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The Effect of Selenium Foliar Application on the Physiological Responses of Edamame under Different Water Treatments

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Abstract: Drought has devastating effects on crops, posing enormous risks to food security. This study investigated the impact of foliar applied selenium [at four concentrations (25, 50 and 75 mg/L)] on the photosynthesis capacity, antioxidative enzyme activities [ascorbate peroxidase (APX) and guaiacol peroxidase (GPX)] and yield parameters of a drought susceptible edamame under optimal watering and drought-stressed conditions. The study was conducted in the greenhouse under controlled conditions with leaf sampling done at vegetative, flowering and pod filling stages. Treatment of drought-stressed plants with selenium selectively induced PIabs and chlorophyll content at the vegetative stage. Ascorbate peroxidase was the only parameter induced at the flowering stage by selenium under drought stress. Selenium had no effect on all parameters under drought stress at pod filling, suggesting that the efficacy of selenium declines with time. In addition, yield parameters were not substantially affected by selenium under drought stress. Although selenium was effective for selected parameters, the application should only be limited to edamame growing under drought stress because, under well-watered conditions, it had negative impacts. Future studies should explore the responses drought stressed edamame after secondary application of selenium (i.e., at vegetative, flowering and pod filling).

Keywords: antioxidants; drought; photosynthesis; reactive oxygen species; selenium; soybean; yield

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1. Introduction

Anthropogenic climate change affects global weather extremes such as drought, which impacts negatively on agricultural systems, especially in Sub-Saharan Africa (SSA) [1]. Drought imposes enormous risk on food security (food availability, accessibility, utilisation and stability), thereby increasing hunger and poverty in vulnerable SSA populations [1,2]. To alleviate malnutrition, twenty-six African countries adopted edamame (*Glycine max* L. Merrill) as a new crop because of its high nutritional and economic benefits (it contributed to income generation in Mauritania) [3].

The effects of drought stress on plants include impairment of germination and seedling stand, reduction of the shoot and/or root fresh and dry weight, and reduction of growth and development, consequently resulting in reduced yields [4]. Drought stress also induces significant yield loss in edamame, affecting the cultivars introduced to South Africa differently, with UVE17 being the most susceptible [5]. Moloi and van der Merwe [6] revealed that reductions in edamame yield under drought stress were associated with the increased production of reactive oxygen species (ROS), which ultimately increased lipid peroxidation. Hlahla et al. [7] further associated such reductions in edamame yield with disruptions in the photosynthetic capacity under drought stress.

Although the physiological and biochemical screening mechanisms useful for edamame drought tolerance breeding programs were identified [6,7], the process is lengthy and does not provide immediate outcomes. As a result, timely drought mitigation strategies are required to secure food production amid changing climatic conditions. Recent studies suggest

selenium as a possible mitigation strategy [8]. Although not an essential element for plants, it is beneficial for the improvement of plant growth [9]. It also improved the photosynthetic capacity, the maximum quantum yield of photosystem II (PSII) and the photochemical quenching of various crops [10]. Application of this micronutrient may also alleviate the damage caused by abiotic stressors, regulate water status in plants and protect plants against oxidative stress by activating the ROS scavenging mechanisms during drought stress [8,11]. Selenium application induced the defence responses of soybeans during salinity stress through increased proline accumulation, increased activities of the antioxidative enzymes [ascorbate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase, catalase, and glutathione reductase], and increased accumulation of non-enzymatic antioxidants (ascorbate, and glutathione) [12,13]. Studies on the involvement of selenium in drought stress indicated its importance in the induction of the photosynthetic capacity of drought-stressed potatoes [13]. In soybeans, Galic et al. [14] showed that selenium mitigated the effects of drought stress. Hasanuzzaman and Fujita [15] indicated that selenium increased the antioxidative potential of drought-stressed grapeseed, which maintained low ROS accumulation and lipid peroxidation.

A drought mitigation strategy using selenium could benefit the small-scale farmers of South Africa, who are the main edamame producers. However, there are no records of such studies. Therefore, this study assessed the effect of foliar application of selenium (at the vegetative stage only) on the photosynthetic capacity, antioxidative enzyme activities (APX and GPX) and yield parameters of a drought-susceptible edamame cultivar. We hypothesised that foliar application of selenium at the vegetative stage improves the physiological responses and associated yield parameters of edamame under drought stress.

