Evaluating the Tolerance of Some Wheat Varieties Towards Water Stress Induced by Sorbitol *

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Abstract

This study was conducted at the Biotechnology Department, Swaida Research Center, GCSAR, and Syria during 2016. Eight wheat genotypes were screened at the seedling stage using Sorbitol to mimic drought in the laboratory. The experiment was performed using Complete Randomized Design and the genotypes were evaluated by Cluster analysis based on the sum of reduction ratios for shoot and root length. The results revealed variability in the experimented genotypes' sensitivity towards water stress in shoot and root length. Cham 10 showed the highest values whereas Doma 1 recorded the lowest. These results referred to the existence of genetic variation among genotypes under study. In addition, Cluster analysis indicated classifying studied genotypes into two groups: tolerant group containing Cham 10 (the most tolerant genotype), Bohuth 11, Cham 3, Bohuth 7, and sensitive group containing Doma 1 (the most sensitive genotype), Jolan 2, Doma 4 and Bohuth 8.

Keywords

Drought, Sorbitol, Wheat, Cluster Analysis

تقييم تحمل بعض طرز القمح

تجاه الإجهاد المائي المحدث باستخدام السوربيتول

ملخص:

نُفذت هذه الدراسة في مخبر التقانات الحيوية، مركز بحوث السويداء، الهيئة العامة للبحوث العلمية الزراعية، سورية خلال العام 2016. تم في هذه الدراسة غربلة ثمانية طرز من القمح في طور البادرة باستخدام سكر السوربيتول لمحاكاة الجفاف في المختبر. نُفذت التجربة باستخدام التصميم العشوائي التام، وقُومت الطرز المدروسة باستخدام التحليل العنقودي المعتمد على مجموع نسب التخفيض لطول السويقة وطول أطول جذر. بينت النتائج اختلاف حساسية الطرز المدروسة تجاه الإجهاد المائي في طول السويقة وطول أطول جذر، حيث امتلك الطراز شام 10 أعلى القيم بينما سجل الطراز دوما 1 أدناها. تشير هذه النتائج إلى وجود تباين وراثي بين الطرز المدروسة. كما أظهرت نتائج التحليل العنقودي فصل الطرز المدروسة إلى مجموعتين: مجموعة الطرز المتحملة التي تضم شام 10 (أكثر الطرز تحملا)، بحوث 11، شام 3 وبحوث 7، ومجموعة الطرز الحساسة التي تضم دوما 1 (أكثر الطرز حساسية)، جولان 2، دوما 4 وبحوث 8.

الكلمات المفتاحية:

الجفاف، السوربيتول، القمح، التحليل العنقودي

Introduction

The 21st century agriculture is facing the daunting challenge of attaining nearly up to 70% increase in crop productivity by 2050 (Friedrich, 2015; Wang et al., 2016). The challenge is to extract and select high-yield, environment stress-tolerant genotypes, especially the drought. Drought results from a decrease in rainfall, which causes a reduction in available soil water and is often associated with high temperature rates. (Singh B. et al., 2015.) With accelerated global climate change, the global drought incidence is likely to swell beyond 20% by the end of this century (Singh B. et al., 2015), especially in the Mediterranean and the Middle East region, which is one of the most vulnerable areas to severe drought. This causes an intense decline in the yield production which leads to the point that sometimes there is no production at all (Barlow et al., 2015). When the plant is exposed to drought, reactive oxygen species accumulate inside the cells, so their temperature increase causing higher viscosity in cell contents and a change in protein interaction, protein accumulation and decomposition (Farooq et al., 2008).

Cell shrinkage is an immediate symptom of cell dryness caused by drought, and the accumulation of solubility may be toxic to some enzymes, which in turn leads to reduced photosynthesis and reduced water efficiency, resulting in a reduction in the final yield. Drought tolerance is a product of many cellular, molecular and physiological processes that include increasing or decreasing the expression of certain genes associated with dehydration, leading to the accumulation of many solvents, improvement of antioxidant systems, reduction of transpiration, inhibition of vegetative growth and reduction of stasis (Pareek et al., 2010).

Farmers selected drought-tolerant genotypes based on certain morphological and physiological characteristics associated with drought tolerance under field conditions (Dhanda et al., 2004).

However, the selection process under field conditions faces many difficulties, and the weather conditions cannot be controlled, which reduces the efficiency of the selection process. In order to study the effects of stress on plants precisely, it is necessary to apply in vitro methods, which are based on the addition of compounds to the growth medium. Drought is usually stimulated by adding mannitol, sorbitol, or polyethylene glycol (Verslues et al., 2006). These substances reduce lower the water potential of the growth medium, making it harder for the roots to extract water, simulating what happens in drying soil. Sorbitol was used as a drought-cultivator for screening and studying many varieties, including maize (Jain et al., 2010), potatoes (Cioloca et al., 2016), apricots (Sorkheh et al., 2011) and bananas (Vanhove et al., 2012). In vitro methods provide many benefits, including controlling the level of stress, the time it occurs, and the ability to use a large number of plants in a small place (Lawlor, 2013).

