African Journal of Biotechnology Vol. 10(55), pp. 11483-11487, 21 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1480
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Enhanced glutathione peroxidases (GPx) activity in young barley seedlings enriched with selenium

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Accepted 27 July, 2011

To assess whether young barley seedlings could be used as a selenium-enriched food material, a field experiment was carried out. Barley seeds were soaked in aqueous solutions at 20°C for 24 h. The solutions contained selenium in the form of sodium selenite and were at 0, 10, 20 and 30 µg/ml, respectively. Then the seeds were sowed in a non-selenium-fertilized field. Seedlings were harvested 2, 3, 4, and 5 weeks after sowing. Results show that selenium treatment did not affect (P>0.05) the biomass yield, height and stem diameter of young barley seedlings but significantly increased (P<0.05) selenium content and enhanced chlorophyll accumulation and glutathione peroxidases (GPx) activity in young barley seedlings. This study indicates that the method of soaking seeds in selenium solutions was feasible in enhancing selenium content in young barley seedlings which could be used as a selenium-enriched food material.

Key words: Selenium, young barley seedlings, growth, chlorophyll, glutathione peroxidases.

INTRODUCTION

Selenium (Se) is an essential trace element for human beings. Se deficiency is associated with some diseases and generally impairs the immune system. The recommended dietary allowance of Se is 55 µg per day for healthy human adults. However, Se content in foods is relatively low and fails to meet the daily dietary requirement. It is estimated that there are 500 to 1000 million Se-deficient people in the world (Combs, 2001). In China, Keshan disease and Kashin-Beck disease in the population have been associated with a relatively low Se status caused by low-Se-food diets. It is necessary to try to increase people's Se intake, and this problem is effectively solved by increasing Se in food. Therefore, Se-enriched plant products have been investigated (Fang et al., 2008; Hu et al., 2002; Smrkolj et al., 2007; Stibilj et al., 2004), but no studies have been done on Se-enriched young barley seedlings to our knowledge. Barley (*Hordeum vulgare* L.) is one of the widely consumed cereal crops.

Traditionally, almost 80 to 90% of barley productions have been used for animal feeds and beer malts. Recently, young barley seedlings have been used as food material for people in Asian countries such as China, Japan, and Korea. Young barley seedlings are rich in dietary fiber, chlorophyll, carotene, vitamins, and such mineral elements as calcium, potassium, iron, and zinc. They also contain some physiologically beneficial enzymes, such as superoxide dismutase, cytochrome oxidase, lipase, protease, catalase, etc. If the Se content in young barley seedlings could be enriched, it will be helpful in solving the Se deficiency problem mentioned earlier. Therefore, this study was to investigate the effect of Se treatment on growth of young barley seedlings, Se and chlorophyll contents and GPx activity in young barley seedlings, which would determine whether young barley seedlings could be used as a type of Se-enriched food material.

MATERIALS AND METHODS

Preparation of Se-enriched young barley seedlings

Barley seeds (variety: gang-2) were soaked in solutions at 20°C for 24 h. The solutions contained Se in the form of sodium selenite at concentrations of 0 (as control), 10, 20, and 30 µg/ml, respectively.

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Table 1. Effect of Se concentration on biomass yield, height and stem diameter of young barley seedlings.

Na_2SeO_3 (μg/ml)	Week after sowing											
	Biomass yield (g/100 plants DW)				Height of seedling (cm)				Stem diameter (mm)			
	2	3	4	5	2	3	4	5	2	3	4	5
0	4.21 (0.36)	8.55 (0.65)	14.25 (1.34)	24.39 (1.98)	14.26 (1.32)	18.71 (2.09)	26.09 (2.04)	40.22 (3.51)	2.08 (0.12)	2.96 (0.15)	3.97 (0.21)	4.70 (0.23)
10	4.32 (0.41)	7.64(080.)	14.77 (0.98)	22.14 (2.08)	13.77 (1.45)	16.87 (1.97)	24.15 (1.99)	38.97 (4.12)	2.13 (0.09)	2.88 (0.15)	3.92 (0.22)	4.87 (0.35)
20	4.09 (0.29)	9.05 (0.77)	15.01 (1.28)	25.61 (2.61)	15.21 (1.29)	18.93 (2.13)	27.09 (2.01)	41.09 (3.19)	2.09 (0.16)	2.79 (0.19)	3.85 (0.30)	4.66 (0.41)
30	4.19 (0.32)	8.63 (0.81)	14.92 (1.01)	25.92 (2.29)	14.09 (1.17)	17.84 (1.53)	25.51 (2.18)	39.11 (4.06)	2.14 (0.11)	3.06 (0.21)	4.01 (0.26)	4.78 (0.31)

The results are expressed as mean ± SD (in parentheses) with three replications. Barley seeds were soaked in solutions at 20°C for 24 h with different concentrations of selenite.

