# SEED PRIMING WITH β-AMINOBUTYRIC ACID (BABA) IMPROVED PRODUCTION OF CHICKPEA GENOTYPES BY OPTIMIZING ANTIOXIDANT ACTIVITY UNDER DIFFERENT MOISTURE CONDITIONS

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(Received 20th Sep 2022; accepted 27th Apr 2023)

Abstract. Chickpea is one of the drought tolerant leguminous crops mostly cultivated in the arid region of Pakistan. Global warming is the main cause of changing rainfall pattern in the arid regions, which indirectly affecting the production of chickpea.  $\beta$ -aminobutyric acid (BABA) is known as the drought mitigating agent mostly used for boosting the production of drought susceptible plants. For checking the effect of BABA on the growth, yield and quality traits of chickpea cultivars against drought stress, a field experiment was conducted at the research field of B.Z.U. Bahadur Sub-Campus Layyah, Pakistan (latitude-32°N, longitude-73°E and altitude-184 msl) during rabi seasons 2016-17. For this purpose chickpea cultivars (Noor 2013 and Bhakkar 2011) were tested under irrigated as well as rainfed condition. Different doses of BABA (Dry seed, Hydropriming, 1 mM, 2 mM and 3 mM) were applied by seed priming method. Findings of this research study elaborate that irrigated condition enhanced all the growth and yield traits as well as total protein contents but decrease the antioxidants in comparison to the rainfed condition. Noor 2013 performed best by increasing germination count, no. of pods plant<sup>-1</sup>, no. of seeds plant<sup>-1</sup>, harvest index, days to anthesis, superoxide dismutase, peroxidase, catalase and total phenolic contents. Whereas, Bhakkar 2011 showed the highest performance for no. of primary branches per plant, biological yield, grain yield, thousand seed weight and total soluble protein. 2 mM of BABA is the best dose for all the growth and yield traits, while maximum antioxidant activity was found by the application of 3 mM of BABA. In short, cultivation of both chickpea cultivars should be promoted in irrigated areas. Priming with 2 and 3 mM of BABA is the best solution for minimizing the impact of moderate drought stress.

Keywords: Cicer arietinum (L), rainfed, economic analysis, enzymes, quality traits

#### Introduction

Among all the leguminous crops pulses are very imperative food crops playing a significant role in the economy of the country. Pulses are widely grown and suitable in all environments including rainfed conditions, arid, semi-arid and temperate climates (Didinger and Thompson, 2021). Pulses not only have a higher protein concentration than other cereal crops but also provide food for about 20% of the world's population (Kumar Shukla et al., 2013). Chickpea (*Cicer arietinum* L.) is globally the third largest and very nutritious cultivated leguminous crop (Yegrem, 2021; Barik, 2021). In Pakistan, two main types of chickpeas are being cultivated. About 85% of the chickpea genotypes are of the Desi type which have smaller seeds having angular shape and dark seed coat, while the remaining 15% are of the Kabuli type which have large seeds having smooth and beige colored seed coat (Mohammadi, 2015). Similarly, it serves as source of protein for peoples who cannot purchase animal meat. As chickpea is a drought tolerant crop, therefore, growers of the Pakistan cultivate this crop at the end of monsoon season or as dobari crop (grown on residual moisture after the harvest of rice

crop) to utilize the residual soil moisture. Chickpea has been demanded for its nutritional grain with 25–28% protein (Soomro et al., 2021).

It is mostly (>80%) cultivated under rainfed conditions, where water deficit owing to uncertainty in rainfall patterns is generally the major yield limiting factor (Khan et al., 2019). Climate models project declining rainfall frequency allied with rising temperatures, which will further increase the gap between actual and potential yield (Rani et al., 2020). Improving the yield under water deficit conditions appears to be a rather difficult undertaking, requiring a solid understanding of the underlying traits. The water deficit-induced reduction in plant growth and productivity is underlain by a diverse range of processes. For instance, water deficit impedes photosynthesis through a reduction in both chlorophyll content and photosynthetic efficiency (Mafakheri et al., 2010). In addition, water deficit triggers osmotic stress, which in the absence of osmotic adjustment (e.g. by proline accumulation) impedes enzyme activity and harms macromolecules' structure (Mwadzingeni et al., 2016; Hassanvand et al., 2019).

Beta amino butyric acid (BABA) are the non-proteaceous molecules used as priming agents in the plants to improve drought stress resistance in the plants under field conditions. These secondary metabolites improve the plant growth in drought stress environments and enhance plant yield attributes under drought stress conditions (Tabassum et al., 2017). A number of studies have found the positive impact of beta amino butyricacid to create resistance against drought stress under field environments. Beta aminobutyric acid is now considered a major resistive agent that improves plant immune system and helps plant to tolerate against drought stress (Wasaya et al., 2021; Abid et al., 2021). Several scientists have revealed that beta aminobutyric acid has ability to create tolerance against various abiotic stresses including drought stress and salt stress as well as oxidative stress (Jisha and Puthur, 2016). We were motivated to evaluate the tolerance mechanisms of BABA in chickpea under drought stress based on the beneficial effects of beta aminobutyric acid in producing resistance against drought stress in other plants. A positive water potential due to beta aminobutyric acid priming was observed in plants under drought stress (Jakab et al., 2005) as well as better yield was obtained in apple and wheat due to BABA application (Tworkoski et al., 2011; Quero et al., 2015). Beta aminobutyric acid application was noticed most effective to increase the plant growth mechanisms under drought stress environments (Jiang et al., 2012). Beta aminobutyric acid primed wheat plants exposed to drought stress displayed decreased aggregation of reactive species, enhanced activity of antioxidant enzymes and minimal osmotic stress damage to cell membrane (Du et al., 2012). Therefore, information regarding the effect of BABA on the physiological, growth, yield and quality traits of chickpea against drought stress is still unknown. The aim of this study was to evaluate the performance of Desi and Kabuli chickpea cultivars under irrigated as well as rainfed condition and the effect of seed priming with BABA on the growth and development of chickpea affected by different drought stress conditions.

## Material and methods

This field experiment was performed at the research field of B.Z.U. Bahadur Sub-Campus Layyah, Pakistan (latitude-32°N, longitude-73°E and altitude-184 msl) during rabi season (winter season) 2016-17. The soil physio-chemical properties of the experimental site were tested, before sowing, to assess fertility status. Sandy loam soil texture of research field having pH (8.5), electrical conductivity (0.18 dS/m), organic matter content (0.57%), Nitrogen content (0.034%), phosphorus (5.8 mg kg<sup>-1</sup>) and potassium (96 mg kg<sup>-1</sup> soil) respectively. Weather data for whole crop season of three years is given in *Figure 1*.

