



# Article Exogenous Selenium Improves Physio-Biochemical and Performance of Drought-Stressed Phaseolus vulgaris Seeded in Saline Soil

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Abstract: Water and salt stresses are among the most important global problems that limit the growth and production of several crops. The current study aims at the possibility of mitigating the effect of deficit irrigation of common bean plants growing in saline lands by foliar spraying with selenium via the assessment of growth, productivity, physiological, and biochemical measurements. In our study, two field-based trials were conducted in 2017 and 2018 to examine the influence of three selenium (Se) concentrations (0 (Se<sub>0</sub>), 25 (Se<sub>25</sub>), and 50 mg  $L^{-1}$  (Se<sub>50</sub>)) on common bean plants grown under full irrigation ( $I_{100} = 100\%$  of the crop evapotranspiration; ETc) and deficit irrigation  $(I_{80} = 80\% \text{ of ETc}, \text{ and } I_{60} = 60\% \text{ of ETc})$ . Bean plants exposed to water stress led to a notable reduction in growth, yield, water productivity (WP), water status, SPAD value, and chlorophyll a fluorescence features (Fv/Fm and PI). However, foliar spraying of selenium at 25 or 50 mg  $L^{-1}$  on stressed bean plants attenuated the harmful effects of water stress. The findings suggest that foliage application of 25 or 50 mg  $L^{-1}$  selenium to common bean plants grown under  $I_{80}$  resulted in a higher membrane stability index, relative water content, SPAD chlorophyll index, and better efficiency of photosystem II (Fv/Fm, and PI). Water deficit at 20% increased the WP by 17%; however, supplementation of 25 or  $50 \text{ mg L}^{-1}$  selenium mediated further increases in WP up to 26%. Exogenous application of selenium  $(25 \text{ mg L}^{-1} \text{ or } 50 \text{ mg L}^{-1})$  to water-stressed bean plants elevated the plant defense system component, given that it increased the free proline, ascorbic acid, and glutathione levels, as well as antioxidant enzymes (SOD, APX, GPX, and CAT). It was concluded that the application of higher levels  $(25 \text{ or/and } 50 \text{ mg } \text{L}^{-1})$  of Se improves plant water status as well as the growth and yield of common beans cultivated in saline soil.

Keywords: common bean; selenium; deficit irrigation; growth; pods yield; osmolytes; antioxidant



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## 1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a prominent legume that is widely cultivated and consumed for its nutritional value [1], and it is considered an important source of proteins and calories [2]. Egypt is one of the world's top producers and exporters of green beans, with a global production of approximately 27 million mg produced from a land area of roughly 1.65 million hectares [3].

Innovative agriculture and plant-based technologies are currently focused on plant growth, productivity, and impedance to environmental stresses [4,5]. Among these stressors, drought, salinity, and alkalinity are the most serious global issues causing substantial crop yield losses [6–9]. The physio-biochemical responses of plants to salinity and drought stress have similar lineaments. These stressors result in osmotic stress and water loss from the cytoplasm into the intercellular space, which causes cellular dehydration, stomatal closure, and a decrease in carbon dioxide fixation [10,11]. Drought stress impedes plant growth and production by accumulating abscisic acid and lowering cell turgor pressure, which inhibits several critical physiological processes that include cell division and elongation [12–14]. Continuous water stress provokes over-accumulation of free radicals, which attack the fundamental building blocks of tissues, such as lipids, Proteins, and DNA, and inhibits the activity of many enzymes [15–17]. Simultaneously, the free radicals disturb cellular redox homeostasis, induce chlorophyll degradation, reduce membrane integrity, inhibit photosynthetic machinery, and affect plant performance [17–19]. Irrigation is necessary at all phases of bean plant growth to obtain a satisfactory yield [20] since beans are water-deficit sensitive crops, mainly because they have a shallow root system [2]. Moreover, this sensitivity may occur during the early stages of growth and even during germination [21].

Salt soil is a serious concern in arid regions like Egypt, where drought episodes and irrigation water demands are higher [5,22,23]. The co-occurrence of water deficit and salt soil conditions may result in severe stress to crops such as beans because of the synergistic interaction of these major stressors [11,19]. Therefore, a line of investigations has been initiated to explore sustainable techniques for reducing the combined effects of environmental stresses. The exogenous application of antioxidative compounds has been proven to promote plant defense mechanisms and improve abiotic stress tolerance at different stages of plant growth [21,24,25].

Selenium is a vital microelement and antioxidant for plants [26], particularly those that are subjected to environmental stressors like drought [27,28], salinity [29], high temperatures [30], and heavy metals [31]. Exogenous selenium decreases oxidative stress in plants under abiotic stress, upregulating antioxidative enzymes, improving ascorbate–glutathione (AsA-GSH) cycles, and increasing osmolyte contents as an effective mechanism involved in scavenging the free radicals [32,33]. Selenium was found to improve photosynthetic efficiency by protecting the chloroplast ultrastructure and chlorophyll from free radicals [29,34]. Furthermore, selenium helps plants maintain their water content by elevating water uptake and lowering water loss from plant cells [35]. Recently, biofortification with selenium has emerged as a viable approach for increasing its content in plant-based foods [36], which is gaining great importance, particularly in selenium-deficient areas like Egypt [26,33].

As far as we know, few studies have examined the impact of foliar spraying of selenium on stressed bean plants under different abiotic stresses, including drought, salinity, and alkalinity. In this research, we examine whether foliar spraying of selenium could lessen the impact of water stress on bean plants cultivated under salt soil conditions. Therefore, this research aimed to study the effect of selenium application on the growth, yield, water status, membrane stability, efficiency of photosystem II (PSII), concentration of osmolytes, and antioxidant activity of bean plants under full and deficit irrigation.

# 2. Materials and Methods

# 2.1. Site of Experimentation

Two open field trials were performed on a private Farm, in El-Fayoum, Egypt (29.5004 N, 30.8767 E) in 2017 and 2018. Before the initiation of each experiment, soil samples were taken at a depth of 25 cm, and the physicochemical parameters of the experimentation site were measured. Soil samples were analyzed according to the standard published procedures [37,38], and the results are presented in Table 1. According to the aridity index, the research region has a hyper-arid climate [39]. Information regarding the meteorological data for El-Fayoum during the study months (September–November) is shown in Table 2.

**Table 1.** Physicochemical characteristics and soil moisture content at a 25 cm depth of the soils in the 2017 and 2018 growing seasons.

Season	EC (dS/m)	рН	OM %	CaCO <sub>3</sub>	Pa D	article Siz istributio	ze on	Soil ρd Texture g.cm <sup>-3</sup>	Ksat	Soil Moisture Content at			
				%	Sand %	Silt %	Clay %		g.cm <sup>-3</sup>	${ m cm}~{ m h}^{-1}$	FC %	WP %	AW %
2017 2018	6.22 6.18	7.66 7.70	1.13 1.15	4.51 4.42	74.2 72.6	12.8 14.5	13.0 12.9	SL SL	1.58 1.55	2.10 1.96	21.03 22.2	10.55 11.4	10.48 10.8

EC = electrical conductivity; OM = organic matter content; SL = sandy loam; Ksat = hydraulic conductivity;  $\rho d$  = bulk density; soil moisture content FC, WP, and AW are field capacity, wilting point, and available water, respectively.

