Research Article

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Grain amino acid composition of barley (Hordeum vulgare L.) cultivars subjected to selenium doses

Selenyum dozlarına maruz bırakılan arpa (Hordeum vulgare L.) çeşitlerinde tane amino asit içeriği

Abstract

Background: Selenium (Se 34) is an essential micronutrient for humans and animals and has growth promoting and antioxidative effects at low concentrations.

Methods: Effects of various sodium selenite (Na2SeO3) doses on grain amino acid content of barley cultivars (Bülbül 89 and Çetin 2000) was investigated using ion exchange liquid chromatography.

Results: Majority of the amino acids could be altered with Selenium (Se) fertilization. Grain Se content of Bülbül 89 (0.175 mg kg−1) and Çetin 2000 (0.171 mg kg−1) were similar and both displayed an increase in proteinogenic, essential, and sulfur amino acids. The response of cultivars was more pronounced for Se accumulation and amino acid content at mid dose (12.5 mg ha−1). The quantities of proteinogenic, essential and sulfur amino acids increased considerably at that dose. Se induced increase in nitrogen content might cause an increase in some of the proteins of grain and consequently can alter amino acid composition. An obvious increase in the limiting amino acids (lysine and threonine) were prominent in response to Se fertilization.

Conclusion: Se treatment influence amino acid composition of barley grains; especially improve the quantity of limiting amino acids and consequently nutritional value of the grain.

Keywords: Amino acid; Na2SeO3; Çetin 2000; Bülbül 89; Hordeum vulgare L.

Özet

Giriş: Selenyum (Se 34) insan ve hayvanlar için esansiyel bir mikronütrient olup düşük dozlarda büyüme teşvik edici ve antioksidatif etki gösterir.

Metod: Çeşitli dozlardaki sodyum selenitin (Na2SeO3) arpa çeşitlerinin (Bülbül 89, Çetin 2000) tane amino asit içeriği üzerindeki etkisi iyon değişimi sıvı kromatografisi ile analiz edilmiştir.

Bulgular: Çeşitlerde amino asitlerin çoğunun selenyum (Se) uygulamasına bağlı olarak değişebilceği gösterilmiştir. Bülbül 89 (0.175 mg kg−1) ve Çetin 2000 (0.171 mg kg−1) çeşitlerinde tane Se içeriği birbirine yakın olup her iki side proteinogenik, esansiyel ve kükürtlü aa içeriğinde artış göstermiştir. Çeşitlerin Se birikimi ve amino asit içeriği bakımından gösterdiği tepki ara dozda (12.5 mg ha−1) daha belirgin olmuştur. Bu doza proteinogenik, esansiyel ve kükürtlü amino asit miktarı belirgin şekilde artış göstermiştir. Tane azot içeriğindeki Se indükledi artış bazı proteinlerin artışına neden olabilir ve buna bağlı olarak amino asit bileşimini değiştirebilir. Çeşitlerde sıvılayıcı amino asitlerdeki (лизин ve treonin) bariz artış Se uygulamasına tepki olarak önem kazanmıştır.

Sonuç: Se uygulaması arpa tanesinde amino asit içeriğini etkilemekle olup özellikle sıvılayıcı amino asitlerin miktarını artırma etkisiyle tanede besin değerinin iyileştirilmesine katkı sağlamaktadır.

Anahtar kelimeler: Amino asit; Na2SeO3; Çetin 2000; Bülbül 89; Hordeum vulgare L.
**Introduction**

Selenium (Se⁴⁺) is an essential micronutrient for humans, animals and many other life forms [1, 2]. It is also necessary for animal growth, fertility and needed for the prevention of several diseases through mainly taking part in selenocysteine (SeCys)-containing proteins [3]. Sec is involved in active site of selenoproteins as glutathione peroxidase (GSH­Px) and iodothyronine deiodinases [3–5]. Selenium exists in multiple oxidation states, with each state having different fates within the environment. Plants take up Se as selenate (SeO₄²⁻) ions or selenite (SeO₃²⁻) ions [6]. Selenium and selenite are more soluble than the reduced forms of Se [7]. Although there is strong evidence that Se is required for the growth of green algae, the essentiality of Se as a micronutrient in higher plants is still controversial [8]. Plants can functionally concentrate selenium in the body, primarily in the seeds [9], which can be toxic at higher concentrations and cause membrane lipid peroxidation in barley [10].

