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Original article

The Effects of Exogenous Glutamine Application on Some Germination Parameters of Different Carrot Cultivars Grown in Salinity Conditions

Hatice Üstüner [a, *], Kamile Ulukapı [b] & Ayşe Gül Nasırcılar [c]

[a] Antalya Science and Art Center, Antalya, Türkiye
[b] Department of Plant and Animal Production, Vocational School of Technical Sciences, University of Akdeniz, Antalya, Türkiye
[c] Department of Mathematic and Science Education, Faculty of Education, University of Akdeniz, Antalya, Türkiye

Abstract

Carrot (*Daucus carota*), which can be orange, yellow, purple, white and red in color due to the difference in the pigments it contains, is a very rich food in terms of carotene and ascorbic acid. In this study, the effects of exogenous Glutamine (Gln) pre-treatments (1, 2, 3, 4 mM) on the some germination parameters of carrot seeds (orange, yellow, purple), which are known to be sensitive to salt stress according to the cultivar, were investigated under salt stress conditions (150 mM NaCl). For this purpose, germination percentage (GP), mean germination time (MGT), germination rate coefficient (CVG), germination rate index (GRI), germination index (GI), which are some of the important germination parameters, were determined. According to the results of analysis of variance, it was determined that there was an interaction between cultivars and applications and all germination parameters under salt stress conditions (***p<0.001). The effect of Gln pre-treatments on germination under salt stress and unstressed conditions differed according to the application dose and cultivar. Glutamine application under salt stress had no effect on germination except for the orange carrot cv. In this cultivar, 1 mM pre-treatment had a positive effect on germination parameters under salt stress conditions. In unstressed conditions, although 3 mM Gln pretreatment in orange carrot cv. and 4 mM Gln in yellow carrot cv. had a positive effect on germination parameters compared to control, the highest germination in purple carrot cv. was obtained in control seeds.

Keywords: Carrot, Germination, Glutamine, Seed.

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* **Corresponding author:**

Üstüner H. is a Dr. Biology Teacher at Antalya Science and Art Center, Antalya, Türkiye. She graduated from Akdeniz University, Faculty of Science, Department of Biology, Department of Botany, PhD program. Her research interests include the Biology, Plant Physiology and Plant Biotechnology . She has lived, worked, and studied in Antalya, Türkiye.
Email: h.ustuner1977@gmail.com

INTRODUCTION

A large part of the irrigated agricultural lands on a global scale is affected by salt stress and is irrigated with waters with high salt content. Every year, about 10 million hectares of land becomes unusable for agriculture due to excessive salinity. This situation causes an average annual economic loss of 12 billion dollars on a global scale. In order to meet the nutritional needs of the rapidly increasing population, more production will be required on salt-affected lands and more irrigation with salt water resources will be required in the coming years (Qadir et al. 2008; Rozema and Flowers 2008; Bolton and Simon 2019).

Carrot, which is an important source of vitamins and minerals from the Apiaceae family, is among the top ten most produced vegetables worldwide (Nijabat et al. 2020). Since it is a glycophytic plant, there is a significant loss of yield in salty conditions and this situation causes production problems of carrot on a global scale. Beside, there are also limited resources available for plant breeders to increase salt tolerance (Bolton and Simon 2019). Germination of carrot seeds under stress conditions is rather slow and uneven **(**Delian and Lagunovschi-Luchian 2015). Morover, there are few studies evaluating the germination parameters of carrots under salt stress. Evaluation of salt tolerance during the germination process, which is a critical stage of plant development, is a fast and economical method (Bolton and Simon 2019). Different priming applications to seeds before seed germination helps to reduce the negative effects of abiotic stresses. In this way, more homogeneous germination and seedling emergence can be achieved (Eisvand et al. 2011). Some applications to reduce the effects of salt stress in carrots are foliar application of jasmonic acid (Smoleń, et al. 2020) and priming of seeds with silicon dioxide (Nasırcılar et al. 2021).

External amino acid applications are a method applied in various plants to develop tolerance to different abiotic stresses and to alleviate the destructive effects of stress (Heuer 2003; Botta 2012; Sadak et al. 2015). Glutamine, a very important amino acid for plant growth and development, has a similar effect and has been applied externally to alleviate the adverse effects of salt stress in sugarcane and potato (Patade et al. 2014; Teixeira and Pereira 2007). Although some external applications have been made in carrots under salt stress conditions, there is no study in which glutamine is used as priming. In this study, it is aimed to determine that the effect of external glutamine application on germination parameters of carrot cultivars under salt stress conditions.

