Nutrient capacity of amino acids from buckwheat seeds and sprouts

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Summary

Buckwheat biodiversity is connected with specific amino acids accumulation, which can be utilized at using buckwheat sprouts for production of functional foods, as a part of gluten-free diet. Buckwheat protein is of high quality regarding amino acid composition and can be recommended for human diet. The experimental buckwheat variants were characterized by different contents of 17 amino acids in seeds together with a similar re-distribution of amino acids in stems and leaves after 2 weeks of cultivation. Buckwheat sprouts are a valuable nutrition source regarding a sulfur-containing amino acid methionine. Novel information regarding high contents of amino acid leucine (25–29 $g\cdot kg^{-1}$ dry weight) in leaves of experimental buckwheat sprouts is presented. During the early stage of buckwheat growth, active accumulation of glutamic acid is observed, which can be connected with accumulation of arginine, proline and leucine.

Keywords

pseudocereals; functional food; buckwheat seedlings; amino acid

Coeliac disease, previously estimated as rare in childhood, is now recognized as a common condition that may be diagnosed at any age. Studies based on serological screening showed the worldwide incidence of 0.3–1.2% in unselected, North American, European, Indian and South American populations [1, 2]. In genetically pre-disposed individuals, factors accountable for this life-long intolerance to this type of compounds exist. These are wheat gliadins and other prolamins, like secalin of rye, avenin of oat and hordein of barley [3]. The only available treatment for coeliac disease is the life-long adherence to a gluten-free diet, while these products are often poor in minerals, vitamins and/or proteins. Investigation of the buckwheat flour incorporation effect to a gluten-free experimental formulation of bread showed positive changes of qualitative parameters of bread, such as specific volume index and loaf size, together with the proportional enrichment in proteins and microelements, especially in copper and manganese [4].

It was found that rice and maize flours are poor regarding their nutritional value (low protein, fibre, folate contents) compared to buckwheat flour [5]. Protein content in buckwheat is higher than in rice, wheat, sorghum or maize [6–8]. Another advantage of buckwheat flour is its positive effect on bread texture [9, 10]. It was shown that consumption of boiled buckwheat groats or bread baked using 50% buckwheat flour significantly reduced post-prandial blood glucose and insulin responses compared to white wheat bread. Positive effects of buckwheat consumption also extend to animal feeding [11].

Gluten is a composite of gliadin and glutenin, which are conjoined with starch in the endosperm of various grass-related grains. Several gluten-free grains, such as the pseudocereals amaranth, quinoa and buckwheat, are characterized by excellent nutrient profiled. Contrary to most common grains, the proteins in amaranth, quinoa and buckwheat are composed mainly of globulins and albumins, and contain very little or no prolamin

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proteins, which are the main storage proteins in cereals, and also the toxic proteins in coeliac disease [12, 13]. The amino acid composition of globulins and albumins differs significantly from that of prolamins, which has implications in relation to their nutritional quality. Globulins and albumins contain less glutamic acid and proline than prolamins, and more essential amino acids such as lysine [13]. By studying the distribution of the seventeen amino acids in the protein fractions (albumin, globulin, prolamin, glutelin) in four buckwheat varieties, the sequence of amino acid content from high to low in albumin was glutamic acid, aspartic acid, lysine, arginine, glycine and valine [14]. Buckwheat proteins have typically a higher biological value than those of cereals because of their high lysine levels, resulting in amino acid score (*AAS*) of 100, one of the highest among plant sources [15].

Buckwheat grains, bran and flour are characterized by high protein content. The protein content varied among different buckwheat species and have similar qualitative characteristics to the protein content in cereal grains [16]. Compared to cereals, the amino acid composition of buckwheat is characterized by higher contents of aspartic acid, lysine and arginine, and less glutamic acid and proline. Due to the high lysine content, buckwheat has a higher nutritional value than other cereal grains because lysine is the first limiting amino acid from this protein source. The lysine content decreased in an order of albumin > glutelin > globulin > prolamin, the threonine content in an order of prolamin > glutelin > albumin > globulin in the protein from kernels of common buckwheat varieties (*F. esculentum*) of Japanese spring buckwheat, Japanese summer buckwheat, Yuqiao No. 1 and tartary buckwheat varieties (*F. tataricum*) [15].

Buckwheat grain is characterized by a high content of starch, protein with an advantageous amino acid composition and a low content of α-gliadin [17, 18]. Since buckwheat has an indeterminate growth habit, seeds could be harvested at various stages of maturity within a single day. During maturation, changes in amino acid composition of cereals and legumes are observed, while the amino acid composition of maturing buckwheat is relatively stable. For limiting amino acids, such as lysine, and amino acids of storage proteins, such as glutamic acid, changes in buckwheat are intermediate between those of true cereals and legumes [19].

Nowadays, not enough information is available about the content of proteins, total amino acids and their composition in buckwheat sprouts during different stages of growth. It was found that total amino acid content of buckwheat sprouts (*F. tataricum*) was higher by approximately 28–38 % than in the buckwheat seed. In this tissue, fourteen kinds of amino acids, including aspartic acid, glutamic acid and lysine, were notably increased. However, arginine and cysteine (a sulfur-containing amino acid) decreased [20]. As multifunctional foods, fermented buckwheat sprouts were studied, showing negative results in allergy tests [21]. The effects of elicitation supported by phenylpropanoid pathway precursor feeding on buckwheat sprouts was shown to have a positive effect on the content of antioxidants useful for human nutrition [22].

