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DETERMINATION OF *IN VITRO* SALT TESTING EFFICIENCY AND SALINITY TOLERANCE OF DIFFERENT PEPPER (C*apsicum annum* L.) GENOTYPES

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This study was conducted to determine salt-tolerance levels of different pepper genotypes and to determine of correlation the efficiency of *in vitro* and hydroponic culture salt-testing procedures. For this purposes, 7 different pepper genotypes were subjected to control (0 mM) and 5 different NaCl levels 50, 100, 125, 150 mM in *in vitro* and hydroponic culture to determine salt-tolerance. Different pepper genotypes exhibited different responses to different NaCl doses and significant correlations were observed between *in vitro* and hydroponic culture testing for some of the investigated parameters. *In vitro* germination had the significant correlation with Shoot Fresh Weight (0.80) and Root Dry Weight (0.85). On the other hand, *in vitro* plant height highly correlated with hydroponic NaCl testing parameters and had the highest correlation with Root Dried Weight (0.71). There were also high correlations between *in vitro* root length and hydroponic NaCl testing parameters such Shoot Fresh Weight (0,84), Root Dry Weight (0.85) and Leaf Area (0.77). Present findings proved that *in vitro* salt-testing was a simple and cheap method. Thus, it could be preferred by the breeders just to get reliable outcomes in a short time.

Keywords: Pepper, Salt screening, Hydroponic culture, Efficiency

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INTRODUCTION

Pepper is among the significant vegetables and intensely produced worldwide in different forms and for various purposes. Pepper belongs to *Solanaceae* family and it is originated from South America. Commonly used *Capsicum annuum* L. is the most widespread pepper species of the world. For *C. annuum*, Mexico and Central America are the primary gene centers and South and Central Europe, Africa, Asia and Latin America are the secondary gene centers (IBPGR, 1984). As it was in the other plant species, abiotic stressors cause significant yield losses in peppers. Soil and irrigation water salinity exert serious abiotic stress on pepper plants.

Especially in arid and semi-arid regions, dissolved salts are leached into groundwater and then together with high groundwater tables, such dissolved salts move upward within the soil profile through capillarity and ultimately is accumulated within the upper soil layers or over the soil surface. Such a case is defined as salinity (GREENE *et al*., 2016; ETIKALA *et al*., 2021). Soil salinization is increasing at an alarming rate with 1.5 million hectares of land becoming unsuitable for agriculture each year and 50% of the cultivable land is predicted to be unsuitable for farming by 2050 (HASANUZZAMAN *et al*., 2014). Soil salinity and alkalinity are greatly influenced by irrigation and drainage practices and soil characteristics (CHHABRA, 2017). Dissolved salts are easily up-taken by the plants. Then, salts entered into plant structure may have negative effects when they exceeds threshold concentrations. Excessive concentrations may have toxic impacts on plants through destroyed nutrient uptake and metabolism. İncreased salt concentrations lead to decline in plant water uptake from the soils due to reduced osmotic potential which cause destruction in soil structure, recess, and hence terminate plant growth and development (MANUEL *et al*., 2017; AMIN *et al*., 2019).

Salinity stress causes a reduction in growth and biomass, chlorophyll degradation, water status modification, malfunctions in stomatal functions, modifications in transpiration and respiration, and disequilibria in ion ratios (EL-ESAWI *et al*., 2019). Plant tolerance or sensitivity to saline conditions is closely related to growth stages. In general, all plants are more sensitive to saline conditions in germination and initial growth stages. Salt-induced damages ultimately end up with insufficient growth and development. Pepper (*C. annuum* L.) is moderately tolerant to saline conditions. AYERS *et al*. (1985), indicated that the yield decrease in peppers started at 1.0-1.5 dS/m salinity levels and about 50% yield loss was expected at EC=3.4 dS/m salinity level. SMITH *et al*. (1991), observed a reduction in germination ratio of pepper seeds at 10 - 100 mM salt concentrations and reported only 5% germination at 200 - 300 mM salt concentrations. The pepper plant is not a salt-tolerant vegetable, and about 14% of fruit yield loss occurs as a result of each increase in salt level of 1.0 dS/m (EL-HIFNY and EL-SAYED, 2011). SONNEVELD *et al*. (1991), carried out a study on tomatoes, peppers and cucumbers and reported threshold salinity levels for decreasing yields as between 2.3-3.5 dS/m and also indicated 2.3 – 7.6% decrease in yields per unit increase (1 dS/m) in salinity. Previous investigations have been conducted to mitigate the harmful impact of salt stress on sweet pepper, but most have not been sufficient or broadly applicable. As a result, the search for cheaper, ecologically-friendly strategies for salinity amelioration which enhance the growth and productivity of sweet pepper has been very important to the agriculture sector (HERNÁNDEZ, 2019). For sustainability of plant production activities over salinized lands, salt resistance of the genotypes should be identified and improved

