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Mitigation of salt stress in *Camelina sativa* by epibrassinolide and salicylic acid treatments

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Salinity stress is a critical abiotic factor that severely limits the growth of crops and agricultural productivity. This study explores the potential of exogenously applied salicylic acid (SA) and 24-epibrassinolide (EbR) to mitigate the adverse effects of salt stress on camelina by enhancing physiological processes critical for plant resilience, such as water retention, membrane stability, and pigment biosynthesis. This research was conducted to determine the effects of two hormones applied under salt stress on the growth and development of the camelina plant (*Camelina sativa*) under greenhouse conditions. Eight parameters were evaluated in this study, including Fresh Weight (FW), Dry Weight (DW), Plant Water Content (PWC), Relative Water Content (RWC), Membrane Permeability (MP), Chlorophyll-a (Ch-a), Chlorophyll-b (Ch-b) and carotenoids. The results revealed that salt stress significantly reduced plant growth and productivity of all genotypes in most parameters. Under salt stress conditions, the addition of SA and the combination of EbR + SA enhanced the performance of both RWC and carotenoid parameters. In contrast, EbR treatment specifically enhanced RWC without significantly affecting carotenoid levels. The highest FW (5.49 g) and DW (1.31 g) were obtained for the NaCl + EbR + SA treatment group after the control group. The highest values of MP were obtained for the NaCl treatment group in Arslanbey genotype and for the NaCl + EbR + SA treatment group for the other two genotypes. Furthermore, when the control and NaCl + EbR + SA treatments were compared, the highest increase in Ch-a, Ch-b and carotenoid contents was obtained in PI-650142 genotype. However, more research is required to fully understand the mechanisms and effects of these compounds when applied together. Overall, SA and EbR have promising potential for improving the productivity of crops grown under salt-stress conditions. Further studies should be performed by considering the sensitivity of genotypes with low salt tolerance.

Keywords *Camelina sativa*, Abiotic stress, 24-Epibrassinolide, Salicylic acid, Plant growth and development

Presently, there is a demand for high crop yield to overcome competition in modern agriculture, achieve profit and meet high food demand due to population growth. To achieve high crop yield, agricultural malpractices are being adopted for crop growth, including the application of poor-quality irrigation water. Salinisation, which occurs due to improper agricultural practices, is increasing worldwide in both intensity and extent[1]. In arid regions, the irrigation of the arable land often exacerbates salinisation due to the evaporation of naturally high-salt groundwater[2]. Therefore, water scarcity and salinity stress following the use of groundwater for irrigation are common in arid and semiarid regions. However, it is estimated that the extent of soil salinisation will increase in the coming years due to climate change[3].

Salinity stress is one of the most critical abiotic factors that plays a role in reducing crop yield and quality. Salinity disrupts the morphology, physiology and biochemical processes of the plant[4]. The accumulation of Na + and Cl − ions in the tissues of cultivated plants in soils containing high levels of NaCl is one of the most harmful effects of salinity. The excessive concentration of Na + in the soil also restricts the uptake of K+, which is an essential macronutrient for plant growth and development. This leads to low agricultural yield and quality and even death of the plant[5]. Salt stress can also decrease photosynthesis by limiting the amount of green pigments[6].

The camelina plant has an effective morphological structure against abiotic stress factors such as high temperature, drought and salinity[7]. The oily and waxy structure of the cuticle layer in camelina prevents water loss from the stomata and thus enables the plant to resist various abiotic stress factors[8]. The development of salt-tolerant crops such as camelina is important to ensure the food security of the rapidly increasing world population. The morphological and biochemical analyses performed on camelina plants grown under NaCl

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concentrations ranging from 50 to 300 mM in vitro to determine their salinity tolerance revealed that the plants produced more biomass as a plant defense mechanism against low salinity levels[9]. Another study demonstrated that Camelina exhibits high adaptability to saline soil conditions; however, the relative water content (RWC), plant water content (PWC), and chlorophyll content decrease under stress conditions, while membrane permeability (MP) increases[10]. Low concentrations (25–50 mM) of NaCl applied at different concentrations during the seedling period of camelina had a positive effect on the fresh weight (FW) and dry weight (DW) of the plant, and the critical salt concentration for seedling growth was 75 mM[11]. To date, only a few studies have been conducted to determine the morphological and photosynthetic changes that occur in the camelina plant under salinity stress. The identification of species that can grow in saline soils is a good approach to exploit such barren lands.

Plants respond to salt stress through intricate physiological and molecular mechanisms, including regulating genes and enzymes involved in water balance, ion homeostasis, pigment biosynthesis, and cell wall integrity[12]. Salicylic acid (SA) and brassinosteroids (e.g., 24-epibrassinolide, EbR) are key hormonal regulators that modulate these processes to enhance stress tolerance[13,14]. SA specifically increases the activity of enzymes that detoxify reactive oxygen species (ROS) (e.g., superoxide dismutase [SOD], peroxidase [POD], catalase [CAT]). It also improves plant tolerance to salt stress by increasing the expression of SOS1 (Salt Hypersensitive 1) and NHX (Na+/H + antiporter) genes that regulate ion homeostasis[15,16]. Exogenous application of brassinosteroids (BRs) alleviates stress by minimizing reactive oxygen species (ROS) accumulation, improving osmotic adjustment and ion homeostasis, and upregulating stress-responsive genes. Additionally, BRs contribute to stress tolerance by modulating the synthesis and activity of proteins involved in stress response pathways[17]. These mechanisms support both the preservation of photosynthetic capacity and the maintenance of ion homeostasis in plants exposed to salt stress.

Many hormones or chemicals, including salicylic acid (SA), melatonin and brassinosteroids (BRs) in particular, are used to increase the tolerance of plants against abiotic stressors. However, in terms of low cost and ease of application to large areas, SA is the most frequently used hormone. SA is found in trace amounts in the natural structure of plants, and it is involved in the coordination of physiological processes such as stomatal closure, nutrient intake and protein synthesis[18]. SA also plays a major role in abiotic tolerance and has the potential to exert protective effects on plants under stress conditions[19]. Many studies have reported that SA treatment shows an increase in salt-stress tolerance in different plant species, such as *Brassica juncea*, *Arabidopsis thaliana*[17,20,21].

