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不同氮浓度和形态比例对水曲柳幼苗叶绿素合成、光合作用以及生物量分配的影响

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摘 要 水曲柳(*Fraxinus mandshurica*)是我国东北林区重要的工业用材树种，在东北林区广泛种植，因而其培育近来日益得到高度重视。在水曲柳的种植区域内，尽管林地内凋落物丰富，但该地区气温低，冬季长，氮素矿化速度低，氮素供给显得不足。本研究采用沙培的方式，在为幼苗提供完全平衡营养液 30 d 后，对幼苗进行 4 种不同的氮素浓度(1、4、8、16 mmol·L^{-1})或 5 种氮素形态(NO_3^--N 和 NH_4^+-N)比例(0、25%、50%、75%、100%)的处理。氮素浓度处理使用 NH_4NO_3，氮素形态处理中 NO_3^--N 使用 KNO_3，NH_4^+-N 使用 NH_4Cl。每一种处理方式重复 15 次。随后对不同的氮浓度和形态比例对水曲柳幼苗的叶绿素合成、光合作用、生物量的累积以及生物量在地下部分和地上部分的分配的影响进行了研究，旨在进一步了解氮素浓度和氮素形态的比例对水曲柳幼苗生理生态学的影响。在氮素浓度处理中，5-氨基-酮戊酸(5-aminolevulinic acid，ALA)的合成速率随氮素浓度呈现不规则变化，在 1 mmol·L^{-1}处达到最大(0.72 μmol·g^{-1} FW·h^{-1})，在 4 mmol·L^{-1}处最小。当氮素形态比例从 0 增加到 50%时，ALA 合成速率逐渐增加，而当氮素形态比例再增加时(75%～100%)，ALA 合成速率反而减少。氮素浓度对胆色原素(Porphobilinogen，PBG)合成酶活性没有显著影响，而当氮素形态比例从 0 增加到 75%时，PBG 合成酶活性逐渐增加，只对幼苗供给 NO_3^--N 时，PBG 合成酶活性又下降。氮素浓度从 1 mmol·L^{-1}增加到 8 mmol·L^{-1}时，叶绿素 a、叶绿素 b 以及总叶绿素含量增加，但 PBG 合成酶活性对这 3 个指标没有影响。当氮素形态比例从 0 增加到 75%时，总叶绿素含量逐渐增加，而其它两个指标则呈现不规则变化，并且总叶绿素含量变化与 PBG 合成酶活性没有显著关系。当氮素浓度从 1 增加到8 mmol·L^{-1}时，叶片总氮和可溶性蛋白增加，而过多的氮素供给(16 mmol·L^{-1})并不能使叶片合成和累积更多的氮和可溶性蛋白。当氮素形态比例从 0 增加到 75%时，叶片总氮和可溶性蛋白含量增加。尽管水曲柳幼苗偏好NO_3^--N，但完全供给 NO_3^--N 时，叶片总氮和可溶性蛋白含量下降。当氮素浓度从 1 增加到 8 mmol·L^{-1}时，净光合速率增加；过多供给氮素(16 mmol·L^{-1})净光合速率反而下降。当氮素形态比例从 0 增加到 75%时，净光合速率增加，而完全供给 NO_3^--N 时，光合速率下降。相似地，随外源氮供给的浓度从 1 增加到 8 mmol·L^{-1}，总生物量也增加；与此同时，总生物量在地下部分的分配减少，在地上部分的分配增加。随 NO_3^--N 比例从 0 增加到 75%，总生物量也增加。在氮素形态处理中，生物量在地下部分的分配随 NO_3^--N 比例增加而增加。无论是氮浓度处理还是氮素形态处理，生物量的累积与光合作用强度有密切的关系，且这种关系与氮素在叶片中的分配有直接的联系。根据实验结果，在水曲柳幼苗的培育过程中，氮素浓度为 8 mmol·L^{-1}为益，氮素形态比例为 75%:25%为益。

关键词 水曲柳 氮 氮形态 叶绿素合成 光合作用 生物量 生物量分配

EFFECTS OF DIFFERENT CONCENTRATIONS AND FORM RATIOS OF NITROGEN ON CHLOROPHYLL BIOSYNTHESIS, PHOTOSYNTHESIS, AND BIOMASS PARTITIONING IN *FRAXINUS MANDSHURICA* SEEDLINGS

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Abstract *Fraxinus mandshurica*, native to northeastern China, is an important timber tree. The tree often suffers from nitrogen (N) deficiency because long winter results in slow decomposition rate of litter. In the present study, effects of different concentrations and form ratios of nitrogen on chlorophyll biosynthesis, total leaf N, soluble protein, and photosynthesis were studied. During the N concentration treatment, biosynthesis rate of 5-aminolevulinic acid (ALA) and activity of porphobilinogen (PBG) synthase showed irregular changes, but total chlorophyll content increased when N concentrations varied from 1 to 8 mmol·L^{-1}, and activity of PBG

