Response of Nitrogen Metabolism in Pak-choi Plants Treated with Different Sodium Selenate (Na$_2$SeO$_4$) Concentrations

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Currently, biofortification breeding programs are being conducted to increase the selenium (Se) content of crops. Se is an element essential to humans and is mostly acquired via consumption of vegetables. However, the effects of Se on the main plant metabolisms such as nitrogen metabolism are unclear. The main objective of this study was to determine the effect of the application of different concentrations of Se (10, 20, and 30 μM) as Na$_2$SeO$_4$ on nitrogen metabolism in Pak-choi grown hydroponically. Supplemental Se increased all enzyme activities analyzed, including nitrate reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT). In 20-μM Se treatment of two cultivars, the fresh weight was significantly increased and the foliar NO$_3^-$ content was significantly decreased. Compared to the control, the data on Se treatments showed stronger activations of NR, GS, and GOGAT, as well as greater concentrations of total ammonium, amino acid, and protein, and a higher nitrogen use efficiency, resulting in increased biomass production. This suggests the mechanism of Se applied to Pak-choi to decrease NO$_3^-$ content was due to increasing nitrogen metabolism and protein synthesis to biomass.

Key Words: biomass, enzyme, hydroponic, nitrate, nitrogen assimilation, nitrogen use efficiency.

Introduction

Food safety and health concerns have become an increasingly important focus for vegetables, in addition to pesticide residue and nitrate (NO$_3^-$) contents. According to the 2012 annual report of agriculture statistics, the total cultivation area of short-term leafy vegetables exceeds 104,000 ha, accounting for 68.8% of the total arable land in Taiwan. Pak-choi is one of the main leafy vegetables cultivated year-round. NO$_3^-$ is part of the nitrogen (N) cycle in nature (Santamaria, 2006), and its accumulation in plants differs among types and varieties owing to genetics (Martignon et al., 1994; Reinink et al., 1994). The accumulation of NO$_3^-$ is most obvious in leafy vegetables such as lettuce, spinach, celery, and Pak-choi.

There is a consensus that dietary nitrates are essentially inert and acquire biological activity. Nitrate is a natural constituent of the human diet and an approved food additive. It can be partially converted to nitrogen monoxide, which induces vasodilation and thereby decreases blood pressure. Animal studies have shown that dietary nitrates also dilate coronary arteries and protect them from ischemia and large infarction. Recent studies indicated that such beneficial health effects due to dietary nitrate may be achievable at intake levels resulting from the daily consumption of nitrate-rich vegetables (Habermeyer et al., 2015). However, NO$_3^-$ also poses a risk to human health because after ingestion, it is rapidly transformed into nitrite and N-nitroso compounds. These forms are toxic and can cause serious pathologies in humans, such as methemoglobinemia and blue-baby syndrome, or can magnify the risk of cancer because the nitrites are transformed into nitrosamines (Mensinga et al., 2003; Santamaria, 2006). Therefore, many organizations in Europe and the U.S., for example, the Joint Expert Committee of Food and Agriculture, the European Commission’s Scientific Committee on Food, and the U.S. Environmental Protection Agency, limit the NO$_3^-$ content in vegetables (Mensinga et al., 2003; Speijers and van den Brandt, 2003).

Crop yields largely depend on the quantity of N available in the growth medium (Lea and Azevedo, 2006) because approximately 30–40% of the N applied can be converted into crop yield by plants. Moll et al.
(1982) defined the nitrogen use efficiency (NUE) as the production of biomass with the available N in the medium. Currently, there is great interest in identifying the processes involved in the regulation of N uptake and metabolism within plants (Andrews et al., 2004), and one of the main aims of optimizing the NUE in plants for human consumption is to reduce the foliar NO$_3^-$ content.

Selenium (Se) has been deemed essential to animal nutrition since 1957, and humans require 50–70 μg of Se daily (U.S. Department of Agriculture, 2003). Dietary Se deficiency in humans is caused by the ingestion of plant foods with imperceptible concentrations of this element due to its low bioavailability in many crop soils (Pedrero et al., 2006; Smorklji et al., 2005). Biofortification is one method by which to solve this problem. Current projects such as those of Chen et al. (2002) on wheat varieties and, more recently, of Pedrero et al. (2006) on radishes, have shown that fertilization with Se increases the content of this trace element in plants; all these projects focused on potential increases in daily Se ingestion in humans.