**Table 2.** The meteorological data include monthly maximum (T. max), minimum (T. min), and average (T. avg) temperature, relative humidity (RH), average monthly wind speed ( $U^2$ ), and the average pan evaporation ( $E_{pan}$ ) registered during both study seasons (2017–2018) at Fayoum, Egypt.

Month	T. max (°C) T. min		T. avg (°C)	RH (%)	$\mathrm{U}^2\mathrm{ms}^{-1}$	E <sub>pan</sub> mm Day <sup>-1</sup>	
			2017				
September	38.3	23.6	30.95	37.0	2.1	5.85	
Öctober	34.0	22.4	28.2	40.0	1.95	4.7	
November	27.8	15.4	21.6	41.5	2.2	2.15	
			2018				
September	37.2	22.4	31.2	38.2	1.99	5.6	
Öctober	33.2	20.3	28.6	396	2.1	4.3	
November	26.4	14.9	20.4	41	2	3.2	

# 2.2. Plant Management, Experimental Design, and Treatments

The common bean 'cv. Bronco' was used in these trials. This cultivar was chosen because of its high productivity and popularity among consumers. Also, it is suitable for adaptation to the climate and soil conditions in the area. Sowing was carried out in the open field on 6 September 2017 and 10 September 2018 in both growing seasons. Each experimental unit was surrounded by a non-irrigated space of 1 m. During the initial irrigation, the plants were appropriately moistened, and one week after complete germination, the irrigation regimes were started. All the experimental units received identical doses of NPK at approximately 150 kg ha<sup>-1</sup>, 60 kg ha<sup>-1</sup>, and 70 kg ha<sup>-1</sup>, respectively. All agricultural practices, pests, and disease control were carried out based on the agricultural bulletin issued by the Egyptian Agricultural Research Center. The experimental design was made up of completely random blocks (RCBD), and each treatment was replicated three times. Each plot area was 9 m<sup>2</sup>, (0.6 m wide  $\times$  15 m long) and contained two planting rows. The spacing between rows in the same bed was 30, whereas the spacing between plants within rows was 10 cm. Each experiment included two factors: three selenium concentrations and three irrigation levels. Selenium concentrations of 0 mg/L (tap water), 25 mg L<sup>-1</sup>,

and 50 mg L<sup>-1</sup> were foliar sprayed until runoff. Before spraying, Tween-20 (0.1%, v/v, as a surfactant) was added to promote optimal penetration into leaf tissues. Selenium supplementations were performed two times, 25 and 35 days after sowing. Three irrigation levels were applied, each representing a different percentage of crop evapotranspiration (ETc) as the following: 100% of ETc (I<sub>100</sub>), 80% of ETc (I<sub>80</sub>), and 60% of ETc (I<sub>60</sub>).

## 2.3. Irrigation Water Application

The daily reference evapotranspiration (ETo, mm day<sup>-1</sup>) was determined using the Class A pan (E<sub>pan</sub>, mm day<sup>-1</sup>) and the pan coefficient (K<sub>p</sub>) as follows [40]:

$$ETo = E_{pan} \times K_p \tag{1}$$

ETc was calculated using the ETo and crop coefficient (Kc) according to the following equation:

$$ETc = ETo \times Kc$$
 (2)

The duration of each crop growth stage was 15 days initially, 25 days for the development stage, 25 days for the mid-season stage, and 10 days for the late-season stage. According to [40], the *Phaseolus vulgaris* plant coefficients (Kc) were 0.50 for the initial, 1.05 for the mid, and 0.90 for the end stages [40]. The growing common bean plants were irrigated every two days. The amount of water added to each experimental unit was calculated using the following equation:

$$IWA = \frac{A \times ETc \times Ii \times Kr}{Ea \times 1000 \times (1 - LR)}$$
(3)

where *IWA* refers to the water used in irrigation (m<sup>3</sup>), *A* denotes the experimental unit area (m<sup>2</sup>), *ETc* denotes crop water needs (mm day<sup>-1</sup>), *li* denotes the intervals between irrigation events (day), *Kr* denotes covering factor, *Ea* denotes irrigation application efficiency (%), and *LR* denotes leaching requirements.

#### 2.4. Bean Growth and Green Pods Yield

Sixty days after seed sowing, three plants were randomly taken from each experimental plot to evaluate the growth traits. Shoot lengths were measured using a meter scale, and the number of leaves of plant<sup>-1</sup> was counted. The leaf area plant<sup>-1</sup> was determined using the leaf disk method as described in [41]. Fresh plant shoot samples were placed in an oven at 70 °C, taken out of the oven when the weight was constant, and the dry weight of the shoots was recorded. During the harvesting period, the green pods of all plants in each plot were picked. For each plot, the green bean pods on each plant (n = 10) were collected, counted, and weighed, and the green pod yield (t ha<sup>-1</sup>) was determined. For the determination of biochemical attributes, three plants were taken from each plot. Water productivity (WP) values were obtained for different treatments after harvesting as kg pods per m<sup>3</sup> of applied water using the following equation [41]:

#### 2.5. Leaf Relative Water Content, Membrane Stability, and Irrigation Use Efficiency

The relative water content (RWC) was determined in the leaves of bean plants [42]. Twenty discs (with a diameter of two cm) from fully expanded fresh leaves were weighed (FW) and then directly placed in water for six hours in the dark, after which the turgid mass was weighed (TW). Thereafter, the discs (DW) were dried in an oven at 65 °C for 48 h, and the dry mass (DM) was recorded. The RWC (%) was calculated as follows:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$
(5)

To evaluate the leaf membrane stability index (MSI) [43], duplicate samples of completely expanded fresh leaf tissue weighing 0.2 g were placed in a tube, and then 10 mL of distilled water was added. The sample was placed in a water bath for 30 min at 40 °C, and then the solution's electrical conductivity (EC1) was recorded. Another sample was boiled for 10 min at 100 °C, and the solution's electrical conductivity (EC2) was also recorded. The percentage of MSI was determined as follows:

$$MSI(\%) = 1 - (EC1/EC2) \times 100$$
(6)

#### 2.6. The Photosynthetic Performance

The relative chlorophyll content SPAD index of the two upper leaves of plants was measured using a SPAD-502 chlorophyll meter (Minolta, Japan). On two separate sunny days, one leaf (of the same age) per plant was selected for the measurement of the chlorophyll fluorescence parameters using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd., Kings Lynn, UK). The PI<sub>abs</sub> as an index of the photosynthetic performance reflect the electron transfer from PSII to PSI, and the energetic communication between PSII complexes was determined as described in [44]. The ratio of Fv/Fm provides an estimation of the maximal quantum efficiency of PSII, which was calculated using the formula [45]:

$$Fv/Fm = (Fm - F0)/Fm \tag{7}$$

where Fm is the maximum value of chlorophyll fluorescence, F0 is the minimum/initial value of chlorophyll fluorescence, and Fv is the variable chlorophyll fluorescence (Fv is the difference between F0 and Fm).

