

## DIFFERENCES OF PHYSIOLOGICAL PARAMETERS AND SUCROSE METABOLISM IN BREAD WHEAT GENOTYPES EXPOSED TO DROUGHT STRESS

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### ABSTRACT

Twelve bread wheat genotypes were assessed for physiological parameters and sucrose metabolism for genotypic variations under drought stress condition. The field experiments were conducted 2013–2014 under irrigated and rain-fed conditions (having extremely dry conditions) to investigate the flag leaf area (FLA), flag leaf chlorophyll content (Chl), relative water content (RWC), loss of turgidity (LT), sucrose content, reducing sugars content, starch content, acid invertase activity (INV) and sucrose synthase activity (SS) in wheat genotypes. Although FLA, Chl, RWC and starch content decreased in all genotypes under drought conditions, LT generally increased, whereas sucrose content, reducing sugars content, acid INV and SS activities varied from genotype to genotype. Based on the results of Chl, RWC, and LT genotypes DH16, DH19 and Kate A-1 were relatively drought-tolerant genotypes. In addition, the genotypes DH16, DH18, DH19, Krasunia-Odeska and Syrena-Odeska adjusted their sucrose metabolism well when exposed to drought stress. Moreover, the genotypes DH19, DH21, Bezostaja-1 and Krasunia-Odeska regulated acid INV activity. The results suggest that Chl, water status of the leaves, reducing sugars and acid INV activity could be an effective selection criterion of drought tolerance for wheat.

**Key words:** Acid invertase, soluble sugar, sucrose synthase, *Triticum aestivum* L., water deficit

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### INTRODUCTION

The principal food grain of Turkey, wheat is growing at the largest area of the country as well as worldwide under single crop. The wheat production is affected by cultivation under dry agricultural areas and marginal lands where limited, irregular precipitation creates water deficits. In prolonged drought conditions, as the water potential of the soil decreases, the stomata of leaves close, after which turgor pressure, leaf growth and the rate of photosynthesis decline, and ultimately, yield reduction occurs (Prasad *et al.*, 2008). Water stress mostly affects the wheat grain yield during flowering and early grain-filling stages by causing either deteriorated pollination and fertilization or the abortion of grains (Barnabas *et al.*, 2008). Reduced endosperm cell counts, carbohydrate supply and amyloplasts in the grain are the likely causes to no grain formation when wheat plants exposed water stress during pollination (Reynolds *et al.*, 2016).

In semi-arid regions, wheat breeding for drought tolerance requires determination of the best selection criteria for drought-tolerant wheat genotypes (Saba *et al.*, 2010). One potential criterion is the ability to preserve the rate of carbon assimilation during photosynthesis, which can sustain plant growth and production throughout dry periods (del Pozo *et al.*, 2020). Other possible criteria

include the chlorophyll content (Chl) in leaves, which affects the photosynthetic ability of plant tissues, and relative water content (RWC) in leaves, which when high lessens the elasticity of tissue cell walls and increases osmotic regulation (Rad *et al.*, 2013). Plants exposed to drought stress regulate their osmotic potential through accumulating organic solutions, organic acids, potassium and sugar alcohols in their cells to preserve turgor. However, the age of plants, especially during generative development, affects their osmotic regulation, which is slow before flowering but accelerates thereafter (Sanders and Arndt, 2012).

Another potential criterion for identifying drought-tolerant wheat is the higher heritable capacity of a plant genotype to store and translocate greater amounts of soluble sugars to grain, which has been positively associated with grain yield (Shearman *et al.*, 2005; Ruuska *et al.*, 2006) and thus proposed as a factor of increased tolerance to drought (Li *et al.*, 2015). Soluble carbohydrates are a major source of the increase of organic solutions under drought stress and the accumulation of reducing and soluble sugars, especially sucrose. Sucrose protects mitochondria and other cellular components from the unfavourable impacts of drought, can forestall structural changes in soluble proteins and protects membrane phospholipids in the liquid–crystalline phase by acting as a substitute for water (Ruan *et*

