

Foliar application of γ -aminobutyric acid (GABA) mitigates drought stress in canola (*Brassica napus* L.): Insights into antioxidant defense, oxidative stress, lipid profiling, and yield attributes

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Abstract

Water stress is a major environmental challenge that impacts plant growth and performance. Gamma-aminobutyric acid (GABA), an important regulatory molecule in metabolic pathways, plays a key role in plant growth and stress tolerance. This study aimed to examine the effect of foliar GABA treatment on antioxidant enzyme activity, physiological traits, growth measures, and both quantitative and qualitative yield of rapeseed (*Brassica napus* L.) under different water stress levels. The experiment was conducted over two consecutive years in a field setting, with three water stress levels (full irrigation, 60%, and 30% of field capacity) and four GABA concentrations (0, 25, 50, and 75 mM). GABA application, especially at 50 and 75 mM, boosted antioxidant enzyme activity and enhanced photosynthetic pigment content, while decreasing levels of proline, malondialdehyde, and hydrogen peroxide. The positive effects of GABA led to increased grain yield, Number of Siliques Per Plant, Number of Seeds Per Silique, and 1000 Kernel Weight. Additionally, GABA treatment raised the percentage, index, and oil yield compared to controls. Water stress reduced linolenic and linoleic fatty acids, while increasing oleic, erucic, and palmitic acids. However, GABA application at 50 and 75 mM alleviated these changes. Overall, the results suggest that GABA application can effectively enhance water stress tolerance and improve rapeseed yield by strengthening the antioxidant system and modulating physiological responses.

Introduction

In human nutrition, oilseed products hold a special place due to their essential fatty acids, high-quality oil, and adequate protein, and their global production is rising. It is estimated that by 2050, the worldwide demand for these products will double¹. Rapeseed (*Brassica napus* L.), with a cultivated area of 37 million hectares worldwide and a 13.4% share of the global oilseed market, is the third-largest oilseed crop and has extensive uses in the food and industrial sectors². The grains of this crop contain about 45 to 50 percent oil and 17 to 26 percent protein, and some of its flour and meal are also used as by-products for animal feed^{2,3,4}. Additionally, non-edible rapeseed oil is suitable for biodiesel production, accounting for 70% of the global output of this type of fuel⁵.

However, the sustainable production of this strategic crop faces a serious threat from climate change. The intensification of climate change in recent years has caused various environmental changes, including increased temperatures, changing rainfall patterns, the spread of droughts, and alterations in growing seasons. One of the significant consequences of these changes is the disruption of the water cycle, which negatively affects plant growth and development⁶. Important morphophysiological changes that occur in response to water stress include alterations in the expansion and size of various plant organs, cell swelling, internal water content, enzymatic activities, photosynthetic pigment contents, stomatal conductance, osmotic adjustments, and photosynthetic activities^{7,8}. Studies on rapeseed show that this plant has developed a diverse range of morphological, physiological, and biochemical adaptations to tolerate water stress^{9,10}. However, like other crops, rapeseed is highly sensitive to drought and climate change, and its survival and productivity are likely to be impacted under such conditions¹¹. Research indicates that water stress can reduce rapeseed yield by affecting various physiological processes, including photosynthesis, respiration, transpiration, and nutrient uptake¹². In this context, reductions such as decreased plant height¹³, weakened root systems¹⁴, fewer secondary branches and siliques¹³, and smaller grain size and weight¹⁵ have been reported. Additionally, water stress induces oxidative stress and lipid peroxidation, which lead to changes in fat content and ultimately affect grain quality¹⁶. These effects are especially notable during flowering and seed filling stages^{17,18}, mainly due to a reduction in the grain filling period¹⁹.

In recent years, the role of amino acids in inducing plant resistance to stresses has garnered significant attention in both fundamental and applied fields of crop physiology²⁰. Gamma-aminobutyric acid (GABA), as a non-protein amino acid²¹ that has no adverse or toxic effects on humans and the environment²², enhances plant resistance to abiotic stresses^{23,24}.

This compound plays a vital role as an essential intermediate in nitrogen metabolism and amino acid biosynthesis and participates in the production of primary and secondary metabolites²². GABA has also been shown to be crucial in boosting antioxidant defense mechanisms²⁵, increasing osmolyte levels, leaf turgor, and promoting growth and development^{24,26}. In this context, studies have revealed that exogenous application of GABA under stress conditions can significantly elevate endogenous GABA levels in plants, subsequently enhancing photosynthetic capacity, maintaining cell membrane integrity, activating enzymes that improve water stress tolerance, and reducing ROS accumulation in treated plants^{27,28,29}.

Rapeseed plants are known for their vital role as a source of edible and industrial oil, as well as their protein-rich meal. Given their importance, in this study we chose rapeseed plants as the target crop. The study investigated the impacts of gamma-aminobutyric acid (GABA) on the water stress tolerance mechanism of rapeseed plants. Furthermore, the research also focused on how GABA influences rapeseed plant stress resistance, growth, and physiology, as well as its impact on this crop productivity under water stress conditions.

Materials and methods

This research was conducted over two consecutive crop years (2022 and 2023) at the Maragheh University Research Field (located at 46°27' E longitude and 37°37' N latitude, at an altitude of 1566 meters) in Maragheh, Iran. The experiment was conducted a split-plot design within a randomized complete block with three replications. The main factor consisted of three irrigation regimes: no stress (complete irrigation), moderate stress (60% of field capacity), and severe stress (30% of field capacity), applied at the start of the flowering stage (BBCH 63). To determine field capacity, permanent wilting point, and soil bulk density, random samples were collected from the 0–30 cm soil layer using 7 cm cylinders from multiple points in the undisturbed soil. These samples were transported to the laboratory, where the apparent specific gravity, volumetric moisture content at field capacity, and permanent wilting point were measured as 1.32 g/cm³, 32.2%, and 13.1%, respectively. The irrigation strips measured 175 microns in thickness, with 20 cm spacing between holes, and had a flow rate of 2 liters per hour at 1 bar pressure. Drip tapes were installed alongside each crop row, each connected to a water tap linked to a supply pipe. The sub-factor involved applying different concentrations of γ -aminobutyric acid (GABA) (Sigma-Aldrich) at four levels: 0, 25, 50, and 75 mM. GABA was applied

through spraying with a five-liter hand sprayer at 5.5 MPa during three key phenological stages stem elongation (BBCH 57), flowering initiation (BBCH 63), and siliques initiation (BBCH 65) using distilled water as the solvent, during the morning hours and under windless conditions.

Field preparation, plant materials, and seed planting were carried out to prepare the land for planting. The field was cleared of weeds using the herbicide Trifluralin (TREFLAN 48% EC) at a rate of 2 liters per hectare, followed by disking and leveling. Rapeseed seeds (Neptune variety, obtained from the Agricultural Office Management, Maragheh, Iran) were sown in six rows, each measuring 2 meters in length, with a row spacing of 30 centimeters. The seeds were planted at a depth of about 2 cm, with plant spacing within the row of 5 cm. The distance between plots was set at 0.5 meters, and the space between blocks was 1.5 meters. Planting was conducted in the first year on September 13, 2022, and in the second year on September 9, 2023. Immediately after planting, the first irrigation was performed, providing sufficient water, and irrigation continued until the beginning of the flowering stage (BBCH 63), based on the plants physiological needs and field conditions. Throughout the experiment, weed control was maintained by hand weeding, and thinning was done after plants were fully established. After emergence, Deltamethrin was applied as a foliar spray at a dose of 750 ml/ha to control Agrotis pests during the stem elongation stage. Additionally, imidacloprid (Confidor) was applied during the pre-flowering stage to control aphids. Prior to planting, a soil sample was collected from a depth of 0 to 30 cm, and its physical and chemical properties were analyzed (Table 1). Based on these results and the fertilizer requirements of the rapeseed plants, fertilization was applied. Meteorological data on average temperature and total monthly precipitation for both years of the experiment are presented in Figs. 1 and 2.

