



The Effect of Ascorbic Acid on Improving Agronomic Traits and Bioactive Compounds in Psyllium under Water Deficit Stress in a Pot Experiment

Abdolkhalegh Kord, Maryam Allahdou*, Mahmood Solouki

Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Zabol, Iran

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*Corresponding author's email: maryam.allahdou@uoz.ac.ir

ABSTRACT

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This study aimed to investigate the role of ascorbic acid in improving the agronomic and biochemical traits of psyllium under water deficit stress conditions. The experiment was conducted in a greenhouse with four irrigation regimes (100, 75, 50, and 25% field capacity) and four concentrations of ascorbic acid (0, 5, 10, and 20 mM) in a factorial design based on a randomized complete block design with three replications. The measurements considered plant height, above-ground dry mass, seed yield, harvest index, seed mucilage content, total phenolic content, total flavonoid content, antioxidant activity (DPPH), phenolic and flavonoid compounds. These compounds were gallic acid, rutin, p-coumaric acid, cinnamic acid, apigenin and kaempferol in seed extracts. The interaction effect of water deficit and application of ascorbic acid was significant on several traits, i.e., seed yield, harvest index, seed mucilage content, total phenolic content and antioxidant activity. Averaged across all ascorbic acid levels, water deficit stress significantly reduced agronomic traits, i.e., plant height, above-ground dry mass, seed yield and harvest index while increasing the mucilage, total phenol, total flavonoid content, and antioxidant activity of the seeds. HPLC analysis indicated that the highest levels of gallic acid were observed under stress conditions at 75% field capacity and 5 mM ascorbic acid treatment (91.467 mg 100 g⁻¹ of seed). Quercetin-3-O-rutinoside (rutin) was found under normal conditions with 10 mM ascorbic acid and at 75% field capacity with 20 mM ascorbic acid (49.50 and 48.49 mg 100 g⁻¹ of seed, respectively). Additionally, p-coumaric acid was highest at 75% field capacity with 0 mM ascorbic acid (8.47 mg 100 g⁻¹ of seed), while cinnamic acid was also highest at 75% field capacity with 0 mM ascorbic acid (3.30 mg 100 g⁻¹ of seed). The metabolites apigenin and kaempferol were not detected in the seeds. The increase in seed yield and quality of *P. ovata*, resulting from the exogenous application of ascorbic acid under water stress and non-stress conditions, could be a promising strategy for enhancing drought tolerance and increasing the production of bioactive compounds in seeds.

Abbreviations: Ascorbic acid (AsA), Plant height (PH), Dry mass (DM), Seed yield (SY), harvest index (HI), Total phenol content (TPC), Total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Normal (N)

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Introduction

Plantago ovata L., a dicot annual herb, belongs to the *Plantaginaceae* family and has been used in folk medicine for many years. It is indigenous to the Mediterranean region of Europe, Southwest Asia, Southeast Spain, and North Africa (Dhar et al., 2005; Talukder et al., 2016). This plant is a well-known medicinal plant because of the laxative nature of its seed mucilage, which is extensively applied for the treatment of intestinal disorders (Kundu et al., 2018; Ltaeif et al., 2021). Additionally, its seeds are effective at inhibiting diarrhea, hypercholesterolemia, constipation, atherosclerosis, diabetes, and obesity and have antioxidant and antibacterial activities (Sahagun et al., 2015; Budzianowska et al., 2024). Aqueous extracts of seeds contain high amounts of hemicellulose and anionic polysaccharides such as D-xylose, L-arabinose, and D-galacturonic acid (Gupta et al., 2015; Khedher et al., 2024).

P. ovata contains bioactive compounds such as polyphenols, which have excellent medicinal characteristics. Polyphenols include simple molecules such as phenolic acids and large and polymerized compounds such as tannins, which are produced during the secondary metabolism of crops. Some natural polyphenols are flavonoids, phenolic acids, xanthenes, lignans, stilbenes, tannins, anthraquinones, hydroxycinnamic acids, and neolignans, which possess antioxidant activity (Talukder et al., 2016). A biochemical analysis of *P. ovata* seeds revealed the presence of flavonoids (plantaovaside, rutin, luteolin 4-glucoside, luteolin 7-glucoside, and apigenin 7-glucoside), phenylethanoids (forsythoside B, acteoside, verbascoside, and plantamajoside) (Budzianowska et al., 2024), kaempferol, organic acids, amino acids, and simple sugars (Frezza et al., 2021).

Water deficit stress can negatively affect the growth and yield of various crops by influencing their physiological and biochemical processes (El-Beltaghi et al., 2020; Farooq et al., 2020; Ghassemi et al., 2023). Under severe or prolonged water stress, a portion of the energy generated by incident photons may be transmitted into processes of reactive oxygen species (ROS) production, such as hydrogen peroxide (H₂O₂), and cause oxidative damage to crop tissues (Jiang and Zhang, 2002). Nevertheless, plants can activate enzyme antioxidant systems, such as superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase or glutathione reductase, and non-enzymatic antioxidant systems, including phenolic compounds, alkaloids, terpenoids, vitamins, glutathione, tocopherols, carotenoids, and ascorbic acid, to decrease oxidative damage (Agarwa and Pandey, 2004; Penella, 2017; Gaafar et al., 2020). Phenolic compounds are effective in the growth and reproduction of plants

and in scavenging free radicals produced under abiotic and biotic stress conditions (Balasundram et al., 2006). Drought stress in medicinal, spice, and aromatic plants can serve as an elicitor, inducing increased production of secondary metabolites (Selmar and Kleinwächter, 2013). It has been reported that medicinal plants grown in semi-arid regions produce more bioactive compounds than similar species in temperate regions. This is because metabolic processes, such as reduced CO₂ fixation in the Calvin cycle, lead to the production of more reduced compounds, including isoprenoids, phenols, and alkaloids (Kleinwächter and Selmar, 2015).

Ascorbic acid, a water-soluble molecule, is a notable nonenzymatic antioxidant that plays an important role in the detoxification of ROS and the regulation of other essential biological processes in plants under stress conditions (Penella et al., 2017). It acts as an antioxidant, a co-factor for several enzymes, a main redox buffer, and a precursor for oxalate and tartrate production and modulates phytohormones, which are involved in signaling routes of cell growth and development, mitosis, the transition from the vegetative stage to the productive stage, and senescence (Beltaghi, 2008; Rigano et al., 2017; Gallie, 2013). Several studies have reported that the application of ascorbic acid improves the growth, yield, and quality of bioactive compounds in various plants, such as quinoa (*Chenopodium quinoa* Wild.) (Aziz et al., 2018), common bean (*Phaseolus vulgaris* L.) (Gaafar et al., 2020), *Ocimum sanctum* L. (Munir et al., 2021), milk thistle (*Silybum marianum* L.) (Zahra et al., 2021), and mung bean (*Vigna radiata* L. Wilczek) (Shahzadi et al., 2023). Drought stress imposes major limitations on crop growth and productivity, particularly in arid regions. To mitigate its adverse effects, various strategies have been explored, among which the application of ascorbic acid has shown promising potential. Given the wide range of medicinal properties attributed to *Plantago ovata* (psyllium), this study investigated the role of ascorbic acid in improving key agronomic traits under drought stress. Furthermore, since limited research has focused on the interaction between stress conditions, ascorbic acid, and the biosynthesis of bioactive compounds in psyllium, the present work also examined its impact on important medicinal metabolites, including rutin, apigenin, kaempferol, p-coumaric acid, and gallic acid.

Materials and Methods

Plant materials and chemicals

Seeds of *Plantago ovata* L. were collected from their natural habitats in the Sangan region of Khash, Iran. All the standards and chemicals used in this study were prepared from Sigma Aldrich (St. Louis, MO, USA).

