



Seed priming-induced modulation of photosynthesis and root system architecture in faba bean under controlled conditions

Shahram Torabian¹ · Theresa J. Nartea² · Salar Farhangi-Abriz³

Received: 30 June 2025 / Revised: 26 January 2026 / Accepted: 28 January 2026 / Published online: 16 February 2026
© The Author(s) 2026

Abstract

Priming is a water-based method that partially hydrates seeds to activate the early germination process. Faba bean (*Vicia faba* L.) seeds typically take 7–14 days to germinate under normal conditions. Delayed germination may reduce faba bean plant establishment and potential yield. A greenhouse pot experiment evaluated the effects of seed priming treatments on photosynthetic traits and root architecture of four faba bean varieties (Windsor, Purple Negreta, Big Organic, and Midnight). Seeds were primed with water (hydropriming), NaCl (osmopriming), bulk ZnO, and nano-ZnO. Results revealed significant variation among varieties and priming treatments for morphological, physiological, and root-related traits. Midnight demonstrated the lowest growth and delayed flowering. Windsor and Purple Negreta exhibited higher shoot biomass and branching. Nano-ZnO significantly enhanced plant height and shoot dry matter, whereas NaCl priming increased root biomass and branching. Gas exchange parameters, including net photosynthesis rate (P_n), transpiration rate (E), and water use efficiency (WUE), were positively influenced by seed priming across growth stages. Water priming notably enhanced P_n and WUE, particularly in the Windsor variety. Chlorophyll content (SPAD) increased under seed priming, especially at the flowering stage. Specific root length decreased under priming, with NaCl resulting in the lowest values. Application of nano-ZnO reduced the number of roots by 11% while increasing root depth by 8% compared to the control. Overall, seed priming may enhance growth, photosynthesis, and root development of faba beans under controlled conditions regardless of seed size.

Keywords Nano-ZnO · Osmopriming · Specific root length · Photosynthesis rate · Transpiration rate

Introduction

Rapid and uniform seed germination is essential for optimal crop establishment, which directly affects plant growth, resource use efficiency, and final yield. Seed priming, a pre-sowing technique, enhances seed vigor by initiating key metabolic processes associated with germination without

allowing radicle emergence (Srivastava et al. 2021). This method improves seed performance by inducing physiological and biochemical changes such as membrane repair, osmotic regulation, enzyme activation, and reduced electrolyte leakage (Ahmad et al. 2021; Sheteiwy et al. 2021). Additionally, priming promotes the activity of hydrolytic enzymes like amylase and nitrate reductase, which facilitate early seedling development and nitrogen assimilation (Anaytullah 2007). Primed seeds often exhibit faster and more synchronized germination, improved emergence under a wider temperature range, deeper root growth, early flowering, and enhanced tolerance to abiotic stresses and soil-borne diseases (Devika et al. 2021; Marthandan et al. 2020; Okello et al. 2022). These advantages ultimately contribute to better stand establishment, resource use efficiency, and yield potential.

Various seed priming methods have been developed, including hydropriming, osmopriming, halopriming (e.g., using NaCl or KCl), solid matrix priming, and

Communicated by A. Gniazdowska-Piekarska

✉ Shahram Torabian
storabian@vysu.edu

¹ Agricultural Research Station, College of Agriculture, Virginia State University, VAPetersburg, USA

² Virginia Cooperative Extension, College of Agriculture, Virginia State University, VAPetersburg, USA

³ Cotton Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Gorgan, Iran

biostimulant-based priming using salicylic acid or ascorbic acid (Khafagy et al. 2017; Khan et al. 2017). Salt-based haloprimering enhances osmotic balance and ion homeostasis (El-Sanatawy et al. 2021), while increasing antioxidant activity (Fu et al. 2020). In recent years, nano-priming, particularly with metal oxide nanoparticles such as ZnO has emerged as a promising strategy to improve seedling vigor, nutrient uptake, and stress resilience (Kapoor et al. 2022; Mickky 2022; Esper Neto et al. 2020; Torre-Roche et al. 2020). Zinc plays a crucial role in seed metabolism by enhancing α -amylase activity, breaking dormancy, and regulating hormone synthesis (Rehman et al. 2012). Zinc priming has been linked to improved grain yield, seed protein content, and total biomass (Gontrani et al. 2023; Itroutwar et al. 2020).

Faba bean (*Vicia faba* L.) is the third most important grain legume globally, valued for its high protein content, nitrogen-fixing ability, and role in sustainable cropping systems (Singh et al. 2013). However, its germination and establishment are often limited by suboptimal environmental conditions, particularly in cold or rainfed regions where low soil temperatures and moisture delay emergence and reduce stand uniformity. Faba bean requires a minimum soil temperature of around 7 °C for optimal germination, and delayed emergence increases vulnerability to biotic and abiotic stresses (Nouairi et al. 2019). Seed priming has shown potential in mitigating such constraints. For example, salicylic acid priming improved salt tolerance (Azooz 2009), while ascorbic acid and nicotinamide enhanced stress tolerance and photosynthetic efficiency (Azooz et al. 2013). Calcium chloride priming has also been found to improve photosynthesis under cadmium stress (Nouairi et al. 2019).

Despite these advances, limited studies have comprehensively evaluated how different seed priming treatments influence key physiological traits such as photosynthetic capacity and root system architecture, both of which are critical for early vigor and resource acquisition. Therefore, this study aimed to investigate the effects of various seed priming methods, including hydropriming, osmopriming with NaCl, and treatments with bulk ZnO and nano-ZnO, on photosynthetic parameters and root traits of four faba bean varieties with contrasting seed sizes under controlled conditions. This research seeks to identify effective priming strategies that enhance early growth, physiological efficiency, and root development, ultimately supporting improved faba bean establishment and yield potential across diverse production environments.

