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BLACK SOYBEAN RESPONSE TO ANTIOXIDANT APPLICATION FOR GROWTH AND YIELD UNDER SALINITY STRESS CONDITIONS

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SUMMARY

Black soybean (*Glycine max* L.) is native to Asia and well adapted to tropical climate countries, such as, Indonesia. The demand for black soybean supply increases due to soy sauce making. Limited fertile land causes efforts to enhance black soybean production to relocate to sub-optimal lands, such as, saline land. However, in saline soils the plant productivity is very low; therefore, technological inputs are required to prevent the crop from salinity stress, one of which is an exogenous application of antioxidants. The presented study aimed to analyze the yield in several black soybean cultivars when applied with antioxidants under salinity stress conditions. The study used a randomized complete block design with a factorial arrangement and three replications. The first factor comprised four cultivars of black soybean, i.e., Malika, Detam 2, Detam 3, and Detam 4, while the second factor was antioxidants (ascorbic acid, salicylic acid, and vitamin E - α-tocopherol) application, and without application of antioxidant (control). The results showed that salinity stress significantly affected the growth and yield of four black soybean cultivars. However, cultivar Detam 2 compared with other cultivars, performed best based on morphological and yield-related traits under saline soil conditions. Furthermore, in antioxidants, salicylic acid gave the best results for morphological characters, while ascorbic acid and vitamin E performed better for yield-related traits. Overall, the antioxidant application increased the tolerance of black soybean genotypes as compared with the control under salinity stress conditions.

Keywords: Black soybean (*Glycine max* L.), antioxidant defense, cultivars, morphological traits, NaCl levels

Key findings: Antioxidants play an essential role and increase the tolerance of black soybeans to salinity stress conditions.

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INTRODUCTION

In East-Asian countries, black soybean (*Glycine max* L.), dubbed as the king of plant protein, contains anthocyanins found in its seed coat. Black soybean is also an important practical food that needs more exploration. Soybean is classifies as a moderately salt-tolerant crop plant with a threshold of 5 dS/m; however, salt-sensitive soybean cultivars severely affected under salt stress did not produce seeds at a soil salinity level of 8 dS/m

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(Papiernik *et al*., 2005; Bustingorri and Lavado, 2011). With declining fertile lands, providing technical inputs is a solution other than using salinity-stress tolerant cultivars. Salinity problems occur when the soil contains the highest dissolved salt content, interfering with plant physiological processes and growth (Machado and Serralheiro, 2017). Soil salinity causes ion toxicity, nutrient deficiency, and osmotic and oxidative stress on plants, eventually, limiting water uptake from soil (Shrivastava and Kumar, 2015). Salinity and poor water management can also condense soil fertility permanently (Nasyirah, 2015).

Efforts to increase black soybean production under stress conditions using crop improvement are often unsuccessful because of their polygenic nature (Bhartiya *et al*., 2012; Rasyad *et al*., 2018). Therefore, a focused approach incorporating physiological, biochemical, and metabolic aspects in salt-tolerant molecules is essential for developing tolerant cultivars. In recent decades, protective exogenous, such as, osmoprotectants (proline, glycine betaine, and trehalose), plant hormones (gibberellic acid, jasmonic acid, brassinosteroids, and salicylic acid), antioxidants (ascorbic acid, glutathione, and tocopherols), signaling molecules (nitric oxide and hydrogen peroxide), polyamines (spermidine, spermine, and putrescine), trace elements (selenium and silicon) are effective in reducing salt-induced damages to plants (Yusuf *et al*., 2012; Kuswantoro *et al*., 2020).

This shield can promote plant growth and yield well under salinity stress conditions. Salicylic acid has a positive effect by protecting the development of antistress activities and accelerating the growth normalization process after disabling the stress factors (Sakhabutdinova *et al*., 2003). Salicylic acid plays a role in detoxification and neutralizing superoxide radicals (Rizwan *et al*., 2011). Several studies have shown that applying salicylic acid (0.5 mM) can promote the formation of ROS (reactive oxygen species) in photosynthetic tissues and increase oxidative damage during salt and osmotic stress conditions (Barba-Espin *et al*., 2011). Besides, administering ascorbic acid with a concentration of 1000 ppm increased rice production by 10%–15% in the sensitive cultivars under salinity stress (Barus *et al*., 2015, 2022). Hassan *et al.* (2021) also reported that exogenous application of ascorbic acid could overcome the negative impact of salt stress on barley plants by increasing the morpho-physiological traits and decreasing oxidative stress in plants through increasing enzymatic antioxidant activity. Furthermore, the foliar application of α-tocopherol with a concentration of 100 and 200 mg/L at earlier stages can mitigate the adverse impact of salt stress on sunflowers (Lalarukh *et al.,* 2022).

