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EFFECT OF DROUGHT ON THE TOTAL PROTEIN COMPOSITION IN SUNFLOWER SEEDLINGS

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Abstract. *The aim of the work was to study the protein composition of seedlings of several sunflower genotypes under the influence of simulated drought. The protein composition was studied using SDS electrophoresis. The drought effect was created using PEG (6000). Drought-induced changes in protein composition were detected in only two of the six studied genotypes. In the Rf 2 genotype, under the influence of PEG (30%), 2 zones with low molecular weights accumulated over 6 days. In the ASC3 genotype, 3 zones of proteins with low molecular weights began to appear already at the first stage of drought, and they accumulated as the drought intensified. The amount of these zones increased under severe drought by almost 5-10 times compared to the control. The molecular weights of the proteins that accumulate during drought (D1, D2 and D3) range from 22 to 20 kDa, which is consistent with the molecular weights of dehydrins, which found in Helianthus annuus. The enhanced accumulation of these proteins under drought conditions may suggest that the ASC3 genotype exhibited greater drought tolerance and could be a promising parental line for breeding purposes.*

Keywords: dehydrins, drought, genotype, protein composition, sunflower.

INTRODUCTION

Drought is one of the main climatic stresses, significantly reducing the yield of many agricultural plants [1]. Like other types of stress, water stress causes oxidative stress associated with an increase in the amount of reactive oxygen species (ROS) in plant cells [2]. ROS are highly reactive and can cause irreversible changes in the structures of proteins, nucleic acids, and membrane lipids [3]. Thus, water stress causes significant morphological, physiological, and biochemical changes in plants, which can lead to delayed plant growth and sometimes to the loss of part of the crop [4]. In plants resistant to various types of stress, the processes of ROS accumulation are usually inhibited. [5]. Plants capable of biosynthesis of various protective compounds have a high antioxidant status, allowing them to withstand unfavorable climatic conditions [6]. Therefore, analyzing breeding material for this trait is a crucial step in for effective selection.

An important role in maintaining plant viability under drought conditions

may be played by hydrophilic proteins, which help protect cells from dehydration [7]. These proteins belong to the group of dehydrins, which are classified as LEA proteins of class II [8]. The structural features of dehydrins include the presence of conserved sequences, designated K-, S- and Y-segments. The highly conserved K-segment is mandatory for all dehydrins [8]. The classification of dehydrins developed by Close T. is based on the number and combination of conserved segments in their structure. There are six classes of dehydrins: YnSKn, Kn, KnS, SKn, YnKn and FnSKn. Among them, the most common class of dehydrins is the YnSK2 class.

Dehydrins usually accumulate in seeds at late stages of plant development, but they are also found in vegetative tissues of plants under the influence of various stress factors [9]. Dehydrins are believed to prevent protein denaturation during dehydration and can also bind significant amounts of water, preventing tissue dehydration [10]. For example, barley dehydrins DHN1-DHN4, DHN6 and DHN9, which have the YSK2 structure, accumulate in seedlings in response to dehydration. [11]. Also, high levels of dehydrins have been found to correlate with plant resistance to drought and other types of stress. For sorghum plants (Sorghum bicolor (L) Moench), a correlation was also found between the drought resistance of varieties and the accumulation of dehydrins in their tissues [12]. Also was discovered that water stress increased the transcriptional activity of the dehydrin gene HaDhn2 of sunflower (*Heliantus annuus* L.) much more strongly in plants of the drought-tolerant variety compared to the drought-sensitive variety [13].

Dehydrins play an important role in alleviating dehydration stress in various rice species [14]. Among the dehydrins studied, the DHN8 gene of O. sativa ssp. japonica showed up to 448-fold increase in expression on the first day of stress treatment. In maize (*Z. mays*) seedlings, the level of transcripts for various dehydrins was significantly elevated under water deficiency [15]. However, the accumulation of dehydrin transcripts does not directly correlate with the content of the corresponding proteins. Therefore, the aim of our study was to investigate changes in the protein composition of seedlings of different sunflower genotypes under the influence of water stress.