Materials and methods

The experiment was conducted in 2025 at the Virginia State University greenhouse located at Randolph Farm (37°13'43" N, 77°26'2" W). Throughout the experimental period in the greenhouse, the temperature ranged from 20 °C to 30 °C, the relative humidity was approximately 60%, and the photosynthetically active radiation (PAR) averaged 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A randomized complete block design (RCBD) with three replications was used. The study included four faba bean varieties: Windsor, Purple Negreta, Big Organic, and Midnight (Fig. 1). Windsor seeds were obtained from Territorial Seed Company (Oregon, USA), while the remaining varieties were sourced online from various commercial seed suppliers. Five seed priming treatments were evaluated: (1) control (non-primed), (2) hydropriming (distilled water), (3) osmopriming with NaCl (100 mM), (4) bulk ZnO (60 mg L^{-1}), and (5) nano-ZnO (60 mg L^{-1}). The NaCl (assay 99.0–100.5% on a moisture-free basis) was obtained from Spectrum Laboratory Products, Inc. (New Brunswick, NJ, USA). Bulk ZnO was purchased from Carolina Biological Supply (Burlington, NC, USA), and nano-ZnO powder (purity 99.8%, particle size 10–30 nm) was sourced from SkySpring Nanomaterials, Inc. (Houston, TX, USA). Seeds were primed by soaking in the respective solutions for 6 h, then air-dried before planting. A total of 60 pots (23 cm diameter $\times$ 23 cm height; $\sim$ 9 L capacity) were filled with commercial potting soil, and three seeds were sown per pot on February 28, 2025. After seedling establishment, one healthy plant per pot was retained; the rest were thinned. Fertilization was done using a 20-20-20 NPK solution at a concentration of 0.5 g L^{-1} , applied twice (30 and 50 days after planting), with each pot receiving 200 mL of the solution. Plants were irrigated weekly using a hose, applying the same amount of water to each pot to maintain uniform soil moisture. To manage thrips, neem oil (organic pesticide) was applied using a hand sprayer at two growth stages: N3 (three nodes) and N6 (six nodes).

Gas exchange parameters were recorded using a CI-340 handheld photosynthesis system (CID Bio-Science, USA) at N3 (22 days after planting), N6 (48 days after planting), and flowering stages (73 days after planting), between 10:00 AM and 2:00 PM. For each evaluation period, the average plant age (days after planting) was recorded and reported, acknowledging that phenological development may vary among varieties due to their distinct growth habits. Three fully expanded leaves from the upper third of each plant were randomly selected per variety and treatment for analysis. Parameters recorded included net photosynthetic rate (P_n), transpiration rate (E), photosynthetically active radiation (PAR), and leaf surface temperature. Water use efficiency (WUE) was calculated as P_n/E . The average leaf

Fig. 1 Seed varieties of faba bean used in the study: (1) Midnight, (2) Big Organic, (3) Windsor, (4) Purple Negreta, shown next to a U.S. penny for scale



temperature during measurements was 26.7 °C. Chlorophyll content was assessed using a SPAD chlorophyll meter (Minolta SPAD 502) at the same growth stages as the gas exchange measurements. At flowering, plants were harvested. Aboveground biomass was oven-dried, while root systems were carefully washed over a sieve to minimize root loss. Root samples were photographed and then dried in a hot room. Root images were analyzed using GiA Roots software (GiA-API; Galkovskyi et al. 2012) to quantify root traits, including total root length, depth, volume, surface area, average width, number of roots, and average root width. Specific root length (SRL) was calculated as total root length divided by root dry mass, providing an index of root thickness.

Data from the RCBD were statistically analyzed using SAS 9.4 software (SAS Institute, Cary, NC, USA). Normality tests were performed before analysis. Treatment effects and interactions were considered significant at $p \leq 0.05$ based on F-tests. Where applicable, mean comparisons were performed using the least significant difference (LSD) test, and all results are presented with the standard error (SE) of the mean. Figures were generated using Microsoft Excel. Principal Component Analysis (PCA) was conducted using R software to explore relationships among physiological and morphological parameters across treatments and varieties.

Results

Growth and phenological parameters

The results revealed significant variation among the faba bean varieties in plant height, shoot dry matter, root-to-shoot ratio, number of branches, and days to flowering (Table 1). The variety Midnight exhibited the lowest plant height, shoot dry matter, and number of branches, but had the longest duration to flowering. Windsor produced the highest shoot dry matter, although it had the lowest root-to-shoot ratio. Purple Negreta recorded the highest number of branches, averaging 4.4 per plant. The highest root-to-shoot ratios were observed in Purple Negreta (0.26) and Big Organic (0.25). However, no significant differences were found among varieties for root dry matter.

Seed priming treatments had significant effects on plant height, shoot dry matter, root dry matter, and number of branches (Table 1). Priming with nano-ZnO resulted in the tallest plants and highest shoot dry matter, with increases of 16% and 36%, respectively, compared to the control. The highest root dry matter was observed in seeds primed with NaCl, which showed a 57% increase over the control. Additionally, NaCl priming increased the number of branches by 31% compared to the control. Seed priming had no significant effect on root-to-shoot ratio or days to flowering.

Table 1 Means of growth parameters in Faba bean varieties in response to seed priming treatments

Source of variation	Plant height (cm)	Shoot dry matter (g)	Root dry matter (g)	Root/shoot ratio	Number of branches	Day to flowering	SPAD at N3	SPAD at N6	SPAD at flowering
<i>Variety</i>									
Windsor	65.7±3.5a	20.3±1.1a	4.15±0.35	0.20±0.01b	3.80±0.27ab	69.6±3.5b	41.7±3.1	40.1±2.4ab	40.3±1.5b
Purple	59.2±4.2a	17.6±1.4ab	4.62±0.41	0.26±0.01a	4.40±0.31a	73.2±4.6b	41.0±1.6	41.3±2.9a	43.2±0.9a
Big Organic	62.7±3.7a	14.4±1.7b	3.83±0.88	0.25±0.01a	3.66±0.38ab	75.6±3.0ab	42.4±1.5	37.2±1.3b	41.8±1.1ab
Midnight	46.3±2.7b	14.3±1.7b	3.12±0.59	0.21±0.01ab	3.46±0.38b	83.0±1.8a	41.1±2.2	41.1±1.2a	41.9±2.0ab
<i>Treatment</i>									
Control	53.5±2.8b	13.7±1.6b	3.21±0.23b	0.23±0.02	3.41±0.40b	79.8±3.2	40.4±1.1	39.0±1.4	39.2±1.3b
Water	61.5±5.6ab	15.6±1.5ab	3.44±0.31ab	0.22±0.01	3.50±0.33b	78.6±4.0	42.6±1.5	38.9±1.1	43.1±1.6a
NaCl	58.8±4.7ab	18.5±1.5ab	5.06±0.69a	0.26±0.01	4.50±0.19a	71.8±4.2	41.6±0.9	41.2±2.0	42.4±2.1a
ZnO	56.1±5.1ab	16.8±1.7ab	3.70±0.88ab	0.22±0.01	3.83±0.26ab	73.5±2.8	42.5±1.6	41.9±1.1	41.9±2.2a
Nano-ZnO	62.4±5.0a	18.7±1.2a	4.25±0.90ab	0.22±0.01	3.91±0.28ab	72.9±3.4	40.6±1.7	38.7±2.1	42.3±0.8a