The 1985 FAO/WHO/UNU protein report [23] defined reference amino acid patterns for infants based on breast milk and for pre-school children, school children and adults from age-specific estimates of dietary indispensable amino acid requirements divided by the safe protein requirement for each age group. It was found that protein quality of a diet should be estimated from its digestibility adjusted by its *AAS* calculated from its limiting amino acid in comparison with the reference amino acid pattern [24].

AAS and indicated first-limiting amino acid for a given protein often differed with data source and choice of reference pattern. Some of the indications at variance with indication data's which are previously validated by bioassays. The variability in protein quality ratings is reduced by expressing the data as essential amino acid contents. *AAS* to show protein quality was calculated for wheat, peanut flours and soy protein isolate [25]. Nowadays are not so many results which present *AAS* of protein quality for different bitter and sweat buckwheat varieties. It is known that protein content and bioavailability in buckwheat is particularly higher compared to other grains such as wheat, rice, maize and sorghum [25–28].

The data regarding amino acid composition and content in buckwheat (*F. esculentum*) grains showed differences depending on variety and origin [29]. Therefore the aim of this study was to determine total protein content, amino acid composition and essential amino acid content in the different varieties of sweet (*F. esculentum*) and bitter (*F. tataricum*) buckwheat plants.

MATERIALS AND METHODS

Plant description

Four different buckwheat species and varieties, namely, *Fagopyrum esculentum* Moench (varieties Rubra and Karadag) and *F. tataricum* Gaertn.

(ssp. *rotundatum* and ssp. *himalaicum*) were collected in 2015 and selected for the present study. Variety Rubra with high anthocyanin content (30.87–40.41 $mg \cdot kg^{-1}$ dry weight (DW)) in the vegetative organ was obtained by family selection method [30] from chemo mutants from Taras Shevchenko National University of Kyiv (Kyiv, Ukraine). Variety Karadag was received from the Institute of Agriculture at the Ukrainian Academy of Agrarian Science (Kyiv, Ukraine). *F. tataricum* Gaertn. is a one-year plant which, among the species studied, has a better pollination of flowers and a higher grain production. The collection of buckwheat germplasm, which is maintained at the Scientific Research Institute of Groat Crops (Kamianec-Podiľskyj, Ukraine), comprises nearly 1 000 samples readily available for breeding research. The buckwheat samples (leaves, stems) were collected after 2 weeks of growth under greenhouse conditions. For each buckwheat variant, 6 plants were grown in six big pots. The leaves and stems were harvested and frozen in liquid nitrogen. Afterwards, the samples were lyophilized. After finishing the freeze-drying process, the material was ground by flint mill (20 000 ×*g*, 2 min). In parallel, estimation of amino acid composition in the seeds of the four varieties was carried out.

Determination of amino acid composition

Acid hydrolysis of amino acids

Direct hydrolysis of samples was carried out with 6 $mol \cdot l^{-1}$ HCl to obtain hydrolysates suitable for analysis of all amino acids except for sulfur amino acids. To the dry samples (0.5 g), 30 ml of 6 $mol \cdot l^{-1}$ HCl were added and the mixture was incubated at 110 °C for 23 h. After finishing the hydroxylation procedure, the mixture was neutralized with 30 ml of neutralizing solution (120 g NaOH in 1 000 ml dilution buffer (3 $mol \cdot l^{-1}$ NaOH), pH 2.2). Then, the mixture was added dilution buffer pH 2.2 till 100 ml. After finishing the procedure of acid hydrolysis, the amino acid composition of tested samples was analysed by ion-exchange chromatography [31]. The content of amino acids was determined, after acid hydrolysis

Tab. 1. Amino acid composition of proteins from the samples of individual types of grains.

Amino acids	Content [$g \cdot kg^{-1}$]			
	F. tataricum ssp. *himalaicum*	*F. tataricum* ssp. *rotundatum*	*F. esculentum* var. Rubra	*F. esculentum* var. Karadag
Non-essential amino acids				
Alanine	5.38 + 0.40	7.94 + 0.21	8.87 + 0.23	6.38 + 0.46
Aspartic acid	14.33 + 1.42	18.25 + 0.34	15.59 + 0.46	14.98 + 1.42
Cysteine	1.88 + 0.19	3.07 + 0.10	3.85 + 0.03	3.30 + 0.03
Glutamic acid	24.28 + 2.20	36.98 + 1.16	36.46 + 0.29	31.94 + 2.84
Glycine	7.06 + 0.59	10.79 + 0.15	9.61 + 0.30	8.57 + 0.52
Proline	4.67 + 0.37	6.10 + 0.50	5.39 + 0.50	5.38 + 0.52
Tyrosine	3.70 + 0.27	5.22 + 0.22	4.26 + 0.45	3.62 + 0.54
Serine	7.45 + 0.43	10.35 + 0.05	9.11 + 2.78	8.16 + 0.57
Essential amino acids				
Threonine	6.02 + 0.33	7.73 + 0.16	7.13 + 0.65	6.48 + 0.53
Valine	5.42 + 0.78	8.13 + 0.12	6.40 + 0.16	5.83 + 0.40
Isoleucine	4.40 + 0.27	6.41 + 0.18	5.23 + 0.42	4.82 + 0.28
Leucine	8.98 + 0.54	12.47 + 0.31	11.12 + 0.40	10.24 + 0.19
Phenylalanine	5.72 + 0.46	8.49 + 0.31	7.25 + 0.30	6.71 + 0.54
Lysine	6.08 + 0.80	10.26 + 0.32	9.07 + 1.14	7.96 + 0.35
Methionine	1.04 + 0.18	1.76 + 0.13	1.88 + 0.18	1.69 + 0.02
Semi-essential amino acids				
Arginine	10.74 + 0.86	17.29 + 0.34	15.57 + 0.01	13.64 + 0.43
Histidine	4.35 + 0.28	5.68 + 0.22	4.91 + 0.50	4.64 + 0.44
Sum of amino acids	116.08 + 10.37	176.92 + 4.29	161.7 + 8.80	144.34 + 10.08

All results are expressed as mean ± standard deviation on dry weight basis with 6 mol·l^{-1} HCl, using an automatic amino acids analyser (AAA 400; Ingos, Prague, Czech Republic).

