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SHORT COMMUNICATION

Effect of organic-inorganic N sources on growth, NPK nutrients and secondary metabolites of *Panax Notoginseng* (Burk.) F. H. Chen

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ABSTRACT

The effects of organic and inorganic N combined application on *P.notoginseng* cultivation are poorly known. For this reason, we have carried out a pot culture experiment in 2010. The treatments respectively were (1) control (no N input, CK), (2) organic N (ON), (3) half organic N and half inorganic N (1/2ON+ 1/2IN), (4) inorganic N (IN). Plant growth, NPK and secondary metabolites concentration were assayed. Results showed that compared to CK, ON increased plant height, N, P concentration and accumulation and total saponins concentration, but reduce total flavonoids concentration. IN increased shoot and root biomass, N concentration and accumulation, K accumulation, but reduce total flavonoids and saponins concentration. 1/2ON+ 1/2IN had the highest hair root length, root diameter, biomass, N concentration and accumulation, K concentration in roots, total saponins concentration, but had the lowest K concentration in shoots, total flavonoids concentration in shoots and roots. Correlation analysis showed total flavonoids concentration was negative correlated with N concentration and accumulation, K concentration in shoots, but was positive correlated K concentration in roots. Total saponins concentration was negative correlated with K concentration in shoots whereas positive in roots, and was negative correlated with total flavonoids concentration. Totally, organic and inorganic N combined application with the ratio of 50/50 is the best way for growth and saponins of *P.notoginseng*.

Keywords: *Panax notoginseng*; Organic and inorganic N; Growth; Nutrients uptake; Secondary metabolites

INTRODUCTION

Nitrogen (N) is one of the main elements required for plant growth and is also essential for the synthesis of amino acid, protein and enzyme, etc. Chemical N fertilizer, as a supplement of nitrogen, has been widely and excessively applied in intensive agriculture system that consequently leads environmental problems and lowly N use efficiency (Ju et al., 2009; Fan et al., 2012), imbalance soil nutrients (Miao et al., 2010), etc. Thus, how to reduce chemical N fertilizer input into agricultural system is a great challenge for us. Organic fertilizer made by crop residual and animal manure, has an advantage of balanced nutrients (Sheikh et al., 2012) that could improve soil organic matter, physiochemical properties, soil microbial activity (Kowaljow et al., 2012; Zhang et al., 2012) as well as crop quality (Onyango et al., 2012), but also has a disadvantage of lowly nutrients concentration that cause largely dosage (Sheikh et al., 2012) and lowly crop production (Onyango et al., 2012). Now the combined application of organic and chemical fertilizers is gradually recognized as a feasible way to address soil fertility decline in some places (Chivenge et al., 2011). For instance, in Kenya maize cultured area, the labor or benefit-costs ratios have not significant difference when organic manures alone is applied or combined with chemical fertilizers, but the combined application has the highest net benefit and improves soil C, N and Ca contents, especially for Ca content improvement (Mucheru-Muna et al., 2007). In addition, soil organic matter is improved by the combined application, thereby to enhance soil DTPA-Zn, Fe and Mn contents (Li et al., 2007).

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Secondary metabolites such as saponins and flavonoids widely existed in plant organs, are playing a significant role in pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides and food additives(Murthy et al., 2014). The number and quantitative variation of plant secondary metabolites are affected by their around growing environment, which including biotic and abiotic factors (Szakiel et al., 2011a; 2011b). The biotic factors contain interactions between plants and their enemies or allies (Szakiel et al., 2011a); and the abiotic factors include temperature, soil fertility, water and light (Szakiel et al., 2011b). The assumptions tested by a number of studies are being approved, reveal that secondary metabolites especially phenolic compounds are stimulated to synthesize under adverse conditions but restrained under good conditions for growth (Liu et al., 2010a; Liu et al., 2010b; Ibrahim et al., 2011). Similarly, several studies have been conducted on the influence of environmental factors (soil fertility) on saponins concentrations, but the relevance of spaonins metabolism and growing conditions are not consistently (Müller et al., 2013; Vijay et al., 2009; Zhu et al., 2009a; 2009b). Müller et al. (2013) showed that leaf centelloside concentration in *C.asiatica* is negatively correlated with herb and leaf yield as well as leaf N concentration, and the respective sapogenins concentrations are negatively correlated with K concentrations. *P. dodecandra* with application of manures would boost the growth and berry yield rather than increase the saponins concentration in the tropical South Africa and Madagascar (Ndamba et al., 1996). These results are consistent with the hypotheses of the CNB and GDB. Nevertheless, couples of results are not in accordance with these hypotheses completely. For instance, the shatavarins concentration in *A. racemosus* root with respectively N 80 $mg\cdot kg^{-1}$, P 160 $mg\cdot kg^{-1}$ and K 160 $mg\cdot kg^{-1}$ of soil application are 1.66, 1.87 and 1.75-fold higher than with no fertilizer, but the tuber with highly N application is not boosted (Vijay et al., 2009). Similarly, the biomass and saikosaponin concentration of *B. chinese* root with medium N and P fertilizers separately application increase significantly compared to those with no N and P application; the highest saikosaponin concentration is gained at medium N and P level combined application, but is reduced with excessively application (Zhu et al., 2009a; 2009b). So, more similarly researches should be carried out to reveal the relationship between saponins metabolism and growing environment.

