Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS
(2022) 36 (3), 349-355
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2022.044

Importance of Priming Application Times on Growth, Relative Water Content and Photosynthetic Pigments of Rapeseed (*Brassica napus* ssp. *oleifera* L.) Cultivars Under Salinity Stress

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ARTICLE INFO

Article history:
Received date: 11.05.2022
Accepted date: 23.08.2022

Keywords:
Carotenoid
Chitosan
Chlorophyll
Germination
Time of priming application

ABSTRACT

Environmental stress factors affect plant production more and more every day. One of these stress factors is salinity. The use of biostimulants is increasing day by day and gaining importance in order to reduce the effects of stress factors and increase the yield and quality in plant production. Chitosan (Ch) is one of the biostimulants whose use in agriculture is increasing day by day. Seeds of rapeseed cultivars were used in this study, and it is an important oil plant. In this study, the times of priming applications with Ch (3 times) [0 (control) (Ch1), 12 hour (Ch2), 24 hour (Ch3)] and different doses of salt stress (S) [0 (control) (S1), 50 mM L^{-1} (S2), 100mM L^{-1} (S3)] in rapeseed cultivars (NK Caravel (C1), Elvis (C2), Champlain (C3) under laboratory conditions were investigated. Germination percentage (GP), seedling length (SL), root length (RL), seedling fresh weight (SFW), root fresh weight (RFW), relative water content (RWC), total chlorophyll (Total Chl), carotenoid (Crt) parameters were examined. As a result of the research, with Ch applications, GP (84.67% to 86.67), SL (7.83 cm to 8.12), RL (6.42 cm to 6.50), SFW (0.10 g to 0.53), RFW (0.02 g to 0.06), RWC (62.84% to 63.30), Total Chl (1.60 mg g^{-1} to 1.90), and Crt (1.60 mg g^{-1} to 1.89) has increased. It has been determined that Ch application times play an important role in reducing salt stress in the investigated parameters.

1. Introduction

Salinity is becoming one of the most important stress factor all over the world (Gürsoy 2020; Mushtaq et al. 2021; Gürsoy 2022a). The salinity causes adverse effects of physiological parameters and a decrease in the yield of a crop (Zahra et al. 2018; Iqbal et al. 2019). Crops can be exposed to salt stress at all stages of development from germination to maturity, but stress is known to be more sensitive for many plant species during the germination and early seedling growth phase (Ali et al. 2020). Today, seed priming methods are widely used to increase the tolerance of plant varieties against abiotic stresses (Lal et al. 2018). Seed priming is an alternative, inexpensive and feasible technique as compared with other agronomical applications for mitigate salt stress (Elsiddig et al. 2022). Rapid and uniform germination is of vital importance in plant production, and it can affect the viability of seedlings as well as yield and quality (Palve et al. 2022). Today, the application of some biopolymers such as chitosan, which has many advantages such as safe, cheap and easy production, is widely used all over the world (Hajihashemi and Kazemi 2022). Chitosan is a natural modified from chitins which act as a potential biostimulant in agriculture (Gürsoy 2020; Gürsoy 2022b; Zhang 2022). Application of chitosan in agriculture due to its biodegradability, antimicrobial activity and plant growth promotion, with seed priming plant defense mechanism, chlorophyll content can be increased (Ahmed et al. 2020; Chouhan and Mandal 2021). The application of exogenous chitosan increases plants tolerance to several forms of stress, such as drought, salt, osmotic, and low-temperature stress (Jabeen and Ahmad 2013; Pongprayoon et al. 2013; Li et al. 2017).

Rapeseed is a very important plant in the production of oil crops due to its high oil content and oil quality (Gürsoy and Kolsarıcı, 2017; Gürsoy 2019; Arslan and Culpan, 2022).

The aim of this study is to determine the effect of priming with chitosan at different times on rapeseed cultivars under salt stress conditions on the germination properties, relative water content and photosynthetic pigments of the cultivars.

