J. Agr. Sci. Tech. (2022) Vol. 24(1): 183-198

Natural Variation for Salt Tolerance among Basil Accessions from Iran Based on Fluorescence Transient and Morphological and Growth Characteristics

S. Shirahmadi[1], M. Esna-Ashari[1*], S. Aliniaeifard[2], and Gh. Abbas Akbari[3]

ABSTRACT

To study salinity tolerance of 15 basil accessions widely distributed across Iran, they were grown under two salt levels including control (no NaCl) and 40 mM NaCl (Hoagland nutrient solutions with EC of 1.1 and 5.5 dS m^{-1}, respectively). The studied parameters included morphological characteristics and chlorophyll a fluorescence transient (OJIP) measurements. According to the results, the accessions were categorized into three clusters under the salt stress. Salinity had significant effects on morphological and growth parameters in all basil plants. Compared to the control, NaCl decreased plant height. The number of leaves in Khash, Zabol, and Orumiyeh accessions was more than the others. Both salt and accession caused a decrease up to 43% in leaf fresh weight, emphasizing the major role of accession when salinity was applied. Salinity influenced negatively the biomass yield in basil plants. These decreases varied from 19 to 45% depending on the accessions. Salt treatment of basil plants decreased photosystem II activity, as evaluated from chlorophyll fluorescence data. The parameters that were most affected by salt treatment were maximal quantum yield of PSII photochemistry (F_V/F_M) and calculated Performance Index (PI_{ABS}). Overall, among the studied basil accessions, genotype Ardabil had superior tolerance to salt stress. Furthermore, the most of accessions can be used for studying the mechanism of salinity tolerance in basil plant.

Keywords: Chlorophyll a fluorescence, Cluster analysis, Growth parameters, *Ocimum* spp., Salinity stress.

INTRODUCTION

Sweet basil (*Ocimum* spp.) is a culinary herb belonged to *Lamiaceae* family (Morales and Simon, 1996). There are about 30 species of *Ocimum*, of which the most commonly cultivated species is *basilicum* (Paton *et al.*, 1999) native to tropical Asia (Paton, 1992). Basil can be used for its essential oils, fresh or dry leaves, and flowers, and also as an ornamental plant (Pushpangadan and George, 2012). The sweet basil is moderately tolerant to salinity. Basil species are tolerant to salinity, especially in germination and emergence phases, but further research is needed to elucidate salt tolerance of sweet basil in the other growth stages and different accessions (Ramin, 2006). Indoor growing in the greenhouse has shown to be better than outdoor condition, as the crops have proper growth and development, and protection against adverse weather conditions (Bisbis *et al.*, 2018). However, there are some limiting factors for greenhouse-grown plants; one of the main factors is poor-quality irrigation

---

[1] Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Islamic Republic of Iran.

[*] Corresponding author; e-mail: m.esnaashari@basu.ac.ir

[2] Department of Horticulture, Aburaihan Campus, University of Tehran, Tehran, Islamic Republic of Iran.

[3] Department of Agronomy and Plant Breeding Sciences, Aburaihan Campus, University of Tehran, Tehran, Islamic Republic of Iran.

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

water that leads to salinization, which in turn is a serious problem for crop productivity (Minhas, 1996). Many adverse effects have been observed in plants exposed to salinity stress. Plants response to environmental stresses is a coordinated action of individual cells and synergistically whole plant organisms (Singh, 2015). Effects of salinity stress on an osmotic equilibrium of plants depend on the source, concentration, and duration of salinity. Sodium and chloride ions cause hyper-osmotic stress, which in turn reduces the availability of water needed for the maintenance of cellular turgor pressure as well as mineral nutrients. Salinity can also interfere with plant growth and development through imposing osmotic stress, the primary stress that ends up with oxidative stress as the main cause of secondary stress (Munns and Tester, 2008). The presence of high NaCl concentration in the root zone mediates several physiological and morphological responses in plants, including the reduction of water uptake that affects plant growth and yield (Tester and Davenport, 2003). Absorption of Na^+ and Cl^- negatively affects growth by impairing metabolic processes and stomata closure along with photo-inhibition and oxidative stress resulting in photosynthetic depression (Zhu, 2001). Photosynthesis initiates with electron transport chain followed by $NADP^+$ and ATP input to Calvin Cycle and the synthesis of the products is carefully adjusted. Changes at any site of sequences mediated to stress can affect photosynthesis efficiency (Kan *et al.,* 2017). Measurement of chlorophyll fluorescence and "JIP-Test" variables in an intact leaf provides a valuable complement to studies of the long-term changes in the electron transport chain. Decreasing plant growth under salt stress may vary depending on plants cultivar (Bie *et al.,* 2004), which directly or indirectly occurs at the presence of salinity. Chlorophyll fluorescence has been used to assess the effects of different environmental stresses on various plant species such as salt stress in tomato (Zushi and Matsuzoe, 2017), drought stress in Acer genotypes (Banks, 2018), and low temperature and water stress in rice (Hasani *et al.*, 2014). Genetic diversity is the basis of selection for different morpho-economic traits and a prelude to potential crop improvement (Singh *et al.*, 2018). Accumulation of phenolic compounds under environmental stresses mainly depends on the plant species and growth condition (Mosadegh *et al.*, 2018). A variation in biochemical traits has been reported by Aghaali *et al*. (2017) who screened 50 accessions of basil plant. They showed that total phenols and antioxidant capacity differed among the studied accessions. Some researchers have investigated different extents of salt tolerance among basil accessions (Rouphael *et al.*, 2018). Akbari *et al.* (2018) classified 19 accessions of sweet basil into a number of cold-tolerant groups based on their cumulative temperature response and seed germination rate and percentage. A range of physiological and morphological variations has been shown in green Iranian and Genovese basil under salt stress conditions. Genovese basil was more tolerant to salinity compared to the Iranian genotype (Bekhradi *et al.*, 2015). Basil plants thrive well in a variety of climatic conditions (Pushpangadan and George, 2012). According to the previous studies in Iran, a number of basil accessions have been clustered based on some different attributes (Aghaali *et al.*, 2017; Akbari *et al.*, 2018; Bekhradi *et al.*, 2015; Moghaddam, 2015). Aghaali *et al*. (2017) stated that the first step in selecting salt-tolerant accessions is to expose the collected basil plants to salinity conditions. To discover the relationships between accessions and their reaction to salinity conditions, a comprehensive study, as well as applications of techniques, is needed. Cluster analysis is an effective method of organizing data and classification problems solving in various fields (Potashev *et al.*, 2014). Application of clustering, in particular K-means, has been conducted for data analysis since 1980 (Govender, 2019). Plant heritability indicates the extent of similarities and determines the potential of

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

Table 1. Geographical attributes of the fifteen collected Iranian basil accessions.