As mentioned above, many studies have focused on Se with the aim of boosting its intake in humans through plant consumption (Cartes et al., 2005; Pedrero et al., 2006). However, very few of these studies analyzed the impact of this element on plant physiology. Ruiz et al. (2007) demonstrated that Se affects plant N metabolism in the lettuce by reducing the NO$_3^-$ content. Supplemental Se can increase the yield of Chinese cabbage, as well as the contents of Se, Vitamin C, soluble sugar, and soluble protein, while decreasing the content of NO$_3^-$ (Zhang et al., 2013). Therefore, the aim of the present study was to determine whether the NUE and foliar NO$_3^-$ content are affected by the application of Se to Pak-choi.

**Materials and Methods**

*Plant material and growing conditions*

Hydroponic experiment: Pak-choi ([Brassica chinensis](https://en.wikipedia.org/wiki/Brassica_chinensis) L. ‘Golden yellow’ (‘GY’) and ‘Four season’ (‘FS’)) seeds were sown and grown for 10 days in sponges (size, 30 cm × 20 cm × 2 cm) saturated with nutrient solution of a 1/4 concentration (Rios et al., 2010). The 10-day-old seedlings were transferred to plastic vessels (35-cm upper diameter, 20-cm lower diameter, 10-cm high), with each vessel containing six seedlings. The seedlings were planted in an experimental growth chamber at National Chung Hsing University (Taichung, Taiwan).

The environmental conditions in the growth chamber were as follows: relative humidity of 70–85%, temperature of 26°C/17°C (day/night), and a 12-h photoperiod under a high-luminosity LED T8 tube (SW-L20WH-120HE; Light Torch Technology, Taiwan) with a 1000 ± 200 μmol·m$^{-2}$·s$^{-1}$ photosynthetic photon flux density (measured from the top of the plants using an LI-250A quantum sensor). Until the end of the experiment, the plants received a growth solution containing 4-mM Ca(NO$_3^-$)$_2$, 6-mM KNO$_3$, 2-mM MgSO$_4$·7H$_2$O, 1-mM Na$_2$HPO$_4$·2H$_2$O, 50-μM H$_3$BO$_3$, 2-μM MnCl$_2$·4H$_2$O, 1-μM ZnSO$_4$·7H$_2$O, 0.1-μM Na$_2$MoO$_4$·2H$_2$O, 0.25-μM CuSO$_4$·5H$_2$O, and 10-μM Fe-ethylenediaminetetraacetic acid (EDTA) (Rios et al., 2010). The nutrient solution (pH 5.5–6.0) was renewed every 7 days. SeO$_4$ was applied at different concentrations (10, 20, or 30 μM) as Na$_2$SeO$_4$ (Panreac, Spain) to the nutrient solution. The experimental design was a randomized complete block with three treatments. Each treatment was administered to six plants and replicated three times.

*Plant sampling*

Pak-choi leaves were sampled 28 days after germination. Leaf samples were standardized using only fully-expanded leaves taken from the middle parts of the plants in each replicate. The material was rinsed three times with distilled water and then blotted on filter paper. The plant material was used to determine the biomass [fresh weight (FW)], NO$_3^-$ concentration, and enzymatic activities [nitrate reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT)]. The rest of the plant material was dried at 70°C for 3 days to determine the dry weight (DW) and total N content, as well as the ammonium, amino acid, and protein contents. These parameters define the NUE (as described below).

*Plant analysis*

NO$_3^-$ was analyzed from an aqueous extract of 0.2 g of fresh and ground leaf material in 10 mL of Millipore-filtered water. A 100-μL aliquot was taken for determination of NO$_3^-$ and was added to 10% (w/v) salicylic acid in sulfuric acid (96%). Then, the NO$_3^-$ content was measured with a spectrophotometer (U-2900; Hitachi High-Technologies Co., Japan) following the method of Cataldo et al. (1975). The results were expressed and recorded as mg·kg$^{-1}$ FW. The NH$_4^+$, amino acid, and protein contents were determined using the methods of Krom (1980), Rosen (1957), and Lowry et al. (1951), respectively.

