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RESEARCH ARTICLE

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Effects of different concentrations of nitrogen and phosphorus on chlorophyll biosynthesis, chlorophyll a fluorescence, and photosynthesis in *Larix olgensis* seedlings

Abstract In our experiments, one-year-old *Larix olgensis* seedlings were cultivated in sand, and supplied with solutions with different concentrations of nitrate or phosphate. The effects of nitrogen and phosphorus supply on chlorophyll biosynthesis, total nitrogen content, and photosynthetic rate were studied. The experimental results are listed below: 1) 5-aminolevulinic acid (ALA) synthetic rate increased as nitrate concentrations supplied to larch seedlings increased from 1 to 8 mmol/L. But the rate decreased by 17% when nitrate concentration increased to 16 mmol/L, in contrast to the control. Under phosphate treatments, ALA synthetic rates were similar to those under nitrate treatments. The activities of porphobilinogen (PBG) synthase reached a maximum when larch seedlings were supplied with 8 mmol/L of nitrate or 1 mmol/L of phosphate. 2) When larch seedlings were supplied with 8 mmol/L of nitrate and 0.5 mmol/L of phosphate, the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids reached a maximum. The total nitrogen contents in leaves increased as nitrate concentrations increased. 3) When phosphate concentrations increased from 0.125 to 1 mmol/L, the total nitrogen contents in leaves slightly increased; however, continuous increase of phosphate concentrations resulted in the decrease in total nitrogen contents in leaves. When nitrate concentrations increased from 1 to 8 mmol/L, soluble protein contents in leaves increased in general, and continuous increase of nitrate concentrations induced a decrease in soluble protein contents in leaves. Under treatment of 0.25 mmol/L of phosphate, the soluble protein contents reached a maximum. 4) In general, F_v/F_m increased as nitrate concentrations increased from 1 to 8 mmol/L, and continuous increase of nitrate concentration resulted in decrease in F_v/F_m. The similar changes occurred under phosphate treatments. As nitrate concentrations increased from 1 to 8 mmol/L, photosynthetic rates gradually increased, but when nitrate concentrations increased to 16 mmol/L, photosynthetic rate reduced by 16%, in contrast to the control. Photosynthetic rates reached a maximum when seedlings were supplied with 1 mmol/L, and an oversupply of phosphate (2 mmol/L) resulted in decrease in photosynthetic rates. The results suggested that supply levels of nitrogen affected ALA biosynthetic rates, activities of PBG synthase, and affected contents of chlorophyll and carotenoids. Moreover, nitrogen supply levels affected contents of total nitrogen and soluble proteins in leaves, and net photosynthetic rates. ALA biosynthesis rates and activities of PBG synthase were affected by phosphate supply, but contents of chlorophyll and carotenoids were not affected. And net photosynthetic rates were affected little by phosphate supply.

Keywords *Larix olgensis*, nitrogen, phosphorus, chlorophyll biosynthesis, photosynthetic rate

1 Introduction

Nitrogen is one of the essential macro elements needed by plants, and has a greater limiting effect on plant productivity than any other elements (Vitousek and Howarth, 1991; Cassman et al., 1993; Crawford and Glass, 1998). In boreal forest systems, some plants acquire organic nitrogen from soils (Näsholm, 1998), but trees prefer inorganic nitrogen forms (e.g., nitrate and exchangeable