Accession code	Collection area	Latitude (°N)	Longitude (°E)	Elevation (m)
1	Isfahan	32/65	51/66	1598
2	Shiraz	29/59	52/58	1472
3	Khash	28/23	61/19	1419
4	Malayer	34/30	48.81	1771
5	Zahedan	29/45	60/88	1334
6	Tonekabon	36/81	50/87	20
7	Birjand	32/86	59/22	1516
8	Zabol	31/03	61/49	481
9	Orumiyeh	37/54	45/07	1332
10	Gorgan	36/84	54/43	155
11	Ardabil	38/25	48/30	1351
12	Kermanshah	34/32	47/07	1304
13	Zanjan	36/68	48/50	1663
14	Pakdasht	35/46	51/68	1013
15	Kerman	30/28	57/08	1760

selective breeding as well as the prediction of many issues. The objectives of the present study were as follows:

a) Investigating the diversity of basil plants collected from different locations and ecological areas of Iran using morphological and growth characteristics.
b) Clustering accessions based on the correct number of clusters and determination of the heritability of the morphological traits of basil accessions.
c) Measurement of the salt-induced changes in OJIP parameters to be compared with the morphological traits.
d) Identification of the superior accession(s) for salt stress tolerance that can be used for the production of sweet basil in greenhouse using saline water.

MATERIALS AND METHODES

Plant Materials

Experiments were carried out in the research greenhouse of Aburaihan Campus (southern Tehran, 35° 28’ N and 51° 41’ E, elevation 1,035 m), University of Tehran, Pakdasht, Iran. Fifteen accessions of basil plants collected from different geographical regions (Table1) were evaluated for a range of morphological and growth traits from January to February for two successive years (2018 and 2019). Characteristics of the collection sites were chosen based on the geographical long distance diversity in climate. Separated seeds from basil plants were sown in 102-cell trays (2.8 L×2.6 W×6 H cm, approx. 25 mL per cell) filled with a mixture of perlite/cocopeat (1:1 v:v) and germinated within 10 days. Seedlings with four leaves were transplanted into 14 cm diameter pots filled with the same mixture as the above cells, 30 days after sowing. In order to synchronize the growth of basil plants, they were grown in a greenhouse with supplemental light provided from LED lamps (Model: IGL-15B, 220-240V, 18W). The average of photosynthetic photon flux density between 8 am to 7 pm was 340 μmol m^{-2} s^{-1} (measured with PAR-FluorPen FP 100-MAX). Air temperature and relative humidity averaged 23˚C and 50%, respectively, which were measured using a temperature/humidity sensor (Clock-Humidity HTC-2).

Salinity Treatments

Plants were grown in a hydroponic system in which a modified Hoagland solution

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

(Table 2) with a pH of 6.5-7 was used. One week after transplanting, salt treatment including 40 mM NaCl was started and compared with a control (no NaCl). The average electrical conductivity of the nutrient solutions was 1.1 and 5.5 dS m^{-1} for the control and NaCl treatment, respectively. Salinity treatment was imposed gradually in three steps with one-day distance, each by adding 10 mM NaCl to the Hoagland solution. For every 10 mM increase in NaCl, the EC of the solution increased by 1.1 dS m^{-1} and, eventually, reached 5.5 dS m^{-1} for 40 mM NaCl treatment. Basil plants were irrigated with the nutrition solution three times a week and harvested 40 days after the beginning of salt application.

Measurements

Plant height was measured from the down to the tip of the main stem using a standard ruler at the harvesting time. At harvest, roots, stems, and leaves were separated. Roots were gently surface dried and immediately weighed using electronic balance (Sartorius TE1502S Talent Analytical Balance). Detached leaves were counted in the leaf blade with petiole and then weighed. The stems were also weighed separately. The dry weight of leaves, stems and roots was determined after placing them in an oven at 72°C for 48 hours. Fresh and dry weights of individual basil plants (with roots) were used to calculate the fresh and dry biomass yield. Leaf area (cm^2) of fully expanded leaves per plant was measured by an image analysis software (Image j, Version 1.46r).

Fluorescence transient (OJIP) measurements

All OJIP transients were measured using fully expanded leaves from the middle of plants following 40 days of exposure to salt stress. The determined parameters are shown in Table 3. Before measuring chlorophyll fluorescence, the leaves were kept in the dark for 20 minutes. OJIP transients were calculated using a portable chlorophyll a fluorometer (Fluorpen FP100 Instruments, PSI, Czech Republic).

Experimental Design

The experiment was set up in a randomized complete block design with three replications and five plants per replication. Analysis of variance of the data as well as the comparison of the means for measured traits was performed using SAS (USA Institute Inc., Cary. NC) and Duncan's multiple range test, respectively. The statistical parameters that were calculated for the measured traits using Microsoft Excel (Version, 2016) through the associated equations are shown in Table 4. Heritability was expressed as the ratio of the genetic variance to the phenotypic variance. Cluster analysis of the accessions was made

Table 2. Compositions of nutrient solution used in the experiment.

Salts used in nutrition solution	Stock solution (g L^{-1} deionized water)	Vol added for final solution (mL L^{-1})
KNO_3	101.11	5
Ca $(NO_3)_2$ 4H2O	236.15	5
$MgSO_4$ $7H_2O$	246.48	2
KH_2PO_4	136.09	1
H_3BO_3	2.86	1
$MnCl_2$ $4H_2O$	1.86	1
$CuSO_4$ $5H_2O$	0.08	1
H_2MoO_4	0.09	1
ZnSO4 7 H_2O	0.22	1
Fe EDTA	5	0.5

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

Table 3. Selected JIP-test parameters measured in this study.

