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TdERF1, an ethylene response factor associated with dehydration responses in durum wheat (Triticum turgidum L. subsp. durum)

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Water deficit and increasing salinization reduce productivity of wheat, the leading crop for human diet. While the complete genome sequence of this crop has not been deciphered, a BAC library screening allowed the isolation of *TdERF1*, the first ethylene response factor gene from durum wheat. This gene is putatively involved in mediating salt stress tolerance and its characterization provides clues toward understanding the mechanisms underlying the adaptation/tolerance of durum wheat to suboptimal growth conditions. *TdERF1* expression is differentially induced by high salt treatment in 2 durum wheat varieties, the salt-tolerant Grecale (GR) and the salt-sensitive Om Rabiaa (OR). To further extend these findings, we show here that the expression of this ERF is correlated with physiological parameters, such as the accumulation of osmo-regulators and membrane integrity, that discriminate between the 2 contrasted wheat genotypes. The data confirm that GR and OR are 2 contrasted wheat genotypes with regard to salt-stress and show that *TdERF1* is also induced by water stress with an expression pattern clearly discriminating between the 2 genotypes. These findings suggest that *TdERF1* might be involved in responses to salt and water stress providing a potential genetic marker discriminating between tolerant and sensitive wheat varieties.

Introduction

Salt and water stress are major problems threatening plant yield. One of the basic plant responses to stress involves ionic homeostasis to circumvent cellular dehydration caused by high soil salinity and drought. This is ensured by the synthesis and accumulation of compatible solutes in the cytoplasm and organelle such as proline and soluble sugars, involved in osmotic adjustment mechanisms.[1,2] These osmo-regulators are also osmo-protectors acting as low-molecular-weight chaperones that stabilize proteins, membranes and macromolecular structures under stressful conditions and also as scavengers of reactive oxygen species (ROS).[3-6] Both biochemical elements can be considered as "biochemical markers" of tolerance to salt stress and thus can be used for early selection of salt tolerant varieties.[7] The osmolyte mannnitol (sugar alcohol) was reported to improve the growth of wheat plants under water and salt stress in callus and in the whole plant via better osmotic adjustment.[8] Despite being one of the most outstanding manifestations of salt stress, the exact role of proline in the resistance to salt stress remains unclear.[9]

Brief summary of recently published article

Since durum wheat is considered as a food crop most indispensable for the survival of human populations, different strategies aiming to improve its productivity have been developed. However, understanding the adaptation and tolerance mechanisms in durum wheat has been hampered by the lack of genomic resources on this species and by the big size and high complexity of its genome. Even

