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UPTAKE AND DISTRIBUTION OF SELENIUM, NITROGEN AND SULFUR IN THREE BARLEY CULTIVARS SUBJECTED TO SELENIUM APPLICATIONS

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□ *Selenium (Se) is an essential micronutrient for humans and animals, and also affects plant metabolism. In this study, the effects of increasing doses of Se application on the uptake of selenium (Se), nitrogen (N), and sulfur (S) content of three barley cultivars were investigated. The analysis indicated that the Se contents were highest in root tissues, intermediate in grain, and lowest in stem tissues in all cultivars. Total Se accumulations (whole plant) were highest in 'Bülbül 89' (0.171 mg kg^{-1}), intermediate in 'Çetin 2000' (0.153 mg kg^{-1}), and lowest in 'Tarm 92' cultivars (0.124 mg kg^{-1}). In all cultivars, the increase in grain Se content resulted in a decrease in the grain N content. While 'Bülbül 89' can be suggested for biofortification purposes, less Se accumulating 'Tarm 92' cultivar can be suggested as a fodder in Se contaminated or high Se containing areas.*

Keywords: barley, *Hordeum vulgare*, nitrogen, selenium, sulfur, 'Tarm 92', 'Çetin 2000', 'Bülbül 89'

INTRODUCTION

Selinium (Se) is an essential micronutrient for human and animal health, with antioxidant, anti-cancer and anti-viral properties (Lyons et al., 2004; Davis et al., 2006; Surai, 2000). Selenium is also considered among essential micronutrient for many organisms, including plants (Zhu et al., 2009; White at al., 2004). Since plants are the main source for Se supply, plant Se metabolism is important for Se nutrition of humans and other animals. It is necessary for growth, fertility and prevention of several diseases in

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animals. It inhibits peroxide formation by affecting the activity of glutathione peroxidase (GSHPx) (Kaneko, 1989; Surai, 2000).

Among the many forms of selenium, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are the most soluble and most mobile forms of the Se in soil (Davis et al., 2006). Plants mainly take up Se as the selenate or selenite ions. When selenite is provided in solution culture, it is more toxic than selenate in higher plants (Carlson et al., 1989; Terry et al., 2000). Selenite is mostly assimilated in the roots (Gissel-Nielsen, 1987), and undergoes faster conversion to selenoaminoacids despite a lower uptake rate (Smith and Watkinson, 1984). Although Se does not appear to be an essential nutrient for most plants, it is incorporated into amino acids and proteins after uptake (Eriksson, 2001).

The physical and chemical similarities of Se and sulfur (S) help explain the intimate association between Se and S metabolism in plants (Sors et al., 2005). Both S and Se forms are part of the VI-A chalcogen group of elements, with the most common valence states of S and Se being -2, 0, $+4$, $+6$. These elements have chemical differences, from which one can infer that some biochemical processes involving Se may be excluded from those associated with S. Most plant species contain less than 25 μg Se g^{-1} dry weight and cannot tolerate high levels of Se in the environment. The non-specific integration of the seleno amino acids, selenocysteine (SeCys) and selenomethionine (SeMet) into proteins is believed to be the major contributor of Se toxicity in plants (Brown and Shrift, 1981, 1982). The existence of Se analogues of S containing metabolites in plants indicates that the biosynthesis of most Se compounds may depend on the enzymes involved in the S assimilation pathway (Brown and Shrift, 1982).

Animals Se status is sensitive to changes in the food supply, and barley can be a good fodder for supplying Se. Deficiency of Se in animals has been linked to Keshan disease, which has been observed in low-Se areas (Fordyce et al., 2000a, 2000b; Johnson et al., 2000; Tan et al., 2002). Se can be toxic to plants, animals and humans at higher concentrations. The rapid bioaccumulation of Se up the food chain can cause toxicity and teratogenic effects in animals, especially in fish and birds (Hamilton, 2004; Hamilton and Buhl, 2004). Toxic plant tissue levels of Se are generally above 5 mg kg^{-1} (Reilly, 1996), but among agricultural crops the phytotoxicity of Se is variable (Mikkelsen et al., 1989). Selenium levels of the soils in different regions of the world are variable.