Panax notoginseng (Burk.) F. H. Chen, a traditional Chinese medicinal plant named Sanqi or Tienchi, with an over 1,000 years domestication history, has been listed as a dietary supplement by US Dietary Supplement Health and Education Act (Xia et al., 2014). The main bioactive compounds of *P.notoginseng* are dammarane-type saponins (Wang et al., 2012), flavonoids and polysaccharides (Kim, 2012). Over 85% total production of *P.notoginseng* is gained from Wenshan Prefecture of Yunnan Province, China (Szakiel et al., 2011b). However, according to a field survey, the content of organic matter, total nitrogen, and total phosphorus in the soil after *P.notoginseng* planting is higher than that in soils without planting, especially for nitrogen and potassium available concentration (Xia et al., 2016). Thus, nutrients in *P.notoginseng* planting soil is seriously imbalanced. Unfortunately, only a few researches have been carried on how to reduce fertilizer input in *P.notoginseng* cultivation, but the results are not so persuasive (Xia et al., 2016). Most studies on *P.notoginseng* mainly focus on phytochemistry (Wang et al., 2016), pharmacy (Uzayisenga et al., 2014) and quality assessment (Toh et al., 2010; Li et al., 2013; Yao et al., 2011; Liu et al., 2011). Few studies have been carried out on fertilization how to influence secondary metabolism of *P.notoginseng*. Therefore, the present study is to examine the effect of organic and inorganic N resources on plant growth of *P. notoginseng* and secondary metabolites. For this objective, we measured the growth characteristics, concentration of N, P, K, total flavonoids and saponins of *P. notoginseng* under pot culture with different nitrogen treatments.

MATERIALS AND METHODS

Materials

Experiment was conducted on in 2010, at the Experimental Farm of Yanshan, Sanqi Research Institute of Wenshan, Yunnan, China, by whom provided 1-yr old seedlings with similar growth. Fresh red loam soil with pH, organic matter, available N, P (P_2O_5) and K (K_2O) respectively were 5.44, 18.2 $g\cdot kg^{-1}$, 49.59 $mg\cdot kg^{-1}$, 3.00 $mg\cdot kg^{-1}$, 52.90 $mg\cdot kg^{-1}$, had been used for pot culture. Organic N resource, which was rapeseed residue after oil extraction, was fermented and the $N\text{-}P_2O_5\text{-}K_2O$ concentration was 5.67%-2.00%-1.39% (Dried base). Inorganic N resource was ammonium nitrate (34% nitrogen). Others are calcium-magnesium phosphate fertilizer (14% P_2O_5), potassium sulfate (K_2O 50%) and microelements with B, Fe, Mn, Cu, Zn, Mo.

Experimental design

The experiment was laid out as a randomized complete block design with the pots containing 18 kg soil replicated five times. The fertilization regimes were: (1) control (no N input, CK), (2) organic N resource(ON), (3)half organic N resource and half inorganic N resource(1/2ON+ 1/2IN), (4) inorganic N resource(IN). The amount of N for all N treatments (without CK) was 0.2 $g\cdot kg^{-1}$ soil. For each treatment, calcium-magnesium phosphate fertilizer and potassium sulfate consider to a supplement of P and K, were applied according with the amount of P_2O_5

0.15 g·kg^{-1} soil and K_2O 0.30 g·kg^{-1} soil, respectively. Each pot was added 5 mL micronutrient solution with H_3BO_3 2.86g/L, $MnCl_2·2H_2O$ 1.81g/L, $ZnSO_4·7H_2O$ 0.22 g/L, $CuSO_4·5H_2O$ 0.08g/L, $Na_2MoO_4·2H_2O$ 0.025g/L, Na_2EDTA-Fe 2.78 g/L. Total organic N resource, P_2O_5, K_2O and micronutrients as basal fertilizer were applied once, 40% of total inorganic N resource was basal applied, and 60% was equally top-dressed on April 8, July 1 and October 1. Each pot was planted eight 1-yr old seedlings of *P.notoginseng* with similar growth on January 6. Others were same with the field management.

Plant sampling

On December 28, 2010, plants in each pot were all harvested and washed by tap water, and then the plant height, leaf length and width, hair root length, middle of root diameter were determined, respectively. After that, all plants were divided into shoots and roots, dried, each part was weighed as the biomass, then grinded for reserving, respectively.