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synthase did not change significantly. Total leaf N, soluble leaf proteins, and photosynthesis rate also increased when N concentrations increased from 1 to 8 mmol·L^{-1}. These results suggested that N concentrations did not affect biosynthesis rates of ALA and activities of PBG synthase and 8 mmol·L^{-1} of N most increased total leaf N, soluble proteins in leaves, and photosynthesis rate. During the N form treatment, biosynthesis of ALA increased when the ratio of NO_3^--N increased from 0 to 50%, but activity of PBG synthase, total chlorophyll concentrations, total leaf N and soluble leaf proteins increased when the ratio of NO_3^--N increased from 0 to 75%. Photosynthesis rate also increased when the ratio of NO_3^--N increased from 0 to 75%. During the two types of treatments, there were significant relationships between total leaf N, soluble protein concentrations and net photosynthesis rates. These results suggested that *F. mandshurica* seedlings showed a preference for NO_3^--N, but the ratio of 75% NO_3^--N to 25% NH_4^+-N promoted an increase in chlorophyll biosynthesis, accumulation of N and soluble proteins in leaves, and net photosynthesis rate. Total biomass increased as exogenous nitrogen concentrations increased from 1 to 8 mmol·L^{-1}. Biomass partitioning to roots decreased, and biomass partitioning to shoots increased. As ratios of NO_3^--N to NH_4^+-N increased from 0 to 75%, total biomass increased, but biomass partitioning to roots increased as ratios of NO_3^--N to NH_4^+-N increased from 0 to 100%. When both nitrogen concentration treatment and form ratio treatment were carried out, biomass accumulation was closely related to photosynthesis rate, and the relationship between biomass accumulation and photosynthesis was related to nitrogen partitioning in leaves. It is proposed that 8 mmol·L^{-1} and 75% NO_3^--N∶25% NH_4^+-N should be used in practical cultivation of *F. mandshurica* seedlings.

Key words *Fraxinus mandshurica*, Nitrogen, Nitrogen form, Chlorophyll biosynthesis, Photosynthesis, Biomass, Biomass partitioning

Nitrogen (N) is an essential mineral element for plants, comprising 1.5% to 2% of plant dry matter and approximately 16% of total plant protein (Frink *et al.*, 1999). Though N is the most abundant element in the atmosphere, it is frequently the limiting factor for plant productivity all around the world (Martins-Loução & Lips, 2000; Crawford *et al.*, 2000). Moreover, N availability limits plant growth and yield more than other nutritional factors (Vitousek & Howarth, 1991; Cassman *et al.*, 1993; Crawford & Glass, 1998), because N affects many physiological processes occurring in plants by regulating more than one hundred genes, including the genes encoding aquaporins, root phosphate and K^+ transporters, nitrate transporters, nitrate and nitrite reductase, and metabolic enzymes such as transaldolase, malate dehydrogenase, asparagine synthetase, histidine decarboxylase (Wang *et al.*, 2001).

Soil inorganic N is often the primary N source (Glass *et al.*, 1999; Murphy *et al.*, 2000), and nitrate and ammonium are the main forms of inorganic N in soils. Nitrate is predominant in agricultural soils (Crawford & Glass, 1998; Hirsch & Sussman, 1999), but in forest soils, ammonium is usually the predominant form of inorganic N (Malagoli *et al.*, 2000). Although most plants grow optimally with a mixture of ammonium and nitrate, some plants show their preference to ammonium (Fried *et al.*, 1965; Minotti *et al.*, 1969; Edwards & Horton, 1982; Peterson *et al.*, 1988; Bassirirad *et al.*, 1997; Kronzucher *et al.*, 1995a; 1995b; 1996). Thus many forest trees show a poor growth in soils with nitrate as the main N source. In contrast, other forest trees that prefer to nitrate show a poor growth in soils with ammonium as the main N source. The preference affects physiological processes in plants, especially photosynthesis, ultimately affecting plant productivity.

Biomass accumulation comes from photosynthetic fixation of CO_2 by source leaves of plants. During photosynthetic fixation of CO_2, light is an important factor. For light energy to be used by any system, the light must be absorbed. This is a significant problem for photosynthetic organisms. Photosynthesis encompasses a complex series of reactions that involve light absorption, energy conversion, electron transfer, and a multistep enzymatic pathway that converts CO_2 and water into carbohydrates. The first step of photosynthesis is light absorption by photosynthetic pigments in source leaves. Thus photosynthetic pigments are very important to photosynthesis. Therefore, biosynthesis of these photosynthetic pigments, especially chlorophyll, is essential to photosynthesis, growth, development, and productivity of plants.

This study focused on the deciduous, broad-leaved tree *Fraxinus mandshurica* that is native to northeastern China and has become one of the main trees for industrial wood. How to cultivate this tree is important because it has been widely planted in the northeastern China. In present study, changes of chlorophyll biosynthesis, total leaf N, soluble leaf proteins and photosynthesis in *Fraxinus mandshurica* seedlings fed with different N concentrations and N form ratios were investigated in order to understand how N supply levels and N form ratios affect chlorophyll biosynthesis and photosynthesis in these seedlings.