#### 2.7. Osmolytes and Antioxidative Compounds Quantification

The leaf-free proline content was calorimetrically measured at 520 nm as described in [46], and the average leaf-free proline content was calculated. The leaf's total free amino acid content was calorimetrically measured at a wavelength of 570 nm, as outlined in [47]. The leaf-soluble sugar content was determined using the anthrone reagent method [48]. The samples were extracted in 96% (v/v) ethanol, and the resulting mixture was boiled for 10 min after reaction with an anthrone reagent. A spectrophotometer (Bausch and Lomb-2000) was used to measure the solution's absorbance at 625 nm.

The leaf bean content of ascorbic acid (AsA) was assessed using the method presented in [49]. The extraction process was performed using trichloroacetic acid (TCA, 6%, w/v) and a leaf sample of 0.1 g. The sample was centrifuged at 15,000× g for 5 min under cold conditions at 4 °C. The supernatant was taken and placed into a reaction vessel, and then a standard mixture of 0.2 M phosphate buffer with pH 7.4, 0.5% (v/v) NEM (Nethylmaleimide), 10 mM DTT, 10% (w/v) TCA, 42% (v/v) H<sub>3</sub>PO<sub>4</sub>, 4% (v/v) 2,2'-dipyridyl, and 3% (w/v) FeCl<sub>3</sub> was inserted. Following that, the tubes were preserved for 40 min at 42 °C before the absorption of the solution was measured using a spectrophotometer at 525 nm. The glutathione (GSH) assessment in bean leaves was performed following the method reported in [50]. The absorbance at 412 nm was measured after adding 10 µL of 50 GSH reductase units mL<sup>-1</sup>.

The crude extract of the enzyme was prepared with 500 mg of fresh leaves and homogenized using 2 mL of 0.1 M potassium phosphate buffer with 7.5 pH, which was previously mixed with 0.1 mM ethylenediaminetetraacetic acid (EDTA). The sample was centrifuged for 30 min at  $12,000 \times g$  and 4 °C. A pure enzyme preparation was utilized to assay the enzymatic antioxidant activity. For the quantification of superoxide dismutase activity (SOD; EC 1.15.1.1), the photochemical method described in [51] was used. The SOD activity (U mg<sup>-1</sup> protein) was assessed as the amount of enzyme required to produce a 50% decrease in the rate of nitroblue tetrazolium reduction at 560 nm. The peroxidase (POD) activity was quantified as described in [52]. For assaying the ascorbate peroxidase, the APX activity (1.11.1.11) method as described in [53] was used by looking for AsA oxidation, which was detected as a decrease in absorbance at 290 nm. The catalase activity (CAT; EC

1.11.1.6) was quantified as described in [54] by observing the decomposition of  $H_2O_2$  in a spectrophotometer at 240 nm for 2–3 min. The CAT activity was measured as the difference in absorbance (A<sub>240</sub>) per unit of time.

## 2.8. Statistical Analysis

All measured data in both growth were analyzed using the two-way ANOVA procedures in the GenStat program (version 12, VSN International Ltd., Oxford, UK). The Student–Newman–Keuls test was used to make comparisons between treatment means at a significance level of  $p \leq 0.05$ .

#### 3. Results

## 3.1. The Photosynthetic Machinery, Cell Membrane Stability, and Water Status of Bean Plants

The analyzed physio-biochemical attributes, namely, SPAD value, Fv/Fm, PI, RWC, and MSI, were affected by irrigation regimes, selenium application, and the integrative application of both factors (Table 3). The deleterious effects of drought-mediated stress on *Phaseolus vulgaris* plants were described as inhibition of the photosynthetic machinery (Table 3). The photosynthetic efficiency in terms of SPAD chlorophyll index, PI, and Fv/Fm gradually decreased in response to increasing drought stress. Compared to non-selenium-treated plants, foliage-applied Se<sub>25</sub> or Se<sub>50</sub> increased all the aforementioned parameters. At all water stress levels, spraying with Se<sub>25</sub> or Se<sub>50</sub> recovered the drought-induced damages in the photosynthetic machinery, showing that they increased the SPAD chlorophyll, PI, and Fv/Fm, and registered values similar to those observed under control conditions without selenium application (I<sub>100</sub> × Se<sub>0</sub>). The integrative I<sub>100</sub> × Se<sub>25</sub> or Se<sub>50</sub> treatment resulted in the highest values (Table 3).

**Table 3.** Modulation in the chlorophyll fluorescence and SPAD chlorophyll value, cell membrane integrity (MSI), and water status of bean plants in 2017 and 2018 in response to foliar supplementation of selenium (Se) and different irrigation regimes (IR).