*al.*, 2010). Monosaccharides support respiration and mitochondrial electron transport as respiratory substrates, which could counter the onset of dormancy and promote metabolism, energy production and the formation of oxygen radicals (Leprince *et al.*, 2017). However, the role of reducing sugars in adapting to stress remains unclear. Bhattacharya and Kundu (2020) argued that their accumulation can harm plant tissues in various ways while Das *et al.* (2018) claimed that increase in soluble sugars under drought stress contribute to enduring the drought due to decreased sugar translocation, growth and starch hydrolysis. Cereals such as wheat use both carbons derived from photosynthetic assimilation and pre-stored carbohydrates from mature leaves for grain filling. After heading, if carbon obtained via photosynthetic accumulation remains low, then soluble sugars and starch in leaves play a significant role in compensating for the carbon supply to grain (Yang and Zhang, 2006).

Another pair of potential criteria for drought tolerance in wheat is the activities of the enzymes acid invertase (INV) and sucrose synthase (SS), which are closely associated with sucrose content. Whereas acid INV functions as a regulatory enzyme (Liu *et al.*, 2014), SS plays a key role in seed maturation and abiotic stress tolerance by converting sucrose to starch (Zhang *et al.*, 2020). Given those properties, both acid INV and SS could be worthwhile selection criteria for drought tolerance in wheat.

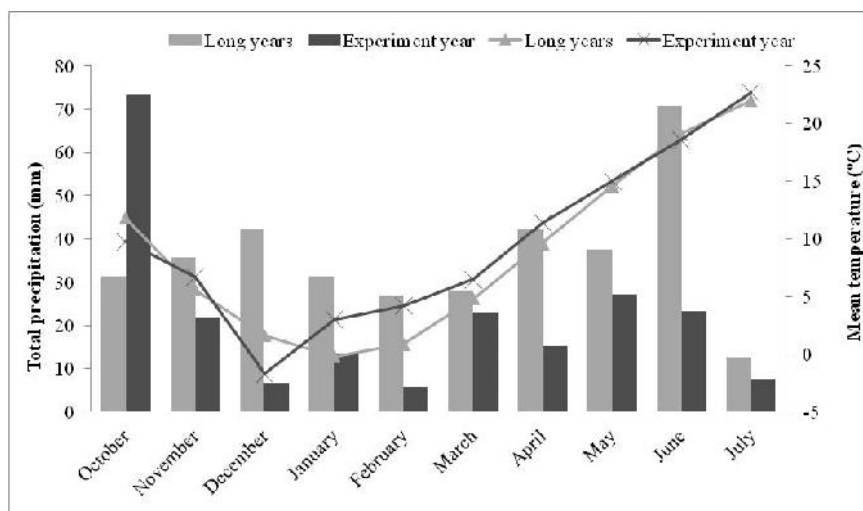
Drought tolerance has long been studied by both plant breeders and physiologists, some of whom have evaluated changes in the morphological (Bürling *et al.*, 2013), physiological (Dwivedi *et al.*, 2018), biochemical (Aidoo *et al.*, 2017) and molecular (Kalaipandian *et al.*, 2018) properties of wheat under drought stress. In addition, it was indicated in our previous study related to antioxidative enzyme activities that drought condition increased catalase (CAT) activity as compared with the control in wheat genotypes (Kutlu *et al.* 2017). However, because breeders seeking to improve the drought tolerance of wheat need to know how its genotypes differently regulate metabolic processes amid stress, it remains necessary to clarify variability in physiological changes and sucrose metabolism among wheat genotypes during stressful conditions. Accordingly, in the study reported here, changes in flag leaf area (FLA), Chl, RWC, loss of turgidity (LT), soluble sugar content and both acid INV and SS activity were formulated in the bread wheat genotypes during early maturity under field conditions exposed to drought stress.

