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REACTION OF HYBRID ORNAMENTAL KALES TO *IN VITRO* SALT STRESS DURING THE GERMINATION AND EMERGENCE PERIODS

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ABSTRACT

This study aimed to determine the reactions of five F_1 hybrid ornamental kale cultivars (Red Pigeon, Victoria Pigeon, Red Chidori, White Kamome, and Pink Kamome) to *in vitro* salt stress during germination and emergence. Sterilized seeds were cultured on Murashige and Skoog (MS) media containing four different NaCl doses (0, 3, 7, and 12 dS/m). According to the obtained results, the germination rate, speed of germination, average germination time, germination index, and sensitivity index values were 42.72-97.09%, 0.76-3.43, 3.08-6.80 days, 6.60-41.28, and 0.81-1.51, respectively. Emergence rate, speed of emergence, the average emergence time, emergence index, hypocotyl length and radicle length values were detected as 11.99-93.78%, 0.16-1.63, 4.97-8.69 days, 1.45-18.75, 0.39-3.27 cm and 0.68-3.43 cm, respectively. The F_1 Red Pigeon and F_1 Victoria Pigeon were the most salt stress resistant cultivars, while the F_1 White Kamome cultivars were the most susceptible to salt stress. Increasing the NaCl dose had a statistically significant negative effect on both the germination and emergence properties of the cultivars.

KEYWORDS:
Brassica; *In vitro*; NaCl dose; Germination; Emergence

INTRODUCTION

Seed germination and emergence are successive events that follow expansion, enzyme activation, the start of embryo growth, and the cracking of the seed coat [1]. Many stress factors such as salt stress [2, 3, 4, 5], heavy metals [6; 7] and drought stress [8]. have also been reported to have an effect on germination, seedling growth and productivity components. As an abiotic danger, salinity can cause various adverse effects by inhibiting seed germination at its higher levels, and encouraging dormancy at its lower levels. Salt stress affects all of the developmental stages of plants, most plant species are more susceptible to salt stress during the germination period [9, 10, 11]. *Brassica* species, which include ornamental kale, have moderate salt stress resistance, but their tolerance to salt varies by species.

Previously, eight different NaCl doses (0, 25, 50, 75, 100, 125, 150, and 200 mM) were studied in winter canola cultivars, and the results showed that the germination rate varied between 65.3% and 100%, the germination time varied between 3.75 days and 8.71 days, the radicle length varied between 0.50 cm and 12.81 cm, and the shoot length varied between 0.59 cm and 8.79 cm. Moreover, the cultivars were resistant to salt stress up to a 125 mM NaCl dose, and the germination rate decreased significantly thereafter [4]. In their study of five ornamental kales (Nagoya, Tokyo, Osaka, Pigeon, and Seagull), Chen et al. [5] determined that, although varying by cultivars, 150 mmol/L of NaCl inhibited germination. The Nagoya cultivar was most resistant to salt stress, while the Pigeon cultivar was most affected by salt. For the Nagoya cultivar, 213.65 mmol/L was determined to be the lethal dose, whereas 87.32 mmol/L was determined to be the lethal dose for the Pigeon cultivar.

Ornamental kales are commonly used as outdoor plants by municipalities for flower planting applications in green fields under winter conditions. In Turkey, curled leaves (Kamome and Chidori series) are more common in regions with a continental climate, and smooth leaves (Pigeon series) are common in the temperate climate regions. Due to the use of hybrid cultivars in production, their seeds are sold at higher prices.

In this study, the effects of *in vitro* salt stress applications on seed germination and the emergence parameters of five hybrid ornamental kale cultivars, which have high adaptability and are widely grown in Turkey, were investigated.

MATERIALS AND METHODS

Plant materials. The seeds of five different F_1 hybrid ornamental kale cultivars (Red Pigeon, Victoria Pigeon, Red Chidori, White Kamome, and

Pink Kamome) that were provided by a seed company were used as the study material.

Sterilization of seeds. The seeds were rinsed with tap water to remove any abiotic materials, put in a sterile cabinet, and kept in a 20% hypochlorite solution containing a few drops of Tween-20 for 15 minutes; then, they were rinsed three times for 6 minutes each with bidistilled water.

***In vitro* seed germination and emergence, salt (NaCl) applications.** The sterilized seeds were cultured on MS media [12] containing four different salt concentrations (0, 3, 7, and 12 dS/m). The pH value of the MS media with 30 g sucrose/L and 7 g agar/L added was adjusted to 5.8. The seeds were planted on glass petri dishes 6 cm in diameter, each containing 7 mL of the medium (10 seeds/petri dish), and the petri dishes were covered with plastic wrap to avoid infection/contamination. The seeds were germinated in climate-controlled chambers at 25°C, with 16/8 hours light/darkness.