Fluorescence parameter	Description (Strasser, 2004)
F_o	Minimal fluorescence yield of the dark adapted state
F_M	Maximal fluorescence yield
F_V/F_o	Oxygen electron complex activity
F_V/F_M	Maximal quantum yield of PSII photochemistry
M_o	Approximated initial slope of the fluorescence transient, expressing the rate of RCs' closure
Ψ_o	Efficiency of electron transfer from Q^-_A to Q_B
Φ_{Eo}	Energy transfer
Φ_{Do}	Heat dissipation
PI_{ABS}	performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
ABS/RC	Specific absorption flux per RC
TR_o/RC	Trapped energy flux per RC
ET_o/RC	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A
DI_o/RC	Energy dissipation flux per RC

Table 4. Statistical parameters calculated for the measured traits using associated equations[a].

Parameter	Equation
Genotypic Variance	$V_G = \mathrm{MSg} - \mathrm{MSe}/\mathrm{r}$
Error Variance	Ve= MSe
Phenotypic variance	Vp= Vg+Ve
Heritability	$H_b = VG/VP$
Genotypic Coefficient of Variation	$CVG = \frac{\sqrt{\mathrm{VG}}}{\mathrm{M}} \times 100$
Phenotypic Coefficient of Variation	$CVP = \frac{\sqrt{\mathrm{VP}}}{\mathrm{M}} \times 100$

[a] MSg: Mean Square genotype, MSe: Mean Square error, r: Repeat, M: Mean.

with the R-Studio program (version 3.5.3, K-means function) through the partitioning clustering (K-means). We performed the clustering with K= 3 and K presents the number of clusters.

RESULTS AND DISCUSSION

Variability Study

According to the obtained results, morphological and growth characteristics were affected by salinity in all accessions compared to the control plants (Table 5). High levels of variation between the control and salt-treated plants in terms of leaf area as well as the number of leaves and plant height were found. However, minimum difference was observed in dry weights of leaves and stems. In a study on Iranian basil accessions, a low level of variation was obtained from essential oil percentage, suggesting a positive correlation between dry weight and the amounts of polyphenol compounds (Akbari *et al.*, 2019; Scagel *et*

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

Table 5. Statistical parameters calculated for the traits under both control and salt treatment.

Trait	Statistical parameter[a]							
Control (No salt)	M	SE	V_G	V_E	V_P	H_B	CV_G	CV_P
Plant Height (cm)	32.07	1.73	42.06	2.79	44.85	93.78	20.21	20.87
Leaf Number (per plant)	36.06	2.4	62.8	26.19	88.99	70.56	21.97	26.15
Leaf Fresh Weight (g)	6.25	0.29	0.76	0.50	1.26	59.95	13.94	18.01
Leaf Dry Weight (g)	0.57	0.03	0.01	0.006	0.01	66.66	18.90	23.15
Stem Fresh Weight (g)	3.49	0.30	1.24	0.164	1.40	88.36	31.97	34.01
Stem Dry Weight (g)	0.31	0.02	0.008	0.002	0.01	76.45	30.14	34.47
Root Fresh Weight (g)	17.36	0.81	6.58	3.58	10.16	64.77	14.77	18.35
Root Dry Weight (g)	1.61	0.09	0.09	0.05	0.14	66.38	19.56	24.01
Fresh shoot Biomass (g)	9.74	0.48	2.51	1.10	3.61	69.55	16.27	19.50
Dry Shoot Biomass (g)	0.89	0.04	0.02	0.01	0.03	64.37	17.55	21.87
Total Biomass (g)	2.50	0.14	0.20	0.1	0.31	65.40	18.13	22.42
Leaf Area (cm^2)	235.93	15.72	2510.54	1232.10	3742.65	67.07	21.23	25.93
NaCl treatment								
Plant Height (cm)	26.8	1.40	25.14	5.49	30.63	82.07	18.71	20.65
Leaf Number (Per plant)	29.63	2.47	76.01	15.71	91.72	82.87	29.42	32.32
Leaf Fresh Weight (g)	4.79	0.26	0.65	0.39	1.04	62.73	16.91	21.35
Leaf Dry Weight (g)	0.41	0.02	0.005	0.007	0.01	44.73	18.36	27.45
Stem Fresh Weight (g)	2.47	0.21	0.52	0.164	0.68	76.09	29.25	33.53
Stem Dry Weight (g)	0.22	0.02	0.005	0.002	0.007	71.42	32.14	38.03
Root Fresh Weight (g)	13.17	0.70	5.23	2.39	7.62	68.66	17.37	20.96
Root Dry Weight (g)	1.09	0.07	0.04	0.04	0.09	47.87	19.46	28.12
Fresh Biomass Shoot(g)	7.26	0.40	1.61	0.87	2.48	65.01	17.51	21.72
Dry Biomass Shoot (g)	0.63	0.04	0.01	0.01	0.03	60.43	21.39	27.51
Total Biomass (g)	1.73	0.11	0.11	0.09	0.21	53.44	19.48	26.65
Leaf Area (cm^2)	177.1	11.33	1188.96	765.89	1954.85	60.82	19.46	24.96

[a] M: Mean, SE: Standard Error, V_G: Genotypic Variance, V_E: Environmental Variance, V_P: Phenotypic Variance, H_B: Heritability, CV_G: Genotypic Coefficients of Variation and CV_P: Phenotypic Coefficients of Variation.

al., 2019). Such high variation in any individual attribute results in a precise selection for many targets and diversity of the accessions is very important for both genetic studies and breeding programs (Singh *et al*., 2018). The highest phenotypic variations were observed in the leaf area as well as the leaf height and number of leaves (Table 5). Under salinity conditions, variation in stem dry weight was higher compared to leaf dry weight. In contrast, leaf dry weight was found lower than stem dry weight in the non-salinized nutrient solution. Environmental effects can adversely influence the adaptation of a plant species. Kainer *et al*. (2015) reported that environmental conditions have greater impact on plant traits than genetics. Among the measured traits and based on the variation coefficients, the weight of fresh and dry stem and number of leaves showed the highest variability followed by the stem dry weight, number of leaves, and stem fresh weight, respectively (Table 5). For the leaf and root fresh weight, variation coefficients were relatively low; suggesting that cultivated plants had sufficient diversity in phenotype. The estimated heritability was relatively high for all of the studied traits, except for root dry weight in NaCl treatment (44.85%). The highest heritability was obtained for plant height (93.78% in the control). The closer heritability to 100 means, the heritance would be greater for that special trait. The accuracy of genomic selection is higher for traits with higher

