

## Role of Brassinosteroids (BRs) in Alleviating the Effect of Irrigation Water Deficiency in “Crimson Seedless” Grape Cultivar

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**Abstract:** Water deficit is one of the most important abiotic stresses limiting grapevine growth, yield, and quality, particularly in arid and semi-arid regions. Brassinosteroids (BRs) are plant hormones known to enhance physiological and biochemical tolerance to water stress in various crops. This study was conducted during the 2024 and 2025 growing seasons, using a randomized complete block design (RCBD), and results were discussed separately for each season. The objective was to evaluate the effects of foliar BR application at three concentrations combined with different irrigation levels on the performance of the ‘Crimson Seedless’ grapevine. Treatments consisted of three BR concentrations (0, 0.75, and 1.5 ppm) combined with three irrigation levels (100%  $ET_0$  as control, 75%, and 50%  $ET_0$ ). Brassinosteroids were applied at the beginning of vegetative growth, after flowering, and two weeks before veraison, while irrigation regimens were scheduled based on crop evapotranspiration ( $ET_0$ ). Results showed that foliar application of BR at 1.5 ppm under 75%  $ET_0$  irrigation deficit exhibited superior performance in most vegetative and reproductive parameters. In contrast, ABA concentration was comparatively higher under BR at 0 ppm combined with 50%  $ET_0$ . These findings highlight the potential of BR application as a practical strategy to optimize water use efficiency and improve grapevine performance under limited water availability.

**Keywords:** “Crimson Seedless” cv., Brassinosteroids, Deficit irrigation, ABA Concentration.

### Introduction

Climate change and competing water demands have intensified water scarcity worldwide, posing serious challenges to agricultural productivity [1]. Grape (*Vitis vinifera* L.) is among crops sensitive to water deficit, where suboptimal irrigation often reduces vegetative growth, yield, and fruit quality [2]. Moreover, Crimson Seedless is considered as one of the most widely cultivated table grape cultivars globally, appreciated for its attractive color and market acceptance; yet it is also susceptible to drought impacts. Deficit irrigation strategies such as regulated deficit irrigation have been shown to improve water use efficiency and enhance specific quality traits like berry coloration without substantially lowering yield [3].

Brassinosteroids (BRs) are a class of plant steroid hormones with well-documented roles in enhancing stress tolerance, including drought, through modulation of antioxidant systems, photosynthetic efficiency, and osmotic adjustment. In grapevines and other crops, BR applications have been associated with reduced oxidative damage and improved physiological performance under drought conditions. However, there remains a gap in literature specifically assessing combined BR and deficit irrigation effects on Crimson Seedless performance under water limited environments [4].

Water deficit is a major abiotic stress limiting grapevine growth and productivity by disrupting water relations, photosynthesis, and hormonal regulation. Abscisic acid (ABA) plays a central role in drought responses through its regulation of stomatal conductance, shoot growth, stress signaling, and irrigation deficit typically increases ABA levels while suppressing vegetative growth [5,6]. In contrast, brassinosteroids (BRs) can mitigate drought stress by enhancing photosynthetic efficiency, maintaining chlorophyll content, and modulating endogenous ABA accumulation [7,8].

Therefore, joint evaluation of ABA concentration and vegetative traits, including leaf area, chlorophyll content, shoot length, and cane maturity, provides insight into BR-mediated alleviation of irrigation-induced stress in grapevines. Despite previous studies on deficit irrigation or brassinosteroids in grapevines, information on their combined effects remain limited. The results of this study provide new insights for optimizing water use and brassinosteroids application under water-limited environments. Therefore, his study aims to assess how foliar BR application at different concentrations can mitigate the effects of water deficit on vine performance and fruit quality in “Crimson Seedless” grape cultivar.

## Materials and Methods

### Experimental Site and Plant Material

The experiment was conducted during the growing seasons of 2024 and 2025, in a commercial Crimson Seedless table grape vineyard located in Sadat city, a Subtropical desert climate region of Egypt. Vines were ten years old, planted at 2 × 3 m spacing, trained on an Overhead Trellising System (Spanish barron). Sixty-three vines were chosen for this study (7 treatments × 3 replicates × 3 vines / replicate), pruned during the 1st week of February, with a bud load of 80 buds/vine (8 canes × 10 buds/cane) in addition to 8 spurs × 2 buds / spur. The experiment was carried out on the same vines for both seasons 2024 and 2025, and received common horticultural practices recommended by Ministry of Agriculture.