Table 1
Physical and chemical characteristics of the soil sample at the research site.

Soil characteristics	Amount	Soil characteristics	Amount
Sampling depth (cm)	0–30	Electrical conductivity of saturated Extract (decsiemens/m)	0.53
Sand (percent)	20.33	Neutralizing substances (percentage)	3.4
Clay (percent)	43.67	Saturated mud reaction (pH)	7.57
Silt (percent)	36	Total nitrogen (percentage)	0.071
Soil texture	Clay loam	Absorbable phosphorus (mg/kg)	9.57

Measuring Traits

To assess physiological and biochemical traits, leaf sampling was conducted at the end of the flowering stage of rapeseed (BBCH 69). In each plot, 10 plants were randomly selected, and leaf samples were separated and wrapped in aluminum foil before being transported to the laboratory in a refrigerator with ice. To prevent metabolic changes, the leaves were then frozen in liquid nitrogen and stored at -18°C until testing.

Photosynthetic pigments

At the flowering stage, 0.2 g of leaf sample was homogenized in 10 ml of 80% acetone, and the homogenate was centrifuged for 15 minutes at 12,000 rpm. Finally, the absorbance at wavelengths of 663 nm, 645 nm, and 470 nm was measured using a spectrophotometer (Shimadzu UV-1800, Japan), and the contents of chlorophyll a, b, and carotenoids were calculated in mg per gram of dry weight based on the following equations³⁰.

Chlorophyll a = (19.3 A663 – 0.86 A645) V/100 W

Chlorophyll b = (19.3 A645 – 3.6 A663) V/100 W

Carotenoids = 100 A470 – 3.27 Chlorophyll a – 104 Chlorophyll b/227

Catalase

A reaction mixture containing potassium phosphate buffer, hydrogen peroxide, double-distilled water, and enzyme solution was used to assess catalase enzyme activity. The activity was estimated by measuring the absorption of the reaction mixture at 290 nm wavelength³¹.

Ascorbate peroxidase

A reaction mixture containing phosphate buffer, ascorbate, EDTA, double-distilled water, hydrogen peroxide, and extracted enzyme solution was used to measure the activity of ascorbate peroxidase. The absorbance of the reaction complex was measured at 290 nm using a spectrophotometer (UV-2100-vis)³².

Guaiacol peroxidase

To measure the activity of the guaiacol peroxidase enzyme, a reaction mixture consisting of phosphate buffer, guaiacol, hydrogen peroxide, EDTA, and enzyme solution was used. The increase in absorbance at 470 nm was recorded using a spectrophotometer (UV-2100-vis) over one minute³³.

Superoxide dismutase

To measure the activity of superoxide dismutase and its isozymes, the method of Sairam et al.³⁴ was used. The reaction involved a mixture of sodium carbonate, methionine, EDTA, potassium phosphate buffer, distilled water, and enzyme extract. The reaction was initiated by adding riboflavin, and after stopping it, the absorbance was measured at a wavelength of 560 nm with a spectrophotometer (UV-2100-vis)³². Malondialdehyde (MDA) was determined using the Stewart and Bewley method³⁵. For this, leaf samples were centrifuged for 10 minutes at 15,000 rpm. The resulting solution was then combined with trichloroacetic acid and thiobarbituric acid, and the mixture was placed in a bain-marie at 100 °C for 30 minutes. The samples were quickly cooled in an ice bath and centrifuged again for 10 minutes at 10,000 rpm. Finally, the optical absorbance at wavelengths of 532 and 600 nm was measured using a spectrophotometer (UV-2100-vis).

Malondialdehyde

Malondialdehyde was measured using the Stewart and Bewley method³⁵. For this procedure, leaf samples were centrifuged for 10 minutes at 15,000 rpm. The resulting solution was then mixed with trichloroacetic acid and thiobarbituric acid, and the mixture was placed in a water bath at 100 °C for 30 minutes. Afterward, the samples were rapidly cooled in an ice bath and centrifuged again for 10 minutes at 10,000 rpm. Finally, the optical absorbance of the solutions at 532 and 600 nm was measured with a spectrophotometer (UV-2100-vis).

Hydrogen Peroxide

To measure hydrogen peroxide (H₂O₂), samples homogenized with trichloroacetic acid were centrifuged at 4 °C and 11,000 rpm for 15 minutes. Then, a reaction solution containing phosphate buffer and potassium iodide was prepared, and its absorbance was measured at a wavelength of 390 nm using a spectrophotometer (UV-2100-vis)³⁶.

Proline

To extract proline, the weighed leaf was first ground in a mortar with 10 ml of 3% sulfosalicylic acid and homogenized, then centrifuged for 5 minutes at 6000 rpm. 2 ml of the supernatant were combined with 2 ml of ninhydrin reagent and 2 ml of acetic acid and placed in a boiling water bath for one hour. Immediately after that, the samples were cooled in an ice bath for a few minutes. Then, 4 ml of toluene was added to each sample and shaken for 15 to 20 seconds to make the solution uniform. After separating the two phases, the upper pink phase containing proline was separated, and its absorbance was measured at a wavelength of 520 nm by a spectrophotometer³⁷.

Morphological and yield traits

At the end of the growing season, when the plants reached maturity and yellowed (BBCH 99), the crop was harvested. Measurements of agronomic traits in the plots including stem height and diameter, number of secondary branches, length and number of siliques, number of seeds per silique, and 1000- kernel weight were taken from 10 randomly selected plants. The height from the ground to the tallest part of each plant was measured with a ruler and recorded in centimeters. The diameter of the main stem was measured near the soil surface with a caliper on the same plants, and the average in millimeters was reported as the stem diameter. The length of the siliques was measured in millimeters using a ruler. All siliques on the plants were counted, and the average was recorded as the number of siliques per plant. All siliques and seeds inside them were manually separated and counted; after averaging, this was reported as the number of seeds per silique. The weight of 1,000 kernels was measured on a three-zero scale. After removing two side rows and half a meter from the beginning and end of each plot as margins, the entire above-ground biomass was harvested and weighed to estimate biological yield (BY) in kg/ha. After separating the seeds from the siliques, the seed weight was measured with a scale and reported as seed yield (SY) or economic yield (EY) per unit area (kg/ha). The harvest index (HI) for each plot was calculated by dividing SY by BY and expressed as a percentage.

Oil extraction and measurement of seed oil percentage were carried out by separating the oil with petroleum ether solvent and using a shaker device³⁸. The oil yield was calculated by multiplying the oil percentage by the seed yield and expressed as kilograms per hectare. Additionally, the biological oil yield index was determined by dividing the oil yield by the biological yield³⁹.

Statistical Analysis

Data were tested for normality before statistical analysis using nonparametric methods with SPSS 26 software. Finally, data analysis and comparison of means were performed using SAS 9.1, and means were compared using the LSD test at a 5% probability level.

