Pol. J. Environ. Stud. Vol. 33, No. 5 (2024), 5367-5479
DOI: *10.15244/pjoes/177182*

Original Research

Exogenous Application of β-aminobutyric Acid Improved Water Relations, Membrane Stability Index, and Achene Yield in Sunflower Hybrids Under Terminal Drought Stress

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Received: 28 August 2023
Accepted: 17 December 2023

Abstract

Sunflower (*Helianthus annuus* L.) is a major oilseed crop grown for edible oil throughout the world, including Pakistan. Drought stress is the most significant constraint to crop production in a changing climate scenario, and its severity is increasing day by day. Less rainfall and rising temperatures, particularly during the seed-filling phase, reduce achene yield in sunflower hybrids. Exogenous application of β aminobutyric acid (BABA) is effective in reducing the severity of terminal drought stress in many crop plants. Therefore, the present study was conducted to assess the effect of foliar BABA application on the membrane stability, growth, and productivity of sunflower hybrids under induced terminal drought conditions. A pot experiment was carried out to compare the growth and yield potential of two sunflower hybrids, Hysun-33 and NK-Senji, to two levels of water application: normal irrigation (NI) and terminal drought (TD). To combat terminal drought, four BABA concentrations (0, 20, 40, and 60 mM) were used. When compared to NK-Senji, Hysun-33 had the highest SPAD-chlorophyll value, head weight, 100-achene weight, and achene yield. In comparison to normal irrigation terminal drought

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increased proline content while lowering relative water content, SPAD-Chlorophyll, 100-achene weight, and achene yield. However, under terminal drought, foliar application of β-aminobutyric acid improved relative water content, SPAD-chlorophyll value, head weight, 100-achene weight, and achene yield. When different levels of BABA were used, the application of 60 mM produced 46% more achene yield than the control. A reduced achene yield was obtained under terminal drought which was improved by foliar spraying of 60 mM β-aminobutyric acid which increased in SPAD-chlorophyll value, membrane stability index, relative water content, and 100-achene weight. Overall, the exogenous application of BABA improved water relations, membrane stability index, and achene yield in sunflower hybrids under terminal drought stress.

Keywords: achene yield, β-amino butyric acid, membrane stability index, sunflower hybrids, terminal drought

Introduction

Sunflower is an important oilseed crop grown for its edible oil in the majority of countries, including Pakistan. However, its productivity is severely hampered by a variety of abiotic stresses including drought. Moisture stress is a frequent limiting factor in the growth and productivity of field crops [1]. Climate change has altered rainfall patterns, causing drought severity in the most semi-arid regions. This has resulted in a drop in soil moisture content, resulting in leaf wilting and significant yield reductions in field crops [2]. Crop yield and related traits were significantly reduced under drought stress circumstances due to disruption of leaf gas exchange properties which restrict the size of source, sink tissues, and affect assimilate translocation and dry matter partitioning [3, 4].

Plant physiological and biochemical responses to drought stress include changes in leaf relative water content [5], photosynthetic efficiency, antioxidant activity, carbohydrate metabolism, protein and lipid peroxidation [5-8], root proliferation [9], and membrane stability [4]. Drought stress significantly impairs the gas exchange characteristics due to reduced leaf area and size, early leaf senescence, and changes in the structure of pigments and proteins [10]. Reduced yield and associated characteristics may also be the result of stomatal closure which decreases CO_2 absorption and results in decreased photosynthesis [11]. Numerous studies have concluded that the decline in photosynthetic activity is mostly due to a disruption in stomatal control caused by drought stress [12, 13]. Additionally, it indicates that when plants are subjected to drought stress their leaf relative water content and transpiration rate decrease dramatically, lowering crop yield [14]. Drought stress adversely affects plant growth and leaf water content [15]. Nonetheless, biomass accumulation and leaf relative water contents (RWC) are the primary indicators of plant performance under drought stress [16].

