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Melatonin seed priming improves early establishment and water stress tolerance of peanut

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ABSTRACT

Water stress is a major cause of yield loss in peanut cultivation. Melatonin seed priming has been used to enhance stress tolerance in several crops, but not in peanut. We investigated the impact of seed priming with melatonin on the growth, development, and drought tolerance of two peanut cultivars, TUFRunner™ '511', a drought tolerant cultivar, and New Mexico Valencia A, a drought sensitive cultivar. Peanut seed priming tests using variable rates of melatonin (0–200 μM), indicated that 50 μM of melatonin resulted in more uniform seed germination and improved seedling growth in both cultivars under non stress conditions. Seed priming with melatonin also promoted vegetative growth, as evidenced by higher whole-plant transpiration, net CO_2 assimilation, and root water uptake under both well-watered and water stress conditions in both cultivars. Higher antioxidant activity and protective osmolyte accumulation, lower reactive oxygen species accumulation and membrane damage were observed in primed compared with non-primed plants. Seed priming with melatonin induced a growth promoting effect that was more evident under well-watered conditions for TUFRunnner™ '511', whereas for New Mexico Valencia A, major differences in physiological responses were observed under water stress conditions. New Mexico Valencia A primed plants exhibited a more sensitized stress response, with faster down-regulation of photosynthesis and transpiration compared with non-primed plants. The results demonstrate that melatonin seed priming has significant potential to improve early establishment and promote growth of peanut under optimal conditions, while also improve stress tolerance during water stress.

1. Introduction

Drought stress can have a devastating impact on peanut production (Kottapalli et al., 2009; Rowland et al., 2012; Bhogireddy et al., 2020). Although peanut is generally considered to be drought-tolerant, unpredictable rainfall patterns and limited availability of groundwater for irrigation in some production areas has raised concerns about the profitability of peanut cultivation in the United States (Abou Kheira, 2009; Rowland et al., 2012; Zurweller et al., 2018b; Bhogireddy et al., 2020). Several strategies have emerged to enhance water stress tolerance in crops. Some approaches target optimizing irrigation practices and environmental management, while others concentrate on enhancing crop stress tolerance through plant breeding (Costa et al., 2007; Ben Abdallah et al., 2017; Dalal et al., 2019; Moshelion, 2020). Recently, increasing emphasis has been placed on manipulating plant physiological processes by stress priming to improve crop water use, stress

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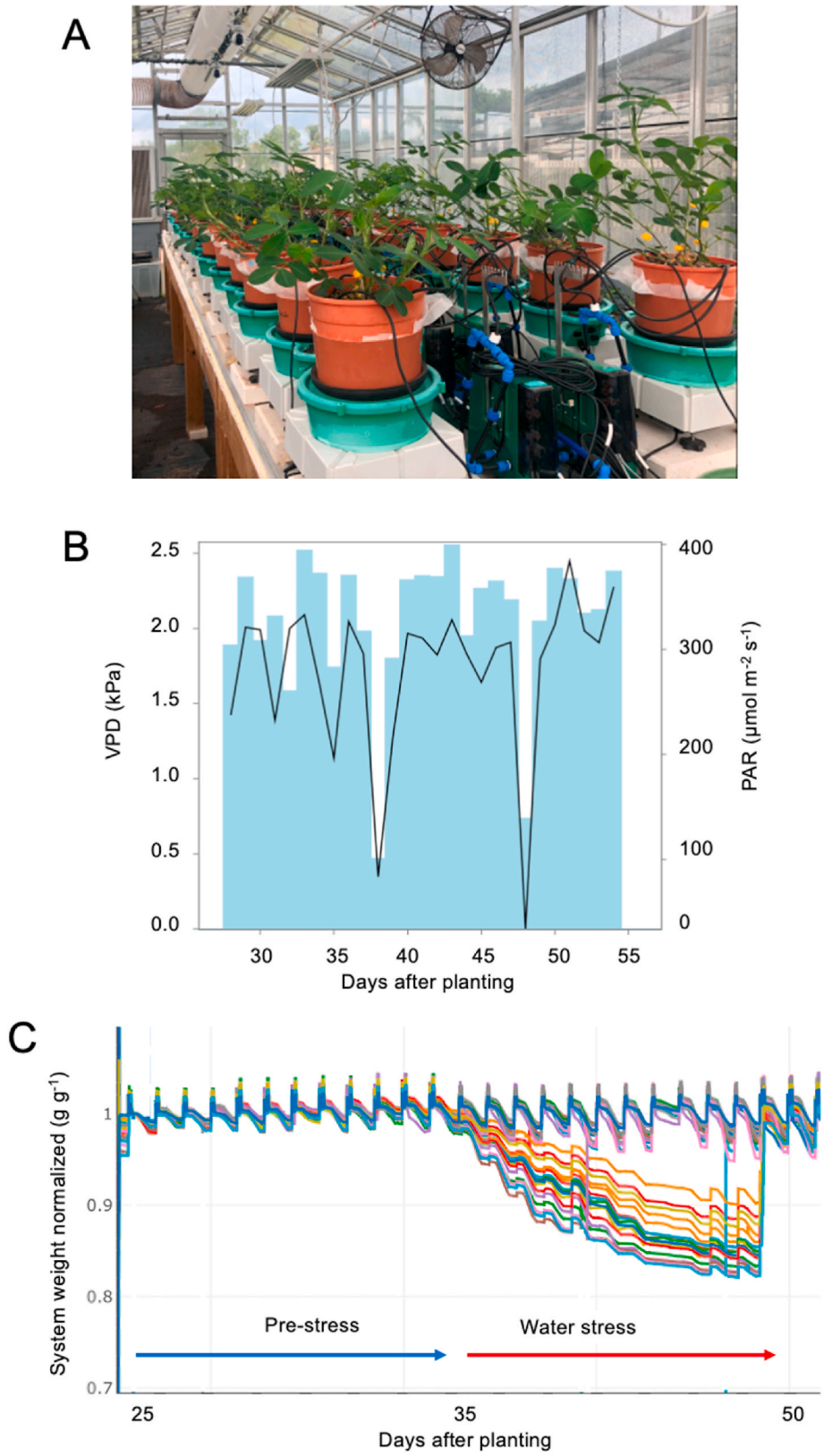

Fig. 1. Experimental setup of the high-throughput phenotyping system (Plant DiTech), and environmental and experimental conditions. A) Image of peanut plants growing on the Plant-DiTech system during the seed priming experiment, B) Daily vapor pressure deficit (VPD) and photosynthetically active radiation (PAR) during the seed priming experiment, C) Normalized system weight during pre-stress and water stress periods. Each line represents a single plant replicate.

Table 1
Initial seed weight, radicle length, and germination percentage of seven-day-old seedlings of New Mexico Valencia A and TUFRunner™ '511' peanuts treated with varying rates of melatonin (0–200 μM).

	Melatonin rate	Initial seed weight	Radicle length	Germination
New Mexico Valencia A	0	0.46 ± 0.05 ns,z,y	13.29 ± 4.13 B	40 ± 16.73 B
	50	0.46 ± 0.05	26.76 ± 4.13 A	80 ± 16.73 AB
	100	0.39 ± 0.05	18.83 ± 4.13 AB	100 ± 16.73 A
	150	0.47 ± 0.05	20.55 ± 4.13 AB	80 ± 16.73 AB
	200	0.51 ± 0.05	21.27 ± 4.13 AB	60 ± 16.73 AB
TUFRunner™ '511'	0	0.86 ± 0.05 AB	23.55 ± 4.13 ns	80 ± 16.73 ns
	50	0.92 ± 0.05 A	31.52 ± 4.13	100 ± 16.73
	100	0.82 ± 0.05 AB	29.79 ± 4.13	100 ± 16.73
	150	0.83 ± 0.05 AB	24.24 ± 4.13	100 ± 16.73
	200	0.75 ± 0.05 B	24.46 ± 4.13	80 ± 16.73

z Least square means ± SE. Means followed by different letters are significantly different among melatonin rates within each cultivar at a $P \leq 0.05$ according to Fisher's LSD test.
ns Indicates no significant difference ($P > 0.05$).
y Units: Initial seed weight = g seed^{-1}, radicle length = mm, germination = %.