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2. Materials and Methods

The research was carried out at the Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). Rapeseed cultivars [NK Caravel (C1), Elvis(C2), Champlain (C3)] were used in this study. Before starting the study, the seeds were weighed and then kept in sodium hypochlorite solution for 5 minutes for sterilization. After this process, they were washed several times with distilled water. They were left to dry at room conditions until they reached their initial weight. Uniform and healthy looking seeds (to decrease errors in seed germination) were selected from each of the 3 cultivars and the seeds subjected to in 3 different time periods [0 (control) (Ch1), 12 hour (Ch2), 24 hour (Ch3)] of chitosan priming treatment were applied to rapeseed cultivars at room temperature. Untreated seeds were used as control. For each chitosan priming application, 50 seeds of all varieties were placed in sterile petri dishes on Whatman No:1 blotting papers and 10 ml of different doses of salt [0 (control) (S1), 50 mM L^{-1} (S2), 100mM L^{-1} (S3)] concentrations were added. Only water was added to the control petri dish. In order to prevent evaporation the petri dishes are wrapped with parafilm. The petri dishes were left to germinate at room temperature. Filter papers were changed every 2 days and 10 ml of salt containing solutions were added. The research randomized plots experimental design were made with 3 replication according to the trial pattern. All germination processes were carried out according to ISTA rules (ISTA 2003). In the study; germination percentage (GP), seedling length (SL), root length (RL), seedling fresh weight (SFW), root fresh weight (RFW), relative water content (RWC), total chlorophyll (Total Chl), carotenoid (Crt) parameters were examined.

Germination percentage (%)

Germination% = (number of germinated seeds/total number of seeds)×100 (Siddiqi et al. 2007)

Determination of relative water contents

In order to determine the relative water content in the leaf samples taken from plants belonging to the rapeseed cultivars in the control and stress groups were weighed and their fresh weight was determined, then they were placed in glass tubes containing 5 ml of distilled water and kept in the light for 24 hours. At the end of this period, the hydrated leaf samples were weighed again and their weight in turgor condition was determined. Later, these leaf samples will be dried in the oven at 80°C for 48 hours and their dry weight will be determined again. Finally, the relative water contents will be found according to the formulas below (Ritchie et al. 1990).

RWC(%) = (FW − DW)/(TW − DW) x 100 (Relative water content)

FW: fresh weight, TW: turgor weight, DW: dry weight

Chlorophyll (mg g^{-1})

Fresh samples (0.25g) were taken from the leaves of the rapeseed seedling and homogenized with 80% acetone. It was filtered and made up to 25 ml with acetone. These samples were read in the spectrometer at 663 and 645 nm wavelengths spectrophotometer. Chlorophyll was calculated with the following formula (Lichtenthaler and Welburn 1983).

Chlorophyll a (mg g^{-1}) = (12.7*663 nm)-(2.69*645 nm)*V/W*10000

Chlorophyll b (mg g^{-1}) = (22.91*645 nm)-(4.68*663 nm)*V/W*10000

Total Chlorophyll = Chlorophyll a + Chlorophyll b

V = volume leaf extract in 80% Acetone

W = fresh weight of leaf material

Carotenoid (mg g^{-1})

Fresh samples (0.25g) taken from young leaves of rapeseed seedlings were homogenized in 80% acetone in a place not directly exposed to light, and then filtered. The amount of carotenoid will be determined according to the following formula by completing the obtained filtered extract with acetone to 25 ml and reading it at 450 nm wavelength (Lichtenthaler and Welburn 1983).

Carotenoid (mg g^{-1})= (4.07 x A450-(0.0435 x Chlorophyll a+0.367 x Chlorophyll b)

Statistical analysis

The data obtained in the research were subjected to statistical analysis using the MSTAT-C program. Duncan test was used to describe the degree of significance between the means.

