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Regulation of Glutathione under Abiotic Stress in Mutant and Wild Type *Arabidopsis thaliana*

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Abstract: The regulation of the GSH metabolic genes under stress was investigated by studying the mRNA expression pattern for the *GGT* genes in *Arabidopsis* plants grown in liquid culture system. Plants were exposed for 6 h to the following treatments: H_2O_2 (5 mM), salicylic acid S.A. (100 μM), jasmonic acid J.A. (100 μM), 2,4-D (100 μM) and $CdCI_2$ (100 μM). *GGT1* messages were found to be upregulated under H_2O_2, S.A, 2,4-D and $CdC1_2$. Meanwhile, GGT3/mRNAs were induced only under jasmonic acid with no further induction under the other stress treatments. In contrast, the *GGT2/mRNAs* were not greatly affected by different stress treatments, however, it showed some minor induction under S.A. and 2,4-D treatments. The investigation of the involvement of *GGT1* gene in stress tolerance by following the behavior of *ggtl* mutants along with their characteristic phenotype under different stress conditions revealed that, the highly stressful conditions of light intensity (200 and 400 μmol $m^{-2}s^{-1}$) or temperature treatments (14 and 25°C) enhanced the GGT1 in the mutant phenotype above or below the optimal degree, rather than the combinations between them. This enhancement is recognized through the wider distribution of the chlorotic regions on the *ggtl* mutant leaves, the reduction in rosette diameter and the early blot pattern, rather than the reduction in the total weight of dry seeds/plant. In addition, the mutants responded more significantly to the high concentrations of 2,4-D (100, 200 and 400 μM) by enhancing the mutant phenotype.

Key words: *Arabidopsis thaliana*, stresses, metabolic genes

Introduction

Glutathione (GSH) can substantially protect plants from either biotic or abiotic stresses. Under biotic stresses GSH showed strong induction and rapid accumulation (Edwards *et al*., 1991; Vanacker *et al*., 2000). For example, under pathogen attack plants generate a cascade of inducible defense systems. This includes the oxidation burst, the synthesis of phytoalexins, the induction of hydrolytic enzymes, such as GSTs and glutathione peroxidases (GPXs), as well as the upregulation of GSH biosynthesis (Apostol *et al*., 1989; Lamb *et al*., 1989; Levine *et al*., 1994; Jabs *et al*., 1996; O'Brien *et al*., 1998). GSH acts as a potential systemic messenger to carry the resistance signal concerning the attack to the unchallenged plant tissues. The increase of GSH during pathogen attack helps to protect cells from excessive damage caused by the accumulation of the Reactive Oxygen Species (ROS) that result during the oxidative burst (May *et al*., 1996).

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Under abiotic stresses, GSH is one of the antioxidative defense systems that are widely distributed in all sub-cellular compartments (Rose and Bode, 1993; Nishikimi and Yagi, 1996). The most relevant function of GSH is the involvement in the ascorbate-GSH cycle. GSH is used as a source of reducing equivalents for the ascorbate GSH cycle by transferring reducing equivalents from NADPH to GSH and subsequently to ascorbate. The important role of this system in protecting plants from the ROS, such as hydrogen peroxide (H_2O_2), superoxide (O_2) and hydroxyl (OH) radicals is that result in the oxidative damage (Larson, 1988; Alscher, 1989; Foyer *et al*., 1994; Noctor *et al*., 1998a; Asada, 1999).

In the ascorbate GSH cycle, GSH serves as a reductant for recycling dehydroascorbate. The resulting ascorbate molecule is involved in reducing O_2 and generating tocopherol (Asada and Takashi, 1987). Furthermore, it serves as a substrate in the enzyme-catalyzed reaction for the detoxification of H_2O_2 (Groden and Beck, 1979; Hossain and Asada, 1984) and for the conversion of violaxathin to zeaxanthin in the xanthophylls cycle (Yainamoto *et al*., 1972). In these reactions, ascorbate is oxidized to the monodehydroascorbate radical. In chloroplasts, the monodehydroascorbate radicals can be recycled by photosynthetic activity via ferredoxin (Miyake and Asada, 1992, 1994) or by monodehydroascorbate radical reductase activity with the consumption of NADH or NADPH (Hossain *et al*., 1984; Borrachino *et al*., 1986), or they can disproportionate spontaneously, yielding ascorbate and dehydroascorbate.

