Effects of foliar application of salicylic acid on pigments, photosynthesis, ion leakage, compatible osmolytes, and antioxidant enzymes of rapeseed (*Brassica napus* L.) under chilling stress

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Abstract

In the present study, foliar application of salicylic acid (SA) was investigated in terms of its effects on a number of physiologic characteristics of rapeseed, Danob cultivar under chilling stress during the crop year 2018-2019 in the farm of Boroujerd Agriculture Research Site using a split plot design with 4 repetitions. Treatments included sowing date at two levels of conventional sowing date (late September) and late sowing (15-day delay, which represents chilling stress condition) as the main plot and 5 levels of sprinkling SA including no sprinkling (control) and treatments with 0, 100, 200, 300, and 400 µM SA as the subplot. Findings revealed that conventional sowing date along with applying 100 µM SA resulted in the maximum chlorophyll (a, b, and total), anthocyanins, and proline contents while late sowing date with applying 100 µM SA resulted in the maximum flavonoids and soluble sugar contents. The highest levels of catalase enzyme activities were recorded for the plants treated with 100 and 400 µM SA, but delayed sowing date along with 200 µM SA led to the highest activity of superoxide dismutase. Non-application of SA (µM) in the rapeseeds sown during the conventional sowing date showed the highest percentage of ion leakage. Applying low concentration of SA to the rapeseed plants sown at the conventional sowing date seems to improve their chlorophyll (a, b, and total) and anthocyanin contents. Increasing the concentration of SA in delayed sowing could reduce the adverse effects of chilling stress in the study. In the case of flavonoids and soluble sugars, applying 100 µM SA in the delayed sowing group compensated for the negative effects of low temperature compared with the control, improving the plant's resistance to this stress. In sum, the most favorable performance in most characteristics of the rapeseeds was obtained with conventional sowing date along with applying 100 µM SA.

Keywords: chilling stress, proline, rapeseed, soluble sugars, sowing date

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Introduction

Rapeseed (*Brassica napus L.*) is the second main oil seed crop in the world composing 12% of the vegetable oil produced worldwide. It not only provides for the oil required for human food in cooking, but also is used in feed as a rich source of protein for farm animals and is a renewable source

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for biodiesel as well as industrial applications (Lei et al., 2019).

Plants face various biotic and abiotic stresses. Among abiotic stresses, chilling stress changes various morphological, cytological, and other biochemical processes in plants (Saleem et al., 2021). In fact, low temperature is an influential environmental factor affecting agricultural plants’ geographical distribution and performance (Zhu et al., 2021). Chilling stress induces physiological and metabolic changes in reactive oxygen species (ROS), malondialdehyde (MAD), sucrose, lipid peroxidation, proline and other metabolites (Lei et al., 2019). Plants’ tolerance when they are faced with stress is a function of the unsaturated fatty acid contents of the lipid membrane and its degree of unsaturation. Low temperature can alter permeability and fluidity of the cell membrane, disturbing its stability, and reducing its unsaturated fatty acid contents. These adverse effects impair the plant’s natural growth. Moreover, chilling stress disturbs the performance of photosynthesis II reactions, speeds up the formation of ROS, and disturbs the accumulation of photosynthesis pigments (Li and Wang, 2021).

In order to grow under this condition, plants adopt special strategies, among which employing plant growth regulators plays an important role in mitigating the effects of stress (Saleem et al., 2021). Growth regulators have a crucial role in monitoring various growth processes and signaling cascades in the plants under stress conditions. Salicylic acid is an important growth regulator in plants with the ability to mitigate biotic and abiotic stresses. It is a main signaling molecule in increasing the plant’s resistance to abiotic stresses (Kohli et al., 2018). Phytohormones such as salicylic acid (SA), which are known for their role in plants’ growth, development, and responses to stress, are natural nontoxic substances produced either by plants or synthetically and can actively modify the plants’ physiological processes (Song et al., 2022). Eisvand et al. (2015) reported that priming maize seeds with 100 ppm SA improved germination and increased the activity of catalase and peroxidase enzymes in leaves. In addition, application of SA can help the plant to tolerate biotic and abiotic stresses through regulation of various physiological, cytological, and biochemical properties. It has shown the potential to reduce and modify various changes in plants due to stress. Endogenous and exogenous salicylic acids play a crucial role in mitigating the changes due to the cold temperature through activating various signaling pathways such as dependent or independent signaling pathways, mitogen activated protein kinase pathways (MAPKs), and ROS. Activation of these pathways through upregulation of antioxidants, osmolytes, and other proteins that respond to cold, e.g. dehydrin and other families of proteins, result in recovering from the chill-induced changes (Saleem et al., 2021).