Different letters in each column indicate a significant difference at $p \leq 0.05$. Values are presented as mean ± standard error (SE) of three replicates

Table 2 Means of root parameters in Faba bean varieties in response to seed priming treatments

Source of variation	Number of roots	Average root width (cm)	Root depth (cm)	Root length (cm)	Root surface area (cm ²)	Root volume (cm ³)	Root width (cm)	Specific root length (cm g ⁻¹)
<i>Variety</i>								
Windsor	129.2±7.2	0.060±0.002ab	67.2±3.29ab	11,060±630ab	2199±221ab	46.9±5.68ab	51.9±1.90a	2665±31.4b
Purple	141.6±6.5	0.064±0.002a	72.8±2.56a	12,390±667a	2594±258a	58.3±6.14a	53.4±3.69a	2681±24.2b
Big Organic	129.6±6.7	0.055±0.003b	63.4±3.04b	10,095±589b	1788±215b	33.2±4.00b	46.5±2.03b	2635±22.0b
Midnight	131.1±6.9	0.055±0.002b	63.2±4.17b	9655±389b	1684±178b	31.0±8.71b	46.5±3.04b	3094±28.5a
<i>Treatment</i>								
Control	135.7±6.2a	0.057±0.002	65.0±3.20ab	11,326±658	2116±409	42.0±2.66	48.6±1.83	3528±21.6a
Water	137.2±4.8a	0.058±0.003	67.1±3.54ab	10,719±742	2006±238	39.3±1.25	49.8±0.99	3097±16.8b
NaCl	132.5±6.8ab	0.061±0.002	67.8±3.08ab	11,043±905	2242±111	49.6±3.06	51.8±2.29	2182±13.3c
ZnO	139.2±5.1a	0.055±0.004	62.7±3.16b	10,572±682	1848±219	33.8±3.82	46.6±1.08	2857±10.8b
Nano-ZnO	119.8±4.9b	0.061±0.002	70.6±4.02a	10,341±472	2118±308	47.0±2.03	51.0±1.52	2433±18.4bc

Different letters in each column indicate a significant difference at $p \leq 0.05$. Values are presented as mean ± standard error (SE) of three replicates

Chlorophyll SPAD

Chlorophyll SPAD values varied significantly among faba bean varieties at the N6 and flowering stages, while no significant differences were observed at the N3 stage (Table 1). At the N6 stage, Purple Negreta and Midnight exhibited the highest chlorophyll SPAD values, whereas Big Organic recorded the lowest. During the flowering stage, Purple Negreta maintained the highest chlorophyll SPAD, while Windsor showed the lowest value (Table 1).

Seed priming significantly affected chlorophyll SPAD only at the flowering stage. Overall, primed seeds exhibited an 8% increase in chlorophyll SPAD compared to the control. However, no significant differences were observed among the four priming treatments at this stage (Table 1).

Root parameters

Average root traits, including root width, depth, length, surface area, volume, and specific root length, varied significantly among faba bean varieties (Table 2), while the number of roots remained statistically similar across all varieties. Purple Negreta exhibited the highest values for average root width (0.064 cm), root depth (72.8 cm), root length (12,390 cm), surface area (2,594 cm²), volume (58.3 cm³), and specific root length (654 cm), followed by Windsor. In contrast, Big Organic and Midnight showed the lowest values for these parameters. Midnight recorded the highest specific root length when expressed per gram of root dry matter (3,094 cm g⁻¹).

Seed priming had a significant effect on the number of roots, root depth, and specific root length (Table 2). Priming with nano-ZnO reduced the number of roots by 11% compared to the control, but increased root depth by 8%. In contrast, bulk ZnO treatment resulted in the lowest root depth (62.7 cm). On average, seed priming significantly

reduced specific root length by 25% compared to the control, with the lowest value ($2,182 \text{ cm g}^{-1}$) observed under NaCl priming.

Gas exchange at the N3 stage

The net photosynthesis rate (Pn) of faba bean varieties at the N3 stage was significantly influenced by the interaction between seed priming treatments and variety (Fig. 2a). Among the treatments, hydropriming (water priming) resulted in the highest net photosynthesis rate in the Windsor variety ($8.50 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), which was significantly higher than all other treatment variety combinations. In contrast, Purple Negreta and Midnight exhibited lower Pn values under both the control and water-primed conditions, with the lowest observed in Midnight under hydropriming (approximately $2.33 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Overall, across all varieties, seed priming increased the net photosynthesis rate by 52% compared to the control.

Transpiration rate (E) at the N3 stage was also significantly affected by the interaction between priming treatment and variety (Fig. 2d). Midnight consistently exhibited higher transpiration rates across treatments, with the highest value recorded under the control condition ($2.36 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Big Organic showed the highest transpiration rates under salt (NaCl) and ZnO priming. In contrast, Windsor and Purple Negreta maintained relatively stable transpiration rates across all treatments. Notably, nano-ZnO priming resulted in uniform transpiration rates across all varieties, with no statistically significant differences among them (Fig. 2d).

Water use efficiency (WUE), calculated as the ratio of net photosynthesis to transpiration, was significantly influenced by the priming $\times$ variety interaction (Fig. 2g). The highest WUE was observed in Windsor under hydropriming ($4.63 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$), followed by Big Organic under the same treatment. Conversely, Midnight recorded the lowest WUE values under both control and hydroprimed conditions. Seed priming did not significantly improve WUE in Purple Negreta (Fig. 2g). Additionally, all varieties exhibited statistically similar WUE under salt and ZnO priming treatments.