3.Results and Discussion

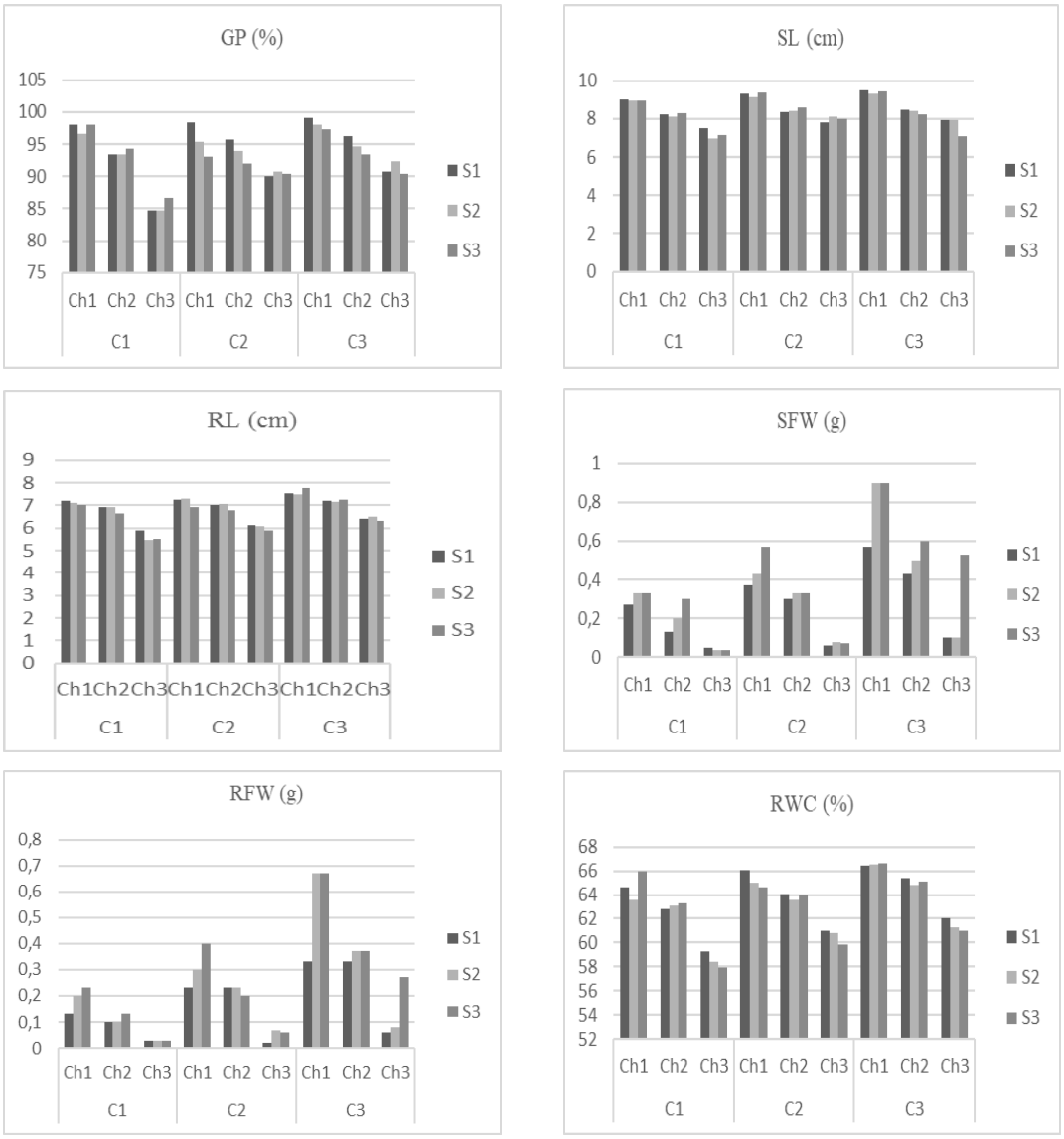
The variance analysis results of this study, which was conducted to determine the effects of chitosan priming application times on the germination parameters, seedling growth, total chlorophyll, relative water content and carotenoid of rapeseed varieties under salt stress, are given in Table 1. When Table 1 is examined, Cultivars × Ch times × S Doses triple interaction is seen to be significant at the level of 5% for root length and 1% for other parameters examined. Besides, Cultivars, Ch times and Salt doses are important at the 1% level. On the other hand, it was determined that the bilateral interactions were statistically significant at the level of 1%. In the RL parameter, the Cultivars × S doses bilateral interaction was found to be statistically insignificant.

