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Reduction of Drought Stress Effects on Guar (*Cyamopsis tetragonoloba* L.) using Ascorbic Acid and Calcium Carbonate

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ABSTRACT

Background: How plants respond to stress conditions can play an important role in the establishment and increasing crop yield. The results of researchers' reports indicate that extensive morphophysiological and biochemical changes occur in plants under drought stress conditions. This study aimed to investigate the effects of ascorbic acid (AA) and calcium carbonate (CC) on guar under different soil moisture conditions.

Methods: The experimental treatments included three drought stress levels (30%, 50% and 70% field capacity, FC) and three foliar application levels (no application, foliar application with CC and AA with 0/003 concentration).

Result: Drought and calcium caused an increase in the sugar content relative to the other treatments. An increase in drought intensity led to an increase in proline content, soluble protein and ascorbate peroxidase (APX) enzyme activity. On the other hand, Leaf relative water content (RWC) decreased significantly. AA and CC increased the soluble protein content and RWC relative to the control condition. In general, moisture availability below 70% FC seems to induce drought stress countermeasures in guar. Foliar application with AA and CC can effectively mitigate drought stress effects on this plant.

Key words: Amino acid, Antioxidant, Free radicals, Osmotic regulation.

INTRODUCTION

Among abiotic stresses, drought is considered the most critical stress affecting the reduced crop yield, especially in arid and semi-arid areas, as well as the major production limitation in these regions (EL Sabagh *et al*., 2019). Drought disrupts the physiological and biochemical balance in the plant, resulting in photoinhibition and the production of reactive oxygen species (ROS) (Gill and Tuteja, 2010). Non-enzymatic defense systems such as carotenoids, ascorbate and enzymatic ones including catalase, superoxide dismutase, ascorbate peroxidase and polyphenol oxidase are activated under ROS conditions and decrease the effects of drought stress in plants. Studies have shown that increasing ascorbic acid (AA) is one of the critical defense mechanisms in plants against different stresses, which plays a role in ROS detoxification (Akram *et al*., 2017). The existence of few millimoles of AA in leaves shows the vital role of this compound as a part of the antioxidant defense system (Noctor and foyer, 1998). AA prevents the oxidation and destruction of protein structures by direct and indirect removal of free radicals. Oxygen free radicals produced during the stress cause destruction of cell membranes, nucleic acids and cell proteins because of their high reactivity with proteins and lipids (Akram *et al*., 2017; Peltzer *et al*., 2002). AA increases the protein content in roots and aerial organs, which contributes to remove oxygen free radicals (Kerepesi and Galiba, 2000). Furthermore, calcium (Ca), despite being immobile, is an essential element for plant growth and has several electrochemical, structural and catalytic tasks in plants (Jones, 1997). Neutralizing the acidic radicals, adjusting cell permeability, acting as a messenger in plant tissues, making pectocellulose walls and activating certain enzymes and their reaction with plant hormones are the electrochemical, structural and catalytic roles of Ca (Grenzi *et al*., 2021).

Plants keep the osmotic potential more negative in growing cells and meristematic zones by accumulating secondary metabolites such as sugars, amino acids and proteins, thereby maintaining water absorption and turgescence for these cells (Chimenti *et al*., 2006). Studies have shown that these compounds can form under drought stress by hydrolyzing polysaccharides and oligosaccharides such as starch, fructan, *etc*. (Chimenti *et al*., 2006).

Guar (*Cyamopsis tetragonoloba* L.) is an annual plant from the Legume family, which can be used as a potential substitute crop in dry plains due to its resistance to salinity and drought (Grover *et al*., 2016). This plant is an essential source of galactomannan extracted from the plant's

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endosperm and jellifies by dissolving in water. For this feature, Guar has various applications in different industries (Pathak and Roy, 2015). Despite some studies on the effects of drought on the Guar plant, there are limited studies on this plant on the effects of AA and Calcium Carbonate (CC) under drought stress. Therefore, this study aimed to investigate the application of AA and CC to improve growth and decrease drought effects on this plant.

MATERIALS AND METHODS

Planting method and experimental treatments

This study was done as a factorial experiment with two factors using a completely randomized design with three replications in the research greenhouse; the experiment was conducted during February to May 2020 at Saravan Higher Education Complex. Experimental treatments included three drought levels (70%, 50% and 30% field capacity, FC) and three foliar application levels (no application, foliar application with CC and CC with 0/003 concentration). Guar seeds were initially sown in seedling trays and then transported to the main pots after germination; drought stress levels were also applied simultaneously. The pots were irrigated with distilled water until the flowering stage. To measure FC using the weight method, three pots were filled with the experimental soil to determine the moisture percentage in FC conditions. The pots were heated in an oven at 90°C for 72 h to measure the dry soil weight and then the pots were saturated with water. Next, pot surfaces were covered to prevent evaporation and the pots were weighed daily until the readings were constant for two days. Moisture percentage at FC was calculated and applied by the difference between the current pot weights and their weights after drying in the oven (Arshadi, 2011). The foliar application was done twice with a one-week interval in the five-leaf stage. At the end of the flowering stage, the plants were collected and transferred to the laboratory to measure the experimental traits.

