ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
First Online: October, 2022. DII 2067-5720 RAR 2022-16

ACCUMULATION OF DEHYDRIN TRANSCRIPTS CORRELATES WITH TOLERANCE TO DROUGHT STRESS IN SUNFLOWER

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ABSTRACT

Present study was focused on the analyses of phenotypic traits (across field and laboratory trials) and transcriptional changes in cotyledons and roots of sunflower hybrids, exposed to different levels of drought stress. By comparing the level of dehydrins genes (DHNs) expression in tolerant and sensitive genotypes as response to drought and of the correlations between physiologic responses in laboratory and field screening, an efficient and easily manageable experimental test system for sunflower seedlings was established. Thus, DHNs genes (Rab18-like, Xero1 and COR47-like) differentially expressed under induced hydric stress could be used as a proceeding for estimation of plant drought survival, hence, improving the pre-screening trials in the breeding programs aimed on plant tolerance to water-deficit stresses.

Keywords: dehydrins, drought stress, gene expression, germination, PEG, seedlings, sunflower.

INTRODUCTION

Climate change, characterized by an increase in temperature and reduced water availability, drastically affects agricultural production and global food security. Among strategic oilseed crops, sunflower, grown as a source of premium oil and dietary fiber throughout the world (Adeleke and Babalola, 2020), with annual production of 47 metric tonnes, is one of the most important (FAOSTAT). In the Republic of Moldova, it occupies 25% of the cultivated land and ranks third after wheat and corn (NBS).

Drought is a major abiotic stress that limits yield and product quality (Skoric, 2009). Although sunflower is a deep-rooted crop, exposure to water stress poses serious challenges for plants and causes the reduction or delay of seed germination, compromises seedlings establishment and their particularities (volume and lengths of root, lengths of shoot and coleoptiles, dry and fresh weight) and, finally, decreases the seed and oil production (Petcu et al., 2001; Ahmad et al., 2009; Saensee et al., 2012; Li et al., 2013; Chachar et al., 2016). According to the warm scenario, in all regions of Eastern Europe, including Moldova, a potential reduction in sunflower production of around 12-14% is expected in 2030 if current climate trends continued (Debaeke et al., 2017).

Genetic improvement of crops with enhanced tolerance to drought is one of the most effective and economically feasible approaches to minimize the adverse effects of stress on yield and to contribute to successful sunflower production, especially in water deficit areas. Evaluation of available germplasm to identify drought-tolerant genotypes using accurate, rapid and effective screening methods is a key element for breeding programs and development of new cultivars. A series of agronomic, morphological, physiological, and metabolic traits, which are modified in response to stress, may serve as a potential approach for selection (Ashraf et al., 2006; Ahmad et al., 2022).

An efficient and rapid way for primary

Received 1 September 2022; accepted 12 October 2022.

screening to drought tolerance in a laboratory is the application of polyethylene glycol (PEG 6000) in order to create osmotic stress consequences of drought condition. Many breeders successfully used the agro-morphological traits, as well as plant responses to PEG induced drought stress and stress tolerance indices derived from germination percentage (GSI), dry matter (DMSI), plant height (PHSI) and root length (RLSI) as significant parameters for the selection of sunflower drought-tolerant genotypes (Kaya et al., 2006; Ahmad et al., 2009; Vassilevska-Ivanov et al., 2014; Razzaq et al., 2017; Saucă et al., 2017; Darbani et al., 2020).

Conventional breeding for drought resistance is time consuming and is greatly constrained by inadequate screening methods and lack of knowledge of the genetics and molecular base of drought resistance. To overcome such barriers, the molecular approach focused on the identification of reliable markers associated with drought tolerance, which could accelerate the genetic enhancement of drought tolerance may be used (Nirmal Raj et al., 2019).

Dehydration stress induces significant molecular responses involving changes in their transcriptome, proteome, and metabolome (Fernandez et al., 2008; Fulda et al., 2011; Hanin et al., 2011; Sun et al., 2021). In recent years, various physiological investigations have highlighted the positive relationship between cellular accumulation of dehydrins (DHNs) and increased plant tolerance to water-deficit stresses, especially cold, drought, and high salinity (Hernández-Sanchez et al., 2017; Zhang et al., 2018; Sun et al., 2021). DHNs share a number of structural features, the most notable being three types of conserved sequence: K-(EKKGIMDKIKEKLPG), Y-([T/V]D[E/Q] YGNP) and S-(serine-track) motifs, among which K- is the core segment of amphiphilic α-helixes located at the C-terminal end of the proteins (Cuevas-Velazquez et al., 2014). Also, biochemical studies indicate the K-segments are the functional parts of DHNs that mediate cellular stress tolerance (Drira et al., 2013), bind the dehydrins to cell membranes (Eriksson et al., 2016), or maintain enzyme activity (Yang et al., 2015). Based on the presence of the K-, S-, and Y-segments, DHNs are classified into the Kn, SKn, KnS, YnKn, and YnSKn structural subgroups (Sun et al., 2021).

