ABSTRACT:
WD40 repeat-containing proteins participate in DNA-protein and protein-protein interactions and positively regulate plant stress responses. GTS1, known as a WD40 repeat-containing protein, works as a scaffold protein and is important in ribosome biogenesis and also biomass accumulation. In this study, we evaluated the GIGANTUS1 (GTS1) gene expression in response to biotic and abiotic stress factors in Arabidopsis thaliana plants. In addition, we grew and characterized A. thaliana gts1 mutant (T-DNA SALK_010647) in order to observe the effects of its absence on plants. According to our results, 100-200 mM abscisic acid (ABA) and 100-200 mM sodium chloride (NaCl) treatment did not cause any changes in GTS1 gene expression, while only 6 h of 1 g/l and 2 g/l yeast extract (YE) treatment negatively affected GTS1 expression in 10-day-old plant explants. After 10 and 30 days of YE treatment, GTS1 gene expression was upregulated, and as a consequence plant growth efficiency was reduced. We thus concluded that through the downregulation of GTS1 transcripts, we could obtain better growth and/or higher biomass, which seems to be a good option for agricultural recruitments.

Keywords: GIGANTUS1, Arabidopsis thaliana, yeast extract, biotic stress, WD40 repeat

INTRODUCTION
Plants are naturally exposed to various stress conditions. In order to cope up with adverse stress conditions, plants have evolved different strategies. In addition to understanding the molecular mechanisms of stress response and adaptation, unravelling the roles of signalling pathways are also important since signalling plays an essential role in stress response. In many signalling pathways, scaffold proteins act as key regulators (JAIN & PANDEY 2018). They bind or interact with other components of signalling cascades. WD40 repeat-containing proteins participate in DNA-protein and protein-protein interactions (SMITH 2008; GACHOMO et al. 2014; CHUANG et al. 2015; KONG et al. 2015). They serve as molecular platforms in diverse cellular processes such as protein trafficking, transcription, ribosome biogenesis, flowering, development and chromatin modification (NEER et al. 1994; SHI et al. 2005; LI et al. 2007; HSIAO et al. 2016). The common and defined feature of these proteins is the WD40 motif which consists of approximately 40 amino acids and generally contains a glycine-histidine (GH) dipeptide at the N-terminus and a tryptophan-aspartic acid (WD) dipeptide at the C-terminal. They are called WD-repeats as they typically end with Tryptophan (W) and Aspartic acid (D) (IOUK et al. 2001; YAFFE et al. 2001; VAN NOCKER et al. 2003; LETUNIC et al. 2009; OUyang et al. 2012).

There are diverse WD40 repeats containing proteins in Arabidopsis. GIGANTUS1 (GTS1), a WD40 repeat-containing protein, has been found in Arabidopsis. It has been shown that via protein interactions it regulates development by affecting seed germination and biomass accumulation and it also takes place in ribosome biogenesis (GACHOMO et al. 2014). Since plant development is strongly related to stress response, unravelling the molecular mechanisms under these biological processes is crucial. However, the underlying mechanisms have not been elucidated completely (GACHOMO et al. 2013).
In theory, it is known that WD40 repeat-containing proteins play key roles in biomass accumulation and ribbon biogenesis as well as plant growth and development (Ramsay & Glover 2005; Ishida et al. 2016). In a study conducted with cotton, it was shown that WD40 repeat proteins are involved in the regulation of cotton fiber development (Salih et al. 2018). The studies of WD40 repeat proteins in Arabidopsis, rice, and foxtail millet have highlighted the diverse roles of WD40 repeat proteins in plant development and responses to the environment (van Nocker & Ludwig 2003; Mishra et al. 2012a; Ouyang et al. 2012). Therefore they are considered to be important in plant stress response mechanisms. Very few WD40 repeat-containing proteins have been reported in crop plants. However, WD40 repeat proteins are increasingly recognized as key regulators of specific developmental events or responses to environmental stresses in plants (Mishra et al. 2012b; Mostafa 2015). In a study conducted by Gachomo et al. (2013), it was shown that the ANGUSTIFOLIA gene also participates in many biochemical pathways related to biotic and abiotic stress response. In Arabidopsis, the WD-repeat protein was shown to coordinate the cellular networks associated with stress response, and light and hormone signalling (Chuang et al. 2015). Furthermore, Ananieva et al. (2008) presented the regulatory role of the WD40 domain of a myo-inositol phosphatase in stress signalling.