2. Materials and Methods

2.1. Plant Material and Experimental Setup

Edamame seeds, UVE17, characterised as being susceptible to drought stress according to van der Merwe et al. [5], Moloi and van der Merwe et al. [6], and Hlahla et al. [7], were germinated in seedling trays filled with Hygromix seedling mixture [Hygrotech (Pty) Ltd., Pretoria, South Africa]. After 14 days, the plants were transplanted (1 seedling per pot) in potting bags (10 L capacity) containing 10 kg of sandy, loamy soil under controlled conditions at 18 °C night and 25 °C day temperatures in a greenhouse of the University of the Free State (29°6′31.94″ S; 26°11′18.95″ E). The soil was maintained at 100% (1.6 L water) water holding capacity (WHC) to avoid drought stress. At the first trifoliate stage (V1), sodium selenite treatment was foliarly applied to individual plants at three concentrations (25 mg/L, 50 mg/L and 75 mg/L). The foliar application was used because it was successfully used in a number of studies [11–15]. Drought stress was induced at the third trifoliate leaf stage (V3) by withholding irrigation to reach 30% WHC (0.48 L of water in the soil). Moloi and van der Merwe [6] established this level as the point of severe drought stress in edamame. A Hydrosense II (Campbell Scientific, Stellenbosch, South Africa, sourced from the US) fitted with a 12 cm sensor rod, CS659, was used to measure the volumetric water content (VWC) of the soil where 20.9% VWC represented 100% WHC (well-watered) and 6.12% VWC represented 30% WHC (drought stressed). In addition, plants were weighed (Optika N 3200, Pontenarica, Italy) daily to ensure that the water level in the soil matched the treatment. The trial design was a randomised, split-plot design where the water level represented the main plot and selenium treatment, the sub-plot. The design included four biological replications with two pots per replication. For all physiological measurements, sampling was done on the top, fully expanded leaves at vegetative, flowering and pod filling stages.

2.2. Measurement of the Physiological Responses

Relative chlorophyll content was measured using a non-destructive chlorophyll content meter (CL-01 Hansatech Instruments, King's Lynn, UK). This device provides an indication of green colour using dual-wavelength optical absorbance (620 and 940 nm

wavelength), and the results were expressed in arbitrary units (a.u). Four measurements were taken on the same leaf to get a representative value.

A pocket PEA chlorophyll fluorimeter (Hansatech Instruments, King's Lynn, UK) was used to collect chlorophyll fluorescence data on a leaf that was dark adapted for 30 min between 10:00 and 12:00 on a clear, sunny day. The parameters included the PIabs (performance index on absorbance basis, which shows the efficiency of PSI) and Fv/Fm (Fv = variable fluorescence and Fm = maximum fluorescence), which indicated the photochemical efficiency of PSII.

A leaf porometer (Li-Cor. ADC Bio-Scientific Ltd., Hoddesdon, UK) was used to measure stomatal conductance. Measurements were taken between 10:00 and 12:00 on a clear, sunny summer day.

Antioxidative enzyme activities were done on the same leaves that were used for the non-destructive measurements. At harvest, the leaves were crushed in liquid nitrogen and stored at −20 °C. Extractions for the determination of ascorbate and guaiacol peroxidases were done according to Pukacka and Ratajczak [16]. The frozen leaf material (0.5 g) was ground to a fine paste on ice in 5 mL 50 mM potassium phosphate buffer, pH 7.0, containing 0.1% (*v*/*v*) Triton X-100, 2% (*w*/*v*) polyvinylpyrrolidone (PVP), 1 mM ascorbate, and 1 mM EDTA). The homogenate was centrifuged (15,000× *g*) at 4 °C for 20 min, and the supernatant obtained served as the enzyme extract.