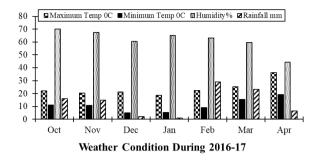


Figure 1. Weather details of experimental site

### **Planting material**

Seeds of two chickpea varieties Noor-2013 (Kabuli type) and NIAB-2016 (Desi type) were obtained from Ayyub Agriculture Research Institute (AARI) Faisalabad, Pakistan and Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, Pakistan respectively.  $\beta$ -Aminobutyric acid (C<sub>4</sub>H<sub>9</sub>NO<sub>2</sub>) was purchased from national dealer of Sigma-Aldrich Pakistan.

## **Experimental details**

The experiment was planned under field conditions in three replicates following randomized complete block design with split-split plot arrangement. The component of drought, which consists of irrigated and rainfed treatments, was kept under main plot. The irrigated treatment received 242.6 mm water (92.6 mm rainfall + 150 mm supplementary irrigation), whereas the rainfed treatment received 192.6 mm water (92.6 mm rainfall + 100 mm supplementary irrigation). Supplementary irrigation was applied in the rainfed treatment at the time of field preparation and in the months of December and January in order to avoid drying of the plants. The varietal component (Noor-2013 and Bhakkar-2011) was randomized in sub plots, whereas seed priming with different levels of BABA (0 mM, 1.0 mM, 2.0 mM and 3.0 mM) were taken in account in sub-sub plots. The data regarding weather attributes which include maximum and minimum temperature, relative humidity and occurrence of rainfall during crop growth period is given in *Figure 1*.

## Crop husbandry

Hand drill was used for sowing purpose with seed rate of 80 kg/ha by maintaining the 30 cm row to row distance. The seed of both chickpea varieties was sown on October 23, 2016. Net plot size was maintained  $1.2 \text{ m} \times 5 \text{ m}$ . Fertilization was done on soil nutrient requirement according to soil analysis report. Di-ammonium phosphate (DAP) with 45% phosphorous and 18% nitrogen was given with 56 kg/ha rate while nitrogen was given in less amount because it fixes atmospheric nitrogen through biological nitrogen fixation with rate of 22 kg/ha and potassium was given in the form of Sulphate of Potash (SOP) having 50% potassium with rate of 60 kg/ha. All the fertilizers were given to crop as basal dose.

#### **Observations and measurements**

### Morphological and yield traits

Germination count was recorded three times after ten days of sowing from each subplot with the help of  $1 \text{ m}^2$  quadrate and their means were calculated. Randomly, ten plants were chosen out of every experimental unit at crop maturity and then data of plant height, no. of primary branches, no. of pods per plant and seeds per pod were collected. Height of theses selected plants was measured by using meter scale and then averaged to measure the average plant height of each treatment. While primary branches, pod per plant and seed per pod were counted manually. After harvesting and before the separation of pods from the plants weight of plants harvested from 1 m<sup>2</sup> was measured by weighing balance and then converted in to t/ha to measure the biological yield of each experimental treatment. After the threshing of pods weight of grains of plants harvested from 1 m<sup>2</sup> was measured by weighing balance and then converted in to t/ha to measure the grain yield of each experimental treatment. After that, thousand seeds were counted by digital seed counter from each experimental treatment and weight of these thousand grains was measured with digital weighing balance to observe the 1000 grain weight. Harvest index (HI) % was measured by the ratio of seed yield to biomass yield by following formula:

Crop was weekly visited till start of anthesis stage and total number of days were counted from sowing to anthesis start to identify the number of days to anthesis start. Similarly, to identify the number of days to pod setting crop was also visually visited at weekly bases and total number of days from sowing to pod setting were counted.

#### Plant growth and development

#### *Leaf area index (LAI)*

LAI was measured from 60 days after sowing (DAS) to 105 DAS with 15 days interval. Plants from 1 m<sup>2</sup> area were harvested at each interval and leaves of these plants were separated and eight to measure the fresh weight of leaves. After this about 3 g of leaves from each experimental treatment were separated and their length and width were measure by scale and then leaf area was determined by multiplying this length and width with coefficient factor 0.92 (Watson, 1947). LAI was calculated by the following equation:

$$LAI = \frac{LA1}{LA2}$$
(Eq.2)

where LA1 designated for leaf-area and LA2 = land-area.

#### *Leaf area duration (LAD)*

LAD was totally dependent on the LAI. After measuring the LAI at each harvest then LAD was obtained following equation (Hunt, 1978):

$$LAD = \frac{LAI1 + LAI2}{2} \times t2 - t1 \tag{Eq.3}$$

where LAI<sub>1</sub> designated for Leaf-area-index of first samples, LAI<sub>2</sub> designated for Leafarea-index of second samples, T1 designated for time of sampling-1<sup>st</sup>, T2 designated for time of sampling-2<sup>nd</sup>.

# Growth rate of crop, $CGR(g.m^{-2} day^{-1})$

Fresh leaves of 1 m<sup>2</sup> area were oven-dried at 75°C till persistent weight was gained and then dry weight of these leaves was measured. Then CGR was measured by ratio of difference of dry weights of two harvests to the time interval. CGR was calculated using under given formula (Hunt, 1978):

$$CGR = \frac{W2 - W1}{t2 - t1}$$
 (Eq.4)

whereas W1 designated for weight of dry leaves at first harvest, W2 designated for weight dry leaves at  $2^{nd}$  harvest,  $t_2$ - $t_1$  designated for interval of time between two consecutive harvests.

### **Biochemical parameters**

### Superoxide dismutase, SOD (IU min<sup>-1</sup> mg of protein<sup>-1</sup>)

Capacity of enzymes to reduce the Photo-chemical reduction of nitro-blue tetrazolium chloride (NBT) is called as superoxide dismutase (SOD) and was measured by Bayer and Fridovich (1987). At 560 nm wavelength waves absorbance of the reaction mixture was notices and about 50% reduction of NBT photoreaction rate is considered as 1 unit of SOD. Unit of SOD was taken as EU mg<sup>-1</sup> of protein.