Results

The application of GABA under different irrigation conditions across both years induced changes in the activity of the ascorbate peroxidase (APX) enzyme. These changes suggested that GABA acts as a potential regulator of antioxidant defense, particularly under water stress conditions. In the first year of the experiment, 75 and 50 mM GABA concentrations resulted in the highest APX activity, while in the second year, the 50 mM treatment produced the highest activity. Moreover, in most treatments, APX levels were generally lower in the second year compared to the first (Table 2). A similar pattern was observed for the activities of catalase (CAT) and guaiacol peroxidase (GPX) enzymes (Table 2). Notably, under moderate stress conditions in both years, a reduction in CAT activity was evident in treatments without GABA. However, GABA foliar spraying helped compensate for this decrease and improved the response relative to non-stress conditions. Regarding superoxide dismutase (SOD) activity, the data showed that although the highest SOD levels were observed under moderate stress in both years, the application of 75 mM GABA in the first year and 50 mM GABA in the second year resulted in the greatest enzyme activity (Table 2). Due to better weather conditions in the second year, the medium GABA concentration exhibited a greater controlling effect than a higher concentration. Additionally, at each stress level, GABA application increased SOD levels compared to treatments without GABA. As shown in Table 2,

hydrogen peroxide (H₂O₂) levels peaked in the treatment without GABA under severe water stress in the first year. Conversely, the lowest H₂O₂ levels were observed in the 50 mM GABA treatment during the second year and under non-stress conditions. Overall, higher stress levels and lower GABA doses resulted in increased H₂O₂, indicating heightened oxidative stress. GABA application, especially at 50 and 75 mM, reduced hydrogen peroxide levels, with a more significant reduction under non-stress conditions (Table 2). The highest malondialdehyde (MDA) levels occurred in the first year under severe stress and without GABA application, signaling increased lipid peroxidation and oxidative damage caused by water stress (Table 2). Application of GABA at different levels (25, 50, 75 mM) generally decreased MDA levels across most stress conditions, with the greatest reduction observed at 50 mM. The effect of 75 mM GABA was weaker, possibly due to negative effects at high doses. Comparing the two years, although some treatments in the second year showed slightly lower MDA levels, the overall trend remained similar. This decline might be attributed to more optimal physiological functions in the second year, driven by favorable weather conditions. Despite GABA's ability to significantly reduce MDA levels, the amount of MDA remained higher under stress than in non-stress treatments, even with GABA application. This suggests that GABA's protective effect is relative and cannot completely eliminate stress effects but can mitigate damage to an acceptable extent.

As water stress became more severe, proline levels increased, with the highest proline observed in the severe stress treatment without GABA application in the first year (Table 2). Additionally, applying GABA in both years of the experiment significantly lowered proline levels under stress conditions (Table 2). In the first year, 75 mM GABA treatment caused the greatest reduction in proline content, while in the second year, 50 mM GABA achieved the largest decrease. The lowest proline content was found in the non-stressed treatment with 50 mM GABA in the second year, highlighting GABA's positive role in reducing metabolic stress and enhancing the plant's physiological health.

As stress intensity increased, chlorophyll a and b concentrations decreased (Table 2). In contrast, treatment with exogenous GABA, especially at concentrations of 50 and 75 mM, significantly increased chlorophyll a and b content (Table 2). The highest amount of carotenoid pigment in the first year was associated with the 75 mM GABA treatment at the moderate stress level, while in the same year, the treatment without GABA application had the lowest amount (Table 2). A similar trend was observed in the second year, but the highest carotenoid amount was linked to the 50 mM GABA treatment at the severe stress level (Table 2). In the first year, as GABA levels increased, canola grain yield also increased (Table 3). The same trend was seen in the second year, though foliar application of 50 mM GABA resulted in slightly higher grain yield than 75 mM GABA (Table 3). This suggests that the optimal GABA level's effect on yield can vary with environmental factors. Additionally, the average from both years indicates that the highest grain yield was under non-stressed conditions, with yield decreasing significantly as water stress increased (Table 3). In this context, the number of siliques per plant increased with higher GABA concentration in the first year. The no GABA treatment had the fewest, while 75 mM GABA had the most. In the second year, the highest number of siliques per plant was observed with 50 mM GABA (Table 3). Also, in the first year, increasing GABA levels improved the number of seeds per silique, with the highest at 75 mM GABA and the lowest without GABA. Similarly, in the second year, the highest seed count per silique was at 50 mM GABA, while 75 mM GABA caused a slight decrease in this trait (Table 3). Based on the combined analysis, the highest number of seeds per silique was under non-stressed conditions, with moderate stress greatly reducing this trait, and severe stress resulting in the lowest value (Table 3). Regarding thousand-kernel weight, increasing GABA concentration enhanced this trait, with the lowest in the no GABA treatment and the highest with 50 and 75 mM GABA, respectively (Table 3). The highest thousand-kernel weight was observed under non-stressed and moderate-stress conditions, decreasing as stress severity increased (Table 3). GABA application also influenced the number of side branches and overall performance. In both years, these traits were lowest without GABA, but the highest values were with 75 mM GABA in the first year and 50 mM GABA in the second (Table 3). The highest harvest index in the first year was with 75 mM GABA, and in the second year with 50 mM GABA, while the lowest was without GABA. Water stress reduced the harvest index overall (Table 3). The oil percentage was affected by the treatments; in the first year, the highest oil percentage was under non-stressed conditions and with GABA at 50 and 75 mM, while the lowest was under severe stress without GABA. GABA, especially at 75 mM, helped mitigate stress effects at moderate levels (Table 4). In the second year, the highest oil percentage was under non-stressed conditions with 50 mM GABA, and the lowest was during severe stress without GABA. GABA at 50 and 75 mM increased oil percentage under stress compared to no GABA (Table 4). Overall, comparing both years shows that although the average oil percentage was slightly higher in the second year, plant responses to GABA levels were consistent. Increasing GABA from 0 to 50 mM boosted oil yield in both years, with higher overall yields in the second year. However, increasing to 75 mM in the second year reduced this component (Table 4). In year one, raising GABA to 50 mM increased the oil harvest index over no GABA and other doses. In year two, the highest index was at 75 mM GABA, with the lowest without GABA (Table 4).

In this study, in both years, as water stress severity increased, the amount of linolenic acid decreased. However, applying GABA was able to lessen this decrease. In the first year, at all water stress levels except severe stress where the 75 mM level performed better than the 50 and 75 mM GABA treatments showed better performance than the no-GABA and 25 mM GABA treatments. In the second year, at all water stress levels, all three GABA levels (25, 50, and 75 mM) increased the percentage of linolenic acid in the grain compared to the treatment without GABA; however, the highest amounts of this fatty acid were mainly observed in the 50 and 75 mM GABA treatments (Table 4). The highest linoleic acid percentage in both years of the experiment was observed under conditions without water stress and with the 50 and 75 mM GABA treatments. Overall, although the amount of linoleic acid decreased with increasing stress intensity, at all stress levels, treatments containing GABA exhibited higher linoleic acid levels than those without GABA application. Additionally, a comparison of the two years showed that the linoleic acid content was lower in the first year across most treatments compared to the second year, although the pattern of response to water stress and GABA application remained consistent in both years (Table 4). In contrast, the percentage of oleic acid increased as water stress intensified (Table 4). The highest oleic acid percentage was observed in both years of the study under severe stress conditions, while the lowest was in non-stress conditions. Conversely, GABA treatment resulted in a decrease in this acid. In both years, the highest levels of oleic acid were found in treatments without GABA and with 25 mM GABA, whereas applying 50 and 75 mM GABA in both years led to a reduction compared to the treatments without GABA. Regarding the percentage of palmitic acid, this fatty acid also increased with rising water stress intensity, reaching the highest level under severe stress conditions. In contrast, applying GABA at concentrations of 50 and 75 mM reduced the percentage of palmitic acid compared to both the treatment without GABA and the treatment with 25 mM GABA (Table 4). Additionally, the percentage of erucic acid increased with higher water stress levels. However, applying GABA in many treatments resulted in a decrease in its percentage compared to treatments without GABA. In the first year, 50 and 75 mM

GABA treatments under moderate stress and 75 mM GABA under both non-stress and severe stress conditions lowered erucic acid levels compared to no GABA application. In the second year, 50 and 75 mM GABA treatments reduced erucic acid levels relative to treatments without GABA across all stress levels, especially under moderate stress (Table 4).