Gas exchange at the N6 stage

The interaction between faba bean variety and seed priming treatment had a significant effect on Pn at the N6 stage (Fig. 2b). The highest Pn were recorded in Big Organic primed with water ($15.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and Purple Negreta primed with NaCl ($14.66 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). In contrast, the lowest rate was observed in Midnight under

the control treatment ($3.26 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Across all varieties, seed priming increased Pn by 12% compared to the control.

Transpiration rate at the N6 stage was also significantly affected by the interaction between variety and priming treatment (Fig. 2e). The highest E ($2.93 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was observed in Midnight primed with ZnO. Across all varieties, no significant differences in transpiration rate were detected among NaCl, ZnO, and nano-ZnO priming treatments. The lowest transpiration rate was recorded in Windsor under water priming ($0.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Under control conditions, Purple Negreta exhibited the lowest transpiration among all varieties.

Water use efficiency at the N6 stage was significantly influenced by the interaction between variety and seed priming treatment (Fig. 2h). The highest WUE was observed in Windsor primed with water, while the lowest was in Midnight under the control treatment. Purple Negreta and Big Organic showed relatively stable WUE across all priming treatments. Notably, hydropriming significantly improved WUE in Midnight compared to the control. Overall, seed priming increased WUE by 29% across all varieties compared to the control.

Gas exchange at the flowering stage

The interaction between faba bean variety and seed priming treatment significantly influenced the Pn at the flowering stage (Fig. 2c). The highest Pn were observed in Windsor ($13.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and Big Organic ($12.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) when primed with water. In contrast, the lowest rates were recorded in Big Organic under the control treatment and Midnight primed with NaCl. No significant differences in Pn were found for Purple Negreta and Midnight across priming treatments compared to their respective controls. On average, seed priming increased the Pn by 18% compared to the control across all varieties.

At the flowering stage, the highest E ($2.93 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was recorded in Windsor under control conditions (Fig. 2f). While seed priming with water increased E by 7.4% compared to control across all varieties, the difference was not statistically significant. No consistent pattern of significant changes was observed among the different priming treatments.

Water use efficiency at flowering was also affected by the interaction between variety and priming treatment (Fig. 2i). The highest WUE values were recorded in Windsor when primed with ZnO and nano-ZnO. In contrast, the lowest WUE was found in Purple Negreta under NaCl priming. WUE in Midnight was not significantly affected by any seed priming treatment. Overall, seed priming increased

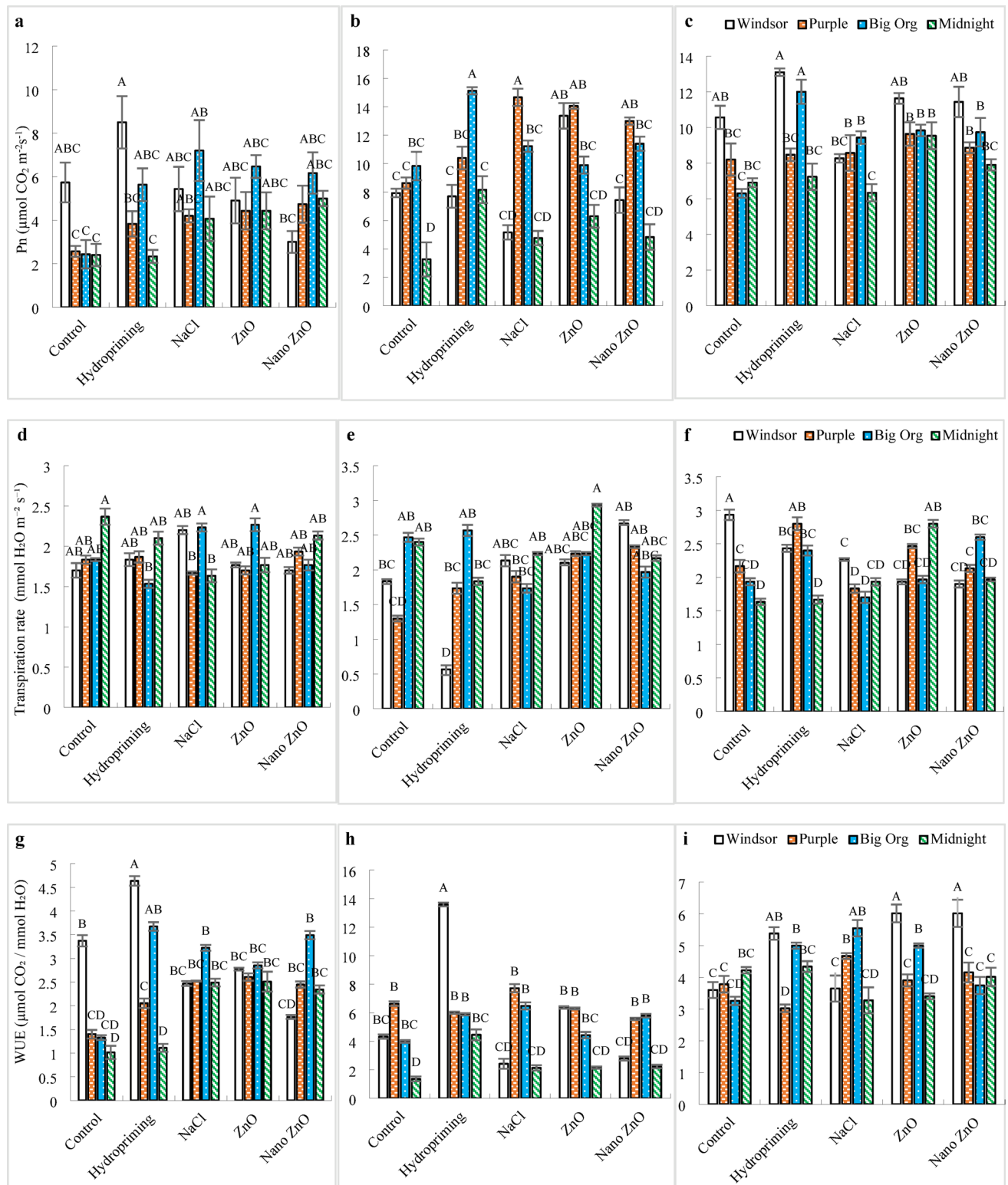


Fig. 2 Photosynthesis rate (Pn) at N3 (a), N6 (b), flowering stage (c); Transpiration rate (E) at N3 (d), N6 (e), flowering stage (f); water use efficiency (WUE) at N3 (g), N6 (h), flowering stage (i) of faba bean varieties under different seed priming treatments. Different letters in

each column indicate a significant difference at $p \leq 0.05$. Bars represent mean values, and error bars indicate the standard error (SE) of three replicates