Sulfur amino acids oxidative hydrolysis

Sulfur amino acids (cysteine, methionine) were oxidized with a mixture of hydrogen peroxide and formic acid, 1:9 (1 part 33% hydrogen peroxide and 9 parts of 85% formic acid.) To the dry samples (0.5 g), 5 ml of the oxidation mixture were added and the mixture was incubated in a refrigerator at 0–4 °C for 16 h. After oxidation, 1 ml of 6 mol·l^{-1} HCl was added to the samples, and they were allowed to stand for 15 min. Then, 80 ml of 6 mol·l^{-1} HCl were added to the samples for hydroxylation process at a temperature of 105 °C for 23 h. After cooling, the hydrolysates were evaporated to 80 ml in a vacuum evaporator at 50 °C. The residue of honey consistency was washed 2 times with 5 ml of deionized water and evaporated to dryness. The dry residues were diluted by 50 ml of dilution buffer pH 2.2 and stored in a refrigerator. The obtained oxidative products (methionine sulfoxide and cysteic acid) were analysed by an automatic amino acid analyser AAA 400.

Essential amino acid index and amino acid score

Essential amino acid index (*EAA*) was calculated according to the procedure of Oser [32] taking into account the ratio of *EAA* in the test protein relative to their respective amounts in whole egg protein.

The amino acid score (*AAS*) parameter was used to describe the protein nutritional quality. [24]. *AAS* is based on comparison of the mean amino acid requirements with milk protein as a reference. Mean amino acid requirement is expressed as percentage of total amino acids.

Statistical analysis

Except for chromatographic analysis, all other experiments were replicated thrice and all measurements were carried out at least twice. Data were analysed using analysis of variance (ANOVA). Differences were considered to be significant at $P < 0.05$ throughout the study.

RESULTS AND DISCUSSION

It is generally accepted that not only the seeds, but also the other parts of buckwheat plants are rich in compounds with a high biological value. The seeds used for food contain proteins, which have high lysine levels and specific amino acid content, giving them a high nutritional value. The absence of gluten in buckwheat flour determines its potential use in gluten-free diets but quality of protein and the antioxidant composition of different varieties of buckwheat can vary, which is important to be known for flour production [29].

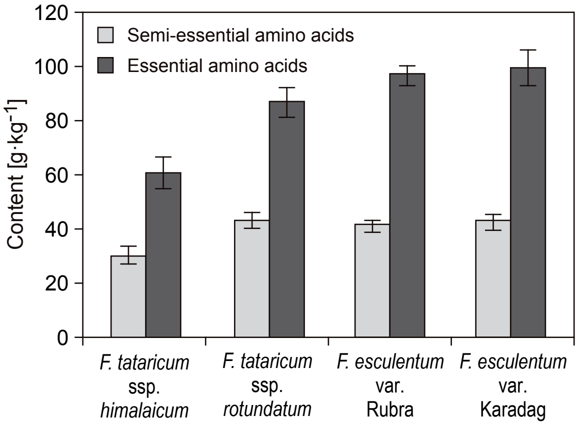

Fig. 1. Total contents of essential and non-essential amino acids in the experimental buckwheat seeds.

Content of amino acids is expressed per kilogram of dry weight.

The results of our experimental analysis confirmed high contents of lysine, isoleucine, tryptophan, valine, histidine and phenylalanine in the seeds of all experimental buckwheat variants. Out of these, seeds of *F. tataricum* var. *Rotundatum* was characterized by the highest content of these essential amino acids – 70.78 g·kg^{-1} DW (Tab. 1). The seeds of *F. esculentum* var. *Rubra* was on the second place regarding the contents of lysine, isoleucine, tryptophan, valine, histidine and phenylalanine, with the total content of essential amino acids of 61.77 g·kg^{-1} DW. The lowest content of lysine, isoleucine, tryptophan, valine, histidine and phenylalanine was determined in seeds of *F. tataricum* ssp. *himalaicum* (48.4 g·kg^{-1} DW). Total content of essential amino acids in the experimental variants varied (from higher to lower level): *F. tataricum* ssp. *rotundatum* > *F. esculentum* var. Rubra > *F. esculentum* var. Karadag > *F. tataricum* ssp. *himalaicum* (Fig. 1). The total content of essential amino acids had a similar trend for investigated experimental variants. Such variation in results of amino acid composition among species of *F. esculentum* and *F. tataricum* suggests that it is important to make pre-screening of buckwheat variety characteristics for recommendation as a sufficient nutritional source to the food processing and agricultural markets.

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Tab. 2. Essential amino acids in the protein of experimental buckwheat varieties and in egg albumin.