# Peroxidase, POD $(m.mol min^{-1} mg of protein^{-1})$

The degree of POD resolved regarding thio-barbituric acid-reactive substances (TBARS), with slight modifications, as per method defined by Carmak and Horst (1991). Leaf samples (approximately 1 g) were taken and homogenized in tri-chloro acetic acid (TCA, 3 ml having 1.0% w/v concentration) at a temperature of 4°C. It was then, centrifuged at 20,000 × for a time of 15 min. After that, supernatant (0.5 ml) was taken and added to thio-barbituric corrosive (TBA) solution (3.0 ml having 0.5% v/v concentration) in tri-chloro acetic acid (20%). Afterward, incubation of solution was completed at a temperature of 95°C by continuous shaking for a time period of 50 min and then cooling the cylinders in an ice-water. It was followed by centrifuging the samples at 9000 × for a period of 10 min. Supernatant was collected and its absorbance was taken out at 532.0 nm. The convergence of thiobarbituric acid-reactive substances was determined by using the method given by Carmak and Horst (1991) by utilizing the assimilation coefficient at 155.0 m.m<sup>-1</sup> cm<sup>-1</sup>.

# Catalase, CAT ( $\mu$ .mol min<sup>-1</sup> mg of protein<sup>-1</sup>)

With slight modifications, as per method of Aebi (1983), the activity of Catalase enzyme was determined. Homogenization of 100 mg leaves tissues in 5 ml of 0.1 molar phosphate buffer having pH of 6.40, in a cold pestle and mortar. Centrifugation of the extract was achieved at  $10,000 \times$  for a time period of 20 min at a temperature of 4°C temperature. For enzyme assay supernatant was obtained. About 3 ml solution was made having 2.6 ml of 0.1 molar phosphate buffer having pH 6.4,

0.2 ml of 0.1 ml enzyme extract and 10 mM solution of hydrogen peroxide to form a reaction mixture. This mixture was stirred at a room temperature of  $25^{\circ}$ C. Variations of absorbance were noticed at 230.0 nm wave length for a time period of 15 s for 2 min on an ultraviolet-visible-spectrophotometer. Decomposition of one nano mole of hydrogen peroxide in 1 min was considered as unit of catalase activity with extinction coefficient 36 mM cm<sup>-1</sup>.

# Total phenolic contents, $TPC(mg.g^{-1})$

According to Folin–Ciocalteu technique (Singleton and Lamuela-Raventos, 1999; Singleton and Rossi, 1965) the total phenolic contents were determined. 0.1 mM to 1.0 mM standard solutions of TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid) were prepared in methanol were prepared. In order to calculate total phenolic contents in seeds (WS), firstly, the seed coat percentage, SCP was measured. To estimate this, seed coat, SC were detached from de-hulled seeds, DS and then weighed. Seed coat % was measured by using the given formula:

$$SCP = seed \ coat \ weight/Ws \times 100$$
 (Eq.5)

TPC were measured by the following formula:

$$TPC = TPC$$
 in seed coat  $\times$  seed coat percentage/100 (Eq.6)

### *Total soluble protein concentration* $(mg.g^{-1})$

Determination of protein (total soluble proteins) was done according to Bradford (1976) method. Homogenization of 2 g leaves was done with 8 ml of ice cold, 10 mM solution of Tris hydro chloric acid, with basic pH, 20 mM solution of magnesium chloride, 10 mM solution of sodium hydrogen carbonate, 1 mM solution EDTA, 2 mM solution of PMSF, 12.5% glycerol, 20 mM solution of beta-mercaptoethanol and 120 mg of Polyclar. After this homogenization centrifugation of subsequent was done at 12000  $\times$  g for 30 min and then spectrophotometrically total soluble proteins were determined.

#### Statistical and economic analysis

The data were analyzed by Statistix 8.1 and means were compared by using LSD test at  $p \le 0.05$ . Graphs were made in Microsoft Office 2016 by using MS-Excel. Economic analysis was done to check the economic value of chickpea cultivation under irrigated and rainfed areas by adding different levels of BABA seed priming. To calculate the total cost of growing chickpeas, various expenses such as land rent, seedbed preparation, seed purchase, BABA and fertilizer application, irrigation, and harvesting were considered. The net income was calculated by subtracting all the expenses from the gross income, while the benefit-cost ratio was obtained by dividing the gross income by the total production cost, which included all the expenses incurred from sowing to harvesting (Shahzad et al., 2017).

## Results

In this experimentation two chickpea cultivars were grown under two water available conditions (irrigated and rainfall). In order to test the effectiveness of BABA to promote

production of chickpea under different water regimes, the seed of both chickpea cultivars was primed with different concentrations of BABA. The analysis of variance (ANOVA) showed that the solo utilization of irrigation resources and BABA dosages exhibit significant differences among all the growth, yield and quality traits of chickpea except harvest index and days to pod setting. These two traits were non-significantly affected by irrigation resources and BABA application, respectively. While, chickpea cultivars showed non-significant differences on their germination count, plant height, no. of pods per plant, days to anthesis and days to pod setting. On the other hand, combined study of all the three factors showed non-significant results except thousand seed weight of chickpea (*Tables 1, 2* and *3*).

Source of variation	df	GM	PH (cm)	PB	PP	BY (t/ha)	SP	GY (t/ha)
Replication (R)	2	0.02	121.20	0.05	14.55	0.02	50.01	0.02
Drought (D)	1	96.27*	$72.40^{**}$	3.36**	385.07**	3.74**	1217.25**	$0.56^{**}$
Error R×D	2	3.02	214.60	0.02	9.12	0.07	20.44	0.00
Varieties (V)	1	3.27 <sup>ns</sup>	16.00 <sup>ns</sup>	$0.64^{**}$	1.07 <sup>ns</sup>	0.17**	$2.30^{*}$	0.09**
D×V	1	0.07 <sup>ns</sup>	0.00 <sup>ns</sup>	0.01 <sup>ns</sup>	1.07 <sup>ns</sup>	0.05 <sup>ns</sup>	0.61 <sup>ns</sup>	0.01 <sup>ns</sup>
Error (R×D×V)	4	1.32	15.80	0.02	2.67	0.14	5.75	0.01
β-aminobutyric acid (B)	4	44.02**	$14.70^{**}$	$8.70^{**}$	71.32**	$1.94^{*}$	336.02**	0.35**
D×B	4	2.52 <sup>ns</sup>	$0.70^{**}$	$0.41^{**}$	$7.07^{**}$	0.05 <sup>ns</sup>	30.54**	$0.02^{**}$
V×B	4	3.43 <sup>ns</sup>	0.10 <sup>ns</sup>	0.11 <sup>ns</sup>	0.82 <sup>ns</sup>	0.04 <sup>ns</sup>	2.14 <sup>ns</sup>	0.00 <sup>ns</sup>
D×V×B	4	0.90 <sup>ns</sup>	0.00 <sup>ns</sup>	0.18 <sup>ns</sup>	2.40 <sup>ns</sup>	0.06 <sup>ns</sup>	8.48 <sup>ns</sup>	0.00 <sup>ns</sup>
Error (R×D×V×B)	32	2.67	0.20	0.10	2.13	0.06	3.67	0.01