Up to now, the relationship between GTS1 gene expression and stress response has not been reported in A. thaliana. In this study, we examined whether abiotic and biotic stressors affect the GTS1 expression which is strongly related to plant growth and development.

**MATERIALS AND METHODS**

**Plant material, seed germination and plant growth conditions.** In this study, Arabidopsis thaliana (Col-0) seeds kindly provided by Dr. Ralf Stracke (Bielefeld University, Center for Biotechnology) and gts1 mutant (T-DNA SALK_010647) seeds from the Arabidopsis Biological Research Center (ARBC) were used. All of the seeds were surface sterilized and placed on Murashige and Skoog (Murashige & Skoog 1962) basal medium. The seeds and plantlets were incubated under fluorescent light (16 h light/8 h dark) at 24°C in the plant growth chamber. While the 10-day-old plants were referred to as ‘10 DAG (day after germination),’ the 30-day-old plants were referred to as ‘30 DAG.’

**Abscisic acid, sodium chloride and yeast extract treatments.** In the first part of the study, almost 2 cm long 10 DAG and 30 DAG Arabidopsis hypocotyl explants were treated with 100 mM, 200 mM abscisic acid (ABA); 100mM, 200 mM sodium chloride (NaCl) and 1 g/l, 2 g/l yeast extract (YE) (Cakir & Ari 2009) for 3 and 6 hours at 24°C in the rotary shaker. After stress treatments, the samples were collected and washed with distilled water and used for RNA isolation.

In the second part of the study, 10 DAG and 30 DAG whole Arabidopsis plants were transferred to MS medium with the addition of 1 g/l, 2 g/l YE. The plant samples were incubated for 24 h and 48 h time periods under fluorescent light (16 h light/8 h dark) at 24°C in the plant growth chamber. After YE treatment, the collected samples were directly used for RNA isolation.

**Genotyping of gts1 mutant seeds.** In order to observe the effects of GTS1 gene absence on plants, gts1 mutant Arabidopsis plants were grown. For the complementation test, T-DNA insertion in the GIGANTUS1 gene was analysed by PCR with specific primers for the T-DNA left border. The primers used in the analysis are given in Table 1.

**RNA isolation, RT-PCR and qPCR analysis.** Total RNA was isolated from the plant samples using TRIzol® Reagent (Invitrogen, 15596026) according to the manufacturer’s instructions. After examining the integrity and purity of the RNA samples, cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems®, 4368814) according to the manufacturer’s instructions.

In the first part of the study, RT PCR analysis was conducted to examine the expression change for the GTS1 gene A. thaliana (Col-0). In the second part of the study, the YE treatment was performed and the expression change of the GTS1 gene was determined via qPCR. The qPCR analysis was carried out using the Roche LightCycler Nano Instrument and the obtained Ct values were evaluated relatively according to the 2^-ΔΔCt method and the expression coefficients were determined. All of the reactions were carried out in a total volume of 25 μl and contained 3 μl of cDNA, 0.5 μl of each primer to a final concentration of 10 μM, and 12.5 μl of SYBR Green mix, up to 25 μl of Nuclease-free water. The PCR conditions were as follows: 50°C for 1 min, followed by 40 amplification cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 30 sec. The actin gene was used as the endogenous control for normalization and the untreated samples were accepted as the control. The primer sequences used in the expression analysis are provided in Table 2.