This study was conducted in Moldova, which is characterized by marginally high Se content in the soil and ground water (Hannigan et al., 2006). In this study our goal was to investigate the differential accumulation of selenium in different plant parts, and its effect on grain nitrogen and sulfur content in three barley cultivars subjected to soil application of increasing selenium doses.

MATERIALS AND METHODS

The study was carried out in Moldova in 2005. Seeds of three different barley cultivars 'Çetin 2000', 'Bülbül 89', and 'Tarm 92' were obtained from TİGEM (Aksaray, Turkey) and used as experimental material. The field experiment was arranged in a completely randomized design with three replications. In each repetition there were 15 different equal areas (2 m^2 each). Four doses (0.00 g ha^{-1}, 6.25 g ha^{-1}, 12.50 g ha^{-1}, 18.75 g ha^{-1}, 25 g ha^{-1}) of sodium selenite (Na_2SeO_3) were applied to the three barley varieties. The soil of the experimental field was analyzed for texture. The saturation, pH, salinity, lime, organic matters were analyzed by modified Walkley-Black method (1934) and nitrogen (N), potassium (K), available phosphorus (P), calcium (Ca), magnesium (Mg), and chlorine (Cl) were analyzed by the method of Olsen et al. (1954).

Seeds were sowed approximately 2 cm deep into the soil. Sodium selenite was dissolved in water and appropriate doses were applied to soil soon after sowing. After maturation 25 plants were uprooted from each parcel and packed in a paper bag for analysis.

Se Analysis

Sample preparation was made by following the procedure of EPA, Perkin Elmer Inc. (Perkin Elmer, 2000). Plant samples were separated on three parts (root, stem, and grain), ground in the laboratory mill and digested in a flask and 0.10 g of sample was added to 20 mL of nitric acid (HNO_3) and let stand overnight. Then 3 mL of perchloric acid ($HClO_4$) was added, refluxes inserted, and heated to 175°C for 60 min. After that, refluxes were removed and continued to heat until dense white fumes were present and evaporated. Deionized water was then added to bring the total volume to 25 mL. A 5 mL aliquot of digested solution was pipetted into a 50 mL volumetric flask to which concentrated hydrochloric acid (HCl) (25 mL) and deionized water (15 mL) were added. The flask was placed in a water bath for 20 minutes at 90°C to reduce selenate to selenite. After cooling, the solution was brought to volume with deionized water. Selinium contents in all samples were analyzed by Hydride Generation (FIAS-400, Perkin Elmer, Waltham, MA, USA) Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst-800, PerkinElmer). The method's detection limit was 0.003 mg kg^{-1}. All samples were analyzed in triplicate. The experimental soil samples were dried at room temperature, sieved using a 1.0 mm screen and prepared using the standard procedures (PerkinElmer, 2000; US Environmental Protection Agency, 1996) and Se content of experiment soil was analyzed.

N and S Analysis

Nitrogen contents of the barley grain samples were determined by the method of Nelson and Sommers (1973). Sulfur contents of grain samples were determined by the method described by Butters and Chenery (1959).

Statistical Analysis

The significance of the difference between mean values obtained from three independent replicates was determined by a one-way analysis of variance (ANOVA) at 95% confidence interval by using SPSS for windows (SPSS, Inc., Chicago, IL, USA). Comparison of means was performed by using Duncan's New Multiple Range Test or standard error of the mean (SEM) at a 5% level of probability.

RESULTS

Soil Properties

The experimental soil comprised of 30% sand, 23% clay and 46% silt. The average Se content of the experimental soil was 0.832 mg kg^{-1} (pH of 8.3). In the experimental soil, the content of organic matter (2.42%) and potassium (97.8 mg g^{-1}) was enough, however, N (0.18%), P (5.56 mg g^{-1}), Ca (0.008%), Mg (0.004) % and Cl (0.003%) were low (Sillanpaa, 1990; Lindsay and Norvell, 1978).

