



Selenium protects rice plants from water deficit stress

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ABSTRACT

Selenium (Se) is essential to humans and animals due to its antioxidant properties. Although it is not considered an essential nutrient for higher plants. Many studies show that Se in low concentrations (up to 0.5 mg kg⁻¹) provides beneficial effects to non-hyperaccumulating plants by participating in antioxidant defense systems and enhancing tolerance to abiotic stress. Therefore, this study aimed to evaluate the effects of Se application rates on rice plants under different soil water conditions. The experiment was conducted on an Oxisol using four Se rates (0, 0.5, 1.0 and 2.0 mg kg⁻¹) and two soil water conditions (irrigated and water deficit). Selenium application via soil up to 0.5 mg kg⁻¹ increased the plant height, chlorophyll index, sulfur and copper accumulation in shoots, carbon dioxide assimilation, superoxide dismutase (EC 1.15.1.1) activity and decreased the hydrogen peroxide concentration in rice leaves. The accumulation of Se in shoot biomass and Se concentration in seeds increased linearly with the applied rates. Water deficit strongly decreased the plant growth and yield. However, rice plants treated with Se showed higher net photosynthesis, water use efficiency and antioxidant system. This study provides useful information about the roles of Se in protecting rice plants from water deficit stress.

1. Introduction

Cultivated crops are frequently exposed to adverse conditions that affect their growth and productivity. Among the various types of environmental stress, drought is considered the most devastating because of crop yield losses, which can reach billions of dollars annually worldwide (Lambers et al., 2008; Tardieu et al., 2014; Aon, 2015). It is estimated that by 2050, when the world population is expected to reach 9.7 billion people, approximately 49% of the global grain production will be cultivated in regions affected by water deficit (Rosegrant, 2016).

Rice (*Oryza sativa* L.) is the second most cultivated cereal in the world and the main food source for more than half of the world population (Reis et al., 2018). Approximately 75% of rice production worldwide comes from planting in an irrigated and/or flooded system

(FAO, 2017). However, these cultivated area expansions are limited due to strong impacts on environmental problems (Sander et al., 2014). Therefore, upland rice cultivation tends to expand into arid regions, where dry spells are more consistent. These regions are characterized by long periods of soil water deficit due to interruptions in rainfall during the rainy season (Joy et al., 2015).

Water deficit reduces soil water potential. This reduction has direct implications for transpiration, photosynthesis, leaf temperature, stomatal opening and antioxidant metabolism, all of which affect the growth, development and especially the yield of economically important crops (Nawaz et al., 2015; Reis et al., 2015). Application of selenium (Se) can increase growth and minimize the effects of abiotic stresses (e.g., drought, salinity, high temperatures and potentially toxic element) in plants (Djanaguiraman et al., 2010; Kumar et al., 2012;

Abbreviations: A, CO₂ assimilation rate; APX, ascorbate peroxidase; Ci, internal CO₂ concentration; CAT, catalase; E, transpiration; EiC, instantaneous carboxylation efficiency; FW, fresh weight; gs, stomatal conductance; H₂O₂, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; WUE, water use efficiency

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Nawaz et al., 2015; Jiang et al., 2017; Reis et al., 2017, 2018). There are conclusive evidences of Se deficiency in Brazilian soils (Reis et al., 2018; Silva et al., 2018). Selenium variation in Brazilian soils range from 10 to 150 $\mu\text{g kg}^{-1}$ (Reis et al., 2017). Therefore, Se supplementation is needed in order to reduce the harmful effects on plant metabolism and physiology caused by drought stress can be decreased by Se supplementation. Selenium increases the activity of antioxidant enzymes and reduces both the generation of reactive oxygen species (ROS) and the lipid peroxidation rates in the leaf cell tissue (Habibi, 2013; Nawaz et al., 2015; Mostofa et al., 2017; Reis et al., 2018).

An increase in CO_2 assimilation rate and stomatal conductance is promoted by application of Se in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) plants exposed to drought stress (Habibi, 2013; Nawaz et al., 2015). Similarly, in olive (*Olea europaea* L.) under water stress, foliar applications of Se can increase photosynthesis, regulate the water status of trees and maintain a sufficiently high level of leaf water content in plants (Proietti et al., 2013). Yao et al. (2009) observed significant increases in root activity, catalase activity and chlorophyll concentration as well as decreased malondialdehyde concentration in wheat seedlings subjected to Se application under drought stress.

Therefore, studies that demonstrate the positive effects of Se application on rice plants under water deficit conditions are highly relevant. This study aimed to validate the effects of Se doses applied to the soil under two soil water conditions on the growth, yield, antioxidant system and nutritional quality status of rice plants.

2. Materials and methods

2.1. Experimental design and rice cultivation

The experiment was conducted in a greenhouse from July to December 2014 at the Soil Science Department of Federal University of Lavras, Brazil. Pots with 4 kg of Oxisol (clay textured) collected from the 0–0.20-m layer were used. The soil was air-dried, sieved using a 4-mm mesh and characterized for its major chemical and physical properties according to the methodology suggested by EMBRAPA (1997). The chemical and physical properties as follows: pH- H_2O = 5.1; organic matter = 46 g kg^{-1} ; P (Mehlich-1) = 0.84 mg kg^{-1} ; K = 1.7 mmolc dm^{-3} ; Ca = 1.5 cmolc dm^{-3} ; Mg = 0.4 cmolc dm^{-3} ; Al = 0.5 cmolc dm^{-3} ; HAl = 6.3 cmolc dm^{-3} ; P-rem = 11.37 mg L^{-1} ; clay = 710 g kg^{-1} ; silt = 140 g kg^{-1} ; and sand = 150 g kg^{-1} . The content of Se was $0.2 \pm 0.04 \text{ mg kg}^{-1}$, which was determined after soil digestion according the USEPA 3051A method (USEPA, 1998).

