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SOME ASPECTS OF THE WINTER WHEAT PHOTOSYNTHETIC APPARATUS RESISTANCE TO DROUGHT

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Summary. Changes in pigments and proteins content of chloroplast membranes in winter wheat varieties of different resistance under the influence of a drought were studied. The loss of pigments and main structural proteins was greater in less resistant varieties. It was established that the more resistant varieties differed in the increased content of low molecular weight proteins already in the control. Under the drought effects, the relative content of low molecular weight protective proteins increased. Thus, it is shown that the content of proteins of 36, 21 and 16 kDa can be one of the molecular markers of drought resistance.

Keywords: *Triticum aestivum* L., photosynthesis, pigments, drought. proteins of the photosynthetic apparatus.

Winter wheat is one of the main strategic crops of mankind [1], the yield of which is affected by various stress factors [2]. Drought occupies a special place among abiotic factors affecting the growth, development and productivity of plants [3]. Wheat harvest losses under different drought conditions can range from 30 to 90% [4]. In case of water deficit, first of all, the stomata are closed, which leads to a violation of transpiration and, as a result, to overheating of the leaf [5]. Photosystem II (PS II), electronic transport, and Rubisco-activase are the most sensitive to the effects of elevated temperatures [5, 6]. As a result of disruption of electron transport, reactive oxygen species are produced by the photosynthetic apparatus, which can lead to the development of oxidative stress and the destruction of a number of photosynthetic structures [7]. So, for example, one of these structures is D1protein, the resynthesis of which is blocked by active forms of oxygen [7]. Meanwhile, maintaining electron transport at a high level can significantly reduce the production of reactive oxygen species and prevent the development of oxidative stress. Plants have several mechanisms capable of maintaining electronic transport under drought conditions. Such mechanisms are photorespiration and cyclic transport of electrons [5, 7]. Also, recently, it is believed that plastoquinol oxidase (PTOX) can act as a certain "valve" that allows you to remove excess electrons from the electron transport chain, thereby keeping electron transport through PS II at a high level [8]. Since it is known that the resistance of plants to stress largely depends on the stability of the photosynthetic apparatus, it can be assumed that the different heat and drought resistance of winter wheat varieties can be determined precisely by the preservation of a high functional state of the photosynthetic apparatus. In this regard, the aim of the work was to study the peculiarities of the loss of pigment-protein complexes of chloroplasts of different drought-resistant varieties of winter wheat under the influence of drought, as well as to determine the production of protective low molecular weight proteins in these varieties.

Materials and methods

Four varieties of winter wheat of Ukrainian selection were used for research. These varieties differed in their resistance to drought. During the variety testing, they received the following rating:

Perlyna Lisostepu - 5-6 points, Dostatok - 7-8 points, Podolyanka - 8 points and Odeska 267 - 8.5-9 points.

Winter wheat of the researched varieties was sown on experimental plots of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine measuring 3x1 m in September. The soil is gray sod-podzolic. Application of NPK is standard according to cultivation technology. After overwintering in open ground, the plants were transplanted into 10 kg vegetation vessels. Control plants were irrigated to ensure 60-70% of the soil's total moisture content. For experimental plants in the flowering phase, drought conditions were created for 7 days at 30% total moisture content (FC). Flag leaves were selected to isolate chloroplasts. The leaves were homogenized for 2 minutes on an MPW - 302 homogenizer (Poland) in a medium containing 50 mM tricin pH 7.6, 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl2. After that, the homogenate was passed through 2 layers of calico and centrifuged on an Eppendorf 5810 centrifuge (Germany) for 5 minutes at a speed of 400g to precipitate large cell fragments. The supernatant was centrifuged a second time for 10 minutes at a speed of 1000 g, obtaining the fraction of chloroplasts in the sediment. The sediment was resuspended, passing it through capron, in 10 mm tricine buffer pH 7.6 with the addition of 0.1 M sucrose, 10 mm NaCl, 5 mm MgCl2.

Electrophoresis of chlorophyll-protein complexes was performed in a polyacrylamide gel [9]. The separating gel contained 12% acrylamide, 0.1% bisacrylamide, 6 M urea, 0.375 M Tris-HCl (pH 8.9), 0.003% ammonium persulfate, 0.0003% TEMED. The concentrating gel contained 8% acrylamide, 0.1% bisacrylamide, 6 M urea, 0.175 M Tris-HCl (pH 6.8), 0.003% ammonium persulfate, 0.0003% TEMED. The gel was stained with the Brilliant Blue R organic dye in an aqueous solution of 10% acetic acid with the addition of 30% ethanol for 12 hours at room temperature. Decolorization of the gel was carried out in an aqueous solution containing 10% ethanol and 10% acetic acid. The gel plates were scanned on the Epson Stylus SX 125 scanner, the registers were processed using the computer gel processing program "ScnImage". A mixture of marker proteins from the company "SIGMA", USA was used to identify the bands.

