


ORIGINAL ARTICLE

Drought Stress

Cross-Generational Effect of Water Deficit Priming on Physiology of Peanut Plants Under Water Stress

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Received: 24 November 2023 | **Revised:** 1 July 2024 | **Accepted:** 2 July 2024

Funding: This work was funded by the checkoff funds from the National Peanut Board and the Florida Peanut Producers Association awarded to Aline de Camargo Santos, Diane Rowland, and Elias Bassil.

Keywords: crop resilience | cross-generational priming | drought stress | peanut | stress acclimation | water deficit priming

ABSTRACT

Water deficit priming through regulated deficit irrigation has been shown to be beneficial for peanut cultivation, leading to improved water-use efficiency during the crop cycle and enhanced stress acclimation. The effects of priming using water deficit can be heritable, but little is known about stress priming effects on the physiology and growth of successive generations undergoing water stress. Two experiments were conducted to assess cross-generational priming by determining physiological and growth responses of offspring of primed and non-primed peanut plants of two genotypes, COC-041 and New Mexico Valencia C (NMV-C), both previously found to be strongly responsive to priming. Seeds were collected from parental plants subjected to mild water stress by regulated deficit irrigation (primed) or adequate irrigation (non-primed). These seeds were then planted, and the offspring were monitored for physiological and growth responses to water stress, including on a whole-plant basis using a high-throughput physiological phenotyping platform and on individual leaves by periodic single-leaf measurements. Measurements included whole-plant transpiration (plant-*Tr*), root water uptake, leaf transpiration, stomatal conductance and net CO₂ assimilation (leaf-*Tr*, leaf-*g_s*, and leaf-*A*), leaf water and osmotic potential (leaf- Ψ_w and leaf- Ψ_o), leaf osmotic adjustment, leaf relative water content (leaf-*RWC*) and cumulative plant-*Tr*. Offspring of both genotypes from primed parent plants had faster early establishment, with more uniform germination, and more rapid initial seedling growth compared to offspring from non-primed parent plants. Although offspring of both non-primed and primed plants of both genotypes exhibited a significant reduction of plant-*Tr*, gas exchange, leaf- Ψ_w , leaf- Ψ_o , and leaf-*RWC* when exposed to water stress, offspring of primed plants showed increased water use efficiency through reduced leaf-*g_s*, leaf-*Tr* and plant-*Tr* while maintaining leaf-*A* under water stress. Despite offspring of both primed and non-primed plants being susceptible to severe water stress, offspring of primed plants exhibited overall enhanced water use efficiency, leading to greater dry biomass production per gram of transpired water and a trend of less growth reduction due to water stress compared to offspring of non-primed plants, especially for the genotype COC-041. This study shows the potential of water deficit priming to promote cross-generational changes in physiological function under limited water availability, by enhancing crop stress acclimation in the next plant generation.

Summary

- The offspring of primed peanut plants exhibited faster initial establishment than those of non-primed plants, suggesting cross-generational phenotypic inheritance of 'stress memory'.
- Genotypic differences in physiological function and water use were observed in the offspring of primed plants when enduring water stress.
- Offspring of primed plants exhibited improved water use efficiency and less negative effects of water stress on physiology and growth compared to non-primed plants.

1 | Introduction

Water scarcity is the main limiting factor for agricultural production worldwide. To meet future food demands of current cultivated areas, growers may need to improve irrigation management and supplement rainfed agricultural systems where feasible. Even with supplemental irrigation, some areas may be limited by the amount and quality of irrigation water (Rosa et al. 2020; FAO 2015; OECD 2017), needing improvements in irrigation efficiency and crop water use to ensure crop production (Rosa et al. 2020; Spangler et al. 2020; Costa et al. 2007). Additionally, the implementation of efficient crop stress management strategies will likely play a key role in minimizing the impact of water stress on crop productivity.

Although peanut (*Arachis hypogaea*) is considered a relatively drought-tolerant crop, water scarcity can drastically minimize yield potential and seed quality (Govind et al. 2009; Nautiyal, Rachaputi and Joshi 2002; Abou Kheira 2009; Dutra et al. 2018). Previous research showed the potential for stress acclimation and improved water use in peanut by exposing plants to temporary mild water deficit priming early in the growth cycle (Rowland et al. 2012; Zurweller, Rowland, et al. 2018; Puangbut et al. 2010; Jongrunklang et al. 2011). The effects of water deficit-induced priming may even be passed on to the offspring of primed plants (Racette et al. 2019). In fact, research has shown that seedlings from stressed parents of some peanut genotypes show improved vigour, accelerated germination, earlier root development and faster early establishment under adequate water conditions compared to seedlings derived from non-primed parent plants (Racette et al. 2019, 2020). These research findings suggest that certain peanut genotypes display signs of cross-generational stress memory, wherein the progeny of stressed plants could undergo genetic reprogramming or maternal provisioning across generations. As a result, regardless of the environmental conditions experienced by seeds of stressed parents, the offspring exhibit a stress phenotype by accelerating germination and early root establishment compared to seeds derived from non-stressed parents (Racette et al. 2019).

Understanding cross-generational stress acclimation responses can have significant implications for improving crop water management and identifying new traits which could be incorporated into the development of stress-tolerant cultivars (Bilichak and

Kovalchuk 2016; Ben Abdallah et al. 2017; Fleta-Soriano and Munne-Bosch 2016). Additionally, stress memory may have practical applications for seed quality and stand establishment in the field (Racette et al. 2019). A growing body of literature on priming and stress memory in a diverse range of crops has shown that plants subjected to reiterated cycles of stress and recovery can alter their photosynthetic capacity, plant water use and status, carbon partitioning between above- and below-ground biomass and vegetative growth (Marcos et al. 2018; Neves et al. 2017; Vincent et al. 2015, 2020; Zhang et al. 2021).

While there is increasing attention to cross-generational stress memory in plants, not all studies expose the progeny of primed and non-primed plants to both non-stress and stress conditions in the next generation, which is essential for understanding and quantifying the impact of priming and stress acclimation across generations (Herman and Sultan 2011; Ben Abdallah et al. 2017; Fleta-Soriano and Munne-Bosch 2016; Hilker et al. 2016; Schwachtje et al. 2019). The present study was conducted to determine if peanut seeds from genotypes previously found to be strongly responsive to priming (Zurweller, Rowland, et al. 2018; Racette et al. 2019) have altered physiological responses to water stress influenced by the water stress history of the parent plants. Here we exposed plants to either vegetative water deficit priming or well-watered conditions during the entire crop cycle, and exposed their offspring to water stress to determine if priming could potentially confer water stress tolerance to the next generation of plants. We hypothesised that the offspring derived from parental plants subjected to water deficit priming will have improved performance, defined as more efficient plant water use and biomass accumulation than non-primed plants, when subjected to water stress.

2 | Materials and Methods

2.1 | Physiological Phenotyping Platform and Experimental Setup

Greenhouse studies were conducted in 2021 and 2022 at the University of Florida, Tropical Research and Education Center in Homestead, Florida, using a high-throughput physiological phenotyping platform (Plant-Ditech Ltd., Yavne, Israel; PDT). The PDT phenotyping system allows continuous monitoring of specific plant performance variables, as well as the ability to schedule and apply customised irrigation treatments precisely and separately to individual plant units. The PDT system is described in detail by Halperin et al. (2017) and Dalal et al. (2020). Briefly, the PDT is an integrated array of specialised 3.9-l growth units placed on highly sensitive, temperature-compensated lysimeters (Tadea-Huntleigh, model 1042 C4; Vishay Intertechnology, Malvern, PA, USA) (Figure 1A). Each array unit has a soil water sensor (5TE, Meter Group, Pullman, WA, USA) that reports soil water content, temperature and electrical conductivity. Each array unit includes a controller module that collects sensor and lysimeter data continuously (every 3 min). The controller also has solenoids that can be triggered to initiate an irrigation for each pot independently according to user specific set points. The data is accessed and visualised online and in real-time using the SPAC-analytics software interface (Plant-Ditech Ltd., Yavne, Israel). The system was installed in a temperature-controlled

