, but a slightly

increase with the late treatment (days 20, 25 and 30). With 400 mg/L YPS applied on day 25 of the cultures, the hairy root biomass was increased to 11.25 gdw/L, about 1.1-fold in comparison with the control culture . For the rutin and quercetin accumulation, they were effectively stimulated by the YPS elicitor, most significantly with 200 mg/L of YPS provided on day 25, and the total flavonoids content was 3.13 mg/g, or about 2.1-fold in comparison with the control of 1.49 mg/g [Figure 3b]. Correspondingly, the total flavonoids yield was as high as 34.52 mg/L, about 2.3-fold compared to the control of 14.80 mg/L [Figure 3c]. The typical HPLC profiles of rutin and quercetin standards, control cultures, and YPS treated hairy root samples are shown in Figure 4.

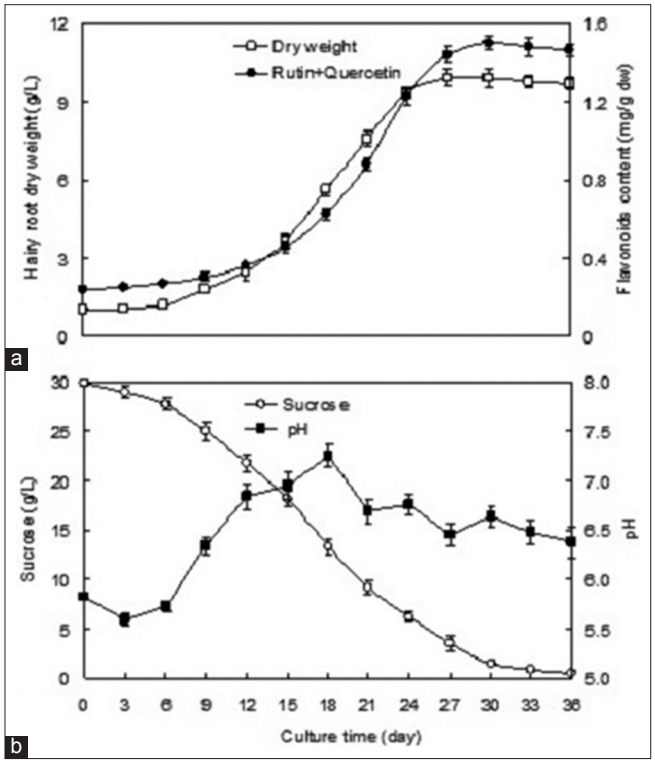

Figure 2: Time courses of biomass and flavonoids content (a), residue sugar (sucrose) and medium pH (b) in the hairy root culture of *F. tataricum*. The error bars represented standard deviations, (n = 3)

Kinetics of *F. tataricum* hairy root growth and flavonoids accumulation after treatment with YPS

According to the previous evaluation, the maximal flavonoids yield was obtained when the hairy root cultures were treated with YPS at 200 mg/L on day 25 [Figure 3]. Therefore, the kinetic studies of biomass growth and flavonoids accumulation in hairy root cultures of *F. tataricum* stimulated by YPS elicitor (200 mg/L) were further investigated, which are shown in Figure 5. The promoting effect of YPS on the hairy growth of *F. tataricum* could be observed after 1 day treatment [Figure 5a] and the maximum root biomass was 11.45 gdw/L obtained on day 28, about 1.2-fold compared to that of the control 9.92 gdw/L. The stimulation effect of YPS on flavonoids accumulation of *F. tataricum* hairy root could be notably noticed after 2 days treatment, and then followed a steady increase to the end of the culture period. The highest flavonoids content of the hairy roots was 3.15 mg/g, about 2.1-fold in comparison with the control of 1.51 mg/g [Figure 5b].

