Pedosphere **17**(5): 595–600, 2007
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier Limited and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Effects of Amino Acids Replacing Nitrate on Growth, Nitrate Accumulation, and Macroelement Concentrations in Pak-choi (*Brassica chinensis* L.)*[1]

WANG Hua-Jing[1,2], WU Liang-Huan[1,*2], WANG Min-Yan[1], ZHU Yuan-Hong[3], TAO Qin-Nan[1] and ZHANG Fu-Suo[4]

[1] *Ministry of Education Key Laboratory of Environmental Remediation and Ecosystem Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310029 (China). E-mail: wanghuajing0631@163.com*
[2] *College of Geography and Resource Sciences, Sichuan Normal University, Chengdu 610066 (China)*
[3] *Department of Crop and Soil Sciences, the Pennsylvania State University (USA)*
[4] *College of Resources and Environment, China Agricultural University, Beijing 100094 (China)*

(Received January 20, 2007; revised June 17, 2007)

ABSTRACT

A hydroponic experiment was carried out to determine the influence of replacing 20% of nitrate-N in nutrient solutions with 20 individual amino acids on growth, nitrate accumulation, and concentrations of nitrogen (N), phosphorus (P), and potassium (K) in pak-choi (*Brassica chinensis* L.) shoots. When 20% of nitrate-N was replaced with arginine (Arg) compared to the full nitrate treatment, pak-choi shoot fresh and dry weights increased significantly ($P \leq 0.05$), but when 20% of nitrate-N was replaced with alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), glycine (Gly), serine (Ser), threonine (Thr), cysteine (Cys), and tyrosine (Tyr), shoot fresh and dry weights decreased significantly ($P \leq 0.05$). After replacing 20% of nitrate-N with asparagine (Asn) and glutamine (Gln), shoot fresh and dry weights were unaffected. Compared to the full nitrate treatment, amino acid replacement treatments, except for Cys, Gly, histidine (His), and Arg, significantly reduced ($P \leq 0.05$) nitrate concentrations in plant shoots. Except for Cys, Leu, Pro, and Met, total N concentrations in plant tissues of the other amino acid treatments significantly increased ($P \leq 0.05$). Amino acids also affected total P and K concentrations, but the effects differed depending on individual amino acids. To improve pak-choi shoot quality, Gln and Asn, due to their insignificant effects on pak-choi growth, their significant reduction in nitrate concentrations, and their increase in macroelement content in plants, may be used to partially replace nitrate-N.

Key Words: amino acids, growth, N, P, and K, nitrate accumulation, pak-choi

Citation: Wang, H. J., Wu, L. H., Wang, M. Y., Zhu, Y. H., Tao, Q. N. and Zhang, F. S. 2007. Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pak-choi (*Brassica chinensis* L.). *Pedosphere*. **17**(5): 595–600.

INTRODUCTION

Nitrogen is an essential element for plant growth, and nitrate is one of the available nitrogen forms for plant uptake. When nitrate uptake by plants exceeds their assimilation, nitrate is accumulated in plant tissues, which may become human food. High human intake of nitrate from food and water may cause health problems, such as cancers and blue baby syndrome (Zhu, 2002; Dich *et al.*, 1996). Vegetables are the main nitrate intake sources for humans. Human daily nitrate intake from vegetables accounts for about 80% of the total dietary nitrate intake (Shen *et al.*, 1982). Therefore, many governments and international organizations have set maximum nitrate levels for vegetables (Gunes *et al.*, 1996; Santamaria and Elia, 1997). To reduce nitrate content in vegetables, researchers have investigated the effects of inorganic fertilizer rates (Kotsiras *et al.*, 2002), inorganic fertilizer ratios (Inal and Tarakcioglu,

*[1] Project supported by the National Natural Science Foundation of China (No. 30370838).
*[2] Corresponding author. E-mail: finm@zju.edu.cn.

2001; Kotsiras *et al.*, 2002), and field management practices (Feller and Fink, 2004) on nitrate content in plant tissues.

Research has demonstrated that plants can absorb and use amino acids (Nasholm *et al.*, 1998; Weigelt *et al.*, 2003; Persson *et al.*, 2003). Because amino acids short-circuit the nitrogen cycle defined in traditional biochemistry, scientists have had to reconsider the nitrogen cycle (Chapin III, 1995). Amino acids also inhibit plant nitrate uptake (Muller and Touraine, 1992; Sivasankar *et al.*, 1997; Vidmar *et al.*, 2000; Aslam *et al.*, 2001). When amino acids are supplied as the sole nitrogen source, different amino acids affect plant growth differently (Liu *et al.*, 2003). However, information on effects of partial replacement of nitrate-N with amino acid-N in nutrient solutions on plant growth is lacking.