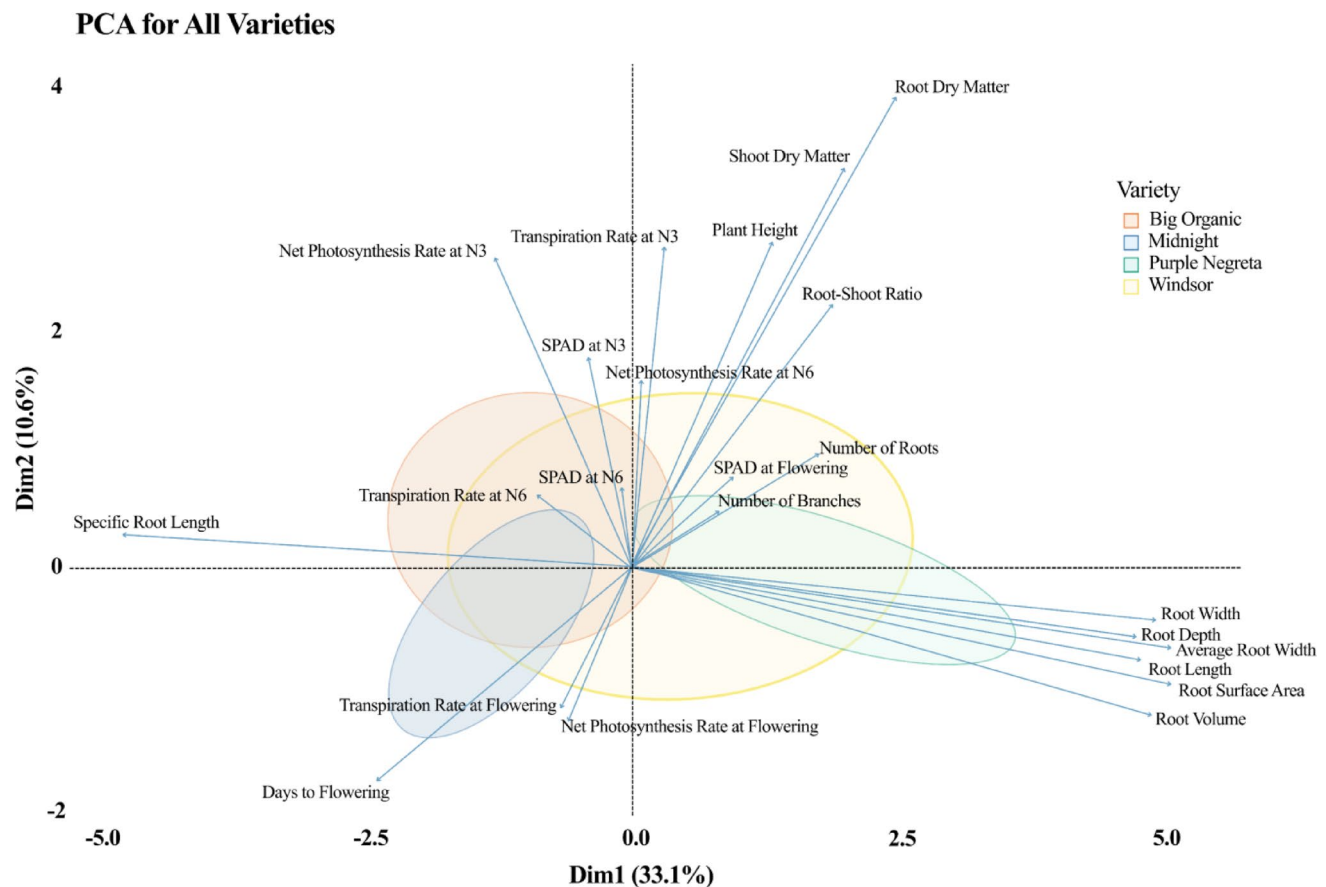


Fig. 3 Principal component analysis (PCA) of the growth, root and photosynthesis parameters of faba bean varieties

WUE by 19% compared to the control across all varieties. Regardless of seed priming, Windsor exhibited the highest average WUE ($4.93 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) among the varieties tested.

Principle component analysis

Principal Component Analysis (PCA) was conducted to assess variation among four faba bean varieties, Windsor, Midnight, Purple Negreta, and Big Organic based on physiological, morphological, and root traits (Fig. 3). Purple Negreta was associated with higher values for root morphological traits, including root width, length, depth, surface area, and volume. Big Organic was positioned on the negative side of Dim1 and was associated with traits such as specific root length and transpiration rate at flowering. Windsor appeared centrally located in the PCA plot. Trait vectors indicated that root dry matter (0.83^{**}), plant height (0.95^{**}), and number of branches (0.78^{**}) were strongly correlated with shoot dry matter. PCA also showed that Pn and E were positively correlated with shoot dry matter at different growth stages: Pn (0.59^{**}) and E (0.68^{**}) at N3, Pn

(0.71^{**}) and E (0.71^{**}) at N6, and Pn (0.57^{**}) and E (0.65^{**}) at flowering. Chlorophyll SPAD values were also strongly positively correlated with shoot dry matter at N3 (0.83^{**}), N6 (0.90^{**}), and flowering (0.84^{**}). Additionally, days to flowering showed a positive correlation (0.71^{**}) with shoot dry matter. Root depth showed a strong negative correlation with average root width (-0.33^{**}) and a strong positive correlation with the number of roots (0.95^{**}), root width (0.99^{**}), root area (0.88^{**}), specific root length (0.77^{**}), root surface area (0.88^{**}), root length (0.96^{**}), and root volume (0.75^{**}). Specific root length was strongly negatively correlated with average root width (-0.33^{**}), indicating that roots with higher specific root length are thinner. Table S1 presents the Pearson correlation coefficients among all measured parameters in the evaluated faba bean varieties.