Plants' acclimation to water deficit is the result of different events that lead to adaptive changes in plant growth and physio-chemical processes, such as changes in plant structure, osmotic potential, and antioxidant defense mechanisms [17]. Plant defensive mechanisms against drought stress are affected by a variety of factors, including the genetic make-up of the cultivar, crop growth stage, and severity and duration of water stress [18]. Numerous studies have shown that drought stress reduces achene yield, oil yield, and oil quality in sunflowers [5, 19]. However, the severity of drought stress was found to be different for various growth stages. It has been discovered that exposure to drought during the anthesis, and achene filling stages is the most critical, causing a 50% reduction in sunflower yield [20, 21]. Although sunflower is a moderately drought-tolerant crop, it is sensitive to drought stress during the reproductive stages, from early flowering to achene filling [22].

Different osmolytes, such as glycine betaine, proline, H_2O_2, γ-aminobutyric acid (GABA), and β-aminobutyric acid (BABA) have been successfully used to make plants resilient to abiotic stresses, especially drought stress [3, 23, 24]. β-Aminobutyric acid (BABA) is a non-protein amino acid that promotes plant tolerance to a variety of abiotic stresses including low potassium stress [25, 26], salt stress [27], heat stress [28], cadmium toxicity [29], and acid rain toxicity [30]. This broad-spectrum application of BABA suggests that it has the potential to activate plant resistance mechanisms against abiotic stresses [31].

Exogenous application of BABA has been shown to improve osmotic balance by promoting the synthesis of osmolytes, which reduces oxidative damage by optimizing the activities of antioxidant enzymes [32]. It has been reported that BABA-treated plants accumulated more abscisic acid (ABA), which is important in reducing drought stress in field crops. BABA pre-treatment is effective in arabidopsis [31, 33], maize [34], and wheat [35] in improving drought tolerance by regulating antioxidant enzymes, ABA accumulation, and stomatal regulation. BABA is an important osmolyte used to mitigate drought through different techniques, such as seed priming, seed coating, and foliar spray. A lot of work has been done on BABA for its use as a pre-treatment drought-mitigating agent in field crops, but its use on crop foliage has not been extensively studied previously, especially in oilseed crops. Keeping in view the importance of BABA as mentioned above, the current study was designed to determine the effect of foliar BABA application on the achene yield of sunflower hybrids under terminal drought stress.

Materials and Methods

Experimental Details

The current experiment was conducted in a wire-house at Bahauddin Zakariya University, Bahadur Sub Campus Layyah (latitude 31.25ºN and longitude 73.09ºE), during the 2018 crop season. The soil used in pots was collected from a nearby field area and analyzed physio-chemically. The soil was sandy loam with a 0.65% organic matter, pH (8.4), available phosphorus (9 ppm), total nitrogen (0.17 g kg^{-1}), available potassium (84 ppm), and electrical conductivity (1.14 dS m^{-1}).

In this study, two sunflower hybrids, Hysun-33 and NK-Senji, purchased from Monsanto (Pvt) Ltd and Syngenta (Pvt) Ltd respectively, were used. The hybrids were chosen based on the majority of cultivated hybrids in the region with good yield and early sown with high oil content, especially in Hysun-33. Seeds were sown on 8th March 2018 in clay pots (27 × 18 cm). The experimental design used for this study was a completely randomized design (CRD) with 3-replications. A total of 48 pots were used for this study and all these pots were filled with 12 kg potting mixture that had a soil-to-farmyard manure ratio of 3:1. The pots were watered and 5 seeds per pot were sown at a depth of 2 cm. Based on the soil analysis report, fertilizer was applied at 60 g N, 40 g P, and 25 g K per pot using urea (46% N), di-ammonium phosphate (DAP) (18% N, 46% P), and sulfate of potash (SOP) (50% K). The entire amount of DAP and SOP was applied at sowing, while urea was applied in two splits at the 5-leaf and flowering stages. After germination, two plants were kept in each pot and raised to maturity. Both hybrids were grown in well-watered conditions until flowering. At the start of the flowering stage, the pots were divided into two sets, and then water stress treatments of well-watered (WW) and terminal drought (TD) were applied. In WW, the water holding capacity (WHC) of the soil was maintained at 80%, whereas in TD, the water stress was imposed by keeping WHC at 45%. The required amount of water for maintaining WHC according to treatments was determined by weighing pots after the application of the calculated amount of water and designated as the target weight. The target weight of the pot was achieved for drought imposition by applying water every alternate day. After one week of drought, a foliar spray of β-Amino Butyric Acid (BABA) (97% purchased from Sigma-Aldrich) was prepared in four different concentrations i.e. 0 (water spray), 20, 40, and 60 mM; and applied on foliage to mitigate drought stress. The whole plant was fully sprayed with BABA solution for BABA treatment and water sprayed for controlled treatment. The drought period was maintained for two weeks after imposition. The crop was harvested on 15th June 2018 when it reached physiological maturity.

