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Screening of paprika (*Capsicum annuum* L.) varieties resistant to NaCl salt stress

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SUMMARY

Salinity stress is one of the environmental factors that negatively affect the growth and production of pepper plants. The 100 seeds' weight was measured. The total fresh weight of five seedlings and the growth rate of one seedling of three paprika varieties were also measured under the influence of NaCl at a salinity level threshold of (3 dS m^{-1}). The proportion of tissue water content in three pepper cultivars was measured according to a mathematical formula at the end of the experiment. In terms of seed weight, the (Carma) cultivar outperformed other types greatly. Except for the superiority of both (Carma, and Bobita F1) over (Fokusz) variety in total fresh seedlings weight under sodium chloride as abiotic stress, there are no significant differences in the total seedlings' dry weight and the rate of seedling growth. The non-drought-resistant type (Bobita F1) loses water the fastest, at 89.61%, compared to drought-tolerant kinds, which lose water at a slower rate (Carma, and Fokusz). The results demonstrate the (Carma) variety's numerical vigor, particularly in the growth rate. More testing is needed to determine the selection of varieties that are resistant to abiotic and biotic stresses.

Keywords: *foliar, immunity, pepper, pests, salt, stress*

INTRODUCTION

Arzani (2008) reported salinity in soil or water is one of the major abiotic stressors, reducing plant growth and productivity all around the world. According to reports, salinity stress affects more than 6% of the world's geographical area. In agricultural areas, the main causes of salinity are poor soil and water management, insufficient rainfall, and high evaporation. Traditional breeding programs are critical for crop improvement. Plant breeding initiatives are working to develop tolerant cultivars for saline fields and identify salt-tolerant genetic resources through screening. Within a crop species, genetic diversity provides a practical technique for screening and breeding enhanced salt-resistant cultivars. Özdemir et al. (2016) studied the tolerance to salinity of 4 landrace pepper cultivars. It was concluded that the Demre Sivrisi cultivar showed a higher tolerance level than other cultivars studied in the study, and hence can be suggested for pepper salt tolerance breeding studies.

Salts get concentrated in the soil solution as the soil dries, increasing salt stress. As a result, salt problems are more severe in hot, dry weather than in cool, wet conditions (Kotuby-Amacher et al., 2000). It's still unknown why environmental stresses cause powdery mildew disease development in pepper and paprika (*Capsicum annuum* L.). Abiotic stressors like drought and salinity have a complicated relationship with plant disease susceptibility (Massimi and Radocz, 2020). Foliar spraying is one of the most effective technical approaches for reducing the negative effects of abiotic and biotic stresses on pepper plants (Massimi and Radocz, 2020). When sweet pepper plants were subjected to sodium chloride salt stress, foliar silicon application enhanced chlorophyll a and b concentrations, mineral nutrients, hydration status, and fruit yield. Lipid peroxidation, electrolyte leakage, superoxide levels, and hydrogen peroxide levels were also lowered by silicon treatments (Abdelaal et al., 2020). Foliar treatment of 10^{-5} M gibberellic acid (GA3) had a growth-promoting impact on unstressed sweet pepper seedlings and was successful in enhancing salinity tolerance up to 50 mmol L^{-1} NaCl (Miceli et al., 2020). Exogenous Alpha-Tocopherol (TOC) foliar spraying improved adaptability to soil salinity issues in pepper plants by improving chlorophyll, enzymatic and non-enzymatic antioxidants, plant growth and production, and salt stress tolerance. This could be related to the cytokinin-mediated greening effect. According to the findings of this study, exogenous spraying of TOC at a concentration of 1 mmol L^{-1} boosts the expression of stress response genes and increases salt stress tolerance in pepper plants (Taha et al., 2018).