Currently, abiotic stress conditions and sustainable agricultural systems for providing healthy food for better human health are of foremost concern. Numerous reports stated using antioxidants to resist abiotic stress, including salinity stress. Based on earlier descriptions, the presented study aimed to determine the effect of the antioxidant application on the agronomic traits and yield of several black soybeans under salinity stress.

MATERIALS AND METHODS

The recent study transpired from April to July 2021 under the field and laboratory conditions of the Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia. The research used black soybean (*Glycine max* L.) cultivars, antioxidants, saline soil, and other supporting materials. This study also employed a randomized complete block design with a factorial arrangement and two factors. The first factor was black soybean cultivars, consisting of Malika, Detam 2, Detam 3, and Detam 4, with the second factor as antioxidants, including ascorbic acid, salicylic acid, vitamin E (α-tocopherol), and without antioxidant (control).

Seed preparation

The seeds of four black soybean cultivars came from the Research Institute for Various Nuts and Tubers, Malang City, East Java, Indonesia.

Saline soil

The saline soil came from Paluh Merbau hamlet, Tanjung Rejo village, Percut Sei Tuan sub-district, Deli Serdang regency, Medan, Indonesia. Before taking the saline soil planting media, analyzing the dirt proceeded in the laboratory. The criteria for the saline soil were to have a salinity level of 4-6 mmhos.

Preparation of planting media

The planting medium was a mixture of saline soil and topsoil with a ratio of 2:1, respectively. Soil analysis for its nutrient content and salinity level preceded placing in polybags.

Planting

Planting ensued with two seeds per polybag, maintaining one plant per polybag after germination and in one-week-old plants.

Application of antioxidants

Antioxidants (ascorbic acid, salicylic acid, and vitamin E) application progressed to the soybean plants at 14, 24, 34, and 42 days after planting at 10-day intervals, respectively. Each antioxidant applied had a concentration of 250 ppm.

Data recorded and statistical analysis

Plant height measured at two, four, six, and eight weeks after planting (WAP) started from the base of the rootstock to the growing point of the plant expressed in centimeters (cm). Counting the number of productive branches followed at eight WAP. Measurement of total leaf chlorophyll content used a chlorophyll meter, namely, SPAD-502 Plus by measuring the absorbance of the leaves in two wavelength regions, from three leaves on one stalk (trifolia) of plants of eight WAP, which were added up to get the average value. Observation for seed weight per plant occurred after the harvest of plants by weighing all the seeds taken from one plant. The air-dried seeds used an analytical balance for weighing. The same procedure continued with the observation of seed weight per plot in each experimental plot. Assessing 100-seed weight also proceeded after harvesting the plants by taking 100 seeds from sample plants per plot taken randomly, with the 100 seeds weighed using an analytical balance, then recording the results.

Statistical analysis

All the recorded data underwent F-test analysis, with the means further compared and separated using Duncan's Multiple Range Test (DMRT) (Hanafiah, 2016).

RESULTS AND DISCUSSION

Soil analysis

The results of the soil analysis of the planting media are in Table 1. The experimental soil analysis showed the dominance of sand (48.60%), followed by dust (32.71%) and clay (18.69%). The soil had a pH of 6.21 and contained organic carbon (1.91%), total nitrogen (0.11%), and available P (12.77 ppm). The soil also has exchangeable K (2.29 me/100), Ca (13.32 me/100), Mg (31.20 me/100), Cu (3 ppm), Zn (30 ppm), Mn (174 ppm), and Fe (1241 ppm). The soil characteristic is high salinity (6.9 mmhos/cm). According to Suud *et al*. (2016), the EC value of the soil has a positive correlation with N content in it, which may refer to the addition of potential nutrients to enhance the ionized nutrients for increasing the EC value of the soil.

Table 1. Soil analysis result.