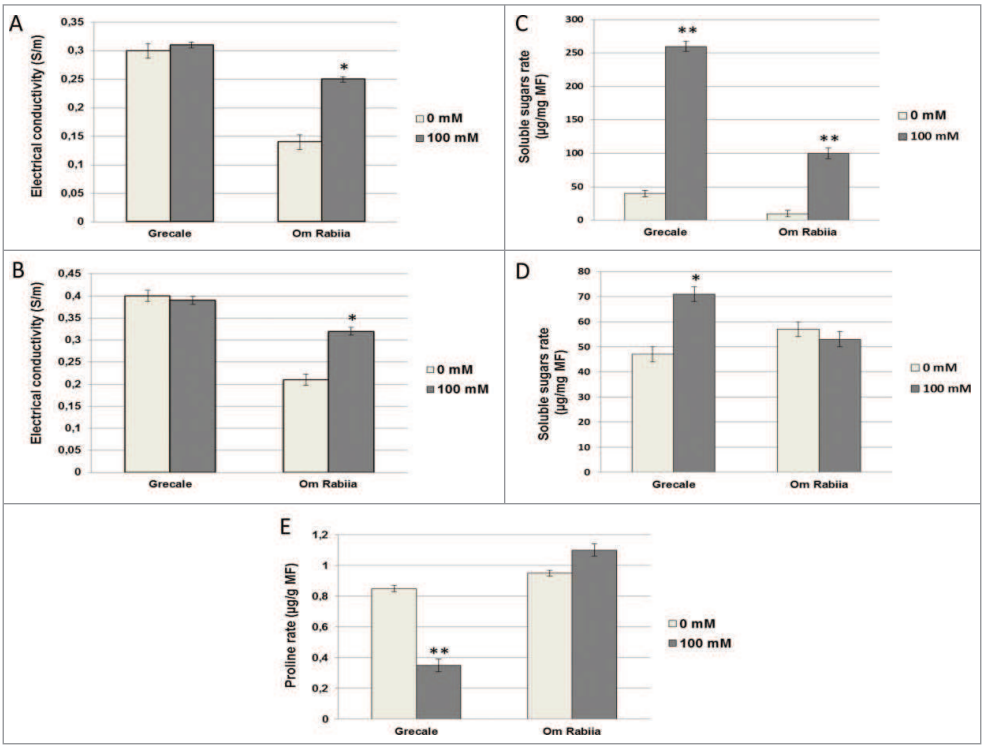

Figure 1. Assessing physiological parameters in Om Rabiia and Grecale, 2 durum wheat genotypes contrasting with regard to salt stress tolerance. Comparative analysis of Electrical conductivity in leaves (**A**) and roots (**B**); soluble sugar content in leaves (**C**) and roots (**D**), and Proline content in leaves (**E**) between Om Rabiaa and Grecale with or without salt treatment (100 of NaCl). The experiment lasted 14 days and was carried out in triplicate for each NaCl concentration and for each analysis. Values are means standard deviation (SD) ($n \geq 30$) of 3 replicates. *$0.01 < P < 0.05$, ***$P < 0.001$ (Student's t-test).

though genes belonging to AP2/ERF family are known to be involved in many stress responses,[10-12] so far, they have not been investigated in durum wheat. In fact, a unique ERF transcription factor named *TdERF1* was recently isolated and its expression has been shown to be associated with salt stress [13]. Two genotypes of *T. durum*, named GR (Grecale) and OR (Om Rabiia), were assessed for their salt-stress tolerance using germination capacity and stomatal conductance tests indicating that GR and OR are salt tolerant and sensitive, respectively. The expression pattern of *TdERF1* was also been shown to be discriminating between the 2 wheat varieties contrasted in terms of salt-stress tolerance.[13] Using physiological criteria, the data presented here confirm that GR and OR durum wheat genotypes display contrasted behavior with regards to salt stress. The data also show that *TdERF1* expression induced under water stress supporting a potential role of this *ERF* gene in responses to both salt and dehydration stress.

Differential responses to salt stress among OR and GR durum wheat genotypes regarding osmo-regulators and sugar accumulation

Membrane stability is considered as a criterion for resistant genotypes identification. Cellular membrane integrity of durum wheat was investigated in GR and OR genotypes by assessing electrolytes electric conductivity as this reflects the level of cell permeability integration. The data show that under salt stress GR genotype maintains higher membrane stability in both leaves and roots (**Fig. 1A and B**) suggesting GR variety is able to regulate the flux of toxic salt ions into the cell probably through its ability to store salt ions. By contrast, the OR genotype showed altered membrane integrity as revealed by electrical conductivity monitoring (**Fig. 1A and B**). Indeed, electrical conductivity of OR genotype is higher than in natural condition, suggesting that membrane stability was altered when compared to non-stress condition. All together, these results suggest that GR variety is more tolerant to salt stress than OR variety due to its ability to control cell flux of toxic ion and to sequestrate those that managed to infiltrate in salt stress condition. The accumulation of osmo-regulators assayed with or without salt treatment (100 mM NaCl) revealed that leaves undergo a dramatic increase in sugar accumulation in both GR and OR genotypes (**Fig. 1C**). However, GR leaves display a significantly higher basal soluble sugar content than OR suggesting that the stress sensitive genotype has a lower capacity to accumulate soluble sugars that can act as osmo-protectors against salt stress. Under the same stress conditions, sugar accumulation was less important in roots though it is more important in GR than in OR (**Fig. 1D**). Surprisingly, monitoring proline content in leaves revealed that the accumulation of this amino acid upon salt treatment is reduced in GR, while, it is enhanced in OR genotype by salt stress (**Fig. 1E**). Recent studies showed that in despite of being the most common osmolyte during salt stress, proline doesn't