BRs are another hormonal group that reduces abiotic stress factors and has recently been preferred in applications for stress tolerance. The main goal of BR application to plants under salt stress is to ensure their normal growth. The use of BRs has been one of the most consequential approaches to increase crop production and quality, as they are non-toxic and environment friendly. BRs also function as plant growth regulators that exhibit structural resemblances to animal steroid hormones[14]. BRs have the potential to promote tolerance in plants against salinity, drought, high or low temperature and other stress factors[22]. Several studies have suggested the curative role of BRs under stressful conditions. BRs increase abiotic stress tolerance by protecting membrane stability[23,24]. 24-Epibrassinolide (EbR) is a naturally occurring plant hormone classified as a type of brassinosteroid (BR).

Both SA and EbR can induce physiological and biochemical changes that play a critical role in plants during abiotic stress conditions. Because salt stress is considered to have a detrimental effect on plant growth, the present research hypothesizes that SA and EbR will mitigate this effect of NaCl stress on the growth and development of camelina. Hence, the present research aims to investigate whether treatment with SA and/or EbR can decrease the detrimental effects of NaCl stress on camelina and promote its growth.

Materials and methods

Plant material and treatments

Three distinct camelina genotypes were used as plant material: two genotypes obtained from the USDA (United States Department of Agriculture) and the Arslanbey variety registered by the Ministry of Agriculture of the Republic of Turkey. The origins of the genotypes are Sweden (PI-650142 or No:402) and Denmark (PI-304269 or Ames 26665).

The study was conducted using a complete randomised plot design under controlled greenhouse conditions in Samsun, Turkey. Seeds were sowed in pots (25 cm diameter and 20 cm height) filled with a mixture of peat: perlite: soil (1:1:1, v: v:v). There were five plants per pot and five replicates per treatment.

Camelina plants were grown under natural light conditions (day length: 7–8 h), day/night temperatures of 26 °C/14°C and relative humidity of 75–80% during the experiment. The plants were irrigated with Hoagland's solution every 2 days for the experimental duration.

Salt tolerance of the camelina genotypes was determined by a preliminary study, and the most effective concentration of 140 mM (salt amount in each treatment: 5.72 g) was selected to Göre (2023)[25]. A control group was included to determine the effects of other factors. EbR (0 and 1.5 µM) and SA (0 and 1.5 µM) treatments were performed with Hoagland's nutrient solution (pH: 7.2; electrical conductivity (EC): 2.2 $dS{\cdot}m^{-1}$) as the irrigant solution[26]. Table 1 shows the standard composition of Hoagland's nutrient solution.

Irrigation was applied to each pot with 100 mL of irrigation water at 2-day intervals when the plants reached the stage of development of approximately eight leaves at 5 weeks after sowing. NaCl, EbR and SA treatments were applied seven times during the growth period at 2-day intervals, and the treatments were terminated when the total amount of each treatment was reached (140 mM NaCl, 1.5 µM EbR and 1.5 µM SA). After the completion of the salt treatment, the plants were irrigated at field capacity (200 mL per day) for 10 days.

Plant samples were collected at 50 days from sowing to assess the content of Ch-a, Ch-b and carotenoids in leaves; MP; PWC; and RWC. Plant FW was weighed separately after the plants were dehydrated by placing them

Compounds	Concentration of stock solution (mM)	Volume of stock solution of final solution (ml/L)	Element	Final concentration of element (µM)	Final concentration of element (ppm)
Macro-nutrients					
KNO_3	1000.00	6.00	N	16,000	224
$Ca(NO_3)_2.4H_2O$	1000.00	4.00	K	6000	235
KH_2PO_4	1000.00	2.00	Ca	4000	160
$MgSO_4.7H_2O$	1000.00	1.00	P	2000	62
			S	1000	32
			Mg	1000	24
Micro-nutrients					
KCL	25.00	2.00	Cl	50	1.77
H_3BO_3	12.50		B	25	0.27
$MnSO_4.H_2O$	1.00		Mn	2	0.11
$ZnSO_4.7H_2O$	1.00		Zn	2	0.13
$CuSO_4.5H_2O$	0.25		Cu	0.5	0.03
MoO_3	0.25		Mo	0.5	0.05
Fe Na EDTA	64.00	1.00	Fe	53.7	3

Table 1. Composition of hoagland nutrient solution.

on blotting papers for 30 min. Plant DW was obtained by weighing the plants after drying in an oven at 72 °C for 48 h[22].

Plant water content (PWC)
Based on the plant FW and DW, PWC was calculated using the following formula[27]:

$$\text{Plant Water Content} = \left(\frac{Fresh\ weight - Dry\ weight}{Fresh\ weight}\right) x\ 100 \quad (1)$$

Relative water content (RWC)
Fresh leaf discs, weighing approximately 0.3 g, were taken from each plant and their initial weight was recorded. The discs were then placed in 25 mL beakers filled with water and incubated overnight in the dark to reach full turgidity. After incubation, the turgid weight of each sample was recorded. The samples were then dried in an oven at 80 °C for 24 h, and their dry weight was measured. RWC of each sample was calculated using the following formula[28]:

$$\text{Relative Water Content (RWC)} = \left(\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgor weight} - \text{Dry weight}}\right) x\ 100 \quad (2)$$

Membrane permeability (MP)
MP was calculated using the EC meter. MP was calculated using the following formula[29]:

$$\text{Membrane Permeability} = \left(\frac{\text{EC1}}{\text{EC2}}\right) x\ 100 \quad (3)$$

Chlorophyll-a, chlorophyll-b and carotenoids
To determine the content of Ch a, Ch b and carotenoids, 1 cm–diameter leaf discs were taken from a leaf in the middle side of the main shoot of each plant. The content of chlorophyll and carotenoids was calculated according to Visser et al.[30] by using the absorbance data.

Statistical and data analysis
Analysis of variance of the experimental data was performed using the JMP (2007) statistical software program ($p < 0.05$). Differences in mean values were compared using Tukey's multiple comparison test[31].

Results
In addition to genotype (G) and salinity factors, the binary interactions of NaCl×SA and G×NaCl and the ternary interactions of G×NaCl + EbR and G×NaCl + SA were determined to be significant for all the evaluated parameters. Among the evaluated parameters, only NaCl + EbR + SA and G×NaCl + EbR + SA interactions for FW; NaCl + EbR, NaCl + EbR + SA and G×NaCl + EbR + SA interactions for RWC; and G×NaCl + EbR + SA interactions for carotenoids were found to be non-significant.