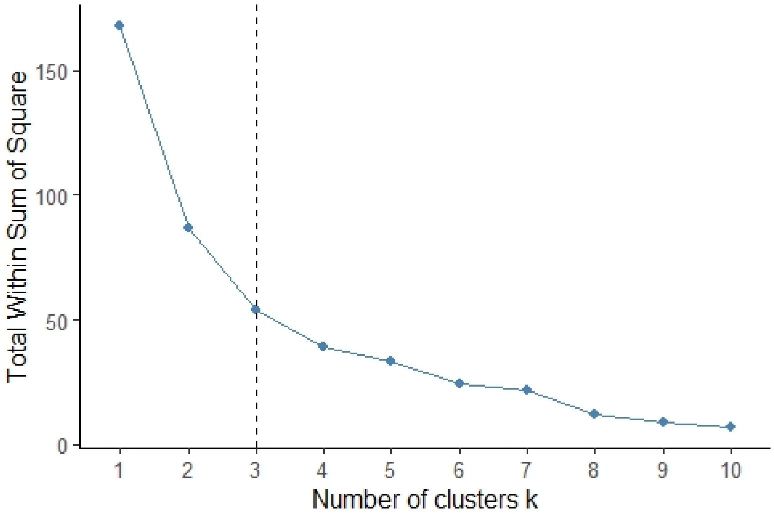

Figure 1. Optimal number of clusters for basil accesions under salinity condition.

heritability (Kainer *et al.*, 2015). In order to improve any trait, including yield, a selection manner is required in which the attributes that evince high heritability are selected. In the present study, two traits including plant height and leaf number are valuable for genetic practices. Yield improvement requires measurement of variation in available accessions, determination of relationship between morphological traits and yield, and evaluation of environmental factors affecting these traits (Bhargava *et al.*, 2012).

Cluster Analysis

To differentiate the accessions based on a similar structure, they were fragmented into separate groups through the cluster analysis. Selection of the number of clusters is one of the most important problems in cluster analysis (Jain, 2010). An optimal and significant number of clusters is 3 (K= 3), suitable for the accession variations in the present study (Figure 1). Within the sum of squares/between the sum of squares (WSS/BSS) is 67.8%. WSS indicate how closely related are objects in a cluster. BSS indicates how distinct a cluster is from the other clusters. The result of 67.8% is a measure of constant variance in our data set that is explained by the clustering. K-means minimize the within-group dispersion and maximize the between-group dispersion. Clusters of sizes were 5, 9, and 1. The clustering vector was special for any accession [Esfahan, Shiraz, Zabol, Kermanshah and Pakdasht (1), Khash, Malayer, Zahedan, Tonekabon, Birjand, Orumiyeh, Gorgan, Zanjan and Gorgan (2) and Ardabil (3)]. Table 6 shows the distinction of basil accessions based on the Euclidean distance clustering strategy, which is achieved from the distinct matrix to predict the distinction of 15 different accessions. The values of distance between the accessions changed from 0.75 to 11.88. The farthest accessions were numbers 11 and 13, while the accessions 1 and 2 were found as the closest. Accessions' grouping was performed based on morphological traits using principal component analysis, which was conducted using the correlation coefficients matrix in the R program. Since the unit of measurement is typically not the same. To eliminate the Scales, variables become standardized. The largest dissimilarity occurred between Ardabil and the other accessions. The result of releasing

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

Table 6. Distance among the clusters in 15 basil accessions under salinity condition.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.75													
3	5.36	4.93												
4	5.47	5.34	4.38											
5	6.66	6.32	2.87	2.84										
6	5.41	4.95	1.36	4.20	2.81									
7	6.46	6.04	2.77	3.48	2.17	2.04								
8	2.94	2.49	2.78	4.89	4.81	2.91	4.46							
9	5.36	4.99	0.88	4.00	2.67	1.88	2.81	3.12						
10	6.66	6.21	2.26	4.42	2.39	1.75	1.87	4.16	2.72					
11	11.88	11.4	6.82	8.05	5.68	6.81	5.92	9.41	6.81	5.67				
12	4.07	3.48	3.61	6.13	5.61	3.91	5.46	2.69	3.90	5.23	9.97			
13	7.14	6.83	3.51	2.99	1.48	3.58	2.69	4.40	3.27	3.21	5.51	6.16		
14	3.52	3.47	3.04	3.97	4.43	3.62	4.54	2.59	2.67	4.78	9.10	4.09	4.72	
15	7.08	6.72	2.18	4.80	2.49	2.61	3.12	4.60	2.20	2.31	5.21	5.44	3.14	4.40

[a] The code for each accession is the same as code of accession in Table 1.

the first cluster through cluster analysis is shown in (Figure 2). In this study, the first goal in cluster analysis was to determine the location and the number of accessions in each cluster. It was evidenced by the results that there was no relationship established between the genetic diversity and geographical diversity. On the other hand, the accessions that originated from different regions were in one cluster. For example, Esfahan, Zabol and Kermanshah accessions were distributed in cluster 1 and Khash, Gorgan, Zanjan, and Orumiyeh accessions in cluster 2. The biggest cluster was cluster 3 with 8 accessions. Accession 11 (Ardabil) was separately clustered and this is a cluster with one part. Moghaddam *et al*. (2011) evaluated the genetic diversity of some Iranian *Ocimum* spp. accessions using AFLP markers and found no relationship between genetic divergence and geographical origins. The highest degree of overlapping was observed between clusters 1 and 2, which means the objects are very close to each other. Accession 3, 6, and 9 were assigned to one more cluster having similar characteristics.

