Pak. J. Bot., 59(1): January 2027.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2027-1(5)

SALICYLIC ACID IMPROVES PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF A CRITICALLY ENDANGERED MEDICINAL PLANT, *RHAPONTICOIDES SCHMIDII* WAGENITZ, UNDER DROUGHT STRESS

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Abstract

This study aimed to evaluate the effects of drought stress and salicylic acid (SA) on the growth parameters and secondary metabolites of *Rhaponticoides schmidii* Wagenitz, a critically endangered medicinal species. The treatments consisted of three irrigation regimes (control: 80% field capacity; moderate stress: 60% FC; and severe stress: 40% FC) combined with three SA concentrations (0, 0.05, and 1 mM). The results revealed that both drought and SA treatments, and their interaction, significantly affected physiological, biochemical, phenological, and yield-related traits. Severe drought stress (40% FC) decreased chlorophyll a, chlorophyll b, total chlorophyll content, and soluble protein by 51, 33, 46, and 28%, respectively, and shortened phenological stages by about eight days. Yield components, including the number of cypselae/plant, percentage of healthy seeds/plant, seed weight, and total number of seeds/plant by 44%, 43%, 35% and 32%, respectively, compared with the control. In particular, the treatment with 1 mM SA significantly enhanced the activities of ascorbate peroxidase (APX) and peroxidase (POD), as well as DPPH radical scavenging capacity, by 31%, 21%, and 52%, respectively, in plants under severe drought stress. This treatment also produced the highest levels of flavonoids (Scutellarin, Visinin, Rutin, Quercetin, Kaempferol, Apigenin, and Luteolin) and phenolic compounds, while the lowest values occurred under well-watered (80% FC) conditions without SA. Overall, salicylic acid effectively mitigated the adverse impacts of drought stress, enhanced antioxidant activity, secondary metabolite accumulation, and yield potential in the critically endangered plant, *Rhaponticoides schmidii* Wagenitz.

Key words: Antioxidant enzymes; Abiotic stress mitigation; *Ex situ* conservation; Flavonoid accumulation; Phenolic compounds

Introduction

Herbal plants have been a source of medicinal compounds, and plant-based medicines still play a significant role in human health and wellbeing (Ashraf, 2020). But medicinal plant resources are under increasing threat from climate change, drought, and other anthropogenic pressures during the Anthropocene (Antonelli *et al*., 2023). Moreover, over-exploitation and habitat destruction have further led to the rapid extinction of many wild medicinal plants (Mykhailenko *et al*., 2025). To overcome these challenges, an increasing proportion of commercial herbal resources are now derived from cultivated plants, and there are concerns about the quality and variability of metabolites of interest (Sun *et al*., 2019). On the other hand, knowledge of the regulation of biosynthesis and accumulation of valuable metabolites by environmental factors is critical for enhancing both sustainability and phytochemical quality (Coelho *et al*., 2020). Drought is a major environmental factor limiting plant growth, development, and productivity (Saeed *et al*., 2024). The response to drought is highly variable among plant species, genotypes, and different stages of plant development, as well as the severity and duration of drought (Saeed *et al*., 2024). Prolonged drought can lead to changes in organelle membranes and damage to macromolecules, thereby affecting plant growth (Miao *et al*., 2015). Salicylic acid (SA) is one of the key plant regulators in response to stress. SA is a signal molecule and is involved in the regulation of antioxidant balance under stress conditions (Saleem *et al*., 2025). SA has been shown to increase tolerance by increasing antioxidant capacity and preventing excessive production of reactive oxygen species (ROS) (Sadia *et al*., 2023). But its application is influenced by many factors, such as the mode of application, time of application, concentration, and plant species (Janda *et al*., 2020). Therefore, the use of exogenous SA has gained much attention as a way to mitigate the adverse effects of water deficits and sustain physiological balance in plants under stress (Simaei *et al*., 2012). *Rhaponticoides schmidii* Wagenitz is a critically endangered perennial medicinal plant belonging to the Asteraceae family, which is endemic to Khorasan Razavi Province in northeastern Iran (Negaresh *et al*., 2015). Earlier research has focused on its chemical constituents, antioxidant and anti-diabetic effects, and as a natural dye (Benli *et al*., 2022). Furthermore, another member of the *Rhaponticoides* genus, *Rhaponticoides wagenitziana,* has been reported for its phytochemical composition, cultivation, and decorative properties (Yücel *et al*., 2022). But the interaction between salicylic acid and water shortage in *Rhaponticoides schmidii* Wagenitz under controlled conditions is poorly understood and has received little attention in previous studies. Thus, assessing the effects of SA under limited water supply is crucial to developing strategies to mitigate the adverse effects of drought stress in this species.

Received: 25-03-2026 | Revised: 18-05-2026 | Accepted: 25-05-2026 | Online: 11-08-2026

Materials and Methods

The seeds of *Rhaponticoides schmidii* were collected from a wild mature plant community, grown in Bar village, located in Neyshabur city, Khorasan Razavi province of Northeast Iran. The plantlets obtained from the germinated seeds were removed from their primary roots and transferred to vials containing 35 ml of MS medium supplemented with 3% sucrose and solidified with 0.8% agar. To achieve further multiplication, the developed plantlets were transferred to fresh medium every 3–4 weeks for a total culture period of 8 weeks. *In vitro* elongated shoots were removed from the culture medium and transferred to vials containing 35 ml of half-strength MS medium (½ MS) supplemented with 3% sucrose and solidified with 0.8% agar, without phytohormones, for 2–3 weeks. After this period, the *In vitro* shoots were transferred to vials containing 35 ml of the same medium supplemented with 2 mg L^{-1} of the phytohormone 1-naphthaleneacetic acid (NAA) and subcultured onto fresh medium every 3–4 weeks. To acclimatize the well-developed rooted plantlets, they were gently removed from the rooting medium, washed to remove adhering agar, and transferred to 250 ml plastic containers filled with a mixture of peat, perlite, and vermiculite in a 1:1:1 (v/v) ratio. The containers were covered with transparent plastic bags to maintain high humidity and placed under controlled conditions at 25°C with a 16/8 h photoperiod. The plantlets were irrigated with a half-strength MS solution, and the plastic bags were gradually removed weekly until completely removed after 4–6 weeks (Fig. 1).