Essential amino acids	Content [g·kg⁻¹]				
	F. tataricum ssp. *himalaicum*	*F. tataricum* ssp. *rotundatum*	*F. esculentum* var. Rubra	*F. esculentum* var. Karadag	Egg albumin
Threonine	4.01	5.15	5.75	5.23	0.57
Valine	3.61	5.54	5.16	4.70	1.03
Isoleucine	2.93	4.27	4.22	3.89	0.71
Leucine	5.98	8.31	8.97	8.26	0.99
Phenylalanine	3.81	5.66	5.85	5.41	0.75
Histidine	2.90	3.78	3.96	3.74	0.24
Lysine	4.05	6.84	7.31	6.42	0.64
Methionine	0.69	1.17	1.52	1.36	0.54

Content is expressed per kilogram of protein.

Expert Consultation's Protein Quality Evaluation Report recommended a next coefficient of nutritive value of protein: the protein digestibility-corrected *AAS* [33]. Content of the essential amino acid arginine in the experimental buckwheat varieties was estimated to be 2 times higher than that in milk and egg proteins. Arginine is classified as a semi-essential or conditionally essential amino acid, depending on the developmental stage and health status of mammals [34]. The role of arginine as an alternative source, besides glutamate, for proline biosynthesis is still under discussion. The role of arginine as a precursor of nitric oxide (NO) is known. Also, conversion of arginine to polyamines plays an important role in regulation of the plant development. Arginine and proline are related to the reduction of plant stress [35, 36]. *F. tataricum* ssp. *rotundatum* and *F. esculentum* var. Rubra contained a higher level essential amino acids compared to *F. tataricum* ssp. *himalaicum* and *F. esculentum* var. Karadag. Threonine and histidine contents were higher in all experimental buckwheat seeds compared to the contents of threonine and histidine in milk and egg proteins by 17–70%. In all experimental buckwheat seeds, phenylalanine was determined (Tab. 2).

AAS is derived from the ratio between the amino acid in a test protein and the corresponding amino acid in a reference amino acid pattern. *AAS* exceeding 100 % are truncated to 100 %. The advantages of *AAS* are its simplicity and direct relationship to human protein requirements [32]. The recent WHO/FAO/UNU report [33] endorsed the 1985 report in recommending the amino acid content of breast milk as the best estimate of infant amino acid requirements [37]. The parameter of *AAS* shows the protein quality of a diet [24, 37]. Tab. 3 shows results of analysis of buckwheat seeds of different varieties. It is visible that *AAS* of seeds of *F. esculentum* var. Rubra, *F. esculentum* var. Ka-

Tab. 3. Amino acid score in the seeds of experimental buckwheat varieties.

Essential amino acids	Amino acid score [%]			
	F. tataricum ssp. *himalaicum*	*F. tataricum* ssp. *rotundatum*	*F. esculentum* var. Rubra	*F. esculentum* var. Karadag
Threonine	107.2	137.7	153.7	139.8
Valine	71.6	109.9	102.4	93.3
Isoleucine	69.1	100.7	99.5	91.8
Leucine	74.5	103.5	111.7	102.9
Phenylalanine	–	–	–	–
Histidine	133.0	173.4	181.7	171.6
Lysine	54.8	92.6	98.9	86.9
Methionine	50.8	86.0	111.8	100.0

Amino acid score is expressed as percentage of total amino acids.

radag and *F. tataricum* ssp. *rotundatum* was almost fully acceptable for the diet, being a source of essential amino acids.

It is known that vegetative mass of buckwheat plants has a high content of biologically active compounds such as phytosterols, flavonoids, fagopyrins, phenolic acids, lignans and vitamins. In our research, we observed changes and re-distribution on amino acids during early stages of buckwheat growth. It was done through amino acid composition analysis of the seeds, and of the stems as well as leaves after 2 weeks cultivation. The biodiversity part of experiment was based on the use of different buckwheat species and varieties with an aim to understand the re-distribution process of amino acids in buckwheat sprouts. The analysis could also confirm nutrition capacities of buckwheat sprouts for food processing needs. All experimental variants, with different contents of amino acids in seeds, were characterized