In the model plant *Arabidopsis thaliana* genome several DHNs genes have been annotated. They include *RAB18* (Y2SK2-type), *RD29B* (Kn-type), *COR47* (SK3-type), *ERD10* (SK3-type), *ERD14* (SK2-type) that may be found in many subcellular locations (Sun et al., 2021), for example in nucleus (Kalemba et al., 2015), cytoplasm (Cui et al., 2020), or in both (Sánchez et al., 2017). Different type of dehydrins, such as *RAB18*, *COR47* and *XERO1* are differential expressed in cotyledon, shoot and leaf apex, inflorescence meristem, petal, petiole and other plant structure as response to cold, heat, water deprivation, being considered abiotic stress marker genes (Hernández-Sánchez et al., 2017).

Aspects of molecular responses of sunflower to drought stress have been analyzed at the gene expression level by many researcher (Cellier et al., 1998, 2000; Kane and Rieseberg, 2007; Roche et al., 2007; Fernandez et al., 2008; Liang et al., 2017). Thus, the increase of two dehydrins, *HaDhn1* and *HaDhn2* was correlated with the decrease of midday leaf water potential during progressive stress and mainly up-regulated in a drought tolerant line (Cellier et al., 1998). An oscillation of *HaDhn1* in a diurnal way with a peak of mRNA at midday was ascertain. In contrast, the increase of *HaDhn2* transcript was independent of day/night cycles (Cellier et al., 2000). Also, an association between physiological response to water stress and differential expression of DHNs genes in leaves, were revealed by assessing of four sunflower recombinant inbred lines and parental lines contrasted in their responses to dehydration and rehydration (Kiani et al., 2007).

Recently, as a result of sunflower (HanXRQr2.0-SUNRISE) genome sequencing, three DHNs genes have been annotated: *Rab18-like* (Gene ID: 118488043), *Xero1* (Gene ID: 110908988) and *COR47-like* (Gene ID: 110910634).

These dehydrins have been the subject of the present transcriptomic study in context of association of the molecular markers with physiological drought tolerance at the seedling stage. The germination is one of the critical growth stages of crop plants that are very sensitive to drought. There will be no germination if osmotic potential reaches up to -5.0 bars (Ahmad et al., 2009).

The research presents the analysis of the data convergence of a field and laboratory trials on sunflower in order to identify contrasting genotypes by the physiological and molecular response (differential DHN gene expression) to water stress for the opportunity to identify of the easy-to-test indices in drought-resistant genotypes screening.

MATERIAL AND METHODS

Plant growth and stress treatments

Thirteen experimental sunflower hybrids created by the Company AMG-Agroselect Comert have been tested in comparative trials in the experimental field during 2019 and 2020 years according to the conditions mentioned by Duca et al. (2022). Data was recorded for ten plants (n=10, 4 repetitions), selected from central rows, for eight quantitative traits: Plant Height (PH, cm), Number of Leaves (NL), Head Diameter (HD, cm), Number of Achene per Head (APH), Achene Weight per Head (AWH, g), 1000-Seed Weight (TSW, g), Hectoliter Mass of Seeds (HMS kg/hl) and Seed yield (SY, kg/ha). Data was collected during vegetative stage (PH and NL) and after being harvested (HD, APH, AWH, TSW, HMS, SY).

Two hybrids were tested against hydric stress at germination and seedling stages under laboratory conditions. The first hybrid, conventionally noted as 413S, presented significant reduction of the majority of analysed agronomic traits in 2020 (characterized by low precipitation quantity, especially in the most critical stages of sunflower development) compared to 2019 (Duca et al., 2022) and supposed to be sensible. The second one (1718R), showed stable productive indices in the same conditions and was supposed to be resistant to drought.

Polyethylene glycol 6000 (PEG-6000) solution of 10% and 20% (osmotic potential of -0.55 and -1.60 MPa, respectively) was used to induce drought stress. The experience has been performed according to the methodology described by Clapco et al. (2018). Number of germinated seeds were counted daily and data was recorded for 8 days. After this period the number of germinated seeds were recorded and the germination rate (GR), promptness index (PI) and germination stress index (GSTI) was calculated according to George (1967), using following formulae:

GR = Germinated seeds/Total Seeds x 100;

PI = nd2(1,0) + nd4(0,75) + nd6(0,50) + nd8(0,25), where: nd2, nd4, nd6, nd8, represent the number of germinated seeds at 2^{nd}, 4^{th}, 6^{th}, and 8^{th} day, respectively.

GSTI = (PI of stressed seeds/PI of control seeds) x 100.

The shoot and root length were measured in cm with a ruler after 8 days of the start of the experiment. Plant dry weights were recorded after drying at 70°C at a constant weight. Based on these measurements the plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) were calculated according to Ashraf et al. (2006), as following:

PHSI = (Plant height of stressed plant/ Plant height of control plants) x 100

RLSI = (Root length stressed plant/Root length of control plants) x 100

DMSI = (Dry matter of stressed plant/Dry matter of control plants) x 100.