Two weeks after *Ex vitro* acclimatization of plantlets, they were transferred to 10 L plastic pots containing 60% field soil + 40% Sand. Then, two weeks after transferring (full establishment), irrigation treatments were applied at three levels based on field capacity (FC): 80% (Control-IR1), 60% (mild drought-IR2), and 40% (severe drought-IR3) FC. Plants were irrigated with Hoagland nutrient solution throughout the growing period to provide essential nutrients for normal growth (Hoagland and Arnon, 1950). The experiment was arranged in a completely randomized design with eight replications. Briefly, drought levels were established by maintaining soil moisture at defined percentages of field capacity through periodic weighing and replenishment of water to the target levels. In addition, three concentration levels of SA, including no SA (SA1), 0.5 mM (SA2), and one mM (SA3), were prepared based on a method reported by Nazar *et al*., (2015). SA was dissolved in absolute ethanol and then was gradually added dropwise to water (ethanol/water: 1/1000 v/v) to dissolve completely. Subsequently, the SA treatments were applied in three foliar applications: first during plant establishment, second during the middle phase of vegetative growth, and finally at the 50% flowering stage.

Fig. 1. *In vitro* propagation of *R. schmidii*: (A) A mature plant, (B) Intact pappus-removed achenes, (C) A germinated seedling on hormone-free MS media, (D) A fully developed seedling on hormone-free MS media, (E) Multiple shoots regeneration from the epicotyl and cotyledonary petiole explant on MS media supplemented with BAP, (F) Root induction of *invitro* regenerated shoots on MS media supplemented with NAA, (G) well-developed rooted *In vitro* propagated shoots on MS media supplemented with NAA, (H) *Ex vitro* acclimatized plantlet, and (I) A 4-week-old *Ex vitro* acclimatized plantlet.

Measurement of physiological and biochemical properties: After full establishment, plants were subjected to drought and salicylic acid (SA) treatments for 30 days. After that, photosynthetic pigments were assessed at the 50% blooming stage. To determine the amounts of carotenoid, chlorophyll a, and chlorophyll b, leaf samples were taken. Pigment contents were calculated using Eqs. 1-3.

Chl-a = (13/36×A664)–(5/19×A648) Formula (1)
Chl-b = (27/43×A648)–(8/12×A664) Formula (2)
Car=((1000×A470)–(2/13×Chl a)–(97/64×Chl b))/209 Formula (3)

Potassium phosphate buffer method was used to measure peroxidase (POD) by taking absorbance at 470 nm (Srinivas *et al*., 1999). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured using the Yamaguchi *et al*., (1995) method, which is based on tracking the decrease in absorbance at 290 nm during ascorbate oxidation. The Bradford method with spectrophotometric measurement was used to quantify the amount of soluble protein (Bradford, 1976).

According to Owen *et al*., (2003), phenolic acids in the leaves were isolated and separated. Methanol was used to extract the samples three times, and the extracts were vacuum-evaporated at 35°C. After suspending the residue in 50 milliliters of acetonitrile, the lipid components were extracted three times using 20 milliliters of hexane. After drying the acetonitrile phase over anhydrous magnesium sulfate, the solvent was once again vacuum-extracted at 35°C. Using an HPLC (Agilent 1260 Infinity, Agilent Technologies, USA), the resultant dry residue was diluted in 5 mL of methanol and examined for phenolic acids. A C18 column (Perfectsil Target ODS-3, 5 µm, 250 × 4.6 mm; MZ Analysentechnik, Mainz, Germany) was used for the separation process.

During the growing season, phenological characteristics such as the number of days to flower initiation, 50% flowering, full flowering, and 50% cypsela opening were noted. The number of cypselae per plant, the percentage of healthy seeds per cypsela, the hundred-seed weight, and the total seed weight per plant were all measured at the conclusion of the growth cycle. SAS software (version 9.3) was used to analyze the data, and Tukey's honestly significant difference (HSD) test was used to compare treatment means at the 5% probability level.

Results

The results revealed a clear decreasing trend in chlorophyll pigments with increasing drought intensity, whereas SA application progressively mitigated these declines (Table 1). IR1 + SA3 treatment showed the highest chlorophyll a (1.66 mg/g fw), b (0.63 mg/g fw), and total chlorophyll content (2.29 mg/g fw), representing an increase of 181%, 91%, and 149%, respectively, compared with the severely stressed IR3 + SA1 treatment (Table 1). Carotenoid levels generally decreased under drought stress, and the highest carotenoid content was achieved in the IR3 + SA3 treatment (0.57 mg/g fw), showing an increase of 84% compared to the IR1 + SA1 treatment (0.31 mg/g fw), while the treatment IR1 + SA1 had the lowest carotenoid levels (Table 1).

Effect of Irrigation and SA levels on Biochemical Parameters: The activities of ascorbate peroxidase (APX), peroxidase (POD), and DPPH radical scavenging capacity varied significantly among treatments (Figs. 2-a, b, and Fig. 2-d). In general, these three parameters increased progressively with rising SA concentration and drought stress intensity, reaching their maximum levels under the IR3 + SA3 treatment and minimum values under the IR1 + SA1 treatment (p<0.05). APX, POD, and DPPH radical scavenging capacity increased by 96%, 43%, and 152%, respectively, under the most stressed and SA-treated condition (IR3 + SA3) compared to the IR1 + SA1 treatment (Fig. 2-a, b, and Fig. 2-d).

