



Phosphorus fertilisation differentially affects morpho-physiological traits and yield in soybean exposed to water stress

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Abstract

Background and aims Soybean plants frequently encounter challenges such as phosphorus (P) deficiency and water stress in many regions. However, the mechanisms governing low P and water stress tolerance in soybeans at different growth stages remain unclear. This study investigates the effect of P availability and water stress on soybean growth, morpho-physiological traits, and seed yield.

Methods We conducted experiments using the soybean genotype (PI 561271) grown under two P levels (10 or 60 mg P kg⁻¹ dry soil) with three watering conditions: well-watered (WW), early water stress (EWS) and terminal water stress (TWS). Plant assessments occurred at the vegetative, flowering, seed formation, and maturity stages.

Results Water stress decreased leaf area, shoot and root dry weights, root length, photosynthetic rate, stomatal conductance, total carboxylates, water use, water use efficiency, shoot, root and seed P contents, seed nitrogen contents, and seed yield compared to WW at the corresponding P level and growth stage. P60 significantly increased all parameters except P use efficiency (PUE) under all water treatments. EWS plants supplemented with P60 exhibited the highest root dry weight, root length and total carboxylates. Notably, flowering and seed formation stages had the highest carboxylate proportions (oxalic, malic, malonic and citric acids) under WW and EWS at P60. P10 had significantly higher PUE than P60, irrespective of water treatments during flowering and seed formation, while the opposite trend occurred at the maturity stage. Seed protein content significantly varied between P treatments regardless of the water stress.

Conclusions Our findings underscore the adverse impacts of combined low P and water stress on soybean growth, morpho-physiological traits, seed yield and protein content. Moreover, increased P availability alleviated the adverse effects of water stress, highlighting the importance of adequate P fertilisation for soybean resilience to water stress.

Keywords Shoot traits · Root traits · Leaf gas exchange traits · Root exudation · Phosphorus use efficiency · Water use efficiency · Seed yield

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Abbreviations

DAS	Days after sowing
FC	Field capacity
DW	Dry weight
SDW	Shoot dry weight
WW	Well-watered
EWS	Early water stress
TWS	Terminal water stress
SWC	Soil water content
WUE	Water-use efficiency
PUE	Phosphorus-use efficiency

Introduction

Soybean (*Glycine max* L.) is a widely grown crop worldwide renowned for its rich protein and edible vegetable oil content. However, soybean plants often encounter water stress and P deficiency in many regions. The availability of P fertilisers, crucial for sustaining soybean productivity, raises concerns due to the limited rock phosphate reserves and projected depletion within the next 50 to 80 years (Cordell and White 2011). Studies have confirmed that inadequate P significantly reduces soybean shoot, root traits, and seed yield (Qingping et al. 2003; Feng et al. 2021a; Salim et al. 2023; Wang et al. 2010). Water stress can impede soil P diffusion and plant P uptake (Suriyagoda et al. 2014), slowing plant growth by decreasing photosynthetic rates and stomatal conductance (Ghannoum and Conroy 2007). Leguminous crops like soybean suffer compromised growth and yield under combined P deficiency and abiotic stress (Sulieman and Tran 2015). Roots have evolved strategies such as carboxylate release to enhance P acquisition from low P soils (Lambers et al. 2006). This root exudation also assists plants in mitigating excess carbon accumulation under water stress, stemming from an imbalance between leaf photosynthetic supply and reduced carbon demand for shoot growth (Williams and de Vries 2020).

Water stress significantly reduces soybean yield (Oya et al. 2004). The anticipated climate changes will intensify this stress, threatening soybean production worldwide (Foyer et al. 2016). Particularly vulnerable are the agricultural areas of Western Australia that heavily rely on farming for economic survival (Siddique et al. 1993). Soybean plants respond to drought by extending their taproot into deeper

soil layers for enhanced water accessibility (Kaspar et al. 1984; Chen et al. 2022). Drought stress also increases biomass partitioning to roots, increasing the root-to-shoot ratio (Manavalan et al. 2009). The initial response to water stress involves stomatal closure, decreasing transpiration and photosynthesis, and ultimately hindering plant growth and development (Arya et al. 2021; Mutava et al. 2015). Traits such as chlorophyll content (Yang et al. 2021), net photosynthetic rate, stomatal conductance, and transpiration rate (Wang et al. 2020) have been investigated for their role in drought tolerance. Efficient water use, reflected in increased water use efficiency (WUE), plays a pivotal role in drought tolerance through reduced transpiration and photosynthesis (Kaler et al. 2017). Plants often respond to reduced water supply with stomatal closure, which decreases leaf transpiration and photosynthesis rate, increases leaf temperature (Tardieu 2005, 2013), and improves WUE (Tardieu 2005). Drought stress during the reproductive stage adversely affects grain yield, seed number and size (Desclaux et al. 2000), with soybean particularly susceptible to drought stress during the flowering and podding stages, decreasing pod set, seed number, and yield (Smiciklas et al. 1992; Sadeghipour and Abbasi 2012; He et al. 2017).

Plants experiencing water stress and low P conditions exhibit distinct root morphological responses for enhanced resource acquisition, such as increased specific root length, decreased root diameter and improved root mass ratio (Suriyagoda et al. 2014; Fan et al. 2015). Under combined water stress and P deficiency, plants increase root mass ratio to enhance nutrient and water acquisition (Shen et al. 2011). Drought (Manavalan et al. 2009) and low P availability (Qingping et al. 2003) are pivotal factors limiting soybean seed yield and its components. Studies have shown that P supplementation alleviated the adverse effects of drought on yield performance in soybean (He et al. 2019; Jin et al. 2006; Feng et al. 2021b) and other crops, such as wheat and barley (Rodriguez et al. 1996; Jones 2003), and cowpea (Jemo et al. 2017). Increased P availability has been linked to improved WUE (Payne et al. 1992) in numerous plant species under drought stress. While applied P counteracted the adverse effects of drought stress soybean on yield (Jin et al. 2006), the underlying mechanisms remain unclear. While root morphological and physiological responses to low P environments and water

stress have been explored in grain legumes (Pearse et al. 2006), pasture legumes (Pang et al. 2010) and canola (Pearse et al. 2006), but limited research has investigated their interaction concerning root systems, root morphological and physiological responses, PUE, WUE, yield and yield-contributing traits across different growth stages in leguminous crops exposed to combined water stress and low P availability (Fan et al. 2015; Suriyagoda et al. 2010). Hence, this study examines individual and combined interactive effects of low P availability and water stress on various traits in a soybean genotype grown in soil columns.

Our hypothesis posits that the combined effect of low P and water stress decreases plant growth, shoot and root traits, seed yield, yield components, physiological PUE, WUE, root exudation, and gas exchange traits across various growth stages. However, we anticipate that P supply will alleviate the detrimental effects of water stress on plant growth, morphological and physiological traits and seed yield.

