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RESEARCH ARTICLE

Modification in amino acid profiles of barley and oat leaves during somaclonal breeding for abiotic resistance

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Abstract

The creation of regenerant plants of oat and barley during somaclonal breeding using stressors (RA_{PEG}, RA_{Cd}, RA_{Al}) and without them ($RA_{Control}$) led to a decrease in the total content of free amino acids relative to the initial genotypes by 15%-38%. At the same time, for barley, *in vitro* conditions were least affected by the content of Glu, Ser and Thr, and for oats-Thr and His. Changes in the amino acid profile in oat plants are more consistent in RA_{PEG}-RA_{Cd} pair (nine of the 15 amino acids had the same content) than in pairs RA_{Cd}-RA_{Al} (3 amino acids total) and RA_{PEG}- RA_{Al} (5 amino acids). For barley plants, the contents coincide of 8 different amino acids in the RA_{PEG}-RA_{Al} and RA_{PEG}-RA_{Cd} comparison pairs and of 7 amino acids in the RA_{Cd}-RA_{Al} pair. It is assumed that the change in the amino acid profile during somaclonal breeding is more determined by the species (cultivar) belonging of the used plant genotype than by the type of stressor.

Keywords: Drought, cadmium, aluminum, regenerant plants, cell breeding, resistance

Introduction

An effective way to increase plant genetic diversity and create sources of resistance to edaphic stressors in addition to widely used mutational breeding (Kailasam Peiter, 2021) is cell selection in vitro under selective conditions based on somaclonal variability. In the laboratory of plant biotechnology and microorganisms of Federal Agricultural Research Center of the North-East named after N.V. Rudnitsky (Kirov, Russia), a technology has been developed for creating cereal regenerant plants on selective media with ionic toxicity of aluminum, cadmium, as well as with water deficiency. Currently, 11 regenerative barley lines obtained at the FARC of the North-East by cell selection are included in the collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (St.-Petersburg, Russia) as genetic sources of tolerance to drought and high soil content of hydrogen and aluminum ions. Based on regenerant lines, new barley cv. Forward and Bionic were obtained, which under conditions of edaphic stress (pH 3.8-4.5; Al^{3+} 0.5-9.6 mg/100 g of soil) exceeded the standard cultivar by 113%-128% in yield (Forward-5.5 t/ha, Bionic-6.6 t/ha) (Shupletsova Shchennikova, 2016). On stressful soil backgrounds, regenerant lines induced on in vitro media with cadmium or aluminum showed low manifestation of oxidative stress symptoms (by intensity of lipid peroxidation), which provides higher seed productivity (1.5-3.6 times by the grain number and 1.5-3.0 times by the grain weight per plant) and adaptive advantages over initial genotype and regenerants induced on drought stress media (Shupletsova et al. 2020). Regenerants induced on selective media with cadmium show a high level of adaptation to stress: increased root barrier functions, minimal accumulation of toxic ions in above-ground organs, high seed productivity (exceeded the original genotype in terms of grain number and grain weight per plant by 35.5%) (Shupletsova & Tovstik 2021).

The use of regenerant plants in breeding involves a comprehensive assessment of their biochemical features, in particular, amino acid metabolism. There are many articles revealed that amino acid metabolism could

be a vital component for plant abiotic stress tolerance (Ali et al. 2019). Most of the attention of researchers is attracted by such amino acids as glutamate, proline (Teixeira et al. 2020), arginine, a group of aromatic amino acids and branched-chain amino acids (Heinemann, Hildebrandt 2021). Free amino acids have been shown to have functional roles in plant stress tolerance. They are involved in metal binding, antioxidant protection and signalling in plants under heavy metal stress (Xu et al. 2012). Amino acid metabolism plays an important role in intracellular pH regulation.