Soluble protein content declined as drought severity increased, but was obviously enhanced with SA application (Fig. 2-c). The treatment IR1 + SA3 showed an increase of 84% compared with the lowest value recorded in the IR3 + SA1 treatment (Fig. 2-c). The concentration of total flavonoid and flavonoid compounds (Scutellarin, Visinin, Rutin, Quercetin, Kaempferol, Apigenin, and Luteolin) as well as their interaction was significantly affected by both drought stress and SA (Fig. 3). Drought stress significantly reduced flavonoid accumulation, while SA application mitigated these reductions across all stress levels. The highest total flavonoid content (203.25 mg.g^{-1} fw) was recorded in the treatment IR3 + SA3, representing around 59% increase compared to the lowest value (127.70 mg.g^{-1} fw) observed in the treatment IR1 + SA1. Similarly, the concentrations of Scutellarin, Visinin, Rutin, Quercetin, Kaempferol, Apigenin, and Luteolin increased progressively with rising SA concentration, with the maximum level detected in the treatment IR3 + SA3 (Fig. 3).

Table 1. Combined effect of drought and SA on photosynthetic pigments of *R. schmidii*. (mean ± SE).

Irrigation	Salicylic acid	Chlorophyll-a (mg/g fw)	Chlorophyll-b (mg/g fw)	Total chlorophyll (mg/g fw)	Carotenoids (mg/g fw)
IR1*	SA1	1.21 ^{e} ± 0.01	0.49 ^{e} ± 0.02	1.70 ^{e} ± 0.01	0.31 ^{h} ± 0.02
	SA2	1.60 ^{b} ± 0.01	0.60 ^{b} ± 0.01	2.20 ^{b} ± 0.07	0.45 ^{e} ± 0.01
	SA3	1.66 ^{a} ± 0.05	0.63 ^{a} ± 0.01	2.29 ^{a} ± 0.03	0.48 ^{d} ± 0.01
IR2	SA1	0.99 ^{f} ± 0.03	0.40 ^{g} ± 0.03	1.39 ^{f} ± 0.02	0.35 ^{g} ± 0.01
	SA2	1.40 ^{d} ± 0.02	0.54 ^{d} ± 0.01	1.94 ^{d} ± 0.01	0.49 ^{c} ± 0.04
	SA3	1.50 ^{c} ± 0.03	0.59 ^{c} ± 0.01	2.09 ^{c} ± 0.01	0.52 ^{b} ± 0.03
IR3	SA1	0.59 ^{h} ± 0.01	0.33 ^{h} ± 0.02	0.92 ^{h} ± 0.02	0.38 ^{f} ± 0.01
	SA2	0.94 ^{g} ± 0.02	0.43 ^{f} ± 0.03	1.37 ^{f} ± 0.01	0.52 ^{b} ± 0.03
	SA3	1.21 ^{e} ± 0.03	0.49 ^{e} ± 0.01	1.70 ^{e} ± 0.01	0.57 ^{a} ± 0.03

*Means in each column followed by the same letters are not significantly different at p<0.05 based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid

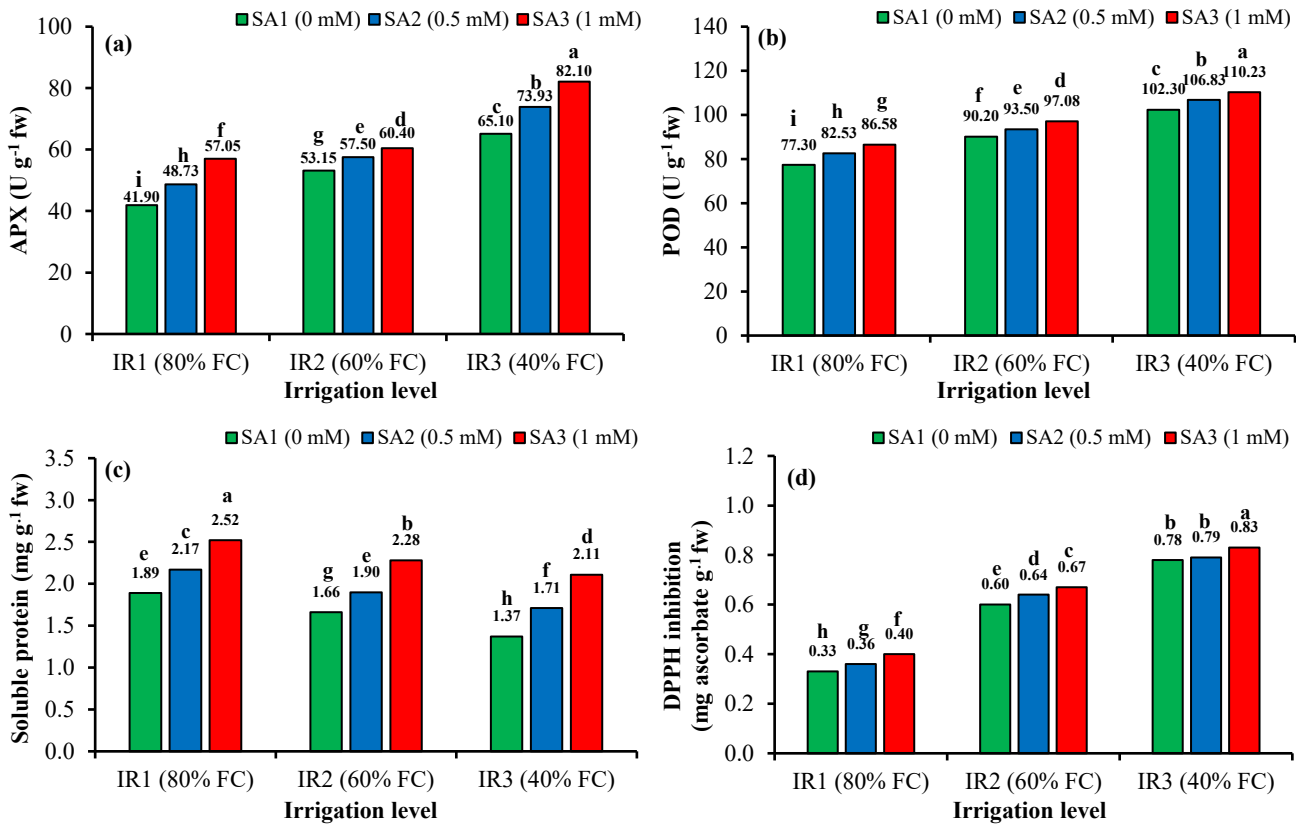

Fig. 2. Combined effect of drought stress and salicylic acid on (a) ascorbate peroxidase (APX), (b) peroxidase (POD), (c) soluble protein, and (d) DPPH inhibition of *R. schmidii*. Means followed by the same letters are not significantly different at $p<0.05$ based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid.

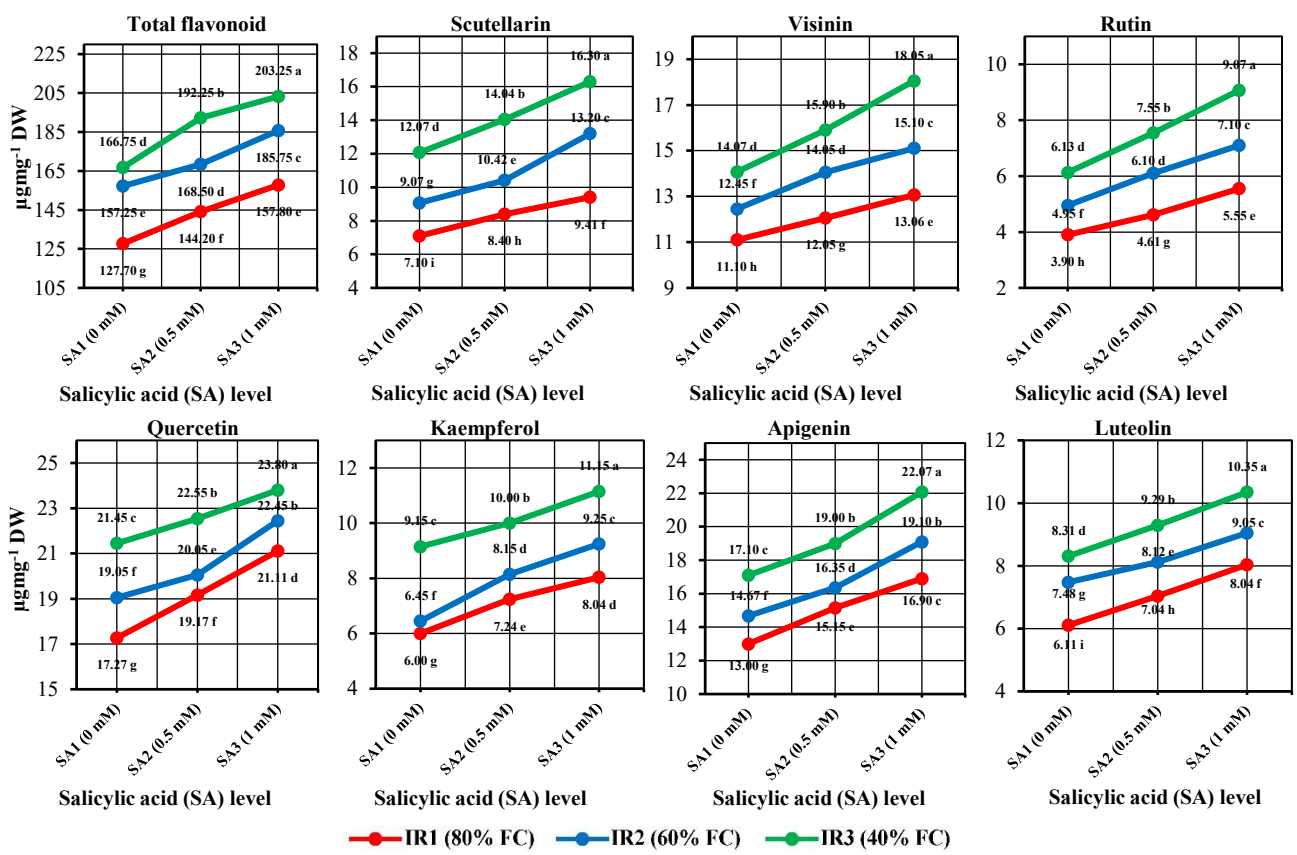

Fig. 3. Combined effect of drought stress and salicylic acid on flavonoid content of *R. schmidii*. Means in each column followed by the same letters are not significantly different at $p<0.05$ based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid.