RNA extraction and cDNA synthesis

The cotyledons and roots of drought-stressed (PEG-treated) and control (distilled water only) sunflower 10-day old seedlings were collected and immediately frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted using TRI Reagent (Ambion, Applied Biosystems) according to the manufacturer's protocol. The quality and

yield of the RNA samples were assessed through gel electrophoresis and UV spectrometry.

For reverse-transcription reactions the purified RNA samples (1 μg) were treated with DNAse I, RNAse-free (Thermo Scientific). First strand cDNA was synthesized using RevertAid RT Kit (Thermo Scientific), oligo (dT)18 and random hexamer primers, following the manufacturer's instructions. The experimental design comprised three biological replicates of each experimental variants.

Quantitative Real-time PCR analysis

Three genes related to drought stress response were analyzed by quantitative real-time PCR (qRT-PCR) using cDNA as template. The specific primers for target and reference genes were designed based on the published *H. annuus* sequences using Primer3Web v.3.0.0. (Untergasser et al., 2012) (Table 1).

Each Real-Time PCR reaction (total volume of 20 μl) included: 2 μl of template (1:6 diluted in RNAse free water cDNA), 2 × Maxima SYBR Green/ROX PCR Master Mix (Fermentas), 0,4 μM of each reverse and forward primers. The thermal cycling conditions programmed at the thermocycler Applied Biosystems QuantStudio 5 (Thermo Fisher Scientific) were: initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 40 s at 60°C. The gene-specific amplification products were assessed by melting curve analysis, followed by agarose gel electrophoresis. All reactions were performed in triplicate, including the non-template controls.

The cycle threshold (Ct) method was used to present relative gene expression. Normalized expression data were computed as $2^{-\Delta Ct}$, where $\triangle Ct = (Ct_{target\ gene} - Ct_{reference\ gene})$. Fold change ($FC= 2^{-\Delta\Delta Ct}$) was used to compare the dehydrin genes expression in two different samples (PEG treated/untreated control, tolerant/sensitive hybrid in response to drought). In case of $2^{-\Delta\Delta Ct}<1$, the negative inverse of $2^{-\Delta\Delta Ct}$ ($-1/2^{-\Delta\Delta Ct}$) was considered as the fold change of reduction in gene expression (Schmittgen and Livak, 2008).

Table 1. Genes and primers used in the study

DHN Genes (GeneBank ID)	Transcript (length, nt)	Primer sequence (5´→3´)	
		Forward	Reverse
Rab18-like (118488043)	XM_035985091.1 (1089)	caaaaccaacccagtgccac	tcctccttgcccatcatcct
Xero1 (110908988)	XM_022153993.2 (843)	aggatgatggagagggaggc	attcaccaccgcctacagtg
COR47-like (110910634)	XM_022155250.2 (1108)	tcagatgttcacgcaccacc	tccgctttcttgtgaccacc
Actin (reference gene)	AF282624.1 (1134)	gctaacagggaaaagatgactc	actggcataaagagaaagcacg

Statistical analysis

Primary data was subjected to descriptive statistical analysis. Difference significance of mean values was analysed by Student's t-test ($n=3$, $p<0.05$). Data was graphed as mean ± SDs. Genes with a p-value lower than 0.05 and $FC\geq1.5$ (upregulated) or $\leq$-1.5 (down regulated) were considered to be differentially expressed.

RESULTS AND DISCUSSION

Growth and yield of sunflower hybrids in the field trial

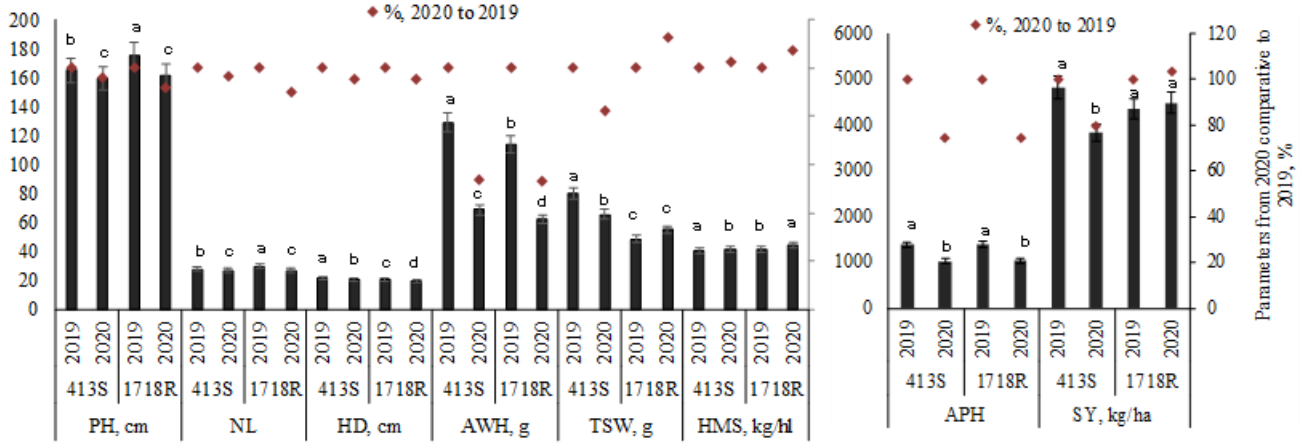
According to observation data (Figure 1), in experimental hybrid 413 all analyzed parameters, except HMS which shown a small increase (by 2.7%), were significantly lower in 2020 compared to 2019. Reduction of plant height, leaves number and head diameter was less pronounced and constituted 4.3%, 3.6% and 4.5%, respectively. Unfavorable environmental conditions especially influenced the yield related traits, such as number and weight of seeds per head, 1000-seeds weight, as well as yield. Thus, in the second year, these parameters have decreased by 17.9-46.3%, the most affected trait being AWH. Consequently, the yield decreased by 20.6%.