Applying SA to improve the rapeseed plants’ tolerance to chilling stress may be dose dependent; therefore, it is imperative to use the optimal concentration. Considering the antioxidant properties of salicylic acid, foliar application of various concentrations of this quasi hormone was investigated in this study in terms of their mitigating effects on rapeseeds under chilling stress.

Materials and Methods

The experiment was conducted as a split plot design with 4 repetitions in the farm of Boroujerd Agriculture Research Site (48° 45’ E, 23° 55 N, and Alt. 1235 m above sea level) during the crop year 2018-2019. Treatments included sowing date at two levels of conventional sowing date (late September) and late sowing (15-day delay, representing chilling stress condition) as the main plot and 5 levels of SA treatment as foliar sprinkling (0, 100, 200, 300, and 400 µM) as the subplot. Analysis of the physical and chemical

Table 1
Physical and chemical characteristics of farm's soil

K (ppm)	P (ppm)	N (ppm)	pH	EC (ds.m-1)	texture	Sample depth (cm)
335	7.1	0.12	7.7	0.51	Silty Clay	0-30

characteristics of the farm's soil is presented in Table 1.

Based on the soil analysis results, 150 kg ha^{-1}, 150 kg ha^{-1}, and 100 kg ha^{-1} triple superphosphate, potassium phosphate, and urea were applied to the soil, respectively as fertilizers. A sprinkler irrigation system was used in the study field, which was divided into plots with randomized complete block design with four repetitions.

After preparation of the farm through plowing, disking, and harrowing, seeds of rapeseed, Danob cultivar were sown in 5x2 m plots and on 6 rows with the intensity of 700,000 plants ha^{-1}, in a depth of 2 cm. The distance between two rows and between two plants in each row were considered as 35 cm and about 4 cm, respectively. The distance between blocks and the main plots was 1 m, and plastic covers were used to avoid sprinkling leakage from reaching the nearby plots. Following the 30-year meteorological data of the study region, foliar application of SA started when the mean temperature approached 7-10 ℃, approximately 50 days after sowing (Table 2).

Photosynthesis pigment assay

Sampling for chlorophyll (a and b) and carotenoids' concentration was done 10 days after foliar application of SA. Leaf tissues (0.5 g) were pulverized using liquid nitrogen and acetone 80%. A spectrophotometer (BT600 Plus, Canada) was used to assay chlorophyll a, chlorophyll b, and carotenoid contents at 663 nm, 465 nm, and 470 nm wavelengths, respectively (LIchten and Babani, 2000):

Chlorophyll a = (19.3 $*$ A663 - 0.86 $*$ A645) V/100W

Chlorophyll b = (19.3 $*$ A645 - 3.6 $*$ A663) V/100W

Carotenoids = 100 (A470) - 3.27(mg chl. a) – 104 (mg chl. b)/227

Anthocyanin assay

Wagner's (1979) method was used to assay anthocyanin contents by a spectrophotometer in µg/fresh weight at 550 nm wavelength, using Equation 1:

A= ebc

where A, e, b, and c are extinction coefficient, absorption, cuvette width, and concentration of the solution, respectively.

Table 2
Temperature (°C) fluctuations of experimental site (2018)

Dates	Minimum	Maximum	Average
23 Sept. – 22 Oct.	2.3	22.8	12.5
23 Oct. – 21 Nov.	-1.95	15.5	6.7
22 Nov. – 21 Dec.	-7.2	7.5	0.1

Flavonoids assay

Absorption of the extracts was read at 270, 300, and 330 nm. The extinction coefficient ε = 3300 mM cm^{-1} was used to assay flavonoid contents, reported in µM g^{-1} (Krizek, 1998).

Relative ion leakage assay

In order to assay relative ion leakage in the samples, their primary electric conductivity (Lt) was measured using a digital EC meter (Metrohm, 644). After measuring their final electric conductivity (LO), the ion leakage was measured using the following formula (Lutts et al., 1995):

LT/LO x 100.

