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GSTF1 gene expression at local Albanian wheat cultivar Dajti under salinity and heat conditions

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Abstract

Plants have evolved effective defense mechanisms against stress-induced oxidative damages, among which an important role play glutathione S-transferases (GSTs). This huge class of proteins have been reported to increase in a number of crops under temperature and saline stresses. However, different wheat cultivars display specific characteristics of expression. In our study we controlled the transcription of GSTF1 gene at leaves of wheat (*Triticum aestivum* L.) of local cultivar Dajti, evaluated previously as resistant toward salt and temperature stresses. Three different concentrations of NaCl, 50, 100, 200mM, were applied at plants germinated in Hoagland culture, and total ARN was extracted from leaves collected at 0-3-6-10-24-72 hrs after treatment. Seeds from the same cultivar were germinated in Hoagland culture under heat treatment, keeping controls at 25°C/20°C and the rest under a 35°C /25°C-day/night regime in a growth chamber. Total RNA was extracted after one week, 30, and 45 days following HT. RT-PCRs were performed using primers specific for *GSTF1*. Concentration of amplicons was evaluated in agarose gels. In conclusion, the transcription of GSTF1 at Dajti cultivar is reduced during the time of exposure on saline conditions, does not depend on salt concentration, and is not affected by prolonged temperature stress.

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Introduction

Wheat is among the most important crops cultivated in Mediterranean Albania, which faces high temperatures related to drought and salinity of the soils. The susceptibility of wheat production from climatic changes from year to year, has driven the engagement of institutions related to crop agriculture in a long-term research for the evaluation of the most tolerant cultivars (1, 2). Dajti is considered as a tolerant cultivar toward environmental conditions, and has been evaluated for its agronomical performance and physiological adaptation toward salinity. According to a previous report (3) it shows a higher capacity for osmoregulation compared to other local wheat cultivars. In this study we report on the expression of *GSTF1* gene from this cultivar grown in saline conditions, and under heat stress.

Based on previous reports, the molecular identities of key ion transport systems that are fundamental to plant salt tolerance were reported to be known (4) since 2000, while the molecular changes in response to heat stress were poorly understood (5). A review (6) summarizes the research on plant ion homeostasis in saline environments, and presents a model that integrates current understanding of salt stress sensing, which leads to the activation of the SOS pathway and the regulation of ion transport systems that facilitate ion homeostasis. According to a number of reports (6, 7) most halophytes and glycophytes tolerate salinity by rather similar strategies, however, a salt tolerant genetic model is required to delineate if salt tolerance is affected most by form or function of genes or more by differences in the expression of common genes due either to transcriptional or post-transcriptional control. A SOS signal pathway is a pivotal regulator of, at least some, key transport systems required for ion homeostasis according to 6, 8, 9; 10. A diagram

of the relevant transporters and Ca^{+}-dependent stress signaling pathway involved in Na^{+} homeostasis was prepared (6), and was concluded that little is known about the mechanistic entities that are responsible for Cl^{-} transport or the regulation of Cl^{-} homeostasis.

A complex set of stress-responsive transcription factors was evaluated (4), accompanied by data on protein interaction analysis and evaluation of allelism, additivity, epistasis, which may allow the determination of ordered relationships between stress signaling components.

Reduction of photosynthesis by HT stress appears to be another major factor that imposes source limitation to yield formation (11) for wheat. Different components of tolerance determined by different sets of genes are critical for heat tolerance at different stages of the life cycle and in various tissues of cereals. According to (12) among major classes of molecules involved in determination of thermotolerance at their vegetative stage of development are the so-called thermotolerance of translation and thermostability of key enzymes**.** A specific study (5) conducted the heat stress responsive transcriptome analysis at wheat cultivars. Based on the genome-wide gene expression profiles in the leaves of wheat genotypes, was concluded that except for heat shock protein and heat shock factor genes, these putative heat responsive genes encode transcription factors and proteins involved in phytohormone biosynthesis/signaling, calcium and sugar signal pathways, RNA metabolism, ribosomal proteins, primary and secondary metabolisms, as well as proteins related to other stresses.