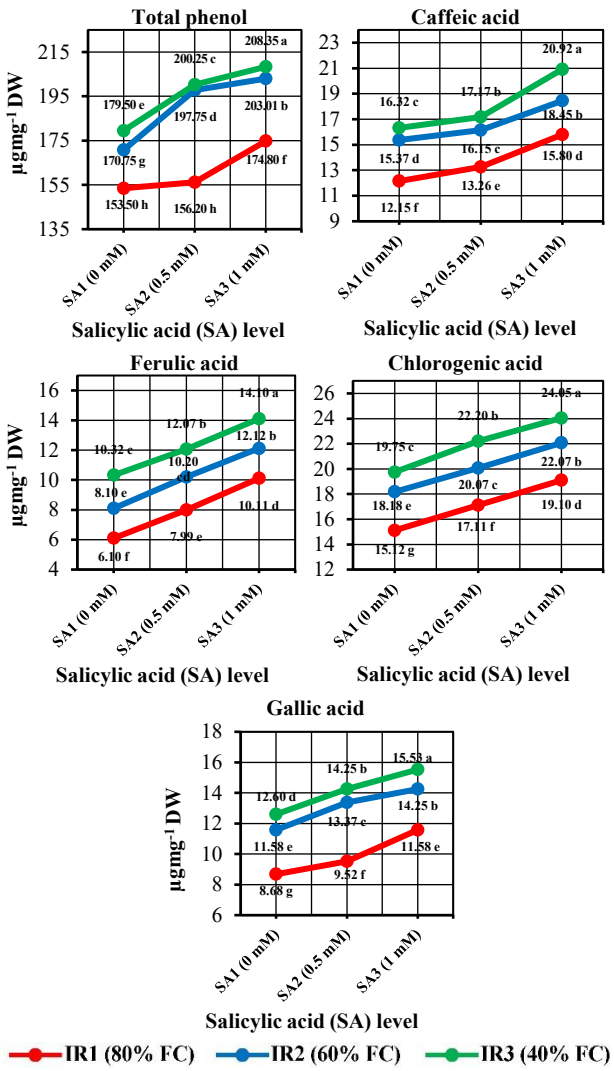

Fig. 4. Combined effect of drought stress and salicylic acid on phenolic content of *R. schmidii*. Means in each column followed by the same letters are not significantly different at $p<0.05$ based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid.

Phenolic compounds followed a comparable pattern (Fig. 4). Total phenolic content, along with caffeic acid, ferulic acid, chlorogenic acid, and gallic acid, increased substantially with SA application under drought conditions. The treatment IR3 + SA3 produced the highest total phenolic content (205.30 mg.g^{-1} fw), indicating around 34% improvement compared to the treatment IR1 + SA1. Among phenolic compounds, chlorogenic acid showed the greatest sensitivity to SA treatment, increasing from 15.12 mg.g^{-1} fw in treatment IR1 + SA1 to 24.05 mg.g^{-1} fw in the treatment IR3 + SA3 (Fig. 4).

The phenological traits of *R. schmidii* were significantly influenced by the interaction between drought stress and SA concentration (Table 2 and Fig. 5-b). The treatment IR1 +SA3 showed the longest developmental periods, with 80.5, 90.5, 98.5, and 114.5 days to flowering initiation, 50% flowering, full flowering, and 50% cypsela opening, respectively. In contrast, the shortest durations (70.75, 79.5, 90.0, and 105.0 days, respectively) were recorded in the IR3 + SA1 treatment (Table 2).

Increasing drought severity led to a clear acceleration of phenological stages (Table 2). Severe drought (IR3 + SA1) reduced developmental periods by approximately 8% compared to the IR1 + SA1 treatment. However, the SA application obviously counteracted these reductions (Fig. 5-b). Under severe drought conditions, applying 1 mM of SA increased the duration of flowering initiation, 50% flowering, full flowering, and 50% cypsela opening by 7%, 9%, 4%, and 6% compared to IR3 + SA1 (Table 2). The radar analysis further confirmed that SA-treated plants, especially under moderate and severe drought, exhibited more stable and extended phenological patterns compared to the untreated treatment IR1 + SA1 (Fig. 5-b).

The yield components of *R. schmidii* were significantly affected by the interaction between drought stress and SA concentration (Table 3 and Fig. 5-c). The highest number of cypselae/plant (13.75), percentage of healthy seeds/plant (40%) and hundred-seed weight (5.50 g) were recorded in IR1 + SA3 treatment, whereas the lowest values (5.00, 21% and 2.53 g, respectively) were observed in severe drought conditions and 0 mM of SA, which represented of decreases approximately 64%, 48%, and 54%, respectively, due to severe drought stress (Table 3). The highest seed weight per plant (1.40 g) was achieved in IR2 + SA3 treatment, showing an increase of about 82% compared to the lowest value (0.77 g) recorded in IR3 + SA1. Across irrigation levels, the application of 1 mM of SA increased the number of cypselae/plant by 53–100%, the percentage of healthy seeds by 8–29%, and the seed weight/plant by 19–44% compared to untreated SA (Table 3).

Table 2. Combined effect of drought and SA on phenological traits of *R. schmidii* (mean ± SE).

Irrigation level	Salicylic acid	Days to flowering initiation	Days to 50% flowering	Days to full flowering	Days to opening 50% of cypselae
IR1*	SA1	76.75 ^{d} ± 2.5	86.25 ^{d} ± 0.5	97.50 ^{c} ± 0.2	114.50 ^{a} ± 0.1
	SA2	79.75 ^{b} ± 1.6	88.25 ^{c} ± 0.2	98.00 ^{b} ± 0.2	112.50 ^{b} ± 0.1
	SA3	80.50 ^{a} ± 0.4	90.50 ^{a} ± 0.1	98.50 ^{a} ± 0.1	114.50 ^{a} ± 0.2
IR2	SA1	73.00 ^{f} ± 1.1	85.00 ^{e} ± 0.1	92.50 ^{f} ± 0.1	112.00 ^{bc} ± 0.2
	SA2	75.50 ^{e} ± 1.1	88.00 ^{c} ± 0.1	95.50 ^{d} ± 0.8	109.00 ^{e} ± 0.1
	SA3	77.75 ^{c} ± 0.1	89.00 ^{b} ± 0.8	95.75 ^{d} ± 0.1	111.00 ^{d} ± 0.2
IR3	SA1	70.75 ^{h} ± 0.9	79.50 ^{g} ± 0.1	90.00 ^{h} ± 0.4	105.00 ^{g} ± 0.1
	SA2	71.25 ^{g} ± 0.5	83.00 ^{f} ± 0.1	91.25 ^{g} ± 0.2	107.00 ^{f} ± 0.1
	SA3	75.75 ^{e} ± 0.1	86.50 ^{d} ± 0.4	93.50 ^{e} ± 0.5	111.50 ^{cd} ± 0.1