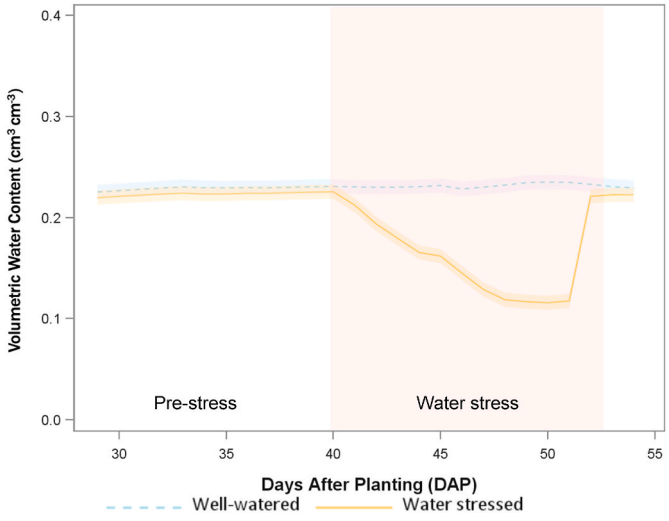

Fig. 2. Volumetric water content during pre-stress, and water stress periods. Values are least square means ± confidence intervals.

tolerance, and resilience in agricultural systems (Balmer et al., 2015; Hilker and Schmulling, 2019; Moshelion, 2020; Vincent et al., 2020).

Several factors play a role in the success of priming techniques in stress acclimation and mitigation of abiotic stress impacts on plant growth and yield. Seed priming has been effective in altering physiological responses to achieve faster and more uniform initial plant establishment, which is positively correlated with improved crop yield (Boukari et al., 2019; Heshmati et al., 2021; Johnson and Puthur, 2021; Rhaman et al., 2021). Seed priming can be achieved with the use of natural or synthetic compounds known to be involved in the activation or amplification of stress defense responses in plants (Savvides et al., 2016; Sako et al., 2020; Rhaman et al., 2021; Guzmán et al., 2022). When applied to seeds, these chemicals enhance stress tolerance via a process called cross-tolerance or cross-priming, in which the exposure to one stress or biochemical cue during priming triggers tolerance mechanisms to subsequent stresses (Bilichak and Kovalchuk, 2016; Hossain et al., 2018; Zhang et al., 2019; Johnson and Puthur, 2021).

Melatonin (N-acetyl-5-methoxytryptamine) is a regulatory molecule found in most plants, with concentrations that vary according to plant species, cultivar within a species, growth stage, plant organ, and environmental conditions where the plant is grown (Janas and Posmyk, 2013; Arnao and Hernandez-Ruiz, 2014; Sharif et al., 2018). Melatonin's role in plants as a natural antioxidant and growth regulator has increased the interest in its application for stress priming, and under non-stress conditions, the benefits of seed priming with melatonin extends to improving photosynthetic activity and promoting growth (Arnao and Hernandez-Ruiz, 2014; Sharif et al., 2018; Agathokleous et al., 2021; Ahmad et al., 2023).

The efficiency of melatonin as an antioxidant is partially attributed to its amphiphilic nature, which allows protection against oxidative stress in different plant organelles and structures (Arnao and Hernandez-Ruiz, 2014; Sharif et al., 2018; Agathokleous et al., 2021; Rajora et al., 2022). Melatonin can enhance the efficiency of redox balance and protect cells against oxidative stress damage by improving plant homeostasis and biomass production during stress (Savvides et al., 2016; Sharif et al., 2018; Agathokleous et al., 2021; Ahmad et al., 2023; Rafique et al., 2023; Zulfiqar et al., 2024). Melatonin-primed seeds can have higher germination rates, more uniform crop establishment, and improved seed vigor and seedling viability under ideal to unfavorable environmental conditions in canola (*Brassica napus*), corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.), safflower (*Carthamus tinctorius* L.), soybean (*Glycine max* L.), *Stevia rebaudiana*, triticale (*Triticale hexaploide* L.), and wheat (*Triticum aestivum* L.) due to increased activity of antioxidant enzymes and sugar and starch metabolism during germination (Wei et al., 2015; Balabusta et al., 2016; Simlat et al., 2018; Cao et al., 2019; Heshmati et al., 2021; Guo et al., 2022; Rafique et al., 2023).

Although melatonin's application for agricultural use might still present some challenges related to economic viability, new techniques for microbial synthesis of melatonin have shown high potential for commercial production (Gao et al., 2022). Furthermore, seed priming with melatonin can be a cost-effective approach for managing crop stress, since it generally utilizes compounds applied at minimal rates (Antoniou et al., 2016; Savvides et al., 2016; Sako et al., 2020; Rhaman et al., 2021; Rafique et al., 2023), while recent findings also highlight the potential of smart delivery of priming agents such as melatonin through the use of nanocarriers, requiring reduced overall chemical usage (Gohari et al., 2024). Nevertheless, preliminary research is necessary to identify the application rates of particular compounds, and application timing/frequency to best prime plants, and promote stress acclimation (Guzmán et al., 2022; Balmer et al., 2015; Filippou et al., 2013; Hilker et al., 2016; Johnson and Puthur, 2021; Sako et al., 2020).

The present study focused on addressing the general lack in understanding of the effects of seed priming using melatonin in peanut and whether melatonin priming resulted in any measurable physiological effects. While most studies have focused on early developmental stages, we also focused on characterizing the residual effects of melatonin on stress occurring later in the crop cycle, specifically at early flowering, a very vulnerable physiological stage for peanut plants to endure water stress (Abou Kheira, 2009; Jongrungklang et al., 2011). We hypothesized that seed priming with melatonin can enhance drought tolerance in peanut in early and subsequent developmental stages via improved antioxidant activity, and in the absence of stress (adequate water conditions), seed priming can benefit the plants as a biostimulant by improving overall crop performance and promoting growth.

2. Materials and methods

2.1. Physiological phenotyping platform and experimental setup

Greenhouse studies were conducted in 2022 at the University of

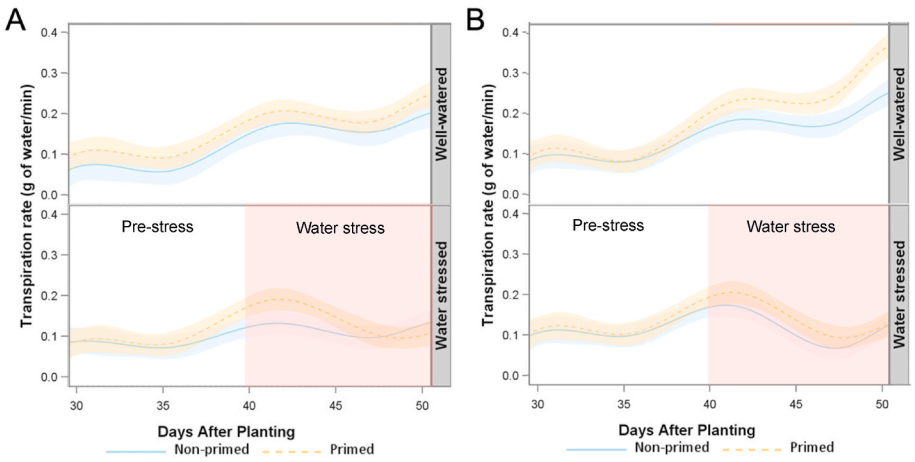

Fig. 3. Midday transpiration rate of melatonin-primed and non-primed New Mexico Valencia A (A) and TUFRunner™ '511' (B) peanut plants, during pre-stress and water stress. Values are least square means ± 95% confidence intervals.

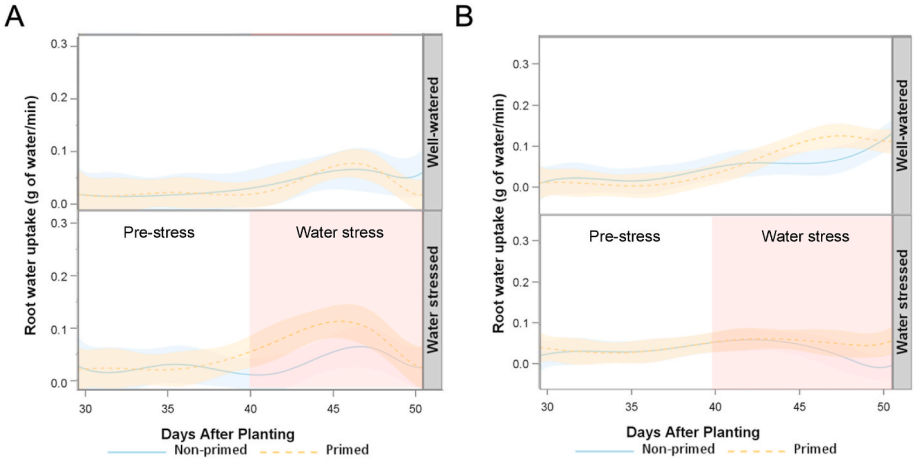

Fig. 4. Root water uptake of melatonin-primed and non-primed New Mexico Valencia A (A) and TUFRunner™ '511' (B) peanut plants, during pre-stress and water stress. Values are least square means ± 95% confidence intervals.

