RESEARCH ARTICLE Legume Research- An International Journal, Volume 46 Issue 12: 1590-1596 (December 2023)

Promote the Yield and Physiological Traits in Guar (*Cyamopsis tetragonoloba* L.) by Application of Salicylic Acid and Potassium under Water Stress Conditions

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10.18805/LRF-753

ABSTRACT

Background: Determination of the most important level of salicylic acid to increase guar yield in drought stress conditions.

Methods: In order to study the effect of potassium and salicylic acid on agricultural and physiological traits of guar in different irrigation treatments, a field experiment was conducted during 2020-2021 under the climatic conditions of Jiroft area (Kerman province, Iran) in the farm of research center and agricultural and natural resources education. The experimental treatments include three levels of irrigation (regular irrigation, stress 60 and 80% of the plant's water requirement) in strip plots, three levels of salicylic acid (no application, 0.1 and 0.5 mM) as foliar spraying and two levels of potassium (no application and use of 100% of the plant's need for potassium) in horizontal plots.

Result: The results showed that drought stress and application of potassium and salicylic acid had a significant effect on all traits. Decreasing the amount of water led to a decrease in the physiological and functional characteristics of guar compared to regular irrigation. But potassium and salicylic acid were able to improve the yield characteristics and yield of guar under stress and non-stress conditions and the use of potassium + 0.5 mM salicylic acid were introduced as the best treatment for both conditions. This treatment at 60% stress led to an increase in plant height (91%), the number of leaves (37%), the number of pods (85%), seed number (70%), seed yield (100%), chlorophyll a (61%), chlorophyll b (59%) and carotenoid (100%) compared to the control treatment.

Key words: Chlorophyll, Fertilization, Grain yield, Yield attributes.

INTRODUCTION

Clusterbean (*Cyamopsis tetragonoloba* L.) is an annual, summer and dicotyledonous plant from the legume family (Clemente and Jimenez-Lopez, 2020), which tolerates drought and salinity stress and increases nitrogen by fixing atmospheric nitrogen and soil organic matter (Singla *et al.,* 2016). Therefore, this plant is a suitable option for grain production in arid and semi-arid regions (Trostle, 2020). Due to its protein content, guar flour is considered the main substitute for soy flour in livestock and poultry feed (Chiofalo *et al.,* 2018). Its gum is also used in food, drilling, cosmetic, health and pharmaceutical industries (Gresta *et al.,* 2018).

Drought stress is one of the most important abiotic stresses (Kehl, 2020), which leads to a decrease in cell expansion and changes in the physiological and biochemical processes of plants and affects growth, production and yield (Akbari Moghadam, 2012). Crop management has been reported to be effective in reducing damages caused by various types of stress in plants (Zhoa *et al.,* 2020). One of the types of management is the use of nutrients for the yield and development of plants. Increasing evidence shows that nutrients play an important role in plant resistance to stress and have been introduced as one of the most promising methods to reduce the effect of drought on plants (Hera *et al.,* 2018). One of these elements is potassium. Although potassium is not involved in the construction of any of the important plant compounds, it plays an important role in many physiological processes such as photosynthesis, transfer of plant material to reservoirs, water retention, stomatal conduction, osmotic regulation, enzyme activity, *etc*., (Wang *et al.,* 2013). In the condition of potassium deficiency, the sensitivity of plants to environmental stresses increases. So that the production of reactive oxygen radicals in plants is strongly stimulated (Cakmak, 2005).

In the study of the effect of potassium on grain yield and the concentration of some low-use nutrients in cowpea under drought stress conditions, it was reported that drought stress led to a decrease in yield indicators and the concentration of elements in beans, but the application of

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How to cite this article: Amiri, A., Aien, A., Afsharmanesh, G. and Koorepaz, S. (2023). Promote the Yield and Physiological Traits in Guar (*Cyamopsis tetragonoloba* L.) by Application of Salicylic Acid and Potassium under Water Stress Conditions. Legume Research. 46(12): 1590-1596. doi: 10.18805/LRF-753.

Submitted: 03-05-2023 **Accepted:** 14-09-2023 **Online:** 11-10-2023

potassium reduced the effect of stress and also It sought to improve yield indicators (Zahedi *et al.,* 2018). Investigating the effect of drought stress on the flow of potassium and anions in the filaments of broad bean showed that in water shortage conditions, the entry of potassium ions causes the maintenance of turgor pressure and the expansion of cell growth (Dauphin *et al.,* 2001). In an experiment that investigated the effect of irrigation and potassium sulfate fertilizer on the yield and yield components of green beans, it was found that the highest yield and yield components are obtained with the application of 50 kg/ha of potassium sulfate fertilizer. Also, examining the trend of changes indicated that the effect of potassium sulfate fertilizer on grain yield and green neem yield can be justified (Sharifi *et al.,* 2013).