**Table 1.** Mean sum of square with significance level of ANOVA for the influence of drought and  $\beta$ -aminobutyric acid on the growth and yield traits of chickpea cultivars

\*\*Highly significant < 0.01, \*Significant < 0.05 and <sup>ns</sup>Non-significant; df = degree of freedom, GM = germination count, PH = plant height, PB = no. of primary branches, PP = no. of pods  $plant^{-1}$ , BY = biological yield, SP = no. of seeds  $plant^{-1}$ , GY = grain yield

**Table 2.** Mean sum of square with significance level of ANOVA for the influence of drought and  $\beta$ -aminobutyric acid on the yield and quality traits of chickpea cultivars

Source of variation	df	TSW (g)	HI	DA	DPS	SOD	POD	CAT
Replication (R)	2	2.07	7.38	19.00	7.50	8.85	0.11	4.58
Drought (D)	1	2124.15**	6.14 <sup>ns</sup>	166.70**	163.40**	2970.88**	41.58**	1964.82**
Error R×D	2	1.40	4.63	1.20	2.40	4.93	0.09	4.46
Varieties (V)	1	984.15**	15.02**	0.10 <sup>ns</sup>	8.80 <sup>ns</sup>	95.76**	4.16**	152.48**
D×V	1	236.02**	11.99 <sup>ns</sup>	9.60 <sup>ns</sup>	10.40 <sup>ns</sup>	0.08 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00 <sup>ns</sup>
Error ( $R \times D \times V$ )	4	10.93	3.94	1.80	9.10	1.22	0.03	1.08
$\beta$ -aminobutyric acid (B)	4	1756.31**	9.75**	9.10**	12.60 <sup>ns</sup>	626.91**	13.86**	$605.48^{**}$
D×B	4	116.11**	1.81 <sup>ns</sup>	2.70 <sup>ns</sup>	9.10 <sup>ns</sup>	109.52**	$0.79^{**}$	46.12**
V×B	4	50.61**	2.66 <sup>ns</sup>	3.50 <sup>ns</sup>	8.90 <sup>ns</sup>	2.83 <sup>ns</sup>	0.07 <sup>ns</sup>	1.75 <sup>ns</sup>
$D \times V \times B$	4	32.14**	3.61 <sup>ns</sup>	1.00 <sup>ns</sup>	8.00 <sup>ns</sup>	0.67 <sup>ns</sup>	0.03 <sup>ns</sup>	1.06 <sup>ns</sup>
Error (R×D×V×B)	32	11.04	3.05	2.90	6.90	2.78	0.07	2.15

\*\*Highly significant < 0.01, \*Significant < 0.05 and <sup>ns</sup>Non-significant; df = degree of freedom, TSW = thousand seeds weight, HI = harvest index, DA = days to anthesis, DPS = days to pod setting, SOD = superoxide dismutase, POD = peroxidase, CAT = catalase

Source of variation	df	ТРС	TSP
Replication (R)	2	4.20	0.20
Drought (D)	1	830.97**	$107.00^{**}$
Error R×D	2	3.35	0.20
Varieties (V)	1	119.88**	10.20**
$D \times V$	1	1.85 <sup>ns</sup>	1.10 <sup>ns</sup>
Error (R×D×V)	4	1.29	0.10
β-aminobutyric acid (B)	4	502.63**	$40.10^{**}$
D×B	4	18.99**	$1.00^{**}$
V×B	4	2.43 <sup>ns</sup>	$0.60^{**}$
$D \times V \times B$	4	1.08 <sup>ns</sup>	0.30 <sup>ns</sup>
Error (R×D×V×B)	32	2.48	0.10

**Table 3.** Mean sum of square with significance level of ANOVA for the influence of drought and  $\beta$ -aminobutyric acid on the quality traits of chickpea cultivars

\*\*Highly significant < 0.01, \*Significant < 0.05 and <sup>ns</sup>Non-significant; df = degree of freedom, TPC = total phenolic contents, TSP = total soluble protein

#### Growth and yield traits

The least significant difference test (LSD), conducted to compare various growth and yield traits of the crop, explains that maximum germination count (22.30 plants/m<sup>2</sup>), plant height (27.90 cm), number of primary branches per plant (3.40), number of pods per plant (29.70), biological yield (4.70 t/ha), number of seeds per plant (50.80), grain yield (1.53 t/ha), thousand seeds weight (270.70 g), days to anthesis (115.57), and days to pod setting (121.17) were found higher under irrigated treatment than in rainfed treatment. While the minimum germination count (19.80 plants/m<sup>2</sup>), plant height (25.70 cm), no. of primary branches per plant (2.90), no. of pods plant<sup>-1</sup> (24.60), biological yield (4.20 t/ha), no. of seeds plant<sup>-1</sup> (41.80), grain yield (1.34 t/ha), thousand seeds weight (258.80 g), days to anthesis (112.23) and days to pod setting (117.87) were observed under rainfed irrigation (*Tables 4* and 5).