No.	Type of analysis	Values	Test method	Status
1	C-Organic (%)	1.91	IK 5.0 (Spectrophotometry)	Low (1-2)
2	N-Total (%)	0.11	IK 6.0 (Kjeldahl)	Low (0.1-0.2)
3	P-Bray I (ppm)	12.77	IK 7.0 (Spectrophotometry)	High (11-15)
4	K-dd (me/100 g)	2.29	IK 8.0 (AAS)	Very high (< 1)
5	Ca (me/100 g)	13.32	IK 8.0 (AAS)	High (11-20)
6	Mg (me/100 g)	31.20	IK 8.0 (AAS)	Very high (> 8)
7	Cu (ppm)	3.00	IK 8.0 (AAS)	Medium
8	Zn (ppm)	30.00	IK 8.0 (AAS)	Medium
9	Mn (ppm)	174.00	IK 8.0 (AAS)	Very high
10	Fe (ppm)	1241.00	IK 8.0 (AAS)	Very high
11	pH	6.21	IK 3.0 (Electrometry)	Neutral
12	Al-dd (me/100g)	0.00	IK 4.0 (Titrimetry)	-
13	DHL (mmhos/cm)	6.90	IK 3.0 (Electrometry)	High
14	Texture			
	Sand (%)	48.60		
	Silt (%)	32.71	IK 9.0 (Hydrometer)	
	Clay (%)	18.69		

Plant height

Salinity affects almost all aspects of plant development, including vegetative growth, such as, plant height. Based on the results, the observed plant height of four black soybean cultivars was significantly affected by the genotypes in the saline soil and not by the antioxidant treatments and their interaction with black soybean cultivars (Figure 1). In black soybean cultivars, the plant height varied, and the cultivar Detam 2 displayed taller plants, whereas the shortest resulted in the cultivar Detam 4. The differences in the genotypes for plant height might be due to the diverse genetic makeup of the cultivars and their response to the growing environment. Sutarman's (2021) findings also revealed the varied reactions of soybean cultivars due to the diverse genetic potential of the soybean genotypes and their response to environmental conditions, including saline stress. Genetic differences in tolerance to saline stress in soybean genotypes also came from other studies (Zuffo *et al.,* 2020; Silva *et al.,* 2021), which may be useful in identifying genotypes more adapted to sowing under abiotic stress conditions.

Apart from the influence of salinity, plant height in each cultivar also differs in the antioxidant applications in saline soil (Figure 2). It is a fact that plant height will decrease under saline stress conditions. However, applying antioxidants can reduce the negative effect of saline stress, and the plant height has increased compared with the control (without antioxidant applications). It means that antioxidants can stimulate and enhance growth traits. It is presumably due to the participation of antioxidants in regulating cell division and growth by enhancement of the cell development cycle (Smirnoff, 2005). Furthermore, Foyer (2005) explained that plants synthesize low molecular weight antioxidants, such as ascorbate, as redox buffers that interact with numerous cellular components and influence plant growth and development.

Results further revealed that the black soybean cultivar Detam 2 produced taller plants with the application of salicylic acid. Generally, salicylic acid provides the best results compared with ascorbic acid and vitamin E. It is because salicylic acid plays a vital role in the defense mechanism of plants, as a signaling molecule under stressful environments (Kang *et al*., 2012; Barus *et al*., 2019). Furthermore, Khan *et al*. (2010) stated that salicylic acid can improve physiological and biochemical processes, as well as enhance macro-nutrient activities, such as N, P, K, and Ca, in antioxidant enzymes. Tarigan and Wardana (2020) reported that the application of salicylic acid 150 ppm/plot on saline soil significantly increased root length, root dry weight, stomata number and thick cuticle of vetiver.

Number of productive branches

Analysis results showed the number of productive branches was significantly affected by the type of antioxidants in the saline soil, while the cultivars and the interactions were not (Figure 3). It is also a fact that salinity stress conditions affect the growth traits, including the number of branches per plant. It might be the inhibition of new branch formation and occurring early senescence at the older branches due to salinity (Islam *et al*., 2012). However, using antioxidants can reduce the terrible effects of salinity, which decrease the number of branches. As observed, the number of branches was higher in samples treated with antioxidants compared with the control. The application of ascorbic acid showed the best results for productive branches in black soybean (Figure 3).