A foliar spray of 1% (w/v) mono-potassium phosphate (KH_2PO_4) on the upper surfaces of lower leaves of greenhouse-grown peppers induced local and systemic control against (*Leveillula taurica*) as compared to control plants. There was a reduction in the leaf area covered with sporulating colonies and conidial production on leaf tissue 24 or 48 hours after MKP was applied to the lower leaves of plants that had been exposed to the source of inoculum. When it came to lowering powdery mildew on greenhouse-grown plants, MKP was compared to a sterol-inhibiting systemic fungicide. Both treatments effectively inhibited powdery mildew when compared to non-treated control plants, though the fungicide-based treatment appeared to be slightly more effective (although not significantly) in controlling the disease. Phosphate solutions were not phytotoxic to plant tissue and did not affect yield when compared to the fungicide treatment (Reuveni et al., 1998).

Tomatoes were sprayed with foliar sprays under saline NaCl conditions, same as peppers. The most successful ways of salicylic acid application to recover the decreased growth characteristics of tomato plants

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under salt stress (100 mmol L^{-1} NaCl) were leaf pretreatment, root pretreatment, and leaf treatment, respectively. Each method was given a dose of 100 mg L^{-1} (Souri and Tohidloo, 2019). GA3 (10^{-5}M) growth regulator foliar spraying promoted growth in unstressed tomato seedlings and was successful in increasing salinity tolerance of tomato seedlings up to 25 mmol L^{-1} NaCl with foliar treatment (Miceli et al., 2020). It was previously concluded that tomato plants were severely harmed by saline stress (50 mmol L^{-1} NaCl); however, the damage was alleviated by foliar spray of copper nanoparticles (250 mg L^{-1}), which improved performance and improved the Na^+/K^+ ratio (Pérez-Labrada et al., 2019). Exogenous salicylic acid sprayed on leaves, on the other hand, did not affect preventing fruit production loss due to excessive salt stress (Kowalska and Smolen, 2013).

Among the scientific and previously tested methods to select seeds that are resistant to environmental conditions and pests, it depends on the size and weight of the seed. For example, Massimi (2018) previously demonstrated the relationship between seed size and weight, with the quality and vigor of cultivars in barley. Renugadevi et al. (2009) found a link between seedling vigor and seed size in cluster beans (*Tetragonoloba* (L.) Taub.) and Anuradha et al. (2009) in chickpea (*Cicer arietinum* L.). In these studies, the strength or vigor of the seeds is usually explained by the fact that the larger size carries higher weight and better nutritional content to produce healthy and strong seedlings. Massimi et al. (2018) confirmed similar trends that the fresh weight and vigor of corn (6640VT3P) were substantially greater than other compared hybrids. However, it was substantially higher in the greenhouse when it came to water use efficiency. Corn (6640VT3P) superiority's can be ascribed to its higher seed weight. As a result, the findings imply that corn (6640VT3P) had a significant impact on seedling establishment under extreme drought conditions.

One of the most important ancient study reviews on the topic of seed size (TeKrony and Egli, 1991) was published in the crop science journal, where ancient reports were collected explaining that one of the most important reasons for improving the quality of vegetable seeds was due to the size of the seeds as expressed by their weight or volume. Cabbage (*Brassica oleracea* var. *capitata*), turnip (*Brassica rapa* L.), lettuce (*Lactuca sativa* L.), and Austrian winter field peas (*Pisum sativum* subsp. *arvense* (L.) Poir) are among the vegetables highlighted in the study.

Returning to the sources, it appears that under salt circumstances, seed cultivars with big sizes and higher initial nutrient content can be relied upon. This choice corresponds to the foliar spraying approach used to get the best results in resisting salt and powdery mildew in both pepper and paprika plants. Especially when considering that salt stress reduces growth and causes crop loss by interfering with photosynthesis and disturbing the antioxidant defense mechanism. This occurs either because of the influence of salinity, which generates osmotic pressure and dehydration, or because of the effect of salinity, which causes nutrient imbalance (ionic homeostasis or stress). The research concept is based on selecting the heaviest paprika seeds for seedling growth, water conservation, and growth speed as markers of optimum resistance to sodium chloride salinity and using them to spray with salicylic acid to activate immunity against fungal pests.