	GM	PH (cm)	PB	PP	BY (t/ha)	SP	GY (t/ha)
Drought stress	LSD=0.83	LSD=1.89	LSD=0.15	LSD=0.83	LSD=0.13	LSD=1.14	LSD=0.03
Irrigated	22.30 A	27.90 A	3.40 A	29.70 A	4.70 A	50.80 A	1.53 A
Rainfed	19.80 B	25.70 B	2.90 B	24.60 B	4.20 B	41.80 B	1.34 B
Chickpea cultivars	LSD=0.83	LSD=1.89	LSD=0.15	LSD=0.83	LSD=0.13	LSD=1.14	LSD=0.03
Noor 2013	21.30	26.20	3.10 B	27.30	4.47 B	46.50 A	1.40 B
Bhakkar 2011	20.80	27.30	3.30 A	27.00	5.57 A	42.10 B	1.47 A
<u>β-aminobutyric acid</u>	LSD=1.31	LSD=1.99	LSD=0.26	LSD=1.31	LSD=0.20	LSD=1.80	LSD=0.05
Dry seed	18.40 D	25.30 C	2.00 E	23.70 D	4.00 D	39.50 D	1.24 D
Hydro-priming	20.00 C	26.10 BC	2.70 D	25.70 C	4.30 C	42.80 C	1.33 C
1 mM	21.90 B	26.80 B	3.20 C	28.30 B	4.50 B	47.10 B	1.45 B
2 mM	23.40 A	27.90 A	4.20 A	29.90 A	5.00 A	53.10 A	1.69 A
3 mM	21.00 B	27.70 AB	3.70 B	28.20 B	4.60 B	48.90 B	1.48 B

**Table 4.** Mean comparison values for the effect of drought and  $\beta$ -aminobutyric acid on the growth and yield traits of chickpea cultivars

Upper case letters next to the mean values indicate the difference is significant as determined by least significant difference (LSD) test performed at  $p \le 0.05$ 

GM = germination count, PH = plant height, PB = no. of primary branches, PP = no. of pods plant<sup>-1</sup>, BY = biological yield, SP = no. of seeds plant<sup>-1</sup>, GY = grain yield

APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 21(4):2863-2880. http://www.aloki.hu • ISSN 1589 1623 (Print) • ISSN 1785 0037 (Online) DOI: http://dx.doi.org/10.15666/aeer/2104\_28632880

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	TSW (g)	HI	DA	DPS	SOD	POD	CAT
Drought level	LSD=1.69	LSD=0.77	LSD=0.85	LSD=1.37	LSD=0.86	LSD=0.13	LSD=0.76
Irrigated	270.70 A	32.10	115.57 A	121.17 A	29.00 B	3.70 B	25.20 B
Rainfed	258.80 B	31.51	112.23 B	117.87 B	43.10 A	5.30 A	36.70 A
<u>Chickpea cultivars</u>	LSD=1.69	LSD=0.77	LSD=0.35	LSD=1.37	LSD=0.86	LSD=0.13	LSD=0.76
Noor 2013	260.70 B	33.50 A	113.93	119.13	37.30 A	4.80 A	32.60 A
Bhakkar 2011	268.80 A	31.33 B	113.87	119.90	34.80 B	4.30 B	29.40 B
<u>β-aminobutyric acid</u>	LSD=2.68	LSD=1.02	LSD=1.35	LSD=2.17	LSD=1.30	LSD=0.20	LSD=1.71
Dry seed	249.30 E	29.60 D	113.83 B	118.83	29.30 C	3.50 D	24.20 D
Hydro-priming	256.40 D	29.70 C	113.92 B	119.08	28.60 C	3.40 D	23.30 D
1 mM	266.80 C	30.40 BC	113.17 B	119.25	35.70 B	4.60 C	31.50 C
2 mM	279. 70 A	31.80 A	115.33 A	121.33	43.10 AB	5.50 B	37.20 B
3 mM	271.70 B	31.20 AB	113.25 B	119.08	43.70 A	5.70 A	38.60 A

**Table 5.** Mean comparison values for the effect of drought and  $\beta$ -aminobutyric acid on the yield and quality traits of chickpea cultivars

Upper case letters next to the mean values indicate the difference is significant as determined by least significant difference (LSD) test performed at  $p \le 0.05$ 

TSW = thousand seeds weight, HI = harvest index, DA = days to anthesis, DPS = days to pod setting, SOD = superoxide dismutase, POD = peroxidase, CAT = catalase

Chickpea cultivar Bhakkar 2011 performed better due to its high performance in different growth and yield traits, such as no. of primary branches (3.30 primary branches), biological yield (5.57 t/ha), grain yield (1.47 t/ha) and thousand seeds weight (268.8 g). While Noor 2013 performed best in seed per plant (46.50 seeds) and harvest index (33.50) (*Tables 4* and 5).

Similarly, application of 2 mM of beta-amino butyric acid (BABA) application through seed priming significantly enhance the germination count, plant height, no. of primary branches, no. of pods plant<sup>-1</sup>, biological yield, no. of seeds plant<sup>-1</sup>, grain yield, thousand seeds weight, harvest index and days to anthesis upto (23.40 plants/m<sup>2</sup>, 27.90 cm, 4.20 branches per plant, 29.90 pods per plant, 5.00 t/ha, 53.10 seeds per plant, 1.69 t/ha, 279.70 g, 31.80 and 115.33 days, respectively) which was (1.27, 1.10, 2.10, 1.26, 1.25, 1.34, 1.36, 1.12, 1.07 and 1.01 times) higher than the unprimed seeds (*Tables 4* and 5).

Interactive effect of drought, cultivar and  $\beta$ -aminobutyric acid on thousand seed weight was found significant and cultivar Bhakkar-2011 attained maximum TSW with BABA seed priming at the rate of 2 mM (*Table 6*) under irrigated conditions the growth and most of the yield traits of chickpea cultivars were not found to be significant (*Table 7*).

## Biochemical parameters and quality traits

LSD mean comparison values for all the studied quality traits showed significant variation among various irrigation resources, chickpea cultivars and BABA dosages. In case of irrigation resources, maximum SOD, POD, CAT and TPC values (43.10 unit mg<sup>-1</sup> of protein min<sup>-1</sup>, 5.30 mM mg<sup>-1</sup> of protein min<sup>-1</sup>, 36.70  $\mu$ M mg<sup>-1</sup> of protein min<sup>-1</sup> and 31.2 mg g<sup>-1</sup>) were obtained under rainfed condition, while highest TSP (10.0 mg.g<sup>-1</sup>) was obtained under irrigated condition. Noor 2013 was found as the best genotype due to its best performance in all the studied quality traits except TSP. High concentration of TSP was obtained in Bhakkar 2011 (*Tables 5* and 8).

BABA priming enhance the antioxidants concentration in chickpea cultivars. 1.49, 1.63, 1.60 and 1.65 times results were found by the application of 3 mM of BABA as compared to the unprimed seeds. On the other hand, maximum TSP was obtained by the application of 2 and 3 mM of BABA which was 1.51 times greater than the dry seeded plots (*Tables 5* and 8).