## MATERIALS AND METHODS

**Agronomic practices and plant material:** The study was conducted during the 2013–2014 crop season at the Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Turkey. The soil containing 1.38% organic matter and 5.8% lime, was unsalted (0.08%), loamy and slightly alkaline (pH 7.8). Total twelve bread wheat genotypes including three Turkish bread wheat cultivars (Altay 2000, Müfitbey and Nacibey), four foreign-origin bread wheat cultivars (Bezostaja-1, Kate A-1, Krasunia-Odeska and Syrena-Odeska) and five doubled haploid (DH) wheat lines were used as genetic material. Bezostaja-1 (recommended for irrigated farming lands) was chosen as control cultivar (Öztürk and Aydın, 2017). The DH lines were developed from F<sub>2</sub> generations of hybrids of 33IBSWN-S-244, Tosunbey and Müfitbey in the Plant Tissue Culture Laboratory of the Transitional Zone Agricultural Research Institute in Eskişehir, Turkey. The experiment was designed in a split-plot design involving both irrigated and rain-fed conditions with three replications. Sowing was performed on 9 October 2013 in four rows with parcels 1 m in length and space between rows of 30 cm. The amount of fertiliser used in irrigated plots was 90 kg ha<sup>-1</sup> N and 120 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 60 kg N ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> in rain-fed plots.

In the case of precipitation, the study period was not an ideal growing season for wheat compared to long term climatic data. Although the total precipitation of long-term year of the experiment in Eskişehir was approximately 358.5 mm, received quantity during the year of the experiment was very low with the value of 141.4 mm (Figure 1). Optimising yield and quality in wheat cultivation requires an average of 500–600 mm water, or at least 400 mm. Wheat plants need the most water during stem elongation, heading and early periods of maturity, typically from March to May; however, those months in the year of the experiment experienced far less precipitation and higher temperatures than in last 30 years (Figure 1). In response to the extreme drought during the experiment, 300 mm of water were applied as irrigation reinforcements during stem elongation and early periods of maturity.

Flag leaves from early periods of maturity were used to analyse RWC, LT, sucrose content, reducing sugars content, starch content and both acid INV and SS activities.



**Figure 1. Total precipitation and mean temperature belong long term and experiment year in 2013-2014 growing season.**

**Flag leaf area and chlorophyll content:** Flag leaf area (FLA) was calculated by multiplying the length and the width of each leaf by 0.75, a specific coefficient for wheat (Kalaycı *et al.*, 1998), whereas Chl was measured using a chlorophyll meter (SPAD-502Plus, Konica Minolta), following Adamsen *et al.* (1999).

**Leaf relative water content and loss of turgidity:** Leaf RWC (%) and LT were determined according to Gulen and Eris's (2003) method; the fresh weights (FW) of leaf discs 1.5 cm in diameter were weighed and released in distilled water in petri dishes for 4 h, after which they were gently blotted, and their turgid weight (TW) was scaled. Thereafter, leaves were placed in an oven at 70 °C for 24 h, after which their dry weight (DW) was determined. Ultimately, RWC and LT were calculated by using the following formulas:

$$\text{RWC (\%)} = \frac{[(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100}{1}$$

$$\text{LT (\%)} = \frac{[(\text{TW} - \text{FW}) / \text{TW}] \times 100}{1}$$

**Soluble sugars:** A method involving anthrone reagent, modified for determining non-reducing sugars content, was used to determine sucrose content (van Handel, 1968). First, a colourimetric estimation of reducing sugars content was performed with dinitrosalicylic acid (Miller, 1959), after which starch was detected by measuring glucose following the digestion of the ethanol-insoluble residue with amyloglucosidase (Dinar *et al.*, 1983). For those measurements, the dried sugars were dissolved in 1 mL of distilled water. In collecting the solution, sugars were extracted by suspending 100 mg of leaves in 5 mL of 80% (v/v) ethanol at 80 °C for 30 min, after which the ethanolic solution was collected. After that procedure was repeated 4 times, the ethanolic solutions were merged and evaporated until dry at 55 °C with the support of continuous ventilation (Turhan, 2012).

**Acid invertase and sucrose synthase enzymes assays:**

The soluble (i.e. cytosolic) activity of acid INV in leaf tissue was determined by following method by Aloni *et al.* (1991). First, leaf samples totalling 500 mg were ground in 5 mL of ice-cold, 25-mM HEPES (N2-2-ethane sulphonic acid) buffer pH 7.2, containing 5 mM of MgCl<sub>2</sub>, 2 mM of DDT (DL-Dithiothreitol) and 3 mM DIECA (diethyldithiocarbamic acid). Next, the mixture was centrifuged at 20,000 g for 20 min at 4 °C, after which aliquots of 100 µL of the supernatant were incubated in 10 mL of 0.1 N phosphate citrate buffer (pH 5.0) and 20 mM sucrose at 37 °C for 30 min. After that, 1 mL of dinitrosalicylic acid reagent was added, and the mixture was boiled for 5 min. The resulting sugars were measured colorimetrically.