The seeds were then sown in a field never amended with Se-containing fertilizers. Field experiment was designed by randomized complete blocks with three replications, 3 × 2 m of each plot. The total Se content of the top soil (30 cm) was 0.17 µg/g, of which the water-soluble Se was 0.014 µg/g. The experiment was carried out in Taizhou City (119°54' N, 32°29' E), Jiangsu Province, China. Young barley seedlings were harvested 2, 3, 4, and 5 weeks after sowing.

Biomass measurement

Biomass production was represented by the dry weight yield of seedlings. 100 young barley seedlings (including stems and leaves, but not the under-ground parts which were not consumed) were harvested from each plot. After drying at 55 ± 1°C for 48 h, the dry weight of samples was measured.

Total Se and protein-bound Se determination

The total Se (T-Se) and protein-bound Se (PB-Se) contents of Se-enriched young barley seedlings were determined according to the method reported by Liu and Gu (2009). Briefly, the fresh leaves were cut into 2 to 3 cm in length and dried for 24 h by a freeze-drying system (Labconco, USA). 1 g of the sample was digested with 5 ml of a mixture of HNO_3 and $HClO_4$ (v/v, 4:1) at 130°C for 1 h. After cooling, 5 ml of concentrated HCl was added and incubated at 115°C for 20 min. A reflux condensation equipment was employed during digestion to avoid the volatilization loss of Se. After digesting, the solution was cooled down to room temperature and transferred into a volumetric flask and made up to 50 ml with ultrapure water. Then, the digested solution was used for total Se determination by hydride generation of atomic fluorescence spectrometer (HG-AFS) method. For the PB-Se determination, the semipermeable membrane device (SPMD) technique and the enzymatic hydrolysis (protease XIV, Sigma) were used (Liu and Gu, 2009).

Chlorophyll determination

1 g of the fresh leaves was added with 4 ml of pre-cooled 80% acetone solution and ground. The homogenized sample was centrifuged (4°C) at 10000 rpm for 10 min. The supernatant was diluted to 100 ml with 80% acetone to extract chlorophyll. The chlorophyll content was measured using a UV/VIS Spectrometer System (Unico, UV-2800, USA) according to Arnon (1949).

GPx activity determination

The GPx (EC 1.11.1.9) activity was examined with the method described by Hartikainen et al. (2000).The total soluble proteins of the enzyme extract were determined spectrophotometrically by the method described by Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

All trials were carried out in triplicate and all the data were given as means ± standard deviation (SD). The statistical significance was evaluated using Duncan's multiple-range test and $P<0.05$ was taken as significant.

RESULTS AND DISCUSSION

Growth of young barley seedlings

The data in Table 1 showed that Se treatments had no significant effect ($P>0.05$) on biomass yield, height and stem diameter of young barley seedlings from 2 to 5 weeks after sowing. These results indicate that the concentrations of selenite ranging from 0 to 30 µg/ml did not inhibit the growth of young barley seedlings. Similar results were observed and previously reported in lettuce (Xue et al., 2001).

Se content in young barley seedlings

Se is apparently not required for growth by a majority of higher plants. In fact, high content of Se in the tissues of plants may result in a toxic effect. However, a small number of plants which are called Se hyper-accumulators are able to accumulate extraordinarily high levels of Se. This study shows that young barley seedlings had a relatively high capacity to accumulate Se. As shown in Table 2, Se contents in young barley

Table 2. Accumulation of T-Se and PB-Se contents in young barley seedlings.