Drought	Cultivars	BABA	TSW	HI	DA	DPS	SOD	POD	CAT
0		Dry seed	247.30 jk	30.50	114.70	117.30	25.70	4.40	29.60
	)13	Hydro-priming	257.30 gh	30.10	116.00	122.00	25.40	4.10	28.10
	Noor 2013	1 mM	264.00 ef	30.30	115.70	121.70	31.10	5.80	39.80
	Noc	2 mM	284.30 b	32.40	117.67	123.60	35.40	6.80	46.30
Irrigated		3 mM	270.60 cd	30.10	116.00	121.30	33.80	7.00	47.10
lmig	1	Dry seed	254.60 hi	29.90	115.00	121.00	34.90	3.20	26.20
	Bhakkar 2011	Hydro-priming	263.00 f	29.90	115.30	121.30	33.10	3.70	25.60
	kar	1 mM	285.30 b	30.30	115.30	121.30	43.60	5.20	36.20
	hak	2 mM	293.30 a	30.90	116.30	122.30	54.50	6.20	42.70
	В	3 mM	287.60 b	30.60	113.70	119.60	56.10	6.50	44.80
		Dry seed	243.70 k	29.60	112.67	118.00	24.00	3.20	21.80
	013	Hydro-priming	252.30 h-j	28.30	111.00	113.60	23.80	2.90	20.50
	Noor 2013	1 mM	255.60 hi	30.60	110.00	116.00	27.40	3.80	26.40
	No	2 mM	269.30 с-е	30.80	113.30	119.30	32.40	4.80	32.20
Rainfed		3 mM	263.00 f	29.80	112.30	118.30	31.50	5.00	33.30
Raiı	1	Dry seed	251.60 ij	28.50	113.00	119.00	32.80	2.70	19.20
	201	Hydro-priming	253.00 hi	30.40	113.30	119.30	32.10	2.80	19.00
	kar	1 mM	262.30 fg	33.00	111.60	118.00	40.90	3.40	23.60
	Bhakkar 2011	2 mM	272.00 c	30.00	114.00	120.00	50.00	4.00	27.80
	E	3 mM	265.60 d-f	34.40	111.00	117.00	53.40	4.30	28.60
		LSD	5.36	3.17	2.70	4.34	2.73	0.41	2.42

**Table 6.** Interactive effect of drought, cultivar and  $\beta$ -aminobutyric acid on the yield and biochemical parameters of chickpea cultivars

Lower case letters next to the mean interaction values indicate the difference is significant as determined by least significant difference (LSD) test performed at  $p \le 0.05$ 

## Crop growth related traits

Drought conditions, BABA priming and chickpea cultivars significantly affected leaf area index (LAI), leaf area duration (LAD) and crop growth ratio (CGR). Under the case of drought regimes, maximum LAI was recorded in irrigated conditions while minimum LAI was recorded in rainfed conditions. In case of chickpea cultivars maximum LAI was recorded in Noor-2013 while minimum LAI was recorded in Bhakkar-2011. Similarly, in the case of BABA priming maximum LAI was recorded where 2 mM solution of BABA was applied while minimum LAI was recorded where dry seeds were grown. The interactive effect of Drought, BABA priming and chickpea cultivars was found significant against LAI. Maximum LAI was recorded in Noor-2013 when it was grown under irrigated conditions with a 2 mM solution of BABA (*Fig. 2*).

Drought	Cultivars	BABA	GM	PH	PB	PP	BY	SP	GY
		Dry seed	18.33	25.80	2.10	25.60	4.18	42.60	1.31
	Noor 2013	Hydro-priming	22.00	26.60	2.80	26.60	4.40	44.30	1.38
	or 2	1 mM	23.00	27.30	3.30	31.00	4.69	51.60	1.49
	Noc	2 mM	25.00	29.00	4.30	33.30	5.29	60.00	1.82
Irrigated		3 mM	22.30	27.90	4.10	32.00	4.88	56.10	1.53
inig	1	Dry seed	20.00	27.10	2.40	25.00	4.39	41.70	1.37
П	Bhakkar 2011	Hydro-priming	22.00	27.70	3.30	29.00	4.62	48.30	1.45
	kar	1 mM	23.30	28.40	3.00	30.60	4.76	51.10	1.52
	hak	2 mM	24.30	29.70	4.90	32.30	5.52	58.00	1.88
	В	3 mM	23.00	29.10	4.10	31.60	4.97	54.60	1.60
		Dry seed	16.30	23.60	2.00	22.30	3.82	37.10	1.07
	013	Hydro-priming	18.00	24.50	2.30	24.30	4.18	40.50	1.22
	Noor 2013	1 mM	21.30	25.20	3.10	25.60	4.20	42.70	1.33
_	Noc	2 mM	22.00	26.10	3.60	27.30	4.61	47.10	1.48
Rainfed		3 mM	20.00	26.40	3.30	25.00	4.43	43.10	1.38
Raiı	1	Dry seed	19.00	24.70	1.80	22.00	3.61	36.60	1.20
	201	Hydro-priming	18.00	25.50	2.40	23.00	4.02	38.20	1.29
	Bhakkar 2011	1 mM	20.00	26.20	3.50	26.00	4.56	43.30	1.47
	hak	2 mM	22.30	26.80	4.00	26.60	4.86	47.50	1.57
	B	3 mM	21.00	27.50	3.50	24.30	4.42	41.90	1.42
		LSD	2.63	5.99	0.50	2.63	0.41	3.61	0.10

*Table 7.* Interactive effect of drought, cultivar and  $\beta$ -aminobutyric acid on the growth and yield traits of chickpea cultivars

GM = germination count, PH = plant height, PB = no. of primary branches, PP = no. of pods plant<sup>-1</sup>, BY = biological yield, SP = no. of seeds plant<sup>-1</sup>, GY = grain yield

	TPC (g)	TSP
<u>Drought level</u>	LSD = 0.81	LSD = 0.19
Irrigated	23.80 B	10.00 A
Rainfed	31.20 A	7.30 B
Chickpea cultivars	LSD = 0.81	LSD = 0.19
Noor 2013	28.90 A	8.20 B
Bhakkar 2011	26.10 B	9.10 A
<u>β-aminobutyric acid</u>	LSD = 1.28	LSD = 0.30
Dry seed	21.00 D	6.90 C
Hydro-priming	20.60 D	6.60 C
1 mM	27.80 C	9.00 B
2 mM	33.20 B	10.40 A
3 mM	34.60 A	10.40 A

**Table 8.** Mean comparison values for the effect of drought and  $\beta$ -aminobutyric acid on the quality traits of chickpea cultivars