Table 2
Impacts of different GABA concentrations on physiological and biochemical traits of rapeseed.

drought stress	Year	GABA	Ascorbate Peroxidase ($\mu\text{mol min}^{-1}\text{mg}^{-1}\text{protein}$)	Catalase ($\mu\text{mol min}^{-1}\text{mg}^{-1}\text{protein}$)	Guaiacol Peroxidase ($\text{U mg}^{-1}\text{Protein}$)	Superoxide Dismutase ($\text{U mg}^{-1}\text{protein}$)	Hydrogen Peroxide ($\text{mmol g}^{-1}\text{FW}$)	Malondialdehyde ($\text{nmol g}^{-1}\text{FW}$)	Proline ($\text{mg g}^{-1}\text{FW}$)	Chlorophyll a ($\text{mg g}^{-1}\text{FW}$)
no stress	First Year	0	2.96	31.00	2.40	25.16	5.90	20.56	31.20	2.3
		25	4.86	45.00	6.46	36.60	4.40	15.66	30.50	2.4
		50	5.43	58.00	4.20	48.73	3.43	13.30	27.10	2.6
		75	5.53	62.00	4.83	50.16	3.10	11.60	25.16	2.7
moderate stress		0	6.56	110.66	8.26	89.33	11.20	25.60	125.06	1.8
		25	7.36	143.00	10.73	112.96	9.43	20.60	112.63	1.9
		50	11.76	189.33	13.30	152.03	8.86	16.50	98.23	2.2
		75	12.10	203.33	14.03	165.16	8.50	15.40	96.66	2.51
severe stress		0	5.66	80.33	6.93	58.33	25.43	76.20	281.66	1.41
		25	6.30	100.00	8.23	80.30	18.36	60.03	254.66	1.4
		50	8.10	134.33	10.93	100.10	16.20	50.50	248.33	1.6
		75	8.80	140.00	11.43	107.73	15.83	47.50	237.66	1.83
no stress	Second Year	0	1.16	15.13	1.36	14.00	4.30	9.70	18.53	2.8
		25	1.30	16.66	1.61	20.36	4.10	9.10	16.60	2.98
		50	3.90	45.86	3.70	38.46	1.10	4.70	5.50	3.6
		75	2.56	30.93	2.90	36.10	3.00	7.30	9.70	3.32
moderate stress		0	5.90	79.63	6.60	70.16	9.10	25.86	95.63	2.3
		25	6.23	85.53	7.03	90.20	8.80	20.43	89.53	2.303
		50	10.30	150.06	12.10	145.66	6.30	13.60	30.66	2.8
		75	8.60	110.80	9.90	129.56	5.00	18.83	51.36	2.64
severe stress		0	5.90	57.10	5.83	44.96	20.90	69.40	197.10	1.54
		25	5.20	63.40	5.83	52.00	18.36	60.00	180.10	1.8
		50	7.60	100.66	9.20	101.06	11.73	48.60	124.00	2.2
		75	6.40	89.73	7.70	79.76	14.93	29.93	154.10	2.19
LSD			0.754	10.24	0.948	10.58	1.31	5.29	8.44	0.316

Table 3
Impacts of different GABA concentrations on morphological and yield traits of rapeseed.

drought stress	Year	GABA	Grain Yield (kg h ⁻¹)	Number of Siliques Per Plant	Number of Seeds Per Silique	1000 Kernel Weight (g)	Number of Side Branches	Harvest Index (%)	Plant Height (cm)	Silique Length (cm)	Stem Diameter (mm)	Biological Yield (kg h ⁻¹)
no stress	First Year	0	2651.2	72.005	20.17	3.46	4.66	59.25	108.067	5.5	8.27	4457.2
		25	2927.2	76.75	21.23	3.4	5.66	63.81	109.027	5.9	8.32	4587.2
		50	3089	77.54	22.48	3.5	6	65.50	111.96	6	9.53	4732.3
		75	3499.9	85.78	22.71	3.63	6.3	67.3	115.61	6.4	10.10	5194.3
moderate stress		0	2090.3	68.23	18.16	3	4.66	55.67	99.99	5.2	7.03	3734.6
		25	2273	70.08	19.05	3.3	5	58.08	102.38	5.36	7.4	3912
		50	2345.3	70.14	19.36	3.4	5.3	58.74	105.13	5.4	7.61	3975.9
		75	2474.1	71.92	20.15	3.43	5.66	59.57	108.05	5.5	7.87	4146.5
severe stress		0	1126.3	52.56	14.74	2.03	3.6	43	87.86	4.1	5.99	2626.3
		25	1689.1	64.20	16.43	2.16	4	52.45	93.03	4.7	6.65	3198.8
		50	1699.1	64.80	16.68	2.86	4.3	51.66	97.1	5.01	6.76	3253.8
		75	1978.3	68.09	18.05	3.06	4.6	56.23	99.12	5.24	7.01	3541.3
no stress	Second Year	0	3559.7	80.34	23.31	3.7	5.6	62.98	125.35	6.4	10.52	5645.7
		25	3830.3	82.38	24.48	3.86	6	67.76	126.57	6.7	10.84	5653.3
		50	4741.6	90.52	27.43	4.23	6.6	77.34	137.30	7.04	12.18	6179.2
		75	3989.3	82.89	25.34	4	6.3	70.08	134.96	6.8	11.47	5687.6
moderate stress		0	2828.7	74.46	20.51	3.3	4.6	55.99	117.81	5.9	9.03	5037.7
		25	2923.9	75.93	20.86	3.4	5.3	55.06	118.5	6.02	9.35	5288.1
		50	3477.8	80.25	23.30	3.63	6	61.7	125.08	6.42	10.47	5628
		75	3236.5	76.60	22.71	3.5	5.6	58.08	121.44	6.22	10.07	5558.1
severe stress		0	1654.3	58.78	16.58	2.56	4	47.05	98.06	4.5	6.98	3513.6
		25	2103	68.12	17.7	2.73	4.3	50.54	105.28	5.26	7.94	4183.7
		50	2722.8	74.45	20.50	3.26	5.3	54.12	113.62	6.14	9.12	5046.1
		75	2355.5	72.55	18.24	3.03	5	52.11	108.65	5.49	8.53	4558.1
LSD			628.28	10.59	2.93	0.59	1.06	7.7	12.75	0.94	1.87	859.11

Table 4
The effect of drought stress on oil and fatty acid profile of rapeseed under different GABA concentrations.