1 Materials and methods

1.1 Plant materials and growth conditions

Low temperature-pretreated seeds were sterilized and were sowed in sterilized soil (soil: sand = 7:3). When seedlings had a couple of real leaves, the young seedlings were transplanted in plastic pots (d, 30 cm; h, 27 cm) with sterilized and washed sand on May 20, 2002, four seedlings in one pot. The potted seedlings were placed in a glasshouse. The seedlings were fed with solution listed below for 30 d: NH_4NO_3, 8 mmol·L^{-1}; KH_2PO_4, 1 mmol·L^{-1}; KCl, 1 mmol·L^{-1}; $CaCl_2 \cdot 6H_2O$, 1 mmol·L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.6 mmol·L^{-1}; $FeCl_3 \cdot 6H_2O$, 0.02 mmol·L^{-1}; $MnCl_2 \cdot 4H_2O$, 6 μmol·L^{-1}; H_3BO_3, 0.016 mmol·L^{-1}; $ZnCl_2$, 0.3 μmol·L^{-1}; $CuCl_2 \cdot 2H_2O$, 0.3 μmol·L^{-1}; $NaMoO_4 \cdot 2H_2O$, 0.3 μmol·L^{-1}. Then the seedlings were treated with different N concentrations and N form ratios (NO_3^--N to NH_4^+-N), respectively. N concentration treatments were done as below: 16 mmol·L^{-1}, 8 mmol·L^{-1}, 4 mmol·L^{-1} and 1 mmol·L^{-1} of NH_4NO_3. 8 mmol·L^{-1} of NH_4NO_3 was used as the control. In all concentration treatments, concentrations of other nutrient elements were as same as above mentioned. N form treatments were done as below: 0 NO_3^--N to 100% NH_4^+-N – 0:8 mmol·L^{-1}; 25% NO_3^--N to 75% NH_4^+-N – 2:6 mmol·L^{-1}; 50% NO_3^--N to 50% NH_4^+-N – 4:4 mmol·L^{-1}; 75% NO_3^--N to 25% NH_4^+-N – 6:2 mmol·L^{-1}; 100% NO_3^--N to 0 NH_4^+-N – 8:0 mmol·L^{-1}. In all N form treatments, NO_3^--N was supplied as KNO_3, NH_4^+-N was supplied as NH_4Cl, and other components and their concentrations were as same as above mentioned. The pH was adjusted to 5.5 – 6.0 using $Ca(OH)_2$ or H_2SO_4 as required. Every treatment was replicated 15 times. From June 20 to August 5, 2002, the seedlings were treated with different concentrations and form ratios of nitrogen. The gas exchange was determined on August 5, 2002, and then leaves were cut off for other experiments described as below.

1.2 Determination of gas exchange

Photosynthesis rate was measured in the young fully-expanded leaves using Photosynthesis System CI-301PS (CID, Inc, Vancouver, Can). Temperature varied between 33 – 36 ℃, and PFD varied between 1 780 μmol·m^{-2}·s^{-1} and 1 800 μmol·m^{-2}·s^{-1}. Relative humidity was 55% – 65%. After determination of gas exchange, the leaves were cut from seedlings and were frozen in liquid nitrogen, then stored in – 80 ℃ or desiccated for 12 h at 80 ℃ and stored in desiccators for other further analyses as described below.

1.3 Assay for the biosynthesis of ALA and activity of PBG

Chlorophyll is one of a group of tetrapyrrole molecules which are essential to plant metabolism and its biosynthesis is also essential. The first committed precursor of the tetrapyrrole pathway is 5-aminolevulinic acid (ALA), two molecules of ALA react to yield porphobilinogen (PBG), and four molecules of PBG form the ring structure of protoporphyrin IX. (Smith & Griffiths, 1993; Malkin & Niyogi, 2000). Protoporphyrin IX is the common branch point for the synthesis of heme and chlorophyll.

Assay of ALA　0.4 g of leaf tissues were placed in a glass Petri dish (d, 5 cm) with 10 ml of 0.1 mol·L^{-1} levulinic acid (pH 6.0, Sigma-Aldrich, Inc, St. Louis, USA) and incubated for 6 h under illumination (50 μmol·m^{-2}·s^{-1}). They were homogenized in 4% TCA and the homogenate was centrifuged at 10 000*g* for 30 min. The resulting supernatant was purified on a column of Dowex 50W-X8 (Sigma-Aldrich, Inc). The volume of the eluate was adjusted 10 ml. The pH of the eluate was adjusted to 4.6 by addition of 4.8 ml of 1 mol·L^{-1} sodium acetate, and then 0.3 ml of acetylacetone (Sigma-Aldrich, Inc) was added. The sample was heated at 100 ℃ for 10 min and cooled to room temperature. An aliquot of the sample and the same volume of Ehrlich's reagent (Sigma-Aldrich, Inc) were mixed together, and the absorbance at 553 nm was measured after 15 min according to Masuda *et al*. (1996).