Proline, soluble sugars, and antioxidant enzyme assays

Leaf proline and soluble carbohydrate contents were assayed following the methods described by Bates (1973) and Kochert (1987), respectively. Also, activities of superoxide dismutase and catalase enzymes were assayed following the methods explained in Giannopolitis and Ries (1977) and Cakmak and Horst (1991), respectively

Statistical Analysis

The data were analyzed using SAS (Version 9.1). Means were compared using Duncan's Multiple Range Test (P≤0.05), and Excel (2019) was employed to draw the graphs.

Table 3
Analysis of variance of some physiological traits of rapeseed under the influence of sowing date and salicylic acid treatments

Source of Variation	Df	Chl. (a)	Chl. (b)	Chl. Total	Car	Flavonoids	Anthocyanins	Proline	Soluble Sugars	Catalase	Superoxide Dismutase	Ion leakage
Replicate	1	0.01^{ns}	0.01^{*}	0.021^{ns}	0.01^{*}	0.002^{ns}	0.0001^{ns}	2.69^{ns}	64.99^{ns}	0.0001^{ns}	1.69^{ns}	12.78^{ns}
SD	2	2.64^{**}	1.2^{**}	7.4^{**}	0.001^{ns}	0.23^{**}	0.26^{*}	473.07^{**}	843.2^{**}	0.0003^{ns}	20.55^{*}	69.9^{*}
Error main	1	0.02	0.002	0.04	0.001	0.001	0.001	3.52	8.92	0.0001	1.39	6.03
SA	1	0.50^{**}	0.50^{**}	1.91^{**}	0.054^{**}	0.032^{**}	0.023^{**}	35.38^{*}	78.36^{ns}	0.001^{**}	26.73^{**}	63.12^{**}
SD × SA	2	0.15^{*}	0.07^{**}	0.37^{**}	0.0001^{ns}	0.008^{**}	0.005^{*}	3.4^{ns}	140.73^{*}	0.0001^{ns}	6.94^{**}	62.85^{**}
Error	4	0.05	0.01	0.07	0.007	0.002	0.002	14	43.2	0.0001	1.65	9.85
Coefficient of Variation (%)		11.7	16.6	10.6	10.3	11.8	9.5	13.84	12.02	7.40	12.80	7.42

**and * indicate significance at the probability level of 1 and 5%, respectively, and ns indicates non-significance; SA: salicylic acid, SD: sowing date

Table 4
Mean comparisons of interaction effects of sowing date × salicylic acid foliar application on some physiological traits of rapeseed

Treatments	Salicylic Acid (µM)	Chl. a (mg/g FW)	Chl. b (mg/g FW)	Total Chl. (mg/g FW)	Flavonoids (mg/g FW)	Anthocyanins (µg/g FW)	Soluble Sugars (mg/g FW)	Superoxide Dismutase (U/mg Protein)	Ion Leakage (%)
Conventional Sowing Date	Control	1.88bcd	0.68c	2.56c	0.21f	0.42bc	51.39cd	11.53ab	44.4ab
	0	1.86bcd	0.49d	2.35c	0.26ef	0.56a	51.98cd	10.66abc	46.6a
	100	2.74a	1.33a	4.06a	0.32de	0.59a	46.90d	6.88ef	35.8c
	200	2.48a	1.01b	3.48b	0.35cd	0.59a	46.6d	6.18f	34c
	300	2.06b	0.51d	2.57c	0.23f	0.56a	51.76cd	9.09cd	40.8b
	400	1.89bc	0.68c	2.57c	0.22f	0.55a	54.23bcd	11.92ab	45ab
Delayed Sowing Date	Control	1.52d	0.36de	1.87d	0.31de	0.35c	52.16cd	11.61ab	46.4a
	0	1.51d	0.31e	1.82d	0.33cd	0.35c	58.18bc	11.80ab	45.4ab
	100	1.87bcd	0.68c	2.56c	0.53a	0.48b	69.57a	9.19cd	42.8ab
	200	1.71bcd	0.73c	2.43c	0.43b	0.45b	58.69bc	8.57de	40.6b
	300	1.89bc	0.42de	2.31c	0.44b	0.38c	51.71cd	12.65a	43.5ab
	400	1.60cd	0.27e	1.86d	0.39bc	0.36c	62.85ab	10.30bcd	43.3ab

Different letters in each column indicate a significant difference (P≤0.05) according to Duncan's multiple range test.