Selenium Contents in Root, Stem and Grain

Selenium contents were determined in root, stem, and grain. The total Se content was higher in root compared to stem and grains in cultivars. In terms of the Se content of root and total plant parts, the effects of Se applications in 'Çetin 2000' were significant ($P < 0.05$), but not significant in 'Bülbül 89' ($P > 0.05$) and 'Tarm 92' ($P > 0.05$) (Figure 1). However, Se content of the stem and the grain were significantly different from the control for all three cultivars ($P < 0.05$). In 'Çetin 2000' and 'Bülbül 89' cultivars, a linear increase in root and grain Se contents was observed at 6.25 and, 12.50 g ha^{-1} Se applications, but decreased at 18.75 and 25 g ha^{-1} doses (Table 1). The root and grain Se contents of 'Tarm 92' showed a significant increase at 6.25 g ha^{-1} Se dose. Also, a gradual increase in stem Se content of this cultivar was observed at 6.25, 12.50, and 18.75 g ha^{-1} applications, but a sharp decrease was realized at 25 g ha^{-1} (Table 1).

The cultivars showed differential accumulation of Se, and assimilation of N and S. Selenium content of 'Çetin 2000' was found to be 0.197 mg kg^{-1} for root, 0.094 mg kg^{-1} for stem, and 0.170 mg kg^{-1} for grain. Se content of 'Bülbül 89' for root, stem and grain was 0.206 mg kg^{-1}, 0.134 mg kg^{-1}, and 0.175 mg kg^{-1}, respectively. Se content of 'Tarm 92' for root, stem and

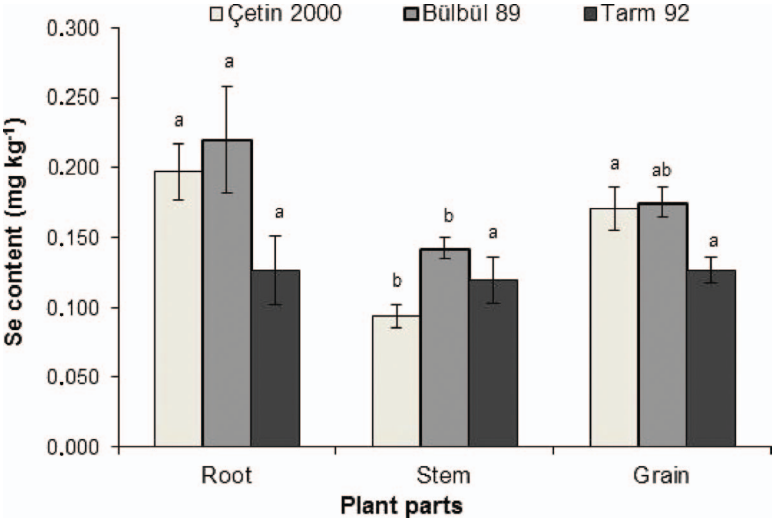


FIGURE 1 Mean total Se content in different parts of cultivars subjected to Se applications. Letters above bars indicate significant differences between plant parts in a cultivar. Bars with the same letter are not significantly different. Error bars indicate SE of means.

grain was 0.126 mg kg^{-1}, 0.119 mg kg^{-1}, and 0.126 mg kg^{-1}, respectively. The root Se content of 'Çetin 2000' was intermediate compared to 'Tarm 92' and 'Bülbül 89'. 'Bülbül 89' accumulated the highest (0.134 mg kg^{-1}) and 'Çetin 2000' accumulated the lowest (0.094 mg kg^{-1}) amount of Se in Stem. The Stem Se content was low in 'Çetin 2000' and 'Bülbül 89' compared to root ($P < 0.05$). Selenium content of 'Çetin 2000' and 'Bülbül 89' was close in their root and grain. 'Tarm 92' is characterized by the least Se level in root and grain compared to other cultivars. Considering the total Se accumulation in the whole plant parts, 'Bülbül 89' accumulated the highest amount with the average of 0.174 mg kg^{-1}, 'Çetin 2000' was the second

TABLE 1 Se content in different parts of 'Çetin 2000', 'Bülbül 89', and 'Tarm 92' cultivars

