plant biology

Plant Biology ISSN 1435-8603

RESEARCH ARTICLE

Modulatory responses of physiological and biochemical status are related to drought tolerance levels in peanut cultivars

M. V. Checchio[1], A. L. Bacha[1], W. C. Carrega[2], G. da Silveira Sousa Júnior[3], P. L. da Costa Aguiar Alves[1] & P. L. Gratão[1]

1 Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brazil
2 Agrárion® LTDA, Brazil
3 Instituto Municipal de Ensino Superior "Victório Cardassi", São Paulo, Brazil

Keywords
Abiotic stress; antioxidant enzymes; *Arachis hypogaea* L.; gas exchange; water deficit.

Correspondence
P. L. Gratão, Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Depto. de Biologia, Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, São Paulo, Brazil.
E-mail: pl.gratao@unesp.br

Editor
B. Hu

Received: 8 March 2024;
Accepted: 6 October 2024

doi:10.1111/plb.13740

ABSTRACT

- Peanut (*Arachis hypogaea* L.) is the fourth most cultivated oilseed in the world, but its cultivation is subject to fluctuations in water demand. Current studies of tolerance between cultivars and physiological mechanisms involved in plant recovery after drought are insufficient for selection of tolerant cultivars.
- We evaluated tolerance of different peanut cultivars to water deficit and subsequent rehydration, based on physiological and biochemical status. Gas exchange, photosynthetic pigments, F_v/F_m, MDA, H_2O_2 and antioxidant enzyme activity were analysed.
- Drought stress and rehydration triggered distinct changes in pigments, F_v/F_m, gas exchange, and H_2O_2 across genotypes, with increased MDA in all cultivars under stress. Based on multivariate analysis, 'IAC Sempre Verde' was identified as most drought sensitive, while 'IAC OL3', 'IAC 503', and 'IAC OL6' exhibited variations in physiological responses and antioxidant activity correlated to their respective tolerance levels. Notably, 'IAC OL3' had higher WUE and enhanced enzymatic defence and was classified as the most drought tolerant in this context.
- The above findings suggest that antioxidant metabolism is a important factor for plant recovery post-rehydration. Our study provides insights into antioxidant and physiological responses of peanut cultivars, which can support breeding programs for selection of drought-tolerant genotypes. Future field studies should be conducted for a better understanding of tolerance of these cultivars, particularly through correlation of these data with crop yield impact.

INTRODUCTION

Drought is the most prevalent stress and bane of global agriculture (Ault, 2020; Gupta *et al.*, 2020; Kaur *et al.*, 2021; Bakry *et al.*, 2024). Recent estimates indicate that among all abiotic stresses, drought alone is responsible for the annual loss of around 6 million tons of global peanut yield (Sarkar *et al.*, 2016; Bakry *et al.*, 2024), one of the main oilseeds of socioeconomic importance (Zhao *et al.*, 2018; Yang *et al.*, 2019).

The vulnerability of peanut plants to water deficiency is dependent on growth stage and phenotype variability (Puangbut *et al.*, 2009). Despite some tolerance, drought particularly affects yield during the flowering and pod formation stages (Koolachart *et al.*, 2013; Kaur *et al.*, 2021). Plants can maintain their functional physiological activities even during periods of severe water deficit through metabolic adjustment and modular adaptations (escape/avoidance/tolerance) (Farooq *et al.*, 2009; Laxa *et al.*, 2019; Kaur *et al.*, 2021). However, drought tolerance, which is linked to the plant phenotype, involves activation of an integrated network of responses at physiological, biochemical, and molecular levels (Farooq *et al.*, 2009; Bakry *et al.*, 2012), being dependent on plant development stage, stress severity and duration (Chakraborty *et al.*, 2015; Kaur *et al.*, 2021). The severity of drought can also limit photosynthesis and metabolism in peanut, affecting the photosynthetic apparatus and its components, and compromisiing the physiological processes of plants (Farooq *et al.*, 2009; Rivas *et al.*, 2016; Pilon *et al.*, 2018).

Within plant drought signalling process occurs at biochemical and molecular levels, influencing synthesis of organic compounds, production of reactive oxygen species (ROS), and the redox antioxidant system. Several transcription factors are involved (such as DREB) as well as activity of specific enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), guaiacol peroxidase (GPOX, EC 1.11.1.7), and glutathione peroxidase (GSH-PX, EC 1.11.1.9) (Yi *et al.*, 2016; Bhalani *et al.*, 2019; Bhogireddy *et al.*, 2020). Studies have suggested a strong correlation between activation of the antioxidant defence system and drought tolerance, which is also relevant for plant recovery after stress periods (Furlan *et al.*, 2016; Laxa *et al.*, 2019). Many of these variables can be measured to detect plant conditioning to stress. However, previous reports do not always account for intraspecific differences between genotypes of the same species, especially when evaluating the water deficiency index and data on physiological and biochemical parameters (Farooq

et al., 2009). Such differences also relate to variations in photosynthesis and stomatal and non-stomatal processes (Pilon *et al.*, 2018) which ultimately correlate with tolerance level.

The drought survival strategy through the capacity for rapid metabolic recovery can be decisive for the productive performance of cultivated species, such as peanuts (Hu *et al.*, 2010; Laxa *et al.*, 2019). However, there are still insufficient comparative studies on this crop in response to drought; especially studies related to the intrinsic physiological and biochemical responses in metabolic recovery after rehydration, as well as studies involving the tolerance of different cultivars to water deficit (Bhogireddy *et al.*, 2020). Thus, the search for tolerant cultivars and the involved mechanisms is essential, given the socioeconomic relevance of this crop. To address this, we hypothesized that peanut genotypes differ in their patterns of metabolic responses to water deficit and subsequent rehydration. Therefore, this study evaluated the tolerance of different peanut cultivars subjected to water deficit and rehydration and the involved physiological and biochemical mechanisms.

MATERIAL AND METHODS

Plant material, treatments and growth conditions

The experiment was carried out in a greenhouse at the São Paulo State University (UNESP), Jaboticabal-SP, Brazil (21°15′17″S, 48°18′20″W, 590 m a.s.l.). Maximum and minimum temperatures and relative air humidity inside the greenhouse were monitored during the experimental period (Fig. S1).

The peanut seeds (cultivars 'IAC 503', 'IAC OL3', 'IAC OL6' and 'IAC Sempre Verde') were acquired from the Agronomic Institute of Campinas—IAC. A completely randomized experimental design was used, and the treatments were arranged in a 4 × 3 factorial scheme (4 cultivars × 3 water conditions), with three replications, totaling 36 experimental units. Each experimental unit consisted of a 25-L pot containing three peanut plants. As substrate, we used a mixture of a eutrophic red Oxisol (clayey texture) and sand (3:1 v/v). Representative aliquots of this mixture were subjected to chemical analysis of pH ($CaCl_2$): 6.1, organic matter: 9.0 g dm^{-3}, P resin: 10 mg dm^{-3}, S: 13 mg dm^{-3}, Ca: 21 mg dm^{-3}, Mg: 7 $mmol_c$ dm^{-3}, Na: ns, K: 1.3 $mmol_c$ dm^{-3}, H^+Al: 13 $mmol_c$ dm^{-3}, sum of bases: 28.5 $mmol_c$ dm^{-3}, cation exchange capacity: 41.1 $mmol_c$ dm^{-3}, and base saturation: 69%.

