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Exogenous GABA Stimulates Endogenous GABA and Phenolic Acid Contents in Tomato Plants under Salt Stress

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Abstract

Gamma aminobutyric acid (GABA) is a non protein amino acid found in various organisms including plants. In recent years, the signal role of GABA in the stress response has of special interest. However, the effects of exogenous GABA on phenolic compounds which have special roles as antioxidants are not well known. In this study, the effects of exogenous GABA on endogenous GABA and phenolic contents were analyzed in tomato plants under salt stress. Tomato plants were grown in a growth chamber under controlled conditions and NaCl and GABA were applied in Hoagland solution. Qualitative and quantitative analysis of GABA and phenolic compounds (Benzoic, caffeic, chlorogenic, gallic, hydroxybenzoic, syringic, rosmarinic, p-coumaric, sinapic, t-cinnamic, t-ferulic acids, catechin, epicatechin, hesperidin and quercetin) were measured by HPLC. Differences were found in chlorogenic acid, coumaric acid and gallic acid among the phenolic substances. We found a significant increase in gallic acid and coumaric acid contents under 200 mM NaCl and GABA applications. Exogenous GABA treatment caused a slight increase in endogenous GABA content. The increase in GABA content under GABA+salt treatments were higher than that of single salt and GABA applications. According to our results we can suggest that exogenous GABA could enhance the stress response by enhancing some phenolic substances and GABA content under salt stress.
Keywords: Gamma-aminobutyric acid, HPLC, Salinity, *Solanum lycopersicon*.

1. Introduction

Gamma aminobutyric acid (GABA) is an important non protein amino acid that is found in various organisms including plants. In animals, GABA has an important role as an inhibitor neurotransmitter. Moreover, in plants it has special impacts on plant metabolism such as pH regulation, and C- N balance [1-3].

In the GABA shunt, α-ketoglutarate is converted to glutamate by the activity of glutamate dehydrogenase (GDH, EC 1.4.1.4). Then GABA is occurred from glutamate by the activity of glutamate decarboxylase (GAD, EC 4.1.1.15). In addition, GABA can be synthesized via polyamine degradation. Abiotic and biotic stresses can cause cytosolic acidification and low pH activates glutamate decarboxylase and GABA can be accumulated rapidly in various parts of plants [3]. Also environmental stress factors can increase cytosolic Ca^{2+} which activates calmodulin-dependent glutamate decarboxylase and GABA synthesis [1,3]. Moreover, in recent years GABA is defined as a signal molecule [4,5].

In previous studies, it has been reported that exogenous application of GABA could alleviate the deleterious effects of stress conditions. Exogenous GABA could stimulate polyamine biosynthesis and degradation under stress [6]. It was also reported that GABA could participate in regulating of gene expression as a signal molecule under stress conditions [7].

Phenolic compounds have also important roles in the defense mechanism of higher plants. Their biosynthesis is often enhanced by various environmental stresses, such as salinity. Moreover, they can act as potential antioxidants by eliminating the deleterious effects of ROS induced by salt stress [8]. However, the impact of exogenous GABA treatments on phenolic compounds in tomato plants under stress conditions is not well known. In this study, our aim was to determine the effects of exogenous GABA on the contents of phenolic substances and GABA in tomato plants under salt stress.

2.Materials and Methods

2.1 Growth conditions and stress treatment

The seeds of tomato plants *Solanum lycopersicum* L. cv. H-2274 were provided from Anatolia Agricultural

Research Institute in Eskisehir, Turkey. First, the seeds were surface sterilized in 5% NaOCl and washed thoroughly with sterile water and then germinated on perlite in a growth chamber under 26°C/22°C, 16⁄8 h light⁄dark, with a relative humidity of 65% and a light intensity of 175 µmol m^{-2} s^{-1}. The seedlings were watered regularly with ½ Hoagland solution (Hoagland, Sigma H2395). After 4 weeks 0, 100, 200 mM NaCl and/or 0.5 mM GABA were applied in Hoagland solution for one week. At the harvest, the leaves were frozen in liquid nitrogen and stored at -80 ºC until analyses.

2.2 GABA analysis

GABA analysis was performed with HPLC (Agilent 1200). 0.2 g of leaf samples were homogenized in 5 mL solution of water:chloroform:methanol (3:5:12). The samples were then centrifuged for 5 min at 10,000 g at 4 ºC, and the supernatant was dried. In order to derivatizate with 2-hydroxynaphthaldehyde (HN), the samples were dissolved in 100 µL of ultrapure water and 150 µL of Borax buffer (pH 8) and 250 µL of HN (0.3%). The mixture was kept at 80 ºC for 30 min and then cooled at room temperature. 1 mL of methanol was added to the samples. Samples were separated by reversed-phase column Supelco LC18 (250x4.6 mm, 5 µm) with the injection volume of 5 µL, flow rate of 1 mL min^{-1}, and mobile phase of methanol:water (62:38). The analysis was carried out at 330 nm wavelength. The retention time was 12 min. The peak areas were compared with the GABA standards and calculated for determining of the GABA content [9].

