

The interactive effects of salinity and drought stress on germination, seedling growth, and physiological parameters of pumpkin

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Abstract

This study aimed to explore the response of pumpkin to salt and drought stress during germination and early plant growth stages. Salt stress was induced with various sodium chloride concentrations (0.00%, 0.25%, 0.50%, and 0.75%) in non-drought (distilled water) and drought (15% PEG-6000) conditions. The seeds of the pumpkin cultivar T28 for snacks were germinated between filter papers using the respective solutions. In the pot experiment, the seedlings at the 2-leaf stage were exposed to these stresses for 30 days. The plant height, fresh weight, dry weight, leaf area, leaf dry matter, chlorophyll content (Chl), relative water content (RWC), and cell membrane stability (CMS) of the plants were inquired. Drought markedly reduced the germination index, plant height, fresh weight, dry weight, leaf area, RWC, and CMS. Conversely, increased mean germination time, Chl, and dry matter were determined in drought conditions. Salinity stress above 0.50% NaCl influenced these traits, with salinity's inhibitory effects surpassing those of drought. Germination percentage dropped from 100% to 46% at 0.75% NaCl under drought, whereas it remained stable under non-drought stress. Pumpkin was more sensitive to drought and salinity stress at the germination stage than at the early growth stage. The correlation between germination and seedling growth parameters indicated that the germination index and mean germination time were substantially associated with nearly all growth traits of pumpkin. The study highlights the germination index as a key indicator of stress tolerance and identifies 0.50% NaCl as a critical threshold level for pumpkin under salt and drought stress.

Keywords: *Cucurbita pepo* L., Drought, Salinity, Germination, Plant growth, Cell membrane stability

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INTRODUCTION

Today, there are five important cucurbit species - *Cucurbita pepo*, *C. moschata*, *C. maxima*, *C. ficifolia*, and *C. argyrosperma* (*C. mixta*) - are cultivated for fresh fruits, flowers, and seeds extensively worldwide (Robinson and Decker-Walters, 1997). Most pumpkins consumed as snacks belong to the *C. pepo* L. species (Gülşen et al., 2016). Its fresh fruits and flowers have been utilized as vegetables and mature seeds as snacks (Maynard and Maynard, 2000). The seeds have a high nutritional value, a high protein content ranging from 28% to 40%, and oil (35% to 50%), with 78% of the oil being unsaturated fatty acids (Turkmen et al., 2015; Dziwulska-Hunek et al., 2021; Aghbolaghi et al., 2021; Aghbolaghi et al., 2022). Moreover, it provides vitamins A, C, and E (Liang et al., 2022; Jahed et al., 2023) and is abundant in essential minerals, including K, P, Ca, Mg, and Fe (Seymen et al., 2019). Along with its nutritional values, it is a valuable alternative crop in arid and semiarid regions in Türkiye because it adapts highly to different ecological conditions (Yasar et al., 2014).

The production of pumpkins for snacks has gained popularity among farmers due to their low water requirements, especially in arid areas where rainfall is insufficient, and their high economic returns. Under these conditions, drought and salinity are common abiotic stresses for pumpkin cultivation, resulting in yield and economic losses (Seymen et al., 2019; Aghbolaghi et al., 2023). Soil salinity occurs naturally in arid regions,

