CONTENTS OF AMINO ACIDS IN VARIOUS CULTIVARS OF BARLEY (*HORDEUM VULGARE* L.)

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Abstract: The twenty barley cultivars were grown under nutrition by Nitrogen dose 60 kg ha^{-1} on field soil (type pseudogley). The grains of barley cultivars were used for study of variability of amino-acid. By qualitative analysis are identified 11 different amino acids. For each of indentified amino acid was used quantitative method to determine their concentration. The total concentration of free amino acids was analyzed for all varieties of barley. The highest total concentration of free amino acids in the analyzed varieties of barley cultivars with Ac Oxbow (11.5%), Sissy (11.3%), Steffi (11.2%), Severa (11.1%), Scarlett (11.0%) and the smallest variety Zaida, Ursa, Seebe, Aren (8.8%) and cultivar Tempera (8.0%). Soil fertility implies the existence of easily accessible nutrients for plants. Out of all the elements absorbed from the soil, the plants need nitrogen in the largest quantities.

Keywords: barley, cultivar, amino acid, soil, nitrogen

Introduction

Due to the long-time cultivation of plants, the soil becomes deficient in natural nutritive elements. The lack of nutrients can also occur due to the nutrients outflow into the deeper layers of soil or creation of forms which the plant cannot access. Plants compete with microorganisms for nitrogen, and considerable amounts of it are removed from the soil by harvest and picking of various products. Plants also absorb organic nitrogen substances, such as urea, for example, but still the largest part of the absorbed nitrogen originates from inorganic compounds. If the plant absorbs nitrogen in the form of NH_4^+ ion, it integrates it into organic compounds immediately, but NO_3^- ion must first be reduced; those are the initial steps of nitrogen assimilation. In the initial development phases, young grains need ammonium ion until they develop enzyme systems for nitrates exploitation. Nitrogen is an integral part of amino acids and many other compounds in which it is always reduced as -NH or -NH_2 group. Amino acids and peptides are transported through the plant, thus accelerating the creation of proteins and regulating the plant hormones synthesis. (Djukić et al., 2006).

In numerous researches, barley nutrition by nitrogen has shown that barley genotypes have such a significant reaction to the increased amounts of nitrogen that they change production properties and seed quality (Knezevic et al., 2011; Glamočlija et al., 2011). According to previous studies, the effect of nitrogen on the studied characteristics depended on N quantity applied. Increasing amounts of nitrogen decrease positive effects on spike lenght, number of grains per spike and yield. However, the protein content in grain kept increasing to the highest nitrogen dose, which lowered the quality of malting barley (Malesevic et al., 2010; Dubis et al., 2012). The effect of nutrition on nitrogen content and protein fractions in barley grain hordeins subject of numerous studies (Qi et al., 2006; Corke and Atsmon, 1998; Pecio and Bichonski, 2003). If there is less precipitation, barley will make better use of - nitrogen used for nutrition (Pagola

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et al., 2009). The optimal quantity of nutrients depends on investigation year, natural soil fertility and plant genotype, as well as on mutual interaction of these factors.
When it comes to nutritional standards, barley is valuable food which had wrongfully been neglected in human nutrition. It is mainly used as raw material in beer and whiskey industry and as food for domestic animals. Specific application of barley is determined by variations of contents of grain chemical components, amino acid composition in particular. The contents of protein should be higher in barley fodder and amino acid contents should be equivalent to animals' nutrition needs (higher contents of threonine, tryptophan, lysine and methionine). Malted barley should contain less protein.
The aim of this paper is study of variability of amino acid composition and contents in grain of different barley cultivars.

Materials and methods

Grain sample of 20 genotypes of *Hordeum vulgare* L. (Alarik, Aren, Ac Oxbow, Astor, Atol, Arapiles, Severa, Skarlet, Steffi, Sissy, Salon, Seebe, Tempera, Terno, Triumph, Thuringia, Ursa, Ursel, Zaida, Michka) was used for analysis of amino acid contents. Amino acids were extracted from complex compound of grain (carbohydrates, lipids, nonorganic salts etc) by using 80% of ethanol and sedimentation of dissolved proteins by chlorophorm (Grujic-Injac, 1962). Identification of some amino acids is conducted by using comparison with test substances. The method of spectrophotometry was used for establishing concentration of identified amino acids. Total concentration of free amino acids was determined by standard curved line for proline.