The physical and chemical similarities of Se and sulfur (S) help elucidate the intimate association between the metabolisms of the two elements in plants [11]. The predominant forms of S and Se available to plants are sulfate, selenate and selenite. 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 selenoamino acids, selenocysteine (SeCys) and selenomethionine (SeMet) into proteins is believed to be the major contributor of Se toxicity in plants [12, 13]. The existence of Se analogs 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 [13].

Content and amino acid composition are important quality criteria for nutritional value of cereal grains used as fodder. The amino acid composition of cereal grains is somewhat unbalanced and the content of essential amino acids in cereal grains is insufficient to meet the needs of livestock [14]. Barley is one of the most important cereal species used as fodder. Barley cultivars satisfy the requirements of livestock for both energy and protein. It is possible to fortify fodder with Se-enriched barley to prevent diseases caused by Se deficiency. Besides, Se-enriched barley may provide additional compounds that may benefit livestock health. Considering difficulties of several approaches for improving nutritional quality of barley grains, we tested the effect of Se fertilization on grain amino acid composition of two barley cultivars. To the best of our knowledge, no detailed research has been conducted on how Se fertilization influences the amino acid composition of barley grains. This study aims to determine amino acid composition and to take an initial look at the effect of different levels of Se fertilization on the amino acid composition in two barley cultivars.

**Materials and methods**

**Experimental design**

The field experiment was arranged in a completely randomized block with three replications. In each repetition, there were 15 different equal plots (2 m² each) and the application of four doses (6.25, 12.50, 18.75, and 25 g ha⁻¹) of Na₂SeO₃ to the two barley cultivars (Çetin 2000, Bülbül 89). The soil texture of the experimental field was analyzed according to the method described by Bouyoucos [15]. Seeds were sowed approximately 2 cm deep in the soil. Na₂SeO₃ was dissolved in water and applied in highly diluted state to the soil for homogenous application soon after the sowing. Controlled irrigation was practiced to prevent leaching of the Se from the soil. After a maturation period of 4 months, 25 whole plants from each plot were harvested manually for analysis.

**Selenium analysis**

Sample preparation was made by following the procedure of EPA, Perkin Elmer Inc. [16]. Grains were ground in the laboratory mill and digested in a flask and 0.10 g of sample was added to 20 mL of nitric acid (HNO₃) and let stand overnight. Then 3 mL of perchloric acid (HClO₄) 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 HCl (25 mL) and deionized water (15 mL) were added. The flask was placed in a water bath for 20 minutes at 900°C to reduce selenate to selenite. After cooling, the solution was brought to volume with deionized water. Selenium contents in all samples were analyzed by Hydride Generation (FIAS-400, PerkinElmer, USA) Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst-800, PerkinElmer,
USA). The method’s detection limit was 0.003 mg kg⁻¹. All samples were analyzed in triplicate.

Amino acid analysis

Amino acids were analyzed according to ion exchange liquid chromatographic method by Amino Acid Analyzer AAA 339 M. Grains of barley cultivars treated with Se was used to determine the amino acid compositions. Column chromatography method was used to determine qualitative and quantitative amino acid compositions [17]. Ground dry tissue was extracted and hydrolyzed by using hydrochloric acid (6 N) in a drying chamber at 103–105°C during 24 h. The moisture content of the samples varied between 10 and 12% and calculations were made considering the dry matter. Amino acids were separated on an AAA 339 M amino acid analyzer (OSTION LG ANB column, 8 mm diameter, 35 mm length). The chromatography conditions included the use of mobile phase, ninhydrin with added sodium citrate buffer (pH 2.2), eluent flow rate of 15 mL h⁻¹ and a chromatography cycle of 120 min [17]. Standard amino acids were chromatographed in parallel, while qualitative amino acid composition was determined from retention times. Mixture of 18 amino acids was used as internal standard. The colorimetric measurement of the complex resulting from the ninhydrin reaction was carried out at 570 nm (440 nm for proline). Quantitative analysis was by automated determination of peak areas for identified acids [17].