Contrary, hybrid 1718 presented a high tolerance to drought and even some parameters, such as plant height, leaves number and head diameter were even lower (by 4.8-10.0%) in

2020, yield related traits (especially TSW and HMS), as well as yield indicated higher values (by 3.1-12.5%) than in 2019, the differences being statistically insignificant at 5% probability level. 1000-seed weight is one of the most important quality factors, of which grain sturdiness and filling (Radić et al., 2013).

According to Abolhasani and Saeedi (2004) seed number was the most important criteria for yield improvement in either stressed or unstressed condition. The reduction in the number of seeds per head and seed weight after water deficit periods was also reported by Göksoy et al. (2004) and Pourtaghi et al. (2011).

In our research, both hybrids showed a similar decrease of APH and AWH (25.5% and 25.7%; 46.3% and 45.7%, for 413 and 1718, respectively) under drought conditions.



Note: *Means with different letters are statistically different between each other at 5% probability level ($p<0.05$, Student's t-test).*

Figure 1. Agronomic traits of hybrid 413S and 1718R during two experimental periods (2019 and 2020)

Stress tolerance indices of sunflower genotypes

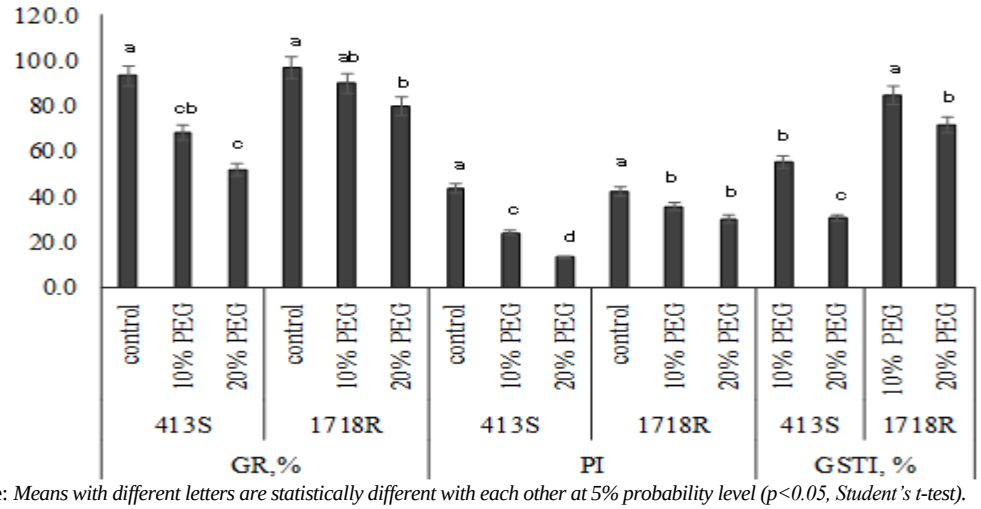
The, germination rate (GR) of sunflower hybrid studied decreased considerably with the increasing of hydric stress severity, (Figure 2).

Thus, if at -0.55 MPa, osmotic potential GR in hybrid 413S decreased by 25% compared to untreated control, in the case of -1.36 MPa, osmotic potential was 41.6% lower. In the drought tolerant hybrid 1718 a reduction of GR by 6.7 (statistically insignificant) and 16.7%, respectively, was observed. Previously, many researchers concluded that increasing drought stress levels progressively reduced seed germination in sunflower genotypes (Kaya et al., 2006; Ahmad et al., 2009; Saensee et al., 2012; Clapco et al., 2018).

Promptness (PI) and germination stress tolerance (GSTI) indices used to interpret differences in the rate of germination due to osmotic stress indicated more pronounced divergences among genotypes. The drought tolerant hybrid showed higher values for both levels of applied osmotic potential: PI - 35.7 and 30.3%, respectively, for -0.55 and -1.36 MPa osmotic potential, compared to 24.1 and 13.3% in the sensible hybrid; GSTI - 84.6 and 71.7%, compared to 55.3 and 30.6%, respectively (Figure 2).