2.3 Phenolic compound analysis

Qualitative and quantitative analyses of phenolic compounds were analyzed by HPLC (Agilent 1200). 0.1 g of leaf samples were homogenized in methanol (HPLC grade), and centrifuged at 10,000 g for 10 min. The samples were filtered with 0.45 µm filters and separated by reversed-phase column Supelco LC18 (250x4.6 mm 2,5 µm) with an injection volume of 20 µL and flow rate of 0.8 mL min^{-1} at 278 nm. 2% of acetic acid and methanol were used as mobile phase and applied gradiently as described by Caponio et al. [10]. Each sample was analyzed for 90 min. Quantifications were done by comparing the peak areas with phenolic compounds standards (Benzoic, caffeic, chlorogenic, gallic, hydroxybenzoic, syringic, rosmarinic, p-coumaric, sinapic, t-cinnamic, t-ferulic acids, catechin, epicatechin, hesperidin and quercetin).

2.4 Statistical analysis

The effects of exogenous GABA application were determined using one-way variance analysis ANOVA. The applications were compared by least significant difference (LSD) test at $p<0.05$. The spread of values was shown in the figures as standard errors of the means.

3. Results and Discussion

Gamma aminobutyric acid is an important metabolite that has essential roles in the primary metabolism of plants [4,11]. In recent years, GABA is mentioned as a signal molecule under various stress conditions. Accumulation of GABA in stressed tissues may activate the regulation of the expression of genes that have roles in the detoxification of ROS [7,12]. Therefore, GABA could help to maintain the cells from oxidative damage and enhance the stress response in plants [13].

Recent studies have mentioned that endogenous GABA level can be increased by exogenous GABA application depending on the GABA concentration [14,15]. In a previous study, GABA treatment caused a remarkable increase in endogenous GABA concentration in the roots of melon plants under both normal and hypoxia stress conditions. The highest increase was reported in stress+GABA application. We are in agreement with these findings with an increase in endogenous GABA concentration by GABA treatment under both stressed and non-stressed conditions (Figure 1). This increase could help to induce defense mechanism against stress conditions in tomato plants.

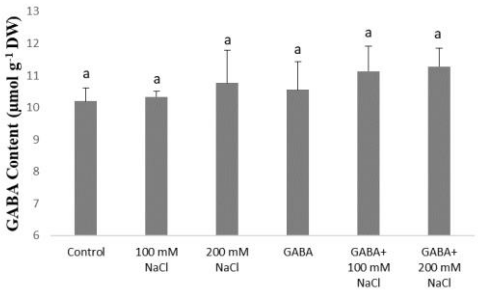

Figure 1. The effects of exogenous GABA application and salt stress on endogenous GABA content in the leaves of *S. lycopersicon*. The bars represent the means ± S.E ($p < 0.05$).

Parallel to our study, endogenous GABA level was induced by NaCl stress, however the application of GABA under salt stress increased endogenous GABA concentration more than single salt treatment [7]. Wang et al. [6] also reported that the increase in GABA concentration could help to inhibit the deleterious effects of stress by maintaining cellular pH and promoting of the TCA cycle. In melon plants, GABA application was mentioned to eliminate the deleterious effects of hypoxia stress by enhancing the polyamine biosynthesis and prevents the degradation of polyamines which have important roles under stress conditions [6]. Alqarawi et al. [16] reported that GABA treatment increased antioxidant enzymes activities and could protect the cells against the deleterious effects of salt stress by maintaining of the hormones and mineral nutrients and by reducing lipid peroxidation. Another important impact of GABA was its preventing role in

the increase in sodium and chloride ion levels under salt stress.

In addition, in the postharvest process GABA application can have positive effects on preserving the quality of the fruits. Sheng et al. [15] reported that exogenous GABA could prevent the decrease of organic acids during postharvest storage and suggested GABA treatment for enhancing the postharvest quality. GABA is also suggested as an important supplement for increasing of the fruit resistance against adverse conditions. In addition, GABA could help to increase the flavor and the quality of the fruits during postharvest. Therefore, the effects of exogenous GABA on plant physiology should be well evaluated for enhancing crop yield and quality.

In our study, we also determined the effects of GABA on phenolic substances in tomato plants under salt stress. Qualitative and quantitative analyses of 15 phenolic compounds (Benzoic, caffeic, chlorogenic, gallic, hydroxybenzoic, syringic, rosmarinic, p-coumaric, sinapic, t-cinnamic, t-ferulic acids, catechin, epicatechin, hesperidin and quercetin) were measured by HPLC. Under these conditions we found differences in chlorogenic acid, coumaric acid and gallic acid among the phenolic compounds (Figure 2, 3, 4).