Results

Chlorophyll (a)

Results of ANOVA showed that the effects of main factors of the study, sowing date, and SA application, as well as the interaction of the effects of sowing date + SA were significant on chlorophyll (a) contents of the rapeseed plants under study both at p≤0.01 and p≤0.05 (Table 3). Comparison of mean chlorophyll (a) contents revealed that the conventional sowing date + 100 µM SA resulted in the highest level of chlorophyll (a), although this was not significantly different from that obtained under the conventional sowing date + 200 µM SA treatment with a mean chlorophyll (a) content of 2.48 mg g^{-1} (Table 4). The combined treatment of delayed planting date ± non-application of salicylic acid (0 µM) with an average of 1.51 mg/g resulted in the lowest amount of chlorophyll a. Also, conventional planting date + 100 µM salicylic acid increased chlorophyll a by 47.31% compared to conventional planting date + non-application of salicylic acid (0) (Table 4). Conventional planting date + 100 µM salicylic acid increased 81.45% of chlorophyll a compared to delayed planting date + non-application of salicylic acid (0) (Table 4). Foliar application of SA had no significant effects on chlorophyll (a) contents of the rapeseeds under the delayed sowing date (chilling stress group) as shown by Table 4.

Chlorophyll (b)

Significant effects of the main factors of the study and the interaction between sowing date + SA were observed on chlorophyll (b) contents at P≤0.01 (Table 3). Maximum and minimum levels of chlorophyll (b) were recorded in the rapeseeds treated with the conventional sowing date + 100 µM SA (1.33 mg g^{-1}) and the delayed sowing date + 400 µM SA (0.27 mg g^{-1}), respectively. Amongst delayed sowing groups, the highest chlorophyll (b) contents were observed with 100 and 200 µM SA treatments (Table 4). Also, conventional planting date + 100 µM salicylic acid increased chlorophyll a by 171/42% compared to the conventional planting date + non-application of salicylic acid (Table 4). Conventional planting date + 100 µM salicylic acid increased chlorophyll a contents by 329% compared to the delayed planting date + non-application of salicylic acid (Table 4).

Chlorophyll (a + b)

Sowing date, SA application, and the interaction of sowing date + SA showed significant effects on chlorophyll (a + b) contents of the rapeseed plants under study at p≤0.01 (Table 3). The conventional sowing date + 100 µM SA resulted in the highest mean chlorophyll (a + b) content (4.06 mg g^{-1}) while the delayed sowing date + non-application of SA resulted in the lowest mean chlorophyll (a + b) content (1.28 mg g^{-1}) as shown in Table 4.

Carotenoids

The effects of SA on carotenoids contents of the experimental plants showed significant difference at p≤0.01 (Table 3). Compared with the control (no foliar application) carotenoid contents increased under SA treatments, and 200 and 0 µM SA resulted in the highest (0.89 mg g^{-1}) and lowest (0.69 mg g^{-1}) carotenoid contents, respectively (Fig. I).

Flavonoids

The effects of main factors of the study and their interactions were significant on flavonoid contents of the rapeseed plants at p≤0.01 (Table 3). Treatments with delayed sowing date + 100 µM SA and the conventional sowing date + control (no foliar spray) resulted in the maximum (0.53 mg g^{-1}) and minimum (0.21 mg g^{-1}) mean flavonoid contents (Table 4). Moreover, similar decreasing effects were observed on carotenoid contents in the conventional sowing date by 300 and 400 µM SA application (Table 4).

Anthocyanins

Effects of sowing date and SA application, and their interactions were significant on anthocyanins content (Table 3). Comparison of mean anthocyanin contents showed that the conventional sowing date along with all concentrations of SA led to increased anthocyanin contents. In addition, conventional sowing date + 100 and 200 µM SA and also the delayed sowing date + either control (no foliar spray) or 0 µM SA application recorded the maximum (0.59 µ g^{-1}) and minimum (0.35 µ g^{-1}) mean anthocyanin contents (Table 4). Also, the delayed sowing group treated with foliar application of 300 and 400 µM SA had similar reducing effects on the production of anthocyanins in the plants under study (Table 4).