Na_2SeO_3 (µg/ml)	Week after sowing							
	T-Se (µg DW)				PB-Se (%)			
	2	3	4	5	2	3	4	5
0	0.04[a] (0.02)	0.05[a] (0.02)	0.04[a] (0.02)	0.07[a] (0.03)	96.0[a] (2.9)	93.6[a] (6.6)	94.1[a] (7.1)	94.3[a] (5.9)
10	0.22[b] (0.04)	0.23[b] (0.06)	0.27[b] (0.06)	0.20[b] (0.05)	81.1[b] (7.0)	86.9[ab] (6.0)	91.1[a] (6.1)	93.5[a] (5.0)
20	0.32[c] (0.04)	0.42[c] (0.06)	0.43[c] (0.05)	0.31[c] (0.04)	81.7[b] (9.2)	89.6[ab] (5.4)	82.1[a] (5.4)	88.4[a] (7.9)
30	0.51[d] (0.09)	0.59[d] (0.09)	0.61[d] (0.11)	0.48[d] (0.08)	68.4[c] (6.1)	79.2[b] (8.6)	84.4[a] (8.6)	86.5[a] (7.7)

The results are expressed as mean ± SD (in parentheses) with three replications. Within the same column, means followed by the same superscript letter are not significantly different (P>0.05). Barley seeds were soaked in solutions at 20°C for 24 h with different concentrations of selenite.

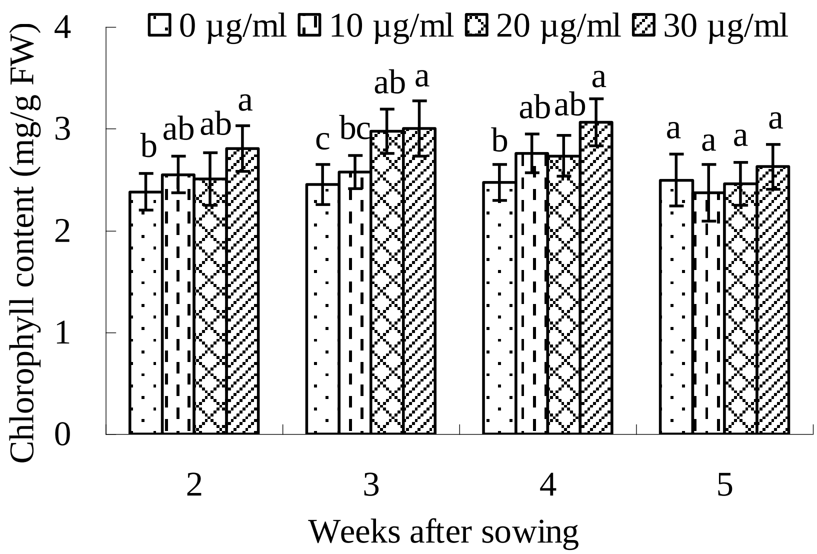

Figure 1. Effect of Se treatment on chlorophyll content (mg/g FW) in young barley seedlings. The results are expressed as mean ± SD with three replications. Columns marked with same letter are not significant at P>0.05. Barley seeds were soaked in solutions at 20°C for 24 h with different concentrations of selenite.

seedlings increased significantly (P<0.05) as the selenite concentration supplied in the soaking solutions increased from 0 to 30 µg/ml. The results were in consistence with those obtained in Se-enriched rice (Hu et al., 2002). In addition, the ratio of PB-Se to T-Se in young barley seedlings increased as the seedlings grew longer (Table 2). 5 weeks after sowing, the proportion of PB-Se to T-Se was ranged from 83 to 93% when treated with various concentrations of selenite. This indicated that the transformation of selenite to PB-Se species for young barley seedlings was rapid and effective.

Chlorophyll content in young barley seedlings

Figure 1 shows chlorophyll contents of young barley seedlings at various Se addition levels and in different growth stages. The effect of Se treatment on chlorophyll content was related to the Se concentration and the growth period of young barley seedlings. A lower Se level of 10 µg/ml did not affect chlorophyll content (P>0.05) at all the growth stages. However, at a higher Se dose of 30 µg/ml, a significant increase (P<0.05) of chlorophyll content in young barley seedlings was detected from 2 to 4 weeks after sowing. Based on the Se content in young barley seedlings (Table 2), the results suggest that Se promoting influence on chlorophyll content was dependent on its content in seedlings. This may be because Se could stimulate the respiration rates and the flow of electrons in respiratory chain and accelerated the chlorophyll biosynthesis (Germ et al., 2005).