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ammonium) because trees have developed very consummate mechanisms to acquire and assimilate inorganic nitrogen forms (Martins-Loução, 2000). All around the world, the inorganic nitrogen forms available for plants and microbes in soils account for 1% of total nitrogen or less (Foth and Ellis, 1997). Plants and microbes also deplete the available nitrogen. In addition, nitrate is prone to leach, and thus the available nitrogen in soils becomes less. In natural conditions, nitrogen available for plants in soils in terrestrial ecosystems originates from the rock weathering, bacteria nitrogen fixation, decomposition of organic matters, and falls of atmospheric nitrogen (Chapin et al., 2002). In some forest systems, sometimes nitrogen falls are high (Ohrui and Mitchell, 1997; Mitchell et al., 1997), which has some effects on photosynthesis, tree growth, and nutrition statuses (Nakaji et al., 2001), but complementarities of available nitrogen mainly come from mineralization of organic matters (Chapin et al., 2002). In northeastern forest ecosystems in China, temperature is the main factor affecting mineralization (Zhang et al., 1999). Mineralization is slow because of about half-year winter; available nitrogen cannot be complemented in the soils, resulting in low concentrations of available nitrogen. Therefore, even though there is a great amount of litter on the surface of the soils, the main forest trees in the forest regions are under deficiency of available nitrogen (Zhang et al., 1999). Much is known about the great effects of nitrogen deficiency on plant growth and photosynthesis, but little is known about its effect on chlorophyll biosynthesis.

Phosphorus is also one of the essential macro elements needed by plants. Even though there is a great amount of phosphate in the lithosphere, phosphate available for plants in soils is very deficient. In soil solutions, the concentrations of available phosphate are usually 0–8 μmol/L. Even if the soils are supplied with phosphate fertilizers, most phosphate is fixated by the soils (Barrow, 1980; Marschner, 1995; Holford, 1997), becoming unavailable phosphate for plants. And more, available phosphate diffuses very slowly in soils, less than 4–5 magnitude orders than nitrate, thus forming the depletion zones around roots (i.e., in the rhizospheres) (Poirier and Bucher, 2002). Therefore, plants are often under the deficiency of available phosphate. Phosphate deficiency has great effects on photosynthesis (Lewise et al., 1994; Warren et al., 2002), growth (de Groot et al., 2001), and biomass partitioning (Wu et al., 2003). But little is known about its effect on chlorophyll biosynthesis.

Larxi olgensis is one of main forest trees used for industry in northeastern China, and the effects of deficiency of available nitrogen and phosphate on productivity of the tree species are great. In the present paper, the effects of nitrogen and phosphate deficiency on chlorophyll biosynthesis, nitrogen contents in the leaves, and photosynthesis in *L. olgensis* seedlings were studied, aiming at increasing productivity in *L. olgensis* plantations or mixed plantations of *L. olgensis* and *Fraxinus mandshurica*.

2 Materials and methods

2.1 Materials and culture

Material culture of one-year-old healthy *L. olgensis* seedlings with the same height and diameter of stem caudex were chosen as experimental materials. The seedlings were planted in plastic pots with a diameter of 30 cm and height of 27 cm. The plastic pots contained sand soaked with HCl solution for few days and then washed with water before, 4 seedlings per pot. The pots with seedlings were transported to a greenhouse. The seedlings were supplied with nutrition solution: NH_4NO_3 8 mmol/L, KH_2PO_4 1 mmol/L, KCl 1 mmol/L, $CaCl_2 \cdot 6H_2O$ 1 mmol/L, $MgSO_4 \cdot 7H_2O$ 0.6 mmol/L, $FeCl_3 \cdot 6H_2O$ 0.02 mmol/L, $MnCl_2 \cdot 4H_2O$ 6 μmol/L, H_3BO_3 0.016 mmol/L, $ZnCl_2$ 0.3 μmol/L, $CuCl_2 \cdot 2H_2O$ 0.3μmol/L, $NaMoO_4 \cdot 2H_2O$ 0.3 μmol/L. If necessary, pH was regulated to 5.5–6.0 with $Ca(OH)_2$ or H_2SO_4 solution. After a month, the seedlings were treated with NH_4NO_3 or KH_2PO_4 concentration gradient. NH_4NO_3 concentration gradient was below: 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L (8.0 mmol/L as a control), and KH_2PO_4 concentration gradient was below: 0.125, 0.25, 0.5, 1.0, and 2 mmol/L (1.0 mmol/L as a control). When the seedlings were treated with NH_4NO_3 or KH_2PO_4 concentration gradient, the concentrations of other nutrients were not changed. The seedlings were supplied with nutrition solutions every two days (100 mL per pot once, AM 8:00–9:00). When seedlings were not supplied with nutrition solutions, they was supplied with water twice (at AM 8:00–9:00 and PM 5:00–6:00), 200 mL per pot once. The temperature in the greenhouse was 30 and 18°C day/night, relative humidity was above 80%, and day length was 14 h/day. After treatment of two months, some determination was carried out and samples were made.