Measurement of traits and analysis of data

The traits of total sugar (McCready *et al.,* 1950), amino acids (Yemm and Cocking, 1955), proline (Bates *et al.,* 1973), soluble protein (Bradford, 1976), ascorbate peroxidase activity (APX) (Chen and Asada, 1992) and relative water content (RWC) were calculated in this study. To this end, a developed young leaf was separated from the top of the plant; its fresh weight was measured and soaked in water for 24 h (at 4°C in the dark). Then, the saturated weight and dry weight (after 24 h at 75°C) of the leaf were determined using Equation 1 (Ritchie and Neguyen, 1990).

$$RWC= \frac{FW\text{-}DW}{TW\text{-}DW} \times 100 \quad .....(1)$$

Data were analyzed using Minitab 17 software. Mean values were compared with the LSD method at a 5% probability level.

RESULTS AND DISCUSSION

Total sugar

The interaction of drought and foliar application significantly affected the plant's total sugar content (TSC) (Table 1). The treatment with 30% FC and Ca foliar application contained the utmost TSC (0.62 mg/g of leaf fresh weight, LFW). In the other treatments, TSC was lower than 0.55 mg/g LFW. In 30% FC, foliar application of AA significantly increased TSC compared to no application. Similarly, Ca foliar application significantly increased the TSC compared to AA application. Generally, TSC also showed an increasing trend with increasing drought stress intensity from 70% to 30% FC (Table 2). These sugars are a valuable source for storing organic carbon and play a role in osmotic regulation and maintaining osmotic pressure (Chimenti *et al*., 2006). In fact, osmotic regulation occurs more effectively in plants that accumulate soluble sugars in response to drought stress (Slama *et al*., 2007).

Proline

The Guar's proline content was significantly affected by the interaction effect of drought and foliar application (Table 1). The proline content (11.4 μg/g LFW) was maximized in the treatment with 30% FC and no spraying, which was the only treatment in which proline content exceeded 11 μg/g LFW. In treatments with 30 and 50% FC and foliar application of Ca and AA, proline content decreased significantly compared to no foliar application conditions and the same FC levels (Table 2). In these two drought treatments, foliar application with AA decreased the proline content more than that of Ca application. Generally, proline content increases in plants parallel to the rise of damage to cell membranes and the plant tries to mitigate the damage to membranes by increasing the proline level in its tissues (Shinde *et al*., 2016; Xu *et al*., 2022).

Amino acids

The interaction of drought and foliar application significantly affected Guar's amino acid content (Table 1), with the

Table 1: ANOVA for studied traits of Guar.

S.O.V	df	Total sugar	Proline	Amino acid	APX activity	Soluble protein	RWC
Drought stress (D)	2	0.041**	96.5**	2028**	87.2**	49.3**	568**
Spraying (S)	2	0.020**	4.21**	20.5ns	5.47**	16.2**	120**
D × S	4	0.002*	4.83**	57.9**	2.10*	0.439 ns	19.4ns
Error	18	0.0006	0.155	7.06	0.595	1.68	8.67

ns: Non-significant, *Significant in 5% level and **Significant in 1% level.

Table 2: Mean comparisons of interaction of drought stress and spraying on traits of total sugar, proline, amino acid and APX activity in Guar.

Drought stress based on FC (%)	Spraying (3%)	Total sugar (mg gFW^{-1})	Proline (µg gFW^{-1})	Amino acid (mg gFW^{-1})	APX activity (µmol min^{-1} gFW^{-1})
70	Non-spraying	0.38f	2.23g	12.1e	2.50e
	AA	0.40ef	3.60f	18.7d	2.87e
	$CaCO_3$	0.43de	3.53f	18.3d	2.77e
50	Non-spraying	0.44cde	8.50bc	41.2b	7.20c
	AA	0.47c	6.26e	32.4c	5.23d
	$CaCO_3$	0.52b	7.00d	36.4c	4.40d
30	Non-spraying	0.46cd	11.4a	49.6a	10.1a
	AA	0.54b	8.23c	42.8b	8.67b
	$CaCO_3$	0.62a	9.10b	44.7b	8.03bc

Means that have a common letter, have not significantly different together at 5% based on LSD test.

uppermost level (49.6 mg/g LFW) measured in the treatment of 30% FC and no foliar application. Generally, amino acid levels increased with the intensity of drought stress (Table 2). With each drought stress level of 30 and 50% FC, AA and Ca application resulted in a significant decrease of amino acids compared to no foliar application conditions. As with the proline content result, the application of AA and Ca significantly increased amino acid amounts in 70% FC treatment compared to no application conditions (Table 2). The decreased levels of amino acids in moderate and high stresses are apparently caused by the plant's reduced ability to perceive the stress condition or to synthesize new proteins to strengthen the plant's ability to resist drought stress. Plants use drought stress mitigating mechanisms by engineering-like changes in the formation or decomposition of primary and secondary metabolites. Plants adapt to stresses because of changes in the amounts of these metabolites (*e.g.*, amino acids) (EL Sabagh *et al.*, 2019).