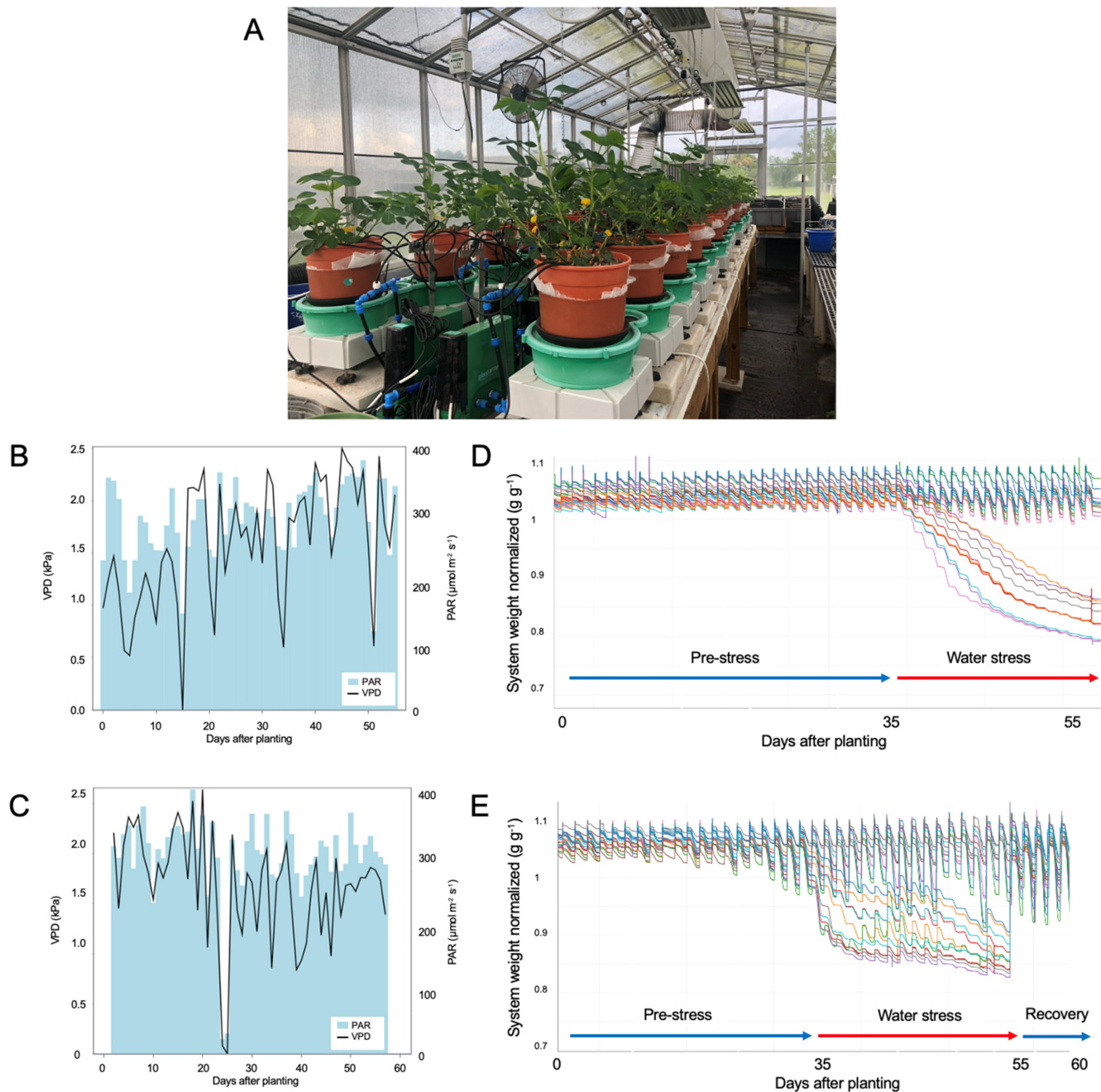


FIGURE 1 | Experimental setup of plants on the Plant Di-Tech system. (A) Overview of plants on the system during one of the peanut experiments, (B) and (C) daily vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) during cross-generational priming Experiment 1 and Experiment 2, respectively, (D) and (E) normalised system weight during pre-stress and water stress periods for Experiment 1 and pre-stress, water stress and recovery periods for Experiment 2, respectively. Each line represents one plant replicate.

greenhouse with a weather station that continuously monitored ambient temperature, relative humidity (RH%), photosynthetically active radiation (PAR) and calculates vapour pressure deficit (VPD) (WatchDog 2800 Weather Station, Spectrum Technologies, Inc., Aurora, IL, USA). Temperature in the greenhouse ranged from 24°C to 32°C, and the RH ranged from 70 to 90% during the experiments. VPD and PAR varied according to the day of the experiment; VPD ranged from 0.5 to 2.5 kPa and PAR ranged from 200 to 400 $\mu\text{mol}^{-2}\text{s}^{-1}$ (Figure 1B,C).

The experimental setup was based on de Camargo Santos et al. (2024) with some modifications. Briefly, before the start

of each experiment, all load-cell units were calibrated, and the initial weight of the system components (drainage container, pot, soil probe, irrigation drippers, substrate and beads used to prevent evaporation from the substrate) was measured and 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 at 70°C, was used as the growing medium. The gravimetric water content of the saturated substrate was 0.77–0.80 g g^{-1} or 77%–80%, and 0.60–0.70 g g^{-1} or 60%–70% after drainage, which translated to a matric potential of –8 to –9 and –10 to –15 kPa, respectively, for the saturated and drained substrate. The average volumetric water

content measurements obtained from the soil water sensor recorded between 07:00 and 08:00 h were used to calculate the daily values presented.

The experiment was set to begin after the pots were inserted in the drainage containers and filled with the substrate, the probes and irrigation drippers were inserted, and 300 cm³ of 6 mm beads were placed on top of the substrate to prevent evaporation from the substrate. The irrigation regime was programmed to occur at night, between 23:00 and 02:00 h, in three to five consecutive irrigation pulses scheduled 30 min apart. 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., Geldermalsen, The Netherlands) with micronutrients and ammonium sulfate (Smith et al. 1983). During water stress, plants in the well-watered treatment received water for irrigation.

2.2 | Plant Material, Experimental Design and Treatments

2.2.1 | Acquired Parental Plant Stress Exposure—Producing Stress Affected Seeds

Peanut seeds of New Mexico Valencia C (Reg. No. 24, PI 565461; NMV-C) and COC-041 (PI 493631) were planted during the crop season in 2021 to produce the two types of progenies according to their acquired parental plant stress exposure history: non-primed offspring and primed offspring (Rowland et al. 2012; Racette et al. 2019). These seeds were then used in the subsequent cross-generational priming experiments. Seeds were surface sterilised with 0.2% sodium hypochlorite for 5 min, rinsed in deionised water for 30 s, and planted on 8 February 2021. The PDT system was used in the production of stress affected seeds to precisely apply the irrigation treatments in non-primed and primed parent plants, and to monitor whole-plant responses to the stress levels applied to the primed parent plants. The experiment consisted of a 2 × 2 factorial deployed in randomised complete block design with eight individual plant replicates per treatment and genotype combination. Each genotype was assigned to one of the following treatments: (i) non-primed, full irrigation during the entire crop cycle or (ii) primed, vegetative water deficit priming. Non-primed plants received daily irrigation based on the previous day's transpiration to restore the substrate to full saturation during the entire crop cycle. The water deficit priming treatment was applied based on a water-saving irrigation strategy previously developed for peanut (Rowland et al. 2012), in which the plants received deficit irrigation during the vegetative growth stage (0–45 DAP) with full irrigation restored for the remaining crop cycle. Water deficit primed plants received ~60% of the irrigation of control plants during the initial 45 days, with the irrigation triggered once the substrate reached 70% saturation. Afterward, primed plants received full irrigation for the remaining crop cycle. Soil volumetric water content (VWC) during seed production in the parental generation for non-primed plants ranged from 0.34 to 0.38 cm³ cm⁻³ during the entire crop cycle, while VWC for primed plants ranged from 0.20 to 0.28 cm³ cm⁻³ during the water deficit priming period and from 0.32 to 0.36 cm³ cm⁻³

when full irrigation was restored for the remaining crop cycle. At pegging, containers filled with the substrate were placed around the pots to aid in peg and pod development. All plants were harvested at 157 DAP, and harvested pods were dried to 10% moisture and stored at 4°C until planting of the cross-generational priming experiments. Seeds harvested from each treatment and genotype combination were bulked across replicates to be used in the cross-generational priming experiments.

2.2.2 | Cross-Generational Priming Experiments

Two consecutive experiments (Experiments 1 and 2) were conducted in March and May 2022 to test cross-generational priming, using seeds from both genotypes, COC-041 and NMV-C, and acquired parental plant stress exposure, non-primed offspring and primed offspring (described above). In Experiment 1, the acquired parental plant stress exposure effect was examined under prolonged water stress, while in Experiment 2, water stress was followed by a recovery period. Each genotype and acquired parental plant stress exposure combination were assigned to either a no water stress treatment (well-watered control conditions during the entire experiment), or a water stress treatment applied at early flowering, a physiological stage that generally has significant impact on pod yield (Abou Kheira 2009; Jongrunklang et al. 2011; Figure 1D,E). Well-watered plants received daily irrigation based on the previous day's transpiration to restore the substrate to full saturation. Water-stressed plants received similar irrigation as well-watered plants up to 30–40 DAP, after which plants were exposed to water stress consisting of no irrigation until plants in the water stress treatment were transpiring less than 30% of control plants in the well-watered treatment. Experiment 1 was harvested at the end of the water stress period at 55 days after planting (DAP), and Experiment 2 was harvested at 60 DAP, after the water stress period and recovery period (after full irrigation was restored for 8 days).

2.3 | Physiological Assessment for the Cross-Generational Priming Experiments

2.3.1 | Whole Plant Continuous Measurements

Whole-plant continuous measurements were obtained from the PDT system for the cross-generational priming experiments. 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 for pot weight over a 30-min period, between 05:00 and 05:30 h for the start of the day, and between 21:00 and 21:30 h for the end-of-the-day. Cumulative transpiration was calculated as the sum of the daily transpiration during the entire experiment. Whole-plant transpiration (plant-*Tr*) was calculated as the difference between consecutive pot weight readings from the lysimeter recorded every 3 min (e.g. plant-*Tr* at 11:00 h is the difference between the readings recorded at 10:57 h and 11:00 h). Root water uptake was calculated as the difference between consecutive soil water content readings from the soil sensor multiplied by the substrate volume,

as described by Halperin et al. (2017). The average of measurements recorded from 11:00 h to 13:00 h were used to calculate the midday transpiration rate and root water uptake.

2.3.2 | Single-Leaf Periodic Measurements

Leaf gas exchange [net CO₂ assimilation (leaf-*A*), transpiration (leaf-*Tr*), stomatal conductance of water vapour (leaf-*g_s*) and water use efficiency (leaf-*WUE*)], leaf water and osmotic potentials (leaf- Ψ_w and leaf- Ψ_o), leaf osmotic adjustment, and leaf relative water content (leaf-*RWC*) were measured on the leaf at the second nodal position of each plant. All variables, except for osmotic potential, were measured at critical time points of the experiment, defined as pre-stress (prior to the application of water stress), early stress (2–5 days into the stress treatment), late stress (10–14 days into the stress treatment) and the end of recovery (after 7 days of daily irrigation restored). Leaf gas exchange was measured with a portable infrared gas analyser (CIRAS-3, PP Systems, Amesbury, MA, USA) from 10:00 to 12:00 h, with the reference CO₂ concentration set to 390 $\mu\text{mol mol}^{-1}$, and light-saturated photosynthetic photon flux of 1000 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Leaf water use efficiency (leaf-*WUE*) was calculated as leaf-*A* divided by leaf-*Tr*.