The goal of this short study was to see how different drought-tolerant and non-drought-tolerant paprika varieties have different seed weights that responded to a simulated NaCl salt stress of a specific threshold level. The final goal is for the researcher to be able to choose the best genotypes for a trial of foliar salicylic acid spraying, which promotes induced systematic resistance (ISR), or SAR immunity in plants against pests, and diseases. Spraying with diluted sodium bicarbonate solution, which is used to treat powdery mildew and some insect pests like whitefly, aphids, leaf miners, worms, and thrips, will also be tested. With the need to recommend the name of the best variety to resist salinity, fungal pathogens, and pests.

MATERIALS AND METHODS

Test weight: the test weight was measured by taking a reading of 3 replicates for each paprika variety containing 100 seeds. Two drought-tolerant sweet pepper varieties (Carma and Fokusz) and one non-drought-tolerant paprika variety (Bobita F1) were chosen at random. The weight was recorded in grams using (OHAUS adventurer) digital scale.

Germination-imbibition under NaCl salinity stress: ten seeds were randomly selected from each paprika variety and seeded in Petri dishes with a 9-mm diameter and two layers of filter papers in each replicate. The seeds were irrigated with NaCl saltwater (purity is 98.5%) at a level of 3 dS m^{-1}. Kotuby-Amacher et al. (2000) reported that a 25% yield loss of pepper starts at a 3.3 dS m^{-1} threshold. The average laboratory temperature and humidity were recorded daily using a model (SENCOR SWS 5051) weather station, with the average temperature and humidity being (23.6 °C) and (33.42%) in the experiment. In a split fully randomized system (CRD), variety and treatment were repeated three times at random.

From the second to the eleventh day, each dish (9-mm diameter) received a total of 16 mm of saltwater has (1597 mg L^{-1}), allocated according to evaporation, and need, and each dish was ventilated for fifteen minutes daily. The total dissolved salts (TDS) in the salty solution were measured using a (Pancellent) electronic sensor with three replicates.

Parameters measured: five normal seedlings were taken from each treatment on the twelfth day, and the total fresh weight was measured. A seedling is classified as abnormal if one or more of its important seedling structures are absent, such as the root, shoot, or terminal bud. A healthy normal seedling will have all the necessary structures for appropriate development. The seedlings were then dried at 70 °C for 48 hours in a ventilated oven, and the total dry weight was measured. In the molecular biology lab, the (OHAUS adventurer) digital scale was used. The

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Association of Official Seed Analysts' seedling growth rate (SGR) concept was imitated (AOSA, 1983). The total dry weight of normal seedlings per treatment was divided by the number of seedlings included obtaining a seedling growth rate of one seedling. Tissue water content (TWC) was calculated using Black and Pritchard's formula (2002) for a total of five seedlings:

$$TWC=[(\text{Fresh Weight}–\text{Dry Weight})/\text{Fresh Weight}]\times100 \quad (1)$$

Statistical analysis: the deductive results were gathered, and their normal distribution was examined using SPSS (version 26). Then, a single factor analysis of variance (ANOVA-One Way) of Excel (365) was used as described by (Carlberg, 2014). At a probability level of 0.05, means were separated based on the Honestly Significant Difference (HSD). While the ANOVA findings revealed significant differences between pairs of means, a Post Hoc Test (Tukey-Kramer Post Hoc Test in Excel) was employed to evaluate differences in several groups' means. At the end of the study, both regression and correlation analyses were conducted to understand the extent to which the growth rate of seedlings depends on their weight before drying.

RESULTS AND DISCUSSION

In *Table 1* the average weight of 100 seeds of the three cultivars. There are significant differences between the three varieties of paprika. The (Carma) variety outperformed the rest of the varieties in the weight of 100 seeds (gram) significantly.