Assay of PBG synthase　The formation of PBG by PBG synthase was measured with ALA (Sigma-Aldrich, Inc) as the substrate by method of Masuda *et al*. (1996). The tissues were homogenized in a buffer that contained 20 mmol·L^{-1} Tris-HCl (pH 8.0), 0.5 mmol·L^{-1} $MgCl_2$ and 1 mmol·L^{-1} β-mercaptoethanol, and the extracts were centrifuged at 10 000 *g* for 15 min at 4 ℃. Each resulting supernatant was diluted with the buffer to an appropriate concentration of protein. The reaction was started by addition of ALA (final concentration is 3 mmol·L^{-1}) and it was allowed to proceed at 25 ℃ in darkness. The amount of PBG formed was calculated from the absorbance of the product of the reaction with Ehrlich's reagent at 553 nm, using a molar extinction coefficient of 6.8×10^4.

1.4 Determination of chlorophyll, total leaf nitrogen and soluble proteins

For determination of chlorophyll and total carotenoids, about 0.1 g of fresh leaf samples was grounded to homogenate with 2 ml of dimethylformamide in a mortar with a pestle. After washing the mortar and pestle three times, 1 ml of dimethylformamide every time, the homogenate was centrifuged at 4 500 rpm for 10 min at 4 ℃. The total supernatant was added to 10 ml and the assay was done as Wellburn (1994). Soluble proteins

were determined according to the method of Bradford (1976), using bovine serum albumin as a standard. Kjelkahl determination was used in determination of total leaf nitrogen (Horneck & Miller, 1998).

1.5 Determination of total biomass and root/shoot ratio

At the same time that gas exchange and chlorophyll fluorescence was determined, other plants used for total biomass and root/shoot ratio were uprooted from the plastic pots, and then were washed with tap water and distilled water. The shoots and roots were cut apart using sharp knifes. The shoots and roots were dried at 80 ℃ for 24 h, and then were weighted.

1.6 Statistic analysis

All chemical experiments were repeated three times. Determinations for photosynthesis rates of each treatment were repeated 10 times. Statistic analysis was performed using SPSS statistical package (v10.0, SPSS Inc, Chicago, Illinois, USA).

2 Results

2.1 Changes of chlorophyll biosynthesis

In the experiments, the activity of PBG synthase was inhibited by the treatment with levulinic acid, and then ALA biosynthesis rates was monitored by measuring the amount of accumulated ALA in tissues. When seedlings were fed with N of 1 mmol·L^{-1}, the rate of accumulated ALA came up to the peak (0.72 μmol·g^{-1} FW·h^{-1}), ALA biosynthesis rate was the lowest in leaves of seedlings fed with N of 4 mmol·L^{-1} (Fig. 1A). When NO_3^--N increased from 0 to 50% ALA accumulated linearly, reaching the peak at 50%, then decreased (Fig. 1B).

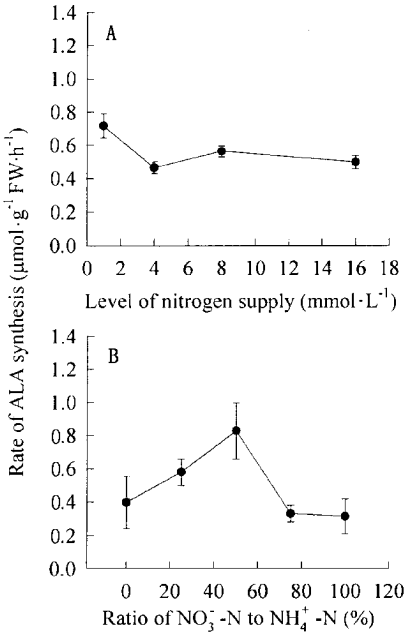

Fig. 1 Rates of ALA in leaves of seedlings fed with nitrogen of different concentrations (A) and form ratio of NO_3^--N to NH_4^+-N (B)
The results are means ± *SD* (n = 3)

PBG synthase catalyzes the formation of PBG from ALA. The activity of PBG synthase in leaves of seedlings fed with N of 1 – 16 mmol·L^{-1} did not change significantly (t test, $p > 0.05$, Fig. 2A), indicating that levels of N supply did not significantly affect PBG synthase activity. When seedlings were fed with N of different form ratios (NO_3^--N to NH_4^+-N ratios varied from 0 to 75%), activity of PBG synthase increased linearly with the ratios, but then decreased at the ratio of 100% of NO_3^--N to 0 NH_4^+-N (Fig. 2B), indicating that the ratio of NO_3^--N to NH_4^+-N (75%∶25%) was appropriate for activity of PBG synthase.