Statistical analysis

The Shapiro-Wilk’s test was used and histogram and q–q plots were examined to assess the data normality. Levene test was applied to test variance homogeneity. A two-way analysis of variance (two-way ANOVA) was performed to investigate the effects of barley cultivars and dose groups on amino acid levels. Both main effects and interaction of these two factors are examined with interaction models. Since, interaction terms were found to be statistically significant for nearly half of the amino-acid levels, one-way analysis was also applied to conduct group comparisons. A two-sided independent samples t test was applied to compare barley cultivars and one-way analysis of variance was applied to compare dose groups. Post-hoc analysis was performed with Tukey test. Multiple tests were adjusted using Benjamini-Hochberg procedure. All values are expressed as mean and standard deviation statistics. Analyses were conducted using R 3.2.3 software (www.r-project.org ) [18]. A p < 0.05 probability level was considered as statistically significant.

Results and discussion

Soil properties

Saturation, pH, salinity, lime, organic matter, nitrogen, potassium, available phosphorus, calcium, magnesium and chlorine were determined in our previous study in all experimental soil samples [19] as in other studies [20, 21]. The loamy soil comprised 30% sand, 23% clay and 46% silt, with a pH of 8.3. Organic matter content (24.2 mg g⁻¹) was within the normal range by FAO standards [22]. While N (1.8 mg g⁻¹), available P (5.56 mg g⁻¹), Ca (0.08 mg g⁻¹), Mg (0.04 mg g⁻¹) and Cl (0.03 mg g⁻¹) were low, the K level (97.8 mg g⁻¹) was considered as normal [23].

Se content of experimental soil in the previous study was estimated as 0.83 mg kg⁻¹, being twice as high as the world’s mean Se content [19]. The Se content in some soils were calculated to be in the range of 0.01–2 mg kg⁻¹, but mean Se content was reported as 0.4 mg kg⁻¹ [24] in the world.

Grain selenium content

Both Bülbül 89 and Çetin 2000 contained significantly higher selenium concentrations at 6.25, 12.5, 18.75 g ha⁻¹ doses compared to the control set up (For cultivar effect: \( F = 79.945, p < 0.001 \); For dose effect: \( F = 0.843, p = 0.369 \); For interaction effect: \( F = 26.062, p < 0.001 \)) as reported in the previous study [19]. In general, grain selenium content increased with treatment doses, but it was more pronounced at 12.5 g ha⁻¹ (mid dose) in both cultivars (Figure 1) (p < 0.001). This study supports the work of Broadley et al. [25] who also reported increases in grain Se concentration of *Triticum aestivum* L. on application

![Figure 1: Grain Se content (mg kg⁻¹ dry weight) in two barley cultivars subjected to various Se doses. Error bars indicate SE of means.](image_url)
of Na$_2$SeO$_4$. Similarly, Lyons [26] reported that a modest application of 10 g Se ha$^{-1}$ can increase Se concentration of wheat grain by around 10-fold. Differential Se accumulation in different plant genotypes is an expected phenomenon, and the uptake, translocation and accumulation of Se may be distinctive for different plant species, even in cultivars of the same species [27]. Both genetic and environmental factors affect Se concentration in cereal grains. Genetic variation in grain Se has been reported for wheat, although Se acquisition and accumulation is strongly dependent upon environmental conditions, cultural practices and selenium fertilization [28–30].

**Effect of Se on amino acid composition**

Amino acids are fundamental ingredients in protein synthesis. Previous studies confirmed that Se affects the physiology of plants and as a result, amino acid metabolism may be directly or indirectly influenced. There are several constraints in improving the amino acid composition of grain storage proteins. Classical breeding or genetic engineering strategies which are typical examples to achieve that purpose, may either not be useful or are very difficult steps for the modification of amino acid composition, considering the limited genetic variations in barley. Some other approaches used for the modification of amino acid composition include the work of Jun-cong et al. [31], who reported significant differences in grain protein and B-hordein content when different sowing dates were considered, and the use of high lysine mutant barley genotypes as reported by Shewry [32]. The increments were between the ranges of a few percentage points to a maximum of 30%. A corresponding decrease in B-hordein content was also reported. Reduced starch and crop yield was also observed in those lysine-rich mutant phenotypes. Thus, in spite of a considerable investment in mutation breeding for high lysine barley cultivars, yields were not correlated with lysine content due to segregation of high lysine character with low grain yield.