In general, plants have evolved very effective defense mechanisms, among which an import role play glutathione S-transferases (GSTs) with catalytic and non-catalytic functions (13,14). Based on their primary structure, the plant GSTs may be grouped into four main classes (phi, zeta, tau and theta) and two outlying groups (14). They are abundant proteins encoded by a highly divergent family mainly involved in stress responses that protect cell components from oxidative damage by scavenging toxic organic hyperoxides, which may be caused by different abiotic factors included high temperature and high salt (15). The investigation of GST activities has shown that wheat has the second highest GST activity among 38 crops (15), and that it is increased under drought and saline stresses (16, 17). GSTs were also reported to be affected by heat stress, and showed higher expression levels during the short-term heat shock than long-term heat treatments (5). According to (18) it is likely that difference in *GSTF1* gene expression patterns between wheat cultivars is due to distinct signaling pathways, which activate GSTF1 pathway, and that this gene is not induced by stress stimuli, while others report that the expression of GST gene raise due to osmotic stress in different wheat cultivars (19; 13). Here we report on the transcription of *GSTF1* gene at leaves of wheat (*Triticum aestivum* L.) of Albanian local cultivar Dajti under salinity and temperature stress, aiming to understand the tolerance of this cultivar toward environmental conditions in molecular level, and to use it as model for the study of other local cultivars.

Materials and Methods

Expression of *GSTF1* at different salinity conditions

Seeds from cultivar Dajti, donated from the Seed Bank of Korca Region, Albania, were selected and kept in dark for 24 hrs at 4°C following (18). Then they were allowed to germinate at 22°C for 3 days, planted to Petri dishes and watered with modified Hoagland solution. After 16 days of the last treatment they were transferred to water cultures (Hoagland) with different NaCl concentrations: Control culture (600 ml Hoagland) without NaCl; 50 mM NaCl in 600 ml Hoagland; 100 mM NaCl in 600 ml Hoagland; 200 mM NaCl in 600 ml Hoagland. From each of the fourth cultures described, leaves were removed 0, 3, 6, 10, 24, and 72hr from the treatment and used to extract total RNA. Until the extraction procedure plants were kept at -20°C.

Expression of GST at heat shock treatment

Seeds of wheat cultivars Dajti (100 seeds) after sterilization were germinated in culture Hoagland for an initial 45 days in a 25°C/20°C-day/night regime in the growth chamber. Half of plants followed for an additional period of 45 days the same regime (controls), while the other half were kept at the stress regime 35°C/25°C-day/night following (11). The effect of temperature stress on *GSTF1* gene expression were controlled after one week (vegetative phase), after 30 days (anthesis phase), and after 45 days (15 days after anthesis) of treatment. The same growth conditions were applied for two more local wheat cultivars named LVS and Progresi, considered as more susceptible toward environmental conditions, which were used to compare the results on *GSTF1* expression with Dajti cultivar.

RNA extraction from wheat leaves

The total RNA extraction was completed following (20) from each of the categories of plants, and were further used to amplify the *GSTF1* fragment using a set of primers reported by (18).

GST-1F ATGGAAAACACTAACGTTGTACTC
GST-1R AACTTATAAGCCGAGTTTCTTCTTC

The master Mix was prepared following the instructions of HS RT-PCR kit from Sigma in a total volume of 50µl (with 20ng of template RNA). Cycling conditions: Initial step of 50 sec/50°C, initial denaturing for 2min/94°C, 35 cycles of 15sec/94°C-30sec/65°C-1min/68°C, a final extension of 5min at 68°C. Concentration of amplicons was evaluated in agarose gels.

Results

Expression of GST at different salinity conditions: In this study we analyzed the expression of GSTF1 (the first phi class) gene under saline and prolonged high temperatures at local cultivar Dajti, considered as tolerant toward environmental conditions and as one of the best bread wheat cultivars in Albania regarding the agronomical performance. The RT-PCR analysis of GSTF1 expression under saline stresses (0-50-100-200mMNaCl) was controlled at six time intervals after treatment (**Fig. 1A, 1B, 1C**). Results show that the main factor, which influences