In general, seed germination and seedling growth stages can be less tolerant to environmental stresses than adult plants, as drought may cause a reduction in the seed germination and a significant damage to the seedling growth plants (Sun et al., 2010), which inevitably causes a reduction in the final yield (Rauf et al., 2006). Thus, the selection in seed germination and seedling growth stages is one of the important criteria for determining stress-tolerant genotypes (Gharoobi et al., 2012).

In this study, we tested eight wheat varieties in the laboratory using sorbitol to induce drought. These varieties were separated according to their ability to tolerate drought during the seedling growth stage.

Materials and Research Methods

-Plant Material and Growth Conditions

In this study, we used eight wheat genotypes obtained from the Crop Research Administration, (GCSAR) Damascus, Syria: Bohuth-7, Bohuth-11, and Doma-1, Cham-3 as hard wheat genotypes and Bohuth-8, Cham-10, Doma-4, Jolan-2 as soft wheat varieties. (Table No.1) In order to mimic in vitro drought, we used several concentrations of Sorbitol: 2%, 4%, 8% and 10% (weight: volume). The seeds were sterilized using a Topsin M (2g /L) fungicide for 10 minutes then 20% sodium hypochlorite for 15 minutes then with distilled water three times. The experiment was carried out in test tubes on the medium of MS (Murashige and Skoog 1962). Eight homogenous seed types of each wheat genotype were planted with an average of eight seeds per treatment and one seed per tube, and the MS medium was used only as a control. After four weeks, the growing plants were collected then the following examined characters were studied. The tubes were placed in the growth chamber at a temperature of 22 ± 1 °C, and a light period of (16/8 light/ dark).

Table (1):
Information on the genotypes used in this study

Genotype	Rainfall Stability Area	Year of Release
Bohuth-7	One	2000
Bohuth-11	One	2004
Doma-1	One	2002
Cham-3	Two	1987
Bohuth-8	Irrigated	2007
Cham-10	Irrigated	2004
Doma-4	Two	2007
Jolan-2	One	2007

Source: The Crop Research Administration, (GCSAR) Damascus, Syria.

Examined Characters

The shoot length and the root length were measured, along with their ratio to each other. Moreover, reduction ratios of the shoot length and the root length were estimated in comparison with the controlled genotypes, in accordance with Al Awdah and others (2005).

$$\text{Reduction Ratio \%} = \frac{\text{Length in the control} - \text{Length in the treatment}}{\text{Length in the control}} \times 100$$

different from those that contribute to the tolerance of moderate stress (Skirycz et al., 2011). Based on the previously mentioned information, the water stress level that causes 50% growth decline should be relied upon as a tool for examining the impact of water stress. Moreover, genotypes should be categorized according to their ability to tolerate this level of water stress.

Table No. 2:

(root/shoot) Reduction ratio in relation with the examined levels of water stress, in comparison with the controlled genotypes

Level of Sorbitol/ Genotypes	2%	4%	8%	10%
Bohuth 7	26.9	42.6	44.8	100.0
Bohuth 11	27.4	34.3	36.8	100.0
Doma 1	43.3	79.9	85.9	100.0
Cham 3	14.5	32.9	76.9	100.0
Bohuth 8	22.0	57.1	68.2	100.0
Cham 10	13.6	21.3	65.2	100.0
Doma 4	60.5	67.5	81.4	100.0
Jolan 2	42.9	56.7	48.0	100.0
Average	31.4	49.0	63.4	100.0

-The impact of the drought on the measured characters

The drought caused a decline in the measured characters in comparison with the controlled genotypes (Table No.3). The decline in the growth is a reaction to the imposed stress which led to a decrease in the water content of the cells. This resulted in cytostasis in the genotypes (Taiz and Zeiger, 2006) due to the loss of the turgor characteristic of the cell. A growth decline was observed in the root system and shoot system as a reaction to the dehydration treatment of the wheat (Ahmad et al., 2013). During the treatment of the controlled genotypes, genotype Doma 4 registered the longest growth of root among the other genotypes (14.3 cm), while Cham 10 registered the longest shoot growth (31.1 cm). On the other hand, in the treatment of the stress, Doma 1 registered the shortest growth of root (2.1 cm) and of shoot (7 cm), while Cham 10 registered the longest growth of root (9.5 cm) and of shoot (21.5 cm). These results show that there

Experiment Design and Data Analysis

The experiment was designed using Complete Randomized Design. Data were expressed as arithmetic mean with ± standard deviation (SD). Examined genotypes were categorized accordingly to their drought tolerance using Cluster analysis, based on the sum of the reduction ratio of the root length and shoot length, through using SPSS (version 19).