resulting from low precipitation, preventing excess salt drainage from the root zone to deeper soil layers. It induces osmotic stress, inhibiting water uptake by roots, and ion toxicity due to excess Na^+ and Cl^- (Maas and Grattan, 1999; Okçu et al., 2005). According to Xu et al. (2017), pumpkin is classified as a plant with low salinity tolerance. Inhibited germination and plant growth of pumpkin under saline conditions are reported by Irik and Bikmaz (2024), with similarities to the findings of Tarchoun et al. (2022) in *C. maxima* Duchesne. In addition, less chlorophyll content (SPAD) (Taratima et al., 2022), smaller leaves (Taratima et al., 2023), and an imbalance of ions between Na, Ca, and K (Kurum et al., 2013) were identified as responses to salinity in pumpkin. Increased ion leakage and enzymatic activity (APX, SOD, CAT, and GR) were also explored (Sevengör et al., 2011; Irik and Bikmaz, 2024). Besides, drought negatively impacts pumpkin plants with similar responses to salinity, such as reduced leaf area, chlorophyll a and b content, net photosynthesis, and stomatal conductance (Aghai and Ehsanzade, 2011), as well as plant height, fruit numbers, seed and fruit yield, photosynthetic activity, and relative water content (Najafi et al., 2021), seed quantity and weight (Naeemi et al., 2012), and ultimately seed and fruit yield (Najafi et al., 2021). However, the combined effects of drought and salinity, which frequently occur together during the life cycle of pumpkin plants, have not been examined and their effects remain unclear. Therefore, this study evaluated the effects of salinity stress, specifically NaCl salt, on seed germination and the early growth stages of pumpkin growth in both drought and non-drought conditions and also examined the correlation between germination traits and plant growth parameters.

MATERIALS AND METHODS

This study assessed the responses of pumpkin (*Cucurbita pepo* L. cv. T28) to salinity and drought stress conditions by considering germination and morpho-physiological parameters. Sodium chloride (NaCl) was used to simulate salinity and polyethylene glycol (PEG-6000) induced drought stress.

Germination test

Drought stress was simulated using 15% PEG 6000, which equals about 2 bar osmotic potential according to Michel and Kaufmann (1973) and Yasar et al. (2014), and distilled water served as a non-drought control treatment. NaCl solutions of 0.00%, 0.25%, 0.50%, and 0.75% were prepared for salinity treatments. Electrical conductivity (EC) values for non-drought treatments were 0.0, 4.8, 9.1, and 13.6 dS m^{-1} ; for drought, they were 0.4, 3.4, 6.5, and 9.3 dS m^{-1} , respectively.

Four replicates of 25 seeds were placed between three filter papers moistened with 7 milliliters of the respective test solutions on each paper. The papers were refreshed every two days to prevent salt and PEG accumulation. After rolling the papers with seeds, they were inserted into a zipped plastic bag to inhibit water evaporation. They were exposed to $25 \pm 1^\circ\text{C}$ in an incubator for 8 days under darkness (ISTA 2018). Daily germination scores were recorded, with a seed having a 2 mm radicle protrusion. Germination percentage and mean germination time (MGT) were calculated following the ISTA (2018) rules and the formula described by Salehzade et al. (2009) for the germination index.

Pot experiment

Seedling growth conditions

The seeds of pumpkin cultivar T28 were sown in seedling trays with a growth medium (peat, perlite, and vermiculite 6:1:1, by volume). The trays were then put in a growth chamber of 24°C day/ 18°C night, a photoperiod of 18/6 hours, and a relative humidity of 65-70%. Uniform and healthy seedlings at the 2-leaf stage after 14 days of planting were used in the study. The seedlings were placed in plastic pots with a volume of 0.5 L, filled with the same growing mixture used for non-drought conditions. To simulate drought stress, 150 g of PEG-6000 was added to one kilogram of the growth medium, achieving a concentration of 15% PEG-6000. Different salinity stresses were arranged using NaCl levels (0.00%, 0.25%, 0.50%, and 0.75% NaCl), as implemented in the germination test.

Plant growth conditions and stress application

The plants were irrigated with Hoagland's nutrient solution after transplantation. Climatic conditions were the same in seedling growth conditions, and they were allowed to grow under these temperature, light, and moisture conditions. After 21 days of stress incubation, the plants were cut above the soil surface, and the fresh biomass was used to determine plant fresh weight and dry weight after drying at 80°C for 24 hours.