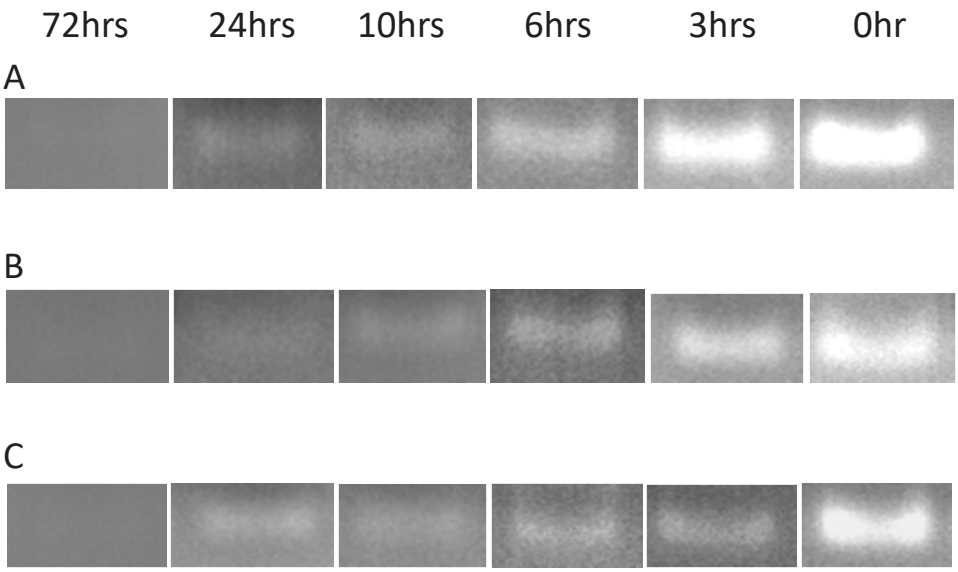

Figure 1. RT-PCR amplicons of *GSTF1* from plantlets grown in Hoagland solution with 50mM NaCl (A), 100mM NaCl (B), 200mM NaCl (C).

Figure 2. Control samples, verified for the GSTF1 transcript in three different time intervals.

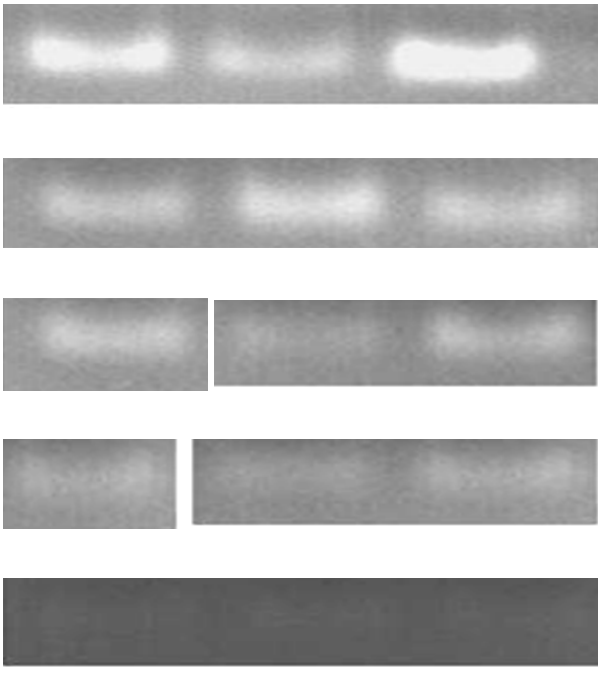

Figure 3. From up down *GSTF1* transcription at wheat cultivar Dajti: 3hrs after treatment; 6 hrs after treatment; 10hrs after treatment; 24hrs after treatment; 72hrs after treatment. From right to left of each picture the concentrations of NaCl were 50Mm, 100Mm, and 200mM.

the amount of transcript is the time of the exposure on saline stress, and that the transcript gets reduced by time.

As seen from the **Fig. 2**, wheat leaves from control plants do produce a constant amount of transcript, in the absence of the saline or HT stresses imposed during the experiment, meaning that GSTF1 serves cells not only during the stress conditions but in a constant manner.

The GSTF1 expression at plants collected within the same time interval after treatment is independent from the concentration of NaCl added at growth culture.

Expression of GSTF1 at wheat cultivar Dajti under temperature stress

In order to verify the expression of GSTF1 at the same cultivar treated with prolonged high temperature, we carried out the treatment for 45 days after an initial growth phase of 45 days in normal conditions. **Fig. 4** shows that the expression of GSTF1 for one week, 30 days and 45 days under treatment is the same with that of the control plants.

There is no difference in GSTF1 expression at Dajti leaves under control conditions and prolonged heat treatment. Two local cultivars considered more susceptible toward high temperatures than Dajti (named LVS and Progresi) were analyzed simultaneously in order to compare the results. Interestingly, for LVS the concentrations of GSTF1 transripts were higher at HT samples compared to the controls, while at cultivar Progresi there was a constant expression of the GSTF1 independently of the temperature regime.