Material and methods

Plant material and growth conditions

We selected soybean genotype (PI 561271) based on its root traits, phenology and yield established in our previous study (Salim et al. 2023). Plants were grown in polyvinyl chloride cylindrical (PVC) columns (100 cm depth and 15 cm diameter). A long, transparent polyethylene sleeve (110 cm × 25 cm, 105 µm thick) with 24 holes was placed inside each PVC column to ease harvest and to avoid root damage at harvest. Each PVC column was filled with 1.0 kg coarse gravel at the bottom and 25 kg sandy loam soil and river sand (3:1 ratio by weight). The soil was collected from UWA's Ridgefield Farm, Pingelly (32°30' S, 116°59' E), which had a sandy loam texture comprising 72% sand, 8% silt and 20% clay. Table 1 lists the soil's chemical properties. Basal nutrients [190 mg N kg⁻¹ dry soil and 130 mg K kg⁻¹ dry soil (Gao et al. 2020)] and P as per the two P treatments [low P (P10) with 10 mg P kg⁻¹ in dry soil and optimum P (P60) with 60 mg P kg⁻¹ in dry soil using single superphosphate as the P source] were applied in the top 20 cm soil layers in each PVC column and equally distributed to mimic field fertiliser application. There were three water

Table 1 Chemical properties of the soil used in this study (before mixing with sand)

Property	Value
Sand (%)	72
Silt (%)	8
Clay (%)	20
Ammonium nitrogen (mg kg ⁻¹)	4.3
Nitrate nitrogen (mg kg ⁻¹)	3.7
Phosphorus Colwell (mg kg ⁻¹)	6.5
Potassium Colwell (mg kg ⁻¹)	74
Sulphur (mg kg ⁻¹)	3.4
Total organic carbon (g kg ⁻¹)	0.47
Conductivity (dS m ⁻¹)	0.04
pH (CaCl ₂)	7.00
pH (H ₂ O)	7.80
Phosphorus buffering index (PBI)	65.3

treatments such as well-watered (WW), early water stress (EWS) and terminal water stress (TWS) (Supplementary Fig. 1). The experiment followed the same procedures for seed sterilisation, rhizobial inoculation, and fertiliser application as described elsewhere (Salim et al. 2022, 2023). This experiment, conducted in a controlled environment glasshouse from October 2022 to March 2023, had a randomised complete block design with two factors (three water treatments and two P levels), three harvests, and four replicates (64 PVC columns + 8 extra columns for initial plant growth data) (Supplementary Fig. 1). At 20 days after sowing (DAS), the PVC columns were covered with a ~ 10 mm layer of glass beads to minimise soil evaporation. Dropped leaves were collected quickly to avoid decay in the PVC columns and oven-dried at 70 °C.

Water treatment determination and calculation

Field capacity (FC) was determined before the experiment following the method in Figueroa-Bustos et al. (2020). Water content in the columns was managed by watering to weight every three days, with water-leaking outlets at the bottom. Until 40 DAS, all PVC columns were hand-watered twice weekly to maintain 80% FC (Supplementary Fig. 2). For 64 PVC columns, two water treatments were imposed at 40 DAS at the vegetative stage: (1) well-watered (WW) by hand watering twice a week to maintain 80% FC and (2) early water stress (EWS) by watering to 40% FC up to the flowering

stage, with the terminal water stress (TWS) treatment imposed from the flowering to maturity stage by withholding watering. All columns were weighed twice weekly between 12:00 and 15:00 on the watering day, with all weights recorded. Total water use for the whole lifecycle was calculated by summing the added from sowing to maturity (Supplementary Fig. 2). The following variables were calculated: (i) Water use efficiency for seed yield (WUE)=seed yield/water use, (ii) WUE for shoot dry=shoot dry weight/water use, (iii) soil water content (SWC) was calculated as $[1 - (W_c - W_n)/(W_c - W_d)] \times 100$, where W_c is the initial column weight at saturation, W_n is the weight of the column on the day of measurements, and W_d is the weight of the column with dry soil (Sharma et al. 2020; Figueroa-Bustos et al. 2019).

Measurements of above-and below-ground traits

At 40 DAS, eight columns were harvested to obtain initial plant growth data (Supplementary Table S1). Sixteen PVC columns (8 from WW and 8 from EWS) were harvested at the appearance of the first flower (65 DAS), 24 PVC columns (8 from WW, 8 from EWS and 8 from TWS) were harvested at the seed formation stage (115 DAS), and 24 PVC columns (8 from WW, 8 from EWS and 8 from TWS) were harvested at maturity when 95% of the pods had turned brown (145 DAS) (Fehr et al. 1971). Plant growth, shoot dry weight, root dry weight, leaf gas exchange traits, root exudation, P acquisition, PUE, and WUE were measured. Immediately after harvesting the shoots, the plastic bag was removed carefully from each PVC column, with plastic sleeves cut longitudinally to remove the root system from the soil. The root system was shaken gently to remove bulk soil before collecting rhizosphere root exudation. After collecting root exudates, the roots were washed, placed in plastic bags and stored in a 4 °C cold room until root scanning using WinRhizo Pro software (v2009, Regent Instrument, Quebec, QC, Canada), with the standard method (Salim et al. 2022) to measure root traits.

Root exudation collection and measurements

Root exudation collection followed an established method routinely used in our laboratory (Chen et al. 2013a; Salim et al. 2023). Briefly, roots

with rhizosphere soil were immersed in a 500 mL beaker containing 50–200 mL of 0.2 mM CaCl_2 , depending on the root volume, for about one minute with frequent gentle shaking to maximise the detachment of rhizosphere soil. After removing the roots from the solution, a subsample of the suspension was drawn and filtered (0.20 μm) into a 1-mL HPLC vial containing 20 μL concentrated orthophosphoric acid. The filled HPLC vials were placed on dry ice during the extraction procedures and stored at -20 °C until carboxylate analysis using the HPLC method developed by Cawthray (2003), except oxalate, which was determined according to Ushio et al. (2015). Carboxylates (oxalic, citric, cis-aconitic, fumaric, malic, and malonic acids) were detected by relating the retention times and absorption spectra of samples with working standards (ICN Biomedicals Inc., Aurora, OH, USA). Total carboxylates are the sum of all organic acids exuded from rhizosphere roots.

Shoot, root and seed P acquisition, physiological PUE, and seed N and protein content

About 12–15 mg subsamples of finely ground dry shoots (stems and leaves), roots and seeds were added to hot concentrated nitric and perchloric acids for acid digestion (3: 1) as detailed elsewhere (Pang et al. 2010). The samples were ground to a fine powder using a coffee grinder. Shoot P, root P and seed P and N concentrations were determined using a UV-VIS spectrophotometer (Shimadzu Corporation, Japan), followed by the malachite green method (Motomizu et al. 1983). Shoot P, root P, seed P and N contents were obtained from the P and N concentrations and corresponding dry weights. PUE was calculated as the ratio of shoot dry weight to shoot P concentration, as described by Hammond et al. (2009). Seed protein content was calculated from the percentage of N concentration multiplied by 6.25 (the conversion factor), as described by Tshering et al. (2022).

Leaf gas exchange traits

Leaf chlorophyll content was determined using a Soil Plant Analysis Development (SPAD) 502+ chlorophyll meter (Minolta, Japan). Photosynthetic rate,

transpiration rate, and stomatal conductance were determined at the initial growth stage (vegetative stage, 40 DAS), flowering stage (65 DAS), and seed formation stage (115 DAS) using a LICOR-6400 gas exchange unit (Li-COR Biosciences, Lincoln, NE, USA). Gas exchange and SPAD readings were measured on the youngest fully expanded leaves on the main stem between 10.00 and 13.00 at each growth stage (Liyana and Dilrukshi 2022). The SPAD meter measured the difference between red (650 nm) and infrared (940 nm) light transmittance through the leaf, generating a SPAD value proportional to leaf chlorophyll content. Photosynthetic photon flux density was set at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface, block temperature at $25 \text{ }^\circ\text{C}$, the flow rate at $500 \mu\text{mol s}^{-1}$, and ambient CO_2 concentration of the incoming gas at $400 \mu\text{mol mol}^{-1}$. Plants were harvested after two days when the stomata of fully expanded young leaves in water-stressed plants were closed. Stomatal conductance was used as an integrative parameter reflecting water-stressed plants (Medrano et al. 2002).