Leaf area per plant was quantified using ImageJ software (Kaya, 2024). Dry matter was determined by calculating the percentage of dry weight relative to fresh weight. The chlorophyll content was measured using the Konica Minolta SPAD-502 (Osaka, Japan) as the SPAD index (Kulan et al., 2021). The relative water content (RWC) and cell membrane stability (CMS) of the uppermost fully expanded leaves were determined using the formulas described by Nijabat et al. (2020). To determine the leaf RWC, the second leaf from the top of the plants was pulled out, and the fresh weight was directly weighed. This leaf was then floated in distilled water in a Falcon tube for 24 h, and its turgor weight was determined after excess water on the leaf surface was gently removed with paper towels. Subsequently, the leaf was dried at 80°C for 24 h to obtain the dry weight.

$\text{RWC (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgor weight} - \text{Dry weight})] \times 100$

Leaf dry matter (%)= (Dry weight / Fresh weight) × 100

Six leaf samples with a diameter of 10 mm were excised from the leaf and immersed in 25 mL of distilled water in a 50 mL glass tube. After 24 h of incubation at 20°C, the electrical conductivity of the soaking solution was measured using an EC meter (WTW 3.15i) and recorded as initial EC_i. The tubes were then transferred to a water bath at 90°C for 1 h, and the final EC value (EC_f) was measured after cooling to room temperature. The cell membrane stability (CMS) was calculated as:

CMS (%)= (1-[(EC_i/EC_f)]×100 (Farooq and Azam, 2006)

Statistical data analysis

Data were subjected to analysis of variance (ANOVA) using the JMP 13.2 software following a completely randomized design (CRD) with two factors and four replications per treatment. The significance levels between means were determined using the Least Significant Differences (LSD) test at $p < 0.05$.

RESULTS AND DISCUSSION

Drought and salinity significantly reduced germination percentage, mean germination time, and germination index of pumpkin seeds (Table 1). Furthermore, analysis of variance revealed a significant interaction between drought and salinity. Under drought stress, the mean germination percentage and the germination index decreased from 97.5% to 79.5% and from 11.5 to 5.5, respectively. However, these stresses prolonged the mean time to germination in pumpkin seeds.

Table 1. Main effects of salinity and drought on germination percentage, mean germination time, and germination index of pumpkin

Factor	Germination percentage (%)	MGT (day)	Germination index
Drought (A)			
Control	97.5 ^a	2.19 ^b	11.5 ^{a†}
Drought	79.5 ^b	4.50 ^a	5.5 ^b
Salinity (B)			
0.00%	99.0 ^a	2.46 ^c	10.7 ^a
0.25%	97.5 ^a	2.86 ^b	9.45 ^b
0.50%	85.0 ^b	3.88 ^a	7.30 ^c
0.75%	72.5 ^c	4.18 ^a	6.44 ^d
<i>A</i>	**	**	**
<i>B</i>	**	**	**
<i>A × B</i>	**	**	**

†: Letters connected with the mean in each column refer to significance level at $p < 0.05$. **: significant at $p < 0.01$.

In non-drought control treatment, salinity did not change the germination percentage of pumpkin seeds. However, it declined at 0.50% NaCl under drought stress (Figure 1). The lowest germination percentage of 46.0% was recorded at 0.75% NaCl under drought. Under non-drought conditions, the germination index reduced slightly from 12.3 to 10.8 with increasing salinity. However, drought significantly impacted the germination index, leading to a drop from 9.06 to 2.07. Drought delayed the mean germination time, while increased salinity significantly prolonged this period. Under drought stress, the hazardous effects of salinity on the mean germination time of pumpkin appeared.

The germination percentage began to decrease at 0.50% NaCl only under drought stress and no significant changes were observed under non-drought conditions. The germination index was also diminished by increasing salinity and a greater reduction was observed under drought stress. However, Tarchoun et al. (2022) found that rising NaCl levels decreased the germination percentage of *C. maxima*. Jahed et al. (2023), Ashraf et al. (2021) and Saadaoui et al. (2023) reported inhibited germination under drought stress. Kaya (2024) demonstrated that mean germination time and germination index were inhibited by increasing drought stress with PEG 6000. Furthermore, increased salinity retarded mean germination time, particularly under drought stress. It was obvious that the combination of drought and salt stress showed inhibitory effects on the germination performance of pumpkin. Similar findings were observed by Irik and Bikmaz (2024), who stated a prolonged germination time and a drop in germination percentage and index due to salinity stress.