The plant Se content was determined by researchers at the National Chung Hsing University by inductively-coupled plasma mass spectrometry (Elan DRC II; PerkinElmer, Inc., USA).

*Enzyme extractions and assays*

Leaves were ground in a mortar at 0°C in 50 mM of KH$_2$PO$_4$ buffer (pH 7.5) containing 2 mM of EDTA, 15 g·L$^{-1}$ of soluble casein, 2 mM of dithiothreitol, and 10 g·L$^{-1}$ of insoluble polyvinylpolypyrrolidone. The homogenate was filtered and then centrifuged at 30000 × g for 20 min. The extract (cytosol and organelle fractions) was used to measure enzymatic activities. The extraction medium was optimized for the enzymat-
ic activities so that all enzymes could be extracted jointly using the same method.

The NR assay followed the method of Kaiser and Lewis (1984). In a final volume of 2 mL, the reaction mixture contained 100 mM of KH$_2$PO$_4$ buffer (pH 7.5), 100 mM of KNO$_3$, 10 mM of cysteine, 2 mM of nicotinamide adenine dinucleotide (NADH), and enzyme extract. Incubation was performed at 30°C for 30 min and stopped by adding 1 M of zinc acetate. The nitrite formed was determined calorimetrically at 540 nm after coupling with sulfanilamide and naphthylethlenediamine dihydrochloride according to the method of Hageman and Huckleby (1971).

GS activity was measured by the hydroxamate synthetase assay adapted from the study of Kaiser and Lewis (1984). The reaction mixture contained 100 mM of KH$_2$PO$_4$ buffer (pH 7.5), 4 mM of EDTA, 1 M of L-sodium glutamate, 450 mM of MgSO$_4$·7H$_2$O, 300 mM of hydroxylamine, 100 mM of adenosine triphosphate, and enzyme extract. Two controls were prepared, one without glutamine and the other without hydroxylamine. Following 30 min of incubation at 28°C, the formation of glutamylhydroxamate was determined calorimetrically at 540 nm after complexing with acidified ferric chloride.

GOGAT activity was measured spectrophotometrically (U-2900; Hitachi High-Technologies Co.) at 30°C by monitoring the oxidation of NADH at 340 nm, which was essentially as indicated by Groat and Vance (1981) and Singh and Srivastava (1986), and always within 2 h of extraction. The reaction mixture contained 50 mM of KH$_2$PO$_4$ buffer (pH 7.5), 1 mM of mercaptoethanol, 1 mM of EDTA, 18.75 mM of 2-oxoglutarate, 75 mM of L-glutamine, and enzyme extract. Control tubes were simultaneously incubated with all reagents, except for the substrates (2-oxoglutarate and L-glutamine) in order to correct for endogenous NADH oxidation. The decrease in absorbance (which was linear for at least 10 min) was recorded for 5 min.

Calculation of the nitrogen use efficiency

To determine the total reduced N, a sample of 0.1 g of DW was digested with sulfuric acid and H$_2$O$_2$. After dilution with deionized water, a 1-mL aliquot of the digest was added to the reaction medium containing a buffer [5% potassium sodium tartrate, 100 μM of sodium phosphate, and 5.4% (w/v) sodium hydroxide], 15% (w/v) sodium silicate, 0.03% (w/v) sodium nitroprusside, and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37°C for 15 min, and the total reduced N was measured by spectrophotometry (U-2900; Hitachi High-Technologies Co.) according to the method of Baethgen and Alley (1989).