A randomized complete block design (RCBD) with three replicates was used. Each replicate comprised a block of vines receiving one of the following foliar Brassinosteroids (BRs) × irrigation treatments (% ET<sub>0</sub>) as follow:

1. Brassinosteroids (BRs) 0 ppm (100% ET<sub>0</sub>) (control)
2. Brassinosteroids (BRs) 0 ppm (50% ET<sub>0</sub>)
3. Brassinosteroids (BRs) 0 ppm (75% ET<sub>0</sub>)
4. Brassinosteroids (BRs) 0.75 ppm (50% ET<sub>0</sub>)
5. Brassinosteroids (BRs) 0.75 ppm (75% ET<sub>0</sub>)
6. Brassinosteroids (BRs) 1.5 ppm (50% ET<sub>0</sub>)
7. Brassinosteroids (BRs) 1.5 ppm (75% ET<sub>0</sub>)

Type and method of BR used for this experiment was: Foliar sprays of 24-epibrassinolide. Preparation & application: BR solutions were prepared at 0, 0.75, and 1.5 ppm in distilled water with 0.1% Tween-20 as surfactant. Each vine received 2L per application, sprayed until runoff.

BR solutions were applied at three phenological stages: start of vegetative growth, post flowering, and two weeks before veraison. Irrigation levels (100, 75, and 50% ET<sub>0</sub>) were maintained based on weekly ET<sub>0</sub> calculations using local meteorological data and crop coefficients.

### Measurements and Data Collection

#### 1. Soil Analysis

Soil sample was collected and analyzed at the beginning of the experiment for both seasons to determine its physical and chemical properties.

#### 2. Yield and Berry Quality Measurements

At harvest, clusters were counted and weighed to determine the following:

- Yield per vine (kg).
- Cluster weight (g)
- Berry weight (g)
- Berry size (cm<sup>3</sup>).

#### 3. Chemical Characteristics of Berries

- Total soluble solids (TSS %).
- Titratable acidity (%).
- TSS/acid ratio according to A.O.A.C [9].
- Anthocyanin content in berry skin: it was determined spectrophotometrically according to established analytical methods [10].

#### 4. Vegetative Growth and Physiological Parameters

Vegetative growth indices were recorded throughout the season. Samples of 20 leaves were collected from each treatment for:

- Leaf area determination at harvest time “using leaf area meter, Model CI 203, USA”.
- Total chlorophyll content: was measured using “Nondestructive Minolta chlorophyll meter model SPAD 502”.

- Abscisic acid (ABA) was extracted from fresh leaf samples following a standard organic solvent extraction method as described by Zhang et al. [11], and ABA concentration was determined on a fresh weight basis.
- Shoot length was measured at the end of the growing season by (cm).
- Cane maturity was evaluated based on the degree of lignification of current-season shoots. At the end of the growing season, the length of the lignified (brown, woody) portion of each shoot was measured and expressed as a percentage of the total shoot length using this equation.

$$\text{Cane maturity (\%)} = (\text{Lignified shoot length} / \text{Total shoot length}) \times 100$$

### Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) according to a randomized complete block design. Means representing the effects of the tested treatments were compared using the New Least Significant Difference (New LSD) test at the 0.05 probability level, as described by Snedecor and Cochran [12]. All analyses were performed using SPSS version 25 (IBM Corp., USA).

## Results and Discussion

### 1. Soil Analysis

Soil physical and chemical analyses showed consistent texture and baseline nutrient levels across seasons. Irrigation water was within acceptable salinity and pH ranges for table grape production. Soil analyses indicated a sandy-loam texture with moderate organic matter and nutrient availability suitable for vine growth in both 2024 and 2025.

