Effect of Amino Acid Application on Physiological and Biochemical Properties of Salt-Stressed Broccoli Seedlings

The effect of exogenous amino acid on some physiological and biochemical properties of broccoli seedlings under salt stress was investigated. Two products containing amino acids (Ga and Pr) were applied from the soil to the root zone of the plant 3 times with one-week intervals in this study. The solutions prepared with 0 and 100 mM NaCl were applied to the plant as irrigation water. In the study, the effects of salt stress and applications on H_2O_2, MDA, proline sucrose, catalase (CAT), superoxide dismutase (SOD), indole acetic acid (IAA), salicylic acid (SA), gibberellic acid (GA) and abscisic acid (ABA) content of seedlings were investigated. The content of H_2O_2, MDA, proline, sucrose, CAT, SOD and ABA increased, while the content of IAA, GA and SA in the plant decreased with salinity. However, with exogenous amino acid applications, the effect of salt stress on these parameters in the plant was alleviated, thus contributing to the increase in the tolerance of broccoli seedlings to salt stress.

Keywords: *Broccoli, antioxidant enzyme, hormone, salinity*

Introduction

Salinity stress is one of the major abiotic stresses threatening agricultural production worldwide. About 20% of the irrigated agricultural lands in the world have salinity problems. It is estimated that the area affected by salinity will be approximately 50% of the total agricultural land by 2050 (Kumar et al., 2020; Zhao et al., 2021).

With salinity, damages occur in plants in terms of various physiological, biochemical and molecular properties, and plant growth is adversely affected. Salt stress reveals its effect on the plant with osmotic and ion stress. With salt stress, high concentrations of Na^+ accumulate in plant cells, reaching toxic levels and causing disruption of ion homeostasis. Ion imbalance and water deficiency in plant cells under salt stress cause osmotic stress, resulting in many changes such as decrease in cell turgor pressure, deterioration of the structure of the plasma membrane (Park et al., 2016). Salinity reveals these stresses by reducing plant water and nutrient uptake. Salt stress also causes various physiological and molecular changes together, suppresses cell division and expansion, inhibits photosynthesis and reduces plant growth. The activities of enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which are effective in photosynthesis, or protein stability are affected by salinity, in addition, salinity changes the levels of sugars such as sucrose, fructose and glycolysis in the plant (Zhu et al., 2002; Zhao et al., 2021). Salinity also disrupts various physiological and biochemical processes such as reactive oxygen species (ROS) (such as O_2, H_2O_2, O_2•- and OH•) formation and membrane leakage (Mushtaq et al., 2020).

Broccoli is known to be tolerant to moderate salinity. It has been stated that salt stress causes a decrease in growth with a two-stage effect in the roots and salt accumulated in the leaves of broccoli. However, the response to salt stress differs depending on the level of salt stress and the cultivars of broccoli (Chevilly et al., 2021).

There are various applications to mitigate of salt stress, which causes significant damage to plants. One of them is the application of amino acids. It is known that salt stress creates various changes in the plant with its effects on the amino acid content, which is also involved in plant metabolism. For this reason, researchers have obtained results that the damage caused by stress on the plant can be alleviated by applying amino acids exogenous to the plant. Amino acids regulate ion transport in the plant, modulate stomatal opening, affect the synthesis and activity of some enzymes, gene expression and redox homeostasis (Rai, 2002). In previous studies, important effects of amino acid applications against various abiotic stress factors, including salt stress, were determined (Wang et al., 2017; Haghighi et al., 2020; Matysiak et al., 2020; Peña Calzada et al., 2022).

The aim of this study is to determine the effect of exogenous amino acid application on some physiological and biochemical properties of broccoli seedlings under salt stress.

Materials and Methods

Plant Material and Experimental Design

A pot study was carried out in the controlled greenhouse conditions. To obtain seedlings, the broccoli (*Brassica oleracea* var. italica) seeds were sown in viols containing peat:perlite (2:1). Seedlings with about 2-3 leaves were planted in 1.3 liter pots in a mixture of soil, peat, sand and manure (2:1:1:1). After planting the seedlings, 30 ml of amino acid solution (Ga: Gluten amin and Pr: Protein) was drenched to each plant root zone.

