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Seed Priming with Ascorbic Acid to Ameliorate the Effects of Salinity Stress on Germination and Growth Traits of Rapeseed (*Brassica napus* L.)

Tuzluluk Stresinin Kolza Tohumunun (*Brassica napus* L.) Çimlenme ve Büyüme Özellikleri Üzerindeki Etkilerini Iyileştirmek için Askorbik Asit ile Tohum Hazırlama

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Abstract: This study was conducted to determine the effects of seed priming with ascorbic acid (AsA) on germination and seedling characteristics of rapeseed (*Brassica napus* L.) under different salinity levels. To this end, the study examined five salinity levels (0.20, 5.0, 10.0, 15.0, and 20.0 dS m<sup>-1</sup> NaCl) and four priming doses (control, 0.5, 1.0, and 2.0 mM ascorbic acid). Salt stress negatively affected germination and some plant growth traits, such as radicle length (RL), plumule length (PL), radicle fresh weight (RFW) and plumule fresh weight (PFW), finally germination percentage (FGP), germination index (GI), mean germination time (MGT) and seedling vigor index (SVI). On the other hand, priming with AsA positively affected all parameters except FGP, PFW and salt tolerance index (STI). According to the interaction of salinity stress and priming, the highest values for GI (125.3), MGT (1.63 days), PFW (0.35 g), and STI (139.3%) were obtained at 5.0 dS m<sup>-1</sup> salinity stress and 1.0 mM AsA dose. However, the highest value was obtained for RL (10.6 cm) and SVI (1170.7) at the control treatment and 0.5 mM AsA dose, whereas the highest value was obtained for PL (1.7 cm) at the control treatment and 1.0 mM) had a positive effect on seedling germination and growth traits, while higher concentrations had a toxic effect on germination. Thus, it was concluded that priming rapeseed seeds with an AsA dose of 0.5 mM could reduce the restrictive impact of salinity stress on seed germination and seedling improvement. Further studies on the yield and growth parameters of rapeseed under salt stress by AsA application are recommended.

Keywords: Ascorbic acid, germination traits, rapeseed, salinity

#### &

Öz: Bu çalışma, farklı tuzluluk seviyeleri altında askorbik asit (AsA) ile tohum hazırlamanın kolza tohumlarının (*Brassica napus* L.) çimlenme ve fide özellikleri üzerine etkilerini belirlemek amacıyla yapılmıştır. Bu amaçla, çalışmada beş tuzluluk seviyesi (0.20, 5.0, 10.0, 15.0 ve 20.0 dS m<sup>-1</sup> NaCl) ve dört priming dozu (kontrol, 0.5, 1.0 ve 2.0 mM askorbik asit) incelenmiştir. Tuz stresi çimlenmeyi ve kökçük uzunluğu (RL), sapçık uzunluğu (PL), kökçük taze ağırlığı (RFW), sapçık taze ağırlığı (PFW), nihai çimlenme yüzdesi (FGP), çimlenme indeksi (GI), ortalama çimlenme süresi (MGT) ve vigor indeksi (SVI) gibi bazı çimlenme ve fide büyüme özelliklerini olumsuz etkilemiştir. AsA ile yapılan priming ise FGP, PFW ve tuz tolerans indeksi (STI) dışındaki tüm parametreleri olumlu etkilemiştir. Tuzluluk stresi ve priming etkileşimine göre en yüksek GI (125.3), MGT (1.63 gün), PFW (0.35 g) ve STI (%139.3) değerleri 5.0 dS m<sup>-1</sup> tuzluluk stresi ve 1.0 mM AsA'de elde edilmiştir. Bununla birlikte, RL (10.6 cm) ve SVI (1170.7) için en yüksek değerler kontrol konusu ve 0.5 mM AsA dozunda elde edilirken, PL (1.7 cm) için en yüksek değer kontrol konusu ve 1.0 mM AsA dozunda elde edilmiştir. Düşük dozlarda (0.5 ve 1.0 mM) askorbik asit, çimlenme ve fide büyüme özellikleri üzerinde olumlu bir etkiye sahipken, daha yüksek konsantrasyonlar çimlenme üzerine toksik etki yapmıştır. Bu nedenle kolza tohumlarının 0.5 mM AsA dozu ile ön uygulamaya tabi tutulmasının tuz stresinin tohum çimlenmesi ve fide gelişimi üzerindeki kısıtlayıcı etkisini azaltabileceği sonucuna varılmıştır. Tuz stresi altında AsA uygulamasının kolzanın verim ve gelişme parametrelerine etkileri ile ilgili ileri araştırmaların yapılmaşı önerilmektedir. **Anahtar Kelimeler**: Askorbik asit, cimlenme özellikleri, kolza, tuzluluk

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## INTRODUCTION

Rapeseed (*Brassica napus* L.) belongs to the Brassicaceae family and is the third-largest oil crop worldwide, which plays an essential role in oilseed production (Wang et al., 2023). Recently, the multifunctional use of canola, including as a vegetable, landscaping, and forage, has developed rapidly (Batool et al., 2022). Brassica crops are frequently cultivated in dry and semi-arid environments, where the build-up of significant salt concentrations has a detrimental effect on germination, early seedling development, and yield (Shah et al., 2018). Rapeseed is a moderately salt-tolerant species (Batool et al., 2021). Moreover, early development stage osmotic stress and ionic imbalance caused by salinity stress negatively impacted rapeseed growth, photosynthesis, and nutrient absorption, significantly reducing production.