Although the effects of amino acids replacing nitrate in nutrient solutions on nitrate concentrations in onion and lettuce have been reported (Gunes *et al.*, 1994, 1996), there is little information regarding the effects of amino acids on growth, nitrate concentrations, and macroelement content in pak-choi (*Brassica chinensis* L.), which is one of the main leafy vegetables grown in the Yangtze River region of China. Research has shown that leafy vegetables easily accumulate nitrate in their tissues (Chen *et al.*, 2004). In addition, there is a need for information on how different amino acids affect vegetables. The objective of this research was to determine the effects of partial replacement of nitrate with different amino acids on pak-choi growth, nitrate accumulation, and macroelement concentrations in pak-choi shoots.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse on Huajiachi Campus of Zhejiang University from March to April in 2003. Pak-choi seeds were soaked for about 8 h and sowed in sand in an illuminated incubator at a photosynthetic photon flux density of 200 μmol m^{-2} s^{-2}. The incubator was set to a photoperiod of 14 h and a day/night temperature of 25/20 °C. Fifteen days after germination, seedlings were transplanted into 2 000 mL plastic pots filled with a 1/2-strength Hoagland's solution. Each pot was planted with five seedlings, and the planted pots were kept in a greenhouse.

There were 126 pots arranged in a randomized complete block design with 21 treatments and six replications. The twenty-one treatments that were applied five days after transplanting included one control treatment receiving 100% nitrate-N and 20 amino acid treatments receiving 80% nitrate-N and 20% amino acid N from one of 20 amino acids. The 20 amino acids used were alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), tryptophane (Trp), methionine (Met), aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), arginine (Arg), histidine (His), glycine (Gly), serine (Ser), threonine (Thr), cysteine (Cys), tyrosine (Tyr), asparagine (Asn), and glutamine (Gln). All treatments had the same total 12.5 mmol L^{-1} N concentration in nutrient solutions.

During the experiment, pH in nutrient solutions was maintained at about 6.0 by addition of either 1 mmol L^{-1} NaOH or H_2SO_4, and the nutrient solutions were replaced every four days. The solutions in pots were aerated throughout the experimental period. Twenty-four days after application of the treatments, pak-choi plants were harvested. Two fresh plants from each pot were used for immediate nitrate determination in shoots. The remaining plants were rinsed with deionized water, and then roots were removed. The shoots were weighed fresh and then oven-dried at 70 °C for 48 h. The dried shoots were used for determining dry matter weight and total N, P, and K contents.

After plant tissues were digested with sulfuric acid and hydrogen peroxide, total N (except nitrate and nitrite) was determined using a Kjeldahl method. Total P was determined colorimetrically using a spectrophotometer, and total K was determined using a flame photometer (Li, 1983). Nitrate-N was determined using the H_2SO_4-salicylic acid method as described by Cataldo *et al.* (1975). Yield and other collected data were subject to analysis of variance (ANOVA) or correlation analysis using data processing system (Tang and Feng, 1997), and the least significant difference (LSD) multiple range test was used for mean separation between treatments ($P \leq 0.05$).

RESULTS AND DISCUSSION

Pak-choi shoot growth

For all amino acid treatments, compared to the full nitrate treatment, only the Arg treatment significantly increased ($P \leq 0.05$) pak-choi shoot fresh and dry weights (Fig. 1). Shoot fresh and dry weights for Asn and Gln treatments were not significantly different from the nitrate treatment (Fig. 1). Fifteen other amino acid treatments, including Gly, Ala, Ser, Asp, Lys, Tyr, Cys, Val, Pro, Ile, Thr, Phe, Glu, Leu, and Met, significantly reduced ($P \leq 0.05$) pak-choi shoot fresh and dry weights (Fig. 1).