Based on soil chemical analysis, liming was carried out to raise the base saturation to 60% using CaCO_3 and MgCO_3 at a ratio of 4:1. After 30 days of soil incubation with a humidity close to 60% of the total pore volume (TPV), doses of Se (sodium selenate, Sigma-Aldrich, Saint Louis, USA) were applied to the soil. Each pot received a macronutrient fertilizer containing: 80 mg of N, 250 mg of P, 90 mg of K, and 50 mg of S kg^{-1} soil. Micronutrient applications consisted of 0.5 mg of B, 1.5 mg of Cu, 0.1 mg of Mo and 5.0 mg of Zn kg^{-1} soil.

Afterward, 15 rice seeds (*O. sativa* L. cv. IAC 202) were sown per pot, and nine days after seedling emergence, the rice seedlings were thinned three plants per pot. In addition, during the rice cultivation period, blanket fertilizations of 473 mg of N and 436 mg of K kg^{-1} soil were carried out and divided into five applications.

The experiments were set up as a completely randomized design in a 4×2 factorial scheme with four Se doses (0, 0.5, 1.0 and 2.0 mg kg^{-1} Se) and two soil water conditions (irrigated and water deficit), with four replicates, during the beginning of the reproductive stage. The control treatment consisted of maintaining the soil near field capacity, and water deficit treatment involved maintaining the soil at approximately -50 kPa , as determined by for rice cultivation. Each experimental unit consisted of two pots: one used for the analyses performed (gas exchange, antioxidant system, SPAD index growth and nutrition) at the end of water deficit, and the other was used only at seed harvest.

To monitor soil water tension, tensiometers were installed at a 0.15-m depth in each experimental plot, and the replenishment of evapotranspired water was performed based on the tensiometric reading, which was performed twice daily at 9:00 and 16:00 h. Appropriate water replenishment was used to determine water retention curves for the soil. From emergence to the flag leaf/collar-formation stage, field capacity was maintained by soil moisture in all pots.

In the flag leaf/collar-formation stage, water deficit treatments were applied to the respective pots, and soil water potential was maintained at -50 kPa for 14 days to simulate dry spells; samples not subjected to water deficit were maintained under normal irrigation conditions. At the end of this period, irrigation to plants under water stress was restored to field capacity until harvest.

2.2. Gas exchange measurements

On the 14th day of water stress at the end of water deficit period, which coincided with the initial panicle exertion stage of rice, gas exchange evaluations were performed using a portable infrared gas analyzer (Infra Red Gas Analyzer - IRGA, brand LI-COR Biosciences, model LICOR 6400). The CO_2 assimilation rate expressed by area (A - $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s - $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration (E - $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and internal CO_2 concentration in the substomatal chamber (C_i - $\mu\text{mol CO}_2 \text{ mol air}^{-1}$) were obtained. With those data, an estimation of both instantaneous carboxylation efficiency [E_iC , (A/C_i - mol air^{-1})] and water use efficiency [WUE , (A/E - $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)] were determined. Readings were performed on a clear day between 9:00 and 11:00 a.m. using the flag leaf as a pattern, including the last fully developed leaf. The photosynthetically active radiation (PAR) was standardized to an artificial saturating light of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and an ambient CO_2 concentration. The average relative humidity was 30%, and the ambient temperature was between 35 and 42 °C.

2.3. Biometric and chlorophyll meter measurements

Using three leaves per pot on the same day, the SPAD index was determined using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan). After SPAD readings, leaves were collected and immediately conditioned in liquid nitrogen, after which they were stored at $-80 \text{ }^\circ\text{C}$ for biochemical analyses. After all these determinations at end of the water deficit period, the height of plants at that time was obtained by measuring from the plant base to the end of last fully expanded leaf. The shoots and roots of plants were collected and dried in a forced-air oven for 72 h in order to obtain and determine the dry biomass and root/shoot relation. The other post treatments aimed at seed production were conducted until the cycle ended, after which the grains were harvested, dried in a forced-air oven, and weighed to obtain the dry mass of seeds in each pot.

2.4. Extraction and quantification of antioxidant enzymes

To quantify antioxidant enzyme activity in leaves, an extract was obtained by maceration of 0.1 g of leaves in liquid nitrogen. The extract was then added to an extraction buffer solution containing 0.1 M potassium phosphate (pH 7.8), 0.1 mM EDTA (pH 7.0), 0.01 M ascorbic acid and 22 mg of polyvinylpyrrolidone (PVPP) (Biemelt et al., 1998). The supernatant was then collected and used for enzymatic analyses of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX).

2.4.1. Superoxide dismutase (SOD; EC 1.15.1.1)

SOD activity was evaluated by the ability of the enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT), as proposed by Giannopolitis and Ries (1977). An aliquot of the supernatant was added to an incubation medium composed of 50 mM potassium phosphate (pH

7.8), 14 mM methionine, 0.1 μM EDTA, 75 μM NBT and 2 μM riboflavin. Tubes with reaction media and samples were illuminated for 7 min with a 20-W fluorescent lamp. For the control, the same reaction medium was illuminated without the sample present. Readings were performed at 560 nm. One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under assay conditions.

2.4.2. Catalase (CAT; EC 1.11.1.6)

CAT was evaluated according to method described by Havir and McHale (1987), in which an aliquot of enzyme extract was added to an incubation medium containing 100 mM potassium phosphate (pH 7.0) that was previously incubated at 30 °C. Immediately prior to absorbance readings, 12.5 mM hydrogen peroxide was added to trigger this reaction. CAT activity was determined by measuring the consumption of hydrogen peroxide (H_2O_2) at 240 nm for 3 min. A molar extinction coefficient of 36 $\text{mM}^{-1} \text{cm}^{-1}$ was used for calculations.