accordingly. Today, classical breeding methods are supported by *in vitro* techniques and thus, it is possible to select salt-tolerant cells in culture media and plants can be generated from these cells (TAL, 1992; ZAIR *et al*., 2003). Plant salt tolerance at germination and seedling stages can be determined with the germination tests assessed through various physiological and biochemical parameters (ALMANSOURI *et al*., 1999; SADAT NOORI *et al*., 2000; SULTANA *et al*., 2000; SOLTANI *et al*., 2004). Pepper is largely produced undercover worldwide. Intensive fertilizers and groundwater irrigations in spring and autumn or single season undercover productions are continuously increasing soil salinity levels. Crop rotations and precipitations are not available in greenhouses as it was in open fields, thus prevention of salinity is a quite costly and troublesome process.

Cultivation of salt-tolerant species/cultivars seems to be the best practice under saline conditions. Therefore, it is quite significant to know about salt-tolerance levels of the plant species and to decide about the type or cultivar to be produced based on soil salinity levels determined through soil analyses. There is a need for practical and cost-effective methods to identify salt-tolerance of the plants like peppers in a short period. Ascorbic acid (AA) application, is also applied to be done in *in vitro* cultures, that may be supplied to confer salt confrontation to calli that are ordained to be removed into saline circumstances, the development of calli of alfalfa *Medicago sativa* L. under salt stress was meaningfully enhanced by adding concentration of Ascorbic acid (AA) that ranges from 0.5 to 2.0 mM, perhaps by means of inhibition and reserve of acid phosphatase action (ARAB *et al*., 2006). Different researchers tried to make salt tolerant plants by means of tissue culture, by using several procedures (i.e. shoot culture, suspension culture and callus) for screening for cells and tissues that show differences in their capability to tolerate fairly high levels of salt (NaCl) in media (WOODWARD *et al*., 2005). Such methods should allow researchers to test a huge sum of materials in a short time. Early-stage testing is possible in hydroponic, sand and soil cultures, but several materials can also be tested under *in vitro* conditions in a short time. As compared to *in vivo* experiments, *in vitro* experiments bring about some advantages including higher degrees of control on test conditions. The method is quite simple, inexpensive and fast. It also provides effective guidance and control over the materials. Therefore, the present study was conducted to determine salt-tolerance levels of different pepper genotypes in tissue culture and hydroponic culture media. The primary target is to present potential use of tissue culture and hydroponic culture to identify salt-tolerance levels of different pepper types and to compare the data obtained from tissue culture and hydroponic culture experiments conducted to determine salt-tolerance levels of pepper genotypes.

MATERIALS AND METHODS

Materials

This study was conducted at greenhouse and laboratories of Erciyes University Agricultural Faculty. Five different pepper types (1 Blocky, 3 Capia, 1 Charleston, 1 Turkish long pepper and 1 Chili) at F_6 generation level were used as the material of the study.

Hydroponic NaCl Testing

Seedling salt tests were conducted in a growth chamber situated in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture. For hydroponic NaCl tests, pepper

seeds were germinated in peat:perlite (2:1) media with an EC of 0.4, then seedlings with 2-3 true-leaves were transplanted into the hydroponic culture in 8 L plastic pots contained nutrient solution aerated continuously with an air pump. The average day/night temperatures were 25/22 °C, the relative humidity was 65-70% and about 350 µmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber.