Yield and yield-contributing traits

At maturity, the plants were cut at ground level. Pods were separated from leaves and stems into pod walls and seeds. Total pod and seed numbers were recorded. Seed weight was recorded after oven-drying at $30 \text{ }^\circ\text{C}$ for 7 d. Leaves, stems, and pod shells were oven-dried at $65 \text{ }^\circ\text{C}$ for 72 h and weighed for shoot dry weight. Harvest index was calculated as the ratio of seed weight to total above-ground dry weight. Seed number per pod was calculated as total seed number divided by total number of filled pods. Mean seed weight was calculated as seed yield divided by seed number.

Statistical analyses

Analysis of variance (ANOVA) was determined to investigate the effect of three water and two P treatment and their interactions on the parameters at flowering, seed formation and maturity stages. The effect of P on plant growth traits was only measured at the vegetative stage. In cases of no significant interaction, the main effects ($P < 0.05$) were presented by pooling the data across water and P treatments. Tukey's honest significance difference test was used for multiple comparisons and determining significant differences

between treatments ($P < 0.05$). Pearson's correlation analysis was performed separately on each vital parameter from the seed formation and maturity stages.

Results

Phenology, shoot and root morphological traits

At 40 DAS at the vegetative stage, all parameters significantly differed in response to P supply (Supplementary Table S1, Supplementary Fig. 3a, $P < 0.01$). Flowering commenced 63–65 DAS in all treatments. For EWS, time to flowering occurred two days earlier than WW conditions in both P treatments (Supplementary Table S4).

At the flowering stage, some parameters did not significantly differ in response to water and P supply interaction effects, including days to flowering, trifoliolate leaf number, SPAD value, root shoot ratio, and specific root length (Supplementary Table S4). Other parameters, including plant height, leaf area, shoot dry weight, root dry weight, total dry weight, average root diameter, total root length and root length density, exhibited individual and interaction effects between water and P supply (Table 2, Supplementary Fig. 3b, $P < 0.05$, $P < 0.01$ and $P < 0.001$). The EWS treatment decreased plant height, leaf area (16%), shoot dry weight (26%), root dry weight (28%), total dry weight, average root diameter, total root length and root length density compared to the WW treatment under P60, but no significant differences in these parameters occurred under P10 (Table 2, Supplementary Fig. 4a).

At the seed formation stage, the water and P supply interactions were not significant for days to 1st pod set, days to seed formation, plant height, trifoliolate leaf number, average root diameter, or specific root length (Supplementary Table S6). In contrast, significant main effects and their interaction occurred for SPAD value, leaf area, shoot dry weight, root dry weight, total dry weight, root: shoot ratio, total root length and root length density (Table 3, Supplementary Fig. 3c, 4b, $P < 0.05$, $P < 0.01$ and $P < 0.001$). The three water treatments had similar SPAD values under P60, while WW had higher SPAD values than EWS and TWS under P10. Under P60, leaf areas decreased under EWS (10%) and TWS (60%), with no significant difference under P10. Under P60, shoot

Table 2 Root and shoot traits of soybean genotype PI 561271 harvested at the flowering stage (64 DAS) grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW) or

early water stress (EWS) conditions. Values mean four replicates (one plant per soil column)

Water treatment	P rate	Plant height (cm)	Leaf area (cm ² plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Total dry weight (g plant ⁻¹)	Average root diameter (mm)	Total root length (m)	Root length density (cm cm ⁻³)
WW	P10	21.7c	579c	5.97c	3.54c	9.51c	0.38c	283c	1.60c
EWS	P10	22.2c	474c	5.30c	3.21c	8.51d	0.37c	275c	1.48c
WW	P60	34.0a	1509a	12.38a	5.63a	18.02a	0.49a	443a	2.51a
EWS	P60	28.7b	1302b	9.81b	4.40b	14.21b	0.45b	350b	1.98b
ANOVA	P	***	***	***	***	***	***	***	***
	W	*	***	***	***	***	***	***	***
	W×P	**	*	***	***	***	*	***	***

Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

dry weights decreased by 22% in EWS and 62% in TWS compared to WW conditions (Table 3, Supplementary Fig. 3c, $P < 0.01$). Under P10, shoot dry weight decreased by 12% in EWS and 32% in TWS compared to WW conditions. EWS plants produced the most root dry weight (14% more than WW and TWS) under P60. Root dry weights ranged from 3.64 g plant⁻¹ (TWS) to 4.27 g plant⁻¹ (EWS) to 4.74 g plant⁻¹ (WW) under P10 (Supplementary Fig. 4b, $P < 0.001$). Under P60, EWS plants also

produced the greatest total root length (14% more than WW and TWS). Under P10, WW plants had the greatest total root length (Table 3). At maturity, WW and EWS produced 26% more shoot dry weight than TWS under P60, with no significant differences observed under P10. Under P60, root dry weights decreased by 14% in EWS and TWS plants compared to WW plants, with no significant differences observed under P10 (Table 4).

Table 3 Root and shoot traits of soybean genotype PI 561271 harvested at the seeding stage (115 DAS) grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil with well-watered (WW), early water stress (EWS) or terminal water stress (TWS) conditions

Water treatment	P rate	SPAD value	Leaf area (cm ² plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Total dry weight (g plant ⁻¹)	Root: shoot ratio	Total root length (m)	Root length density (cm cm ⁻³)
WW	P10	31.67b	776d	15.92 cd	4.74d	20.66 cd	0.30a	393c	2.22c
EWS	P10	27.20c	751d	13.96de	4.27e	18.23de	0.31a	359d	2.03d
TWS	P10	27.10c	652d	10.79e	3.64f	14.42e	0.34a	309e	1.75e
WW	P60	41.07a	1737a	31.96a	6.56b	38.51a	0.21b	510b	2.89b
EWS	P60	41.07a	1570b	26.17b	7.21a	33.38b	0.28a	569a	3.22a
TWS	P60	42.10a	1085c	19.64c	6.04c	25.67c	0.31a	486b	2.75b
ANOVA	P	***	***	***	***	***	***	***	***
	W	*	***	***	***	***	***	***	***
	W×P	**	***	**	***	**	*	***	***

Values mean four replicates (one plant per soil column). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Table 4 Dry matter accumulation, yield and yield contributing traits of soybean genotype PI 561271 harvested at physiological maturity (145 DAS) grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW), early water stress (EWS) or terminal water stress (TWS) conditions