2.4.3. Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity was determined by monitoring the oxidation rate of ascorbate at 290 nm for 3 min. For this measurement, an aliquot of enzyme extract was added to an incubation buffer composed of 100 mM potassium phosphate (pH 7.0) and 0.5 mM ascorbic acid, which was previously incubated at 30 °C. Prior to measuring the oxidation rate, 0.1 mM H_2O_2 (Nakano and Asada, 1981) was added to the sample. A molar extinction coefficient of 2.8 $\text{mM}^{-1} \text{cm}^{-1}$ was used for calculations.

2.5. Hydrogen peroxide

Hydrogen peroxide was quantified from an extract made by macerating 0.2 g of leaves in liquid nitrogen. The extract was then homogenized in 1.5 mL of trichloroacetic acid (TCA) and centrifuged at 12,000g for 15 min at 4 °C. Hydrogen peroxide levels were determined by measuring absorbance at 390 nm. Aliquots of the supernatant were added to a reaction medium containing 2.5 mM potassium phosphate buffer (pH 7.0) and 500 mM potassium iodate (Velikova et al., 2000). Quantification of H_2O_2 was performed based on a standard curve with known concentrations of H_2O_2 .

2.6. Lipid peroxidation

Lipid peroxidation was determined by the quantification of thiobarbituric acid reactive species (TBA), as described by Buege and Aust (1978). A total of 0.2 g of leaves was macerated in liquid nitrogen and 20% PVPP (w/v), which was homogenized in 0.1% (w/v) TCA. The homogenate was then centrifuged at 10,000g for 10 min. Aliquots of the supernatant were added to a reaction medium containing 0.5% (w/v) TBA and 10% (w/v) TCA, after which the samples were incubated at 95 °C for 30 min. The reaction was stopped by cooling the samples on ice. Absorbance was measured using a spectrophotometer at 535 nm and 600 nm, and the results were expressed in nanomoles of malondialdehyde (MDA) per milligram of fresh weight (FW).

2.7. Chemical analysis of plant tissue

Shoot dry biomass and grains were ground in a Willey mill equipped with a 40-mesh screen. Subsequently, in the aerial parts of plants, chemical analyses were performed to determine macro and micro-nutrient concentration in accordance with the methodology described by Malavolta et al. (1997). A turbidimetric method was used to quantify total sulfur in plant tissue was previously described by Malavolta et al. (1997). Se concentration in these tissues, 0.5 g of tissue was used for perchloric-nitric digestion. Extracts sat for 15 min, after which 10 mL of deionized water was added. For analytical quality control, analyses were certified using the BCR 402 (white clover) standard (Sigma-

Aldrich). Blank and certified reference samples were analyzed along with the digestion of every batch, which presented a minimum of 95% ($n = 7$) recovery. The extracts were measured Ca, Mg, K, Cu, Fe, Mn, Se and Zn using atomic absorption spectroscopy with electrothermal atomization in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada) as previously described by Silva Junior et al. (2017).

2.8. Statistical analysis

When all data sets were considered, normality was analyzed using the Anderson-Darling test, and homoscedasticity was verified using the variance equation test (or the Levene test). The data obtained for each variable were subjected to the analysis of variance ($p \leq 0.05$), and the factors were compared using the Scott-Knott test in R 3.2.3 statistical program (R Development Core Team, 2011).

3. Results

3.1. Gas exchange and antioxidant metabolism

Interactions ($p \leq 0.05$) between soil water conditions and Se doses applied to *A*, *gs*, *E*, *EiC* and *WUE* (Fig. 1A-F) were observed. Internal CO_2 concentration (Table 1 and Fig. 1D), lipid peroxidation (Table 1), SOD activity (Fig. 2A) and H_2O_2 concentration (Table 1 and Fig. 2B) were affected ($p \leq 0.05$) when analyzed individually by water condition and Se doses. Ascorbate peroxidase and CAT activity were not affected by soil water condition or by Se doses.

Water deficit promoted the reduction of *A*, *gs*, *E* and *EiC* (Fig. 1A-C and E) by all Se doses applied, except when *gs* were checked without Se application. For *WUE* estimation (Fig. 1F), the highest dose of Se (2.0 mg kg^{-1}) enabled an increase of this variable when plants were subjected to water deficit, and these results differed from those of irrigated plants. The *Ci* increased by 16.52% in plants under water deficit (Table 1).

Application of 1.0 mg kg^{-1} Se to irrigated plants usually promoted the highest values of *A*, *gs*, *E* and *EiC* (Fig. 1A-C and E), unlike plants that did not receive Se. For *WUE* (Fig. 1F), there were no differences among Se doses in irrigated plants. However, at the highest dose (2.0 mg kg^{-1}), there was reduction in *A*, *gs*, and *EiC*. In plants subjected to water deficit, application of Se promoted an increase in *A*, *E*, *EiC* and *WUE* (Fig. 1A, C, E and F) compared to plants that did not receive Se. The *gs* in plants subjected to water deficit did not vary with the doses of Se applied (Fig. 1B).

The *A* and *E* of plants under water deficit that received a dose of 1.0 mg kg^{-1} Se in the soil were 76.0% and 46.2% higher than those of plants that did not receive Se (Fig. 1A and C). The *Ci* decreased accordingly with increasing doses of Se applied, regardless of whether plants were under water stress or not (Fig. 1D). A dose of 0.5 mg kg^{-1} Se promoted a *Ci* that was approximately 15% lower than that of plants that did not receive Se application, although the results were not different compared to those of other doses.