Moreover, the effect of the binary interaction NaCl + EbR on RWC; the ternary interaction NaCl + EbR + SA on FW and RWC; and the quadruple interaction G×NaCl + EbR + SA on FW, RWC and carotenoid content was

Source of variation	df	FW	DW	PWC	RWC	MP	Ch-a	Ch-b	Carotenoids
Genotype (G)	2	329.75**	212.12**	4.29*	215.96**	316.95**	1345.81**	349.40**	1253.56**
Treatment									
NaCl	1	11398.42**	789.94**	707.70*	2212.15**	4779.88**	70.24**	17.18**	2741.87**
NaCl + EbR	1	45.14**	1303.70**	511.45**	2.89 ns	1176.60**	2156.53**	572.98**	3029.47**
NaCl + SA	1	780.93**	1500.23**	181.42**	949.12**	36.65**	336.49**	253.28**	1684.49**
NaCl + EbR + SA	1	0.78 ns	12.16 **	7.33**	0.25 ns	46.02**	150.54**	39.73**	15.56**
Interactions									
G*NaCl	2	766.93**	741.86**	166.67**	351.18**	430.38**	496.50**	202.23**	2970.24**
G*NaCl + EbR	2	1049.21**	751.72**	417.75**	67.21**	1672.05**	1569.43**	400.60**	1058.87**
G*NaCl + SA	2	151.99**	784.67**	149.14**	496.98**	449.66**	1329.67**	205.91**	1481.58**
G*NaCl + EbR + SA	2	1.26 ns	23.27**	5.86**	0.05 ns	45.48**	143.22**	57.03**	2.57 ns

Table 2. Analysis of variance of parameters of three camelina genotypes and various treatment interactions. *Indicate significant differences at a specified level ($p < 0.05$), **Indicate significant differences at a specified level ($p < 0.01$) while "ns" indicates no significant difference.

		FW (g)	DW (g)	PWC (%)	RWC (%)	MP (%)	Ch-a (mg g^{-1})	Ch-b (mg g^{-1})	Carotenoids (mg g^{-1})
Genotype (G)*	Arslanbey (G1)	8.34 a	1.38 a	86.05 a	13.63 b	16.05 a	0.94 a	0.39 a	1.04 a
	PI-304269 (G2)	6.56 b	1.01 b	82.69 b	13.90 b	16.62 ab	0.42 b	0.17 c	0.56 c
	PI-650142 (G3)	4.92 c	1.01 b	84.81 ab	14.84 a	16.97 b	0.46 b	0.23 b	0.92 b
Treatment	Control**	12.17	1.68 a	86.52 b	11.66	12.27 a	0.90 a	0.36 a	0.81 c
	NaCl***	5.37	0.88 d	88.27 a	16.24	4.55 b	0.46 d	0.20 d	0.64 d
	NaCl + EbR^{+}	4.69	1.04 c	83.26 d	15.46	16.47 c	0.58 c	0.24 c	0.80 c
	NaCl + SA^{++}	5.39	0.80 e	84.49 c	14.13	17.94 d	0.48 d	0.24 c	0.88 b
	NaCl + EbR + SA^{+++}	5.49	1.31 b	80.04 e	13.15	21.64 e	0.61 b	0.30 b	1.14 a

Table 3. Effects of NaCl, SA, and EbR treatments on various parameters of three Camelina sativa genotypes, genotype-specific responses and treatment averages. $^{*}Q_{0.05\ G}$:2.381; $^{**}Q_{0.05}$ Control: 4.893; $^{***}Q_{0.05\ NaCl}$:2.908; $^{+}Q_{0.05\ NaClx+EbR}$:2.615; $^{++}Q_{0.05\ NaClx+SA}$:2.615; $^{+++}Q_{0.05\ NaClx+EbRx+SA}$:3.099. Mean values followed by the same letter are not significantly different according to Tukey's multiple range test ($P < 0.05$). The absence of letters next to the data indicates no significant differences among the values in that column.

non-significant (Table 2). Regarding the genotypes, Arslanbey variety performed better in almost all parameters, except RWC (Table 3).

Fresh weight (FW) and dry weight (DW)

The highest values of FW and DW (8.34 and 1.38 g, respectively) were obtained for Arslanbey variety (Table 3). The highest FW was obtained for the NaCl + EbR + SA (5.49 g) treatment group as compared to the control group, although the difference was not statistically significant. The highest DW was obtained for the combined treatment (NaCl + EbR + SA) group (1.31 g) as compared to the control group ($p < 0.01$). The genotypes differed in their sensitivity to abiotic stress factors. Arslanbey, PI-304269 and PI-650142 genotypes are each being compared to themselves under control conditions versus NaCl stress, and they showed a significant decrease in FW (26.44%, 84.76% and 53.24%, respectively). Following the treatment with EbR and/or SA, the FW again increased except for that of Arslanbey variety (Fig. 1a). The NaCl + EbR + SA treatment led to a higher DW (1.83 g) only for PI-650142 genotype as compared to that for the control group (Fig. 1b). Furthermore, compared to the control group, Arslanbey variety showed the highest DW under NaCl (2.01 g) treatment. For the genotypes other than Arslanbey, single or combined treatment of hormones (SA or EbR) removed the reducing effect of salt on DW.

Plant water content (PWC)

Arslanbey variety showed the highest PWC value (86.05%) among the genotypes, while the NaCl treatment showed the highest PWC value (88.27%) among the treatments (Table 3). The highest PWC values for each genotype under different treatments were also estimated. Arslanbey genotype showed the highest value (88.86%) in the control group, PI-304269 genotype showed the highest value (93.66%) in the salt treatment group and PI-650142 genotype showed the highest value (85.54%) in the NaCl + EbR treatment group. All genotypes showed the lowest PWC values for the NaCl + EbR + SA treatment group (Fig. 2a). The greatest change in the PWC value (22.48%) occurred in the PI-304269 genotype (from 93.66 to 71.18%). The PWC value of this genotype was 93.66% in the NaCl treatment and 71.18% in the NaCl + EbR + SA treatment.