Prior to sowing, the seeds were treated with insecticide and fungicide. After emergence, thinning was carried out, retaining three plants per pot. All plants were conditioned to full irrigation (CT treatment—control) until 65 days after sowing (DAS). At 66 DAS, the rehydrated treatment (RH) started by suspending irrigation for 9 days, following by rehydration for 3 days. At 69 DAS, irrigation of the water deficit (WD) group was also suspended. Total suspension of irrigation in both groups was maintained for 9 days, until reaching 30% of pots water retention capacity (WRC). After 9 days, only plants from RH were irrigated again to 80% WRC for 3 days. The control treatment was maintained at 80% WRC throughout. To replace sufficient water to maintain the desired moisture level, water availability was controlled using the gravimetric method, after replacement of evapotranspired water. After this period, at 78 DAS, evaluations were undertaken for all treatments, in which leaves were also collected for the biochemical analyses described below.

Photosynthetic pigment content

The chlorophyll and carotenoid content were assayed spectrophotometrically following Lichtenthaler (1987). Fresh leaves immersed in acetone (80% v/v) in a tube and incubated in the dark (72 h at 4°C), then measurements taken at: Chlorophyll *a* = 663 nm, Chlorophyll *b* = 647 nm, Carotenoids = 470 nm. The content was expressed in micrograms pigment per gram fresh weight (μg g fresh $weight^{-1}$).

Quantum efficiency of PSII (F_v/F_m)

The photosystem II (PSII) quantum efficiency was monitored using a plant efficiency analyser (Hansatec, model PEA). At excitation of 650 nm and after dark-adapting leaves for 30 min in a leaf clip followed by exposure to light of 3000 μmol m^{-2} s^{-1}, maximum fluorescence (F_m) was measured, followed by calculation of maximum photochemical efficiency F_v/F_m (Janka *et al.*, 2015).

Gas exchange

Net CO_2 assimilation (A—μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s—mol H_2O m^{-2} s^{-1}), transpiration (E—mmol H_2O m^{-2} s^{-1}), intracellular CO_2 (C_i—μmol mol^{-1}) and leaf temperature (Tleaf—°C) were evaluated between 09:30 and 10:30 h, using and infrared gas analyser (IRGA model LI 6400; Li-Cor®) at 400 μmol CO_2 mol^{-1}, 14 mmol H_2O mol^{-1}, chamber temperature 25°C, flow rate 400 μmol s^{-1}, atmospheric pressure 1000 KPa and photosynthetically active radiation (PAR) of 1500 μmol m^{-2} s^{-1}. PAR was standardized according to geographic location and specific time at which the analyses were conducted. From the collected data, the instantaneous carboxylation efficiency (A/C_i—μmol CO_2 m^{-2} s^{-1}) and water use efficiency (WUE—μmol CO_2 mmol H_2O^{-1}) were calculated.

Hydrogen peroxide (H_2O_2) content

The H_2O_2 content was determined following Alexieva *et al.* (2001) using the potassium iodide reaction read in 390 nm. Leaves were homogenized in trichloroacetic acid (0.1%) and centrifuged at 10,000 × *g* for 10 min. The supernatant was added to 100 mM potassium phosphate buffer (pH 7.5) and 1 M potassium iodide solution. Samples were then kept on ice for 1 h. H_2O_2 content was assessed using a standard curve (Gratão *et al.*, 2015). The results are expressed in μmol mg^{-1} fresh weight (FW).

Lipid peroxidation (MDA)

Lipidic peroxidation was analysed as content of thiobarbituric acid (TBA) reactive substances (TBARS) (Mihara *et al.*, 1980). Fresh samples were mixed with 20% (w/v) polyvinylpyrrolidone (PVP) and 0.1% trichloroacetic acid (TCA). The content was homogenized and centrifuged at 11,000 × *g* for 15 min at

4°C. The supernatant was added to 20% TCA and 5% TBA and incubated in a water bath (95°C) for 30 min. Samples were then incubated in an ice bath for 10 min to stop the reaction, then centrifuged at 11,000 × *g* for 5 min. The concentration of malondialdehyde (MDA) equivalents was measured spectrophotometrically (535 and 600 nm) and results expressed as μmol mg^{-1} FW.

Protein extraction and determination of antioxidant activity

The leaves were homogenized in a chilled mortar with a pestle with extraction buffer of 100 mM potassium phosphate (pH 7.5), 1 mM ethylenediamine tetraacetic acid (EDTA), 3 mM DL-dithiothreitol, and 5% (w/v) insoluble PVPP in 3:1 vol/FW (Azevedo *et al.*, 1998). The homogenate was centrifuged at 10,000 × *g* for 30 min, and the supernatant stored at −80°C for further enzyme activity determination, expressed as mg protein. The protein concentration was assayed following the method of Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase assay (SOD, EC 1.15.1.1)
Activity of SOD (U mg protein^{-1}) was analysed according to Giannopolitis & Ries (1977) as inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT), conducted in a reaction chamber, under a 15 W fluorescent lamp at 25°C, for 5 min. The assay medium contained 50 mM sodium phosphate-buffered saline (pH 7.8), 50 mM methionine, 10 mM EDTA, 1 mM nitrotetrazolium blue chloride, and 0.1 mM riboflavin. The absorbance was read at 560 nm.

Ascorbate peroxidase assay (APX, EC 1.11.1.11)
The APX activity (μmol min^{-1} mg^{-1} protein) was assayed by monitoring the rate of ascorbate oxidation at 30°C and 290 nm (Cakmak & Horst, 1991). The reaction consisted of plant extraction in 80 mM potassium phosphate buffer (pH 7.0) including 5 mM ascorbate, 1 mM EDTA, and 1 mM H_2O_2.

Guaiacol peroxidase assay (GPOX, EC 1.11.1.7)
The GPOX activity (U g protein^{-1}) was monitored at 450 nm. The reaction mixture contained phosphate–citrate buffer (0.2 M dibasic sodium phosphate and 0.1 M citric acid) pH 5.0, guaiacol (0.5%), and plant extract. Subsequently H_2O_2 was added, and the mixture vortexed and incubated at 30°C for 15 min. The reaction was stopped by immediately transferring samples to an ice water bath, followed by addition of sodium metabisulphite solution (2%) (Monteiro *et al.*, 2011).

Glutathione peroxidase assay (GSH-PX, EC 1.11.1.9)
The GSH-PX activity (μmol min^{-1} mg^{-1} protein) was determined in a plant extract, potassium phosphate buffer (100 mM), EDTA (3 mM), 0.24 U GR mL^{-1}, 10 mM GSH, and 1 mM sodium azide. The mixture was kept in 37°C for 10 min and finally the reaction was catalysed by addition of 1.5 mM NADPH and 1.5 mM H_2O_2. Oxidation was monitored for 5 min at 340 nm (Anderson & Davis, 2004).

Statistical analysis

A multiple comparison between means was performed using Tukey's test followed by ANOVA for each characteristic (a = 0.05). The statistical analysis was performed using AGROESTAT® software (Barbosa & Maldonado Jr, 2015). To identify the behaviour of cultivars in response to the treatments, and to integrate all data from physiological and biochemical evaluations, a principal components analysis (PCA) was performed. This process was developed by reducing the multivariate data matrix to an interpretable two-dimensional biplot that explains the most variation in the data obtained in WD and RH conditions, separately. The graphics were made using Origin® software. 9.0 (Microcal®).