The total plant nitrogen accumulation (TNA; expressed in mg N) was calculated using both the total nitrogen content (TNC) and the DW of all leaves (Sorgona et al., 2006) as follows:

\[ \text{TNA} = \text{TNC} \times \text{DW of all leaves} \]

TNC was assumed to represent the sum of the organic N and NO$_3^-$ contents. The NUE and nitrogen transport efficiency (NTE) were calculated as follows:

\[ \text{NUE} = \frac{\text{DW/N taken up per unit}}{} \]

\[ \text{NTE} = \frac{\text{N accumulation in the shoot/TNA in the whole plant}}{} \]

Statistical analysis

All data were subjected to analysis of variance, and significant differences in the means were evaluated using Fisher’s least significant difference test (Fisher’s LSD test) or the Student’s t-test. Data are presented as the mean ± standard error (n = 3).

Results

Shoot biomass differed significantly with different Se treatments (Fig. 1). The FW was significantly increased under 20-μM Se treatment, the FW of the ‘GY’ and ‘FS’ increasing by 24.1% and 15.5%, respectively. However, the FW of both the ‘GY’ and ‘FS’ decreased under 30-μM treatment, indicating that intermediate concentrations of Se are less toxic to plants because the plants grew better with 20-μM Se treatment than with
any other concentration.

The leaf blade NO$_3^-$ content was lower in the plants treated with 20-μM Se than in the control (Table 1). With this treatment, the leaf blade NO$_3^-$ content decreased in the ‘GY’ and ‘FS’ by 14.5% and 6.1%, respectively. This concentration of Se also induced enzymatic activity of NR, explaining the reduction in NO$_3^-$ . In general, the level of NO$_3^-$ was lower in the Se-treated plants than in the controls.

Se treatment increased the enzymatic activity, including NR, GS, and GOGAT (Table 1; Fig. 2). GS activity increased as the Se concentration increased, the highest activity being observed with 20-μM Se treatment. In addition, GOGAT activity was also affected by this trace element, the activity increasing with increasing Se concentration.

The NH$_4^+$ , amino acid, and protein contents increased significantly in the plants treated with 10-μM and 20-μM Se (Table 2), but not with 30-μM Se. The main amino acid groups identified in the Pak-choi included aspartic acid, asparagine, glutamic acid, arginine, threonine, alanine, and valine (Table 3), the levels of all of which increased in the ‘GY’ and ‘FS’. When Se was applied, the TNA and NUE$_{shoot}$ increased, but the NUE$_{root}$ decreased (Table 4). These measurements were in agreement with the biomass results (Fig. 1), in that the biomass increased in plants treated with 20-μM Se. This result indicated that this trace element can improve the NUE and, in turn, biomass production, in Pak-choi plants. The Se content was 4.67–4.89 ppm in the hydroponically-grown plants of two cultivars treated with 20 μM Se in a while the amount of Se in plants without Se treatment was undetectable (0 ppm). The two cultivars assimilated Se similarly.

Discussion

Although there are several factors that affect Pak-choi growth, N is the most important element in terms of the effect on the yield of this crop. For cultivation, which is highly dependent on N availability, apart from the NO$_3^-$ and reduced N contents, it is important to determine the NUE to assess the need for nitrogenous fertilizers (Lawlor, 2002). The NO$_3^-$ content is important in plants grown for human consumption because a high intake of this nutrient can be harmful to human health (Santamaria, 2006), and a high NO$_3^-$ content diminishes the quality of the products obtained.

Rios et al. (2010) reported that Se affected plant N metabolism; however, application of an optimal amount of Se increased the enzymatic activities of GS and GOGAT in lettuce plants, and thereby improved the