Unlike the results for *Ci*, water deficit increased the concentrations of H_2O_2 and MDA by 19.4% and 28% in rice plants (Table 1), respectively. Application of 0.5 mg kg^{-1} Se promoted an increase in SOD activity and a decrease in H_2O_2 concentration (Fig. 2A and B) compared to those of plants that did not receive Se regardless of the water regimen to which the rice plants were subjected.

3.2. Biometric measurements, chlorophyll readings and nutrient accumulations

Interactions ($p \leq 0.05$) between the water conditions of the soil and doses of Se regarding SPAD index and the accumulation of sulfur (S) (Fig. 3A and C) were observed. Plant height and copper (Cu) accumulation (Table 2 and Fig. 3B and D) were affected ($p \leq 0.05$) individually by water conditions and Se doses. Dry shoot, root, overall biomass and

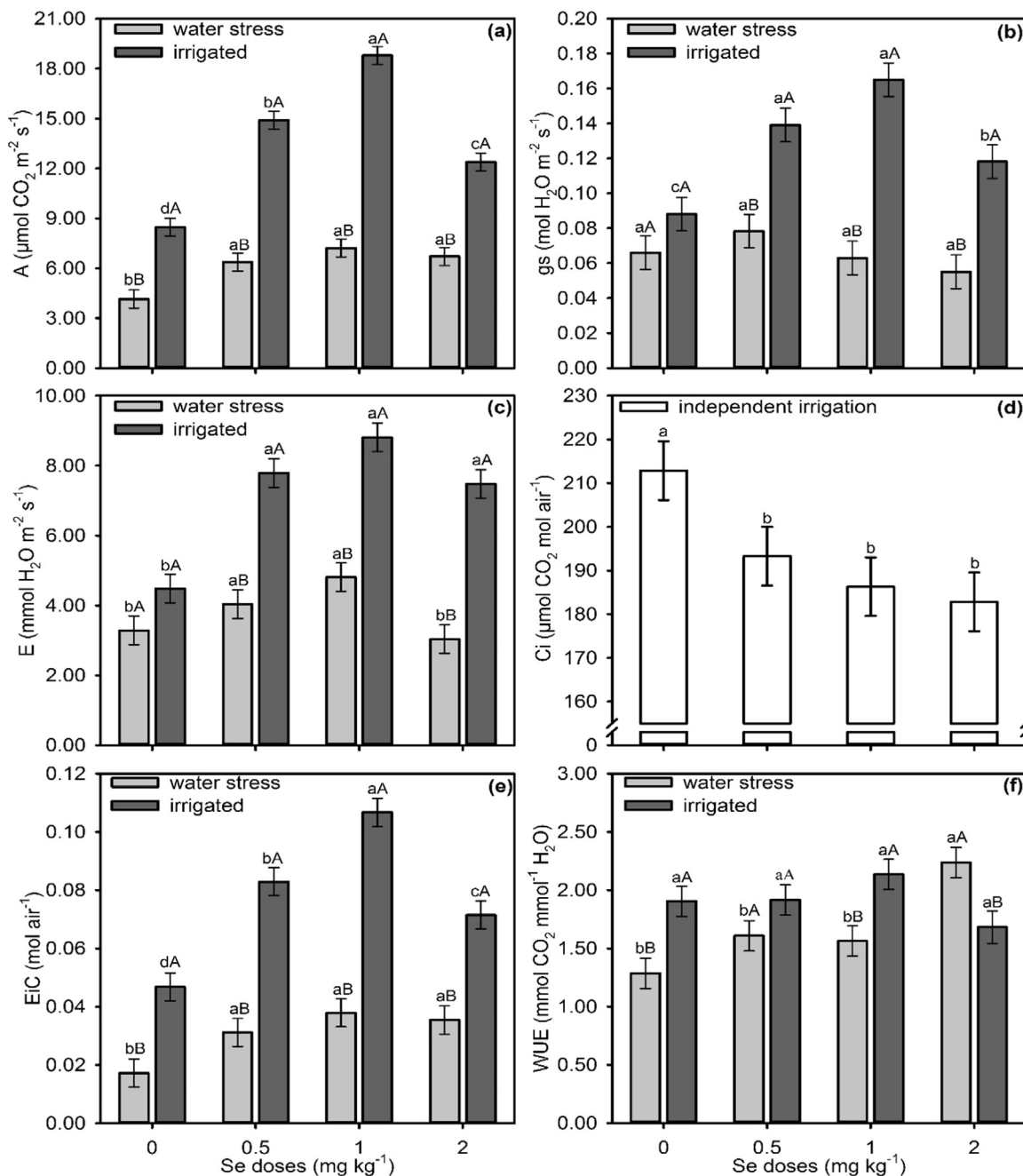


Fig. 1. Effects of soil water conditions and Se application rates on the CO₂ assimilation rate (a), stomatal conductance (b), transpiration rate (c), internal CO₂ concentration (d), instantaneous efficiency of carboxylation (e) and water use efficiency (f) of rice plants. Uppercase letters compare the means soil water conditions in each Se application rate, while lowercase letters compare the means of the Se application rate at each soil water conditions; lowercase letters alone at bars, compare the means of the Se application rate independent of soil water conditions, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of the average values (n = 4).

Table 1

Effects of water conditions on internal CO₂ concentration (Ci), hydrogen peroxide (H₂O₂) and lipid peroxidation (MDA) in rice plants.

Water condition	Ci (μmol m ⁻² s ⁻¹)	H ₂ O ₂ (μmol H ₂ O ₂ mg ⁻¹ FW)	MDA (nmol mg ⁻¹ FW)
Irrigated	179.03 ^b ± 3.94	11.84 ^b ± 0.63	82.45 ^b ± 4.93
Water deficit	208.59 ^a ± 7.09	14.69 ^a ± 0.82	114.62 ^a ± 4.22

Means not sharing the same letter, a and b, differ significantly (p < 0.05) within each column. ± standard error of average values (n = 4).

seed biomass as well as the accumulations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn) and zinc (Zn) (Table 2) were affected by soil water conditions. The root/shoot ratio was not affected by soil water conditions or Se doses.