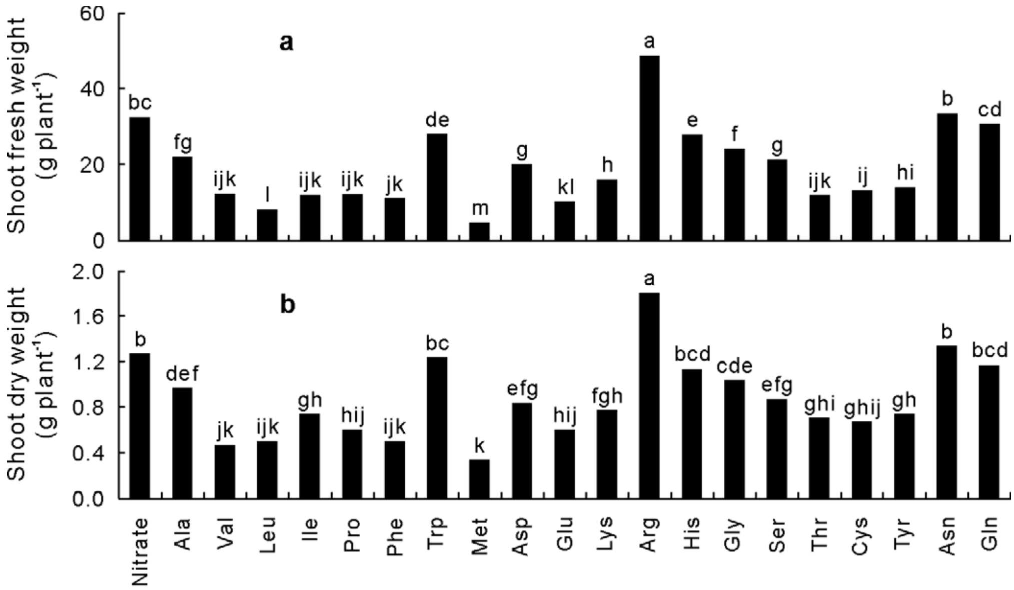

Fig. 1 Effects of replacing 20% of nitrate-N by amino acids on pak-choi shoot fresh (a) and dry (b) weights. Data shown are treatment means ($n = 6$). Bars labeled with the same letter(s) are not significantly different at $P \leq 0.05$ by LSD multiple range test. Ala = Alanine, Val = valine, Leu = leucine, Ile = isoleucine, Pro = proline, Phe = phenylalanine, Trp = tryptophane, Met = methionine, Asp = aspartic acid, Glu = glutamic acid, Lys = lysine, Arg = arginine, His = histidine, Gly = glycine, Ser = serine, Thr = threonine, Cys = cysteine, Tyr = tyrosine, Asn = asparagine, and Gln = glutamine.

These results indicated that different nitrogen sources for pak-choi had different effects on growth. This may be related to different chemical properties and functions of nitrate and individual amino acids in the nitrogen cycle. Nitrate increases production of physiologically active forms of cytokinins and cytokinins stimulate leaf growth (Rahayu *et al.*, 2005). Nitrate also functions as osmotica in vacuoles for cell extension (Marschner, 1995). In this research, a significantly ($P \leq 0.05$) positive relation ($r = 0.420$) between shoot fresh weights and shoot nitrate contents was also found, indicating that shoot nitrate concentrations had a positive effect on plant growth. The addition of amino acids to nutrient solutions may reduce nitrate uptake by plants (Muller and Touraine, 1992; Aslam *et al.*, 2001). The reduction of nitrate uptake may be the reason for the reduction of plant growth for most of the amino acid treatments. In addition, a significantly ($P \leq 0.05$) positive correlation ($r = 0.436$) was found between the shoot fresh weight and shoot K concentration in this study.

Gunes *et al.* (1994, 1996) working with winter onion and winter lettuce reported that replacing 20% of the nitrate with Gly or a mixture of amino acids did not affect fresh and dry weights. In another instance, appropriate rates of Trp and Met applied to soil had a positive impact on plant growth (Frankenberger *et al.*, 1990; Arshad and Frankenberger, 1990; Arshad *et al.*, 1995). The authors attributed this positive impact to the auxin and ethylene production in the soil or plant tissues, or a change in the rhizosphere's microflora that benefited plant growth. The possible reasons for the discrepancies between our results and the others may be the differences in plant species and culture media (hydroponic *vs.* soil) used for the experiments.

Nitrate accumulation in pak-choi shoots

Except for the treatments receiving Cys, Gly, His, and Arg, compared to the full nitrate treatment, all other amino acids significantly reduced ($P \leq 0.05$) nitrate concentrations in plant shoots (Fig. 2). The reduction was greatest in the Met treatment, which reduced nitrate concentration in plants 43% compared to the nitrate treatment. Asn and Gln, which for plant growth was not significantly different from the control, significantly reduced ($P \leq 0.05$) nitrate concentrations in pak-choi shoots (24% and 22%, respectively). However, Arg, which significantly increased ($P \leq 0.05$) plant growth, was not significantly different from the control for nitrate concentration in pak-choi shoots (Fig. 2).