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Se concentration (μM)</th>
<th>Nitrate content (mg·kg$^{-1}$)</th>
<th>NR activity (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blade</td>
<td>Petiole</td>
<td>Whole plant</td>
</tr>
<tr>
<td>‘GY’</td>
<td>0</td>
<td>2476 a</td>
<td>6708 a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2250 a</td>
<td>6600 a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1738 b</td>
<td>6244 b</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1856 b</td>
<td>5842 c</td>
</tr>
<tr>
<td>‘FS’</td>
<td>0</td>
<td>2263 a</td>
<td>5550 a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1950 b</td>
<td>5225 ab</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1978 b</td>
<td>5450 a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1718 c</td>
<td>5150 b</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences among treatments at $P<0.05$ by Fisher’s LSD test (n = 3).
The amino acid and protein contents were both elevated (Table 2), and the leaf blade NO$_3^-$ (Table 1). In addition, the biomass of Pak-choi increased in enzymatic activity and improvement in NUE (Blasco et al., 2008; Moran et al., 1994). We found that Pak-choi plants under 30-μM Se treatment showed a negative impact on plant growth and development, and could also generate reactive oxygen species, which damage a number of macromolecules and cell structures. Numerous studies have reported inter- and intra-species differences in Se accumulation (Barberon et al., 2008; Terry et al., 2000; White et al., 2007). Typical Se concentrations in field-grown hyper-accumulators range from 1000 to 10000 mg·kg$^{-1}$ DW, whereas in non-accumulators the concentrations are usually <20 mg·kg$^{-1}$ (Galeas et al., 2007). Pak-choi is a non-accumulator because it does not readily accumulate excess Se. Therefore, the application of 20-μM Se by hydroponic culture not only improved the protein and amino acid contents, but also increased the Se content of Pak-choi without reaching toxic levels.

### Table 2. NH$_4^+$, amino acid, and protein contents in two cultivars of Pak-choi [Brassica chinensis L. ‘Golden yellow’ (‘GY’) and ‘Four season’ (‘FS’)] treated with different concentrations of Se.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Se concentration (μM)</th>
<th>NH$_4^+$ (mg·g$^{-1}$ DW)</th>
<th>Amino acid (μmol alanine·g$^{-1}$ FW)</th>
<th>Protein (mg·g$^{-1}$ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘GY’</td>
<td>0</td>
<td>2.05 b$^*$</td>
<td>25.5 c</td>
<td>9.17 b</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.31 a</td>
<td>33.3 a</td>
<td>11.28 a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.29 a</td>
<td>31.1 a</td>
<td>11.04 a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.09 b</td>
<td>28.3 b</td>
<td>9.33 b</td>
</tr>
<tr>
<td>‘FS’</td>
<td>0</td>
<td>2.15 b</td>
<td>23.7 c</td>
<td>9.32 b</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.48 a</td>
<td>26.5 b</td>
<td>14.31 a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.46 a</td>
<td>31.2 a</td>
<td>13.88 a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.26 ab</td>
<td>29.6 a</td>
<td>11.47 b</td>
</tr>
</tbody>
</table>

$^*$ Different letters indicate significant differences among treatments at $P<0.05$ by Fisher’s LSD test (n = 3).

### Table 3. Amino acid contents in two cultivars of Pak-choi [Brassica chinensis L. ‘Golden yellow’ (‘GY’) and ‘Four season’ (‘FS’)] treated with Se.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Se concentration (mg/100 g)</th>
<th>Threonine (mg/100 g)</th>
<th>Serine (mg/100 g)</th>
<th>Alanine (mg/100 g)</th>
<th>Valine (mg/100 g)</th>
<th>Aspartic acid (mg/100 g)</th>
<th>Asparagine (mg/100 g)</th>
<th>Glutamic acid (mg/100 g)</th>
<th>Arginine (mg/100 g)</th>
<th>Lysine (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘GY’</td>
<td>0</td>
<td>112.0$^*$</td>
<td>104.3</td>
<td>243.3</td>
<td>129.4</td>
<td>112.3$^*$</td>
<td>114.2</td>
<td>37.4</td>
<td>117.8</td>
<td>79.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>130.7$^{**}$</td>
<td>92.9</td>
<td>273.6$^*$</td>
<td>142.45$^*$</td>
<td>106.9</td>
<td>209.93$^{**}$</td>
<td>57.14$^{**}$</td>
<td>123.9</td>
<td>82.3</td>
</tr>
<tr>
<td>‘FS’</td>
<td>0</td>
<td>111.1</td>
<td>67.5</td>
<td>312.2</td>
<td>137.7</td>
<td>72.6</td>
<td>89.3</td>
<td>80.3</td>
<td>103.2</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>151.1$^*$</td>
<td>80.6</td>
<td>387.7$^{**}$</td>
<td>168.0$^{**}$</td>
<td>113.3$^*$</td>
<td>159.1$^{**}$</td>
<td>145.0$^{**}$</td>
<td>154.3$^{**}$</td>
<td>92.6</td>
</tr>
</tbody>
</table>

$^*$ Data are the means of 3 replications.