Table 1: **The average weight of 100 seeds weight for several paprika varieties**

Genotype	100 Seeds weight (gram)
Carma	1.017 A
Fokusz	0.837 B
Bobita F1	0.758 C
Significance	S

$P \leq 0.05$. NS: Non-Significant & S: Significant.

Further, the sweet pepper varieties (Carma, and Bobita F1) outperformed the third type (Fokusz) for the fresh weight of the five seedlings together under the treatment of NaCl saltwater (*Table 2*). No significant differences were recorded for the studied measures between the two varieties (Carma, and Bobita F1). *Table 2* shows the significant superiority of the (Carma) variety over the (Fokusz) variety concerning the fresh weight of the five seedlings under NaCl salt stress of (3 dS m^{-1}), as well as the significant difference between the two varieties of (Bobita F1) and (Fokusz).

Table 2 indicates there are no significant differences between the three cultivars regarding the biometrics of the weight of the five dry seedlings together or the weight of one seedling expressed in seedling growth rate and its unit is grams for one seedling. Despite this, (Carma) variety is mathematically superior in these readings over the rest of the varieties under study.

Table 2: **Means of germination-imbibition seedlings traits for pepper varieties planted in NaCl (3 dS m^{-1})**

Genotype	Seedling fresh weight [(gram) seedlings^{-5}]	Seedlings dry weight [(gram) seedlings^{-5}]	Seedling growth rate (gram seedling $^{-1}$)
Carma	0.183 A	0.0203 A	0.0041 A
Fokusz	0.123 B	0.0163 A	0.0033 A
Bobita F1	0.180 A	0.0187 A	0.0037 A
Significance	S	NS	NS

$P \leq 0.05$. NS: Non-Significant & S: Significant.

The statistical treatment method of regression and correlation (*Appendix A*) was used to predict the mathematical relationship in which the growth rate of one seedling depends on the weight of five fresh seedlings. *Table 3* shows a regression of 0.607 due to the response of seedling dry weight (seedling growth rate) to seedlings' fresh (wet) weight when cultivating three paprika varieties under saline conditions of NaCl level of 3 dS m^{-1}. There is a positive high-strength correlation (0.779) that can be deduced from this mathematical forecasting relationship.

$$Y = 0.011\,X+0.0019 \quad (2)$$

Y: Seedling growth rate (gram seedling^{-1}).
X: 5 Seedlings fresh weight (gram seedlings^{-5}).

Table 3: **The independent variable of 5 seedlings' fresh weight (X) and the dependent variable of seedling growth rate (Y) were studied using regression and correlation analysis**

Genotype	Seedling growth rate (gram seedling $^{-1}$) Dependent variable	Seedling fresh weight [(gram) seedlings^{-5}] Independent variable
Fokusz	0.003	0.12
Bobita F1	0.004	0.171
Carma	0.004	0.202
Fokusz	0.0036	0.125
Bobita F1	0.0034	0.174
Carma	0.0046	0.199
Fokusz	0.0032	0.124
Bobita F1	0.0038	0.196
Carma	0.0036	0.147
Regression	0.607	
Correlation	0.779	
Interpretation	Strong positive correlation	

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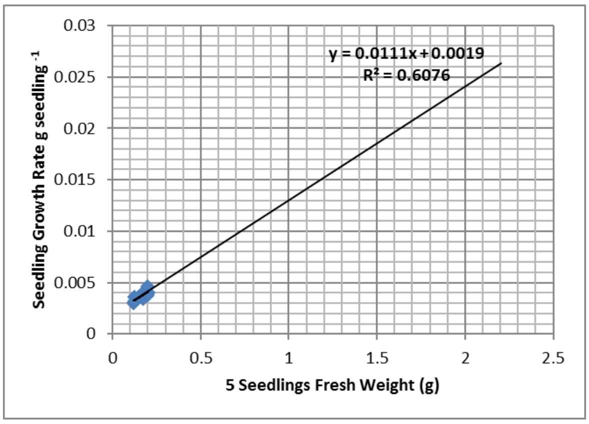
The linear prediction equation in *Figure 1* can be used to determine the weight of the dry seedling when knowing the weight of 5 wet seedlings in the case of planting paprika varieties of different seed weights under saline NaCl water of 3 dS m^{-1}.