Both of these products contain aspartic acid, gamma-aminobutiric acid, glutamic acid, alanine, arginine, phenylalanine, glycine, hydroxyproline, histidine, isoleucine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophan and valine (Ga total %45 and Pr total 41%) in different ratios.

The amino acid treatments were repeated three times at one-week intervals. Salinity application was started with irrigations after the first amino acid application. The solutions prepared with 0 and 100 mM NaCl were applied to the plants as irrigation water. Experiments were conducted with randomized plots design: a total of 108 plants were used with three replications and 6 plants per repeat. The experiment was terminated 40 days after the seedling planting. Some physiological and biochemical analyses were made.

Hydrogen peroxide (H_2O_2), Malondialdehyde, Sucrose and Proline

H_2O_2 and MDA content of leaf tissues were determined according to method of Liu et al. (2014). The MDA content was determined by spectrophometrically at 532 and 600 nm absorbance (Sahin et al., 2018). H_2O_2 was determined at 390 nm in spectrophotometer (Sahin et al., 2018).

The content of sucrose in the samples was determined in the spectrophotometer at a wavelength of 620 nm (Wu et al., 2011). Proline extraction and proline content were determined according to method of Bates et al. (1973) and the samples were measured at 520 nm with spectrophotometer.

CAT and SOD Enzyme Activities

Fresh leaf samples were homogenized in the extraction solution according to the method specified by Angelini et al. (1990) and Angelini vand Federico (1989) and the obtained supernatant was used to determine enzyme activities. CAT activity was determined by the decrease in absorbance of H_2O_2 at 240 nm. SOD activitiy at 560 nm by spectophotometrically (Liu et al., 2014).

Hormone Content

Extraction and purification processes were performed as described by Battal and Tileklioglu (2001) and Kuraishi et al. (1991). The hormones were determined by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC). Abscisic acid (ABA), gibberellic acid (GA), indole acetic acid (IAA) and salicylic acid (SA) were defined at 265 nm with a UV detector (Turan et al., 2014).

Statistical Analysis

A two-way ANOVA was used for data analysis and means comparison was made according to the Duncan multiple comparison test using SPSS program.

Results and Discussion

The effects of salt stress and amino acid application on H_2O_2, MDA, proline, sucrose, CAT, SOD, IAA, GA, SA and ABA content of broccoli seedlings are given in Figure 1, 2, 3, 4, 5 and 6.

With salinity, the amount of H_2O_2, MDA (Figure 1), proline (Figure 2), sucrose (Figure 3), CAT, SOD (Figure 4) and ABA (Figure 6) increased significantly, while the content of IAA, GA and SA (Figure 5) decreased. The increase in plant H_2O_2 and MDA content with salinity was quite high compared to the control. The amount of proline, sucrose, CAT, SOD and ABA increased by 95%, 26%, 207%, 324% and 224%, respectively, compared to the control in plants under salt stress without treatment. The content of IAA, GA and SA decreased with salinity by 26%, 43% and 32%, respectively. However, the effects of exogenous amino acid applications to plants and salinity in terms of these parameters have changed. When plants in salty conditions are compared with each other; H_2O_2, MDA, proline, CAT, SOD and ABA contents of treated (Pr and Ga) plants were lower by 64-52%, 71-40%, 60-47%, 7-40%, 2-37% and 30-44% (respectively) than control plants under the same conditions. On the other hand, sucrose, IAA, GA and SA contents treatments (Pr and Ga) were increased by 82-113%, 25-259%, 95-121% and 61-30% (respectively) compared to control in salty conditions.

It was determined that H_2O_2, MDA, proline, sucrose, CAT and SOD increased in broccoli seedlings with salinity, in this study. Thus, the plant started to react to salt and damage symptoms appeared in plant morphological features. Similarly, in another study, increased salinity in broccoli decreased leaf area, shoot length, root length, shoot and root dry weights, and increased antioxidant enzyme activities such as SOD, CAT and ascorbate peroxidase (APX) (Ali et al., 2022). Also, according to Akram et al. (2020) stated that the activities of total phenolics, H_2O_2, MDA, glycine betaine, proline, ascorbic acid, CAT, SOD and peroxidase (POD) enzymes increased with increasing salt stress in broccoli. With salt stress, excessive H_2O_2 production occurs in the plant and it oxidizes amino acids such as methionine and cysteine from Calvin cycle enzymes, causing programmed cell death (Mushtaq et al., 2020). In salt stress, the plant antioxidant defense system detoxifies ROS and protects the plant against oxidative damage caused by stress by providing the balance of ROS formation. Excessive ROS production caused by salt stress is one of the reasons that prevent morphological, physiological and biochemical activities in plants by strengthening the antioxidant defense system (Hasanuzzaman et al., 2021). Oxidative stress in plants occurs with enzymatic (SOD, CAT etc.) and non-enzymatic (ascorbic acid, phenolic alkaloids, flavonoids, carotenoids etc.) antioxidant defense mechanisms and endogenous protection mechanism (Hasanuzzaman et al., 2020).