2.2 Experimental methods

2.2.1Chemical analyses

Chlorophyll was extracted with dimethyl-fromamide solution. After centrifugation, the spectrophotometric analyses were carried out as the methods of Wellburn (1994). The biosynthesis rates of δ -aminolevulinic acid (ALA) and activities of porphobilinogen (PBG) synthase were determined as the method of Wu et al. (2003). Total nitrogen contents in leaves of the seedlings were determined with the Kjeldahl method (Horneck and Miller, 1998), and the content of solvable proteins was determined by Bradford (1976).

2.2.2 Gas exchange and chlorophyll fluorescence determination

Gas exchange was determined between AM 9:00–11:00, using CI-301PS photosynthesis analyzer (CID Ltd, USA), and at the same time, chlorophyll fluorescence parameters were determined using FMS fluorescence analyzer (Hansatech Instruments Ltd, UK) (Wu et al., 2003). Other conditions were below: temperature 33%–36°C PPFD 1,800 μmol/(m^2·s), relative humidity 55%–65%. After determination of gas exchange and chlorophyll fluorescence, the leaves were cut off the seedlings for analyses of biosynthesis rates of ALA, soluble protein contents, and chlorophyll contents. Some leaves were treated with liquid nitrogen and stored under –80°C for analyses of activities of PBG synthase, and other leaves were dried under 80°C and then were used for analyses of total nitrogen contents.

2.2.3 Statistical analyses

All chemical analyses in the experiments were repeated thrice, and the determinations of gas exchange and chlorophyll fluorescence were repeated 10 times. The statistical analyses carried out by SPSS software (v. 10.0, SPSS Inc, Chicago, Illinois, USA).

3 Results

3.1 Changes in chlorophyll biosynthesis rates and its contents under different nitrogen or phosphorus concentration treatments

As the concentrations of ammonium nitrate increased from 1 to 8 mmol/L, the biosynthesis rates of ALA increased. But when NH_4NO_3 increased to 16 mmol/L, the rate decreased by 17% (Fig. 1A), not significant (p>0.05, LSD). The effects of phosphate treatment on ALA biosynthesis rates were similar to the effects of nitrate treatment. When phosphate concentration increased to 2 mmol/L, ALA biosynthesis rate also decreased (not significant, p>0.05, LSD). Under the treatment of nitrate and phosphate, changes in PBG synthase activities were irregular, but activities of PBG synthase also reached the peak when biosynthesis rates of ALA reached (the concentrations of nitrate and phosphate were 8 and 1 mmol/L, respectively). When nitrate concentration was 16 mmol/L, the activity of PBG synthase decreased by 17% (Fig. 1C), but the decrease was not significant (p>0.05, LSD). And when phosphate concentration was 2 mmol/L, the activity of PBG synthase also decreased by 16% (Fig. 1D), and the decrease was also not significant (p>0.05, LSD).

As for changes in chlorophyll concentrations and carotenoids, concentrations of Chl a, Chl b, and carotenoids reached the peaks when nitrate concentration was 8 mmol/L, but the ratios of Chl a to Chl b reached the peak value when nitrate concentration was 4 mmol/L (Table 1). When seedlings were treated with phosphate concentration gradient, the contents of Chl a, Chl b, total Chl, and carotenoids reached the peak values at phosphate concentration of 1 mmol/L. And the ratio of Chl a to Chl b reached the peak value at phosphate concentration of 0.5 mmol/L (Table 1).

