Efficacy of benzyladenine for compensating the reduction in soybean productivity under low water supply

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Abstract. Undoubtedly, drought is a negative consequence of climate change. Farmers have to deal with this issue and may be forced to irrigate their crops with less water than required, however reduction in productivity is anticipated. Thus, two–year field trials were conducted to assess the impact of irrigation regimes (60, 80 and 100% of crop evapotranspiration, denoted ET 60, ET 80 and ET 100, respectively) and benzyladenine rates (0, 50, 100, 150 and 200 mg L −1, symbolized as BA 0, BA 50, BA 100, BA 150, BA 200, respectively) on soybean. Findings clarified that the maximum increases in plant height and net assimilation rate were obtained with the interactions of ET 100 or ET 80 x BA 200 or BA 150 in both seasons. ET 80 x BA 200 (in both seasons) and ET 100 x BA 150 (in the first season) were as similar as ET 100 x BA 200 for enhancing pods number plant −1. Irrigation water use efficiency progressively increased with decreasing irrigation water amount and increasing benzyladenine rate. In conclusion, the reduction in seed yield due to lowering water supply up to 80% of crop evapotranspiration (with saving 20% of irrigation water) could be compensated using benzyladenine, 150 mg L −1, thus it should be involved in soybean irrigation programs.

Keywords: deficit water, growth promoters, relative growth rate, soybean oil.

INTRODUCTION

Soybean [Glycine max (L.) Merr.] occupies a prestigious position in the global economy, since its seeds are considered a major source of protein (approximately, 38–47%) and oil (approximately, 18–22%) (Nakagawa et al. 2020; Tian et al. 2020), besides its importance in sustainable agriculture. In water rationalization patterns, the increase of water use efficiency should be pursued in a sustainability prospective, increasing crop productivity per unit of water used (Oweis and Hachum 2003). In this respect, farmers are obligated to irrigate their crops using less water than required (El–Bially et al. 2018; Salem et al. 2021). However, soybean production is affected by abiotic
pressures, i.e. water deficit. Water stress is a major factor in determining crop productivity, particularly in arid and semi-arid areas. In Egypt, based on the local farmers’ irrigation practice for the soybean cropping system, the optimized water consumption is 550 mm/total growing period (Sheteiwy et al. 2021). Water deficit conditions affect the water potential and turgor pressure of the cells and this can disturb the normal plant physiological mechanisms inducing various impacts on growth and yield parameters of the crops (Reisdorph and Koster 1999; El-Metwally et al. 2021). Soybean under drought stress at beginning of pod, beginning of seed and full seed stages resulted in substantial yield losses (Dogan et al. 2007). The photosynthetic activity of soybean plants was severely decreased (Zhang et al. 2016) and photoinhibition increased (Sofo et al. 2009) in drought conditions. Because of severe drought stress during flowering and pod-setting stage, losses in leaf area, dry matter and seed yield per soybean plant reached 61, 67 and 77%, respectively (Wei et al. 2018).

Due to application of plant growth regulators, alternations in metabolic and physiological processes in plants reflecting on flowering, fruiting, maturation, fruit drop, and defoliation were observed (Rademacher 2015). One of the most important plant growth promoters is cytokinins group which play a crucial role in plant differentiation and development (Yeh et al. 2015). In soybean, enhancement in yield was evident because of cytokinin foliar application (Soares et al. 2017). Borges et al. (2014) proved that benzyladenine as a compound of cytokinin can reduce pod abortion and improve yield of soybean. Moreover, application of benzyladenine significantly improved growth, yield, yield attributes and quality of soybean (Khaswa et al. 2014). Still, the knowledge about the potency of benzyladenine and its demeanor in soybean under drought is not sufficiently available.

Therefore, the objective of this study was to evaluate the efficacy of benzyladenine to alleviate the impacts of low water supply in soybean.

MATERIALS AND METHODS

Site attributes

The experiments were carried out under open field conditions during two years (2015 and 2016) at the experimental research and production station of National Research Centre, El–Nubaria region, Egypt (latitude 30°38’N, and longitude 31°16’E, and mean altitude 21 m above sea level). Location is typified as arid region having cool winters as well as hot dry summers with no rainfall. Mean values of weather status prevailing during soybean life cycle are provided in Table 1. Physical and chemical properties of the experimental soil are shown in Table 2.