The SS activity was determined in method proposed by Aloni *et al.* (1996). First, following the same extraction procedure described for acid INV, the mixture was dialysed overnight to remove internal sugars. Next, enzymatic activity was determined as the breakdown of sucrose on aliquots of 200 µL incubated in 0.1 M of phosphate-citrate buffer (pH 7.0) containing 200 mM of sucrose and 5 mM of UDP (Uridine 5'-diphosphate). Following incubation at 37 °C for 30 min, the resulting fructose was detected by a dinitrosalicylic acid reaction, and data were recorded as FW. Bradford (1976) assay was used to determine the total soluble protein content of the crude extracts.

**Statistical analyses:** Analysis of the variance (ANOVA) using the general linear model (GLM) to evaluate variation among genotypes and effects of application and the classification of genotypes according to Duncan ( $P < 0.01$ ) were performed using the IBM SPSS statistical package program (Version 20.0 for Windows, SPSS, Chicago, USA).

## RESULTS AND DISCUSSION

Variance analysis revealed the significant ( $p < 0.01$ ) effects of application, genotype and genotype  $\times$  application interactions on FLA, Chl, RWC, LT, reducing

sugars content, starch content and both acid INV and SS activities. Although the application did not affect the sucrose content, differences among the genotypes and genotype  $\times$  application interactions were significant (Table 1).

**Table 1. Results of variance analysis (ANOVA) of genotype, application and their interactions with examined properties in flag leaves of bread wheat genotypes under drought stress. Numbers represent  $F$  values at 0.01 level.**

Dependent variable	Independent variable		
	Genotype	Application	Genotype x Application
FLA	15.80**	4374.52**	16.49**
Flag Chl	16.82**	977.17**	19.87**
Leaf RWC	22.07**	627.22**	12.19**
LT	19.04**	792.71**	11.50**
Sucrose content	7.25**	0.15 <sup>ns</sup>	5.84**
Reducing sugars content	6.35**	12.67**	6.69**
Starch content	38.09**	569.18**	38.09**
Acid INV activity	31.25**	74.11**	29.29**
SS activity	72.81**	110.23**	61.52**

\*\*Significant at  $P < 0.01$ , <sup>ns</sup> Non-significant.