Ascorbate peroxidase activity was determined using a modified method by Mishra et al. [17]. The reaction (1 mL) contained 550 µL 50 mM phosphate buffer (pH 7.0), 200 µL 100 mM H_2O_2, 150 µL 0.5 mM ascorbate, 50 µL 0.1 mM EDTA, and 50 µL enzyme extract. The absorbance was measured at 290 nm (Cary 100 Bio, Varian, Australia) for 5 min at 20 °C. The ascorbate activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Guaiacol peroxidase activity was determined using the method by Zieslin and Ben-Zaken [18]. The assay solution (1 mL) contained 500 µL 80 mM phosphate buffer (pH 5.5), 50 µL 200 mM H_2O_2, 100 µL 50 mM guaiacol, 340 µL distilled H_2O, and 10 µL enzyme extract. Absorbance was measured at 470 nm (Cary 100 Bio, Varian, Australia) for 3 min at 30 °C. The guaiacol peroxidase activity was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1}.

The protein content from enzyme extracts was determined according to the method by Bradford [19] using gamma-globulin as a standard (0.5 mg/mL). The absorbance was read at 595 nm on a microplate detector (Anthos Labtech Inc. GmbH, Salzburg, Austria) using a microplate (Greiner Bio-One, Kremsmunster, Austria).

2.3. Yield Parameters

At reproductive stage eight (R8), the following parameters were recorded to establish the effect of selenium on drought-stressed plants: number of branches per plant, number of pods per plant, number of seeds per plant and seed mass per plant. Although edamame is a crop that is consumed at the R6-R7 stage, sampling was done at the R8 stage, which is important for the seed bank (the focus of the study was not on the nutritional quality).

2.4. Statistical Analysis

Statistical analysis was done using TIBCO Statistica [20] and R Core Team statistics [21]. Data analysis included: multivariate analysis of covariance (MANCOVA) to establish significant differences at different growth stages; analysis of variance (ANOVA) to establish the effect of water treatment, selenium treatment, and their interactions at different growth stages separately; the Tukey test for homogenous groups at alpha = 0.05 to establish the differences between treatments.

3. Results

Table 1 represents the effect of water and selenium treatments on the different stages of edamame development. Changing water levels had an impact on all stages of edamame development (vegetative, flowering and pod filling) ($p \leq 0.001$). This shows that water affects most of the studied physiological traits irrespective of the developmental stage in

edamame. In contrast, different selenium concentrations had no effect on all growth stages ($p > 0.05$). Selenium treatment was more effective when exposed to water treatment at the vegetative stage ($p \leq 0.001$), showing that it can only affect the physiological traits during the early developmental stages.

Table 1. The effect of water and selenium treatments on the different growth stages of edamame (vegetative, flowering and pod filling).

Variate	Vegetative	Flowering	Pod Filling
Water	0.0001 ***	0.0001 ***	0.0001 ***
Selenium	0.2624	0.1017	0.2948
Selenium × Water	0.0002 ***	0.2450	0.0723

*** $p \leq 0.001$. Numbers represent p values.

The effect of water and selenium treatments on the physiological traits of edamame at vegetative, flowering and pod-filling stages are given in Table 2. At the vegetative stage, the water treatment only affected the photosynthesis traits; Fv/Fm ($p \leq 0.05$), PIabs ($p \leq 0.05$), chlorophyll content ($p \leq 0.01$), and stomatal conductance ($p \leq 0.001$). As growth progressed, the number of photosynthesis traits affected by water treatments was reduced; with only stomatal conductance affected at flowering ($p \leq 0.001$), while PIabs and stomatal conductance ($p \leq 0.05$) were influenced at pod filling. None of the antioxidative enzymes was influenced by water treatment ($p \geq 0.05$) at any developmental stage. Although selenium application alone was not effective, it had a substantial effect under the influence of water treatment. It had an impact on PIabs ($p \leq 0.001$), chlorophyll ($p \leq 0.01$) and guaiacol peroxidase ($p \leq 0.05$) at the vegetative stage. With increased plant age, APX was the only parameter influenced by this interaction ($p \leq 0.05$) at flowering. There were no significant interactions at pod filling, which shows that selenium was not effective at this stage.

Table 2. The effect of water and selenium treatments on the physiological traits of edamame at vegetative, flowering and pod-filling stages.