Leaf-*RWC* was measured according to Barrs and Weatherley (1962) by collecting one leaf disc (0.785 cm²) per plant from 11:00 to 12:00 h, measuring the fresh weight, floating the disc on deionised water for 24 h, reweighing, and oven-drying the disc at 70°C for 24 h. The oven-dry leaf disc weight was then determined, and the leaf-*RWC* was calculated as:

$$\%RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Leaf- Ψ_w , leaf- Ψ_o and osmotic adjustment were measured in leaves sampled from 11:00 to 12:00 h. Leaf- Ψ_w was measured using a Scholander-type pressure chamber (Model 1515D Pressure Chamber Instrument, PMS Instrument Company, Albany, OR, USA). Leaf- Ψ_o was measured on the same leaf used for the determination of the leaf- Ψ_w , and osmotic adjustment was measured on adjacent leaves at the same stage as the late stress measurement in the first experiment. To determine the leaf osmotic potential, the plant tissue was macerated in liquid nitrogen using a ceramic mortar and pestle, followed by filtration and centrifugation of the macerated leaf tissue at 10,000 g for 15 min at 4°C. A 10- μL aliquot of the resulting supernatant was analysed for osmolality using a vapour pressure osmometer (Model 5600, VAPRO, ELITechGroup Inc., San Jose, CA, USA). The osmometer readings were then converted to osmotic potential using the Van't Hoff equation (Callister et al. 2006). Osmotic adjustment was measured according to the methodology described by Lins et al. (2018). Briefly, three leaves were sampled per plant and immediately saturated with deionised water for 24 h at 4°C in the dark. After reaching full turgor, leaves were gently dried, macerated, filtered, and centrifuged, using a similar methodology as described above for the osmotic potential. The total osmotic adjustment was calculated as the difference between the osmotic potentials at full turgor of the plants in the well-watered conditions and water-stressed treatments (Blum 1989).

At the end of the cross-generational priming experiments, plants were harvested, total plant leaf area was measured using a leaf area meter (model LI-3100, Li-Cor, Inc., Lincoln, NE, USA), plant tissue was separated into shoot and root tissues, and then dried in an oven at 60°C until they reached a constant weight to determine the dry weight of above (above-ground DW) and below-ground (below-ground DW) tissues and root: shoot partitioning. Plant water use efficiency (plant-*WUE*) was calculated as the total plant DW (above-ground and below-ground DW) divided by cumulative transpiration.

2.4 | Statistical Analyses

Data from cross-generational priming were analysed independently for Experiment 1 and Experiment 2, with genotype, acquired parental plant stress exposure, irrigation treatment, and their interactions with the day of the measurement (DAP) considered fixed effects, and block nested with DAP treated as a random effect. Response variables were analysed using the generalised linear mixed model methodology as implemented in SAS PROC GLIMMIX (SAS/STAT 14.1, SAS Institute Inc., Cary, NC, USA). Akaike's information criterion corrected for small sample size (AICc) was used to select the covariance structure of the model (Bedrick and Tsai 1994; Brewer, Butler and Cooksley 2016). A three-way analysis of variance (ANOVA) and 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 for single leaf periodically taken measurements. 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 generalised linear mixed models' structure.

3 | Results

In cross-generational priming Experiment 1, VWC ranged from 0.33 to 0.35 cm³ cm⁻³ during the pre-stress phase and for plants in the water stress treatment and dropped to approximately 0.12–0.14 cm³ cm⁻³ by the end of the stress treatment after 15 days of water deficit (Figure 2A). In cross-generational priming Experiment 2, VWC for plants in the water stress treatment ranged from 0.24 to 0.28 cm³ cm⁻³ during the pre-stress phase, dropped to approximately 0.08 to 0.14 cm³ cm⁻³ by the end of the stress treatment, and increased to 0.24 to 0.28 cm³ cm⁻³ during the recovery period (Figure 2B).

The main differences measured in the offspring from primed and non-primed parents were detected in whole plant measurements under well-watered conditions for both genotypes. Primed offspring of COC-041 were transpiring 25% more than non-primed offspring while NMV-C plants were transpiring 10% more than non-primed offspring during initial plant establishment (0–35 DAP) in Experiment 1 (Figure 3A). Root water uptake of both genotypes was higher during the initial 15 days of seedling development for offspring plants from primed parents compared to plants from non-primed parents in Experiment 1 (Figure 3B).

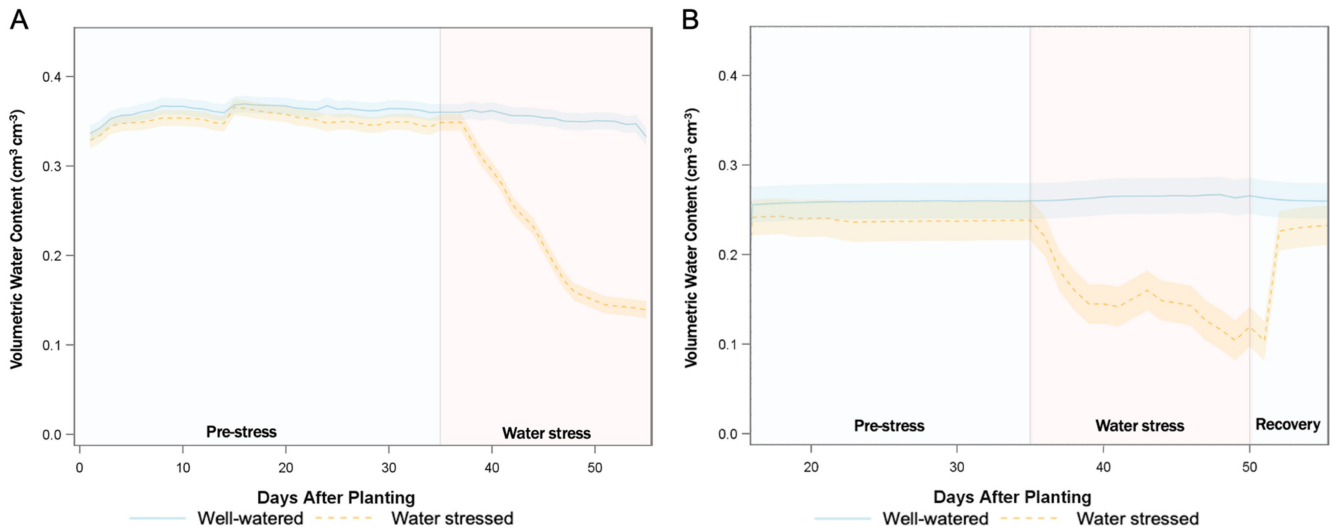


FIGURE 2 | Volumetric water content during pre-stress, water stress and recovery periods in cross-generational priming Experiment 1 (A) and Experiment 2 (B). Values are means \pm 95% confidence intervals from the soil water sensor's measurements.

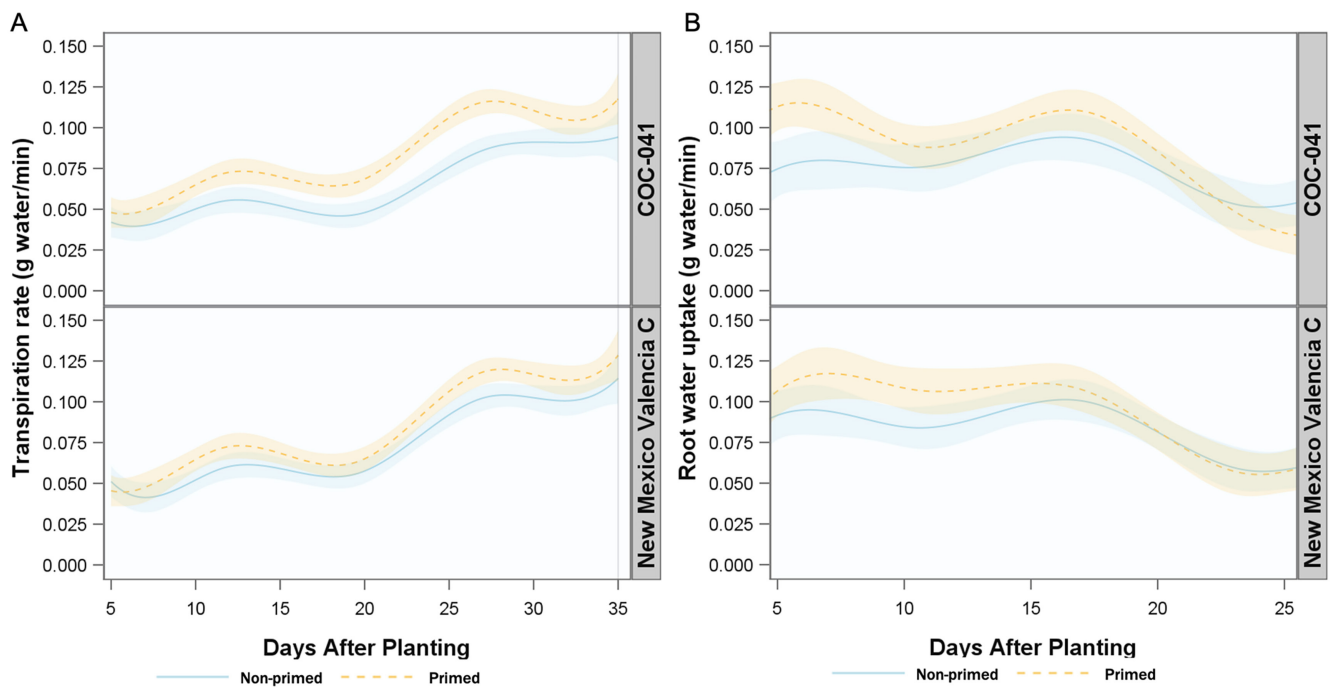


FIGURE 3 | (A) Midday transpiration rate and (B) midday root water uptake of the offspring of primed and non-primed plants displayed by genotype, COC-041 and New Mexico Valencia C, pre-stress under well-watered conditions during cross-generational priming Experiment 1. Values are means \pm 95% confidence intervals.