Water treatment	P rate	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Total dry weight (g plant ⁻¹)	Mature pods (plant ⁻¹)	Empty pods (plant ⁻¹)	Pod dry weight (g plant ⁻¹)	Seeds number (pod ⁻¹)	Seeds number (plant ⁻¹)	100 seed weight (g)	Harvest index (%)
WW	P10	11.2c	7.7b	18.9d	19.0b	1.3bc	11.7b	2.3b	44.0b	10.6c	29.3b
EWS	P10	10.4c	7.7b	18.1de	13.3bc	2.0ab	11.6bc	2.1bc	28.3 cd	9.8c	31.3b
TWS	P10	9.5c	7.1b	16.7e	9.0c	3.3a	7.8c	1.8c	16.1d	9.4c	19.5c
WW	P60	17.9a	8.8a	26.6a	32.6a	0	23.9a	3.1a	102.3a	14.0a	42.5a
EWS	P60	16.0a	7.9b	24.0b	31.3a	0	21.9a	2.8a	88.4a	13.1ab	41.8a
TWS	P60	13.4b	7.5b	20.9c	18.0b	0.7bc	11.5bc	2.2b	40.8bc	11.3bc	27.5b
ANOVA	P	***	***	***	***	***	***	***	***	***	***
	W	***	***	***	**	**	***	***	***	***	***
	W × P	*	*	**	**	ns	***	ns	***	ns	ns

Values mean four replicates (one plant per soil column). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Turkey's test. ns, non-significant; *, **, and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

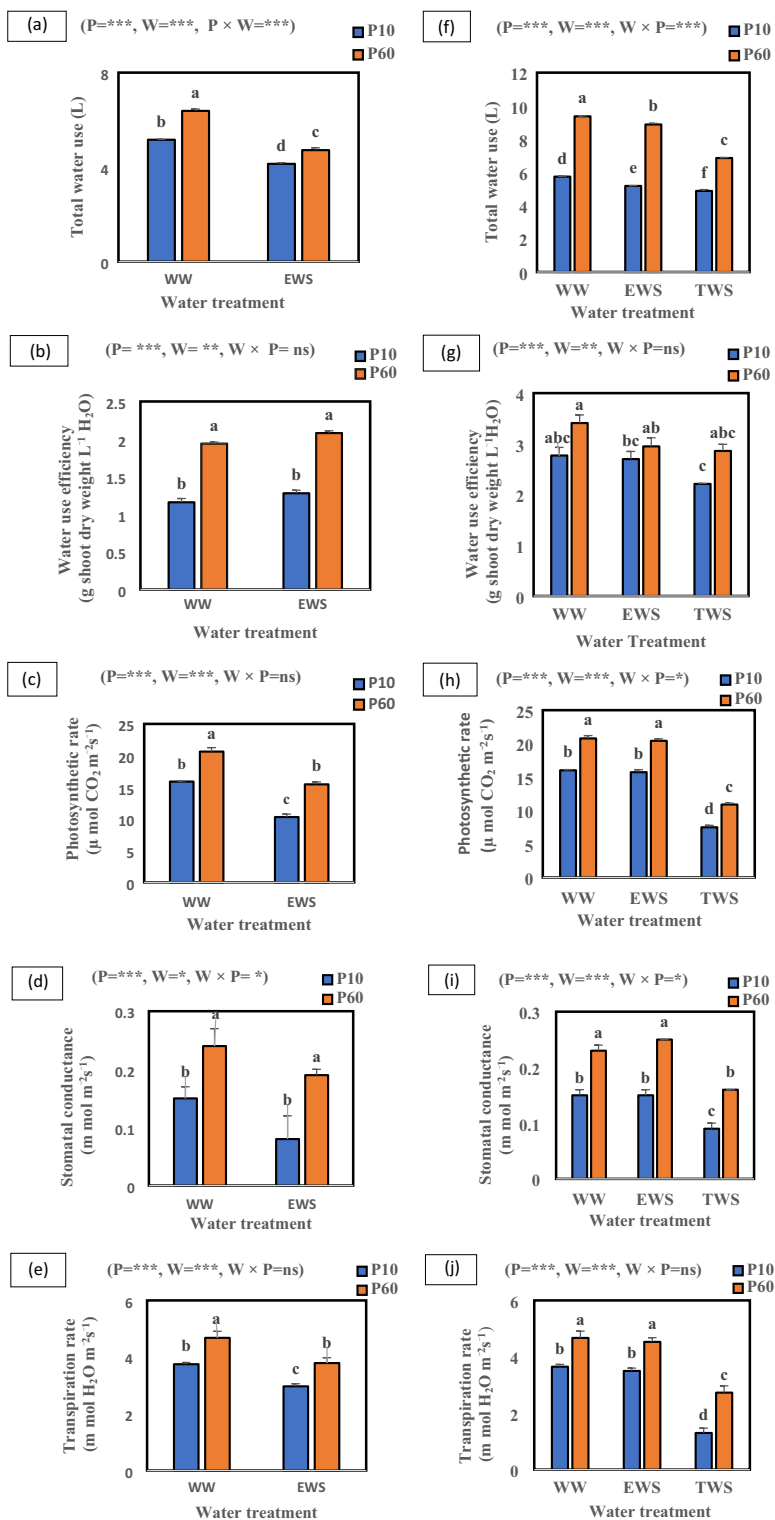
Leaf gas exchange

At the vegetative stage, WW conditions significantly increased the photosynthetic rate, stomatal conductance, and transpiration rate under P60 compared to P10 (Supplementary Table S2). At the flowering stage, individual water and P supply effects occurred for photosynthetic and transpiration rates, but no interaction effects occurred (Fig. 1c, e). However, stomatal conductance significantly differed between the P10 and P60 treatments, with similar values in WW and EWS plants (Fig. 1d). At the seed formation stage, WW and EWS plants exhibited significant water and P interaction effects on photosynthetic rate and stomatal conductance (Fig. 1h, i, $P < 0.05$). Significant main effects for water and P occurred for transpiration rate (Fig. 1j, $P < 0.001$). The photosynthetic rate of TWS plants significantly decreased by 89% under P60 and 113% under P10 compared to WW and EWS, which had statistically similar values for P60 and P10 (Fig. 1h, $P < 0.05$). Similar trends occurred for stomatal conductance. TWS plants decreased stomatal conductance by 50% under P60 and 67% under P10 compared to WW and EWS (Fig. 1i, $P < 0.05$).

Rhizosphere carboxylates

At the flowering stage, oxalic acid, malic acid, malonic acid, citric acid, fumaric acid, and total carboxylates exhibited significant water and P supply interaction effects (Supplementary Table S5, Fig. 2a, b, $P < 0.05$, $P < 0.01$ and $P < 0.001$) while cis-aconitic acid had individual effects for P supply (Supplementary Table S5, Fig. 2b). Under P60 EWS plants decreased total carboxylates (111%) compared to WW plants, while under P10, EWS plants secreted more total carboxylates (119%) than WW plants. Under P60, WW plants secreted more oxalic acid, malic acid, malonic acid, citric acid and fumaric acid than EWS plants. Under P10 and P60, all water treatments produced higher proportions of oxalic acid, citric acid, malic acid and malonic acid in the rhizosphere than other carboxylates. Under P10, WW plants had higher proportions of citric acid (60%) and oxalic acid (25%) than EWS plants. Under P60, EWS plants had a higher proportion of oxalic acid (25%) than WW plants (Supplementary Table S5, Fig. 2b). At the seed formation stage, malic acid, malonic acid, fumaric acid, and total carboxylates exhibited significant