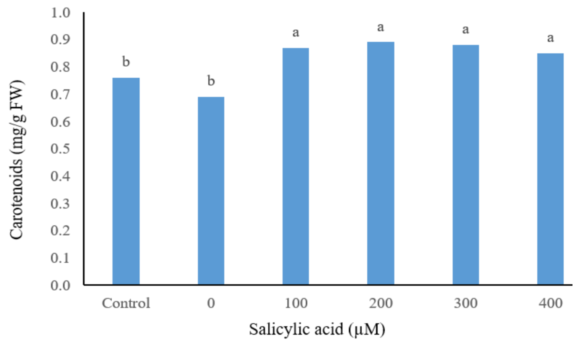

Fig. I. Effect of different levels of salicylic acid on leaf carotenoids; different letters indicate a significant difference (P≤0.05) according to Duncan's multiple range test.

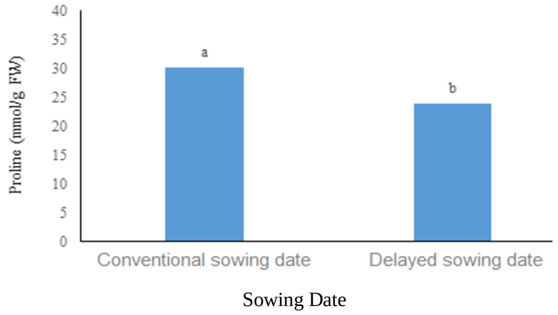

Fig. II. Mean comparison of leaf proline content under the influence of sowing date treatment; different letters indicate a significant difference (P≤0.01) according to Duncan's multiple range test.

Proline

ANOVA showed that the effects of main factors of the study, i.e. sowing date and SA application, were significant on proline content of the plants (Table 3). Compared with the delayed sowing, conventional date had an increase by 26.27% in the proline level (Fig. II). Also, comparison of mean proline contents revealed that applying 100 µM SA led to 23.64% increase in proline contents as compared with non-application of SA (Fig. III).

Soluble sugars

Based on the ANOVA the effects of sowing date and interaction of sowing date × SA application were significant on soluble carbohydrates of the rapeseed (Table 3). Comparison of means showed that the delayed sowing date + 100 µM SA resulted in an increase by 49.29% in the soluble sugars in comparison with the combined treatment of the conventional sowing date + 200 µM SA (Table 4). The delayed planting date + salicylic acid (100 µM) compared to the delayed planting date + non-application of salicylic acid increased the level of soluble sugars by 19.57% (Table 4). The delayed planting date + salicylic acid (100 µM) compared to conventional planting date + salicylic acid (100 µM) increased the level of soluble sugars by 48.33% (Table 4).

Catalase

Results of ANOVA showed that SA treatment had a significant effect ($p \leq 0.01$) on catalase contents of the rapeseed plants (Table 3). Foliar application with 400 µM SA resulted 44.44% increase in catalase antioxidant enzyme activities compared with 100 µM SA treatment (Fig. IV). The application of the highest and lowest concentrations of salicylic acid, 400 and 0 µM, increased the activity of catalase compared to the control by 18.18% (Fig. IV).

Superoxide dismutase

The effects of main factors of the study and their interaction (sowing date × foliar SA) were significant on the activities of superoxide dismutase enzyme (Table 3). Comparison of means revealed that the delayed sowing date + 300 µM SA increased activity of this enzyme by 104.69% compared with the combined treatment of the conventional sowing date + 200 µM SA (Table 4). The interaction effect of delayed planting date + salicylic acid (300 µM) compared to delayed planting date + non-application of salicylic increased the activity of this antioxidant by 7.20%. Finally, the delayed planting date + salicylic acid (300 µM) compared to conventional planting date + non-application of salicylic increased the activity of this antioxidant by 18.66%.