Discussion

This study revealed substantial variation among faba bean varieties in morphological, physiological, and root architectural traits, consistent with previous reports on varietal differences in faba bean growth (Azooz 2009; Belachew et

al. 2018; Muktadir et al. 2020). Midnight exhibited the lowest vegetative growth, characterized by the shortest plant height, minimal shoot dry matter, and fewer branches. In contrast, Windsor showed the highest shoot dry matter but the lowest root-to-shoot ratio, suggesting a growth preference toward shoot biomass rather than root development, as reported shoot allocation can reduce root investment (Robinson 2023). Purple Negreta stood out for its high number of branches and elevated chlorophyll content at both the N6 and flowering stages, along with a well-developed root system, including superior depth, width, surface area, and volume. Extensive root systems are associated with greater nutrient and water uptake, which in turn promote shoot growth and chlorophyll retention (Holz et al. 2024). Big Organic exhibited a root-to-shoot ratio comparable to Purple Negreta but had lower values for overall root morphological parameters. Notably, root dry matter did not differ significantly among the varieties, suggesting that root mass alone may not fully reflect root system architecture, as emphasized by de Dorlodot et al. (2007). PCA results highlighted that shoot dry matter was strongly associated with root dry matter, plant height, chlorophyll SPAD, net photosynthetic rate, and transpiration rate. Root depth was positively associated with most root traits, including number of roots, root width, root area, specific root length, root surface area, root length, and root volume, but negatively correlated with average root width. The negative correlation between specific root length and average root width indicates that thinner roots tend to have higher specific root length. Notably, photosynthesis contributed positively to shoot biomass accumulation (Fig. 3), consistent with previous studies highlighting the importance of photosynthetic efficiency for overall growth (Li et al. 2023).

Seed priming had pronounced effects on faba bean growth and physiological performance. Seed priming significantly increased plant height, as well as shoot and root dry matter, compared to the control. It did not affect the root-to-shoot ratio, as it proportionally enhanced both root and shoot growth. Seed priming enhances germination uniformity and vigor, resulting in stronger seedlings and higher yields (Ceritoglu et al. 2024; Hussain et al. 2016). This process involves physiological and biochemical changes that improve seed performance. Priming shortens the lag phase of water uptake, allowing seeds to absorb water more quickly when sown (Debbarma and Das 2017). It also activates key metabolic processes, such as respiration and starch breakdown, before germination begins. Primed seeds often show higher respiration rates, providing more ATP to support seedling growth (Hussain et al. 2016). Additionally, priming boosts starch metabolism by increasing the activity of enzymes like α -amylase, which breaks down starch into

soluble sugars that fuel early growth (Debbarma and Das 2017).

Among treatments, nano-ZnO priming significantly increased plant height, shoot, and root dry matter by 16, 36, and 32%, respectively, relative to the control, suggesting that nano-ZnO may enhance nutrient availability and stimulate physiological processes that promote overall plant growth. Salam et al. (2022) observed increased biomass in maize under cobalt stress with nano-ZnO priming. Donia and Carbone (2023) found enhanced germination and chlorophyll content in wheat with nano-ZnO priming. Li et al. (2021) reported improved seedling vigor in rice with ZnO nanoparticle priming. Similarly, Waqas Mazhar et al. (2022) documented seed priming with nano-ZnO increases total chlorophyll, plant height, and biomass of rice under drought stress. Although nano-ZnO priming reduced root number by 11%, it increased root depth by 8%, potentially enhancing access to deeper soil resources. While deeper rooting is generally regarded as an adaptive strategy for plants under drought stress (Li et al. 2022), its advantages may be limited in pot experiments due to spatial constraints. Previous studies support these findings and indicate that ZnO nanoparticle priming increases root biomass and root extension in wheat (Al-Salama 2022; El-Shazoly et al. 2025). These benefits are likely due to the biostimulant effect of nanoscale ZnO, which offers controlled zinc release, enhances seed metabolism, and triggers hormonal and transcriptional responses that promote cell division and elongation (Akbar and Yalçın 2025; Donia and Carbone 2023). ZnO also enhances photosystem II function by improving photon capture and electron transport efficiency, which in turn supports higher photosynthesis and biomass accumulation (Wang et al. 2020).

In our study, all priming treatments reduced specific root length, with NaCl showing the most pronounced reduction (2182 cm g^{-1}), possibly indicating a shift toward thicker or more compact roots. NaCl priming had the most significant impact on root development, increasing root dry matter by 57% and the number of branches by 31%. This is consistent with findings in *Brassica napus*, where NaCl priming improved root biomass (BiBi et al. 2024), and in chickpea, where it enhanced antioxidant responses that stabilized photosystem II and indirectly promoted root growth (Paul et al. 2025). Paul et al. (2023) further showed that NaCl priming modulates ion uptake and osmoregulation to improve root development in legumes. Mechanistically, NaCl priming activates seed metabolism during imbibition, upregulating enzymes involved in energy production and root cell division (Khan et al. 2022). It also alters hormone levels, notably auxins and ABA, triggering root system remodeling even under mild salt stress (Habibi et al. 2023). Moreover, NaCl priming supports osmotic adjustment and ionic balance, especially favorable Na^+/K^+ ratios, allowing for

improved water uptake, cell turgor maintenance, and root elongation (Khan et al. 2022).

Chlorophyll content was influenced by seed priming only at the flowering stage, with an average 8% increase over the control. Although no significant differences were found among individual priming treatments, the improvement suggests enhanced photosynthetic pigment accumulation. Net photosynthesis increased with seed priming by 52%, 12%, and 18% at the N3, N6, and flowering stages, respectively. These improvements are supported by prior studies showing that seed priming enhances pigment synthesis and photosystem activity (Sherin et al. 2022). Priming triggers early gene activation involved in chlorophyll biosynthesis and photosystem assembly, alongside increased Rubisco activity, leading to improved photosynthetic efficiency (Rhaman et al. 2020; Sherin et al. 2022; Zhang et al. 2021). Windsor consistently displayed superior photosynthetic performance under water priming, especially during early (N3) and late (flowering) stages. Water priming also moderately increased E (7.4%) across varieties, although these changes were not always significant. Water use efficiency was positively influenced by seed priming at all stages. Across varieties, WUE increased by 52%, 29%, and 19% at N3, N6, and flowering stages, respectively. Notably, Windsor consistently showed the highest WUE values, especially when primed with water, ZnO, or nano-ZnO treatments, highlighting its efficient gas exchange regulation and photosynthetic capacity under primed conditions.