	Variate	Fv/Fm	PIabs	Chl	g_s	APX	GPX
Vegetative	Water	0.00125 *	11.4 *	5.58 **	364.045 ***	0.000013	0.252
	Selenium	0.00006	0.421	1.93	19.412	0.000033	0.161
	Selenium × Water	0.00054	4.01 ***	4.87 **	10.251	0.000079	0.382 *
Flowering	Water	0.00195	57	0.0338	1.131.985 ***	0.00045	0.112
	Selenium	0.00006	1.35	3.32	10.297	0.00044	1
	Selenium × Water	0.00077	0.939	2.25	14.565	0.00113 *	0.456
Pod filling	Water	0.00001	42.5 *	8.1	298.938 *	0.00211	0.302
	Selenium	0.00004	2.54	0.591	12.068	0.00157	0.772
	Selenium × Water	0.00012	7.36	2.41	13.997	0.00045	0.602

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Fv/Fm = ratio of variable fluorescence to maximum fluorescence, PIabs = performance index on absorbance basis, Chl = relative chlorophyll, g_s = stomatal conductance, APX = ascorbate peroxidase, and GPX = guaiacol peroxidase.

Table 3 shows that water treatment influenced the number of branches per plant ($p \leq 0.01$), number of pods per plant ($p \leq 0.001$), number of seeds per plant ($p \leq 0.001$), and total seed mass per plant ($p \leq 0.001$). Selenium, with or without consideration of the water treatment, did not affect the yield parameters.

Table 3. The effect of water and selenium treatments on the yield parameters of edamame at reproductive stage eight.

Variate	Branch	Pods	Seed	Seed Mass
Water	15.1 **	282 ***	790 ***	521 ***
Selenium	0.917	9.78	16.1	11.6
Selenium × Water	2.71	9.87	16.1	10.3

** $p \leq 0.01$, *** $p \leq 0.001$. Branch = number of branches per plant, pods = number of pods per plant, seed = number of seeds per plant, and seed mass = total seed mass per plant.

Drought treatment led to a significant reduction ($p \leq 0.05$) in the Fv/fm (0.788 under 30% WHC compared to 0.818 under 100%WHC), PIabs (1.43 under 30% WHC compared to 4.28 under 100% WHC), chlorophyll (4.39 under 30% WHC compared to 6.98 under 100% WHC), and stomatal conductance (321 mmol/m^2 s^2 at 30% WHC compared to 633 mmol/m^2 s^2 at 100% WHC) at the vegetative stage, showing the downregulation of photosynthesis. As growth progressed to flowering and pod filling, there was less reduction in the photosynthesis capacity, where stomatal conductance was the only parameter that was significantly ($p \leq 0.05$) reduced. At flowering, drought stress reduced the stomatal conductance from 690 to 227 mmol/m^2 s^2, while it reduced from 505 to 312 mmol/m^2 s^2 at pod filling. Water treatment had no significant effect ($p \geq 0.05$ on the activities of the antioxidative enzymes. Drought stress negatively affected yield because it further reduced the number of branches per plant, number of pods per plant, number of seeds per plant and seed mass per plant ($p \leq 0.05$) (Table 4).

Table 4. The effect of drought stress on the photosynthesis of edamame at vegetative, flowering and pod filling and on the yield traits.

	Vegetative		Flowering		Pod Filling		Yield Traits		
WHC	100%	30%	100%	30%	100%	30%	100%	30%	
Fv/Fm	0.808 ^{a}	0.796 ^{b}	0.792 ^{a}	0.774 ^{a}	0.826 ^{a}	0.823 ^{a}	6.44 ^{a}	4.93 ^{b}	Branch
PIabs	3.25^a	2.01 ^{b}	2.7 ^{a}	2.5 ^{a}	7.38 ^{a}	9.74 ^{b}	13.6 ^{a}	7.6 ^{b}	Pods
Chl	5.9^a	5.06 ^{b}	7.12 ^{a}	7.19 ^{a}	12.4 ^{a}	13.2 ^{a}	21.7 ^{a}	11.7 ^{b}	Seed
g_s	605^a	379 ^{b}	664 ^{a}	379 ^{b}	505 ^{a}	312 ^{b}	12.5 ^{a}	4.41 ^{b}	Seed mass

For each growth stage, different row letters represent significant ($p \leq 0.05$) differences between the water treatments. Values represent means (n = 16). Fv/Fm = ratio of variable fluorescence to maximum fluorescence, PIabs = performance index on absorbance basis, Chl = relative chlorophyll, g_s = stomatal conductance, branch = number of branches per plant, pods = number of pods per plant, seed = number of seeds per plant, seed mass = total seed mass per plant, WHC = water holding capacity of the soil, 100% WHC = well-watered plants, 30% = drought-stressed plants.