These results were in accordance with those of Ahmad et al. (2009) who reported that water stress (induced with PEG solution) at germination and seedling growth stages reduced the GSTI in six sunflower hybrids/breeding lines. Decrease in tolerance indices was explained by the delay in seedling emergence due to slower cell division and plant metabolism disturbance under water deficiency (Ayaz et al., 2000; Ahmad et al., 2009).

Promptness index and germination stress tolerance index were also found as reliable. indicators to screen drought tolerant maize hybrids at seedling stage (Partheeban et al., 2017; Nirmal Raj et al., 2019).



Note: *Means with different letters are statistically different with each other at 5% probability level ($p<0.05$, Student's t-test).*

Figure 2. Effect of different PEG concentrations on final germination rate (GR), promptness index (PI) and stress tolerance index (GSI) in sunflower hybrids

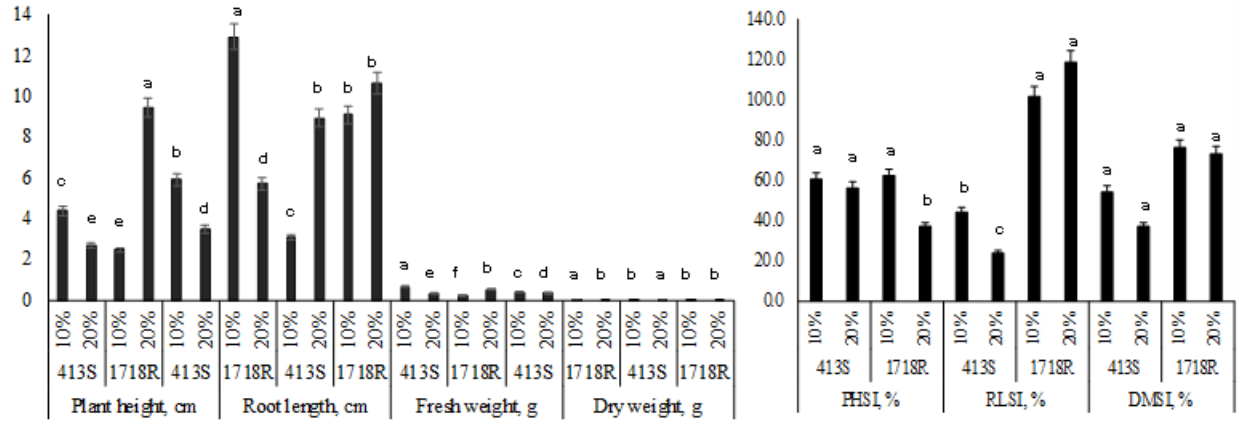
The physiological indices such as plant height index (PHSI), root length stress index (RLSI) and dry matter stress index (DMSI), also were used to evaluate the response of sunflower hybrids to PEG-induced water stress.

A significant reduction of all analyzed parameters, except root length of 1718R hybrid, was observed with the increase of PEG concentration in both sunflower genotypes (Figure 3).

In the case of hybrid 413S the most affected traits were root length (by 55.7 and 76.0% lower comparative to control for -0.55, and -1.60 MPa osmotic potential, respectively), followed by plant height (39.1%). Contrary to the aforementioned, the effect of hydric stress on root elongation in drought tolerant hybrid 1718R was insignificant and greater values were observed in PEG treated samples as compared to control (by 18.6% at -1.60 MPa osmotic potential). In the same time, a significant decrease (by 37.4 and 63.0% lower comparative to control for -0.55, and -1.60 MPa osmotic potential, respectively) in seedling length was observed in the resistant hybrid. A similar extension in root length concomitant to the reduction in shoot length has been also described in other agronomic species (Guoxiong et al., 2002; Shabbir et al., 2015).

It is known that higher root growth is linked with better drought tolerance, helping plants to explore deeper soil layers and ensure a supply of water under dry conditions. Sharp and Davies (1989) noted that the development of the root system in dry soil is usually less inhibited than shoot growth, and may even be promoted. This trait is under genetic control and constitutes a benefit to maintain an adequate water supply in plants (Sponchiado et al., 1989). According to Nejad (2011) better root growth at seedling stage would result in perfect root architecture at maturity.

Both hybrids responded similarly to drought stress by decrease of dry matter values, the effect being more pronounced in drought tolerant genotype (by 31.1% lower) comparative to sensible one (-19.4%) at 20% PEG6000 treatments. It should be noted that if seedling dry weight did not change significantly, the fresh weight decreased along with the increase of PEG concentration, ranging between 73.5-76.3% and 37.6-54.6% to the control in resistant and sensible hybrid, respectively. The results are in accordance with those reported by Clapco et al. (2018), Ahmad et al. (2009) and Saensee et al. (2012) and suggest that water stress induces especially a reduction of water content in seedling, not the seedling mass accumulation.