**Statistical analysis.** The qPCR results and analysis of gene expression (RT-PCR) were statistically evaluated using the one-way ANOVA with post-hoc Tukey’s test and two-way ANOVA via GraphPad Prism® 5.01 computer program. p values <0.05 were considered significant. The experiments were performed with 3 biological and 2 technical repetitions.
RESULTS

Change in GTS1 expression based on stress treatment.

Surface sterilized *A. thaliana* seeds were germinated on MS medium in the plant growth chamber. In the first part of the study, GTS1 gene expression was analysed after 100 mM, 200 mM ABA; 100 mM, 200 mM NaCl and 1 g/l, 2 g/l YE treatments to 10 DAG and 30 DAG Arabidopsis plants after 3 and 6 hour treatments. In order to examine the gene expression, the total RNA was isolated from the ABA, NaCl and YE treated plants. After checking RNA quantity and quality, cDNA was synthesized and used in the RT-PCR analysis. There was no significant change in the GTS1 gene expression for the ABA and NaCl treated samples for the tested concentrations and time periods (Fig. 1). Therefore, based on our results, it was decided to continue to the experiments only with YE treatment for a longer period of time.

The concentrations of all the plant treatments are written above the 1.5% agarose gel images. DAG refers to ‘days after germination’. The actin gene was used as an internal control. Fig. 1 shows that there was no expression change in the ABA and NaCl treated plants in contrast to the YE treated explants belonging to 10 DAG only in 6 hours of treatment.

Phenotypic characterization. It is known that the GTS1 gene participates in biomass accumulation and it negatively regulates growth. It is also associated with ribosome biogenesis, again playing an important role in molecular interactions (GACHOMO et al. 2014). That is why, before further analysing the expression of the GTS1 gene, the

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**Table 1.** The primer sequences used in PCR analysis

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-DNA-LB</td>
<td>5’CCGTCTCACTGGTGAAGAAAGA3’</td>
<td>GACHOMO et al. 2014</td>
</tr>
<tr>
<td>GTS1-R2</td>
<td>5’CTATGGTGGCAGAAGTGAG3’</td>
<td>GACHOMO et al. 2014</td>
</tr>
</tbody>
</table>

**Table 2.** The primer sequences of the genes used in the expression analysis of Col-0 plants.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTS1-F1</td>
<td>5’GAGGAGCTGAGGTTATT3’</td>
<td>GACHOMO et al. 2014</td>
</tr>
<tr>
<td>GTS1-R1</td>
<td>5’CAAGACGGGTATCTGGGTA3’</td>
<td>GACHOMO et al. 2014</td>
</tr>
<tr>
<td>actin-F</td>
<td>5’TGCTGAGCGATGAGC3’</td>
<td></td>
</tr>
<tr>
<td>actin-R</td>
<td>5’GAGGAGCTGAGGTTATT3’</td>
<td></td>
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**Fig. 1.** GTS1 gene expression analysis after ABA, NaCl and YE treatment to *Arabidopsis thaliana* (Col-0) plants for 3 and 6 hour time periods.

**Fig. 2.** The gts1 mutant. (A) The T-DNA insertion in the gene causes an ineffective gene. The actin gene was used as an internal control. (B) Wt and gts1 mutant Arabidopsis plants, showing different phenotypes consistent with the presence and absence of the gene.
effects due to the absence of the gene on the phenotypes are observed. Hence, we planted gts1 mutant Arabidopsis seeds (SALK_010647). As can be seen in Fig. 2b, the gts1 mutant Arabidopsis plants showed better growth. In addition to phenotypic characterization, the T-DNA insertion in the GTS1 gene was also tested. T-DNA was inserted in one of the exon regions of the gene (Fig. 2). Also, GTS1 gene expression was tested in the gts1 mutant and wt Arabidopsis plants and it was shown that there was no expression of the GTS1 gene in the mutant plants according to the primers in Table 2 (Gachomo et al. 2014).