drought stress	Year	GABA	Oil percentage (%)	Oil Yield (kg h ⁻¹)	Oil Harvest Index (%)	Linolenic Acid (%)	Linoleic Acid (%)	Oleic Acid (%)	Palmitic Acid (%)	Erucic Acid (%)
no stress	First Year	0	39.4 ± 1.25	1047.93 ± 227.69	23.38 ± 2.60	6.01 ± 0.09	16.79 ± 0.19	61.98 ± 1.4	4.19 ± 0.13	0.202 ± 0.02
		25	39.7 ± 1.51	1162.51 ± 68.801	25.34 ± 1.16	6.07 ± 0.1	16.8 ± 0.16	61.58 ± 0.78	4.11 ± 0.14	0.18 ± 0.01
		50	42 ± 1.42	1298.25 ± 82.27	27.47 ± 0.73	6.77 ± 0.18	17.91 ± 0.41	60.1 ± 0.95	3.88 ± 0.17	0.168 ± 0.01
		75	42.1 ± 1.22	1475.24 ± 175.87	28.34 ± 1.38	6.8 ± 0.23	18.00 ± 0.81	60 ± 0.70	3.6 ± 0.12	0.12 ± 0.02
moderate stress		0	36.9 ± 1.49	768.61 ± 117.09	20.51 ± 1.18	5.02 ± 0.15	15.36 ± 0.45	63.2 ± 0.73	4.73 ± 0.06	0.39 ± 0.01
		25	37.2 ± 1.25	844.69 ± 29.07	21.60 ± 0.47	5.10 ± 0.12	15.65 ± 0.32	63.12 ± 0.25	4.7 ± 0.11	0.371 ± 0
		50	38 ± 1.21	888.61 ± 119.21	22.29 ± 1.03	5.54 ± 0.09	16.44 ± 0.38	62.38 ± 0.27	4.49 ± 0.1	0.273 ± 0.01
		75	39.1 ± 1.39	968.77 ± 120.42	23.31 ± 1.57	5.61 ± 0.1	16.51 ± 0.38	62.006 ± 0.46	4.29 ± 0.03	0.26 ± 0.01
severe stress		0	32.8 ± 1.13	370.26 ± 66.14	14.42 ± 3.76	4.3 ± 0.08	14.03 ± 0.33	64.19 ± 0.65	5.5 ± 0.06	0.54 ± 0.01
		25	32.6 ± 1.30	547.46 ± 96.30	17.07 ± 0.5	4.33 ± 0.08	14.03 ± 0.2	64.03 ± 0.22	5.47 ± 0.09	0.5 ± 0.03
		50	35 ± 1.25	594.37 ± 152.22	18.94 ± 7.29	4.4 ± 0.12	14.67 ± 0.35	63.92 ± 0.82	5.38 ± 0.07	0.41 ± 0.02
		75	36.8 ± 1.42	731.07 ± 145.70	20.92 ± 5.03	4.76 ± 0.14	15.11 ± 0.18	63.30 ± 0.9	5.27 ± 0.06	0.39 ± 0.01
no stress	Second Year	0	40.9 ± 1.49	1450.55 ± 204.36	25.76 ± 0.92	5.98 ± 0.05	17.97 ± 0.06	60.91 ± 0.76	3.22 ± 0.12	0.123 ± 0.01
		25	41.8 ± 1.61	1597.97 ± 57.37	28.38 ± 1.74	5.99 ± 0.21	17.99 ± 0.09	60.57 ± 0.46	3.17 ± 0.08	0.111 ± 0.01
		50	45.1 ± 1.65	2138.38 ± 186.32	34.60 ± 2.86	6.91 ± 0.07	18.61 ± 0.07	56.97 ± 0.16	3.03 ± 0.07	0.06 ± 0.04
		75	42.9 ± 1.39	1713.47 ± 209.13	30.06 ± 1.11	6.87 ± 0.06	18.51 ± 0.53	57.1 ± 1.42	3.08 ± 0.09	0.08 ± 0.02
moderate stress		0	38.2 ± 1.51	1085.65 ± 254.02	21.45 ± 3.16	4.73 ± 0.32	16.4 ± 0.04	62.03 ± 0.08	4.43 ± 0.13	0.311 ± 0.01
		25	38.7 ± 1.66	1127.78 ± 175.08	21.27 ± 0.9	4.73 ± 0.26	16.44 ± 0.05	61.90 ± 0.74	4.35 ± 0.1	0.3 ± 0.03
		50	40.5 ± 1.39	1409.12 ± 192.06	24.97 ± 1.74	5.19 ± 0.06	17.11 ± 0.05	61.08 ± 0.27	3.59 ± 0.08	0.20 ± 0.03
		75	39.3 ± 1.18	1273.68 ± 229.58	22.82 ± 2.36	5.07 ± 0.1	17.06 ± 0.16	61.18 ± 1	3.62 ± 0.06	0.227 ± 0.01
severe stress		0	36.7 ± 1.56	607.84 ± 66.66	17.27 ± 0.76	4.37 ± 0.05	14.11 ± 0.22	63.75 ± 1.12	5.08 ± 0.08	0.44 ± 0.02
		25	37 ± 1.48	779.79 ± 94	18.68 ± 1.15	4.41 ± 0.12	14.14 ± 0.09	63.51 ± 0.66	5.04 ± 0.08	0.41 ± 0.01
		50	38 ± 1.71	1036.90 ± 128.45	20.54 ± 0.8	4.61 ± 0.37	16.18 ± 0.23	62.38 ± 1.06	4.81 ± 0.08	0.34 ± 0.01
		75	37.7 ± 1.31	886.01 ± 74.95	19.66 ± 2.61	4.63 ± 0.32	16.2 ± 0.06	62.42 ± 0.58	4.78 ± 0.08	0.34 ± 0.02
LSD			1.92	250.79	4.22	0.254	0.509	1.191	0.151	0.025

Values are means ± standard error (± ER) of three replications (n = 3).

Discussion

Physiological characteristics, as well as the quantity and quality of plant production, can be influenced by changes in environmental factors, especially the occurrence of various stresses. Conversely, the use of some exogenous and environmentally friendly compounds can lessen the severity of such adverse factors by promoting positive effects on plant growth and development. In this study, the activities of antioxidant enzymes SOD, CAT, GPX, and APX increased significantly at both stress levels compared to non-stressed conditions, regardless of GABA application. Notably, under adverse environmental conditions, ROS production in plant cells increases, making plants more reliant on antioxidant defense mechanisms, including enzymes involved in ROS neutralization⁴⁰. Our findings showed that in rapeseed, water stress caused a significant rise in H₂O₂ and MDA concentrations compared to normal irrigation conditions. However, as shown in other studies^{41, 42, 43, 44}, exogenous GABA application helped plants more effectively remove these compounds by stimulating antioxidant pathways. It is important to note that H₂O₂ and MDA are produced through cellular metabolic activities and serve as indicators for assessing plant defense capacity against environmental stresses^{45, 28, 46}. Research indicates that GABA application reduces H₂O₂ levels by enhancing the regulation of antioxidant enzymes such as APX, CAT, and GPX^{47, 48}. There is ample evidence that these enzymes increase activity in various plant species exposed to water stress, especially at high levels^{49, 50, 51}. Additionally, SOD, an important antioxidant enzyme, helps reduce oxidative damage caused by O₂²⁻ by catalyzing its conversion to O₂ and H₂O₂⁵². Various studies have shown that exogenous GABA under stress conditions enhances plants' ability to withstand oxidative stress by upregulating antioxidant enzyme gene expression⁵³, stabilizing enzyme structures, improving ROS balance^{44, 54}, and increasing endogenous GABA content through GABA-shunt metabolism regulation⁵⁵. In our experiment, under severe stress conditions in the first and second years, the lowest enzyme activity was recorded in treatments without GABA, indicating the destructive effects of severe stress on these activities, consistent with other studies^{7, 41}. Furthermore, enzyme activity was reduced under severe stress compared to moderate stress, possibly due to decreased photosynthesis, increased cell membrane damage, enzyme inactivation, or disrupted antioxidant gene expression at high stress levels^{56, 57}. This is mainly because maintaining enzyme activity demands more energy, which the plant struggles to supply during increased water stress⁵⁸. Also, under such conditions, plants may degrade non-essential proteins, including antioxidant enzymes that can no longer control ROS, activating proteases to generate amino acids for respiration or essential protein synthesis⁵⁹. However, GABA application, especially at high concentrations (50 and 75 mM), increased antioxidant enzyme activity regardless of these fluctuations. Nonetheless, under severe water stress, GABA's stimulating effect on the antioxidant system was diminished, and the plant's defense response declined, aligning with findings by Yang et al.⁷. The lesser improvement in enzyme activity under severe stress, despite GABA application, may be because the plant's capacity to respond is severely limited, hindered further by energy shortages and ribosomal damage, preventing extensive new protein synthesis. Therefore, the observed enzyme activity increase likely results from activating dormant enzymes and reducing their degradation, rather than new synthesis⁶⁰. Overall, these results highlight that the effectiveness of biochemical interventions like GABA depends heavily on plant-specific conditions and is most beneficial when the plant retains enough metabolic capacity to mount defense responses, rather than during late stress stages. In fact, plant reactions to water deficit appear more aligned with the severity of stress perceived rather than the deficit itself^{7, 41}.