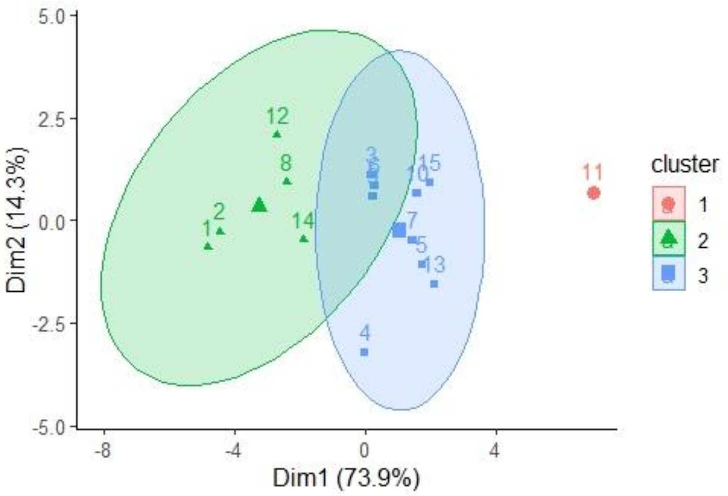

Figure 2. Cluster analysis of basil accessions under salinity condition based on principal component analysis. The code for each accession is the same as code of accession in Table 1.

Growth Attributes

The basil accessions evaluated in this study had a range of low to high tolerance to salinity in terms of morphological and growth characteristics when grown under NaCl treatment. The range of plant height started from 14.33 to 40.83 cm in the control and 14.16 to 36.5 cm in salinity treatment, in which the shortest and the longest height belonged to Kermanshah and Kerman accessions, respectively (Figure 3-a). The height of Khash accession decreased from 33.83 to 23.16 cm under salt treatment, confirming that salinity limits the growth of some basil plants. The number of leaves is another important growth parameter for the basil plant. Ardabil accession performed better in terms of this trait compared to the other accessions, showing relatively low negative effects of salinity on its number of leaves (Figure 3-b). Leaf fresh weight of the 15 basil accessions decreased due to salinity. Khash, Orumiyeh, and Kerman accessions had the greatest leaf fresh weight, but they were more affected by salt treatment in this regard (Figure 3-c). Ardabil showed high leaf fresh weight having the least impact from salinity on this attribute. The least amount of leaf fresh weight was recorded for Kermanshah accession with 5.48 g per plant. Difference between the accessions in terms of leaf dry weight was more or less the same as their leaf fresh weight (Figure 3-d). In this study, stem fresh weight and stem dry weight was higher in the control plants than salinized ones (Figure 3-e, f). In the present study, NaCl caused decrease in root fresh weight by 42% (Khash), 39% (Esfahan, Zabol, Pakdasht), 36% (Orumiyeh), 33% (Shiraz), 24% (Kerman), 21% (Malayer), 17% (Zahedan), 15% (Birjand), 14% (Kermanshah, Zanjan), 11% (Tonekabon), 2% (Ardabil), and 1% (Gorgan) (derived from data, data are not shown) (Figure1-g). Root dry weight data indicated that this trait decreased with salt content in nutrition solution, which was the lowest weight in Khash accession at 48% compared to the control (Figure 1-h). Maia *et al.* (2017) reported two basil varieties (Roxo and Verdi) as tolerant to saline water, without a change in their dry weight. One of the main discrepancies about the basil accessions is related to their leaf area. After 45 days of salt treatment, the control plants had a larger leaf area than the salinized plants, indicating the negative influence of NaCl on leaf area. At the end of the experiment, all control plants showed high leaf area and only Zanjan accession had the same leaf area as the control (Figure 3-i). In Khash and Orumiyeh accessions, the plants had greater shoot fresh and dry weight, respectively, than the others (Figure 3-j, k). In view of commercial performance, shoot fresh weight is a very important trait that is considered as the marketing value of a leafy vegetable like basil. Shoot dry weight was less sensitive to salinity (Shannon and Grieve, 1998). Plant tolerance during exposure to some level of salinity stress can be considered as a successful plant selection procedure beneficial for recommending proper accession for saline regions. In this study, difference in the response to NaCl treatment was recorded for the fifteen studied accessions. NaCl application (40 mM) in nutrient solution decreased the fresh weight of the whole plant, particularly in Khash accession, where the reduction was about 50% compared to the other accessions (Figure 3-l). A similar result was obtained for total dry weight in Khash accession. Another important attribute to evaluate the morphological, physiological, and growth performance of plants is their biomass production. In this study, basil plants grown at the presence of salinity showed lower total biomass 45 days after the salt treatment compared to the controls (Figure 3-l). Bernstein *et al.* (2010) have also reported that leaf biomass of basil (Perrie cultivar) decreases as a result of exposure to 25 mM NaCl. In the present study, basil accessions had a moderate tolerance to salinity imposed by 40 mM NaCl in terms of leaf biomass. These results emphasized the clear role of