The SPAD index was lower in plants cultivated under water deficit in the absence of Se and with application 1.0 mg kg⁻¹ (Fig. 3A) when compared to plants grown under normal water conditions. The highest SPAD index in irrigated plants was recorded at the Se dose of 1.0 mg kg⁻¹, although no difference was observed in plants that did not receive Se. When comparing the Se doses in treatments of water stress, plants that received a 0.5 mg kg⁻¹ Se dose had an 8% higher SPAD

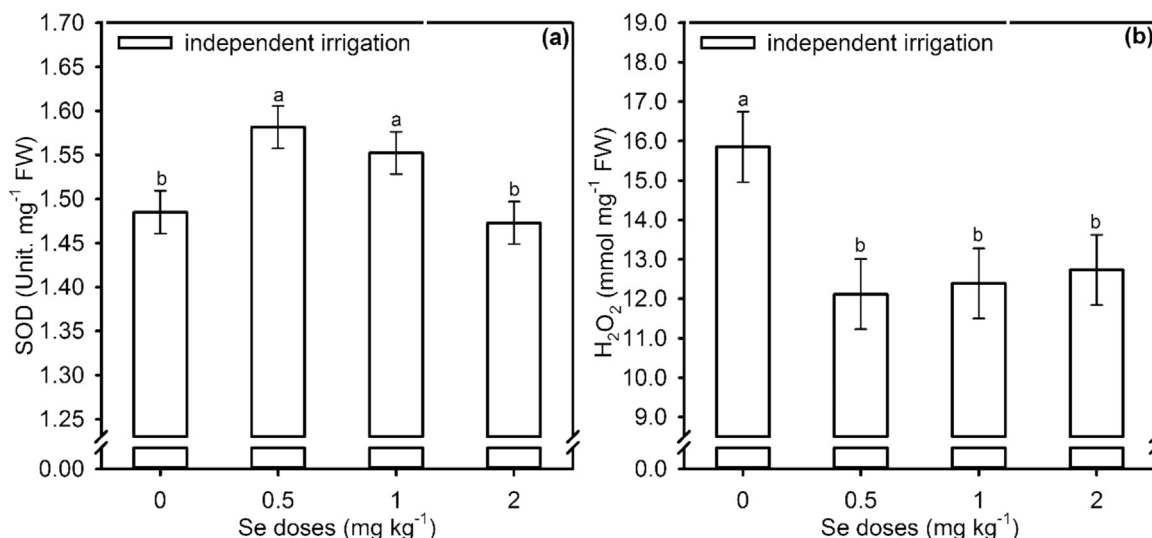


Fig. 2. Effect of Se application rates on the superoxide dismutase activity (a) and hydrogen peroxide concentration (b) in rice leaves. Means followed by same lowercase letter do not differ from each other according to the Scott-Knott test (5%). The vertical bars indicate the standard errors of the average values (n = 4).