The antioxidants act as a plant defense system against saline stress for improving yield traits through an increased number of productive branches. The productive branches in various cultivars of black soybean interacting with several types of antioxidants in saline soil appear in Figure 4. Cultivar Detam 2, applied with ascorbic acid, responded better, followed by salicylic acid and vitamin E. According to Akhlaghi *et al*. (2018), ascorbic acid is a major non-enzyme antioxidant playing an important role in mediating certain oxidative pressures caused by biotic and abiotic stresses. Besides, ascorbic acid is a powerful antioxidant and the most common in almost all crop plant tissues (Hasanuzzaman *et al*., 2013), functioning as an enzyme cofactor for the biosynthesis of plant phytohormones and their transduction pathways (Smirnoff, 2005; Hossain *et al*., 2018; Semida *et al*., 2018). Ascorbic acid also has a key role in regulating cell division and elongation, thus, growth and development (Horemans *et al*. 2000; Potters *et al*. 2002). Some past studies also mentioned that it may influence the expression of many defense genes and the genes responsible for plant growth (Zhang *et al*., 2013, 2019).

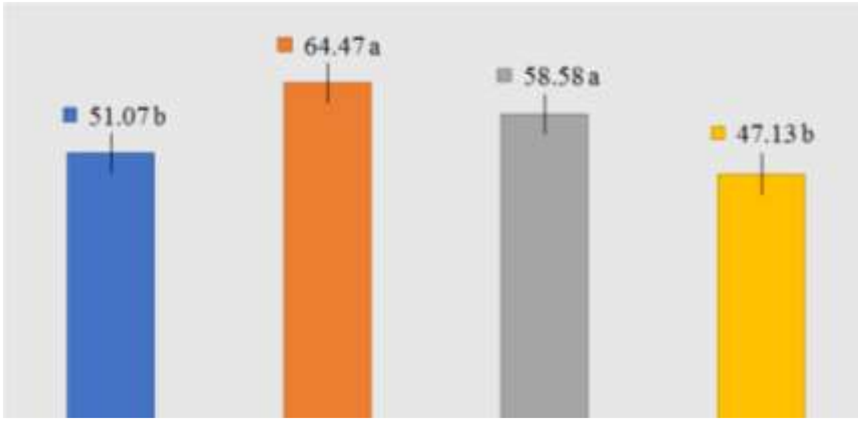

Figure 1. Plant height of black soybean cultivars under salinity stress conditions.

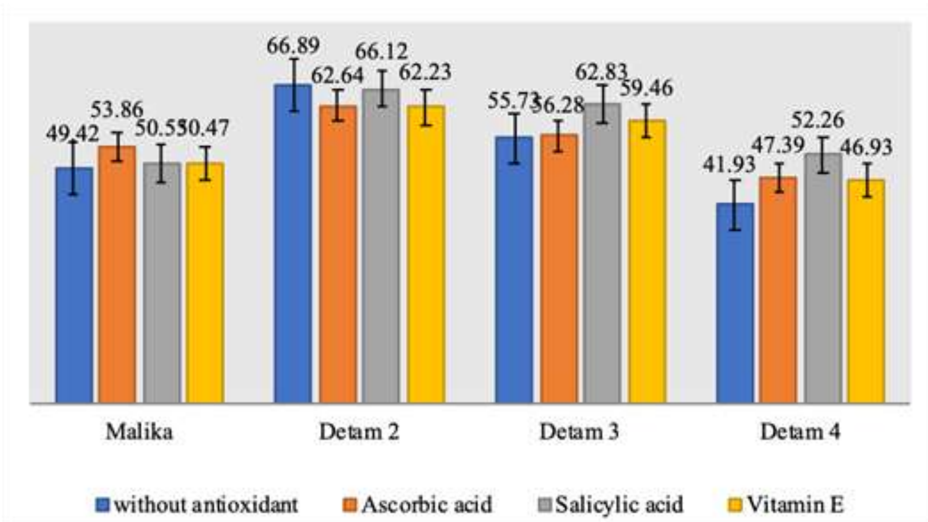

Figure 2. Plant height of black soybean cultivars with antioxidant application under salinity stress conditions.