N, P, K concentration measurement

Plant samples were dried at 70°C and then digested in sulfuric acid (H_2SO_4), the concentration of N was determined by Kjeldahl method; the concentration of P was determined by Mo-Sb colorimetry; the concentration of K was determined by flame atomic absorption spectrophotometry.

Total flavonoids concentration measurement

Plant samples were extracted in 70% ethanol by sonication for 30 min and the total flavonoids content was determined by colorimetric assay (Liu et al., 2010b).

Saponins concentration measurement

Plant samples were extracted with methanol (MeOH) by sonication for 30 min and the saponins contents were determined by HPLC (Xia et al., 2016). Agilent 1100 series HPLC apparatus (Agilent, USA) was used for saponins contents determination. A Shim-pack PREP-ODS(H). Kit column(250 mm × 4.6 mm, 5 μm) was used at 25 °C. A binary gradient elution system consisted of water (A) and acetonitrile (B) and separation was achieved using the following gradient program: 0~12 min, 18% B, 12~35 min, 18%~38% B, 35~45 min, 38% B. The flow-rate was 1.0 mL·min^{-1} and sample injection volume was 20 μL and monitored at 203 nm.

Statistical analysis

The datas were evaluated by one-way analysis of variance with treatment differences among means being tested at $P<0.05$ with LSD (Least-significant difference) multiple range test. Pearson correlation was done to determine correlations among variables.

RESULTS

Plant growth

Results showed ON and 1/2ON+1/2IN with the plant height of 11.3 cm and 10.7 cm, were significantly higher than CK and IN with 9.4cm and 9.5cm. However, leaf length was significantly ($p<0.05$) reduced after N application, but not affected by different N resources. There was no significant difference with leaf width among different treatments. 1/2ON+1/2IN with the longest hair root length of 12.5cm, was obviously longer than ON (11.5cm), IN(11.1cm) and CK(11.6cm), but the difference between ON and IN was not statistically significance. Root diameter was significantly increased after N application, and 1/2ON+1/2IN attained the biggest diameter of 21.1 mm, but no significant difference was observed between ON and IN (Fig. 1).

Compared to CK, ON could not increase the biomass of shoots and roots, whereas IN and 1/2ON+1/2IN had a significant increment of shoots and roots biomass with 24.5% and 18.4%, 34.3% and 23.3%, respectively. IN got a 9.3% and 18.6% higher biomass of shoots and roots than ON, and the difference of shoot biomass between IN and ON was statistically different ($p<0.05$) (Fig. 2).

N, P, K concentration and accumulation

Compared to CK, ON, 1/2ON+1/2IN and IN increased N concentration respectively by 36.4%, 55.4% and 50.0% in shoots, 37.7%, 73.3% and 47.5% in roots. Meanwhile, N concentration in shoots and roots with combined application of organic and inorganic N resources was significantly higher than separated application, was 13.9% and 25.8% higher than ON, was 3.6% and 17.5% higher than IN, respectively. Organic N resource applied alone could remarkably increase P concentration both in shoots and roots by 28.8% and 20.1%, but inorganic N resource separated or combined with organic N resource application could not increase P concentration comparing with CK. There was no significant difference with K concentration in shoots among CK, ON and IN, which respectively were 12.0%, 11.8% and 12.0% significantly higher than 1/2ON+1/2IN. However, the roots K concentration of 1/2ON+1/2IN increased by 17.4%, 4.1% and 15.0% compared to CK, ON and IN, respectively. In addition, K concentration in roots of ON was significantly higher than CK, but IN was not significant difference with CK (Fig. 3AB).

Fig. 3CD showed that all N application treatments could increase N accumulation compared to CK, the increment of ON, 1/2ON+1/2IN and IN in shoot respectively were 49.6%, 93.5% and 82.8%, in root respectively were 57.6%, 98.9% and 74.6%. Meanwhile, combined application