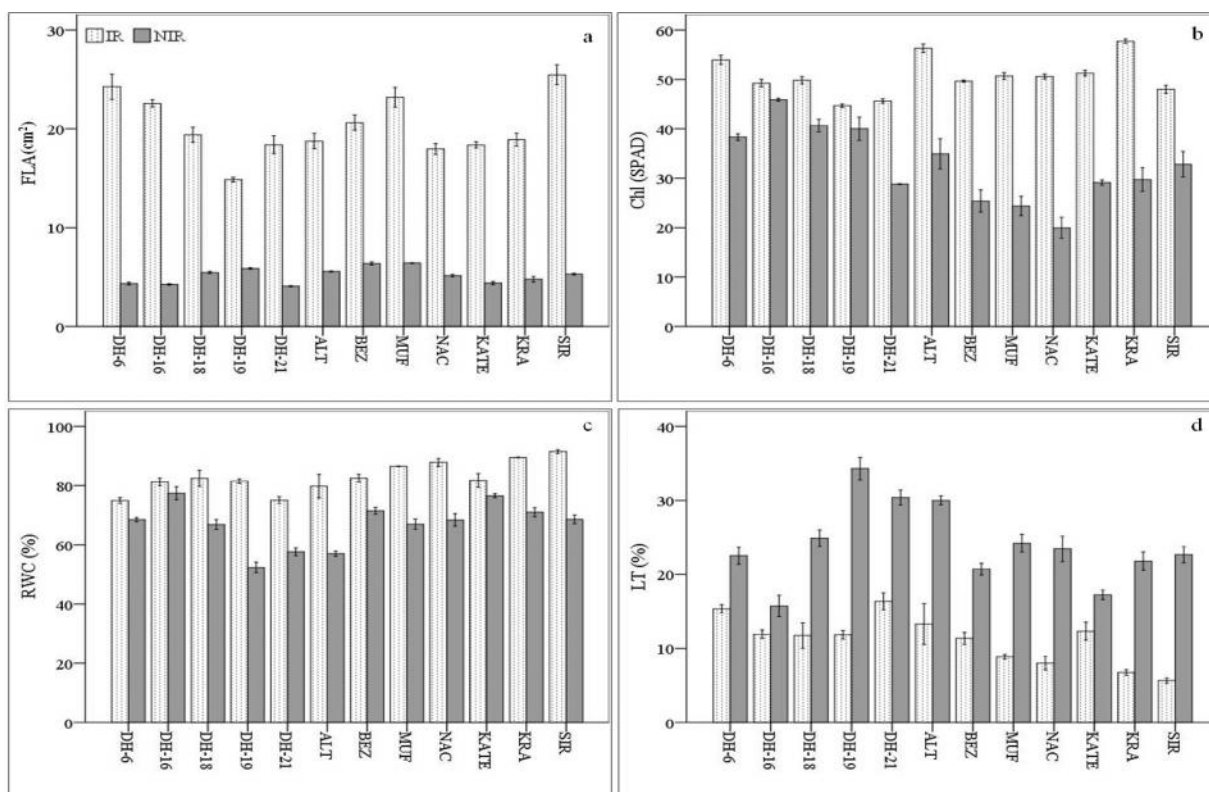
**Flag leaf area, chlorophyll content, relative water content and loss of turgidity:** Flag leaf area significantly increased among the genotypes depending on the application to irrigation (Figure 2a). In irrigated conditions, the Syrena-Odeska genotype gave the greatest FLA and the DH6 genotype followed it. However, the DH19 genotype had the smallest FLA, showing the least decrease when exposed to drought conditions. In drought conditions, the Müfitbey and Bezostaja-1 genotypes were prominent in terms of FLAs. Generally, a large leaf area contributes to yield of genotypes positively; however, based on the selecting approach for FLA in wheat breeding, broad-leaved genotypes are preferable in places without water shortages, whereas narrow-leaved ones are the more appropriate in places with drought conditions (Fellahi *et al.*, 2018). In that sense, genotype DH19, with its small FLA, can better resist drought and heat.

Because flag leaves transfer 30–50% of photosynthetic assimilates to kernels during plant maturity (Abid *et al.*, 2017), their photosynthesis is a significant factor of grain yield in wheat. In irrigated conditions, Chl ranged from 44.67 SPAD (DH19) to 57.73 SPAD (Krasunia-Odeska), whereas in drought conditions it varied between 19.97 SPAD (Nacibey) and 45.90 SPAD (DH16) (Figure 2b). Under drought conditions, genotypes DH16 and DH19 protected their Chl, whereas the genotypes Nacibey, Müfitbey, Bezostaja-1 and Krasunia-Odeska lost the most of Chl. Genotypes with high Chl, identifiable by their dark green colour, are desirable in wheat breeding to develop varieties for irrigated conditions. Higher Chl indicates a greater rate of photosynthesis, which is essential for high yields, and when water stress does not occur, stomata remain open for a long period to make

photosynthesis. However, because genotypes with high Chl also have high canopy temperatures, which make them susceptible to drought in extremely arid areas, genotypes with low Chl may be advantageous for wheat breeding in arid areas (Reynolds *et al.*, 2009).