To increase the objectivity of the evaluation of plant adaptive potential, it is advisable to conduct studies on regenerant lines induced by the general initial genotype, which allows eliminating the factor of genotypic influence on the results. Since the mechanisms of plant resistance to excess metals in the medium are quite universal: delay of excessive number of ions in roots or outside metabolically important organs (accumulation in vacuoles, removal through the cell wall), conversion of excess ions into inert forms, and the realization of adaptive plant reactions to drought is due to mechanisms of another nature and is associated, first of all, with the accumulation of osmoprotectants, the purpose of present article was to identify differences in the content of some free amino acids in oats and barley plants of the initial genotypes and regenerant lines obtained in the presence of various stressors.

Materials and Methods

The subjects of the study were plants of barley cv. Luch, oats selection line 2h15 and their regenerant forms obtained as a result of cell somaclonal selection: 1. regenerant RA_{Al} lines induced by initial genotypes in callus culture on a selective medium with 40 mg/l Al^{3+}; 2. regenerant RA_{PEG} lines induced by initial genotypes in callus culture on a selective medium with 15% PEG (the advantages of PEG as a drought stress inducer is well-known; see for example (Frolov et al. 2017); 3. regenerant RA_{Cd} lines induced by initial genotypes in callus culture on a selective medium with 15 mg/l Cd^{2+}. Induction of callus with subsequent regeneration of plants was carried out according to previously developed methods (Shupletsova Shirokikh, 2015). Plants were grown in artificial climate chambers with a temperature of 22°C (day), 16 (night); illumination of 10,000 lx; air humidity 80% to the phase of leaf-tube formation on natural sod-podzolic soil at pH 6.0. The high performance liquid chromatomass spectrometry method was used to evaluate the amino acid composition of the leaves of the test plants (with triple replication of the experiment). Estimation of amino acid content was carried out on a tandem liquid chromatomass spectrometer LCMS-8040 Triple Quadrupole LCMS System (Shimazu, Japan) according to the manufacturer's instructions. The recorded m/z transitions were determined during the optimization of MRM parameters using the LabSolutionsLCMS 5.86 software. Statistical data processing was performed in Microsoft Office Excel 2013. The tables present average data from three analytical replications; the significance of the differences was assessed by the Duncan criterion at $p \leq 0.05$.

Results and Discussion

The content of selected amino acids varied greatly depending on the plant species and the composition of the selective medium. By itself, conducting cell selection in the absence of stress agents ($RA_{Control}$) significantly altered

Table 1. The concentrations of free amino acids (mg/g dry matter) in the leaf biomass of barley plants.

Amino acid	Initial genotype	Regenerant plant			
		$RA_{Control}$	RA_{PEG}	RA_{Al}	RA_{Cd}
Alanine (Ala)	0.0085 c	0.0030 b	0.0025 b	0.0005 a	0.0025 b
Arginine (Arg)	0.0305 b	0.0200 a	0.0230 a	0.0120 a	0.0195 a
Aspartic Acid (Asp)	0.0020 c	0.0020 c	0.0010 b	0.0010 b	0.0004 a
Glutamic Acid (Glu)	0.0035 ab	0.0060 b	0.0090 c	0.0020 a	0.0050 b
Histidine (His)	0.0009 b	0.0004 a	0.0010 b	0.0003 a	0.0003 a
Leucine+Isoleucine (Leu+Ile)	0.4075 d	0.3050 c	0.2685 bc	0.2515 b	0.2435 a
Lysine (Lys)	0.0080 c	0.0020 a	0.0025 ab	0.0030 b	0.0040 b
Methionine (Met)	0.0295 b	0.0450 c	0.0405 c	0.0195 a	0.0270 b
Phenylalanine (Phe)	0.6700 c	0.3615 a	0.6535 c	0.4105 a	0.4405 b
Proline (Pro)	0.0040 b	0.0030 a	0.0030 a	0.0025 a	0.0030 a
Serine (Ser)	0.0010 b	0.0010 b	0.0009 ab	0.0010 b	0.0006 a
Threonine (Thr)	0.0050 b	0.0030 a	0.0035 ab	0.0045 ab	0.0030 ab
Tyrosine (Tyr)	0.3815 c	0.2810 b	0.2900 ab	0.2505 ab	0.2370 a
Valine (Val)	0.0190 c	0.0180 c	0.0090 b	0.0070 a	0.0095 b
In total	1.5709 c	1.0509 a	1.3079 b	0.9658 a	0.9958 a

Note: for each cultivar values followed with the same letter don't differed statistically according to the Duncan criterion at $p \leq 0.05$.

the processes of amino acid synthesis in regenerant barley plants compared to the original genotype (Tab. 1).