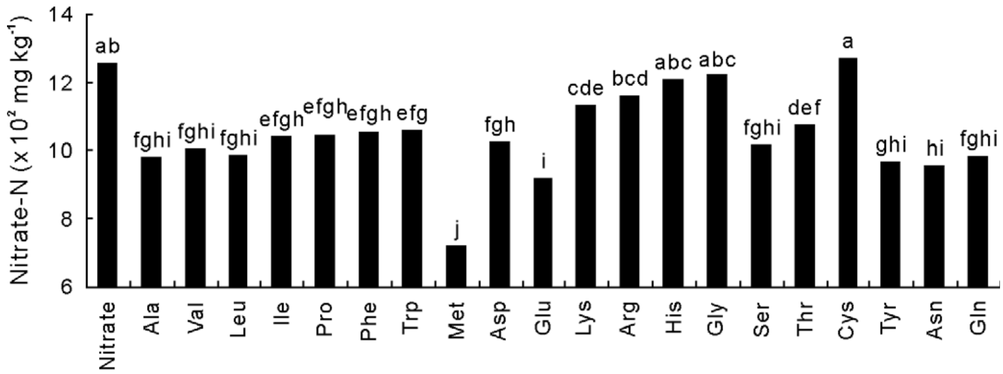

Fig. 2 Effects of replacing 20% of nitrate-N by amino acids on nitrate-N concentrations in fresh pak-choi shoots. Data shown are treatment means ($n = 6$). Bars labeled with the same letter(s) are not significantly different at $P \leq 0.05$ by LSD's multiple range test. Ala = Alanine, Val = valine, Leu = leucine, Ile = isoleucine, Pro = proline, Phe = phenylalanine, Trp = tryptophane, Met = methionine, Asp = aspartic acid, Glu = glutamic acid, Lys = lysine, Arg = arginine, His = histidine, Gly = glycine, Ser = serine, Thr = threonine, Cys = cysteine, Tyr = tyrosine, Asn = asparagine, and Gln = glutamine.

These results were consistent with those for winter onion (*Allium cepa* L.) and winter lettuce (*Lactuca sativa* L.) (Gunes *et al.*, 1994, 1996). The reduction of nitrate concentrations in plant tissues after addition of amino acids may result from reduction in nitrate uptake in plant roots (Muller and Touraine, 1992; Sivasankar *et al.*, 1997; Vidmar *et al.*, 2000; Aslam *et al.*, 2001). Vidmar *et al.* (2000) reported that amino acids inhibited expression of *HvNRT2* transcript in roots, thus blocking synthesis of the mRNA encoding nitrate transporter that is directly related to nitrate uptake. Alternatively, Aslam *et al.* (2001) discovered that induction of a nitrate transporter in the presence of amino acids may be normal, but the turnover of mRNA encoding the nitrate transporter may have increased. They also suggested that synthesis of mRNA may decrease when amino acids are supplied for plant growth.

N, P, and K concentrations in pak-choi shoots

Amino acids had different effects on N, P, and K concentrations in plant tissues. Compared to a full nitrate treatment, and except for the Cys, Leu, Pro, and Met treatments, the other amino acids significantly increased ($P \leq 0.05$) N concentrations in pak-choi shoots (8%–23%) (Table I). For P concentrations in plant shoots, Thr, Ser, and Asp treatments were not significantly different from the nitrate treatment; Tyr, Leu, and Val treatments significantly decreased ($P \leq 0.05$, 4%); and Pro, Met, Glu, Phe, Asn, Lys, Trp, Gly, Ala, His, Ile, Gln, Arg, and Cys significantly increased ($P \leq 0.05$, 5%–30%) (Table I). K concentrations in pak-choi shoots of the Gln, Phe, Ala, His, Lys, Ser, and Arg treatments were not significantly different from the nitrate treatment; Gly, Asn, Pro, and Trp treatments were significantly higher ($P \leq 0.05$, 10%–17%); and Thr, Cys, Asp, Glu, Ile, Leu, Met, Val, and Tyr treatments were significantly lower ($P \leq 0.05$, 6%–49%) (Table I).