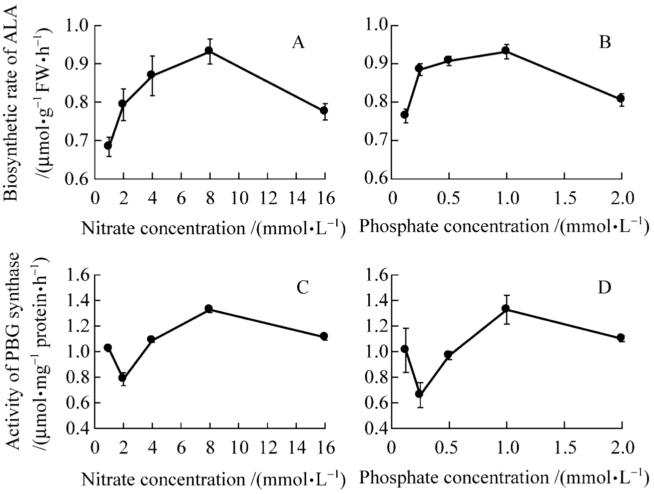

Fig. 1 Effects of different nitrate and phosphate concentration treatment on biosynthesis rates of ALA (A and B) and activity of PBG synthase (C and D) in leaves of seedlings (mean±s.d., n=3)

3.2 Changes in total leaf nitrogen contents and soluble proteins

The total nitrogen in the leaves on the seedlings under the treatment of nitrate increased as the nitrate concentrates increase from 1 to 16 mmol/L, reaching the peak value (18.9 mg/g DW) at the nitrate concentration of 16 mmol/L (Fig. 2A). As phosphate concentrations increased from 0.125 to 1 mmol/L, total nitrogen in the leaves on the seedlings treated with phosphate slightly increased, but decreased by 9% at the phosphate concentration of 2 mmol/L (Fig. 2B). When nitrate concentrations increased from 1 to 8 mmol/L, soluble protein concentrations gradually increased, but decreased by 17% at nitrate concentration of 16 mmol/L (Fig. 2C). Under the treatment of phosphate, soluble protein concentration in leaves reached the peak value, and then decreased (Fig. 2D).

3.3 Changes in fluorescence parameters and net photosynthetic rates

F_v/F_m indicates the maximal photon production, and is the parameter that shows an intrinsic rate in PSII (i.e., photon efficiency when all PSII reaction centers open) (Maxwell and Johnson, 2000). In general, F_v/F_m increased as nitrate concentrations increased from 1 to 8 mmol/L, but decreased

Table 1 Changes of chlorophyll and carotenoids in leaves of seedlings supplied with different nitrate or phosphate concentrations (unit: mg/g FW)*

Treatment		Chl a	Chl b	Total chlorophyll	Chl a/b	Carotenoids
Nitrate /(mmol·L^{-1})	1	0.140,2 a	0.048,2 a	0.188,4 a	2.888,3 a	0.034,6 a
		(±0. 027,4)	(±0.006,4)	(±0.033,7)	(±0.207,2)	(±0.004,2)
	2	0.119,8 a	0.039,6 a	0.159,4 a	3.039,4 a	0.028,7 a
		(±0.029,2)	(±0.010,3)	(±0.039,2)	(±0.196,9)	(±0.006,2)
	4	0.130,8 a	0.042,3 a	0.173,1 a	3.103,6 a	0.032,3 a
		(±0.047,3)	(±0.016,0)	(±0.063,3)	(±0.085,4)	(±0.009,1)
	8	0.202,3 b	0.071,7 b	0.274,0 b	2.831,2 a	0.045,5 b
		(±0.008,8)	(±0.007,1)	(±0.015,8)	(±0.172,5)	(±0.004,8)
	16	0.173,6 a	0.058,2 a	0.231,8 a	2.997,3 a	0.041,0 b
		(±0.025,7)	(±0.010,2)	(±0.035,7)	(±0.162,0)	(±0.006,7)
Phosphate /(mmol·L^{-1})	0.125	0.151,4 a	0.053,2 a	0.204,6 a	2.854,8 a	0.034,7 a
		(±0.016,2)	(±0.007,0)	(±0.023,1)	(±0.101,8)	(±0.004,3)
	0.25	0.194,7 a	0.069,5 a	0.264,2 a	2.897,9 a	0.046,1 a
		(±0.066,1)	(±0.030,1)	(±0.096,0)	(±0.342,3)	(±0.014,2)
	0.50	0.135,2 a	0.044,4 a	0.179,7 a	3.061,8 a	0.033,3 a
		(±0.022,4)	(±0.009,4)	(±0.031,7)	(±0.161,9)	(±0.005,3)
	1.00	0.202,3 a	0.071,7 a	0.274,0 a	2.831,2 a	0.045,5 a
		(±0.008,8)	(±0.007,1)	(±0.015,8)	(±0.172,5)	(±0.004,8)
	2.00	0.149,3 a	0.050,6 a	0.199,9 a	2.991,9 a	0.035,8 a
		(±0.018,1)	(±0.010,2)	(±0.027,6)	(±0.337,9)	(±0.005,0)