Fig. 1 (a, f) Total water use, (b, g) water use efficiency, (c, h) photosynthetic rate, (d, i) stomatal conductance and (e, j) transpiration rate of soybean genotype PI 561271 grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW) or early water stress (EWS) conditions. Values are the mean of four replicates (one plant per soil column) ± SE. Plants were harvested at the flowering stage (64 DAS) (a–e) and at the seed formation stage (115 DAS) (f–j). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. ns, non-significant; *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively



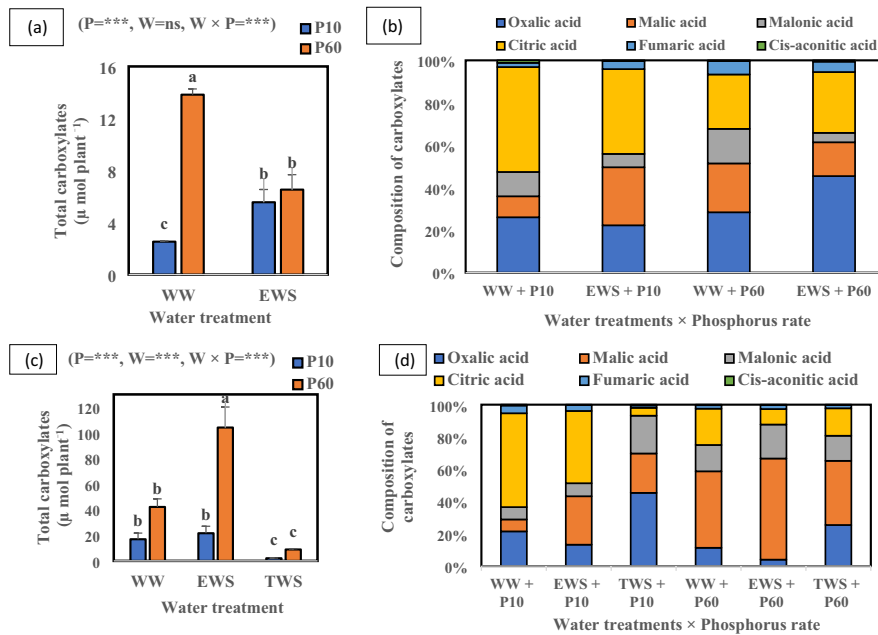


Fig. 2 (a, c) Total carboxylates per plant and (b, d) carboxylate proportions (oxalic acid, malic acid, malonic acid, citric acid, fumaric acid and cis-aconitic acid) of soybean genotype PI 561271 grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW) or early water stress (EWS) conditions. Values are the mean of four replicates (one plant per soil column) ± SE. Plants were harvested at the flowering stage (64 DAS) (a–b) and at the seed formation stage (115 DAS)

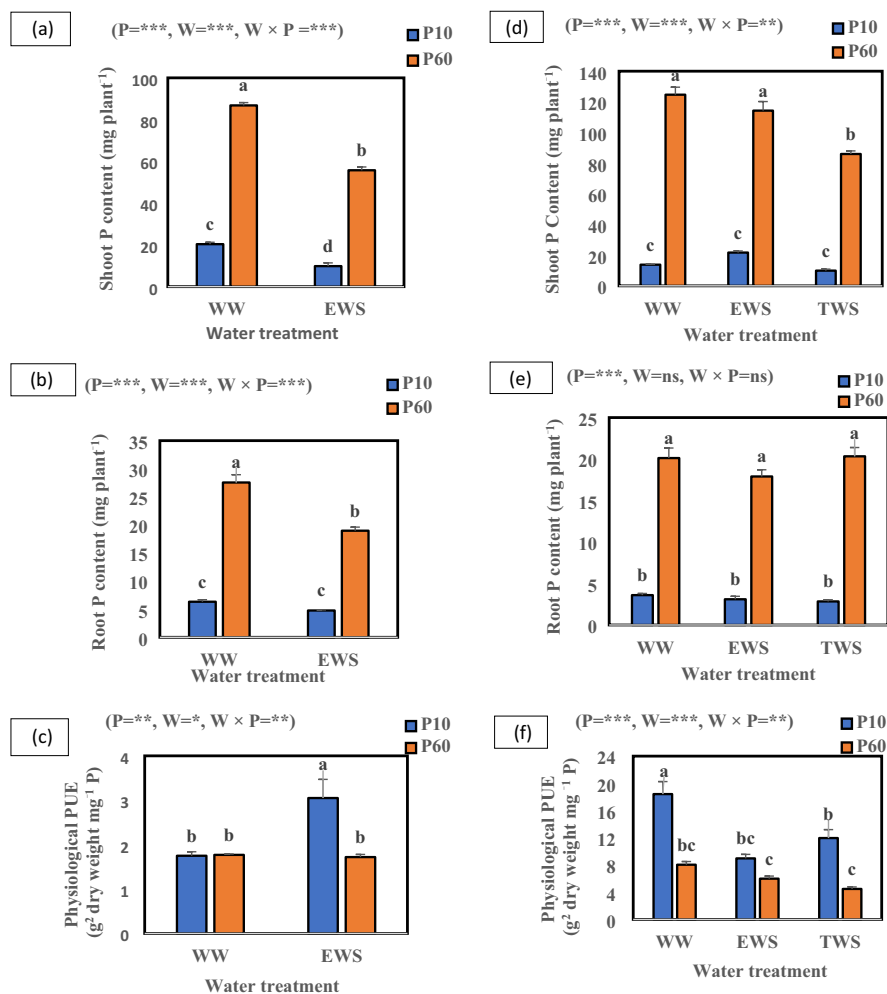
water and P supply interaction effects (Supplementary Table S7, Fig. 2c, d, $P < 0.05$ and $P < 0.001$), while oxalic acid, citric acid and cis-aconitic acid had individual effects for P supply (Supplementary Table S7, Fig. 2c, d). Under P60, compared to EWS and WW, TWS plants decreased total carboxylate exudation (10.7) and (3.7) folds, respectively. Similarly, under P10, TWS plants decreased total carboxylate exudation (862%) compared to EWS and WW. Under P10 and P60, EWS plants comprised higher proportions of malic acid and malonic acid than other carboxylates. Under P10, EWS and TWS plants decreased the proportion of citric acid by 50% and 5% compared to WW plants. Under P60, EWS had the highest proportion of malic acid compared to other carboxylates, followed by WW and TWS (Supplementary Table S7, Fig. 2c, d).