In response to different abiotic stresses, GSH biosynthesis and accumulation were enhanced (Xiang and Oliver, 1998; Foyer and Noctor, 2001). In catalase-deficient mutants, dramatic increases in leaf GSH are observed under high light when H_2O_2 is generated via photorespiration. Moreover, under the hypersensitive stress response and heat shock, GSH has been shown to stimulate the transcription of various genes, including those encoding cell wall hydroxyproline-rich glycoproteins, phenyl of anine-ammonia lyase and chalcone synthase (Dron *et al*. 1988). In this study we report the effect of various abiotic stress treatments by different chemicals as well as heat and light on the regulation of glutathione in wild and mutant lines of *Arabidopsis thaliana.*

Materials and Methods

Plant Material and Stress Treatments by Chemical Agents

Two hundred mg of seeds of wild type ecotype Lansberg and the *ggt1* mutant were sterilized in 2 mL of 50% bleach with 20 µL of 10% triton with shaking for 15 min. The bleach/triton mixture was removed and seeds were extensively rinsed with sterile water for 4-5 times. The seeds were grown up in 50 mL of ½ strength Murashige and Skoog medium (2.2 g of M.S. powder, 1 mL of B5 vitamins, 20 g sucrose and 0.5 g of 2,4 morpholino ethane sulfonic acid powder [MES] dissolved in 1 L) under controlled growth conditions of 22°C and 24 h of white fluorescent illumination of 50 µmol $m^{-2}s^{-1}$. Two weeks old seedlings were exposed for 6 h to the following provide ref of this stresses: 5 mM hydrogen peroxide, H_2O_2, 100 µM of salicylic acid S.A., 100 µM of jasmonic acid, J.A. 100 µM of 2,4 Dichlorophenoxy-acetic acid (2,4-D), 100 µM of cadmium chloride CdCl. Plant tissues were briefly rinsed with water and stored at -80°C in aluminum foil till use for RNA analysis.

RNA Extraction, Separation and Blotting

Total RNA was isolated from the frozen tissues as described by Kirk and Kirk (1985) and Dron *et al*. (1988). Hybridization was performed using P^{32} radiolabelled cDNA probes for the specific selected genes that include GST6, GSH1, GGT1, GGT2 and GGT3. The washing conditions and exposure time were differed according to the messages signal strength.

Selection of gg1 Mutant Lines Subjected to Stress of Different Light and Temperature Treatments

Five to ten seeds of either *Arabidopsis thaliana* wild type ecotype Lansberg or *ggt1.1* mutant line and *ggt1.3* mutant lines were cultivated in individual soil pots (8.5×8.5 cm). The seeds were incubated for two days at 4°C and then transferred to the *Arabidopsis* growth chamber to grow for two-weeks under optimal growth conditions at constant temperature of 21 °C and continuous light intensity of 75 µmol $m^{-2}s^{-1}$. After the former two primary leaves have emerged, the seedlings were carefully thinned to two-three uniform healthy seedlings/pot. These seedlings that have not showed the mutation phenotype yet, were transferred to different controlled growth chambers. Five pots treatment for each line was arranged in one tray in Latin Squares to allow homogenous exposure of light. Three different temperature treatments of 14, 21 and 25°C were applied respectively. For each temperature treatment, three light intensities were processed, by adjusting the full light intensity of the growth chamber at 400 µmol $m^{-2}s^{-1}$ and applying shading conditions with several layers of cheese cloth to obtain 100, 200 µmol $m^{-2}s^{-1}$ light intensities.