Results and discussion

Results of qualitative analysis of free amino acids in investigated barley cultivars were achieved based on 20 chromatograms.The total concentration of free amino acids varied between 8% in cultivar Tempera and 11.5% in cultivar Ac Oxbow (*Table 1.*) According to the potential purpose of the analyzed cultivars, based on total concentrations of free amino acids, two groups of analyzed cultivars were defined. The first group comprised 13 cultivars with concentration above 10%; the second group consisted of cultivars with concentration under 10%. (*Table 1.*)
Glutamic acid is very important for nitrogen metabolism in cell, especially in primary synthesis of amino acids (Knežević et al., 2009). In hordeine storage proteins glutamic acid present as glutamine and have important source of nitrogen for germ nutrition. From biochemical point of view, nitrogen storage in form of glutamine is the most economic way of using it. Glutamic acid is synthesized due to Krebs cycles. In the process of synthesis, ammoniac (produced by reduction of nitrites) is introduced in α-ketoglutar acid. Glutamic acid serves as a precursor for proline formation. Proline is important stress factors redactor for plants. Proline was identified in 9 of 20 chromatograms. (*Table 2.*) Oxyproline is produced by oxidizing of proline. It is known that oxyproline is present in cell walls and plant membranes or glume that envelopes the seed. 5 essential amino acids were present: valine - identified in 14 cultivars with concentration from 1.9 (Steffi) to 5.4% (Severa and Sissy); Tryptophan- in 14 cultivars. Concentration of tryptophan was within the interval from 1% in

cultivars Scarlet and Thuringia to 2% in Alarik and Steffi; leucine - in 10 out of 20 analyzed cultivars. The concentration of leucine varied between 1.2% (Aren) and 3.2% in cultivar Seebe; methionine - was found in 7 cultivars. The contents of methionine went from 1.2% in cultivar. Salon to 1.5% in cultivar Astor; threonine - in three cultivars. The determined concentrations of threonine were - 1% (Thuringia and Michka) and 2.75% in cultivar Tempera.

Table 1. Total concentration of free amino-acids in grain of barley genotypes

Total concen. of free amino-acids (>10 %)				Total concen. of free amino-acids (<10 %)	
Genotype	Concentration (%)	Genotype	Concentration (%)	Genotype	Concentration (%)
Alarik	10.7	Steffi	11.2	Aren	8.8
Ac Oxbow	11.5	Sissy	11.3	Salon	9.5
Astor	11.0	Triumph	10.6	Seebe	8.8
Atol	10.2	Thuringia	10.7	Tempera	8.0
Arapiles	10.2	Ursel	10.7	Terno	9.5
Severa	11.1	Michka	10.7	Ursa	8.8
Skarlet	10.2			Zaida	8.8

Table 2. Qualitative and quantitative analysis of amino acids on 21 chromatograms

Amino acids / Barley lines	proline	oksi-proline	glutamic acid	valine	norvaline	Tryptophan	metionine	leucine	norleucine	Treonine	glicine
Alarik	6.5		20		2.8	2.0					
Aren			23	5.3		1.5		1.2			
Ac Oxbow	5.5		22	3.5		1.8	1.4	1.5	2.0		
Astor	5.2		20	3.0		1.6	1.5				
Atol		4.2	23	4.2	1.7	1.5					
Arapiles		10.7	22	4.0		1.2	1.3	1.6	0.95		
Severa	3.6		26	5.4	1.9						
Skarlet		6.3	22	4.2		1.0	1.3	2.1			
Steffi		7.8	22	1.9		2.0		2.0			
Sissy	3.6		22	5.4		1.8					
Salon		12.0	26	3.8			1.2	3.0			
Seebe			25		3.0			3.2			
Tempera		11.0	25			1.5		1.4		2.7	
Terno		12.2	23	3.8		1.5					
Triumph	4.0		24	4.0		1.2	1.3				
Thuringia	3.8			3.2		1.0	1.3	1.7		1.0	
Ursa	8.5		28		3.2	1.4					
Ursel			22		2.0			2.5			1.0
Zaida	4.6		25	3.2							
Michka			23							1.0	

It was determined that the essential amino acids present in the first group of cultivars (with the total concentration above 10%) tryptophan and valine - existed in 10 cultivars, leucine and methionine - in 6 cultivars, while threonine existed in 2 barley cultivars. The analysis of the existing essential amino acids in the second group of cultivars (with the total concentration under 10%) indicated that the amino acids - leucine, tryptophan

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and valine - were present in 4 cultivars from this group, and methionine and threonine in single cultivars from this group.

Conclusions

The study was established differences among analyzed bareley cultivars according to determined amino acid composition and their contents. Qualitative analysis identified 11 different amino acids, and quantitative methods, was determined for each amino acids concentration and the total concentration of free amino acids were analyzed for all varieties of barley. According to the potential purpose of the analyzed cultivars, based on total concentrations of free amino acids, two groups of analyzed cultivars were defined. Five essential amino acids were identified (valine, triptophan, treonine, metionine and leucine) Barley grain is a reservoir of nutrients and synthesis products which accumulate in the plant throughout its lifetime. Because of that, balanced mineral nutrition of the plant, as a precondition of barley grain quality, is of extreme importance.

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