### Table 4. Specific parameters of the NUE in two cultivars of Pak-choi [Brassica chinensis L. ‘Golden yellow’ (‘GY’) and ‘Four season’ (‘FS’)] treated with Se.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Se concentration (μM)</th>
<th>N concentration (%)</th>
<th>TNA</th>
<th>NUEs</th>
<th>NU/Er</th>
<th>NTE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘GY’</td>
<td>0</td>
<td>3.33$^*$</td>
<td>2.87</td>
<td>120.2</td>
<td>108.4</td>
<td>240.4$^*$</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.25</td>
<td>2.55</td>
<td>136.1$^*$</td>
<td>137.1$^{**}$</td>
<td>223.1</td>
</tr>
<tr>
<td>‘FS’</td>
<td>0</td>
<td>3.49</td>
<td>3.02</td>
<td>143.4</td>
<td>117.8</td>
<td>270.6$^{**}$</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.33</td>
<td>2.85</td>
<td>151.2$^*$</td>
<td>136.3</td>
<td>212.9</td>
</tr>
</tbody>
</table>

$^*$ Data are the means of 3 replications.

* *, **: significantly different at $P<0.05$ or $P<0.01$ by Student’s t-test.
N is taken up by plants primarily in the form of NO$_3^-$ (Barneix, 2007). Once transported to the leaves, this anion is stored in the vacuoles and/or is assimilated into products such as amino acids and proteins required for biomass formation (Ruiz and Romero, 1999). The reduction of NO$_3^-$ produces NO$_2^-$, which is catalyzed by NR. In addition, NH$_4^+$ is transformed into organic nitrogenous compounds by the GS/GOGAT enzymatic cycle, followed by ammonium assimilation into amino acids (Sivasankar and Oaks, 1996). Moreover, there are few reports on the influence of Se application on N metabolism. For example, Rios et al. (2010) reported that the application of Se induced activities of NR, GS, and GOGAT, as well as leading to a greater concentration of total reduced N in lettuce plants. These results could be explained by the coordination between N and S metabolisms. Thus, the application of Se induces assimilation of S and Se. In this way, it stimulates the metabolism of N with the aim of maintaining a proper equilibrium between nitrogenous organic compounds and sulfates (Rios et al., 2008).

In this study, the lower NO$_3^-$ content of the Pak-choi plants observed after Se application may be explained in two ways (Table 1). First, there may be an antagonistic effect of Se root uptake or an inhibitory effect of Se on the root transporters of NO$_3^-$. Second, NO$_3^-$ stimulating the expression of the NR gene appears to be the primary signal for induction. The induction of NO$_3^-$ assimilation by NR was stimulated by the application of selenate (Aslam et al., 1990). In this study, results also showed activations of NR, GS, and GOGAT, as well as increasing concentrations of total ammonium, amino acid, and protein (Fig. 2; Table 2), resulting in increased biomass production (Fig. 1) under Se treatment. However, many detailed mechanisms about how Se affects various enzymes are still unclear, and further investigation is required.

Conclusion
Se increased N metabolism by increasing the activities of NR, GS, and GOGAT enzymes, along with increasing the content of total reduced N in two cultivars of Pak-choi. According to the different parameters analyzed in this study, Se application affected the NUE in the Pak-choi plants and reduced the NO$_3^-$ uptake, thereby lowering the NUE$_{root}$ and the NO$_3^-$ content. In addition, the application of Se increased N utilization, reflected by an increase in the NUE$_{shoot}$ and coinciding with elevated biomass production. The application of 20-μM Se would be highly beneficial for the cultivation of Pak-choi because in addition to lowering the NO$_3^-$ content, it significantly improves the NUE.

Literature Cited
Ruiz, J. M., R. M. Rivero and L. Romero. 2007. Comparative effect of Al, Se and Mo toxicity on NO\textsubscript{3}\textsuperscript{−} assimilation in sunflower (Helianthus annuus L.) plants. J. Environ. Manage. 83: 207–212.