Expression profile of GTS1 gene after YE treatment. Due to RT-PCR results in the first part of the study, the experiment was continued only with the YE treatment which caused a change in the GTS1 gene expression. Accordingly, 10 DAG and 30 DAG Arabidopsis plants were treated with different concentrations of YE. While GTS1 expression was upregulated in both concentrations in the 24 h YE treatment of 10 DAG plants, GTS1 expression was not upregulated in the 48 h YE treatment for both YE concentrations in 10 DAG plants (Fig. 3). Even in the 48 hour treatment of 1 g/l YE GTS1 was down regulated. The plant morphologies are also consistent with the gene expression changes. Plant growth was reduced in accordance with the upregulation of the GTS1 gene. In 30 DAG plants, the 24 hour treatment of both 1 and 2 g/l YE changes almost nothing in the expression of GTS1, however the 48 hour treatment of both 1 and 2 g/l YE upregulates GTS1. The plant morphology was also shown to be consistent with the GTS1 deficiency morphology.

DISCUSSION

There are still many genes associated with plant stress responses yet to be discovered. GTS1 is a recently discovered gene which has been shown to be important in plant growth and development. In this study, we have analysed the expression pattern of the GTS1 gene under biotic and abiotic stress conditions.

Many protein families participate in diverse protein-protein interactions acting as scaffolding proteins (Ekman et al. 2006; Xu & Min 2011; Pesch et al. 2015). Transducin/WD40 repeat proteins belong to one of these families which play central roles in biological processes such as apoptosis, flowering, meristem organization, protein trafficking, signalling, chromatin modifications, cell division and transcriptional mechanisms (van Nocker & Ludwig 2003; Zhu et al. 2004; Suginuma et al. 2008; Zeng et al. 2009; Stirnemann et al. 2010).

WD40 repeat-containing proteins are important for stress tolerance and adaptation in plants. For example, in a study conducted with Triticum aestivum L., the TaWD40D gene was identified and the overexpression of this gene in Arabidopsis was shown to increase tolerance to abscisic acid (ABA) and salt stress (Kong et al. 2015). In another study conducted with rice, it was suggested that for the assembly of the fertility complex, the DUF1620-containing WD40-like repeat protein RFC3 is required (Qin et al. 2016). Zhu et al. (2008) showed that HOS15, a WD40 repeat protein, plays a role in cold stress response through the histone deacetylation mechanism. In a similar study conducted with rice, it was shown that OsRACK1A, a WD40 repeat protein, was regulated by a circadian clock and negatively affects salt tolerance (Zhang et al. 2018).

Gachomo et al. (2014) identified a gene named GTS1, which also contains WD40 repeats. They carried out a comprehensive analysis of the expression profile of GTS1 in various plant tissues as well as a mutational-based phenotypic characterization in Arabidopsis. This gene also regulates growth development in plants. Thus, analyzing its role in plant development and biomass accumulation...
is crucial. GTS1 upregulation negatively affect growth, making it a good candidate for yield improvement. In our study, we wondered whether or not GTS1 was downregulated under stress conditions in Arabidopsis. Hence, in the first part of our study we treated 10 DAG and 30 DAG Arabidopsis explants with 100-200 mM ABA, 100-200 mM NaCl and 1-2 g/l YE for 3 and 6 hours. According to our results, 100-200 mM ABA and 100-200 mM NaCl treatments did not affect GTS1 gene expression in contrast to 1-2 g/l YE. Based on the results from these treatments, we decided to continue with YE treatment for further analysis. YE contains many elements associated with growth including carbohydrates, lipids, proteins, cytokinins, and vitamins, and it has been used for the enhancement of growth and yield in agriculture for many years (Lonhiene et al. 2014). So, it is important to observe its effects under stress conditions in plants. In the second part of our study, we treated 10 DAG and 30 DAG Arabidopsis plants with 1 and 2 g/l YE for 24 and 48 hours. As a consequence, while GTS1 gene expression was upregulated in 10 DAG Arabidopsis plants in the 24 hour treatment, in 30 DAG Arabidopsis plants GTS1 gene expression was upregulated in the 48 hour treatment. Consequently, plant growth was reduced. Similarly, in a study conducted by Gao et al. (2012), it was proven that OsLIS-L1 encoding a lissencephaly type-1-like protein with WD40 repeats is necessary for plant height. Yousef & Ali (2019), reported that the application of YE significantly increased the vegetative growth, yield and fruit quality of tomatoes. Furthermore, in a study conducted with sweet potato plants, where the researchers investigated the effects of YE on growth, yield and water status, the results showed that YE application has positive effects on plant growth and could be used for improving the yield (El-Tohamy et al. 2015). Moreover, Darwesh (2013) claimed that the growth of date palm plantlets under salt stress could be improved via yeast and amino acids applications. YE application has been shown to increase drought tolerance in wheat (Hammad & Ali 2014). Also, Nassar et al. (2016) showed that active YE application to Leucaena plants exposed to salinity induced a significant recovery in the vegetative growth. A study with maize indicated that extracts from carrot roots and yeast have positive impacts on salt stressed plants (Abdel Lalef et al. 2019).