Hydroponic culture medium is composed of 1500 µM $Ca(N0_3)_2$, 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H3BO3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O_4$, and 80 µM Fe EDDHA (electrical conductivity of the control culture was 1.30 dS/m). The NaCl doses of control (0 mM), 50, 100, 125 and 150 mM were applied to seedlings. Salt treatments were initiated 3 days after planting; applied in every two days and the maximum dose of 150 mM was reached on the 6th day of experiment. Besides salt tests, the same genotypes were also grown under control conditions. Following the initiation of salt stress, hydroponic culture media were replaced in every 7 days. Two seedlings were planted into each pot. Twelve pots were used for each genotype (3 controls x 4 treatments). Prior to harvest, SPAD measurements were performed (SPAD-502, Minolta corporation, Ltd., Osaka, Japan) on fully-developed leaves in the middle of stress period and a day before the termination of experiments. Also, the leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) measurements were done by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) before harvest. At the end of the experiment plants were harvested by separating them into shoot and roots. Main stem length (cm) was measured by using ruler and caliper. For the fresh weight determination plant organs were fractioned into the leaf, stem and roots and then weighed. Each fully developed leaf was counted and recorded as leaf numbers (LN)/plant. The root length of the plants were measured by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) after recording the root fresh weight. Leaf area of the plants was measured destructively during the harvesting process by using a portable leaf-area meter (LI-3100, LI-COR. Inc., Lincoln, NE, USA). Total leaf area was recorded as cm^2. To determine the dry biomass, plant tissues were dried in a forced-air oven at 70 °C for 72 h. They were then weighed on an electronic digital scale.

In vitro NaCl Testing

Seeds of 7 pepper genotypes were initially subjected to surface sterilization through 4% sodium hypochlorite and they were germinated in 7% agar MS media with different salt concentrations (control, 50 mM, 100 mM, 125 mM, 150 mM NaCl). Experiments were conducted in 3 replicates with 10 seeds in each replication. Experimental seeds were germinated in growth chambers at 25 ± 2 °C under dark conditions for the initial 3 days and light during the rest of the germination period. Germinations were monitored daily and at the end of the 12^{th} day of experiment, germination ratio (%), shoot length (cm) and root length (cm) were recorded.

Statistical Assessment

The findings obtained from the study were assessment by using variance analysis in SAS (SAS University Ed.) Statistics program (F-Test). * Marked F values 5%, ** marked F values 1%, F marks marked *** were considered significant at 0.1% probability limit. Small

alphabetic letters were used to compare the means between applications (Tukey Test). Principal component analysis was used PAST 4.03 program.

RESULTS AND DISCUSSION

Hydroponic NaCl Testing

The highest performance genotypes in the control and all salt applications are shown in the tables respectively. Considering the plant heights of the genotypes under control and 4 different NaCl treatments, the greatest values were obtained from Chili (15.36 cm), Capia-2 (14,00 cm) and the lowest values were obtained from Blocky (8.41 cm) and Capia-3 (3.00 cm). Regarding total fresh weight, the lowest fresh weight (57.73 g) was recorded in Blocky and the highest plant fresh weight (71.73 g) was determined in Capia-1. With regard to shoot dry weight of the genotypes, the lowest value was obtained from Blocky (4.32 g) and the highest shoot dry weight was obtained from Long pepper (9.57 g). The greatest root fresh weights were obtained from Capia-1 (33.01 g) and Capia-2 (33.87 g) and the lowest root fresh weight were recorded in Chili (23.74 g) and Blocky (26.60 g) treatments. With regard to root dry weight of the genotypes, the lowest value was obtained from Blocky (1.20 g) and the highest root dry weight was obtained from Capia-2 (1.77 g) treatments (Table 1). In other studies with enhanced salinity levels, shoot and root lengths, fresh weights, and dry weights declined (SIVAKUMAR *et al*., 2020). Several methods and techniques have been employed to screen plants for their salt tolerance. Under saline conditions, various changes are experienced in plant growth, physiological, ionic, biochemical and enzymatic attributes of the plants. Initial screening at seedling stage can be carried out with the aid of mean germination time, time to 50% germination, germination index, embryo axis length, seedling fresh and dry biomass, seedling shoot and root length. Among these parameters, biomass (fresh and dry) is a quiet discriminative parameter since it is related to several other parameters such as photosynthesis, transpiration, stomatal conductance, water use efficiency, proline, glycinebetaine, protein content, sugar accumulation, antioxidant enzyme activities (SOD, POD, CAT, GPX), ionic attributes (Na, K, Ca and Cl) (ABBAS *et al*., 2014). In case all these attributes and processes go on positively, then sufficient biomass, plant growth and development will be achieved (MUNNS *et al*., 2008; ABBAS *et al*., 2014).