Barley cultivar	Plant part	Se dose (g ha^{-1})				
		0.00	6.25	12.50	18.75	25.00
Çetin 2000	Root	0.096 ± 0.006a	0.227 ± 0.018b	0.277 ± 0.025b	0.255 ± 0.015b	0.129 ± 0.011a
	Stem	0.110 ± 0.020a	0.118 ± 0.056a	0.097 ± 0.047a	0.100 ± 0.043a	0.043 ± 0.021b
	Grain	0.107 ± 0.005a	0.224 ± 0.006c	0.252 ± 0.004d	0.154 ± 0.003b	0.116 ± 0.012a
Bülbül 89	Root	0.190 ± 0.077a	0.218 ± 0.063a	0.287 ± 0.153a	0.263 ± 0.094a	0.141 ± 0.052a
	Stem	0.122 ± 0.048a	0.175 ± 0.010b	0.165 ± 0.101ab	0.125 ± 0.045a	0.124 ± 0.037a
	Grain	0.142 ± 0.006a	0.138 ± 0.004a	0.249 ± 0.007c	0.174 ± 0.008b	0.173 ± 0.013b
Tarm 92	Root	0.059 ± 0.012a	0.170 ± 0.090a	0.150 ± 0.055a	0.119 ± 0.068a	0.133 ± 0.048a
	Stem	0.049 ± 0.024a	0.126 ± 0.080b	0.127 ± 0.045b	0.217 ± 0.089c	0.075 ± 0.020a
	Grain	0.084 ± 0.005a	0.165 ± 0.005c	0.154 ± 0.007bc	0.137 ± 0.009b	0.091 ± 0.006a

Means followed by the same letter in rows are not significantly different at $P < 0.05$ (ANOVA followed by LSD test).

(0.154 mg kg^{-1}), and 'Tarm 92' was the least(0.124 mg kg^{-1}) accumulating cultivar (Table 1, Figure 1).

Grain Nitrogen Content

The effects of Se application on the grain N content of 'Bülbül 89', 'Çetin 2000' and 'Tarm 92' was found to be statistically significant ($P < 0.05$) compared to control, but differences on the grain N content among these cultivars were not significant ($P > 0.05$). In 'Çetin 2000', N accumulation reached to a maximum level (4655.03 mg kg^{-1}) at 12.50 g ha^{-1} Se application, but in subsequent Se doses a decrease was observed. In 'Bülbül 89' the N content showed an increase in both 6.25 and 12.50 g ha^{-1} Se applications compared to control group. But at higher Se applications, significant decrease was evident in grain N content ($P < 0.05$). Although a fluctuation was observed in grain N content of 'Tarm 92', an increase was noticeable at 12.50, 18.75, and 25 g ha^{-1} applications compared to control group. Maximum amount of N was observed in 'Tarm 92' (3536.21 mg kg^{-1}), moderate amount in 'Çetin 2000' (2899.35 mg kg^{-1}) and least amount in 'Bülbül 89' (2892.68 mg kg^{-1}) in average (Figure 2).

Sulfur Content in Grain of the Three Barley Cultivars

The effects of Se applications on grain S content was found to be significant for 'Çetin 2000' ($P < 0.05$), and 'Tarm 92' ($P < 0.05$), but not significant for 'Bülbül 89' ($P > 0.05$). The mean S content reached to maximum level

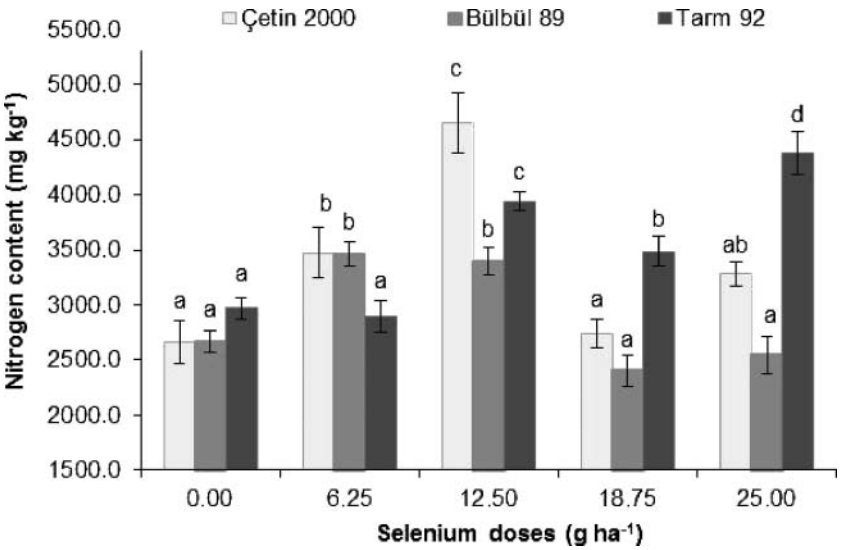


FIGURE 2 N content in grains of barley cultivars subjected to Se applications. Letters above bars indicate significant differences between doses in a cultivar. Bars with the same letter are not significantly different. Error bars indicate SE of means.