PAL activity, medium pH and conductivity changes of *F. tataricum* hairy root cultures induced by YPS

Figure 6 shows the changes of intracellular PAL activity, medium pH and conductivity in the *F. tataricum* hairy root cultures elicited by YPS. The gradual decrease of medium pH and conductivity with time in the control culture could be attributed mainly to the consumption of nutrients and production of metabolites, *e.g.* the consumption of

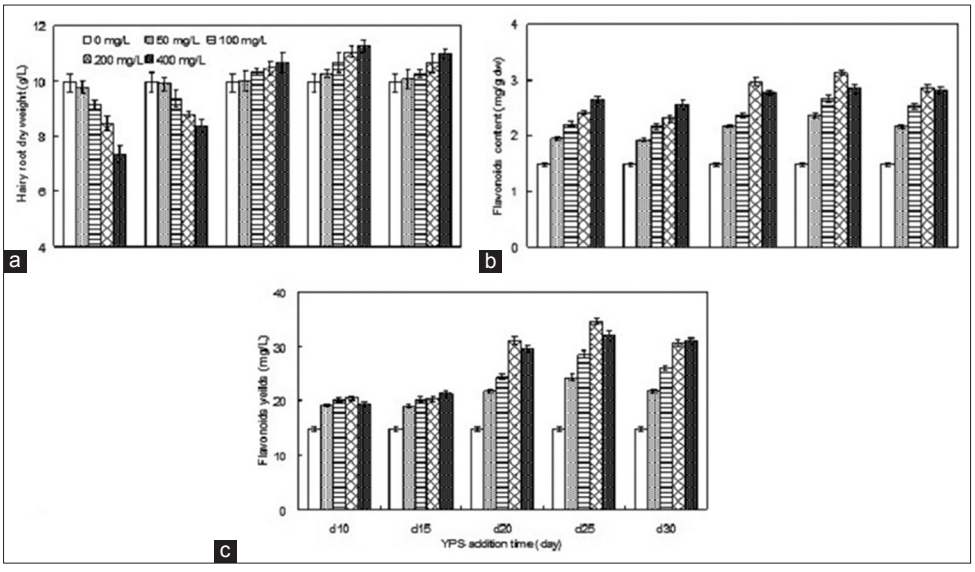

Figure 3: Effects of YPS (0, 50, 100, 200 and 400 mg/L) on the hairy root biomass (a), flavonoids content (b), and flavonoids yield (c) of *F. tataricum* hairy root cultures. The error bars represented standard deviations, (n = 3)

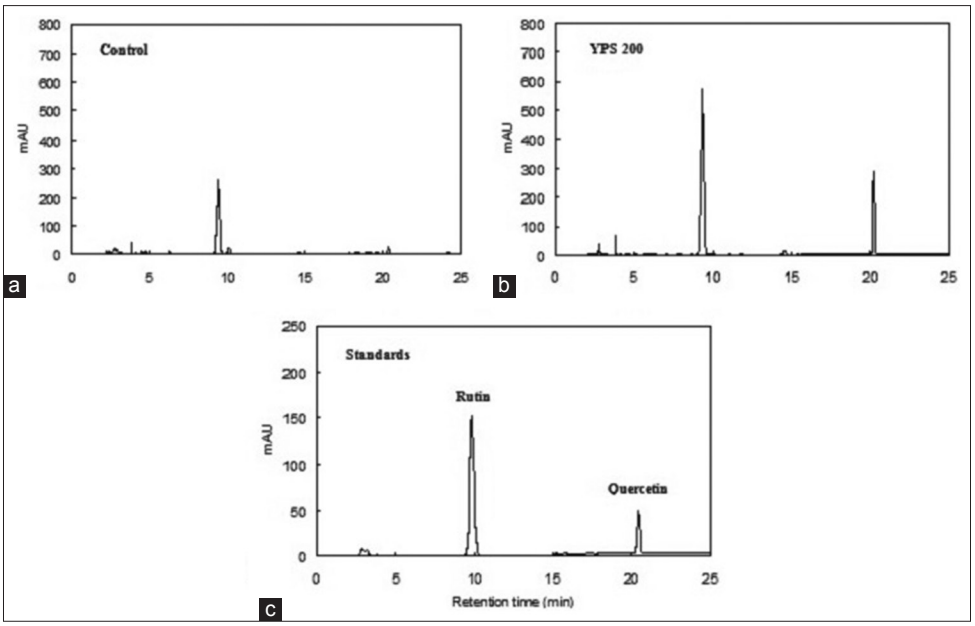

Figure 4: HPLC profiles for flavonoids analysis: Control cultures (a), YPS (200 mg/L) treated hairy roots (b), standards of rutin and quercetin (c)