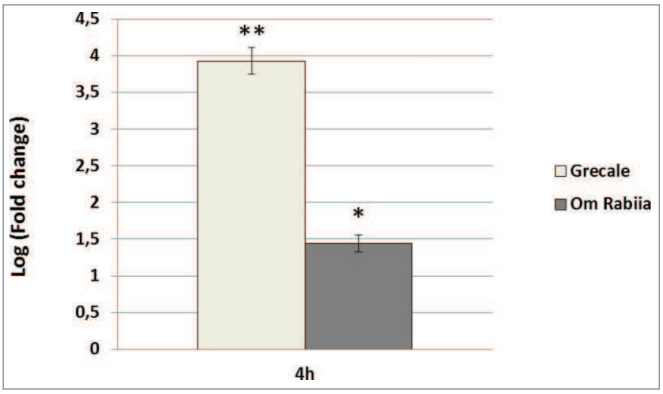

Figure 2. Expression pattern of *TdERF1* in response to water stress. Expression profile of *TdERF1* in leaves from Grecale and OmRabiia durum wheat genotypes following dehydration condition. The levels of TdERF1 transcripts were assessed by real-time quantitative PCR. mRNA accumulation was monitored in 10 days old roots and leaves, after 4 hours under ambiant air condition. For each sample, relative fold changes were determined by normalizing the Ct value of *TdERF1* gene in different tissues in both sensitive and tolerant varieties to the Ct value of Td26S, used as internal control, and by calculating relatively to a calibrator using the formula $2^{-\Delta\Delta Ct}$. ΔΔCt refers to fold differences of TdERF1 expression relative to untreated tissues. The experiment was carried out in triplicate. Values are means standard deviation (SD) ($n \geq 30$) of 3 replicates. *$0.01 < P < 0.05$, ***$P < 0.001$ (Student's t-test).

seem to be the main responsible for salt stress tolerance since the most tolerant plant taxa are typically accumulators of Glycine Betaine.[14] Moreover, being present at very low concentrations, it is unlikely that proline may have any osmotic effect, but it may contribute to stress tolerance mechanisms through its role as a low-molecular-weight chaperone and/or a ROS scavenger.[14] The data suggest that GR genotype may gain its salt stress tolerance by accumulating Glycine Betaine while maintaining a low-molecular-weight of proline. Overall, the physiological criteria assessed here are in line with GR genotype being tolerant and OR genotype sensitive to salt stress.

OR and GR genotypes of durum wheat display contrasting tolerance to water stress

The expression of *TdERF1* in response to dehydration was assessed at the transcript level in OR and GR genotypes of durum wheat using specific primers non-discriminating between the allelic forms of *TdERF1*.[13] in a quantitative real-time PCR. The data indicate that after 4h of water stress (**Fig. 2**), the transcript levels of *TdERF1* were dramatically increased in GR (16-fold) whereas they are moderately enhanced in OR (2-fold). This further supports the hypothesis that *TdERF1* is involved in water stress tolerance and confirm that it may provide a good marker for discriminating tolerant and sensitive wheat varieties.

Altogether, our study confirm that GR and OR are 2 durum wheat varieties contrasted with respect to salt stress tolerance and define *TdERF1* as putative marker in selection programs aiming to discriminate among tolerant and sensitive wheat varieties in response to abiotic stress.