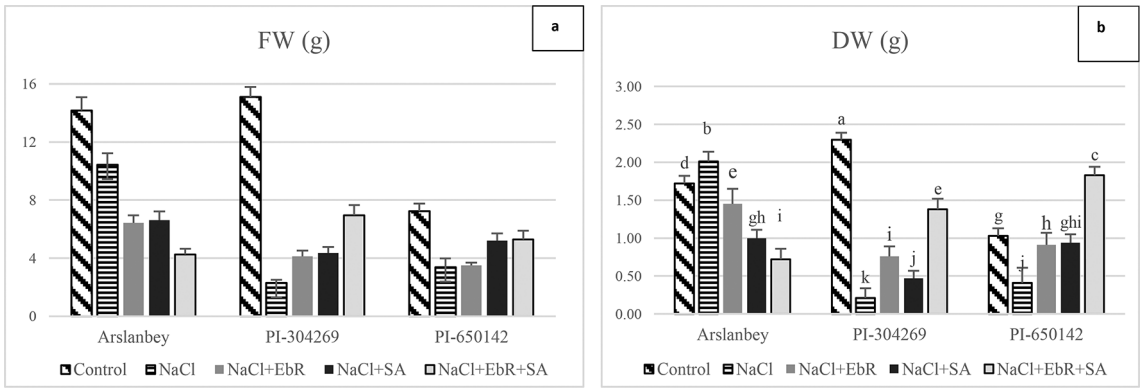

Fig. 1. Effect of EbR and SA on salt-stressed camelina plants (**a**) Fresh Weight (FW) and (**b**) Dry Weight (DW).

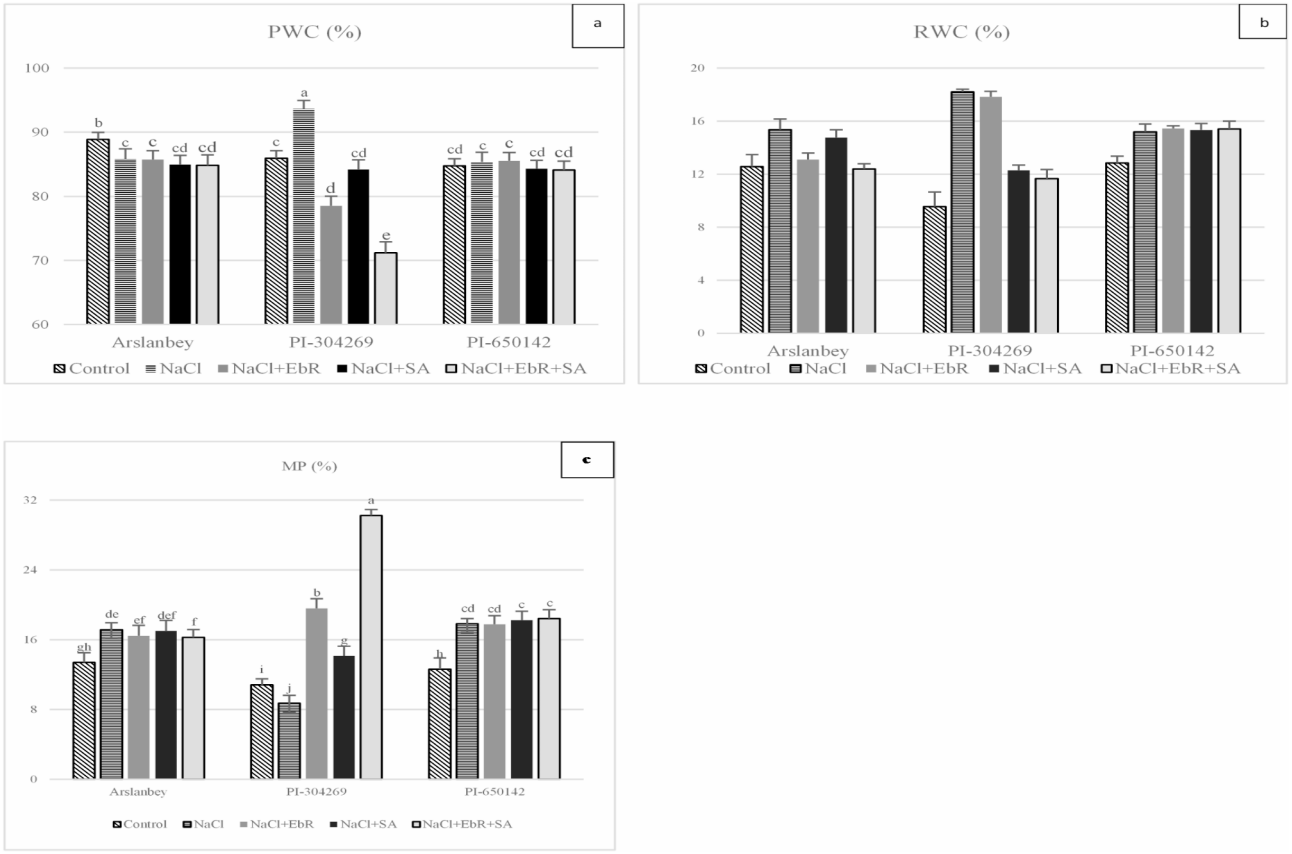

Fig. 2. Effect of EbR and SA on salt-stressed camelina plants (**a**) Plant water content (PWC), (**b**) Relative water content (RWC) and (**c**) Membrane permeability (MP).

Relative water content (RWC)

RWC indicates the plant's hydration status relative to its maximum capacity for water uptake. It is used to monitor changes in the plant's hydration status over time and to evaluate the effect of environmental factors on plant health. RWC can also be used to diagnose the causes of plant stress and as an indicator of plant health. High RWC values indicate that the plant is well hydrated, while low RWC values show that the plant is experiencing stress due to dehydration or other environmental factors.

The highest RWC value (14.84%) was detected in the PI-650142 genotype. The lowest RWC value (13.63%) was noted in the Arslanbey variety. Thus, the susceptibility of Arslanbey variety to stress is higher than that of PI-650142 genotype according to this parameter (Table 3).

A multiple comparison test for the effects of different treatments on RWC could not be performed due to the lack of significant difference among the treatments (Table 3). For NaCl treatment, Arslanbey and PI-304269 genotypes showed the highest RWC value of 15.35% and 18.20%, respectively. PI-650142 genotype showed the highest RWC value (15.44%) for NaCl + EbR treatment (Fig. 2b).

Membrane permeability (MP)

NaCl treatment significantly increased MP as compared to the control group. Arslanbey showed the lowest MP value (13.63%) among the genotypes. Among the treatments, the control group (12.27%) showed the lowest MP value, followed by the NaCl treatment group (14.55%). For the combinations of genotypes and treatments, the control group showed the lowest MP value in all genotypes, except PI-304269. The highest MP values were obtained for the NaCl treatment group for Arslanbey genotype and in the NaCl + EbR + SA treatment group for the other two genotypes (Fig. 2c). Furthermore, the lowest MP value (8.71%) and highest MP value (30.25%) were both shown by the PI-304269 genotype. For PI-304269 genotype, the NaCl + EbR + SA treatment increased the MP value (30.25%) by approximately threefold as compared to that of the control group (10.82%). This further confirmed that PI-304269 genotype is highly affected by salinity and is less responsive to hormone treatment.