Table 1
Analysis of variance on the investigated parameters in rapeseed cultivars of chitosan application times and salt stress

V.S.	D.F.	GP	SL	RL	SFW	RFW	RWC	Total Chl	Crt
		F Value							
Cultivars	2	40.07**	7.83**	10.63**	44.72**	40.07**	8.22**	6.66**	66.36**
Ch times	2	244.04**	112.10**	130.66**	204.09**	244.04**	147.54**	121.16**	330.91**
Cultivars ×Ch times	4	10.14**	17.83**	6.38**	11.15**	10.14**	3.69**	28.60**	21.08**
S Doses	2	331.68**	1441.33**	731.05**	291.82**	331.68**	891.35**	374.07**	100.99**
Cultivars ×S Doses	4	15.44**	11.31**	1.23^{ns}	4.16**	15.44**	6.72**	68.91**	7.47**
Ch times ×S Doses	4	25.13**	21.89**	7.86**	12.61**	25.13**	6.74**	27.85**	59.57**
Cultivars ×Ch times ×S Doses	8	5.84**	9.32**	2.25*	6.81**	5.84**	3.82**	19.17**	11.74**
Error	54	0.53	0.01	0.01	0.004	0.002	0.228	0.001	0.003
CV%		0.78	1.31	1.88	1.54	1.5	0.75	1.62	5.63

**: significance level at $p<0.01$, *: significance level at $p<0.05$. ns: non significant, VS: Variation source, DF: Degrees of Freedom, GP: Germination Percentage, SL: Seedling Length, RL: Root Length, SFW: Seedling Fresh Weight, RFW: Root Fresh Weight, RWC: Relative Water Content, Total Chl: Total Chlorophyll, Crt: Carotenoid

Duncan test results according to variance analysis results are given below as both figures and tables.



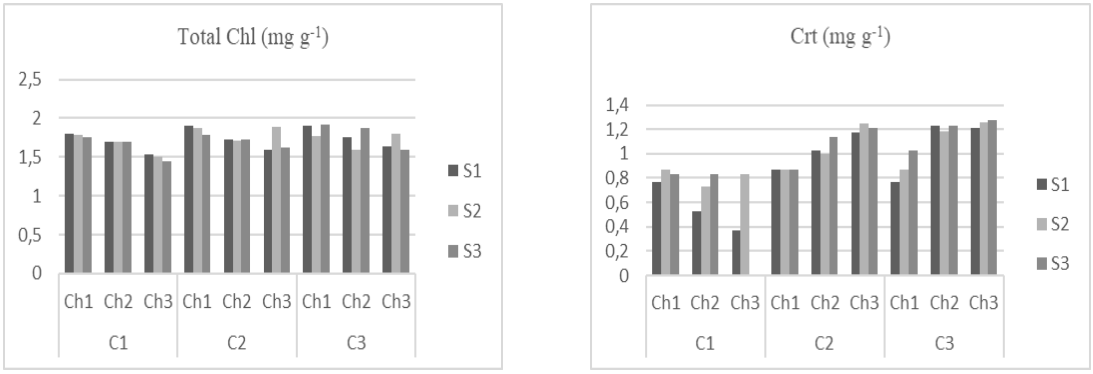


Figure 1
Mean values of germination characteristics, RWC and carotenoid content of rapeseed cultivars under salt stress of different chitosan priming times (triple interaction).

