Australian Journal of Crop Science AJCS
AJCS 7(5):555-560 (2013) ISSN:1835-2707

Exogenous benzoic acid (BZA) treatment can induce drought tolerance in soybean plants by improving gas-exchange and chlorophyll contents

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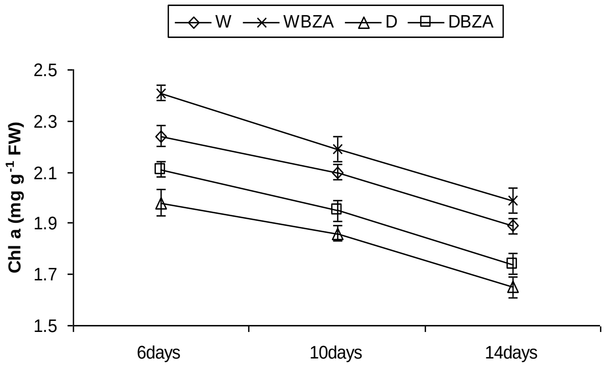
Abstract

In order to reveal the mechanism involved in adaptation to drought, pot study investigated the morphological and physiological responses of soybean in growth, yield, photosynthetic capacity, and chlorophyll contents to drought stress under exogenous benzoic acid (BZA) application. Two levels of soil moisture, well-watered control and drought-stressed were imposed at blooming stage of soybean. BZA was applied at the rate of 0.5 mM after one week of imposition of moisture treatments. Drought stress exhibited a notable decline in net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), water use efficiency (WUE), intercellular CO_2 (Ci) and Ci/Ca ratio. Furthermore, drought stress caused a considerable decline in chlorophyll contents leading to substantial reduction in plant growth, and productivity. Interestingly, exogenous application of BZA remarkably improved the net photosynthesis 11.54% and chlorophyll *a* contents 6.57% at 5% probability level, reduced due to water deficiency. These BZA-induced beneficial effects in gas exchange and chlorophyll contents improvement, ultimately resulted in improved growth, development, yield and yield components in terms of plant height, stem diameter, biological yield, grain yield and harvest index in water-stressed soybean plants.

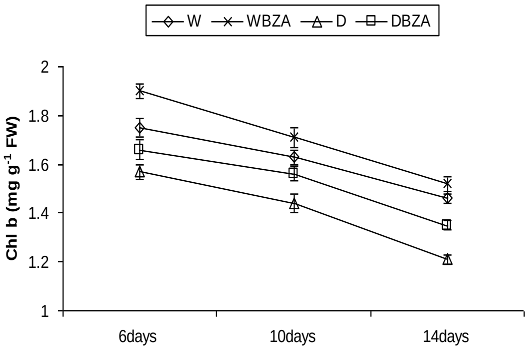
Keywords: Chlorophyll contents; Drought stress; Gas exchange; Exogenous benzoic acid; Soybean; Yield.
Abbreviations: A- net photosynthetic rate, E- transpiration rate, gs- stomatal conductance, WUE- water use efficiency, Ci-intercellular CO_2, BZA- benzoic acid, CRD- completely randomized design.