MATERIALS and METHODS

Material

Orange, yellow and purple carrot cultivars purchased from a commercial company were used in this study, which was carried out in the Laboratory of the Department of Plant and Animal Production, Vocational School of Technical Sciences at Akdeniz University.

Surface Sterilization and Priming with Glutamine

The seeds were kept in 10% sodium hypochlorite solution for surface sterilization by continuously mixing for 10 minutes. After soaking in 70% ethanol for 2 minutes, rinsing in sterile distilled water for 3 times for 5 minutes, priming was done with glutamine. After sterilization, the seeds were kept in sterile jars in 1, 2, 3, 4 mM glutamine solutions at room temperature (24±2ºC) for 24 hours in the dark. Control seeds were kept in sterile distilled water under the same conditions.

Germination Conditions and Parameters

The seeds primed with glutamine were taken to germinate in petri plates. For this process, seeds were placed on double-layer sterile blotters in petri plates (90 x 15 mm). Control seeds were irrigated with distilled water and the seeds used in all other applications were irrigated with brine containing 150 mM NaCl and maintained in a climate room with a photoperiod of 16/8 hours at 24±1ºC. During the experiment, the seeds were checked every day and the control seeds were irrigated with distilled water and the others with salt water to prevent them from drying out, and the number of germinated seeds was recorded daily. Germination trials continued for 14 days and at the end of this period, germination percentage (GP), mean germination time (MGT), germination rate coefficient (CVG), germination rate index (GRI), germination index (GI) were calculated. Calculations were made using the formulas given below.

GP (%) = Number of germinated seeds / Total number of seeds X 100 (Gosh et al. 2014**)**

MGT: Σ Dn / Σn D = days counted from the beginning of the test, n = number of seeds germinated on day D (Ellis and Roberts, 1981; Sivritepe, 2012).

CVG: N1+N2+….+Nx/100xN1T1+……+NxTx (Kotowski, 1926) T: number of days corresponding to N, N: number of seed germinated each day

GRI (%/day): G1/1+G2/2+……+Gx/x (Esechie, 1994)

G1: Germination percentage x100 at the first day after sowing, G2: Germination percentage x100 at the second day after sowing, GI: (14xn1) + (13xn2) +…..+ (1xn14) n1, n2,…, n14: number of germinated seeds on the first, second and subsequent days until the 14th; 14, 13…., and 1 are weights given to the number of germinted seeds on the first, second and subsequent days respectively (modified from Benech et al., 1991).

Statistical Analysis

Experiments were made in 4 repetitions and 25 seeds were used per trial. The evaluation of germination parameters data were made with the analysis of variance in the MINITAB 17 package program and the differences were determined by the Tukey test.

RESULTS and DISCUSSION

In this study, the effects of glutamine pretreatment under salt stress on the germination parameters of three different carrot cultivars were determined. According to the analysis of variance results; cultivar, glutamine applications and the combination of these had a statistically significant effect on all germination parameters at the $p<0.001$ level.

Table 1. Descriptives and variance analysis results of germination parameters of carrot cultivars.

Variation source	df	GP (%)	MGT (mm)	CVG (mm)	GI (mm)	GRI
C	2	***	***	***	***	***
T	9	***	***	***	***	***
C*T	18	***	***	***	***	***
Mean		0.14	3.81	4.70	0.041	6.24
Minimum		0.00	0.00	0.00	0.00	0.00
Maximum		0.90	11.28	52.11	0.25	14.28
Standart deviation		0.21	4.05	10.97	0.06	6.61

GP: Germination Percentage, MGT: Mean Germination Time, CVG: Germination Rate Coefficient, GRI: Germination Rate Index, GI: Germination Index (***p<0.001)

It was determined that there was a statistically significant ($p<0.001$) positive correlation between germination parameters (Table 2). There was a strong positive association between CVG and GP (r=0.939), GI and GP (r=1.000), GI and CVG (r=0.939), and GRI and MGT (r=0.933).