Upper case letters next to the mean values indicate the difference is significant as determined by least significant difference (LSD) test performed at  $p \leq 0.05$ 

TPC = total phenolic contents, TSP = total soluble protein

Interactive effect of drought, cultivar and  $\beta$ -aminobutyric acid on biochemical parameters and quality traits was found to be non-significant (*Tables 6* and 9)

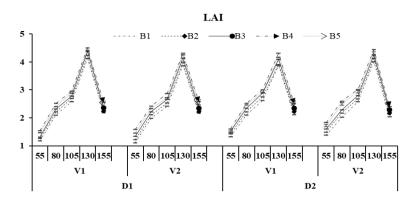
Under case of drought regimes maximum LAD was recorded in irrigated conditions while minimum LAD was recorded in rainfed conditions. In the case of chickpea cultivars maximum LAD was recorded in Noor-2013 while minimum LAD was recorded in Bhakkar-2011. Similarly, in case of BABA priming maximum LAD was recorded where 2 mM solution of BABA was applied while minimum LAD was recorded where dry seeds were grown. The interactive effect of Drought, BABA priming and chickpea cultivars was found significant against LAD. Maximum LAD was recorded in Noor-2013 when it was grown under irrigated conditions with a 2 mM solution of BABA (*Fig. 3*).

Drought	Cultivars	BABA	ТРС	TSP
		Dry seed	25.60	9.00
	013	Hydro-priming	24.20	9.10
	or 2	1 mM	33.60	11.10
7	Nod	2 mM	39.90	11.60
ated		3 mM	40.80	12.20
inig	[]	Dry seed	22.10	8.10
Ι	201	Hydro-priming	21.60	7.40
	kar	1 mM	30.50	9.10
	hak	2 mM	36.30	11.20
	B	3 mM	37.80	11.60
		Dry seed	20.20	5.50
	013	Hydro-priming	18.40	5.40
	or 2	1 mM	Dry seed     25.60     9.       hro-priming     24.20     9.       1 mM     33.60     11       2 mM     39.90     11       3 mM     40.80     12       Dry seed     22.10     8.       hro-priming     21.60     7.       1 mM     30.50     9.       2 mM     36.30     11       3 mM     37.80     11       Dry seed     20.20     5.       hro-priming     18.40     5.       1 mM     24.50     8.       2 mM     30.30     9.       3 mM     31.80     9.       Dry seed     17.90     5.       hro-priming     18.20     4.       1 mM     22.40     7.       2 mM     30.30     9.       3 mM     28.10     8.	8.40
_	Noc	2 mM		9.60
ufec	[	3 mM	31.80	9.20
Raiı	[]	Dry seed	17.90	5.10
Image: Second system Image: Second system   Image: Second system Ima	Hydro-priming	18.20	4.80	
	kar	1 mM	22.40	7.50
	hak	2 mM	26.40	9.20
	B	3 mM	28.10	8.80
		LSD	2.56	0.61

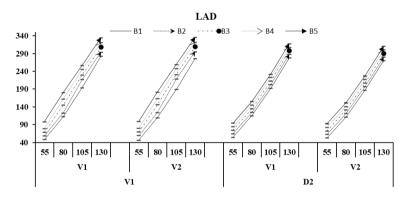
*Table 9.* Interactive effect of drought, cultivar and  $\beta$ -aminobutyric acid on the quality traits of chickpea cultivars

TPC = total phenolic contents, TSP = total soluble protein

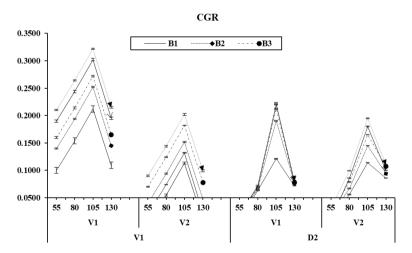
Under the case of drought regimes maximum CGR was recorded in irrigated conditions while minimum CGR was recorded in rainfed conditions. In the case of chickpea cultivars maximum CGR was recorded in Noor-2013 while minimum CGR was recorded in Bhakkar-2011. Similarly, in the case of BABA priming maximum CGR was recorded where 2 mM solution of BABA was applied while minimum CGR was recorded where dry seeds were grown. Interactive effect of Drought, BABA priming and chickpea cultivars was found significant against CGR. Maximum CGR was recorded in Noor-2013 when it was grown under irrigated conditions with a 2 mM solution of BABA (*Fig. 4*).



**Figure 2.** Effect of seed priming with BABA under rainfed and irrigated conditions on leaf area index of chickpea cultivars. ( $V_1 = Noor-2013$ ,  $V_2 = Bhakar-2011$ ,  $D_1 = irrigated$  conditions,  $D_2 = rainfed$  conditions,  $B_1 = Dry$  seeds,  $B_2 = Hydropriming$ ,  $B_3 = 1$  mM solution of BABA,  $B_4 = 2$  mM and  $B_5 = 3$  mM)



**Figure 3.** Effect of seed priming with BABA under rainfed and irrigated conditions on leaf area duration (days) of chickpea cultivars. ( $V_1 = Noor-2013$ ,  $V_2 = Bhakar-2011$ ,  $D_1 = irrigated$  conditions,  $D_2 = rainfed$  conditions,  $B_1 = Dry$  seeds,  $B_2 = Hydropriming$ ,  $B_3 = 1$  mM solution of BABA,  $B_4 = 2$  mM and  $B_5 = 3$  mM)



**Figure 4.** Effect of seed priming with BABA under rainfed and irrigated conditions on crop growth ratio  $(g/m^2/day)$  of chickpea cultivars.  $(V_1 = Noor-2013, V_2 = Bhakar-2011, D_1 = irrigated conditions, D_2 = rainfed conditions, B_1 = Dry seeds, B_2 = Hydropriming, B_3 = 1 mM solution of BABA, B_4 = 2 mM and B_5 = 3 mM)$ 

## Economic analysis

Economic analysis report showed that maximum benefit-cost ratio was calculated where hydro-priming was done in Noor-2013 under rainfed conditions 3.47 while minimum benefit-cost ratio rainfed condition where BABA was applied with 3 mM solution in Bhakkar-2011 (*Table 10*).