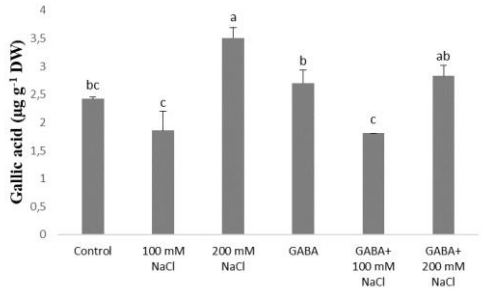

Figure 2. The effects of exogenous GABA application and salt stress on gallic acid content in the leaves of *S. lycopersicon*. The bars represent the means ± S.E. Means with different letters are significantly different ($p < 0.05$).

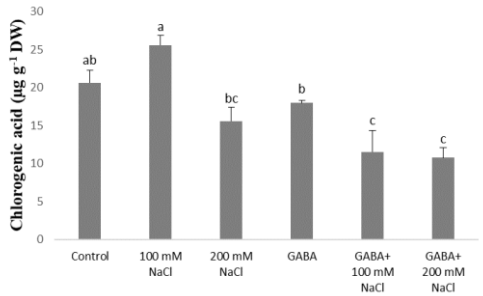

Figure 3. The effects of exogenous GABA application and salt stress on chlorogenic acid content in the leaves of *S. lycopersicon*. The bars represent the means ± S.E. Means with different letters are significantly different ($p < 0.05$).

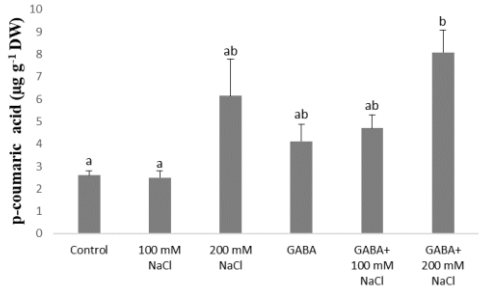

Figure 4. The effects of exogenous GABA application and salt stress on p-coumaric acid content in the leaves of *S. lycopersicon*. The bars represent the means ± S.E. Means with different letters are significantly different ($p < 0.05$).

The roles of phenolic acids as antioxidants have been mentioned because of their properties related to the H-donating ability of the phenols. Therefore, the enhancement in the phenolic substances can help to scavenge the reactive oxygen species [17]. Gallic acid, chlorogenic acid and coumaric acid are known to have the ability of radical scavenging. We found a significant increase in gallic acid and coumaric acid contents under 200 mM NaCl application when compared to control plants. Gallic acid and coumaric acid contents were also enhanced by GABA application. However, chlorogenic acid content was decreased by GABA and GABA+ salt applications as compared to control plants. 100 mM and 200 mM NaCl applications caused an increase in GABA content as compared to control plants. We can suggest that exogenous GABA could enhance the stress response by enhancing gallic acid and coumaric acid contents under salinity (Figure 2, 4).

4.Conclusion

According to our results, we can conclude that exogenous GABA application can enhance some phenolic substances and GABA content under salt stress. The increase in these substances could help to enhance defense strategies in tomato plants under salinity. However, further studies should be done to understand the role of exogenous GABA on the signal and defense mechanisms under stress conditions.

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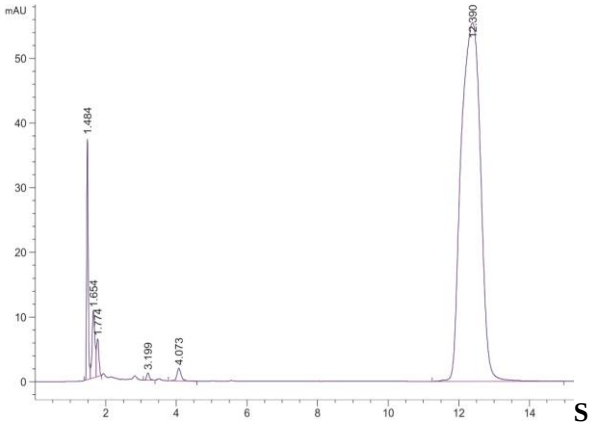
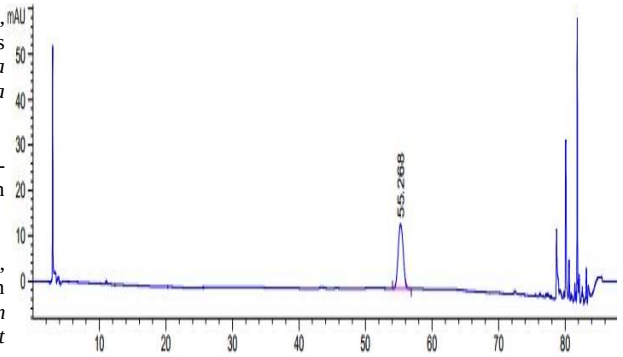
Supplementary materials



S P 1. HPLC chromatogram of GABA. The retention time was 12 min.



SP 2. HPLC chromatogram of one standard of a phenolic compound. Each sample was analyzed for 90 min.