Experiment setup and irrigation treatments

A pot experiment was performed in a greenhouse located in the Sangan region of Khash, Iran (23°34'56" N, 61°19'11" E, 1633 m), with temperatures at 29/16 ± 2 °C D/night, a relative humidity of 35–50%, and a natural photoperiod of 11 h. The experiment was conducted as a factorial design on the basis of a randomized complete block design with three replicates in February 2024. Four ascorbic acid concentrations (0, 5, 10, and 20 mM) were used according to the literature (Jahanbakhsh et al., 2022; Eskandarzadeh et al., 2025) as well as four irrigation treatments (100, 75, 50, and 25% of water requirement (mild, moderate, and severe water stress, respectively). To apply drought stress, a gravimetric method was used, involving a digital scale with an accuracy of 0.001 grams.

The seeds were surface sterilized in 10% (v/v) sodium hypochlorite solution for 10 min and then washed with distilled water four times. The plants were subsequently sown at a depth of 0.5 cm in plastic pots that were 13 cm in height and 13 cm in diameter. These pots contained 1 kg of soil from the plant growth region on February 4, 2024, and were kept in a greenhouse. Three weeks after sowing, the plants were thinned, and two plants remained in each pot. For each experimental unit, two pots were used. The soil texture was sandy (sand 70%, silt 12%, and clay 18%), the pH was 7.97, and the electrical conductivity was 1.1 dSm⁻¹.

All the pots were irrigated to field capacity from the beginning of the experiment to the establishment of the seedlings. After seedling establishment, irrigation regimes were applied and continued until physiological maturity. To apply the irrigation regimes, the pots were weighed daily to maintain the soil moisture content at the levels corresponding to each irrigation regime. Ascorbic acid foliar spraying was performed three times: at the beginning of blooming, flowering, and seed formation. The control plants were sprayed with distilled water. The seeds were harvested at physiological maturity.

Agronomic parameters

Agronomic parameters, including plant height (PH), aboveground dry mass (DM), seed yield (SY), and the harvest index (HI), were measured in two plants in each experimental unit at the physiological maturity stage, and the average values were used in the subsequent analyses. The plants from each experimental unit were harvested and air-dried in the shade, after which the DM was weighed. For the evaluation of seed yield, the seeds were separated from the single plant, weighed, and expressed in grams per plant (g plant⁻¹). Harvest index was determined on the basis of the ratio of seed yield to above-ground dry mass (DM) and reported as a percentage.

Biochemical parameters

Seed mucilage content

The mucilage content was measured as described by Gupta et al. (2015), with slight modifications. 0.1 N HCl (10 mL) was heated to boil in a 100 mL flask. After that, the flask was removed from the flame, and dried seeds (1 g) were added to it. The solution was again heated, and the dissolution of the seed husk was observed. The flask was then removed from the flame when all the seeds had changed color, and the obtained solution was filtered. To separate residual traces of mucilage, the seeds were washed twice with hot water (5 mL), after which they were filtered. The filtered solution was mixed with 95% ethyl alcohol (60 mL), shaken, and incubated for 5 h. Then, the supernatant was discarded, and the flask containing the precipitate was dried in an oven at 50 °C. The dried precipitate was weighed, and the amount of seed mucilage was expressed as a percentage.

Total phenol content (TPC)

To prepare the extract, 10 mL of 80% methanol was added to 1 g of the dried and powdered seeds, which were shaken at 110 rpm for 8 h, after which the samples were filtered. Folin–Ciocalteu reagent was used to measure the amount of TPC, as explained by Ardestani and Yazdanparast (2007), with some changes. In a 10 mL test tube, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin–Ciocalteu's reagent and 2 mL of 7.5% sodium carbonate, and then, the mixture was vigorously shaken for 15 min at 45 °C in a hot water bath. The absorbance of each sample was measured at 765 nm via a spectrophotometer. By using the gallic acid calibration curve, the amount of TPC was calculated and reported as mg of gallic acid equivalent per g of dry weight (GAE mg g⁻¹ DW).

Total flavonoid content (TFC)

The TFCs were estimated as described by Zhang et al. (2015), with slight modifications. A total of 125 µL of the sample extract and 75 µL of 5% NaNO₂ solution were mixed. After 6 min, 150 µL of 10% AlCl₃ was added to the mixture, and after 5 min, 750 µL of 1 M NaOH was mixed with it. Finally, the sample volume was increased to 2500 µL by adding distilled water. After 15 min, the absorbance was read at 510 nm. The value of the TFC was measured via a quercetin calibration curve and expressed as mg of quercetin equivalent per g of dry weight (QE mg g⁻¹ DW).

Radical scavenging activity via the DPPH assay

Antioxidant activity was measured by evaluating the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (Girenavar et al., 2007) with some modifications. Seed extracts (0.1 mL) were

added to 2 mL of DPPH methanolic solution (0.1 mM) and kept in the dark at 25 °C for 30 min. Then, the absorbance of the control sample (DPPH methanolic solution (0.1 mM)) (A_c) and samples containing the seed extract (A_s) were read at 517 nm. The percentage of inhibition was estimated via the following formula:

$$\% \text{inhibition} = \left[\frac{A_c - A_s}{A_c} \right] \times 100$$

Where A_s and A_c are the absorbance values of the plant samples and the control sample, respectively.

Analysis of phenolic and flavonoid compounds via HPLC

Phenolic and flavonoid compounds were determined via an Agilent 1260 Infinity II high-performance liquid chromatography (HPLC) system (Agilent, USA). To obtain the extract, 0.1 g of dried and milled seeds was added to a 10 mL solution of HPLC-grade methanol (80%). The mixture was shaken at 110 rpm and 25 °C for 8 h and then filtered through a 0.22 µm acrylic disk of nylon. Flavonoid compound separation was carried out with a reverse-phase C18 column (250 × 4.6 mm, 5.0 µm) as the stationary phase and a combination of two solvent gradient systems containing water (solvent A) and acetonitrile (solvent B) as the mobile phase, which was injected into the column at a flow rate of 1.000 mL min⁻¹, and the absorbance was read at 330 nm (Mattila et al., 2000). Phenolic compounds were separated with an aqueous formic acid solution of 0.1% (A) and

methanol (B) as the mobile phase at a flow rate of 0.3 mL min⁻¹, and the absorbance was read at 280 nm (Goupy et al., 1999). The compound concentrations of the seed extracts were measured via standard curves of phenolic and flavonoid substances through comparisons of retention times and peak areas and recorded as mg per 100 g of dry weight of sample.

Statistical analysis

A factorial experiment was conducted based on a completely randomized block design with three replications. First, the data normality test was performed using the Kolmogorov-Smirnoff method, and after confirming the normality of the data, the errors were also examined for normality. using Minitab statistical software (Version 14). Subsequently, variance analysis and mean comparisons were conducted using the LSD test at a 5% significance level via the SAS statistical program (Version 9.1). Charts were created using Excel software.

Results

The simple effect of each factor (irrigation regime and exogenous ascorbic acid) had significant effects at the 1% level on all the measured traits in the greenhouse experiment, except for the effect of exogenous ascorbic acid on the total flavonoid content. The interaction effect of irrigation regime and external ascorbic acid was significant for seed yield, harvest index, mucilage, total phenol content, and antioxidant activity (Table 1).

Table 1. Analysis of variance (mean squares) for different traits of *Plantago ovata* L. evaluated at four levels of irrigation regimes (IR) and four levels of exogenous ascorbic acid (AsA) in three replicates (R).

	DF	PH	DM	SY	HI	Muc.	TPC	TFC	AA
R	2	0.813 ^{ns}	0.593 ^{ns}	0.009 ^{ns}	5.037 ^{ns}	1.538 ^{ns}	0.025 ^{ns}	0.001 ^{ns}	19.295 ^{ns}
IR	3	31.833 ^{**}	44.064 ^{**}	3.042 ^{**}	52.378 ^{**}	10.370 ^{**}	44.967 ^{**}	2.495 ^{**}	549.146 ^{**}
AsA	3	19.500 ^{**}	38.050 ^{**}	1.964 ^{**}	18.634 ^{**}	12.900 ^{**}	12.606 ^{**}	0.0009 ^{ns}	1235.058 ^{**}
IR × AsA	9	0.481 ^{ns}	1.009 ^{ns}	0.209 ^{**}	13.936 ^{**}	3.154 ^{**}	0.708 [*]	0.008 ^{ns}	49.960 ^{**}
Error	30	0.835	0.490	0.009	3.665	0.719	0.245	0.007	11.433

DF, degree of freedom; PH, plant height; DM, above-ground dry mass; SY, seed yield; HI, harvest index; Muc., mucilage; TPC, total phenolic content; TFC, total flavonoid content; AA, antioxidant activity. ^{ns}, non-significant; * and **, significant at $P \leq 0.05$ and at $P \leq 0.01$, respectively.