Results and Discussion

-Determining the level of the water stress that is utilized to screen examined genotypes

The examined genotypes showed a decline in all examined characters in correlation with an increase in the concentration of Sorbitol within the growth medium. The level of Sorbitol was only 4% and it was utilized as a tool to screen examined genotypes and their tolerance towards drought in the laboratory. This specific level of Sorbitol was utilized because it showed to cause 50% decline in the growth process, in comparison with the controlled genotypes, in terms of the root length (Al Awdah, 1999). It is also shown to be the average of the reduction ratio in all examined genotypes (49%, Table No. 2). It should be noted that when the level of Sorbitol was only 2%, it caused 31.4% reduction ratio, while when the level of Sorbitol was 8%, it caused 63.4% reduction ratio. Moreover, when the level of Sorbitol was 10% in the treatment, it caused 100% reduction ratio (Table No.2). Many researchers adopted the level of the water stress which causes 50% growth decline as a tool to screen examined genotypes (Al Awadah et al., 2009; Abbas et al., 2012). This level corresponds with a moderate water stress intensity, which commonly takes place in reality. It should be highlighted that many researchers use severe water stress intensity in laboratory experiments, which leads to obtaining inaccurate results. The severe intensity of water stress causes a different response among plants in comparison with using moderate levels (Claeys and Inzé, 2013). The reason could be that genes which contribute to severe water stress tolerance are is a genetic variation between the examined genotypes. This variation can be utilized to select the top genotypes that can tolerate drought the most during the first stages of the plant growth cycle, especially in the areas where rainfall is at its minimum at the onset of the rainy season.

Table No. 3:

Examined characters in the controlled genotypes and in the treated genotypes (Sorbitol 4%). Values are expressed as arithmetic mean with ± standard deviation (n=8). (So) is an indicator for Sorbitol.

	The Length of the Longest Root (cm)			The Length of the Shoot (cm)		
Genotype	Controlled Genotypes	So 4%	Reduction Ratio	Controlled Genotypes	So 4%	Reduction ratio
Bohuth 7	13.8 ± 4	7.9 ± 3.1	42.6	25.4 ± 3	16.7 ± 1.5	34.5
Bohuth 11	10.4 ± 2.3	6.9 ± 3.5	34.3	27.2 ± 3	12.9 ± 3.9	52.4
Doma 1	10.3 ± 1	2.1 ± 1.1	79.9	20 ± 3.4	7 ± 3.2	65.2
Cham 3	10.9 ± 1.1	7.3 ± 3.1	32.9	30.1 ± 2.9	18.3 ± 4.4	39.4
Bohuth 8	11.9 ± 1.6	5.1 ± 3.1	57.1	30.4 ± 4.3	13.3 ± 7.3	56.2
Cham 10	12 ± 1.7	9.5 ± 1.4	21.3	31.1 ± 3.6	21.5 ± 4.3	30.9
Doma 4	14.3 ± 1.9	7 ± 0.9	67.5	27.5 ± 5.9	13.2 ± 5.5	52.1
Jolan 2	14.1 ± 3.6	6.1 ± 1.9	56.7	22.4 ± 7.2	12.6 ± 3.7	43.7

Cluster Analysis

Cluster analysis is used in screening the examined genotypes according to their tolerance to water stress depending on the total reduction ratios (Albiski et al,2012) ; Murshed et al, 2015). In this study, we relied on the sum of the reduction ratios in both the long shoot and root as a criterion for screening the examined genotypes and sorting them according to their level of drought tolerance. Roots are affected by drought first (Osman et al., 2015), therefore the root length is considered a selective trait for distinguishing the potential genotype of the wheat (Abdel-Raheem et al, 2007). However, some studies showed that the shoot system of the plant is considered sensitive against stress (Claeys et al. 2014), knowing that water stress causes a reduction of water in the cells. This will result in repressing the growth of cells in the whole plant (Taiz and Zeiger, 2006). As a result, drought tolerance needs establishing a deep and strong root and a suitable green space. This is why in this study we relied on the longest strands of the root and the shoot as a scale to sort the examined genotypes. The results of the cluster analysis showed that the examined genotypes were sorted into two clear groups (figure1). The first group of the potential genotypes which in turn was divided into two groups. The first includes Cham 10 genotype which took the first class, and the second includes Cham 3 genotype, Bohuth 7 and Bohuth 11. The second group is divided into two groups including the first genotype Doma 1 which took the last class while the second includes Jolan 2, Doma 4 and Bohuth 8.