Salinity is an important abiotic stress factor affecting plant productivity in both irrigated and non-irrigated agricultural lands all over the world (Çirka et al., 2021). Salinity has a negative impact on plant development at all stages. The seed germination phase is the first and most crucial phase of a plant's development. The germination stage of a seed is one of the most responsive to salinity (Wan et al., 2018). For this reason, the salinity tolerance of seeds at the germination stage is critical for growth in saline soils (Ajmal Khan and Gulzar, 2003). Due to an external osmotic potential that limits water uptake, or the deleterious effects of Na<sup>+</sup> and Cl<sup>-</sup> ions, or both, on germinating seeds, seed germination and emergence are reduced under saline conditions (Mahdavi and Rahimi, 2013). With the use of new research techniques, the deleterious effects of salt on plants are being attempted to be improved. Seed priming is one of the methods to promote the germination of seeds in the face of environmental challenges (Fujikura et al., 1993). Priming seeds is an extremely efficient method of increasing germination rates. According to Tanur and Yorgancılar (2020), canola seeds primed with ascorbic acid showed higher germination rates when exposed to increasing doses of salt. Several studies have confirmed the positive effects of seed priming on germination under salt stress (Perveen and Hussain, 2021; Azeem et al., 2023).

To accelerate and improve the uniformity of germination and emergence, seed priming is a popular practise nowadays (Moreno et al., 2018). Seed priming can be defined as the controlled hydration of seeds immersed in a low osmotic potential solution to initiate germination metabolism without prolonging radicals. Seed priming is an inexpensive and easy-to-use method that helps increase germination by regulating enzymatic reactions and water uptake (Ceritoğlu and Erman, 2020). There are many techniques for seed priming, such as hydropriming, halopriming, and Osmo-priming. Many seed priming methods and strategies have been developed with different solutions to improve seed germination under different stress conditions (Mahdy et al., 2020; Ali et al., 2020; Bahrabadi et al., 2022).

When plants are exposed to stress, the production and accumulation of toxic reactive oxygen species, including  $O_2$ ,  $H_2O_2$ , and OH, increases (Mittler, 2002). Plants have evolved various protective mechanisms to prevent oxidative damage caused by salt stress. The increased activity of antioxidant enzymes contributes to the prevention of stress-induced damage by plants (Ahmad et al., 2015). Both enzymatic (catalase (CAT), peroxidases (POD), etc.) and non-enzymatic (ascorbic acid, etc.) antioxidants play an important role in scavenging toxic ROS. Ascorbic acid (AsA) is an important water-soluble compound and a non-enzymatic antioxidant that stimulates plant growth and ameliorates and adequately reduces the harmful effects of excess salt (Azeem et al., 2023). AsA can increase a plant's growth and its ability to withstand stress (Bilska et al., 2019). Numerous studies have reported that plant growth under salt stress improves with the use of AsA (Niu et al., 2022). Ghoohestani et al. (2012) reported that seed preparation with ascorbic acid reduced the negative effects of salinity in tomato seeds, and this was achieved with an ascorbic acid dose of 150 mg L<sup>-1</sup>. AsA seed priming at different concentrations improved seed germination under saline conditions in rapeseed (Molnár et al., 2020), wheat (Baig et al., 2021), barley (Hozayn and Ahmed, 2019), bean (Azooz et al., 2013).

Rapeseed is an important source of vegetable oil worldwide and is moderately salinity tolerant. Therefore, it is very important to determine different applications and dosages that help to improve salt tolerance to increase the yield of rapeseed. However, there are few studies in the literature on the effects of interactions between salt and ascorbic acid on germination and growth characteristics of rapeseed. Therefore, this study



was conducted to investigate the effects of seed treatment with ascorbic acid on germination of rapeseed under salt stress conditions.

## MATERIAL AND METHOD

To evaluate the effect of seed priming with ascorbic acid on the germination of rapeseed under salt stress, laboratory experiments were conducted in February 2023 at Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, Turkey. The winter rapeseed cultivar United Genetic-Es Neptune was used as material.

## Treatments and Experimental Design

The experiments were carried out in sterilized petri dishes under controlled laboratory conditions. The experiment was arranged in a completely randomized design with a factorial arrangement with three replications. In this study, 0.20 (control), 5.0, 10.0, 15.0 and 20.0 dS m<sup>-1</sup> NaCl solution and 0 (control), 0.5, 1.0 and 2.0 mM ascorbic acid (AsA) concentrations were used.

The seeds of rapeseed (*Brassica napus* L.) were surface sterilized in a 5% NaOCl (sodium hypochlorite) solution for 10 min and then washed five times with distilled water. The surface-sterilized seeds were placed in AsA solutions of various concentrations for 10 hours for priming and then dried on filter paper at room temperature for 24 hours until they regained their original moisture content. Twenty seed samples primed with different concentrations of each ascorbic acid were placed in sterile 9-cm standard glass petri dishes on filter paper, and 10 ml of five different salt concentrations were added. Petri dishes were covered and incubated at 20°C with a 16-hour photoperiod in an incubator. The filter papers were changed every two days, and 10 ml of saline solutions was added. The seeds were considered germinated when the radicle emerged. The number of germinated seeds in each petri dish was counted for seven days, starting on the second day at every 24 h.

## **Calculated Germination Indices**

After the 7th day, germinated seeds were taken out from the petri dishes, and final germination percentage and some other primary germination and seedling growth properties such as germination index, mean germination time, radicle length, plumule length, radicle fresh weight and plumule fresh weight were determined. 10 random seedlings were used to measure radicle length, plumule length, radicle fresh weight and plumule fresh weight. The vigor index was calculated using the obtained radicle and plumule lengths. (Abdul-Baki and Anderson, 1970).

The finally germination percentage, germination index and mean germination time were calculated by the following equations;

Finally Germination percentage (FGP) = 
$$\frac{Number \ of \ germinated \ seeds}{Total \ number \ of \ seed} x100$$
 (1)

$$Germination Index (GI) = \frac{Germination \% in each treatment}{Germination \% in the control} x100$$
(2)

Mean germination time (MGT) = 
$$\frac{\sum (N_i T_i)}{\sum N_i}$$
 (3)

Where N<sub>i</sub> is the number of germinated seeds on day T<sub>i</sub>, and T<sub>i</sub> is the number of days counted from the beginning of the germination test.

Using the equation from Fathi and Gaafar (2015), the salt tolerance index (STI) was calculated.