Chlorophyll-a, chlorophyll-b and carotenoid

Arslanbey genotype showed the best results for the contents of Ch-a, Ch-b and carotenoids (0.94, 0.39 and 1.04, respectively). Among the treatments, the NaCl + EbR + SA group showed the highest content of carotenoids (1.14 mg g^{-1}). The highest Ch-a and Ch-b values were obtained from the NaCl + EbR + SA application (0.61 and 0.30 mg g^{-1} respectively) except for the control.

Arslanbey and PI-304269 genotypes had the highest Ch-a content (1.72 and 0.63, respectively) and Ch-b content (0.72 and 0.25, respectively) in the control groups. PI-650142 genotype showed the highest Ch-a content (0.66) in the NaCl + EbR treatment group and the highest Ch-b content (0.42) in the NaCl + EbR + SA treatment group (Fig. 3a and b). This implies that the PI-650142 genotype experiencing salt stress is less affected by the stress following hormonal treatment (SA and/or EbR + SA). Arslanbey and PI-650142 genotypes showed the highest carotenoid content (1.53 and 1.55, respectively) in the NaCl + EbR + SA treatment group. PI-304269 genotype could not stabilise and NaCl treatment indicated the highest carotenoid content after the control group (Fig. 3c). This increase in carotenoid content can be considered a response to salt stress. These findings indicates that the hormonal treatment of plants under NaCl stress led to a decrease in stress levels.

Arslanbey and PI-650142 genotypes showed the highest total chlorophyll content (3.01 and 2.53) in NaCl + EbR + SA treatment group (Fig. 3d). PI-304269 genotype showed a lower chlorophyll content in all treatment groups as compared to the control group. NaCl + EbR + SA treatment led to 170% and 263% increase in chlorophyll content in plants of Arslanbey and PI-650142 genotypes, respectively. In contrast, for the same treatment group, PI-304269 genotype plants showed a 60% decrease in the total chlorophyll content.

Discussion

Parameters such as RWC, DW and chlorophyll contents showed a strong response to hormonal treatments. Especially under NaCl + EbR + SA treatment, a significant increase in RWC value was observed in PI-650142 genotype (Table 3). This finding shows that hormonal applications are effective in alleviating the osmotic effects of salt stress. In previous studies, the combination of SA and brassinosteroids in species such as rice and wheat has yielded similar effects to those observed in this study. It has been reported that it increases water retention capacity in the SA or brassinosteroids under salt stress[32,33]. However, the performance of PI-650142 in this study suggests that certain genotypes of *Camelina sativa* may respond differently to hormonal treatments.

The research demonstrated significant changes in FW and DW across different genotypes under salt stress and hormonal treatments. The FW of all genotypes decreased due to salinity stress, but EbR or SA application alone or EbR + SA interaction treatments improved the FW in two genotypes (PI-650142 and PI-304269). These variations can be attributed to the differential regulation of cell wall synthesis, primarily driven by Cellulose Synthase (CesA) and Pectin Methylesterase (PME) genes. CesA plays a critical role in the synthesis of cellulose microfibrils, which form the structural backbone of the cell wall, enabling sustained cell expansion and growth. Similarly, PME modulates the methylation status of pectin, enhancing cell wall elasticity and mechanical stability under stress conditions. Genes like Cellulose Synthase (CesA) and Pectin Methylesterase (PME) support cell wall integrity and growth, directly affecting FW and DW[34]. In addition, nitrogen assimilation enzymes such as glutamine synthetase optimize nitrogen use efficiency, enhancing biomass accumulation[35]. The increase in FW and DW observed in PI-650142 following SA and EbR treatments suggests that these hormones may enhance CesA, PME, and nitrogen assimilation enzyme activities, collectively promoting cell wall integrity, nitrogen use efficiency, and cellular expansion despite salt-induced osmotic stress. Conversely, the limited improvement in FW and DW in PI-304269 under the same treatments could indicate a lower capacity for cell wall remodeling and nitrogen assimilation, potentially due to reduced activation of these critical genes. These findings emphasize the importance of cell wall synthesis and remodelling in maintaining plant growth and stress resilience, highlighting CesA and PME as potential molecular targets for improving salt tolerance in *Camelina sativa*. Similar results have been reported for SA treatment in different plants such as *Triticum aestivum*, *Helianthus annuus*[36,37]. Several studies have reported that EbR treatment can improve the FW and DW of different plants under saline conditions, although similar research on the camelina plant is yet to be reported[38–40]. A previous study conducted on canola, a close relative of camelina, showed that the growth of canola was remarkably inhibited by 150 mM NaCl, and this adverse effect was significantly alleviated by foliar spraying with EbR[41]. Similar results were noted for *Brassica juncea* treated with 24-EbR or 28-HBL (Homo-Brassinolide)[33,42]. The high tolerance against salt stress in plants might be due to the administration of SA and EbR, which complement the activity of each other