Although primed and non-primed offspring of both genotypes had similar midday root water uptake during the initial 25–30 DAP under well-watered conditions in Experiment 2 (Figure 4A), primed compared to non-primed COC-041 offspring showed a trend of increased root water uptake, while primed NMV-C offspring had higher root water uptake compared to non-primed NMV-C offspring after 29 DAP (Figure 4B).

Primed offspring of both genotypes showed a trend of higher midday plant-*Tr* during the initial days of water stress compared to non-primed offspring in

Experiment 1. However, after 10 days of water stress exposure, all plants tended to have lower plant-*Tr* compared to pre-stress plant-*Tr*, with decreases greater for NMV-C compared to COC-041 (Figure 5A). Primed and non-primed offspring of both genotypes had similar root water uptake under water stress in Experiment 1 (Figure 5B).

In Experiment 2, primed and non-primed offspring of both genotypes had a similar reduction in midday plant-*Tr* during the water stress period and were able to recover midday plant-*Tr* to pre-stress levels after 5 days of restored irrigation (Figure 6A). Genotypic differences in response to parental priming

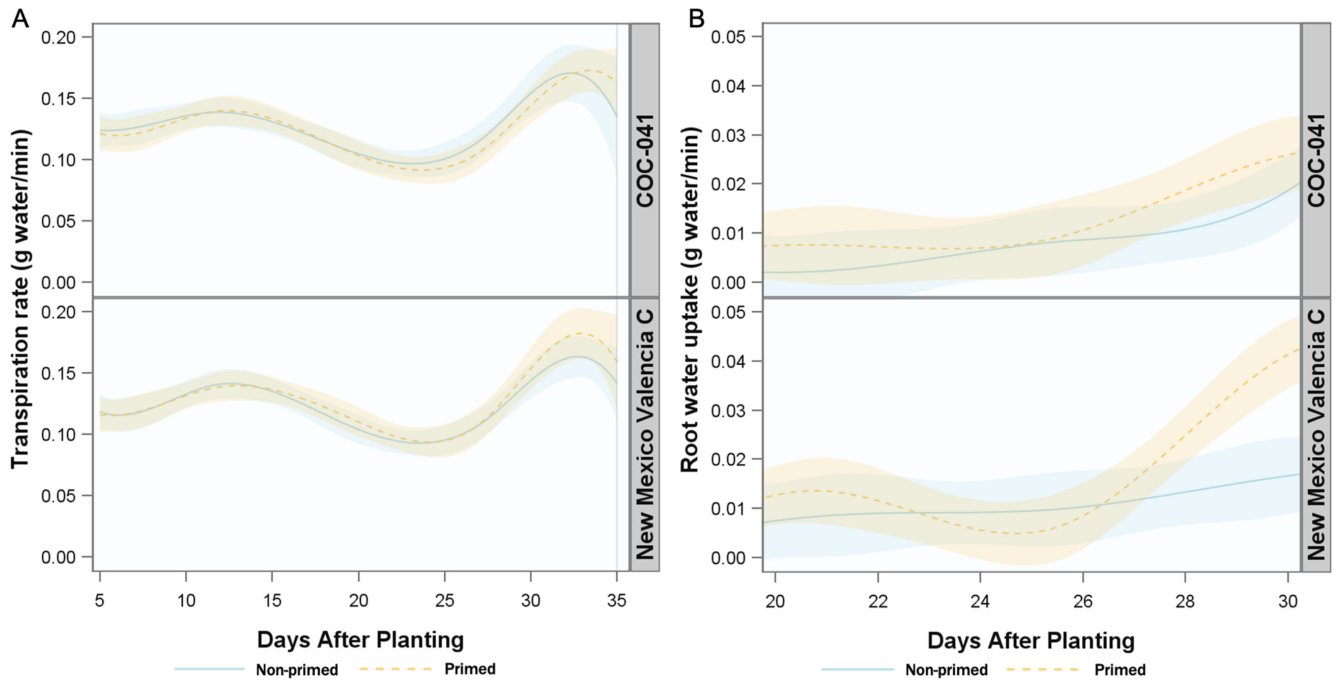


FIGURE 4 | (A) Midday transpiration rate and (B) midday root water uptake of the offspring of primed and non-primed plants displayed by genotype, COC-041 and New Mexico Valencia C, pre-stress under well-watered conditions during cross-generational priming Experiment 2. Values are means \pm 95% confidence intervals.

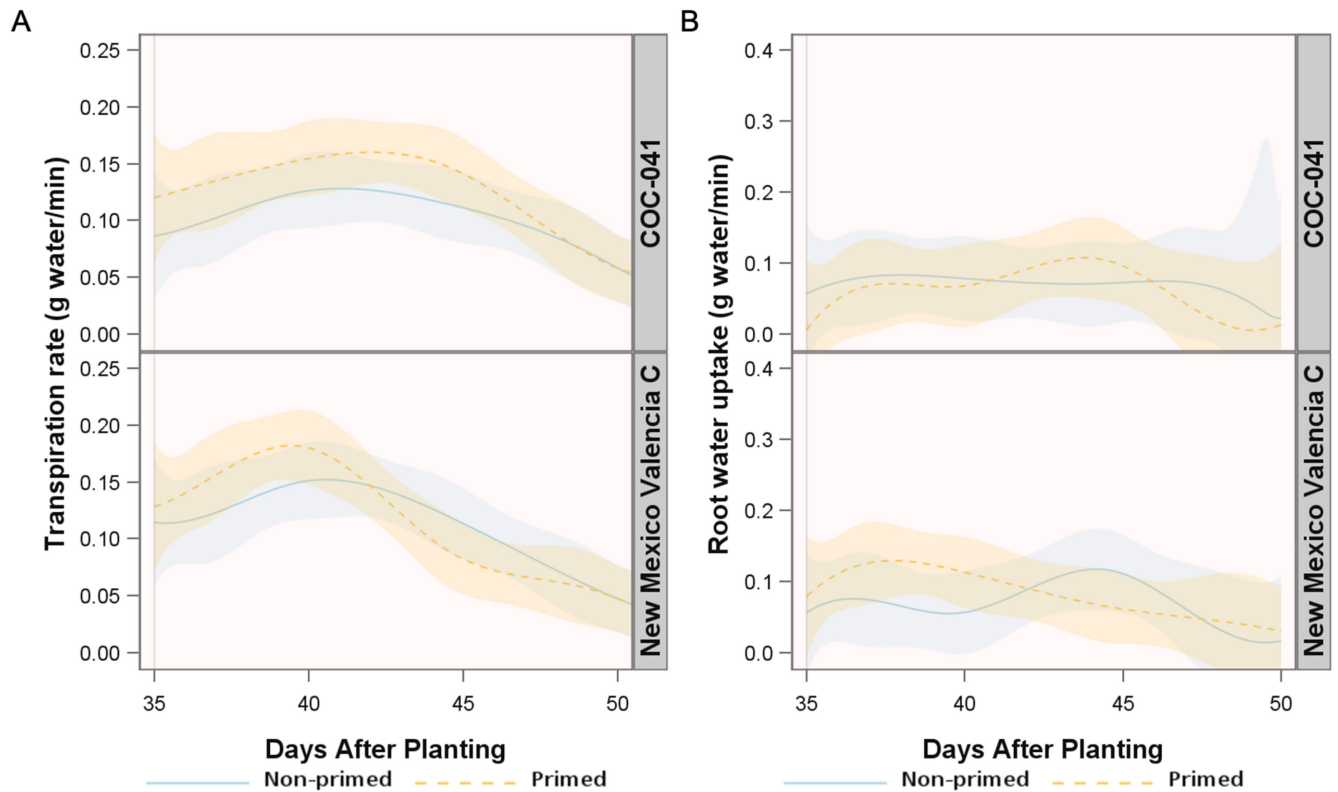


FIGURE 5 | (A) Midday transpiration rate and (B) midday root water uptake of the offspring of primed and non-primed plants under water stress displayed by genotype, COC-041 and New Mexico Valencia C during early flowering water stress in cross-generational priming Experiment 1. Values are means \pm 95% confidence intervals.

background were detected for root water uptake during stress in Experiment 2 (Figure 6B). Whereas both primed and non-primed offspring of COC-041 had a similar decrease in root

water uptake after a few days of water stress, primed offspring of NMV-C had greater root water uptake than non-primed offspring during mid-stress, from 40 to 45 DAP.