Data Collection

At the flowering stage, the SPAD-chlorophyll value was estimated non-destructively after five days of BABA foliar spray. The reading was taken from three different locations on the flag leaf using a portable SPAD-502 Chlorophyll Meter (Minolta Co. Ltd.), and then averaged. To determine the leaf's relative water content, fresh leaves were collected from each pot and transported to the laboratory to be measured for fresh weight (FW) of the leaf. The leaves were then immersed in distilled water for 24 hours at room temperature in the dark. After 24 hours leaves were taken out from water and weighed to determine the turgid weight (TW). Following that, leaf samples were dried in an oven at 70ºC until they reached a constant weight and then weighed to obtain dry weights (DW). The relative water content (RWC) was calculated using the formula proposed by Barrs and Weatherley [36].

$$\mathrm{RWC} = \left[\frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})}\right] \times 100$$

Leaf samples were collected from each treatment for measuring membrane stability index (MSI), and 0.2g leaf was used for further testing. Two sets of test tubes were prepared, each containing 10 ml of distilled water and 0.2g leaf material. One set of test tubes was placed in a water bath for half an hour at 40ºC and the electrical conductivity of each test tube sample was measured using an EC meter (C_1). The second set of test tubes was kept in a water bath at 100ºC for 15 minutes and then their electrical conductivity was measured (C_2). The following formula was used to determine the membrane stability index (MSI).

$$MSI = 1 - \frac{C1}{C2} \times 100$$

Proline concentration was determined following Bates et al. [37]. About 0.5 g of fresh leaves were collected and homogenized in 10 ml of 3% sulfosalicylic acid solution before centrifugation at 13800 rpm for 10 minutes. The homogenate was then filtered and 2 ml of filtrate was mixed in a test tube with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. All the samples in test tubes were incubated for one hour at 100ºC before being cooled in an ice bath. Following cooling, 4 ml of toluene was added to each test tube and vigorously shaken for 1 minute. A spectrophotometer is used to measure the intensity of the light. At 520 nm, the absorbance of the chromophore was measured. On a fresh weight basis, the proline from a standard curve was determined and recorded.

Yield-related Traits and Yield

For head diameter, two plants were taken from each pot and their diameter was measured with a Vernier caliper and averaged. The stem diameter of two plants from each pot was measured and averaged using a Vernier caliper. Two plants were manually harvested from each pot, sundried for one week, and weighed to determine biological yield per pot. Following that, the same plants were manually threshed and the achene yield (g/pot) from each pot was recorded using an electric balance. A random sample was collected from each pot for 100 achene weight, and 100 achenes were manually counted and weighed, to obtain 100 achene weight. The achenes from two heads in each pot were counted and averaged to determine the number of achenes per head. For head weight, two heads were taken from each pot and weighed on a weight balance. At maturity, the plant height of two plants was measured with a measuring tape from the stem base to the tip of the head and averaged. The harvest index (HI) was calculated using the following formula shown below:

$$H.I = \frac{Economic\ yield}{Biological\ yield} \times 100$$

Statistical Analysis

The data collected on all parameters were analyzed statistically using the Fisher's analysis of variance technique with the program software Statistix-8.1 and the means of treatments were compared using the least significant difference (LSD) test at the 5% probability level [38].