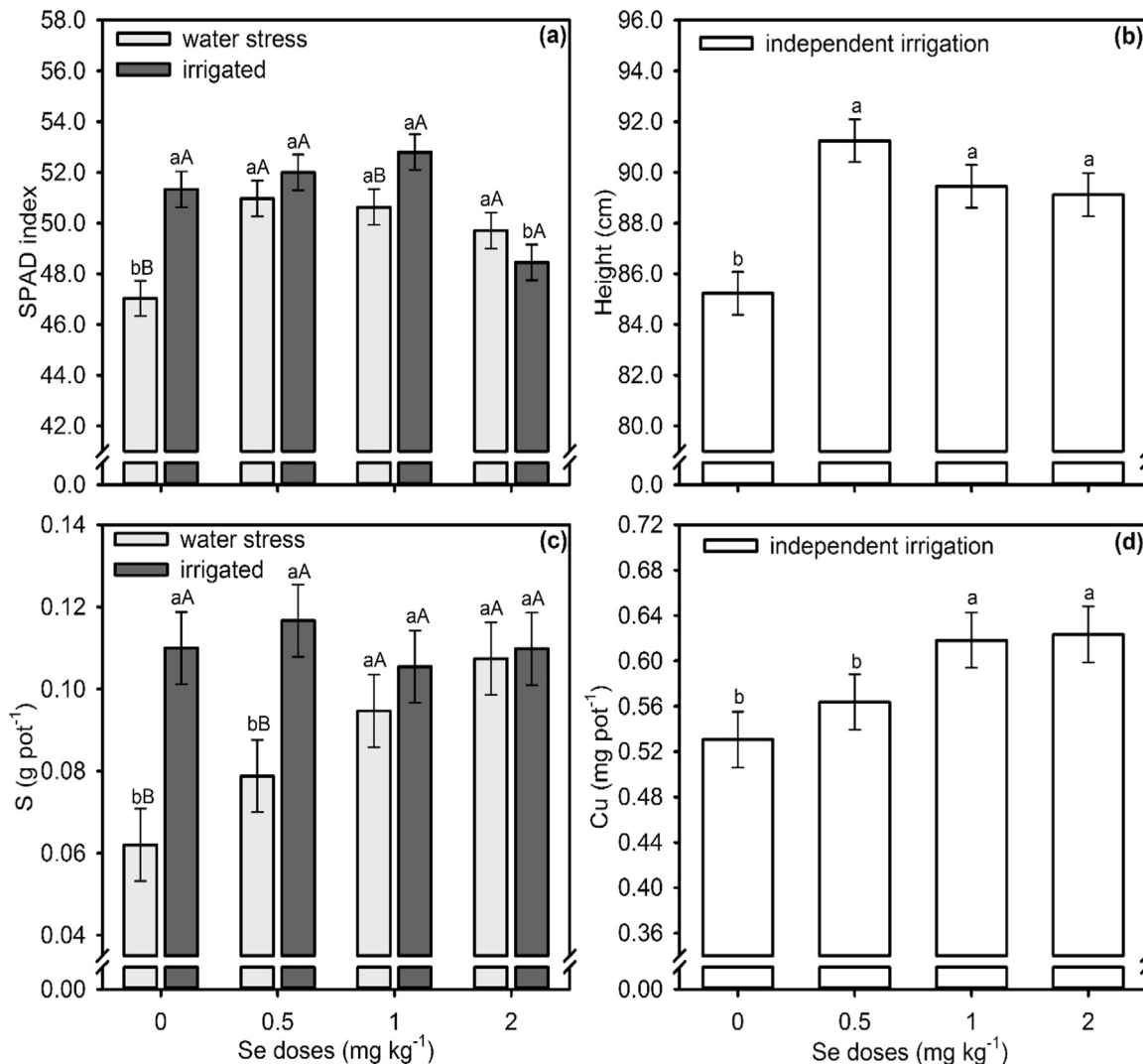


Fig. 3. Effects of soil water condition and Se application rates on the SPAD index (a), height (b), and accumulations of sulfur (c) and copper (d) in rice plants. Uppercase letters compare the means soil water conditions in each Se application rate, while lowercase letters compare the means of the Se application rate at each soil water conditions; lowercase letters alone at bars, compare the means of the Se application independent of soil water conditions, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of average values (n = 4).

Table 2
Growth, grain biomass and nutrient accumulation in rice plants under different soil water conditions.

Growth		Shoot dry biomass	Root dry biomass	Overall dry biomass	Height of plants	Grain biomass
Water condition	(g pot ⁻¹)	(g pot ⁻¹)	(g pot ⁻¹)	(cm)	(g pot ⁻¹)	
Irrigated	79.25 ^a ± 0.84	60.63 ^a ± 2.72	139.88 ^a ± 2.76	93.60 ^a ± 0.84	75.26 ^a ± 1.42	
Water deficit	46.75 ^b ± 0.52	42.63 ^b ± 3.31	89.38 ^b ± 3.40	83.93 ^b ± 0.77	31.19 ^b ± 1.60	
Nutrient accumulation						
Water condition	N	P	K	Ca	Mg	
	g pot ⁻¹					
Irrigated	1.487 ^a ± 0.031	0.127 ^a ± 0.005	1.720 ^a ± 0.044	0.194 ^a ± 0.011	0.249 ^a ± 0.011	
Water deficit	1.136 ^b ± 0.039	0.083 ^b ± 0.003	1.125 ^b ± 0.027	0.149 ^b ± 0.008	0.171 ^b ± 0.011	
Water condition	B	Cu	Fe	Mn	Zn	
	mg pot ⁻¹					
Irrigated	1.097 ^a ± 0.048	0.74 ^a ± 0.021	9.81 ^a ± 0.451	84.39 ^a ± 4.82	3.48 ^a ± 0.111	
Water deficit	0.799 ^b ± 0.044	0.42 ^b ± 0.013	7.24 ^b ± 0.326	59.95 ^b ± 1.75	2.63 ^b ± 0.122	

Means not sharing the same letter, a and b, differ significantly ($p < 0.05$) within each column. ± standard error of average values ($n = 4$).

index than did control plants (Fig. 3A).

The accumulation of S in plants under water stress was similar to S accumulation in irrigated plants that received 1.0 and 2.0 mg kg⁻¹ Se (Fig. 3C). Increasing doses of Se did not cause significant accumulations of S in plants under the normal water regimen. In plants subjected to water deficit, there was significant increase in S accumulation in rice biomass (Fig. 3C).

Plant height increased with Se doses, regardless of water conditions. The 0.5 mg kg⁻¹ dose of Se the provided the highest height, which was a 6.6% increase compared to that of untreated plants (Fig. 3B). The accumulation of Cu in rice plants increased linearly with increasing doses of Se applied to the soil (Fig. 3D). The dose of 2.0 mg kg⁻¹ Se increased Cu accumulation in the shoot biomass by 17.5% compared to that of plants that did not receive Se application.

Water deficit in rice plants caused reductions of 41%, 29.6%, 36.1%, 58.5% and 10.3% in shoot dry biomass, root dry biomass, overall dry biomass, seed production and plant height, respectively. Similarly, the accumulations of N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn decreased under water deficit conditions in rice plants (Table 2).

3.3. Se accumulation and concentration in seeds

Selenium accumulation in shoots (Fig. 4A) was affected ($p \leq 0.05$) only by the applied doses of Se. For Se concentration in grains, interactions ($p \leq 0.05$) between soil water conditions and Se doses (Fig. 4B) were observed. The accumulation of Se in the biomass of rice plants increased linearly with increasing application of Se doses regardless of water regimen (Fig. 4A). The highest accumulation of Se in the biomass (2.10 mg pot⁻¹) was recorded in the treatment consisting of the highest dose of Se. Water deficit promoted a reduction in the Se concentration in the seeds of plants that received doses of 1.0 and 2.0 mg kg⁻¹ Se. Selenium concentration in rice seeds increased linearly from 0 to 20.78 mg kg⁻¹ under water deficit conditions and from 0 to 23.58 mg kg⁻¹ under irrigated conditions (Fig. 4B).