The largest leaf area (2018,53 cm^2) was measured in Capia-1 and the lowest leaf area (1042.64 cm^2) was measured in Capia-3. With regard to the number of leaves, the greatest values (103.00 leaves) were obtained from Capia-1 and the lowest leaf number (71.60) was counted in Capia-3. With regard to photosynthesis rates of the genotypes (6.13 μmol CO_2 cm^{-2} s^{-1}), the lowest values were measured in Blocky and the highest photosynthesis was observed in Chili (10,16 μmol CO_2 cm^{-2} s^{-1}). The largest SPAD (50.73) was measured in Capia-1 and the lowest SPAD (38.22) was measured in Capia-3. The greatest root lengths were obtained from Capia-2 (3983 cm) and Capia-1 (3861) and the lowest root lengths were recorded in Blocky (3209 cm) and Capia-3 (3376 cm) (Table 2). Salinity tolerance of some open pollinated pepper cultivars including Demre Sivrisi, Çalabi, Yağlık, Yalova Çarliston 341 and Kandil Dolma was investigated under 0, 50, 100, 250 mM NaCl treatments. Effects of salt treatments on germination ratios, relative growth rates, total chlorophyll contents, lipid peroxidation, relative

water content and osmotic potential were investigated. The findings revealed that all of the investigated cultivars were negatively influenced by salt stress. Demre Sivrisi cultivar had relatively higher tolerance level than the other cultivars and therefore recommended for pepper breeding studies as a salt tolerance source (ÖZDEMIR *et al*., 2016). KAOUTHER *et al*. (2013), investigated the impacts of NaCl on plant growth, mineral content and solutes synthesis in five Tunisian chili pepper (*Capsicum frutescens* L.) cultivars exposed to salt stress through irrigation water with different NaCl levels (0, 2, 4, 6, 8, 10 and 12 g/l). Researchers reported decreasing plant heights, biomass (dry and fresh) and relative water contents with increasing salinity stress levels. Genotypes were exposed to different salinity treatments (2, 4, 6 and 8 dS/m) along with control (0 dS/m). Incubator germination tests revealed that salinity stress significantly decreased final germination percentage, germination index and embryo axis length of tested genotypes. On the other hand, mean germination time and time to 50% germination increased with increasing salinity levels from 2 to 8 dS/m (TEHSEEN *et al*., 2016). In the present study, similar to the previous studies presented above, reductions in plant biomass and physiological porcesses were determined based on salt stresses and pepper genotypes. Different results for different pepper genotypes were mainly attributed to plant genetics.

Table 1. Plant height, Shoot Fresh Weight, Shoot Dried Weight, Root Fresh Weight and Root Dried Weight total mean values of different pepper genotypes under 5 different NaCl stress levels at Hydroponic Culture

Genotypes	Parameters	P. H. (cm)	S. F. W. (g/plant)	S. D. W. (g/plant)	R. F. W. (g/plant)	R. D. W. (g/plant)
	N	Mean	Mean	Mean	Mean	Mean
Capia-1	15	13.68a-c	71.81a	5.95ab	33.01a	1.59b
Capia-2	15	14.00ab	68.90a	6.77ab	33.87a	1.77a
Long Pepper	15	12.20bc	68.61a	9.57a	27.12b	1.61b
Charleston	15	11.42c	64.94b	6.11ab	25.05c	1.34c
Chili	15	15.36a	59.53c	5.71ab	23.74d	1.25d
Blocky	15	8.41d	52.73d	4.32b	26.60d	1.20e
Capia-3	15	9.06d	45.66e	3.38b	23.20d	1.04f
LSD		***	***	***	***	***
Genotype		***	***	*	***	***
Salt*Genotype		*	***	N.I.	***	***

* Marked F values 5%, ** marked F values 1%, *** Marked values 0.1% was considered significant at the limit of probability, means that do not share a letter are significantly different. N.I: Not important, P.H: Plant heigh, S.F.H: Shoot Fresh Weight, S.D.W: Shoot Dried Weight, R.F.W: Root Fresh Weight, R.D.W: Root Dried Weight