Results

This study indicated that sunflower genotypes differed significantly on all morphological, physiological, and yield parameters (SPAD-chlorophyll, relative water content RWC, plant height, head diameter, number of achene per head, 100-achene weight, and achene yield). The effect of terminal drought stress on SPAD-chlorophyll, relative water contents (RWC), membrane stability index (MSI), and proline content were significant (Table 1). When compared to well-watered treatments, terminal drought decreased SPAD-chlorophyll, RWC, and MSI levels while increasing proline levels. Similarly, stem diameter (SD), head diameter (HD), head weight (HW), hundred achene weight (HAW), and achene yield were all reduced when compared to a well-watered treatment during terminal drought stress (Table 2-3).

When compared to NK-Senji, Hysun-33 performed better in terms of SPAD-chlorophyll value, RWC, MSI, and proline content (Table 1). Similarly, Hysun-33 produced more SD, HD, HW, HAW, and achene yield than NK-Senji (Table 2-3). Foliar application of BABA improved physiological and yield attributes, resulting in increased achene yield of sunflower. When compared to other treatments, 60 mM application of BABA produced more SPAD-chlorophyll value, RWC, MSI, and proline contents, whereas the minimum value of these parameters was recorded in the control treatment, which was sprayed with water only (Table 1). Also, 60 mM BABA application resulted in higher achene yield (AY) and yield-related traits viz. SD, HD, HW, and HAW, while the controlled treatment resulted in the lowest value of these attributes (Tables 2-3).

For SD, HAW, and AY, there was a substantial interaction effect for hybrid × BABA. When compared to other combinations, Hysun-33 produced a higher SD with 40 mM BABA application. In comparison to other combinations, Hysun-33 with 60 mM BABA application produced higher HAW and AY (Table 4). However, in the control treatment, NK-Senji had the lowest AY and HAW (Table 4). For the physiological and yield traits (RWC, MSI, SD, HD, HW, HAW, and AY), hybrid × drought had a significant interaction effect. It was also observed that Hysun-33 performed well under both well-watered and drought-stressed conditions, demonstrating its drought tolerance ability when compared to NK-Senji. When compared to NK-Senji, Hysun-33 produced more RWC, MSI, SD, HD, NOA, HW, and AY under well-watered and drought-stressed conditions (Table 5; 6).

NOA, HAW, AY, and BY all showed a significant interaction effect for drought × BABA. Foliar application of 60 mM BABA resulted in more BY and AY because of increased HAW, and NOA whereas, the minimum value of these attributes was recorded under drought-stressed grown sunflower with water spray (Table 7). However, under terminal drought conditions, higher AY and BY were recorded with 60mM BABA application compared to the control, indicating that the application of BABA solution improved AY and BY compared to the controlled treatment (Table 7). Similarly, for hybrid × BABA × drought only HAW and AY showed a significant interaction. When compared to other combinations, Hysun-33 with 60mM BABA produced the highest AY and HAW, whereas NK-Senji in the control treatment produced the lowest AY and HAW recorded under terminal drought conditions (Table 8). Furthermore, when compared to the control treatment, BABA application increased AY under terminal drought conditions (Table 8).

Discussion

Sunflower is usually cultivated in semi-arid to arid climates, and its success is largely dependent on supplemental irrigation. As a result, it is subjected to a variety of abiotic stresses, the most prominent of which is drought stress [39, 40]. The current study found that terminal drought stress significantly reduced

Table 1. Effect of BABA levels on SPAD-Chlorophyll, relative water contents, membrane stability index, and proline content in sunflower hybrids under terminal drought stress.

	SPAD-chlorophyll value	RWC (%)	MSI (%)	Proline content (mg g^{-1})
Drought environment (D)				
Well-Watered	41.627a	69.813 a	41.138 a	0.3066 b
Drought Stress	37.406 b	61.402 b	35.472 b	0.3856 a
LSD	0.66	1.85	0.87	9.59
Hybrids (H)				
Hysun-33	40.050 a	67.716 a	39.087 a	0.3539 a
NK-senji	38.983 b	63.499 b	37.523 b	0.3383 b
LSD	0.66	1.85	0.87	9.59
Foliar application of BABA				
0 mM	38.546 c	62.778 b	35.786 c	0.3045 c
20 mM	39.000 bc	65.399 ab	38.104 b	0.3385 b
40 mM	39.967 ab	66.772 a	38.724 b	0.3627 a
60 mM	40.554 a	67.480 a	40.606 a	0.3787 a
LSD	1.24	4.48	1.64	0.02
Statistical significance				
Drought level (D)	*	*	*	*
Hybrid (H)	*	*	*	*
BABA	*	*	*	*
H×BABA	NS	NS	NS	NS
H×D	NS	*	*	NS
BABA×D	NS	NS	NS	NS
H×BABA×D	NS	NS	NS	NS