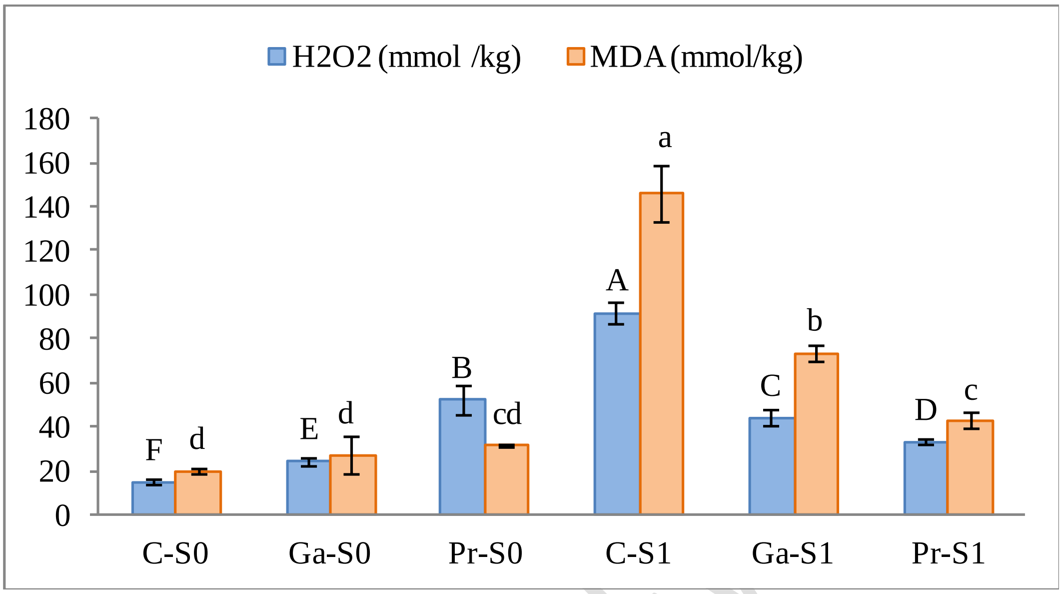
It is stated that various hormones (such as cytokinins, abscisic acid, auxin, jasmonic acid, gibberellin and ethylene) play a role in the improvement of salt stress in plants (Mushtaq et al., 2020; Raza et al., 2022; Vaishnav and Chowdhury, 2023). In this study, the effect of salinity on plant hormone content was significant. GA improves plant metabolism processes by regulating membrane permeability, enzymatic activity, various osmolytes and ion uptake in the plant, thus increasing plant tolerance to abiotic stresses (Sharma et al., 2015; Raza et al., 2022). We investigated that GA content of broccoli seedling showed a significant decrease with salinity. IAA is a versatile phytohormone that has an active role in plant growth and development, especially in stressful conditions. IAA improves the antioxidant defense system and plant tolerance in the plant and thus reduces the oxidative damage caused by abiotic stress (Raza et al., 2022). In this study, IAA content of broccoli seedling was decreased under salt stress. SA is a phenolic compound that modulates pathogenesis-related protein expression and plays a role in plant growth, development and abiotic stress response as well as plant defense responses. Salicylic acid helps the plant respond to and counteract various abiotic stresses (Kang et al., 2014; Raza et al., 2022). SA contents showed a significant decrease with salinity, in this study. ABA is a well-known phytohormone with its effect and role in the adaptation of plants to various abiotic stresses, and it is also called stress hormone. In response to abiotic stress in the plant, endogenous ABA level rises, triggering specific signaling pathways and altering gene expression levels (Danquah et al., 2014; Albacete, 2020; Raza et al., 2022). There was a significant increase in the ABA content of broccoli seedlings with salinity in this study.