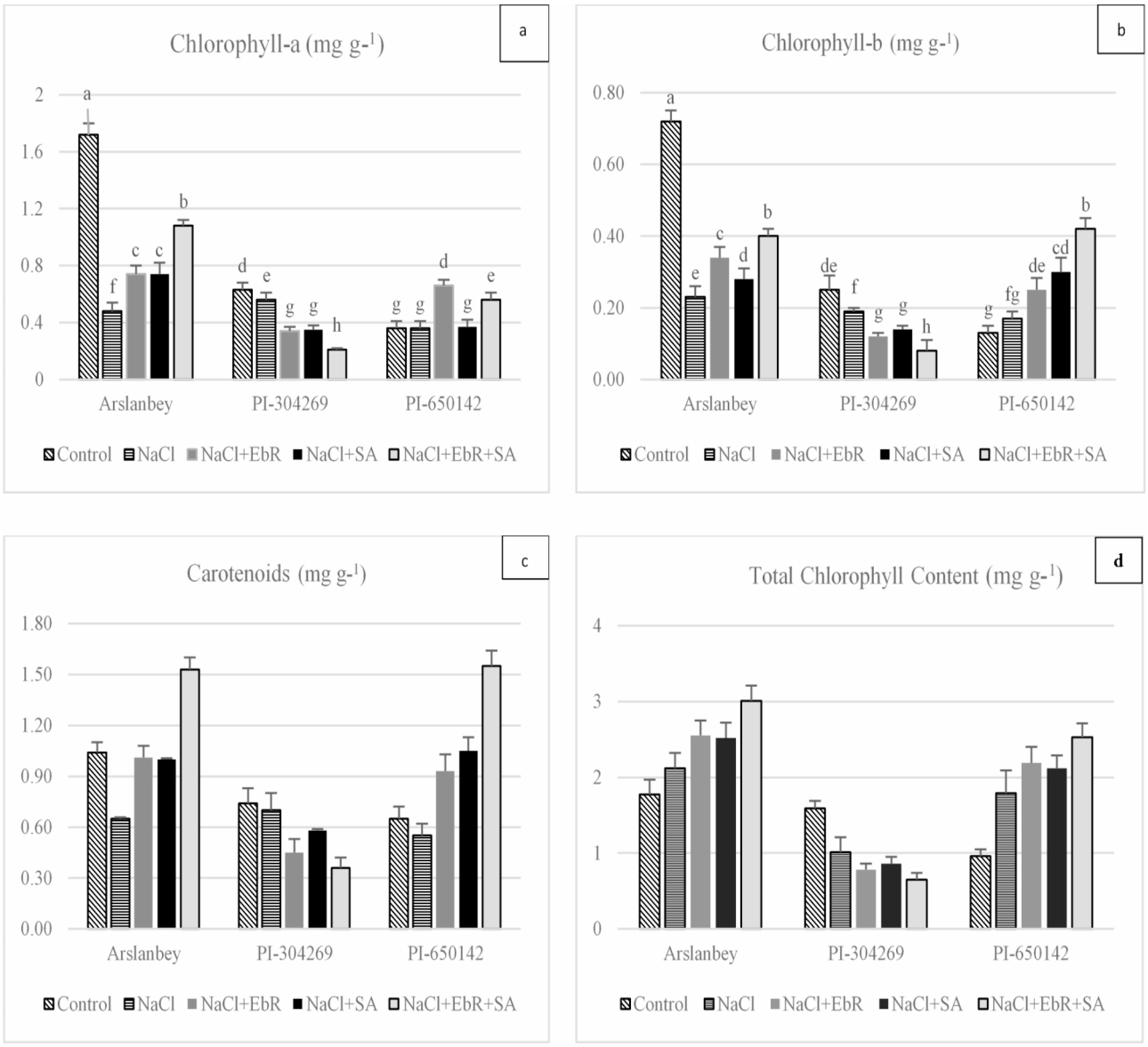

Fig. 3. Effect of EbR and SA on salt-stressed camelina plants (**a**) Chlorophyll-a (Ch-a), (**b**) Chlorophyll-b (Ch-b), (**c**) Carotenoids and (d) Total Chlorophyll Content.

through different mechanisms. Studies on species such as *Brassica nigra* and *Capsicum annuum* have shown that SA and EbR applications support plant growth by improving fresh weight and dry weight parameters[43,44]. It has been stated that this improvement is related to mechanisms such as detoxification of reactive oxygen species (ROS), maintenance of ion homeostasis and increase in membrane stability. Similarly, in *Zea mays*, it has been reported that the combined application of SA and EbR increases the resistance of plants to salt stress[45]. These findings align with our results obtained in *Camelina sativa*, and improvements in fresh weight and dry weight parameters may indicate a synergistic effect of SA and EbR. The combination of these two hormones may lead to a higher potency, resulting in higher FW and DW of the plants in the current study. This suggests that the combination of these two hormones provides a more comprehensive solution to the negative effects of salt stress on plant growth and development.

PWC provides vital information regarding the plant's hydration status, growth conditions and overall health. PWC is affected by several factors, including plant genotype and growth conditions. High PWC values generally indicate that the plant is well hydrated and in good health, while low PWC values represent dehydration or stress due to environmental factors such as high temperature, drought or salinity. PWC also provides information regarding the plant's ability to uptake and utilise water, which is essential for growth and survival. A high PWC value indicates optimal conditions for growth and development, while a low PWC value shows that the plant is experiencing stress in the process of tolerating adverse environmental conditions or other factors.

Salt treatment can decrease PWC due to osmotic potential[46]. When salt is applied to the soil, it increases osmotic pressure, which can draw water from the roots to the shoots, leading to changes in plant water content.

However, salt stress typically reduces the plant's ability to maintain water content, as high salinity leads to osmotic and ionic imbalances that prevent proper water uptake, resulting in a decrease in plant water content rather than an increase.

The reason that plants treated with salt and SA have higher water content than those treated with salt and EbR could be the different effects of these compounds on the plant's water balance. SA is a naturally occurring hormone in plants, and it is involved in various stress responses, including osmotic stress. SA can increase plant tolerance to salinity by increasing water uptake and reducing water loss. EbR, alternatively, is a plant growth regulator that promotes growth and development. The effects of EbR on plant growth and water balance probably differ from those of SA, thereby leading to a lower water content in plants treated with salt and EbR than in those treated with salt and SA. Further research is necessary to confirm these findings and identify the underlying mechanisms.

The exposure of plants to high levels of salt tends to decrease their RWC[47]. This reduction in RWC could indicate diminished turgor pressure, which can subsequently limit the availability of water for the cellular expansion process. Plants exposed to high salt concentrations may experience reduced turgidity in their leaves. However, this effect can be mitigated by treatment with EbR and SA. These compounds help to retain water within the leaves and increase solute accumulation in the cytosol, thereby preventing further loss of turgidity. Ultimately, this enhances the plant's ability to cope with salt stress and maintain healthy growth. RWC emerges as a critical indicator, reflecting a plant's water retention capacity and hydration status, directly influencing its tolerance to salt stress. Low RWC values observed under stress conditions highlight the sensitivity of specific genotypes to dehydration. In contrast, the high RWC values in the PI-650142 genotype demonstrate its enhanced tolerance, emphasizing the significance of RWC in assessing salt stress resilience. This study identified that RWC could be improved through hormonal treatments (SA and EbR), underscoring its pivotal role in evaluating genotypic differences and the efficacy of treatments. Consequently, RWC stands out as the most critical parameter for assessing physiological responses and stress tolerance mechanisms in plants under salt stress. Treatment of the plants with SA or EbR in the presence of NaCl improved the RWC of the plants. Agarwal et al. (2005)[48] showed that the treatment of wheat with SA increased its RWC capacity. Previous studies on different plant species have also indicated that EbR and/or SA could enhance the water retention capacity by promoting water uptake and increasing RWC[20,49,50]. This finding suggests that EbRs may also contribute to an increase in RWC capacity, potentially through their positive effects on water uptake and retention.