Introduction

Food productivity is decreasing due to deleterious effects of numerous biotic and abiotic stresses; therefore minimizing these losses is a major area of concern to ensure food security under climate change scenario. Drought stress is one of the most important abiotic stress factors limiting growth and productivity of crop plants more than any other stress (Ghannoum, 2009), and whose irreversibility depends on the genotype, duration, intensity and plant development stage. Moreover, as a result of limited water supply, a significant percentage of agricultural lands are subjected to degradation if farmers decide to completely abandon agricultural practice on them (Dagar et al., 2006). Enhancing plant resistance to drought stress would be the most economical strategy to sustain agricultural productivity in areas prone to water scarcity (Xiong et al., 2006). How drought affects plant's physiological processes, growth, development and performance, is a burning issue of current research. The plants respond to drought stress through phenological responses, morphological adaptations, physiological changes, and biochemical adaptations. Drought affects the water status, growth, development, yield, membrane integrity, and osmotic adjustment (Praba et al., 2009). The plant's ability to sustain integral physiological processes such as photosynthesis and gas exchange during drought stress, especially in the phases regarded sensitive to the crop, is a potential indication for maintaining productivity under water limiting conditions (Silva et al., 2007). As the key process of primary metabolism, photosynthesis plays a key role in plant performance under drought stress (Pinheiro and Chaves, 2011). The balance between light capture and energy use are of great relevance to studies concerning the responsiveness of the photosynthetic apparatus to drought stress (Chaves et al., 2009; Aranjuelo et al., 2011). Photosynthesis is directly affected by drought and stomatal closure allows the plants to limit transpiration, but it also reduce CO_2 absorption, which ultimately results in limited photosynthetic activity (Nayyar and Gupta, 2006). Other variables related to gas exchange such as stomatal conductance (gs) and transpiration (E), being very sensitive to drought stress and having a good correlation to photosynthesis, have also been identified as promising attributes for induction of drought tolerance in plants (Endres et al., 2010). Loss of chlorophyll contents under drought stress is considered a major cause of inactivation of photosynthesis (Blackburm, 2007). The alteration in chlorophyll contents has been employed to explore the effect of drought stress on plant growth, development, and productivity, and earlier findings revealed that chlorophyll concentration generally decreased under drought stress due to their slow synthesis or fast breakdown (Majumdar et al., 1991). The efficiency of light captured to drive photosynthesis is directly correlated to the chlorophyll contents in the leaf (Netondo et al., 2004). Both reduction in the formation of chlorophyll contents and increase in decomposition under water deficit contributed towards the reduction of chlorophyll under drought stress.



(a)



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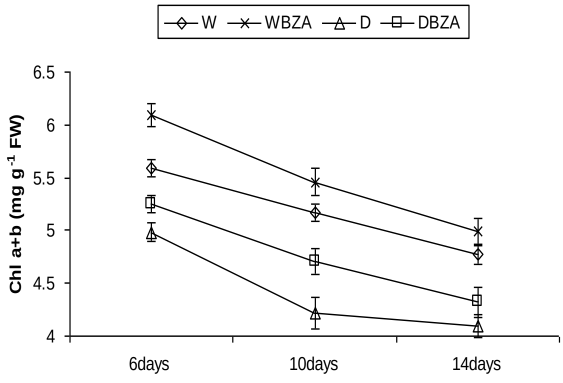

(c)

Days after BZA treatment

Fig 1. Influence of benzoic acid (BZA) application on (a) Chl a, (b) Chl b and (c) Chl a+b contents of soybean under drought. W: well-water, WBZA: benzoic acid (BZA) in well water conditions, D: drought stress, DBZA: benzoic acid (BZA) in drought conditions.

Soybean (*Glycine max* L. Merrill.) is considered a unique crop due to its specific features. It has a high protein and fat content, and also has industrial uses. Its worldwide production and consumption are increasing every year. It is considered highly desirable in human food, and is grown widely in the world and often experiences drought in many regions (Arshad et al., 2006). It has become imperative to improve the drought tolerance of plants under climate changing circumstances. Several techniques are being employed to enhance crop productivity under water limiting conditions. These have been somewhat successful, but have several limitations. Exogenous application of plant growth regulators and compatible solute has been considered a vital strategy to induce drought tolerance in plants. Benzoic acid (BZA) is potentially known to provide abiotic stress tolerance (Senaratna et al., 2003) but benzoic acid-induced drought tolerance has not been widely studied until now. Therefore, the present study was undertaken to investigate the possible role of BZA in improvement of drought tolerance, based upon modulation in growth, development, yield, and physiological attributes. This study explored a clear pattern of drought-induced alteration on above systems and the ameliorative effects of BZA.

Results

Growth and development

There was considerable reduction in growth and development of soybean plants subjected to drought stress as compared to well water control. Exogenous treatment with 0.5 mM benzoic acid (BZA) noticeably improved the growth and yield parameters of drought-stressed soybean plants, which clearly reflect the positive role of BZA in drought tolerance. BZA-treatment under water-deficit conditions improved the plant height (3.81 %), leaf area (11.90 %), lowest node height (20.71 %), stem diameter (23.50 %), number of leaves per plant (11.62 %), and number of stem nodes (4.05 %) (Table 1). Whereas, improved the pod number/plant (10.02 %), one-seeded pod number (4.66 %), two-seeded pod number (3.56 %), three-seeded pod number (12.58 %), four-seeded pod number (13.26 %) under drought stress conditions (Table 2). Foliar application of BZA also elevated the growth and development under well water conditions but this improvement was not promising. However, growth and development stimulatory effect of BZA was more pronounced in drought conditions as compared to well water conditions. In drought conditions, BZA treatment elevated the growth and yield of soybean plants almost up to well water control. During the experimental period, no seedling mortality, leaf shedding or leaf burn was observed in any drought stress treatment.