In present study, it was observed that the main effect of doses on amino acids except for His, Pro, and Glu in cultivars were significant (Table 1). The increase in most limiting amino acids (lysine and threonine) is especially noteworthy after Se fertilization. The lysine level increased to 27% in Çetin 2000 and 8% in Bülbül 89. On the other hand, threonine level increased 19% in Bülbül 89 and 18% in Çetin 2000 (Table 2). Similar results have also been reported by Duma and Karklina [33] indicating

| Amino acids | Main effects | Interaction effect |
|-------------|--------------|--------------------|
| Gly         | F = 4.536; p = 0.046 | F = 5.361; p = 0.004 |
| Ala         | F = 0.359; p = 0.556 | F = 20.313; p < 0.001 |
| Val         | F = 5.092; p = 0.035 | F = 5.679; p < 0.001 |
| Leu         | F = 10.309; p < 0.004 | F = 12.086; p < 0.001 |
| Ile         | F = 7.654; p = 0.012 | F = 3.424; p = 0.027 |
| Phe         | F = 61.334; p < 0.001 | F = 17.033; p < 0.001 |
| Tyr         | F = 0.645; p = 0.431 | F = 9.818; p < 0.001 |
| Trp         | F = 2.859; p = 0.106 | F = 5.033; p = 0.006 |
| Lys         | F = 7.678; p = 0.012 | F = 16.540; p < 0.001 |
| Arg         | F = 123.786; p < 0.001 | F = 10.397; p < 0.001 |
| Thr         | F = 12.200; p = 0.002 | F = 26.581; p < 0.001 |
| Ser         | F = 31.665; p < 0.001 | F = 4.668; p = 0.008 |
| Asp         | F = 1.643; p = 0.215 | F = 18.912; p < 0.001 |
| Glu         | F = 56.623; p < 0.001 | F = 1.086; p = 0.390 |
| Pro         | F = 0.146; p = 0.706 | F = 1.487; p = 0.244 |
| Cys         | F = 6.287; p = 0.021 | F = 3.946; p = 0.016 |
| Met         | F = 46.267; p < 0.001 | F = 12.892; p < 0.001 |
| His         | F = 6.523; p = 0.019 | F = 12.728; p < 0.001 |
| Sulfur aa’s | F = 0.083; p = 0.776 | F = 0.021; p = 0.690 |
| Proteinogenic aa’s | F = 2.623; p = 0.121 | F = 0.987; p = 0.437 |
| Essential aa’s | F = 0.683; p = 0.418 | F = 0.116; p = 0.975 |

Bold values indicate a statistical significant difference after adjusting multiple tests with Benjamini-Hochberg procedure.
that Se treatment caused an increase of 16.2% in lysine and 22.7% in threonine.

Genetic engineering was used to balance amino acid composition in barley in a study by Hansen et al. [34] in which antisense technology was used to suppress C-hordein biosynthesis and leading to 12 and 18% increases in cysteine and methionine, respectively. Our approach was also seemed to be efficient for the fact that the strategy resulted in 15 and 40% increases in cysteine and methionine levels, respectively. While Hansen et al. [34] obtained 15 and 19% increases in lysine and threonine, we obtained 19 and 27% increases, respectively.

The response of cultivars was especially highlighted at the mid dose ranges (except a few amino acids altered at other doses) (Table 2). Grain Se content was also significantly higher in mid Se dose (Figure 1). At 18.75 g ha⁻¹ Se application, grain Se contents were close between cultivars, but amino acid contents were not as homogenous as grain Se content (p = 0.086). Such a fluctuating result in amino acid content depending on the treated Se doses was also reported by Duma and Karklina [33]. For example, while they have obtained a prominent increase in aspartic acid content in 5 mg mL⁻¹ Se dose, arginine was the highest in 10 mg mL⁻¹ treatment. In the current study, the level of methionine, one of the important sulfur amino acids, increased 40% in Bülbül 89 and 22% in Çetin 2000 (Table 2). Grain methionine content was highest at 6.25 g ha⁻¹ Se dose for Bülbül 89. In Çetin 2000, the highest level was observed at the mid dose and the highest dose (Table 3). Se application at that dose might be utilizable to increase the methionine content in those cultivars. Although cysteine level was also significantly increased, the response to Se application was not as remarkable as methionine as a consequence of interaction effect between cultivars in response to doses (Table 1). The cysteine level increased 9% in Çetin 2000 and 15% in Bülbül 89 at the 12.50 g ha⁻¹ dose (Table 2).