Many researchers have proposed RWC as a good indicator for discriminating drought-tolerant genotypes (Bayoumi *et al.*, 2008; Dwivedi *et al.*, 2018). In the irrigated conditions, RWC changed between 74.96% (DH6) and 91.53% (Syrena-Odeska), whereas it peaked at 77.43% (DH16) under rain-fed conditions. Kate A-1 followed this line with 76.57% RWC value. Leaf RWC decreased under water stress in all examined genotypes (Figure 2c), likely due to decreased water supplies transported from the roots to the leaves that can occur when water reserves in the soil decrease. Other researchers have reported that the RWC of certain genotypes, which can deepen their roots and use soil water optimally or control water loss with stomata, are higher (Bayoumi *et al.*, 2008; Hafez and Gharib, 2016); such genotypes, which protect their RWC under stress conditions, can sustain cell turgor and provide higher yields than genotypes with decreased water content. Genotypes could also accumulate solutions and metabolites as well as adjust their osmotic potential (Rad *et al.*, 2013). Loss of turgidity in the genotypes DH19, DH21 and Altay 2000 was approximately 30 to 35% in drought conditions yet was lowest, at nearly 16%, in the genotypes DH16 and Kate A-1 (Figure 2d). As water stress suppresses cell development due to increased LT, leaf turgor pressure and total surface area reduce, which limits the photosynthetic capacity of flag leaves and ultimately lowers grain yield (Chaves *et al.*, 2009). For that reason, it can be said that the genotypes

DH16 and Kate A-1 are preferable for dry conditions due to their high RWC and ability to sustain turgor.



**Figure 2.** Flag leaf area (FLA) (a), flag leaf chlorophyll content (Chl) (b), leaf relative water content (RWC) (c) and loss of turgidity (LT) (d) of bread wheat genotypes under irrigated (IR) and non-irrigated (NIR) conditions. Error bars represent  $\pm$  SE of three replications.

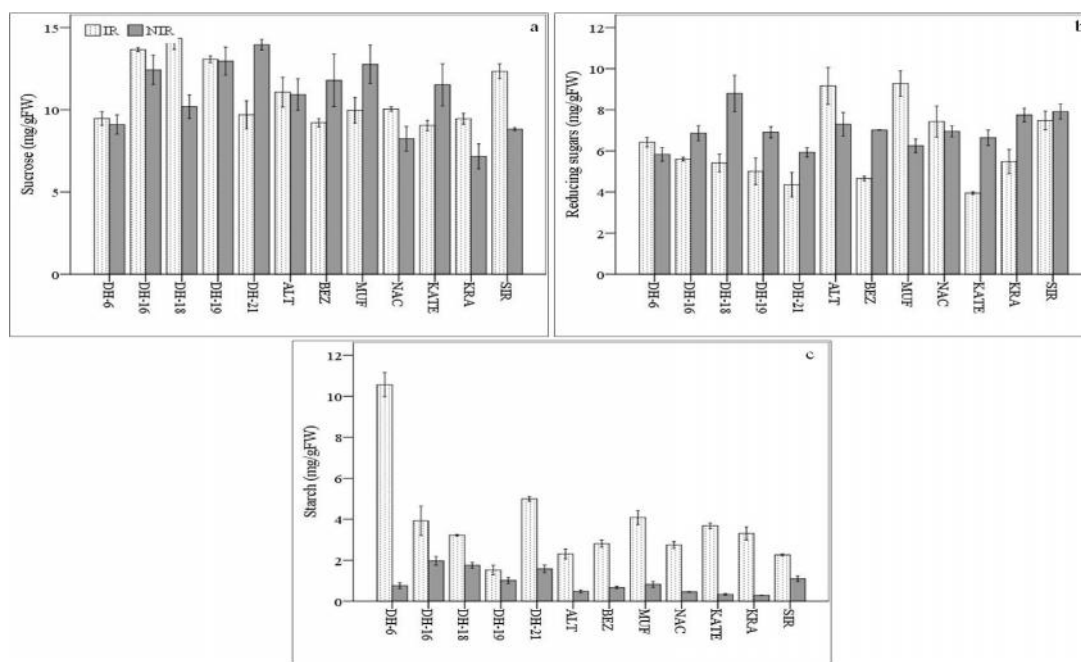
**Soluble sugars:** Under stress conditions, sugars released in plants have properties that support plant growth and mitigate the adverse impacts of stress (Krasensky and Jonak, 2012). The ability of plants to maintain growth and development under drought stress can be measured by the accumulation of sugar in plant tissues; as indicators, low sugar content generally induces leaf senescence, while leaf yellowing accelerates amid a decreased rate of photosynthesis. By contrast, leaves with high sugar content also experience senescence as the transport of sugars to grain accelerates (Shi *et al.*, 2016). Amid severe stress, total soluble sugar content generally increases in leaves (Ruan *et al.*, 2010; Das *et al.*, 2018; Khan *et al.*, 2019). In drought conditions of the experiment reported here, sucrose content was variable in the genotypes (Figure 3a); however, the effects of application were not significant (Table 1). Such results suggest that sucrose content might be stable in drought conditions, which could prove vital for wheat under drought stress. Because stress depresses the rate of photosynthesis, stable sucrose content under drought stress may require plants to reduce their synthesis of starch and canalize carbohydrates to sucrose synthesis (Kondrák *et al.*, 2012). In the experiment, drought stress caused in sucrose