Florida, Tropical Research and Education Center in Homestead, Florida. A high-throughput physiological phenotyping platform (Plant-DiTech Ltd., Yavne, Israel; PDT) was used to continuously monitor the plants' performance, and to precisely schedule and apply irrigation treatments. A detailed description of the PDT phenotyping platform and its functionality is provided by Halperin et al. (2017). The PDT system is an integrated array of 3.9-L pots placed on individual highly sensitive, temperature–compensated load cells used as lysimeters (Tadea-Huntleigh, model 1042 C4; Vishay Intertechnology, Malvern, PA, USA) (Fig. 1a). A soil water sensor (model 5 TE, Meter Group, Pullman, WA, USA) was incorporated into each pot for monitoring soil water content, temperature, and electrical conductivity. Each unit was connected to a controller (model CR1000 data logger, Campbell Scientific, Logan, UT, USA), which continuously collected data (every 3 min) and controlled the irrigation treatment for each plant individually. All the controllers were interconnected, and the data was recorded and sent to a server in real-time (Plant-Ditech Ltd., Yavne, Israel). The data was retrieved using SPAC-analytics (Plant-Ditech Ltd., Yavne, Israel), an online web-based software that enables real-time evaluation of the data collected from the PDT system. The system was installed in a climate-controlled greenhouse with a weather station (WatchDog 2800 Weather Station, Spectrum Technologies, Inc., Aurora, IL, USA), that continuously monitored and recorded the daily variations in photosynthetically active radiation (PAR), and vapor pressure deficit (VPD) (Fig. 1b). During the experiments, the temperature ranged from 24 to 32 °C and the relative humidity ranged from 70 to 90%.

The experimental setup was based on Halperin et al. (2017) with some modifications. Briefly, before the start of the experiments, all load-cell units were calibrated, and the initial weight of the system components (drainage container, pot, soil probe, irrigation drippers, substrate, and plastic beads used to prevent evaporation from the substrate) were recorded. A 1:1 (v/v) mixture of Turface MVP® and Turface Profile Greens Grade® (Profile Products LLC, Buffalo Grove, IL, USA), an inert calcined clay oven-dried for 48 h, was used as the growing medium. The gravimetric water content of the saturated substrate was 0.77 g g^{-1} or 77%, and 0.60 g g^{-1} or 60% after drainage (field capacity). The volumetric water content (VWC) of the saturated Turface was between 0.30 and 0.35 cm^3 cm^{-3}, and 0.20–0.28 cm^3 cm^{-3} after drainage, which translated to a matric potential of −10 to −14 kPa, and −15 to −18 kPa, respectively for the saturated and drained substrate. A layer of 300 cm^3 of 6 mm plastic beads was placed on top of the substrate to prevent any water loss by soil evaporation, thus enabling water mass balance calculations and accurate estimates of plant transpiration. The irrigation regime was programmed to occur during the night to avoid confounding effects with the plants water use during the day, between 2300 and 0200h, in 2–3 sequential pulses scheduled every 30 min. Plants were irrigated with a fertigation solution consisting of a modified Ruakura nutrient solution using Peter's Excel 15-5-15 CAL-MAG (Everris NA Inc.,

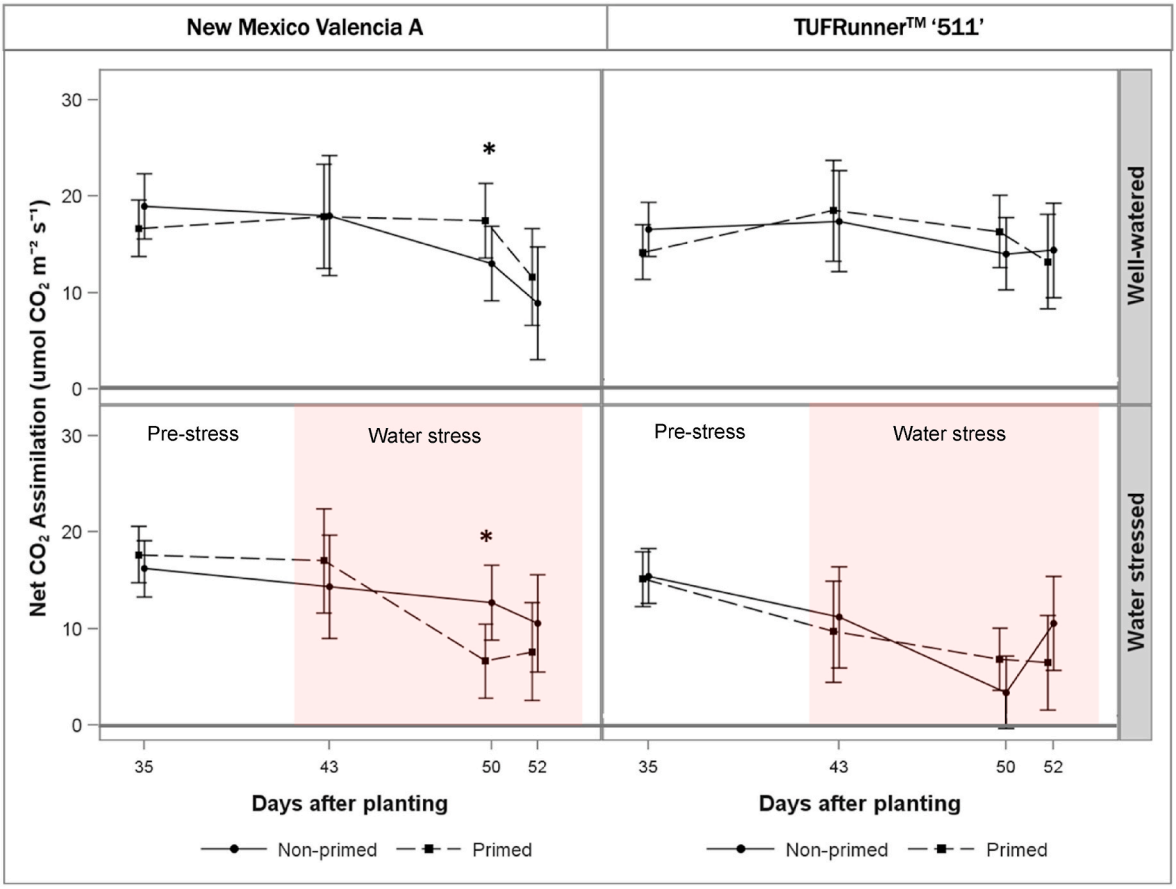

Fig. 5. Leaf net CO_2 assimilation (Leaf-*A*) of non-primed and primed peanut plants displayed by cultivar, New Mexico Valencia A and TUFRunner™ '511', during pre-stress and water stress. Measurements were made on the same day for each time point with values offset between primed and non-primed for each measurement to avoid overlap of bars. Values are least square means ± 95% confidence intervals.

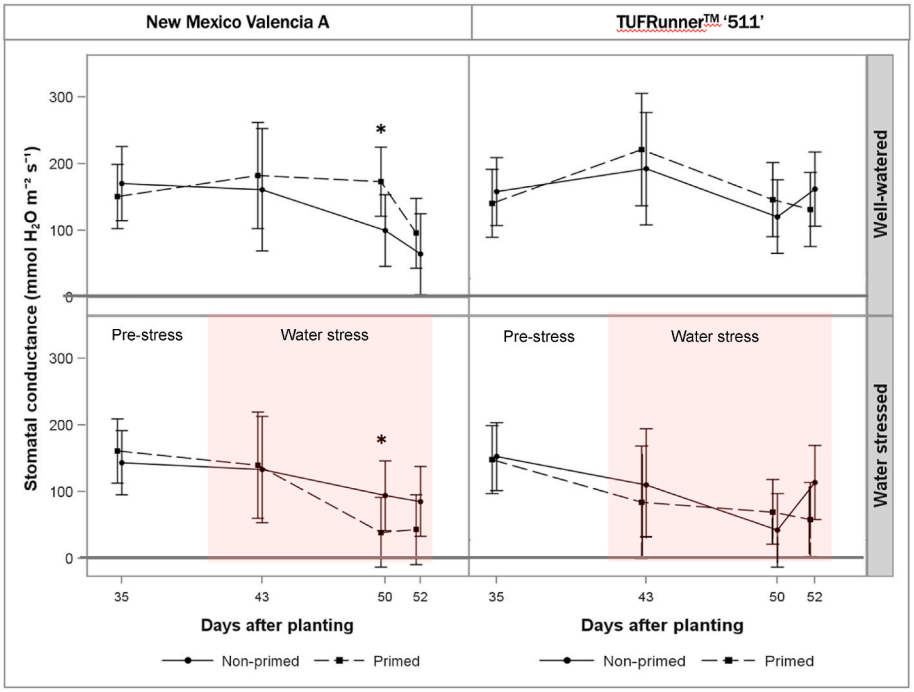

Fig. 6. Leaf stomatal conductance (Leaf-g_s) of non-primed and primed peanut plants displayed by cultivar, New Mexico Valencia A and TUFRunner™ '511', during pre-stress and water stress. Measurements were made on the same day for each time point with values offset between primed and non-primed for each measurement to avoid overlap of bars. Values are least square means ± 95% confidence intervals.