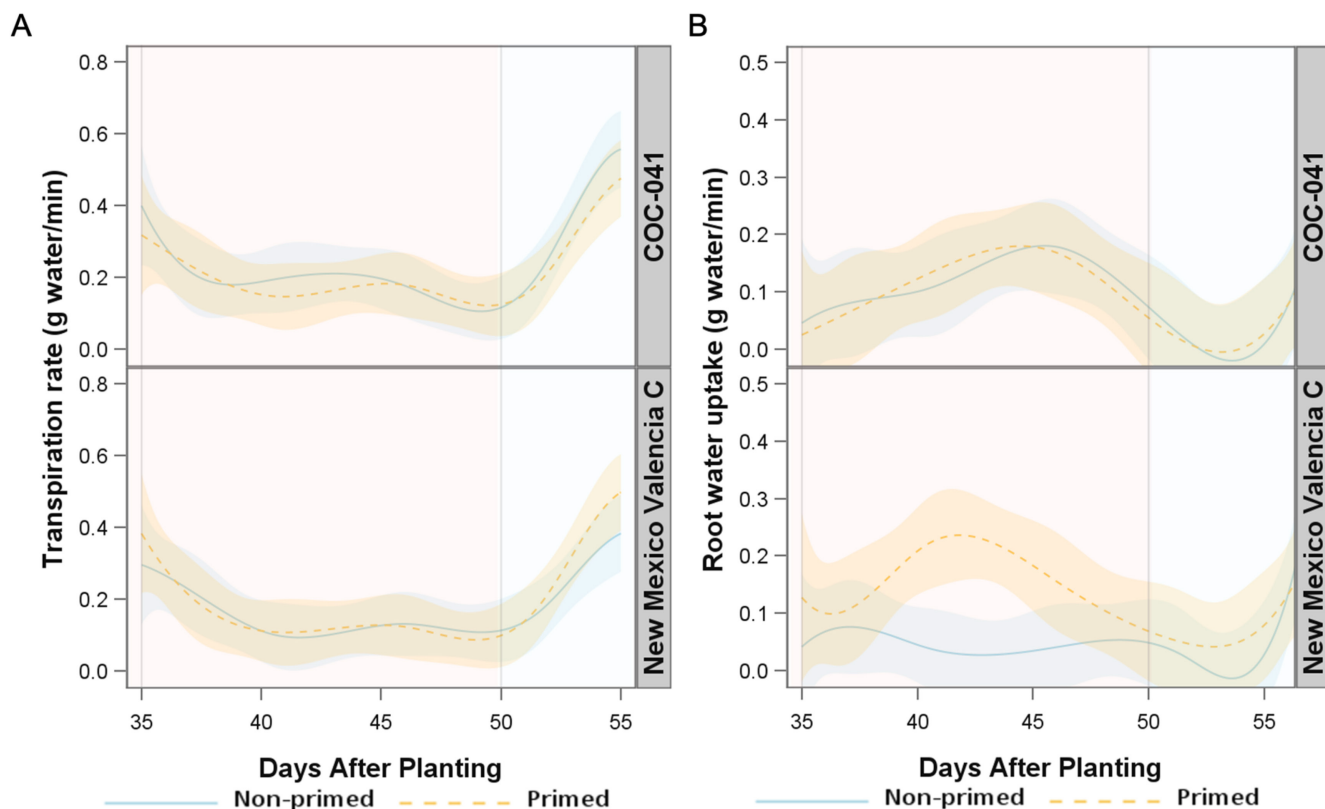


FIGURE 6 | (A) Midday transpiration rate and (B) midday root water uptake of the offspring of primed and non-primed plants displayed by genotype, COC-041 and New Mexico Valencia C during early flowering water stress in cross-generational priming Experiment 2. Values are means \pm 95% confidence intervals.

The parental priming background did not significantly impact leaf- RWC , leaf- Ψ_w or leaf- A under non-water stress or water stress conditions in both experiments (Tables 1 and 2). Although leaf- Tr and leaf- g_s tended to be lower in primed than non-primed offspring of both genotypes under stress (data not shown), there was no significant effect of the acquired parental plant stress exposure ($p > 0.05$) on those variables in Experiment 1. There were significant interactions between acquired parental plant stress exposure \times irrigation treatment and between acquired parental plant stress exposure \times genotype for leaf- WUE in Experiment 1 (Table 1). Leaf- WUE was higher for primed offspring than non-primed offspring during early and late stress for NMV-C and for late stress for COC-041 in Experiment 1 (Figure 7A). The three-way interactions among genotype \times acquired parental plant stress exposure \times irrigation treatment for leaf- WUE and acquired parental plant stress exposure \times genotype \times DAP for leaf- Tr and leaf- g_s were significant in Experiment 2 (Table 2). Leaf- WUE tended to be higher in primed than non-primed offspring of both cultivars during early water stress and similar afterwards, and leaf- WUE was higher for non-primed than primed offspring of NMV-C in the non-water-stress treatment in Experiment 2 (Figure 7B). Leaf- Tr and leaf- g_s were higher in non-primed than primed offspring of COC-041 plants after 5 days of stress exposure, and leaf- g_s was higher in primed compared to non-primed offspring of NMV-C plants in the non-water-stress treatment in Experiment 2 (Supplemental Figures S1 and S2).

The interaction between irrigation treatment and DAP was significant for most variables in both experiments (Tables 1 and 2).

Non-primed and primed offspring of both genotypes had lower leaf- A , leaf- Tr , leaf- g_s , leaf- RWC , and leaf- Ψ_w after 10–15 days of water stress in both experiments (data not shown). Leaf- Ψ_o and osmotic adjustment measured by the end of stress period in Experiment 1 were impacted only by the irrigation treatment (Table 1). All plants under water stress had a significant reduction in leaf- Ψ_o , and non-primed offspring of both genotypes showed a trend of higher osmotic adjustment than primed offspring (data not shown), although the differences were not statistically significant ($p > 0.05$). In Experiment 2, both non-primed and primed offspring of both genotypes recovered most of the measured physiological variables to pre-stress levels 7 days after irrigation was restored.

Although there were no significant interactions between acquired parental plant stress exposure and irrigation treatment for plant dry weight, root: shoot ratio, total leaf area or plant- WUE in both experiments, the impact of water stress on those variables varied according to genotype and acquired parental plant stress history. Water stress impacted all plant growth variables in both experiments, except below-ground DW in Experiment 1 (Figure 8A). Above-ground DW was reduced due to water stress for non-primed offspring of both genotypes and primed offspring of NMV-C in Experiment 1, and for non-primed offspring of COC-041 for Experiment 2 (Figure 8B). Below-ground DW was only reduced by water stress for primed offspring of NMV-C in Experiment 2 (Figure 8B). The root: shoot ratio was significantly higher in water stressed than non-stressed plants in Experiment 1 but not in Experiment 2. Plant leaf area was significantly lower

TABLE 1 | Factorial analysis of variance of leaf net CO₂ assimilation (leaf-*A*), transpiration rate (leaf-*Tr*), stomatal conductance of water vapour (leaf-*g_s*), water use efficiency (leaf-*WUE*), relative water content (leaf-*RWC*), water potential (leaf- Ψ_w) measured at pre-, early- and late-stress measurements, and of the late-stress measurement of leaf osmotic potential (leaf- Ψ_o) for cross-generational priming Experiment 1.

Source/Effect	df	Leaf- <i>A</i>	Leaf- <i>Tr</i>	Leaf- <i>g_s</i>	Leaf- <i>WUE</i>	Leaf- <i>RWC</i>	Leaf- Ψ_w		Leaf- Ψ_o	
		<i>p</i> -Value					df	<i>p</i> -Value	df	<i>p</i> -Value
GT	1	0.4535	0.5962	0.5075	0.5239	0.2119	1	0.0565	1	0.1705
IR TRT	1	0.0278^a	0.1116	0.2630	0.2874	>0.0001	1	>0.0001	1	>0.0001
GT*IR TRT	1	0.1023	0.0098	0.0123	0.8162	0.7721	1	0.6244	1	0.3446
APPSE	1	0.2703	0.3623	0.4057	0.1301	0.7717	1	0.3987	1	0.5967
GT*APPSE	1	0.6077	0.9848	0.9087	0.0057	0.5444	1	0.7192	1	0.5052
APPSE*IR TRT	1	0.7228	0.2020	0.4138	0.0031	0.6804	1	0.8737	1	0.4946
GT*APPSE*IR TRT	1	0.4558	0.3078	0.5009	0.6016	0.9729	1	0.7192	1	0.2521
DAP	2	0.0142	>0.0001	>0.0001	>0.0001	>0.0001	1	>0.0001	—	—
GT*DAP	2	0.2326	0.4067	0.2986	0.1669	0.9019	1	0.1504	—	—
DAP*IR TRT	2	0.0292	0.1064	0.0903	0.6070	>0.0001	1	>0.0001	—	—
GT*DAP*IR TRT	2	0.9481	0.8670	0.6402	0.4459	0.5143	1	0.7526	—	—
DAP*APPSE	2	0.2547	0.2161	0.2186	0.0671	0.4235	1	0.2480	—	—
GT*DAP*APPSE	2	0.9941	0.8727	0.9607	0.3360	0.6562	1	0.5307	—	—
DAP*APPSE*IR TRT	2	0.9586	0.8531	0.9757	0.2364	0.3962	1	0.9921	—	—
GT*DAP*APPSE*IR TRT	2	0.8347	0.8586	0.6576	0.7677	0.8824	1	0.4672	—	—

Abbreviations: APPSE, acquired parental plant stress exposure; DAP, days after planting; GT, genotype; IR TRT, irrigation treatment.

^a*p*-Value ≤ 0.05 for main effects and ≤ 0.10 for interactions are shown in bold.

in water stressed plants of primed offspring of COC-041 and non-primed offspring of NMV-C in Experiment 1, and in water stressed plants of non-primed offspring of COC-041 and primed offspring of NMV-C in Experiment 2 (data not shown). Overall, plant-*WUE* tended to be higher in primed offspring than in the non-primed offspring regardless of the irrigation treatment and higher for primed offspring of COC-041 under stress in Experiment 1 (Figure 9A). Plant-*WUE* was higher for non-primed offspring of COC-041 than primed while higher for well-watered primed offspring of NMV-C than non-primed under well-watered conditions (Figure 9B). Plant-*WUE* was similar between well-watered and water stressed plants regardless of the cultivar or priming treatment in Experiment 1, with similar results observed for Experiment 2, except for primed offspring of COC-041 that showed higher plant-*WUE* under water stress compared to well-watered conditions.