Note: Means with different letters differ significantly at $p \leq 0.05$. Here LSD = Least significant difference; D = drought environments; H = hybrid; BABA = β-aminobutyric acid; NS = non-significant; RWC = relative water contents; MSI= membrane stability index.

achene yield and yield-related traits due to a decrease in RWC and MSI (Table 1-3), which is consistent with previous findings that drought stress severely reduces crop growth and biomass [1], primarily due to decreased cell division and cell elongation [41]. This physiological condition further disrupts nutrient balances due to decreased mineral uptake [42]. According to our findings, the exogenous application of BABA at various concentrations significantly improves the physiological functioning of the plants and is beneficial in achieving higher achene yield and related attributes.

Drought stress, genotype, and foliar spray of BABA all had a significant effect on SPAD-chlorophyll levels (Table 1). The decrease in chlorophyll value under terminal drought stress at the flowering stage (Table 1) could be attributed to increased chlorophyll destruction and disturbed chloroplast structure under stressed conditions [43]. Ghobadi et al. [44] reported a similar reduction of up to 25% in sunflowers. Furthermore, under water stress conditions, the green color decreased resulting in a reduction in SPAD-Chlorophyll value which may lead to the production of reactive oxygen species (ROS) in leaf cells [45, 46]. Previous research has also found a significant decrease in chlorophyll content and the membrane stability index [6, 47]. In our study, drought stress reduces the RWC and MSI of sunflower leaves (Table 1). It has been observed that increased electrolyte leakage under drought stress leads to a reduction in MSI, which could be attributed to a loss in cellular functioning as well as changes in lipoprotein configuration [48]. Drought stress has also been linked to a decrease in cell membrane stability in Kentucky bluegrass [49]. Plants subjected to abiotic stresses frequently suffer cell membrane damage, which reduces membrane stability, chlorophyll content, and photosynthetic performance ultimately resulting in a decrease in leaf RWC and stomatal conductivity [50]. Reduced RWC was also reported in chickpeas (*Cicer arietinum* L.) due to extensive membrane damage in terms of membrane leakage [51], as leaf membrane

Table 2. Effect of BABA levels on stem diameter, head diameter, number of achenes, and head weight of sunflower hybrids under terminal drought stress.

	SD (cm)	HD (cm)	NOA	HWT (g)
Drought environment (D)				
Well-Watered	8.7197 a	10.946 a	503.04 a	43.786 a
Drought Stress	7.8114 b	8.302 b	376.30 b	35.264 b
LSD	0.35	0.46	6.43	1.75
Hybrids (H)				
Hysun-33	8.3615 a	9.9708 a	432.24 b	40.727 a
NK-senji	8.1697 a	9.2773 b	447.09 a	38.323 b
LSD	0.35	0.46	6.43	1.75
Foliar application of BABA				
0 mM	7.7597 b	8.783 b	401.25 d	36.385 c
20 mM	7.7532 b	9.647 a	414.25 d	38.928 bc
40 mM	8.7914 a	9.910 a	454.57 b	40.225 ab
60 mM	8.7579 a	10.155 a	488.62 a	42.562 a
LSD	0.65	0.86	12.13	3.30
Statistical significance				
Drought level (D)	*	*	*	*
Hybrid (H)	NS	*	*	*
BABA	*	*	*	*
H×BABA	*	NS	NS	NS
H×D	*	*	*	*
BABA×D	NS	NS	*	NS
H×BABA×D	NS	NS	NS	NS

Note: Means with different letters differ significantly at $p \leq 0.05$. Here LSD = Least significant difference; D = drought environments; H = hybrid; BABA = β-aminobutyric acid; NS = non-significant; SD = stem diameter; HD = head diameter; NOA= number of achenes; HWT = head weight.

stability is an important characteristic in plants screened for drought tolerance [52]. The decrease in MSI could be attributed to an increase in electrolyte leakage and lipid peroxidation [53].