Yield and yield components

Water deficit conditions disrupted the yield and yield related attributes in terms of grain number/pod, grain number/plant, 100 grain weight, biological yield/plant, grain yield/plant, and harvest index in comparison to well water conditions. BZA-treatment in water-deficit conditions improved the grain number/pod (2.98 %), grain number/plant (6.06 %), 100 grain weight (5.72 %), biological yield/plant (9.31 %), grain yield/plant (11.11 %), and harvest index (3.81 %). The greater effectiveness of exogenous BZA application was found in drought-stressed treatments in the entire yield and yield related parameters as compared to the well-watered (Table 3). Exogenous application of BZA substantially improved the soybean plants yield and yield component in drought stressed as well as well watered plants. This increased effect was more pronounced in water stressed plants than well watered control. BZA treatment resulted in a significant improvement that elevated yield and yield parameters near to the level of the control.

Table 1. Influence of exogenous application of benzoic acid (BZA) on growth and development of soybean under drought.

Treatments	Plant height (cm)	Leaf area (cm^2)	Lowest node height (cm)	Stem diameter (mm)	No. of leaves/plant	No. of nodes on main stem
W	49.87±0.35 a	21.67±0.14 a	10.13±0.09 a	5.07±0.03 b	15.32±0.18 b	14.45±0.05 a
WBZA	50.24±0.62 a	22.56±0.20 a	10.49±0.12 a	5.17±0.07 a	16.15±0.14 a	14.59±0.07 a
D	43.86±1.02 b	16.44±0.23 c	7.89±0.08 c	3.91±0.05 d	11.79±0.20 d	11.79±0.06 c
DBZA	45.54±0.58 b	18.39±0.29 b	9.52±0.01 b	4.83±0.09 c	13.16±0.06 c	12.32±0.06 b

Treatment notations indicate W: well-water, WBZA: benzoic acid (BZA) application under well water conditions, D: drought stress, DBZA: benzoic acid (BZA) application under drought conditions. Values in the table are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman–Keuls test (P < 0.05).

Table 2. Influence of exogenous application of benzoic acid (BZA) on growth development of soybean under drought**.**

Treatments	Pod number/plant	Nil-seed pod number	One-seeded pod number	Two-seeded pod number	Three-seeded pod number	Four-seeded pod number
W	28.81±0.71 a	4.47±0.04 a	7.27±0.05 b	10.61±0.06 a	8.41±0.04 ab	2.20±0.03 b
WBZA	29.24±0.67 a	4.24±0.02 a	7.44±0.04 a	10.85±0.03 a	8.71±0.05 a	2.47±0.03 a
D	21.33±1.04 b	3.15±0.04 b	4.93±0.05 d	7.59±0.07 c	5.72±0.06 c	1.81±0.02 d
DBZA	23.46±0.74 b	2.56±0.03 c	5.76±0.03 c	8.76±0.08 b	6.44±0.08 bc	2.05±0.03 c

Treatment notations indicate W: well-water, WBZA: benzoic acid (BZA) application under well water conditions, D: drought stress, DBZA: benzoic acid (BZA) application under drought conditions. Values in the table are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman–Keuls test (P < 0.05).

Table 3. Influence of exogenous application of benzoic acid (BZA) on yield and yield related traits of soybean under drought.

Treatments	Grain number/pod	Grain number/plant	100 grain weight (g)	Biological yield/plant (g)	Grain yield/plant (g)	Harvest index (%)
W	1.78±0.09 ab	49.13±0.55 a	21.89±0.10 a	44.03±0.49 b	14.09±0.31 ab	32.18±0.23 a
WBZA	1.81±0.04 a	50.22±0.46 a	21.96±0.06 a	46.66±0.73 a	15.15±0.57 a	33.31±0.21 a
D	1.68±0.04 c	39.63±0.77 c	18.87±0.59 b	37.17±1.16 d	11.88±0.48 c	30.45±0.57 b
DBZA	1.73±0.03 bc	42.03±0.59 b	19.95±0.47 b	40.63±0.52 c	13.20±0.23 bc	31.61±0.28 b

Treatment notations indicate W: well-water, WBZA: benzoic acid (BZA) application under well water conditions, D: drought stress, DBZA: benzoic acid (BZA) application under drought conditions. Values in the table are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman–Keuls test (P < 0.05).