Table 2. Leaf Area, Leaf Number, Photosynthesis, SPAD and Root Length total mean values of different pepper genotypes under 5 different NaCl stress levels at Hydroponic Culture

Genotypes	Parameters	L. A. (cm^2)	L. N. (Total)	P. (µmol cm-2s-1)	SPAD	R. L. (cm)
	N	Mean	Mean	Mean	Mean	Mean
Capia-1	15	2018.53a	103.00a	9.40c	50.73a	3861b
Capia-2	15	1732.73c	74.40e	9.66b	46.88ab	3983a
Long Pepper	15	1778.40b	92.20b	7.96e	43.40bc	3530e
Charleston	15	1737.66c	103.20a	8.51d	43.88b	3562d
Chili	15	1481.66c	89.00c	10.16a	47.41ab	3684c
Blocky	15	1416.00e	77.86d	6.13g	46.04ab	3209g
Capia-3	15	1042.64f	71.60f	6.45f	38.22c	3376f
LSD		***	***	***	***	***
Genotype		***	***	***	***	***
Salt*Genotype		***	***	***	N.I	***

* Marked F values 5%, ** marked F values 1%, *** Marked values 0.1% was considered significant at the limit of probability, means that do not share a letter are significantly different. N.I: Not important, L. A: Leaf Area, L. A: Leaf Number, P: Photosynthesis, R.L: Root Length

In vitro NaCl Testing

In vitro experimentation presents advantages over currently used *in vivo* experiments. Such advantages include better control of test conditions. The method is simple, inexpensive, fast, and allows useful guidance during development of the material as well as control of production. Standardized, well-designed methods should be used so that meaningful assessments could be done (SPÅNGBERG, 1978). Screening for salt tolerance at germination or early seedling stage is less laborious, quick responsive, cost effective and trustworthy than at mature plant stages. The preference of plants at an early stage of growth under saline conditions has been considered highly predictive of the response of adult plants to salinity (MUNNS and TESTER, 2008; ABBAS *et al*., 2014). Thus, plants at early stage of growth screened for salinity tolerance could show considerable salinity tolerance at the later stages of growth. So, on the basis of germination and emergence tests, available germplasm can be categorized effectively into salt tolerant and sensitive groups (TEHSEEN *et al*., 2016). In the present study, different pepper types were germinated in tissue culture environment under control and 4 different (50, 100, 125 and 150 mM) NaCl doses and germination ratios (%), plant height (cm) and root length (cm) are provided in (Table 3, Figure 1).

Considering the germination of the genotypes under control and 5 different NaCl treatments, the greatest values were obtained from Capia-1 (79%), Long pepper (77%) and the lowest values were obtained from Blocky (59%) and Chili (62%). Germinated pepper plants were then grown under *in vitro* saline conditions for 15 days. Considering the plant heights of the genotypes under control and 5 different NaCl treatments, the greatest values were obtained from Long pepper (3.78 cm), Capia-1 (3,44 cm) and the lowest values were obtained from Chili (2.72 cm) and Blocky (2.79 cm). The greatest root lengths were obtained from Capia-1 (5.20 cm) and

Capia-2 (5.13) and the lowest root lengths were recorded in Blocky (3.46 cm) and Chili (3.78 cm). According to germination, plant heights and root lengths measured under *in vitro* conditions, a high correlation was observed between the parameters measured in hydroponic testing and *in vitro* germination.

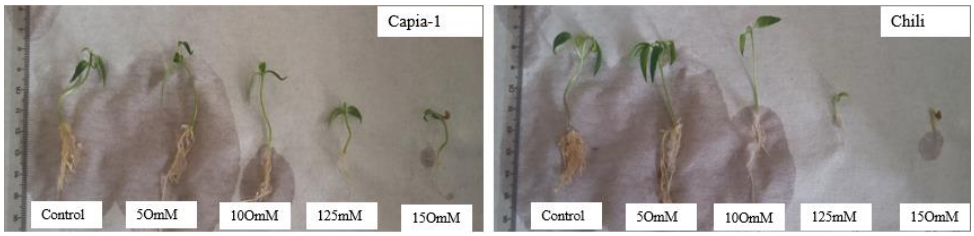

Figure 1. *In vitro* NaCl Testing of Capia-1 and Chili (Control, 50, 100, 125 and 150 mM)