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

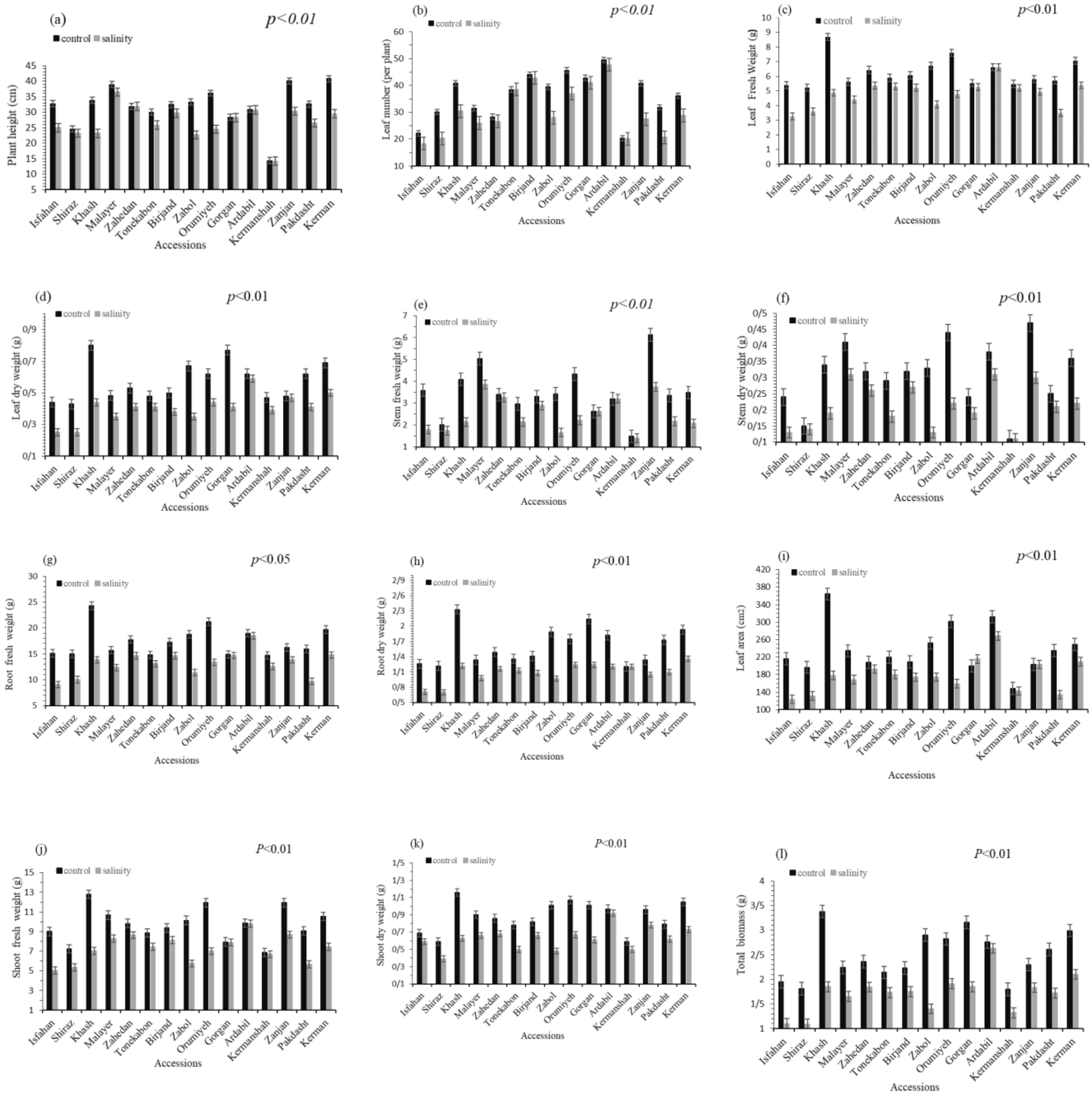

Figure 3. Morphological and growth parameters estimated in 15 basil accessions under both control and salt treatment. Error bars represent Standard Errors.

both salinity and the basil accession in determining tolerance to salt stress. In some studies, basil accessions have been compared through producing polyphenol compounds in response to NaCl, because salinity alters polyphenol production depending on the variety (Scagel *et al*., 2019). Plant breeding program first entails the presence of an extensive variability in the available germplasm for different traits, the most important one being the yield (De Santis *et al*., 2016). Irrigation of sweet basil grown in a hydroponic system fed by Hoagland's solution containing NaCl (5.5

dS m^{-1} EC) influenced plants' total biomass. In the present study, the difference in salinity tolerance was observed among basil accessions. Overall, Ardabil and Zahedan accessions reached more than 8.5 g shoot fresh weight (per plant) after harvest, which is a very important commercial trait under salinity conditions.

Chlorophyll a Fluorescence Transient

In this study, salt concentration and different accessions had significant effects on fluorescence indices (F_o, F_M, M_o, and F_V/F_o), specific energy fluxes (DI_o/RC, ET_o/RC, TR_o/RC and ABS/RC), quantum yield (ΦD_o, ΦE_o, Ψ_o and F_V/F_M), and vitality index (PI_{ABS}) (Table 7). In many of the estimated parameter, the 'spider plot' diagrams were constructed to show interaction between salinity and accession (Figure 4). There was a clear increase in F_o, which could be suggested as a result of the disconnection of the Light Harvesting Complex from PSII (Figure 4-a). A structural damage can cause a decrease in excitation energy transfer from the antenna to the reaction center, which leads to high F_o, and a damage to the reaction center may provoke a drastic reduction in photochemistry (Oukarroum *et al*., 2009). Fluorescence value (F_M) by basil plants grown under salt condition increased to ~13% in Ardabil accession, but it remained unchanged in Isfahan and Tonekabon accessions. However, the salt-treated and control plots of many of accessions are normalized both at F_o and at F_M (Figure 4-a, b). The efficiency of the water-splitting complex on the donor of PSII (as inferred from F_V/F_o) significantly decreased to ~ 8% (Figure 4-c). This ratio is a sensitive component in electron transport chain. The ratio of F_V/F_o decreases as a result of photosynthetic electron transport impairment under salt stress due to an inhibition of osmotically driven uptake of water under salinity (Pereira *et al*., 2000; Frick and Peter, 2002). The maximal quantum efficiency of PSII (calculated from F_V/F_M) values in plants grown under salinity stress for 40 days was less that noted in the control plants. In most accessions, F_V/F_M was close to 0.81, and under controlled condition, this parameter was often proportional to photosynthesis rate. No obvious large difference in the fluorescence indices rise was observed, suggesting that the few reaction centers that are untouched behave normally. However, there was a large increase in the number of inactive reaction centers. A reduction of F_V/F_M (5%) was observed in Kermanshah accession, but Ardabil accession was clearly much more tolerant to salinity stress than the others. The F_V/F_M ratio in three accessions including Khash, Malayer, and Orumiyeh decreased by about 2 and 3% under 40 mM NaCl (Figure 4-d). These values were all below 0.8, indicating that basil plants grown under salt treatment experienced certain degrees of stress state. Our experiment revealed similar results between growth parameter and fluorescence indices. Compared to F_V/F_M, the leaf area significantly decreased in three accessions under salinity (50% of the control). On the other hand, the minimal leaf area directly causes lower light interception. This result showed that salinity affects plant growth due to changes in photosynthesis process (Kalaji and Guo, 2008). It was also

Table7. Analysis of variance (ANOVA) of the effect of salt treatment and accession on chlorophyll fluoresence parameters.