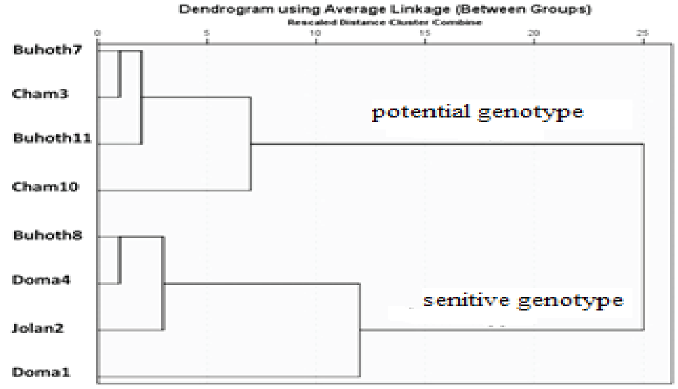

Figure (1)

The cluster analysis based on the reduction ratios for the root and shoot length

Although hard wheat is more tolerant to water stress than soft wheat (Waines, 1994), Cham 10 was classified as the best potential genotype. Furthermore, Cham 10 genotype is used in Syria in irrigated agriculture (table 1), and this contradicts the fact of its nature as a genotype tolerant to drought. The superiority of Cham 10 genotype is due to its ability to absorb sorbitol from the medium of the plant. Sorbitol plays an important role in the osmotic adjustment as a response to drought and it constitutes a source of energy (Bianca et al., 2000). Sorbitol is produced in the growing points of the plant and in the grown leaves (Zhang et al., 2010) and is redistributed in the plant as a response to drought (Jain et al., 2010). It is responsible for making 50% from the osmotic adjustment inside the cells of the plant (Li et al., 2012). Hard wheat genotypes: Cham 3, Bohuth 7, Bohuth 11were classified as tolerant genotypes, and this doesn't contradict with the fact that they are used in Syria for agriculture purposes in the first stability areas (Bohuth 7, Bohuth 11) as well as the second stability areas. (Cham 2) (table 1). Regarding the sensitive genotypes, Doma 1 genotype (hard wheat) was classified as one of the most sensitive genotypes .This does not conflict with the fact that it is used in Syria for agriculture in the first stability areas. Similarly Golan 2 and Bohuth 8 being classified as sensitive genotypes does not conflict with the fact that they are designated for agriculture in the first stability zones and the irrigated areas, respectively On the other hand, the classification of the genotype Doma 4 as a sensitive genotype contradicts with the fact that it is designated for agriculture in the second stability areas (Table 1). Based on our findings, we suggest that Doma 4 genotype to always be provided with complementary irrigations when planted in the second stability areas.

Generally speaking, the root ratio to the shoot ratio is one of the important ratios that gives an estimation of the distribution of the dry matter between the parts of the plant (Hunt, 1990). It is also considered a good pointer to examine the influence of drought on the root and the shoot. The tolerant genotypes allocate more dry matter to build an in-depth root system, while preserving the size of an appropriate vegetative population, while the sensitive genotypes cannot build a deep, in-depth root system. In this study, the length of the root / length of the shoot was measured. The higher the percentage, the deeper the total root and vice versa. In Cham 10(the most tolerant) genotype, we found that the rate of the root/ shoot length increased from 0.39 in the control to 0.44 in the treatment of the stress. The potential genotypes: Bohuth 11 and Cham 3 were the same as Cham 10 genotype, while Bohuth 7 genotype was the opposite. On the other hand, the root/ shoot length in Doma 1 (the most sensitive type) decreased from 0.52 in the control to 0.30 in the treatment of stress (table 3). The other sensitive genotypes Jolan 2 and Doma 4 and Bohuth 8 were the same as Doma 1 genotype.

Table (3)

The ratio of the root/shoot length in the control and treatment groups with 4% sorbitol. (n=8) So (sorbitol)

genotype	control group	4% So
	Root/shoot length	Root/ shoot length
Bohuth 7	0.54	0.47
Bohuth 11	0.38	0.53
Doma 1	0.52	0.30
Cham 3	0.36	0.40
Bohuth 8	0.39	0.38
Cham 10	0.39	0.44
Doma 4	0.52	0.35
Jolan 2	0.63	0.49

Conclusions

1. The examined genotypes were classified into two groups according to their tolerance to drought in the germination phase basing on cluster analysis: tolerant genotypes and sensitive genotypes.
2. Cham1 genotype occupied the first class in the tolerant genotypes, and Doma 1 the last class in the sensitive genotypes
3. In case there was rainfall decline, Doma 4 genotype should be provided in the stable stability areas with complementary irrigations at the beginning of the season.
4. When using sorbitol to mimic drought in the lab, we have to consider the plant ability to

it absorb from the medium, knowing that sorbitol plays an important role in the osmotic adjustment.

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