(c–d). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. ns, non-significant; *** significant at $P < 0.001$

Shoot, root and seed P contents, physiological PUE, and seed N and protein content

At the vegetative stage, P60 and TWS P10 plants significantly differed for shoot P concentration, shoot P content, root P concentration, root P content and physiological PUE (Supplementary Table S3, $P < 0.001$), decreasing more under P10 than P60 for all treatments. At the flowering stage, shoot P content, root P content and physiological PUE exhibited significant water and P supply interaction effects (Fig. 3a–c, $P < 0.01$ and $P < 0.001$). The EWS plants had reduced shoot P content (55%), root P content (45%) and physiological PUE (3%) compared to WW under P60. The EWS plants under P10 had the highest physiological PUE compared to the other treatments. At the seed formation stage, shoot P content and physiological PUE exhibited significant water and P supply

Fig. 3 (a, d) Shoot P content, (b, e) root P content, and (c, f) physiological P-use efficiency of soybean genotype PI 561271 grown with 10 (P10) or 60 (P60) mg kg^{-1} dry soil under well-watered (WW) or early water stress (EWS) conditions. Values are the mean of four replicates (one plant per soil column) \pm SE. Plants were harvested at the flowering stage (64 DAS) (a–c) and at the seed formation stage (115 DAS) (d–f). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. ns, non-significant; *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively



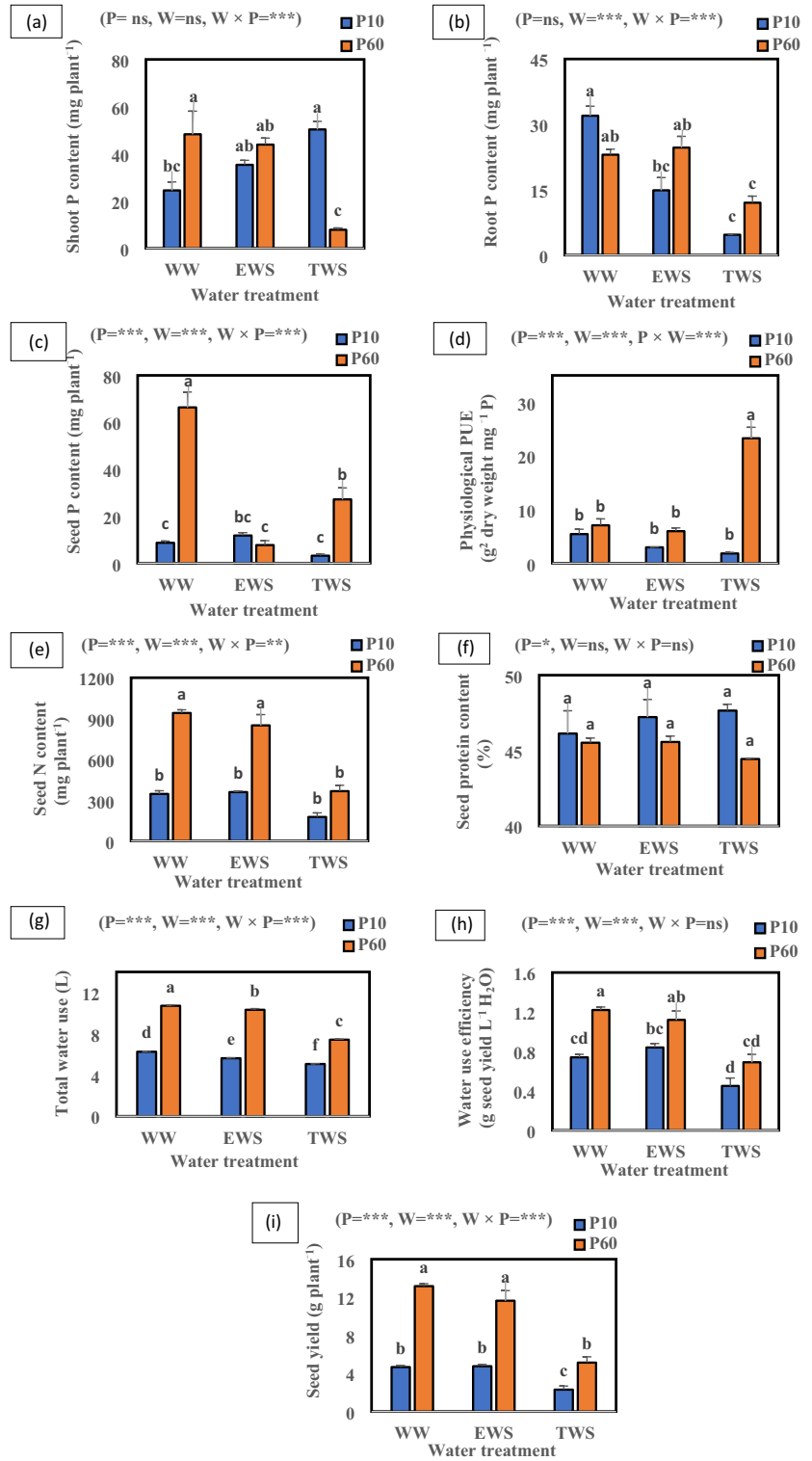
interaction effects (Fig. 3d, f, $P < 0.01$), and root P content had individual effects for water and P supply (Fig. 3e). Under P60, TWS plants decreased shoot P content (38%) and physiological PUE (45%) compared to WW and EWS. Under P10, WW plants had 71% higher physiological PUE (71%) than EWS and TWS. WW plants under P10 and P60 had the highest physiological PUE values (Fig. 3d, f, $P < 0.01$). At the maturity stage, shoot P content, root P content and physiological PUE, seed P content, and seed N content exhibited significant water and P supply interactive effects (Fig. 4a–e, $P < 0.001$), seed protein content had individual effects for P (Fig. 4f, $P < 0.05$). Under P60, TWS plants significantly decreased shoot P content (475%), root P content (95%), seed P content (35%), and seed N content (144%) and increased physiological PUE (253%) compared to WW and EWS. Under

P10, WW plants increased physiological PUE (71%) compared to EWS and TWS. The WW plants under P60 had the highest seed P content, TWS plants under P60 had the highest physiological PUE (Fig. 4c, d, $P < 0.001$), and P10 produced significantly higher seed protein contents than P60 (Fig. 4e, $P < 0.05$).

Soil water content, water use and WUE

The water consumption rate of EWS and TWS plants varied between P treatments, with a significant water and P supply interaction effect ($P < 0.001$). The SWC in WW plants from sowing to maturity was maintained at 80% FC (Supplementary Fig. 2a, b) in both P treatments. The SWC in EWS plants decreased linearly, reaching ~36% under P60 and ~39% under P10 (Supplementary Fig. 2a). The SWC in TWS

Fig. 4 **a** Shoot P content, **b** root P content and **c** seed P content, **d** physiological P-use efficiency, **e** seed nitrogen content, **f** seed protein content (%), **g** total water use, **h** water - use efficiency and **i** seed yield per plant of soybean genotype PI 561271 grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW), early water stress (EWS) or terminal water stress (TWS) conditions. Values mean four replicates (one plant per soil column) ± SE. Plants were harvested at physiological maturity (145 DAS). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. Mean data for each trait followed by different letters differ significantly at $P < 0.05$ using Tukey's test. ns, non-significant; *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively



plants declined to ~25% under P60 and ~28% under P10 (Supplementary Fig. 2b). At the vegetative stage, WW plants significantly increased water use and WUE under P60 compared to P10 (Supplementary Table S2, $P < 0.001$). Water use had significant water and P supply interaction effects at the flowering stage, but no interaction effects occurred for WUE despite individual water and P supply effects (Fig. 1a, b, $P < 0.001$). Similarly, at the seed formation stage, water use exhibited significant water and P supply interactive effects, and WUE had individual water and P supply effects but no interactive effects (Fig. 1f, g, $P < 0.01$ and $P < 0.001$). The WW plants used more water (19%) under P60 and P10 (14%) than the EWS and TWS plants. At the maturity stage, water use exhibited significant water and P supply interactive effects, and WUE had individual water and P supply effects but no interactive effects. Under P60, shoot WUE declined by 9% in EWS and 76% in TWS compared to WW. Under P10, shoot WUE decreased by 13% in WW and 46% in TWS compared to EWS (Fig. 4g, h, $P < 0.001$).