Table 3. Seed Germination, Plant height and Root Length total mean values different pepper genotypes under different salt stress of tissue culture environment

Genotypes	Parameters	S.G. (%)	P. H. (cm)	R. L. (cm)
	N	Mean	Mean	Mean
Capia-1	15	79.00a	3.44b	5.20a
Capia-2	15	76.00c	3.30c	5.13ab
Long Pepper	15	77.00b	3.78a	4.95b
Charleston	15	66.00d	3.02e	4.68c
Chili	15	62.00f	2.72g	3.78e
Blocky	15	56.00g	2.79f	3.46f
Capia-3	15	64.00e	3.05f	4.00d
LSD		***	***	***
Genotype		***	***	***
Salt*Genotype		***	***	***

* Marked F values 5%, ** marked F values 1%, *** Marked values 0.1% was considered significant at the limit of probability, means that do not share a letter are significantly different. N.I: Not important, S. G: Seed germination (%), P.H: Plant heigh, Root Length (cm).

Germination tests assessed with different physiological and biochemical parameters reveal significant information about salt tolerance of the plants at germination and seedling phases (ALMANSOURI *et al*., 1999; SADAT NOORI *et al*., 2000; SULTANA *et al*., 2000; SOLTANI *et al*., 2004). However, plants mostly are not able to sustain early-stage resistance levels throughout the entire growth season (ASHRAF *et al*., 2009). Tomato and pepper are cultivated by more expensive hybrid seeds and most of the commercial cultivars are susceptible to salinity at seed germination and early seedling growth stage (FOOLAD, 2004). Therefore, screening for salinity stress during these stages is important for better germination in direct-seeded crops and enhanced adaptability of the seedlings to saline environments after transplantation (LI *et al*., 2011). Field

screening is critical as salinity levels vary with geographical locations, soil types and environmental conditions (ARZANI, 2008). *In vitro* screening is a promising and cost-effective method for rapid evaluation of germplasm under controlled, uniform, disease-free conditions, in limited space throughout the year (RAI *et al*., 2011). Moreover, seedlings germinated *in vitro* are akin to *in vivo* plants and are expected to respond to salinity stress in a similar manner (TAL, 1992; MILLS, *et al*., 2004; SHIBLI *et al*., 2007). NIKNAM *et al*. (2004), experiential that NaCl affects in vitro development growth and also proteins, sugars and free proline in the seedlings and leaf explants of *Nicotiana tabacum* cultivar Virginia. The fresh and dry mass, form of the seedlings reduced under salinity. Free proline gratified in both seedlings and leaf explants enhanced and polysaccharide content reduced incessantly by enhancing in concentration of NaCl. NIKNAM *et al*. (2006), investigated about the consequences of NaCl on development, proline and proteins contents, and polyphenol oxidase, catalase and peroxidase actions in seedlings and callus cultures of *Trigonella foenum-graecum* L. and *T. aphanoneura* Rech. F. Seeds and hypocotyl explants were cultured on MS medium supplemented with concentrations (0, 50, 100, 150 and 200 mM) NaCl. Seed germination and the fresh and dry mass of the seedlings reduced meaningfully below salinity. JAIN *et al*. (1990), observed, *in vitro* collection of salt tolerant plants of *Brassica juncea* L. (Indian mustard) cultivar Prakash has been done by showing extremely morphogenic cotyledon explant cultures on high NaCl (90 m mhos/cm) media. On revelation to osmotic stress, one of the most usual responses in plants is accretion of different compatible solutes of low molecular mass (ASHRAF *et al*., 2007). Testing micro-propagated plantlets in *in vitro* saline media can overcome some of these *in vivo* problems of variability and repeatability. Field screening in saline sites to select salt-tolerant genotypes of sweet potato (*Ipomoea batatas* (L.) Lam.) is complicated owing to the large spatial variations in the soil salinity level, differential ontogenic reactions of the plant to salinity and a large genotype × environment interaction. *In vitro* culture has been known to be a useful and rapid method to evaluate salt resistance and it provides a controlled and stable medium for studying physiological and biochemical pathways in plants, especially at the molecular level under different salt concentration levels (LOKHANDE *et al*., 2010). *In vitro* techniques were shown to be useful in identifying relatively salt-tolerant genotypes. It has been suggested that *in vitro* culture was a stressful environment that could be responsible for the induction of salt stress during new cultivar improvement (BANG *et al*., 2015; JO *et al*., 2016). There are limited number studies about *in vitro* NaCl screening of peppers. RASHED *et al*. (2016), conducted a study to characterize phenotype response to salt stress under *in vitro* conditions of fourteen tomato genotypes at 50, 100, 150, 200 and 250 mM NaCl levels and indicated that *in vitro* screening was useable in determination of salt tolerant tomato genotypes with a significant potential for further hybridization programs.