According to reports, salicylic acid has a significant effect on plant morphology and physiology and plays a role in stimulating protective mechanisms and increasing resistance against living and non-living stresses (Khalvandi *et al.,* 2021). It seems that in water shortage conditions, the use of plant yield regulators such as salicylic acid can be effective as a solution to prevent the destructive effect of drought stress (Parveen *et al.,* 2021). In an experiment on the effect of drought stress and salicylic acid on yield and yield components of red bean genotypes, it was reported that salicylic acid treatment had a significant effect on stem height, number of pods per plant, number of grains per pod and yield of bean grains. Thus, the application of stress caused a decrease in yield, but the application of salicylic acid improved the growth, yield and yield components of red bean genotypes (Sepehri *et al.,* 2015). The results showed that salicylic acid had a significant effect on the number of pods per plant, grain weight, grain yield and biological yield and led to an increase in these parameters (Rajabi *et al.,* 2013).

Since Iran is a dry and semi-arid country, cultivation of industrial plants suitable for hot and dry climate, relatively short growing season, production cost and low water requirement is recommended for establishing a low-input farming system. The resistance of guar plant against harsh weather conditions such as lack of water, stony land and intense light radiation is in accordance with the conditions of Jiroft region and therefore, the purpose of this experiment is to investigate the effect of salicylic acid and potassium on the agronomic and morphological characteristics of guar plant and to introduce the best fertilizer treatment.

MATERIALS AND METHODS

Site description and planting

The experiment was conducted at the Agricultural Research Center in Southern Kerman, Iran (28.54°N, 57.85°E) as a split plot on strips of randomized complete block designs containing three replicates over two years (2020-2021). Experimental treatments included three levels of drought stress: no drought stress regular irrigation:(ND), moderate drought stress: 80% of the plant's water requirement (MD) and high drought stress: 60% of the plant's water requirement (HD), salicylic acid solution according to the doses [0 (NS), 0.1 mM: 138.12 mg l^{-1} (0.1S) and 0.5 mM: 690.6 mg l^{-1} (0.5S)] was applied by foliar application and potassium (no application (NK) and 100 kg/ha application (100K). Drought stress was determined by determining the irrigation cycle and duration based on KC coefficients, crop evaporation and 10-year meteorological statistics. The exact duration of release was calculated using the formula:

$$\text{Moisture demand} = \frac{\text{Evapotranspiration}}{\text{Time}}$$

The planting date was July 20 in both years, the growing season was approximately 100 to 120 days and irrigation was by mechanized drip. The experiment consisted of 18 plots (treatments) in 3 iterations (a total of 54 treatments on land 60 meters long and 15 meters wide (900 square meters). The dimensions of each plot were 2 × 2 square meters (4 square meters), the spacing between each plot was 1.5 meters and the spacing between replicates was 2 meters. Each plot consisted of 6 rows with 30 cm row spacing and 10 cm grain spacing within rows.

Laboratory analysis

Plant height, pod length, leave number, pod number., seed number, seed yield were measured in this research Protein (Pro) content by Kjeldahl apparatus and leaf potassium (K leaf) content by Hemke and Sparks method (1996) were measured. Using Arnon's equation (Arnon, 1949), the amount of chlorophyll was obtained from Equation 1. The amount of carotenoid was also calculated based on the Gross formula (Gross, 1991) and from Equation 2.

Equation 1:

Chl. a (mg/g FW)= [12.7 (A663)-2.69 (A645)]×V/W
Chl. b (mg/g FW)= [22.9 (A645)-4.68 (A663)]×V/W

In these formulas, A is the optical absorbance of the samples, V is the final volume of used acetone and W is the wet weight of the tissue.

Equation 2:

$$\text{Carotenoids (mg/g)} = \frac{A \times V \times 10^6}{A_{1cm}^{1\%} \times 100 \times W}$$

Statistical analysis

All parameters were tested using analysis of variance (ANOVA). A two-way ANOVA was used to determine the effect of two concentrations of potassium and salicylic acid on guar at different irrigation levels (three levels). In addition, we performed parameter correlation analysis using linear regression models and PCR. Some datasets were log-corrected to meet the ANOVA requirements for normality and homogeneity of variance. Several comparisons were made across sub datasets using Duncan's test.