*Means in each column followed by the same letters are not significantly different at $p<0.05$ based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid

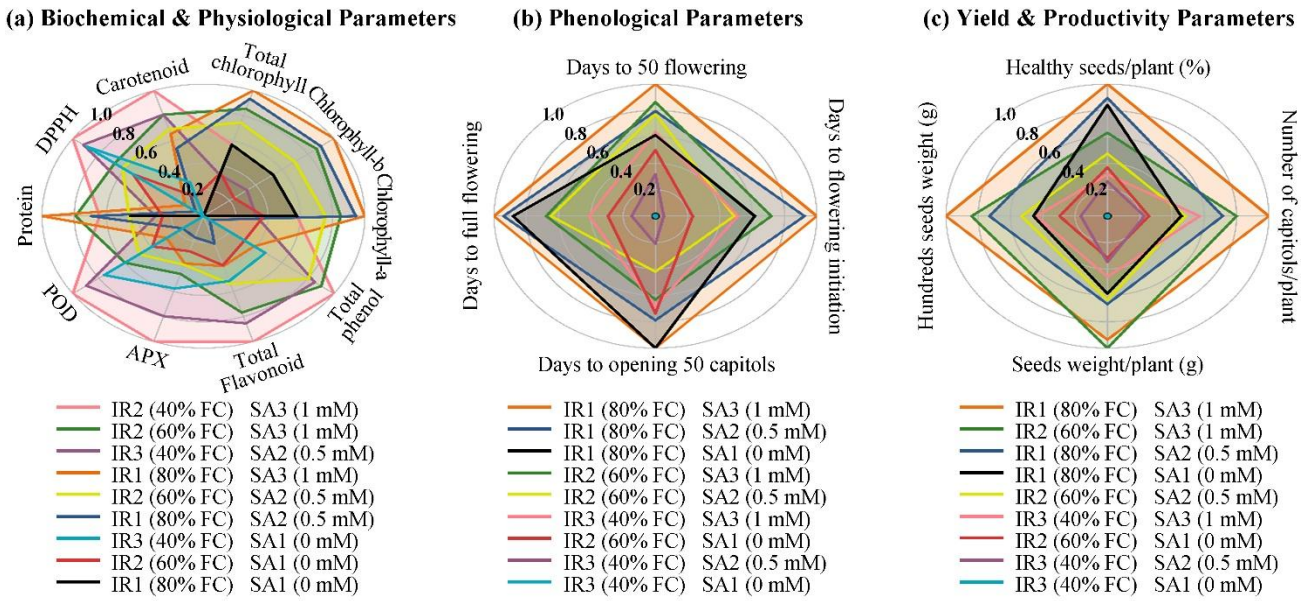

Fig. 5. Combined effects of drought stress (IR; field capacity) and salicylic acid on (a) biochemical & physiological traits, (b) phenological traits, and (c) yield and productivity traits of *R. schmidii*. Values are min–max normalized (0–1) within each panel; polygons show treatment means for the nine IR × SA combinations, and the same color denotes the same treatment across panels. IR1: 80% field capacity; IR2: 60% field capacity; IR3: 40% field capacity. SA1: 0 mM salicylic acid; SA2: 0.5 mM salicylic acid; SA3: 1 mM salicylic acid.