Correlation between hydroponic and in vitro NaCl testing

The data obtained from hydroponic and *in vitro* salt tests were subjected to correlation analysis (Pearson Correlation Coefficients) and results are provided in Table 4. Significant correlations were observed between *in vitro* seed germination and hydroponic NaCl testing results, *in vitro* germination had the significant correlation with S.F.W (0.80) and R.D.W (0.85). On the other hand, *in vitro* plant height highly correlated with hydroponic NaCl testing

parameters and had the highest correlation with R.D.W (0.71). There were also high correlations between *in vitro* root length and hydroponic NaCl testing parameters such S.F.W (0.84), R.D.W (0.85) and L.A (0.77). These stress parameters can provide reliable indication to evaluate the genotypic performance of a crop on the basis of the production biomass or yield (GHOLINEZHAD *et al*., 2014; MORTON *et al*., 2019; DERBALI *et al*., 2020). Stress tolerance parameters also successfully utilized in other crops such as tomato (PAILLES *et al*., 2020), wheat (GRZESIAK *et al*., 2018), barley (NARIMANI *et al*., 2020), and alfalfa (TAVAKOLI *et al*., 2019).

Table 4. Correlations between in vitro salt screening and Hydroponic salt screening with regard to measured seed germination and plant parameters

In vitro salt screening parameters	Hydroponic salt screening parameters									
	P. H. (cm)	S. F. W. (g/plant)	S. D. W. (g/plant)	R. F. W. (g/plant)	R. D. W. (g/plant)	L. A. (cm2)	L. N. (Total)	P. (µmol cm-2s-1)	SPAD	R. L. (cm)
S.G. (%)	0.22	0.80*	0.67	0.72	0.85*	0.72	0.35	-0.32	0.31	0.75
P. H. (cm)	-0.1	0.63	0.75	0.51	0.71	0.57	0.26	-0.51	0.038	0.36
R. L. (cm)	0.15	0.84*	0.64	0.72	0.85*	0.77*	0.44	-0.42	0.29	0.74

* Marked F values 5%, ** marked F values 1%, *** Marked values 0.1% was considered significant at the limit of probability, P.H: Plant height, S.F.W Shoot Fresh Weight, S.D.W: Shoot Dry Weight, R.F.W: Root Fresh Weight, R.D.W: Root Dry Weight, L. A: Leaf Area, L. N: Leaf Number, P: Photosynthesis, R.L: Root Length and Seed germination (%)

Principal component analysis between hydroponic and in vitro NaCl testing

PCA was performed to determine the relationship between seven different pepper cultivars grown in hydroponic and *in vitro* conditions in which five different salt levels were applied, according to plant height and root length parameters (Figure 1). PCA was best documented for distinguishing salt tolerance potentials of genotypes (RAZA *et al*., 2017; SIVAKUMAR *et al*., 2020). According to the analysis of plants grown under hydroponic conditions, the total variation was defined as approximately 99% of the two main components, while *in vitro* conditions the two components were defined as approximately 99% of the total variation. According to the PCA table, the plant height and root length parameters in both growing conditions are located in the same place, although there are some deviations in the graphics. Capia 1 and Capia 2 genotypes are located in the lower right part of the graph in both growing conditions, while Blocky variety is located in the upper left part of the graph in both growing conditions. While the Long pepper genotype is located in the upper region of both graphs, the Charleston genotype is located in the lower region of the graphs. According to the PCA tables, the genotypes grown *in vitro* and in hydroponic conditions are located in the same places in the graphs, although there are some deviations. This result reveals the usability of *in vitro* conditions in plant selection processes. Similar observations for screening salt-tolerant genotypes in rice in PCA and cluster analysis were also demonstrated by (RASEL *et al*., 2021).