When Figure 1 and Table 1 of the results of the averages is examined, it is seen that the germination is decreased with S applications in the GP feature, but it is seen that Ch priming applications are effective in reducing the negative effects of salt stress. It is seen that the highest germination percentage was obtained from the C3 varieties from the Ch2 priming time application. Guan et al. (2009) reported that chitosan priming resulted in development maize germination and seedling growth under low temperature stress. Mahdavi et al. (2011) reported that germination decreased in high chitosan dose applications in their study in which they applied osmotic stress to safflower seeds. However, they reported that the germination percentage increased up to 0.4% chitosan dose. Hameed et al. (2013) reported that seed priming with chitosan enhanced the germination rate compared with non-primed seeds. Chitosan priming under stress resulted in very developed germination index and decreased germination time to promote early seedling establishment in maize. Jabeen and Ahmad (2013) applied chitosan to safflower and sunflower cultivars under salinity stress and they reported that small dose of chitosan application caused boost in germination parameters of both cultivars. When the SL feature is examined (Figure 1, Table 1), it is seen that the seedling length is prolonged in C1 and C2 varieties in Ch2 application at the S3 dose, where salt stress is the highest. Ch application seems to be effective in suppressing salt stress and increasing seedling height. On the other hand, in the RL feature, it is seen that especially Ch2 application in S2 application is effective in extending the root length (Figure 1). Guan et al. (2009) primed corn seeds with chitosan at low temperatures. As a result of the study, all priming treatments with chitosan significantly increased the shoot height and root length as compared with the control. Sheikha and Al-Malki (2011) chitosan application developed growth characteristics such as shoot and root length in case of bean. Hasanah and Sembiring (2018) found that application of salicylic acid and chitosan to leaves of soybean cultivars increased plant height, seedling, and root dry weights. Bakhoum et al. (2020) applied chitosan to reduce salt stress in sunflower plant. As a result of the study, they determined that chitosan applications increased seedling height, fresh weight and dry weight. When the SFW parameter is examined, it is seen that the effect of Ch application (Ch2 and Ch3) is clearly revealed as the doses of salt stress increase. Therefore, it was determined that chitosan application times were effective in reducing the effect of salt stress. Seraj et al. (2021) applied chitosan and salicylic acid to the seeds of the milk thistle plant under water stress conditions. As a result of the study, they reported that when chitosan applications were compared with the control, especially 200 mg L^{-1}application was important in increasing fresh and dry weight. Zhang et al. (2021) applied chitosan to lettuce seeds under salt stress. At the end of the application, they reported that chitosan increased the seedling fresh weight. Although the salt stress increased in the RFW parameter, it was observed that the root length increased with Ch applications. This is particularly evident in the C3 variety. Even at the highest salt application, root length increased 4 times in Ch3 application compared to control (Figure 1). Sen and Mandal (2016) reported that chitosan application to mung bean plant under salt stress increased the root length with Ch application. Harfoush et al. (2017) reported that the application of humic acid and chitosan to the potato plant caused significant increases in the growth parameters of the plant. It was determined that the RWC parameter (Figure 1) increased at the S3 dose, where the salt stress was the highest, especially in the Ch2 application. Abdelaal et al. (2021) reported that the RWC increased by 36.8% in the study they applied chitosan and yeast extract to the garlic plant under water stress conditions. Mazrou et al. (2021) reported that in a 2 year study in which they applied chitosan nanoparticles to *Matricaria chamomilla* plant, they provided an increase in RWC compared to the control, and they achieved the maximum value especially in the application of 300 mg L^{-1}. Photosynthesis is the most important process affected in plants under saline conditions (Zayed et al. 2017). Chlorophyll content in plants exposed to abiotic stress is an important feature in determining the tolerance of plants to stress. When plants are exposed to stresses such as salinity, their chlorophyll content decreases and growth retards (Safikhan et al. 2018).