The plants were examined over the next three weeks. An arbitrary method was created to determine the chlorotic symptoms in the leaves, whereas, the green leaves were represented by five and the yellow leaves by zero. The chlorosis was detected in the cotelydonary leaves, 1st, 2nd and 3rd primary leaves for each line plants. In addition, ten replicates of the four weeks old plants were used to measure the rosette leaves diameter and the inflorescent height. Two weeks later, the dry seeds were collected from each individual plant and weighed to represent the line yield.

Results and Discussion

Exposure of *Arabidopsis* plants to H_2O_2, 2,4-D and salicylic acid did not increase the mRNA levels of *GSH1, GSH2* or *GR1*. In contrast, J.A increased the mRNA level of the three investigated metabolic genes. These data suggest that J.A has a role in the defense against abiotic stresses by increasing expression of GSH metabolic genes. Although the transcript levels for all the genes involved in GSH metabolism were increased by J.A. treatment, GSH content did not increase. these data suggest that, myriad pathways control the cellular GSH concentration. To obtain some insights about the transcriptional control of the enzymes involved in the glutathione metabolic reactions under stress conditions a northern analysis was performed. The blot was hybridized with the individual cDNA probes of *GGT1*, *GGT2* and *GGT3*. To reveal the signal of each gene, the auto radiograms were exposed for various times. For *GGT1* and *GGT3* genes, the blots have been exposed for 15-17 h. However, for *GGT2* gene, a one-week exposure period was required. The expression patterns of *GGT1, GGT2* and *GGT3*/mRNAs are shown in Fig. 1.

To reveal the effect of stresses on the expression level of other genes involved in the GSH metabolic pathways. The cDNA probes of Glutathione-S-transferase (*GST6*) and γ-glutamyl cysteine synthetase (*GSH1*) were used to hybridize the same RNA samples in 2-weeks old seedlings grown in liquid culture and treated with the same stresses for 6 h. For *GST6* gene the signal was strongly detected on the autoradiogram after 30 min. The gene was highly induced under all treatments in both the wild type plants and the *ggt1* mutants. This indicated a wide distribution of *GST6* in *Arabidopsis* plants and an active role of this gene in stress tolerance. In addition, *GSH1* signal was mainly recognized under Jasmonic acid treatment. Data shown in Fig. 2.

The treatment of *Arabidopsis* plants with Cd^{+2} resulted in increasing the transcriptional levels of the genes encoding the GSH biosynthesis enzymes, for example, γ-ECS, GS and GR. The response was specific for Cd^{+2} as they mediate the PCS scavenging pathways. Willekens *et al*. (1997) working