### Experimental set-up

Each experiment was established with a strip-plots design having four replicates. Three irrigation levels (60, 80 and 100% of crop evapotranspiration, ETc, denoted ET<sub>60</sub>, ET<sub>80</sub> and ET<sub>100</sub> respectively) were practiced. As well, five benzyladenine (BA) rates (0, 50, 100, 150 and 200 mg L<sup>-1</sup>, symbolized as BA<sub>0</sub>, BA<sub>50</sub>, BA<sub>100</sub>, BA<sub>150</sub> and BA<sub>200</sub> respectively) were sprayed twice at 45 and 60 days after sowing (DAS).

After harvesting the preceding crop (wheat), the experimental field was ploughed and ridged (60 cm width) before planting. During land preparation, single super-phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was applied at a rate of 357 kg ha<sup>-1</sup>. Moreover, the field was divided into experimental units of about 3.5 m x 3.0 m each. Soybean seeds (cv Giza–111) were inoculated with the specific Rhizobi-
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um strain and immediately sown in hills (2–3 seeds), 20 cm apart on both sides of the ridge. Sowing dates were May 18th and 25th in 2015 and 2016 seasons, respectively. At 20 and 35 DAS, all plots received 52.5 g ammonium nitrate (33.5 % N) and 157.5 g potassium sulphate (48% K₂O), respectively. Plants were watered through trickle irrigation system which comprised of emitters spaced 30.0 cm apart with discharge of 2.0 L h⁻¹.

Based on the information provided in Table 1, FAO Penman–Monteith equation (Allen et al. 1998) was exploited to estimate the daily reference evapotranspiration (ET₀) of soybean along the growing season. Thereafter, crop evapotranspiration (ETc) was calculated with the water budget and irrigation decision support model by FAO–56. Accordingly, the quantity of irrigation water requirement was computed as elucidated by Keller and Bliesner (1990). Seasonal irrigation water applied for 2015 and 2016 seasons are shown in Table 3.

Crop parameters

At 90 DAS, plant height was measured. Also, total chlorophyll content (SPAD value) of the 4th leaf was measured using chlorophyll meter (SPAD 502) according to Soil Plant Analysis Department Section, Minolta Camera Co., Osaka, Japan as reported by Minolta (1989). Moreover, ten plants were randomly selected from the inner rows of each plot at 60 (t₁) and 90 (t₂) DAS to determine relative growth rate (RGR) and net assimilation rate (NAR) as described by Hunt (1982) as follow:

\[ RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \text{ (g g}^{-1} \text{ week}^{-1}) \]  

(1)

Where, \( \ln \) = Natural log, \( W_1 \) = Dry weight of plant m⁻² recorded at time \( t_1 \), \( W_2 \) = Dry weight of plant m⁻² recorded at time \( t_2 \), \( t_1 \) and \( t_2 \) are the interval of time.

\[ NAR = \frac{(W_2 - W_1)}{(LA_2 - LA_1)} \frac{(log LA_2 - log A_1)}{(LA_2 - LA_1)(t_2 - t_1)} \text{ (g cm}^{-2} \text{ week}^{-1}) \]  

(2)

Table 3. Seasonal irrigation water applied in soybean (m³ ha⁻¹) in 2015 and 2016 seasons under various irrigation levels.

| Irrigation level | 2015 season | 2016 season |
|------------------|-------------|-------------|
| ET₆₀             | 2394        | 2436        |
| ET₈₀             | 3192        | 3248        |
| ET₁₀₀            | 3990        | 4060        |

Note: ET₆₀, ET₈₀, and ET₁₀₀: irrigation level at 60, 80 and 100% of crop evapotranspiration, respectively.

Where: \( W_1 \) and \( W_2 \) are total plant dry weight as well as \( LA_1 \) and \( LA_2 \) are total leaf area at time \( t_1 \) and \( t_2 \) respectively.

At full maturity (29th September in 2015 and 6th October in 2016), plants of the experimental plots were harvested to assess pods number plant⁻¹, seed weight plant⁻¹ and seed yield ha⁻¹. According to AOAC (2012), seed oil and protein contents were estimated.