Effects of water stress and exogenous ascorbic acid (AsA) on agronomic parameters

Water stress, 75, 50, and 25% field capacity (mild, moderate, and severe water stress, respectively) led to significant decreases in the mean plant height (PH), plant aboveground dry mass (DM), seed yield (SY), and harvest index (HI). The plant height, plant

aboveground dry mass (DM), seed yield (SY), and harvest index (HI) values gradually decreased with increasing stress intensity until they reached their lowest values under severe stress conditions. Compared with those under the control (non-stress) conditions, the average amounts of plant height, plant aboveground dry mass (DM), seed yield (SY), and harvest index (HI) decreased by 11.84, 10.35,

21.91, and 4.93%, respectively, under mild stress conditions; by 17.76, 29.25, 41.03, and 15.34%, respectively, under moderate stress conditions; and

by 30.94, 46.67, 60.62, and 23.74%, respectively, under severe stress conditions (Table 2).

Table 2. Mean comparison of the irrigation regimes (IR) and exogenous ascorbic acid treatments (AsA) for different traits of *Plantago ovata* L.

	PH (cm)	DM (g plant ⁻¹)	SY (g plant ⁻¹)	HI (%)	Muc. (%)	TPC (mg GAE g ⁻¹ DW)	TFC (mg QE g ⁻¹ DW)	AA (%)
IR								
100% WR	12.66 ^a	9.61 ^a	1.94 ^a	19.63 ^a	15.32 ^c	7.58 ^d	1.182 ^d	58.82 ^c
75% WR	11.167 ^b	8.16 ^b	1.51 ^b	18.67 ^a	16.08 ^b	9.25 ^c	1.37 ^c	69.92 ^b
50% WR	10.41 ^b	6.80 ^c	1.14 ^c	16.62 ^b	16.51 ^b	10.62 ^b	1.68 ^b	71.98 ^{ab}
25% WR	8.75 ^c	5.13 ^d	0.76 ^d	14.97 ^c	17.55 ^a	12.12 ^a	2.22 ^a	73.93 ^a
AsA								
0 mM	9.00 ^c	5.17 ^c	0.83 ^d	16.21 ^b	14.92 ^c	8.73 ^d	1.60 ^a	57.19 ^d
5 mM	10.66 ^b	6.86 ^b	1.21 ^c	16.89 ^b	16.54 ^b	9.55 ^c	1.61 ^a	63.98 ^c
10 mM	11.41 ^{ab}	8.61 ^a	1.55 ^b	17.68 ^{ab}	16.59 ^b	10.13 ^b	1.62 ^a	73.22 ^b
20 mM	11.91 ^a	9.06 ^a	1.76 ^a	19.11 ^a	17.40 ^a	11.16 ^a	1.62 ^a	80.26 ^a

PH, plant height; DM, above-ground dry mass; SY, seed yield; HI, harvest index; Muc., mucilage; TPC, total phenolic content; TFC, total flavonoid content; AA, antioxidant activity; WR, water requirement. In each column and within irrigation regimes and exogenous ascorbic acid treatments, means with similar letters indicate non-significant difference at $P \leq 0.05$ based on the least significant differences.

The values of plant height, plant aboveground dry mass (DM), seed yield (SY), and harvest index (HI) increased as the concentration of external AsA increased and reached a maximum at the highest concentration (20 mM). On average, over all the irrigation regimes, foliar-applied AsA at a concentration of 5 mM increased the plant height, plant aboveground dry mass (DM), seed yield (SY), and harvest index (HI) by approximately 15.63, 24.71, 31.00, and 4.07%, respectively; 10 mM exogenous AsA promoted them by approximately 21.17, 40.01, 46.08, and 8.35%, respectively; and 20 mM AsA enhanced these traits by approximately 24.48, 42.97, 52.47, and 15.18%, respectively, in comparison with the non-application of AsA (Table 2).

Moreover, the increase in seed yield (SY) and harvest index (HI) caused by exogenous AsA depended on the irrigation treatment. Compared with those under the other irrigation regimes, the greatest increases in seed yield (SY) and harvest index (HI) were associated with all the exogenous AsA concentrations obtained under non-stress conditions. The highest amounts of seed yield (SY) and harvest index (HI) were recorded under non-stress

conditions and 20 mM concentrations of external AsA (2.69 g plant⁻¹ and 22.22%, respectively). In this treatment, the amounts of increase in seed yield (SY) and harvest index (HI) were 65.43 and 33.48%, respectively, compared with those in the control (Fig. 1).

Effects of water stress and exogenous AsA on the biochemical parameters of seeds

Our results indicated that both factors (water stress and exogenous AsA) at all water stress levels and concentrations of external AsA had significantly positive effects on the accumulation of mucilage, total phenol content (TPC), and total flavonoid content (TFC), and increased antioxidant activity compared with the control conditions, except for the effect of exogenous AsA on TFC. The contents increased with increasing stress intensity and external AsA concentration. Compared with those under the control (non-stress) conditions, the percentages of mucilage, TPC, TFC, and antioxidant activity increased by 4.97, 18.06, 14.10, and 15.87%, respectively, due to mild water stress; 7.21, 28.63, 29.93, and 18.28%, respectively, due to moderate

water stress; and 12.69, 37.44, 46.91, and 20.44%, respectively, due to severe water stress (Table 2). Compared with the non-application of AsA, foliar application of AsA at a concentration of 5 mM increased mucilage, TPC, and antioxidant activity by approximately 9.78, 8.54, and 10.60%, respectively.

Compared with the non-application of AsA, the 10 mM concentration promoted these traits by approximately 10.03, 13.86, and 21.88%, respectively, and the 20 mM concentration increased these traits by approximately 14.22, 21.82, and 28.74%, respectively (Table 2).