Treatment	F_o	F_M	Fv/F_o	Fv/F_M	Ψ_o	ΦE_o	ΦD_o	PI_{ABS}	ABS/RC	TR_o/RC	ET_o/RC	DI_o/RC
Salinity	**	NS	**	**	*	**	**	**	**	**	NS	**
Accession	**	**	**	**	NS	NS	**	**	**	**	NS	**
Interaction	**	**	**	**	NS	*	**	**	**	**	NS	**
CV	10.16	7.38	7.16	2.77	8	9.03	11.76	27.28	6.59	5.39	10.71	11.6

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

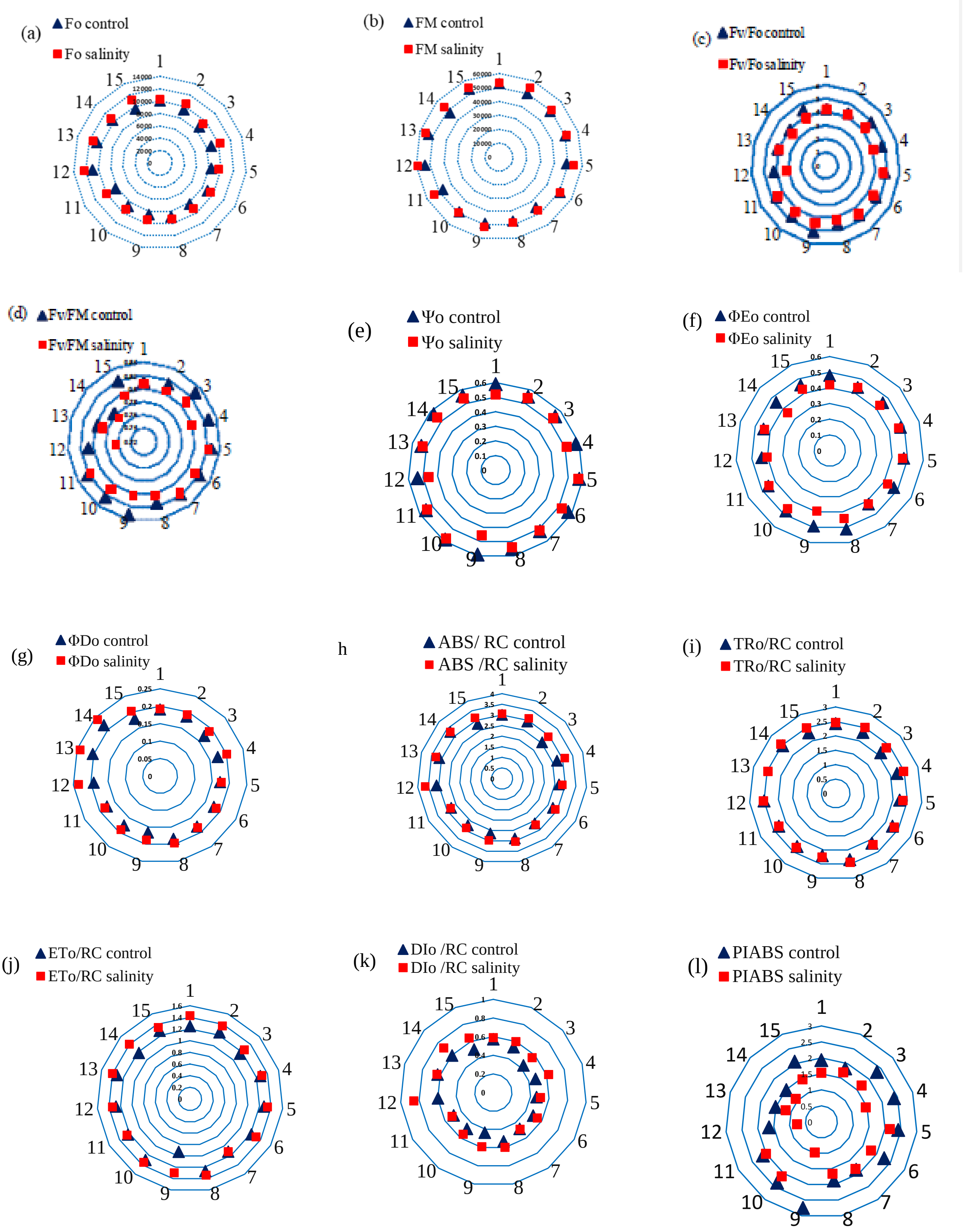

Figure 4. Significant differences in the OJIP parameter values among accessions. The values represent the average of interaction accession-salt measurements in different accessions.

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

found that the root FW and DW decreased directly as fluorescence indices decreased. Besides, compared to leaf area and root biomass, shoot FW and DW of basil plants dramatically decreased with PSII activity. The increase of F_V/F_M value signifies greater light utilization efficiency and a stronger ability of plant to adapt to the condition (Shao *et al*., 2020). Ψ_o (probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-) markedly were higher than in the control (14%) (Figure 4-e). In Malayer, Orumiyeh, and Kermanshah accessions, the values of several parameters per reaction center including ΦE_o (Electron transport yield) was lower (0.8 to 21%) (Figure 4-f), ΦD_o (thermal Dissipated yield) and ABS/RC (Absorption flux per Reaction Center) were higher (0 to 14%) (Figure 4-g), (1 to 18%) (Figure 4-h) when compared to the control plants. Our results showed that TR_o/RC (Trapping of excitation energy) and ET_o/RC (Electron Transport per Reaction Center) decreased with increasing salt concentration in basil leaves, because active reaction centers are converted into inactive RCs, which reduces the energy trapping efficiency and electron transport from PSII (Figure 4-i, j). Zabol accession did not show difference between the control and salinity in ETo/RC index (Figure 4-i) and in DI_o/RC (Dissipated energy flux per Reaction Center) (0.9% to 30%) (Figure 4-k). Accumulation of inactive reaction centers is associated with the increased efficiency of dissipation of absorbed light. In this study, 40 days after treatment, plant biomass in different accessions had greater differences, and it tended to decrease linearly with the chlorophyll fluorescence parameters. Performance index is a multiple parameter that is related to photosynthetic machinery potential. Performance Index (PI_{ABS}) decreased (about 23%) as compared to the control plants (Figure 4-l). As compared to Orumiyeh, where it had dropped to ~ 64%, Ardabil accession had an extremely low value of ~ 1% (Figure 4-l). In wheat leaves, the values of almost all parameters related to the electron donor and acceptor sites of PSII were inhibited by salt stress (Mehta *et al*., 2010). A study has reported the effect of salt stress on tomato leaves using various parameters derived from OJIP curves (Zushi and Matsuzoe, 2017). Similarly, in our study, the values of JIP parameters significantly decreased with salinity stress.