accumulation for only the genotypes DH21, Bezostaja-1, Müfitbey and Kate A-1. Besides, it was determined that DH6, DH19 and Altay 2000 maintained the sucrose content in drought condition. Sink tissues of plants represent the chief source of carbon and energy, not sucrose, which is transported by phloem to tissues for storage by being synthesised in the cytoplasm of leaves (Shi *et al.*, 2016). Consequently, decreased sucrose in leaves might be transported to grain to compensate for the lack of carbohydrate supply due to a decrease in the rate of leaf photosynthesis during drought conditions.

For most genotypes, reducing sugars content was greater in dry conditions than in irrigated ones (Figure 3b). The greatest reducing sugars content occurred in the genotype DH18, followed by two other genotypes Syrena-Odeska and Krasunia-Odeska. Reducing sugars content of the genotypes DH6, Altay 2000, Müfitbey and Nacibey decreased in drought conditions. Such results suggest that the genotypes DH16, DH18, DH19, Krasunia-Odeska and Syrena-Odeska better tolerate drought than other genotypes in terms of the sucrose and reducing sugars contents in their leaves. Nemati *et al.* (2018) have reported that those contents increased in sensitive genotypes under drought

stress; however, findings on that claim are contradictory. Most likely, the increase in reducing sugars content depends on the decrease of starch content (Figure 3c). Starch is a key molecule that, amid drought stress, seems to mediate plant responses to abiotic stress. If photosynthesis is limited under adverse environmental conditions, then plants

generally remobilise starch to provide energy and carbon and it was reported that starch content in leaves decreased significantly as stress increased (Thalmann and Santelia, 2017). The results of the experiment reported here clearly indicated that starch content was quite low in drought conditions compared to irrigated ones (Figure 3).



**Figure 3. Sucrose (a), reducing sugars (b) and starch (c) contents of bread wheat genotypes under irrigated (IR) and non-irrigated (NIR) conditions. Error bars represent  $\pm$  SE of three replications.**

In irrigated conditions, the genotype DH6 had the greatest starch content, whereas in drought conditions the genotype DH16 had the most. Interestingly, the genotype DH19, which had the lowest starch content in irrigated conditions, conserved its starch content in drought conditions. Starch degradation under stress in leaves is often associated with improved stress tolerance (Thalmann and Santelia, 2017), and most researchers who have examined carbohydrate metabolism in stress conditions have reported that leaf starch content decreased in response to abiotic stress (Villadsen *et al.*, 2005; Damour *et al.*, 2008; Sattar *et al.*, 2020). Gonzalez-Cruz and Pastenes (2012), observing that a drought-resistant bean (*Phaseolus vulgaris* L.) variety degraded more starch than a drought-sensitive one, concluded that starch consumed in the bean's leaves accumulated in pods in response to drought stress. If such conclusions about leaf starch content under stress conditions can be extended, then it may be that all genotypes examined in the experiment reported here was drought tolerant.