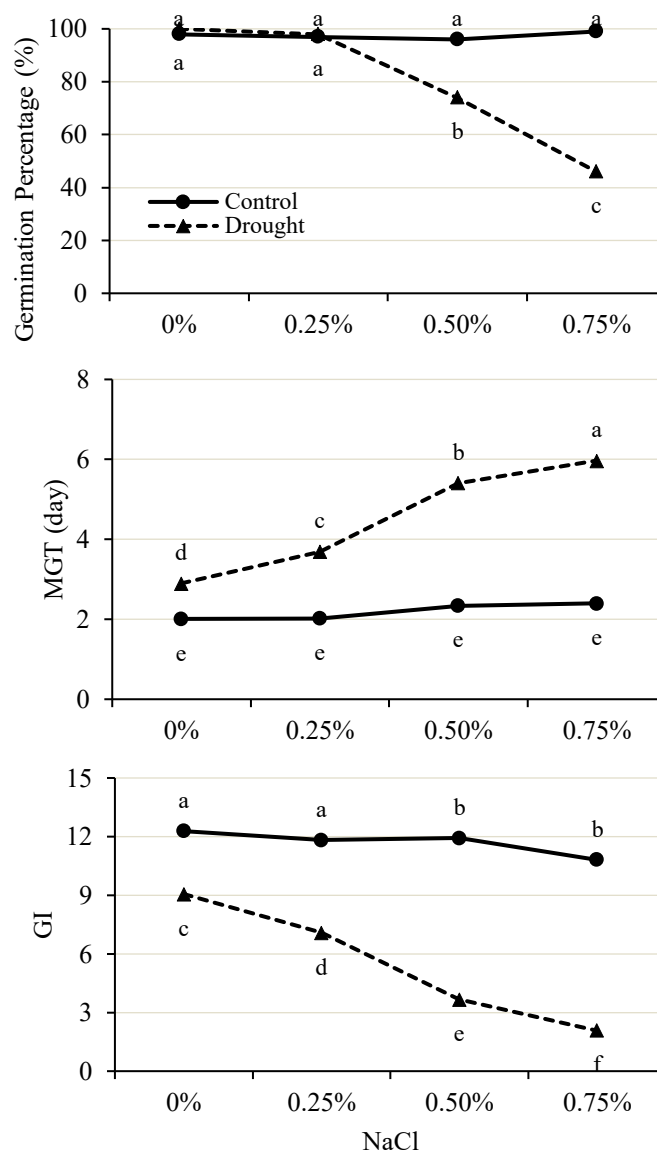


Figure 1. Changes in the germination percentage, mean germination time (MGT), and germination index (GI) of pumpkin seeds subjected to different salinity levels under drought conditions

As shown in Table 2, drought and salinity caused a significant difference in all morpho-physiological parameters of the pumpkin. The interaction between drought and salinity stress was significant, except for dry weight. Under drought stress, plant height, fresh weight, dry weight, leaf area, RWC, and CMS decreased. The interaction effects on morphological and physiological characteristics of pumpkin plants are displayed in Figure 2.