The content of pigmen in leaves was determined according to the Wellburn method [10].

Biological and analytical repeatability of experiments is three times.

Results and discussion

Table 1 presents the results of measuring the content of pigments in the leaves of various varieties of winter wheat under the influence of a 7-day drought. From the above results, it can be seen that the chlorophyll content in the leaves of all varieties decreased under the influence of drought. For more resistant varieties, Podolyanka and Odeska 267, this decrease was insignificant. In less resistant varieties, Dostatok and Perlyna Lisostepu, it was observed significant decrease in chlorophyll content.

Table 1

Changes in the content of pigments in the leaves of different varieties of winter wheat under the effects of drought

Sample		Chlorophyll, mg/g	Carotenoids, mg/g	Chl/Car
Odeska 267	Control	3,59±0,04	0,43±0,02	8,35±0,03
	Drought	3,26±0,04	0,45±0,02	7,24±0,03
Podolyanka	Control	3,79±0,05	0,41±0,02	9,24±0,05
	Drought	3,49±0,01	0,43±0,02	8,11±0,02
Dostatok	Control	3,69±0,07	0,39±0,05	9,46±0,07
	Drought	2,39±0,09	0,57±0,10	4.19±0,10
Perlyna Lisostepu	Control	3,73±0,17	0,38±0,10	9,81±0,12
	Drought	2,12±0,05	0,64±0,14	3,31±0,22

The content of carotenoids, on the contrary, slightly increased in more resistant varieties. In less resistant varieties, the increase in the content of carotenoids was quite significant. The ratio of chlorophylls and carotenoids decreased in all varieties due to the effect of drought, both due to the loss of chlorophylls and due to the increase in the content of carotenoids. An increase in the content of carotenoids is associated with aging or exposure to stress factors [11]. That is, from the obtained data it can be concluded that unstable varieties of winter wheat felt more significant stress than resistant varieties. Table 2 presents changes in the content of the main protein complexes of photosystem II by action 7th day of drought. The analysis of differences by varieties showed different levels of proteins in the photosynthetic system of chloroplasts. The primary photoreaction of PS II is localized on the D1/D2 heterodimer. It is proteins D1/D2 that form the active reaction center of FS II. The zone of filling with these proteins in the control versions of the chloroplasts of wheat plants is numerically close to the values. It is believed that the cooperation of the internal and peripheral antennas (CP47 and CP43) is mandatory for the stabilization of separated charges in the reaction center of FS II. It can be seen from the table that the ratio of internal and peripheral antennae (zones CP47 and CP43) fluctuates within close limits of ≈1.5 for all studied varieties.

Table 2

Changes in the relative content of the main protein complexes of photosystem II in chloroplasts of different varieties of winter wheat under the influence of a 7-day drought (% of total chloroplast protein)

Sample		CP 47	CP 43	D_1/D_2	C3K II
Odeska 267	Control	3,70±0,27	2,20±0,21	6,38±0,28	18,53±0,21
	Drought	3,52±0,30	2,07±0,22	6,08±0,30	17,73±0,30
Podolyanka	Control	3,73±0,21	2,45±0,16	7,32±0,35	18,67±0,21
	Drought	3,53±0,24	2,15±0,20	6,92±0,35	17,77±0,29
Dostatok	Control	4,80±0,28	3,18±0,12	6,81±0,28	21,57±0,20
	Drought	4,00±0,38	2,28±0,18	6,01±0,33	19,29±0,28
Perlyna Lisostepu	Control	5,99±0,16	3,85±0,14	7,00±0,31	23,98±0,19
	Drought	4,87±0,18	2,05±0,20	5,88±0,33	20,16±0,27

The effect of a 7-day drought on plants led to changes in the content of chloroplast proteins. Analysis of the content of chloroplast proteins showed that the decrease in functional activity is mainly due to the destruction of the protein of the reaction center D1, as well as the antenna protein SR 47 in all studied varieties, but to a different degree, in the chain from larger to smaller: Perlyna Lisostepu-Dostatok-Podolyanka-Odeska 267 Also, the content of antenna protein SR 43 and SZK II proteins decreased in all varieties. The table does not show proteins that belong to the FS I complex, since their content practically did not change.