Salt tolerance index (%) = 
$$\left(\frac{TFW \text{ at } S_x}{TFW \text{ at } S_0}\right) x100$$
 (4)

Where TFW, total fresh weight, S<sub>0</sub>; control, S<sub>x</sub>; a given concentration out of four concentration.

To calculate germination indices, germinated seeds were counted daily. Measurements were conducted on ten randomly selected seedlings in each petri dish. A millimeter ruler was used to measure the radicle and plumule lengths accurately. Then, these two pieces were weighed to determine fresh weight. Following that, they were carefully weighed using a sensitive balance of 0.001.

### Statistical Analysis

All statistical analyses were performed using the JMP (SAS institute). For each group classification, the mean value, the standard deviation (SD), the minimum, and the maximum were calculated. Two-way analysis of variance (two-way ANOVA) and least significant difference (LSD) tests for pair-wise multiple comparisons was used for statistical analysis of the data. The statistical significance was defined at (p < .05).

### **RESULTS AND DISCUSSION**

The results of the analysis of the variance of germination traits of rapeseed under salinity stress and ascorbic acid priming treatments are shown in Table 1, and the results of seedling growth traits are shown in Table 2. The germination index (GI) and mean germination time (MGT) were significantly affected by both salinity and priming treatments. However, finally germination percentage (FGP) was significantly affected only by salinity treatments but not by priming treatments (Table 1). The interaction between priming and salinity was significant for GI (p<.01) and MGT (p<.001).

Treatment	df	FGP (%)		GI		MGT (day)		
Treatment		Mean Square	LSD0.05	Mean Square	LSD0.05	Mean Square	LSD0.05	
Salinity	4	515.625***	3.283	3596.933***	3.847	4.151***	0.013	
Priming	3	33.889 n.s.	2.937	122.728**	3.440	0.092*	0.115	
Salinity * Priming	12	25.903 n.s.	6.566	80.978**	7.693	0.111***	0.257	
Error	40	15.83		21.73		0.02		
CV (%)			4.381		4.562		6.487	

**Table 1.** Analysis of variance on the impact of salinity and ascorbic acid on germination properties of rapeseed. *Çizelge 1. Tuzluluk ve askorbik asidin kolza'nın çimlenme özellikleri üzerindeki etkisine ilişkin varyans analizi.* 

\*\*\*:p < .0001, \*\*: p < .01, \*:p < .05, n.s.: non-significance.

Table 2 shows that salinity significantly affected all growth traits (p<.001). In addition, RL, PL, RFW and SVI were significantly affected by the priming treatments. Finally, the interaction between priming and salinity was significant for all seedling growth traits studied (p<.001).

Seed priming with ascorbic acid to ameliorate the effects of salinity stress on germination and growth traits of rapeseed (Brassica napus L.)

<b>Table 2.</b> Analysis of variance on the impact of salinity and ascorbic acid on seedling growth of rapeseed.
Çizelge 2. Tuzluluk ve askorbik asidin kolza'nın fide büyümesi üzerindeki etkisine ilişkin varyans analizi.

		RL	_	Р	PL		RFW		PFW	
Treatment	df	Mean	LSD	Mean	LSD	Mean		Mean		
		Square	0.05	Square	0.05	Square	LSD0.05	Square	LSD0.05	
Salinity	4	250.009***	0.100	6.741***	0.031	0.015***	0.002	0.076***	0.006	
Priming	3	0.397***	0.100	0.011**	0.028	0.000017**	0.002	0.00002 n.s.	0.005	
Salinity*										
Priming	12	0.363***	0.199	0.015***	0.062	0.00007***	0.004	0.0008***	0.011	
Error	40	0.015		0.001		0.000001		0.00005		
CV (%)			2.152		3.331		4.076		2.788	

\*\*\*:p < .0001, \*\*: p < .01, \*:p < .05, n.s.: non-significance.

**Table 2.** (cont.) Analysis of variance on the impact of salinity and ascorbic acid on seedling growth of rapeseed.

 *Çizelge 2.* (devamı) Tuzluluk ve askorbik asidin kolza'nın fide büyümesi üzerindeki etkisine ilişkin varyans analizi.

Treatment	df	SVI		STI		
Treatment	u	Mean Square	LSD0.05	Mean Square	LSD0.05	
Salinity	4	3267844***	24.035	13984.2***	1.623	
Priming	3	4963**	21.498	2.97 n.s.	1.451	
Salinity * Priming	12	5151***	48.071	86.46***	3.245	
Error	40	33943		3.87		
CV (%)			4.564		2.143	

\*\*\*:p < .0001, \*\*: p < .01, \*:p < .05, n.s.: non-significance.

The comparison of mean values of germination and seedling growth traits of rapeseed under five different salinity stress levels and priming treatment with four different levels of ascorbic acid (AsA) is presented in Table 3.

<b>Table 3.</b> Effects of salinity and ascorbic acid treatments on germination properties and seedling growth of rapeseed.	
Çizelge 3. Tuzluluk ve askorbik asit uygulamalarının kolza tohumunun çimlenme özellikleri ve fide büyümesi üzerine etkileri.	