A low NaCl level of 0.25% promoted the leaf area under non-drought conditions and did not considerably decrease up to 0.75% NaCl (Figure 2). Under drought stress, a similar trend was observed, and low leaf size was obtained. However, 0.75% NaCl markedly inhibited the leaf growth of pumpkin plant. Drought also inhibited the leaf growth of pumpkin plants, resulting in a significant reduction in leaf area, which is supported by the findings of Cui et al. (2019) in cucumber. Furthermore, the leaf area was only severely affected by 0.75% NaCl under both control and drought conditions. This result demonstrates that the leaf area exhibited no sensitivity to NaCl concentrations up to 0.75%. The findings of Horuz et al. (2022) confirm the results of the present study, which indicate a reduction in pumpkin leaf area due to salinity. Moreover, the current study reports here that drought exerts a greater inhibitory effect on leaf growth compared to salinity, and the adverse effects of salinity are exacerbated under drought stress. Sucre and Suarez (2011) demonstrated that the impact of salinity on the leaf growth of *Ipomoea pes-caprae* was more detrimental under water deficit conditions.

Table 2. Analysis of variance and mean values of morpho-physiological characteristics of pumpkin plants subjected to salinity and drought stress.

Factor	Plant height (cm)	Plant fresh weight (g plant ⁻¹)	Plant dry weight (g plant ⁻¹)	Leaf dry matter (%)	Leaf area (cm ²)	Chlorophyll content (SPAD)	RWC (%)	CMS (%)
Drought (A)								
Control	17.1 ^a	33.3 ^a	3.32 ^a	10.76 ^b	576 ^a	32.1 ^b	88.1 ^a	63.2 ^{a†}
Drought	12.6 ^b	23.7 ^b	2.40 ^b	11.33 ^a	420 ^b	39.7 ^a	75.3 ^b	52.8 ^b
Salinity(B)								
0.00%	17.2 ^a	30.1 ^b	3.57 ^a	12.98 ^a	507 ^b	43.8 ^a	83.6 ^a	66.6 ^a
0.25%	15.8 ^b	32.8 ^a	3.25 ^a	11.36 ^b	541 ^a	34.9 ^b	86.6 ^b	59.3 ^b
0.50%	14.9 ^b	27.1 ^c	2.59 ^b	10.47 ^c	492 ^b	33.6 ^b	81.7 ^b	53.5 ^c
0.75%	11.4 ^c	23.2 ^d	2.02 ^c	9.36 ^d	453 ^c	31.2 ^c	74.9 ^c	52.7 ^c
<i>A</i>	**	**	**	**	**	**	**	**
<i>B</i>	**	**	**	**	**	**	**	**
<i>A × B</i>	**	**	<i>ns</i>	**	**	**	*	**

†: Letters connected with the mean in each column refer to significance levels at $p < 0.05$. *, **: significant at $p < 0.05$ and $p < 0.01$, respectively.

Under non-drought stress, plant height decreased from 19.9 cm at 0.00% NaCl to 13.8 cm at 0.75% NaCl, while it dropped to 14.4 cm when plants were subjected to drought (Figure 2). Increased salinity inhibited stem elongation and caused a reduction in plant height, with a minimum reduction of 9.12 cm in plants exposed to 0.75% NaCl under drought stress. Shorter plant height under drought stress was confirmed by the findings of Wang et al. (2024), who determined plant height was significantly lower in cucumber plants exposed to 10% PEG drought. Esan et al. (2023) also reported similar results in soybean. Plant height was shorter in pumpkin plants subjected to increasing salinity levels, regardless of the availability of drought stress. However, drought promoted the severity of salinity stress to reduce the plant height, a decline from 19.9 cm to 14.5 cm. This result confirms the findings of Kurum et al. (2013) and Santos et al. (2018), who indicated that both shoot and root lengths of pumpkin were shortened by increasing salinity.

Increased NaCl concentrations under both non-drought and drought conditions resulted in lower plant fresh weight. In drought, it reduced from 36.4 g to 25.3 g, while in 0.75% NaCl, it decreased to 27.6 g. This indicates that drought exerted a greater negative effect on fresh weight than salinity and that fresh weight was more influenced by salinity under drought stress. The application of 0.25% NaCl stimulated plant fresh weight in non-drought conditions, and higher salinity levels led to a significant decrease in fresh weight. This finding is corroborated by Sevgör et al. (2011) and Kurum et al. (2013), who documented a reduction in the fresh weight of pumpkin as a consequence of salinity.

