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Reserve Mobilization, Total Sugars and Proteins in Germinating Seeds of Durum Wheat (*Triticum durum* Desf.) under Water Deficit after Short Period of Imbibition

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Abstract

Imbibition during the first few hours is crucial for the success of seed germination process. The effect of water deficit after 2 and 8 hours (h) of imbibition of seeds of durum wheat was studied in terms of reserve mobilization, total sugars and proteins at 24, 48 and 72 h post treatment. Imbibition for 2 and 8 h was not enough to support normal mobilization of seed reserves and solubilization of sugars. But, a significant increase in total proteins was shown after 24 and 48 h of water deficit for the two imbibition periods (2 and 8 h). Our results indicate that most of the biochemical and molecular changes are intensified during the first 2-8 h of imbibition. Therefore, biochemical and molecular dissection of this phase of seed germination is of great value in the improvement of our understanding of plant's response to water deficit.

Keywords: Durum wheat, imbibition, reserve mobilization, water deficit.

1. Introduction

One of the most detrimental factors that plants face is water deficit. Water deficit is one of the major abiotic stresses that threaten food security worldwide (Chaves *et al.*, 2003; Passioura, 2006); hence, there is an urgent need for multilevel investigations and dissection for better and comprehensive understanding of how plants respond to water deficit. In addition, this will help find effective ways to increase crop yield without consuming too much water.

Plants are exposed to drought at all stages of their growth and development. Plant's response to water deficit is dependent on the developmental stage (Blum *et al*., 1980). No correlation was found between the response of seeds and photosynthesizing seedlings to osmotic stress induced by polyethylene glycol (PEG) in ten cultivars of bread wheat, *Triticum aestivum* L. and one cultivar of durum wheat, *Triticum durum* Desf. (Blum *et al*., 1980). Therefore, one cannot extrapolate results about stress response from one developmental stage to another stage, which necessitates independent studies of the response for each developmental stage.

The first stage in plant development is seed germination, which starts by water uptake (imbibition), followed by reserve mobilization and protein synthesis and ends with the emergence of the radicle from seed tissues (Bewley, 1997). Water uptake by seeds was shown to occur in three phases: initial rapid water uptake, then a plateau phase, which is followed by rapid water uptake during which the radicle elongates and emerges (Bewley, 1997). In germinating wheat seeds, it was shown that protein synthesis starts to increase 30 min after imbibition, and it keeps increasing in the next 6 hours of imbibition (Marcus *et al*., 1966). This coincides with the first phase of imbibition, which is characterized by the rapid water uptake. In addition, this suggests a crucial role for this phase in the success of the germination process.

Seeds of bread wheat (*Triticun aestivum* L.) were imbibed for 24 hours, and then they were dehydrated for the subsequent days. The results showed a decrease in reserve mobilization started at day 5 of germination (Miazek *et al*., 2001). Indeed, germinating seeds of bread wheat showed tolerance to dehydration up to the 4th day post-imbibition (Miazek *et al*., 2001). Whereas, in other studies, the effect of osmotic stress on the germination process of seeds revealed a highly significant decrease in the germination percentage, germination rate and reserve mobilization (Almansouri *et al*., 2001; Sayar *et al*., 2010; Soltani *et al*., 2006). This indicates the differential response of germinating seeds to the different water deficit treatments. Indeed, exposing *Arabidopsis thaliana* plants

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to two different water deficit regimes resulted in significant differences in the transcriptome profiles of the two treatments (Harb *et al*., 2010). Therefore, it is of high importance to study the response of germinating seeds to water deficit that simulates to some extent the real stress in the field. This will lead to a better understanding of how water deficit affect the germination process of seeds, and help in the development of strategies to protect plants from the negative effect of water deficit as early as possible.

In this study, the effect of water deficit after the first hours of imbibition was tested in terms of reserve mobilization, total sugars and proteins during the first 3 days of germination after the onset of water deficit.

2. Materials and Method

2.1. Plant Material

Seeds of durum wheat (*Triticum durum* Desf. cv. Hourani 27) were provided by the National Center for Agriculture Research and Extension (NCARE), Jordan.

