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

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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 ≤ 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 ≤ 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 ≤ 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 ≤ 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

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,
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$^{-1}$. 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 2000 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$_2$SO$_4$, 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$_2$SO$_4$-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).

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).

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.

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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.
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

<table>
<thead>
<tr>
<th>Treatment $^a$</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>48.0ijk $^b$</td>
<td>7.9 hi</td>
<td>81.7cd</td>
</tr>
<tr>
<td>Nitrate + Ala</td>
<td>55.0bcde</td>
<td>8.6 ef</td>
<td>82.5c</td>
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<tr>
<td>Nitrate + Val</td>
<td>59.0a</td>
<td>7.6j</td>
<td>54.0j</td>
</tr>
<tr>
<td>Nitrate + Leu</td>
<td>48.6hij</td>
<td>7.6j</td>
<td>66.1i</td>
</tr>
<tr>
<td>Nitrate + Ile</td>
<td>53.3defg</td>
<td>8.6ef</td>
<td>71.1h</td>
</tr>
<tr>
<td>Nitrate + Pro</td>
<td>47.4jk</td>
<td>10.3a</td>
<td>92.1ab</td>
</tr>
<tr>
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<td>52.6efg</td>
<td>9.0c</td>
<td>83.0c</td>
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<td>Nitrate + Trp</td>
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<td>8.8de</td>
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<tr>
<td>Nitrate + Met</td>
<td>45.4k</td>
<td>9.8b</td>
<td>65.7i</td>
</tr>
<tr>
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<td>57.4ab</td>
<td>7.8ij</td>
<td>75.1gh</td>
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<tr>
<td>Nitrate + Glu</td>
<td>51.5gh</td>
<td>9.0c</td>
<td>73.0gh</td>
</tr>
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<td>8.8de</td>
<td>80.7de</td>
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<td>8.3g</td>
<td>77.6def</td>
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<td>77.7def</td>
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<td>77.0efg</td>
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<tr>
<td>Nitrate + Gln</td>
<td>55.6bcde</td>
<td>8.6f</td>
<td>84.4c</td>
</tr>
</tbody>
</table>

$a$) 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**