The increase in proline content observed in this study under terminal drought stress (Table 2) could be attributed to the accumulation of osmolytes, such as proline, which protects the cell from dehydration injury via solute pressure balance between the cytosol and the external environment [54]. Proline functions as an osmolyte and a radical scavenger, and it is synthesized in various cell compartments to combat stress conditions [55]. It also detoxifies cells by scavenging ROS [56]. Previous research has documented the accumulation of proline and soluble sugars as osmoprotectants against drought [57, 58]. Under stress conditions, compatible solutes, such as amino acids, proline, and glycine betaine, are produced in higher concentrations in plant cells, where they accumulate and cause a decrease in osmotic potential [59, 60].

In the current study, terminal drought stress from flowering to anthesis resulted in lower achene yield, less HAW, HW, and head diameter (Tables 2-3). This decrease in achene yield under drought stress could be related to pollen sterility, which decreased HD and AY [61, 62]. Reduced AY during terminal drought may be due to a lower SPAD-chlorophyll value, a lower RWC, a lower MSI, and less HAW (Table 1-3). Drought reduces plant transpiration efficiency, which raises leaf temperature and, as a result, decreases chlorophyll fluorescence and relative water content [63, 64]. The fluorescence of chlorophyll is an important trait that directly affects plant photosynthetic activity, whereas drought stress significantly reduces photosynthetic activity [65]. Drought stress disrupts stomatal regulation, which reduces CO_2 diffusion into the leaves and the rate of photosynthesis, reducing sunflower growth and yield

Table 3. Effect of BABA levels on hundred achene weight, achene yield, biological yield, and harvest index of sunflower hybrids under terminal drought stress.

	HAW (g)	AY (g)	BY (g)	HI (%)
Drought environment (D)				
Well-Watered	4.0150 a	20.313 a	61.676 a	32.896 a
Drought Stress	2.6665 b	10.113 b	37.779 b	26.726 b
LSD	0.04	0.25	2.13	1.40
Hybrid (H)				
Hysun-33	3.4781 a	15.509 a	49.772 a	30.634 a
NK-senji	3.2033 b	14.918 b	49.683 a	28.989 b
LSD	0.04	0.25	2.13	1.40
Foliar application of BABA				
0 mM	2.8158 d	11.642 d	41.663 c	27.300 b
20 mM	3.2829 b	13.938 c	45.736 b	29.858 ab
40 mM	3.5129 b	16.429 b	54.030 a	29.811 ab
60 mM	3.7512 a	18.844 a	57.480 a	32.277 a
LSD	0.07	0.48	4.02	2.65
Statistical significance				
Drought level (D)	*	*	*	*
Hybrid (H)	*	*	NS	*
BABA	*	*	*	*
H×BABA	*	*	NS	NS
H×D	NS	*	NS	*
BABA×D	*	*	*	NS
H×BABA×D	*	*	NS	NS

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; D = drought environments; H = hybrid; BABA = β-aminobutyric acid; NS = non-significant; HAW= hundred achene weight; AY = achene yield; BY = biological yield; HI = harvest index.

Table 4. Effect of foliar application of BABA levels on stem diameter, hundred achenes weight, and achene yield of sunflower hybrids.

BABA levels (mM)	SD (cm)		HAW (g)		AY (g)	
	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji
0	7.59 c	7.93 bc	3.04 d	2.5 e	12.19 d	11.09 e
20	7.49 c	8.00 bc	3.46 c	3.10 d	14.32 c	13.56 c
40	9.34 a	8.24 a-c	3.59 b	3.44 c	16.74 b	16.12 b
60	9.01 ab	8.49 a-c	3.82 a	3.68 b	18.78 a	18.91 a
LSD	1.10		0.12		0.81	

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; BABA = β-aminobutyric acid; SD = stem diameter; HAW = hundred achene weight; AY = achene yield.