TABLE I

Effects of replacing 20% of nitrate-N by amino acids on N, P, and K concentrations in pak-choi shoots

Treatment[a)]	N	P	K
	mg g^{-1}		
Nitrate	48.0ijk[b)]	7.9hi	81.7cd
Nitrate + Ala	55.0bcde	8.6ef	82.5c
Nitrate + Val	59.0a	7.6j	54.0j
Nitrate + Leu	48.6hij	7.6j	66.1i
Nitrate + Ile	53.3defg	8.6ef	71.1h
Nitrate + Pro	47.4jk	10.3a	92.1ab
Nitrate + Phe	52.6efg	9.0c	83.0c
Nitrate + Trp	54.1cdef	8.8de	89.4b
Nitrate + Met	45.4k	9.8b	65.7i
Nitrate + Asp	57.4ab	7.8ij	75.1fgh
Nitrate + Glu	51.5fgh	9.0c	73.0gh
Nitrate + Lys	53.9cdef	8.8de	80.7cde
Nitrate + Arg	54.0cdef	8.3g	77.6def
Nitrate + His	56.0abcd	8.6ef	81.9cd
Nitrate + Gly	55.0bcde	8.7ef	95.4a
Nitrate + Ser	54.1cdef	7.8hi	77.7def
Nitrate + Thr	56.8abc	8.0h	77.0efg
Nitrate + Cys	50.6ghi	8.3g	76.1efg
Nitrate + Tyr	53.7cdef	7.6j	41.5k
Nitrate + Asn	54.5bcdef	9.0cd	93.1ab
Nitrate + Gln	55.6bcde	8.6f	84.4c

[a)] Ala = alanine, Val = valine, Leu = leucine, Ile = isoleucine, Pro = proline, Phe = phenylalanine, Trp = tryptophane, Met = methionine, Asp = aspartic acid, Glu = glutamic acid, Lys = lysine, Arg = arginine, His = histidine, Gly = glycine, Ser = serine, Thr = threonine, Cys = cysteine, Tyr = tyrosine, Asn = asparagine, and Gln = glutamine.
[b)] Mean values followed by the same letter(s) within each column are not significantly different at $P \leq 0.05$ level by LSD multiple range test ($n = 6$).

The above results indicated that partially replacing nitrate in nutrient solutions with most amino acids increased nitrogen concentration in pak-choi shoots, which is consistence with Gunes *et al.* (1996). However, Gunes *et al.* (1994) found that amino acids partially replacing nitrate had no effect on nitrogen concentrations in plants. The discrepancies from different studies may be partially related to the different plant species used in the experiments.

CONCLUSIONS

Replacement of 20% of nitrate in nutrient solutions with pak-choi using each of 20 amino acids affected shoot growth, nitrate accumulation, and N, P, and K concentrations. Partially replacing nitrate with Asn and Gln significantly reduced nitrate concentrations in plants, but did not significantly reduce yield. Amino acid treatments also affected P and K concentrations in plants, but the effects differed depending on different amino acids. However, Asn and Gln treatments significantly increased N and P concentrations in pak-choi shoots. The study indicated that to improve crop quality, including reducing nitrates and increasing mineral nutrients in shoots, without significantly reducing crop yield, Gln and Asn may be used to partially replace nitrates in pak-choi hydroponic production.

REFERENCES

Arshad, M. and Frankenberger, W. T. Jr. 1990. Response of *Zea mays* L. and *Lycopersicon esculentum* to the ethylene precursors, L-methionine and L-ethionine applied to soil. *Plant and Soil.* **122**(2): 219–227.

Arshad, M., Hussain, A. and Shakoor, A. 1995. Effect of soil applied L-tryptophan on growth and chemical composition of cotton. *Journal of Plant Nutrition.* **18**(2): 317–329.

Aslam, M., Travis, R. L. and Rains, D. W. 2001. Differential effect of amino acid on nitrate uptake and reduction systems in barley roots. *Plant Science.* **160**(2): 219–228.

Cataldo, D. A., Haroon, M., Schrader, L. E. and Youngs, V. L. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communication in Soil Science and Plant Analysis.* **6**: 71–80.

Chapin III, F. S. 1995. New cog in the nitrogen cycle. *Nature.* **377**: 199–200.

Chen, W., Luo, J. K., Jiang, H. M. and Shen, Q. R. 2004. Effects of different NO_3^--N/NH_4^+-N ratios on the biomass and nitrate content of different cultivars of Chinese cabbages. *Acta Pedologica Sinica* (in Chinese). **41**(3): 420–425.

Dich, J., Järvinen, R., Knekt, P. and Penttilä, P. L. 1996. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. *Food Additives and Contaminants.* **13**(5): 541–552.