4 | Discussion

The hypothesis that water deficit priming can improve drought tolerance in the next generation of plants under water stress was supported by the results of this study. Our findings indicate that there was a residual effect of cross-generational water deficit priming on water use and carbon partitioning between above- and below-ground plant organs during plant early establishment of the next generation of plants. The effects of water deficit priming on the next generation can be

advantageous for seedling development under adequate water conditions, by promoting more rapid root system development and more uniform crop stands compared to seedlings from non-primed parents. Furthermore, if the plants endure subsequent water stress, cross-generational priming can benefit the following generation of plants by improving *WUE* and reducing the negative impact of water stress on plant growth and yield.

Although the offspring of primed plants had no prior exposure to stress during their crop cycle, they still showed a stress phenotype by rapidly developing their root systems even under adequate water conditions, a typical priming response already reported for other crops (Suter and Widmer 2013; Blodner et al. 2007; Fleta-Soriano and Munne-Bosch 2016; Hilker et al. 2016). The cumulative data from this research showed that as the water stress levels became more severe, cross-generational priming effects might become less evident, and both primed and non-primed offspring of both genotypes reacted to water stress by drastically reducing plant-*Tr*, leaf- Ψ_w , relative water content, and consequently leaf-*A*. However, primed offspring of both genotypes tended to show improved plant water use efficiency, as demonstrated by the plant-*WUE* results, in which primed offspring showed more dry mass produced per gram of water used for transpiration. Priming benefits were more evident for the genotype NMV-C than COC-041 under well-watered conditions with enhanced growth compared to the offspring of non-primed plants.

TABLE 2 | Factorial analysis of variance of leaf net CO₂ assimilation (leaf-*A*), transpiration rate (leaf-*Tr*), stomatal conductance of water vapour (leaf-*g_s*), water use efficiency (leaf-*WUE*), relative water content (leaf-*RWC*), water potential (leaf- Ψ_w) measured at pre-, early- and late-stress measurements, and of the late-stress measurement of leaf osmotic potential (leaf- Ψ_o) for cross-generational priming Experiment 2.

Source/Effect	df	Leaf- <i>A</i>	Leaf- <i>Tr</i>	Leaf- <i>g_s</i>	Leaf- <i>WUE</i>	Leaf- <i>RWC</i>	Leaf- Ψ_w	
				<i>p</i> -Value			df	<i>p</i> -Value
GT	1	0.2050	0.6818	0.2608	0.0273	0.6749	1	0.2310
IR TRT	1	0.0033^a	0.0096	0.0603	0.7244	0.0023	1	>0.0001
GT*IR TRT	1	0.2050	0.0850	0.0471	0.0441	0.3020	1	0.3404
APPSE	1	0.3759	0.3938	0.2209	0.8209	0.8767	1	0.8385
GT*APPSE	1	0.1218	0.1252	0.0771	0.2465	0.8256	1	0.9382
APPSE*IR TRT	1	0.9509	0.5305	0.7317	0.5007	0.5883	1	0.5270
GT*APPSE*IR TRT	1	0.4427	0.1106	0.2593	0.0421	0.2540	1	0.9077
DAP	2	>0.0001	>0.0001	>0.0001	0.0016	>0.0001	3	0.0001
GT*DAP	2	0.6886	0.5747	0.4617	0.3265	0.3150	3	0.0378
DAP*IR TRT	2	0.0077	0.0008	0.0011	0.5795	0.0005	3	>0.0001
GT*DAP*IR TRT	2	0.2241	0.0924	0.0569	0.7293	0.4955	3	0.0756
DAP*APPSE	2	0.4831	0.9258	0.7809	0.3759	0.8126	3	0.3737
GT*DAP*APPSE	2	0.4866	0.5889	0.2354	0.9363	0.5566	3	0.5771
DAP*APPSE*IR TRT	2	0.2503	0.0347	0.0655	0.0835	0.7953	3	0.2692
GT*DAP*APPSE*IR TRT	2	0.4501	0.8440	0.5573	0.4582	0.5866	3	0.2420

Abbreviations: APPSE, acquired parental plant stress exposure; DAP, days after planting; GT, genotype; IR TRT, irrigation treatment.

^a*p*-Value ≤ 0.05 for main effects and ≤ 0.10 for interactions are shown in bold.

Under water stress, priming was more advantageous for the genotype COC-041 than for NMV-C, as water stress had less of a negative effect on primed offspring of COC-041 compared to primed offspring of NMV-C plants.

In the present study, water deficit priming impacted plant water use and biomass accumulation of the offspring generation, with genotypic differences identified for plant early establishment and plant water use throughout the crop season. In previous studies, the peanut genotypes NMV-C and COC-041 showed similar strategies to overcome water stress and were very sensitive to variations in soil water content (Zurweller, Rowland, et al. 2018; Zurweller, Xavier, et al. 2018; Bhogireddy et al. 2020; Qin et al. 2011). In those studies, both genotypes exhibited rapid stomatal closure once soil water content decreased to a critical level, and when experiencing water stress, both genotypes reallocated carbon to produce deeper root systems compared to non-water stressed plants. Several studies (Bennett, Boote and Hammond 1981; Stansell et al. 1976; Rowland et al. 2012; Zurweller, Rowland, et al. 2018; Jongrunklang et al. 2011, 2012) showed that increased rooting depth and root growth are common drought avoidance mechanisms in peanut. Earlier evidence of cross-generational phenotypic inheritance of 'stress memory' in peanut, showed that the progeny of stressed plants of both NMV-C and COC-041 had faster germination and earlier root establishment under adequate water conditions compared to the progeny of plants that never experienced stress (Racette et al. 2019, 2020). Likewise, in the current study, the offspring of primed plants had faster germination by 3–7 days

(data not shown) and higher root water uptake during early establishment under adequate water conditions compared to plants derived from non-primed parents. Although the seeds might never experience stress in their growth cycle, it could still be a strategic compromise between optimising the fitness of future generations in case of resource constraints in the offspring generation versus prioritising parental fitness and survival (Fleta-Soriano and Munne-Bosch 2016; Vincent et al. 2020; Crisp et al. 2016; Hilker et al. 2016).

In the current study, the major differences in plant water use between plants from distinct acquired parental plant stress exposure were detected during early vegetative growth under adequate water conditions and early mild to moderate levels of stress. Interestingly, early in the growth cycle, increased plant-*Tr* rates of primed compared to non-primed offspring were more evident in the genotype COC-041 than in NMV-C. Those differences in early growth and water use of the offspring of stressed parents could be partially explained by the differences in drought tolerance between the two genotypes (Zurweller, Rowland, et al. 2018; Qin et al. 2011; Kottapalli et al. 2009; Zurweller, Xavier, et al. 2018) and long-lasting adjustments induced during priming of the parent plant. The ability to rapidly establish a uniform stand can play a major role in the final yield of a crop (Finch-Savage and Bassel 2016). Rapid seedling establishment and higher investment in below-ground partitioning during early development observed in the progeny of water-stressed parent plants can be signs of stress acclimation