RESULTS

Quantification of photosynthetic pigments

The lowest concentrations of chlorophylls were in cultivar IAC OL3 under WD and in IAC Sempre Verde (SV) under RH (Fig. 1A). Regarding the control, no cultivar had a reduced chlorophyll content after exposure to stress. Only cultivar IAC SV showed this reduction after rehydration. In contrast, cultivar IAC 503 had an increased chlorophyll content under stress, maintaining this after rehydration (Fig. 1A).

Carotenoid data were similar to chlorophyll data under WD and RH (Fig. 1B). Cultivars IAC 503 and IAC OL3 had a

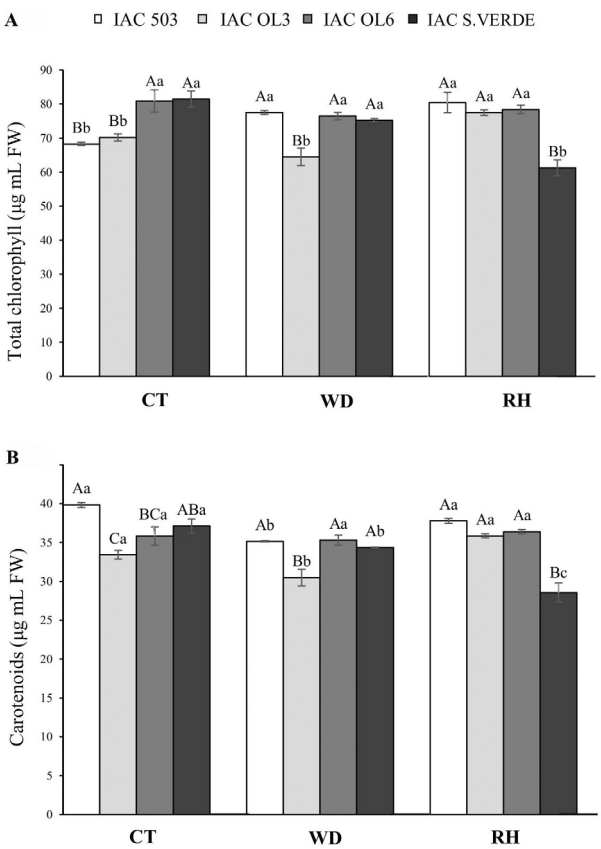

Fig. 1. Total chlorophyll (A) and carotenoid (B) contents in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppercase letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) compare means between conditions within the same cultivar. Error bars indicate ± SD.

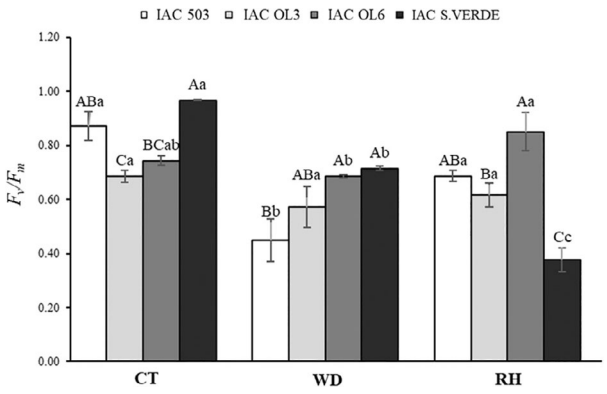

Fig. 2. PSII quantum efficiency (F_v/F_m) in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppe-case letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) compare means between conditions within the same cultivar. Error bars indicate ± SD.

reduced carotenoid content in response to stress, while recovering to levels of controls after rehydration. In contrast, cultivar IAC SV was the most affected, showing a continuous decrease in carotenoid content between conditions. Cultivar IAC OL6 had no changes in either chlorophyll or carotenoid content across the water availability levels (Fig. 1).

Photochemical efficiency (F_v/F_m)

The quantum efficiency of PSII (F_v/F_m) varied between cultivars, regardless of the water condition (Fig. 2), with lowest values in WD and RH conditions in cultivars IAC 503 and IAC SV, respectively. Compared to the control, stress decreased F_v/F_m in IAC 503 and IAC SV plants. However, these values increased in cultivars IAC 503 and OL6 after rehydration, unlike IAC SV in which there was a significant decrease (Fig. 2).

Gas exchange

The net CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) showed an analogous response pattern to water conditions. IAC 503 had highest values for these parameters among cultivars under water deficit, while IAC SV had the lowest values on rehydration (Fig. 3A–C). Conversely, C_i, instantaneous carboxylation efficiency (A/C_i), and water use efficiency (WUE) varied between cultivars and water conditions (Fig. 3D–F).

Compared to control plants, A, g_s, and E showed a constant reduction between water conditions only in IAC 503 and IAC SV, where values were not reestablished after rehydration (Fig. 3A–C). The C_i and A/C_i for IAC 503 fell only during rehydration (Fig. 3D,E), without any statistical difference for WUE (Fig. 3F). Cultivar IAC SV showed a significant reduction in C_i between water conditions, while A/C_i fell only under RH when compared to the control (Fig. 3D,E). In contrast, WUE for this cultivar increased with rehydration compared to control plants (Fig. 3F).

Cultivar IAC OL6 showed a drop in A, g_s, E (Fig. 3A–C) and A/C_i (Fig. 3E) when subjected to water deficit, but had equivalent (A, g_s, A/C_i) and partial (E) recovery when compared to controls after rehydration. In IAC OL3, A was not compromised by water deficit despite a decrease in g_s, E, and C_i (Fig. 3A–D); however, these values were reestablished to control levels after rehydration. A/C_i and WUE (Fig. 3E,F) showed the same response pattern in this cultivar, with an increase under WD, demonstrating best resource use under this condition and consequent adaptation to stress, with values were reestablished to controls after rehydration.

Oxidative metabolism and antioxidant enzymes

The highest MDA contents, an indication of lipid peroxidation, were in cultivars IAC OL6 and IAC SV, regardless of water condition. Compared to controls, water deficit increased MDA in all peanut varieties. MDA concentration was reduced in all cultivars after rehydration, not differing from controls (Fig. 4B).

The concentration of H_2O_2 increased in cultivars IAC OL6 and IAC SV under WD and RH. Regarding control treatment, the increase in H_2O_2 in most stressed plants was fell only in IAC 503 and IAC OL3 with rehydration. In contrast, H_2O_2 concentration increased in IAC OL6 and IAC SV under RH (Fig. 4A).

Activity of antioxidant enzymes SOD, APX, GSH-PX and GPOX differed between cultivars regardless of the treatment (Fig. 5A–D). The most significant SOD activity under WD and RH was in IAC OL3, while the lowest activity was in IAC 503 and IAC SV (Fig. 5A). All cultivars (except IAC 503) showed an increase in SOD activity under the WD. After rehydration, only IAC OL3 had reduced SOD activity (Fig. 5A).

Specific activity of APX was more significant in cultivar IAC OL3 in CT and WD treatments (Fig. 5B). IAC 503 showed increased APX activity under RH, similar to IAC OL3. Lowest APX activity among cultivars was in IAC SV under WD, together with IAC OL6 in RH. Stress caused activation of APX in all cultivars. Only IAC OL3 and IAC OL6 had reduced the APX activity after rehydration (Fig. 5B).