Figure 1: **Mathematical formula of seedlings' fresh weight and growth rate**



The dependent factor (seedling growth rate) is significantly affected by an independent factor (fresh weight of 5 seedlings). The best expectation of regression is the value of F (0.013255786). R-Square is the regression, and multiple R is the correlation. The intercept and X variable coefficients are represented in the forecasting formula (*Figure 1*).

The findings of this study clearly show the vigor of (Carma) paprika under sodium chloride stress compared to other varieties. One explanation for that is the ability of (Carma) genotype to grow in conditions of salinity above the critical threshold for (*Capsicum annuum* L.), which was previously determined at (1.3 dS m^{-1}) (Kotuby-Amacher et al., 2000). Peppers start with a 10% yield loss at 2.2 dS m^{-1} and reach a 50% loss at 5.1 dS m^{-1}, where a quarter of the crop is lost at 3.3 dS m^{-1} (Kotuby-Amacher et al., 2000).

Similar outcomes were acquired and documented ahead of time (Özdemir et al., 2016). The soil salinity threshold value for pepper is 1.5 dS m^{-1}, and the irrigation water salinity threshold value is 1.0 dS m^{-1}. Demre Sivrisi, Calabi Iri Yaglik, Yalova Carliston 341, and Kandil Dolma, all landrace pepper cultivars, were tested for salinity tolerance. The effects of 0, 50, 100, and 250 mmol L^{-1} NaCl on germination %, relative growth rate, total chlorophyll content, lipid peroxidation criterion, relative water content, and osmotic potential were studied for this aim. The results reveal that salt stress had a negative influence on all cultivars; the more salt stress level used, the more negative impact occurred. However, among the cultivars studied, Demre Sivrisi showed a higher tolerance level than the others and can thus be recommended for pepper salt tolerance breeding studies (Özdemir et al., 2016). Salt stress treatments slowed the relative growth rate of all genotypes. RGR was reduced by 19% in Calabi Iri Yaglik seedlings treated with 50 mmol L^{-1} NaCl, whereas the largest reduction was seen in Kandil Dolma cultivars treated with 250 mmol L^{-1} NaCl (Özdemir et al., 2016).

Appendix A: **Regression statistics and ANOVA using Excel (365)**

Regression Statistics		
Multiple R	0.779515119	
R Square	0.607643821	The square root of the regression value equals the correlation value
Adjusted R Square	0.551592938	√ (0.607) = 0.779
Standard Error	0.000321919	
Observations	9	

ANOVA						
	Df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1.12E-06	1.12E-06	10.84093	0.013255786 *	*If significance F- value < than 0.05,
Residual	7	7.25E-07	1.04E-07			
Total	8	1.85E-06				the result is significant (S)

	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**	**Lower 95.0%**	**Upper 95.0%**
Intercept	0.001894003	0.000556	3.40896	0.011304	0.000580228	0.003208	0.00058	0.003208
X Variable 1	0.011079545	0.003365	3.292557	0.013256	0.00312252	0.019037	0.003123	0.019037

Some previous research has shown that heavier seeds give stronger seedlings in barley (Massimi, 2018), and corn (Massimi et al., 2018). The research team of Massimi et al. (2018), experimentally proved the existence of a relationship between seed weight and seedlings' fresh weight of corn. Because of the positive correlation between growth rate and fresh seedling weight in this brief article, it can be noted that there is

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an implicit relationship between seed vigor and weight and growth rate under saline conditions. Similar findings were observed for a positive impact of seed size (weight) on seedling growth of other vegetables such as cabbage (*Brassica oleracea* var. *capitata*), and turnip (*Brassica rapa* L.). Those old studies were previously done by (Hanumaiah and Andrews, 1973) cited in a literature article by (TeKrony and Egli, 1991). (Hanumaiah and Andrews, 1973) found that large seeds had a significantly higher fresh and dry weight for 36 old days seedlings in both turnip and cabbage.