Irrigation water use efficiency and regression analysis

Depending on the computed irrigation water amounts for each irrigation level in 2015 and 2016 seasons (Table 3), irrigation water use efficiency (IWUE) for soybean crop was estimated as follow:

\[ IWUE = \frac{\text{Seed yield}}{\text{Irrigation water amount}} \text{ (kg m}^{-3}) \]  

(3)

Thereafter, simple regression analysis between IWUE (dependent variable) and benzyladenine rate (independent variable) under different irrigation water regimes was derived as explained by Draper and Smith (1998).

Statistical analysis

Data of each season were subjected to analysis of variance (ANOVA) according to Casella (2008), using Costat software program, Version 6.303 (2004). Means separation was performed only when the F–test indicated significant (P<0.05) differences among the treatments, according to the Fisher’s protected LSD test.

RESULTS

Plant growth

Available results in Table 4 show the significant influence of each irrigation and benzyladenine on the investigated growth traits of soybean in 2015 and 2016 seasons. In this respect, with each increase in water amount, there were progressive increases in plant height, SPAD value, RGR and NAR. Application of ET₁₀₀ recorded the maximum values of RGR and NAR surpassing ET₈₀ or ET₆₀. The differences between ET₁₀₀ and ET₆₀ did not reach the P<0.05 level of significance for plant height and NAR in 2016 season as well as RGR in 2015 season. Moreover, the minimal values of such traits were obtained with ET₆₀.
Spraying soybean plants with benzyladenine (BA) caused significant increases in plant height, SPAD value, RGR and NAR (Table 4). With increasing BA rate up to 150 mg L\(^{-1}\), all soybean growth traits progressively increased in both seasons, except RGR in 2015 season. In this respect, BA\(_{200}\) achieved the highest values significantly leveling with BA\(_{150}\).

The significant interaction of irrigation level x benzyladenine rate presented in Table 5 revealed that the most effective combinations for enhancing plant height and NAR were ET\(_{100}\) or ET\(_{80}\) whether with BA\(_{200}\) or BA\(_{150}\) in both seasons. Also, ET\(_{100}\) x BA\(_{100}\) interaction statistically equaled the former remarkable interactions for plant height in 2016 season and NAR in both seasons.

**Yield traits and oil and protein percentages**

All yield traits of soybean statistically responded to irrigation and benzyladenine treatments in 2015 and 2016 seasons (Table 6). ET\(_{100}\) was the effective practice for producing higher values of pods number plant\(^{-1}\), seed weight plant\(^{-1}\), seed yield, oil % and protein %. There were no significant variations between ET\(_{100}\) and ET\(_{80}\) for seed yield (in 2015 seasons) and protein % (in 2016 season). Remarkable reductions in all crop traits were obviously obtained with ET\(_{60}\).

Data presented in Table 6 show the significant increases in pods number plant\(^{-1}\), seed weight plant\(^{-1}\), seed yield, oil % and protein % with increasing BA rates from 0 to 200 mg L\(^{-1}\). The highest values of all these traits were obtained from BA\(_{200}\) along with BA\(_{150}\), except pods number plant\(^{-1}\) in both seasons and oil % in the second season. The relative enhancements due to BA\(_{150}\) compared to untreated control (BA\(_{0}\)) reached 25.5% for seed weight plant\(^{-1}\), 14.3% for seed yield, 9.2% for oil % and 7.2% for protein % (as averages of the two seasons).

Concerning the interaction of irrigation level x benzyladenine rate, the presented values in Table 7 clarify that under ET\(_{100}\), whether with BA\(_{200}\) or BA\(_{150}\) possessed the favorable effects on pods number plant\(^{-1}\) and seed yield ha\(^{-1}\) in both growing seasons of 2015 and 2016. ET\(_{80}\) x BA\(_{200}\) (in both seasons) and ET\(_{100}\) x BA\(_{150}\) (in the first season) were as similar as ET\(_{100}\) x BA\(_{200}\) for enhancing pods number plant\(^{-1}\). Moreover, in 2015 season, ET\(_{60}\) x BA\(_{150}\) or BA\(_{200}\) and ET\(_{100}\) x BA\(_{150}\) recorded seed yield values like that of ET\(_{100}\) x BA\(_{200}\).