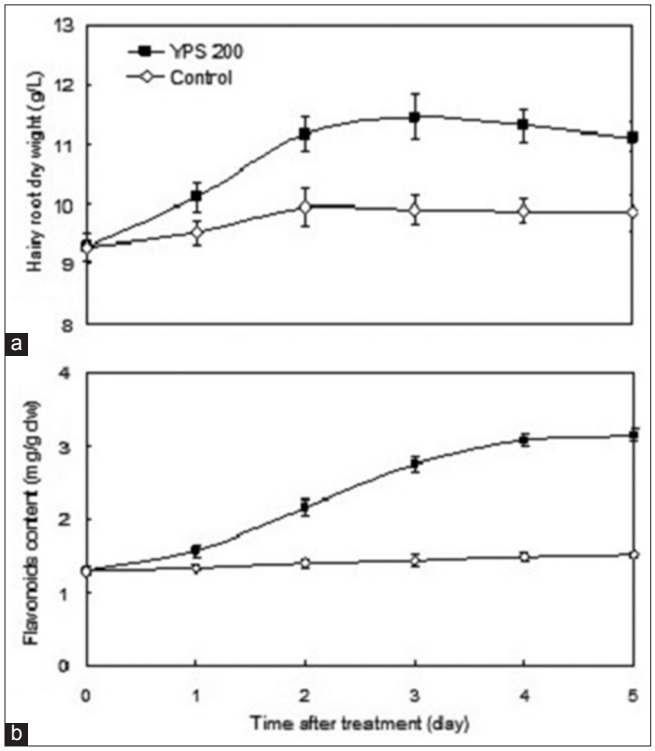

Figure 5: Kinetic studies of biomass growth (a) and flavonoids accumulation (b) of *F. tataricum* hairy root cultures after treatment with 200 mg/L of YPS. YPS was applied to the hairy root cultures on day 25, and the total period of culture was 30 days; The error bars represented standard deviations, (n = 3)

mineral nutrients in the culture medium for the conductivity decrease. Compared with those of the control culture, both the intracellular PAL activity [Figure 6a] and medium conductivity [Figure 6c] were increased, and the pH of culture medium [Figure 6b] was decreased after treatment with YPS (200 mg./L). The notable increase in medium conductivity and the more rapid drop in medium pH in the early treatment period (Days 0-2) after the addition of YPS may be a consequence of increasing membrane K^+ and H^+ effluxes which are well-known early events in the elicitation of plant cell responses.[27] As shown in Figure 6a, the intracellular PAL activity of *F. tataricum* hairy root cells was notably stimulated by the YPS elicitation, from 1.1- to 1.5-fold of the control level over 5 days. PAL is a key enzyme at the entrance step in the phenylpropanoid pathway in plants, and its activity increase stimulated by the elicitors is suggestive of an enhanced secondary metabolism in the plant cells.[28,29] On the basis of these results obtained from this study, it could be speculated that the phenylpropanoid pathway was closely associated with the flavonoid biosynthesis in the hairy root culture of *F. tataricum*. That was concordant with the findings of previous reports.[30,31] Moreover, it also implied that the exogenous yeast polysaccharide (YPS), as an efficient biotic elicitor, may be taken up by receptors on the surface of the root cells or transformed to a stress signal in stimulating the functional metabolites accumulation of *F. tataricum* hairy roots. Anyway, these valuable results provide further evidence for the elicitor activity of YPS in stimulating the stress responses and secondary metabolism of *F. tataricum* hairy root cultures.