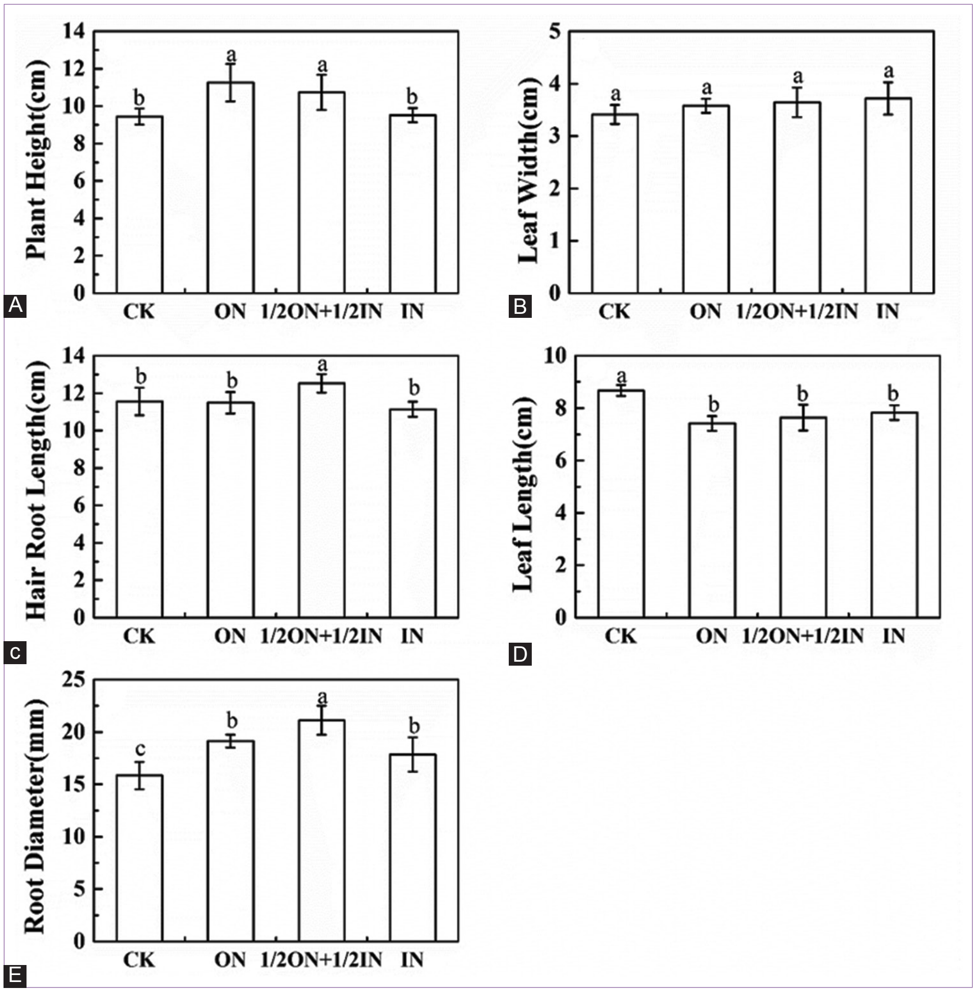

Fig 1. Effect of organic-inorganic N resource on the growth of P. notoginseng CK control; ON total organic N resource; 1/2ON+1/2IN half organic N and half inorganic N; IN total inorganic N; (A) plant height; (B) leaf length;(C) leaf width; (D) hair root length; (E) root diameter; Small letters in different bars mean significant difference at p<0.05.

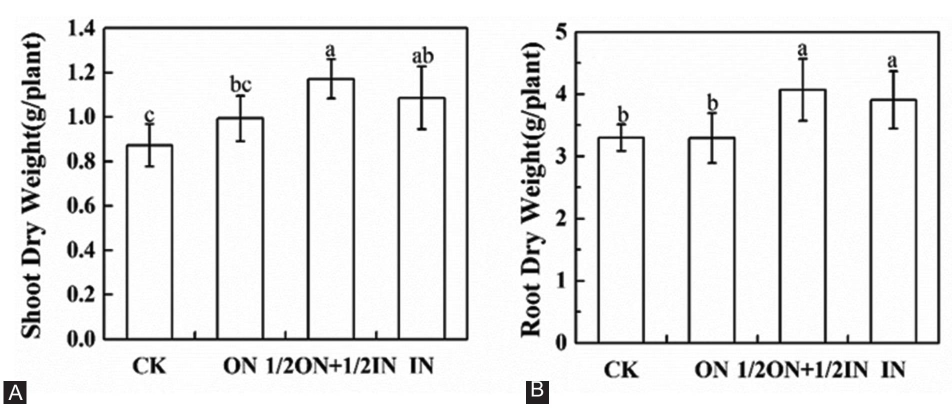

Fig 2. Effect of organic-inorganic N resource on the biomass of P. notoginsengv CK control; ON total organic N resource; 1/2ON+1/2IN half organic N and half inorganic N; IN total inorganic N; (A) shoot dry weight; (B) root dry weight. Small letters in different bars mean significant difference at p<0.05.

organic and inorganic N resources had a significant higher N accumulation than separated application. In addition, N accumulation between ON and IN was not significantly different, though IN was higher than ON. Compared to CK, organic N alone or combined with inorganic N application significantly increased P accumulation by 22.5% and 15.3% in shoots, and by 27.0% and 22.8% in roots, respectively. However, inorganic N resource separated

Table 1: Correlation analysis between nutrients concentration and secondary metabolites