In the present study, in most treatments, the levels of antioxidant enzymes were generally lower in the second year compared to the first year. This may be linked to higher rainfall, increased humidity, and lower temperatures during the second year (refer to table or meteorological data). However, the overall trend of plant response to GABA treatments remained consistent and reliable across both years, indicating the effectiveness and stability of the plant's physiological responses to this application. Notably, the positive effects of GABA were not limited to stress conditions; even under fully irrigated conditions, foliar application of this substance increased antioxidant enzyme activity compared to treatments without GABA. This suggests that GABA, besides protecting against stress, can also serve as a primary stimulator of the antioxidant defense system under normal conditions. The beneficial effects of GABA foliar spraying have been confirmed in black pepper (*Piper nigrum* L.)⁶¹, creeping bentgrass (*Agrostis stolonifera*)⁶², and beans (*Phaseolus vulgaris*)⁶³.

In our study, increasing stress intensity led to higher proline levels. Proline is known as a stress-related amino acid whose levels typically change with stress intensity and plant tolerance^{64, 65}. However, in most cases, continued water stress results in a significant increase in proline levels⁶⁶. Proline often plays a key role in boosting stress tolerance by participating in osmotic regulation, maintaining cellular water balance, stabilizing protein structures, and scavenging reactive oxygen species (ROS)^{45, 67}. In this study, the increase in proline content aligned with the plant's adaptive response to stress, reflecting the accumulation of an osmolyte. Additionally, when comparing years, proline content in the second year was lower than in the first year, even under similar stress conditions. This difference may be due to variations in climatic conditions or a greater ability to cope with stress in the second year, possibly due to more effective rainfall. The application of GABA in both crop years of this experiment significantly reduced proline levels under stress conditions. Research indicates that treating plants with GABA regulates proline and other compatible compounds such as glycine betaine under various stress conditions and in different plant species^{44, 47, 54}. In crop plants, proline not only acts as an effective osmotic stabilizer to maintain cell pressure but also functions as a non-enzymatic antioxidant, neutralizing free radicals within the cell and stabilizing proteins and membrane structures⁶⁸. Since proline synthesis is highly energy-consuming and requires large amounts of nitrogen and carbon⁶⁹, it appears likely that GABA reduces the need for proline accumulation through several parallel pathways. First, GABA decreases the antioxidant burden of proline by enhancing the antioxidant enzyme defense system. Second, GABA itself may directly contribute to osmotic regulation by helping control cell water potential through cation balance, possibly partially replacing proline's osmotic function. Third, GABA may drastically reduce the initial need for osmotic adaptation by lowering stress signals (such as hormonal changes) or improving plant water status via stomatal regulation²². In this scenario, the plant recognizes that with GABA, it no longer needs to allocate resources toward producing a costly compound like proline. Instead, it can direct stored energy and nitrogen to repair processes, maintaining membrane integrity, and supporting basal metabolism⁷⁰.

The results of the present study also indicated a decrease in chlorophyll a and b levels as stress intensity increased and highlighted the role of GABA in maintaining the stability of photosynthetic pigments and in creating protective responses against the negative effects of water stress on the photosynthesis process. Similarly, a comparable trend was observed for carotenoids. In general, changes in the amounts of photosynthetic pigments, which are known as one of the main indicators of the decline in leaf photosynthetic capacity in response to environmental stresses, including water stress⁷¹, are mainly caused by processes such as activation of the chlorophyllase enzyme, increase in reactive oxygen species (ROS), lipid peroxidation, and damage to internal

membranes, ultimately leading to a decrease in chlorophyll content through intensified electrolyte leakage⁷². Numerous pieces of evidence also indicate that oxidative stress caused by water deficit directly results in the destruction of photosynthetic pigments and a reduction in photosynthesis^{73, 74, 75}. However, in the present study, the application of different levels of GABA significantly reduced these effects, likely due to the reduction of pigment degradation, modulation of chlorophyll synthesis, improvement of antioxidant system efficiency, and regulation of stomatal and intracellular CO₂^{76, 77}. This suggests that although water stress caused a decrease in photosynthetic pigments, the application of GABA before exposure to stress significantly improved these pigments⁷³. Regarding carotenoids, under conditions of increased water stress, these pigments serve as vital protective factors in maintaining chloroplast membrane structure and inhibiting chlorophyll photooxidation, thereby preventing further degradation of photosynthetic pigments⁷⁸. In this context, GABA not only accelerates the biosynthesis of polyamines and their precursors but also prevents their degradation, thereby increasing carotenoid content in the plant⁷⁹. Moreover, it has been reported that exogenous GABA helps maintain the structure and function of photosystem II under stress conditions⁸⁰. Several studies have also shown that GABA application under environmental stress significantly increases carotenoid levels in various plant species, including pepper (*Capsicum annuum* L.) and soybean (*Glycine max* L.), which aligns with our findings and underscores GABA's strong potential in mitigating stress effects on photosynthetic pigments^{26, 73, 81}.