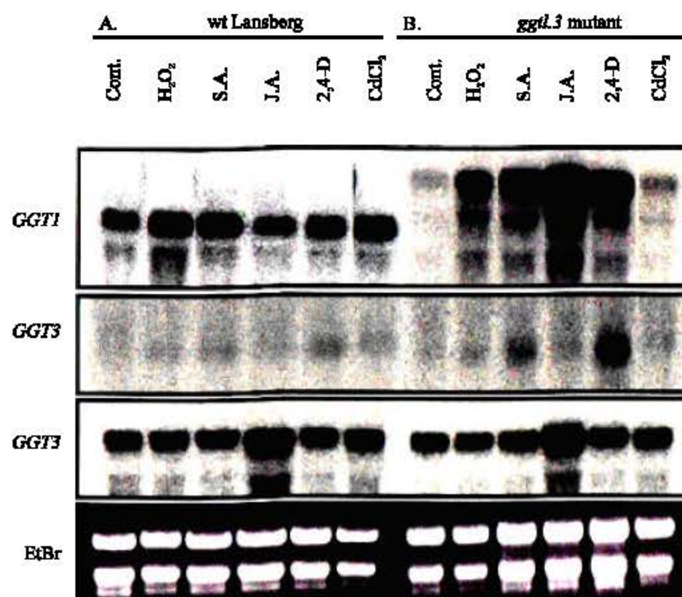

Fig. 1: Stress-dependent mRNA expression pattern of *GGT1, GGT2* and *GGT3 genes* in *Arabidopsis thaliana* plants of : A. The wild type ecotype Lansberg. B. The *ggtl* mutants. Plants were grown for two weeks in the liquid culter system and exposed for 6 h to the following treatments: Hydrogen peroxide H_2O_2 5 mM; Salicylic acid S.A., 100 μM; Jasmonic acid J.A., 100 μM; 2,4-Dichlorophenoxy-acetic acid 2,4-D, 100 μM; Cadmium chloride $CdCl_2$ 100 μM. Ten microgram of the denatured RNA were separated on 1.2% formaldehyde agarose gel. The ethidium bromide staining of the gel is shown for equal loading. The blot was hybridized with the radioactive cDNA probes of *GGT1*, *GGT2* and *GGT3* genes

on catalyses deficient mutants suggested that, GSH synthesis is driven by the increased demand for GSH in response to the oxidative stress and GSH conversion to PCS.

Jasmonic Acid (JA) also activated the transcription of the same genes suggesting that the molecules were involved in the signal transduction pathway for the phytochelatins synthesis. Moreover, the response of these genes to heavy metals and J.A was coordinately processed. Jasmonate acts as a stress hormone and plays a role in plant growth and development (Parthier, 1990, 1991; Creelman and Mullet, 1997). It was found that jasmonate induces the expression of jasmonate-induced proteins (JIP) in plant tissues treated with jasmonate (Weidhase *et al*, 1987a, b; Muller-uri *et al*., 1988), most of these proteins, are stress proteins or proteins that protect and defend plants under stress. Cadmium induces the synthesis of PCS that bind metals in the cytosol and sequester them in the vacuole (Rauser, 1999; Mehra and Tripathi, 2000).

Glutathione, the phytochelatin precursor, was found to decrease after Cd^{2+} exposure (Rauser, 1999; Zenk, 1996). Some genetic studies have confirmed that GSH-deficient mutants of *Arabidopsis* that lacked the γ-ECS activity were also PCS deficient and hypersensitive to Cd^{2+} (Cobbett *et al*., 1998). Moreover, Yong *et al*. (1999) and Zhu *et al*. (1999a, b) proposed that the regulation of GSH biosynthesis is an endogenous mechanism, which PCS might modulate its

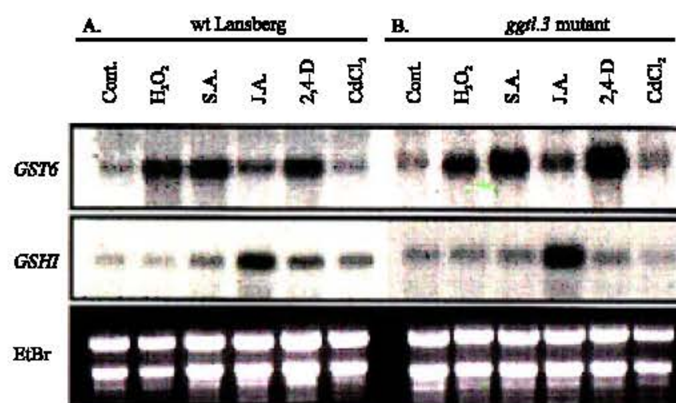

Fig. 2: Stress-dependent mRNA expression pattern of *GST6* and *GSH1 genes* in *Arabidopsis thaliana* plants of : A. The wild type ecotype Lansberg. B. The *ggtl* mutants. Plants were grown for two weeks in the liquid culter system and exposed for 6 h to the following treatments: Hydrogen peroxide H_2O_2 5 mM; Salicylic acid S.A., 100 μM; Jasmonic acid J.A., 100 μM; 2,4-Dichlorophenoxy-acetic acid 2,4-D, 100 μM; Cadmium chloride $CdCl_2$ 100 μM. Ten microgram of the denatured RNA were separated on 1.2% formaldehyde agarose gel. The ethidium bromide staining of the gel is shown for equal loading. The blot was hybridized with the radioactive cDNA probes of *GST6* and *GSH1* genes