More than 10% increase was observed in the level of all amino acids other than serine, cysteine and glutamate in Çetin 2000 cultivar. The level of alanine, arginine, proline and methionine increased 40, 34, 31 and 28%, respectively. In Bülbül 89, methionine, arginine and proline exhibited the highest response to Se treatment with 40, 36, and 24% increases, respectively. The glutamate was the least responsive amino acid with only 4% increase for that cultivar. A parallel increase in serine and cysteine content of Çetin 2000 and Bülbül 89 was observed with 4 and 8% rise for cysteine, and 9 and 15% rise for serine, respectively (Table 2). The reason for this observation might be the synthesis of serine from 3-phosphoglycerate and its usage as a precursor for cysteine biosynthesis in plants [35].

The rate of increase in aromatic amino acids phenylalanine and tyrosine was almost parallel in the cultivars studied. This can be explained by the fact that chorismate is the precursor in the biosynthesis of those aromatic amino acids. On the other hand, tryptophan biosynthesis did not increase in both cultivars. It was clear that Se fertilization can be used to manipulate the level of phenylalanine, tyrosine and tryptophan, since these amino acids could be converted into other amino acids and compounds. Aspartate is used as a precursor in methionine biosynthesis in plants. A similar impact of Se on aspartate and methionine levels in Çetin 2000 and Bülbül 89 was observed at 11 and 16% in aspartate, and 28 and 40% in methionine, respectively. Glutamate is a very important amino acid and plays a crucial role in nitrogen metabolism and as expected, the amount of glutamate was the highest in both cultivars (Table 3). Glutamate level was also found to be highest in the study of Asween [14]. It is the precursor for the biosynthesis of proline, which is the cyclic form of glutamic acid. Proline synthesis is affected by many abiotic stress factors and although not significant, it was the second in quantity compared to other proteinogenic amino acids in our cultivars (Tables 1 and 3). Se applications resulted in 31

| Amino acid | Çetin-2000 | Bülbül-89 |
|------------|------------|------------|
|            | % increase | Se dose | % increase | Se dose |
| Glycine    | 17         | 12.5     | 9           | 12.5    |
| Alanine    | 40         | 12.5     | 10          | 12.5    |
| Valine     | 20         | 12.5     | 8           | 12.5    |
| Leucine    | 14         | 18.75    | 17          | 12.5    |
| Isoleucine | 15         | 18.75    | 15          | 12.5    |
| Methionine | 22         | 12.5     | 40          | 6.25    |
| Phenylalanine | 25   | 18.75    | 14          | 12.5    |
| Tyrosine   | 25         | 18.75    | 13          | 12.5    |
| Tryptophan | a          | b        | a           | b       |
| Lysine     | 27         | 12.5     | 8           | 12.5    |
| Arginine   | 34         | 12.5     | 36          | 18.75   |
| Histidine  | 23         | 12.5     | 17          | 12.5    |
| Threonine  | 18         | 12.5     | 19          | 12.5    |
| Serine     | 4          | 12.5     | 8           | 12.5    |
| Proline    | 31         | 12.5     | 24          | 18.75   |
| Cysteine   | 9          | 12.5     | 15          | 12.5    |
| Aspartate  | 11         | 12.5     | 16          | 12.5    |
| Glutamate  | 9          | 12.5     | 4           | 12.5    |

aIncrease was not observed.
bAll applied doses.
Table 3: Mean amino acid distribution levels (mg 100 mg⁻¹ dry weight) and one-way analysis results between barley cultivars and doses.