The yield and its components of rapeseed, like those of other crops, are influenced by internal and external factors, especially water stress, which reduces both the quantity and quality of production by disrupting physiological and biochemical processes related to growth²⁴. In rapeseed, water stress at different stages, particularly during reproductive growth, decreases water and assimilate availability, resulting in pollen grain sterility, flower shedding, a lower percentage of flowers developing into carpels, and seed loss, ultimately lowering seed number and weight^{82, 83, 84}. One key trait affecting yield is the number of seeds per silique; a higher count can provide a larger reservoir of photosynthetic materials, leading to increased yield^{83, 85}. It has also been noted that silique shedding after formation mainly results from insufficient photosynthetic materials and resources for developing siliques⁸⁶. Consequently, water shortage impacts numerous physiological activities, severely reducing the number of siliques and seeds^{87, 88}. Furthermore, water stress can negatively affect grain yield by decreasing thousand-grain weight⁸⁹, due to reduced production of photosynthetic assimilates, impaired transport of these materials to the grains, shortened flowering to maturity period, or decreased grain filling duration^{90, 91}. Since grain number and grain weight are interconnected, under stress conditions, fewer reservoirs result in more processed material per seed, leading to larger grains with higher weight per thousand grains an effect that depends on stress severity^{92, 93}. Comparison of two crop years in the present study showed that the values of yield and yield components were lower in the first year than in the second year, which could be due to lower rainfall and higher temperature in the first year than in the second year, especially during the pollination and grain filling stages (Figs. 1 and 2). These conditions increase evaporation and transpiration, reduce mineral uptake from the soil, and accelerate leaf aging⁹⁴, shortening this period and damaging reproductive structures. Conversely, several studies have demonstrated that GABA application can enhance performance traits, ultimately raising productivity^{95, 96}. GABA can induce positive morphological and physiological changes, such as improving photosynthesis, gas exchange, chlorophyll synthesis, maintaining cell structure, membrane stability, and strengthening enzymatic defenses, all of which can significantly increase grain yield^{95, 97}. Mekontso et al.⁹⁸ and Kumari et al.⁹⁹ noted that GABA application can improve nutrient absorption under stress. Additionally, GABA can enhance thousand- kernel weight and seed number per inflorescence under both stressed and non-stressed conditions, acting as a moderator⁹⁷. In this study, increased antioxidant enzyme activity, which reduced water stress effects, highlighted the beneficial role of GABA in stress conditions, leading to higher grain and biological yields. Plants treated with GABA also showed increases in silique length, seed number per silique, and silique number per plant all directly contributing to higher final grain yield. Water stress negatively impacted both grain and biological yields, as noted by Niknam et al.¹⁰⁰ and Khalili et al.¹⁰¹, who found significant reductions in rapeseed biological performance. Specifically, drought-induced premature leaf senescence and diminished ongoing photosynthesis are primary factors in biomass decline¹⁰², compounded by reduced stomatal conductance and metabolic disruption, which impair growth¹⁰³. Environmental stresses can also damage the photosynthetic electron transport chain, further reducing growth of green tissues. Conversely, Xiang et al.⁸⁰ showed that exogenous GABA application can mitigate these effects, boosting photosynthesis and biomass. Recent research confirms GABA's role in protecting plants under environmental stresses by enhancing photosynthetic efficiency and resource use^{96, 104, 105}. Regarding product quality, primarily related to seed oil trait, our study found that this component were affected by treatments. Stress often reduces oil percentage and yield by impairing oil droplet formation and accumulation^{106, 107}. These findings highlight the importance of sufficient moisture for oil biosynthesis, with deficits particularly detrimental at the season's end, impacting seed oil quantity and yield^{108, 109, 110}. Water and heat stress lead to decreased oil percentage by speeding up ripening and reducing thousand-grain weight¹¹¹. Moreover, Seyyedi et al.¹¹² indicated that oil yield correlates directly with grain yield, whereas oil percentage is less affected by environmental or genetic factors because it is controlled by many genes.

In the present study, the effects of drought stress levels and different GABA concentrations varied on fatty acid levels. As in previous research, increased water stress led to a decrease in linolenic and linoleic acids^{113, 114} and a rise in oleic and palmitic acids^{115, 116, 117}. These changes might be due to the shortened growth period caused by water shortage and disruption in unsaturated fatty acid synthesis¹¹⁸. Specifically, as this period shortens, enzyme activity involved in producing linolenic and linoleic acids diminishes, along with NADPH deficiency. Consequently, the conversion of oleic acid to linolenic and linoleic acids decreases, increasing oleic acid while reducing the others¹¹⁹. Additionally, the increased presence of ROS under water stress has less impact on oleic and palmitic acids but a greater effect on linolenic and linoleic acids, which contain multiple double bonds¹²⁰. Moreover, this situation may trigger an adaptive response, increasing oleic and palmitic acids to reduce membrane fluidity and prevent ion leakage by packing lipid molecules more tightly¹²¹. However, Zunun-Pérez et al.¹²² noted that fluctuations in fatty acid levels can be influenced by the timing and severity of water stress at different plant growth stages. In our study, under higher water stress conditions, erucic acid percentage increased, aligning with findings from Ullah et al.¹²³ and Safavifard et al.¹¹³. It appears that, under water stress, reduced internal energy levels and heightened activity of fatty acid elongase (FAE1) facilitate oleic acid synthesis, setting the stage for the accumulation of long-chain fatty acids like erucic acid. Other reasons for the rise in erucic acid (with one double bond) include the need for membrane lipid stability and decreased vulnerability to oxidative damage¹²⁴.

The results of this study showed that the application of exogenous GABA influences fatty acid composition under water stress conditions. Kumar et al.¹²⁵ reported that exogenous GABA treatment enhances the metabolic pathways of unsaturated fatty acids, especially linoleic acid and alpha-linolenic acid, by increasing the conversion of carbon skeletons and energy from sucrose. Additionally, considering GABA's effect on reducing oxidative stress²⁵, which was also observed in this study, the changes in fatty acid composition were expected. Furthermore, Palabıyık et al.¹²⁶ reported that activation of the GABA-shunt pathway under water stress conditions and the subsequent expression of fatty acid desaturase genes will affect "cell membranes, and unsaturated fatty acids".

Data from correlation tables and regression analyses showed that the selected traits were significantly related to performance. Grain yield had a positive and significant correlation with biological yield, plant height, stem diameter, number of side branches per plant, number of siliques per plant, silique length, number of seeds per plant, thousand-kernel weight, oil percentage, and harvest index (Table 5).

Grain yield showed a strong and significant relationship with biological yield ($r = 0.95$) and stem diameter ($r = 0.98$) (Table 6). It appears that conditions or treatments that enable plants to perform optimally and increase dry matter production also positively influence grain production. Increasing stem diameter may provide more storage space for photosynthetic materials before flowering and facilitate remobilization, leading to increases in the number of siliques per plant ($r = 0.93$), seeds per silique ($r = 0.96$), and seed weight ($r = 0.87$). Additionally, the correlation between the number of side branches and biological yield ($r = 0.85$), grain yield ($r = 0.92$), and harvest index ($r = 0.91$) indicates that the plant's radial growth and production of side branches significantly affect biomass accumulation, grain production, and overall yield. This trait also showed a positive and significant relationship with the harvest index, reinforcing its greater impact on grain production and yield compared to biomass. In other words, in rapeseed, breeding and management efforts should focus on increasing the number of secondary branches. Grain yield also showed a strong and significant relationship with the number of seeds per silique ($r = 0.99$, $R^2 = 0.98$), plant height ($r = 0.97$, $R^2 = 0.93$), and the relationship between plant height and the number of side branches ($r = 0.85$), as well as between the number of side branches and siliques per plant ($r = 0.93$) (Table 6). This information suggests that increasing plant height can promote the development of more secondary stems, thereby increasing silique production, seed number per plant, and ultimately grain yield. These findings indicate that applying treatments that enhance plant height and radial growth can lead to higher yields.

Table 5
Correlation coefficients between traits measured of rapeseed under different GABA concentrations.

Characteristics	Oil Harvest Index (%)	Oil Yield (kg h ⁻¹)	Oil percentage (%)	Stem Diameter (mm)	Plant Height (cm)	Number of Side Branches Per Plant	Silique Length (cm)	Biological Yield (kg h ⁻¹)	Harvest Index (%)	1000 kernel weight (g)	Number of Seeds Per Plant	Number of Siliques Per Plant
Oil Harvest Index (%)	1.00											
Oil Yield (kg h ⁻¹)	0.95	1.00										
Oil Percentage (%)	0.96	0.95	1.00									
Stem Diameter (mm)	0.87	0.97	0.91	1.00								
Plant Height (cm)	0.84	0.95	0.89	0.98	1.00							
Number of Side Branches Per Plant	0.93	0.92	0.93	0.88	0.85	1.00						
Silique Length (cm)	0.89	0.96	0.91	0.96	0.96	0.92	1.00					
Biological Yield (kg h ⁻¹)	0.80	0.93	0.88	0.97	0.97	0.85	0.96	1.00				
Harvest Index (%)	0.99	0.92	0.91	0.82	0.80	0.91	0.87	0.76	1.00			
1000 kernel weight (g)	0.92	0.92	0.94	0.87	0.89	0.92	0.93	0.87	0.90	1.00		
Number of Seeds Per Plant	0.95	0.99	0.95	0.96	0.96	0.93	0.97	0.94	0.92	0.94	1.00	
Number of Siliques Per Plant	0.92	0.95	0.92	0.93	0.91	0.93	0.97	0.93	0.91	0.92	0.95	1.00
Grain Yield (kg h ⁻¹)	0.94	1.00	0.94	0.98	0.97	0.92	0.98	0.95	0.91	0.93	0.99	0.97

Table 6

Simple linear regression analysis showing relationships among morphological and yield traits of rapeseed under different GABA concentrations.