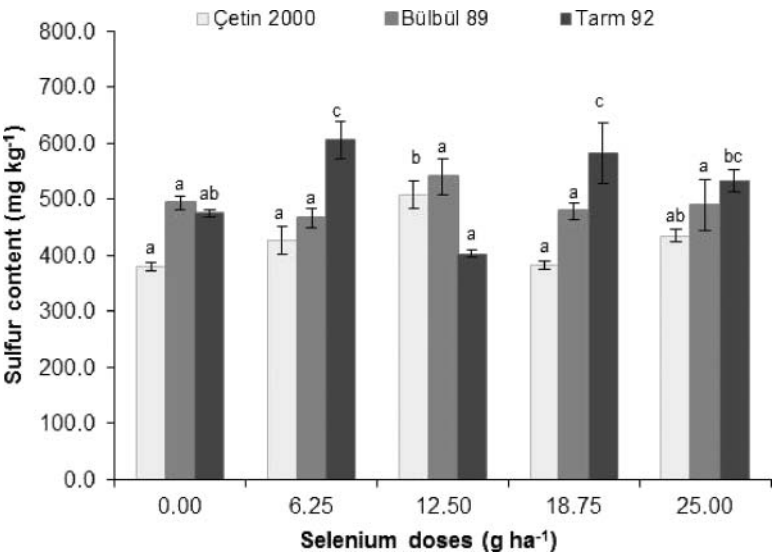


FIGURE 3 S content in grains of barley cultivars subjected to Se applications. Letters above bars indicate significant differences between doses in a cultivar. Bars with the same letter are not significantly different. Error bars indicate SE of means.

at 12.50 g ha^{-1} Se in 'Çetin 2000', however, a decrease was observed at subsequent Se doses. In 'Bülbül 89', S content increased gradually at 6.25, 12.50, and 18.75 g ha^{-1} Se applications. In 'Tarm 92', although a fluctuation was observed, maximum S accumulation was realized at 6.25 g ha^{-1}. The mean S levels in 'Çetin 2000', 'Bülbül 89' and 'Tarm 92' cultivars were 503 mg kg^{-1}, 521 mg kg^{-1} and 514 mg kg^{-1}, respectively (Figure 3).

Yield

The yields (1000 grains) for 'Çetin 2000' were 376.7, 323.3, 316.7, 406.7, 303.3 g for control, 6.25, 12.5, 18.75 and 25 g ha^{-1}, respectively. The yields (1000 grains) for 'Bülbül 89' were 476.6, 530.0, 540.0, 433.3, and 476.6 g for control, 6.25, 12.5, 18.75 and 25 g ha^{-1}, respectively. The yield (1000 grains) for 'Tarm 92' was 513.3, 413.3, 500.0, 430.0, and 446.7 g for control, 6.25, 12.5, 18.75 and 25 g ha^{-1}, respectively (Figure 4). Increasing Se doses did not affect the yield significantly in all cultivars ($P > 0.05$). However mean yield between 'Çetin 2000' and the other two cultivars was found to be statistically significant ($P < 0.05$). There was no significant difference among the yields of control groups in all cultivars ($P > 0.05$).

DISCUSSION

Se was unevenly distributed among the analyzed tissues of 'Çetin 2000' and 'Bülbül 89' cultivars in such a manner that Se contents were highest in root tissues, intermediate in grain, and lowest in stem tissues. However