**Irrigation water use efficiency and regression analysis**

As depicted in Fig. 1, irrigation water use efficiency (IWUE) progressively increased with decreasing irrigation water amount and increasing benzyladenine rate. In this regard, the maximum values of IWUE were obtained with ET\(_{60}\) x BA\(_{200}\) in both seasons and ET\(_{60}\) x BA\(_{150}\) in

**Table 5.** Plant height and net assimilation rate (NAR), of soybean as affected by irrigation level and benzyladenine rate interaction in 2015 and 2016 seasons.

| Variable   | Plant height (cm) | NAR (g cm\(^{-2}\) week\(^{-1}\)) |
|------------|-------------------|-----------------------------------|
|            | ET\(_{60}\)  | ET\(_{80}\)  | ET\(_{100}\)  | ET\(_{60}\)  | ET\(_{80}\)  | ET\(_{100}\)  |
| 2015       |                 |                                   |
| BA\(_{0}\)  | 67.9i           | 75.0b                      | 82.3f         | 3.50c         | 3.70de        | 3.90bcd       |
| BA\(_{50}\) | 70.3i           | 83.3df                     | 85.5de        | 3.70de        | 3.80cde       | 3.92bcd       |
| BA\(_{100}\)| 75.0b          | 87.0bd                     | 89.0c         | 3.80cde       | 3.80cde       | 4.10abc       |
| BA\(_{150}\)| 77.0bf         | 90.3bh                     | 92.0a         | 3.80cde       | 4.10abc       | 4.20ab        |
| BA\(_{200}\)| 78.0f          | 91.7bh                     | 93.0a         | 3.90bcd       | 4.20ab        | 4.30a         |
| 2016       |                 |                                   |
| BA\(_{0}\)  | 79.0f           | 80.7def                    | 83.1cde       | 3.50c         | 3.60df        | 3.70def       |
| BA\(_{50}\) | 79.3f           | 82.8def                    | 84.2bed       | 3.70def       | 3.80def       | 3.92de        |
| BA\(_{100}\)| 80.4def        | 84.1bed                    | 86.4bc        | 3.70def       | 4.00def       | 4.30b         |
| BA\(_{150}\)| 83.0cdef       | 87.7bc                     | 89.2e         | 3.90cde       | 4.20abc       | 4.40a         |
| BA\(_{200}\)| 83.2ed         | 88.5a                      | 90.1a         | 4.00bcd       | 4.30b         | 4.50a         |