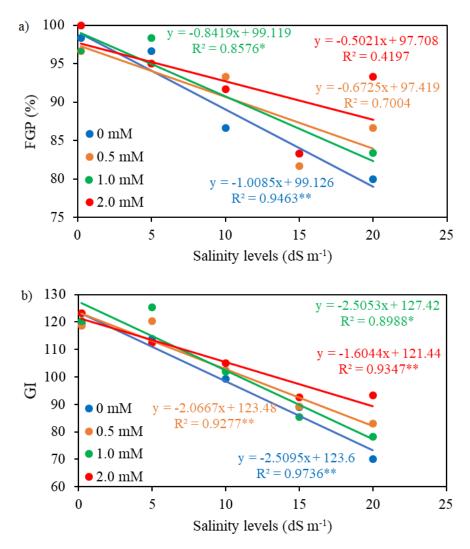
<u> </u>	$\mathbf{ECD}(0/)$	CI	$MCT(1, \cdot)$	DI (and)	<b>DI</b> (	$\mathbf{DEM}(\cdot)$		CVI	CTT (0/)
Treatment	FGP (%)	GI	MGT (day)	RL (cm)	PL (cm)	RFW (g)	PFW (g)	SVI	STI (%)
Salinity (dS m <sup>-1</sup> )									
0.20	97.92a	120.50a	1.85d	10.258a	2.22a	0.084b	0.245c	1221.96a	100.00c
5.0	96.67a	118.00a	1.90d	9.897b	1.58b	0.098a	0.325a	1110.33b	130.74a
10.0	90.83b	102.17b	2.37c	5.875c	0.74c	0.055c	0.300b	601.12c	109.49b
15.0	82.92c	89.08c	2.63b	1.642d	0.56d	0.032d	0.209d	182.33d	74.74d
20.0	85.83c	81.17d	3.28a	0.363e	0.52e	0.014e	0.124e	75.70e	42.59e
AsA (mM)									
0	89.00	98.40b	2.51a	5.473c	1.09c	0.057a	0.242	616.69c	92.41
0.5	91.00	102.73a	2.38b	5.835a	1.14ab	0.057a	0.239	660.00a	91.54
1.0	90.67	102.27a	2.4ab	5.611b	1.15a	0.055b	0.240	643.45ab	91.28
2.0	92.67	105.33a	2.33b	5.509c	1.11bc	0.057a	0.241	633.00bc	92.28

Different letters within a column indicate significant differences.

FGP decreased significantly with increasing salinity, decreasing by 15.32% at 15.0 dS m<sup>-1</sup> compared with the control (Table 3). A relative increase was also observed for FGP at 20.0 dS m<sup>-1</sup>. However, no statistically significant difference was observed for FGP between 0.20 and 5.0 dS m<sup>-1</sup> and between 15.0 and 20.0 dS m<sup>-1</sup>.

The decrease in germination percentage with increasing salinity was due to the negative effects of salinity on physiological processes (Shahzad et al., 2019). However, the effects of priming with AsA and the interactions between salinity and priming on the FGP trait were not statistically significant (Figure 1a).

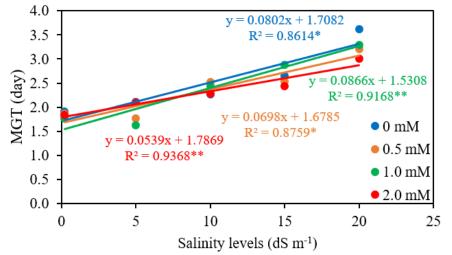
Increasing salinity significantly reduced GI (Table 1). At GI, no statistically significant difference was found between 0.20 and 5.0 dS m<sup>-1</sup>. At 20.0 dS m<sup>-1</sup>, a decrease of up to 33% was observed compared with the control. When the salinity × AsA interaction was examined, GI values of plants under salinity stress were significantly affected by AsA (Figure 1b). The highest GI value (125.33) was observed at 5.0 dS m<sup>-1</sup> and 1.0 mM AsA interaction, while the lowest GI value (70.0) was observed at 20.0 dS m<sup>-1</sup> salinity and 0 mM AsA interaction (Figure 1b). At 5.0 dS m<sup>-1</sup> salinity level, the application of 1.0 mM AsA resulted in a 9% increase in GI value compared to the control. However, when 2.0 mM AsA doses were applied at the highest salinity, the value of GI increased by 25% compared with the control. These results indicated that priming with AsA could reduce the negative effects of salinity stress in relation to GI. Baig et al. (2021) found that the GI value of wheat seeds significantly increased with AsA treatments under salinity conditions. These results agree well with those of Tanur and Yorganclar (2020). A high GI indicates that the strength of the seed is high (Maguire, 1962). This result means that treatments with AsA showed earlier and more synchronized germination than non-primed seeds (Baig et al., 2021).



**Figure 1.** Impact of different salinity levels on primed rapeseed GI and FGP (\*\* p < .01, \*p<.05). *Şekil 1. Farklı tuzluluk seviyelerinin astarlanmış kolza tohumunun GI ve FGP üzerindeki etkisi* (\*\* p < .01, \*p<.05).

GI was more affected by salinity than FGP. GI decreased by almost 42% at 20.0 dS m<sup>-1</sup>, while FGP decreased by only 19% (Figure 1). An important reason for the higher sensitivity of GI to salinity than FGP could be the damage caused by salinity stress to the enzymes involved in germination (Feghhenabi et al., 2020). Flowers (1972) reported that 0.33 m NaCl inhibited malate and glucose-6-phosphate dehydrogenase activities by up to 66% and 60%, respectively. On the other hand, El-Hawary et al. (2023) reported that treatment of seeds with AsA improved dehydrogenase and  $\alpha$ -amylase activities compared with the control. The enzyme amylase in seeds mainly serves to initiate seed germination and activate seedling growth (Li et al., 2017).

Increasing salinity stress resulted in a significant prolongation of MGT (Table 3). Compared to the control (0.20 dS m<sup>-1</sup>), an increase in salinity stress to 20.0 dS m<sup>-1</sup> prolonged the germination time by 77%. Gulzar and Khan, (2001) reported that increases in salinity generally delayed seed germination. Priming treatments also contributed to the shortening of germination time compared to the control. Considering the salinity × AsA interaction, at low salinity levels (0.2 and 5.0 dS m<sup>-1</sup>), the average germination time of the treatments with 1.0 mM AsA dose was shorter than the control, while at other salinity levels, 2.0 mM dose significantly shortened the average germination time. The shortest MGT was 1.63 days at 5.0 dS m<sup>-1</sup> salinity and 1.0 mM AsA dose, whereas the longest MGT was obtained at 20.0 dS m<sup>-1</sup> salinity and 0 mM AsA dose (Figure 2). These results indicated that priming treatments applied under salinity conditions contributed significantly to reducing germination time. Seeds primed with different doses of AsA improved germination rate and uniformity with significantly higher GI and shorter MGT without affecting FGP compared to non-primed seeds.