*Table 10.* Impact of BABA application on the economic returns of chickpea cultivars under rainfed and irrigated conditions

Treatments	Total expenditure	Grain yield (kg)	Gross income	Net income	BCR
$D_1V_1B_0$	40215.00	1542.50	126485.00	86270.00	3.15
$D_1V_1B_1$	40215.00	1623.20	133104.70	92889.79	3.31
$D_1V_1B_2\\$	77715.00	1738.90	142594.50	64879.58	1.83
$D_1V_1B_3$	115215.00	2123.30	174113.30	58898.33	1.51
$D_1V_1B_4\\$	145891.60	1779.10	152715.00	6823.33	0.96
$D_2V_1B_0\\$	34215.0	1263.20	103585.20	69370.24	3.03
$D_2V_1B_1 \\$	34215.00	1446.60	118626.60	84411.67	3.47
$D_2V_1B_2 \\$	71715.00	1553.30	127373.30	55658.33	1.78
$D_2V_1B_3\\$	109215.00	1738.80	142588.70	33373.75	1.31
$D_2V_1B_4\\$	131449.90	1603.00	146715.00	15265.04	0.90
$D_1V_2B_0$	40215.00	1373.90	112660.00	72445.09	2.80
$D_1V_2B_1$	40215.00	1455.50	119356.70	79141.75	2.97
$D_1V_2B_2$	77715.00	1522.90	124879.10	47164.17	1.61
$D_1V_2B_3$	115215.00	1880.10	154160.00	38945.00	1.34
$D_1V_2B_4$	131370.80	1602.00	152715.00	21344.17	0.86
$D_2V_2B_0$	34215.00	1200.80	98468.30	64253.33	2.88
$D_2V_2B_1 \\$	34215.00	1289.80	105769.20	71554.21	3.09
$D_2V_2B_2$	71715.00	1467.30	120326.00	48611.01	1.68
$D_2V_2B_3$	109215.00	1577.50	129358.60	20143.60	1.18
$D_2V_2B_4$	116589.20	1421.80		-30125.75	0.79

 $D_1$  = irrigated,  $D_2$  = rainfed,  $V_1$  = Noor-2013,  $V_2$  = Bhakkar-2011,  $B_0$  = dry seeds,  $B_1$  = hydropriming,  $B_2$  = 1 mM solution of BABA,  $B_3$  = 2 mM and  $B_4$  = 3 mM

#### Discussion

From the results of this study, it was revealed that drought stress significantly reduced chickpea seed germination while priming with BABA at the rate of 2 mM produced highest germination, whereas BABA application at the rate of 1 mM and 3 mM were statistically at par with each other and superior than control treatments. BABA has been shown to involve in the developmental regulation of several plants. A high dose of BABA affected adversely seed germination of pearl millet, whereas seed germination of Arabidopsis was not inhibited (Zimmerli et al., 2008). In rice, seed priming with BABA was known to cause an increase in seedling growth parameters (Goswami et al., 2013), and it was also reported that seed priming treatments reduced the time taken to initiate the germination process, improved the rate of germination and synchronization, enhanced the lengths of shoot and root and thus increased the fresh and dry weight of the seedlings (Farooq et al., 2006).

Drought stress is a global problem that adversely affects the performance of different crop plants. The development of drought tolerant cultivars is a prerequisite to encountering the prevailing drought stress on a sustainable basis. Evaluation of the chickpea responses to drought stress showed significant variations for different traits of early growth stages, physio-chemical nature and grain yield. Significance of the variations depicted the differential responses of the genotypes to variable environments at different growth stages. These variations could be attributed to the differences in their genetic makeup of the studied genotypes. Different researchers have also reported the differential responses of the chickpea genotypes under different environments and at different growth stages (Maqbool et al., 2015). Results of this study revealed that beta amino-butyric acid alleviated drought stress and improved the growth and yield of chickpea of this improvement may be that exogenous application of plant growth regulators (PGRs) b-aminobutyric acid (BABA) might play an important role in regulating growth attributes like LAI, LAD and CGR as well as in enhancing abiotic and biotic stress tolerance in plants (Ton et al., 2009). BABA induces drought tolerance and constitutes an effective strategy to mitigate drought damage in plants like Arabidopsis thaliana and ultimately increases yield (Jakab et al., 2005). Within plant cell, SOD acts as the first line of defense against oxidative stress as its activity directly modulates the amount of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>, the two important Haber-Weiss reaction substrates (Bowler et al., 1992). Results of this study revealed that priming with the 3 mM solution of BABA improved the POD, CAT and SOD. On exposure to drought, the primed plants showed comparatively a high SOD, POD and CAT activity over the unprimed primed seeds. When faced with environmental stresses, plants tend to activate their own self-defense mechanism by producing an excessive amount of proline, APX, CAT, SOD, and GR. These substances help to enhance the plant's natural defense system (Ahmad et al., 2016).

Nevertheless, the magnitude of increase was more in primed plants with BABApriming than unprimed seeds. However, the oxidative damage to lipid membranes only occurred when the soil water was almost depleted, not when the ROS began to increase at moderate levels of water depletion. The data suggest that the antioxidant enzymes SOD, CAT, which all increased in concert with ROS may have mitigated the effects of the ROS. SOD catalyses the dismutation of O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>, while CAT, ACT, and are responsible for the removal of H<sub>2</sub>O<sub>2</sub> (Yasir eta al., 2021). The antioxidant enzymes may have played a role in mitigating the effects of ROS that were higher in chickpea cultivar Noor-2013 than in the Bhakkar-2011, and maybe a partial explanation for the reduced level of membrane damage at severe water shortage when the plants were primed with BABA. Alternatively, BABA may have reduced the level of ROS such as peroxides, singlet oxygen, superoxide, and hydroxyl radical in the leaves which may also be a reason for the reduced lipid damage, as expressed by MDA concentration, at severe water shortage. These results are similar to those observed in winter wheat and spring wheat subjected to soil drying (Seifikalhor et al., 2022).

#### Conclusion

In this experiment, two chickpea cultivars were tested under two water regimes and BABA was applied to improve the growth and production of chickpea cultivars in arid regions. By summarizing the whole experimental findings, it was found that rainfall provided low moisture to the plant and created drought stress condition. This situation

negatively affected the growth and production of chickpea cultivars. Chickpeas when subjected to drought stress in a rainfed environment, they activated their defense system by ramping up antioxidant levels in their leaf tissues. By priming seeds with BABA at concentrations of 2 mM and 3 mM, we observed significant improvements in both growth and yield attributes. This suggests that using BABA for seed priming is a promising solution for mitigating the effects of drought stress.

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