Effects of medium renewal and YPS elicitation on biomass growth and flavonoids production in *F. tataricum* hairy root cultures

Table 1 summarizes the effects of medium renewal and YPS elicitation on root biomass growth and flavonoids production in the hairy root culture of *F. tataricum*. In general, the single medium renewal process effectively promoted the hairy root growth but not benefit for

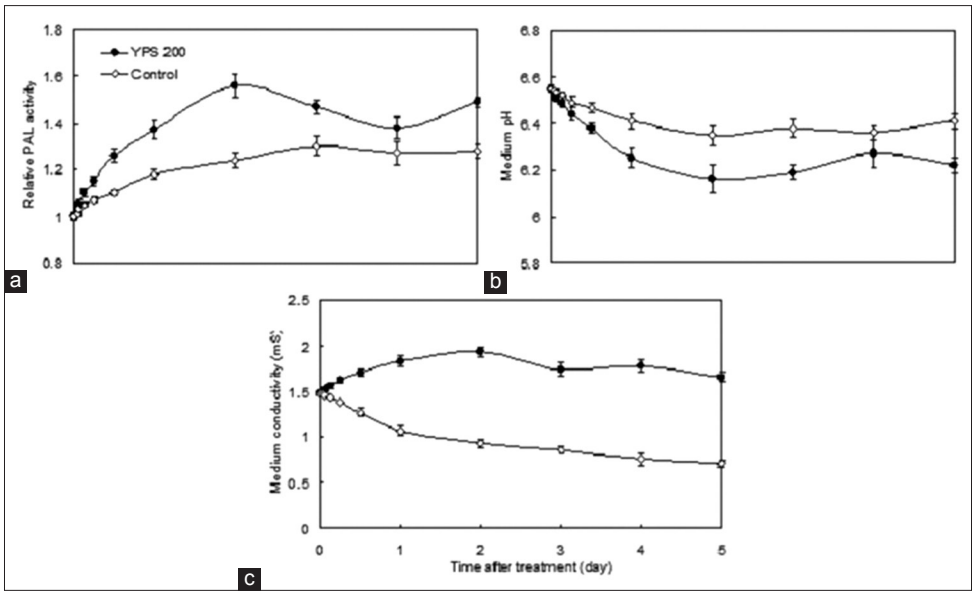

Figure 6: Time courses of PAL activity (a), medium pH (b), and conductivity (c) of *F. tataricum* hairy root cultures after elicitation treatment with 200 mg/L of YPS in comparison with the control. YPS was applied to the hairy root cultures on day 25, and the treatment period was 5 days; The error bars represented standard deviations, (n = 3)

Table 1: Root biomass and flavonoids production in *F. tataricum* hairy root cultures with YPS elicitation, medium renew and their synergistic effects

Treatment	Root biomass (g dw/L)	Rutin content (mg/g dw)	Quercetin content (mg/g dw)	Flavonoids Content (mg/g dw)	Flavonoidsyield (mg/L)
Control	9.92±0.28^{e}	1.48±0.04^{d}	0.02±0.00^{c}	1.50±0.04^{d}	14.88±0.40^{e}
Medium renew on day 25	13.36±0.46^{d}	1.44±0.03^{d}	0.02±0.00^{c}	1.46±0.03^{d}	19.51±0.41^{d}
YPS addition on day 25	11.08±0.32^{c}	3.07±0.04^{b}	0.05±0.01^{a}	3.12±0.05^{b}	34.57±0.40^{c}
Medium renew on day 25+YPS addition on day 25	13.68±0.51^{b}	3.02±0.05^{c}	0.04±0.01^{b}	3.06±0.06^{c}	41.86±0.82^{b}
Medium renew on day 25+YPS addition on day 25+YPS addition on day 30	13.82±0.48^{a}	3.36±0.06^{a}	0.05±0.01^{a}	3.41±0.07^{a}	47.13±0.96^{a}