The results presented in (Tab. 1) indicate that, in general, the synthesis of free amino acids in $RA_{Control}$ barley decreased by 33% compared to the initial genotype. At the same time, an increase in the content of Met was noted (by 52%). Four amino acids remained at the control level (Asp, Glu, Ser and Val), for the remaining amino acids there was a decrease in the content from 25% (Pro) to 65% (Ala). Content of aromatic AA (Phe and Tyr, which are necessary for generation of antioxidant metabolites (Tzin Galili, 2010) in RAControl plants relative to initial genotype was strongly decreased (by 46% and 26 % respectively). Content of branched-chain AA (Ile, Leu, and Val) had 25% depression for Leu+Ile but zero for Val. The branched-chain amino acids have already been identified as essential factors for dehydration tolerance (Pires et al. 2016).

The drought stress in the callus medium (RA_{PEG}) had a smaller overall effect (a decrease in the total content of free amino acids was only 17%). An increase in the content was noted for Met (by 37%) for Glu (almost three times). Aromatic amino acids reacted differently: the Phe content remained at the level of the initial genotype, and the Tyr content decreased by 24%. Phenylalanine (Phe) and tyrosine (Tyr) are known to be essential for protein synthesis and production of aromatic secondary metabolites such as anthocyanin, which are essential for maintaining cell wall extensibility (Zemanová et al. 2017). The synthesis of branched-chain AA decreased sharply: Val-by 53%, Leu+Ile-by 34%. At the same time, callus culture conditions with PEG did not affect the contents of His, Phe, Ser and Thr.

Callus media with aluminum RA_{Al} and cadmium RA_{Cd} led to a qualitatively similar change in amino acid profile; their overall level decreased relative to the initial genotype by 37%-39%. The content of aromatic amino acids was almost the same in these variants and decreased by 34%-39% relative to the initial genotype. The depression of branched-chain AA synthesis was slightly higher 39%-41%. Branched-chain amino acids (Ile, Leu, and Val) have previously been shown to increase dramatically in Cd-sensitive plants (Zemanová et al. 2017). Thr and Glu content remained at the level of the initial barley genotype.

The results (Tab. 2) indicate that in oat leaves content of aromatic AA (Phe and Tyr) in $RA_{Control}$ plants relative to initial genotype was decreased by 35% and 7% respectively.

Reaction of branched-chain AA (Ile, Leu, and Val, which are the building blocks of proteins) in oat was different to barley: 22% depression for Val but zero for Leu+Ile. The accumulation of Leu, Ile and Val may serve to promote stress-induced protein synthesis and may act as signaling molecules to regulate gene expression (Joshi et al. 2010). The reduction of these amino acids content may indicate a lack of stress in the *in vitro* medium. For the $RA_{Control}$, there was a decrease in the content of seven amino acids in different degree (by 8% for Tyr up to 78% for Lys), an increase of 50% in Ser. For seven more amino acids, no differences were noted with the initial genotype. The total level of amino acid content in $RA_{Control}$ was 79% of the initial genotype.

In the RAPEG, the content of aromatic amino acids was depressed by 26%-37%, branched-chain Leu+Ile-by 52%, but the synthesis of another branched-chain amino

Table 2. The concentrations of free amino acids (mg/g dry matter) in the leaf biomass of oat plants.