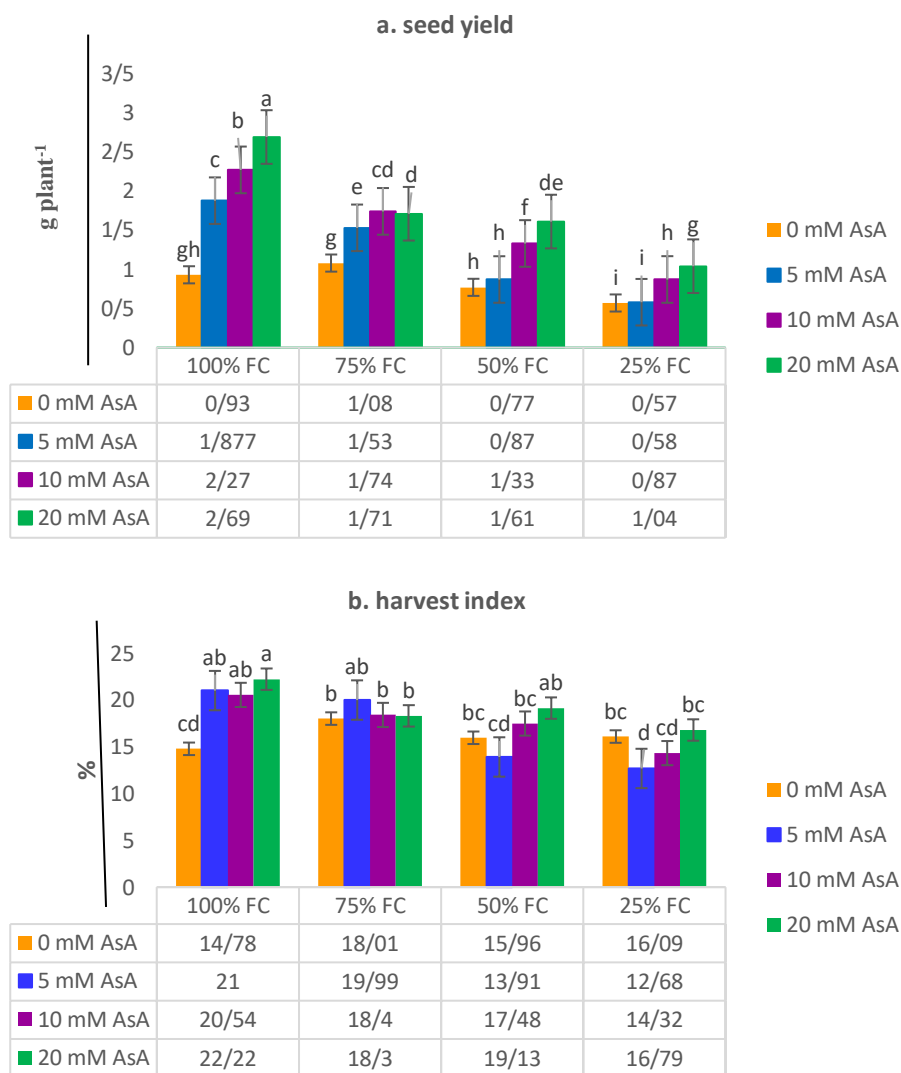


Fig. 1. Mean comparison for interaction of irrigation regime and exogenous ascorbic acid (AsA) on seed yield and harvest index of *Plantago ovata* L. FC, field capacity; means with similar letters indicate non-significant difference at $P \leq 0.05$ based on the least significant differences.

Moreover, the amount of increase in mucilage, TPC, and antioxidant activity caused by exogenous AsA depended on the irrigation regime. The lowest and highest were recorded in non-stresses (13.04%, 5.79 mg GAE g⁻¹ DW, and 44.08%, respectively), and severe water stress conditions and 20 mM foliar-applied AsA (18.87%, 12.85 mg GAE g⁻¹ DW, and 86.13%), respectively (Fig. 2).

Effects of water stress and exogenous AsA on phenolic and flavonoid compounds in seeds

The HPLC analysis detected six phenolic and flavonoid compounds, including gallic acid, rutin, *p*-coumaric acid, and cinnamic acid in seed extract. Apigenin and kaempferol were not detected in the seed extracts at any concentration of AsA.

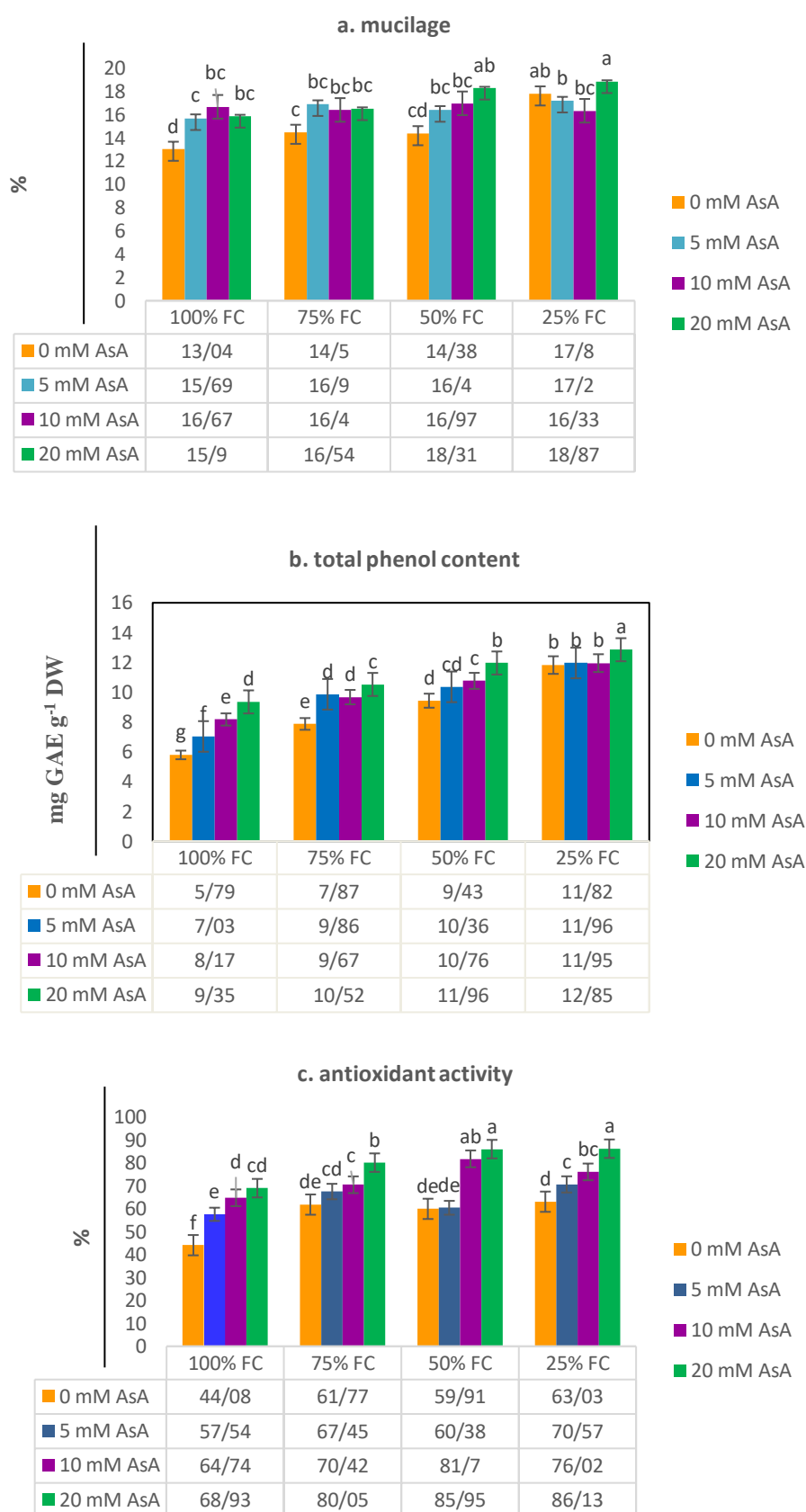
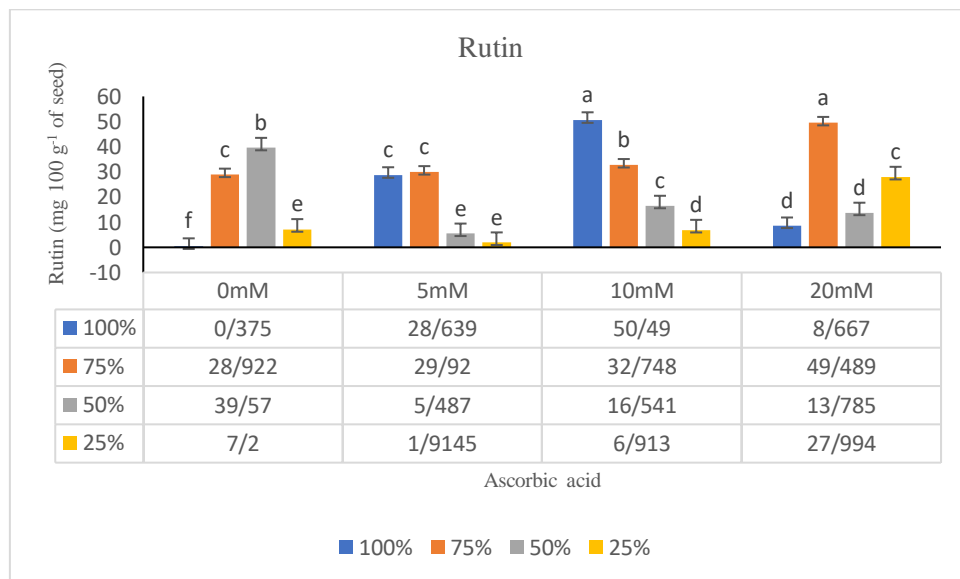
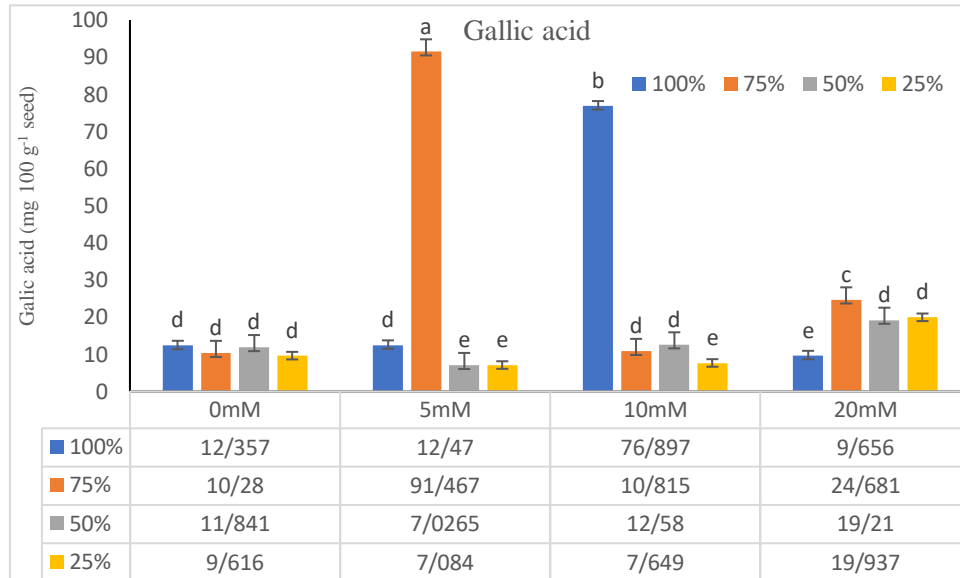