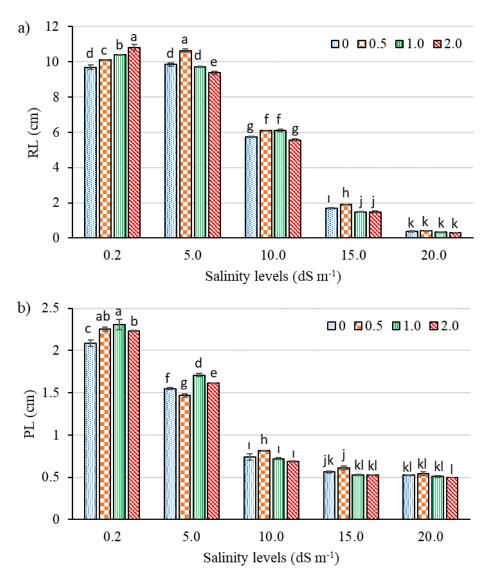


**Figure 2.** Impact of different salinity levels on primed rapeseed MGT (\*\* p < .01, \* p<.05). *Şekil 2. Farklı tuzluluk seviyelerinin astarlanmış kolza tohumunun MGT üzerindeki etkisi* (\*\* p < .01, \* p<.05).

In the study, the values of RL, PL, RFW, PFW, SVI, and STI decreased significantly with increasing salinity. This could be due to the inhibition of water uptake by the saline conditions (Abdollahi and Jafari, 2012). This can also be caused by the toxic effect of Na<sup>+</sup> and Cl<sup>-</sup> ions on germination (Khajeh-Hosseini et al., 2003). At the highest salinity, the values of RL and PL decreased by 96.5% and 76.6%, respectively, compared to the control. Jamil et al (2006) reported that RL and PL are two important parameters in determining the sensitivity of seeds to salinity stress. Decreased root and stem growth with increasing salt stress may be attributed to ion toxicity and disproportionate nutrient uptake by seedlings (Bybordi and Tabatabaei, 2009). Several studies have reported that increasing salinity stress significantly reduces nutrient uptake and root and stem growth rates (Ajmal Khan and Gulzar, 2003). However, priming treatments with AsA significantly improved RL and PL. The highest improvement was observed at AsA doses of 0.5 mM for RL and 1.0 mM for PL. The higher AsA dose (2.0 mM) was toxic for both parameters. Regarding the interaction between salinity and priming, the effect of salinity on primed seeds was lower than the effect on non-



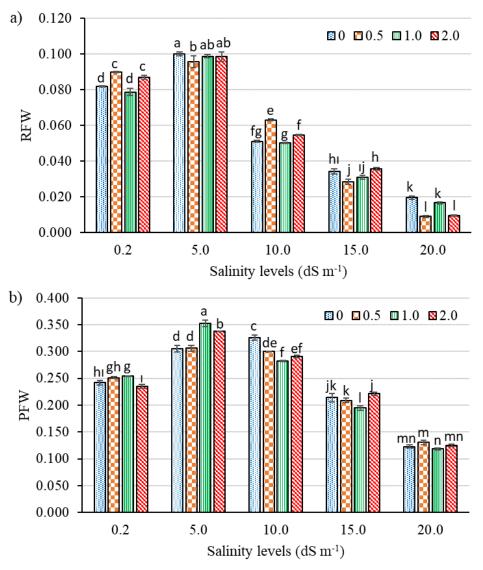
primed seeds. The results also showed that radicle length was more responsive to salinity stress than plumule length (Figure 3a, Figure 3b). In conclusion, low concentrations of AsA (0.5 mM) had a more beneficial effect on seedling growth than high concentrations. In general, treatment with AsA improved the growth characteristics of seedlings under salt stress by reducing oxidative stress.



**Figure 3.** Impact of different salinity levels on primed rapeseed's RL and PL. *Şekil 3. Farklı tuzluluk seviyelerinin astarlanmış kolza tohumunun RL ve PL üzerindeki etkisi.* 

RFW and PFW values increased by 16.7% and 32.7%, respectively, at salinity level 5.0 dS m<sup>-1</sup> and decreased significantly at higher salinities (Table 3). Priming treatments with different doses of AsA had no significant effect on RFW and PFW traits. The interaction between salinity stress and ascorbic acid on RFW and PFW traits of rapeseed seedlings was highly significant (p<.001) (Table 2). The results show that all priming treatments improved RFW at a salt concentration of 5.0 dS m<sup>-1</sup> but not at higher NaCl concentrations. Similarly, PFW increased at all priming concentrations at 5.0 dS m<sup>-1</sup> compared to control salinity (0.20 dS m<sup>-1</sup>), but decreased at other higher salt concentrations (Figure 4). It can be said that osmotic stress due to salinity stress causes this situation by preventing cell division and proliferation (Sezer et al., 2021). Anaya et al. (2018) reported that increasing salinity significantly decreased the fresh weight of broad bean

seedlings compared to the control, while 0.5 mM salicylic acid treatment improved fresh weight compared to salinity treatment.