Note: ET\(_{60}\), ET\(_{80}\), and ET\(_{100}\): irrigation level at 60, 80 and 100% of crop evapotranspiration, respectively; BA\(_{0}\), BA\(_{50}\), BA\(_{100}\), BA\(_{150}\), BA\(_{200}\): benzyladenine rate of 0, 50, 100, 150 and 200 mg L\(^{-1}\), respectively; SPAD: total leaf chlorophyll content; RGR: relative growth rate; NAR: net assimilation rate. Means followed by different letters in each column are significantly different (\(P<0.05\)).
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the second season, while ET_{100} x BA_0 or BA_{50} recorded the lowest value in both seasons. Despite the similarity of benzyladenine behavior under different irrigation regimes, its relationship strength with IWUE varied (Fig. 2). In this respect, regression analysis showed that benzyladenine rate was more correlated with IWUE under low water supply (ET_{60}), since R^2 value was higher compared to other irrigation regimes (ET_{80} or ET_{100}).
As well known, supplying plants by water amounts less than required almost caused disorders in plant growth and development. Findings of the current research confirmed that reducing irrigation water by 40% in soybean mightily caused reduction in growth traits. Accordingly, irrigating soybean by 60% of crop evapotranspiration (ET 60) caused reductions (averages of the two seasons) could be amounted to 11.8% for plant height, 7.8% for SPAD value, 10.3% for RGR and 8.4% for NAR (Table 4). These results might be ascribed to that water stress causes changing in patterns of plant growth and development (Ouda et al. 2010) and disturbance of metabolites transportation within plant (Tayel and Sabreen, 2011). Also, subjecting plants to water amount less than needed may caused reduction in accumulation of assimilates in seeds leading to decreases in yield traits (Table 6). In this respect, De Souza et al. (1997) reported that water stress shortened the seed–filling period resulting 32 and 44% reductions in seed size and seed yield, respectively. Soybean plants exposed to water stress were characterized by significant decline in photosynthetic rate, stomatal conductance and transpiration rate (Ohashi et al. 2006). Excessive energy in reaction center of photosynthesis apparatus (PSII or PSI) can cause pigment bleaching in sun leaves, as well as can induce photo inhibition, thereby damaging pigments through oxidative stress (Kim et al. 2011; El-Metwally and Saudy 2021). Moreover, plants subjected to drought exhibit large reductions in relative water content of leaves (RWC) and water potential (Dekov et al. 2001; Nayyar and Gupta 2006), leading to dehydration of plant tissue as a result of high light intensity and soil water deficit (Nicolás et al. 2005). Additionally, Zhang et al. (2016) reported that stomata are sensitive to RWC and tend to close with decreasing RWC, which can result in lower stomatal conductance levels in drought–exposed plants. Stomatal closure primarily causes a decline in the photosynthesis rate (Mahajan and Tuteja 2005). Recent studies confirmed the adverse impacts of drought on crop growth and yields (Saudy and El-Metwally 2019; Saudy et al. 2020; Mubarak et al 2021; Saudy et al. 2021). Contrariwise, supplying soybean plants with adequate water requirement might enhance the plant to absorb a larger quantity of nutrients, which leads to increased vegetative growth of plants and increase the plant’s ability to intercept the radiation, hence increase the rate of photosynthesis (Tayel and Sabreen 2011, Gkaswa et al. 2014). Accordingly, yield and its attributes of soybean were enhanced by applying ET 100 followed by ET 80 (Table 6). Furthermore, the differences between ET 100 and ET 80 were slight and did not exceed 5.1% for pods number plant−1, 9.6% for seed weight plant−1, 5.4% for seed yield ha−1, 4.6% for oil % and 3.3% for protein % (averages of the two seasons).

Motivational effect of benzyladenine toward growth and yield of soybean was more evident with elevating its concentration up to BA 200 or BA 150, improving the performances (Tables 4 and 6). In this regard, BA plays a great role in cell division and cell differentiation, senescence, photosynthetic pigment (Sarwat and EL–Sherif 2007). Plant growth regulators can enhance the source–sink ratio and catalyze the translocation of photosynthesis assimilates, thereby helping in efficacious flower genesis, fruit and seed development and eventually boost crop productivity. Photosynthetic potentiality and effective partitioning of accumulated substances from source to sink were improved because of growth regulators application (Solamani et al. 2001). A correlation between endogenous levels of cytokinins and the degree of flower abortion and set was reported by Nooden et al. (1990). It is noted that cytokinins regulate flower and pod development in soybean, thus application of exogenous benzyladenine (individual of cytokinins) has been shown to prevent abortion of flowers and/or pods (Reese et al., 1995). The positive effect of benzyladenine spraying may be due to ability to adjust several biological processes owing their ability to influence almost all plant development and growth stages (Plihalova et al. 2016). benzyladenine substantially induced accumulation of total N and protein synthesis (Taiz and Zeiger 2006; Ayad and Gamal El–Din 2011).
Despite soybean plants differentiate plentiful floral buds, most of them fail to grow into pods and abort during development. Remarkable abortion of flowers and pods commonly occurs in soybean (Abernethy et al. 1997). Such phenomenon is exactly induced with presence of water stress. Seed abortion began earlier in stressed plants and the duration of the maturation period was significantly reduced, leading to accelerated senescence (Desclaux and Roumet 1996). Because of impairment of ovule function as result of water deficit, soybean flowers aborted (Kokubun et al. 2001). By examining the data in Tables 5 and 7, we can obtain that ET<sub>100</sub> x BA<sub>0</sub> interaction (farmer common practice) is more effective for enhancing crop growth and yield traits than ET<sub>80</sub> x BA<sub>0</sub> one. However, the values of plant height, NAR, pods number plant<sup>-1</sup> and seed yield ha<sup>-1</sup> due to ET<sub>80</sub> x BA<sub>200</sub> or BA<sub>150</sub> treatments were higher than those of produced with common practice (ET<sub>100</sub> x BA<sub>0</sub>). Such finding refers to the beneficial impact of benzyladenine under low water supply conditions. Sharma and Walia (1996) reported that BA may be involved in the development of leaves and branches in plants under adverse conditions such as low drought. Exogenous application of plant hormones i.e. 6-benzyladenine (individual of cytokinins) have been used to improve vegetative growth and yields by foliar applications (Baschauadhuri 2016). The improvements in plant growth under water stress as a result of benzyladenine application could be attributed to its importance for inducing the antioxidant compounds in treated plants. The application of cytokinins could raise the antioxidants such as phenolics and flavonoids levels (Rahneshan et al. 2018). The potential high antioxidant capacity in plants treated by BA was attributed to the high amounts of phenolics (Mangena 2020). Phenolics are highly potent antioxidants and free–radical scavengers owing to their capacity as strong reducing agents (Chandra et al. 2014). Similar result was obtained by Hassanein et al. (2005) and Ahmed et al. (2016).