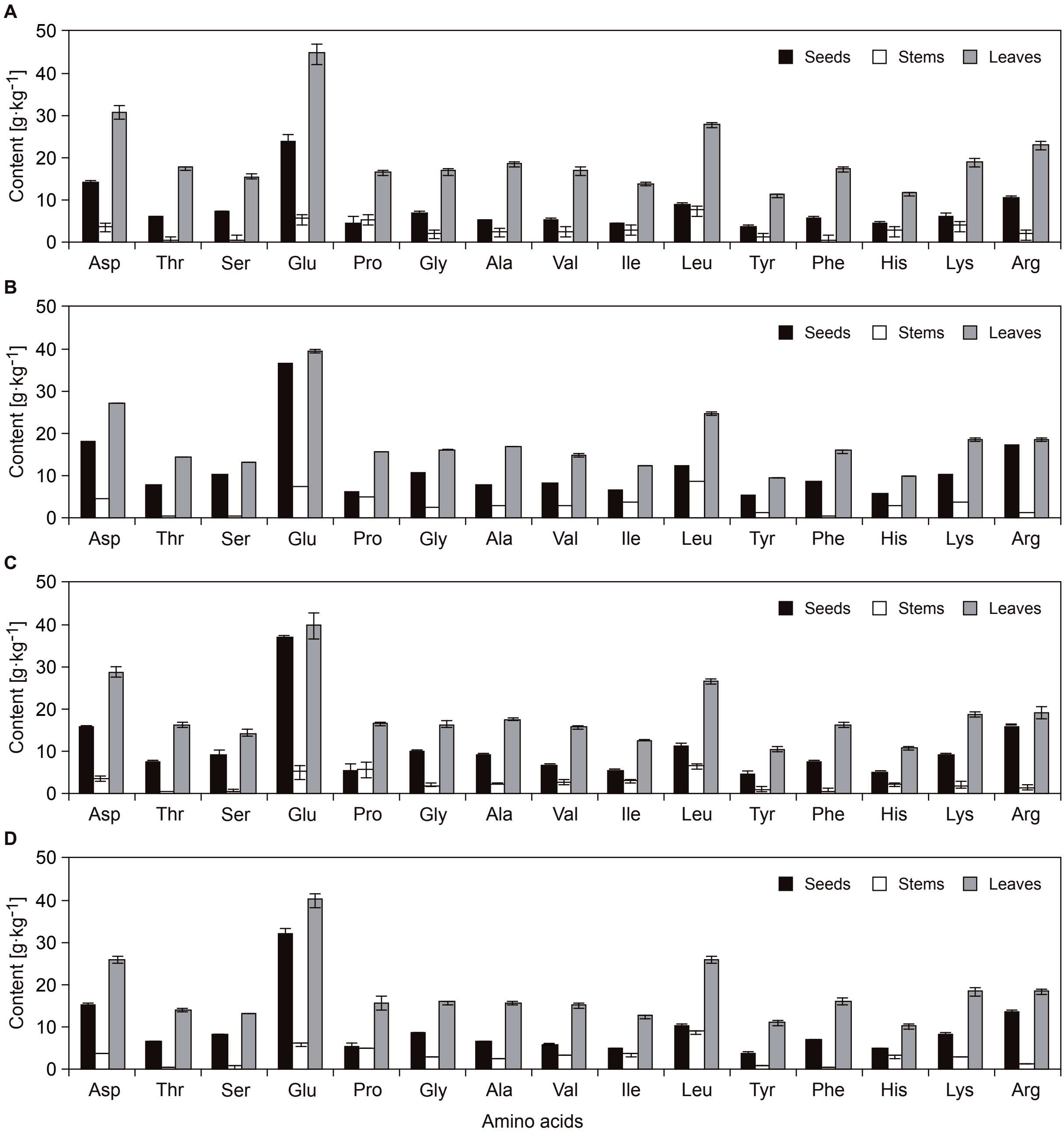

Fig. 2. Amino acid composition in different buckwheat species and varieties in seeds, stems and leaves.

A – *F. tataricum* ssp. *himalaicum*, B – *F. tataricum* ssp. *rotundatum*, C – *F. esculentum* var. Rubra, D – *F. esculentum* var. Karadag. Content of amino acids is expressed per kilogram of dry weight.

by a similar tendency of re-distribution of amino acids in the stems and leaves after 2 weeks of cultivation. The significantly high increasing content of all amino acids was observed in all experimental variants of buckwheat leaves (Fig. 2). In the stems, a lower content of amino acids was determined, compared to seeds and leaves, which evidenced a more active amino acid biosynthesis in leaves. At the same time, buckwheat sprouts were characterized by a high content of leucine in stems (Fig. 2).

In the early stage of growth, active accumulation of glutamic acid is observed, which is connected with accumulation of arginine, proline and leucine in leaves and stems (Fig. 2). Proline content in leaves and stems of *F. tataricum* ssp. *himalaicum* was higher than in seeds by 73 % and 13 %, respectively. In stems of *F. esculentum* var. Rubra, *F. esculentum* var. Karadag and *F. tataricum* ssp. *rotundatum*, the same level as in seeds was determined. At the same time, leaves of *F. esculentum* var. Rubra, *F. esculentum* var. Karadag and *F. tataricum* ssp. *rotundatum* were characterized by increased proline contents by more than 2-fold compared to the investigated seeds of buckwheat varieties. The proline content was on a high level in stems and significantly increased in leaves compared to the other estimated amino acids. It was observed that methionine content was increased in leaves of *F. tataricum* ssp. *himalaicum* by 72 %, in leaves of *F. tataricum* ssp. *rotundatum* by 46 %, in leaves of *F. esculentum* var. Karadag by 42 % and in leaves of *F. esculentum* var. Rubra by 36 %

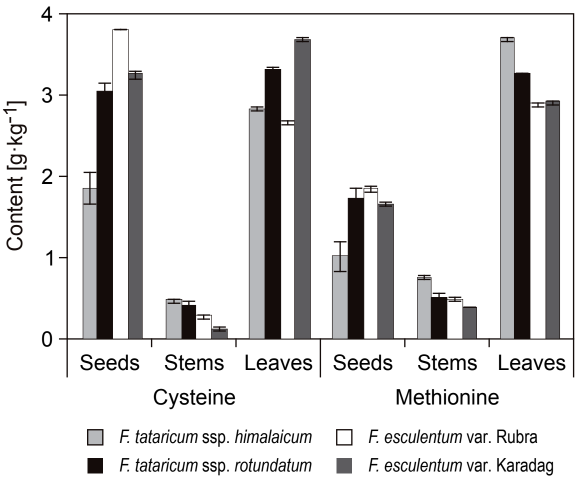

Fig. 3. Content of sulfur-containing amino acids in different buckwheat species and varieties in leaves, stems and seeds.

Content of amino acids is expressed per kilogram of dry weight.

compared to the methionine content in the investigated seeds (Fig. 3). The content of cysteine increased in all experimental variants but this tendency varied in leaves compared to seeds of different buckwheat varieties. Cysteine content was higher by 35 % in leaves of *F. tataricum* ssp. *himalaicum,* by 8 % in leaves of *F. tataricum* ssp. *rotundatum* and by 12 % in leaves of *F. esculentum* var. Karadag. On the other hand, cysteine content in leaves of *F. esculentum* var. Rubra was lower than in seeds by 42 % (Fig. 3).