Dependent Variable	Independent Variable	Slop	Standard Error	Path Coefficient	T-statistic	P-value	Coefficient of Determination
Grain Yield (Kg h ⁻¹)	Number of Seeds Per Plant	272.66	6.85	0.99	39.81	0.00	98.60
Number of Seeds Per Plant	Silique Length (cm)	4.10	0.22	0.97	18.97	0.00	94.20
Silique Length (cm)	Number of Siliques Per Plant	0.08	0.00	0.97	20.29	0.00	94.90
Number of Siliques Per Plant	Number of Side Branches Per Plant	9.72	0.79	0.93	12.36	0.00	87.40
Number of Side Branches Per Plant	1000 kernel weight (g)	1.438	0.131	0.920	10.998	0.000	84.6
1000 kernel weight (g)	Harvest Index (%)	0.061	0.006	0.901	9.746	0.000	81.2
Harvest Index (%)	Plant Height (cm)	0.482	0.077	0.802	6.297	0.000	64.3

Conclusion

This study demonstrates that foliar application of γ -aminobutyric acid (GABA) is an effective strategy for mitigating drought-induced damage in canola (*Brassica napus* L.). In this context, the dose used can vary according to environmental condition. Under water deficit conditions, GABA (particularly at 50 and 75 mM) significantly enhanced the antioxidant defense system, elevating the activity of key enzymes (SOD, CAT, APX, GPX) and consequently reducing oxidative stress markers (H₂O₂ and MDA). This mitigation of oxidative damage was associated with the preservation of photosynthetic pigments (chlorophyll a, b, and carotenoids) and a reduced reliance on proline accumulation, indicating improved cellular homeostasis. Crucially, GABA application translated these physiological benefits into agronomic gains, improving yield components (siliques per plant, seeds per silique, thousand-kernel weight) and final grain yield under both moderate and severe stress. Furthermore, GABA positively influenced seed quality by modulating fatty acid composition, increasing the proportion of linoleic and linolenic acids while decreasing oleic, palmitic, and erucic acids under stress, thereby enhancing oil nutritional profile. The optimal concentration was context-dependent, with 75 mM more effective in a more severe drought year and 50 mM sufficient under milder stress. Therefore, GABA acts as a multifaceted plant biostimulant, integrating antioxidant enhancement, physiological protection, and metabolic reprogramming to bolster drought tolerance and secure yield and quality in canola, presenting a promising tool for sustainable crop management.

Declarations

Ethics approval and consent to participate

The manuscript is an original work that has not been published in other journals.

Consent for publication

All authors agreed to the publication.

Competing interests

The authors declare no competing interests

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Author Contribution

Kh.N has done all the practical work, F.Sh and A.A wrote the main manuscript text and M.J has done the editing of the manuscript and N.S performed data analysis. All authors reviewed the manuscript.

Data Availability

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Figures

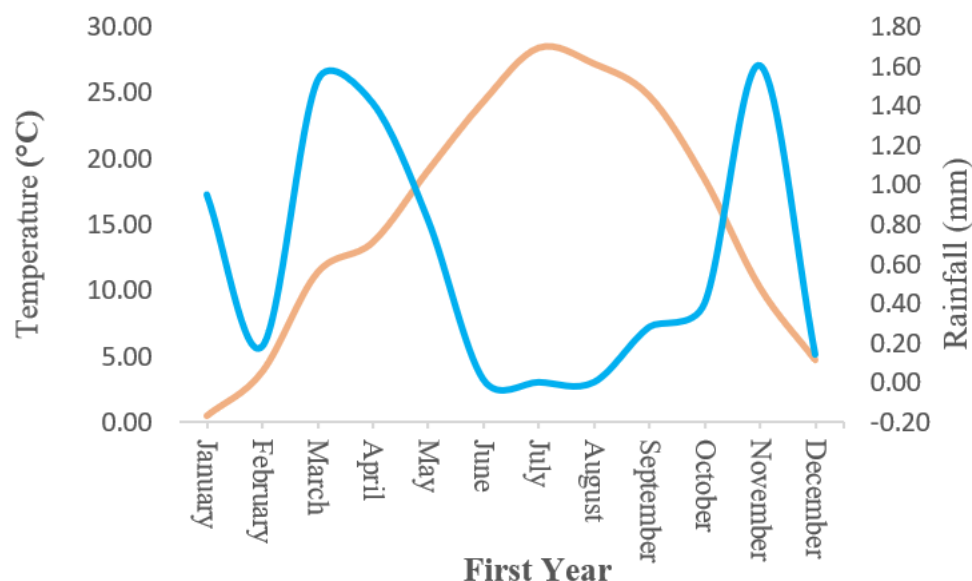


Figure 1

Average temperature and rainfall during the plant growth period in 2022-2023 cropping year.

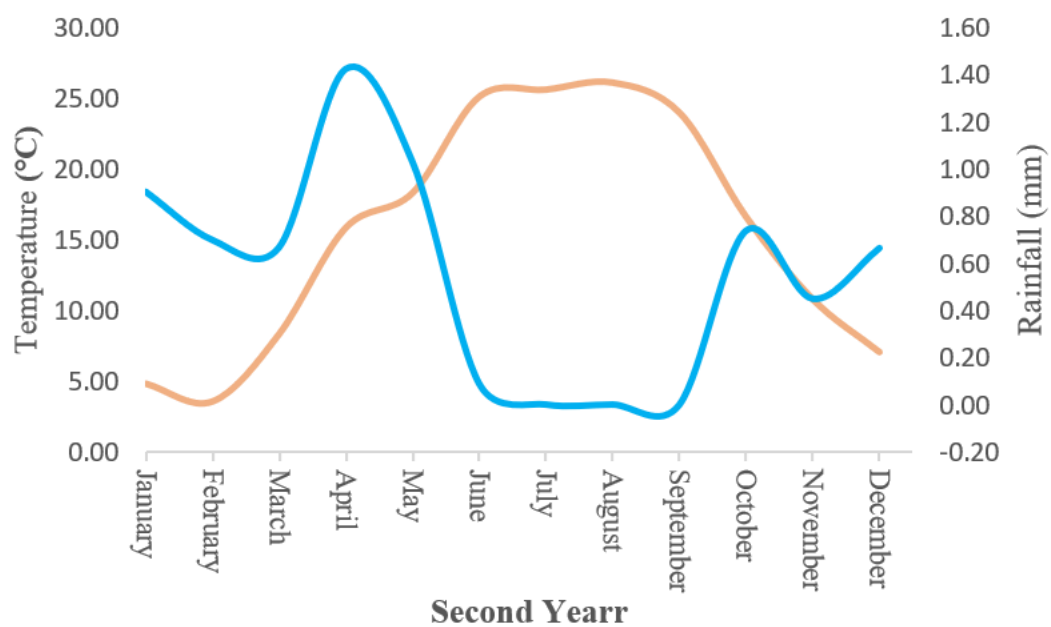


Figure 2

Average temperature and rainfall during the plant growth period in 2023-2024 cropping year.