Variables	SN	SP	SK	RN	RP	RK
SF	−0.665**	0.052	0.792**	−0.706**	0.227	−0.588**
RF	−0.816**	−0.067	0.651**	−0.875**	−0.038	−0.723**
R_1	0.505*	0.321	0.135	0.390	0.340	0.220
R_{g1}	−0.062	−0.070	−0.661**	0.160	−0.044	0.311
R_{b1}	0.384	−0.016	−0.822**	0.477*	−0.160	0.551*
R_d	0.476*	0.072	−0.828**	0.578**	−0.012	0.666**
Total saponins	0.290	−0.004	−0.845**	0.445	−0.077	0.551*

SN nitrogen concentration in shoot, SP phosphorus concentration in shoot, SK potassium concentration in shoot, RN nitrogen in root, RP phosphorus concentration in root, RK potassium concentration in root, SF total flavonoids concentration in shoot, RF total flavonoids concentration in root, R_1 concentration of notoginsenoside R_1 in root, R_{g1} concentration of ginsenoside R_{g1} in root, R_{b1} concentration of ginsenoside R_{b1} in root, R_d concentration of ginsenoside R_d in root, Total saponins sum of notoginsenoside R_1+ginsenoside R_{g1}+R_{b1}+R_d. "*" and "**" mean correlated significantly and highly significantly (p<0.05 and p<0.01)

Table 2: Correlation analysis between nutrients accumulation and secondary metabolites

Variables	SAN	SAP	SAK	RAN	RAP	RAK
SF	−0.663**	−0.373	−0.134	−0.748**	−0.461*	−0.829**
RF	−0.755**	−0.424	−0.220	−0.842**	−0.585**	−0.693**
R_1	0.459*	0.404	0.403	−0.397	0.321	−0.165
R_{g1}	−0.050	0.274	−0.479*	0.066	0.382	0.310
R_{b1}	0.357	0.300	−0.111	0.438	0.299	0.600**
R_d	0.453	0.477*	−0.067	0.534*	0.531*	0.620**
Total saponins	0.275	0.388	−0.249	0.376	0.438	0.536*

SAN nitrogen accumulation in shoot, SAP phosphorus accumulation in shoot, SAK potassium accumulation in shoot, RAN nitrogen accumulation in root, RAP phosphorus accumulation in root, RAK potassium accumulation in root. "*" and "**" mean correlated significantly and highly significantly (p<0.05 and p<0.01)

Table 3: Correlation analysis between total flavonoids and saponins

Variables	R_1	R_{g1}	R_{b1}	R_d	Total saponins
SF	−0.073	−0.442	−0.811**	−0.835**	−0.759**
RF	−0.413	−0.291	−0.648**	−0.789**	−0.627**

"*" and "**" mean correlated significantly and highly significantly (p<0.05 and p<0.01)

application had little effects on P accumulation compared to CK, which had a significant decreasing compared to organic N resource separated application. The difference of K accumulation was not significant between CK and ON, of which were significantly lower than IN and 1/2ON+1/2IN. Moreover, the highest K accumulation in shoots was gained with IN, whereas in roots was gained with 1/2ON+1/2IN.

Secondary metabolites concentration

Fig. 4 AB showed total flavonoids concentration in shoots and roots respectively were 3.96% and 0.88% when no N application, but N application significantly decreased it. In addition, combined application organic and inorganic N resources had the lowest concentration with 2.47% in shoots and 0.56% in roots. Moreover, the difference of total flavonoids concentration with separated application organic or inorganic N resource was not significant, but ON and IN was 0.45% and 0.32% lower than CK in shoots while was lower 0.21% and 0.14% in roots.

Figure 4C indicated that N application had a positive effect on notoginsenoside R_1, while separated application organic N resource attained the highest concentration with 0.47%, which was 0.12% higher than CK, 0.06% higher than 1/2ON+1/2IN, 0.03% higher than ON. Compared to CK with the ginsenoside R_{g1} concentration of 3.13%, separated application inorganic N resource significantly decreased it by 0.35%, while organic N separated or combined with inorganic N resource application had a concentration of 3.04% and 3.30%, but were not significantly different with CK. Separated application organic or inorganic N resource had little effects on concentration of ginsenoside R_{b1} compared to CK, while combined application would significantly increase it by 0.56%. Organic N separated or combined with inorganic N application respectively had a 0.08% and 0.19% higher concentration of ginsenoside R_d than CK, while separated application inorganic N resource would slightly but not significantly decrease it. In addition, total four saponins concentration reached the highest with 6.70% when combined application organic and inorganic N resources, which was 0.97% higher than CK. However, separated application inorganic N would decrease the total four saponins concentration by 0.39% compared to CK.