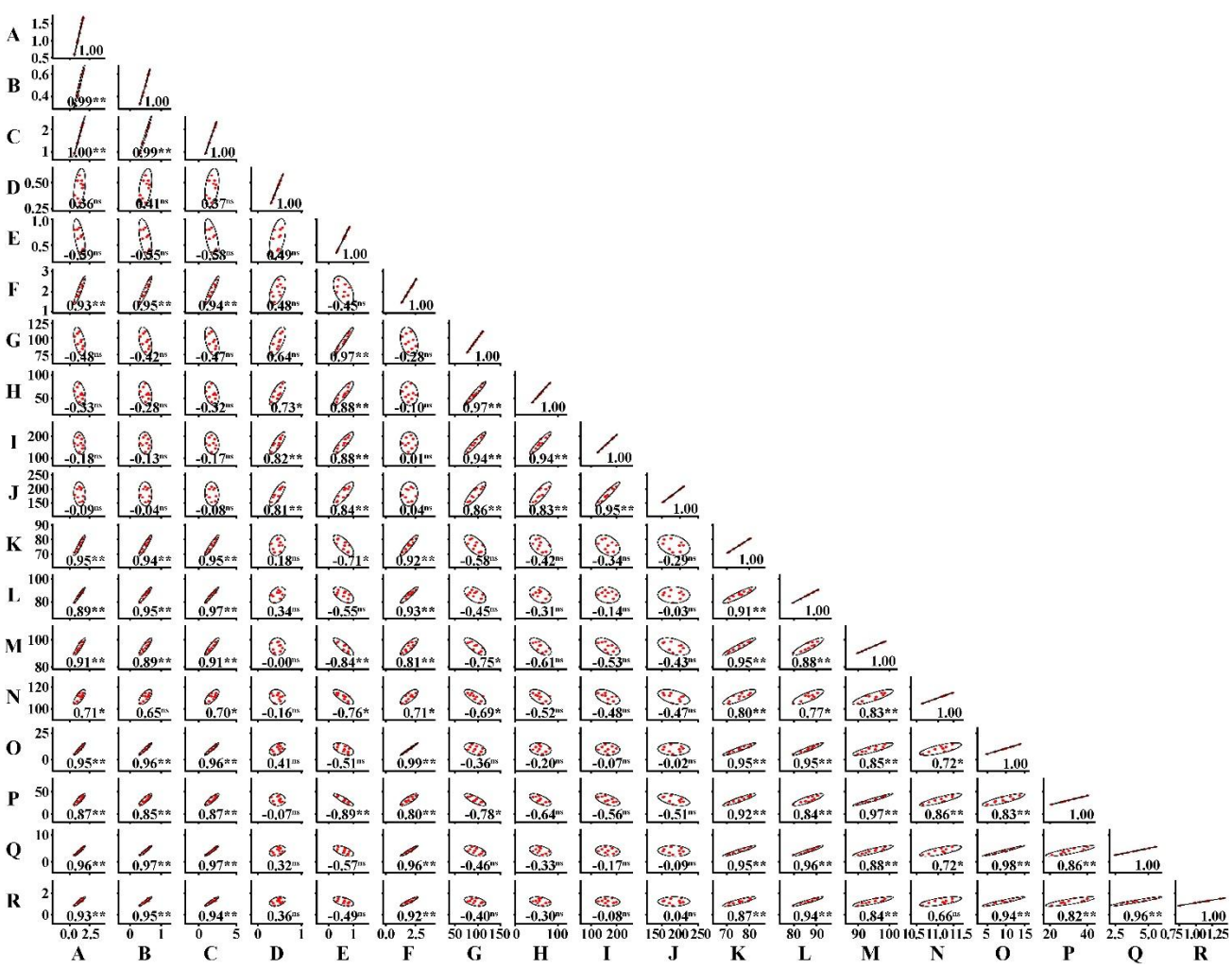

Fig. 6 Pearson's correlation coefficients among physiological, biochemical, phenological, and yield-related parameters of *Rhaponticoides schmidii* Wagenitz at different salicylic acid concentrations under drought stress. A: Chl-a, B: Chl-b, C: Total Chl content, D: Carotenoids, E: DPPH, F: Soluble protein, G: POD, H: APX, I: Total flavonoid, J: Total phenol, K: days to flowering initiation, L: days to 50% flowering, M: days to full flowering, N: days to 50% cypsela opening, O: number of cypselae per plant, P: percentage of healthy seeds per plant, Q: 100-seed weight, and R: total seed weight per plant.

Table 3. Combined effect of drought and SA on yield components of *R. schmidii* (mean±SE).

Irrigation level	Salicylic acid	Number of cypselae /plant	Healthy seeds/ plant (%)	Hundred seeds weight (g)	Seeds weight/ plant (g)
IR1*	SA1	9.00 ^{e} ± 0.1	37 ^{c} ± 0.5	3.89 ^{e} ± 0.1	1.14 ^{e} ± 0.10
	SA2	11.25 ^{c} ± 0.1	38 ^{b} ± 0.2	4.70 ^{c} ± 0.2	1.19 ^{c} ± 0.03
	SA3	13.75 ^{a} ± 0.2	40 ^{a} ± 0.2	5.50 ^{a} ± 0.1	1.36 ^{b} ± 0.01
IR2	SA1	7.25 ^{f} ± 0.1	28 ^{f} ± 0.11	3.41 ^{g} ± 0.1	0.97 ^{g} ± 0.10
	SA2	9.25 ^{e} ± 0.1	30 ^{e} ± 0.1	4.11 ^{d} ± 0.1	1.17 ^{d} ± 0.08
	SA3	12.00 ^{b} ± 0.1	33 ^{d} ± 0.9	5.03 ^{b} ± 0.2	1.40 ^{a} ± 0.02
IR3	SA1	5.00 ^{g} ± 0.1	21 ^{i} ± 0.1	2.53 ^{i} ± 0.2	0.77 ^{i} ± 0.03
	SA2	7.00 ^{f} ± 0.1	26 ^{h} ± 0.1	3.02 ± 0.1 ^{h}	0.99 ^{h} ± 0.02
	SA3	10.00 ^{d} ± 0.1	27 ^{g} ± 0.1	3.84 ^{f} ± 0.1	1.06 ^{f} ± 0.01

*Means in each column followed by the same letters are not significantly different at $p<0.05$ based on Tukey's range test (HSD). IR1: 80% field capacity, IR2: 60% field capacity, IR3: 40% field capacity, SA1: 0 mM Salicylic acid, SA2: 0.5 mM Salicylic acid, and SA3: 1 mM Salicylic acid

The radar plot further illustrated that IR2+SA3 and IR1+SA3 treatments formed the broadest polygons, indicating superior overall productivity and stronger drought resilience compared to untreated or severely stressed plants (Fig. 5-c).

Discussion

Exogenous SA application enhanced chlorophyll a, chlorophyll b, and total carotenoid contents in *R. schmidii* under drought conditions (Table 1). This finding indicated that SA might have supported the photosynthetic ability under drought stress. The positive correlations between chlorophyll and yield related traits, such as number of cypselae per plant, seed weight and percentage of healthy seeds per plant, also suggested that the retention of photosynthetic pigments was associated with increased yield (Fig. 6). On the other hand, the negative correlation between pigments and antioxidants or phenolic content indicated that plants with improved pigment stability were less affected by oxidative stress, and so had less secondary compound accumulation. Similar effects of SA application upon drought stress were reported in Satureja hortensis (Ghojavand *et al*., 2023) and in two Thymus species (Mohammadi *et al*., 2019). While this was not directly studied in the current study, previous studies indicated that SA can help maintain pigment content by maintaining chloroplast activity and antioxidant defence under stress (Sedaghathoor *et al*., 2017; Decsi *et al*., 2025; Zhao *et al*., 2025).