In this study, the effects of the above-mentioned salt stress on broccoli seedlings changed significantly with amino acid treatments. The increase in H_2O_2, MDA, proline, sucrose, ABA, CAT and SOD activities with salinity was less with amino acid treatments The reduction in IAA, SA and GA content caused by salt stress was lower with amino acid application.Amino acids are biostimulants that have a positive effect on plant growth and reduce damage caused by abiotic stresses (Ali et al., 2019). Similarly, it was determined that the amino acid could reduce the harmful effects of ROS and increase the tolerance of wheat seedlings under salt stress conditions (Bahari et al., 2013). Peña Calzada et al. (2022) determined that the amino acid mixture sprayed from the leaves increases the accumulation of K+ in the plant under salt stress, and affects biological processes such as osmolytes, photosynthetic pigments, and eliminates the morphological and physiological damages caused by salinity. In addition, it was stated that it caused a decrease in Na+ accumulation and MDA concentration. The role of amino acids in abiotic stress tolerance are involved with mechanisms that compatible osmolytes, regulate pH and act as nitrogen and carbon reserves (Ali et al., 2019). It has been determined that amino acids can alleviate the salt stress membrane by showing similar effects in broccoli seedlings, thus providing tolerance against salt stress with less damage to plant development.

Conclusion

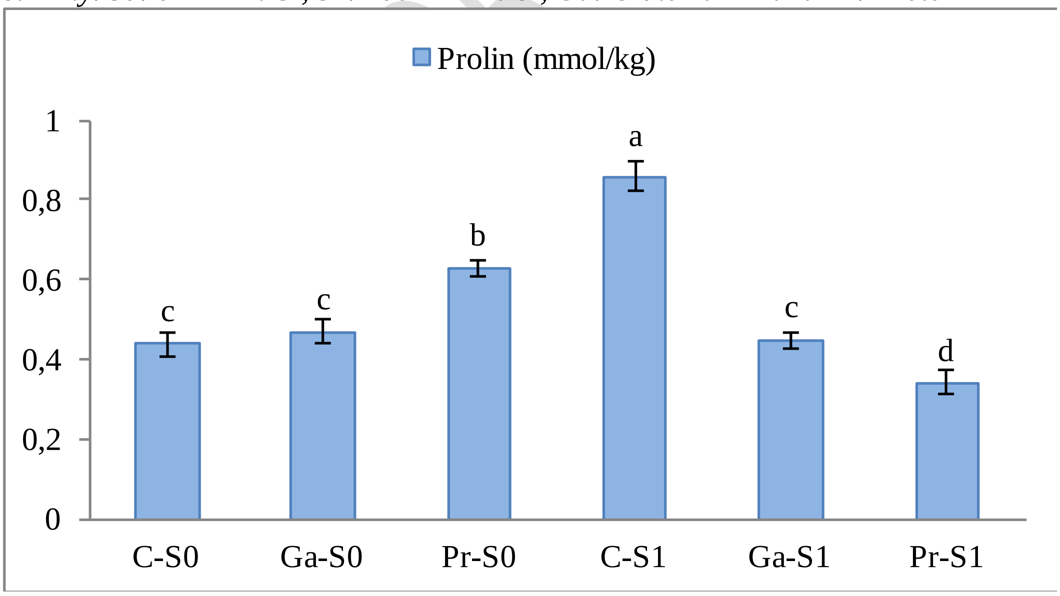
In this study, the effect of salt stress on some physiological and biochemical properties of broccoli seedlings was investigated, and significant effects were observed on these properties with salt stress and thus damage to the plant occurred. On the other hand, it was determined that amino acid application could alleviate the damage in broccoli seedlings by changing the effect of salt stress on these parameters. However, further research is needed to determine the precise role of amino acids in this effect of salt stress on broccoli seedlings.