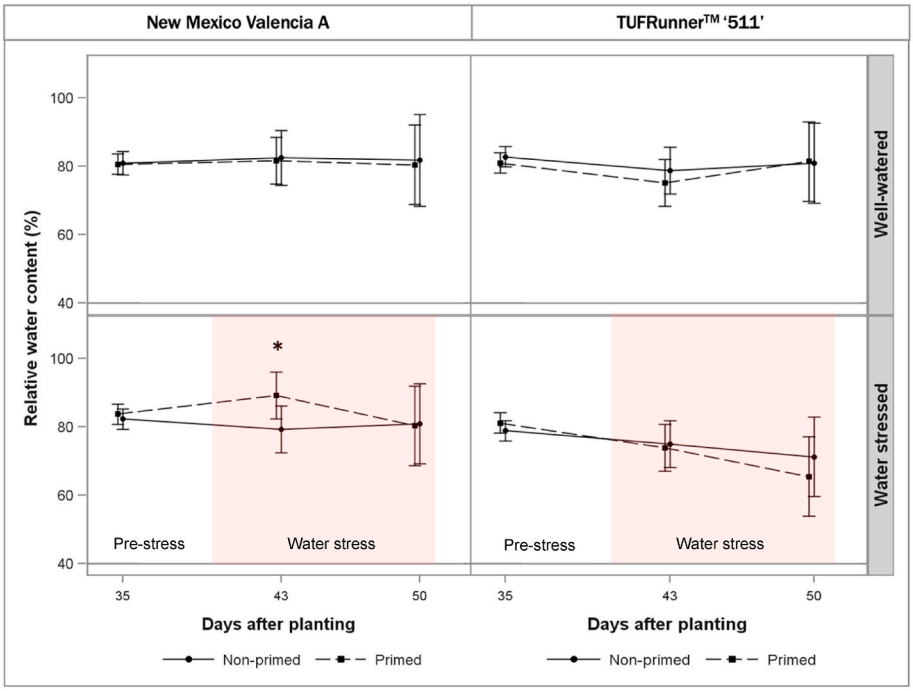

Fig. 7. Leaf relative water content (Leaf-*RWC*) of non-primed and primed peanut plants displayed by cultivar, New Mexico Valencia A and TUFRunner™ '511', during pre-stress and water stress. Measurements were made on the same day for each time point with values offset between primed and non-primed for each measurement to avoid overlap of bars. Values are least square means ± 95% confidence intervals.

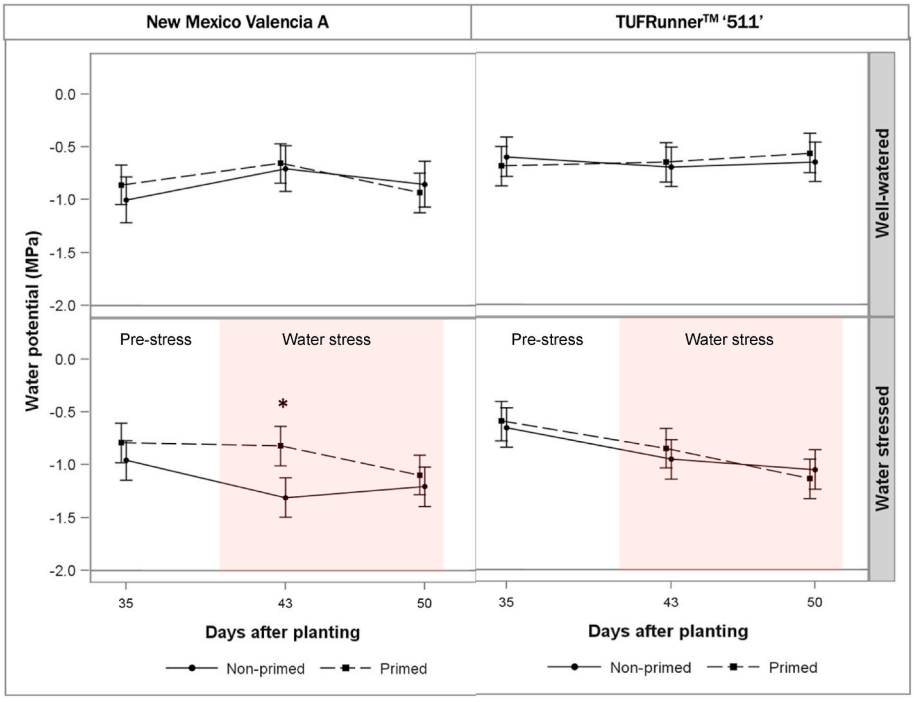

Fig. 8. Leaf water potential (Leaf-Ψ_W) of non-primed and primed peanut plants displayed by cultivar, New Mexico Valencia A and TUFRunner™ '511', during pre-stress and water stress. Measurements were made on the same day for each time point with values offset between primed and non-primed for each measurement to avoid overlap of bars. Values are least square means ± 95% confidence intervals.

Geldermalsen, The Netherlands) containing micronutrients and ammonium sulfate (Smith et al., 1983). During water stress, plants in the control treatment received water for irrigation.

2.2. Plant material, experimental design, and treatments

2.2.1. Melatonin rates study and seed priming protocol

Peanut seeds of New Mexico Valencia A [(Hsi and Finkner, 1972); Reg. No. 14] and TUFRunner™ '511' [(Tillman and Gorbet, 2017); Reg. No. CV-131, PI 674432] were used in a study to identify the most

Table 2
Antioxidant enzymatic activities, lipid peroxidation, reactive oxygen species (ROS), and protective osmolyte concentrations of primed and non-primed of New Mexico Valencia A and TUFRunner™ '511' peanut plants at early stress, 40 days after planting (DAP).

	New Mexico Valencia A				TUFRunner™ '511'				Analysis of Variance						
	Well-watered		Water-stressed		Well-watered		Water-stressed		IR	P	IR*P	CV	CV*IR	CV*P	CV*IR*P
Antioxidants	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed							
APX[a]	2.68 ± 0.24[b]	4.14 ± 0.21	3.53 ± 0.21	4.55 ± 0.21	4.63 ± 0.21	5.81 ± 0.21	6.37 ± 0.21	7.60 ± 0.21	***[c]	***	ns	***	**	ns	ns
CAT	0.08 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.21 ± 0.01	0.30 ± 0.01	***	***	ns	***	**	ns	ns
DHAR	0.14 ± 0.02	0.25 ± 0.02	0.35 ± 0.02	0.45 ± 0.02	0.24 ± 0.02	0.38 ± 0.02	0.48 ± 0.02	0.66 ± 0.02	***	***	ns	***	ns	ns	ns
GPX	2.21 ± 0.18	3.41 ± 0.16	3.54 ± 0.16	4.32 ± 0.16	3.87 ± 0.16	4.60 ± 0.16	5.31 ± 0.16	6.04 ± 0.16	***	***	ns	***	ns	ns	ns
GTR	1.23 ± 0.18	2.03 ± 0.15	2.21 ± 0.15	3.92 ± 0.15	2.62 ± 0.15	3.82 ± 0.15	3.50 ± 0.15	4.98 ± 0.15	***	***	ns	***	ns	**	*
MDHAR	0.14 ± 0.02	0.32 ± 0.02	0.36 ± 0.02	0.45 ± 0.02	0.29 ± 0.02	0.43 ± 0.02	0.48 ± 0.02	0.71 ± 0.02	***	***	ns	***	*	ns	**
POD	0.25 ± 0.03	0.37 ± 0.03	0.34 ± 0.03	0.45 ± 0.03	0.54 ± 0.03	0.65 ± 0.03	0.63 ± 0.03	0.90 ± 0.03	***	***	ns	***	ns	ns	ns
SOD	1.81 ± 0.22	2.57 ± 0.19	3.17 ± 0.19	3.96 ± 0.19	3.67 ± 0.19	3.80 ± 0.19	4.92 ± 0.19	6.01 ± 0.19	***	**	ns	***	ns	ns	ns
Lipid peroxidation indicator															
MDA	1.36 ± 0.17	1.28 ± 0.15	6.20 ± 0.15	3.69 ± 0.15	0.86 ± 0.15	1.09 ± 0.15	4.17 ± 0.15	2.19 ± 0.15	***	***	***	***	***	ns	ns
Reactive oxygen species															
O_2	1.91 ± 0.17	1.82 ± 0.15	5.66 ± 0.15	4.19 ± 0.15	2.02 ± 0.15	1.78 ± 0.15	3.47 ± 0.15	2.37 ± 0.15	***	***	***	***	***	**	*
H_2O_2	6.49 ± 0.64	6.00 ± 0.55	16.92 ± 0.55	10.04 ± 0.55	3.70 ± 0.55	3.38 ± 0.55	7.88 ± 0.55	4.37 ± 0.55	***	***	**	***	***	ns	ns
Protective osmolytes															
PRO	0.78 ± 0.14	1.31 ± 0.13	1.59 ± 0.13	2.27 ± 0.13	1.20 ± 0.13	1.90 ± 0.13	2.24 ± 0.13	3.46 ± 0.13	***	***	*	***	**	**	ns
GB	1.25 ± 0.23	1.96 ± 0.20	1.40 ± 0.20	3.29 ± 0.20	2.29 ± 0.20	3.84 ± 0.20	2.60 ± 0.20	4.39 ± 0.20	**	***	*	***	ns	ns	ns