In the present study, the combination of 40% field capacity and 1 mM SA produced the highest APX, POD, and DPPH radical scavenging activity in *R. schmidii* (Fig. 2). These parameters were positively correlated with total phenolic and flavonoid contents, showing that enzymatic and nonenzymatic antioxidant responses increased together under drought stress (Fig. 6). In this study, the increase in APX, POD, and DPPH activity under SA treatment is consistent with the protective role of SA in improving stress tolerance by modulating antioxidant enzyme activity and maintaining cellular homeostasis. Earlier work has shown that SA can enhance ROS scavenging capacity by stimulating hydrogen peroxide detoxifying enzymes and thereby reducing oxidative damage under drought stress (Huang *et al*., 2022). Similar responses have been reported in *Scophularia striata*, where foliar application of SA increased APX activity under drought stress (Sobhani *et al*., 2023). In *Silybum marianum*, SA application under drought conditions also increased proline, soluble carbohydrates, sucrose, total phenolic compounds, and DPPH radical scavenging activity (Estaji and Niknam, 2020). Recent molecular evidence further suggested that SA may regulate antioxidant defense through stress-responsive pathways involving transcription factors such as WRKY and NAC (Salinas *et al*., 2025).

The highest soluble protein content in *R. schmidii* was recorded under 80% field capacity combined with 1 mM SA. Under drought stress, protein synthesis is often reduced, while protein degradation may increase because of enhanced protease activity. In addition, the production of superoxide and hydroxyl radicals under stress conditions causes oxidative damage to amino acids, thereby affecting protein structure and function (Sharma & Dubey, 2005). Salicylic acid plays a critical role in protecting cell membranes and organelles, including the protein synthesis machinery, by reducing oxidative stress and increasing proline accumulation. It also prevents protein degradation and promotes protein synthesis by enhancing nitrate reductase activity and preventing its inactivation (Huang *et al.*, 2022). Correlation analysis revealed strong positive relationships between soluble protein content, chlorophyll concentration, and yield components, indicating that protein stability supports both photosynthetic capacity and reproductive performance (Fig. 6). Similar findings were reported in *Plectranthus tenuiflorus* grown under water stress, where SA application alleviated oxidative damage caused by severe drought (40% FC) and increased soluble protein content (Jalal *et al.*, 2012). Moreover, recent studies have shown that exogenous SA application under drought stress can upregulate genes involved in nitrogen assimilation, such as nitrate and nitrite reductases, as well as protein turnover pathways, including components of the ubiquitin–proteasome system, thereby sustaining protein homeostasis and reducing proteolytic degradation (Zhang *et al.*, 2025).

Based on the present analysis, severe water deficit (40% FC) combined with 1 mM SA resulted in the highest concentrations of flavonoid and phenolic compounds in *R. schmidii* leaves, whereas the lowest levels were observed under 80% FC without SA application (Figs. 3 and 4). Salicylic acid treatments enhanced the accumulation of these metabolites under both well-watered and drought conditions, confirming its regulatory role in secondary metabolite biosynthesis. Correlation analysis revealed strong positive associations between total phenolic and flavonoid contents

and DPPH radical-scavenging activity (r>0.90), as well as antioxidant enzyme activities (APX and POD), indicating that these metabolites play key roles in mitigating oxidative stress by scavenging free radicals (Fig. 6).

The observed increases suggest that SA promoted the accumulation of phenolic and flavonoid compounds under drought stress. This response may be related to stimulation of the phenylpropanoid pathway, as reported previously for salicylic acid-treated plants (Abbaspour & Ehsanpour, 2016). Similar effects were observed in Panax ginseng, where SA application increased both phenolic and flavonoid contents (Ali *et al*., 2007). Because these compounds contribute to antioxidant protection by scavenging free radicals and limiting oxidative damage, their accumulation may have helped maintain cellular redox balance in *R. schmidii* under stress (Waśkiewicz *et al*., 2013; Wu *et al*., 2023). These findings are also consistent with earlier reports describing SA as an inducer of defense-related secondary metabolism in medicinal plants exposed to drought (Nazar *et al*., 2015).

Drought stress accelerated phenological development and reduced yield-related parameters in *R. schmidii* (Tables 2 and 3). In contrast, the SA application, especially at 1 mM, helped moderate these effects and improved both phenological and yield responses under water-limited conditions. The positive correlations between flowering duration and yield parameters showed that plants maintaining better physiological performance under SA treatment were also able to sustain stronger reproductive performance (Fig. 6). Similar effects of SA on flowering and reproductive traits under drought have been reported in other studies (Ghasemi Pirbalouti *et al*., 2014; Luo *et al*., 2022). Previous research showed that drought stress during reproductive stages can reduce 100-seed weight and total yield through negative effects on carbon metabolism and photosynthetic activity (Song *et al*., 2022). In addition, studies on medicinal plants in the Asteraceae family showed that elicitors such as SA can influence stress responses and secondary metabolite production under abiotic stress (Nazar *et al*., 2015).

This study showed that salicylic acid influenced physiological, biochemical, phenological, and yield-related parameters, as well as secondary metabolite accumulation, in *R. schmidii* under drought stress. Overall, SA application helped reduce the negative effects of water deficit, mainly through improved antioxidant responses and greater accumulation of phenolic and flavonoid compounds. It also mitigated the effects of drought on plant development and overall performance, particularly at 1 mM. Given the endangered status of *R. schmidii*, these findings showed that SA could be useful in improving stress tolerance and supporting *ex situ* conservation of this species. Future studies should investigate the combined effects of SA with other elicitors and environmental factors, while also optimizing propagation protocols for conservation and sustainable utilization of this species.

Acknowledgment

The authors sincerely thank the Department of Agrotechnology, Ferdowsi University of Mashhad, Iran, for supporting this research.

Author's Contribution: Hessamoddin Solouki: Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing original draft. Mohammad Kafi: Conceptualization, Resources, funding acquisition, Writing, review, editing and Supervision. Ahmad Nezami: Conceptualization, Resources, Funding acquisition, Writing, review, editing and Supervision. Jafar Nabati: Investigation, Methodology, Writing, review, editing and scientific advice.

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

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