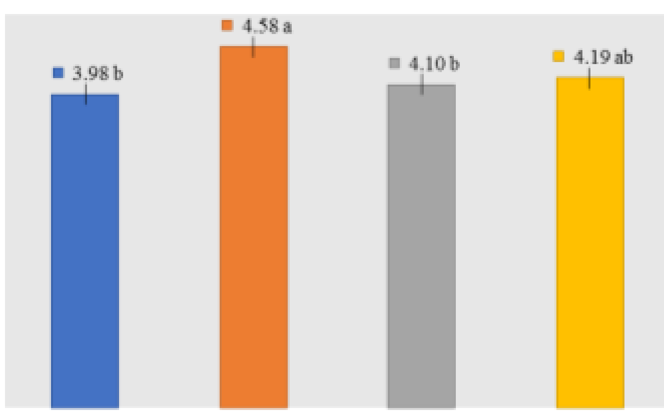

Figure 3. Productive branches of black soybeans in saline soil with the antioxidant applications.

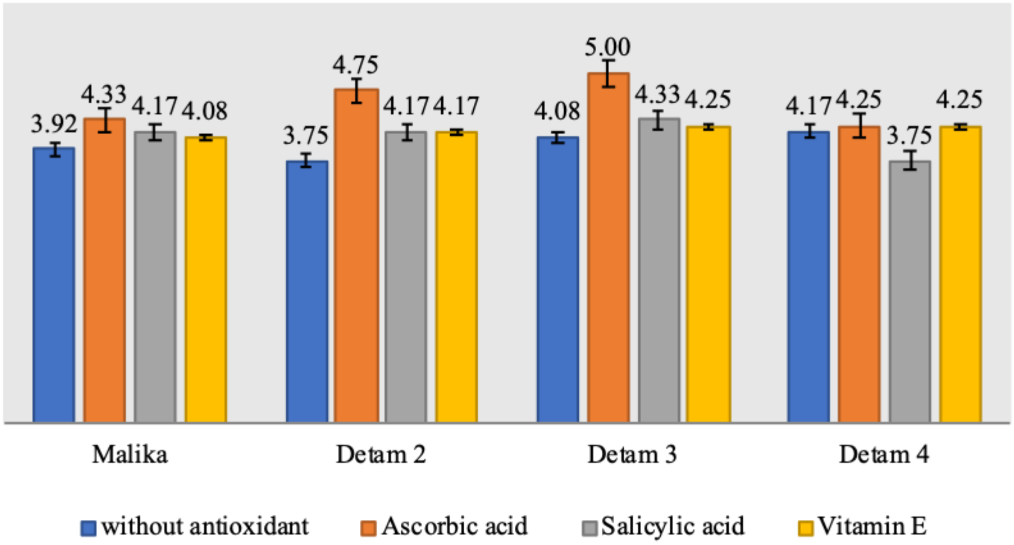

Figure 4. Productive branches of black soybean cultivars with antioxidant applications under salinity stress conditions.

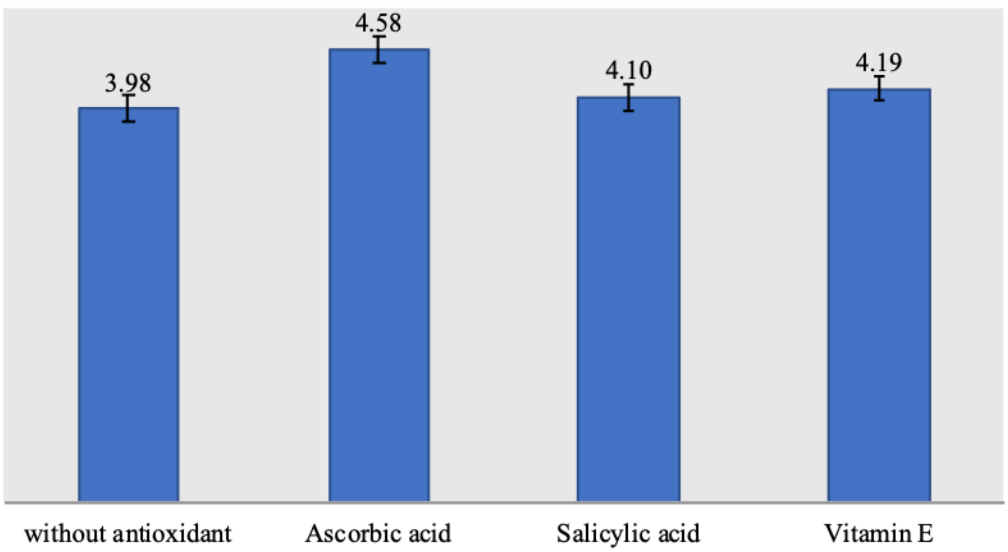

Figure 5. Chlorophyll content of black soybeans in saline soil with the antioxidant applications.