Table 2. Correlation of germination parameters of the carrot cultivars.

	GP	MGT	CVG	GI	GRI
GP	—				
MGT	0.672	—			
	< .001	—			
CVG	0.939	0.439	—		
	< .001	< .001	—		
GI	1.000	0.672	0.939	—	
	< .001	< .001	< .001	—	
GRI	0.676	0.933	0.438	0.676	—
	< .001	< .001	< .001	< .001	—

GP: Germination Percentage, MGT: Mean Germination Time, CVG: Germination Rate Coefficient, GRI: Germination Rate Index, GI: Germination Index

The effects of salt stress on carrots vary according to the genotype, and germination was also obtained in some cultivars at very high salt concentrations (Basma et al. 2014). Consistent with this study, germination rates in saline conditions differed according to cultivar, and while no germination could be obtained under 150 mM salt stress in purple cv., orange was the most germinated cultivar. In a study in which the salt tolerance of a total of 294 carrot accessions was determined, the total number of germinated seeds and germination rate were greatly reduced at increasing salt concentrations. The cultivars were classified as tolerant or sensitive according to the germination rate of the seeds in the range of 125 - 150 mM salt concentration (Bolton and Simon 2019). In this study, in the orange cultivar, 3 mM glutamine pre-treatment significantly increased the germination parameters GP, CVG, GI and GRI values when compared to control plants in without salt stress condition. Especially, it provided an increase of approximately 2 fold on the germination percentage. It also decreased the germination time by causing a decrease in the MGT value. A similar effect was obtained with 1 mM glutamine pre-treatment under salt stress conditions. In the other three glutamine concentrations, germination did not occur in saline conditions (Figure 1).