Various environmental stressors, such as salt, impede plant growth and development. Different NaCl concentrations were applied to the cultivars Maha, Tata Puri, and Hot Queen (2 [control], 4, 6, and 8 dS m^{-1}). Higher saline levels significantly reduced root and shoot length, dry matter contents, relative growth rate, leaf area, specific leaf area, and leaf area ratio (6 and 8 dS m^{-1}). The latter conclusion was reported by (Ziaf et al., 2009).

Table 4 shows that when cultivating under simulated salt conditions, there are considerable changes in tissue water content amongst the cultivars. This is a decreasing equation that explains how much water is removed from tissues when they are dried. The non-drought-resistant variety (Bobita F1) loses water at the fastest rate of 89.61%, whereas drought-tolerant cultivars (Carma, and Fokusz) lose water at slower rates. A similar approach has already been obtained previously (Özdemir et al., 2016). The 250 mmol L^{-1} NaCl treatment resulted in the greatest drop in the leaf relative water content (RWC) of all cultivars. Kandil Dolma had the greatest decline in RWC, while Demre Sivrisi had the smallest decrease in the leaf relative water content (RWC). Demre Sivrisi had the smallest drop in relative water content when compared to the other genotypes and salt treatments. The change in the leaf relative water content (RWC) levels could indicate an increase in membrane permeability (Özdemir et al., 2016).

The relative growth rate and relative leaf water content are the best indicators of hindered pepper growth under salt stress. Furthermore, because pepper is a Na^{+} excluder, lower pepper plant development under saline circumstances is mostly due to osmotic stress rather than ionic toxicity. As a result, RLWC can be utilized to detect salt stress in peppers. Furthermore, this simple feature (RLWC) can be utilized to test the salt tolerance of pepper germplasm (Ziaf et al., 2009).

Table 4: **Tissue water content (%) for three paprika varieties germinated in NaCl salt solution**

Genotype	Tissue water content (%)
Carma	88.77 % AB
Fokusz	86.73 % B
Bobita F1	89.61 % A
Significance	S

$P \leq 0.05$. NS: Non-Significant & S: Significant.

Further experimentation is required to ascertain the research selection of varieties tolerant to salinity (Carma, and Fokusz), with other measures such as shoot and seedling length in the stage of two to three true leaves, or the first set of true leaves. Foliar spraying with the salicylic acid solution at a reduced dose of 50 mg L^{-1}, and sodium bicarbonate (0.52%) (w/v) will give more accurate results on varieties that are better selected for environmental conditions such as salinity.

CONCLUSIONS

As compared to other varieties of paprika, the results of this study demonstrate (Carma) paprika's vigor under sodium chloride tension of a threshold level of 3 dS m^{-1}. The reason for this is due to the vigor of genotype under saline conditions and the higher primary weight of the seeds.

Under salt stress, the relative growth rate and relative leaf water content are the best predictors of stunted pepper growth. Furthermore, because pepper is a sodium ion excluder, osmotic stress rather than ionic toxicity causes decreased pepper plant development in saline conditions. After comparing salicylic acid spraying (50 mg L^{-1}) and sodium bicarbonate spraying (0.52%) (w/v) on drought-resistant and non-drought resistant cultivars and studying the significant differences in the interaction, it is recommended to pick cultivars that are resistant to salinity and fungal diseases.

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