expression. Their studies on *Brassica juncea* that over expressed γ-ECS revealed that the PCS biosynthesis and Cd^{2+} tolerance were also increased. Also, exposure of the wild type *Brassica juncea* to Cd^{2+} was found to increase the level of GS transcripts (Schäfer *et al.*, 1998). Scützendübel *et al.* (2001) have demonstrated that cells challenged by Cd^{2+} at concentrations exceeding the detoxification capacity, resulted in H_2O_2 accumulation because of imbalance of the redox system.

On the other hand, salicylic acid, which is a plant phenolic hormone, plays a crucial role in stress resistance in plants (Durner *et al.*, 1997; Alvarez, 2000) and is endogenously required to activate the transcription of defense genes (Gaffney *et al.*, 1993; Delaney *et al.*, 1994; Andersson *et al.*, 1998; Chamnongpol *et al.*, 1998). One of the major inducible defense genes under salicylic acid treatment is the glutathione-S-transferase GST (Edwards *et al.*, 2000).

In plants, it has been postulated that a common effect of all the stresses treatments is the generation of ROS produced during the oxidative stress (Levine *et al.*, 1994; Tenhaken *et al.*, 1995; Ulmasov *et al.*, 1994, 1995). In *Arabidopsis* protoplast treated by H_2O_2, the expression of genes involved in defense against oxidative stress was induced and that of genes involved in plant growth was suppressed (Jasmieson and Storz, 1997; Kovtun *et al.*, 2000). H_2O_2 plays vital role as a signal molecule during the Hypersensitive Response (HR) (Lamb and Dixon, 1997; Grant and Loake, 2000). May and Leaver (1993) have demonstrated that exogenously applied and endogenously produced H_2O_2increase GSH concentrations. Also, Xiang and Oliver (1998) recorded the same observation in *Arabidopsis* liquid culture treated with 5 μM H_2O_2, which support the role of GSH to protect cells from oxidative damage. However, they demonstrated that treatment of *Arabidopsis* liquid culture with exogenous H_2O_2 up to 20 μM for one hour failed to induce the accumulation of GSH metabolic gene transcripts.

Table 1: The illustration of the enhancement of different criteria of the *ggt1* mutant phenotype under the stress of light and temperature treatments

	Temperature					
	25°C			21°C		
	Rosette diameter		t-test	Rosette diameter		t-test
Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	wt. Lansberg	*ggt1.3*	wtvs *ggt1.3*	wt lansberg	*ggt1.3*	wtvs *ggt1.3*
100	10.05±1.02	8.56±0.81*	0.0022	5.10±0.32	5.08±0.08	0.1124
200	10.50±0.87	8.79±0.82*	0.0008	6.03±0.23	5.48±0.15*	0.0008
400	9.54±0.97	8.12±0.95*	0.004	5.55±0.28	4.97±0.26*	0.0039
	Inflorescent height		t-test	Infloresent height	t-test	
100	11.38±0.92	11.05±0.83*	0	4.89±0.63	5.77±1.67	0.3211
200	12.94±1.04	13.32±1.16*	0	2.47±0.95	5.73±0.94*	0.0001
400	12.82±1.46	14.33±0.92*	0	4.70±0.66	11.28±0.56*	0
	Yield		t-test	Yield		t-test
100	0.15±0.04	0.14±0.04	0.6133	017±0.05	0.12±0.04	0.1096
200	0.14±0.03	0.12±0.04	0.228	0.18±0.06	0.15±0.06	0.4887
400	0.19±0.04	0.12±0.05*	0.0253	0.19±0.03	0.10±0.01*	0.0016