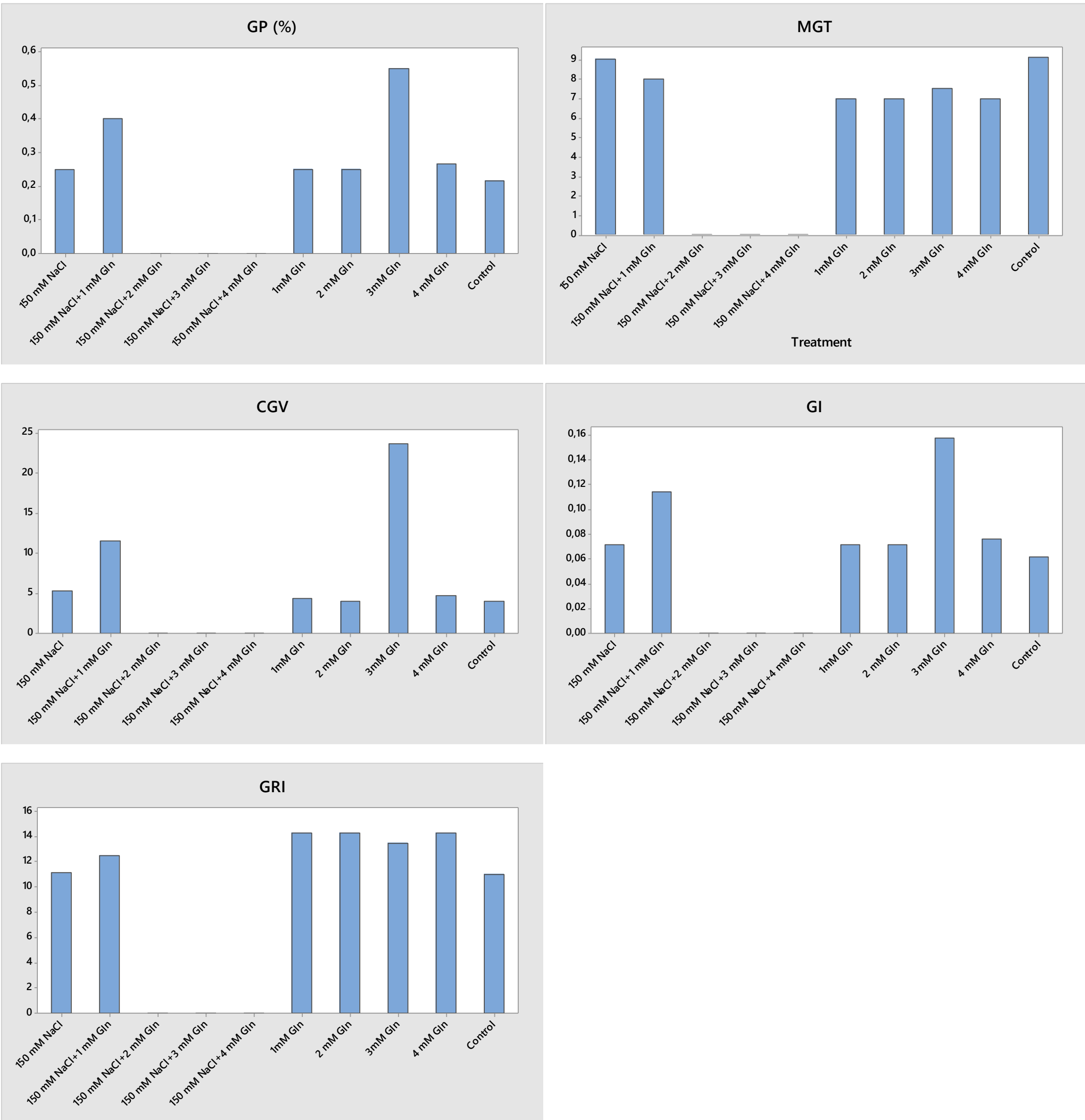

Figure 1. The effect of glutamine pretreatment on germination parameters of orange cultivar under saline and unstressed conditions.

In the yellow cultivar, glutamine pretreatment was effective only in non-stress conditions, while it was uneffective on germination parameters under salt stress conditions. 4 mM pre-application had an inducing effect on all parameters except MGT and shortened the germination time in unstressed conditions (Figure 2).