4. Discussion

Our results demonstrated that water stress reduces photosynthesis, growth, nutrient absorption and rice yield. However, Se application increased *A*, *g_s*, *E* and *WUE* in plants under water stress. The reductions

of *A*, *g_s*, *E* and *EiC* (Fig. 1A-C and E) in plants subjected to water deficit at all doses of Se are related to the negative effects of water restriction on these variables. On the other hand, an increase in *WUE* at the dose of 2.0 mg kg⁻¹ Se (Fig. 1F) was also observed in these plants. Water deficit significantly reduces the growth of cultivated plants due to the direct influence of water deficit on various physiological and biochemical processes (R. Ahmad et al., 2016b).

Decreases in *A* and *g_s* in plants experiencing water deficit may be due to chlorophyll degradation and fragmentation, the suppression of rubisco activity and stomatal closure (Jáki et al., 2017; Zhang et al., 2017). Transpiration is an essential process for plants during photosynthesis. A reduction in transpiration to maintain internal water balance when plants are subjected to water deficit, as described by Yan et al. (2016), was verified in this study. Zain et al. (2014) reported a decrease in *A*, *g_s* and *E* as water deficit increased while subjecting rice plants to water deficit, which was also observed in this study.

Although water stress decreased *A*, *g_s*, *E*, *EiC* and *WUE*, Se application attenuates these reductions, which was verified in our study. This is related to the protective Se effects that minimizes damage to chloroplast structure and promotes greater activity of the electron transport system compared to the control. Nawaz et al. (2015) and Proietti et al. (2013) reported positive effects of Se application on *A*, *g_s* and *E* in wheat and olive plants under both normal water and water deficit conditions.

The lowest *EiC* in plants subjected to water stress in the present study is associated with a limitation in CO₂ assimilation (Fig. 1A) caused by stomatal closure (Fig. 1B). Stomatal closure is considered the main cause of decreasing rates of photosynthesis in C3 plants subjected to moderate water deficit (Flexas and Medrano, 2002). However, under more severe stress conditions, *C_i* increases, which indicates a predominance of non-stomatal limitations to photosynthesis, as reported by Mafakheri et al. (2010).

The increase in *WUE* with increasing Se doses in rice plants under water deficit is related to the activation of osmotically active compounds by this element. Nawaz et al. (2016) reported the accumulation of osmotically active molecules in corn (*Zea mays* L.) under conditions of water restriction associated with Se applications, which resulted in the greatest *WUE* by the plant.

The highest concentrations of H₂O₂ and lipid peroxidation (Table 1) in rice plants subjected to water deficit could be related to the disturbance between generation and extinction of reactive oxygen species. This is because water stress reduces the water content in plants, which leads to the degradation of pigments (Fig. 3A), reduction of the photochemical phase, reduction of CO₂ assimilation (Fig. 1A), breakage of homeostasis and elevation of ROS generation (Faize et al., 2011). Habibi (2013) and Zain et al. (2014) reported increases in the concentrations of lipid peroxidation and H₂O₂ in barley and rice, respectively, in plants subjected to water deficit.

An increase in SOD enzyme activity associated with decreased *C_i* and H₂O₂ in rice plants due to Se application in the present study shows the protective role of this element, as reported by Feng et al. (2012) and Yao et al. (2013). Even though SOD is not a selenoenzyme, Se increases gene expression and activity levels of the SOD enzyme. Jiang et al. (2017) reported that genes involved in SOD activation mechanisms are significantly upregulated in maize roots 24 h after applications of 1 μM Se. Nawaz et al. (2016) reported an increase in SOD activity in corn plants under normal irrigation conditions after the addition of Se to soil.

A reduction in H₂O₂ content is related to increased SOD activity (Fig. 2A) as a result of Se application, as SOD is the first line of anti-oxidative defense against ROS. An increase in SOD activity after Se application provides evidence that this element may be directly involved in the extinction of superoxide (O₂⁻) and hydroxyl radicals (OH⁻) in cells (Nawaz et al., 2016).

Water deficit decreased the height, biomass and grain yield of rice plants, and these changes may be related to numerous processes in the plant in which water participates, with an emphasis on maintaining

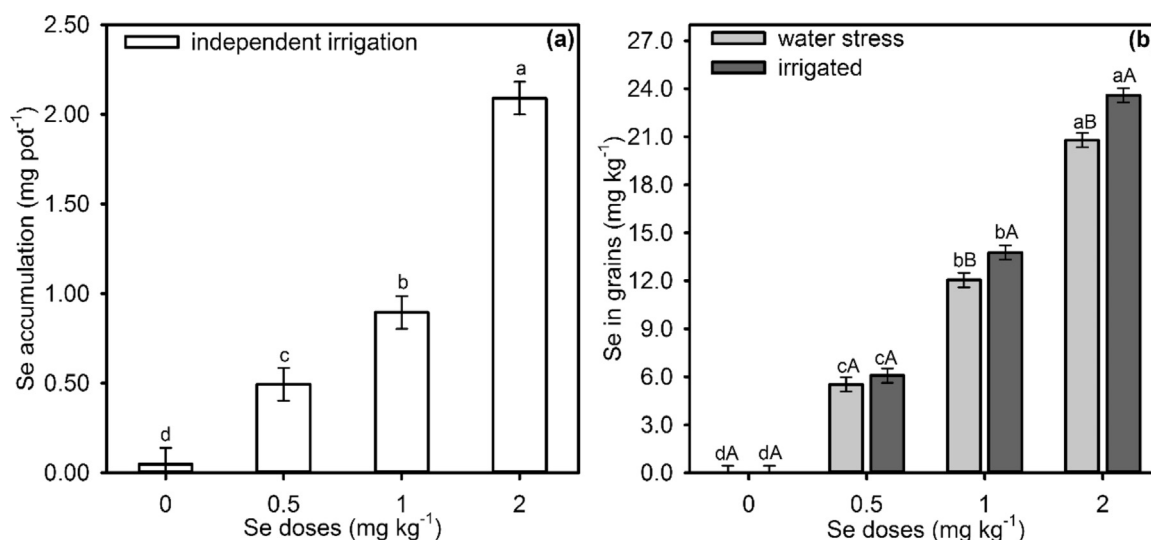


Fig. 4. Selenium accumulation in shoot biomass (a) and Se concentration in seeds (b) due to Se application rates and soil water condition. Uppercase letters compare the means soil water condition in each Se application rate, while lowercase letters compare the means of the Se application rate at each soil water condition; lowercase letters alone at bars, compare the means of the Se application rate independent of soil water condition, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of average values ($n = 4$).