Regardless of the presence of drought, salinity consistently reduced the dry weight of pumpkin plants (Figure 2). A lower dry weight was recorded in pumpkin plants subjected to drought stress than in those not exposed to drought. Salinity caused a 40% reduction in dry weight under non-drought conditions and a 47% reduction under drought conditions. A linear decrease in dry weight due to increased salinity suggests that it is more sensitive to salinity than fresh weight. Kuşvuran et al. (2021) found a decline in root and shoot dry weight of melon seedlings exposed to salinity stress. Leaf dry matter was also reduced by drought and salinity, but this reduction was minimal compared to the other traits, implying that it was the least affected trait by drought and salinity. Similarly, the findings of Oliveira et al. (2014) and Santos et al. (2018) are consistent with this reduction in total dry matter associated with increasing salinity. A negative effect of drought on dry matter was also determined by Wang et al. (2024) in cucumber. This study indicates that the differences between non-drought and drought conditions were not prominent in dry matter, suggesting that it was not a reliable indicator of drought tolerance in pumpkin.

The chlorophyll content of pumpkin plants exposed to drought stress exceeded that of the control group. Increased salinity reduced chlorophyll content in pumpkin plants under both control and drought conditions. A NaCl level of 0.75% led to a severe depression in chlorophyll content. These results agreed with those of Sevgör et al. (2011) and Taratima et al. (2023), who found that pumpkin plants lost more total chlorophyll when they were stressed by salinity.

Pumpkin plants exposed to drought stress exhibited a lower RWC than those in non-drought conditions. Drought stress resulted in reduced RWC at all NaCl levels. This result is similar to the findings of Cui et al. (2019) in cucumbers, who reported a decrease in RWC due to drought. However, salinity levels of up to 0.50% NaCl did not considerably influence RWC under both control and drought conditions, while the lowest RWC was recorded at 0.75% NaCl. This finding indicates that drought had a more significant impact on reducing RWC than salinity. Horuz et al. (2022) reported that pumpkin plants subjected to salt stress exhibited a reduction in relative water

content (RWC) in comparison to plants that were not exposed to stress. This finding was subsequently corroborated by Rehman et al. (2024) in a study examining melon plants under conditions of drought stress.