MATERIALS AND METHODS

The material for the study was seedlings of 6 sunflower genotypes: three lines with cytoplasmic androsterility (ASC1-ASC3) and three androfertile lines with restored fertility (Rf1-Rf3). To induce drought stress, a solution of PEG (6000) was used in a concentration of 20% and 30%. Plants watered with distilled water served as a control. Experimental samples at the stage of the first true leaf pair were treated with PEG for 3 or 6 days. Some of the plants were rehydrated after 3 days of PEG treatment, while in other samples the process of creating drought was

continued. Thus, 4 variants of experimental samples were obtained, differing in the degree of drought: 20% PEG (3 days), 30% PEG (3 days), 20% PEG (6 days), and 30% PEG (6 days).

For protein extraction from plant material was used 0.1 M Tris-HCl buffer pH 8.0 (100 mM EDTA, 0.2% ascorbic acid). The sample was ground in a mortar and extracted by stirring for 1 hour in a sample/buffer ratio of 1:4. After centrifugation at 12000 for 10 minutes, a sample was precipitated using TCA. The precipitated protein was washed from impurities three times: with 70% ethanol, 96% ethanol and acetone. Then the protein was dissolved in protein buffer for SDS-electrophoresis. Proteins were separated by denaturing SDS PAGE [16]. A Mini-PROTEAN Tetra Cell chamber (Bio-Rad Laboratories, Inc., USA) was used for electrophoresis. The concentration of the separating gel was 12.5% AA, and the stacking gel was 4%. All tracks were aligned by the amount of protein added. Protein concentration was measured using Bradford protein assay [17]. Protein Marker IV peqGOLD (Life Science) were used as markers. Stained and washed electrophoregrams were scanned on an Epson Expression 10000XL scanner (GE Healthcare, USA). The evaluation of each sample (Rf value of all bands in the lane, calculation of molecular weights based on the markers, and determination of bands intensity) was carried out using the Phoretix 1D Advansed software (TotalLab, Ltd., Great Britain).

RESULTS AND DISCUSSIONS

The study of the total protein composition in sunflower seedlings leaves under drought stress of varying severity showed significant polymorphism of the response in different genotypes. Electrophoretic analysis of the studied sunflower genotypes under drought conditions showed that only two genotypes revealed noticeable changes in the total protein composition (Fig. 1). In the Rf 2 genotype, under the influence of severe drought, 2 protein zones (provisionally designated D1 and D3) with molecular masses of 21.7 kD and 19.6 kD accumulate. The abundance of zone D1 increases by 36%, and zone D3 - by 21% (Fig. 2).

In another sunflower line (ASC3), more significant changes in the protein composition were observed (Fig. 1). In this genotype, as water deficiency increased, three protein zones accumulated: D1 (Mr 21.7 kD), D2 (Mr 20.8 kD), and D3 (Mr 19.6 kD). The dynamics of accumulation of these zones, reflected in the diagram, indicates that in this genotype, already at the first stage of water stress (20% PEG/3 days), sharply increased synthesis of proteins with low molecular weight (Fig.2). Further intensification of drought stress resulted in even greater accumulation of these zones. The level of D1 protein level increased threefold at the first stage and further rose 4.5-fold as the drought intensified. Similarly, the amount of D2 protein also was accumulated 3.5-4.9 times. Zone D3 accumulates more than all others, it

increases 6.5 times at the first stage and 10 times at the last stage. The massive accumulation of low molecular weight proteins under drought stress in these two genotypes, as well as molecular weights of proteins, fluctuating from 22 to 20 kDa, may indicate that these proteins are dehydrins and play an important role in plant resistance to water stress.