Chlorophyll content

Types of antioxidants significantly affected the chlorophyll content in black soybean cultivars in saline soil, while the cultivars and their interaction with antioxidants showed non-significant effects (Figure 5). Results further revealed that the chlorophyll content of soybean cultivars was higher with the use of antioxidant application than the control under salinity stress conditions. Therefore, it proved that applying antioxidants to soybean plants can overcome the adverse effects of salt stress. Antioxidants boost the activities by stabilizing and protecting photosynthetic pigments from oxidative damage caused by abiotic stress conditions (Praveena and Murthy, 2014).

Generally, the decrease in leaf chlorophyll content can occur due to disrupting chlorophyll formation in chloroplasts under saline stress conditions. The Na^+ and Cl^- ions that accumulate in the chloroplast will inhibit photosynthesis because chlorophyll is the major photosynthesis material directly associated with plant growth and development (Dawood and El-Awadi, 2015; Saleem *et al*., 2020). Besides, high salt concentrations in soil and water create a high osmotic potential, decreasing the plant water supply. A decrease in water potential causes osmotic stress, which reversibly inactivates the photosynthetic electron transport by intercellular space shrinkage (Nongpiur *et al*., 2016; Jabeen and Ahmad, 2017).

In the presented study, ascorbic acid is the most effective antioxidant in increasing chlorophyll content (Figure 5). Saline stress conditions also induced water deficit and increased ionic and osmotic effects, leading to oxidative stress and the formation of reactive oxygen species (Bohnert and Jensen, 1996). According to Bilska *et al*. (2019), ascorbic acid is the first line of plant defense against oxidative stress by removing several free radicals, often as a substrate of APX and an essential enzyme of the ascorbate–glutathione pathway (Sharma *et al*., 2019). Furthermore, ascorbate also acts as a cofactor for several cellular enzymes, such as violaxanthin de-epoxidase, essential for photoprotection by the xanthophyll cycle and other enzymes directly involved in the removal of reactive oxygen species, thus, improving the antioxidant ability of plant tissues (Zhou *et al*., 2016).

In addition to environmental factors, the genetic makeup of crop plants is crucial in determining the chlorophyll content. Cultivar Detam 3 had the highest chlorophyll content compared with other cultivars, but it was not significantly different from Detam 2 when applied with ascorbic acid under saline stress conditions (Figure 6). However, cultivars Malika and Detam 4 had almost the same chlorophyll content. Based on these results, black soybean cultivars Detam 2 and Detam 3 can effectively benefit the breeding of genotypes with high chlorophyll content. Higher chlorophyll content indicates more active photosynthetic activities, eventually increasing crop production. Past studies also confirmed that increased photosynthetic activity correlates with increased biomass and grain yield (Brestic *et al*., 2012; Baslam *et al*., 2020).

Seed weight plant^{-1} and plot^{-1} and 100-seed weight

Results expressed that black soybean cultivars significantly affected the seed weight per plant and per plot and 100-seed weight under saline soil conditions (Table 2). Soybean cultivar Detam 2 showed the highest seed weight per plant and plot and 100-seed weight compared with the other three cultivars, namely, Malika, Detam 3, and Detam 4. It indicated that the cultivar Detam 2 has the highest adaptation to salinity stress. Taufiq's *et al*. (2020) findings stated that the tolerant cultivars allocate more energy to promote the yields by reducing energy allocation for high growth. In addition, Detam 2 cultivar shows the most positive interaction with the antioxidants compared with other black soybean genotypes in the studies (Figure 7).