When levels of N supply varied from 1 to 8 mmol·L^{-1}, the levels of chlorophyll a increased linearly, and the changes of chlorophyll b and carotenoids were similar to chlorophyll a (Table 1). Though the changes of chlorophyll a, chlorophyll b and carotenoids were not regular when seedlings were fed with N of different ratios of NO_3^--N to NH_4^+-N, their levels reached the peak value at the ratio of 75%∶25% (Table 1), indicating leaves of the seedlings under the ratio of N form synthesized and accumulated more chlorophyll and carotenoids. The ratios of chlorophyll a to chlorophyll b changed irregularly during the two types of treatments (Table 1).

When seedlings were fed with N of different concentrations, biosynthesis rates of ALA in leaves were affected by activities of PBG synthase (Fig. 1A and Fig. 2A), because ALA was converted to PBG by activity of PBG synthase. When seedlings were fed with N of 4 mmol·L^{-1}, the highest activity of PBG synthase resulted in the highest level of chlorophyll a and chlorophyll b (Table 1). Although chlorophyll biosynthesis is controlled by PBG synthase in some extent, there was not significant relationship between total chlorophyll and activity of PBG synthase when seedlings were fed with different ratios of NO_3^--N to NH_4^+-N (Sig. 0.462 > 0.05, 1-tailed, Fig. 2B and Table 1). Moreover, there was not relationship between levels of chlorophyll a and activity of PBG synthase (Sig. 0.423 > 0.05, 1-tailed). When seedlings were fed with N of different ratios of NO_3^--N to NH_4^+-N, ALA biosynthesis rates were not related to activity of PBG synthase (Sig. 0.322 > 0.05, 1-tailed, Fig. 1B and Fig. 2B). Levels of chlorophyll a were not significantly related to the activity of PBG synthase (Sig. 0.068 > 0.05, 1-tailed, Table 1 and Fig. 2B). Although the changes of total chlorophyll were consistent with that of PBG synthase activity, total chlorophyll did not show significant correlation with activity of PBG synthase (Sig. 0.053 > 0.05, 1-tailed).

2.2 Changes of total nitrogen and soluble proteins

Total leaf N levels increased linearly as N concentrations varied from 1 to 8 mmol·L^{-1}, and total leaf N decreased at 16 mmol·L^{-1} (Fig. 3A), indicating 8 mmol·L^{-1} was the most favorite for N accumulation in

Table 1　Levels of chlorophyll a, chlorophyll b and carotenoids in leaves of seedlings fed with different N concentrations and N form ratios

Treatment type		Chlorophyll a ($mg \cdot g^{-1}$ FW)	Chlorophyll b ($mg \cdot g^{-1}$ FW)	Total chlorophyll ($mg \cdot g^{-1}$ FW)	Chl a/Chl b	Carotenoids ($mg \cdot g^{-1}$ FW)
NO_3^--N:NH_4^+-N	0:100	0.150 ± 0.024	0.061 ± 0.013	0.211 ± 0.038	2.48	0.031 ± 0.003
	25:75	0.182 ± 0.009	0.068 ± 0.004	0.249 ± 0.012	2.70	0.038 ± 0.002
	50:50	0.172 ± 0.002	0.068 ± 0.003	0.240 ± 0.003	2.54	0.035 ± 0.003
	75:25	0.189 ± 0.024	0.071 ± 0.011	0.261 ± 0.035	2.67	0.039 ± 0.004
	100:0	0.156 ± 0.014	0.063 ± 0.023	0.219 ± 0.087	2.46	0.036 ± 0.014
N concentrations	1 $mmol \cdot L^{-1}$	0.137 ± 0.009	0.054 ± 0.008	0.191 ± 0.017	2.51	0.028 ± 0.002
	4 $mmol \cdot L^{-1}$	0.178 ± 0.026	0.066 ± 0.008	0.244 ± 0.034	2.67	0.037 ± 0.005
	8 $mmol \cdot L^{-1}$	0.176 ± 0.027	0.066 ± 0.001	0.256 ± 0.017	2.66	0.036 ± 0.004
	16 $mmol \cdot L^{-1}$	0.166 ± 0.037	0.063 ± 0.015	0.229 ± 0.052	2.63	0.035 ± 0.005

The results are the means ± *SD* ($n = 3$)

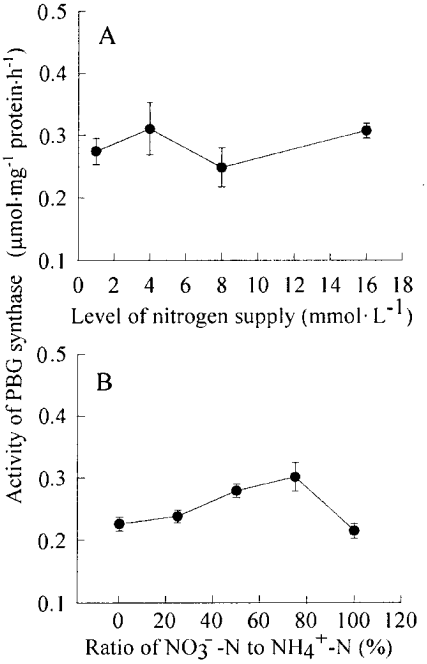

Fig.2　Changes of activity of PBG synthase in leaves of seedlings fed with nitrogen of different concentrations and ratio of NO_3^--N to NH_4^+-N
The results are mean ± *SD* ($n = 3$)