2.2. *Water Uptake*

Six groups of seeds of durum wheat (*Triticum durum* Desf. cv. Hourani 27) each group with 20 seeds was prepared. Then, their initial weight (W1) was taken before imbibition. After that, the seeds were put on wet filter paper in 9 cm plates and the plates were kept in an incubator set at 25ºC under dark conditions for different time intervals: 1, 2, 3, 4, 6, 7, and 8 h. Final weight (W2) was taken every one hour after imbibition, and water uptake percentage was calculated:

Water uptake% = (W2 – W1)/ W1 * 100

2.3. Reserve Mobilization

Dry seeds without imbibition were oven dried at 104ºC overnight. After that, their weight was taken and considered the original seed dry weight. The effect of water deficit after few hours of imbibition was tested; wheat seeds were kept on wet filter paper in 9 cm plates at 25ºC under dark conditions for 2 and 8 h. Then, wheat seeds were transferred to dry filter paper in 9 cm plates after blotting the excess water. Samples were taken from treated and control (well-watered) plates at: 24, 48 and 72 h. Samples were oven dried at 104ºC overnight, and their dry weights were taken (dry weight of seed remnants).

Reserve mobilization was calculated as follows:

Weight of mobilized reserve (mg seed^{-1}) = (Original seed dry weight – Dry weight of seed remnants)/Number of seeds

Reserve depletion %= (Weight of mobilized reserve / Original seed dry weight) *100

2.4. Germination Under Water Deficit

Seeds were considered germinated when the radicle is 1 mm long. The germination percentage of wheat seeds exposed to imbibition for 2 and 8 h, followed by water deficit was calculated at 48 and 72 h of treatment.

2.5. Quantification of Sugars

Sugars were quantified by anthrone method (Yemm and Willis, 1954). Briefly, seeds were ground in 80% ethanol. Then, a volume of the supernatant was reacted with anthrone reagent under boiling for 5-10 min. Glucose was the standard, and absorbance was read at 630 nm.

2.6. Quantification of Total Proteins

Total proteins in wheat seeds were quantified by Bradford's method (1976). Briefly, seeds were ground in distilled water, and then the mixture was centrifuged. Four (4) µL of the supernatant was mixed with 200 µL of Bradford reagent. The absorbance of the resultant product was read at 575nm. Standard curve of bovine serum albumin was run with the samples.

2.7. Statistical Analysis

All data were analyzed by Student's - T test using Excel software. Differences with p-value less than 0.05 were considered significant.

3. Results

3.1. Water Uptake

To determine at what time point water uptake by seeds will reach 25 and 50%, the water uptake of wheat seeds was monitored for the first 8 hours of imbibition. During this time period, an increase in the accumulated water content in seeds was shown (Fig. 1). After 2 h of imbibition, the accumulative water uptake was about 25%, and then it increases to reach 50% after 8 h of imbibition (Fig. 1).

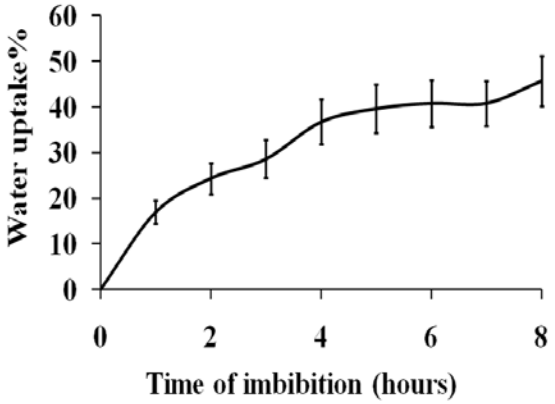

Figure 1. Water uptake during the first hours of imbibition in seeds of durum wheat. Error bars represent the standard errors of the means (n=8). The experiment was repeated with the same results.

3.2. Reserve Mobilization in Response to Water Deficit

To test if water absorbed during the first 8 h is enough to support all the phases of the germination process; wheat seeds imbibed for 2 and 8 h were dehydrated for 24, 48, and 72 h. After that, reserve mobilization was determined for both treatments at the three time points of water deficit. The results showed no change in reserve mobilization between the treated and the control seeds after 24 h of treatment. Whereas, the amount of water absorbed after 2 and 8 h was not enough for normal reserve mobilization after 48 and 72 h of water deficit. After 48 h of water deficit, reserve mobilization was 54% and 61% of the well-watered control at 2 and 8 h of imbibition, respectively

(Fig. 2A). Whereas, after 72 h of water deficit reserve mobilization decreased to 44% and 25% at 2 and 8 hours of imbibition, respectively (Fig. 2A).