Fig. 2. Mean comparison for interaction of irrigation regime and exogenous ascorbic acid (AsA) on biochemical traits of seed extracts of *Plantago ovata* L. FC, field capacity; means with similar letters indicate non-significant difference at $P \leq 0.05$ based on the least significant differences.

Gallic acid: The highest concentration of gallic acid was observed at 75% of field capacity (mild stress) with a 5 mM ascorbic acid concentration. In the absence of ascorbic acid, the amount of this metabolite decreased as stress increased, with a

recorded level of 22.18 under severe stress (25% of field capacity). Therefore, mild stress combined with a small amount of ascorbic acid (5 mM) is recommended to enhance this secondary metabolite (Fig. 3a).



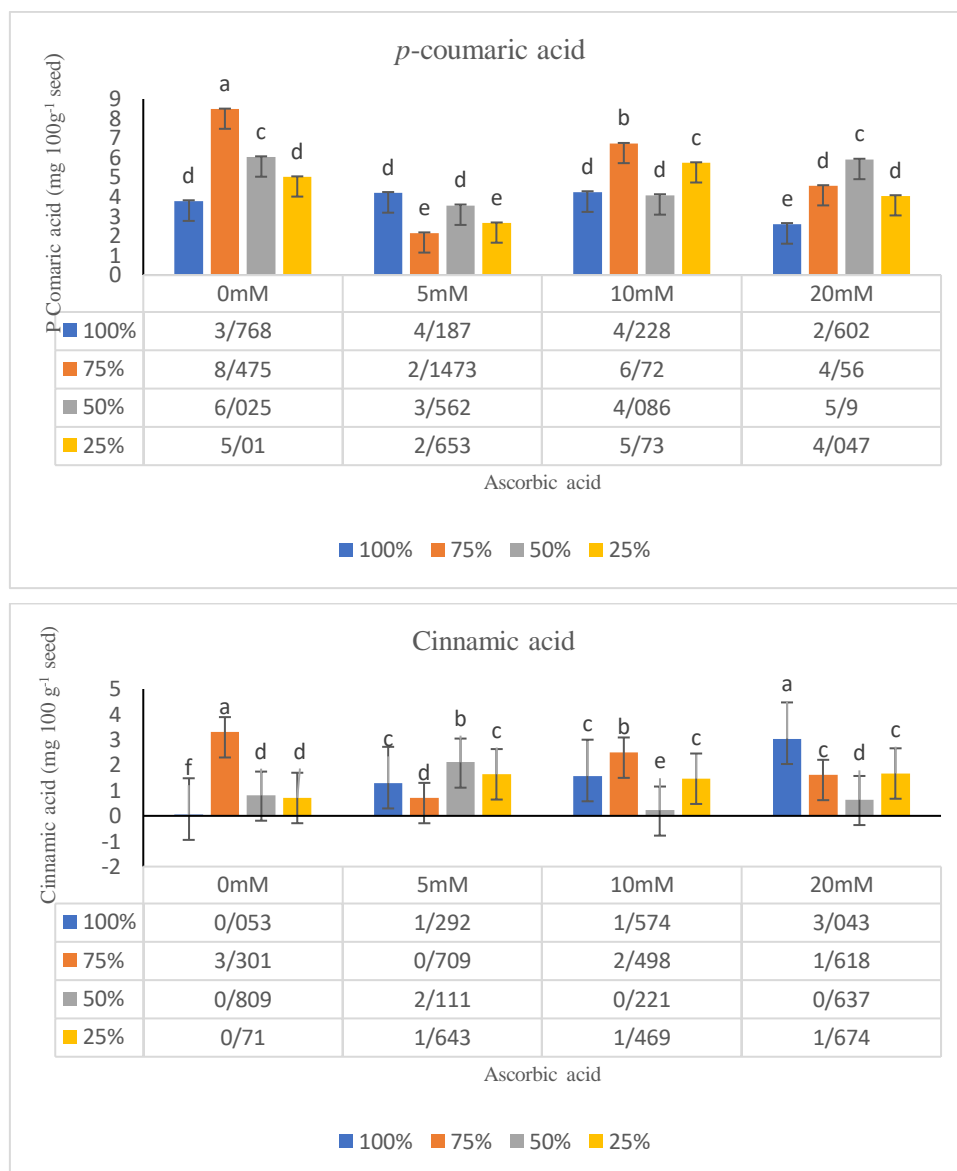


Fig. 3. Mean comparison for interaction of irrigation regime and exogenous ascorbic acid (AsA) on phenolic and flavonoid compounds in seeds, Gallic acid, Rutin, -p-coumaric acid and Cinnamic acid respectively.

Rutin: The amount of rutin increased with higher stress levels and a lack of ascorbic acid; however, severe stress (at 25% of field capacity) resulted in a decrease in its concentration. Under severe stress conditions, increasing ascorbic acid concentration led to an increase in this secondary metabolite. Specifically, at a concentration of 20 mM ascorbic acid, the amount of rutin increased from 2.7 to 27.99 (Fig. 3b). The highest concentration of this compound was observed under normal conditions with 10 mM ascorbic acid, as well as under mild stress (75% of field capacity) with 20 mM ascorbic acid (49.50 and 49.40, respectively).

p-coumaric acid: The application of ascorbic acid did not positively affect p-coumaric acid levels. In most conditions, increasing the concentration of ascorbic acid reduced the amount of this compound compared

to the control (non-application) (Fig. 3c). The highest concentration of this secondary metabolite was observed under mild stress conditions (75% field capacity) without the application of ascorbic acid (47.8 mg 100 g⁻¹ of seed).

Cinnamic acid: Stress increased the concentration of cinnamic acid. Under mild stress, the amount of this compound increased significantly (60.45-fold greater than that in the control). The use of ascorbic acid also improved cinnamic acid levels under severe stress conditions (Fig. 3d). The highest concentration of this secondary metabolite was noted under mild stress conditions without ascorbic acid application (3.30 mg 100 g⁻¹ of seed).