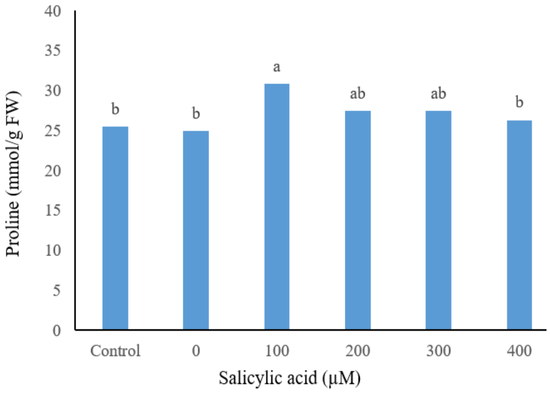

Fig. III. Mean comparison of leaf proline content among different levels of salicylic acid; different letters indicate a significant difference (P≤0.05) according to Duncan's multiple range test.

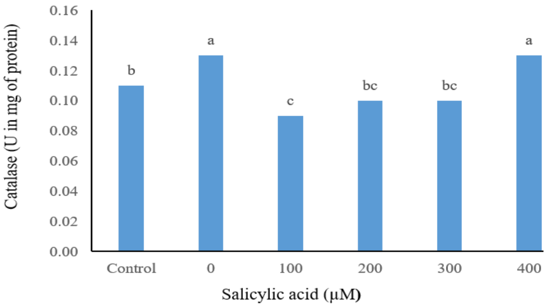

Fig. IV. Mean comparison of catalase levels under different levels of salicylic acid treatment; different letters indicate a significant difference (P≤0.05) according to Duncan's multiple range test.

Ion leakage

The main factors of the study (sowing date and SA application) and their interaction had significant effects on the ionic exchange of the plants in experimental groups at $p \leq 0.01$ and $p \leq 0.05$ (Table 3). Maximum levels of ion leakage were observed in the conventional sowing date + non-application of SA and the delayed sowing date + control groups as 46.6% and 46.4%, respectively while the minimum levels were recorded in the conventional sowing date + 200 µM SA and the conventional sowing date + 100 µM SA as 34% and 35.8%, respectively (Table 4).

Discussion

Chilling stress disturbs chloroplast enzymatic activates and membrane status which gives rise to photo inhibition in PSII and PSI photosystem and reduced photosynthesis performance (Liu et al., 2018). The adverse effects of this stress on PSII are higher than those in PSI (Adamski et al., 2020). Plant growth regulators, salicylic acid, and its derivatives play an important role in plants' growth, photosynthesis, stomatal conductivity, perspiration, and tolerance to stress conditions (Heydarnejadiyan et al., 2020). Early sowing date + 200 µM SA resulted in the highest level of chlorophyll (a) contents of flax plants (Shenavaei Zare et al., 2021). Low temperature due to the production of ROS, disrupts photosynthesis (Sharma et al., 2020). The destructive effect of low temperature was reported to reduce stomatal conductivity or photochemical activity, which led to photo inhibition and reduced P_N and chlorophyll contents of Camelina (Xu et al., 2022). Shenavaei Zare et al. (2021) reported that early sowing date in flax increased chlorophyll (b) contents of flax by 66.87% compared with late sowing group, and SA (100 µM) resulted in higher chlorophyll (b) contents in comparison with glycine betaine and sodium nitroprusside treatments.

Applying SA in soybean resulted in the maximum chlorophyll (a, b, and total) contents in comparison with the control and jasmonic acid groups (Farhangi-Abriz and Ghassemi-Golezani, 2018). In addition, 100 µM salicylic acid increased chlorophyll (a+b) contents of *Mentha piperita* L. by 4.89% in comparison with non-application of SA (Jahani et al., 2021).

Salicylic acid is a soluble phenolic compound and an endogenous growth regulator that contributes as a signaling molecule in regulation of physiological processes in plants, improving their growth parameters such as shoot length, biomass, chlorophyll pigments and carbohydrates contents, stomatal adjustments, nutrient uptakes, chlorophyll and protein synthesis, inhibition of ethylene biosynthesis, perspiration, and photosynthesis (Parveen et al., 2021). The highest amount of carotenoid was obtained with the application of salicylic acid (50 Mm) in white mustard (mustard sprouts) (Artés-Hernández et al., 2022). By increasing the level of concentration of salicylic acid (6 mM), the amount of carotenoid in sunflower plant increased (Parveen et al., 2021).