Table 1: Continued

	14°C		
	Rosette diameter		t-test
Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	wt. Lansberg	*ggt1.3*	wtvs *ggt1.3*
100	5.00±0.11	4.90±0.06*	0.0003
200	5.78±0.18	5.22±0.17*	0.0003
400	4.43±0.24	3.85±0.10*	0.0011
	Inflorescent height		t-test
100	2.78±0.45	3.98±0.53*	0
200	3.35±1.01	6.0±0.60*	0.0005
400	3.37±0.55	6.73±0.24*	0
	Yield		t-test
100	0.12±0.02	0.12±0.02	0.7171
200	0.11±0.03	0.11±0.03	0.8132
400	0.12±0.05	0.18±0.02	0.2629

Means with * are significantly different from the control wild type plants, $p>0.05$ indicates insignificant difference, $p = 0.05$ indicates significant difference

In soybean, H_2O_2 induced the expression of the defense-related gene, glutathione-S-transferases (GST) and Glutathione Peroxidase (GPX) (Levine *et al*., 1994). In *Arabidopsis* suspension cultures, H_2O_2 induced the expression of *GST* that comprises a family of enzymes involved in cellular detoxification process following oxidative stresses, Xiang and Oliver (1998). Also, H_2O_2 was found to induce the expression of genes encoding proteins required for peroxisome biogenesis Lopez-Huertas *et al*., 2000). Several gene sets were upregulated under H_2O_2 treatment. This includes the genes encode for a senescence related protein, a protein kinase and a DNA repair protein (Lopez-Huertas *et al*., 2000).

Numerous compounds can induce the regulation of *GSTs* in plants. These include the strong auxins like 2,4-D and salicylic acid, various electrophilic substrates and heavy metals. It has been postulated that the induced *GSTs* respond to oxidative stress to protect cellular compartments from damage (Levine *et al*., 1994; Tenhaken *et al*., 1995; Ulmasov *et al*., 1994, 1995). It has been also found that the plant GST gene family is the only plant gene that contains the octopine synthetase (OCS) elements in their proniotor. The OCS elements have been identified to confer inducibility by strong auxin, salicylic acid as well as cadmium, glutathione, hydrogen peroxide and methyl jasmonate

Table 2: Estimation of chlorosis on the 2nd rosette leaf of the *ggtl* mutants as compared to the wild type ecotype lansberg under light intensities of 400, 200 and 100 µmol m^{-2} s^{-1} at 14, 21 and 25°C at different days. The chlorosis was arbitrary detected from the 26th to 36th days. Five represents the full green leaf and zero represents the complete yellow leaf

		Light intensities					
		400 µmol m^{-2} s^{-1}		t-test	200 µmol m^{-2} s^{-1}		t-test
Temperature	Days	wt	*ggt1.3*	wtvs *ggt1.3*	wt	*ggt1.3*	wtvs *ggt1.3*
14°C	29th	5.00±0.00	4.50±0.00	0.000	5.00±0.00	4.50±0.00	0.000
	32th	3.39±0.22	2.00±0.71	0.027	3.57±0.35	2.69±0.37	0.19
	35th	2.94±0.30	1.13±0.85	0.021	3.29±0.27	1.94±0.56	0.016
	40th	2.28±0.26	0.63±0.75	0.019	2.64±0.24	1.44±0.78	0.035
	43th	1.89±0.42	0±0.00	0.000	2.36±0.24	1.13±0.58	0.014
21°C	26th	4.85±0.24	4.75±0.35	0.471	4.95±0.16	4.67±0.43	0.094
	29th	4.67±0.35	4.45±0.69	0.395	4.78±0.36	4.10±0.42	0.002
	33th	2.83±0.43	2.75±0.89	0.796	3.33±0.43	2.50±1.17	0.073
	36th	1.56±0.46	1.00±0.67	0.049	2.44±0.53	0.95±0.98	0.002
25°C	26th	5.00±0.00	4.20±0.75	0.021	5.00±0.00	4.61±0.55	0.065
	27th	4.72±0.36	3.60±0.78	0.002	4.85±0.24	4.22±0.62	0.020
	28th	4.13±0.35	3.11±0.89	0.010	4.30±0.26	3.27±0.26	0.000
	33th	3.19±0.59	1.05±0.63	0.000	3.94±0.17	2.16±0.25	0.000
	36th	2.88±0.74	0.55±0.53	0.000	3.67±0.25	1.22±0.26	0.000