Discussion

In the present study, water stress decreased plant height (PH), plant aboveground dry mass (DM), seed yield (SY), and the harvest index (HI). These results are similar to those reported for *P. ovata* (Bannayan et al., 2008), fennel (Gholami Zali and Ehsanzadeh, 2018), safflower (Alizadeh Yeloojeh et al., 2020), common bean (Gaafar et al., 2020), and summer savory (Rasouli et al., 2023). However, the use of exogenous AsA improved the growth parameters and seed yield under all irrigation regimes (water stress and non-stress conditions). These observations agree with Dolatabadian et al. (2010), who reported the efficiency of exogenous AsA in increasing plant height (PH), stem and leaf dry weights, and seed yield (SY) under water stress in corn. A study by El-bially et al. (2018) revealed that crop parameters such as the leaf area index, head weight, and seed yield (SY) of sunflower under water stress decreased with increasing stress intensity and that the application of exogenous AsA improved these parameters. In another study, an improvement in yield attributes caused by AsA was observed (Noreen et al., 2021). Zahra et al. (2021) reported that AsA treatment increased the quantity of milk thistles, including the main panicle length, number of flowers, achene yield, and achene 1000 weight. It seems that AsA improves cell division and thus increases growth. Moreover, AsA, as an antioxidant, neutralizes the reactive oxygen species produced due to water deficit stress and, as a result, reduces the deleterious effects of water deficit stress (Dolatabadian et al., 2010). In addition to negatively affecting biomass reduction, growth retardation, or behavioral changes, drought stress can alter the source-sink properties of the entire plant, thereby affecting the overall biosynthetic function and the allocation and accumulation of secondary metabolites by changing the physiological characteristics of specific organs or tissues (Kleinwächter and Selmar, 2015).

The accumulation of phenols in plants usually occurs due to environmental stresses (Rodriguez et al., 2011). In the present study, water stress increased the levels of secondary metabolites such as total phenol content (TPC) and total flavonoid content (TFC) and increased antioxidant activity. Similarly, an increase in secondary metabolites with antioxidant properties under water stress has been reported in other studies (Caser et al., 2018; Farooq et al., 2020; Mumivand et al., 2021). These metabolites are free radical scavengers, such as DDPH; therefore, they protect plants against oxidative stress (Kedare and Sing, 2011). Since stress-related responses broadly affect overall metabolism, the synthesis and accumulation of secondary metabolites are also influenced by these responses (Selmar, 2008). Researchers have reported that under stress conditions, stomata close in

response to water deficiency, which greatly reduces carbon dioxide uptake. Consequently, the consumption of reducing equivalents (NADPH + H⁺) for carbon dioxide fixation via the Calvin cycle decreases significantly, leading to a large surplus of NADPH + H⁺. As a result, metabolic processes are redirected toward the synthesis of highly reduced compounds such as isoprenoids, phenols, and alkaloids (Selmar and Kleinwächter, 2013a, b).

Our results revealed that exogenous AsA increased the contents of TP and TF and antioxidant activity. These observations agree with those of Gaafar et al. (2020), who reported positive impacts of water stress and foliar-applied AsA on the TP, TF, and tannin contents and antioxidant activity in common bean. They also reported that the degree of increase in these metabolites depended on the irrigation regime. Farooq et al. (2020) reported that the total phenolic content increased in all four safflower cultivars under water stress as well as under foliar application of AsA. The accumulation of TP and TF and the improvement in antioxidant activity due to the exogenous application of AsA in *Ocimum sanctum* L. have also been reported (Munir et al., 2021). These results indicate that foliar application of AsA activates some gene regulatory mechanisms involved in the production of such non-enzymatic antioxidants (Farooq et al., 2020). These antioxidants can act as cell component stabilizers that may decrease the harmful effects of water deficit stress (Gaafar et al., 2020). Additionally, in our study, the external application of AsA led to an increase in both seed yield and secondary metabolites under both water stress and non-stress conditions, which indicates that AsA may reduce the harmful effects of water stress and improve seed yield through these metabolites. Phenolic compounds are associated with plant antioxidant ability (Gaafar et al., 2020; Zaman et al., 2020). In the present study, with increasing phenol and flavonoid contents, the antioxidant activity increased, which shows that these compounds can inhibit free radicals. Kainama et al. (2020) reported that secondary metabolites, including phenolics and flavonoids, have antioxidant effects through free radical scavengers, electron donors, and chain breakers. In this study, the mucilage content of seeds increased under water stress and exogenous AsA conditions. These results have already been reported by other researchers (Rahimi et al., 2013; Zare et al., 2023).

The results of our study revealed a positive impact of exogenous AsA on phenolic and flavonoid compounds in the seed extracts, but the effect of exogenous AsA on bioactive compounds depended on the concentration of AsA and the type of compounds. Similarly, Abed et al. (2023) reported that the effects of methyl jasmonate at different concentrations on the bioactive compounds of

Moringa oleifera (Lam.) were different. They reported that the highest amount of baicalin was found in the 30 mg L⁻¹ MeJa group, whereas the maximum accumulation of rutin, hesperidin, rosmarinic acid, quercetin, and kaempferol was obtained in the 20 mg L⁻¹ MeJa group. In the present study, the most abundant flavonoid compound was rutin, which increased under normal and mild stress conditions, as well as with the application of 10 and 20 mM ascorbic acid, respectively. Rutin has many medicinal uses, including antimicrobial, antifungal, and antiallergic properties, and it has been reported to provide medicinal benefits for treating diseases such as cancer, diabetes, high blood pressure, and high cholesterol. Additionally, it has an advantage over other flavonoids, as it is a non-toxic, non-oxidizable molecule (Sharma et al., 2013). This study observed that ascorbic acid plays a significant role in increasing this beneficial flavonoid compound. Therefore, mild stress combined with a concentration of 10 mM ascorbic acid can elevate rutin levels up to 134 times compared to normal stress conditions without ascorbic acid application (Fig. 3b). Ascorbic acid also positively affected the levels of other compounds, such as p-coumaric acid and cinnamic acid. Thus, the use of ascorbic acid under mild stress conditions is recommended to enhance bioactive compounds in psyllium. The positive effects of ascorbic acid in increasing secondary metabolites under drought stress conditions have also been reported in other plants, such as common bean (Gaafar et al., 2020), quinoa (Aziz et al., 2018), and jute (Sharma et al., 2024).

Conclusion

The deleterious effects of water deficit stress on the growth parameters and seed yield of *P. ovata* L. were reduced by foliar application of ascorbic acid (AsA). Water deficit stress and exogenous AsA increased the total phenol and flavonoid contents, mucilage percentage, and antioxidant activity of the seeds. The 20 mM concentration of AsA had a relatively positive effect on the agronomic and biochemical parameters under all the irrigation regimes. External AsA improved most of the phenol and flavonoid compounds in the seed extracts, but the increase in these compounds was dependent on the AsA concentration and compound type. Whereas in rutin, under severe stress conditions, increasing ascorbic acid concentration led to an increase in this secondary metabolite. Specifically, at a concentration of 20 mM of ascorbic acid has its maximum level. The increase in growth, seed yield, and seed bioactive compounds under both water stress and non-stress conditions is a promising method for improving the quantity and quality of seeds under both water conditions and the biochemical properties of seeds.