Treatments	SP.	AD	Fv/	Fm	Ι	Υ	М	SI	RWC		
11 cutilite 1100	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	
IR											
$I_{100}$	$38.4 \pm 0.5a$	$38.2 \pm 0.4a$	$0.82\pm0.0a$	$0.83\pm0.0a$	$2.7\pm0.2a$	$2.9\pm0.1a$	$58.6 \pm 1.1a$	$59.8\pm2.4a$	$89.0\pm0.6a$	$89.4 \pm 1.1a$	
I <sub>80</sub>	$34.4 \pm 1.1b$	$33.7 \pm 1.4b$	$0.80\pm0.01 \mathrm{ab}$	$0.81\pm0.0$ ab	$2.3 \pm 0.3b$ $2.7 \pm 0.2b$		$55.1 \pm 2.3b$	$55.8 \pm 1.9b$	$86.2 \pm 0.8a$	$86.9 \pm 1.0a$	
I <sub>60</sub>	$27.3 \pm 1.7c$	$25.9\pm2.1c$	$0.77 \pm 0.01b$ $0.78 \pm 0.05b$		$2.0 \pm 0.2c$	$2.0 \pm 0.2c$ $2.1 \pm 0.2c$		$42.9 \pm 2.1c$ $43.4 \pm 2.9c$		$80.4 \pm 3.3b$	
Se (mg $L^{-1}$ )											
Se <sub>0</sub>	$27.9 \pm 2.2c$	$27.9 \pm 1.8c$	$0.77\pm0.01b$	$0.77\pm0.01b$	$1.9\pm0.1c$	$2.3 \pm 0.2b$	$46.1 \pm 2.1b$	$46.4 \pm 3.3b$	$81.7 \pm 1.1c$	$82.5\pm1.1b$	
Se <sub>25</sub>	$37.4 \pm 1.1a$	$35.5\pm0.8a$	$0.81\pm0.00a$	$0.83\pm0.0a$	$2.3 \pm 0.1b$	$2.7\pm0.01a$	$55.3\pm2.7a$	$56.1 \pm 1.9a$	$87.6 \pm 1.1a$	$87.4 \pm 1.0a$	
Se <sub>50</sub>	$34.9 \pm 1.0b$	$34.5 \pm 1.1b$	$0.80\pm0.00a$	$0.82\pm0.0a$	$2.8 \pm 0.1a$	$2.7 \pm 0.1a$	$55.2 \pm 2.7a$ $56.5 \pm 1.7a$		$85.4 \pm 1.0b$	$86.8 \pm 1.0a$	
$IR \times Se$											
$I_{100} \times Se_0$	$38.4 \pm 1.4a$	$37.9 \pm 0.9a$	$0.82\pm0.00a$	$0.83\pm0.0$ ab	$2.7 \pm 0.2b$	$2.9\pm0.1a$	$58.4 \pm 2.9a$	$59.7 \pm 2.4a$	$88.8 \pm 1.4a$	$89.4 \pm 2.3a$	
$I_{100} \times Se_{25}$	$38.8 \pm 0.3a$	$38.6 \pm 0.5a$	$0.82\pm0.00a$	$0.84\pm0.0a$	$2.3 \pm 0.1 d$	$2.9\pm0.1a$	$58.8 \pm 2.9a$	$59.7 \pm 1.3a$	$89.1 \pm 0.7a$	$90.1 \pm 0.9a$	
$I_{100} \times Se_{50}$	$38.0 \pm 0.4a$	$38.2 \pm 0.2a$	$0.82 \pm 0.01a$ $0.84 \pm 0.0a$		$3.2 \pm 0.3a$	$2.9\pm0.2a$	$58.6 \pm 2.7a$	$59.9 \pm 0.4a$	$88.9 \pm 0.6a$	$88.7 \pm 1.5a$	
$I_{80} \times Se_0$	$26.2 \pm 1.7c$	$25.9 \pm 1.9c$	0.7800.01ab	$0.77\pm0.01 \mathrm{bc}$	$1.8\pm0.2 \mathrm{f}$	$2.3 \pm 0.3b$	$48.6\pm1.9b$	$48.8\pm2.2b$	$82.9 \pm 1.6 bc$	$83.3 \pm 0.4b$	
$I_{80} \times Se_{25}$	$38.4 \pm 1.2a$	$37.9 \pm 0.4a$	$0.81\pm0.00a$	$0.82\pm0.0$ ab	$2.1 \pm 0.2e$	$2.9\pm0.2a$	$57.8 \pm 1.1a$	$58.9 \pm 2.4a$	$87.8 \pm 1.2a$	$88.7\pm0.7a$	
$I_{80} \times Se_{50}$	$38.6 \pm 0.4a$	$37.4 \pm 0.5a$	$0.82 \pm 0.00a$	$0.83\pm0.0$ ab	$3.1 \pm 0.3a$	$2.9 \pm 0.2a$	$58.8 \pm 2.0a$	$59.6 \pm 2.4a$	$87.8 \pm 1.3a$	$88.5 \pm 2.8a$	
$I_{60} \times Se_0$	$19.0 \pm 1.9$ d	$20.0 \pm 0.2 d$	$0.73 \pm 0.02b$	$0.73 \pm 0.02c$	$1.3 \pm 0.2 \mathrm{g}$	$1.72 \pm 0.2c$	$31.2 \pm 2.3c$	$30.6 \pm 0.9c$	$73.5 \pm 0.2d$	$74.8\pm0.6c$	
$I_{60} \times Se_{25}$	$34.9 \pm 0.7b$	$29.9 \pm 0.8b$	$0.81\pm0.00a$	$0.82\pm0.01$ ab	$2.5\pm0.1c$	$2.38 \pm 0.3b$	$49.3 \pm 2.4b$	$49.6 \pm 2.2b$	$85.7\pm2.4ab$	$83.4 \pm 1.2b$	
$I_{60} \times Se_{50}$	$28.0 \pm 1.1c$	$27.8 \pm 1.3 bc$	$0.77\pm0.01$ ab	$0.80\pm0.0$ ab	$2.1 \pm 0.02e$	$2.35\pm0.3b$	$48.3 \pm 1.3b$	$49.9 \pm b$	$79.5 \pm 1.2c$	$83.1 \pm 0.2b$	

Different lowercase letters adjacent to the mean values (n = 5) in the same column indicates significant difference according to Tukey's HSD test at  $p \le 0.05$ .

Reducing irrigation from 80% ETc to 60% ETc markedly decreased the MSI by 14% and 40% and the RWC by 1% and 9% compared to full irrigation ( $I_{100}$ ) as an average for the two seasons, respectively (Table 3). However, the foliar application of 25 or 50 mg L<sup>-1</sup> selenium increased the MSI (by 21%; seasonal average) and RWC (by 6%; seasonal average) compared to untreated control plants. Nevertheless, exogenously applied selenium relieved the negative influence of drought stress on bean plants grown under salt soil, in the sense that the foliar application of selenium (25 or 50 mg L<sup>-1</sup>) mediated increases in the MSI by 21% and 59% (seasonal average) and RWC by 6% and 12% (seasonal average) of the stressed plants, respectively, compared to the respective control (Table 3).

#### 3.2. Osmolytes and Antioxidative Compounds of Bean Plants

Osmolytes and antioxidants are part of the plant defense system constituents that were identified in this study (Figures 1 and 2). Significant differences were observed among all the combined treatments. Foliar-applied selenium enhanced proline accumulation in water-stressed beans grown in salt soil. The highest proline value corresponded to the integrative  $I_{60} \times Se_{50}$  treatment, followed by  $I_{60} \times Se_{25}$ . Fully irrigated bean plants, whether or not treated with selenium, had the highest values of free amino acid content. The highest TSS content was observed under full irrigation without selenium supplementation ( $I_{100} \times Se_0$ ), followed by the combined application of full irrigation with selenium application at 25 mg L<sup>-1</sup> ( $I_{100} \times Se_{25}$ ). The non-enzymatic antioxidant analyses (AsA and GSH) in stressed beans were enhanced by selenium supplementation, as shown in Figure 2. Exogenous 25 or 50 mg L<sup>-1</sup> selenium to water-stressed bean plants at 20% increased the AsA by 38% and 41%, and GSH by 45% and 49%, respectively, compared to the corresponding control ( $I_{80} \times Se_0$ ). However, these increases in the ASA and GSH by foliar-applied selenium at 25 or 50 mg L<sup>-1</sup> to drought-stressed plants at 40% were 28% and 25%, and 64% and 43%, respectively, in relation to the corresponding control ( $I_{60} \times Se_0$ ).