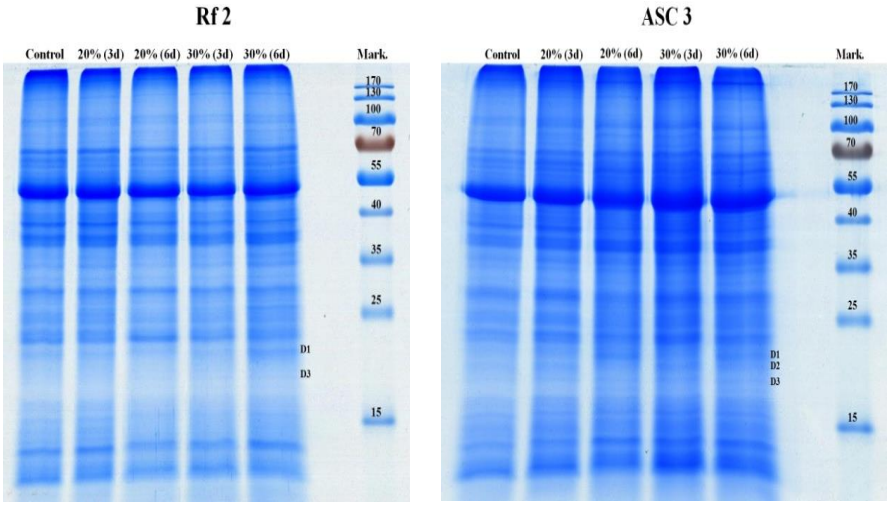


Fig. 1. Electrophoretic profiles of total protein in samples obtained during drought stress in sunflower genotype Rf 2 and ASC 3. The drought stress conditions are indicated above the tracks, the rightmost lane is the molecular mass markers (170-15 kDa).

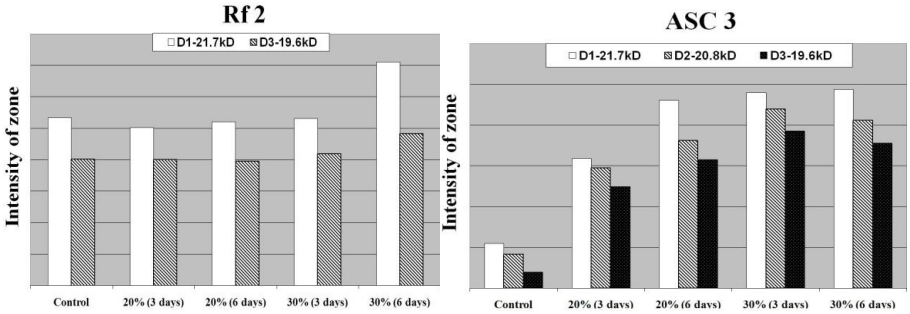


Fig. 2. Diagram of changes in the intensity of zones D1-D3 during increasing drought stress in the sunflower genotypes Rf 2 and ASC3.

In the drought-resistant sunflower genotype, the genes *HaDhn1* and *HaDhn2*, which accumulate under drought conditions, were identified [18]. The proteins encoded by these genes belong to the dehydrin family. The accumulation of *HaDhn1*, *HaDhn2* transcripts was compared in the resistant R1 genotype and in

the sensitive S1 genotype subjected to progressive drought. The results of the study confirmed that under drought conditions in the leaves of the resistant genotype R1, the accumulation of transcripts of dehydrins *HaDhn1, HaDhn2* was much higher than in the drought-sensitive genotype S1 plants [13]. According to the UniProtKB database, the molecular weights of dehydrin proteins found in sunflower range from 26 to 16 kDa [19].

CONCLUSIONS

Accurate assessment of resistance of plant genotypes is crucial for the successful development of drought-tolerant varieties. Coordinated approaches involving traditional plant breeding as well as molecular approaches should be used to identify and develop drought-tolerant varieties. To speed up the selection process, laboratory methods for early diagnosis of seeds and seedlings are used, which makes it possible to carry out assessment all year round and study large volumes of selection material.

This study identified two sunflower genotypes exhibiting noticeable alterations in seedling protein composition under drought conditions. These two lines also displayed significant differences in both the intensity and the timing of this response. In the Rf 2 genotype, changes in protein composition were observed only under very severe drought conditions (30% PEG/6 days), with the increase in the quantity of two protein zones (D1 and D3) being insignificant, at 36% and 21%, respectively. In contrast, the ASC 3 line showed an increase in the amount of D1, D2, and D3 proteins even under mild drought conditions (20% PEG/3 days). The accumulation of these three protein zones continued to increase with escalating water stress, reaching an almost fivefold increase at the severe stress stage (30% PEG/6 days).

The molecular weights of the proteins that accumulated during water stress (D1, D2, and D3) ranged from 22 to 18 kDa, consistent with the molecular weights of dehydrins reported in *Helianthus annuus* [18, 19]. The enhanced accumulation of proteins under drought conditions suggests that the ASC3 genotype possesses greater drought tolerance and may serve a promising parental line for breeding purposes. To further substantiate these findings, subsequent studies should focus on evaluating the antioxidant activity in seedlings of the investigated genotypes under drought stress conditions. A concurrent analysis of antioxidant responses alongside other components of the plant defense system will provide a more robust and comprehensive assessment of genotype-specific drought tolerance.

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