Table 4. Influence of exogenous application of benzoic acid (BZA) on leaf gas-exchange attributes of soybean under drought

Treatments	Photosynthesis (A) (μmol m^{-2} s^{-1})	Transpiration rate (E) (mmol m^{-2} s^{-1})	Stomatal conductance (gs) (μmol m^{-2} s^{-1})	Water use efficiency (WUE) (μmol $mmol^{-1}$)	Intercellular CO_2 (Ci) (μmol mol^{-})	Ci/Ca
W	15.92±0.14 b	7.90±0.10 b	0.293±0.003 b	2.07±0.04 ab	239±1.68 b	0.65±0.011 ab
WBZA	17.66±0.22 a	8.21±0.02 a	0.309±0.005 a	2.11±0.02 a	243±1.44 a	0.68±0.013 a
D	13.51±0.23 d	7.04±0.07 d	0.251±0.005 d	1.81±0.06 c	225±2.19 d	0.59±0.009 c
DBZA	15.07±0.12 c	7.55±0.06 c	0.269±0.008 c	1.97±0.05 bc	232±1.53 c	0.63±0.010 bc

Treatment notations indicate W: well-water, WBZA: benzoic acid (BZA) application under well water conditions, D: drought stress, DBZA: benzoic acid (BZA) application under drought conditions. Values in the table are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman–Keuls test (P < 0.05).

Leaf gas-exchange

Photosynthesis is one of the most vital physiological processes contributing to growth and productivity of crop plants for food. Water-deficit conditions caused noticeable reduction in the gas exchange attributes of soybean plants under drought stress. BZA treatment enhanced the gas exchange traits in drought stressed as well as well watered plants, however, this increment was more pronounced in water stressed plants. BZA-treatment substantially enhanced the net photosynthesis (11.54 %), transpiration rate (7.24 %), stomatal conductance (7.57 %), water use efficiency (8.83 %), intercellular CO_2 (3.11 %), and ambient CO_2 ratio (6.78 %) under drought conditions, whereas, improved these traits by 10.93 %, 3.92 %, 5.46 %, 1.93 %, 1.67 % and 4.61 %, respectively under well water conditions (Table 4). BZA-induced enhancement of gas exchange traits was intermediate in combination of DBZA treatment than that of well water control.

Chlorophyll contents

Chlorophyll pigments play a key role in light-capturing for photosynthesis, whose content forced a direct impact on the intensity of photosynthesis. Photosynthetic pigments (Chl *a*, Chl *b* and Chl *a+b*) were reduced by drought stress as compared to well water control, and the extent of this reduction was obviously less in the benzoic acid treatment than that in the non-benzoic acid treatment, manifesting that application of BZA could mitigate the decomposition of photosynthetic pigments of soybean plants under drought stress. Consequently, chlorophyll content of BZA-treated plants was noticeably higher than non-treated (Figure 1). BZA-treatment led to increment in Chl a contents by 7.59, 4.28 and 5.29% under well water and 6.57, 4.83 and 5.45% under drought conditions on 6, 10 and 14 days after BZA-treatment, respectively. Foliar application of BZA led to increase in Chl b contents up to 8.57, 4.91 and 4.11% under well water and 5.73, 8.33 and 11.57% under drought conditions after 6, 10 and 14 days of BZA application, respectively. BZA-treatment led to elevation in Chl *a+b* contents 8.94, 5.61 and 4.61% under well-water and 5.42, 11.61 and 5.62% under drought conditions on 6, 10 and 14 days, respectively.