The results of seed reserve depletion showed the same trend as that shown for reserve mobilization. No change in seed reserve depletion was shown after 24 h (Fig. 2B). Seed reserve depletion was reduced by about 5 and 4 % after 48 h of water deficit for the two imbibition periods (2 and 8 h), respectively (Fig. 2B). After 72 h of water deficit, the reductions were 13 and 11 % after 2 and 8 h of imbibition, respectively (Fig. 2B).

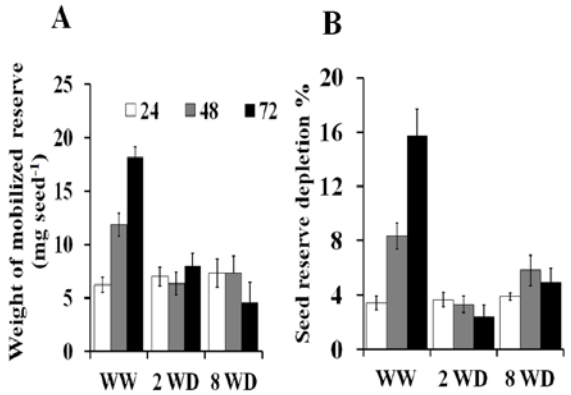

Figure 2. The effect of water deficit on reserve mobilization of seeds of durum wheat. A) Weight of mobilized reserve (mg.seed^{-1}) in wheat seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered (WW) control. B) Seed reserve depletion % in wheat seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered (WW) control. Error bars represent the standard errors of the means (n = 6). The experiment was repeated with the same results.

Reserve mobilization was determined after 7 days of water deficit at 2 and 8 h of imbibition. Both 2 and 8 h of imbibition showed a reduction in reserve mobilization and depletion of 84% compared to the well-watered control (Fig. 3A and B).

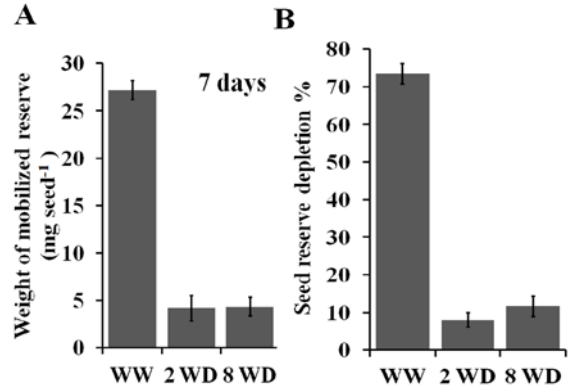

Figure 3. Reserve mobilization after 7 days of germination of seeds of durum wheat under water deficit (WD) compared to well-watered control (WW). A) Weight of mobilized reserve (mg.seed^{-1}) in seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered control (WW). B) Reserve depletion percentage in seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered control (WW). Error bars represent the standard errors of the means (n= 6 for WD and 10 for WW). The experiment was repeated with the same results.

3.3. Total Sugars in Response to Water Deficit

Soluble sugars were quantified in dehydrated seeds imbibed for 2 and 8 h of imbibition after 24, 48 and 72 h of water deficit. No change in soluble sugars was shown after 24 h of water deficit for the two imbibition periods (2 and 8 h) (Fig. 4). After 48 h of water deficit, soluble sugars decreased to 76 and 78 % compared to the control at 2 and 8 h of imbibition, respectively (Fig. 4). The reductions were 73 and 47 % at 2, and 8 h of imbibition after 72 h of water deficit, respectively (Fig. 4).

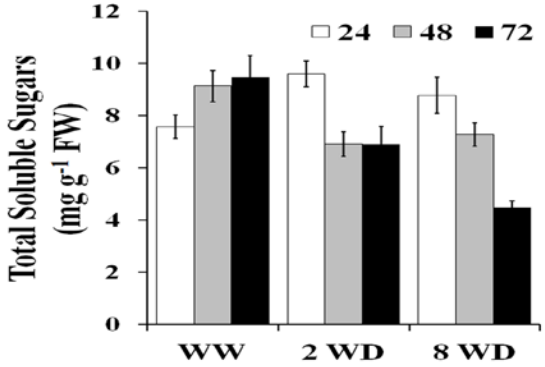

Figure 4. The effect of water deficit on the total soluble sugars in durum wheat seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered (WW) control. Error bars represent the standard errors of the means (n = 6). The experiment was repeated with the same results.