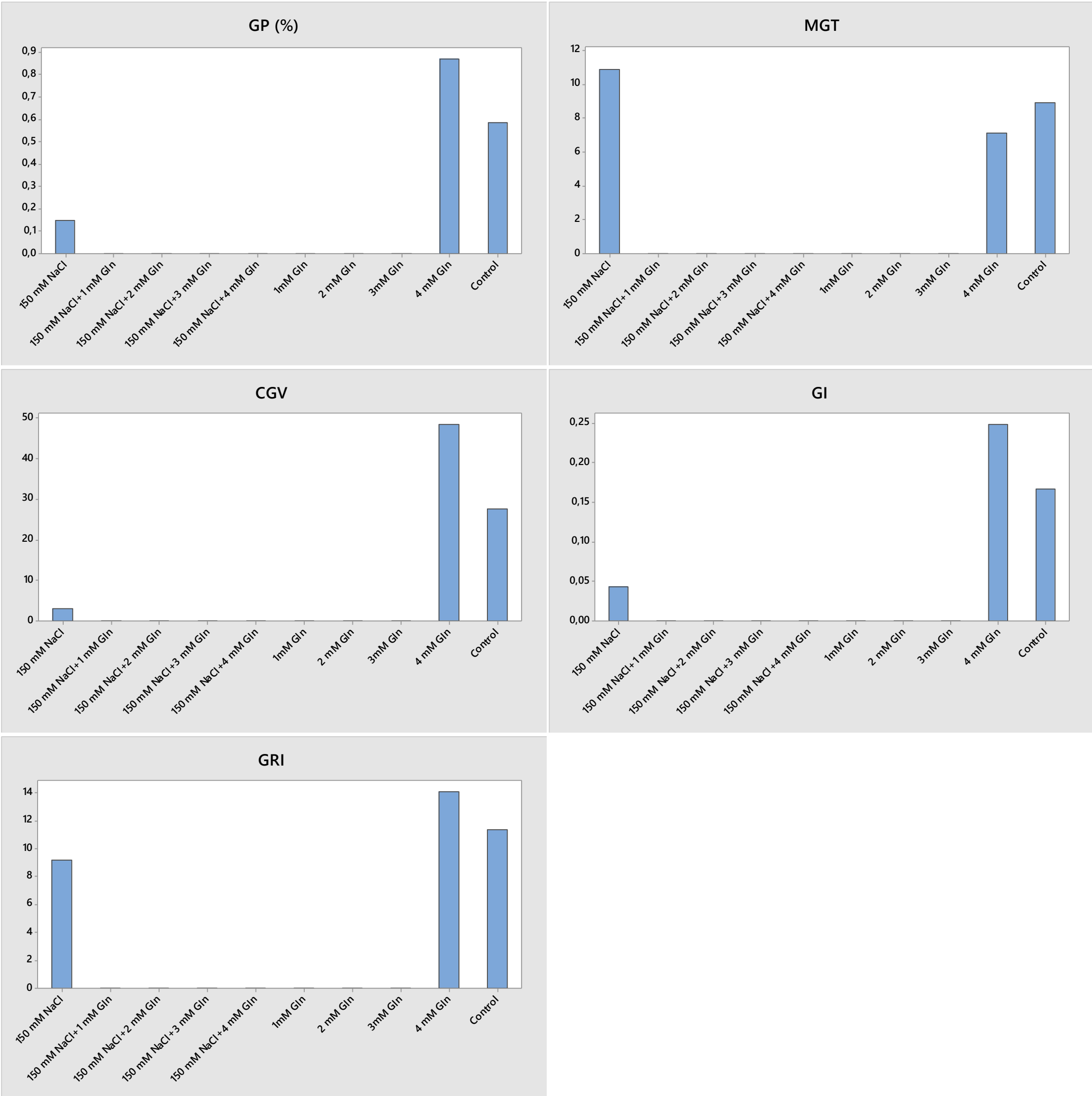

Figure 2. The effect of glutamine pretreatment on germination parameters of yellow cultivar under saline and unstressed conditions.

Purple cv. was the most affected cultivar by salt stress and none of the seeds germinated under salt stress conditions. Glutamine pretreatment was also ineffective in saline conditions and germination was not achieved at any concentration. In this context, the purple cultivar in which no germination could be obtained at 150 mm salt concentration, can be classified as the most sensitive cultivar. In unstressed conditions, the seeds germinated in all doses of glutamine, but the highest germination rate occurred in control plants. Only 4 mM pre-treatment had a healing effect in terms of shortening the germination time (Figure 3).