Note: *Means with different letters are statistically different with each other at 5% probability level (p<0.05, Student's t-test)*

Figure 3. Effect of different PEG concentrations on plant height, root length and dry matter stress tolerance indexes (PHSI, RLSI and DMSI) of some sunflower hybrids

Analysis of physiological indices showed significant difference among genotypes only for root length stress tolerance index (RLSI) that suggests this trait is an important criterion for selection.

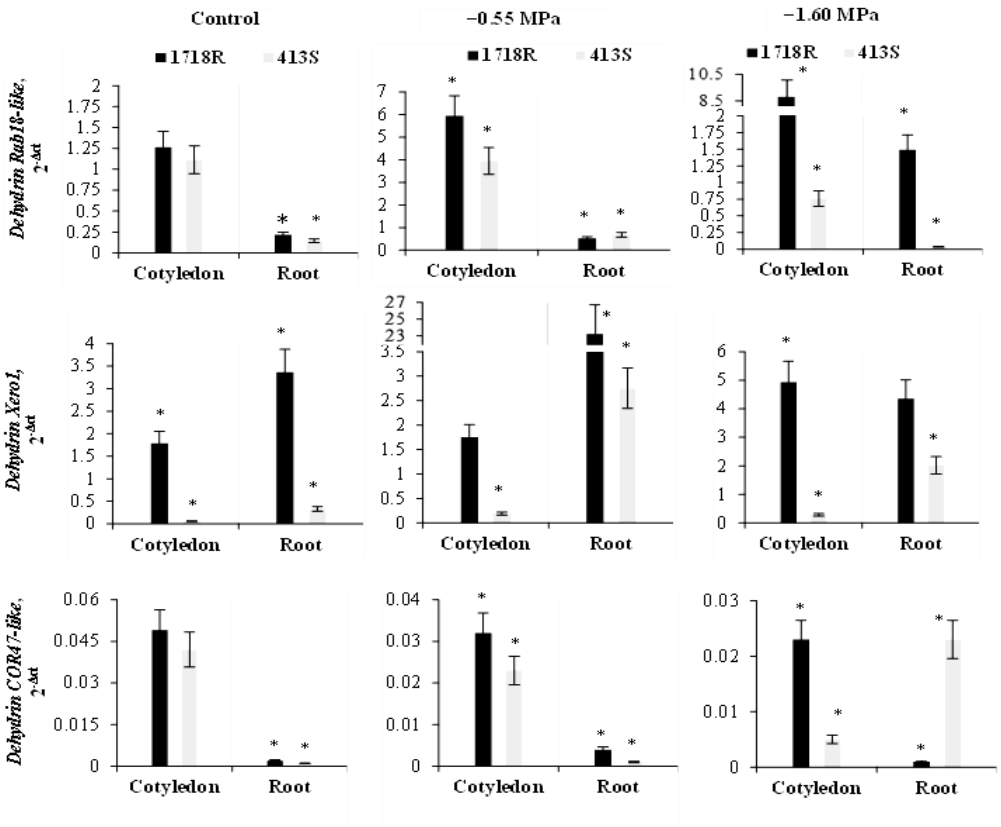
Dehydrins gene expression assay by qRT-PCR

Two experimental hybrids of sunflower (1718R and 413S) with different sensitivity to drought showed a various steady-state of DHN transcripts amount in normal, unstressed seedlings and much more differentiated level in response to induced hydric stress. The differences of mRNA quantities are determined not only of biological contrasting responses of plants, but also by the type of studied dehydrins. Thus, in both untreated hybrids the dehydrin *Xero1* gene is more transcriptionally active in roots than in cotyledons, compared to *Rab18-like* and *COR47-like* whose transcripts accumulate much more in cotyledons than in roots (Figure 4).

It is also important to note that in control plants the highest content of transcripts (except those of dehydrin Rab18-like in cotyledons) was those of the *dehydrin Xero1* (maxim values of relative expression 1.79 for cotyledons and 3.37 for roots). In contrast, the lowest values were identified in the case of the dehydrin *COR47-like* gene (maximum values of 0.049 for cotyledons and 0.002 for roots) compared to the other two DHNs investigated.

Quantitative changes in expression of DHN genes of drought tolerant plants 1718R *versus* sensitive 413S plants were detected. Thus, analysis of the relative gene expression ratio of control plants 1718R versus 413S revealed a higher concentration of *Xero1* transcripts in cotyledons (19.65-fold) and roots (10.28-fold). Also, the drought-tolerant hybrid differs from the sensitive one by the higher content of the transcripts of the other two genes studied *Rab18-like* (1.51-fold) and *COR47-like* (2-fold), but only in roots. The differences in transcript amounts in cotyledons of 1718R versus 413S are not significant for *Rab18-like* gene (FC=1.13; p=0.29) and *COR47-like* gene (FC=1.15; p=0.38) (Figure 5).