Based on Figure 7, the responses of the black soybean cultivars applied with antioxidants varied under salinity stress, whereas the response of various genotypes to salinity stress was different. According to Aini *et al*. (2014), genotypes may respond much better to soil salinity, but it also depends on the genetic makeup of the genotypes. Tigabu *et al*. (2013) reported a significant genotypic variation in salinity tolerance of the sorghum genotypes at the early growth stages. Barus *et al*. (2015, 2019) also indicated that the response of each cultivar was considerably different to salinity stress conditions.

Black soybean cultivar Detam 2 emerged as a salinity-tolerant soybean genotype, as confirmed by its higher growth and yield compared with other test cultivars. According to Martinezballesta and Carvajal (2014), plant salinity tolerance receives regulation by various mechanisms, including water uptake regulation and transport, sodium ion (Na^+) exclusion, and tissue Na^+ tolerance. Investigations on the transport of ions, primarily Na^+, potassium (K^+) and chloride (Cl^-), and water in plants are essential for understanding these mechanisms. The Na^+ transport processes include calcium (Ca^{2+}) sensitive and Ca^{2+} insensitive Na^+ influxes to the plant roots across the plasma membrane. However, Na^+/H^+ antiporters-mediated Na^+ efflux to the soil solution and other transports, such as Na^+ transport from the plant roots interface into the xylem, and compartmentalizing the accumulated Na^+ in individual cells of crop plants (Deinlein *et al*., 2014; Cao *et al*., 2017).

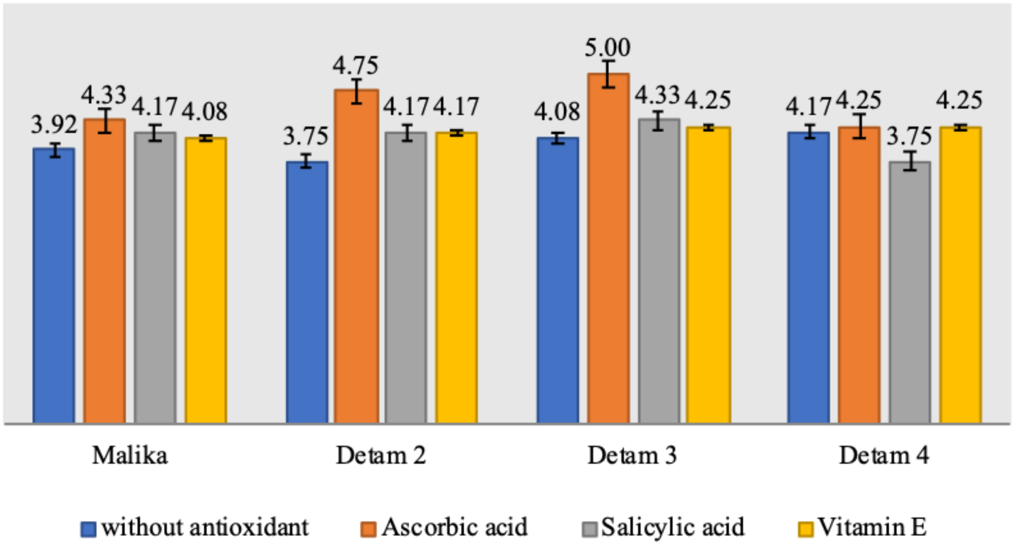

Figure 6. Chlorophyll content of black soybean cultivars with antioxidant applications under salinity stress conditions.

Table 2. Seed weight per plant and plot and 100-seed weight of black soybean cultivars under salinity stress conditions with the antioxidant applications.

Cultivars	Seed weight plant^{-1} (g)	Seed weight plot^{-1} (g)	100-seed weight (g)
Malika	13.92 b	54.13 b	14.14 b
Detam 2	17.52 a	70.10 a	15.87 a
Detam 3	11.65 b	46.61 b	14.40 b
Detam 4	11.60 b	46.41 b	13.32 b

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

Figure 7. The 100-seed weight of black soybean cultivars with an antioxidant application under salinity stress conditions.

CONCLUSIONS

Salinity stress significantly affected the growth and yield of black soybean (*Glycine max* L.) cultivars. Based on morphological characters and yield, the soybean cultivar Detam 2 provided the best growth under saline soil conditions compared with other cultivars. Furthermore, salicylic acid produced the best results for morphological traits, while ascorbic acid and vitamin E gave the best results for yield-related traits. Antioxidant application increased the tolerance of black soybean cultivars compared with control under salinity stress conditions.

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