Materials and Methods

Membrane integrity assay was performed on leaf discs emerged in distilled water at low temperature. Electrolyte loss was measured as increase in the electrical conductivity of the medium exsorption compared to control, the total conductivity being the ratio of the conductivity before and after autoclaving leaves and roots. Proline rates were measured according to Troll et Lindsley,[15] modified by MONNEVEUX et NEMMAR.[16] Soluble sugars were assessed according to Shields et Burnett.[17] These physiological experiments were performed on 14-day-old plants. For water stress treatment, seedling growth and gene expression analyses were performed as described previously.[13]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Chen H, Jiang J-G. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environ Rev 2010; 18:309-19; http://dx.doi.org/10.1139/A10-014
2. Kronzucker HJ, Britto DT. Sodium transport in plants: a critical review. New Phytol 2011; 189:54-81; PMID:21118256; http://dx.doi.org/10.1111/j.1469-8137.2010.03540.x
3. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exper Bot 2007; 59:206-16; http://dx.doi.org/10.1016/j.envexpbot.2005.12.006
4. Chen THH, Murata N. Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci 2008; 13:499-505; PMID:18703379; http://dx.doi.org/10.1016/j.tplants.2008.06.007
5. Flowers TJ, Colmer TD. Salinity tolerance in halophytes*. New Phytol 2008; 179:945-63; PMID:18565144; http://dx.doi.org/10.1111/j.1469-8137.2008.02531.x
6. Szabados L, Savouré A. Proline: a multifunctional amino acid. Trends Plant Sci 2010; 15:89-97; PMID:20036181
7. Belfakih M, Ibriz M, Zouahri A, Hilali S. Effet de la salinité sur la croissance des deux variétés de bananier «grande naine» et «petite naine» et leur nutrition minérale au Maroc. J Appl Biosci 2013; 63:4689-702; http://dx.doi.org/10.4314/jab.v63i1.87244
8. Shen B, Hohmann S, Jensen RG, Bohnert HJ. Roles of Sugar Alcohols in Osmotic Stress Adaptation. Replacement of Glycerol by Mannitol and Sorbitol in Yeast. Plant Physiol 1999; 121:45-52; PMID:10482659; http://dx.doi.org/10.1104/pp.121.1.45
9. Ben Rejeb K, Abdelly C, Savouré A. La proline, un acide aminé multifonctionnel impliqué dans l'adaptation des plantes aux contraintes environnementales. Biol Aujourdhui 2012; 206:291-9; PMID:23419256; http://dx.doi.org/10.1051/jbio/2012030
10. Shinozaki K, Dennis ES. Cell signalling and gene regulation: global analyses of signal transduction and gene expression profiles. Curr Opin Plant Biol

2003; 6:405-9; PMID:12972039; http://dx.doi.org/10.1016/S1369-5266(03)00093-1
11. Chen WJ, Zhu T. Networks of transcription factors with roles in environmental stress response. Trends Plant Sci 2004; 9:591-596; PMID:15564126; http://dx.doi.org/10.1016/j.tplants.2004.10.007
12. Yamaguchi-Shinozaki K, Shinozaki K. Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci 2005; 10:88-94; PMID:15708346; http://dx.doi.org/10.1016/j.tplants.2004.12.012
13. Makhloufi E, Yousfi F-E, Marande W, Mila I, Hanana M, Bergès H, Mzid R, Bouzayen M. Isolation and molecular characterization of ERF1, an ethylene response factor gene from durum wheat (Triticum turgidum L. subsp. durum), potentially involved in salt-stress responses. J Exp Bot 2014; 65:6359-71; PMID:25205575; http://dx.doi.org/10.1093/jxb/eru352
14. Gil R, Bautista I, Boscaiu M, Lidón A, Wankhade S, Sánchez H, Llinares J, Vicente O. Responses of five Mediterranean halophytes to seasonal changes in environmental conditions. AoB Plants 2014; 6:plu049; PMID:25139768; http://dx.doi.org/10.1093/aobpla/plu049
15. Troll W, Lindsley J. A photometric method for the determination of proline. J Biol Chem 1955; 215:655-60; PMID:13242563
16. Monneveux P, Nemmar M. Contribution à l'étude de la résistance à la sécheresse chez le blé tendre (Triticum aestivum L.) et chez le blé dur (Triticum durum Desf.) : étude de l'accumulation de la proline au cours du cycle de développement. Agronomie 1986; 6:583-90; http://dx.doi.org/10.1051/agro:19860611
17. Shields R, Burnett W. Determination of protein-bound carbohydrate in serum by modified anthrone method. Anal Chem 1960; 32:885-6; http://dx.doi.org/10.1021/ac60163a053