The molecular responses, of two divergent sunflower genotypes by their physiological response to PEG-induced and naturally, in the field hydric stress, are similar in some cases and different in others, depending on seedling tissues and dehydrin genes (Figure 4). According to fold changes used to compare the dehydrin genes expression in studied hybrids due to PEG-treatment, the 1718R showed increased amount of *Rab18-like* transcript in both seedlings' tissues, in cotyledons (4.70- and 6.98-fold in variants with -0.55 and -1.60 MPa, respectively) and roots (2.40- and 6.87-fold in case of -0.55 and -1.60 MPa, respectively). Interestingly, another response expressed by *Rab18-like* mRNA relative amount variation due to PEG treatment is ascertained in case of the sensitive genotype 413S.



Note: *Bars represent the mean ± standard deviations (SDs) of three independent biological replicates (*p<0.05, Student's t-test)*

Figure 4. Relative expression levels of DHN genes in seedlings cotyledons and roots under normal and induced hydric stress conditions

Thus, in this hybrid, level of *Rab18-like* expression revealed upregulation in samples treated with 10% PEG (3.95-fold in cotyledons and 4.8-fold in roots) and down regulation under 20% PEG treatment (-1.5-fold in cotyledons and -3.6-fold in roots).

A differentiated expression of genes was, also, highlighted at *Xero1* mRNA level, which is upregulated in all stressed samples of 413S, up to 6-fold related to untreated samples. In contrast, the drought-tolerant genotype 1718R showed a variable molecular response for cotyledons and roots depending of intensity of induced hydric stress. Increased amounts of dehydrin *Xero1* transcript were identified, only, in the cotyledons (2.76-fold) under 20% PEG and roots (6.87-fold) of samples treated with 10% PEG, revealing a higher hydric stress sensitivity of the roots comparative to cotyledons. In the other analyzed variants (cotyledon and roots under 10 and 20% PEG respectively) the change of the dehydrin *Xero1* transcript concentration is statistically insignificant.

A molecular response of the seedlings under hydric stress different than that revealed at the genes *Rab18-like* and *Xero1*, have been established in the case of the *COR47-like* - the gene that showed the smallest amounts of transcript in control samples of both genotypes. Thus, in the cotyledons of both hybrids analyzed under stress, there is a lower content of dehydrin *COR47-like* transcript, especially in samples treated with 20% PEG, 2-fold in the resistant genotype and 8.6-fold in the sensitive one. In contrast, in the roots of the treated sensitive hybrid, this gene showed a higher expression level of 5.6-fold (-0.55 MPa) and 20.2-fold (-1.60 MPa). In the seedling roots of the resistant hybrid, the mild intensity stress causes upregulation (FC=2.0), while the one of higher intensity induce down regulation (FC=-2.0) of the expression of dehydrin *COR47-like* mRNA (Figure 5).

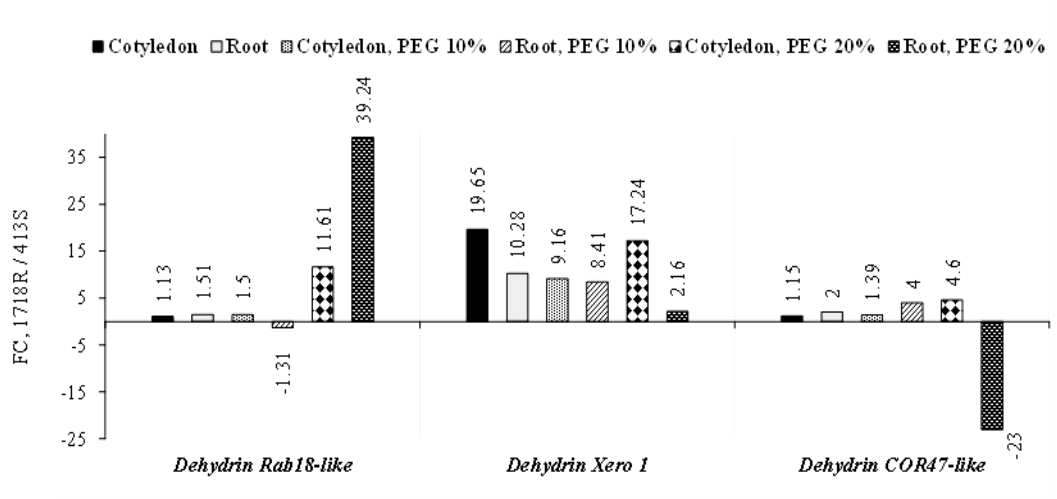


Figure 5. Quantitative changes of DHN genes expression in drought tolerant (1718R) versus sensitive (413S) plants under normal and hydric stress conditions

In conclusion, all three dehydrins analyzed changed their transcript content due to PEG treatment, both in the cotyledons and in the roots. However, there is a higher response reaction in the roots compared to cotyledons, especially in the 413S sensitive genotype. Also, it is obvious the general character (the same change trend for all treated samples of the same genotype) of upregulation in dehydrin expression of *Rab18-like* in case of tolerant genotype and of *Xero1* in case of the sensitive one. A more contrasting response due to the induced stress is highlighted for expression of dehydrin *COR47-like* depending on all experimental factors (genotype, PEG treatment, seedlings tissues).