Among cultivars, IAC OL3 had the highest GSH-PX activity, regardless of the treatment. On the other hand, IAC OL6 under WD and IAC SV under RH had the lowest values. All cultivars showed the same response pattern regarding water condition, increasing activity under water deficit and not changing after rehydration (Fig. 5C).

The highest GPOX activity was in IAC OL3 under WD and IAC OL6 under RH (Fig. 5D). All cultivars increased GPOX activity under stress (except IAC 503) compared to controls. After rehydration, all cultivars reduced the GPOX activity compared to WD plants (except IAC OL6) (Fig. 5D).

Principal components analysis

The PCA separated all physiological and biochemical variables in the water deficit (Fig. 6A) and rehydration (Fig. 6B) conditions. The models used three components, evidencing 89.1% and 93.6% of the data, respectively. In general, antioxidant enzymes (SOD, APX, GPOX, GSH-PX) showed a direct correlation with IAC OL3 regardless of cultivation condition, being a important factor in antioxidant defence against stress (Fig. 6A). After rehydration, together with antioxidant metabolism, g_s, C_i and E were also determining factors in cellular

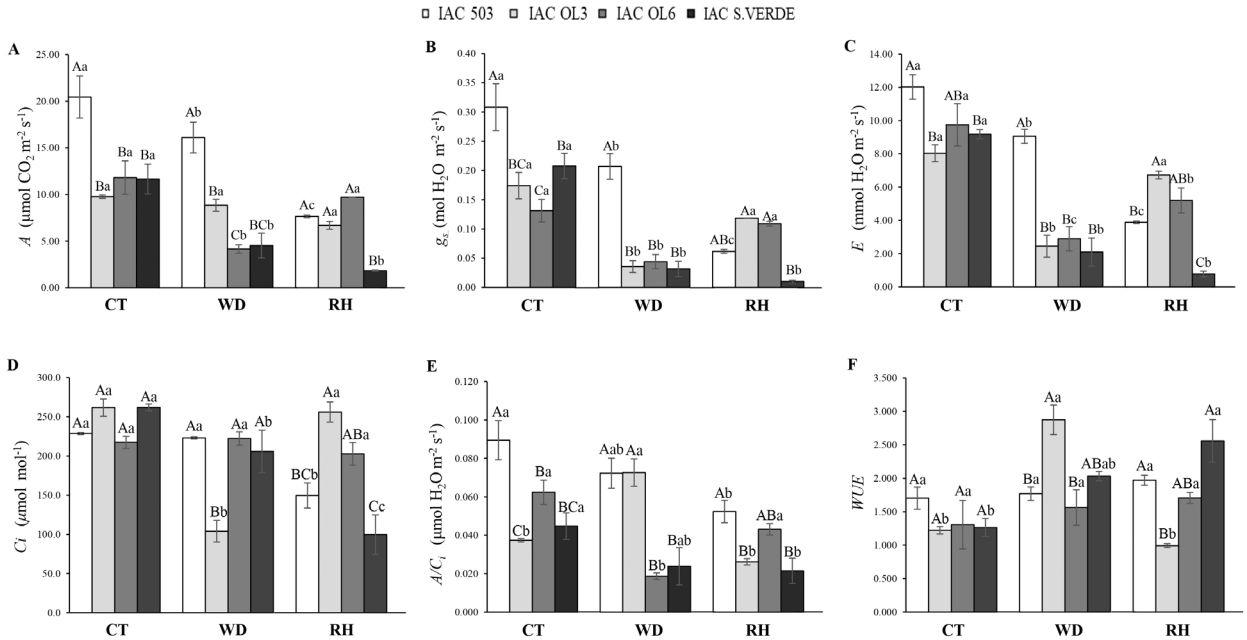

Fig. 3. Net CO_2 assimilation rate (A), stomatal conductance (B), transpiration (C), intracellular CO_2 (D), instantaneous carboxylation efficiency (E) and water use efficiency (F) in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppercase letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) compare means between conditions within the same cultivar. Error bars indicate ± SD.

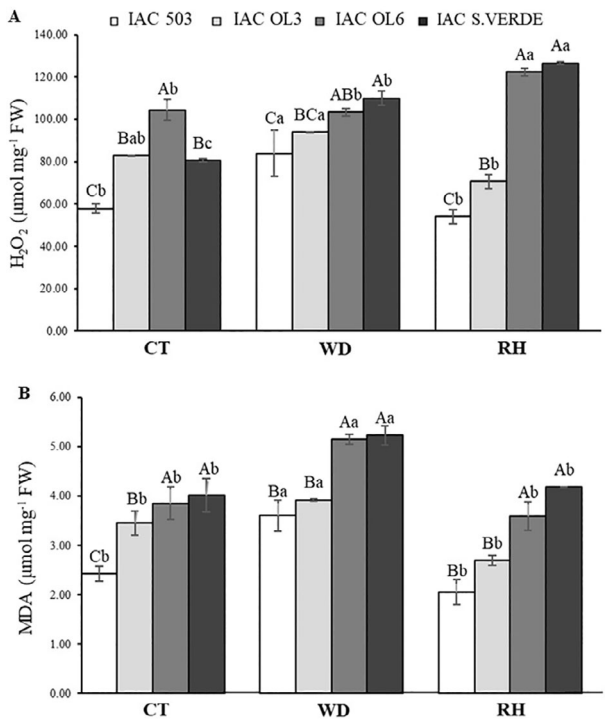

Fig. 4. Quantification of H_2O_2 (A) and MDA (B) content in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppercase letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) compare means between conditions within the same cultivar. Error bars indicate ± SD.

homeostasis and plant recovery (Fig. 6B), also being directly related to PC1 and IAC OL3.

Oxidative damage (MDA, H_2O_2) was strongly associated with IAC SV regardless of water condition (Fig. 6). IAC 503 correlated mainly with g_s under stress, while IAC OL6 was correlated with oxidative damage, together with IAC SV (Fig. 6A). For rehydration, all pigments, APX, and A/C_i were also associated with IAC 503 recovery, while antioxidants and E, C_i and g_s, were associated with IAC OL6, but at lower intensity compared to IAC OL3 (Fig. 6B).

DISCUSSION

Several studies on plant drought tolerance have found that metabolic impairment is usually affected by a decrease in g_s, pigment content, and limitations to photosynthetic activity, influencing CO_2 fixation, and accumulation of ROS. However, results vary, as does plant tolerance or sensitivity. In this context, changes in all these parameters, in addition to oxidative damage evidenced by ROS accumulation, linked to antioxidant metabolism (SOD, APX, GPOX e GSH-PX), were evaluated in this study, with the most significant data discussed below.