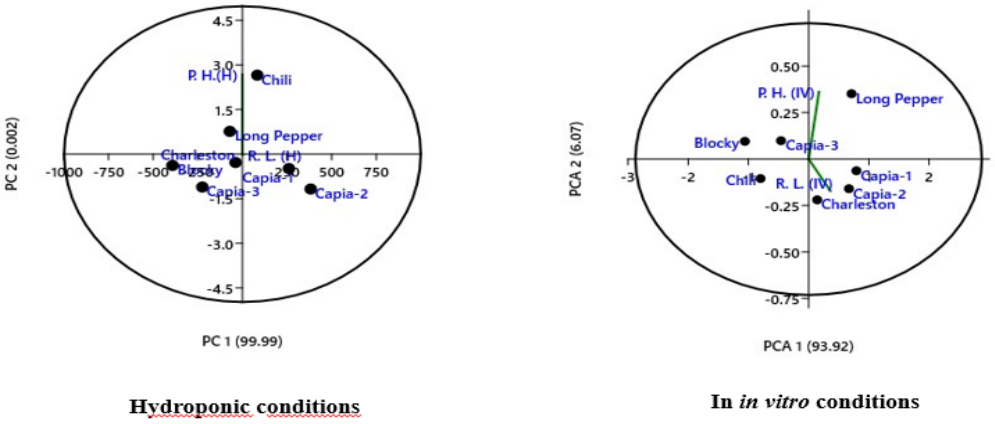

Figure 2. Principal component analysis (PCA) with plant height (P. H.) and root length (R. L.) parameters of pepper genotypes grown at seven different salt levels in hydroponic (H) and *in vitro* (IV) conditions.

CONCLUSION

In hydroponic conditions, Capia-1, Capai-2 and Long pepper were the first 3 in all measured parameters, whereas in genealogy, plant height and root length parameters measured in *in vitro* conditions, these genotypes were the first three. In hydroponic conditions, the lowest performansion genotypes were Blocky and Chili, respectively. In *in vitro* conditions, these are also the lowest performance of genotypes. Blocky and Chili pepper genotypes with low germination ratio, plant height and root length had parallel plant height values from both testing procedures. For the other genotypes, although not overlapped one-to-one, still parallel results were observed in both testing procedures. It was concluded that with *in vitro* tests, large populations could be screened for abiotic and biotic stressors in a shorter time and in a cost-effective fashion. With the resultant correlations from *in vitro* conditions, number of genotypes to be used in *in vivo* tests could be reduced by *in vitro* hydroponic, sand and soil-like media culture.

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NEKE ODREĐIVANJE EFIKASNOSTI *IN VITRO* ISPITIVANJA NA UTICAJ SOLI I TOLERANCIJE NA SALINITET RAZLIČITIH GENOTIPOVA PAPRIKE (*Capsicum annum* L.)

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Izvod

Ovo istraživanje je sprovedeno da bi se utvrdili nivoi tolerancije soli različitih genotipova paprike i da bi se utvrdila korelacija efikasnosti *in vitro* i hidroponskih kultura za ispitivanje uticaja soli. U tu svrhu, 7 različitih genotipova paprike je podvrgnuto kontroli (0 mM) i tretmanu 5 različitih nivoa NaCl 50, 100, 125, 150 mM u *in vitro* i hidroponskoj kulturi radi određivanja tolerancije na so. Različiti genotipovi paprike su pokazali različite odgovore na različite doze NaCl i uočene su značajne korelacije između *in vitro* i hidroponskog testiranja za neke od ispitivanih parametara. *In vitro* klijavost je imala značajnu korelaciju sa težinom svežeg izdanka (0,80) i masom osušenog korena (0,85). S druge strane, *in vitro* visina biljke je u visokoj korelaciji sa parametrima ispitivanja hidroponskog NaCl i imala je najveću korelaciju sa osušenom masom korena (0,71). Postojale su i visoke korelacije između *in vitro* dužine korena i parametara hidroponskog NaCl testiranja, kao što su težina svežeg izdanka (0,84), težina osušenog korena (0,85) i površina lista (0,77). Dobijeni rezultati su pokazali da je *in vitro* ispitivanje na uticaj soli bila jednostavna i jeftina metoda. Stoga bi ga oplemenjivači mogli primenjivati da bi dobili pouzdane rezultate u kratkom vremenu.

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