[a] Ascorbate peroxidase (APX) (μmol H_2O_2 g^{-1} fresh weight), Catalase (CAT) (units mg^{-1} protein), Dehydroascorbate reductase (DHAR) (μmol min^{-1} mg^{-1} protein), Guaiacol peroxidase (GPX) (μmol H_2O_2 g^{-1} fresh weight), Glutathione reductase (GTR) (units g^{-1} fresh weight), Monodehydroascorbate reductase (MDHAR) (μmol min^{-1} mg^{-1} protein), Peroxidase (POD) (units mg^{-1} protein), Superoxide dimustase (SOD) (units mg^{-1} protein), Malondialdehyde (MDA) (μmol MDA ml^{-1} g^{-1} dry weight), Oxide (O_2) (μmol min^{-1} g^{-1} protein), Hydrogen peroxide (H_2O_2) (μmol min^{-1} g^{-1} protein), Proline (PRO) (units mg^{-1} protein), Glycine betaine (GB) (units mg^{-1} protein), Irrigation treatment (IR), Priming treatment (P), Cultivar (CV).

[b] Least square means ± SE.

[c] P-value ≤ * 0.05, ** 0.001, *** <0.0001 and [ns] non-significant for main effects and their interactions.

effective rate of melatonin that could alter seedling growth and thus potentially prime peanut seeds. Preliminary trials were performed to define whether the control treatment would be a non-treated unprimed seed or hydroprimed seed (no melatonin) as a solvent control. Since we did not find any significant differences in seedling establishment between unprimed and hydroprimed with distilled water seeds (data not shown), we proceeded with hydroprimed seeds in water as the control, non-primed seeds. The seeds were surface sterilized with 0.2% sodium hypochlorite for 5 min, rinsed in deionized water for 30 s, and then soaked in a solution of either only distilled water (control, non-primed) or distilled water with increasing rates of melatonin (N-acetyl-5-methoxytryptamine; Sigma-Aldrich, Burlington, MA, USA). The treatments consisted of 0 μM (control – distilled water only, non-primed), 50, 100, 150, and 200 μM of melatonin, with fifteen replicates per treatment. The ratio of seed weight to solution volume (w/v) was 1:5, and the seeds were primed for 12 h at 25 °C in the dark with constant mild agitation. Subsequently, the seeds were gently dried and transferred to a laminar flow hood (EdgeGARD model EG-4252, The Baker Co. Inc. Sanford, ME, USA) at 28 ± 2 °C for 48–72 h to decrease the seed moisture content before planting. Measurements included initial seed weight, radicle length and germination percentage after seven days of planting (DAP).

2.2.2. Seed priming effects on water stress tolerance

Non-primed and primed seeds of New Mexico Valencia A and TUFRunner™ '511' were planted on June 08, 2022. The experiment consisted of a 2 × 2 x 2 factorial randomized complete block design with four replicates. Each cultivar and priming combination were assigned to a no water stress treatment (well-watered conditions during the entire experiment), or water stress treatment at early flowering (from approximately 35 to 50 DAP), a physiological stage that generally has significant impact on pod yield (Fig. 1c). Well-watered plants received daily irrigation consisting of 110% of the previous day's transpiration to restore the substrate to full saturation and account for increases in growth and water use. Water-stressed plants received similar irrigation as well-watered plants up to 35 DAP, but then exposed to water stress by limiting irrigation to 20% of the previous day transpiration until plants in the water stress treatment were transpiring less than 30% of plants in the well-watered treatment.

2.2.3. Physiological assessment

2.2.3.1. Whole plant continuous measurements. Whole plant continuous measurements were obtained from the lysimeter-based Plant-Ditech system. Daily transpiration for each plant was determined by calculating the difference between the system's start and end-of-the-day reference points obtained for each individual lysimeter. These reference points were obtained by averaging the lysimeter's readings over a 30-min period, between 05:00 and 05:30h for the start-of-the-day, and between 21:00 and 21:30h for the end-of-the-day. Whole-plant transpiration (plant-*Tr*) and root water uptake were calculated from the lysimeter and soil sensor readings (according to the substrate volume) temporal series as described by Halperin et al. (2017). In short, those variables

Table 3
Antioxidant enzymatic activities, lipid peroxidation, reactive oxygen species (ROS), and protective osmolytes concentrations of primed and non-primed New Mexico Valencia A and TUFRunner™ '511' peanut plants at late stress, 50 days after planting (DAP).

	New Mexico Valencia A				TUFRunner™ '511'				Analysis of Variance						
	Well-watered		Water-stressed		Well-watered		Water-stressed		IR	P	IR*P	CV	CV*IR	CV*P	CV*IR*P
Antioxidants	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed							
APX[a]	3.14 ± 0.24[b]	3.95 ± 0.21	3.98 ± 0.21	5.82 ± 0.21	5.24 ± 0.21	6.05 ± 0.21	5.68 ± 0.21	8.29 ± 0.21	***[c]	***	***	***	ns	ns	ns
CAT	0.10 ± 0.02	0.14 ± 0.01	0.16 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.32 ± 0.01	0.41 ± 0.01	0.47 ± 0.01	***	***	ns	***	***	ns	ns
DHAR	0.22 ± 0.02	0.29 ± 0.02	0.38 ± 0.02	0.55 ± 0.02	0.32 ± 0.02	0.50 ± 0.02	0.57 ± 0.02	0.81 ± 0.02	***	***	*	***	*	**	ns
GPX	3.07 ± 0.18	3.20 ± 0.16	3.56 ± 0.16	5.21 ± 0.16	4.70 ± 0.16	5.06 ± 0.16	4.92 ± 0.16	6.47 ± 0.16	***	***	***	***	*	ns	ns
GTR	1.29 ± 0.18	2.25 ± 0.15	2.47 ± 0.15	4.13 ± 0.15	3.19 ± 0.15	4.03 ± 0.15	5.23 ± 0.15	5.74 ± 0.15	***	***	**	***	ns	ns	ns
MDHAR	0.18 ± 0.02	0.40 ± 0.02	0.49 ± 0.02	0.64 ± 0.02	0.40 ± 0.02	0.62 ± 0.02	0.65 ± 0.02	0.71 ± 0.02	***	***	**	***	**	ns	ns
POD	0.31 ± 0.03	0.34 ± 0.03	0.43 ± 0.03	0.81 ± 0.03	0.66 ± 0.03	0.76 ± 0.03	0.91 ± 0.03	1.00 ± 0.03	***	***	***	***	ns	**	***
SOD	3.17 ± 0.22	3.83 ± 0.19	3.39 ± 0.19	5.01 ± 0.19	4.65 ± 0.19	5.37 ± 0.19	4.80 ± 0.19	6.89 ± 0.19	***	***	***	***	ns	ns	ns
Lipid peroxidation indicator															
MDA	1.67 ± 0.17	1.69 ± 0.15	7.39 ± 0.15	3.94 ± 0.15	1.23 ± 0.15	1.19 ± 0.15	4.32 ± 0.15	2.58 ± 0.15	***	***	***	***	***	**	**
Reactive oxygen species															
O_2	2.60 ± 0.17	2.01 ± 0.15	6.81 ± 0.15	3.82 ± 0.15	2.02 ± 0.15	1.91 ± 0.15	4.17 ± 0.15	2.19 ± 0.15	***	***	**	***	**	**	ns
H_2O_2	8.61 ± 0.64	4.49 ± 0.55	20.18 ± 0.55	10.29 ± 0.55	5.36 ± 0.55	2.52 ± 0.55	11.36 ± 0.55	5.88 ± 0.55	***	***	***	***	***	**	ns
Protective osmolytes															
PRO	1.61 ± 0.13	2.48 ± 0.13	3.89 ± 0.13	4.52 ± 0.13	2.35 ± 0.13	3.39 ± 0.13	4.56 ± 0.13	5.13 ± 0.13	***	***	ns	***	ns	ns	ns
GB	1.44 ± 0.23	2.04 ± 0.20	3.30 ± 0.20	3.84 ± 0.20	2.61 ± 0.20	2.96 ± 0.20	4.47 ± 0.20	5.02 ± 0.20	***	**	ns	***	ns	ns	ns

[a] Ascorbate peroxidase (APX) (μmol H_2O_2 g^{-1} fresh weight), Catalase (CAT) (units mg^{-1} protein), Dehydroascorbate reductase (DHAR) (μmol min^{-1} mg^{-1} protein), Guaiacol peroxidase (GPX) (μmol H_2O_2 g^{-1} fresh weight), Glutathione reductase (GTR) (units g^{-1} fresh weight), Monodehydroascorbate reductase (MDHAR) (μmol min^{-1} mg^{-1} protein), Peroxidase (POD) (units mg^{-1} protein), Superoxide dimustase (SOD) (units mg^{-1} protein), Malondialdehyde (MDA) (μmol MDA ml^{-1} g^{-1} dry weight), Oxide (O_2) (μmol min^{-1} g^{-1} protein), Hydrogen peroxide (H_2O_2) (μmol min^{-1} g^{-1} protein), Proline (PRO) (units mg^{-1} protein), Glycine betaine (GB) (units mg^{-1} protein), Irrigation treatment (IR), Priming treatment (P), Cultivar (CV).