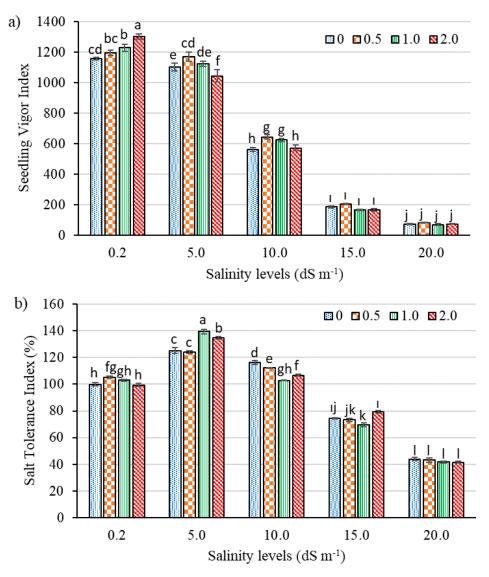
**Figure 4.** Impact of different salinity levels on primed rapeseed's RFW and PFW. *Şekil 4. Farklı tuzluluk seviyelerinin astarlanmış kolza tohumunun RFW ve PFW üzerindeki etkisi.* 

The increase in salinity from 5.0 to 20.0 dS m<sup>-1</sup> resulted in a gradual decrease in SVI (Figure 5a). The highest SVI value was obtained at the control, while the lowest SVI mean value (75.70) was obtained at the highest salt concentration of 20 dS m<sup>-1</sup>. Kandil et al. (2015) reported that the vigor index of soybean seeds gradually decreased with increasing salinity levels. However, priming treatments with AsA resulted in significant improvement in seedling vigor (SVI). The highest improvement was obtained at 0.5 mM AsA concentration. It tended to decrease again with increasing AsA concentration (Table 3). Regarding salinity × AsA interactions, the highest SVI value (1303.95) was observed at control salinity and 0.5 mM AsA interaction, while the lowest SVI value (75.15) was observed at 20.0 dS m<sup>-1</sup> salinity and 1.0 mM AsA interaction (Figure 5a). Under salinity stress conditions, the highest SVI value (1170.74) was obtained from 0.5 mM dose AsA and 5.0 dS m<sup>-1</sup> treatments. At 5.0 dS m<sup>-1</sup> salinity, 0.5 mM AsA treatment increased SVI by 6.2% compared to control treatments, while at 20.0 dS m<sup>-1</sup> the increase was 14.5% compared to control. These results indicate that priming treatments on rapeseed seeds alleviated the negative effects of not very high salt stress on SVI compared to the control treatment. However, the effects of priming treatments on



seed vigor traits such as GI and SVI were more significant than their effects on FGP. This suggests that seed vigor, rather than the amount of germinating seeds under salt stress, is improved by priming with AsA (Chen et al., 2021). According to Chen et al. (2021), priming sorghum seeds with CaCl<sub>2</sub> improved seed vigor, which in turn contributed to the development of vigorous seedlings under salt stress.

Figure 5b shows the effect of different salinity concentrations on the STI of rapeseed seedlings for seeds primed with AsA. The STI value initially increased relatively with increasing salinity stress (at 5.0 dS m<sup>-1</sup>) and decreased at higher salinity levels. The highest STI value was observed at 5.0 dS m<sup>-1</sup>, and the lowest STI value was at 20.0 dS m<sup>-1</sup>. Kiremit et al. (2017) investigated the germination characteristics of flax seeds under different salt concentrations and determined that salt tolerance indices decreased with increasing salt concentration. The effect of priming treatments on STI value was not statistically significant. When the interaction between salinity and priming was analyzed, the highest value for STI was 130.74% at a salt concentration of 5.0 dS m<sup>-1</sup> and an AsA dose of 1.0 mM (Figure 5b). The lowest value was 41.46% when treated with 20.0 dS m<sup>-1</sup> x 2.0 mM AsA. Accordingly, when the salinity level was increased from 5.0 dS m<sup>-1</sup> to 20.0 dS m<sup>-1</sup>, the salt tolerance index of rapeseed seeds decreased by 93.0%. Overall, applying AsA at a dose of 0.5 mM increased the STI at all salinity levels for the canola preparation. As a result, seeds primed with 0.5 mM AsA produced seedlings with better tolerance to salt stress than the control. This finding is consistent with those of Mahdy et al. (2020).



**Figure 5.** Impact of different salinity levels on primed rapeseed's SVI and STI. *Şekil 5. Farklı tuzluluk seviyelerinin astarlanmış kolza tohumunun SVI ve STI üzerindeki etkisi.* 

In general, germination and growth traits were found to be negatively affected by increasing salt concentration. 5.0 dS m<sup>-1</sup> NaCl concentration did not cause significant damage to seedling germination and growth traits but contributed to the improvement of some traits (PFW, RFW and STI). However, when the salinity stress increased to 10.0 dS m<sup>-1</sup>, most of the investigated traits were significantly reduced.

## CONCLUSION

Considering the general results of this study, salt stress was found to have an inhibitory effect on germination and some growth characteristics of rapeseed seeds. However, it was found that priming seeds with AsA improved seed germination and growth characteristics at different levels of salt stress. It was found that priming with AsA at appropriate doses improved seed growth traits, such as radicle and leaflet length, fresh weight, and seedling vigor index of rapeseed seeds. It was also concluded that priming could lead to the better and faster seedling establishment at the early stage, thus increasing the plant's tolerance to various stress conditions. Based on the results of this study, it is recommended to prime rapeseed with low AsA concentrations (0.50 mM) under low to high salinity stress conditions. However, to confirm the results of the present study and to investigate the effects of AsA application on yield and growth parameters of rapeseed under salt stress, field-level experiments are strongly recommended.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

## DECLARATION OF AUTHOR CONTRIBUTION

Conceptualization, methodology, data acquisition, visualization, writing original draft writing review, and editing by author Sevda Taşan.