The physiological responses of edamame treated with four selenium concentrations under two water levels and three growth stages are given in Table 5. These results are important to show how the physiological parameters were affected by selenium under drought stress. Data presented in Table 2 highlighted that selenium was only effective under interaction with water treatments. All significant effects are represented by different row letters ($p \leq 0.05$). For the vegetative stage, the application of selenium (50 and 75 mg/L) on the drought-stressed plants substantially increased PIabs (2.37 and 2.52, respectively) compared to the control (1.43). The PIabs of well-watered plants were significantly reduced under selenium treatment (2.59, 50 mg/L selenium and 2.35, 75 mg/L). The application of selenium at 75 mg/L on drought-stressed plants significantly increased chlorophyll (5.76) compared to the drought-stressed control (4.39). In contrast, selenium treatment on well-watered plants led to a substantial reduction in chlorophyll (6.98, 0 mg/L to 4.82, 75 mg/L). At the flowering stage, the only physiological trait that significantly affected the interaction of selenium with water treatment was APX under drought stress. Compared to no-selenium treated plants at 30% WHC (0.015), APX activity increased substantially when the plants were treated with 50 mg/L (0.0586) and 75 mg/L (0.045) selenium. There was no substantial effect on the APX activity when selenium was applied to the well-watered plants.

Table 5. The effect of selenium on the physiological responses of edamame under two water levels at vegetative, flowering and pod filling.

			Selenium Concentration (mg/L)			
	Variate	WHC	0	25	50	75
Vegetative	Fv/Fm	100%	0.818 ^{a}	0.818 ^{a}	0.803 ^{a}	0.798 ^{a}
		30%	0.788 ^{a}	0.795 ^{a}	0.8 ^{a}	0.803 ^{a}
	PIabs	100%	4.28 ^{c}	3.79 ^{bc}	2.59 ^{ab}	2.35 ^{ab}
		30%	1.43 ^{a}	1.91 ^{a}	2.37 ^{ab}	2.52 ^{ab}
	Chl	100%	6.98 c	6.82 ^{bc}	4.96 ^{ab}	4.82 ^{a}
		30%	4.39 ^{a}	5.25 ^{abc}	4.84 ^{ab}	5.76 ^{abc}
	g_s	100%	633 ^{c}	524 ^{bcd}	592 ^{cd}	671 ^{c}
		30%	321 ^{a}	382 ^{ab}	400 ^{ab}	464 ^{abd}
	APX	100%	0.028 ^{a}	0.028 ^{a}	0.020 ^{a}	0.023 ^{a}
		30%	0.023 ^{a}	0.028 ^{a}	0.030 ^{a}	0.023 ^{a}
	GPX	100%	1.8 b	1.66 ^{ab}	1.47 ^{ab}	1.15 ^{ab}
		30%	1.36 ^{ab}	1.01 ^{a}	1.57 ^{ab}	1.42 ^{ab}
Flowering	Fv/Fm	100%	0.798 ^{a}	0.798 ^{a}	0.788 ^{a}	0.786 ^{a}
		30%	0.778 ^{a}	0.765 ^{a}	0.781 ^{a}	0.784 ^{a}
	PIabs	100%	3.35 ^{a}	3.08 ^{a}	2.44 ^{a}	1.93 ^{a}
		30%	2.77 ^{a}	2.82 ^{a}	2.01 ^{a}	2.85 ^{a}
	Chl	100%	8.29 ^{a}	7.58 ^{a}	6.11 ^{a}	6.52 ^{a}
		30%	7.74 ^{a}	6.49 ^{a}	6.81 ^{a}	7.71 ^{a}
	g_s	100%	690 ^{cd}	641 ^{cd}	585 ^{bcd}	739 ^{d}
		30%	227 ^{a}	286 ^{ab}	318 ^{ab}	400 ^{abc}
	APX	100%	0.030 ^{a}	0.035 ^{a}	0.020 ^{a}	0.030 ^{a}
		30%	0.015 ^{a}	0.028 ^{ab}	0.058 ^{b}	0.045 ^{ab}
	GPX	100%	1.4 ^{a}	1.75 ^{a}	1.29 ^{a}	1.72 ^{a}
		30%	1.25 ^{a}	1.42 ^{a}	1.46 ^{a}	2.48 ^{a}
Pod filling	Fv/Fm	100%	0.828 ^{a}	0.823 ^{a}	0.82 ^{a}	0.825 ^{a}
		30%	0.818 ^{a}	0.825 ^{a}	0.828 ^{a}	0.83 ^{a}
	PIabs	100%	8.79 ^{a}	8.22 ^{a}	5.85 ^{a}	6.68 ^{a}
		30%	9.38 ^{a}	8.93 ^{a}	9.66 ^{a}	10.8 ^{a}
	Chl	100%	12.7 ^{a}	13 ^{a}	11.4 ^{a}	12.4 ^{a}
		30%	13.2 ^{a}	12.8 ^{a}	13.7 ^{a}	14 ^{a}
	g_s	100%	544 ^{b}	516 ^{ab}	516 ^{ab}	444 ^{abd}
		30%	230 ^{c}	333 ^{acd}	393 ^{abcd}	291 ^{cd}
	APX	100%	0.080 ^{a}	0.055 ^{a}	0.068 ^{a}	0.060 ^{a}
		30%	0.093 ^{a}	0.053 ^{a}	0.090 ^{a}	0.093 ^{a}
	GPX	100%	2.43 ^{b}	2.21 ^{ab}	2.82 ^{ab}	2.4 ^{ab}
		30%	2.04 ^{ab}	2.51 ^{a}	2.79 ^{ab}	3.3 ^{ab}