Discussions

In general, plants have evolved very effective defense mechanisms against stress-induced oxidative damages, among which

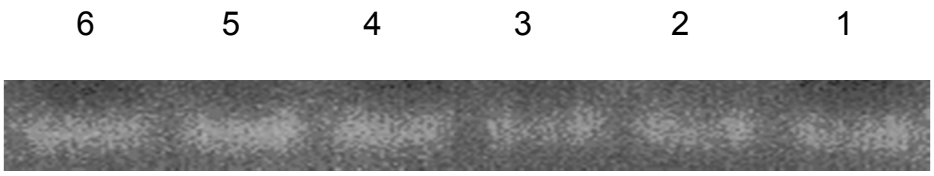

Figure 4. RT-PCR amplicons of *GSTF1* transcripts from plantlets grown in Hoagland solution and treated with prolonged temperature. 1-Dajti control; 2-Dajti after one week HT; 3-4.Dajti after 30 days HT; 5-6. Dajti after 45 days HT.

an import role play glutathione S-transferases (GSTs) (13, 14). Previous reports have demonstrated that GSTs can be differentially regulated by different abiotic stress factors, such as herbicides, hydrogen peroxide, dehydration, ultraviolet light, cold, phosphate starvation, ozone exposure, high temperature, saline stresses, hormone treatments, and draught. GSTs activities have been reported to increase in a number of crops under drought and saline stresses (16, 17, 15, 21), while there are reports on different expression patterns between wheat cultivars (18). At plants NaCl stress can damage photosynthetic mechanism via combination of superoxide and H_2O_2 mediated oxidation (4; 18), and a number of strategies are determined as protective mechanisms plant develop in response to it. Our results prove that GSTF1 expression at cultivar Dajti reaches its maximum immediately after treatment, as a protective response toward the increased salinity, however, not related to the concentration of salt. This is in accordance to (18) results on two wheat cultivars respectively tolerant and sensitive to salt stress. A previous research (3) on the effect of salinity on early seedling growth for cultivar Dajti compared to two other cultivars, showed that while sodium and potassium accumulation were similar, the chloride accumulation in plant tissues was much lower at Dajti cultivar, which at the same time displayed a much higher capacity for osmoregulation. The conclusions of (3) report that the solutes involved in the osmotic adjustment of Dajti tissues are not known. However, a number of studies have shown a negative correlation between Cl - concentrations in leaves and salt tolerance (22, 23), which is also the case for Dajti cultivar. The increased transcription of GSTF1 after NaCl treatment might be connected to the specific osmoregulation and more specifically to the inhibition of chloride accumulation at the roots of this cultivar. Previous reports conclude that expression of GST genes raise due to osmotic stress in different wheat cultivars (19,13), which might be the case for Dajti cultivar with a very good capacity for osmoregulation and timely reduction of GSTF1 expression.

GSTs involved in cellular detoxification seem to be produced in response to different stress factors, and furthermore are plant specific (18, 24, 25, 26, 27). According to previous reports (5) the genes encoding enzymatic components of ROS-scavenging pathways including glutathione—transferase (GST) are also affected by heat stress, suggesting a link between these two stress signaling pathways. In general, the genetic basis of wheat heat adaptation is poorly understood and no heat-tolerance genes have been cloned so far (28, 29). The most affected by high temperatures is Calvin cycle, at which the *Rubisco* activation by *rubisco activase* are believed to be reduced by high temperature stress, however, a number of reports describe differences in the heat tolerance of Rubisco synthesis even within a species (11, 29). According to (29) cultivar Dajti did not display suppression of the expression of *Rubisco* genes during vegetation and anthesis phase under HT, which brings to the conclusion that Rubisco activase a nuclear encoded protein that is responsible for the regulation of the activity of Rubisco might be responsible for the resistance. A simultaneous study of the synthesis of rubisco activase and GSTF1 at cultivar Dajti under HT could help to understand a possible link between heat-driven signaling pathways. Heat stress-responsive transcriptome analysis in wheat has shown a higher expression of glutathione peroxidases during short-term heat shock than the long-term heat treatments. The comparison of GSTF1 expression at Dajti cultivar with that of two more susceptible local cultivars LVS and Progresi under HT showed that, at LVS the expression was modified (increased), while at Progresi similarly to Dajti the expression remains constant. According to (5), plants can scavenge the ROS generated by heat shock, but the oxidative stress induced by long–term heat stress may impair their ability to generate these beneficial molecules.

In conclusion, the transcription of GSTF1 at Dajti cultivar increases immediately after salt treatment, is reduced during the time of exposure on saline conditions, does not depend on salt concentration, and is not affected by prolonged temperature stress. Based on the above we believe that this cultivar with a natural higher capacity for osmoregulation compared to other cultivars, and resistance toward long HT should be studied further in order to understand better the factors influencing the resistance displayed in both cases.

Conflict of Interest Statement

The authors confirm that this article content has no conflict of interest.

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