Quantitative variations of DHN transcripts, in normal conditions and as response to drought, mostly upregulated than downregulated in a tissue-specific manner and depending on severity of stress. This indicates that studied genes may have different functions as part of distinct mechanisms for hydric stress tolerance. The previous investigations showed that in *Arabidopsis*, *AtCOR47* is expressed constitutively in all tissues in response to low temperature (Puhakainen et al., 2004). The biological processes have been established for *AtCOR47*, such as ion and water-binding, cryoprotective activity, metal-binding (Hara, 2010; Sun et al., 2021). The Arabidopsis DHN *AtRAB18* is also upregulated and accumulates under low temperatures, drought, salinity, and ABA, revealing that it is involved in these stress responses (Nylander et al., 2001; Alsheikh et al., 2005; Hundertmark and Hincha, 2008).

The amounts of *COR47-like* transcripts are less compared to the other two dehydrins (*Rab18-like* and *Xero1* mARN), analyzed in control conditions. This is evidence that suggests variations in dehydrin accumulation patterns with respect to plant development stage. For example, it has been established that the dehydrin profiles of winter wheat at seedling, tillering, jointing, and anthesis stages differ from each other (Zhang et al., 2013).

Another important conclusion is revealed from the analysis of quantitative changes of DHN genes expression in drought tolerant (1718R) versus sensitive (413S) plants under hydric stress. Thus, the genotype that showed resistance to hydric stress in the laboratory and field trials differs essentially by the higher content of dehydrin *Xero1* transcripts, both under normal conditions and in those of induced hydric stress (-0.55 and -1.60 MP osmotic potential). Moreover, the 1718R genotype differs from the 413S by the mRNA amounts of the *Rab18-like* and *COR47-like* dehydrins which are higher in the roots of untreated seedlings and remained unchanged or higher (with one exception) despite the variations in steady-state level of DHN genes

expression under hydric stress conditions (Figure 5).

The obtained data are in agreement with other comparative studies on plant varieties with different stress tolerance that provide evidence for a positive correlation between DHNs gene expression or protein accumulation and physiological responses (Hanin et al., 2011). Thus, a correlation between drought tolerance and accumulation of dehydrin proteins were found in *Populus popularis* (Pelah et al., 1997). Similarly, positive correlations between *Dhn3* and *Dhn4* transcript accumulation and several traits associated with drought tolerance were revealed in a set of Korean barley cultivars (Park et al., 2006). Other examples that can be mentioned are the correlations between the level of dehydrin transcript and drought tolerance in two differently tolerant cultivars of durum wheat (Labhilili et al., 1995).

Evidence that high levels DHNs can be used as a plant molecular marker in screening of tolerance at various related water-deficit stresses are obvious from transgenic studies that have widely demonstrated the positive effect of DHNs expression in order to survive changing conditions. Thus, some studies (Brini et al., 2007) highlighted that the expression of the durum wheat DHN-5 in *A. thaliana* led to an increase in salt and osmotic stress tolerance. Also, have been shown that overexpression of the wheat dehydrin PMA80 enhances rice tolerance to drought and salt stress (Cheng et al., 2002).

It should be noted that the positive relation between plants overexpressing DHNs and high tolerance to abiotic stress is not always found. For example, the overexpression of *AtRAB18* in Arabidopsis did not enhance the freeze and drought tolerance (Lang and Palva, 1992), but the co-expression together with *AtCor47* led to improved freeze tolerance but not drought tolerance (Puhakainen et al., 2004). These findings suggest that under certain physiological conditions, synergistic activity rather than individual activity of some DHNs will improve stress tolerance.

It is also important to mention that for most plants, including sunflowers, the level of comparability between the research results on resistance to abiotic factors is so far quite low due to the different study approaches (microarrays, qPCR, proteome analysis techniques), with different materials (immature embryos, seedlings, plants in different ontogenetic phases), in different conditions (*in vitro*, greenhouse or field tests, different stress intensities, etc.).

CONCLUSIONS

The presented evidence confirms that drought adaptive responses result from differences in the regulation of expression of specific genes. By comparing the level of DHN genes expression of tolerant and sensitive genotypes in response to drought, and identifying the additional correlations between physiologic responses in laboratory and field screening was established an efficient and easily experimental test system for sunflower seedlings. Thus, it is suggested to use differential gene expression of DHNs *Rab18-like*, *Xero1* and *COR47-like* in sunflower plants grown under induced hydric stress as a proceeding for estimation of plant drought survival, hence, improving the pre-screening trials in the breeding programs aimed on plant tolerance to water-deficit stresses.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution of the Government of the Republic of Moldova for supporting the implementation of this research, which was done in the framework of the project within the National Program 20.80009.5107.01 - *Genetico-molecular and biotechnological studies of sunflower in context of sustainable management of agricultural ecosystems*, and the seed company „AMG-Agroselect Comerţ" LLC, for conducting field experiments and data recording.

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