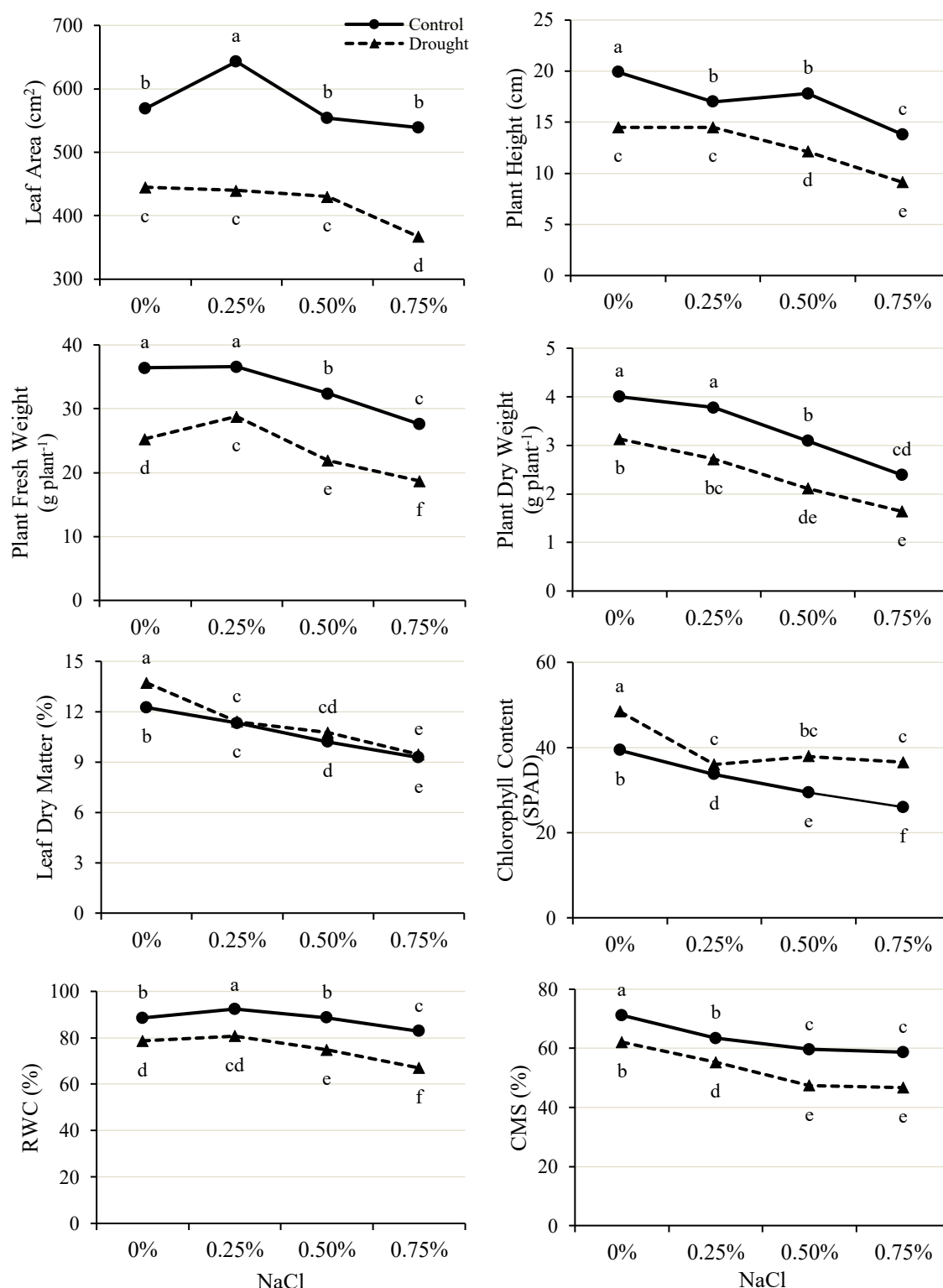


Figure 2. Changes in leaf area, plant height, plant fresh weight, plant dry weight, leaf dry matter, chlorophyll content (SPAD), relative water content (RWC), and cell membrane stability (CMS) of pumpkin plants exposed to different salinity levels under drought conditions.

The cell membrane stability was higher in pumpkin plants grown under non-drought conditions than under drought. However, it decreased with increasing salinity. Under drought conditions, the cell membrane stability decreased by 25% at a NaCl level of 0.75% but was reduced by 17.5% under non-drought conditions. This reduction due to salinity was 15.6% in control and 25.8% under drought stress, indicating that salinity exerted a detrimental effect under drought stress. Electrolyte leakage is a stress tolerance indicator showing cell membrane stability (Irik and Bikmaz, 2024). Increased electrolyte leakage or decreased cell membrane stability were declared under saline conditions by Jamil et al. (2012) in sugar beet, Farooq and Azam (2006) in wheat, Ashraf and Ali (2008) in canola, Elsheery et al. (2020) in cucumber and under drought conditions by Bajji et al. (2002) and ElBasyoni et al. (2017) in wheat and Irik and Bikmaz (2024) in pumpkin. In addition, a decreasing trend in cell membrane stability was observed with increasing salinity under both drought and non-drought conditions.