Mean values represent selenium × water interactions (n = 4) for physiological parameters at different growth stages. Different row letters show that the means are significant ($p \leq 0.05$), Fv/Fm = ratio of variable fluorescence to maximum fluorescence (arbitrary units (a.u.), PIabs = performance index on absorbance basis (a.u.), Chl = relative chlorophyll (a.u.), g_s = stomatal conductance (mmol m^{-2} s^{-1}), APX = ascorbate peroxidase (mmol ascorbate mg prot./min), GPX = guaiacol peroxidase (mmol tetraguaiacol mg prot./min), WHC = water holding capacity of the soil, 100% WHC = well-watered plants, 30% = drought stressed plants.

4. Discussion

Drought stress negatively impacts photosynthesis and plants' electron transport chain [22]. Hlahla et al. [7] associated reductions in edamame yield with disruptions in the photosynthetic capacity under drought stress. The current study was in agreement with these findings, where drought substantially reduced the photosynthesis performance of drought-susceptible edamame at vegetative (reduced Fv/Fm, PIabs, chlorophyll and stomatal conductance), flowering (stomatal conductance) and pod filling (PIabs and stomatal conductance) along reductions in the yield traits. Such reductions in the photosynthesis efficiency under drought strongly suggest the need for drought mitigation strategies in order to avoid interruptions at the metabolic level with ultimate effects on the yield. For this reason, the current study also reports on the effect of selenium application as

a foliar treatment on the physiological and yield responses of edamame under different water treatments.

This is important because a few studies indicated the beneficial effects of selenium in combating the negative effects of drought stress in plants [8] through improved photochemistry of PSII, which could result in improved photosynthesis, growth and yield [10,23]. We needed to establish whether the foliar application of selenium in this study could upregulate photosynthesis under different water treatments. Application of selenium (50 and 75 mg/L) on drought-stressed edamame at the vegetative stage substantially increased PIabs, strongly suggesting that selenium improved the overall functioning of the electron flow through PSII in agreement with Custers et al. [24]. These results corroborated the analysis reported in Tables 1 and 2, which showed that selenium selectively affected photosynthesis (PIabs and chlorophyll) under the influence of water treatments at the vegetative stage. This could result from the one-time application of selenium at the vegetative stage, suggesting that when the plants reached flowering and pod filling, the efficacy of selenium was reduced. The results further suggest that selenium could only upregulate photosynthesis at the vegetative stage when plants are experiencing drought stress because the treatment of well-watered plants with selenium downregulated PIabs. The non-significant increase in Fv/Fm for selenium-treated plants under drought stress was in agreement with Špela and Mateja [25], who found that selenium had no effect on the quantum yield of PSII in soybeans. This shows that selenium selectively induces the photosynthesis parameters in drought-susceptible edamame.