The essentiality of photosynthetic pigments in energy metabolism of plants is undeniable, especially when exposed to unfavourable environmental conditions (Sun *et al.*, 2022). Changes in total chlorophyll and carotenoid content as a result of water deficit have been reported, contributing to the distinction between stress-tolerant and stress-sensitive cultivars (Farooq *et al.*, 2009; Ashraf & Harris, 2013; Anjum *et al.*, 2017; Morey *et al.*, 2021). The decrease in total chlorophyll content for cultivar IAC SV after rehydration (Fig. 1A) suggests sensitivity

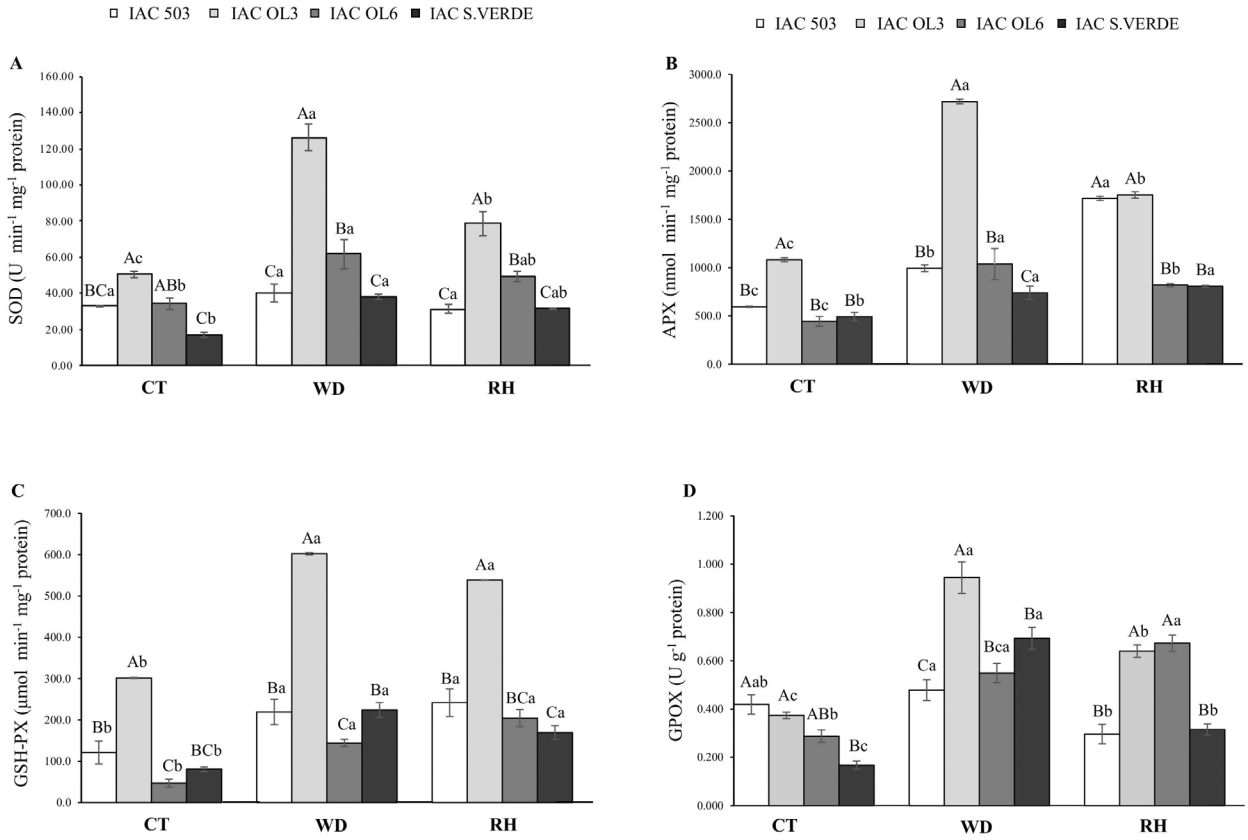

Fig. 5. Enzyme activity of SOD (A), APX (B), GSH-PX (C) and GPOX (D) in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppercase letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) compare means between conditions within the same cultivar. Error bars indicate ± SD.

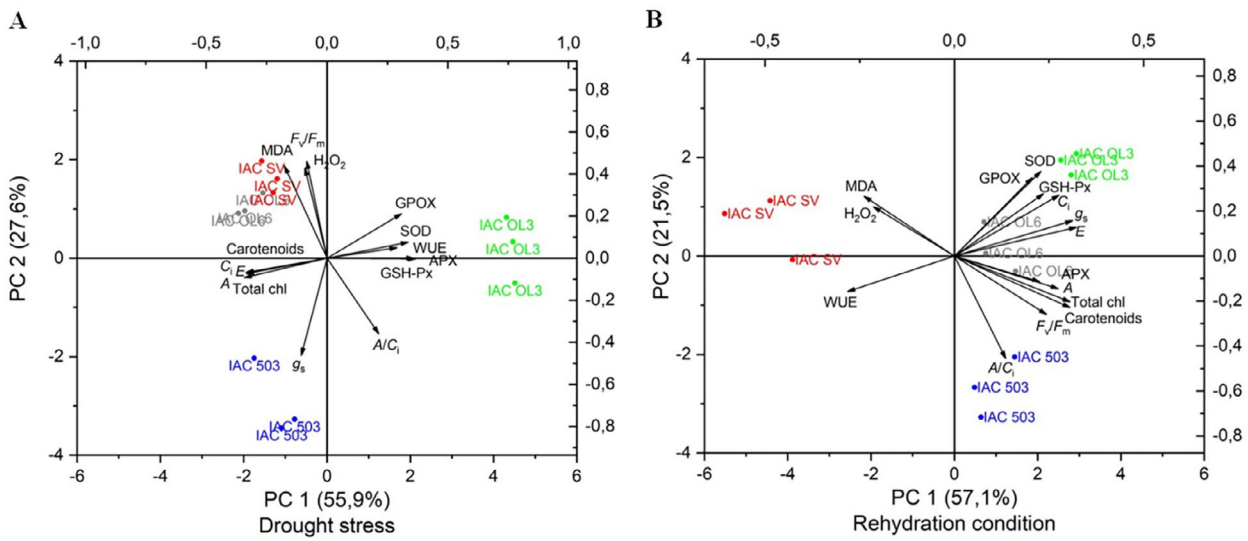

Fig. 6. Analysis of main components by differences among peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under water deficit (A) and after rehydration (B).

to stress. This reduction is a common indication of photooxidation of pigments and denaturation of chlorophyll caused by ROS synthesis in response to water deficit (Fathi & Tari, 2016; Bhalani *et al.*, 2019). On the other hand, maintenance of high total chlorophyll content in cultivars IAC OL3 and IAC OL6 under stress suggests an adaptive response of these genotypes

through regulation of chlorophyll synthesis (Sun *et al.*, 2022), also probably related to activation of protective mechanisms for photosynthetic machinery, resulting in maintenance of quantum efficiency of PSII. (F_v/F_m; Fig. 2). Similarly, Chakraborty *et al.* (2015) have reported an increase in total chlorophyll content in peanut cultivars under drought.

Water deficit also reduced carotenoid content in most cultivars (Fig. 1B), suggesting that this decrease is associated with an increase in ROS promoted by the stressful condition (Yu *et al.*, 2007; Sadak & Bakhoum, 2022), as reflected in high MDA (Fig. 3B). Our data corroborate previous studies with peanut under drought, which found a reduction in carotenoid content, with relatively lower values in tolerant cultivars (Chakraborty *et al.*, 2015; Morey *et al.*, 2021). Carotenoids have multiple functions in the photosynthetic apparatus, also acting to protect against oxidative damage (Jaleel *et al.*, 2009; Sun *et al.*, 2022). Carotenoids act as a defence against singlet oxygen ($^1O_2$), through interference with the triplet state of chlorophyll, preventing excess excitation energy transfer and consequent formation of ROS (Farooq *et al.*, 2009; Foyer & Noctor, 2011). Here, plant recovery capacity was also observed after rehydration, in which IAC 503 and IAC OL3 had restored carotenoid content to values similar to control plants, unlike IAC SV (Fig. 1B).