Evaluation of germination and emergence parameters. The germination trials were finalized on the 10th day by counting the radicle that reached 2 mm in length. In the germination trials, the values for the germination rate (GR) and speed, average germination time (AGT), germination index, and sensitivity index were calculated. The emergence criterion was based on the period in which the cotyledon leaves were completely parallel to the ground. In the emergence trials, the emergence rate and speed, average emergence time, emergence index, and lengths of the hypocotyl and radicle were evaluated. The hypocotyl and radicle lengths were determined by calculating the average values of 10 plantlets. The following formulas were used in the calculations for the germination and emergence parameters [13, 14]:

Germination/Emergence Rate (%) = (G/T) x 100

Germination/Emergence Speed = n1/t1 + n2/t2 + ... + nn/tn [15]

Average Germination/Emergence Time (day) = [(1. day G x 1. day) + (2. day G x 2. day) + ... + (n. day G x n. day)] / Total G

Germination Index/Emergence Index = (1. day GR/Dt1) + (2. day GR/Dt2) + ... + (n. day GR/Dtn)

Sensitivity Index = AGT in the salt application / AGT in the control application [16]

In the equations: G represents the Germinated/ Emerged seed number, T represents the total number of seeds, AGT represents the average germination time, Dt represents the day of counting, and n represents the last day of counting, GR represents the germination rate/emergence rate.

TABLE 1

The effects of *in vitro* salt stress on the germination parameters of the hybrid ornamental kale cultivars.

Accessions	NaCl (dS/m)	Germination rate	Germination speed	Average Germination Time	Germination index	Sensitivity index
	0	96.64^{ab}	3.43	3.08^{h}	41.28	-
F_1 Red	3	95.88^{ab}	3.67	3.28^{gh}	38.46	1.08
Pigeon	7	94.09^{ab}	2.92	3.69^{gh}	28.80	1.21
	12	90.33^{a-c}	2.06	4.85^{de}	20.56	1.51
Average		94.24^{1}	3.02^{1}	3.72^{5}	32.28^{1}	0.95^{1}
	0	95.49^{ab}	3.19	3.93^{fg}	30.33	-
F_1 Victoria	3	97.09^{a}	2.74	3.95^{fg}	28.01	1.04
Pigeon	7	94.54^{ab}	2.54	4.59^{ef}	23.08	1.21
	12	82.11^{c-e}	1.69	5.43^{cd}	15.50	1.48
Average		92.31^{1}	2.54^{2}	4.47^{4}	24.23^{2}	0.93^{1}
	0	91.69^{a-c}	2.74	4.77^{de}	23.87	-
F_1 Red	3	86.58^{a-e}	2.24	5.15^{c-e}	20.32	1.08
Chidori	7	76.59^{d-f}	1.76	5.36^{cd}	16.70	1.16
	12	53.61^{g}	0.95	6.59^{a}	10.59	1.45
Average		77.12^{2}	1.92^{3}	5.47^{2}	17.88^{3}	0.93^{1}
	0	87.49^{a-d}	2.05	5.40^{cd}	19.06	-
F_1 White	3	75.27^{ef}	1.66	5.71^{bc}	15.31	1.07
Kamome	7	66.59^{f}	1.28	6.17^{ab}	12.16	1.22
	12	42.72^{h}	0.76	6.80^{a}	6.60	1.33
Average		68.02^{4}	1.44^{4}	6.02^{1}	13.28^{4}	0.94^{1}
	0	85.03^{b-e}	2.41	4.75^{de}	23.54	-
F_1 Pink	3	85.45^{a-e}	2.26	4.79^{de}	21.12	1.02
Kamome	7	68.98^{f}	2.40	3.95^{fg}	21.05	0.81
	12	52.27^{gh}	1.06	5.70^{bc}	10.22	1.22
Average		72.93^{3}	2.03^{3}	4.80^{3}	18.98^{3}	0.76^{2}
	0	91.27^{A}	2.76^{A}	4.39^{C}	27.62^{A}	-
NaCl	3	88.05^{A}	2.51^{B}	4.58^{BC}	24.65^{B}	1.05^{B}
Average	7	80.16^{B}	2.18^{C}	4.75^{B}	20.36^{C}	1.12^{B}
	12	64.21^{C}	1.30^{D}	5.87^{A}	12.69^{D}	1.39^{A}

Different capital letters in a same column show significant differences among the salt doses ($p<0.05$)
Different small letters in a same column show significant differences among the species x salt doses ($p<0.05$)
Different figures in a same column show significant differences among the species ($p<0.05$)

TABLE 2
The effects of salt stress on the emergence parameters of the hybrid ornamental kale cultivars.