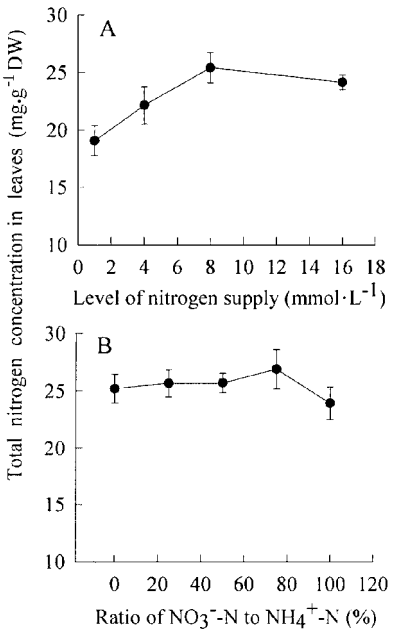

Fig.3　Changes of total nitrogen in leaves of seedlings fed with nitrogen of different concentrations and ratios of NO_3^--N to NH_4^+-N
The results are means ± *SD* ($n = 3$)

leaves of the seedlings. When seedlings were fed the mixture of NO_3^--N and NH_4^+-N, total leaf N levels in leaves increased as the ratios of NO_3^--N to NH_4^+-N increased from 0 to 75%, and then decreased at 100% of NO_3^--N (Fig. 3B).

The similarity of the changes of soluble protein concentrations in leaves occurred when seedlings were fed with different N concentrations and N form ratios (Fig. 4). The data in Fig. 4A showed that when seedlings were fed with N of 8 $mmol \cdot L^{-1}$ they biosynthesized the most of soluble proteins in leaves, N overfeeding decreased the levels of soluble proteins in leaves, suggesting N overfeeding is not profitable for seedlings and cause economic loss. When the ratio of NO_3^--N to NH_4^+-N is 75% vs. 25% seedlings biosynthesized and accumulated the most of soluble proteins though the seedlings are preferential to NO_3^--N (Fig. 4B).

Thus, according to the results above mentioned, it is seen that N supply of 8 $mmol \cdot L^{-1}$ is profitable for reduction of expense for the maintenance of growth and development of *F. mandshurica* seedlings, and the ratio of 75% NO_3^--N to 25% NH_4^+-N is the most appropriate ratio of N forms for growth and development of *F. mandshurica* seedlings.

2.3　Changes of photosynthesis, total biomass and biomass partitioning

Net photosynthesis and rate increased as the N concentrations increased from 1 to 8 $mmol \cdot L^{-1}$ and then decreased at N of 16 $mmol \cdot L^{-1}$ (Fig. 5A). The similar changes of total biomass occurred (Fig. 6A). The results indicated that net photosynthesis rate and total biomass increased as levels of N supply increased to some extent and 8 $mmol \cdot L^{-1}$ was profitable for *F. mandshurica* seedlings. Net photosynthesis rate increased as ratios of NO_3^--N to NH_4^+-N increased from 0 to 75%, but when

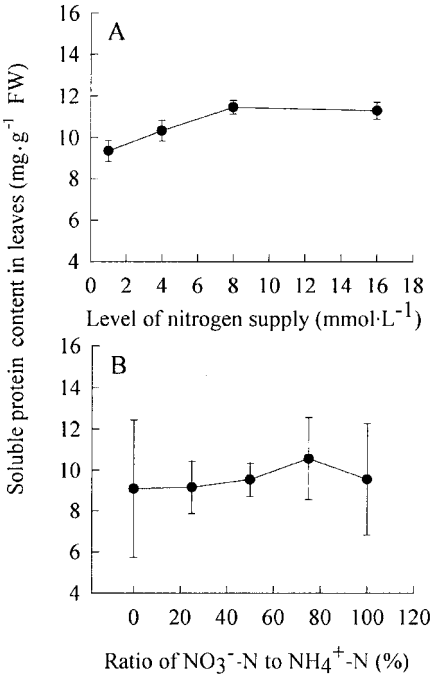

Fig.4 Changes of soluble protein concentrations in leaves of seedlings fed with nitrogen of different concentrations and ratios of NO_3^--N to NH_4^+-N
The results are means ± *SD* ($n = 3$)