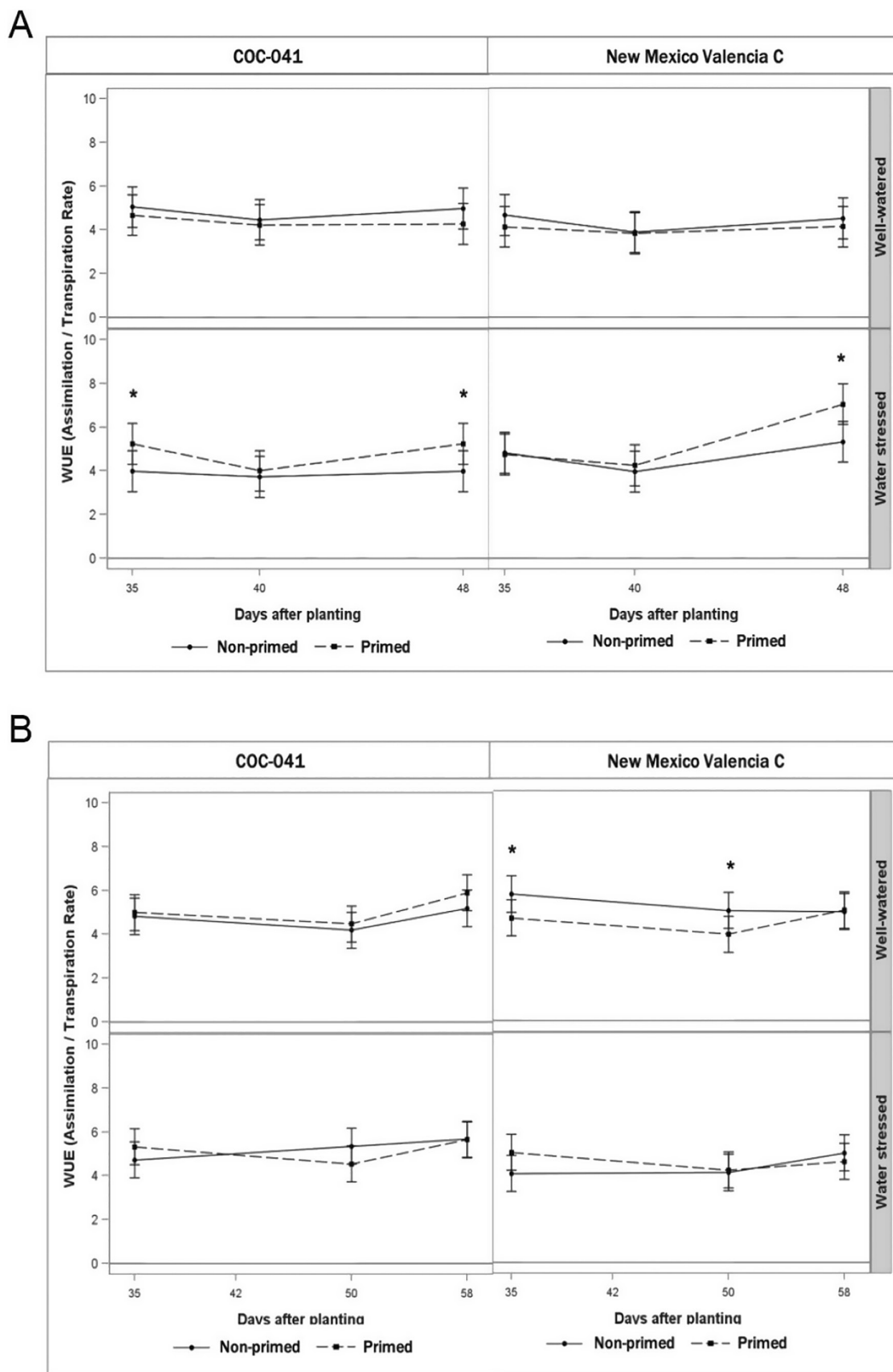


FIGURE 7 | Leaf water use efficiency (Leaf-WUE) of primed and non-primed plants displayed by genotype and irrigation treatment for cross-generational priming Experiment 1 (A) and Experiment 2 (B). Values are means \pm 95% confidence intervals.

mechanisms that can aid the progeny under future stress events, or part of drought avoidance or escape by accelerating life cycle completion (Negin et al. 2019; Herman and Sultan 2011; Blodner et al. 2007).

Although alterations in root water uptake and plant water use were detected during early establishment according to the genotype and the stress exposure experienced by parental plants, these differences became less evident once the plants

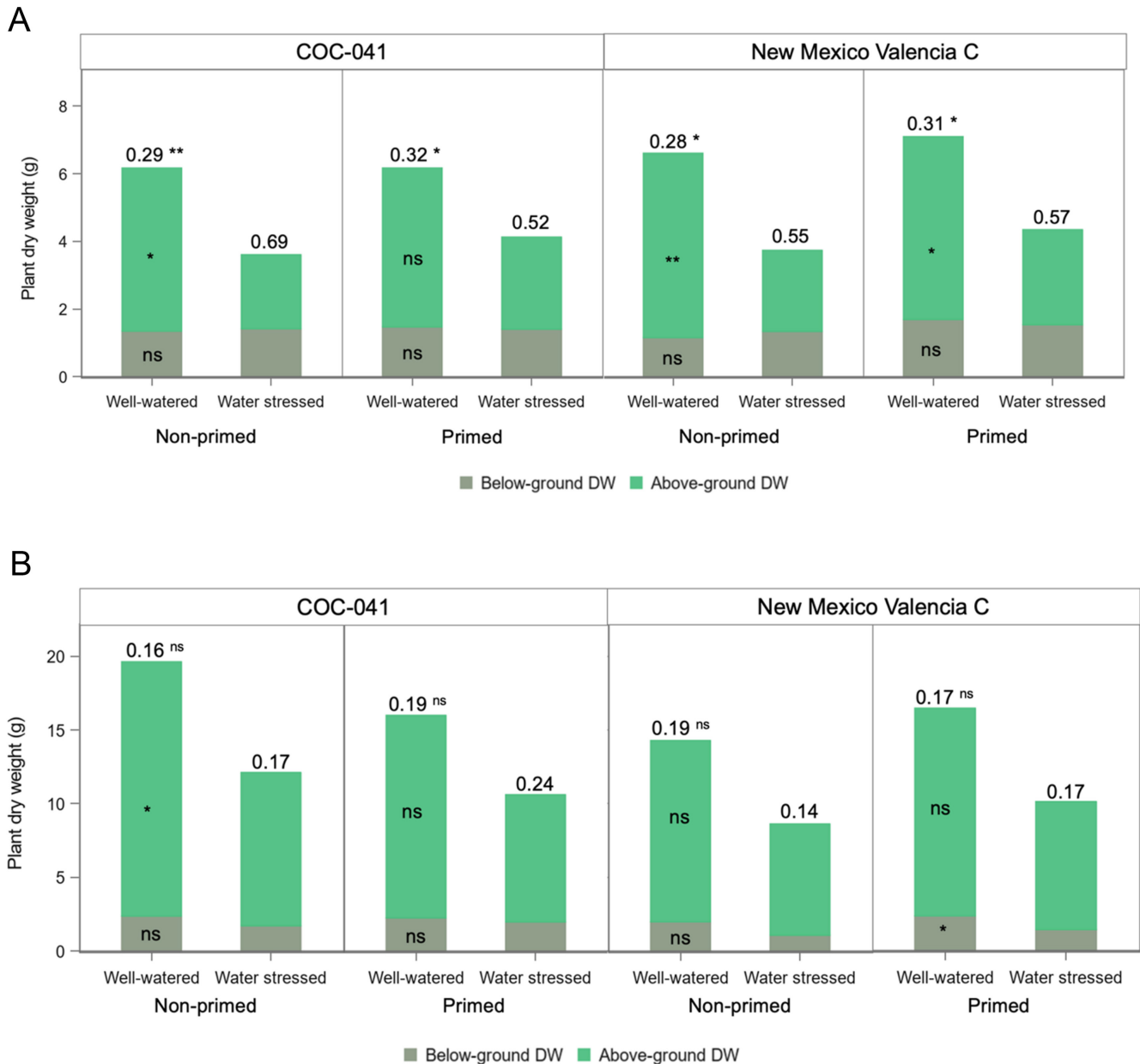


FIGURE 8 | Above-ground (green) and below-ground dry biomass (brown) and root:shoot ratio of primed and non-primed plants under well-watered and water-stressed conditions displayed by genotype for Experiment 1 (A) and Experiment 2 (B). Bars represent data collected at the harvest 55 days after planting (DAP). Numbers on top of the bars are the root:shoot ratio. *Indicates significance at $p \leq 0.05$; **Indicates significant at $p \leq 0.001$; ns indicates not significant ($p > 0.05$) for the main effect of irrigation treatment.

were subjected to subsequent more severe water stress during early flowering (approximately 35–40 DAP), in both experiments. Plant-*Tr* of both genotypes and acquired parental plant stress exposure combinations was reduced to approximately 25% of the control (well-watered) plants around 10 days after irrigation ceased. Major differences in plant water use and root water uptake between genotypes were more evident at mild to moderate stress levels. These findings suggest that major adjustments in plant transpiration and growth may occur at mild to moderate levels of stress, and as water stress levels increase plants become susceptible to reduced transpiration and growth regardless of previous stress exposure or genotype as shown previously for peanut (Zurweller, Xavier, et al. 2018; Abou Kheira 2009). In the field at noon on a sunny day the magnitude the differences between drought and control

treatments may be greater than results observed in the greenhouse due to stomatal conductance perhaps being higher in the control treatment under higher PAR.

Bennett et al. (1981, 1984) reported that peanut leaves decreased their midday turgor potential to zero and closed stomata when water potential was below -1.6 MPa, and leaf-*RWC* was below 82% after 11 days of withholding water under field conditions. Those authors also reported significant decreases leaf-*A* and leaf- g_s in peanut once leaf-*RWC* dropped from 85% to 70%. In the current study, plants under adequate water conditions had midday leaf- Ψ_w ranging from -0.8 to -1.0 MPa and leaf-*RWC* ~80%–90%. Stressed peanut plants from both genotypes and acquired parental plant stress exposure had similar leaf-*RWC*, leaf- Ψ_w , and leaf-*A* compared to well-watered plants at the early stress