PWC and RWC results obtained in this study highlight significant differences among the genotypes under salt stress and hormonal treatments. The higher PWC and RWC values observed in the PI-650142 genotype, particularly following SA and EbR treatments, suggest improved water uptake and retention capacity, which are critical for maintaining hydration status and turgor pressure under stress conditions. Water balance and osmotic regulation, critical for PWC and RWC, are mediated by Aquaporins (PIP1, PIP2), which facilitate water transport across cell membranes, and Na+/H + antiporters (NHX), which maintain ion homeostasis and osmotic balance; the expression of these genes is enhanced by SA and EbR, contributing to the preservation of these parameters under adverse conditions[15,51]. In contrast, the PI-304269 genotype showed significant reductions in PWC and RWC, even with hormonal treatments, indicating a limited capacity to activate these key molecular pathways. These results underscore the importance of aquaporins and NHX genes in regulating water content and maintaining osmotic stability, further demonstrating the potential of SA and EbR to mitigate the adverse effects of salt stress on *Camelina sativa*.

MP refers to the loss of cellular content from a plant cell because of cell membrane damage. MP can occur due to various biotic and abiotic stress factors such as diseases, physical damage, high temperature and salinity stress. Stress factors could drastically affect the permeability of the plant cell membrane, leading to decreased growth, decreased yield and even plant death. Hence, it is critical to measure MP to assess the stress status of the plant and to determine the severity of the effects of environmental factors on plant health. A high level of MP indicates that the plant is experiencing stress, while a low level of MP shows the normal course of the vital activities of the plant. In this study, the PI-304269 genotype exposed to NaCl stress showed a significant increase in the MP parameter (Fig. 2). In contrast, a decrease in MP values was observed in the PI-650142 genotype with hormonal treatments, indicating that membrane stability was maintained. It has been reported in the literature that SA and EbR regulate the Na+/K + pump to maintain membrane integrity and ion balance[52]. The lower MP values observed in PI-650142 are likely associated with the activity of key enzymes such as Ascorbate peroxidase (APX) and Peroxidase (POD), which are known to contribute to membrane stability under stress conditions. SA and EbR have been shown to enhance the activities of these enzymes, effectively mitigating oxidative stress and preserving cell membrane integrity[53]. Ben-Ahmed et al. (2009)[54] demonstrated that the addition of SA to the growth medium of tomato plants grown under salt stress considerably reduced the harmful effects of stress by maintaining MP (as measured by ion permeability) and photosynthetic pigments. Another study reported that MP increased as salinity stress increased in rapeseed plants but decreased when 1 mM SA was applied to these plants[49]. Similarly, Stevens et al. (2006)[55] showed that the treatment of tomatoes with 150 mM NaCl and 0.1 mM SA reduced the MP value by 44% and 200 mM NaCl and 0.1 mM SA decreased the MP value by 32% and thus SA treatment was also shown to reduce MP-related damage caused by salt stress. In general, MP can provide critical information about the overall health of a plant and its ability to survive and sustain under different environmental conditions. This information can be useful to understand the impact of environmental factors on plant growth and survival and to develop strategies to protect plants from stress. BRs modify the structure and stability of the plant cell membrane under stress conditions[56]. Thus, in this study, higher MP values were seen in the control group and the EbR-treated plants (especially PI-304269) under stress with NaCl treatment. It can be stated that the PI-304269 genotype is very sensitive to NaCl + EbR and NaCl + EbR + SA treatments compared to other genotypes and is the only genotype that shows a significant increase in MP value.

Ch-a, Ch-b and carotenoids are three types of pigments found in plants, and they play important roles in photosynthesis. The contents of Ch-a, Ch-b and carotenoids in a plant can reflect its photosynthetic ability, overall health and response to environmental stress. For example, a decrease in the contents of Ch-a or Ch-b can indicate that the plant is experiencing stress or disease, while an increase in the content of carotenoids can indicate that the plant is responding to environmental stress or high light levels. In general, estimating the contents of Ch-a, Ch-b and carotenoids in a plant can provide critical information regarding the plant's ability to conduct photosynthesis and respond to environmental stress. This information can be useful to monitor plant growth and health and to develop strategies to protect plants from environmental stress and diseases. The increase in the chlorophyll content of camelina plants treated with salt is probably due to salt stress–induced changes in the physiology and metabolism of the plant. Salt stress can stimulate the plant's defense mechanisms, leading to the increased production of antioxidants and other protective compounds, including chlorophyll. Salt can also stimulate the activity of enzymes involved in chlorophyll synthesis, thereby further contributing to the increase in chlorophyll content[32]. However, it is important to note that excessive salinity stress can harm the plant, leading to reduced plant height and productivity.

Chlorophyll biosynthesis genes (CHLH, CHL1) and carotenoid biosynthesis genes such as PSY (Phytoene Synthase) and LCY (Lycopene Cyclase) are essential for pigment stability and photosynthetic performance. SA and EbR treatments have been shown to enhance the expression of these genes, leading to improved pigment accumulation and stress tolerance[57–59]. These molecular processes are critical in supporting the increases observed in Ch-a, Ch-b, and carotenoid content, particularly under NaCl + EbR + SA treatments.

Chlorophylls are the critical components of photosynthesis, the process by which plants produce energy from sunlight. Therefore, alterations in chlorophyll content can substantially affect plant growth and development. Carotenoids are crucial molecules that act as antioxidants and help neutralise harmful free radicals. Treatment of salt stress–exposed plants with SA can increase their carotenoid content, which subsequently enhances the plants' ability to mitigate the negative effects of the stress[60]. This may occur because SA treatment helps limit the toxic effects of chloride and sodium ions and exerts a protective effect against oxidative stress induced by salinity[61]. SA treatment could increase chlorophyll content, which could also help protect plants from the harmful effects of salt stress. NaCl + EbR + SA application provided an increase of up to 170% in chlorophyll-a and carotenoid contents, especially in the PI-650142 genotype (Fig. 3). This increase made a significant contribution to the protection of photosynthetic pigments and the maintenance of the metabolic activity of the plant. Arslanbey genotype also showed similar improvements in pigment contents, but in the PI-304269 genotype, pigment contents remained low in all treatment groups. A previous study reported that the chlorophyll content of camelina under salt stress differed according to the variety[9]. These results show that genotypes may respond differently to hormonal treatments and that the combined application of SA and EbR is effective in increasing pigment stability.

The combined application of SA and EbR has also been reported to increase the expression of genes responsible for chlorophyll synthesis in species such as pepper and sorghum[44,62]. These findings align with the observed pigment stability in PI-650142 and Arslanbey genotypes under NaCl + EbR + SA treatments, further emphasizing the role of these hormones in activating key biosynthetic pathways.