Seed yield and yield components

Mature pod number, pod dry weight, seed number per plant and seed yield exhibited significant individual effects for water and P supply and their interaction (Table 4, Fig. 4i, $P < 0.05$, $P < 0.01$ and $P < 0.001$). However, non-significant interactions occurred for empty pod number, seed number per pod, 100-seed weight and harvest index (Table 4). Under P60 and P10, TWS plants significantly reduced mature pod numbers by 75% and 77%, respectively, but WW and EWS plants produced similar mature pod numbers in both P supply treatments (Table 4, Supplementary Fig. 3c, $P < 0.01$). TWS plants decreased seed numbers per plant by 138% under P60 and 100% under P10 compared to WW and EWS plants, with the highest numbers for WW plants (Table 4, $P < 0.001$). The TWS plants under P10 produced the most empty pods, while WW and EWS plants had no empty pods under P60. The TWS plants had significantly reduced seed yields per plant under P60 (143%) and under P10 (117%) than WW and EWS plants. Seed yield per plant ranged from 5.14 g in TWS to 13.17 g in WW under P60 and 2.30 g in TWS to 4.67 g in WW under P10 (Table 4, $P < 0.001$).

Pearson's correlation (r) for 14 traits

Shoot dry weight, root dry weight, seed yield, photosynthetic rate, stomatal conductance, transpiration rate, total carboxylates, root P content, seed P content, seed N content and WUE (grain) parameters positively correlated with each other, while shoot P content, physiological PUE and seed protein (%) did not (Fig. 5). However, shoot P content positively correlated with seed protein (%) and negatively correlated with physiological PUE. Moreover, physiological PUE negatively correlated with seed protein (%) (Fig. 5).

Discussion

Interaction of water and P treatments on plant growth and shoot and root traits

The findings of this study underscore the intricate interplay between water availability and phosphorus (P) supply on various plant growth parameters and shoot and root traits. Specifically, our results demonstrate that EWS reduced leaf area, shoot dry weight, root dry weight, and total root length at the flowering stage in response to P60 supply. In contrast, a previous study reported that P application increased soybean leaf area under well-watered and water-deficit conditions (He et al. 2019). In the seed formation stage, leaf area, root dry weight and shoot dry weight decreased in EWS and TWS plants compared to WW plants in both P treatments. However, P supply increased leaf area, root dry weight, shoot dry weight and other traits under water stress compared to WW. Applying P to soils stimulates a series of physiological and morphological adjustments in plants that play an essential role in water-deficit tolerance (Faustino et al. 2013; Jin et al. 2006).

In another study, soybean maintained greater root and shoot growth, leaf area, and photosynthetic rate under drought stress (Fatema et al. 2023). The EWS plants under P60 produced the highest root dry weight and total root length at seed formation and maturity stages. Earlier studies have shown that P and water supply affect shoot and root traits, water use and seed yield (Feng et al. 2021b; He et al. 2017). In soybean, water stress significantly decreased root length and surface area, while the opposite trend occurred

Root dry wt.	0.75***																	
Seed yield	0.95***	0.79***																
Photosynthetic rate	0.75***	0.76***	0.86***															
Stomatal conductance	0.91***	0.71***	0.93***	0.88***														
Transpiration rate	0.74***	0.75***	0.84***	0.97***	0.86***													
Total carboxylates	0.64***	0.37	0.73***	0.70***	0.82***	0.66***												
Shoot P content	0.12	0.25	0.29	0.23	0.15	0.16	0.33											
Root P content	0.42*	0.45*	0.48**	0.74***	0.54**	0.75***	0.4	-0.09										
Seed P content	0.69***	0.76***	0.63***	0.46*	0.49**	0.45*	0.01	-0.008	0.17									
Seed N content	0.69***	0.82***	0.69***	0.56**	0.52**	0.54**	0.12	0.38	0.22	0.88***								
Physiological PUE	0.24	-0.01	0.01	-0.17	0.07	-0.1	-0.14	-0.79***	-0.12	0.26	-0.07							
Water use effi.(grain)	0.85***	0.77***	0.94***	0.91**	0.87***	0.89***	0.69***	0.28	0.56**	0.57**	0.64***	-0.058						
Seed protein (%)	-0.38	-0.07	-0.24	-0.26	-0.31	-0.24	-0.12	0.49**	-0.38	-0.26	-0.07	-0.55**	-0.22					
	Shoot dry wt.	Root dry wt.	Seed yield	Photosynthetic rate	Stomatal conductance	Transpiration rate	Total carboxylates	Shoot P content	Root P content	Seed P content	Seed N content	Physiological PUE	Water use effi.(grain)					

Fig. 5 Pearson's correlation (r) for 14 traits of soybean genotype PI 561271 grown with 10 (P10) or 60 (P60) mg kg⁻¹ dry soil under well-watered (WW), early water stress (EWS) or terminal water stress (TWS) conditions. Values mean four replicates (one plant per soil column). Plants were harvested at physiological maturity (145 DAS). Early water stress was imposed at the vegetative stage (40 DAS) with 40% FC until

the flowering stage and rewatered to 80% FC at the flowering stage until harvest. Terminal water stress was imposed at the flowering stage (64 DAS) by withholding watering until harvest. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ (without colour). Colour indicates non-significant correlation values among tested traits

for P addition (He et al. 2019). We found that TWS exerted a more detrimental effect on plant growth and dry matter accumulation than EWS. However, EWS plants produced more root and shoot dry weight and root length than WW plants at the seed formation stage, rebounding on re-watering, indicating a degree of resilience once stress conditions were alleviated, as described by Esan et al. (2023). These results suggest that water stress imposed before the flowering stage is not as harmful as water stress imposed after flowering, irrespective of P supply. Moreover, water restriction and P deficiency reduced plant growth as reflected by low shoot, root, and dry weights, more so under the combined stress, as observed elsewhere (Oukaltouma et al. 2022).

Several studies have shown that low P significantly reduced shoot and root dry weights in mungbean (Meena et al. 2021), chickpea (Pang et al. 2022), soybean (Salim et al. 2023), lentil (Gahoonia et al. 2006), and green gram (Pandey et al. 2014), indicating that P supply alters root architectural traits such as total root length, and root-to-shoot ratio to cope with P deficiency and water stress, such as in narrow-leaved

lupin (Chen et al. 2013). We also confirmed the positive impact of P supplementation on dry matter accumulation, leaf area and root length, underscoring the importance of adequate P levels for mitigating the adverse effects of water stress. These results echo existing research demonstrating adjustments in root architecture, root-to-shoot ratio and total root length to cope with P deficiency and water stress in various leguminous crops, including soybean (Feng et al. 2021a).

Water and P supply interactions on gas exchange traits, PUE and WUE

It is well-established that water stress impairs plant growth by inducing stomatal closure, which limits CO₂ assimilation and reduces photosynthesis (Antolín et al. 2010). We found that P60 supply significantly increased photosynthetic rate, stomatal conductance and transpiration rate compared to P10 under WW conditions during the vegetative stage. These findings echo Pang et al. (2018), who observed reduced gas exchange traits in low-P chickpea plants.