Accessions	NaCl (dS/m)	Emergence Rate	Emergence Rate	Average Emergence Time	Emergence index	Hypocotyl length	Radicle lenght
	0	89.65^{a}	1.62^{a}	4.97^{f}	18.75^{a}	2.17^{c}	2.05^{c-e}
F_1 Red	3	93.78^{a}	1.63^{a}	5.72^{ef}	17.09^{a}	1.98^{cd}	1.73^{e-g}
Pigeon	7	87.10^{ab}	1.41^{ab}	6.23^{def}	13.04^{b}	1.18^{gh}	1.36^{gh}
	12	62.38^{d-f}	0.79^{ef}	8.32^{ab}	7.78^{ef}	0.49^{ij}	1.57^{f-h}
Average		83.23^{1}	1.36^{1}	6.31^{3}	14.18^{1}	1.46^{2}	1.68^{3}
	0	81.66^{a-c}	1.43^{ab}	6.61^{c-e}	13.24^{b}	1.33^{f-h}	1.96^{c-f}
F_1 Victoria	3	87.81^{ab}	1.21^{bc}	7.77^{a-d}	11.16^{bc}	1.21^{f-h}	1.92^{d-f}
Pigeon	7	65.98^{d-f}	0.78^{ef}	8.69^{a}	8.65^{de}	0.72^{ij}	1.40^{gh}
	12	23.41^{jk}	0.30^{hi}	7.93^{a-c}	2.69^{hi}	0.47^{j}	0.68^{i}
Average		64.72^{2}	0.93^{2}	7.75^{1}	8.93^{2}	0.93^{4}	1.49^{3}
	0	74.45^{b-d}	1.17^{cd}	7.19^{a-e}	10.74^{cd}	3.27^{a}	2.39^{c}
F_1 Red	3	55.45^{e-h}	0.79^{ef}	7.52^{a-d}	7.75^{ef}	2.72^{b}	2.24^{cd}
Chidori	7	45.91^{g-i}	0.59^{fg}	7.92^{a-c}	5.79^{fg}	1.63^{d-f}	1.94^{d-f}
	12	18.18^{k}	0.21^{i}	7.70^{a-d}	1.87^{hi}	1.14^{gh}	1.37^{gh}
Average		48.50^{3}	0.69^{3}	$7.58^{1,2}$	6.54^{3}	2.19^{1}	1.99^{2}
	0	58.55^{e-g}	0.86^{e}	6.81^{b-e}	8.74^{de}	1.76^{de}	1.46^{gh}
F_1 White	3	43.18^{hi}	0.52^{gh}	7.59^{a-d}	5.52^{fg}	1.48^{e-g}	1.48^{gh}
Kamome	7	33.18^{ij}	0.46^{gh}	8.30^{ab}	4.23^{gh}	0.99^{hi}	1.14^{h}
	12	11.99^{k}	0.16^{i}	8.32^{ab}	1.45^{i}	0.39^{j}	0.71^{i}
Average		36.73^{4}	0.50^{4}	$6.91^{2,3}$	4.98^{4}	1.16^{3}	1.20^{4}
	0	67.99^{c-e}	1.16^{cd}	7.18^{a-e}	10.18^{c-e}	3.27^{a}	3.42^{a}
F_1 Pink	3	60.90^{d-f}	1.02^{c-e}	6.76^{b-e}	9.18^{c-e}	2.72^{b}	3.43^{a}
Kamome	7	52.67^{f-h}	0.96^{de}	6.63^{c-e}	8.27^{e}	1.35^{e-h}	2.99^{b}
	12	21.74^{jk}	0.29^{hi}	8.45^{a}	2.65^{hi}	0.67^{ij}	1.17^{h}
Average		50.83^{3}	0.86^{2}	$7.26^{1,2}$	7.57^{3}	2.00^{1}	2.75^{1}
	0	74.46^{A}	1.25^{A}	6.55^{B}	12.35^{A}	2.36^{A}	2.26^{A}
NaCl	3	68.22^{B}	1.03^{B}	7.07^{AB}	10.14^{B}	2.02^{B}	2.16^{A}
Average	7	56.96^{C}	0.84^{C}	7.55^{A}	7.99^{C}	1.17^{C}	1.77^{B}
	12	27.54^{D}	0.35^{D}	7.47^{A}	3.28^{D}	0.63^{D}	1.10^{C}

Different capital letters in a same column show significant differences among the salt doses ($p<0.05$)
Different small letters in a same column show significant differences among the species x salt doses ($p<0.05$)
Different figures in a same column show significant differences among the species ($p<0.05$)

Statistical analysis. The germination and emergence trials were carried out in 3 repetitions, each involving 40 seeds. The data were evaluated by analyses of variance, and the means were compared by Duncan's multiple range test ($P<0.05$) using the SPSS statistical package program.