RESULTS AND DISCUSSION

The results of comparing the averages showed that the height of the plant, leave number and pod number decreases with the decrease in the amount of available water (Table 1). So that the highest height of the plant with an average of 121.5 cm belongs to the treatment of regular irrigation × 100% requirement of potassium and 0.5 mM salicylic acid. While the lowest height with an average of 45.8 cm is related to the stress 60% of the water requirement along with no application of potassium and salicylic acid. Therefore, despite guar plant being resistant to drought, lack of water had a negative effect on the height of the plant, moreover leave number and pod number followed this pattern (Table 1). Consumption Application of potassium and salicylic acid reduced the negative effect of dryness. So that the application of 100% of potassium requirement in combination with 0.5 mM salicylic acid in the treatments of 80% and 60% of water requirement led to an increase of 74% and 91% respectively in the height of guar plant compared to no application. The results indicated that the height of the plant was not significant in the control irrigation and the lack of salicylic acid and potassium consumption with 80 and 60% water requirement stress and the application of potassium and 0.1 mM salicylic acid (Table 1). This shows the importance of using potassium and salicylic acid to withstand stress in guar plant. It has been reported that the accumulation of potassium on the cell surface leads to the osmotic absorption of water and the production of turgor pressure required for the opening of stomata and growth (Shabbir Dar *et al.,* 2021). The increase in the height of the chickpea plant due to spraying with salicylic acid has also been reported. So that the increase in the height of the plants has been attributed to the positive effect of salicylic acid in increasing the cell division of the terminal meristem of the stem and root (Salek Meraji and Hatami, 2020). The effectiveness of salicylic acid in inducing tolerance to stress depends on the type of plant or its concentration (Zafar *et al.,* 2021) and in the present study, the use of 0.5 mM salicylic acid compared to 0.1 mM was superior in all levels of irrigation and potassium. The number of leaves in plants depends on the height of the plant and the number of branches (Darvizheh *et al.,* 2019). In the present study, with the increase in water stress, while the height of the plant and the number of branches decreased, the number of leaves decreased compared to the control irrigation treatment. The highest number of leaves with an average of 213.5 was assigned to the treatment of regular irrigation × 100% requirement of potassium × 0.5 mM salicylic acid.

Table 1: Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on plant hieght, leave number, pod number, leaf potassium and pod length (mm) in guar during 2020-2021.

Treatment	Hieght (cm) Mean 2020-21	Leave number Mean 2020-21	Pod number Mean 2020-21	Leaf potassium Mean 2020-21	Pod length (mm) Mean 2020-21
ND+NK+NS	86.87	169	45.43	1.76	9.24
ND+NK+0.1S	97.80	186	56.25	1.45	9.73
ND+NK+0.5S	106.68	178.5	68.03	1.64	10.33
ND+100K+NS	99.46	187.5	67.21	1.04	9.71
ND+100K+0.1S	114.99	197.5	76.38	1.11	10.69
ND+100K+0.5S	121.50	213.5	86.25	1.24	11.22
MD+NK+NS	45.87	134.5	30.88	2.99	4.02
MD+NK+0.1S	56.21	144.5	37.93	2.74	4.64
MD+NK+0.5S	70.13	153	46.23	3.18	5.33
MD+100K+NS	57.92	163	40.28	3.28	4.68
MD+100K+0.1S	78.89	173	46.35	3.44	5.77
MD+100K+0.5S	87.54	184.5	56.95	3.65	6.18
ND+NK+NS	55.69	147	35.85	2.04	6.95
HD+NK+0.1S	63.86	157.5	45.30	2.10	7.64
HD+NK+0.5S	74.55	166	54.91	2.25	8.26
HD+100K+NS	67.87	175.5	44.75	2.44	7.66
HD+100K+0.1S	87.05	184	59.30	2.62	8.70
HD+100K+0.5S	96.22	192.5	66.38	2.83	8.92
Mean Squar	17*	131*	15.6*	0.02n.s	0.04*
LSR	4.62	4.95	4.95	-	0.19
CV (%)	2.8	1.4	4.6	5.6	1.4

Note 1. ND: No drought; MD: Moderate drought; HD: High drought; NK: No pottasium application; NS: No salicylic acid; 0.1S: 0.1 Milli Molar salicylic acid; 0.5S: 0.5 milli molar salicylic acid.

2. Mean within columns followed by different letter are significantly different (Duncan's multiple test, P≤0.05).

3. n.s: Non-significant; *: Significant at 5% probability levels; **: Significant at 1% probability levels.