Table 2: Continued

		Light intensities		
		100 µmol m^{-2} s^{-1}		t-test
Temperature	Days	wt	*ggt1.3*	wtvs *ggt1.3*
14°C	29th	5.00±0.00	4.60±0.00	0.000
	32th	3.22±0.36	2.72±0.36	0.887
	35th	3.06±0.30	2.00±0.61	0.047
	40th	2.83±0.25	1.67±0.56	0.027
	43th	2.56±0.17	1.17±0.56	0.031
21°C	26th	4.94±0.17	4.65±0.47	0.092
	29th	4.63±0.44	4.00±0.537	0.015
	33th	3.38±0.23	2.95±0.50	0.032
	36th	3.19±0.37	2.00±0.62	0.000
25°C	26th	5.00±0.00	4.71±0.39	0.103
	27th	4.95±0.16	4.50±0.50	0.058
	28th	4.45±0.16	3.42±0.35	0.000
	33th	4.15±0.14	3.08±0.49	0.000
	36th	3.70±0.35	2.58±0.49	0.000

Means with * are significantly different from the control wild type plants, $p>0.05$ indicates insignificant difference, $p = 0.05$ indicates significant difference.

(Ulmasov *et al*., 1994, 1995). In the present study, the disappearance of the *ggt1* mutant phenotype under the non-stressfull conditions on the MBS-agar media leads to correlate the mutation phenotype with the stress conditions. To investigate the involvement of *GGT1* gene in stress tolerance, the behavior of *ggt1* mutants along with their characteristic phenotype was followed under different stress conditions. To investigate that GGT1 gene is functionally involved in stress tolerance, the ggt1 mutant lines were stressed under various combinations of light and temperature treatments.

The mutant plants were exposed to various combinations of light intensities (100, 200 and 400 µmol $m^{-2}s^{-1}$) and temperatures degrees 14, 21 and 25°C) in the controlled growth chambers. The results showed that, in general, the high stressful conditions of light intensity (200 and

400 μmol $m^{-2}s^{-1}$) or the temperature treatments (14 and 25°C) occurred above or below the optimal degree (21°C), rather than the combinations between them, showed an enhancement in the *ggt1* mutation phenotype. The chlorotic regions in the *ggt1* mutant leaves revealed a wider distribution under the high levels of light intensity at 200 and 400 μmol $m^{-2}s^{-1}$) and the temperature degrees of 14 and 25°C (Table 1). The yellowing phenotype was more significantly detected under the highest level of treatments. In addition, the more the stress applied, the more the tendency of the mutants to show the chlorosis pattern on the leaves. For example, under a combination of 25°C and 400 μmol $m^{-2}s^{-1}$, the *ggt1* mutant's rosette leaves were totally tuned yellow (i.e showed zero value in the arbitrary method for screening ch!orosis) by the day 36. However, at the same timing under 21°C (optimal temperature) and 400 μmol $m^{-2}s^{-1}$, the *gg1* mutant leaves did not show a complete discoloration.