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Author Contributions

Greenhouse experiments and callus production, AK; data collection, data analysis and writing, MA; editing the article and help in writing, MS. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

References

- Abed AS, Ismail FN, Majeed DM, Al-Jibouri AMJ. 2023. Increasing amounts of secondary metabolites and medicinal compounds in callus culture of *Moringa oleifera* (Lam) using abiotic elicitors. Iraq Journal of Sciences 64, 3902-3913. [https://doi: 10.24996/ij.s.2023.64.8.17](https://doi.org/10.24996/ij.s.2023.64.8.17)
- Agarwa S, Pandey V. 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biological Plant 48 (4), 555-560. [https://doi:10.1023/B:BIOP.0000047152.07878.e7](https://doi.org/10.1023/B:BIOP.0000047152.07878.e7)
- Alizadeh Yeloojeh Kh, Saeidi Gh, Sabzalian M. R. 2020. Drought stress improves the composition of secondary metabolites in safflower at the expense of reduction in seed yield and oil content. Industrial Crop Production 154, 112496. <https://doi.org/10.1016/j.indcrop.2020.112496>
- Ardestani A, Yazdanparast R. 2007. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food Chemistry 104, 21-29. [https://doi: org/10.1016/j.foodchem.2006.10.066](https://doi.org/10.1016/j.foodchem.2006.10.066)
- Aziz A, Aisha Akram N, Ashraf M. 2018. Influence of natural and synthetic vitamin C (ascorbic acid) on primary and secondary metabolites and associated metabolism in quinoa (*Chenopodium quinoa* Willd.) plants under water deficit regimes. Plant Physiology and Biochemistry 123, 193-203. <https://doi.org/10.1016/j.plaphy.2017.12.004>
- Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and uses. Food Chemistry 99 (1), 191-203. <https://doi.org/10.1016/j.foodchem.2005.07.042>
- Bannayan M, Nadjafi F, Aziz M, Tabrizi L, Rastgoo M. 2008. Yield and seed quality of *Plantago ovata* and *Nigella sativa* under different irrigation

- treatments. *Industrial Crops Production* 27, 11-16. [https://doi: 10.1016/j.indcrop.2007.05.002](https://doi.org/10.1016/j.indcrop.2007.05.002)
- Beltaghi MS. 2008. Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chickpea (*Cicer arietinum* L.) plants. *African Journal of plant Science* 2 (10), 118-123. <http://doi.org/105897/AJPS.9000124>
- Budzianowska A, Kikowska M, Budzianowski J. 2024. Phenylethanoid glycosides accumulation and antiradical activity of fractionated extracts of *Plantago ovata* Forssk. Callus cultures lines. *Plant Cell Tissue Organology* 156, 54. <https://doi.org/10.1007/s11240-023-02635-y>
- Caser M, Angiolillo F, Chitarra W, Lovisolio C, Ruffoni B, Pistelli L, Scariot V. 2018. Eco-physiological and phytochemical responses of *Salvia sinaloensis* Fern. to drought stress. *Plant Growth Regulation* 84, 383-394. [https://doi: 10.1007/s10725-017-0349-1](https://doi.org/10.1007/s10725-017-0349-1)
- Dhar MK, Kaul S, Sareen S, Koul AK. 2005. *Plantago ovata*: genetic diversity, cultivation, utilization, and chemistry. *Plant Genetic Resource* 3 (2), 252-263. <https://doi.org/10.1079/PGR200582>
- Dolatabadian A, Modarres Sanavy SA, Sadat Asilan K. 2010. Effect of ascorbic acid foliar application on yield, yield component and several morphological traits of grain corn under water deficit stress conditions. *Notulae Scientia Biologicae* 2, 45-50. <https://doi.org/10.15835/nsb234717>
- El-Bially M, Saady H, El-Metwally I, Shahin M. 2018. Efficacy of ascorbic acid as a cofactor for alleviating water deficit impacts and enhancing sunflower yield and irrigation water-use efficiency. *Agricultural Water Management* 208, 132-139. <https://doi.org/10.1016/j.agwat.2018.06.016>
- El-Beltaghi HS, Shah S, Sulaiman SU, Mansour AT, Shalaby TA. 2022. Impacts of ascorbic acid and alpha-tocopherol on chickpea (*Cicer arietinum* L.) grown in water deficit regimes for sustainable production. *Sustainability* 14, 8861. <https://doi.org/10.3390/su14148861>
- Eskandarzadeh M, Janmohammadi M, Sabaghnia N, Kheshtpaz N. 2025. Exogenous Application of Growth-Stimulating Substances Alleviated the Effects of Water-Deficit Stress on the Spring Camelina sativa. *Agrotechniques in Industrial Crops* 5(1), 70-80. <https://doi.org/10.22126/ATIC.2024.10928.1155>
- Farooq A, Bukhari SA, Akram N.A, Ashraf M, Wijaya L, Alyemeni MN, Ahmad P. 2020. Exogenously applied ascorbic acid-mediated changes in osmoprotection and oxidative defense system enhanced water tolerance in different cultivars of safflower (*Carthamus tinctorious* L.). *Plants* 9, 104. [https://doi:10.3390/plants9010104](https://doi.org/10.3390/plants9010104)
- Frezza C, Sciubba F, Tomai P, Gntili A, Bianco A, Serafini M, Golkar P. 2021. Phytochemical analysis on the seeds of a new Iranian *Plantago ovata* Forssk. Population specimen. *Natural Product Resources* 36 (2), 1-4. <https://doi.org/10.1080/14786419.2021.1881960>
- Gaafar AA, Ali SI, El-shawadfy MA, Salama ZA, Sekara A, Ulrichs Ch, Abdelhamid MT. 2020. Ascorbic acid induces of secondary metabolites, antioxidant activity, growth and productivity of common bean under water stress conditions. *Plants* 9, 627. [https://doi:10.3390/plants9050627](https://doi.org/10.3390/plants9050627)
- Gallie DR. 2013. L-ascorbic acid: a multifunctional molecule supporting plant growth and development. *Scientifica* 795964, 1-24. <http://dx.doi.org/10.1155/2013/795964>
- Ghassemi M, Zahedi M, Gheysari M, Sabzalian M.R. 2023. Effects of inoculation with four mycorrhizal species on seed phenolic and fatty acids of sesame plants grown under different irrigation regimes. *Scientific Reports* 13, 16482. <https://doi.org/10.1038/s41598-023-42375-9>
- Gholami Zali A, Ehsanzadeh P. 2018. Exogenous proline improves osmoregulation, physiological functions, essential oil, and seed yield of fennel. *Industrial Crop Production* 111, 133-140. <http://doi.org/10.1016/j.indcrop.2018.07.067>
- Girenavar B, Jayaprakasha G, Jadegoud Y, Gowda G. N, Patill BS. 2007. Radical scavenging and cytochrome P450 3A4 inhibitory activity of bergaptol and geranylcoumarin from grapefruit. *Bioorganic and Medicinal Chemistry* 15, 3684-3691. <https://doi.org/10.1016/j.bmc.2007.03.047>
- Goupy P, Hugues M, Boivin P, Amiot MJ. 1999. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *Journal of Sciences Food Agriculture* 79, 1625-1634. [https://doi.org/10.1002/\(SICI\)1097-0010\(199909\)79:12<1625::AID-JSFA411>3.0.CO;2-8](https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1625::AID-JSFA411>3.0.CO;2-8)
- Gupta M, Kour B, Kaul S, Dhar M.K. 2015. Mucilage synthesis in callus cultures of *Plantago ovata* Forsk. *National Academy Science Letter* 38, 103-106. [https://doi:10.1007/s40009-014-0303-y](https://doi.org/10.1007/s40009-014-0303-y)
- Jahanbakhshi M, Sadeghi M, Tohidi M Fotouhi F, Fazel zadeh S.A. 2022. Effect of foliar application of ascorbic acid on antioxidant enzyme activities of mung bean (*Vigna radiate* L.) cultivars under water-deficit Stress. *Journal of Plant Environmental Physiology* 65(2), 126-140. [https://doi: 10.30495/iper.2021.679565](https://doi.org/10.30495/iper.2021.679565)