The lowest number of leaves was also obtained in the treatment of 60% water requirement × no potassium and salicylic acid consumption, an average of 134.5 (Table 1). Decreasing the amount of water by reducing the water potential reduces the number of plant leaves. It seems that drought stress affects the formation of primary leaf cells and their differentiation and causes a decrease in the number of leaves (Ghorbani *et al.,* 2019). In the research on the use of chemical fertilizers with high potassium percentage, an increase in the number of leaves of the sweet medicinal plant was observed (Arvin, 2019). The reason for this can be related to the potassium element, which plays an important role in the formation and production of the number of leaves of plants under stress conditions. By influencing the yield and development process, potassium can improve the yield characteristics of plants due to the positive role of K^+ in the stability of enzymes and proteins under stress conditions (Haider Bukhari *et al.,* 2021).

The minimum pod length with an average of four centimeters was observed in the treatment of 60% water requirement × no potassium consumption × no salicylic acid consumption (4.02 mm). The maximum pod length with an average of 11.2 mm was recorded for the treatment of regular irrigation × 100% plant requirement for potassium × 0.5 mM salicylic acid. The reduction in the pod length in the treatments of 60 and 80% of water requirement and the absence of potassium and salicylic acid compared to their application (0.5 mM salicylic acid and potassium) for the treatment of 60 and 80% of water requirement, 28 and 50%. It was recorded in order and percentage (Table 1). The highest leaf potassium was found in the high drought stress under high application of potassium and salicylic acid in two years. Moreover, the lowest leaf potassium content was observed in the no drought stress and salicylic acid.

According to the comparison results, the average number of grains was affected by water deficit stress. So, with the decrease in the amount of available water, the amount of these traits decreased. On the other hand, with the use of potassium and salicylic acid, improvement of the number of seed was observed under water stress conditions. The lowest number of grains with an average of 3.38 (No.) was observed in the treatment of 60% water requirement without application of potassium and salicylic acid. The highest number of grains was also recorded with an average of 7.5 (No.) for regular irrigation treatment × 100% requirement with application of potassium and 0.5 mM salicylic acid (Table 2). Guar seed yield, as the most important trait, was affected by drought stress. So that the

Table 2: Average comparison of the three effects of drought stress, salicylic acid and potassium treatment on seed yield, seed number, chlorophyll a, chlorophyll b and cartnoied in guar during 2020-2021.

Treatment	Seed yield (kg/ha^{-1}) Mean 2020-21	Seed number Mean 2020-21	Chlorophyll a (mg/gr) Mean 2020-21	Chlorophyll b (mg/gr) Mean 2020-21	Cartnoied (mg/gr) Mean 2020-21
ND+NK+NS	1740	4.53	0.55	0.76	4.40
ND+NK+0.1S	2085	4.98	0.62	0.85	5.40
ND+NK+0.5S	2415	5.71	0.69	0.94	5.95
ND+100K+NS	2025	5.38	0.53	0.78	4.76
ND+100K+0.1S	2850	6.22	0.78	1.02	6.48
ND+100K+0.5S	3860	7.50	0.88	1.17	7.45
MD+NK+NS	1165	3.38	0.41	0.58	2.83
MD+NK+0.1S	1485	3.43	0.48	0.68	3.68
MD+NK+0.5S	1815	4.63	0.53	0.78	3.71
MD+100K+NS	1395	3.51	0.44	0.61	2.73
MD+100K+0.1S	2250	4.68	0.60	0.83	4.71
MD+100K+0.5S	2850	5.60	0.66	0.92	5.80
HD+NK+NS	1470	3.70	0.43	0.70	3.65
HD+NK+0.1S	1785	4.60	0.50	0.77	4.58
HD+NK+0.5S	2120	5.20	0.57	0.78	4.95
HD+100K+NS	1850	4.38	0.51	0.71	3.90
HD+100K+0.1S	2630	5.61	0.63	0.95	5.66
HD+100K+0.5S	3130	6.23	0.78	1.09	6.67
Mean Squar	0.7*	0.2*	0.005**	0.002**	0.2*
LSR	280	0.52	0. 03	0.04	0.34
CV(%)	7	5.4	3.4	3	3.6

Note 1. ND: No drought; MD: Moderate drought; HD: High drought; NK: No pottasium application; NS: No salicylic acid; 0.1S: 0.1 Milli Molar salicylic acid; 0.5S: 0.5 Milli molar salicylic acid.