| Amino acids    | Dose (g ha⁻¹) | p*  |
|----------------|---------------|-----|
|                | 0  | 6.25 | 12.5 | 18.75 | 25  |
| Nonpolar aliphatic aa’s |     |      |      |       |     |
| Glycine        |     |      |      |       |     |
| Çetin 2000     | 0.44±0.04a | 0.46±0.03ab | 0.52±0.03b | 0.46±0.01bc | 0.46±0.02bc | 0.035 |
| Bülbül 89      | 0.43±0.01 | 0.45±0.01 | 0.47±0.02 | 0.47±0.02 | 0.43±0.04 | 0.106 |
| p*             | 0.510 | 0.810 | 0.048 | 0.419 | 0.302 |     |
| Alanine        |     |      |      |       |     |
| Çetin 2000     | 0.42±0.01a | 0.45±0.02a | 0.59±0.01b | 0.45±0.03c | 0.53±0.03d | <0.001 |
| Bülbül 89      | 0.46±0.03 | 0.47±0.01 | 0.48±0.01 | 0.49±0.02 | 0.51±0.03 | 0.159 |
| p*             | 0.939 | 0.095 | <0.001 | 0.104 | 0.455 |     |
| Valine         |     |      |      |       |     |
| Çetin 2000     | 0.52±0.04a | 0.54±0.03ab | 0.62±0.02a | 0.54±0.04bc | 0.57±0.03cd | 0.025 |
| Bülbül 89      | 0.51±0.01a | 0.58±0.01b | 0.54±0.02bc | 0.54±0.02bc | 0.51±0.02a | 0.001 |
| p*             | 0.725 | 0.086 | 0.009 | 0.947 | 0.041 |     |
| Leucine        |     |      |      |       |     |
| Çetin 2000     | 0.64±0.04a | 0.66±0.02a,b | 0.71±0.03ab | 0.74±0.03ab | 0.64±0.03a | 0.009 |
| Bülbül 89      | 0.65±0.02a | 0.73±0.02b | 0.76±0.03b | 0.72±0.02b | 0.70±0.03ab | 0.001 |
| p*             | 0.983 | 0.011 | 0.076 | 0.291 | 0.077 |     |
| Isoleucine     |     |      |      |       |     |
| Çetin 2000     | 0.29±0.01 | 0.31±0.01 | 0.31±0.02 | 0.34±0.04 | 0.27±0.03 | 0.065 |
| Bülbül 89      | 0.3±0.04  | 0.34±0.02 | 0.35±0.02 | 0.33±0.01 | 0.32±0.02 | 0.197 |
| p*             | 0.716 | 0.080 | 0.502 | 0.689 | 0.096 |     |
| Methionine     |     |      |      |       |     |
| Çetin 2000     | 0.03±0.01a | 0.04±0.01b | 0.04±0.01b | 0.03±0.01c | 0.04±0.01b | <0.001 |
| Bülbül 89      | 0.03±0.01a | 0.04±0.01b | 0.02±0.01b | 0.03±0.01a | 0.03±0.01a | <0.001 |
| p*             | 0.089 | 0.037 | <0.001 | 0.959 | 0.010 |     |
| Aromatic aa’s  |     |      |      |       |     |
| Phenylalanine  |     |      |      |       |     |
| Çetin 2000     | 0.51±0.02abc | 0.53±0.01a | 0.51±0.03a,b | 0.64±0.03b | 0.46±0.03c | <0.001 |
| Bülbül 89      | 0.55±0.02a | 0.62±0.02a | 0.63±0.03b | 0.58±0.02bc | 0.58±0.02ab | 0.003 |
| p*             | 0.073 | 0.001 | 0.007 | 0.027 | 0.002 |     |
| Tyrosine       |     |      |      |       |     |
| Çetin 2000     | 0.26±0.01abc | 0.26±0.01abc | 0.28±0.01a | 0.33±0.02b | 0.23±0.02c | 0.001 |
| Bülbül 89      | 0.25±0.02 | 0.28±0.02 | 0.29±0.01 | 0.27±0.01 | 0.25±0.02 | 0.093 |
| p*             | 0.518 | 0.172 | 0.689 | 0.016 | 0.275 |     |
| Tryptophane    |     |      |      |       |     |
| Çetin 2000     | 0.04±0.01 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 | 0.300 |
| Bülbül 89      | 0.04±0.01a | 0.03±0.01b | 0.03±0.01b | 0.04±0.01a | 0.04±0.01a | 0.019 |
| p*             | 0.069 | 0.802 | 0.464 | 0.316 | 0.494 |     |
| Basic aa’s     |     |      |      |       |     |
| Lysine         |     |      |      |       |     |
| Çetin 2000     | 0.33±0.02a | 0.37±0.01b | 0.42±0.01c | 0.38±0.02b | 0.38±0.01b | <0.001 |
| Bülbül 89      | 0.35±0.02 | 0.37±0.01 | 0.38±0.01 | 0.36±0.01 | 0.36±0.01 | 0.086 |
| p*             | 0.188 | 0.675 | 0.008 | 0.121 | 0.029 |     |
| Arginine       |     |      |      |       |     |
| Çetin 2000     | 0.38±0.01abc | 0.39±0.01abc | 0.51±0.06b | 0.41±0.02ac | 0.47±0.01bc | <0.001 |
| Bülbül 89      | 0.29±0.02abc | 0.35±0.02ab,b | 0.34±0.04ab,b | 0.39±0.02ab,b | 0.26±0.01bc | <0.001 |
| p*             | 0.001 | 0.008 | 0.016 | 0.370 | <0.001 |     |
| Histidine      |     |      |      |       |     |
| Çetin 2000     | 0.19±0.04 | 0.22±0.01 | 0.23±0.02 | 0.22±0.03 | 0.20±0.03 | 0.327 |
| Bülbül 89      | 0.22±0.01 | 0.23±0.01 | 0.26±0.02 | 0.23±0.01 | 0.23±0.02 | 0.115 |
| p*             | 0.181 | 0.599 | 0.189 | 0.732 | 0.264 |     |
| Polar neutral aa’s |     |      |      |       |     |
| Threonine      |     |      |      |       |     |
| Çetin 2000     | 0.42±0.03abc | 0.47±0.02abc | 0.50±0.02b | 0.48±0.01b | 0.41±0.02c | 0.001 |
| Bülbül 89      | 0.46±0.01abc | 0.49±0.01abc | 0.54±0.02b | 0.49±0.01b | 0.44±0.02c | <0.001 |
and 24% increase in grain proline level in Çetin 2000 and Bülbül 89, respectively. Cultivars exhibited a slight decrease in both Se and proline content at 25 g ha⁻¹ dose compared with that of mid dose (Table 3). The amount of Se (0.252 mg kg⁻¹) and proline (1.247 mg 100 mg⁻¹ dry weight) reached the highest level in Çetin 2000 at the mid Se dose. Although the level of glutamate was lower in our cultivars, the levels of proline and arginine were higher. This indicates that glutamate was used as a precursor for proline and arginine biosynthesis. Histidine biosynthesis is not directly connected to the biosynthesis of other amino acids [35] and its response was comparable in both cultivars.