Other criteria that were distinguished for the mutation phenotype, including the reduction in rosette diameter and the early blot pattern were greatly enhanced under the extreme stressful treatments. The ggt1 mutants reduced their leaves area up to 15% under the light intensities (200 and 400 μmol $m^{-2}s^{-1}$). Moreover, under such levels of light intensity, the mutants greatly accelerated the flowering process. The *ggt1* mutant inflorescence height recorded a major increase (up to 2.5 fold) more than the wild type plants. Further phenotypic patterns that were revealed for the mutant under higher treatments of light and temperature include reduction in the total weight of dry seeds $plant^{-1}$. Similarly, the *ggt1* mutants reported much significant reduction in the yield under the extreme levels of the investigated treatments (25°C and 400 μmol $m^{-2}s^{-1}$) (Table 2). Light was characterized to be a major source of stress damage in the plant cells. The effect of light stress is expressed as a decrease in the photosynthetic capacity and plant growth. However, the major target of light stress in green plant tissues is the chloroplasts (Barber and Andersson, 1992; Prasil *et al.*, 1992; Aro *et al.*, 1993; Polle, 1997). Other cellular compartments can be affected as a result of photo oxidative damage. Under excess light, photo system II involve over production of O_2 in the chloroplasts. This can result in the release of reactive oxygen species ROS , like superoxide radicals. $O\text{-}_2$ hydroxyl radicals. OH-, hydrogen peroxide H_2O_2 or singlet oxygen $1O_2$. This leads to imbalance in the redox homeostasis in the cells and eventually the photooxidative damage (Asada, 1999). Plants maintain their functions under light stress conditions by developing various protection systems. Enzymes, such as superoxide dismutase, ascorbate peroxidase, catalaze, glutathione-S-transferase, peroxidase. In addition to various non-enzymatic molecules such as carotinoids, α-tocopherol, all can be involved in ROS detoxification in plants (Bohnert and Sheveleva, 1998; Russel *et al.*, 1995; Niyogi, 1999). Exposure to excess light results in the photoinhibition of the photosynthetic apparatus (Andresson and Styring, 1991), a mechanism that leads to an inhibition of the electron transport through PSII.

The effects of ROS can be the oxidation of lipids, proteins and chloroplasts enzymes (Foyer *et al.*, 1994). Vallelian-Bindschedler *et al.* (1998) showed that plants exposed to short heat pulses could induce such bursts of $O\text{-}_2$ and H_2O_2. On the other hand, low temperatures and high light can cause photooxidation, a light and oxygen bleaching (Halliwell, 1984) in the leaves, in addition to chlorosis (Van-Hasselt, 1972). Other symptoms of chilling injury in the light include the rapid dysfunction of photosynthesis (Van-Hasselt and Van-Berlo, 1980; Powles *et al.*, 1983), altered chloroplast ultra structure (Wise *et at.*, 1983) and result in cellular lipid degradation (Van-Hasselt, 1974; Dekok and Kuiper, 1977).

In plants, GSH is an important route for stress tolerance. GSH has multiple roles in plant defense mechanisms against both abiotic and biotic stresses (Foyer *et al.*, 1997).. GSH is also a major route of H_2O_2 destruction in plants. Glutathione peroxidases are induced in plants in response to stress (Eshdat *et al.*, 1997). These enzymes are involved in the detoxification of lipid peroxides rather than

hydrogen peroxide. In plants, the major substrate for reductive detoxification of H_2O_2 is ascorbate, which must therefore be continuously regenerated from its oxidized forms. A major function of glutathione in protection against oxidative stress is the re-reduction of ascorbate in the ascorbate-glutathione cycle (Foyer and Halliwell, 1976; Nakano and Asada, 1980). In this pathway, glutathione acts as a recycled intermediate in the reduction of H_2O_2 using electron derived, ultimately from H_2O. Efficient recycling of glutathione is ensured by GR activity. The components of this cycle exist in both chloroplast and cytosol (Foyer, 1993; Foyer *et al*., 1995). The phenotypic changes were more enhanced with the higher concentrations of 2,4-D (600, 800 and 1000 μM).

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