[11]. When sunflower hybrids are subjected to terminal drought stress, achene yield is significantly reduced (Table 3). Many reports agree with the current findings, indicating that drought stress significantly reduces yield. It depicts that skipping irrigation at flowering significantly reduces achene yield compared to well-watered sunflowers due to low water use efficiency under drought conditions [15, 66]. Initiation of flowering

Table 5. Effect of terminal drought stress on relative water content, membrane stability index, stem diameter, and head weight of sunflower hybrids

Drought Environment	RWC (%)		MSI (%)		SD (cm)		HD (cm)	
	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji
Well-Watered	73.74a	65.89 b	42.40a	39.87 b	9.12a	8.31 b	11.64a	10.25 b
Drought Stress	61.69 c	61.11 c	35.76 c	35.18 c	7.59 c	8.02 bc	8.30 c	8.30 c
LSD	3.48		1.64		0.65		0.86	

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; BABA = β-aminobutyric acid; NS = non-significant; RWC = relative water contents; MSI= membrane stability index; SD= stem diameter; HD = head diameter.

Table 6. Effect of terminal drought stress on the number of achenes, head weight, achene yield, and harvest index of sunflower hybrids.

Drought Environment	NOA		HWT (g)		AY (g)		HI (%)	
	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji
Well-Watered	490.36 b	515.71 a	46.57 a	41.03 b	20.39 a	20.24 a	32.53 a	33.26 a
Drought Stress	374.12 c	378.48 c	34.89 c	35.64 c	10.63 b	9.59 c	28.73 b	24.72 c
LSD	12.13		3.30		0.48		2.65	

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; BABA = β-aminobutyric acid; NS = non-significant; NOA= number of achenes; HWT = head weight; AY= achene yield; HI = harvest index.

Table 7. Effect of foliar application of BABA on number of achenes, hundred achene weight, achene yield, and biological yield of sunflower under terminal drought stress.

BABA levels (mM)	NOA		HAW (g)		AY (g)		BY (g)	
	WW	DS	WW	DS	WW	DS	WW	DS
0	465.42 c	337.08 f	3.39 d	2.24 g	15.73 d	7.56 h	50.90 b	32.43 e
20	473.00 c	355.46 f	3.91 c	2.65 f	18.45 c	9.43 g	55.80 b	35.67de
40	514.14 b	395.00 e	4.29 b	2.74f	22.03 b	10.82 f	67.93a	40.14 cd
60	559.58a	417.66 d	4.47a	3.03 e	25.04a	12.64 e	72.08a	42.88 c
LSD	20.52		0.12		0.81		6.81	

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; BABA = β-aminobutyric acid; NS = non-significant; NOA= number of achenes; HAW = hundred achene weight; AY= achene yield; BY = biological yield.

Table 8. Interaction effect of foliar application of BABA on hundred achene weight and achene yield of sunflower hybrids under terminal drought stress

BABA levels (mM)	HAW (g)				AY (g)			
	Well-watered		Drought stress		Well-watered		Drought stress	
	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji	Hysun-33	NK-senji
0	3.61 d	3.17 e	2.47 i	2.01 j	16.11 e	15.35 e	8.27 k	6.84 l
20	4.18 c	3.64 d	2.74 f-h	2.57 hi	18.94 d	17.96 d	9.71 ij	9.16 jk
40	4.33 bc	4.24 bc	2.84 f	2.64 g-i	22.33 c	21.73 c	11.14 gh	10.50 hi
60	4.53 a	4.42 ab	3.23 e	2.83 fg	25.91 a	24.17 b	13.39 f	11.90 g
LSD	0.19				1.31			

Note: Means with different letters differ significantly at p≤0.05. Here LSD = Least significant difference; BABA = β-aminobutyric acid; NS = non-significant; HAW = hundred achene weight; AY= achene yield.

and anthesis are critical stages that determine achene yield, because more fertile flowers and good achene filling lead to higher achene yield while drought stress negatively affects these important traits at these stages [22, 67].