RESULTS AND DISCUSSION

Findings of germination parameters. The variance analysis results of the germination parameters of the *in vitro* salt stress applications on the hybrid ornamental kale cultivars are shown in Table 1. The cultivar x salt interactions of the germination rates and average germination times were statistically significant ($p<0.05$). In addition, the germination rates of the ornamental kale cultivars were significantly affected by the increasing NaCl doses, and varied between 42.72% and 97.09% for all of the cultivars.

The highest germination rate (97.09%) was observed for the F_1 Victoria Pigeon cultivar at the 3 dS/m NaCl dose, followed by the control group (96.64%) of the F_1 Red Pigeon cultivar and the F_1 Red Pigeon cultivar at the 3 dS/m NaCl dose (95.88%). The lowest germination rates were observed for the F_1 White Kamome (42.72%) and F_1 Pink Kamome (52.27%) cultivars at the 12 dS/m dose and the F_1 Red Chidori cultivar (53.61%), respectively. The germination rate decreased with the increasing salt concentrations in all of the cultivars except for the F_1 Victoria Pigeon cultivar at the 3 dS/m dose and the F_1 Pink Kamome cultivar, when compared with the control group (Table 1). The F_1 Red Pigeon (94.24%) and F_1 Victoria Pigeon (92.31%) cultivars were the most salt stress resistant cultivars, whereas the F_1 White Kamome (68.2%) and F_1 Pink Kamome (72.93%) cultivars were the most sensitive to *in vitro* salt stress. Regarding the difference between the salt averages, the germination rates of all of the cultivars were significantly decreased in the salt levels above 3 dS/m (Table 1). Jamil et al. [2] studied three different *Brassica* species (head cabbage, cauliflower, and rapeseed) at four different NaCl doses (0, 4.7, 9.4, and 24.2 dS/m) and reported that the germination rate significantly decreased with the increasing salt concentrations. The decrease in germination was most significant in the cauliflower, while the root and shoot lengths of all three species decreased significantly at the 14.1 dS/m salt level.

Ayaz et al. [17] reported that metabolic disorders and the inhibition of the germination regulatory protein synthesis in plants under salt stress can cause a decrease in the germination rate.

When considering the average germination time, the cultivars were significantly affected by the NaCl doses (Table 1), and varied between 3.08 days and 6.80 days at the different doses. The fastest germination was observed in the F_1 Red Pigeon cultivar control group (3.08 days), followed by the same cultivar at the 3 dS/m (3.28 days) and 7 dS/m (3.69 days) doses (Table 1). The slowest germinations were observed in the F_1 White Kamome (6.80 days) and F_1 Red Chidori (6.59 days) cultivars at the 12 dS/m dose. With the exception of the F_1 Pink Kamome cultivar at the 7 dS/m dose (3.95 days), the average germination time was prolonged for the cultivars at all of the doses above 3 dS/m (Table 1). Regarding the average germination time, the differences in all of the cultivar averages were statistically significant ($p<0.05$); the F_1 Red Pigeon cultivar (3.72 days) had the fastest germination, while the F_1 White Kamome (6.02 days) had the slowest germination. The results from our findings are similar to the data from Jamil et al. [2], Kaya et al. [3], Al-Thabet et al. [18], and Bybordi and Tabatabaei [19].

In consideration of the germination speed, except for the F_1 Red Chidori and F_1 Pink Kamome cultivars, the differences in all of the cultivar averages were statistically significant ($p<0.05$). The germination index shows the pattern of the seed germination, and a decrease in the index value indicates a loss in the germination abilities of the seeds. The highest index values were observed for the control groups of all of the cultivars (Table 1).

Findings of emergence parameters. The cultivar x salt interaction was statistically significant for all of the emergence properties (Table 2). When considering the emergence rate, the differences in the cultivar averages were statistically significant for all of the cultivars, except the F_1 Red Chidori (48.50%) and F_1 Pink Kamome (50.83%) ($p<0.05$). The F_1 Red Pigeon cultivar (83.23%) had the highest emergence rate, whereas the F_1 White Kamome cultivar (36.73%) had the lowest emergence rate. The emergence speed was statistically significant for all but two of the cultivars (F_1 Pink Kamome and F_1 Victoria Pigeon), while the emergence rate, emergence speed, and emergence index decreased significantly with the increasing salt concentrations (Table 2).