Discussion

Among the abiotic stresses, drought stress is by far the most complex, detrimental, and devastating on a global scale and its frequency is likely to increase as a consequence of climate changes (Ceccarelli et al., 2010). Improving drought tolerance capacity of crop plants by exogenous application of some potential growth regulators is considered effective technique to attenuate the deleterious effects of drought on crop production. Tolerance to drought stress results from a series of integrated events occurring at morphological, physiological and biochemical levels. Cell growth is considered one of the most drought-sensitive physiological processes due to the reduction in turgor pressure. Growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Drought stress disrupts mitosis resulting in reduced growth and yield traits (Kaya et al., 2006). Our results exhibited that scarcity of water led to severe decline in growth and yield traits of soybean plants mainly by disrupting leaf gas exchange properties which not only limited the size of the source and sink tissues but also impaired the phloem loading, assimilate translocation and dry matter partitioning. BZA-induced enhancement in growth and yield was in accordance with earlier findings of Christen and Lovett (1993) that BZA increased growth, kernels per ear, 1000-grain weight of spring barley; this was related to the ability of BZA to improve gas exchange that protected them from damage. Photosynthesis is an inevitable process to sustain crop growth and development, and it is well known that photosynthetic machinery in higher plants is most sensitive to drought stress (Falk et al., 1996). In our study, water stress severely hampered the gas exchange parameters of soybean and this could be due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Sgherri and Navari-Izzo, 1995). The past results of many experiments have showed that photosynthesis rate drops when stomatal conductance decreases (Nilsen and Orcutt, 1996). As the stress progressed, biochemical constraints might limit photosynthetic CO_2 fixation more directly. Therefore, it might be thought that photosynthesis rate decreased because stomata were closed before the leaf water potential decreased by the effect of root physiology or soil water conditions. Farquhar and Sharkey (1982) indicated that with decline of both Ci and gs, the A decline is mainly caused by the stomata limitation. If A reduced with the increase of Ci, the main limiting factor affects photosynthesis is non-stomal factors. Reductions in gs and E show a generalized reaction under stress, which is stomatal closure in order to minimize water loss through transpiration (Inman-Bamber and Smith, 2005). Improved gas exchange attributes in soybean plants with BZA treatment is in agreement with Fariduddin et al. (2003) that salicylic acid, which analogue of BZA, treatment enhanced the net photosynthetic rate, intercellular CO_2, water use efficiency, stomatal conductance and transpiration rate in *Brassica juncea*. Stomatal conductance and transpiration rate were increased with increase in photosynthetic rate due to BZA treatment, which suggests that BZA-induced increase in photosynthesis might have been due to stomatal factors. Chloroplast pigments play an important role for the light absorption and conversion in the photosynthesis process. Leaves could change the proportionality between chloroplast pigments dynamically for proper distribution and dissipation of light energy under various environments, which could ensure the normal function of photosynthetic system (Chen et al., 2012). It was reported that the approach for measuring chlorophyll parameters might estimate influence of the environmental stress on growth and yield, since these traits were closely correlated with the rate of carbon exchange (Guo and Li, 2000; Fracheboud et al., 2004). Therefore, analysis of chlorophyll content is considered as vital approach for evaluating the health or integrity of the internal apparatus during photosynthetic process within a leaf (Clark et al., 2000), and provides a rapid and accurate technique of detecting and quantifying plants tolerance to drought stress (Percival and Sheriffs, 2002). The marked reduction in Chl *a*, Chl *b* and Chl *a+b* in drought stressed soybean plants is in line with Egert and Tevini (2002) who revealed that stresses increase the formation of ROS that oxidize photosynthetic pigments. Analysis on photosynthetic pigment showed that, A decline was due to drought stress in soybean plant which resulted in a low content in chlorophyll. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets (Kaiser et al., 1981). BZA-induced increase in pigments contents is in accordance with Fariduddin et al. (2003) who reported that the pigment content was enhanced in wheat seedlings, treated with salicylic acid which is analogue of BZA.

Materials and methods

Plant material, growth conditions and treatments

The study was carried out at research area of Southwest University, Chongqing, China. The experimental area located between latitudes 29° 49′ 32″ N, longitudes 106° 26′ 02″ E and height from sea level 220 m. The experiment was performed in rain-protected wire-house during summer 2010; soybean "Xidou-7" seeds were sown in plastic pots. This variety was developed by Southwest University with approval number of 2007001, it is early maturing and considered moderately tolerant to drought. Its plant is compact type, indeterminate growth habit, brown hair, oval color concentrated leaves, brown leather yellow cotyledons, light-colored umbilical, indehiscent pod, and lodging resistant. Altogether, 8 soybean seeds were planted per plastic pot and thinning was carried out at first true leaf stage to maintain 3 plants per pot. Total weight of each pot was 12 kg after filling with sandy loam soil containing organic matter 25.76 g kg^{-1}, total nitrogen 1.98 g kg^{-1}, total phosphorus 1.77 g kg^{-1}, total potassium 22.33 g kg^{-1}, available nitrogen 55.71 mg kg^{-1}, available phosphorus 27.55 mg kg^{-1}, available potassium 88.47 mg kg^{-1} and pH 6.42.