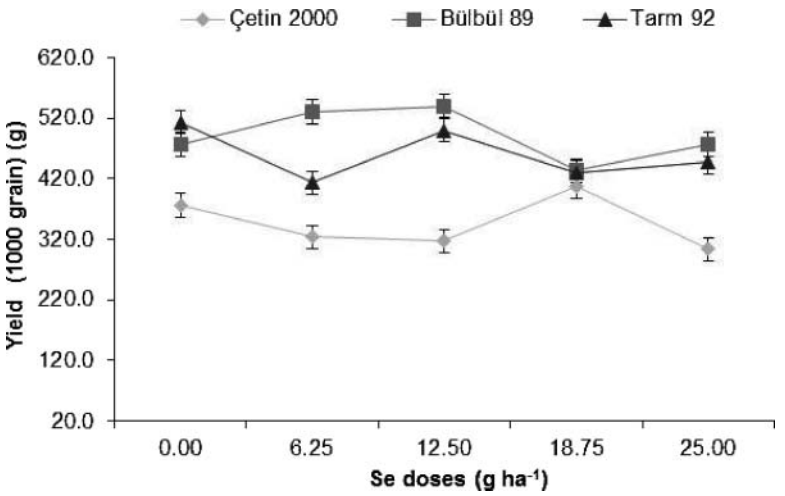


FIGURE 4 Yield of cultivars. Error bars indicate SE of means.

'Tarm 92' cultivar showed an even distribution in its mean Se content in root and grain. The highest and lowest amount of root Se content was observed in 'Bülbül 89' and 'Tarm 92' cultivars, respectively. The cultivars showed significant decrease in their stem Se content when compared with that of their roots. Our results were consistent with those of Huang (1994) who reported that the rate of uptake was highest in root about 10 times than those of grain and stem. However, Zayed et al. (1998) stated that plants usually accumulate more selenium in shoot and leaf than in root tissues. Higher selenium accumulations in root might be associated with the form of Se used. Although selenate is readily translocated from root to shoot tissues, selenite is mostly accumulated in root tissue. Total Se content were highest in 'Bülbül 89' (0.171 mg kg^{-1}), intermediate in 'Çetin 2000' (0.153 mg kg^{-1}), and lowest in 'Tarm 92' (0.124 mg kg^{-1}). Johnsson (1991a) reported that Se content of plants vary among cultivars, but Lyons et al. (2005) asserted that variation of grain Se content was associated with spatial variation in soil Se in Mexico and Australia rather than plant cultivars.

Considering the mean total values in all Se levels, a negative correlation was observed in terms of the Se and N content among the three barley cultivars. Our results indicated that the higher Se concentrations might interfere with the N assimilations in the barley cultivars studied, because the inverse relationship between Se and N contents were obvious in higher Se applications. This may be because of the interference of higher Se doses with nitrogen metabolism. Fluctuations in N content exhibit the cultivar's response to Se stress.

Sulfur accumulation in grains of 'Çetin 2000', 'Bülbül 89' and 'Tarm 92' cultivars differed in response to applied Se concentrations. Total S content was highest in 'Bülbül 89', intermediate in 'Tarm 92', and lowest in 'Çetin

2000' cultivar. In 'Bülbül 89' cultivar the S content showed a linear increase in response to increasing doses of Se application including the 18.75 g ha^{-1} dose. But at 25 g ha^{-1} Se application dose, a decrease was seen. 'Çetin 2000' and 'Tarm 92' cultivars showed a fluctuation in their grain S content in response to applied Se doses. The reason for the fluctuation may be the plants ability to incorporate Se into S-based chemical pathway and assimilate them into cysteine and methionine amino acids.

In this study the effects of Se application on the S content of barley cultivars was found to be insignificant which was consistent with those of Mikkelsen and Wan (1990), who stated that Se had a lesser effect on the S content in plant root. It can be concluded that 'Tarm 92' cultivar stores less Se in its tissues and shows best resistance to Se stress. It is known that high Se containing fodders causes alkali disease in animals. In this study among the cultivars we used, less Se accumulating 'Tarm 92' cultivar can be suggested as fodder for the prevention of alkali disease in animals. Also it can be advantageous to produce 'Tarm 92' cultivar in Se contaminated or high Se containing areas.

Although the yield difference was not significant within the groups among the doses, it was significantly different between 'Çetin 2000' and the other two cultivars. We did not observe an association between grain Se concentration and yield, which has been reported previously (Johnsson, 1991b; Lyons et al., 2004). This result indicates the genotype affects on selenium uptake of different cultivars which might be important for biofortification and phytoremediation purposes. Further research on different cultivars may elucidate the importance of cultivar affect on selenium uptake, translocation and accumulation.

Lyons et al. (2005) stated that, the grain Se concentration in the surveys and field trials including diverse wheat germplasm as well as other cereals were found to be in the range of 5–720 μg kg^{-1}, but much of this variation was associated with spatial variation in soil Se content in Mexico and Australia.

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