Correlation analysis

Total flavonoids concentration in shoots(SF) and in roots(RF) were strongly negative correlated with N concentration in shoots (r=-0.665 and -0.816, p<0.01) and in roots(r=-0.706 and -0.875, p<0.01), were significantly positive correlated with K concentration in shoots (r=0.792 and 0.651, p<0.01) while were negative correlated in roots (r=-0.588 and -0.723, p<0.01), but had no correlation with P concentration. In addition, a significant positive correlation was found between N concentration in shoots and notoginsenoside R_1 and ginsenoside R_d concentration(r=0.505 and 0.476, p<0.05), and between N concentration in roots and ginsenoside R_{b1} concentration(r=0.477, p<0.05). Furthermore, K concentration in shoots negatively correlated with

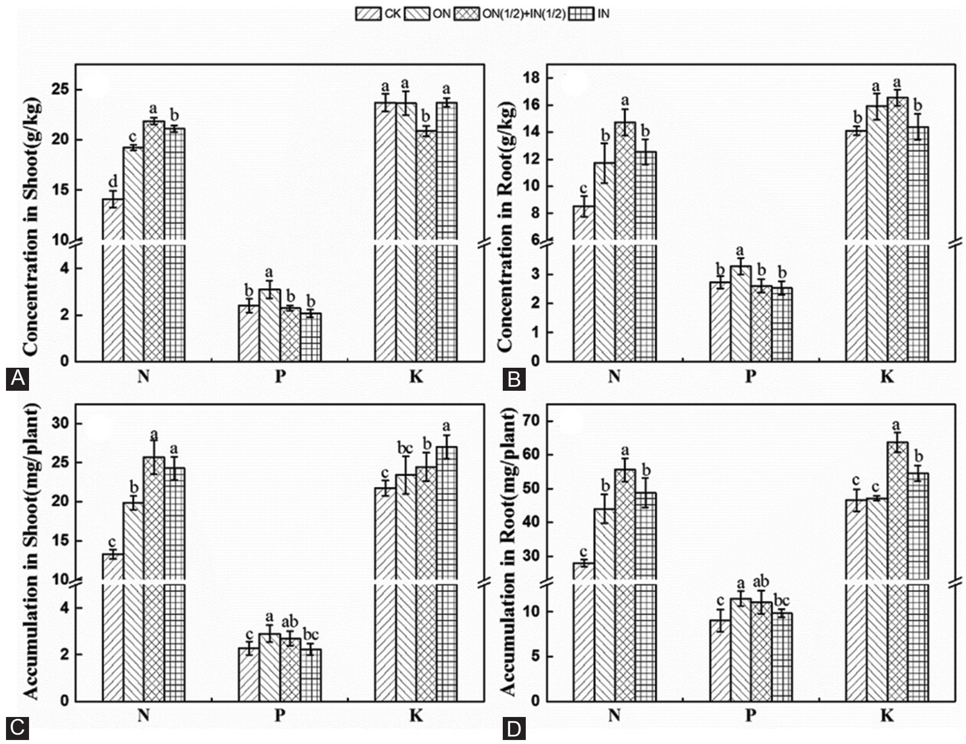

Fig 3. Effect of organic-inorganic N resource on NPK uptake and accumulation of P. notoginseng (A) N, P, K concentration in shoot; (B) N, P, K concentration in root; (C) N, P, K accumulated in shoot; (D) N, P, K accumulated in root. Small letters in different bars mean significant difference at p<0.05.