Amino acid	Initial genotype	Regenerant plant			
		$RA_{Control}$	RA_{PEG}	RA_{Al}	RA_{Cd}
Alanine (Ala)	0.0050 b	0.0045 ab	0.0040 a	0.0075 c	0.0035 a
Arginine (Arg)	0.0315 d	0.0170 a	0.0205 b	0.0230 b	0.0265 c
Aspartic Acid (Asp)	0.0010 b	0.0010 b	0.0010 b	0.0020 c	0.0002 a
Glutamic Acid (Glu)	0.0055 b	0.0045 b	0.0025 a	0.0025 a	0.0030 a
Histidine (His)	0.0010 a	0.0010 a	0.0010 a	0.0010 a	0.0010 a
Leucine+Isoleucine (Leu+Ile)	0.3745 d	0.3460 cd	0.1830 a	0.3120 c	0.2150 b
Lysine (Lys)	0.0040 c	0.0010 a	0.0030 b	0.0040 c	0.0025 b
Methionine (Met)	0.0820 b	0.0580 a	0.0765 ab	0.0780 b	0.0615 a
Phenylalanine (Phe)	0.6535 c	0.4265 a	0.4105 a	0.5215 b	0.4160 a
Proline (Pro)	0.0015 a	0.0025 a	0.0040 b	0.0055 c	0.0020 a
Serine (Ser)	0.0005 ab	0.0010 c	0.0004 a	0.0010 c	0.0004 ab
Threonine (Thr)	0.0035 ab	0.0040 b	0.0035 ab	0.0075 c	0.0025 a
Tyrosine (Tyr)	0.4270 c	0.3960 b	0.3155 a	0.3720 b	0.3155 a
Valine (Val)	0.0150 b	0.0125 a	0.0215 c	0.0195 bc	0.0150 ab
In total	1.6065 a	1.2755 b	1.0469 a	1.3570 b	1.0646 a

Note: for each cultivar values followed with the same letter don't differed statistically according to the Duncan criterion at $p \leq 0.05$

acid (Val) increased by 34%. For four amino acids (Asp, His, Met and Thr), no changes in content were noted, the Pro content almost tripled, for the remaining amino acids the degree of decrease varied from 20%-55%.

Unlike barley, significant differences were noted for oat plants in variants of metal ion (RA_{Al} and RA_{Cd}) action. If in the RA_{Cd} no synthesis enhancement was noted for any of the amino acids, then in the RA_{Al} Ala content was increased by 1.5 times, Thr and Asp-doubled, Pro synthesis-by 3.6 times. The decrease in aromatic amino acids was 17.5% for the RA_{Al}, 32.3% for the RA_{Cd}; for branched-chain AA, the content depression was 15.1% and 41.1%, respectively.

Conclusions

Thus, the study showed that somaclonal variability of oat and barley plants under in vitro conditions leads to significant qualitative and quantitative changes in the content of free amino acids. The total number of amino acids decreased in all variants relative to the original genotypes by 15.5%-34.8% for oats and by 16.7%-38.5% for barley. Despite the fact that the physiological reactions of plants to drought and to heavy metals differ significantly, changes in the amino acid profile in oat plants are more similar in pair RA_{PEG}-RA_{Cd} (nine of 15 amino acids had the same content) than in pairs RA_{Cd}-RA_{Al} (3 amino acids in total) and RA_{PEG}-RA_{Al} (5 amino acids). In all three RA variants, the same content of three amino acids is noted: Glu, His and Met. For barley plants, the contents of eight different amino acids in RA_{PEG}-RA_{Al} and RA_{PEG}-RA_{Cd} comparison pairs, and seven amino acids in the RA_{Cd}-RA_{Al} pair coincide. In all three RA variants, the content of six amino acids coincided, but others than those of oats: Arg, Lys, Pro, Ser, Thr, and Tyr. In general, the change in the amino acid profile during somaclonal breeding is most likely determined not so much by the type of stressor as by the species (cultivar) belonging of the used plant genotype.

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