* For the same treatment, the same letters in a list indicate insignificant differences (p>0.05, LSD)

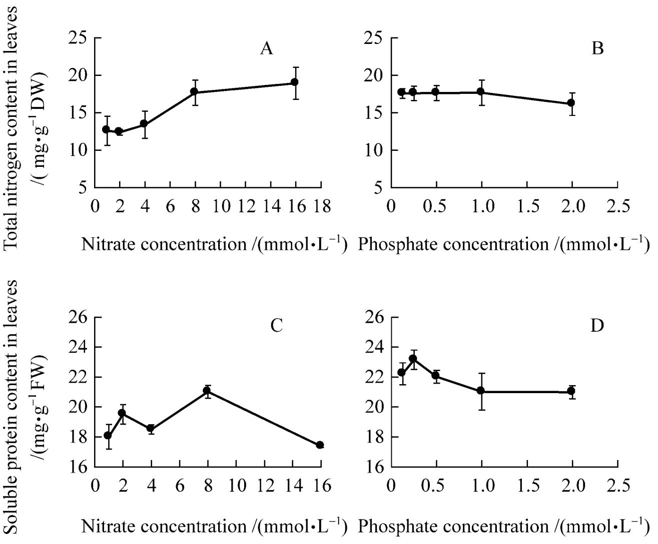

Fig. 2 Effects of different nitrate and phosphate concentration treatment on total nitrogen content (A and B) and soluble protein content (C and D) in leaves of larch seedlings (mean±s.d., n=3)

at the concentration of 16 mmol/L (Fig. 3A). F_v/F_m reached the peak value at the phosphate concentration of 1.0 mmol/L (Fig. 3B). When the seedlings were treated with nitrate, net photosynthetic rates gradually increased as nitrate concentration increased from 1 to 8 mmol/L, but decreased by 16% at nitrate concentration of 16 mmol/L (Fig. 3C). Under treatment of phosphate, net photosynthetic rates reached the peak value at a phosphate concentration of 1 mmol/L (Fig. 3D), but decreased the lowest value at a phosphate concentration of 2 mmol/L (Fig. 3D), suggesting that high phosphate concentrations induced decrease in net photosynthesis rates.

4 Discussions

Nitrogen and phosphorus are two nutrients needed by plants, and their supply to plants directly affects some physiological processes involved in growth and development, especially photosynthesis. The first step in photosynthesis is light capture by plant leaves, and it is carried out by photosynthetic pigments (such as Chl a, Chl b, and carotenoids). Thus, the biosynthesis of photosynthetic pigments is of importance to light capture and photosynthesis. In the chlorophyll biosynthesis, ALA is the first committed precursor. In plants and cyanobacteria, ALA is produced from glutamate in a series of reactions involving

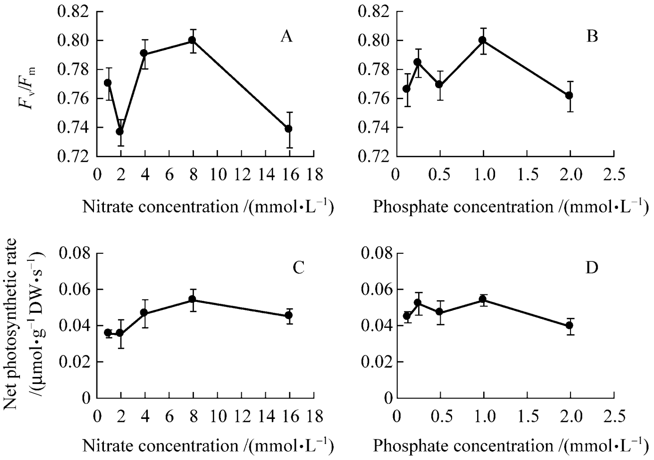

Fig. 3 Effects of different nitrate and phosphate concentration treatment on a fluorescent parameter (A and B) and photosynthetic rates (C and D) in leaves of larch seedlings (mean±s.d., *n*=10)