A previous study reported that the chlorophyll content of camelina under salt stress differed according to the variety. The total chlorophyll content started to decrease at 100 mM salinity in Cheyenne and Suneson cultivars and at 150 mM salinity in Blaine Creek cultivar[8]. Literature regarding the specific effects of EbR and/or EbR + SA on salinity stress in the camelina plant is particularly scarce. It seems that this topic has not yet been studied extensively in camelina plants or the related studies are yet to be published. However, we found some studies that investigated the effect of EbR on salt stress in related plants such as mustard and rapeseed; the findings of these studies may provide some insights into the potential effects of EbR on camelina. For example, foliar application of SA (1 mM) and EbR (0.1 mM) on black mustard increased chlorophyll content, particularly under moderate and severe salinity conditions. Baghizadeh et al. (2014)[63] reported that SA treatment (1 mM) increased the contents of photosynthetic pigments (Ch a, Ch b and carotenoids) as compared to that in rapeseed plants under salinity stress. Furthermore, EbR enhanced photosynthesis and increased chlorophyll content in stressed plants, while SA enhanced plant stress tolerance and improved plant growth[43]. The combination of these two compounds may exert synergistic effects and enhance their individual effects, thereby leading to an increase in chlorophyll content in the plant and potentially improving its growth and stress tolerance under salt-stress conditions.

The application of EbR through the root growth medium is reported to be an effective method to mitigate the harmful effects of salt stress on the growth and development of various plant species, ultimately leading to improved crop yields. For instance, Kagale et al. (2007)[64] found that the addition of EbR (1 and 2 mM) to the germination medium markedly reduced the negative effect of salt stress on the seedling growth of *Brassica napus*. Similarly, the addition of 1 mM EbR to the root growth medium significantly accelerated the growth of sorghum (*Sorghum bicolor*) seedlings under salinity stress (150 mM)[65]. These studies suggest that the supplementation of the plant growth medium with EbR can substantially alleviate the adverse effects of salinity on plants grown under salt stress by regulating their critical physiological processes. However, it is important to note that this conclusion is based on a limited number of studies, and optimising EbR concentrations and application periods might be necessary to achieve these effects. Ashraf et al. (2008)[66] highlighted other issues that should be considered while using EbRs as root supplements. A critical issue is that the addition of EbRs to the field soil may not be effective as they can be degraded partially or entirely by soil microorganisms. Moreover, the addition of EbRs to the soil at optimal concentrations may not be practical from an economic perspective because of their high cost.

For most agronomic crops, the cost of using EbRs as soil supplements outweighs their yield benefits. Hence, more research is needed to optimise the efficiency of using EbRs as soil supplements for mitigating the negative effects of abiotic stresses such as salinity. The supplementation of the plant growth medium with SA

is also an effective method to mitigate the harmful effects of salt stress in various plant species. For instance, in Arabidopsis, the addition of 0.5 mM SA to a salt medium of 100 mM led to improved seed germination[67]. Similarly, in tomatoes, SA treatment resulted in a fourfold increase in the growth rate and significantly higher photosynthetic activity under saline conditions. SA treatment was also shown to reduce MP-related damage caused by salt stress[42]. Another study demonstrated that the addition of 1 mM SA to soil with salinity ranging from 0 to 150 mM increased the photosynthetic capacity of wheat[68]. Thus, treatment of the soil with SA seems to have a beneficial effect on improving seed germination as well as early and late vegetative growth in diverse plant species cultivated in saline environments. Therefore, SA could be considered a safe and eco-friendly compound that could enhance crop protection and promote crop yield in the agricultural sector. However, a limited number of studies have investigated the advantageous effects of SA on crop production under field conditions.

In this study, the responses of different *Camelina sativa* genotypes to salt stress were evaluated both with and without hormonal treatments. PI-650142 showed the highest RWC, and chlorophyll increase with SA and EbR treatments, while reducing MP and maintaining membrane stability, making it the most tolerant genotype. Without treatment, PI-650142 maintained RWC levels and preserved water retention capacity. Arslanbey displayed moderate tolerance, retaining chlorophyll and carotenoid content and improving FW and DW with treatments. Conversely, PI-304269 showed significant declines across all parameters, including high MP and reduced pigment content, even with treatments, identifying it as the most sensitive genotype. These findings highlight the genotype-specific effects of SA and EbR, with PI-650142 emerging as the most resilient to salt stress. Particularly, the positive response of the PI-650142 genotype to hormonal applications indicates that this genotype is a potential candidate for increasing stress tolerance.

Conclusion

The present study showed that the application of SA to camelina plants exposed to salt stress increased RWC and carotenoid levels as compared to those in the control group. This increase in RWC was observed in all genotypes, while the increase in the carotenoid content was noted only in PI-650142 genotype. Furthermore, the treatment of plants under salt stress with EbR mitigated the negative effects of the stress by improving plant growth, water content and plant survival. EbR treatment increased PWC and the contents of Ch-a, Ch-b and carotenoids in PI-650142 genotype as compared to those in other genotypes. Moreover, all genotypes showed an increase in RWC as compared to the control group.

The combined treatment of SA and EbR can potentially have synergistic effects on the camelina plant exposed to salt stress. The synergistic effects of these compounds could lead to even higher water content in the plant as well as improved growth and survival under salt-stress conditions. However, further studies are required to fully understand the mechanisms and effects of these compounds when applied together. The present study showed that the carotenoid content of the stress-induced plants treated with the EbR + SA combination increased as compared to that of the control group. Moreover, PI-650142 genotype showed an increase in DW and Ch-a and Ch-b contents. The RWC was increased in PI-304269 and PI-650142 genotypes, while the carotenoid content was increased in Arslanbey and PI-650142 genotypes.

In conclusion, SA and EbR have different but complementary effects on the camelina plant exposed to salt stress. When applied together, these compounds have the potential to provide even greater benefits in terms of PWC, growth and plant survival under these stress conditions. Further studies are recommended considering the sensitivity of genotypes with low salt tolerance.

Data availability

Data is provided within the manuscript.

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Author contributions

M.G. confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

Author declares and certify that all the work done in this research is the author's original work and has not been submitted to any other journal for publication and that the paper is not considered for publication elsewhere. All the data in this paper are original and reflect the active contribution of the author and coauthor leading to the manuscript. The experiment complied with relevant institutional, national, and international guidelines and legislation. this material has not been deposited in a publicly available herbarium.

Additional information

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