The pots were arranged in a completely randomized design (CRD) with two factors; benzoic acid (BZA) treatments (none and BZA-treated) and soil water levels (well-watered and drought-stressed) with 20 pots per treatment and three replications of each experimental unit. All the pots were supplied with fertilizer at the rate of 8 g pot^{-1} using NPK compound fertilizer with N: 15%, P_2O_5: 5%, K_2O: 5% purchased from Jiuhe Gufen Youxiang Company, China. Well-watered and drought-stressed treatments were maintained at 80% and 35% soil field capacity respectively, by withholding water following the methods of Desclaux and Roumet (1996). The pots were weighed daily to maintain the desired soil water levels by adding appropriate volumes of water. A single most suitable level of benzoic acid (BZA) (0.5 mM) was foliar applied after one week of withholding water at blooming stage of soybean. In order to maintain uniformity in the treatment the BZA was applied uniformly by using aliquot of 30 mL per plant until runoff.

Data measurement

Gas exchange attributes were assessed after 10 d of BZA treatment. For determination of chlorophyll contents the soybean plants were sampled after 6, 10 and 14 days of BZA-treatment. The remaining soybean plants were harvested at maturity to assess growth, development, yield and yield related traits.

Photosynthesis

Net rate of photosynthesis (A), transpiration rate (E) and stomatal conductance (g_s) were measured with a portable infrared gas exchange analyzer based photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) during 8:15–10:15 am. Fully expanded leaves were selected for leaf measurements. Eighteen leaves were selected for each treatment with the following adjustments: molar flow of air per unit leaf area 389.62 mmol lol^{-1} m^{-2} s^{-1}, water vapour pressure into leaf chamber was 3.13 mbar, (photosynthetically active radiation) PAR at leaf surface was up to 1199 mol m^{-2} s^{-1}, temperature of leaf ranged from 36.67 to 41.91 °C, ambient temperature was 39.71 to 42.68 °C and relative humidity (RH) was 52.32 %. Water use efficiency (WUE) was calculated as ratio between net photosynthesis (A) and transpiration rate (E).

Chloroplastic pigments

Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and chlorophyll *a+b* (Chl *a+b*) were determined by method as described by Arnon (1949). Leaf samples of 0.1 g was ground and placed in 15 mL centrifuge tube, along with 10 mL of miscible liquids by 95.5 % acetone and absolute ethyl alcohol in 1:1 ratio. Then covered with black plastic bag and kept at dark place until the samples changed into white. Chl *a*, Chl *b* and Chl *a+b* contents were determined using UV-visible spectrophotometer at 645 nm, 652 nm and 663 nm wavelengths.

Growth, development, and yield traits

Leaf area of soybean plants was measured with LI-3100 leaf area meter (Li-Cor, Lincoln, NE) CI-203 (CID, Inc., USA) 13 days after BZA application. At harvest 33 plants (11 plants from each replicate) representing each treatment were sampled randomly and quantified to assess the growth, development, yield and yield components. Harvest index (HI) was calculated as the ratio of gain yield to biological yield and expressed in percentage by using following formula.

$$HI = \frac{\text{grain yield}}{\text{biological yield}} \times 100$$

Statistical analysis

Data presented as the mean ± SE for each treatment, were tested with analysis of variance (ANOVA), Newman–Keuls test, marked by letters, where the values sharing the same letters are not significantly different at 5 % level, using SPSS 16.0.

Conclusion

Stress tolerance resulting in adaptation mechanisms allows plants to interact with their environment and to maintain photosynthesis to survive the stress period. In sum, BZA treatment improved the growth, yield, gas exchange and chlorophyll contents of soybean under drought stress.

Acknowledgements

The research work was supported by the National Natural Science Foundation of China (Grant No. 31271673). The authors are grateful to the Key Laboratory of Biotechnology and Crop Quality Improvement of Ministry of Agriculture, China.

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