Table 3 shows changes in the content of low molecular weight protective proteins of 36, 21, 16 and 14 kDa. The 36 kDa protein is the so-called plastoquinol oxidase, the 21 kDa protein is a water-soluble chlorophyll protein that stabilizes FS II, the 16 kDa protein contributes to the trimerization of FS II and its stabilization, and the 14 kDa protein is necessary to ensure the necessary conformation for tight binding of Ca++ and Cl-. The filling of the zones of these proteins in the control variants shows a decrease in their content along the chain: Odeska 267 – Podolyanka – Dostatok – Perlyna Lisostepu.

It was found that the pool of plastoquinone can participate in the transfer of electrons by the mechanism of chloroplast respiration (chlororespiration) with the participation of plastid terminal oxidase (PTOX - Plastid Terminal Oxidase) [12]. It has been shown that PTOX content increases in the chloroplasts of tomato and Arabidopsis plants under high temperature stress [13]. Activation of cyclic transport can be caused by structural reorganization of the thylakoid system of chloroplasts under stress.

Table 3

Changes in the content of low-molecular-weight proteins in chloroplasts of various varieties of winter wheat under the influence of a 7-day drought (% of total chloroplast protein)

Sample		36 kDa, %	21 kDa, %	16 kDa, %	14 kDa, %
Odeska 267	Control	10,24±0,12	9,68±0,38	11,04±0,34	5,92±0,33
	Drought	11,22±0,30	12,6±0,53	13,06±0,21	7,11±0,43
Podolyanka	Control	7,83±0,11	9,44±0,22	8,14±0,09	5,67±0,20
	Drought	8,54±0,20	11,22±0,38	10,22±0,51	7,23±0,41
Dostatok	Control	7,65±0,23	8,03±0,21	7,82±0,30	5,82±0,23
	Drought	8,13±0,15	11,8±0,25	9,40±0,21	7,55±0,12
Perlyna Lisostepu	Control	5,99±0,32	6,6±0,31	4,05±0,30	3,38±0,34
	Drought	5,91±0,23	11,20±0,22	6,07±0,52	4,55±0,32

To date, PTOX has been identified as a novel redox component associated with cyclic and linear transport. Data from literary sources indicate a dual role of PTOX: 1 – its activity is necessary for the effective desaturation of carotenoids in leaves at certain stages of the development of the plant organism, but not at all, which implies the existence of a pathway independent of plastid terminal oxidase for the re-oxidation of plastoquinol in combination with phytoene desaturase ; 2 - PTOX is involved in the mechanism of photorespiration in green plant tissues [14].

Analysis of the content of this protein in chloroplasts from wheat plants exposed to a 7-day drought showed an increase in the content of the 36 kDa protein zone, but to a different degree. As for the more drought-resistant varieties, the relative content of this protein (36 kDa) in the chloroplasts from the flag leaves of winter wheat plants of the Podolyanka and Odeska 267 varieties after 7 days of drought was 10% higher than in the control variants.

In the chloroplasts of the leaves of plants of the Dostatok variety, this indicator increased by 6% of the total chloroplast protein, and in the Perlyna Lisostepu variety, despite the fact that the increase was observed up to 21%, in the variant of the 7th day of drought, the relative protein content of 36 kDa still was lower than control variants of drought-resistant varieties. It follows from this that, indeed, the indicator of the content of this protein refers to the parameters that are related to the level of tolerance to water deficit, and can be used in selection works for drought resistance.

Studies of plant adaptations to osmotic stresses have revealed the presence of 20-22 kDa proteins that have homology with WSCP (water-soluble chlorophyll-protein) - a water-soluble chlorophyll-protein induced by salt stress. It was previously shown that the accumulation of mRNA-22 polypeptide is induced by salt stress and drought [15]. It is assumed that this protein reduces protease activity in drought-adapted leaves [16]. The table shows that drought also increases the synthesis of 16, 14 kDa proteins. The labeled polypeptides belong to the early light-induced proteins, which can be functionally involved in the mechanisms of resistance to drought and high temperatures. In the control versions, the content of these proteins, as well as for PTOX proteins, is located in a chain from smaller to larger: Perlyna Lisostepu - Dostatok - Podolyanka - Odeska 267. Under stressful conditions, including hyperthermia, energy absorbed by chlorophyll, which is not can be fully used in photosynthetic reactions, causes oxidative damage to cells with the participation of reactive oxygen species and free radicals. Therefore, the content of such protective proteins as PTOX, which supports electronic transport at a high level, is of great importance for maintaining high functional activity under stress conditions.

Conclusions

Drought-resistant varieties of winter wheat are characterized by an increased content of low molecular weight protective proteins, 36, 21, 16 and 14 kDa, in control plants. Due to drought, these varieties lose less pigments and the main structural proteins of photosystem II. At the same time, the

relative content of low molecular weight protective proteins increases. Thus, it is shown that the content of proteins of 36, 21 and 16 kDa can be one of the molecular markers of drought resistance.

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