Gas exchange governs metabolism and biomass production in plants and is constantly affected by environmental fluctuations, being an indication of stress (Lichtenthaler & Miehé, 1997; Kaur *et al.*, 2021). In this study, damage to the photosynthetic apparatus was more significant for IAC SV, reflected by the low photochemical efficiency (Fig. 2) and *A* (Fig. 3A), which were not reestablished after irrigation. Net CO_2 assimilation is one of the parameters most sensitive to drought, especially through stomatal closure (Reddy *et al.*, 2004), as also observed here (Fig. 3B).

Pilon *et al.* (2018) reported that g_s can reflect water status in drought-stressed peanut plants. A reduction in g_s and *E* was observed in all evaluated cultivars (Fig. 3B,C), suggesting sensitization to water deficit. However, a decrease in g_s reduces CO_2 absorption and impairs photosynthetic processes in plants (Dutra *et al.*, 2015), as seen in Fig. 3A.

Changes resulting from water deficit led to reductions in g_s, *E* (in addition to *A*), C_i and A/C_i, without reestablishment after rehydration in IAC 503 and IAC SV (Fig. 3A–E). However, unlike cultivar IAC SV, F_v/F_m data from IAC 503 (Fig. 2) suggest that other adaptive mechanisms are involved and allowed photosynthetic functioning under stress (Fig. 3). Rosas-Anderson *et al.* (2014) concluded that recovery mechanisms and tolerance of peanut to drought are interdependent on leaf water maintenance and water saving and highlighted that a cultivar from the Virginia accession had limited recovery capacity after drought, although maintaining tissue hydration under stress. Drought tolerance mechanisms are crucial, not only during the stress phase but also for recovery post-rehydration (Laxa *et al.*, 2019). However, these mechanisms have high metabolic and energetic costs, as observed in antioxidant metabolism (Fig. 5B,C; APX and GSH-PX) and leaf temperature (Fig. S2), potentially limiting photosynthetic recovery, as in IAC 503 (Fig. 3A).

Some plants can resist drought stress and progressively recover photosynthetic and metabolic functions after rehydration (Lechner *et al.*, 2008). All parameters were reestablished after rehydration for IAC OL6, including F_v/F_m (Fig. 2), despite the reduction in *A*, g_s, *E* as well as A/C_i due to water deficit (Fig. 3). However, drought-tolerant cultivars tend to recover photosynthetic capacity more rapidly than those sensitive during rehydration (Rivas *et al.*, 2016). Hence, photosynthetic metabolism of IAC OL3 was little changed and there was no impairment to *A* under drought (Fig. 3A) despite decreases in g_s, *E* and C_i (Fig. 3B–D). Moreover, unlike the other cultivars, IAC OL3 had higher efficiency in instantaneous carboxylation (Fig. 3E) under stress, which allowed plants to optimize use of resources (as in *WUE*; Fig. 3F). Reviews on drought tolerance have reported that drought-adapted species maintain high *WUE* (Farooq *et al.*, 2009), corroborating the results obtained here.

Physiological responses, such as the decrease in *A* and damage to photosystems after water deficit are mainly related to high redox potential led by ROS, which are overproduced and cause oxidative stress when they exceed cellular antioxidant capacity (Gill & Tuteja, 2010; Gratão *et al.*, 2015; Kaur *et al.*, 2021). These ROS are cytotoxic and responsible for lipid peroxidation and oxidation of essential macromolecules, such as nucleic acids and proteins. Lipid peroxidation generates an increase in the MDA, an indicator of damage to membrane lipids (Fig. 4B) (Gratão *et al.*, 2015; Checchio *et al.*, 2021). In this context, there was an accumulation of H_2O_2 in most cultivars subjected to stress (Fig. 4A). Therefore, oxidative damage was verified through MDA accumulation in leaves of all cultivars under water deficit (Fig. 4B). However, despite damage caused by lipid peroxidation being higher in IAC OL6 and IAC SV, the latter was more susceptible to stress in terms of photosynthetic data. Therefore, the oxidative damage in this cultivar may have been enhanced by excess ROS production as a result of limitation to CO_2 assimilation (Fig. 3A), as well as reduced transfer of electrons in the thylakoid membranes (see low F_v/F_m; Fig. 2A), generating excess reducing power and triggering ROS (Yi *et al.*, 2016). Other studies have also reported this drought-induced ROS accumulation in peanut (Celikkol *et al.*, 2010; Chakraborty *et al.*, 2015; Bhalani *et al.*, 2019).

In contrast, despite H_2O_2 accumulation under water deficit, cultivars IAC 503 and IAC OL3 showed a reduction in H_2O_2 after rehydration, reaching values equivalent to controls (Fig. 4A). Studies have shown that plants that maintain low ROS are more tolerant to eventual stress (Chakraborty *et al.*, 2015), being strongly correlated with increased antioxidant defence systems (Bhalani *et al.*, 2019; Laxa *et al.*, 2019). As enhanced antioxidant enzyme activity can reflect plant metabolism against stress (Gratão *et al.*, 2015; Checchio *et al.*, 2021), elevated activity of SOD, APX, GPOX and GSH-PX observed here indicated differences among cultivars (Fig. 5). The antioxidant process triggered by SOD is the first barrier to oxidative stress, acting on dismutation of $O_2^{-\bullet}$ to O_2 and H_2O_2 (Gratão *et al.*, 2015), while APX, GPOX, and GSH-PX are fundamental in detoxification of accumulated H_2O_2, reducing it to water (Gill & Tuteja, 2010; Gratão *et al.*, 2015). In general, differences in response were confirmed between defence systems in cultivars after oxidative damage from water deficit. This was verified mainly as activation of antioxidants and decreased H_2O_2 and MDA after stress (except for IAC SV), showing the efficiency of these systems in removing ROS. These results are in accordance with other studies reporting modulation of enzymatic defence systems in peanut under water restriction

(Celikkol *et al.*, 2010; Chakraborty *et al.*, 2015; Furlan *et al.*, 2016; Bhalani *et al.*, 2019).

The reduction in APX activity (Fig. 5B) in IAC OL3 and IAC OL6 in the rehydration conditions seems to be related to the re-establishment of cell homeostasis, as MDA levels were reduced to values equivalent to the control. These data corroborate those of Furlan *et al.* (2016) in peanut under water absence and subsequent rehydration. The magnitude of activation of antioxidant systems and minimal change in photosynthetic parameters of IAC OL3 (Figs. 3 and 5), suggest a better ability to tolerate stress. Dysfunction in the photosynthetic apparatus and electron transport chain caused by drought result in excess production of active oxygen via the Mehler reaction (Reddy *et al.*, 2004). In other words, enhancement of defence systems under stress possibly increased dissipation of toxic species, preventing impaired photosynthetic activity (Fig. 3A), thus maintaining membrane integrity. Therefore, the high enzyme modulation is directly correlated with drought tolerance in this cultivar (Figs. 5 and 6).