Calculation of IWUE confirmed the beneficial effect of benzyladenine under severe stress, moderate stress or well–watered circumstance (Fig. 1), since its application regulate the ratio of soybean seed yield to applied water. In this situation, the increase in IWUE associated increasing benzyladenine rates refers to better exploiting each water drop to produce dry biomass. By comparing the efficiency of benzyladenine under different irrigation regimes, regression analysis (Fig. 2) forecasted that the more the rate of benzyladenine increases by one unit the more the IWUE increases by 0.0008, 0.0011 and 0.0008 units with ET<sub>60</sub>, ET<sub>80</sub> and ET<sub>100</sub> respectively. From R<sup>2</sup> value of each, it is noticed that the importance of benzyladenine is more evident in exhibiting changes in IWUE under severe stress (ET<sub>60</sub>), since 98.2 % of these changes occurred. Accordingly, application of benzyladenine at a rate of 150 or 200 mg L<sup>-1</sup> is so useful under moderate water supply.

**CONCLUSION**

Our findings proved that benzyladenine had the potentiality to enhance crop growth and yield under severe drought (ET<sub>60</sub>), moderate drought (ET<sub>80</sub>) and non drought (ET<sub>100</sub>) conditions. Moreover, the values of plant height, net assimilation rate, pods number plant<sup>-1</sup> and seed yield ha<sup>-1</sup> obtained with ET<sub>80</sub> x BA<sub>200</sub> or BA<sub>150</sub> treatments were higher than those of produced with common farmers practice (ET<sub>100</sub> x BA<sub>0</sub>). As well, the reduction in seed yield due to lowering water supply by 20% (moderate drought) can be compensated using benzyladenine, 150 mg L<sup>-1</sup>. Thus, benzyladenine should be involved in the various irrigation programs of soybean. Also, even if there is more available water in the area, farmers are exhorted to use moderately water–stressed plus benzyladenine pattern viz ET<sub>80</sub> x BA<sub>150</sub> treatment with saving 20% of irrigation water quantity which can be exploited for irrigating other lands. Hence, the treatment of ET<sub>80</sub> x BA<sub>150</sub> may consider an auspicious practice under areas suffering from water shortage for soybean crop production. However, several studies should be implemented to confirm the potency of benzyladenine in altering the physiological/biochemical response involving complex and variable metabolic pathways forming different metabolites in soybean.

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**AUTHOR CONTRIBUTIONS**

Conception and design of the study: Ibrahim Mohamed El–Metwally and Hani Saber Saudy. Acquisition of data for the study: Ibrahim Mohamed El–Metwally and Magdi Tawfik Abdelhamid. Analysis of data for the work: Hani Saber Saudy. Interpretation of data for the work: Ibrahim Mohamed El–Metwally, Magdi Tawfik Abdelhamid. Manuscript revision and approval: Ibrahim Mohamed El–Metwally, Magdi Tawfik Abdelhamid and Hani Saber Saudy.
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