Figure 1. *The effects of amino acid treatments on H_2O_2 and MDA content of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein*



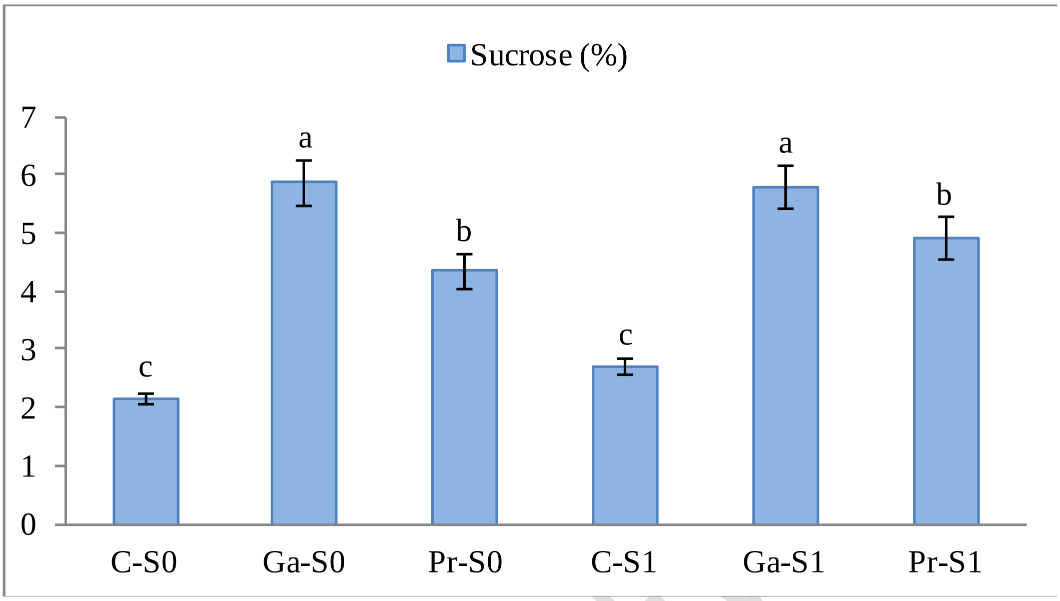
The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

Figure 2. *The effects of amino acidt treatments on prolin content of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein*



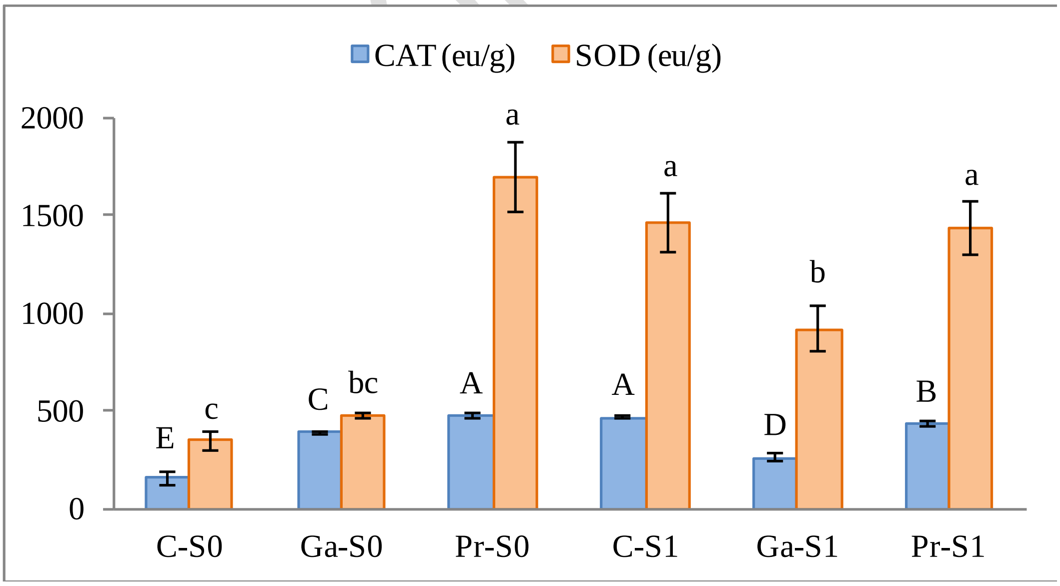
The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

Figure 3. *The effects of amino acid treatments on sucrose content of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein*