Note: The values are expressed as means ± standard deviations (n=3); Different letters (i.e., a-e) Indicated significant differences among the data values; YPS: Yeast polysaccharide

the flavonoids accumulation. With medium renewal in *F. tataricum* hairy root cultures on day 25, the root biomass was increased by about 35% in comparison with the control culture of 9.92 gdw/L. The combination of medium renewal and YPS elicitation dramatically enhanced the hairy root growth and flavonoids production. With medium renewal and YPS (treatment on day 25 and harvesting on day 35) elicitation, the hairy root biomass was increased by more than 38% compared to the control (13.68 gdw/L versus 9.92 gdw/L), and the flavonoids production was also notably increased to 41.86 mg/L, that was about 2.8-fold in comparison with the control of 14.88 mg/L. With medium renewal and YPS (feeding on days 25 and 30 respectively, and harvesting on day 35) elicitation treatments, the root biomass concentration was increased to 13.82 gdw/L, about 1.4-fold in comparison with the control (9.92 gdw/L), and the maximal flavonoids yield was effectively enhanced to 47.13 mg/L, about 3.2-fold compared to the control culture of 14.88 mg/L.

CONCLUSION

This is the first report on the effects of yeast polysaccharide elicitation and medium renewal process on the growth and flavonoid accumulation in *F. tataricum* hairy root cultures. Without obvious changes in the appearance of the hairy roots, the YPS effectively stimulated the hairy root growth and flavonoids production of *F. tataricum*, and the stimulation effect was concentration- dependent. With application of 200 mg/L of YPS to the hairy root cultures on day 25, the total rutin and quercentin content was notably increased to 3.13 mg/g, or about 2.1-fold in comparison with the control of 1.49 mg/g. Combination with the YPS elicitation and medium renewal process, the

maximal flavonoids yield was dramatically enhanced to 47.13 mg/L, about 3.2-fold compared to the control of 14.88 mg/L. Moreover, the present study revealed that the accumulation of these bioactive metabolites was caused by the stimulation of the phenylpropanoid pathway by YPS treatment. The results obtained from this study also suggest that the *F. tataricum* hairy root cultures may be an effective system for rutin and quercetin production. As the yeast polysaccharide is commercially available or can be readily prepared and easily administered to the hairy root cultures, it is suitable for practical applications in the laboratory or large-scale production in the future.