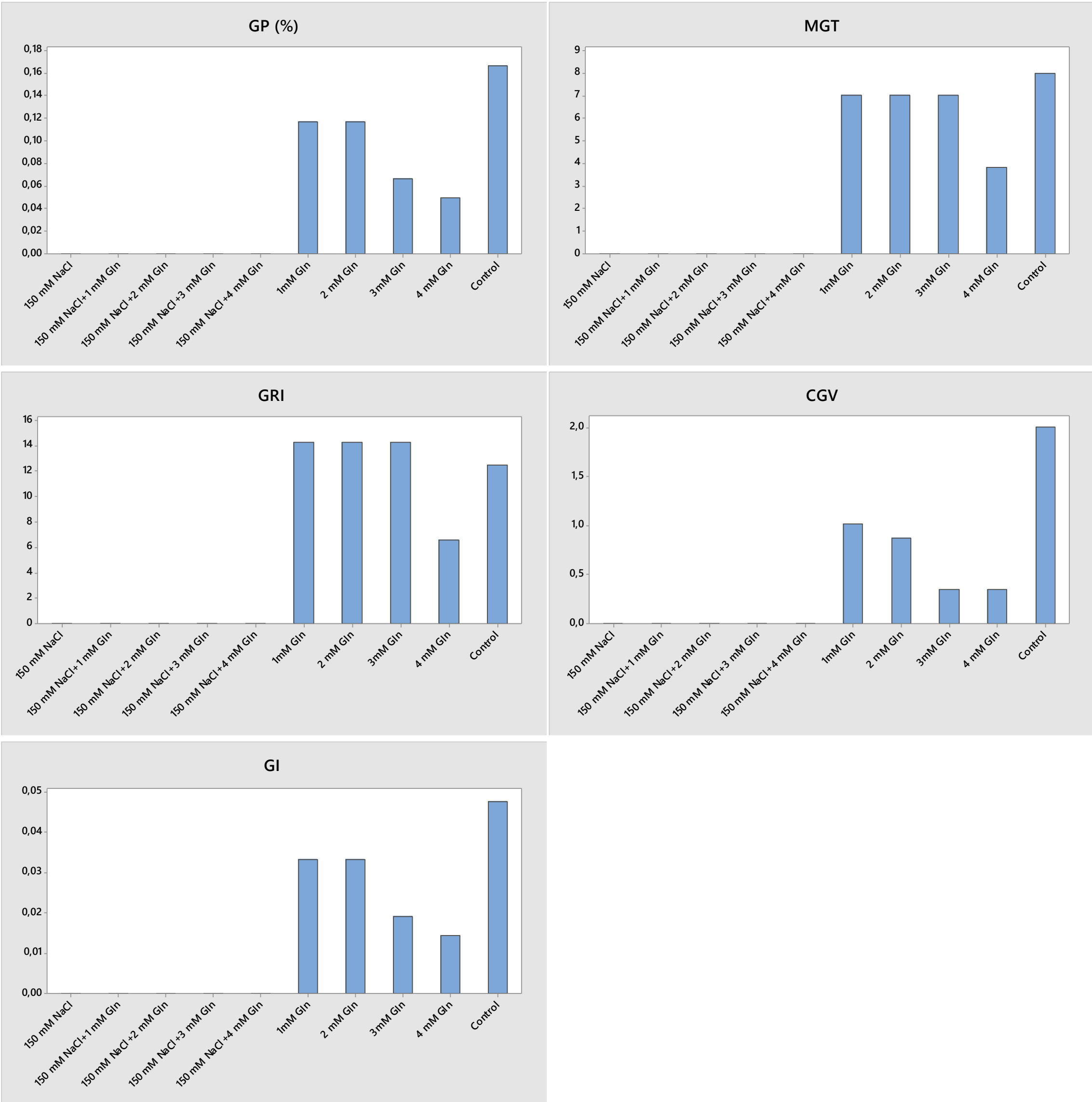

Figure 3. The effect of glutamine pretreatment on germination parameters of purple cultivar under saline and normal conditions.

Priming is one of the techniques applied to prevent germination problems caused by adverse environmental conditions in plants. With this method, seeds can germinate more homogeneously and seedlings obtained from germinated seeds are more durable in field condition. Priming applications can be done to minimize the effects of abiotic stress, as well as applications made under stress-free conditions. It varies from hydropriming applications made by keeping the seeds in water only, to hormonal priming applications made by keeping them in solutions such as gibberellic acid and salicylic acid (Eisvand et al. 2011).

Different priming applications were made for carrots under abiotic stress conditions. Hydropriming with distilled water, halopriming with different concentrations of NaCl, nutripriming and vitamin priming applications were performed on carrots under salt stress conditions. Among these applications, it has been reported that potassium phosphate and ascorbic acid can be used as alternative carrot seed preparation processes to provide the highest germination percentage and early vigorous seedling growth (Delian and Lagunovschi-Luchian 2015). However external application of jasmonic acid had no effect on the growth of salt-sensitive and resistant genotypes under salt stress (Smoleń et al. 2020). On the other hand, application of silicon dioxide to carrot seeds had a positive effect on germination parameters of yellow and black carrot cultivars under saline conditions. Especially in the yellow carrot cv., which did not germinate at a high salt concentration (200 mM), germination was obtained after silicon dioxide application (Nasırcılar et al. 2021). There were no studies of exogenous glutamine pre-treatment in carrots so far, but glutamine application reduced the inhibitory effect of salt stress on germination in onions (Çavuşoğlu et al. 2020). Similarly, in this study, pretreatment of glutamine stimulated germination parameters in two cultivars (orange, yellow) under stress-free conditions. In salt stress conditions, the effect of reducing the harmful effects of stress on all germination parameters was determined only in orange variety.

Conclusion

In recent years, there is also information that glutamine, which has very important functions on plant growth and development, reduces the effects of abiotic stress factors. However, the mechanism by which glutamine is effective in plants under stress conditions has not yet been fully elucidated. The effects of glutamine pre-treatment under different stress conditions have been studied in only the few plants, and no studies have investigated the effect of glutamine on the germination parameters of carrots, which is a glycophytic plant, under salt stress conditions. The effects of glutamine pretreatment showed a cultivar-dependent effect in both non-stress and salt stress conditions, and it had an encouraging effect on germination parameters in the orange variety under saline conditions. Considering that salt stress will have a more restrictive effect on plant production in the future, there is a need for more such studies to develop alternative preparations that can be applied to increase tolerance to salt stress.

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