### Essential amino acid content

In this study, the modification of the essential amino acid level in barley grains was realized by Se fertilization. Modification of essential amino acid level, especially limiting ones, might be important for feeding animals since the barley grains are valuable as a fodder. Although statistical significance was not realized in essential amino acid contents of cultivars, they exhibited an increase up to the mid dose application rate and gradually decreased after that rate (For Çetin 2000, p=0.771; For Bülbül 89, p=0.600). Considering the essential amino acid and grain selenium contents, the cultivars showed similar
response to increasing Se doses. Apart from the highest dose, considerable increase in essential amino acid content was observed and reached a maximum level at the mid dose rate (Figure 2). Se might be interfering with amino acid metabolism at the highest dose, while it was promoting the biosynthesis of some amino acids at the mid dose.

**Proteinogenic amino acid content**

Although the amount of total proteinogenic amino acid content was different in the cultivars, their response was similar at 6.25, 18.75 and 25 g ha⁻¹ doses (Figure 3). Bülbül 89 did not show a significant change at 6.25, 12.5, 25 g ha⁻¹ doses, but Çetin 2000 showed a sharp increase at the mid dose rate (For Çetin 2000, p = 0.829; For Bülbül 89, p = 0.944). At 25 g ha⁻¹ both cultivars exhibited a decrease in proteinogenic amino acid content (Figure 3).

**Sulfur amino acid content**

Sufficient supplies of sulfur amino acids in seeds help the accumulation of sulfur-rich proteins up to a level adequate to meet the nutritional requirement of livestock and poultry [36]. An increase in sulfur amino acid content was observed at 12.5 g ha⁻¹ in Çetin 2000 and up to 18.75 g ha⁻¹ in Bülbül 89 (Figure 4) (For Çetin 2000, p = 0.901; For Bülbül 89, p = 0.864). Total sulfur amino acid of Çetin 2000 was lower compared to Bülbül 89 (p = 0.864). Similar results were observed in the study carried out by Lee et al. [37]. They reported that free amino acid content of *Brassica oleracea* cv. Majestic increased in response to increasing Se doses.

In conclusion, our study indicates that Se fertilization alters the amino acid content in grains of barley. Total sulfur amino acid content of grains can be increased by applying the appropriate dose of Se. However, this depends on cultivars; for instance, methionine level was the highest in Bülbül 89 cultivar at the lowest dose. Moreover, limiting amino acid (lysine and threonine) content of grain can also be increased by Se fertilization. Considering the disadvantages of mutation breeding and the difficulties in genetic manipulation of barley for increasing the limiting amino acid content, Se fertilization might be an effective way to improve the limiting amino acids of grains.

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