Genotypes with higher chlorophyll content and RWC are more resistant to drought stress, and their yield also remains stable [68]. Multiple factors influence crop yield including genotype, climatic conditions, and farm management practices [22, 67]. During the current study, the genotype Hysun-33 produced the highest achene yield and yield-related parameters. The increased achene yield in Hysun-33 was due to increased HD, HAW, and more APH (Tables 2-3). Second, when compared to NK-Senji, this variety retains more RWC and MSI, and produces more SPAD-Chlorophyll (Table 1). As observed in the current experiment, a genotype with more leaves develops an effective and early plant canopy, which improves photosynthetically active radiation interception and grain partitioning, resulting in increased crop yield.

Terminal drought stress decreased AY and related traits due to decreases in RWC, MSI, and SPAD-chlorophyll value (Tables 1-3), whereas BABA application improved AY, RWC, MSI, proline contents, and SPAD-chlorophyll value (Tables 1-3). BABA is an important osmoprotectant, and its foliar application improved SPAD-chlorophyll value in the current study (Table 1), which could contribute to improved photosynthesis and other gas exchange resulting in increased achene yield [58]. Exogenous application of BABA resulted in a significant improvement in gas exchange parameters, such as photosynthesis rate, stomatal conductance, and transpiration rate [35]. The current study found a significant reduction in RWC under drought stress, whereas foliar application of BABA improved RWC in drought-grown sunflowers and mitigated the adverse effects of drought, possibly due to improved osmotic potential and turgor pressure in plant leaves [58]. According to Ali and Hassan [69], BABA can overcome the intensity of various abiotic stresses by maintaining RWC and chlorophyll. More proline contents were observed under drought stress compared with well-watered conditions; however, BABA application improved proline contents even further [Table 1, 70, 71] which enhanced the antioxidant defense system in plants [72] and helped the plant to perform better under stress conditions.

Exogenous applications of osmoprotectants can alleviate drought stress in sunflowers [19, 73]. Interestingly, BABA, as an osmoprotectant, also protects plants from a variety of biotic and abiotic stresses [74, 75]. The current study suggests that a foliar spray of BABA may help mitigate the negative effects of drought in sunflowers. Increased achene yield appears to be a direct result of increased head diameter, number of achenes per head, and 100-achene weight. This increase in yield and yield-related factors could be attributed to the maintenance of higher RWC, SPAD-chlorophyll value, and photosynthetic activity as a result of BABA application. BABA foliar application reduced the negative effects of drought stress by lowering reactive oxygen species (ROS) and malondialdehyde (MDA) levels and increasing the antioxidants [76]. Under drought stress, membrane damage and MDA levels increase; however, BABA application effectively reduces membrane damage and increases proline accumulation [77]. BABA application improves plant defense mechanisms by lowering ROS production and increasing higher CAT and SOD enzyme activities, resulting in improved drought tolerance [78].

Conclusions

Our findings revealed that Hysun-33 performed well under terminal drought stress due to higher SPAD-chlorophyll values, higher relative water contents, and yield-related traits. When compared to well-watered conditions, terminal drought stress decreased the SPAD-chlorophyll value, membrane stability index, and relative water contents, while increasing proline content. Under these conditions, achene yield, 100-achene weight, and head diameter were all also reduced. Foliar application of BABA improved the membrane stability index, maintained higher RWC, and increased chlorophyll content, resulting in increased achene yield in drought-stressed sunflowers. Improved chlorophyll and proline content from foliar BABA application under drought stress leads to increased capitulum, 100-achene weight, and head diameter, which ultimately enhanced achene yield under terminal drought stress. Based on the findings of this study, foliar application of BABA @ 60 mM may be a viable option for increasing achene yield in sunflower hybrids grown under terminal drought stress.

Declaration of Funding

This research was funded by the Researchers Supporting Project Number (RSP2024R298), King Saud University, Riyadh, Saudi Arabia.

Acknowledgments

This research was funded by the Researchers Supporting Project Number (RSP2024R298), King Saud University, Riyadh Saudi Arabia.

Conflict of Interest

The authors declare no conflict of interest.

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