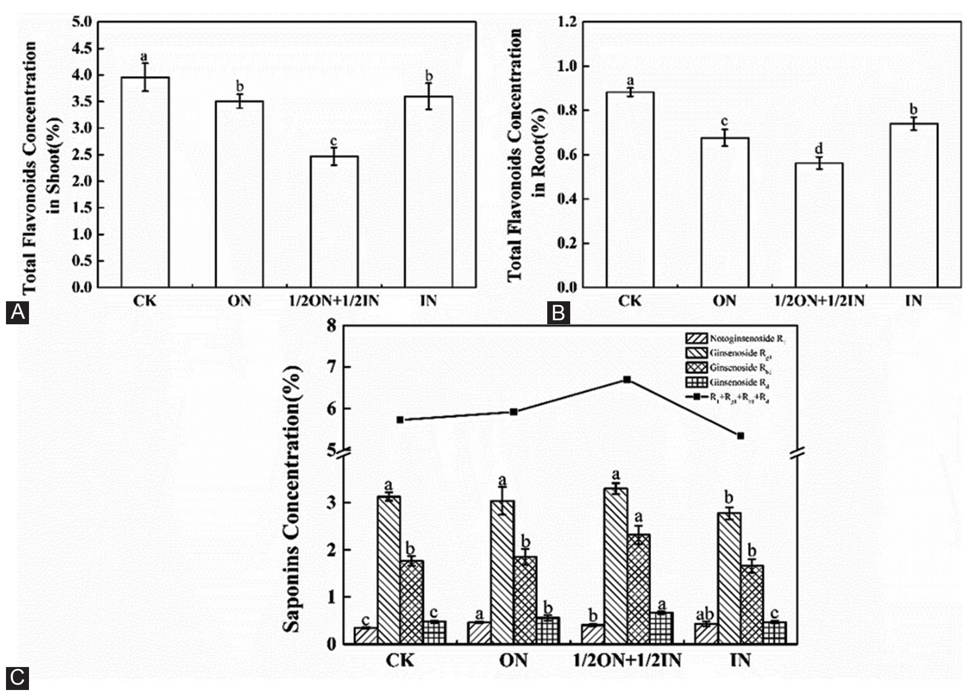

Fig 4. Effect of organic-inorganic N resource on the secondary metabolites of P. notoginseng CK control; ON total organic N resource; 1/2ON+1/2IN half organic N and half inorganic N; IN total inorganic N; (A) total flavonoids concentration in shoot; (B) total flavonoids concentration in root; (C) saponins concentration in root. Small letters in different bars mean significant difference at p<0.05.

ginsenoside R_{g1}, R_{b1}, R_d and total four saponins concentration (r=-0.661,-0.882,-0.828 and -0.845, respectively, p<0.01), but K concentration in root positively correlated with ginsenoside R_{b1} concentration and total four saponins concentration(r=0.666, p<0.01). Finally, P concentration was not correlated with saponins concentration (Table 1).

Similarly, total flavonoids concentration was negatively correlated with N accumulation (r=-0.663 and -0.748 in shoots, -0.755 and -0.842 in roots, p<0.01), with P accumulation in roots (r=-0.461, p<0.05, -0.585, p<0.01), with K accumulation in roots (r=-0.829 and -0.693, p<0.01), but was not correlated with P and K accumulation in shoots. For saponins concentration, N accumulation in shoots only correlated with notoginsenoside R_1 concentration (r=0.459, p<0.05), P accumulation in shoots only correlated with ginsenoside R_d concentration (r=0.477, p<0.05), and K accumulation in shoots only correlated with ginsenoside R_{g1} concentration (r=-0.479, p<0.05). Nevertheless, N and P accumulation in roots were only correlated with ginsenoside R_d concentration (r=0.534 and 0.531, p<0.05), K accumulation in roots was correlated with ginsenoside R_{b1} and R_d as well as total four saponins concentrations (r=0.600 and 0.620, p<0.01; 0.536, p<0.05) (Table 2).

Correlation analysis also indicated that total flavonoids concentration had no correlation with notoginsenoside R_1 and ginsenoside R_{g1} concentration, but negatively correlated with ginsenoside R_{g1}, R_d and total four saponins concentration (p<0.01) (Table 3).

DISCUSSION

Organic and inorganic N on the growth of *P.notoginseng*

Sileshi et al.(2011) consider that N application could improve the plant height of *C. annum*, and this plant receiving either organic fertilizer alone or treating with combined application of organic and chemical fertilizer has significantly higher stem, leaf and fruit yield than that with no N application, but fruit yield with chemical fertilizer alone is lower than with organic fertilizer alone. Chivenge et al. (2011) demonstrate that maize yield is positively correlated with N application, i.e. maize yield with the addition of organic fertilizer(ORs), or chemical fertilizer (CN) or ORs+ CN, respectively increases by 60%, 80% and 114% compared with no N application. Maize yield with sole ORs is lower than with sole CN. Compared with sole application of ORs or CN, the combined application of ORs+ CN increases maize yield by 33% or 17% respectively. So, all the results indicate that N application is essential to improve crop growth and yield, but the effects vary with crops. Combined application of organic and inorganic fertilizer results in a higher crop production than separately application, mainly because of the direct interactions between the two resources that temporary immobilization of N from fertilizers by ORs may result in improving synchrony between supply and demand of nutrients (Palm et al., 2001), thereby the use efficiency of the two N resources are enhanced (Vanlauwe et al., 2001). In addition, combined application of organic and inorganic fertilizer might alleviate other growth limiting factors such as micronutrients (Palm et al., 1997), because combined application has a positive effect on enhancing micronutrients concentration in soil (Li et al., 2007). In the present study, combined application of organic and inorganic N resource leads to a higher N concentration and accumulation than separate application (Fig. 3), and that could supply more available N for plant growth and biomass accumulation of *P.notoginseng* (Fig. 1,2). Organic fertilizer application reduces P sorption by soil (Palm et al., 1997) and then enhances P available in soil (Li et al., 2007), which might improve P absorption of *P.notoginseng* (Fig. 3). But relative low biomass leads by low N concentration (Figure 2,3), also leads application organic N alone have less P accumulation than combined application organic and inorganic N resource(Fig. 4). Besides, application of inorganic N resource alone has a higher concentration and accumulation of N in shoots and roots than application organic N resource alone (Fig. 3). So application inorganic N resource alone leads to a higher biomass than application of organic N resource alone (Fig. 2). This result is similar to Chievnge et al.(2011), but different with Sileshi et al. (2011). This difference might be attributed to higher organic carbon in soil as a long term application of organic fertilizer, so inorganic fertilizer application might have little effects on improving crop yield (Bhattacharyya et al., 2007; Bi et al., 2009). All in all, combined application organic and inorganic N resource is more effective to improve the growth and biomass of *P.notoginseng*.