Conclusion

This study highlights significant varietal differences in growth, root architecture, and physiological responses in faba bean. Purple Negreta and Windsor exhibited strong performance in root traits and shoot biomass, respectively, while Midnight showed limited vegetative growth. Our results demonstrated that seed priming significantly enhanced key morphological and physiological traits in faba bean, including shoot and root biomass, Pn, and WUE. Additionally, no significant differences were detected between the nano and bulk forms of ZnO. Overall, all seed priming treatments, regardless of type, positively influenced faba bean growth. Since no interaction effects of variety and seed priming were observed on faba bean growth, we conclude that the response to seed priming was consistent across the tested varieties. We recommend conducting further research on seed priming using a broader range of techniques and evaluating faba bean performance under field conditions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11738-026-03888-8>.

Funding This work is supported by the Virginia Department of Agriculture and Consumer Services, Specialty Crop Block Grant Program, project award no. 2024–592, from the U.S. Department of Agriculture’s National Institute of Food and Agriculture.

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

Declarations

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ahmad F, Kamal A, Singh A, Ashfaq F, Alamri S, Siddiqui MH, Khan MIR (2021) Seed priming with gibberellic acid induces high salinity tolerance in *Pisum sativum* through antioxidants, secondary metabolites and up-regulation of antiporter genes. *Plant Biol* 23:113–121
- Akbay B, Yalçın FS (2025) Effect of ZnO nano priming on germination and root length of soybean seeds (*Glycine max* L). *Int J Second Metab* 12(1):204–215
- Al-Salama Y (2022) Effect of seed priming with ZnO nanoparticles and saline irrigation water in yield and nutrients uptake by wheat plants. *Environ Sci Proc* 16(1):37
- Anaytullah BB, Bose B (2007) Nitrate-hardened seeds increase germination, amylase activity and proline content in wheat seedlings at low temperature. *Physiol Mol Biol Plants* 13:199–207
- Azooz MM (2009) Salt stress mitigation by seed priming with Salicylic acid in two Faba bean genotypes differing in salt tolerance. *Int J Agric Biol* 11(4):343–350
- Azooz MM, Alzahrani AM, Youssef MM (2013) The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*Vicia Faba* L). *Aust J Crop Sci* 7(13):2091–2100
- Belachew KY, Nagel KA, Fiorani F, Stoddard FL (2018) Diversity in root growth responses to moisture deficit in young Faba bean (*Vicia Faba* L.) plants. *PeerJ* 6:4401
- BiBi R, Elahi NN, Danish S, Alahmadi TA, Ansari MJ (2024) Enhancing germination and growth of Canola (*Brassica Napus* L.) through hydropriming and NaCl priming. *Sci Rep* 14(1):14026
- Ceritoglu M, Erman M, Çığ F (2024) Seed priming boosts plant growth, yield attributes, seed chemical and antioxidant composition in lentil under low-phosphorus field conditions. *Int J Plant Prod* 18(4): 513–530.
- Debbarma M, Das SP (2017) Priming of seed: enhancing growth and development. *Int J Curr Microbiol Appl Sci* 6(1):2390–2396