The hypocotyl length of all of the cultivars significantly decreased with the increasing salt concentrations ($p<0.05$), which can be explained by the ion toxicity-inhibited water uptake and osmotic pressure resulting from the salt stress. These results are similar to the results obtained by, Zamani [11] and Saied et al. [20], who studied rapeseed and other cultivars.

The differences in the emergence rates of the cultivars at the end of the 10th day at the different salt concentrations are shown in Figure 1.

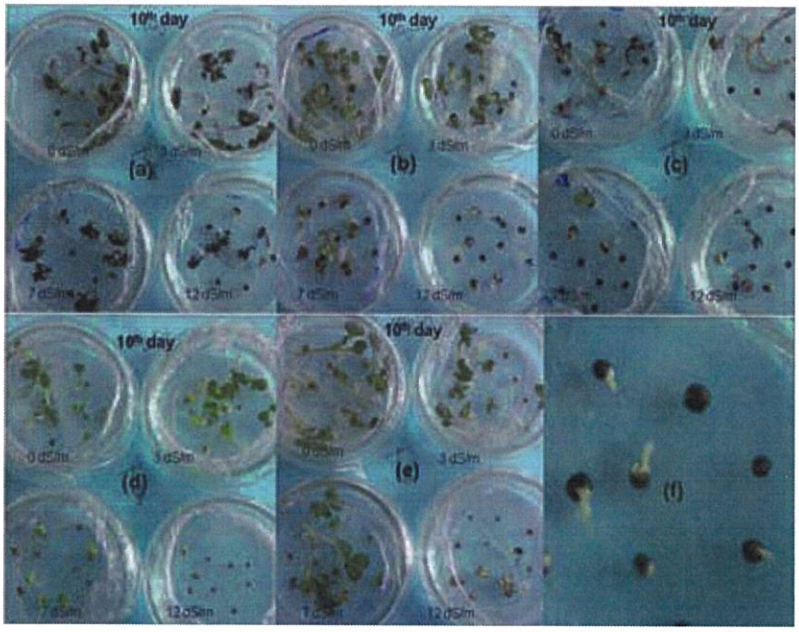

FIGURE 1
Differences in the emergence rates of the cultivars at the end of the 10th day at the different salt concentrations: (a) F_1 Red Pigeon, (b) F_1 Victoria Pigeon, (c) F_1 Red Chidori, (d) F_1 White Kamome, (e) F_1 Pink Kamome, and (f) germinated seeds.

The examination of the averages of the cultivars revealed that the F_1 Red Chidori (2.19 cm) and F_1 Pink Kamome (2.00 cm) cultivars had the highest hypocotyl lengths. In addition, the radicle lengths of the cultivars differed with the salt concentrations; however, the lengths did not decrease significantly until reaching the 3 dS/m dose. A significant decrease in the radicle length was observed in those doses above 3 dS/m ($p<0.05$). With regard to the radicle length, the F_1 Pink Kamome cultivar (2.75 cm) was the least affected by the salt concentrations (Table 2). Kaya et al. [3] studied the effects of different salt concentrations (0, 5, 10, and 20 dS/m) on the germination and early seedling growth of rapeseed (*Brassica napus* ssp. *oleifera* L.), turnip rape (*Brassica campestris* L.), and cabbage (*Brassica oleracea* L.). They determined that the turnip rape was least affected by the NaCl doses, the salt levels did not cause a significant decrease in the germination and seedling growth up to 10 dS/m, and the salt levels inhibited seedling growth rather than germination.

Jamil et al. [2] emphasized that the radicle length was one of the parameters that were significantly affected by salinity. This may be the result of NaCl toxicity and irregularities in the nutrient uptake of the roots due to salinity. Accordingly, Khan and Gulzar [21] reported that salinity adversely affected the root development by reducing the nutrient uptake. Our study findings are also supported by those of Saied et al. [20] and Mahmoodzadeh [22], who reported that the rapeseed root length decreased with increasing salt levels.

CONCLUSION

This study investigated the reactions of five hybrid ornamental kales to salt stress during the germination and emergence periods. It was determined that the adverse effects of the increasing NaCl doses on all of the growth parameters were statistically significant. A general examination of both the germination and emergence parameters revealed that the ornamental kales of the Pigeon cultivar (F_1 Red Pigeon and F_1 Victoria Pigeon) were the most salt stress resistant, whereas the F_1 White Kamome cultivar was the most affected by the salt.

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