**Table 1:** Physical and chemical analysis of the soil at experimental site.

Soil properties	Values	
	2024	2025
Sand (%)	64.4	64.8
Clay (%)	19.7	20.1
Silt (%)	14.8	13.7
<b>Soil texture</b>	<b>Sandy-loam</b>	
pH	7.34	7.22
EC Mmhos/cm	1.92	1.83
Field capacity (%)	16.3	12.4
Organic matter (%)	1.46	1.55
CaCO <sub>3</sub> (%)	0.61	0.58
Total N %	0.95	1.22
Available P (ppm)	0.91	0.84
Available K (ppm)	0.40	0.38

### 2. Yield and Berry Quality Measurements

#### Yield Per Vine, Cluster weight, Berry Weight, and Berry Size

Data of Table (2) display the value of the yield per vine, cluster weight, berry weight and berry size which showed that Brassinosteroids at 1.5 ppm under 75% ET<sub>0</sub> produced significantly higher values compared with other deficit treatments followed by Brassinosteroids at 1.5 ppm under 50% ET<sub>0</sub>. Under Brassinosteroids at 1.5 ppm combined with 50% ET<sub>0</sub>, yield and berry weight and size were slightly reduced compared with the 75% ET<sub>0</sub> treatment; however, they remained significantly higher than the other deficit treatments without BR application. However, there is no significant difference between BR at 0.75 ppm (50% ET<sub>0</sub>) and BR at 0 ppm (100% ET<sub>0</sub>) (control), whereas BR at 0 ppm (50% ET<sub>0</sub>) gave the least values. This response is consistent with the established role of brassinosteroids in promoting berry growth, cell expansion, and yield formation in grapevines [13]. In addition, the moderate water deficit combined with a high BR concentration appeared to balance stress adaptation and productive capacity, a response previously reported under regulated deficit irrigation when BRs were applied exogenously [14,15,7].

This indicates that higher BR concentrations can partially compensate for severe water limitation, as brassinosteroids have been shown to maintain growth and yield under drought stress by improving physiological efficiency [16].

In line with these findings, recent studies further demonstrated that 24-epibrassinolide significantly increased antioxidant enzyme activity and improved carbon and nitrogen metabolism under water deficit conditions in grapevines, contributing to improved growth and yield performance under stress [4].

**Table 2:** Effect of (BRs) application under different irrigation water regimes ( $ET_0$ ) on yield per vine, berry weight, and berry size of ‘Crimson Seedless’ grapevines in 2024 & 2025 seasons.

Characteristic	Yield/vine (kg)		Cluster weight (g)		Berry weight (g)		Berry size (cm <sup>3</sup> )	
	2024	2025	2024	2025	2024	2025	2024	2025
BRs at 0 ppm (100% $ET_0$ ) (control)	17.2	17.5	508.8	513.8	4.83	4.89	4.68	4.74
BRs at 0 ppm (50% $ET_0$ )	15.6	15.9	460.4	468.8	4.38	4.47	4.23	4.31
BRs at 0 ppm (75% $ET_0$ )	16.5	16.7	485.8	489.9	4.61	4.67	4.46	4.51
BRs at 0.75 ppm (50% $ET_0$ )	16.9	17.2	497.6	505.4	4.73	4.80	4.59	4.65
BRs at 0.75 ppm (75% $ET_0$ )	18.3	18.4	536.9	540.2	5.11	5.14	4.96	4.96
BRs at 1.5 ppm (50% $ET_0$ )	20.0	20.7	582.4	609.1	5.54	5.80	5.40	5.64
BRs at 1.5 ppm (75% $ET_0$ )	20.5	21.5	602.8	633.2	5.75	5.98	5.61	5.83
New L.S.D. at 0.05	0.42	0.51	11.3	9.8	0.12	0.11	0.13	0.13

### 3. Chemical Characteristics of Berries

#### Total Soluble Solids (TSS %), Titratable Acidity (TA %), and TSS/Acid Ratio

Data presented in Table (3) followed a different trend where the values indicated that moderate deficit irrigation combined with a high Brassinosteroids concentration (1.5 ppm at 75%  $ET_0$ ) significantly increased total soluble solids (TSS %) and maintained balanced titratable acidity (TA), resulting in higher TSS/acid ratios followed by Brassinosteroids concentration (0.75 ppm at 75%  $ET_0$ ) then BR (1.5 ppm at 50%  $ET_0$ ) compared with the fully irrigated control (BR at 0 ppm (100%  $ET_0$ )) and deficit irrigation treatments without BR application. Although water deficit is often associated with increased sugar concentration due to reduced berry size and concentration effects, this response is strongly dependent on stress severity. Previous studies have demonstrated that moderate water deficit enhances sugar accumulation through both concentration effects and stimulation of carbohydrate metabolism, whereas severe water deficit can limit photosynthetic activity and assimilate availability, thereby reducing sugar accumulation [14,17]. The superior TSS and TSS/acid ratio observed under 75%  $ET_0$  combined with BR application therefore reflect an optimal balance between water limitation and metabolic activity, rather than a simple concentration effect.