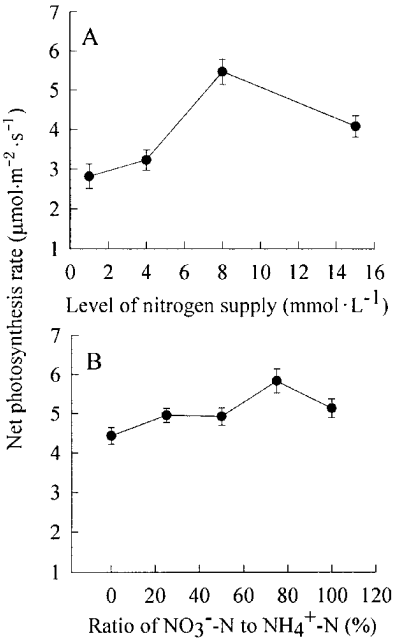

Fig.5 Changes of photosynthesis rate in leaves of seedlings fed with nitrogen of different concentrations (A) and ratios of NO_3^--N to NH_4^+-N (B)
The results are means ± *SD* ($n = 10$)

seedlings were only fed with NO_3^--N, net photosynthesis rate decreased (Fig. 5B). Seedlings supplied with 75% NO_3^--N to 25% NH_4^+-N accumulated the most biomass, but there were not significant differences among all nitrogen form treatment (LSD, $p > 0.05$). The results suggested that although *F. mandshurica* seedlings are preferential to NO_3^--N, only supply of NO_3^--N to the seedlings is not profitable for photosynthesis.

Ratios of root to shoot decreased as supply level increased from 1 to 8 mmol·L^{-1}, and then increased as nitrogen supply was 16 mmol·L^{-1} (Fig. 7A). There were significant differences between 1, 4, and 8 mmol·L^{-1} of nitrogen supply (LSD, $p < 0.05$), suggesting that nitrogen supply levels greatly affected biomass partitioning between roots and shoots. Similarly, ratios of nitrogen forms also affected biomass partitioning between roots and shoots (Fig. 7B), and there were significant differences between 0 and 75% and 100% (LSD, $p < 0.05$).

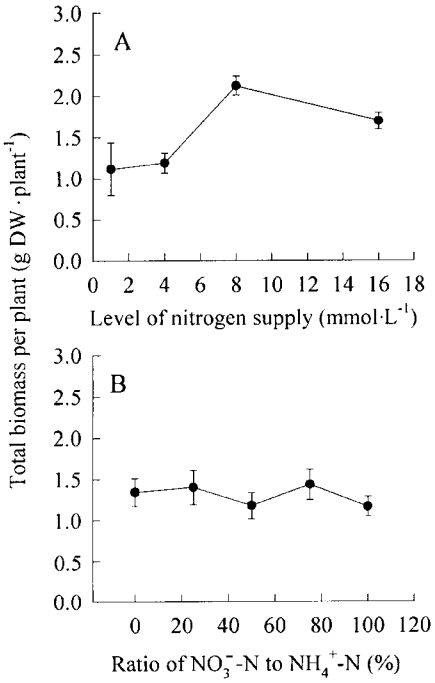

Fig.6 Changes in total biomass per plant supplied with different concentrations (A, mean ± *SD*, $n = 10$) and ratios of NO_3^--N to NH_4^+-N (B, mean ± *SE*, $n = 10$)

3 Discussion

3.1 Response of chlorophyll biosynthesis to nitrogen supply

ALA is first committed precursor of chlorophyll. In higher plants, ALA is formed through C_5 pathway composed of three chloroplast *t*RNA Glu-dependent steps and biosynthesis of ALA begins from glutamic acid. Then ALA is converted into PBG by the activity of PBG synthase (Smith & Griffiths, 1993; Malkin & Niyogi, 2000). Thus chlorophyll biosynthesis may be affected by biosynthesis rate of ALA and activity of PBG synthase. When level of N supply varied from 1 to 8 mmol·L^{-1}, activity of PBG synthase did not change significantly (Fig. 2A) and total chlorophyll concentrations increased (Table 1). Thus PBG synthase did not play a determinant role in chlorophyll biosynthesis when seedlings were fed with N of different concentrations (i.e. the step that PBG synthase catalyses is not limiting). The result is consis-

tent with "control of flux" theory (Small & Kacser, 1993a; 1993b), because PBG is converted into chlorophyll through several steps (Smith & Griffiths, 1993; Malkin & Niyogi, 2000). Similarly, activity of PBG synthase did not play a significant role in chlorophyll biosynthesis as the ratios of NO_3^--N to NH_4^+-N increased from 0 to 100% (Fig. 2B and Table 1).