The data recorded in Figure 2 show that selenium supplementation rebalanced the enzymatic antioxidants in bean plants exposed to different environmental stresses. Seleniumtreated plants grown under both drought stress levels registered higher activities of all analyzed enzymatic antioxidants but did not make a difference when applied to fully irrigated plants. However, selenium supplementation (Se<sub>25</sub> or Se<sub>50</sub>) to drought-stressed plants at 20% upregulated the activity of SOD by 44% and 46%, APX by 23% and 26%, GPX by 65% and 65%, and CAT by 22% and 20%. These increases in SOD, APX, GPX, and CAT were 78% and 73%, 40% and 39%, 33% and 41%, and 31% and 20%, respectively, when selenium with 25 or 50 mg L<sup>-1</sup> was applied to drought-stressed plants at 40% compared to fully irrigated plants that were not treated with selenium (I<sub>100</sub> × Se<sub>0</sub>). Exogenous selenium ameliorated the drought-induced damage via the reinforcement of proline and the antioxidative machinery.

## 3.3. Growth and Productivity Parameters of Beans Plants

The results in Table 4 illustrate that bean plants were influenced by irrigation levels, selenium concentrations, and the interaction between both factors. Bean plants were exposed to deficit irrigation at a rate of 20% (I<sub>80</sub>) and 40% (I<sub>60</sub>), resulting in a substantial decline in all growth parameters compared to full irrigation treatment ( $I_{100}$ ). Compared to non-selenium-treated plants, selenium spraying on bean plants resulted in a significant increase in all growth characteristics. Regarding the interaction between the two factors, the best values for all growth characteristics corresponded to the integrative  $I_{100} \times Se_{25}$ and  $I_{100} \times Se_{50}$  treatments. Selenium-treated (25 or 50 mg L<sup>-1</sup>) bean plants grown under 20% water stress had increased shoot length by 8.1% and 7.7%, leaf numbers by 13.7% and 14.1%, leaf area by 22.4% and 21.9%, and shoot dry weight by 9.9% and 22.3% as an average for both growing seasons, respectively, in comparison to the respective control ( $I_{80} \times Se_0$ ). Also, foliar-applied selenium (25 or 50 mg  $L^{-1}$ ) to severely drought-stressed bean plants  $(I_{60})$  produced substantial increases in shoot length by 9.8% and 10.3%, leaf numbers by 19.0% and 12.4%, leaf area by 35.8% and 25.3%, and shoot dry weight by 35.0% and 22.6% (seasonal average), respectively, compared to the corresponding control ( $I_{60} \times Se_0$ ). Overall, the growth traits of *Phaseolus vulgaris* plants grown under water deficit conditions were promoted by exogenously applied selenium (25 or 50 mg  $L^{-1}$ ).



**Figure 1.** Interactive effect of foliar supplementation of selenium (Se) and different irrigation regimes (IR) on osmoprotectants (e.g., free proline content, total free amino acid (TFAA), and soluble sugar content) of beans plants cultivated in salt soil. The vertical bar represents the standard error. Different letters on the bars refer to significant differences among means based on Tukey's HSD (honestly significant difference) test at the p < 0.05 level.



**Figure 2.** Interactive effect of foliar supplementation of selenium (Se) and different irrigation regimes (IR) on antioxidant non-enzymes; ascorbic acid (AsA) and glutathione (GSH) on leaf antioxidant enzymes; superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT) of bean plants cultivated in saline soil. The vertical bar represents the standard error. Different letters on the bars refer to significant differences among means based on Tukey's HSD (honestly significant difference) test at the p < 0.05 level.

As shown in Table 4, water deficits at 20% ( $I_{60}$ ) and 40% ( $I_{80}$ ) induced a significant decrease in the number of green pods by 13% and 42%, green pod weight by 8% and 31%, and green pod yield  $ha^{-1}$  by 7% and 25% (seasonal average), respectively, compared to full irrigation ( $I_{100}$ ). Regarding selenium supplementation, selenium-treated plants with 25 or 50 mg  $L^{-1}$  exhibited a higher number of pods per plant, green pod weight plant<sup>-1</sup>, and green pod yield in comparison to non-selenium-treated plants. However, exogenous selenium alleviated the impact of drought stress on bean production by producing significant increases in the bean yield and its components. In this respect, water-stressed plants at 20% treated with 25 or 50 mg  $L^{-1}$  selenium increased the number of pods in each plant by 44% and 41%, green pod weight per plant by 31% and 26%, and green pod yield by 11% and 9% (seasonal average), respectively, compared to the respective control ( $I_{80} \times Se_0$ ), and recorded similar values to those observed under full irrigation ( $I_{100}$ ). However, spraying 25 or 50 mg  $L^{-1}$  of selenium on bean plants grown under 40% of water deficit elevated the abovementioned traits by 83.8% and 84.6%, 59.6% and 56.1%, and 58.6% and 57.7% (seasonal average), respectively, compared to the control treatment ( $I_{60} \times Se_0$ ) (seasonal average). The integrative  $I_{100} \times Se_{25}$  and  $I_{100} \times Se_{50}$  treatments yielded the greatest values in both seasons. However, deficit irrigation at 20% ( $I_{80}$ ) increased WP by 17% (seasonal average), and severely deficit irrigation (I<sub>60</sub>) decreased WP in comparison to full irrigation  $(I_{100})$ . Foliar-applied selenium at 25 or 50 mg L<sup>-1</sup> increased WP by 20% and 19%, respectively, compared to the control. Interestingly, selenium supplementation to water-stressed bean plants improved WP, highlighting that bean plants grown under 20% water stress treated with selenium at 25 or 50 mg  $L^{-1}$  resulted in higher WP by 35% and 30% (seasonal average), respectively, than those grown under full irrigation without selenium application  $(I_{100} \times Se_0)$ . However, under severe water deficits  $(I_{60} \times Se_0)$ , selenium application at 25 or 50 mg  $L^{-1}$  improved WP to a lesser extent by 11% and 12%, respectively, compared to fully irrigated plants that were not treated with selenium ( $I_{100} \times Se_0$ ; Table 4). Therefore, in areas where water is not a limiting factor, it is better to apply the  $I_{80} \times Se_{25}$  treatment to achieve the highest WP and similar yield of fully irrigated plants that did not receive selenium. In areas where water is scarce, it is better to apply  $I_{60} \times Se_{25}$  treatment to save 40% of water and increase WP (by up to 17%).