Feller, C. and Fink, M. 2004. Nitrate content, soluble solids content, and yield of table beet as affected by cultivar, sowing date and nitrogen supply. *HortScience.* **39**(6): 1 255–1 259.

Frankenberger, W. T. Jr., Chang, A. C. and Arshad, M. 1990. Response of *Raphanus sativus* to the auxin precusor, L-tryptophan applied to soil. *Plant and Soil.* **129**(2): 235–241.

Gunes, A., Inal, A. and Aktas, M. 1996. Reducing nitrate content of NFT grown winter onion plants (*Allium cepa* L.) by partial replacement of NO_3 with amino acid in nutrient solution. *Scientia Horticulturae.* **65**(2–3): 203–208.

Gunes, A., Post, W. N. K., Kirkby, E. A. and Aktas, M. 1994. Influence of partial replacement of nitrate by amino acid nitrogen or urea in the nutrient medium on nitrate accumulation in NFT grown winter lettuce. *Journal of Plant Nutrition.* **17**(11): 1 929–1 938.

Inal, A. and Tarakcioglu, C. 2001. Effects of nitrogen forms on growth, nitrate accumulation, membrane permeability, and nitrogen use efficiency of hydroponically grown bunch onion under boron deficiency and toxicity. *Journal of Plant Nutrition.* **24**(10): 1 521–1 534.

Kotsiras, A., Olympios, C. M., Drosopoulos, J. and Passam, H. C. 2002. Effects of nitrogen form and concentration on the distribution of ions within cucumber fruits. *Scientia Horticulturae.* **95**(3): 175–183.

Li, Y. K. 1983. Conventional Soil Chemical Nutrient Analysis Techniques (in Chinese). Science Press, Beijing. 457pp.

Liu, W., Li, S. J. and Chen, D. K. 2003. Use of amino acid nitrogen for growth by pak-choi under sterile culture. *Acta Horticulturae.* (627): 131–138.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Harcourt Brace and Company, London. 889pp.

Muller, B. and Touraine, B. 1992. Inhibition of NO_3^- uptake by various phloem-translocated amino acids in soybean seedlings. *Journal of Experimental Botany.* **43**(250): 617–623.

Nasholm, T., Ekblad, A., Nordin, A., Giesler, R., Hogberg, M. and Hogberg, P. 1998. Boreal forest plants take up organic nitrogen. *Nature.* **392**: 914–916.

Persson, J., Hogberg, P., Ekblad, A., Hogberg, M. N., Nordgren, A. and Nasholm, T. 2003. Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. *Oecologia.* **137**(2): 252–257.

Rahayu, Y. S., Walch-Liu, P., Neumann, G., Romheld, V., Wiren, N. and Bangerth, F. 2005. Root-derived cytokinins as long-distance signals for NO_3^--induced stimulation of leaf growth. *Journal of Experiment Botany.* **56**: 1 143–1 152.

Santamaria, P. and Elia, A. 1997. Producing nitrate-free endive heads: Effect of nitrogen form on growth, yield, and ion composition of endive. *Journal of the American Society for Horticultural Science.* **122**(1): 140–145.

Shen, M. Z., Zhai, B. J., Dong, H. R. and Li, J. G. 1982. Study on nitrate content of vegetables I. Estimate on nitrate and nitrite content of different vegetables. *Acta Horticulturae Sinica* (in Chinese). **9**(4): 41–48.

Sivasankar, S., Rothstein, S. and Oaks, A. 1997. Regulation of the accumulation and reduction of nitrate by nitrogen and carbon metabolites in maize seedlings. *Plant Physiology.* **114**(2): 583–589.

Tang, Q. Y. and Feng, M. G. 1997. DPS Data Processing System for Practical Statistics (in Chinese). China Agriculture Press, Beijing. 528pp.

Vidmar, J. J., Zhuo, D., Siddiqi, M. Y., Schjoerring, J. K., Touraine, B. and Glass, A. D. M. 2000. Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. *Plant Physiology.* **123**(1): 307–318.

Weigelt, A., King, R., Bol, R. and Bardgett, R. D. 2003. Inter-specific variability in organic nitrogen uptake of three temperate grassland species. *Journal of Plant Nutrition and Soil Science.* **166**(5): 606–611.

Zhu, Y. 2002. Tillage and corn-soybean rotation effects on nitrate leaching measured with passive capillary fiberglass wick and zero-tension pan lysimeters. Ph.D. Dissertation. The Pennsylvania State University, Univeristy Park, PA 16802. 208pp.