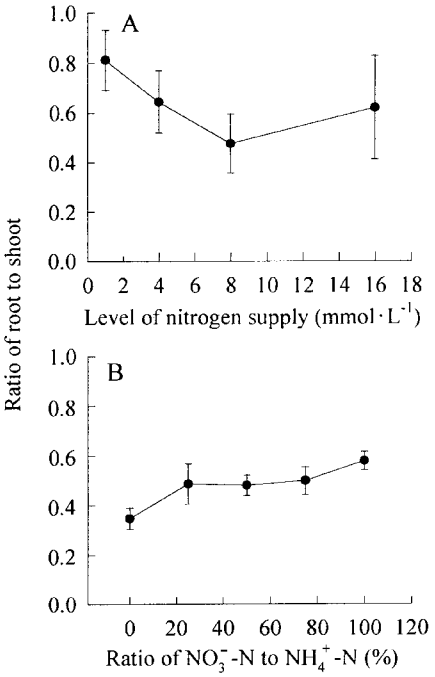

Fig.7 Changes in ratios of root to shoot of plants supplied with different concentrations (A, mean ± *SD*, $n = 10$) and ratios of NO_3^--N to NH_4^+-N (B, mean ± *SE*, $n = 10$)

3.2 Response of net photosynthesis and total biomass to nitrogen supply

Plants absorb nitrate via transporters localized to plasma membrane of the root epidermal and cortical cells over a wide nitrate concentration range using several different transport mechanisms, including constitutive and nitrate-inducible high-affinity transport systems and low-affinity transporters (Stitt, 1999; Hirsch & Sussman, 1999; Forde & Clarkson, 1999; Daniel-Vedele *et al.*, 1998). Absorbed nitrate by roots is reduced to nitrite by nitrate reductase (NR, EC 1.6.6.1), and then reduced to ammonium by nitrite reductase (NiR, EC 1.7.7.1) in both roots and shoots. Ammonium is converted in organic N-containing compounds, such as glutamine, glutamate, asparagine, aspartate. Subsequently these amino acids can also be converted to other amino acids. All of them provide maintenance for plant growth and development. Therefore, the effects of exogenous N supply on growth and development are usually monitored by determining total leaf nitrogen or soluble proteins in leaves (Lusk *et al.*, 1997; Niinemets, 1997).

In seedlings fed with N of different concentrations, there was positive relationship between net photosynthesis rate and total leaf N (Fig. 3A and Fig. 5A) and soluble protein concentrations (Fig. 4A and Fig. 5A). Moreover, the relationships were significant (Sig. 0.047 and 0.021, respectively, < 0.05, 1-tailed). The significant relationship is related to Rubisco (EC 4.1.1.39). It is known that Rubisco is the most important enzyme involved in CO_2 fixation, and its content is thought to be a rate-limiting factor for the light-saturated rate of photosynthesis at present atmospheric CO_2 pressure (Makino *et al.*, 1985). N in leaves is largely allocated into Rubisco protein, accounting for 15% – 35% of total leaf N in C_3 higher plants (Evans, 1989); and moreover, Rubisco represented a larger proportion of leaf soluble protein (Makino *et al.*, 1983; 1984; Wittenbach, 1978; 1979; Lauerer *et al.*, 1993; Bhagwat, 2002), thus causing the fact that plant capacity to fix CO_2 is strengthened when N concentrations and soluble protein concentrations in leaves is high (Field & Mooney, 1986; Evans, 1989; Reich *et al.*, 1994; 1995a; 1995b). Therefore, Our results are reasonable, and are consistent with that of many studies (Field & Mooney, 1986; Evans, 1989; Sinclair & Horie, 1989). The similar cases occurred in seedlings fed with N of different ratios of NO_3^--N to NH_4^+-N. When ratios of NO_3^--N to NH_4^+-N varied from 0 to 75%, total leaf N concentrations, soluble protein concentrations, and net photosynthesis increase. (Fig. 3B, Fig. 4B and Fig. 5B). Total leaf N concentrations, soluble protein concentrations, and net photosynthesis rates were affected by exogenous N supply, therefore exogenous nitrogen supply affected growth and development of the seedlings. In the two types of treatments, the relationships between net photosynthesis and total leaf nitrogen concentration and soluble protein concentration were affected by exogenous nitrogen supply (Fig. 3, Fig. 4, and Fig. 5).

Photosynthesis rates greatly affected biomass accumulation of seedlings supplied with different concentrations and form ratios of nitrogen (Fig. 5 and Fig. 6). The influence resulted from nitrogen partitioning in leaves, because nitrogen levels in leaves are closely related to photosynthesis rate (Fig. 3 and Fig. 5).

Here it is proposed that 8 mmol·L^{-1} of N and the ratio of 75% NO_3^--N to 25% NH_4^+-N should be used in practical cultivation of *F. mandshurica* seedlings.

3.3 Conclusion

In *F. mandshurica* seedlings, N concentration treatments did not affect biosynthesis of ALA and activity of PBG synthase. 8 mmol/L nitrogen supply increased total leaf nitrogen, soluble leaf proteins, photosynthesis rate, biomass accumulation, and biomass partitioning to the most extent.

F. mandshurica seedlings showed preference to NO_3^--N, but the ratio of 75% NO_3^--N to 25% NH_4^+-N was the most profitable to chlorophyll biosynthesis, total leaf nitrogen and soluble protein, photosynthesis, biomass accumulation.

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