#### Anthocyanin content

Similarly, anthocyanin content (Table 3) was highest under BR application at 1.5 ppm combined with moderate deficit irrigation (75%  $ET_0$ ), followed by the lower BR concentration 0.75 ppm under moderate deficit (75%  $ET_0$ ). This pattern suggests that BR application under water limited conditions enhances secondary metabolite accumulation, particularly when stress intensity remains within a physiologically tolerable range. Water deficit is known to activate phenylpropanoid and flavonoid biosynthetic pathways in grape berries, promoting anthocyanin accumulation and improving color and quality attributes [18,17]. Moreover, exogenous application of brassinosteroids has been shown to enhance anthocyanin and phenolic accumulation in grape berry skins, including up-regulation of biosynthetic genes and enzyme activities involved in flavonoid pathways [19].

**Table 3:** Effect of (BRs) application under different irrigation water regimes ( $ET_0$ ) on chemical characteristics of berries of ‘Crimson Seedless’ grapevines in 2024 & 2025 seasons.

Characteristic	TSS %		Acidity %		TSS/Acid Ratio		Anthocyanin mg/100g	
	2024	2025	2024	2025	2024	2025	2024	2025
BRs at 0 ppm (100% $ET_0$ ) (control)	15.8	16.0	0.62	0.64	25.5	25.0	28.4	26.9
BRs at 0 ppm (50% $ET_0$ )	15.1	15.3	0.66	0.68	22.9	22.5	25.7	23.5
BRs at 0 ppm (75% $ET_0$ )	16.2	16.5	0.58	0.60	27.9	27.5	30.6	29.1
BRs at 0.75 ppm (50% $ET_0$ )	16.6	16.9	0.54	0.56	30.7	30.2	33.2	31.8
BRs at 0.75 ppm (75% $ET_0$ )	17.4	17.7	0.46	0.43	37.8	41.2	37.9	39.6
BRs at 1.5 ppm (50% $ET_0$ )	17.1	17.4	0.49	0.47	34.9	37.0	36.5	38.1
BRs at 1.5 ppm (75% $ET_0$ )	17.8	18.1	0.43	0.39	41.4	46.4	39.8	41.4
New L.S.D. at 0.05	0.18	0.24	0.02	0.03	1.9	2.1	1.3	1.1

#### 4. Vegetative Growth and Physiological Parameters

##### Leaf Area cm<sup>2</sup>, Chlorophyll Content SPAD, ABA Concentration, and Shoot Length (cm).

###### Leaf area

Data displayed in Table (4) showed significantly larger leaf area in the 1.5 ppm BR (75% ET<sub>0</sub>) treatment than in other combinations, consistent with the notion that BRs enhance growth under moderate stress while reducing water loss and sustaining photosynthesis as stated by Khan et al. [20] and Singh et al. [21]. Moreover, the leaf is the chief part of the plant where most of the photosynthetic products are synthesized as a result the number of leaves decreased when subjected to water stress [22].

###### Chlorophyll content

Chlorophyll content, which is of utmost importance for photosynthesis, is another photosynthetic attribute strongly influenced by water deficit that has been recognized as a distinctive indication of photo oxidation and degradation of chlorophylls. Therefore, in this study we found that the SPAD readings followed a similar ranking, with the moderate deficit and high BR concentration 1.5 ppm BR (75% ET<sub>0</sub>) treatment followed by Brassinosteroids at 1.5 ppm under 50% ET<sub>0</sub> maintaining higher chlorophyll content as previously stated by Khan et al., [20].