GPx activity in young barley seedlings

The activity of GPx in different growth periods is shown in

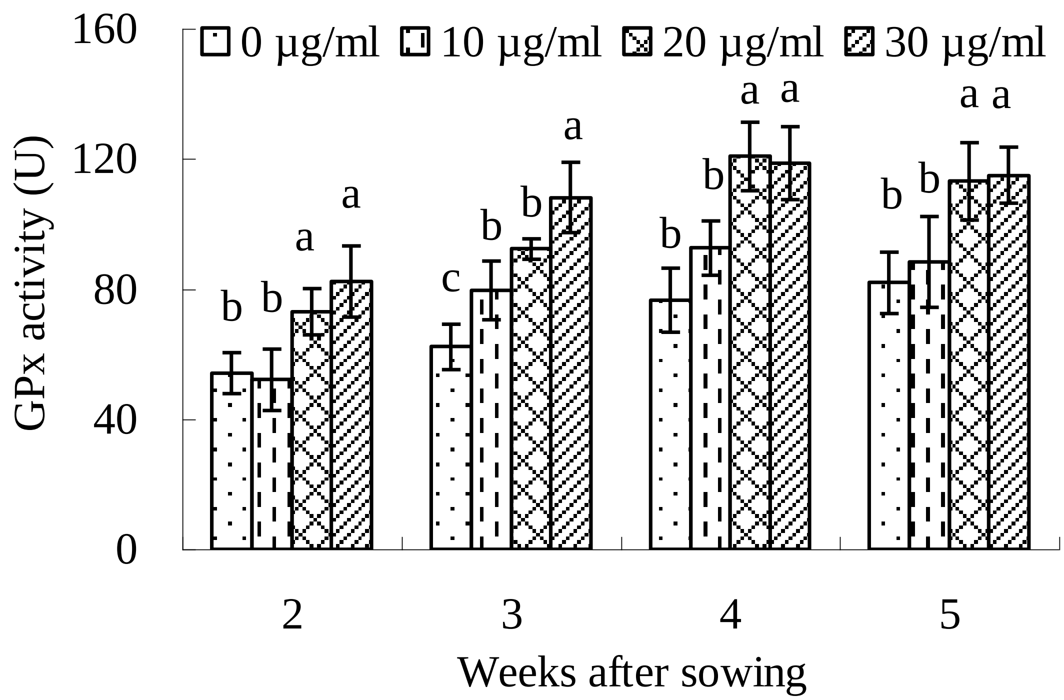

Figure 2. Effect of Se treatment on chlorophyll content (mg/g FW) in young barley seedlings. The results are expressed as mean ± SD with three replications. Columns marked with same letter are not significant at P>0.05. Barley seeds were soaked in solutions at 20°C for 24 h with different concentrations of selenite. One unit (U) of the enzyme activity was calculated as the amount of enzyme catalyzing the conversion of 1 µmol of substrate per min as compared to that in the non-enzyme reaction.

Figure 2. At all the four harvest time points, the GPx activity was found to be increasing ($P<0.05$) with higher Se concentration supplied in the soaking solutions, which suggested that Se treatment had a positive effect on the enzyme activity. GPx is one of the main hydrogen peroxide scavenging enzymes in higher plants. Though, Se was not thought to be an essential element for higher plants, increasing findings revealed that Se could increase the tolerance of plants to oxidative stress (Hartikainen et al., 2000), as well as enhanced the growth of lettuce (Xue et al., 2001) and ryegrass (Hartikainen et al., 2000). In fact, all the aforementioned growth-promoting functions of Se were related to the improved Se-dependent GPx activity.

Conclusions

This study investigates the effects of Se treatment on growth, Se and chlorophyll contents and GPx activity in young barley seedlings. The results show that Se treatment did not affect the growth of young barley seedlings, but it significantly increased the chlorophyll content and enhanced GPx activity. Young barley seedlings possessed a relatively high capacity of Se accumulation and transformation. Se treatment might help young barley seedlings to become a Se-enriched food material.

ACKNOWLEDGEMENTS

This work was supported by the Doctor Research Fund of Henan University of Technology (No. 2010BS051).

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