#### Activities of acid invertase and sucrose synthase:

Various enzymes associated with sugar metabolism engage in various reactions following sucrose transport and accumulation in tissues for storage. Acid INV regulates the

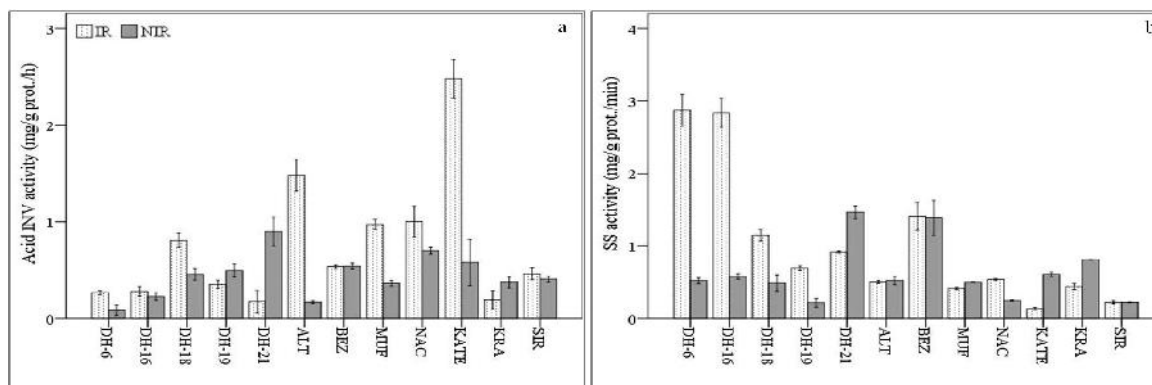
sucrose accumulation of sink organs, especially during plant development, when it adjusts osmotic pressure and sugar signals in response to environmental changes (Koch, 2004). The acid INV activity in the genotypes DH19, DH21 and Krasunia-Odeska was greater in dry conditions, remained the same in the genotype Bezostaja-1 in both irrigated and dry conditions and rose in other genotypes exposed to irrigation (Figure 4a).

Sucrose synthase is responsible for synthesising sucrose in plant leaves and degrading it in sink tissues (Lu *et al.*, 2005). Higher SS activity in leaves reflects their ability to convert assimilates of photosynthesis into sucrose (Wang *et al.*, 2015). In irrigated conditions of the experiment reported here, SS activity was greatest for the genotypes DH6 and DH16 and dramatically decreased in rain-fed conditions (Figure 4b).

However, for the genotypes Altay 2000, Bezostaja-1 and Syrena-Odeska it remained nearly identical in both conditions. In the genotypes DH21, Kate A-1, Müfitbey and Krasunia-Odeska, SS activity increased with drought, whereas in the genotypes DH18, DH19 and Nacibey, it increased with irrigation. The SS activity of the genotypes was generally compatible with sucrose content. Despite increased SS activity in the genotype Krasunia-Odeska, its decrease in sucrose content is noteworthy. In the

case of drought, increased SS and acid INV activity may be protective; therefore, the genotypes DH21, Bezostaja-1 and

Krasunia-Odeska, which can maintain both activities in dry conditions, should be considered to be tolerant to drought.



**Figure 4. Acid invertase (INV) (a) and sucrose synthase (SS) (b) activities of bread wheat genotypes under irrigated (IR) and non-irrigated (NIR) conditions. Error bars represent  $\pm$  SE of three replications.**

**Conclusion:** The characters examined in the study were found to be good criteria for identifying drought-tolerant genotypes in wheat. The genotypes DH16, DH19 and Kate A-1 were outstanding in terms of their physiological properties, whereas the genotypes DH16, DH18, DH19, Krasunia-Odeska and Syrena-Odeska adjusted their sucrose metabolism well when faced with drought. In addition, the genotypes DH19, DH21, Bezostaja-1 and Krasunia-Odeska might have protected acid INV activity associated with sucrose metabolism, which is a unique property of tolerance for wheat. Despite the fact that involved in drought tolerance of wheat plant, SS may not be related to the discrimination of genotypes.

Considering all of the features examined, the genotypes DH16, DH19, Bezostaja-1 and Krasunia-Odeska should be used to breed wheat with greater drought tolerance. The results of the study, performed under field conditions, can afford insights into the changing physiological properties and sucrose metabolism in wheat under drought condition. However, since other environmental factors may affect the trial results under natural conditions, the results should be also evaluated in molecular studies and trials conducted under controlled conditions.

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