## REFERENCES

- Abdollahi, F., & Jafari, L. (2012). Effect of NaCl and KNO<sub>3</sub> priming on seed germination of canola (*Brassica napus* L.) under salinity conditions. *International Journal of Agriculture, Research and Review*, 2(5), 573-579.
- Abdul-Baki, A. A., & Anderson, J. D. (1970). Viability and Leaching of Sugars from Germinating Barley 1. *Crop science*, 10(1), 31-34.
- Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., & Gucel, S. (2015). Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Frontiers in Plant Science*, 6, 868. https://doi.org/10.3389/fpls.2015.00868.
- Ajmal Khan, M., & Gulzar, S. (2003). Germination responses of Sporobolus ioclados: a saline desert grass. Journal of Arid Environments, 53(3), 387-394. https://doi.org/10.1006/jare.2002.1045.
- Ali, L. G., Nulit, R., Ibrahim, M. H., & Yien, C. Y. S. (2020). Enhancement of germination and early seedling growth of rice (*Oryza sativa*) var. FARO44 by seed priming under normal and drought stressed conditions. *Journal of Plant Nutrition*, 43(11), 1579-1593. https://doi.org/10.1080/01904167.2020.1739298.
- Anaya, F., Fghire, R., Wahbi, S., & Loutfi, K. (2018). Influence of salicylic acid on seed germination of *Vicia faba* L. under salt stress. *Journal of the Saudi Society of Agricultural Sciences*, 17(1), 1-8. https://doi.org/10.1016/j.jssas.2015.10.002.
- Azeem, M., Sultana, R., Mahmood, A., Qasim, M., Siddiqui, Z. S., Mumtaz, S., Javed, T., Umar, M., Adnan, M. Y., & Siddiqui, M. H. (2023). Ascorbic and Salicylic Acids Vitalized Growth, Biochemical Responses, Antioxidant Enzymes, Photosynthetic Efficiency, and Ionic Regulation to Alleviate Salinity Stress in Sorghum bicolor. *Journal of Plant Growth Regulation*, 1-14. https://doi.org/10.1007/s00344-023-10907-2.
- Azooz, M. M., Alzahrani, A. M., & Youssef, M. M. (2013). The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*'Vicia faba*'L.). *Australian Journal of Crop Science*, 7(13), 2091-2100.
- Bahrabadi, E., Tavakkol Afshari, R., Mahallati, M. N., & Seyyedi, S. M. (2022). Abscisic, gibberellic, and salicylic acids effects on germination indices of corn under salinity and drought stresses. *Journal of Crop Improvement*, 36(1), 73-89. https://doi.org/10.1080/15427528.2021.1908474.
- Baig, Z., Khan, N., Sahar, S., Sattar, S., & Zehra, R. (2021). Effects of seed priming with ascorbic acid to mitigate salinity stress on three wheat (*Triticum aestivum* L.) cultivars. *Acta Ecologica Sinica*, 41(5), 491-498. https://doi.org/10.1016/j.chnaes.2021.08.010.
- Batool, M., El-Badri, A. M., Hassan, M. U., Haiyun, Y., Chunyun, W., Zhenkun, Y., Jie, K., Wang, B., & Zhou, G. (2022). Drought stress in *Brassica napus*: effects, tolerance mechanisms, and management strategies. *Journal of Plant Growth Regulation*, 42, 21–45. https://doi.org/10.1007/s00344-021-10542-9.



- Batool, N., Noor, T., Ilyas, N., & Shahzad, A. (2021). Salt stress impacts on seed germination and seedling growth of Brassica napus L. Pure and Applied Biology, 4(3), 398-406. http://dx.doi.org/10.19045/bspab.2015.43016.
- Bilska, K., Wojciechowska, N., Alipour, S., & Kalemba, E. M. (2019). Ascorbic acid The little-known antioxidant in woody plants. *Antioxidants*, 8(12), 645. https://doi.org/10.3390/antiox8120645.
- Bybordi, A., & Tabatabaei, J. (2009). Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2), 71-76. https://doi.org/10.15835/nbha3723299.
- Ceritoğlu, M., & Erman, M. (2020). Mitigation of salinity stress on chickpea germination by salicylic acid priming. International Journal of Agriculture and Wildlife Science, 6(3), 582-591. https://doi.org/10.24180/ijaws.774969.
- Chen, X., Zhang, R., Xing, Y., Jiang, B., Li, B., Xu, X., & Zhou, Y. (2021). The efficacy of different seed priming agents for promoting sorghum germination under salt stress. *PloS one*, 16(1), https://doi.org/10.1371/journal.pone.0245505.
- Çirka, M., Kaya, A. R., & Eryiğit, T. (2021). Influence of temperature and salinity stress on seed germination and seedling growth of soybean (*Glycine max L.*). Legume Research, 44(9), 1053-1059. doi: 10.18805/LR-628.
- El-Hawary, M. M., Hashem, O. S. M., & Hasanuzzaman, M. (2023). Seed Priming and Foliar Application with Ascorbic Acid and Salicylic Acid Mitigate Salt Stress in Wheat. Agronomy, 13(2), 493. https://doi.org/10.3390/agronomy13020493.
- Fathi, N. O., & Gaafar, A. A. (2015). Growth Performance and Chemical Composition of Corn Seedlings (*Zea mays* L.) Under Salt Stress and Priming Conditions. *Alexandria Science Exchange Journal*, 36(3), 226–235. doi: 10.21608/asejaiqjsae.2015.2905.
- Feghhenabi, F., Hadi, H., Khodaverdiloo, H., & Van Genuchten, M. T. (2020). Seed priming alleviated salinity stress during germination and emergence of wheat (*Triticum aestivum L.*). Agricultural Water Management, 231, https://doi.org/10.1016/j.agwat.2020.106022.
- Flowers, T. J. (1972). The effect of sodium chloride on enzyme activities from four halophyte species of chenopodiaceae. *Phytochemistry*, 11(6), 1881-1886. https://doi.org/10.1016/S0031-9422(00)90147-X.
- Fujikura, Y., Kraak, H. L., Basra, A. S., & Karssen, C. M. (1993). Hydropriming, a simple and inexpensive priming method. Seed Science and Technology, 21(3), 639-642.
- Ghoohestani, A., Gheisary, H., Zahedi, S. M., & Dolatkhahi, A. (2012). Effect of seed priming of tomato with salicylic acid, ascorbic acid and hydrogen peroxideon germination and plantlet growth in saline conditions. *International Journal of Agronomy Plant Production*, 3, 700-704.
- Gulzar, S., & Khan, M. A. (2001). Seed germination of a halophytic grass *Aeluropus lagopoides*. *Annals of Botany*, 87(3), 319–324. https://doi.org/10.1006/anbo.2000.1336.
- Hozayn, M., & Ahmed, A. A. (2019). Effect of magneto-priming by tryptophan and ascorbic acid on germination attributes of barley (*Hordeum vulgare*, L.) under salinity stress. *EurAsian Journal of BioSciences*, 13(1), 245-251.
- Jamil, M., Deog Bae, L., Kwang Yong, J., Ashraf, M., Sheong Chun, L., & Eui Shik, R. (2006). Effect of salt (NaCl) stress on germination and early seedling growth of four vegetable species. *Journal of Central European Agriculture*, 7(2), 273–282. https://doi.org/10.5513/jcea.v7i2.370.
- Kandil, A. A., Sharief, A. E., & Ahmed, Kh. R. (2015). Performance of some soybean Glycine max (L.) Merrill. cultivars under salinity stress to germination characters. *International Journal of Agronomy and Agricultural Research*, 6(3), 48-56.
- Khajeh-Hosseini, M., Powell, A. A., & Bingham, I. J. (2003). The interaction between salinity stress and seed vigour during germination of soyabean seeds. *Seed Science and Technology*, 31(3), 715-725. https://doi.org/10.15258/sst.2003.31.3.20.
- Kiremit, M. S., Hacıkamiloğlu, M. S., Arslan, H., & Kurt, O. (2017). The effects of different irrigation water salinity levels on germination and early seedling development of flax (*Linum usitatissimum L.*). Anadolu Tarım Bilimleri Dergisi, 32(3), 350-357.
- Li, Z., Xu, J., Gao, Y., Wang, C., Guo, G., Luo, Y., Huang, Y., Hu, W., Sheteiwy, M. S., Guan, Y., & Hu, J. (2017). The synergistic priming effect of exogenous salicylic acid and H<sub>2</sub>O<sub>2</sub> on chilling tolerance enhancement during maize (*Zea mays* L.) seed germination. *Frontiers in Plant Science*, 8, 1153. https://doi.org/10.3389/fpls.2017.01153.
- Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, *2*, 176-177.
- Mahdavi, B., & Rahimi, A. (2013). Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *EurAsian Journal of BioSciences*, 7, 69-76. http://dx.doi.org/10.5053/ejobios.2013.7.0.9.
- Mahdy, A. M., Sherif, F. K., Elkhatib, E. A., Fathi, N. O., & Ahmed, M. H. (2020). Seed priming in nanoparticles of water treatment residual can increase the germination and growth of cucumber seedling under salinity stress. *Journal of Plant Nutrition*, 43(12), 1862-1874. https://doi.org/10.1080/01904167.2020.1750647.