The relationship between all the characteristics examined was determined using simple correlation analysis (Table 3). All parameters showed a negative relationship with mean germination time, excluding Chl. The highest significant negative correlation coefficient ($r = -0.985^{**}$) was found between the germination index and mean germination time. The weakest correlations were noted between Chl and other traits, while the strongest was obtained with plant height. The results revealed that germination traits were strongly associated with plant growth parameters under salt and drought stress. Among germination traits, the most promising trait for estimating plant growth parameters was germination index because it was significantly correlated with plant height ($r = 0.857^{**}$), fresh weight ($r = 0.881^{**}$), dry weight ($r = 0.824^{**}$), RWC ($r = 0.893^{**}$), CMS ($r = 0.902^{**}$) and leaf area ($r = 0.846^{**}$), suggesting that germination index should be considered as a predictor evaluating the response of pumpkin to salinity and drought stress.

Table 3. Correlation coefficients among the investigated parameters of pumpkin exposed to salinity and drought stress.

	CHL	PH	FW	DW	DM	RWC	CMS	LA	GP	MGT	GI	
CHL	1.000	ns	ns	ns	**	ns	ns	*	ns	ns	ns	
PH	-0.071	1.000	**	**	*	**	**	**	**	**	**	
FW	-0.212	0.908	1.000	**	ns	**	**	**	**	**	**	
DW	0.125	0.890	0.913	1.000	**	**	**	**	**	**	**	
DM	0.782	0.410	0.272	0.581	1.000	ns	**	ns	*	ns	ns	
RWC	-0.291	0.855	0.934	0.844	0.207	1.000	**	**	**	**	**	
CMS	0.096	0.859	0.848	0.886	0.506	0.794	1.000	**	**	**	**	
LA	-0.373	0.722	0.869	0.747	0.044	0.887	0.726	1.000	**	**	**	
GP	-0.057	0.731	0.710	0.683	0.415	0.754	0.732	0.607	1.000	**	**	
MGT	0.185	-0.826	-0.850	-0.801	-0.316	-0.882	-0.878	-0.799	-0.850	1.000	**	
GI	-0.215	0.857	0.881	0.824	0.287	0.893	0.902	0.846	0.845	-0.985	1.000	

Chl: Chlorophyll content, PH: Plant height, FW: Plant fresh weight, DW: Plant dry weight, DM: leaf dry matter, RWC: Relative water content, CMS: Cell membrane stability, LA: Leaf area, GP: Germination percentage, MGT: Mean germination time, GI: Germination index

*, ** show significance levels at $p < 0.05$ and $p < 0.01$, respectively. ns: non-significant.

CONCLUSION

Germination and morphophysiological characteristics of pumpkin were significantly affected by salt under drought stress. A NaCl concentration of 0.50% reduced and delayed germination when there was drought stress, but it did not affect germination percentage under non-drought stress. The germination index was more sensitive to drought and salinity and clearly distinguished salinity levels. This means that any increase in salinity under drought caused the germination index to drop, which could be recommended as a valuable criterion for salt and drought stress during germination. The difference between drought and non-drought conditions was evident as the salt level increased. In the pot experiment, the inhibitory effects of salinity on plant height, plant fresh and dry weight were evident. These parameters could be used as the selection criteria for salt- and drought-tolerant pumpkin genotypes. Conversely, compared to non-saline conditions, RWC decreased in the presence of 0.75% NaCl. Under drought stress, cell membrane stability decreased, and increased salinity led to a further reduction, which should therefore be considered when assessing drought tolerance in pumpkin. On the other hand, germination parameters gave significant correlations with morphological and physiological traits, and they distinguished a more precise separation between salinity levels under drought stress. The germination index was evaluated as a predictor of the response of pumpkin plants to salt and drought stress, serving as a primary selection criterion. It was evident that pumpkin showed greater tolerance to salt and drought stress during the early growth stage compared to the germination stage. In conclusion, this study demonstrated that salinity was more harmful

when there was drought stress than in non-drought conditions, with a NaCl level of 0.50% identified as a critical threshold for pumpkin growth.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest. The paper is original unpublished work, and it is not under consideration for publication anywhere else.

Author Contribution

Conceptualization, methodology, and final version were done independently by Gamze Kaya

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