- Jiang M, Zhang J. 2002. Water stress-induce abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany* 53 (379), 2401-2410. <https://doi.org/10.1093/jxb/erf090>
- Kainama H, Fatmawati S, Santoro M, Papilaya PM, Ersam T. 2020. The relationship of free radical scavenging and total phenolic and flavonoid contents of *Garcinia lasoar* PAM. *Pharmaceutical Chemistry Journal* 53, 1151-1157. <https://doi.org/10.1007/s11094-020-02139-5>
- Kedare SB, Sing R.P. 2011. Genetics and development of DPPH method of antioxidant assay. *Journal of Food Sciences Technology* 48 (4), 412-422. <https://doi.org/10.1007/s13197-011-0251-1>
- Khedher A, Dhibi S, Bouzenna H, Akermi S, El Feki A, Tales PHV, Almeida JRGS, Hfaiedh N. 2024. Antiulcerogenic and antioxidant activities of *Plantago ovata* ethanolic extract in rats. *Brazilian Journal of Biology* 84, e255120. <https://doi.org/10.1590/1519-6984.255120>
- Kleinwächter M, Selmar D. 2015. New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. *Agronomic Sustainable Development* 35, 121-131. <https://doi.org/10.1007/s13593-014-0260-3>
- Kundu D, Dey S, Raychaudhuri SS. 2018. Chromium (VI) – induced stress response in the plant *Plantago ovata* Forsk in vitro. *Genes Environment* 40, 21. <https://doi.org/10.1186/s41021-018-0109-0>
- Ltaeif HB, Sakhraoui A, Gonzalez-Orenga S, Faz A.L, Boscaiu M, Vicente O, Rouz. 2021. Responses to salinity in four *Plantago* species from Tunisia. *Plants* 10, 1392. <https://doi.org/10.3390/plants10071392>
- Mattila PH, Astola J, Kumpulainen J. 2000. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *Journal of Agricultural Food Chemistry* 48, 5834-5841. <http://doi:10.1021/jf000661f>. PMID: 11312761
- Mumivand H, Ebrahimi A, Shayganfar A, Hassaneian Khoshro H. 2021. Screening of tarragon accessions based on physiological and phytochemical responses under water deficit. *Scientific Reports* 11, 17839. <https://doi.org/10.1038/s41598-021-97388-z>
- Munir N, Khilji S.A, Shabir M, Sajid Z.A. 2021. Exogenous application of ascorbic acid enhances the antimicrobial and antioxidant potential of *Ocimum sanctum* L. grown under salt stress. *Journal of Food Quality* 4977410, 8. <https://doi.org/10.1155/202/4977410>
- Noreen S, Sultan M, Akhter M.S, Shah KH, Umara U, Manzoor H. 2021. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (*Hordeum vulgare* L.) grown under salt stress. *Plant Physiology Biochemistry* 158, 244-254. <https://doi.org/10.1016/j.plaphy.2020.11.007>
- Penella C, Calatayud A, Melgar J.C. 2017. Ascorbic acid alleviates water stress in young peach trees and improves their performance after rewatering. *Frontiers in Plant Science* 8, 1627. <https://doi:10.3389/fpls.2017.01627>
- Rahimi A, Sayadi F, Dashti H, Tajabadi A. 2013. Effects of water and nitrogen supply on growth, water-use efficiency and mucilage yield of isabgol (*Plantago ovata* Forsk). *Journal of Soil Science Plant Nutrient* 13, 341-354. <http://dx.doi.org/10.4067/S0718-95162013005000028>
- Rasouli F, Amini T, Skrovankova S, Asadi M, Hassanpouraghdam MB, Ereisli R. 2023. Influence of drought stress and mycorrhizal (*Funneliformis mosseae*) symbiosis on growth parameters, chlorophyll fluorescence, antioxidant activity, and essential oil composition of summer savory (*Satureja hortensis* L.) plants. *Frontiers in Plant Science* 14, 1151467. <https://doi:10.3389/fpls.2023.1151467>
- Rigano D, Sirignano C, Tagliatalata-Scafati O. 2017. The potential of natural products for targeting PPAR α . *Acta Pharmaceutica Sinica B* 7, 427-438. <https://doi:10.1016/j.apsb.2017.05.005>
- Rodriguez ES, Moreno DA, Ferreres F, Rubio-Wilhelmi MDM, Ruiz JM. 2011. Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. *Phytochemistry* 72, 723-729. <https://doi.org/10.1016/j.phytochem.2011.02.011>
- Sahagun AM, Vaquera J, Garcia JJ, Calle AP, Diez MJ, Fernandez N, Loro JF, Portilia HO, Sierra M. 2015. Study of the protective effect on intestinal mucosa of the hydrosoluble fiber *Plantago ovata* husk. *BMC Complementary and Alternative Medicine* 15 (1), 298. <http://dx.doi.org/10.1186/s12906-015-0827-9>
- Selmar D. 2008. Potential of salt and drought stress to increase pharmaceutical significant secondary compounds in plants. *Landbauforsch Völk* 58, 139-144.
- Selmar D, Kleinwächter M. 2013a. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crop Production* 42, 558-566. <https://dx.doi.org/10.1016/j.indcrop.2012.06.020>

- Selmar D, Kleinwächter M. 2013b. Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation. *Plant Cell Physiology* 54(6), 817-826. <http://dx.doi.org/10.1093/pcp/pct054>
- Shahzadi E, Nawaz M, Iqbal N, Ali B, Adnan M, Saleem M.H, Okla MK, Abbas ZKh, Al-Qahtani SM, Al-Harbi NA, Marc R.A. 2023. Salicylic acid and ascorbic acid induced modulations in photosynthetic, mineral uptake, and yield attributes of mung bean (*Vigna radiate* L. Wilczek) under ozone stress. *ACS Omega* 8, 13971-13981. <https://doi.org/10.1021/acsomega.3c00376>
- Sharma S, Ali A, Ali J, Sahni JK, Baboota S. 2013. Rutin: therapeutic potential and recent advances in drug delivery. *Expert Opinion Investigation Drugs* 22(8), 1063-79. [https://doi: 10.1517/13543784.2013.805744](https://doi.org/10.1517/13543784.2013.805744)
- Sharma L, Roy S, Satya P, Alam M, Goswami Dhananjay T. 2024. Exogenous ascorbic acid application ameliorates drought stress through improvement in morpho-physiology, nutrient dynamics, stress metabolite production and antioxidant activities recovering cellulosic fiber production in jute (*Corchorus olitorius* L.). *Industrial Crops and Products* 217, 118808. <https://doi.org/10.1016/j.indcrop.2024.118808>.
- Selmar D, Kleinwächter M. 2013. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops and Products* 42, 558-566. <https://doi.org/10.1016/j.indcrop.2012.06.020>
- Talukder P, Talapatra Sh, Ghoshal N, Raychaudhuria S.S. 2016. Antioxidant activity and HPLC analysis of phenolic compounds during in vitro callus culture of *Plantago ovata* Forsk and effect of exogenous additives on accumulation of phenolic compounds. *Journal of Science Food Agriculture* 96 (1), 232-244. <https://doi.org/10.1002/jsfa.7086>
- Zahra N, Wahid A, Hafeez MB, Alyemeni MN, Shaha T, Ahmad P. 2021. Plant growth promoters mediated quality and yield attributes of milk thistle (*Silbyum marianum* L.) ecotypes under salinity stress. *Scientific Reports* 11, 23200. <https://doi.org/10.1038/541598-021-02435-4>
- Zaman MAK, Azzeme AM, Ramle LK, Normanshah N, Ramli SN, Shaharuddin NA. 2020. Induction, multiplication and evaluation of antioxidant activity of *Polyalthia bullata* callus, a woody medicinal plant. *Plants* 9, 1772. [https://doi: 10.3390/plants9121772](https://doi.org/10.3390/plants9121772)
- Zare S, Mirlohi A, Sabzalian M.R, Saeidi Gh, Kocak MZ, Hano Ch. 2023. Water stress and seed color interacting to impact seed and oil yield, protein, mucilage and secoisolariciresinol diglucoside content in cultivated flax (*Linum usitissimum* L.). *Plants* 12, 1632. <https://doi.org/10.3390/plants12081632>
- Zhang DY, Yao XH, Duan MH, Wei FY, Wu GH, Li L. 2015. Variation of essential oil content and antioxidant activity of *Lonicera* species in different sites of China. *Industrial Crops Production* 77, 772-779. [https://doi: org/10.1016/j.indcrop.2015.09.048](https://doi.org/10.1016/j.indcrop.2015.09.048)