Phenolic and flavonoid contents increase because of abiotic stresses in the environment such as chilling stress, heavy metals, drought, and nutrient deficiency to protect plant cells against ROS (Tavallali et al., 2020). Applying SA and brassinolid in *Brassica nigra* increased flavonoid contents (Ghassemi-Golezani et al., 2020). Gene expression and activities of phenylalanine ammonia lyase (PAL) enzyme in leaves of *Crocus sativus* pretreated with SA increased, which in turn resulted in accumulation of phenolics and flavonoids (Tajik et al., 2019). Also, Abdelhameed et al. (2021) found increased accumulation of proline, total phenols, and flavonoids in *Trigonella foenum-graecum* L. treated with SA.

Delayed sowing in rapeseeds was found to reduce anthocyanin significantly in comparison with early sowing date, and the synthesis and accumulation of anthocyanins in epidermic layers of the plants can affect their antioxidant potentials and reduce the adverse effects of oxidative stress (Davami et al., 2022). SA treatment in safflower improved photosynthesis speed, anthocyanin content, and activities of phenylalanine ammonia lyase enzyme (Chavoushi et al., 2020). Also, Kohli et al. (2018) found maximum anthocyanin contents in *Brassica juncea* L. under combined treatment with SA + 24-epibrassinolide.

Low temperature shock causes huge changes in the membrane permeability, free proline contents, and malondialdehyde levels of plants (Nesterova et al., 2019). In their study on rapeseed, Teymoori et al. (2021) found that early sowing date increased proline contents of the plants by 74.36% and 37.82% during 2015-2016 and 2016-2017 crop years, respectively in comparison with the delayed sowing. Unlike the present study, delayed sowing date in the study by Shenavaei Zare et al. (2021) increased proline contents of flax compared with early sowing. As a multipurpose amino acid, proline content of leaves accumulates in many plants in response to abiotic stresses and protects cell membranes, proteins, and cytoplasmic enzymes from the adverse stress conditions by scavenging oxygen free radicals, which damage cell membranes (Davami et al., 2022). Application of SA in *Triticum aestivum* under freezing stress through regulation of sucrose and free proline concentrations (via regulating carbon and nitrogen metabolisms) reduced cell necrosis and therefore, improved the plant's tolerance to freezing stress (Wang et al., 2022).

Plants adapt to low temperature through a number of morphological, physiological, biochemical, and molecular changes (Bhattacharya, 2022). Through metabolic changes (betaines, polyamines, and amino acids) plants resist chilling stress (Qu et al., 2019). Chilling stress was reported to affect carbohydrate metabolism in maize plants, and many carbohydrate metabolites including retene, trehalose-6-phosphate, fructose, fructose-6-phosphate, and glucose accumulated in this plant to confront combined chilling + draught stress (Guo et al., 2021). Soluble sugars and alcoholic sugars function as osmotic regulators signal molecules, and chilling stress protectors in plants and through scavenging ROS in low temperature increase plants' resistance against chilling stress (Hussein et al., 2018). Teymoori et al. (2020) reported that delayed sowing of rapeseeds in two consecutive crop years improved soluble carbohydrate contents of leaves as compared with early sowing date. Applying SA in *Magnolia wufengensis* under freezing stress resulted in the increased accumulation of soluble sugars such as glucose, fructose, and retene through increased activities of amylase, sucrose-P-synthase, and sucrose synthase enzymes, and eventually these changes in metabolites improved the plants' tolerance to stress (Duan et al., 2022). Temperature stress (-2 °C) resulted in synthesis of the highest levels of soluble sugars in rapeseeds (Lei et al., 2019). Applying SA was also found to have increased carbohydrate levels in safflower (Shaki et al., 2018). This plant growth regulator at 2 µM concentration was reported to increase proline, soluble carbohydrate, and chlorophyll (a, b, and total) contents of olive compared to the control plants (Shafiei, et al., 2019). Priming *Pisum sativum* seeds with SA improved growth, photosynthesis, antioxidants (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase), increasing accumulation of osmolyte regulators including soluble sugars and proline (Ahmad et al., 2020). Applying lower concentrations of SA (0, 0.5, 1, and 1.5 mM) resulted in the highest activities of catalase enzyme in wheat (Ignatenko et al., 2019). Also, lower levels of salicylic acid through regulation of antioxidant defense system, played a key role in mitigating the chilling damage in beans (Soliman et al., 2018).