Organic and inorganic N on flavonoids concentration of P.notoginseng

Many studies show that flavonoids concentration in plants is negatively correlated with N concentration in the growing environment (Liu et al., 2010a; Liu et al., 2010b; Ibrahim et al., 2011), which is support the carbon/nutrient balance and the growth differentiation balance hypothesis. In nitrogen deficient plants, the activity of phenyl alanine lyase (PAL) which is a key compound in flavonoids synthesis pathway, is positively correlated with carbon-base compounds (Zhang & Liu, 2015; Xu et al., 2014), is always higher than that in nitrogen enough plants and has more polyphenolic compounds to synthesis flavonoids (Kováčik & Bačkor, 2007). Therefore, plants with nitrogen deficiency will increase flavonoids concentration (Liu et al.,2010a; Liu et al., 2010b; Ibrahim et al., 2011). In our study, the results also indicate that N fertilizer increases N concentration in *P.notoginseng* but decreases total flavonoids concentration (Fig. 4), and there is a significant negative correlation between total flavonoids concentration and N concentration and accumulation in *P.notoginseng* (Table 1,2). The results support the hypothesis of CNB and GDB and other findings (Liu et al., 2010a; Liu et al., 2010b; Ibrahim et al., 2011). Total flavonoids concentration is positive correlation with K concentration in shoots, but with negative correlation in roots (Table 1). The reasons for that are :1) potassium is an activator for many enzymes especially for Rubisco, so more K might be distributed to the shoots of *P.notoginseng* to active some enzymes which could alleviate the negative influence caused by N deficiency (Fig. 3); 2) *P.notoginseng* is a root plant that needs more potassium to boost root, so combined application organic and inorganic N resource has higher K concentration in roots but lower K concentration in shoots (Fig. 3). Therefore, organic and inorganic N resources influence flavonoids concentration of *P.notoginseng* by N and K uptake.

Organic and inorganic N on saponins concentration of P.notoginseng

N application will improve saponins content in each part of *C.asiatica*, and combined application of organic and inorganic fertilizer also will result in a higher madecassoside and asiaticoside concentration than organic or inorganic fertilizer application alone (Siddiqui et al., 2011). Our study also suggests *P.notoginseng* of combined application organic and inorganic N has higher saponins concentration than that with application organic or of inorganic N alone (Fig. 4C). Saponins are mainly bioactive compounds in *Panax* plants, belong to pentacyclic triterpenoids, are synthesized by isoprenoid pathway (Szakiel et al., 2011b) which is affected by mineral nutrients (Müller et al., 2013; Ndamba et al., 1996; Vijay et al., 2009; Zhu et al., 2009a; 2009b). Up to now, which step is regulated by mineral nutrients in saponins synthesis pathway is still unclearly (Müller et al., 2013). Several researches demonstrate that saponins synthesis support the carbon/nutrient balance and the growth differentiation balance hypothesis (Müller et al., 2013; Ndamba et al., 1996). However, several results suggest N application is beneficial to saponins metabolism, but excessive N inhibits saponins synthesis (Vijay et al., 2009; Zhu et al., 2009a; 2009b). Our results demonstrate that N, P can not influence saponins synthesis, but K in shoots has a negative effect on total saponins concentration whereas K in roots has a positive effects (Tables 1, 2). In addition, flavonoids concentration is negative correlated with saponins concentration (Table 3), that might be caused by the competition between flavonoids and saponins synthesis. Thus, we suggest that N application could indirectly affect saponins synthesis by influencing K absorption and distribution. Totally, combined application organic and inorganic N resource is beneficial for improving saponins concentration of *P.notoginseng*.

CONCLUSION

Organic N resource application can increase saponins concentration but has little effects on growth. Inorganic N resource application can improve plant growth but decrease saponins concentration. Combined application of organic and inorganic N resources can both improve plant growth and saponins concentration. In addition, flavonoids concentration is negative correlated with N concentration, and N application indirectly influence saponins concentration. Therefore, we suggest that organic and inorganic N combined application with the ratio of 50/50 is the best way for growth and saponins of *P.notoginseng*.

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Contributions

Ou Xiaohong performed the experiments and wrote the manuscript; Yang Ye and Guo Lanping revised the manuscript; Liu Dahui and Zhu Duanwei convinced and designed the experiments.

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