Treatments _	Shoot Length (cm)		Leaf No. Plant <sup>-1</sup>		Leaf Area Plant <sup>-1</sup> (dm <sup>2</sup> )		Shoot Dry Weight (g Plant <sup>-1</sup> )		No. of Pods $Plant^{-1}$		Pods Weight Plant <sup>-1</sup>		Pods Yield (ton $ha^{-1}$ )		WP (kg pods/m <sup>3</sup> of Water)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
IR																
I <sub>100</sub>	$80.3\pm2.7a$	$84.5\pm3.6a$	$28.9\pm0.44a$	$29.30 \pm 1.2 \mathrm{a}$	$24.3\pm0.8a$	$23.1\pm0.8a$	$23.9\pm0.8a$	$22.8\pm0.5a$	$32.9\pm0.9a$	$32.3 \pm 1.3a$	$52.9 \pm 1.0a$	$52.5\pm0.9a$	$10.6\pm0.2a$	$10.66\pm0.2a$	$2.79\pm0.1b$	$3.55\pm0.1b$
I <sub>80</sub>	$77.8\pm2.9b$	$77.5 \pm 1.8b$	$27.6\pm1.06\mathrm{b}$	$28.2 \pm 0.7 b$	$22.1 \pm 1.4b$	$21.8\pm0.6b$	$20.7 \pm 1.4b$	$21.5\pm0.6b$	$28.3\pm0.8b$	$28.1 \pm 0.8b$	$48.5 \pm 1.4b$	$48.5 \pm 1.2b$	$9.9\pm0.2b$	$9.84 \pm 0.2b$	$3.28\pm0.1a$	$4.15\pm0.1a$
I <sub>60</sub>	$71.9 \pm 1.4c$	$72.6 \pm 4.2c$	$23.8\pm0.91\mathrm{c}$	$23.1 \pm 1.4c$	$17.8\pm0.7c$	$19.1 \pm 1.4c$	$17.8\pm0.7c$	$19.4 \pm 1.6c$	$19.0 \pm 1.7c$	$18.5\pm2.4c$	$36.4 \pm 2.0c$	$35.9 \pm 1.2c$	$8.1 \pm 0.3c$	$7.97\pm0.2c$	$2.66 \pm 0.1c$	$3.66 \pm 0.2b$
Se (mg $L^{-1}$ )																
Se <sub>0</sub>	$73.2\pm2.0b$	$74.13 \pm 3.5b$	$25.1\pm1.41\mathrm{b}$	$25.3 \pm 0.8c$	$19.5\pm0.8b$	$19.2 \pm 1.0c$	$18.6 \pm 0.9c$	$19.3 \pm 1.3c$	$22.5\pm2.1b$	$21.7\pm3.3b$	$40.1 \pm 3.6b$	$39.6\pm3.0b$	$8.5\pm0.6b$	$8.37 \pm 0.7b$	$2.52\pm0.1b$	$3.42\pm0.1b$
Se <sub>25</sub>	$78.2 \pm 3.3a$	$80.12 \pm 2.9a$	$27.5\pm1.67a$	$28.1 \pm 1.2a$	$22.3 \pm 1.2a$	$23.0 \pm 0.7a$	$20.9 \pm 1.2b$	$23.0 \pm 0.4a$	$29.0 \pm 1.3a$	$28.7 \pm 1.8a$	$48.8\pm3.1a$	$49.0 \pm 2.5a$	$10.2 \pm 0.5a$	$10.00 \pm 0.6a$	$3.10\pm0.1a$	$4.02\pm0.1a$
Se <sub>50</sub>	$78.6 \pm 3.3a$	$80.42 \pm 2.2a$	$27.7\pm1.19a$	$27.2 \pm 0.4b$	$22.4 \pm 1.1a$	$21.8\pm0.4b$	$22.9 \pm 1.2a$	$21.5\pm0.7b$	$28.8 \pm 1.3a$	$28.5 \pm 1.3a$	$48.8 \pm 3.3a$	$48.4 \pm 3.1a$	$9.9\pm0.7a$	$10.10\pm0.6a$	$3.12\pm0.1a$	$3.93\pm0.1a$
$IR \times Se$																
$I_{100} \times Se_0$	$78.9 \pm 1.6 \mathrm{ab}$	$80.13 \pm 3.8b$	$28.6\pm0.57a$	$29.0 \pm 2.1a$	$24.1 \pm 0.6a$	$23.1 \pm 1.6a$	$21.7\pm0.6c$	$22.5\pm0.4a$	$32.6 \pm 2.3$ ab	$31.8\pm2.0 \mathrm{ab}$	$52.2 \pm 1.1a$	$52.0 \pm 0.8a$	$10.4\pm0.2ab$	$10.23 \pm 0.1b$	$2.57\pm0.1c$	$3.35\pm0.1ef$
$I_{100}  imes Se_{25}$	$80.4 \pm 1.2a$	$86.33 \pm 3.1a$	$29.0\pm0.57a$	$29.3 \pm 0.3a$	$24.2 \pm 0.6a$	$23.3 \pm 0.3a$	$24.6\pm0.6ab$	$23.1 \pm 0.3a$	$32.9\pm0.3a$	$32.7\pm0.8a$	$52.7\pm0.3a$	$52.3 \pm 1.2a$	$10.9\pm0.1a$	$10.63\pm0.2ab$	$2.84 \pm 0.0b$	$3.54\pm0.1$ de
$I_{100} \times Se_{50}$	$81.6 \pm 0.5a$	$87.1 \pm 3.5a$	$29.0 \pm 0.52a$	$29.7\pm0.6a$	$24.4 \pm 1.0a$	$22.9 \pm 0.6a$	$25.4 \pm 1.0a$	$22.8\pm0.6a$	$33.3 \pm 0.8a$	$32.3 \pm 1.8 \mathrm{ab}$	$53.7\pm0.3a$	$53.3\pm0.6a$	$10.6\pm0.1b$	$11.10\pm0.1a$	$2.95\pm0.0b$	$3.76 \pm 0.1c$
$I_{80} \times Se_0$	$73.4 \pm 0.0c$	$74.2 \pm 2.3 d$	$25.1\pm0.66b$	$26.0 \pm 0.0b$	$18.6\pm0.1b$	$19.7\pm0.0b$	$18.9\pm0.4d$	$19.2\pm0.0b$	$22.2 \pm 1.0c$	$21.8\pm0.0c$	$41.8\pm0.6b$	$40.8\pm0.5b$	$9.4 \pm 0.1$ cd	$9.13\pm0.1c$	$2.90 \pm 0.0b$	$3.76 \pm 0.1$ cd
$I_{80} \times Se_{25}$	$79.8 \pm 2.3a$	$79.7 \pm 1.4 \mathrm{bc}$	$28.8\pm0.82a$	$29.3 \pm 0.6a$	$23.9 \pm 0.4a$	$22.9 \pm 0.9a$	$19.2 \pm 0.4 d$	$22.7\pm0.9a$	$31.7\pm1.7ab$	$31.7\pm1.4$ ab	$51.7\pm0.3a$	$53.3 \pm 0.3a$	$10.4\pm0.1 \mathrm{ab}$	$10.23 \pm 0.1b$	$3.55\pm0.0a$	$4.43\pm0.0a$
$I_{80}  imes Se_{50}$	$80.20\pm0.3a$	$787\pm0.0bcd$	$29.0\pm0.88a$	$29.3 \pm 0.6a$	$23.8 \pm 0.3a$	$22.8\pm0.8a$	$24.0\pm0.7b$	$22.6 \pm 0.8a$	$31.0 \pm 1.1b$	$30.9 \pm 0.8b$	$51.9 \pm 1.4a$	$51.3 \pm 0.3a$	$10.0 \pm 0.2 bc$	$10.17\pm0.4b$	$3.40\pm0.1a$	$4.27\pm0.2ab$
$I_{60} \times Se_0$	$67.4 \pm 0.6d$	$68.0\pm2.6\mathrm{e}$	$21.6 \pm 1.40c$	$20.8\pm0.0d$	$15.9\pm0.7c$	$14.8\pm0.5c$	$15.1\pm0.4e$	$16.1 \pm 0.5c$	$12.6 \pm 0.8 d$	$11.4 \pm 1.0 \mathrm{d}$	$26.3\pm1.5$	$25.9\pm0.6c$	$5.83 \pm 0.3e$	$5.73 \pm 0.1 d$	$2.08 \pm 0.1d$	$3.14\pm0.1 \mathrm{f}$
$I_{60} \times Se_{25}$	$74.3 \pm 0.0 \mathrm{bc}$	$74.3 \pm 2.7 d$	$24.7\pm0.82b$	$25.7\pm0.8b$	$18.7\pm0.4b$	$22.8\pm0.9a$	$19.0 \pm 0.3 d$	$23.2 \pm 0.8a$	$22.4 \pm 1.5c$	$21.7\pm0.7c$	$42.0 \pm 0.3b$	$41.3 \pm 1.6b$	$9.2 \pm 0.5 d$	$9.13 \pm 0.3c$	$2.90 \pm 0.2b$	$4.08 \pm 0.2 \mathrm{bc}$
$I_{60} \times Se_{50}$	$74.0 \pm 1.5c$	$75.3 \pm 2.7$ cd	$25.0 \pm 0.57b$	$22.7 \pm 0.3c$	$18.8 \pm 0.3b$	$19.6 \pm 0.8b$	$19.2 \pm 0.3d$	$19.0 \pm 1.0b$	$22.0 \pm 0.8c$	$22.3 \pm 0.8c$	$40.9 \pm 1.5b$	$40.6 \pm 0.3b$	$9.2 \pm 0.1$ d	$9.03 \pm 0.1c$	$3.00 \pm 0.0b$	$3.75 \pm 0.0d$