3.4. Total Proteins in Response to Water Deficit

After 24 and 48 h a highly significant increase in the concentration of total proteins was shown (Fig. 5). At 2 h of imbibition, the increases were 24 and 22 mg.g^{-1} fresh weight (FW) after 24 and 48 h of water deficit, respectively (Fig. 5). At 8 h of imbibition, the increases were 20 and 32 mg.g^{-1} FW after 24 and 48 h of water deficit, respectively (Fig. 5).

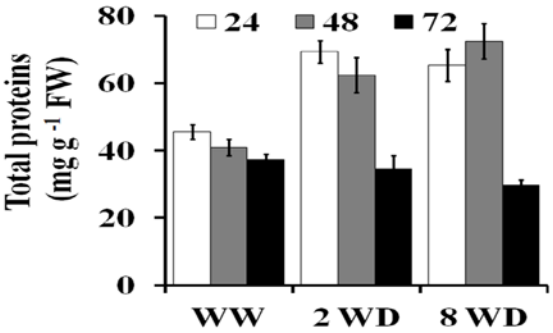

Figure 5. The effect of water deficit on the total proteins in durum wheat seeds exposed to water deficit at 2 and 8 hours of imbibition (2 WD and 8 WD, respectively) compared to the well-watered (WW) control. Error bars represent the standard errors of the means (n = 5). The experiment was repeated with the same results.

3.5. Seed germination in response to water deficit

Water deficit at 2 and 8 h of imbibition resulted in a complete inhibition of germination after 48 and 72 h of water deficit.

4. Discussion

The results of water uptake during the first 8 h of imbibition showed an increase in the accumulative water with time. This is consistent with the study of water uptake in barley (*Hordeum vulgare*) and bread wheat (*Triticum aestivum* L.) (Davidson *et al*., 1976; Clarke, 1980, respectively). Factors related to the sowing medium and to the seed were found to affect water uptake by seeds (Davidson *et al*., 1976; Clarke, 1980). Water potential and hydraulic conductivity of the soil impose limiting factors on the water uptake process (Ward and Shaykewich, 1972). Moreover, the hydraulic conductivity of the seeds is also playing a major role (Ward and Shaykewich, 1972). To exclude the effect of the initial seed size and other factors on the imbibition process, wheat seeds of the same size and from the same batch were used for the determination of water uptake in this study.

A reduction in reserve mobilization was revealed after 48, 72 h and after day 7 of water deficit at 2 and 8 h of imbibition. These results suggest that water deficit during the first hours of imbibition is detrimental to the biochemical and molecular changes needed for seed germination. In agreement, a study on durum wheat showed a highly significant decrease in reserve mobilization in response to osmotic stress after 7 days of imbibition (Soltani *et al*., 2006).

Soluble sugars are one biochemical indicator of the efficiency of seed reserve mobilization. Water deficit at 2 and 8 h of imbibition inhibited the solubilization of sugars after 48 and 72 h of the treatment. This is in agreement with the results of the effect of osmotic stress on the degradation of sugars in durum wheat, which revealed a drastic decrease of soluble sugars after 48 h of treatment (Almansouri *et al*., 2001). In contrast with our results, studies of germinating seeds of bread wheat showed an increase in soluble sugars started at day 2 of water deficit up to day 5 after 24 h of imbibition (Miazek *et al*., 2001).

Proteins are major components of cereals' grain (Shewry and Halford, 2002; Šramková *et al*, 2009; Triboï *et al*., 2003). Seed proteins can be classified into: storage, structural, metabolic, and protective proteins (Shewry and Halford, 2002). In this study, the concentration of total proteins was increased after 24 and 48 h of water deficit following a short period of imbibition of 2 and 8 h. This increase in protein content might be explained as an acclimation strategy to water deficit (Nakashima *et al*., 2009). It is known that structural and functional proteins are induced by environmental stresses such as water deficit (Bartels and Sunkar, 2005; Ramanjulu and Bartels, 2002). Indeed, in a study on two wheat cultivars with different tolerance to salinity, protein synthesis under salt stress was found as an adaptation strategy adopted by the salinity-tolerant cultivar (Delláquila and Spada, 1993). In germinating wheat seeds, albumins and globulins started to increase at day 2 of water deficit under light conditions (Miazek *et al*., 2001). In addition, in germinating wheat seeds, RNA synthesis started as early as 3 h of germination (Rejman and Buchowicz, 1973). This coincides with the first phase of imbibition, which is characterized by the rapid water uptake. This may suggest a crucial role for this phase in the success of the germination process.