[b] Least square means ± SE.

[c] P-value ≤ * 0.05, ** <0.001, *** <0.0001 and [ns] non-significant for main effects and their interactions.

were determined by multiplying the derivative of the initial recorded measurement time series by −1. The average of measurements recorded from 11h00–13h00 were used to calculate the midday transpiration rate and root water uptake.

2.2.3.2. Single-leaf periodic measurements. Leaf gas exchange [net CO_2 assimilation (leaf-*A*), transpiration (leaf-*Tr*), and stomatal conductance of water vapor (leaf-g_s)], leaf water potential (leaf-Ψ_w), and leaf relative water content (leaf-*RWC*) were measured on the leaf at the second nodal position of each plant. All variables were measured at critical time points of the experiment, defined as pre-stress (five days prior to the application of water stress), early stress (five days after the stress treatment was first imposed), late stress (ten to twelve days after the stress treatment was firsts imposed).

Leaf gas exchange was measured with a portable infrared gas analyzer (CIRAS-3, PP Systems, Amesbury, MA, USA) from 1000 to 1200 h, in the same day under similar light conditions for all treatments, with the reference CO_2 concentration set to 390 μmol mol^{-1}, and a light-saturated photosynthetic photon flux of 1000 μmol quanta m^{-2} s^{-1}.

Leaf-*RWC* was measured according to Barrs and Weatherley (1962) by collecting one leaf disc (0.785 cm^2) per plant from 1100 to 1200 h, measuring the fresh weight, submerging the disc in deionized water for 24 h, reweighing, and oven-drying the disc at 70 °C for 24 h. The oven-dried leaf disc weight was then determined, and the leaf-*RWC* was calculated as:

$$\%RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{ x } 100 \quad (4\text{-}1)$$

Leaf-Ψ_w was measured in leaves sampled from 1100 to 1200 h. Water potential was measured using a Scholander-type pressure chamber (model 1515D, PMS Instrument Company, Albany, OR, USA).

2.2.3.3. Biochemical analyses. Antioxidant activities, lipid peroxidation, reactive oxygen species (ROS) levels, and protective osmolyte concentrations were determined in three leaves collected at the second nodal position of each plant in early and late stress, at 40 and 50 DAP, respectively. After collection, leaves were stored in aluminum foil and immediately placed in a cooler with liquid nitrogen until transfer to more permanent storage in a −80 °C freezer. Samples were analyzed at the University of Florida, North Florida Research and Education Center (Quincy, FL, USA). Biochemical analyses included antioxidant activities of ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), guaiacol peroxidase (GPX), glutathione reductase (GTX), monodehydroascorbate reductase (MDAR), peroxidase (POD), and superoxide dismutase (SOD); concentrations of the lipid peroxidation indicator malondialdehyde (MDA); ROS: superoxide (O_2^-) and hydrogen peroxide (H_2O_2); and protective osmolytes proline (PRO) and glycine betaine (GB) concentrations in the leaves. Detailed extraction and spectrophotometric methods for these assays have been previously described by McGee et al. (2021).

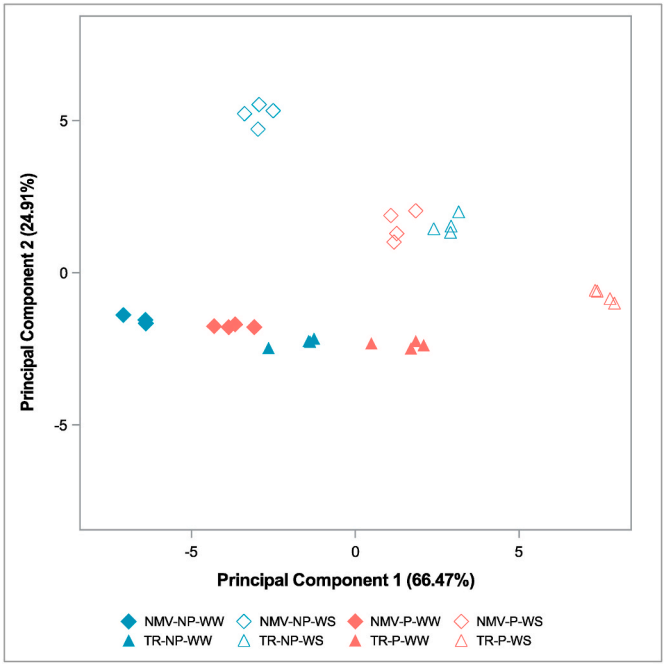

Fig. 9. Principal component analysis (PCA) scatterplot displaying clustering of cultivars, priming, and irrigation treatments based on antioxidant activity, reactive oxygen species, lipid peroxidation and osmolytes at early (40 DAP) and late (50 DAP) stress of peanut plants. Filled symbols indicate plants in the well-watered treatment (WW) and open symbols indicate plants in the pre-flowering water stress treatment (WS). Diamond symbols denote the cultivar New Mexico Valencia A (NMV), and triangle symbols denote the cultivar TUFRunner™ '511' (TR). Light blue symbols represent non-primed plants (NP) and rose symbols represent primed plants (P).

2.2.3.4. Biomass measurements. Plants were harvested at the end of the seed priming experiment at 55 DAP during the flowering and initial pegging stages of peanut. The total leaf area of each plant was measured using a leaf area meter (model LI-3100. Li-Cor, Inc., Lincoln, NE, USA). Tissues were separated into shoot and root portions, fresh weight was measured, and then dried in an oven at 60 °C until they reached a constant mass to determine the dry weight of above and below-ground tissues as well as the root to shoot biomass ratio.

2.2.3.5. Statistical analyses. Data were analyzed as a randomized complete block design with cultivar, priming treatment, and irrigation treatment considered fixed effects, and the day of the measurement (DAP), and replicates treated as random effects. Response variables were analyzed using the generalized linear mixed model's methodology as implemented in SAS® PROC GLIMMIX (SAS/STAT 14.1, SAS Institute Inc., Cary, NC, USA). The minimum Akaike information criterion correction for small sample sizes (AICc) was used to select the covariance structure of the model (Bedrick and Tsai, 1994; Brewer et al., 2016). Pairwise or multiple comparison analyses were performed using Fisher's protected least significant difference (LSD) test for significant differences and interactions among the fixed effects. Given the complexity of the sources of variation within continuous repeated measurements (measurements recorded daily or every 3 min), piecewise polynomial splines were incorporated in the generalized linear mixed models' structure. Principal Component Analysis (PCA) was performed using PROC PRINCOMP to compare biochemical mechanisms of stress response between cultivars and between priming treatments.

3. Results

Initial seed weight, radicle length, and germination percentage were measured in the melatonin rate study (Table 1). A cultivar effect on initial seed weight and radicle length after seven days of planting was detected, while initial seed weight within each melatonin rate was similar for both cultivars. Seed priming with 50 μM of melatonin resulted in higher germination rates and early root development among

Table 4
Above-ground, below-ground and whole-plant biomass, leaf area and the root:shoot biomass ratio of primed and non-primed New Mexico Valencia A and TUFRunner™ '511' peanut plants harvested 55 days after planting (DAP).