Although many plants have WD40 repeat-containing proteins, unfortunately the exact roles of these proteins remain unclear and have yet to be deciphered. So far, it has been proven that these proteins have many regulatory functions including plant stress response and growth in particular. From this point of view, it seems possible that the genes which encode these proteins can be used for further studies in order to obtain stress tolerant and high yield plants. As a consequence, the downregulation of GTS1 by stress treatment seems to be a good option for obtaining plants with a higher growth rate via GTS1 transcription regulation. According to our results, we observed this only during the application of YE to the Arabidopsis explants but not the whole plants. This could provide insights into potential usage. Further research and a detailed function analysis of the GTS1 gene is required in order to fully understand its relationship with stress response.

Acknowledgements – This research was funded by the TUBITAK grant 2209/A (Research Project Support Programme for Undergraduate Students).

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Hsiao YC, Hsu YF, Chen YC, Chang YL & Wang CS. 2016. A WD40 protein, AtGHS40, negatively modulates abscisic acid degrading and signaling genes during seedling growth under high


Proteini sa WD40 ponavljanjem uključeni su u DNK-protein i protein-protein interakcije i pozitivno regulišu odgovor na stres kod biljaka. GTS1, poznat kao protein sa WD40 ponavljanjem, deluje kao protein skele i važan je u biogenezi ribozoma, a takođe i u akumulaciji biomase. U ovoj studiji smo procenili ekspresiju gena GIGANTUS1 (GTS1) u odgovoru na faktore biotičkog i abiotičkog stresa kod vrste Arabidopsis thaliana. Dodatno, gajili smo i okarakterisali A. thaliana gts1 mutanta (T-DNA SALK_010647) kako bi uočili efekte njegovog odsustva kod biljaka. Prema našim rezultatima, tretman sa 100-200 mM abscisinske kiseline (ABA) i 100-200 mM natrijum-hlorida (NaCl) nije izazvao bilo kakve promene u ekspresiji GTS1 gena, dok nakon samo 6 sati tretman sa 1 g/l and 2 g/l ekstrakta kvasca (YE) negativno utiče na ekspresiju GTS1 kod desetodnevnih biljaka. Nakon 10 i 30 dana tretmana ekstraktom kvasca, ekspresija gena GTS1 je bila regulisana, i kao posledica toga smanjena je efikasnost rasta biljaka. U tom smislu može se zaključiti da bi smanjenjem regulacije GTS1 transkriptata mogli postići bolji rast i / ili veću biomasu i čini se da je to dobra opcija za primenu u poljoprivredi.

**Ključne reči:** GIGANTUS1, Arabidopsis thaliana, ekstrakt kvasca, biotički stres, WD40 ponavljanje