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- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410. https://doi.org/10.1016/S1360-1385(02)02312-9.
- Molnár, K., Biró-Janka, B., Nyárádi, I. I., Fodorpataki, L., Varga, B. E., Bálint, J., & Duda, M. M. (2020). Effects of Priming with Ascorbic Acid, L-Cystein and Triacontanol on Germination of Rapeseed (*Brassica napus L.*). Acta Biologica Marisiensis, 3(2), 48-55. https://doi.org/10.2478/abmj-2020-0010.
- Moreno, C., Seal, C. E., & Papenbrock, J. (2018). Seed priming improves germination in saline conditions for Chenopodium quinoa and Amaranthus caudatus. Journal of Agronomy and Crop Science, 204(1), 40-48. https://doi.org/10.1111/jac.12242.
- Niu, J., Chen, Z., Yu, S., & Wang, Q. (2022). Ascorbic acid regulates nitrogen, energy, and gas exchange metabolisms of alfalfa in response to high-nitrate stress. *Environmental Science and Pollution Research*, 29, 24085–24097. https://doi.org/10.1007/s11356-021-17672-3.
- Perveen, S., & Hussain, S. A. (2021). Methionine-induced changes in growth, glycinebetaine, ascorbic acid, total soluble proteins and anthocyanin contents of two Zea mays L. varieties under salt stress. Journal of Animal & Plant Sciences, 31(1), 131-142.
- Sezer, I., Kiremit, M. S., Öztürk, E., Subrata, B. A. G., Osman, H. M., Akay, H., & Arslan, H. (2021). Role of melatonin in improving leaf mineral content and growth of sweet corn seedlings under different soil salinity levels. *Scientia Horticulturae*, 288, 110376. https://doi.org/10.1016/j.scienta.2021.110376.
- Shah, N., Anwar, S., Xu, J., Hou, Z., Salah, A., Khan, S., Gong, J., Shang, Z., Qian, L., & Zhang, C. (2018). The response of transgenic *Brassica* species to salt stress: a review. *Biotechnology Letters*, 40, 1159-1165. https://doi.org/10.1007/s10529-018-2570-z.
- Shahzad, B., Fahad, S., Tanveer, M., Saud, S., & Khan, I. A. (2019). Plant responses and tolerance to salt stress. In *Approaches for enhancing abiotic stress tolerance in plants* (pp. 61-78). CRC Press.
- Tanur, M. & Yorgancılar, M. (2020). Tuz Stresine Maruz Bırakılan Kanola (Brassica napus L.)'da Priming Uygulamalarının (Salisilik Asit ve Askorbik Asit) Çimlenme Üzerine Etkisi . Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 10 (4), 3109-3121. https://doi.org/10.21597/jist.757788.
- Wan, H., Wei, Y., Qian, J., Gao, Y., Wen, J., Yi, B., Ma, C., Tu, J., Fu, T., & Shen, J. (2018). Association mapping of salt tolerance traits at germination stage of rapeseed (*Brassica napus* L.). *Euphytica*, 214(190). https://doi.org/10.1007/s10681-018-2272-6.
- Wang, Z., Song, L., Wang, C., Guo, M., El-Badri, A. M., Batool, M., Kuai, J., Wang, J., Wang, B., & Zhou, G. (2023). Rapeseed-maize double-cropping with high biomass and high economic benefits is a soil environment-friendly forage production mode in the Yangtze River Basin. *European Journal of Agronomy*, 142, 126675. https://doi.org/10.1016/j.eja.2022.126675.