	New Mexico Valencia A				TUFRunner™ '511'				Analysis of Variance						
	Well-watered		Water-stressed		Well-watered		Water-stressed								
	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed	Non-primed	Primed	IR[d]	P	IR*P	CV	CV*IR	CV*P	CV*IR*P
Above-ground FW[a]	23.42 ± 4.10[b,c]	25.00 ± 3.55	15.10 ± 3.55	18.34 ± 3.55	30.31 ± 3.55	46.58 ± 4.10	22.83 ± 3.55	25.86 ± 3.55	**	*	ns	**	ns	ns	ns
Below-ground FW	4.51 ± 1.54	5.20 ± 1.35	4.68 ± 1.35	4.36 ± 1.35	8.95 ± 1.35	11.77 ± 1.54	6.13 ± 1.35	6.74 ± 1.35	*	ns	ns	**	*	ns	ns
Whole plant FW	28.31 ± 4.76	30.20 ± 4.12	19.78 ± 4.12	22.70 ± 4.12	39.26 ± 4.12	58.35 ± 4.76	28.96 ± 4.12	32.59 ± 4.12	**	*	ns	***	ns	ns	ns
Above-ground DW	3.85 ± 0.84	4.45 ± 0.71	2.76 ± 0.71	3.61 ± 0.71	5.35 ± 0.71	8.39 ± 0.84	4.64 ± 0.71	5.25 ± 0.71	*	*	ns	**	ns	ns	ns
Below-ground DW	0.84 ± 0.22	0.89 ± 0.19	0.67 ± 0.19	0.83 ± 0.19	1.41 ± 0.19	1.66 ± 0.22	1.28 ± 0.19	1.51 ± 0.19	ns	ns	ns	**	ns	ns	ns
Whole plant DW	4.69 ± 0.93	5.34 ± 0.81	3.43 ± 0.81	4.44 ± 0.81	6.77 ± 0.81	8.73 ± 0.93	5.92 ± 0.81	6.75 ± 0.81	*	*	ns	***	ns	ns	ns
Plant leaf area	5.92 ± 1.03	7.29 ± 0.89	4.01 ± 0.89	4.86 ± 0.89	8.43 ± 0.89	12.55 ± 1.03	6.28 ± 0.89	7.63 ± 0.89	**	**	ns	***	ns	ns	ns
Root: shoot	0.21 ± 0.05	0.20 ± 0.04	0.24 ± 0.04	0.23 ± 0.04	0.27 ± 0.04	0.19 ± 0.05	0.31 ± 0.04	0.28 ± 0.04	ns	ns	ns	ns	ns	ns	ns

[a] FW = fresh weight, DW = dry weight, plant-Tr = whole plant transpiration.
[b] Least square means ± SE.
[c] Units: Above-ground FW, Below-ground FW, Whole plant FW, Above-ground DW, Below-ground DW, Whole plant DW = g plant, Plant leaf area = $cm.^2$.
[d] P-value ≤ * 0.05, ** <0.001, *** <0.0001 and [ns] non-significant for main effects and their interactions.

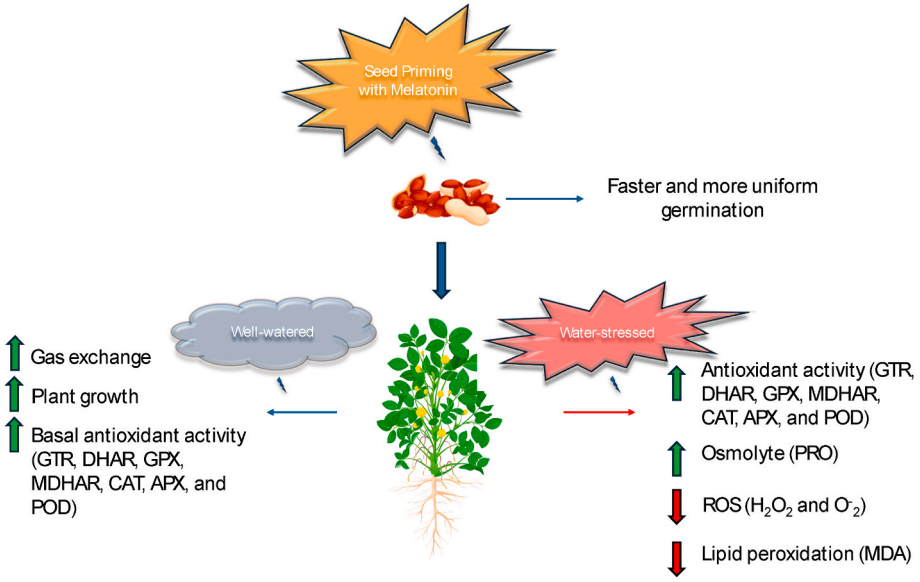

Fig. 10. Proposed mechanisms of melatonin seed priming effects as a growth promoter under well-watered conditions, with higher gas exchange and basal antioxidant activity, and improving stress tolerance under water stress by increasing antioxidant activity and osmolyte accumulation, while reducing reactive oxygen species levels and membrane damage.

all treatments for both cultivars and was selected as the rate to be used in the subsequent seed priming experiment.

Soil volumetric water content (VWC) ranged from 0.22 to 0.24 cm^3 cm^{-3} during the pre-stress phase for all the plants and decreased to approximately 0.12–0.14 cm^3 cm^{-3} for the water-stressed plants, after 12 days of reduced irrigation (Fig. 2).

Both cultivars showed similar responses to seed priming with 50 μM of melatonin for whole-plant measurements. Primed plants had increased midday plant-*Tr* during early development compared with non-primed plants, and midday plant-*Tr* in primed and non-primed plants of both cultivars significantly decreased immediately after exposure to water stress (Fig. 3). Root water uptake increased in TUFRunner™ '511' primed plants under adequate and water stress conditions from early flowering to the end of the experiment, whereas primed plants of New Mexico Valencia A had increased root water uptake only under water stress compared with non-primed plants (Fig. 4).

Genotypic differences were detected in leaf-*A*, leaf-g_s, leaf-*RWC*, and leaf-Ψ_w in response to priming under control and water stress conditions (Figs. 5–8). Both primed and non-primed TUFRunner™ '511' plants had similar leaf gas exchange, leaf-*RWC*, and leaf-Ψ_w under control and water stress conditions during the entire experiment. Primed New Mexico Valencia A plants under non-stressed conditions had higher leaf-*A* and leaf-g_s, and under water stress had higher leaf-*RWC*, and leaf-Ψ_w at the early stress measurement period (43 DAP), and lower leaf-*A* and leaf-g_s at the late stress measurement period (50 DAP) compared to non-primed plants.

Regardless of the priming treatment, water stress significantly impacted leaf gas exchange, leaf-*RWC* and leaf-Ψ_w. Non-primed and primed plants of both cultivars had lower leaf gas exchange, leaf-*RWC*, and leaf-Ψ_w under water stress compared with plants under no water stress. Water-stressed TUFRunner™ '511' plants had a slightly greater reduction in most variables compared with New Mexico Valencia A plants.

Seed priming, irrigation treatment, and cultivar impacted the activity of the enzymatic antioxidants, protective osmolytes and ROS accumulation, and lipid peroxidation in both early stress and more evidently in late stress (Tables 2 and 3). Overall, regardless of the cultivar or irrigation treatment, primed plants showed enhanced antioxidant activity and protective osmolyte accumulation at 40 and 50 DAP (early and late stress assessments) compared with non-primed plants. Primed and non-primed plants of both cultivars under water stress showed higher enzymatic antioxidant activity, protective osmolytes and ROS accumulation, and lipid peroxidation compared to non-water stressed plants in both early and, more evidently, in late stress (Tables 2 and 3). TUFRunner™ '511' plants showed higher antioxidant activity and protective osmolyte accumulation, but lower ROS accumulation and lipid peroxidation compared with New Mexico Valencia A. Principal component analysis generated two distinct separations, one by the irrigation treatment and another by cultivar (Fig. 9). Within the clusters, primed plants showed higher values for Principal Component 1 and lower values for Principal Component 2 than non-primed plants. Over 90% of the biochemical variation was explained by the two first principal components. Principal Component 1 represented 66.47% of the variation and was strongly impacted by the activities of GTR, DHAR, and GPX at both early and late stress, MDHAR, CAT, and PRO accumulation at early stress, and APX, and POD at late stress. Principal Component 2 explained 24.91% of the variation and was strongly impacted by the reactive oxygen species (H_2O_2 and O^-_2) and lipid peroxidation measured as MDA at both early and late stress.

Seed priming with 50 μM of melatonin significantly impacted above ground and whole plant biomass, as well as leaf area (Table 4). The irrigation treatment and cultivar affected most variables, except for below-ground dry weight (not impacted by the irrigation treatment) and the root:shoot ratio (not affected by any of the main effects or their interactions). Regardless of the irrigation treatment, primed plants of both cultivars had higher biomass accumulation and plant leaf area than non-primed plants. Both non-primed and primed plants exposed to water stress had a significant reduction of all whole plant biomass variables, with the exception of below-ground dry weight and the root:shoot ratio compared to plants under non-stress conditions. Non-primed and primed New Mexico Valencia A plants had lower biomass production than both non-primed and primed TUFRunner™ '511' plants.