There was no decline in the H_2O_2 concentration (Fig. 4A) despite activation of enzyme systems in cultivar IAC SV under drought (Fig. 5). This is probably related to excess ROS production beyond IAC SV antioxidant capacity to deal with it, making IAC SV more susceptible to stress. The range of enzymatic modulation and activated antioxidants is decisive in oxidative generated by drought (Laxa *et al.*, 2019), which requires high detoxification capacity of APX, for example (Liebthal *et al.*, 2018). When linked to significant damage to the photosynthetic apparatus (Figs. 1–3), we can infer that the rehydration for 3 days was not enough to mitigate stress damage, possibly requiring a longer recovery time for this cultivar (even though there was a slight reduction in MDA after rehydration; Fig. 4B). Non-enzymatic defence components, such as compatible osmolytes, are possibly involved in reestablishing IAC SV cell homeostasis. However, the lower H_2O_2 level correlated with better adaptation to water deficit suggests that enzymatic detoxification mechanisms were more efficient in cultivar IAC OL3, and less efficient in IAC SV (Figs. 4–6).

The above responses influence not only plant metabolism but also cultivar agronomic performance. Field studies evaluating drought tolerance from peanut cultivars selected here are ongoing. Further studies on peanut yield are essential for a broad understanding of the fine line between susceptibility and tolerance to drought.

In summary, our results showed differential drought tolerance between peanut cultivars, with activity of antioxidant metabolism being strongly correlated in cultivar IAC OL3, as seen by the PCA (Fig. 6). Therefore, the physiological and biochemical responses in stress conditions obtained in this research provide important information for improvement of peanut crops, providing a basis for selection of drought-tolerant cultivars.

CONCLUSION

Damage to plant physiology and biochemistry was more significant in cultivar IAC Sempre Verde, which was identified as the most drought sensitive. Cultivars IAC 503, IAC OL3, and IAC OL6 showed variation in physiological response correlated with their respective tolerance. The most of evaluated parameters were reestablished by rehydration, with antioxidant metabolism being critical in plant recovery. However, higher WUE and enhanced enzymatic defence were confirmed in IAC OL3cultivar, which was classified as drought tolerant. The antioxidant and physiological responses of peanut cultivars found here can aid breeding programs by identifying key traits for development of drought-tolerant plants. Further studies are needed to determine in-depth tolerance mechanisms, especially field experiments correlating physiological and biochemical data with crop yield impact.

AUTHOR CONTRIBUTIONS

The experiment was designed, performed and writed by MVC and PLG. Data collected and analyzed were conducted by MVC, ALB, WCC and GSSJ. The original manuscript was revised by ALB, PLCAA and PLG. All authors aprroved the final version and publication of the manuscript.

ACKNOWLEDGEMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a scholarship (Grant n° 156210/2019-3) (MVC)—Brazil. PLG also thanks CNPq for the research fellowship (Grant n° 303846/2021-6).

DATA AVAILABILITY STATEMENT

Available upon reasonable request.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Maximum (T Max) and minimum (T Min) temperatures and relative humidity in the greenhouse throughout the experimental period (March–May).

Fig. S2. Tleaf—Leaf temperature in peanut cultivars (IAC 503, IAC OL3, IAC OL6, IAC SV) under three water conditions (CT, WD, RH). Different uppercase letters indicate statistical differences ($P < 0.05$ Tukey test) among cultivars within the same condition. Different lowercase letters indicate statistical differences ($P < 0.05$ Tukey test) among means between conditions within the same cultivar. Error bars ± SD.

REFERENCES

Alexieva V., Sergiev I., Mapelli S., Karanov E. (2001) The effect of drought and ultravioleta radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*, **24**, 1337–1344.

Anderson J.V., Davis D.G. (2004) Abiotic stress alters transcript profiles and activity of gluthatione S-transferase, gluthatione peroxidase and gluthatione reductase in *Euphorbia esula*. *Physiologia Plantarum*, **120**, 421–433.

Anjum S.A., Ashraf U., Tanveer M., Khan I., Hussain S., Shahzad B., Zohaib A., Abbas F., Saleem M.F., Ali I., Wang L.C. (2017) Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. *Frontiers in Plant Science*, **8**, 69.

Ashraf M., Harris P.J.C. (2013) Photosynthesis under stressful environments: An overview. *Photosynthetica*, **51**, 163–190.

Ault T.R. (2020) On the essentials of drought in a changing climate. *Science*, **368**, 256–260.

Azevedo R.A., Alas R.M., Smith R.J., Lea P.J. (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-

deficient mutant of barley. *Physiologia Plantarum*, **104**, 280–292.

Bakry B.A., El-Hariri D.M., Sadak M., El-Bassiouny H. (2012) Drought stress mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil. *Journal of Applied Sciences Research*, **8**, 3503–3514.

Bakry B.A., Sabra D.E., Younis A.S.M., Sadak M. (2024) Impact of calcium carbonate and chitosan as signal molecule on modulating the negative effects of drought stress on peanut (*Arachis hypogaea* L.). *Egyptian Journal of Chemistry*, **67**, 1–12.

Bakry B.A., Sadak M.S., Al Ashkar N.M., Ibrahim O.M., Okla M.K., El-Tahan A.M. (2024) The role of carbon nanotubes in improving drought tolerance via upregulation of the physiological processes of peanut plants grown in sandy soils. *Agronomy*, **14**, 611.

Barbosa J.C., Maldonado W., Jr. (2015) *Experimentação agronômica & AgroEstat: Sistemas para Análises Estatísticas de Ensaios Agronômicos*. FCAV/UNESP, Jaboticabal, Brasil, pp 396.

Bhalani H., Thankappan R., Mishra G.P., Sarkar T., Bosamia T.C., Dobaria J.R. (2019) Regulation of antioxidant mechanisms by AtDREB1A improves soil-moisture deficit stress tolerance in transgenic peanut (*Arachis hypogaea* L.). *PLoS One*, **14**, 1–20.

Bhogireddy S., Xavier A., Garg V., Layland N., Arias R., Payton P., Nayak S.N., Pandey M.K., Puppala N., Varshney R. (2020) Genome-wide transcriptome and physiological analyses provide new insights into peanut drought response mechanisms. *Scientific Reports*, **10**, 4071.

Bradford M.M.A. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Cakmak I., Horst W.J. (1991) Effect of alumnium on lipid-peroxidation, superoxide-dismutase, catalase and peroxidase-activities in root-tips of soybean (*Glycine max*). *Physiologia Plantarum*, **834**, 463–468.

Celikkol A.U., Ercan O., Kavas M., Yildiz K., Yilmaz C., Oktem H.A., Yucel M. (2010) Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogaea* L) seedlings. *Plant Growth Regulation*, **61**, 21–28.

Chakraborty K., Singh A.L., Kalariya K.A., Goswami N., Zala P.V. (2015) Physiological responses of peanut (*Arachis hypogaea* L.) cultivars to water deficit stress: Status of oxidative stress and antioxidant enzyme activities. *Acta Botanica Croatica*, **74**, 123–142.

Checchio M.V., Alves R.C., Oliveira K.R., Moro G.V., Santos D.M.M., Gratão P.L. (2021) Enhancement of salt tolerance in corn using *Azospirillum brasilense*: An approach on antioxidant systems. *Journal of Plant Research*, **134**, 1279–1289.

Dutra F., Melo A.S., Filgueiras L.M.B., Silva A.R.F., Oliveira I.M., Brito M.E.B. (2015) Parâmetros fisiológicos e componentes de produção de feijão-caupi cultivado sob deficiência hídrica. *Revista Brasileira de Ciências Agrárias*, **10**, 189–197.