The content of leucine was high in the leaves, while mostly on the same level in the stems, with leucine content in seeds of all experimental variants. The leaves were characterized by the highest content of glutamic acid compared to the contents of other amino acids. Glutamine is biosynthesized from glutamic acid and ammonia [38]. Utilizing glutamine and 2-oxoglutarate by the action of glutamate synthase, glutamate is formed. It plays a central role in amino acid metabolism in plants. The α-amino group of glutamate may be transferred to other amino acids by the action of a wide range of multi-specific aminotransferases. In addition, both the carbon skeleton and α-amino group of glutamate form the basis for the synthesis of γ-aminobutyric acid, arginine and proline [39]. The contents of aspartic acid, glutamic acid, leucine, phenylalanine, lysine and arginine in the leaves were more than 2-fold higher compared to their contents in seeds. In the previous research, information about the high content of essential amino acid lysine in the buckwheat plants was presented [14]. In the present research, we also observed high leucine content in seeds, stems and leaves of the investigated buckwheat varieties.

Proline accumulates in many plant species in response to environmental stress. It was also suggested that proline may also play a role in flowering and development both as a metabolite and as a signal molecule [40]. Connections of proline metabolism to the oxidative pentose phosphate pathway and glutamate-glutamine metabolism are of particular interest [5]. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress [41]. We found an increased content of proline during the early stage of buckwheat seedlings growth, in the buckwheat stems and leaves, which is in concordance with its possible influence on cell proliferation during early stages of buckwheat growth [40]. Accumulation of free proline was also noticed during various stages of *Arabidobsis thaliana* plant development, being dependent on the developmental stage of the plant and type of plant organs [42, 43].

An increased methionine level was observed in leaves compared to its level in seeds of experimental variants. The content of cysteine increased in all experimental variants but this tendency was different in leaves of different buckwheat varieties compared to seeds. It is possible to suggest that methionine is more intensively accumulating during the early stage of buckwheat growth compared to the cysteine content. Recent studies demonstrated that methionine metabolism is under stringent regulatory control and can be regulated differently in various plant species [44]. Our results confirm that buckwheat sprouts can be a valuable nutrition source of methionine, lysine an leucine.

The results of our research show high indices of essential amino acids for argenine, threonine and histidine.

AAS presented in this paper show that quality of buckwheat protein is high and that it can be recommended for human diet. Our results are in concordance with those of KATO et al. [45] estimating the amino acid composition of buckwheat proteins as well balanced and of a high biological value.

CONCLUSION

Nowadays, many mechanisms are being discovered, on molecular and metabolomics levels, to increase the contents of essential and semi-essential amino acids for needs of functional food industry. The topic of buckwheat biodiversity regarding amino acid nutrient capacities is presented in this research work. Among the studied buckwheat varieties, seeds of *F. tataricum* ssp. *rotundatum* and *F. esculentum* var. Rubra were characterized by the highest contents of essential amino acids. It was found that leaves of buckwheat sprouts contain high amounts of leucine and methionine, which is a novel information. During the early stage of buckwheat growth, active accumulation of glutamic acid was observed in sprouts, which can be connected with accumulation of arginine, proline and leucine in leaves and stems. This knowledge may be used to develop new functional foods, which can help in the prevention and treatment of some non-communicable diseases.

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REFERENCES

1. Fasano, A. – Berti, I. – Gerarduzzi, T. – Not, T. – Colletti, R. B. – Drago, S. – Elitsur, Y. – Green, P. H. – Guandalini, S. – Hill, I. D. – Pietzak, M. – Ventura, A. – Thorpe, M. – Kryszak, D. – Fornaroli, F. – Wasserman, S. S. – Murray, J. A. – Horvath, K.: Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Archives of Internal Medicine, *163*, 2003, pp. 286–292. DOI: 10.1001/archinte.163.3.286.
2. Dubé, C. – Rostom, A. – Sy, R. – Cranney, A. – Saloojee, N. – Garritty, C. – Sampson, M. – Zhang, L. – Yazdi, F. – Mamaladze, V. – Pan, I. – Mcneil, J. – Mack, D. – Patel, D. – Moher, D.: The prevalence of coeliac disease in average-risk and at-risk Western European populations: A systematic review. Gastroenterology, *128*, 2005, pp. S57–S67. DOI: 10.1053/j.gastro.2005.02.014.
3. Vader, L. W. – Stepniak, D. T. – Bunnik, E. M. – Kooy, Y. M. – De Haan, W. – Drijfhout, J. W. – Van Veelen, P. A. – Konig, F.: Characterization of cereal toxicity for coeliac disease patients based on protein homology in grains. Gastroenterology, *125*, 2003, pp. 1105–1113. DOI: 10.1016/S0016-5085(03)01204-6.
4. Krupa-Kozak, U. – Wronkowska, M. – Soral-Śmietana, M.: Effect of buckwheat flour on microelements and proteins contents in gluten-free bread. Czech Journal of Food Sciences, *29*, 2011, pp. 103–108. ISSN: 1805-9317. <http://www.agriculturejournals.cz/publicFiles/37206.pdf>
5. Mattioli, R. – Costantino, P. – Trovato, M.: Proline accumulation in plants: Not only stress. Plant Signaling and Behavior, *4*, 2009, pp. 1016–1018. DOI: 10.4161/psb.4.11.9797.
6. Hager, A.-S. – Wolter, A. – Jacob, F. – Zannini, E. – Arendt, E. K.: Nutritional properties and ultrastructure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, *56*, 2012, p. 239–247. DOI: 10.1016/j.jcs.2012.06.005.
7. Ikeda, K. – Kishida, M.: Digestibility of proteins in buckwheat seed. Fagopyrum, *13*, 1993, pp. 21–24. ISSN: 0352-3020.
8. Ikeda, K. – Oku, M. – Kusano, T. – Yasumoto, K.: Inhibitory potency of plant antinutrients towards the in vitro digestibility of buckwheat protein. Journal of Food Science, *51*, 1986, pp. 1527–1530. DOI: 10.1111/j.1365-2621.1986.tb13851.x.
9. Ikeda, K. – Sakaguchi, T. – Kusano, T. – Yasumoto, K.: Endogenous factors affecting protein digestibility in buckwheat. Cereal Chemistry, *68*, 1991, pp. 424–427. ISSN: 0009-0352. <https://www.aaccnet.org/publications/cc/backissues/1991/Documents/68_424.pdf>
10. Wronkowska, M. – Haros, M. – Soral-Śmietana, M.: Effect of starch substitution by buckwheat flour on gluten-free bread quality. Food and Bioprocess Technology, *6*, 2013, pp. 1820–1827. DOI: 10.1007/s11947-012-0839-0.
11. Alvarez-Jubete, L. – Wijngaard, H. – Arendt, E. K. – Gallagher, E.: Polyphenol composition and in vitro

antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, *119*, 2010, pp. 770–778. DOI: 10.1016/j.foodchem.2009.07.032.

12. Tahir, I. – Farooq, S.: Grain composition in some buckwheat cultivars (*Fagopyrum* spp.) with particular reference to protein fractions. Plant Foods for Human Nutrition, *35*, 1985, pp. 153–158. DOI: 10.1007/BF01092142.
13. Drzewiecki, J. – Delgado-Licon, E. – Haruenkit, R. – Pawelzik, E. – Martin-Belloso, O. – Park, Y. – Jung, S. T. – Trakhtenberg, S. – Gorinstein, S.: Identification and differences of total proteins and their soluble fractions in some pseudocereals based on electrophoretic patterns. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 7798–7804. DOI: 10.1021/jf030322x.
14. Gorinstein, S. – Pawelzik, E. – Delgado-Licon, E. – Haruenkit, R. – Weisz, M. – Trakhtenberg, S.: Characterisation of pseudocereal and cereal proteins by protein and amino acid analysis. Journal of the Science of Food and Agriculture, *82*, 2002, pp. 886–891. DOI: 10.1002/jsfa.1120.
15. Wei, Y. – Hu, X. – Zhang, G. – Ouyang, S.: Studies on the amino acid and mineral content of buckwheat protein fractions. Nahrung/Food, *47*, 2003, pp. 114–116. DOI: 10.1002/food.200390020.
16. Ikeda, K.: Buckwheat: composition, chemistry and processing. Advances in Food and Nutrition Research, *44*, 2002, pp. 395–434. DOI: 10.1016/S1043-4526(02)44008-9.
17. Bonafaccia, G. – Marocchini, M. – Kreft, I.: Composition and technological properties of the flour and bran from common and tartary buckwheat. Food Chemistry, *80*, 2003, pp. 9–15. DOI: 10.1016/S0308-8146(02)00228-5.
18. Dziadek, K. – Kopeć, A. – Pastucha, E. – Piątkowska, E. – Leszczyńska, T. – Pisulewska, E. – Witkowicz, R. – Francik, R.: Basic chemical composition and bioactive compounds content in selected cultivars of buckwheat whole seeds, dehulled seeds and hulls. Journal of Cereal Science, *69*, 2016, pp. 1–8. DOI: 10.1016/j.jcs.2016.02.004.
19. Dziedzic, K. – Górecka, D. – Kucharska, M. – Przybylska, B.: Influence of technological process during buckwheat groats production on dietary fibre content and sorption of bile acids. Food Research International, *47*, 2012, pp. 279–283. DOI: 10.1016/j.foodres.2011.07.020.
20. Pomeranz, Y. – Marshall, H. G. – Robbins, G. S. – Gilbertson, J .T.: Protein content and aminoacid composition of maturing buckwheat (*Fagopyrum esculentum* Moench). Cereal Chemistry, *52*, 1975, pp. 479–484. ISSN: 0009-0352. <https://www.aaccnet.org/publications/cc/backissues/1975/Documents/chem52_479.pdf>
21. Maejima, Y. – Nakatsugawa, H. – Ichida, D. – Maejima, M. – Aoyagi, Y. – Maoka, T. – Etoh, H.: Functional compounds in fermented buckwheat sprouts. Bioscience, Biotechnology and Biochemistry, *75*, 2011, pp. 1708–1012. DOI: http://dx.doi.org/10.1271/bbb.110241.
22. Świeca, M.: Potentially bioaccessible phenolics, antioxidant activity and nutritional quality of young buckwheat sprouts affected by elicitation and elicitation supported by phenylpropanoid pathway precursor feeding. Food Chemistry, *192*, 2016, pp. 625–632. DOI: 10.1016/j.foodchem.2015.07.058.
23. Livesey, G.: Energy and protein requirements the 1985 report of the 1981 Joint FAO/WHO/UNU Expert Consultation. Nutrition Bulletin, *12*, 1987, pp. 138–149. DOI: 10.1111/j.1467-3010.1987.tb00040.x.
24. Schaafsma, G.: Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. British Journal of Nutrition, *108*, 2012, pp. S333–S336. DOI: 10.1017/S0007114512002541.
25. Seligson, F. H. – Mackey, L. N.: Variable predictions of protein quality by chemical score due to amino acid analysis and reference pattern. Journal of Nutrition, *114*, 1984, pp. 682–691. ISSN: 0022-3166.
26. Gupta, H. S. – Agrawal, P. K. – Mahajan, V. – Bisht, G. S. – Kumar, A. – Verma, P. – Srivastava, A. – Saha, S. – Babu, R. – Pant, M. C. – Mani, V. P.: Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. Current Science, *96*, 2009, pp. 230–237. ISSN: 0011-3891. <http://www.currentscience.ac.in/Downloads/article_id_096_02_0230_0237_0.pdf>
27. Fabian, C. – Ju, Y. H.: A Review on rice bran protein: its properties and extraction methods. Critical Reviews in Food Science and Nutrition, *51*, 2011, pp. 816–827. DOI: 10.1080/10408398.2010.482678.
28. Tomotake, H. – Yamamoto, N. – Yanaka, N. – Ohinata, H. – Yamazaki, R. – Kayashita, J. – Kato, N.: High protein buckwheat flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility. Nutrition, *22*, 2006, pp. 166–173. DOI: http://dx.doi.org/10.1016/j.nut.2005.01.012.
29. Kim, S. M. – Son, Y. K. – Hwang, J. J. – Kim, S. K. – Hur, H. S. – Park, C. H.: Development and utilization of buckwheat sprouts as functional vegetables. Fagopyrum, *18*, 2001, pp. 49–54. ISSN: 0352-3020.
30. Sytar, O. – Kosyan, A. – Taran, N. – Smetanska, I.: Antocyanins as marker for selection of buckwheat plants with high rutin content. Gesunde Pflanzen, *66*, 2014, pp. 165–169. DOI: 10.1007/s10343-014-0331-z.
31. Llames, C. – Fontaine J.: Determination of amino acids in feeds: Collaborative study. Journal of AOAC International, *77*, 1994, pp. 1362–1402. ISSN: 1060-3271.
32. Oser, B. L.: Methods for integrating essential amino acid content in the nutritional evaluation of protein. Journal of the American Dietetic Association, *27*, 1951, pp. 396–402. ISSN: 0002-8223.
33. Energy and protein requirements: Report of a Joint FAO/WHO/UNU Expert Consultation : WHO Technical Report Series 724. Geneva : World Health Organization, 1991. ISSN: 0512-3054, ISBN:

9241207248. <http://www.fao.org/docrep/003/aa040e/AA040E01.htm>

34. Morris, S. M. Jr.: Enzymes of arginine metabolism. Journal of Nutrition, *134*, 2004, pp. 2743S–2747S. ISSN: 0022-3166.
35. Winter, G. – Todd, C. D. – Trovato, M. – Forlani, G. – Funck, D.: Physiological implications of arginine metabolism in plants. Frontiers in Plant Science, *6*, 2015, Article 534. DOI: 10.3389/fpls.2015.00534.
36. Teixeira, W. F. – Fagan, E. B. – Soares, L. H. – Umburanas, R. C. – Reichardt, K. – Neto, D. D.: Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop. Frontiers in Plant Science, *8*, 2017, Article 327. DOI: 10.3389/fpls.2017.00327.
37. Sytar, O. – Brestic, M. – Zivcak, M. – Tran, L. S.: The contribution of buckwheat genetic resources to health and dietary diversity. Current Genomics, *17*, 2016, pp. 193–206. DOI: 10.2174/1389202917666160202215425.
38. Biel, W. – Jaroszewska, A. – Stankowski, S. – Sadkiewicz, J. – Bośko, P.: Effects of genotype and weed control on the nutrient composition of winter spelt (*Triticum aestivum* ssp. *spelta* L.) and common wheat (*Triticum aestivum* ssp. *vulgare*). Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, *66*, 2016, pp. 27–35. DOI: 10.1080/09064710.2015.1062533.
39. Gamborg, O. L.: The effects of amino acids and ammonium on the growth of plant cells in suspension culture. Plant Physiology, *45*, 1970, pp. 372–375. ISSN: 1532-2548.
40. Forde, B. G. – Lea, P. J.: Glutamate in plants: metabolism, regulation, and signalling. Journal of Experimental Botany, *58*, 2007, pp. 2339–2358. DOI: 10.1093/jxb/erm121.
41. Verslues, P. E. – Sharma, S.: Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book, *8*, 2010, Article e0140. DOI: 10.1199/tab.0140.
42. Szabados, L. – Savouré, A.: Proline: a multifunctional aminoacid, Trends in Plant Science, *15*, 2010, pp. 89–97. DOI: 10.1016/j.tplants.2009.11.009.
43. Maggio, A. S. – Miyazaki, P. – Veronese, T. – Fujita, J. I. – Ibeas, B. – Damsz, M. L. – Narasimhan, P. M. – Hasegawa, R. – Joly, J. – Bressan R. A.: Does proline accumulation play an active role in stress-induced growth reduction? Plant Journal, *31*, 2002, pp. 699–712. DOI: 10.1046/j.1365-313X.2002.01389.x.
44. Kreft, O. – Maimann, S. – Zeh, M. – Hoefgen, R.: Current understanding of the regulation of methionine biosynthesis in plants. Journal of Experimental Botany, *55*, 2004, pp. 1799–1808. DOI: 10.1093/jxb/erh139.
45. Kato, N. – Kayashita, J. – Sasaki, M. : Physiological functions of buckwheat protein and sericin as resistant proteins. Journal of the Japanese Society of Nutrition and Food Science, *53*, 2000, pp. 71–75. DOI: 10.4327/jsnfs.53.71. In Japanese.

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