- de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends Plant Sci* 12(10):474–481
- De La Torre-Roche R, Cantu J, Tamez C, Zuverza-Mena N, Hamdi H, Adisa IO, Elmer W, Gardea-Torresdey J, White JC (2020) Seed biofortification by engineered nanomaterials: a pathway to alleviate malnutrition? *J Agric Food Chem* 68(44):12189–12202
- Devika OS, Singh S, Sarkar D, Barnwal P, Suman J, Rakshit A (2021) Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. *Front Sustain Food Syst* 5:654001
- Donia DT, Carbone M (2023) Seed priming with zinc oxide nanoparticles to enhance crop tolerance to environmental stresses. *Int J Mol Sci* 24(24):17612
- El-Sanatawy AM, Ash-Shormillesy SM, Qabil N, Awad MF, Mansour E (2021) Seed halo-priming improves seedling vigor, grain yield, and water use efficiency of maize under varying irrigation regimes. *Water* 13(15):2115
- El-Shazoly RM, Othman AA, Zaheer MS, Al-Hossainy AF, Abdel-Wahab DA (2025) Zinc oxide seed priming enhances drought tolerance in wheat seedlings by improving antioxidant activity and osmoprotection. *Sci Rep* 15(1):3863
- Esper Neto M, Britt DW, Lara LM, Cartwright A, dos Santos RF, Inoue TT, Batista MA (2020) Initial development of corn seedlings after seed priming with nanoscale synthetic zinc oxide. *Agronomy* 10(2):307
- Fu Y, Zhang Z, Liu J, Chen M, Pan R, Hu W, Guan Y, Hu J (2020) Seed priming with spermidine and trehalose enhances chilling tolerance of rice via different mechanisms. *J Plant Growth Regul* 39:669–679
- Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, Harer J (2012) GiA roots: software for the high throughput analysis of plant root system architecture. *BMC Plant Biol* 12:116
- Gontrani L, Donia DT, Bauer EM, Tagliatesta P, Carbone M (2023) Novel synthesis of zinc oxide nanoparticles from type IV deep eutectic solvents. *Inorg Chim Acta* 545:121268
- Habibi N, Aryan S, Amin MW, Sanada A, Terada N, Koshio K (2023) Potential benefits of seed priming under salt stress conditions on physiological and biochemical attributes of micro-tomato plants. *Plants* 12:2187
- Holz M, Zarebanadkouki M, Benard P, Hoffmann M, Dubbert M (2024) Root and rhizosphere traits for enhanced water and nutrients uptake efficiency in dynamic environments. *Front Plant Sci* 15:1383373
- Hussain S, Khan F, Hussain HA, Nie L (2016) Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. *Front Plant Sci* 7:116
- Itroutwar PD, Govindaraju K, Tamilselvan S, Kannan M, Raja K, Subramanian KS (2020) Seaweed-based biogenic ZnO nanoparticles for improving agro-morphological characteristics of rice (*Oryza sativa* L.). *J Plant Growth Regul* 39:717–728
- Kapoor P, Dhaka RK, Sihag P, Mehla S, Sagwal V, Singh Y, Langaya S, Balyan P, Singh KP, Xing B, White JC (2022) Nanotechnology-enabled biofortification strategies for micronutrients enrichment of food crops: current understanding and future scope. *NanoImpact* 26:100407
- Khafagy MA, Mohamed ZAA, Farouk S, Amrajaa HK (2017) Effect of pre-treatment of barley grain on germination and seedling growth under drought stress. *Adv Appl Sci* 2:33–42
- Khan A, Shafi M, Bakht J, Anwar S (2017) Effect of salinity and seed priming on growth characters of wheat varieties. *Sarhad J Agric* 33:435–446
- Khan MO, Irfan M, Muhammad A, Ullah I, Nawaz S, Khalil MK, Ahmad M (2022) A practical and economical strategy to mitigate salinity stress through seed priming. *Front Environ Sci* 10:991977
- Li B, Zhang X, Morita S, Sekiya N, Araki H, Gu H, Han J, Lu Y, Liu X (2022) Are crop deep roots always beneficial for combating drought: A review of root structure and function, regulation and phenotyping. *Agric Water Manag* 271:107781
- Li R, He Y, Chen J, Zheng S, Zhuang C (2023) Research progress in improving photosynthetic efficiency. *Int J Mol Sci* 24(11):9286
- Li Y, Liang L, Li W, Ashraf U, Ma L, Tang X, Pan S, Tian H, Mo Z (2021) ZnO nanoparticle-based seed priming modulates early growth and enhances physio-biochemical and metabolic profiles of fragrant rice against cadmium toxicity. *J Nanobiotechnol* 19:1–19
- Marthandan V, Geetha R, Kumutha K, Renganathan VG, Karthikeyan A, Ramalingam J (2020) Seed priming: a feasible strategy to enhance drought tolerance in crop plants. *Int J Mol Sci* 21:8258
- Mickky BM (2022) Seed priming as a strategy to improve wheat productivity under abiotic stress: global meta-analysis. *J Plant Growth Regul* 41:1397–1410
- Muktadir MA, Adhikari KN, Merchant A, Belachew KY, Vandenberg A, Stoddard FL, Khazaei H (2020) Physiological and biochemical basis of Faba bean breeding for drought adaptation—a review. *Agronomy* 10(9):1345
- Nouairi I, Jalali K, Zribi F, Barhoumi F, Zribi K, Mhadhbi H (2019) Seed priming with calcium chloride improves the photosynthesis performance of Faba bean plants subjected to cadmium stress. *Photosynthetica* 57:397–406
- Okello D, Komakech R, Gang R, Rahmat E, Chung Y, Omujal F, Kang Y (2022) Influence of various temperatures, seed priming treatments and durations on germination and growth of the medicinal plant *Aspilia africana*. *Sci Rep* 12:14180
- Paul A, Mondal S, Mitra D, Chakraborty K, Biswas AK (2025) Seed priming with NaCl boosted the glutathione-ascorbate pool to facilitate photosystem-II function and maintain starch in NaCl-primed chickpea under salt stress. *Plant Physiol Biochem* 222:109746
- Paul A, Mondal S, Pal A, Biswas S, Chakraborty K, Mazumder A, Biswas AK, Kundu R (2023) Seed priming with NaCl helps to improve tissue tolerance, potassium retention ability of plants, and protects the photosynthetic ability in two different legumes, chickpea and lentil, under salt stress. *Planta* 257:111
- Rehman HU, Aziz T, Farooq M, Wakeel A, Rengel Z (2012) Zinc nutrition in rice production systems: a review. *Plant Soil* 361:203–226 <https://doi.org/10.1007/s11104-012-1346-9>
- Rhaman MS, Imran S, Rauf F, Khatun M, Baskin CC, Murata Y, Hasanuzzaman M (2020) Seed priming with phytohormones: an effective approach for the mitigation of abiotic stress. *Plants* 10:37
- Robinson D (2023) Opt-ing out: root–shoot dynamics are caused by local resource capture and biomass allocation, not optimal partitioning. *Plant Cell Environ* 46(10):3023–3039
- Salam A, Khan AR, Liu L, Yang S, Azhar W, Ulhassan Z, Zeeshan M, Wu J, Fan X, Gan Y (2022) Seed priming with zinc oxide nanoparticles downplayed ultrastructural damage and improved photosynthetic apparatus in maize under cobalt stress. *J Hazard Mater* 423:127021
- Sherin G, Aswathi KR, Puthur JT (2022) Photosynthetic functions in plants subjected to stresses are positively influenced by priming. *Plant Stress* 4:100079
- Sheteyi MS, Shao H, Qi W, Daly P, Sharma A, Shaghaleh H, Hamoud YA, El-Esawi MA, Pan R, Wan Q, Lu H (2021) Seed priming and foliar application with jasmonic acid enhance salinity stress tolerance of soybean (*Glycine max* L.) seedlings. *J Sci Food Agric* 101:2027–2041
- Singh AK, Bharati RC, Manibhushan NC, Pedapati A (2013) An assessment of faba bean (*Vicia Faba* L.) current status and future prospect. *Afr J Agric Res* 8:6634–6641

- Srivastava AK, Suresh Kumar J, Suprasanna P (2021) Seed 'primeomics': plants memorize their germination under stress. *Biol Rev* 96:1723–1743
- Wang Z, Li H, Li X, Xin C, Si J, Li S, Li Y, Zheng X, Li H, Wei X, Zhang Z (2020) Nano-ZnO priming induces salt tolerance by promoting photosynthetic carbon assimilation in wheat. *Arch Agron Soil Sci* 66:1259–1273
- Waqas Mazhar M, Ishtiaq M, Hussain I, Parveen A, Hayat Bhatti K, Azeem M, Thind S, Ajaib M, Maqbool M, Sardar T, Muzammil K (2022) Seed nano-priming with zinc oxide nanoparticles in rice mitigates drought and enhances agronomic profile. *PLoS One* 17:e0264967
- Zhang Y, Zhou X, Dong Y, Zhang F, He Q, Chen J, Zhu S, Zhao T (2021) Seed priming with melatonin improves salt tolerance in cotton through regulating photosynthesis, scavenging reactive oxygen species and coordinating with phytohormone signal pathways. *Ind Crops Prod* 169:113671

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.