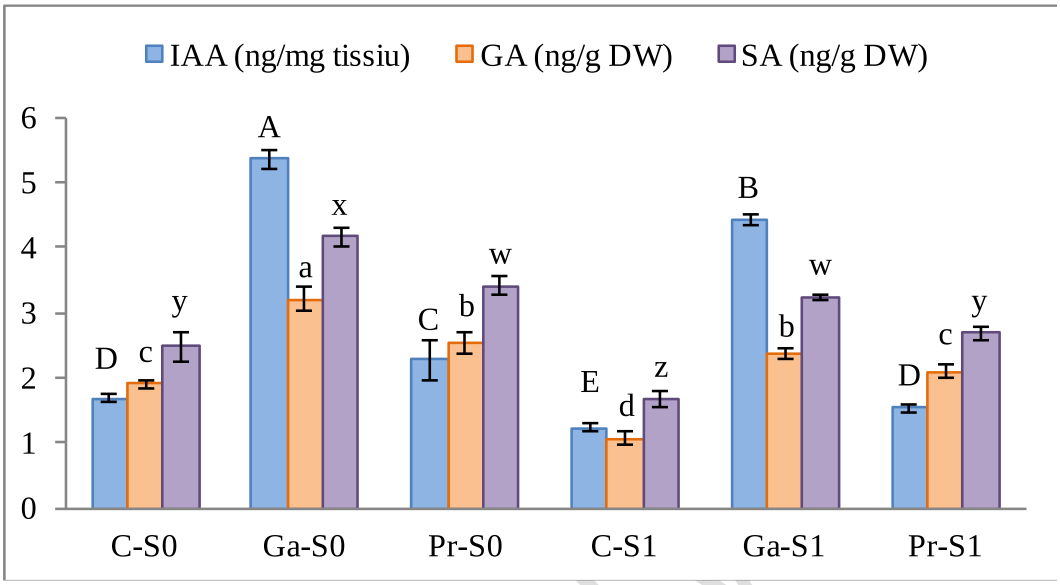
The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

Figure 4. *The effects of amino acid treatments CAT and SOD activity of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein*



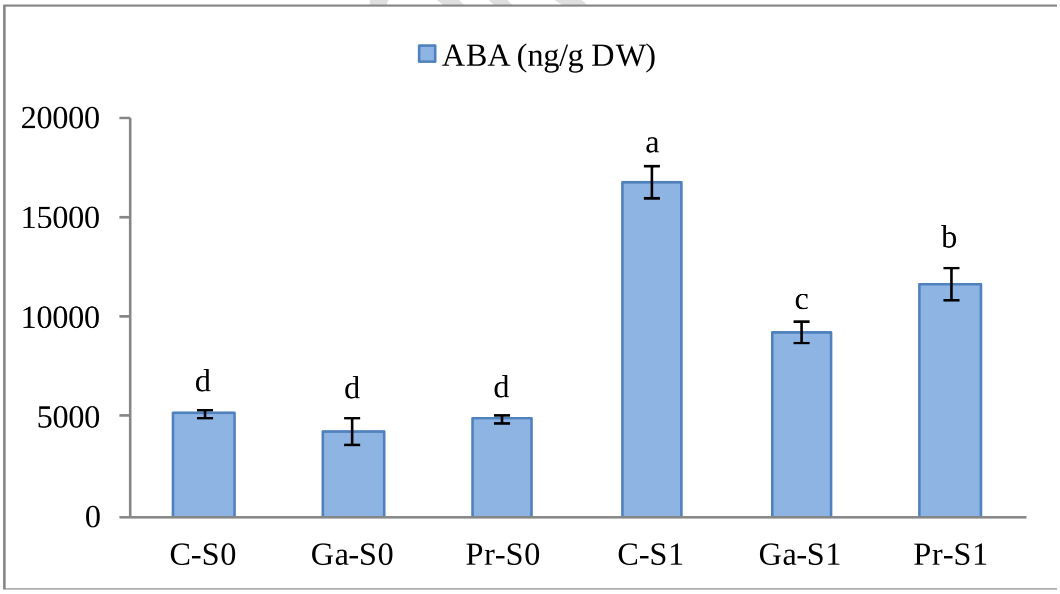
The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

Figure 5. The effects of amino acid treatments on IAA, GA and SA content of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein



The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

Figure 6. *The effects of amino acid treatments on ABA content of broccoli under salinity. S0: 0 mM NaCl, S1: 100 mM NaCl, Ga: Gluten amin and Pr: Protein.*



The difference between the means indicated by different uppercase letters and lower case letters in the same bar is statistically significant (Duncan multiple comparison test, P<0,001).

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