turgor and mitotic impairment (Jaleel et al., 2009). When water is reduced due to water deficit, turgescence severely reduces cell growth (Flexas, 2016). Water deficit affects both stretching and cell growth and inhibits cell growth rather than cell division (Jaleel et al., 2009; Shao et al., 2008).

A decrease in plant height subjected to water deficit is related to leaf primordia formation, which is a process that is sensitive to water restriction. The negative effects of water deficit on rice yield are due to low shoot and root biomass production, decreased soil nutrient utilization and decreased plant photosynthesis, which were both shown in this study and reported by Feng et al. (2012). A decrease in rice yield, growth and biomass was reported by Zain et al. (2014) and Nawaz et al. (2015) in rice and wheat plants subjected to water restrictions.

Selenium has beneficial effects on the growth of plants, especially in plants exposed to stress conditions. Strong growth of plants that received Se is related to improvements promoted by photosynthesis (Fig. 1) and by the decreased formation of reactive oxygen species, lipid peroxidation and formation of H₂O₂ in plant cells (Fig. 2B). Hashem et al. (2013) reported an increase in canola (*Brassica napus* L.) growth when the plants received foliar applications of 2.5 and 5.0 mg L⁻¹ Se.

Drought stress is usually characterized by a reduction of chlorophyll and is associated with a progressive decline in the photosynthetic capacity of plants, which explains the lower SPAD values in plants experiencing water deficit compared to those of plants irrigated regularly. An increase of this index in plants under both water deficit conditions and Se treatment is associated with an increase in chlorophyll content, which is in agreement with the results of Iqbal et al. (2015). A decrease in SPAD index at the highest dose of Se is caused both by an adverse effect on porphobilinogen synthase production, which is necessary for chlorophyll biosynthesis, and the inhibition of biosynthetic enzymes through lipid peroxidation (Saffaryazdi et al., 2012).

An increase in S biomass accumulation in plants that were subjected to water deficit and that received doses of Se may be related to synergistic interactions between selenate and sulfate ions due to their similar chemical properties and use of the same membrane transporter and route of assimilation (Pilon-Smits, 2015; White, 2016). An increase in S content in plants that received 1.0 g kg⁻¹ Se under water deficit conditions may have occurred because the stress promoted an increase in glutathione synthesis due to the need to reduce reactive oxygen species (Table 1). Se has a direct effect on enzyme synthesis. Thus, the synthesis requirement of Se for decreased S may have led to the

accumulation of this element (N. Ahmad et al., 2016a; Sajedi et al., 2011). This accumulation was equal to that in plants under a normal water regimen at those doses.

The increase in Cu accumulation in rice plants due to Se doses differed from the results reported by Schiavon et al. (2013), who detected no effect on Cu accumulation in the shoots of tomato (*Solanum lycopersicon* L.). Arvy et al. (1995) reported increases in Cu in the biomass of *Catharanthus roseus* (L.) plants as a result of Se treatment. Cu, Fe, Zn and Mn are important SOD cofactors (Yao et al., 2013) that may have caused an increase in plant growth because of the high activity of the SOD enzyme (Fig. 2A).

The accumulation of nutrients in the shoots of rice plants under water deficit conditions decreased because of the reduction in mobility and absorption of nutrients. Under water stress conditions, roots are unable to absorb many nutrients from the soil due to decreased root activity, which is associated with decreased water movement toward the roots and slower ion diffusion (Alam, 1999). Sardans and Peñuelas (2004) reported that a 22% reduction in soil moisture decreased the amount of P accumulated in plants by 40%; this reduction mainly occurred because there was no decrease in shoot biomass. In addition, Dejong and Phillips (1982) reported a decrease of 50% in the accumulation of N in clover (*Trifolium subterraneum* L. cv. Woogenellup).

An increase in Se concentration in the shoots and grains of rice plants subjected to the application of this element was demonstrated by Hu et al. (2002), Boldrin et al. (2013), Hu et al. (2014) and Nothstein et al. (2016). This increase is related to the Se concentration mainly in the soil. The accumulation of Se in rice plant biomass is an important content predictor of this element in the seeds, as indicated in the study by Zhang et al. (2006) and confirmed in this present study.

In addition, the intended outcome of biofortification programs is an increase in Se concentration in plants and seeds without harming crop yield. In this regard, the doses applied in the present study did not promote a significant reduction in plant biomass or seeds. The results showed photosynthesis improvements and increased water use efficiency in plants subjected to water deficit, which demonstrates that Se is important for enhancing the development of plants under water deficit conditions.

5. Conclusion

Se application to rice plants improves their CO₂ assimilation rate,

transpiration rate, instantaneous carboxylation efficiency, water use efficiency estimation, and physiological capacity to withstand water deficit. There was a decrease in H₂O₂, and increases in SOD activity, accumulation of Se in shoot biomass and rice seeds due to applied Se doses. Further studies, should be developed under field conditions to establish suitable Se concentration in rice seeds for human consumption.

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