###### ABA concentration

ABA is a stress hormone that increases when the plant experiences water deficit, and higher stress levels lead to higher ABA. Therefore, data presented in Table (4) demonstrate that ABA concentration increased markedly under deficit irrigation without BRs application (0 ppm), particularly at 50% ET<sub>0</sub>, reflecting enhanced drought stress perception and growth inhibition. In contrast, BR-treated vines, especially at 1.5 ppm combined with moderate deficit irrigation (75% ET<sub>0</sub>), exhibited lower ABA accumulation, accompanied by significant improvements in leaf area, chlorophyll content, shoot length, and cane maturity across both seasons. This suggests that BRs application partially counteracted drought-induced ABA signaling, allowing sustained vegetative growth while maintaining stress adaptation.

These findings align with previous reports indicating that BRs enhance drought tolerance by regulating hormonal crosstalk, particularly through moderating ABA biosynthesis and sensitivity under water-limited conditions [23,24].

###### Shoot length

Shoot length measurements indicated that BR application at 1.5 ppm combined with moderate deficit irrigation (75% ET<sub>0</sub>) maintained healthier vegetative growth compared to more severe deficit levels, supporting better overall vine development. Treatments with lower BR concentrations or severe water deficits showed reduced shoot elongation, suggesting that BRs help mitigate the inhibitory effects of water stress on vegetative growth [25].

###### Cane maturity

A similar trend was observed for cane maturity values (Table 4), where cane lignification was most advanced under BRs 1.5 ppm (75% ET<sub>0</sub>), followed by 0.75 ppm under moderate deficit. Fully irrigated vines without BR or severe deficit treatments exhibited slower lignification, indicating that exogenous BRs promote cane maturation under moderate water stress [25]. This result is consistent with findings that water deficit alters wood tissue characteristics linked to structural development [26] and that lignin biosynthesis contributes to drought resistance and structural reinforcement in grapevines [27]. Reviews on biostimulants also indicate that hormone-related treatments modulate morphological and anatomical stress responses in vines [28].

**Table 4:** Effect of (BRs) application under different irrigation water regimes (ET<sub>0</sub>) on the vegetative Growth and Physiological Parameters of ‘Crimson Seedless’ grapevines in 2024 &2025 seasons.

Characteristic	Leaf area (cm <sup>2</sup> )		Chlorophyll content (SPAD)		ABA concentration (ng g <sup>-1</sup> FW)		Shoot length (cm)		Cane maturity %	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
BRs at 0 ppm (100% ET <sub>0</sub> ) (control)	143.1	146.5	32.0	33.1	3.00	2.97	162.5	164.7	74.2	72.5
BRs at 0 ppm (50% ET <sub>0</sub> )	109.8	112.0	28.4	26.8	4.22	4.32	105.4	100.2	64.4	61.3
BRs at 0 ppm (75% ET <sub>0</sub> )	111.6	117.6	30.5	31.5	4.01	4.05	117.8	119.4	69.5	71.8
BRs at 0.75 ppm (50% ET <sub>0</sub> )	142.5	145.1	31.7	33.8	3.90	3.95	160.7	162.9	71.6	70.4
BRs at 0.75 ppm (75% ET <sub>0</sub> )	147.0	149.5	34.5	36.0	3.23	3.28	165.0	168.4	77.0	75.1
BRs at 1.5 ppm (50% ET <sub>0</sub> )	150.4	152.9	37.2	38.4	3.60	3.65	171.3	174.5	82.5	83.6
BRs at 1.5 ppm (75% ET <sub>0</sub> )	155.1	158.3	39.9	41.5	3.07	3.02	175.4	182.1	93.7	94.2
New L.S.D. at 0.05	2.8	2.5	1.2	1.7	0.08	0.05	3.1	2.8	3.1	2.2

## Conclusion

In conclusion, the study demonstrated that foliar application of brassinosteroids (BRs) at 1.5 ppm combined with deficit irrigation at 75% ET<sub>0</sub> is the most effective treatment for enhancing the growth, yield, and quality of “Crimson Seedless” table grapes under limited water conditions. Whereas, ABA concentration was comparatively higher under BR at 0 ppm combined with 50% ET<sub>0</sub>. These findings highlight the potential of brassinosteroids (BRs) to improve drought tolerance and optimize water use efficiency in grapevine cultivation. Such strategies are valuable for grape growers in arid and semi-arid regions aiming for sustainable production amidst water scarcity challenges.

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