Farooq M., Wahid A., Kobayashi N., Fujita D., Basra S.M.A. (2009) Plant drought stress: Effects mechanisms and management. *Agronomy for Sustainable Development*, **29**, 185–212.

Fathi A., Tari D.B. (2016) Effect of drought stress and its mechanism in plants. *International Journal of Life Sciences*, **10**, 1–6.

Foyer C.H., Noctor G. (2011) Ascorbate and glutathione: The heart of the redox rub. *Plant Physiology*, **155**, 2–18.

Furlan A., Bianucci E., Tordable M.C., Kleinert A., Valentine A., Castro S. (2016) Dynamic responses of photosynthesis and the antioxidant system during a drought and rehydration cycle in peanut plants. *Functional Plant Biology*, **43**, 337–345.

Giannopolitis C.N., Ries S.K. (1977) Superoxide Dismutases. *Occurence in higher plants. Plant Physiology*, **59**, 309–314.

Gill S.S., Tuteja N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, **48**, 909–930.

Gratão P.L., Monteiro C.C., Tezotto T., Carvalho R.F., Alves L.R., Peter L.J., Azevedo R.A. (2015) Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. *Biometals*, **28**, 803–816.

Gupta A., Rico-Medina A., Caño-Delgado A.I. (2020) The physiology of plant responses to drought. *Science*, **368**, 266–269.

Hu L., Wang Z., Huang B. (2010) Diffusion limitations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C3 perennial grass species. *Physiologia Plantarum*, **139**, 93–106.

Jaleel C.A., Manivannan P., Wahid A., Farooq M., Al-Juburi H.J., Somasundaram R., Panneerselvam R. (2009) Drought stress in plants: A review on morphological characteristics and pigments composition. *International Journal of Agriculture and Biology*, **11**, 100–105.

Janka E., Körner O., Rosenqvist E., Ottosen C. (2015) Using the quantum yields of photosystem II and the rate of net photosynthesis to monitor high irradiance and temperature stress in chrysanthemum (*Dendranthema grandiflora*). *Plant Physiology and Biochemistry*, **90**, 14–22.

Kaur H., Kohli S.K., Khanna K., Bhardwaj R. (2021) Scrutinizing the impact of water deficit in plants: Transcriptional regulation, signaling, photosynthetic efficacy, and management. *Physiologia Plantarum*, **172**, 935–962.

Koolachart R., Jogloy S., Vorasoot N., Wongkaew S., Holbrook C.C., Jongrungklang N. (2013) Rooting traits of peanut genotypes with different yield response to terminal drought. *Field Crops Research*, **149**, 366–378.

Laxa M., Liebhtal M., Telman W., Chibani K., Dietz K. (2019) The role of the plant antioxidant system in drought tolerance. *Antioxidants*, **8**, 94.

Lechner L., Pereyra-Irujo G.A., Granier C., Aguirrezabal L.A. (2008) Rewatering plants after a long water-deficit treatment reveals that leaf epidermal cells retain their ability to expand after the leaf has apparently reached its final size. *Annals of Botany*, **101**, 1007–1015.

Lichtenthaler H.K. (1987) Chlorophylls and carotenoids; pigments of photosynthetic biomembranes. *Methods in Enzymology*, **148**, 350–382.

Lichtenthaler H.K., Miehé J.A. (1997) Fluorescence imaging as a diagnostic tool for plant stress. *Trends in Plant Science*, **2**, 316–320.

Liebthal M., Maynard D., Dietz K.J. (2018) Peroxiredoxins and redox signaling in plants. *Antioxidants & Redox Signaling*, **28**, 609–624.

Mihara M., Uchiyama M., Fukuzawa K. (1980) Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging, ccl4 intoxication, and vitamin E deficiency. *Biochemical Medicine*, **23**, 302–311.

Monteiro C.C., Carvalho R.F., Gratão P.L., Carvalho G., Tezoto T., Medici L.O., Peres L.E.P., Azevedo R.A. (2011) Biochemical responses of the ethylene-insensitive never ripe tomato mutant subjected to cadmium and sodium stresses. *Environmental and Experimental Botany*, **71**, 306–320.

Morey R., Farber C., McCutchen B., Burow M.D., Simpson C., Kurouski D., Cason J. (2021) Raman spectroscopy-based diagnostics of water deficit and salinity stresses in two accessions of peanut. *Plant Direct*, **5**, e342.

Pilon C., Snider J.L., Sobolev V., Chastain D.R., Sorensen R.B., Meeks C.D., Massa A.N., Walk T., Singh B., Earl H.J. (2018) Assessing stomatal and non-stomatal limitations to carbon assimilation under progressive drought in peanut (*Arachis hypogaea* L.). *Journal of Plant Physiology*, **231**, 124–134.

Puangbut D., Jogloy S., Vorasoot N., Akkasaeng C., Kesmalac T., Palanothai A. (2009) Variability in yield responses of peanut (*Arachis hypogaea* L.) genotypes under early season drought. *Asian Journal of Plant Sciences*, **8**, 254–264.

Reddy A.R., Chaitanya K.V., Vivekanandan M. (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, **161**, 1189–1202.

Rivas R., Falcão H.M., Ribeiro R.V., Machado E.C., Pimentel C., Santos M.G. (2016) Drought tolerance in cowpea species is driven by less sensitivity of leaf gas exchange to water deficit and rapid recovery of photosynthesis after rehydration. *South African Journal of Botany*, **103**, 101–107.

Rosas-Anderson P., Shekoofa A., Sinclair T.R., Balora M., Isleib T.G., Tallury S., Rufty T. (2014) Genetic variation in peanut leaf maintenance and transpiration recovery from severe soil drying. *Field Crops Research*, **158**, 65–72.

Sadak M.S., Bakhoum G.S. (2022) Selenium-induced modulations in growth, productivity and physiochemical responses to water deficiency in quinoa (*Chenopodium quinoa*) grown in sandy soil. *Biocatalysis and Agricultural Biotechnology*, **44**, 102449.

Sarkar T., Thankappan R., Kumar A., Mishra G.P., Dobaria J.R. (2016) Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (*Arachis hypogaea* L.) conferred tolerance to soil-moisture deficit stress. *Frontiers in Plant Science*, **7**, 935.

Sun T., Rao S., Zhou X., Li L. (2022) Plant carotenoids: Recent advances and future perspectives. *Molecular Horticulture*, **2**, 1–21.

Yang X., Luo L., Yu W., Mo B., Liu L. (2019) Recent advances in the acclimation mechanisms and genetic improvement of Peanut for drought tolerance. *Agricultural Sciences*, **10**, 1178–1193.

Yi X., Zhang Y., Yao H., Luo H., Gou L., Chow W.S., Zhang W. (2016) Rapid recovery of photosynthetic rate following soil water deficit and re-watering in cotton plants (*Gossypium herbaceum* L.) is related to the stability of the photosystems. *Journal of Plant Physiology*, **194**, 23–34.

Yu M.M., Schulze H.G., Jetter R., Blades M.W., Turner R.F. (2007) Raman microspectroscopic analysis of triterpenoids found in plant cuticles. *Applied Spectroscopy*, **61**, 32–37.

Zhao X., Li C., Wan S., Zhang T., Yan C., Shan S. (2018) Transcriptomic analysis and discovery of genes in the response of *Arachis hypogaea* to drought stress. *Molecular Biology Reports*, **45**, 119–131.