REFERENCES

1. Fabjan N, Rode J, Košir IJ, Wang ZH, Zhang Z, Kreft I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. J Agric Food Chem 2003;51:6452-5.
2. Guo XD, Ma YJ, Parry J, Gao JM, Yu LL, Wang M. Phenolics content and antioxidant activity of tartary buckwheat from different locations. Molecules 2011;16:9850-67.
3. Qin PY, Wu L, Yao Y, Ren GX. Changes in phytochemical compositions, antioxidant and α-glucosidase inhibitory activities during the processing of tartary buckwheat tea. Food Res Int 2011;50:562-7.
4. Peng LX, Zou L, Zhao JL, Xiang DB, Zhu P, Zhao G. Response surface modeling and optimization of ultrasound-assisted extraction of three flavonoids from tartary buckwheat (*Fagopyrum tataricum*). Pharmacogn Mag 2013;35:210-5.
5. Ikeda K. Buckwheat: Composition, Chemistry and Processing. Adv Food Nutr Res 2002;44:395-434.
6. Brunetti C, Ferdinando MD, Fini A, Pollastri S, Tattini M. Flavonoids as antioxidants and developmental regulators: Relative significance in plants and humans. Int J Mol Sci 2013;14:3540-55.
7. Zhao G, Zou L, Wang ZG, Hu HL, Hu YB, Peng LX. Pharmacokinetic profile of total quercetin after single oral dose of tartary buckwheat extracts in rats. J Agric Food Chem 2011;59:4435-41.
8. Peng LX, Wang S, Zou L, Zhao JL, Zhao G. HPLC fingerprint of buckwheat from different habitats and varieties. Pharmacogn J 2012;31:5-10.
9. Khadem S, Marles RJ. Chromone and flavonoid alkaloids: Occurrence and bioactivity. Molecules 2012;17:191-206.
10. Zhou LG, Wu JY. Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. Nat Prod Rep 2006;23:789-810.
11. Wang JP, Zhou YM, Zhang YH. Kirenol production in hairy root culture of *Siegesbeckea orientalis* and its antimicrobial activity. Pharmacogn Mag 2012;30:149-55.
12. Srivastava S, Srivastava AK. Hairy root culture for mass production of high-value secondary metabolites. Crit Rev Biotechnol 2007;27:29-43.
13. Lee SY, Cho SI, Park MH, Kim YK, Choi JF, Park SU. Growth and rutin production in hairy root cultures of Buckwheat (*Fagopyrum esculentum* M.). Prep Biochem Biotechnol 2007;37:239-46.
14. Kim YK, Li XH, Xu H, Park NI, Uddin MR, Pyon JY, *et al*. Production of phenolic compounds in hairy root culture of tartary buckwheat (*Fagopyrum tataricum* Gaertn). J Crop Sci Biotech 2009;12:53-8.
15. Ionkova I. Effect of methyl jasmonate on production of ariltetralin lignans in hairy root cultures of *Linum tauricum*. Pharmacogn Res 2009;3:102-5.
16. Smetanska I. Production of secondary metabolites using plant cell cultures. Adv Biochem Eng Biotechnol 2008;111:187-228.
17. Chen H, Chen F. Effect of yeast elicitor on the secondary metabolism of Ti-transformed *Salvia miltiorrhiza* cell suspension cultures. Plant Cell Rep 2000;19:710-7.
18. Putalun W, Luealon W, De-Eknamkul W, Tanaka H. Improvement of artemisinin production by chitosan in hairy root cultures of *Artemisia annua* L. Biotechnol Lett 2007;29:1143-6.
19. Prakash G, Srivastava AK. Statistical elicitor optimization studies for the enhancement of azadirachtin production in bioreactor *Azadirachta indica* cell cultivation. Biochem Eng J 2008;40:218-26.
20. Broeckling CD, Huhman DV, Farag MA, Smith JT, May GD, Mendes P, *et al*. Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. J Exp Bot 2005;56:323-36.
21. Zhao J, Zhou L, Wu J. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. Appl Microbiol Biotechnol 2010;87:137-44.
22. Sun YX, Wang YH, Chen Y, Zhang HY. Study on hairy-roots induction of *Fagopyrum tataricum* Gaertn. Acta Agric Boreali Occident Sin 2012;21:91-4.
23. Leung PH, Zhao SN, Ho KP, Wu JY. Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of *Cordyceps sinensis* fungus Cs-HK1. Food Chem 2009;114:1251-6.
24. Zhao G, Zhao JL, Peng LX, Zou L, Wang JB, Zhong LY, *et al*. Effects of yeast polysaccharide on growth and flavonoid accumulation in *Fagopyrum tataricum* sprout cultures. Molecules 2012;17:11335-45.
25. Wu JY, Lin LD. Ultrasound-induced stress responses of *Panax ginseng* cells: Enzymatic browning and phenolics production. Biotechnol Prog 2002;18:862-6.
26. Morard P, Fulcheri C, Henry M. Kinetics of mineral nutrient uptake by *Saponaria officinalis* L. suspension cell cultures in different media. Plant Cell Rep 1998;18:260-5.
27. Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 2005;23:283-333.
28. Zhao JL, Zhou LG, Wu JY. Promotion of *Salvia miltiorrhiza* hairy root growth and tanshinone production by polysaccharide-protein fractions of plant growth-promoting rhizobacterium *Bacillus cereus*. Process Biochem 2010;45:1517-22.
29. Kim HJ, Park KJ, Lim JH. Metabolomic analysis of phenolic compounds in buckwheat (*Fagopyrum esculentum* M.) sprouts treated with methyl jasmonate. J Agric Food Chem 2011;59:5707-13.
30. Liu JF, Li XY, Meng R. Preliminary studies on the factors for promoting flavonoids production during the germination process of tatary buckwheat. Sci Technol Food Ind 2006;27:106-8.
31. Li XH, Thwe AA, Park NI, Suzuki T, Kim SJ, Park SU. Accumulation of phenylpropanoids and correlated gene expression during the development of tartary buckwheat sprouts. J Agric Food Chem 2012;60:5629-35.

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