Table 1
Mean values of germination characteristics, RWC and carotenoid content of rapeseed cultivars under salt stress of different chitosan priming times

Cultivars × Ch times S doses	GP(%)									
	NK Caravel			Elvis			Champlain			Mean
S1	98.00 abc	93.33 hıj	84.67 n	98.33 ab	95.67d-g	90.00 l	99.00 a	96.33 c-f	90.67 kl	97.07 A
S2	96.67 b-e	93.33 hıj	84.67 n	95.33 efg	94.00 ghı	90.67 kl	98.00 abc	94.67 fgh	92.33 ıj	94.11 B
S3	98.00 abc	94.33 gh	86.67 m	93.00 hıj	92.00 jk	90.33 kl	97.33 a-d	93.33 hıj	90.33 kl	88.93 C
Mean	92.00 C	94.67 A	95.33 A	91.56 C	93.33 B	95.00 A	93.00 B	91.78 C	93.67 B	
LSD%1	1.589									
Cultivars × Ch times S doses	SL (cm)									
	NK Caravel			Elvis			Champlain			Mean
S1	9.03 c	8.25 ef	7.517 ı	9.29 ab	8.36 def	7.83 h	9.48 a	8.460 de	7.950 gh	9.213 A
S2	8.97 c	8.14 fg	6.953 j	9.11 bc	8.43 de	8.12 fg	9.297 ab	8.417 de	7.923 gh	8.358 B
S3	8.95 c	8.29 ef	7.163 j	9.35 ab	8.61 d	7.99 gh	9.453 a	8.263 ef	7.067 j	7.612 C
Mean	8.26 C	8.49 B	8.63 AB	8.02 D	8.55 AB	8.55 AB	8.136 CD	8.649 A	8.261 C	
LSD%1	0.2388									
Cultivars × Ch times S doses	RL (cm)									
	NK Caravel			Elvis			Champlain			Mean
S1	7.20 cde	6.90 fg	5.90 l	7.25 cd	7.00 efg	6.11 kl	7.55 b	7.20 cde	6.42 j	7.29 A
S2	7.10 c-f	6.92 fg	5.49 m	7.28 c	7.07 c-f	6.07 l	7.50 b	7.17 cde	6.50 ıj	6.99 B
S3	7.03 d-g	6.65 hı	5.52 m	6.92 fg	6.80 gh	5.89 l	7.77 a	7.24 cde	6.32 jk	6.02 C
Mean	6.67 BC	6.79 B	7.06 A	6.50 CD	6.81 B	7.06 A	6.40 D	6.53 CD	7.11 A	
LSD%5	0.2071									
Cultivars × Ch times S doses	SFW (g)									
	NK Caravel			Elvis			Champlain			Mean
S1	0.27 fgh	0.13 hıj	0.05 ıj	0.37 def	0.30 efg	0.06 ıj	0.57 bc	0.43 cde	0.10 ıj	0.52 A
S2	0.33 efg	0.20 ghı	0.04 j	0.43 cde	0.33 efg	0.08 ıj	0.90 a	0.50 bcd	0.10 ıj	0.35 B
S3	0.33 efg	0.00 efg	0.04 ıj	0.57 bc	0.33 efg	0.07 ıj	0.90 a	0.60 b	0.53 bc	0.12 C
Mean	0.15 G	0.23 DEF	0.37 C	0.19 FG	0.28 CDE	0.50 B	0.23 EFG	0.32 CD	0.68 A	
LSD%1	0.1379									
Cultivars × Ch times S doses	RFW (g)									
	NK Caravel			Elvis			Champlain			Mean
S1	0.13 fgh	0.10 gh	0.03 h	0.23 def	0.23 def	0.02 h	0.33 bcd	0.33 bcd	0.06 h	0.35 A
S2	0.20 efg	0.10 gh	0.03 h	0.30 b-e	0.23 def	0.07 h	0.67 a	0.37 bc	0.06 h	0.23 B
S3	0.23 def	0.13 fgh	0.03 h	0.40 b	0.20 efg	0.06 h	0.67 a	0.37 bc	0.27 cde	0.07 C
Mean	0.09 F	0.16 DE	0.24 C	0.11 EF	0.20 CD	0.37 B	0.13 EF	0.22 CD	0.43 A	
LSD%1	0.09749									
Cultivars × Ch times S doses	RWC (%)									
	NK Caravel			Elvis			Champlain			Mean
S1	64.67 d-g	62.84 ıj	59.30 no	66.07 abc	64.07 e-h	61.00 kl	66.40 ab	65.37 bcd	62.03 jk	65.50 A