tRNA$^{\text{Glu}}$. Two molecules of ALA react to yield PBG, and PBG synthase is the important enzyme in charge of the conversion of ALA to PBG (Smith and Griffiths, 1993; Malkin and Niyogi, 2000). Four molecules of PBG form the ring structure of protoporphyrin IX, the common branch point for the synthesis of heme and chlorophyll. Therefore, the biosynthesis rates of ALA and activities of PBG synthase may affect chlorophyll biosynthesis (Masuda et al., 1996). When *L. olgensis* seedlings were supplied with 8 mmol/L of nitrate, contents of Chl a, Chl b, total chlorophyll, and carotenoids reached their peak values, but oversupply made their contents reduced (Table 1), and at the same time, the biosynthesis rates of ALA and activities of PBG synthase also reached the maximal values at the nitrate concentration of 8 mmol/L, suggesting that the effects of the biosynthesis rates of ALA and activities of PBG synthase on contents of Chl a, Chl b, and carotenoids were direct. In linear reactions, the key enzymes have a strong control over product formation (Small and Kacser, 1993a). Therefore, the results in the present experiments were in accordance with the flux control theory (Small and Kacser, 1993a, 1993b). But under the treatment of phosphate, even though the biosynthesis rates of ALA and activities of PBG synthase reached their maximal values at the phosphate concentration of 1 mmol/L, the contents of Chl a, Chl b, total chlorophyll, and carotenoids did not reach their peak values at the same time, suggesting that the effects of phosphate supply levels on chlorophyll biosynthesis were less than nitrogen supply levels.

Under the treatment of nitrate, the total nitrogen contents in leaves increased as nitrogen supply levels increased (Fig. 2A), but the contents of soluble proteins in leaves only reached their peak values at the nitrate concentration of 8 mmol/L (Fig. 2C), suggesting that the partitioning of nitrogen in leaves to soluble proteins did not reach the maximal values when *L. olgensis* seedlings were supplied with 16 mmol/L of nitrate. Such nitrogen partitioning in leaves directly affected net photosynthetic rates (Fig. 3C), because content of Rubisco, the most key enzyme in Calvin cycle, is the greatest part of soluble proteins in leaves (Wittenbach, 1979; Makino et al., 1983; Lauerer et al., 1993; Bhagwat, 2002). Under the treatment of phosphate, total nitrogen contents reached the peak value when *L. olgensis* seedlings were supplied with 1 mmol/L, but at the same time the contents of soluble proteins in leaves were the lowest (2D), suggesting that the partitioning of nitrogen in leaves to soluble proteins was not great at the phosphate concentration of 1 mmol/L. However, net photosynthetic rates in the seedlings supplied with 1 mmol/L of phosphate reached the peak value (Fig. 3D), suggesting that Rubisco taken the great part of soluble proteins, even though contents of soluble proteins in leaves were the lowest (Warren and Adams, 2002).

5 Conclusions

ALA biosynthesis rate and activity of PBG synthase in *L. olgensis* seedlings were affected by the levels of nitrate used in the seedlings, thus affecting the contents of chlorophyll and carotenoids in the leaves of the seedlings. Nitrate supply also affected the total nitrogen and soluble protein contents in the leaves and as well as photosynthesis rates. When *L. olgensis* seedlings were treated with phosphate, ALA biosynthesis rate and activity of PBG synthase were affected by phosphate levels, but the effects of phosphate levels on contents of chlorophyll and carotenoids, as well as on photosynthesis rates were relatively negligible.

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