In this study, germination was inhibited by water deficit after 2 and 8 h of imbibition. In bread wheat, dehydrated seeds after 24 h of imbibition were tolerant to water deficit up to the fifth day of imbibition (Miazek *et al*., 2001). The effect of osmotic stress using polyethylene glycol (PEG) and/or mannitol on seed germination of wheat, showed a drastic inhibition of the percentage and rate of germination staring at day 2 of imbibition (Almansouri *et al*., 2001; Blum *et al*., 1980; Sayar *et al*., 2010). The complete inhibition of germination as a result of water deficit can be explained by the complete absence of moisture, whereas, under osmotic stress, some moisture is available to allow low germination.

Intensive seedling growth occurs during the first 4 days of imbibition due to the intensive mobilization of seed reserves (Miazek *et al*., 2001). Imbibition during the first hours of the germination process is crucial to provide the germinating seed with enough moisture for the biochemical and molecular changes during normal germination process. Hence, understanding the biochemical and molecular basis of water-seed relationship during the first hours of imbibition is invaluable for the improvement of plant's resistance to water deficit.

References

Almansouri M, Kinet J and Lutts S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil*, **231**: 243–254.

Bartels D and Sunkar R. 2005. Drought and salt tolerance in plants. *Crit Rev Plant Sci*., **24**:23–58.

Bewley J. 1997. Seed germination and dormancy. *Plant Cell*, **9**:1056–1066.

Blum A, Sinemina B and Ziv D. 1980. An evaluation of seed and seedling drought tolerance screening tests in wheat. *Euphytica*, **29**: 727–736.

Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochem*., **72**:248 – 254.

Chaves M, Maroco J and Pereira J. 2003. Understanding plant responses to drought- from genes to the whole plant. *Functional Plant Biol*., **30**: 239 –264.

Clarke J. 1980. Measurement of relative water uptake rates of wheat seeds using agar media. *Canad J Plant Sci*., **60**:1035–1038.

Davidson D, Eastman M and Thomas J. 1976. Water uptake during germination of barley. *Plant Sci Lett*., **6**:223–230.

Dellaquila A and Spada P. 1993. The effect of salinity stress upon protein synthesis of germinating wheat embryos. *Annals of Botany*, **72**:97–101.

Harb A, Krishnan A, Ambavaram MM and Pereira A. 2010. Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiol*., **154**:1254–1271.

Marcus A, Feeley J and Volcani T. 1966. Protein synthesis in imbibed seeds III. Kinetics of amino acid incorporation ribosome activation, and polysome formation. *Plant Physiol*., **41**:1167–1172.

Miazek A Bogdan J and Zagdanska B. 2001. Effects of water deficit during germination of wheat seeds. *Biologia Plantarum* , **44**:397–403.

Nakashima K Ito Y and Yamaguchi-Shinozaki K. 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiol*., **149**: 88–95.

Passioura J. 2006. Increasing crop productivity when water is scarce-from breeding to field management. *Agricult Water Manag.*, **80**:176–196.

Ramanjulu S and Bartels D. 2002. Drought- and desiccation induced modulation of expression in plants. *Plant Cell Environ*., **25**:141–151.

Rejman E and Buchowicz J. 1973. RNA synthesis during the germination of wheat seed. *Phytochem.*, **12**: 271–276.

Sayar R Bchini H Mosbahi M and Ezzine M. 2010. Effects of salt and drought stresses on germination, emergence and seedling growth of durum wheat (*Triticum durum* Desf.). *J Agricult Res*., **5**:2008–2016.

Shewry PR and Halford NG. 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. *J Experi Botany*, **53**: 947–958.

Soltani A Gholipoor M and Zeinali E. 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environ Experi Botany*, **55**:195–200.

Šramkováa Z , Gregováb E and Šturdíka E. 2009. Chemical composition and nutritional quality of wheat grain. *Acta Chimica Slovaca* ,**2**:115–138.

Triboï E Martre P and Triboï –Blondel AM. 2003. Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *J Experi Botany*, **54**: 1731-1742.

Ward J and Shaykewich C. 1972. Water absorption by wheat seeds as influenced by hydraulic properties of soil. *Canad J Soil Sci.*, **52**:99–105.

Yemm E and Willis A. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem J*., **57:**508–514.