S2	63.57 ghı	63.07 hıj	58.45 op	65.00 c-f	63.60 ghı	60.83 lm	66.50 ab	64.85 def	61.28 kl	64.01 B
S3	66.00 abc	63.30 hı	57.92 p	64.67 d-g	63.92 f-ı	59.85 mn	66.63 a	65.12 cde	61.00 kl	60.19 C
Mean	62.27 EF	63.71 BC	64.60 A	61.69 F	63.14 CD	64.21 AB	62.41 E	62.81 DE	64.25 AB	
LSD%1	1.041									
Cultivars × Ch times S doses	Total Chlorophyll (mg g^{-1})									
	Maximus			Sirena			Reyna			Mean
S1	1.80 bcd	1.70 fgh	1.53 jk	1.90 a	1.73 d-g	1.60 ıj	1.90 a	1.76 c-g	1.64 hı	1.834 A
S2	1.78 cde	1.70 fgh	1.51 kl	1.88 a	1.71 e-h	1.89 a	1.77 c-f	1.59 ıj	1.81 bc	1.72 B
S3	1.76 c-g	1.69 gh	1.45 l	1.78 cde	1.73 d-g	1.62 ı	1.92 a	1.87 ab	1.59 ıj	1.63 C
Mean	1.68 EF	1.74 CD	1.77 BC	1.66 FG	1.83 A	1.72 D	1.63 G	1.71 DE	1.79 AB	
LSD%1	0.06894									
Cultivars × Ch times S doses	Crt (mg g^{-1})									
	Maximus			Sirena			Reyna			Mean
S1	0.77 e	0.53 f	0.37 g	0.87 e	1.03 cd	1.17 ab	0.77 e	1.23 ab	1.21 ab	0.86 C
S2	0.87 e	0.73 e	0.83 e	0.87 e	1.00 d	1.25ab	0.87 e	1.18 ab	1.26 ab	0.99 B
S3	0.83 e	0.83 e	1.03 cd	0.87 e	1.14 bc	1.21ab	1.03 cd	1.23 ab	1.28 a	1.07 A
Mean	0.56 F	1.02 C	1.07 BC	0.81 E	1.04 BC	1.10 B	0.90 D	1.07 BC	1.18 A*	
LSD%1	0.1194									

* Dissimilar letters in the column show different groups

In this study, despite the increase in S stress, increases in chlorophyll content were determined, especially in C2 and C3 varieties, with Ch2 application. On the other hand, chlorophyll content increased in the same cultivars with S2 salt dose in Ch3 application compared to the control (Figure 1). When the carotenoid parameter was examined, with the increase in salt strain in all cultivars, increases in Crt were also observed with the Ch application times. Zayed et al. (2017) applied Ch to bean plant under salt stress. They reported that they found increases in the relative water index and chlorophyll content as a result of the study. Gerami et al. (2020) reported an increase in the chlorophyll and carotenoid content of the plant in their study where they applied chitosan to the stevia plant.

4.Conclusion

In this study, the effects of chitosan priming application times on the germination parameters, RWC, chlorophyll and carotenoid content of rapeseed cultivars under salt stress conditions were investigated. Chitosan application times, especially Ch2, and C3 cultivar gave more positive results in terms of the parameters examined. Besides, applications should be made in other plants and under various stress conditions and their results should be evaluated.

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