While the transpiration rate exhibited no interaction effects at the flowering stage, stomatal conductance and photosynthetic rate significantly differed across water and P treatments. Similarly, P fertiliser application enhanced photosynthetic activity in cluster bean under drought stress (Burman et al. 2009). Stomatal conductance also significantly declined in TWS plants by 50% under P60 and 67% under P10 compared to WW and EWS plants. Drought-induced stomatal closure and reduced photosynthesis are common plant drought responses (Mak et al. 2014). We found that TWS plants had lower photosynthetic rates and stomatal conductance than WW and EWS at the seed formation stage, consistent with others (Liyanage and Dilrukshi 2022; Fatema et al. 2023; Wu et al. 2022).

Other studies report reductions in gas exchange traits in crops exposed to drought stress, such as common bean (Kusvuran and Dasgan 2017), cluster bean (Shubhra et al. 2004), faba bean (Abid et al. 2017), chickpea (Mafakheri et al. 2010) and soybean (Hao et al. 2013). The combined water stress and low P stress had a more significant impact on soybean than individual stresses, with similar findings reported in mungbean (Meena et al. 2021) and soybean (Feng et al. 2021b). Moreover, a direct association between shoot traits, such as photosynthetic rate and leaf area, and dry matter accumulation has been established (Roucou et al. 2018). In the present study, increased photosynthetic rate due to increased P supply may have contributed to enhanced dry matter accumulation—a phenomenon reported in other studies (Feng et al. 2021b; Meena et al. 2021; Sharma et al. 2020).

The interaction between water and P treatments influenced shoot P content, root P content and physiological PUE during various growth stages. These observations align with a collective body of research demonstrating that water deficit and P deficiency can lead to decreased shoot P content, root P content and PUE, with the effect often amplified under combined stress conditions, for example, in soybean (He et al. 2019; Feng et al. 2021b), mungbean (Meena et al. 2021), faba bean (Oukaltouma et al. 2022), hemp (Islam et al. 2023) and chickpea (Sharma et al. 2020).

Our results indicated that water use significantly decreased under stress conditions, particularly the combined stress, compared to the control. Numerous studies support our findings, including those on soybean (He et al. 2019), faba bean (Oukaltouma et al.

2022), alfalfa (Fan et al. 2015), chickpea (Sharma et al. 2020) and mung bean (Meena et al. 2021). Shoot WUE and seed yield declined in EWS and TWS plants compared to WW plants at all growth stages. In contrast, shoot WUE and seed yield increased under P60 compared to P10 at all growth stages. Figueroa-Bustos et al. (2020) showed that wheat grain WUE decreased under terminal drought stress compared to well-watered plants, which aligns with our findings. However, a few studies reported increased WUE under drought stress, including in chickpea (Sharma et al. 2020) and mungbean (Meena et al. 2021).

Water and P supply treatment interactions on organic acid exudation

The interplay between water availability and P supply significantly influenced organic acid exudation, shedding light on the multifaceted responses of plants under combined stress. Within the context of P supply, our findings showcased distinct patterns of organic acid exudation under different stress scenarios. Notably, total carboxylates varied across different stress levels and growth stages. Specifically, EWS and TWS decreased total carboxylates compared to WW at the flowering and seed formation stages. Under P10, EWS increased total carboxylates at the flowering stage compared to WW, and TWS decreased total carboxylates at the seed formation stage compared to WW and EWS. Other studies have reported genotypic variability in organic acid exudation in response to low P in soybean (Salim et al. 2023; Krishnapriya and Pandey 2016), industrial hemp (Islam et al. 2023), pasture legumes (Kidd et al. 2016; Tshewang et al. 2020) and chickpea (Pang et al. 2018). We identified that, under the combined stress, oxalic acid, citric acid, malic acid, and malonic acid had the highest carboxylate proportions in the rhizosphere at the flowering stage. In contrast, malic acid and malonic acid had the highest proportions at the seed formation stages. Henry et al. (2007) reported similar findings in wheatgrass.

Other studies reported that total organic acid exudation increased under low P, drought, and combined stresses compared to control, such as mungbean (Meena et al. 2021) and chickpea (Sharma et al. 2020). However, we found that total organic acid exudation decreased under water stress and low P supply

at the flowering and seed formation stages, except EWS increased total organic acid exudation under low P supply at the flowering stage. Root rhizosphere-released organic acids are a critical physiological adaptation strategy to mobilise P in soils containing low bioavailable P (Alloush 2003; Lambers et al. 2015). The organic acids exuded under EWS and low P at the flowering stage could mobilise P in the rhizosphere, improving plant potential for enhanced P contents and PUE.

Water and P supply treatment interactions on yield and yield components

The selection of soybean genotype PI 125671 for this study was based on its demonstrated capacity to yield an extended root system genotype—a trait that positioned it on par with or even ahead of other genotypes under well-watered conditions and adequate P supply (Salim et al. 2023; Salim et al. 2022). Under the combined stress, TWS significantly decreased pod and seed numbers per plant compared to WW and EWS, reducing seed yield per plant. This observation suggests that EWS plants quickly recovered seed yield and yield components kin to WW plants after re-watering from the flowering to maturity stage. Moreover, TWS plants under P60 increased seed yield and mature pod and seed numbers per plant compared to P10, with similar results reported in soybean (He et al. 2019) and mungbean (Meena et al. 2021). Several studies reported that filled pods and seed numbers determine soybean seed yield (He et al. 2019; Feng et al. 2021b), aligning with the observations in EWS and TWS plants.

We found that water stress significantly decreased pod number, seed number, seed yield and 100-seed weight at the maturity stage, consistent with other studies (Liyanage and Dilrukshi 2022; Fatema et al. 2023; Meier et al. 2021). The detrimental effect of P and/or water limitation on plant growth and development decreased grain yield in wheat (Meier et al. 2022; Meier et al. 2021) and cowpea (Uarrotta 2010), also aligning with our findings. An increased linear relationship has been reported under different P fertilisation and grain yield levels in chickpea (Neenu et al. 2014) and soybean (Jin et al. 2006; He et al. 2021), supporting our findings. The study

demonstrated expedited recovery upon re-watering and improved grain yield under combined drought stress and P supply compared to no P supply in cluster bean (Burman et al. 2009). He et al. (2017) reported a positive correlation between P accumulation and grain yield under WW but not WS conditions. Correspondingly, the present study revealed that the increase in grain yield facilitated by applied P was primarily attributed to enhanced P accumulation in the WW treatment, as reported in earlier soybean studies (He et al. 2017; Jin et al. 2006).

Conclusion

This study underscores the significant threat of combined water and P deficits on soybean seed yield and its components. The interplay of insufficient P availability and water stress, specifically TWS imposed during the flowering stage, has more detrimental effects on plant growth, morpho-physiological traits and seed yield than EWS and WW. An increased P supply alleviates the adverse effects of water stress on plant growth, morphology, physiology and seed yield traits. The adaptive resilience of the extended root system of genotype PI 125671 surfaced when EWS plants quickly recovered growth, seed yield and other traits following re-watering. Future studies are warranted to reveal the mechanisms related to water and P deficit adaptation in soybean and to develop improved resilience to adverse conditions.

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Data availability All data generated or analysed during this study are included in this published article [and its supplementary information files]. However, the corresponding author can provide more detailed information on this current study at reasonable request.

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