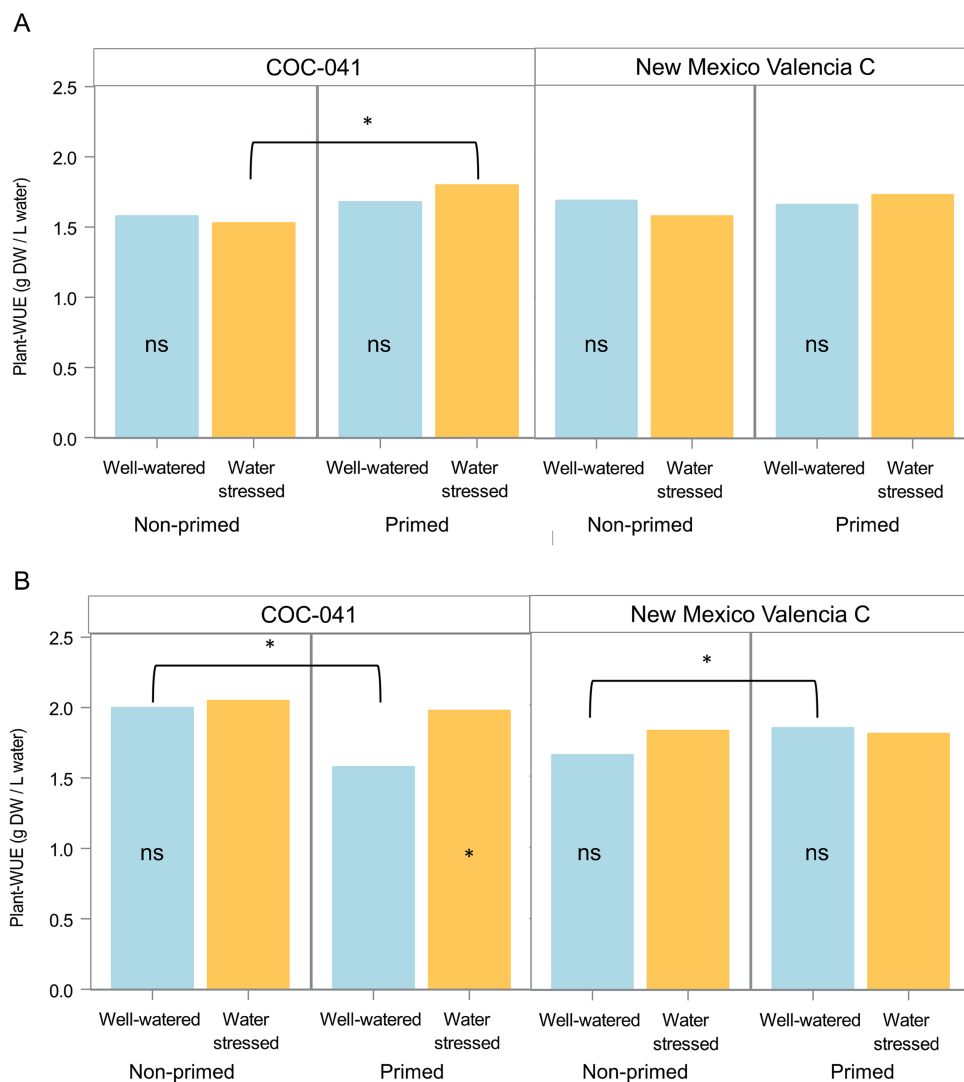


FIGURE 9 | Plant-WUE of primed and non-primed plants under well-watered and water-stressed conditions displayed by genotype for Experiment 1 (A) and Experiment 2 (B). Bars represent data collected during the entire experiments for the cumulative transpiration and biomass data collected at the harvest 55 days after planting (DAP). *Indicates significance at $p \leq 0.05$; ns indicates not significant ($p > 0.05$) for the main effect of irrigation treatment within the bars and the main effect of priming on the brackets on top of the bars.

assessment period, after 5–7 days of withholding irrigation. Under the most severe levels of water stress, at the late stress assessment period, after 14 days of water stress, leaf- Ψ_w and leaf-RWC were reduced to as low as -2.5 MPa and 50%–60% RWC, which translated to a 20%–50% reduction in leaf-A.

Leaf- Ψ_o at full turgor can be used to estimate the potential for osmotic adjustment under environmental stress (Dourado et al. 2022; Lins et al. 2018). In a recent review, Blum (2017) indicated that the inherent capability for osmotic adjustment can vary among plant species and even among genotypes within a species. Previous research on water relations in peanut showed that leaf- Ψ_o decreased from -1.1 to -2.0 MPa after 26 days of moderate to severe stress exposure, while leaf-RWC slightly decreased from 95% to 82%, indicating daily osmotic regulation in response to the water deficit (Bennett et al. 1984). Although osmotic adjustment can be an important aspect of stress adaptation and protection under stress, it can come with a growth ‘cost’ to the plant, since it requires

photoassimilates to be diverted to the production of osmoprotectants (Blum 2017; Mejri et al. 2016). In the current study, measurements of physiological variables on the last day of stress, when differences in leaf- Ψ_o and leaf-RWC were significant (well-watered plants with leaf- Ψ_o of -1.0 to -1.3 MPa and leaf-RWC of 75%–85% compared to stressed plants at -2.10 to -2.90 MPa and leaf-RWC as low as 45%–65%), indicated no significant osmotic adjustment in peanut. Osmotic adjustment is a responsive mechanism that requires time, and when dehydration occurs too quickly, there is insufficient time for osmotic adjustment to occur (Blum 2017; Zhang, Nguyen and Blum 1999; Mejri et al. 2016). Perhaps this is why results here somewhat diverge from those of previous reports of osmotic adjustment in peanut. Furthermore, in the present study, osmotic adjustment was measured in the leaves, but osmotic adjustment can also occur in the roots and result in increased root growth and root water uptake when plants are exposed to a water deficit period (Velazquez-Marquez et al. 2015). Root osmotic adjustment was not measured in the current study.

Single-leaf measurements of WUE showed improved leaf water use efficiency for primed compared to non-primed offspring of both genotypes under water stress in Experiment 1 due to higher reductions in leaf- g_s and leaf- Tr compared to the reductions in leaf- A . Primed plants exhibited a reduction in leaf- g_s under water stress, whereas leaf- A did not decrease, which might be explained by the assumption that primed plants show a partial stomatal closure and changes in photochemical efficiency that allow the plants to assimilate more CO_2 with reduced transpiration to conserve water use during stress (Vincent et al. 2015, 2020).

When crop plants are exposed to water stress, isolating a single trait that is responsible for sustaining yield under stress can be challenging because several complex plant traits contribute to biomass accumulation and yield. Generally, drought stress tolerance requires a coordinated combination of both above- and below-ground traits to sustain productivity under water stress (Zurweller et al. 2021), and adaptation mechanisms that enable plant recovery and stress resilience (Moshelion 2020; Gosa et al. 2019). Plant stress resilience provides information on how well a plant can withstand drought stress and regain its original productivity level once re-watering occurs, indicating how the severity of the damage caused by water deficit affects growth after stress (Dalal et al. 2019; Moshelion 2020; Chen et al. 2016). The acclimation to water deficit can be expressed by a more rapid recovery of photosynthetic functions and reactivation of photosynthetic machinery upon the alleviation of the stress (Vincent et al. 2020). To assess drought resilience in this study, we evaluated recovery rates from stress by measuring plant- Tr , leaf- Tr , leaf- g_s and leaf- A , leaf- RWC , and leaf- Ψ_w once irrigation was restored after water stress. In the present research, regardless of the acquired parental plant stress exposure and genotype, all plants exposed to early flowering water stress recovered similarly once full irrigation was restored.

Water stress impact on plant growth varied according to the genotype, acquired parental plant stress exposure, and the experiment. In Experiment 1, the major impact of water stress was a reduction of above-ground partitioning of biomass in primed offspring of COC-041 and non-primed offspring of NMV-C plants that resulted in an increased root: shoot ratio. In Experiment 2, both above and below-ground biomass of non-primed offspring of COC-041 and primed offspring of NMV-C plants were reduced by water stress, and the root: shoot ratio was similar for both well-watered and water-stressed plants. Environmental conditions were similar in both experiments, except for the longer day length, higher temperatures and the longer recovery period following water stress in the second experiment.

The relationship between whole plant dry biomass and cumulative transpiration, a correlation that explains the plant agronomic WUE (Dalal et al. 2019; Moshelion 2020), was highly significant ($R^2 > 0.85$ and $p < 0.002$) for all the genotype and acquired parental plant stress exposure combinations in both experiments. The impact of acquired parental plant stress exposure on water use and biomass accumulation was more evident in Experiment 1, with non-primed offspring of both genotypes using more water per gram of biomass accumulated than primed offspring under water limited conditions, while water use was similar for both genotypes and non-primed offspring and primed offspring backgrounds in Experiment 2.

Abou Kheira (2009) reported a 28% reduction in yield from water stress at early flowering compared to plants under adequate irrigation throughout the crop cycle, and that maximum evapotranspiration was recorded during pre-flowering. Although crop yield was not measured in the current study, based on whole plant dry matter accumulation, plant growth reductions from the water stress relative to plants under well-watered conditions according to the genotype and acquired parental plant stress exposure combinations were estimated. Whole plant growth was reduced under water stress by about 38%–43% for non-primed offspring of both genotypes and primed offspring of NMV-C plants, while growth of primed offspring plants of COC-041 was reduced by about 33–34% during both experiments. Reductions in cumulative transpiration were similar for all acquired parental plant stress exposure and genotype combinations ranging from 37% to 41% during both experiments. These findings indicate that water deficit priming of the parental generation was more beneficial to COC-041 plants that had improved water use by accumulating more dry matter per gram of water and less growth reduction under water stress, compared to NMV-C plants, for which the differences between primed and non-primed plants were less evident.

5 | Conclusion

This study explored the potential for regulated water deficit priming of parental peanut plants to promote stress acclimation and tolerance in subsequent generations. Results indicated a residual effect of cross-generational priming on water use and carbon partitioning in the offspring of parent plants exposed to water deficit priming. Offspring of primed plants exhibited a stress phenotype by more rapidly developing their root systems early in the crop cycle than non-primed plants, even when receiving adequate irrigation. While offspring of both primed and non-primed plants were susceptible to severe water stress, offspring of primed plants demonstrated improved water use efficiency by producing more dry mass per gram of transpired water and showed a trend of lower growth reduction due to water stress compared to non-primed plants. Thus, peanut plants appear to exhibit stress memory from one generation to the next. Better understanding of these mechanisms and whether they are in part genetically controlled, could be used in priming plants across generations to improve their tolerance to water deficit.

Author Contributions

Aline de Camargo Santos: conceptualization, investigation, funding acquisition, writing—original draft, writing—review and editing, methodology, formal analysis, data curation, project administration. **Bruce Schaffer:** investigation, project administration, formal analysis, writing—original draft, writing—review and editing. **Diane Rowland:** conceptualization, funding acquisition, methodology, project administration, writing—review and editing. **Matthew Bremgartner:** methodology, investigation, formal analysis, writing—review and editing. **Pamela Moon:** investigation, methodology, writing—review and editing, formal analysis. **Barry Tillman:** methodology, investigation, writing—review and editing. **Edivan Rodrigues de Souza:** methodology, investigation, writing—review and editing. **Elias Bassil:** conceptualization, project administration, funding acquisition, writing—review and editing, methodology.

Acknowledgements

We thank Dr Monaliza Santos (UFRPE) for measuring sap osmolarity to determine osmotic potential and adjustment.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, AdCS, upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.