4. Discussion

The hypothesis that seed priming with melatonin can promote drought tolerance in peanut, as well as act as a growth promoting biostimulant in the absence of stress, was supported by this study. Seed priming with 50 μM of melatonin improved peanut seed germination and seedling growth of both tested cultivars. Regardless of the irrigation

treatment, melatonin promoted greater vegetative growth in New Mexico Valencia A and TUFRunner™ '511', which was evidenced by higher whole-plant transpiration, net CO_2 assimilation, and root water uptake. Genotypic differences were observed in plant growth response to seed priming with melatonin. Melatonin had a growth-promoting effect in a drought-tolerant cultivar, TUFRunner™ '511' (Zurweller et al. 2018a, 2021), under both non-stressed and stressed conditions, which was more evident under non-stressed conditions. For New Mexico Valencia A, a drought-sensitive cultivar (Qin et al., 2011), major improvements in plant growth occurred under water stress. Additional benefits from melatonin priming were observed for New Mexico Valencia A in that plants maintained higher relative water content and leaf water potential during water stress. Promotion of growth under both adequate water and water stress conditions can be explained by the enhanced antioxidant activity and osmolyte production, that presumably resulted in reduced ROS accumulation and membrane peroxidation in primed compared with non-primed plants of both cultivars (Foyer and Noctor, 2016; Foyer, 2018; Hasanuzzaman et al., 2020).

The potential of priming with chemical agents applied directly to the leaves or the roots to enhance stress tolerance has been demonstrated in previous studies with peanut (Tian et al., 2019, El Sayed et al., 2020). Peanut responded positively to the application of green leaf volatiles against salt stress by adjusting photosynthetic activity, enhancing the efficiency of antioxidant systems, and improving root morphology (Tian et al., 2019). Root treatment of peanut seedlings with 150 μM of melatonin resulted in protection against oxidative damage from salinity stress by activating both enzymatic and non-enzymatic systems, which resulted in lower ROS levels and lipid peroxidation (ElSayed et al., 2020).

Although chemical priming with foliar application and root incubation can improve stress tolerance in peanut, seed priming is often more practical and therefore conducive to its implementation in agricultural practices. Furthermore, seed priming with melatonin can have the added benefits of stimulating germination and accelerating plant establishment in the absence of stress (Savvides et al., 2016; Rhaman et al., 2021; Huang et al., 2020; Rajora et al., 2022). Improved seed vigor and germination is particularly important for peanut, a crop in which seed quality issues persist and are likely related to the effects of harvest timing on seed maturity (Song et al., 2022), mechanical damage during processing and transportation (Barbosa et al., 2014), and improper storage conditions (de Oliveira et al., 2022). Previous studies have demonstrated that seed priming with melatonin with a dose as low as 5 μM can improve germination rates, seed vigor, and promote faster root establishment in several crops, including canola (*Brassica napus*), waxy corn (*Zea mays* L. var. ceratina), cucumber (*Cucumis sativus* L.), safflower (*Carthamus tinctorius* L.), triticale (*Triticale hexaploide* L.), and wheat (*Triticum aestivum* L.), under both non-stress and stress conditions. However, plant responses can be dose-, crop- and even cultivar-dependent (Wei et al., 2015; Kolodziejczyk et al., 2016; Savvides et al., 2016; Simlat et al., 2018; Cao et al., 2019; Heshmati et al., 2021; Guo et al., 2022; Rafique et al., 2023).

Dose experiments designed to test a range of melatonin seed priming treatments have not been reported in peanut previously. We provided evidence that seed priming with 50 μM of melatonin resulted in more uniform and faster seed germination and early root establishment in both a drought-sensitive and a drought-tolerant peanut cultivar, New Mexico Valencia A and TUFRunner™ '511' respectively. In an extensive review of melatonin application as a crop growth regulator, melatonin has exhibited a concentration-dependent growth-inhibitory effect on root growth in treatments with rates above 100 μM in a wide range of crops (Arnao and Hernandez-Ruiz, 2014). The melatonin rate study was consistent with previous reports, where seeds treated with more than 100 μM of melatonin had less root growth than non-treated seeds or seeds treated with up to 100 μM of melatonin (Guo et al., 2022; Rajora et al., 2022; Sharif et al., 2018; Simlat et al., 2018; Wei et al., 2015).

Most research on seed priming with melatonin focused on understanding its effect on stress responses during early plant establishment (Balabusta et al., 2016; Kolodziejczyk et al., 2016; Simlat et al., 2018; Cao et al., 2019; Guo et al., 2022). Although the ability to rapidly develop a uniform stand can play a key role in the final yield of the crop (Finch-Savage and Bassel, 2016), there are still important unanswered questions regarding the residual effects of melatonin priming on plant growth and stress tolerance during the crop cycle. The current study evaluated whether seed priming with melatonin can potentially enhance drought tolerance when peanut plants are water-stressed during early flowering, one of the most vulnerable physiological stages for adequate water acquisition in this crop (Abou Kheira, 2009; Boote, 1982; Jongrungklang et al., 2011). Many physiological traits can be used as stress tolerance indicators in crops, such as adjustment of photosynthetic capacity (Vincent et al., 2017), stomatal control (Antoniou et al., 2017), root function (Zurweller et al., 2021), efficiency of antioxidant systems in the control of ROS and membrane integrity (Foyer and Noctor, 2016, Cao et al., 2019; Vincent et al., 2020; Heshmati et al., 2021; Zulfiqar et al., 2024), and maintenance of vegetative growth under stress (Chen et al., 2016). In addition to the benefits observed during seedling establishment, primed plants showed overall increased photosynthetic activity, whole-plant transpiration and root water uptake compared to non-primed plants under adequate water conditions that persisted as the plants entered the vegetative stage. Interestingly, seed priming with melatonin stimulated plant growth of TUFRunnner™ '511', a previously described drought-tolerant cultivar (Zurweller et al. 2018a, 2021), inducing a growth promoting effect that was more evident under well-watered conditions. For New Mexico Valencia A, a drought-sensitive cultivar (Qin et al., 2011), major differences in physiological traits were observed under water stress, in which primed plants had a more sensitized stress response, with faster down-regulation of photosynthesis and transpiration compared with non-primed plants. Although the maintenance of photosynthesis during stress can be an indication of stress tolerance in peanut (Zhen et al., 2022), in the current study, even with faster reductions in net CO_2 assimilation, transpiration, and stomatal conductance under stress, primed plants still had higher biomass accumulation than non-primed plants. Furthermore, primed plants of New Mexico Valencia A consistently showed higher relative water content and water potential during stress compared to non-primed plants. Our results indicated that the basal activity levels of antioxidant enzymes in TUFRunner™ '511' under well-watered conditions were higher than in New Mexico Valencia A, which might be one of the factors that explains the higher stress tolerance of the TUFRunner™ '511' cultivar. Melatonin-primed plants of both cultivars also had their basal activity levels of antioxidant enzymes elevated compared with non-primed plants, and under water stress those differences became even more evident combined with lower ROS levels and membrane damage, which can explain the enhanced stress tolerance of primed plants (Cao et al., 2019; Rafique et al., 2023). Results from the principal component analysis showed that the increased activity of the enzymes GTX, DHAR, GPX, MDAR, CAT, APX, and POD, and PRO accumulation played a key role in controlling ROS and reducing membrane damage in water-stressed plants of both cultivars.

A few scenarios can be drawn from our results, with the first being that higher growth promoted in early development of primed plants possibly compensated for the rapid shutting down of metabolic processes involved in growth during stress (Antoniou et al., 2017; Schwachtje et al., 2019; Vincent et al., 2020). A second scenario could be that primed plants became more sensitized to water stress with enhanced basal activity of antioxidant systems and protective osmolyte accumulation, showing a faster stress response that resulted in more efficient control of ROS and less membrane damage from stress (Schwachtje et al., 2019; Rafique et al., 2023; Zulfiqar et al., 2024). A third scenario could be that the accelerated growth promoted by the melatonin treatment resulted in plants with larger root systems that could use more efficiently the residual soil water under limiting conditions. All scenarios are plausible according to typical responses to

priming with melatonin reported in previous studies, which are increased growth and redox potential, osmoregulation of melatonin under abiotic stress, and increased partitioning of assimilates to roots (Arnao and Hernandez-Ruiz, 2014; Sharif et al., 2018; Cao et al., 2019; El Sayed et al., 2020; Agathokleous et al., 2021; Ahmad et al., 2023; Zulfiqar et al., 2024) (Fig. 10).

5. Conclusions

This study provides strong evidence to support the hypothesis that melatonin seed priming can enhance drought tolerance in peanuts, with added benefits in overall crop performance and growth under non-stress conditions. Seed priming with 50 μM of melatonin resulted in more uniform germination, early root development, enhanced antioxidant activity and protective osmolyte accumulation while reducing ROS accumulation and membrane damage, which resulted in a dual role of melatonin in plant growth stimulation and stress tolerance. Genotypic differences were observed, in which seed priming of a drought-tolerant cultivar had a more evident growth promoting effect under well-watered conditions, whereas for a drought-sensitive cultivar, major adjustments in physiological and biochemical activity were observed under water stress that enhanced drought tolerance. Further research should focus on testing the efficacy of our findings under field conditions.

CRediT authorship contribution statement

Aline de Camargo Santos: Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bruce Schaffer:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis. **Andreas G. Ioannou:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Pamela Moon:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Muhammad Shahid:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Diane Rowland:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Barry Tillman:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Matthew Bremgartner:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Vasileios Fotopoulos:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Elias Bassil:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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