**Table 4.** Modulation in vegetative growth characteristics in pod yield and water productivity (WP) of bean plants in 2017 (SI) and 2018 (SII) in response to foliar supplementation of selenium (SE) and different irrigation regimes.

Different lowercase letters adjacent to the mean values (n = 5) in the same column indicates significant difference according to Tukey's HSD test at  $p \le 0.05$ .

## 3.4. Relationships

Pearson's correlation and hierarchical analyses were performed to examine the relationship between measured variables measured under foliar applications of selenium on bean plants grown under drought stress (Figure 3). The obtained results showed a significant positive correlation ( $p \le 0.05$ ) between shoot dry weight, shoot dry weight, shoot length, leaf area, number of leaves, and pod yield with the total soluble sugars, relative water content, membrane stability index, pod weight, SPAD, and performance index. Pearson's correlation analysis also showed a significant positive correlation ( $p \le 0.05$ ) between the parameters of the contents of APX, SOD, AsA, GPX, GSH, and CAT (Figure 3). Moreover, the levels of total free amino acids and proline had a significantly negative correlation ( $p \le 0.05$ ) with the above-mentioned traits.

The hierarchical analysis divided the applied treatments into three main groups (Figure 4). The treatments of  $I_{100} + Se_{25}$ ,  $I_{100} + Se_0$ ,  $I_{100} + Se_{50}$ , and  $I_{80} + Se_0$  were clustered together and showed higher performance compared to that of the second group ( $I_{60} + Se_{50}$ ,  $I_{60} + Se_{25}$ ,  $I_{80} + Se_{50}$ , and  $I_{80} + Se_{25}$ ). These two groups had higher performance than the third group ( $I_{60} + Se_0$ ) (Figure 4). The overall results showed that selenium treatments achieved higher performance than non-selenium treatments under drought stress, as well as under no-stress conditions. Therefore, using selenium alleviated the adverse effects of drought stress, as well as improved the growth and physio-biochemical parameters.



**Figure 3.** Pearson's correlation analysis among the different studied parameters. The colors represent variations in Pearson's correlation value. \* indicates the significance at  $p \le 0.05$ . LN: number of leaves per plant, MSI: membrane stability index, SDW: shoot dry weight, SL: shoot length, LA: total leaf area, LN: leaf number, PW: pod weight, NP: number of pods, PI: performance index, RWC: relative water content, PY: pod yield, FP: free proline, Fv/Fm: photosystem II quantum efficiency, TFAA: total free amino acids, TSS: total soluble sugars, AsA: ascorbate, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, APX: ascorbate peroxidase, and WP: water productivity.



**Figure 4.** The heat map shows an analysis of the hierarchical clustering among the different studied parameters and treatments of leaf spray on bean plants with selenium (Se) under drought-stress conditions. The scale bar represents the Z-score values of data of each parameter. MSI: membrane stability index, PI: performance index, RWC: relative water content, *Fv/Fm*: photosystem II quantum efficiency, TFAA: total free amino acids, TSS: total soluble sugars, AsA: ascorbate, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, APX: ascorbate peroxidase, and WP: water productivity.

Due to the high variation impact caused by foliar applications of Se on bean plants grown under drought-stress conditions on the studied parameters, a principal component analysis (PCA) biplot was performed to represent the impact of the studied applications on the studied variables. PCA-diminution 1 (Dim 1) and -diminution 2 (Dim 2) showed 67.5% and 27.7% of data variability, respectively (Figure 5). The high variability between the non-selenium (Se<sub>0</sub>) treatment and selenium (Se<sub>25</sub> and Se<sub>50</sub>) treatments under drought-stress conditions indicated the role of selenium application in improving the growth parameters and physio-biochemical traits of bean plants. Exogenous selenium enhanced the contents of *Fv/Fm*, RWC, leaf number, shoot dry weight, SPAD, shoot length, pod weight, leaf area, number of pods, pod yield, and PI (Figure 5). Moreover, the PCA biplot indicated that selenium treatments had a positive impact on the activities of SOD, CAT, and APX, as well as on the levels of proline, AsA, and GSH in bean plants under drought-stress conditions (Figure 5). Therefore, selenium treatment played a significant role in improving the growth indices and overcoming stresses in bean plants.



**Figure 5.** Biplot graph of studied parameters and treatments, showing the first two principal component analysis (PCA) dimensions (Dim1 and Dim2). LN: number of leaves per plant, MSI: membrane stability index, SDW: shoot dry weight, SL: shoot length, LA: total leaf area, LN: leaf number, PW: pod weight, NP: number of pods, PI: performance index, RWC: relative water content, PY: pod yield, FP: free proline, *Fv/Fm*: photosystem II quantum efficiency, TFAA: total free amino acids, TSS: total soluble sugars, AsA: ascorbate, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, APX: ascorbate peroxidase, and WP: water productivity.