2. Mean within columns followed by different letter are significantly different (Duncan's multiple test, P≤0.05).

3. n.s: Non-significant; *: Significant at 5% probability levels; **: Significant at 1% probability levels.

yield of the plant in the stresses of 80 and 60% of water requirement was reduced by 16 and 33%, respectively, compared to regular irrigation and in the condition of not using potassium and salicylic acid. The highest seed yield with an average of 3860 kg per hectare belonged to regular irrigation treatment along with application of potassium and 0.5 mM salicylic acid. The lowest seed yield was also observed in the treatment of 60% water requirement without application of potassium and salicylic acid. with an average of 1165 kg per hectare (Table 2). According to a research, water stress had an effect on all stages of mung bean plant growth and development. So that its yield decreased under stress conditions by reducing the number of grains, the number of grains and the weight of one hundred grains (Ghallab *et al.,* 2007). In addition, in another study, the lowest number of pod and the number of red bean grains were observed in the treatment of no application of salicylic acid and the highest amount was observed in the application of 0.7 mM salicylic acid (Shoghian and Rozbahani, 2017).

Salicylic acid has a positive effect on photosynthesis and plant growth indicators on yield components, which is especially beneficial for plants under water stress conditions (Nezhad *et al.,* 2014). Potassium, like salicylic acid, has an effect on nitrogen and protein metabolism, the activation of many enzymes, especially Rubisco, the amount of chlorophyll and the formation of a strong structure of chloroplasts and dozens of other physiological processes leading to increased yield. Also, researches showed that in the conditions of water shortage, potassium consumption can prevent the reduction of plant yield and yield components (Shabbir Dar *et al.,* 2021; Gomaa *et al.,* 2021). It has been stated that due to the growth of the guar plant in tropical regions, excessive heat or water stress, the affinity of Rubisco enzyme with oxygen has increased compared to carbon dioxide, causing an increase in photorespiration and a decrease in net photosynthesis production. Finally, the grain yield decreases (Pedersen and Lauer. 2004). It has also been reported that yield reduction is due to the negative effect of water stress on the number of branches and leaf surface, which leads to a decrease in carbon fixation and photosynthesis (Khan *et al.,* 2002). The study of the effect of salicylic acid foliar spraying on water stress showed that salicylic acid leads to improvement of chlorophyll index, relative water content of leaves, improvement of electrolyte leakage and as a result increase of grain yield (Tarigholeslami *et al.,* 2016).

Dehydration stress on the one hand leads to a decrease in the amount and efficiency of photosynthetic pigments, which consequently leads to a decrease in plant yield and yield components and on the other hand, with the simultaneous application of salicylic acid and potassium in both regular irrigation and stress conditions improved the growth and yield characteristics of guar by increasing the content of chlorophyll. Considering the increase in grain yield and morphological characteristics of cluster bean plant in regular irrigation treatment and in combination with 100% requirement of potassium and 0.5 mM salicylic acid, the highest amount of chlorophyll a, b and carotenoid with an average of 88. 0, 1.17 and 7.45 mg per gram of leaf fresh weight were observed in the mentioned treatment (Table 2). Stress treatments of 80 and 60% of water requirement and no application of potassium and salicylic acid compared to the mentioned treatment, respectively 51 and 53% reduction in the amount of pigment a, 41 and 50% reduction in pigment b and 51 and 62% reduction in showed carotenoid content. While the application of 100% potassium requirement and 0.5 mM salicylic acid increased the amount of chlorophyll a, b and carotenoid in 80% water requirement stress to 81, 58 and 82% respectively and in 60% water requirement stress to 61, 59 and 100% compared to their non-use in both conditions increased the tension (Table 2).

Since the physiological activities of the plant are disturbed directly or indirectly under water stress conditions, it affects the amount of photosynthesis of the plant and the photosynthetic chlorophyll, especially the chlorophyll pigment that is used in plants as it reduces one of the most important factors of maintaining photosynthetic capacity (Sattar *et al.,* 2020). According to the present results, it is reported that the content of photosynthetic chlorophyll of guar plant decreases under water stress conditions. While plant nutrition management in these conditions seeks to improve photosynthetic capacity (Norouzi *et al.,* 2021). Increasing the content of photosynthetic chlorophyll in stress conditions and with the use of potassium (Gomaa *et al.,* 2021) and salicylic acid (Zafar *et al.,* 2021; Nezhad *et al.,* 2014) in other researches. It has been reported to be sharp.

CONCLUSION

In general, potassium and salicylic acid improve physiological activities such as playing an important role in improving photosynthetic pigments, improve growth and yield in stress-free conditions and increase resistance to drought stress.

Conflict of interest

All authors declare that they have no conflicts of interest.

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