Excessive generation of ROS due to chilling stress can cause severe oxidative damage in plants. Therefore, antioxidant defense mechanisms such as superoxide dismutase and peroxidases and also nonenzymatic antioxidants such as phenolic compounds (phenolic acids and flavonoids) scavenge ROS (Rezaie et al., 2020). Zand-Silakhoor et al. (2021) reported that under delayed sowing date, the percentage of antioxidant activities in *Hibiscus sabdariffa* L. increased so that the lowest antioxidant activity (71.57%) was recorded in plants sown on 4th June while 2nd July and 18th June sowing dates resulted in the highest antioxidant activities, 78.62% and 78.20%, respectively. Compared to early sowing date, delayed sowing group of flax plants showed an increase by 2.16% in the activities of superoxide dismutase enzyme, and the activity of this hormone increased under lower concentration of SA (1 mM) in comparison with the higher concentration of SA and other phytohormones (Shenavaei Zare et al., 2022). Biareh et al. (2022) found that salicylic acid can affect the activities of hydrogen peroxide, catalase, and peroxidases in a dose-dependent manner.

Biotic and abiotic stresses can make changes such as withering leaf, reducing leaf surface area, changes in relative water contents, electrolyte leakage, and generation of ROS (Hasanuzzaman et al., 2020). Salicylic acid plays its role in plants in terms of various physiologic and growth responses such as seed germination, stomatal movements, pigment accumulation, photosynthesis, ethylene biosynthesis, heat generation, enzymatic activities, nutrient uptake, florescence, cell membrane performance, nodulation in legumes, and overall growth and development of plants (Ali, 2021). Application of salicylic acid during chilling stress in *rassica oleracea* L. resulted in reduced ion leakage (Min et al., 2021). Similarly, the level of ion leakage in freezing-stressed spinach reduced upon applying ion SA (Shin et al., 2018).

Conclusion

Conventional sowing date along with the low concentration of SA (100 µM) resulted in the highest levels of chlorophyll (a, b, and total), anthocyanin, and proline in rapeseeds. Higher level of SA (200 µM) yielded the highest level of carotenoids. In addition, the highest production and accumulation of flavonoid nonenzyme antioxidant and soluble carbohydrates were recorded with 100 µM SA application. On the other hand, the highest levels of catalase and superoxide dismutase enzymatic antioxidants under delayed sowing date were recorded in the rapeseed plants treated with moderate and high concentrations of SA. The minimum ion leakage was recorded under conventional sowing date and application of SA. Based on the findings, conventional sowing date along with applying low concentration of SA seems to have higher effects on photosynthesis pigments. Considering the appropriate sowing date and lack of chilling stress, this level of SA is probably sufficient as the high concentrations of SA (300 and 400 µM) left negative effects on the plants under study. In contrast, under chilling stress (delayed sowing date), higher concentration of SA in the enzymatic antioxidants improved plants' tolerance. Production of more soluble sugars in the delayed sowing group shows the plant's appropriate use of mechanisms to resist chilling stress. The role of salicylic acid in reducing ion leakage confirms the importance of this phytohormone in mitigating the negative effects of cell membrane fluidity and maintaining its stability. Finally, the conventional sowing date seems to be more appropriate for the rapeseed crop compared with the delayed sowing, and 100 µM concentration of salicylic acid has more favorable effects under this condition while this concentration of SA compared to the control treatment under chilling stress also played an effective role through producing soluble carbohydrates and activating enzymatic and non-enzymatic antioxidant mechanisms.

Chilling stress disrupts the integrity of the cell membrane and intracellular organelles, decreasing photosynthesis and the accumulation of proline, soluble carbohydrates, and antioxidants. This leads to a decrease in cell activities and finally its destruction. On the other hand, the application of some plant growth regulators, including salicylic acid can reduce the adverse effects of chilling stress. The conventional sowing date along with the application of salicylic acid (100 µM) increased chlorophyll and photosynthetic pigments while in delayed cultivation, chilling stress increased the activity of some antioxidants and soluble sugars. Using higher concentrations of salicylic acid was more effective on the activities of catalase and superoxide dismutase under delayed sowing conditions.

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