# 4. Discussion

Recently, selenium has been identified as a plant growth regulator that may modulate various physio-biochemical mechanisms, as well as improve plant stress tolerance [26,31]. Therefore, the selenium biofortification approach was used as an exogenous protectant for plants against environmental stresses [55]. However, there is less information available regarding the application of selenium to bean plants subjected to combined abiotic stresses (drought and salt). In this study, *Phaseolus vulgaris* plants were exposed to reduced irrigation at different levels from 80% of ETc to 60% of ETc synchronized with cultivation in salt soil (EC = 6.20 dS m<sup>-1</sup> and pH = 7.68; Table 4). These stressors markedly decreased the cell membrane integrity, tissue water content, photosynthetic efficiency of PSII, and consequently the growth and yield of bean plants. However, foliar-applied selenium ameliorated stress-induced damages, given that selenium enhances the aforementioned parameters by upregulating the antioxidative components, osmolyte concentration, and antioxidant activities.

Water deficits induce stomatal closure mediated by abscisic acid, reduce water uptake, and induce oxidative stress that has effects on various metabolic processes, such as reduction in the tissue water content (RWC) and membrane stability (MSI; Table 3) [56–58], and plant growth and development are also impaired. Water deficits may cause a high transpiration rate over water absorption, which disturbs the tissue water status [22,59–61]. Nevertheless, the exogenous application of selenium ameliorated the deleterious effects of water stress on bean plants, since it induced improvements in growth and yield-related traits (Tables 2 and 3).

In this study, water stress-induced reduction in the SPAD chlorophyll, chlorophyll fluorescence (*Fv/Fm*), and performance index (PI; Table 3). Chlorophyll degradation and photoinhibition in PSII in the thylakoid membranes occur due to the damaging action of ROS under water stress [61,62]. Under drought stress, the accumulation of ROS hampered the biosynthesis of the PSII core D1 protein and downregulated the photosynthetic electron

transport chain in apples [63]. However, the leaf relative chlorophyll content and the efficiency of PSII in drought-stressed bean plants (Table 3) were substantially enhanced by exogenously applied selenium. These enhancements may be linked to increasing cellular water content and maintaining the membrane stability of *Phaseolus vulgaris* plants via selenium supplementation [64]. Exogenous selenium allowed preservation/reconstruction of the chloroplast ultrastructure and the thylakoids and stroma structure, besides increasing the chloroplast size; thus, selenium could induce restoration of the photosynthetic capacity [26,33]. Recently, Jiang et al. [29] found that selenium supplementation upregulated the antioxidative defense system in maize plants under stress, which may be responsible for

the improvements in photosynthetic efficiency and preservation of membrane stability. The accumulation of osmolytes is an effective defense mechanism of plants to combat environmental stresses, particularly drought stress [27]. These compatible solutes, jointly with selenium, help in maintaining cell turgor by osmotic adjustment [65] and act as scavengers for ROS [33,66]. In the current experiment, selenium-treated bean plants exposed to water stress exhibited higher free proline concentrations compared to the respective control (Figure 1). Our results suggest that selenium regulates the accumulation of osmolytes (proline) to maintain tissue water status and increase the RWC, MSI, and photosynthetic machinery of drought-stressed beans [27,67]. The beneficial effects of selenium in modulating the amounts of proline in plants to withstand abiotic stress have been reported [28,68]. According to [69], selenium application increased proline content via upregulating the activity of proline-synthesizing enzymes (glutamyl kinase) concomitant with reducing the proline-catabolic oxidase enzymes (proline oxidase). Nawaz et al. [27] reported that selenium stimulates many enzymes like amylase activity, which increases the hydrolysis of starch, thus increasing soluble sugars under water deficit conditions.

Our data showed that foliage-applied selenium elevated the GSH and AsA contents of water-stressed bean plants cultivated in saline soil (Figure 2). These positive results indicate an improvement in the glutathione-ascorbate cycle, which plays an important role in ROS detoxification for ameliorating oxidative stress under water deficit conditions [70]. The activity of enzymatic antioxidants, including SOD, APX, GPX, and CAT, of drought-stressed bean plants was elevated via selenium supplementation (Figure 2), resulting in enhanced antioxidative capacity in plants [13]. Several studies have shown the protective role of selenium as an antioxidant against oxidative stress in plants, which could act as a ROS scavenger along with increasing the activity of antioxidant enzymes and downregulating the oxidative stress biomarkers ( $H_2O_2, O_2^{\bullet-}$ ). The activating effect of selenium in stimulating the production of antioxidative compounds is due to its main role in upregulating related gene expressions [29].

In the current research, water stress had inhibitory effects on growth traits (shoot length, number of leaves per plant, leaf area per plant, and shoot dry weight) and productivity (number of pods per plant, green pod weight per plant, green pod yield ha<sup>-1</sup>, and WP) of bean plants (Table 4). Exposing bean plants to abiotic stress leads to the inhibition of cell division and expansion via the downregulation of cyclin-dependent protein kinase expression, leading to a reduction in the number of leaves and leaf area [13,71]. Moreover, our study showed that applying deficit irrigation at 20% increased WP by 17%, whereas selenium supplementation led to further increases in WP by up to 26%. This growth stimulation revealed the role of selenium in coping with abiotic stress. Selenium regulates physio-biochemical signals to withstand drought and salt stress, such as improving the plant's water status (RWC), increasing membrane stability and photosynthetic capacity [72], and upregulating the antioxidative machinery [68,73,74]. Moreover, externally applied selenium increased the RWC of water-stressed olives by stimulating root water uptake from the soil without reducing the transpiration rate [75].

To sum up, it must be well understood that the plant as a living organism, when faced with one or a combination of environmental stresses, begins to mobilize its weapons, which are the components of the antioxidant defense system, as possible mechanisms to defend itself against the harmful effects of stresses [76–78]. However, in most cases,

these endogenous antioxidative components are not enough (especially under severe stress) [79,80], so the producer of this stressed plant provides it with more antioxidants that are applied exogenously, including selenium, to support the plant so that it can defend itself efficiently and provide a satisfactory yield to support sustainable agriculture.

## 5. Conclusions

The results of the present study suggest that selenium (Se) foliar spraying may be a potential method for enhancing drought stress tolerance in beans grown in saline soil. These positive effects mainly arise from the improvement in leaf relative water content, membrane stability, and photosynthetic efficiency (SPAD chlorophyll, Fv/Fm, and PI), as well as from an increase in the plant defense system. The protective action of 25 or 50 mg L<sup>-1</sup> of selenium might be due to an increase in the proline concentration with the upregulation of non-enzymatic (glutathione and aFscorbic acid) and enzymatic (super peroxidase and ascorbate peroxidase) antioxidants. Furthermore, the results demonstrate the utility of the integrative application of 25 mg L<sup>-1</sup> of selenium and water stress at 20%; however, if water is a limiting factor, applying 25 mg L<sup>-1</sup> of selenium and water stress at 40% would be better for ameliorating the drought stress impact and increasing the water productivity in bean plants cultivated in saline soil.

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