Drought stress may also reduce leaf chlorophyll content, which is a critical factor in reducing plant growth and yield [26]. In the current study, selenium treatment (75 mg/L) at the vegetative stage alleviated the negative effects of drought stress by increasing chlorophyll accumulation, further suggesting the upregulation of photosynthesis. Similarly, evidence for chlorophyll increase after treatment with selenium was observed in sorghum [27] and wheat [11]. The observed chlorophyll increase after selenium application in the drought-susceptible edamame indicates that selenium may minimise chloroplast damage, which helps in the maintenance of photosynthetic pigment levels under abiotic stress conditions. Furthermore, selenium may also protect chloroplast enzymes and increase cell metabolic rate, leading to enhanced chlorophyll synthesis and reduced chlorophyll degradation [28]. Moreover, our findings suggest that increased PIabs and chlorophyll under selenium treatment could support higher NADPH and ATP production [29], which drive the carbon dioxide fixation process, resulting in the upregulation of photosynthesis in the drought-stressed edamame. As with PIabs, the results showed a reduction in chlorophyll under well-watered conditions, further showing that selenium upregulates photosynthesis only under drought stress.

Although drought reduced the stomatal conductance of edamame, the application of selenium did not increase it significantly. This implies that there could be more oxidative stress resulting from stomatal closure, causing overproduction of ROS in drought-stressed edamame, in agreement with Guo et al. [30]. In addition, continuous absorption of light by chlorophylls generates ROS molecules [31], further suggesting oxidative stress and lipid peroxidation in drought-stressed edamame in agreement with Moloi and van der Merwe [6]. The authors also showed that drought tolerance was linked to high activities of the antioxidative enzymes. Antioxidative enzymes inhibit lipid peroxidation and maintain the integrity of cellular and subcellular membranes of cell organelles like chloroplast, which are vital for the synthesis and localisation of leaf pigments [28]. In this study, it was also important to establish if selenium could increase the activities of the antioxidative enzymes under drought stress. The results showed that APX was the only antioxidative enzyme that was substantially upregulated by selenium at the flowering stage under drought stress, suggesting that selenium application on drought-stressed edamame could contribute to the fight against oxidative stress in edamame [6]. In agreement, Jóźwiak and Politycka [32] indicated that selenium application on drought-stressed cucumber increased oxidative stress tolerance through increased activities of the antioxidant enzymes and

limited damage of plasma membranes. Similarly, in rice, selenium enhanced drought tolerance by increasing the activities of the antioxidative enzymes APX [28]. Like the photosynthesis parameters, selenium application on well-watered plants appeared to have inhibitory effects on the activity of APX.

Although drought stress reduced all of the studied yield traits, treatment with selenium could not translate into increased yield. This further suggests that the efficacy of selenium under drought stress in edamame decreases with growth stages.

5. Conclusions

Drought stress reduced the photosynthetic capacity, antioxidative enzymes and yield of susceptible edamame. Foliar application of selenium upregulated photosynthesis (PIabs and chlorophyll) selectively at the vegetative stage. At the flowering stage, selenium increased the antioxidative capacity of drought-stressed edamame through increased APX activity. The application of selenium on edamame under well-watered conditions downregulated the physiological responses of edamame, showing that it is efficient only under stress conditions. The application of selenium at the vegetative stage did not affect the yield parameters under drought stress. Efficacy of selenium decreased with the age of the plant (more responses induced at the vegetative stage, followed by flowering with no increases at pod filling and R8), which could explain the non-responsiveness of the yield traits. Therefore, future studies should explore the drought-stressed edamame responses after secondary selenium application.

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