APX activity

The Guar's APX activity was significantly influenced by the interaction effect of drought and foliar application (Table 1). There was no significant difference between foliar application levels in the treatment with 70% Fc and at this stress level, APX activity did not reach even 3 µmol/min/g LFW (Table 2). APX activity also increased significantly with an increase in drought stress from 70% to 30% FC. The utmost activity of APX was observed in the treatment with 30% FC and no foliar application (Table 2). APX activity decreased significantly in 50% FC with AA and Ca application. A similar trend to the 50% FC was observed in 70% FC. APX activity decreased significantly with the foliar application of AA and Ca, but there were no significant differences between AA and Ca application levels (Table 2). Based on our findings, AA and Ca application under drought stress conditions probably reduces APX activity. The explanation of this observation is that the foliar application causes the uptake of these compounds through stomata and their accumulation in the apoplast and extracellular environment (Akram *et al.*, 2017; Atta *et al.*, 2022). The AA and/or extracellular Ca form the first line of defense against external oxidants (Zechmann, 2011). Thus, this induces a signal in the plant that the presence of these compounds in intercellular space assists in confronting the oxidants and the plant reduces the expression of the APX gene (Akram *et al.*, 2017). This phenomenon can result in less expense for the plant to express the gene and synthesize less APX to focus more on producing and storing more dry matter.

Soluble protein

The interaction of drought significantly affected Guar's soluble protein content (SPC) (Table 1), which was maximized (>11.5 mg/g LFW) in the 30% FC treatment. In the other two treatments, SPC was lower than 10.2 mg/g LFW (Table 3). SPC was significantly lower in 50% and 70% FC treatments (40.7%) than 30% FC (63.6%). SPC significantly increased by 28.6% in 50% FC relative to 70% (Table 3). The increase of soluble protein can indicate the drought-resistance mechanism in the plant. The effect of AA and Ca foliar application significantly increased Guar's SPC (Table 1 and 4). It has been shown that the external application of some antioxidants such as AA effectively increases the resistance of plants against drought and subsequent secondary stresses such as oxidative stress (Alayafi, 2020).

RWC

The drought stress significantly influenced Guar's RWC (Table 1), with the highest level (>86%) observed in the 70% FC treatment (Table 3). Significantly lower RWC was recorded in 50% (9.36%) and 30% (18.4%) FC treatments than in 70% FC. Moreover, RWC decreased significantly (9.94%) in 30% FC treatment relative to 50% (Table 3). The decreased RWC under drought stress may result from a decrease in leaf water potential, an increase in leaf evaporation and a decrease in root water uptake so that the plant has to face a reduction in RWC in such conditions (Ahmadizadeh, 2013; Jincy *et al.*, 2022) (Table 4). Studies have shown that Ca consolidates the cell membrane and causes more negative osmotic potential by accumulating cytosol-compatible osmolytes and improving RWC (Naeem *et al.,* 2017).

Table 3: Mean comparisons of drought stress on traits of soluble protein and RWC in Guar.

Drought stress based on FC (%)	Soluble protein (mg gFW^{-1})	RWC (%)
70	7.21c	86.5a
50	10.1b	78.4b
30	11.8a	70.6c

Means that have a common letter, have not significantly different together at 5% based on LSD test.

Table 4: Mean comparisons of spraying on traits soluble protein and RWC in Guar.

Spraying (3 %)	Soluble protein (mg gFW^{-1})	RWC (%)
Non-spraying	8.2b	74.4b
AA	10.1a	79.8a
$CaCO_3$	10.8a	81.4a

Means that have a common letter, have not significantly different together at 5% based on LSD test.

CONCLUSION

Altogether, this study showed that a reduction in soil moisture led to increased soluble sugar, proline and APX activity and the application of CC significantly increased the TSC. Proline content and APX activity significantly decreased with decreasing soil moisture and the application of AA and CC. Reduced soil moisture was followed by a significant increase in SPC and a significant decrease in RWC. These results demonstrate that foliar application with AA and CC is useful in mitigating the effects of drought stress on this plant.

Conflict of interest: None.

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