CONCLUSIONS

In this study, we conducted an experiment by growing 15 different Iranian basil accessions under salinity stress and applied the K-means clustering method to identify and distribute the accessions. Plants varied in their morphology and growth parameters depending on their different origins, and cluster analysis classified them into three groups with clear distance under salinity. Functional characterization of basil showed high yield-related traits for Khash, Zanjan, and Orumiyeh and low-yield traits (low fresh and dry weight in leaf) for Kermanshah, Shiraz, and Gorgan. Also, a wide range of variation was recognized in the collection of Iranian basil accessions under salinity conditions. Salinity stress negatively influenced PSII activity in basil plants, and this depended on the studied accessions. Our experiments allowed us to determine how salinity stress could significantly alter the chlorophyll a fluorescence parameter as well as morphological indices. Based on these results, basil producers can choose the proper salt-tolerant basil accession with high market value potential for successful production in a greenhouse using saline water. In this study, 'Ardabil' accession showed high yield and tolerance to salinity in terms of both morphological and physiological parameters. Furthermore, the other accessions had high potential to be developed as new salt-tolerant basil varieties.

ACKNOWLEDEMENTS

The authors would like to thank Dr. G. Akbari for providing basil seeds and information on the origin of accessions.

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

REFERENCES

1. Aghaali, Z., Darvishzadeh, R. and Aghaei, M. 2017. Association Analysis for Morphological Traits in Iranian Basil Accessions Using ISSR Marker. *Iran. J. Biotechnol*., **9(1):** 93-102.
2. Akbari, G.A., Soltani, E., Binesh, S. and Amini, F. 2018. Cold Tolerance, Productivity and Phytochemical Diversity in Sweet Basil (*Ocimum basilicum* L.) Accessions. *Ind Crops Prod*., **124:** 677-684.
3. Akbari, G. A., Binesh, S., Ramshini, H., Soltani, E., Amini, F. and Mirfazeli, M. S. 2019. Selection of Basil *(Ocimum basilicum L.*) Full-Sib Families from Diverse Landraces. *Jarmap*., **12:** 66-72.
4. Banks, J. M. 2018. Chlorophyll Fluorescence as a Tool to Identify Drought Stress in Acer Genotypes. *Exp. Bot.*, **155:** 118-127.
5. Bekhradi, F., Luna, M., Delshad, M., Jordan, M., Sotomayor, J., Martínez-Conesa, C. and Gil, M. 2015. Effect of Deficit Irrigation on the Postharvest Quality of Different Genotypes of Basil Including Purple and Green Iranian Cultivars and a Genovese Variety. *Postharvest Biol. Tec.*, **100:** 127-135.
6. Bernstein, N., Kravchik, M. and Dudai, N. 2010. Salinity-Induced Changes in Essential Oil, Pigments and Salts Accumulation in Sweet Basil (*Ocimum basilicum*) in Relation to Alterations of Morphological Development. *Ann. Appl. Biol.,* **156(2):** 167-177.
7. Bhargava, A., Shukla, S. and Ohri, D. 2012. Implications of Direct and Indirect Selection Parameters for Improvement of Grain Yield and Quality Components in *Chenopodium quinoa* Willd. *Int J Plant Prod.*, **2(3):**183-192.
8. Bie, Z., Ito, T. and Shinohara, Y. 2004. Effects of Sodium Sulfate and Sodium Bicarbonate on the Growth, Gas Exchange and Mineral Composition of Lettuce. *Sci. Hortic.*, **99(3-4):** 215-224.
9. Bisbis, M. B., Guda, N. and Blanke, M. 2018. Potential Impacts of Climate Change on Vegetable Production and Product Quality–A Review. *J. Clean. Prod.,* **170**: 1602-1620.
10. De Santis, G., D'Ambrosio, T., Rinaldi, M. and Rascio, A. 2016. Heritabilities of Morphological and Quality Traits and Inter Relationships with Yield in Quinoa (*Chenopodium quinoa* Willd.) Genotypes in the Mediterranean Environment. *Cereal Sci.*, **70:** 177-185.
11. Flowers, T. 2004. Improving Crop Salt Tolerance. *J. Exp. Bot.*, **55(396):** 307-319.
12. Flowers, T. and Yeo, A .1995. Breeding for Salinity Resistance in Crop Plants: Where Next? *Funct. Plant Biol.,* **22(6):** 875-884.
13. Fricke, W. and Peters, W. S. 2002. The Biophysics of Leaf Growth in Salt-Stressed Barley. A Study at the Cell Level. *Plant Physiol.*, **129(1)**: 374-388.
14. Govender, P. and Sivakumar,V. 2019. Application of K-means and Hierarchical Clustering Techniques for Analysis of Air Pollution. *Atmos. Pollut. Res*., **11:** 40-56.
15. Hasani, Z., Pirdashti, H., Yaghoubian, Y. and Zaman Nouri, M. 2014. Application of Chlorophyll Fluorescence Technique to Evaluate the Tolerance of Rice (*Oryza sativa* L.) Genotypes to Cold Temperature and Water Stresses. *Iranian Journal of Cell and Tissue.*, **5(2):** 195-206.
16. Jain, A. K. 2010. Data Clustering: 50 Years beyond K-means. *Pattern Recogn. Lett.*, **31(8):** 651-666.
17. Kan, X., Ren, J., Chen, T., Cui, M., Li, C., Zhou, R., . . . PROVID ALL AUTHORS' NAMES!! Yin, Z. 2017. Effects of Salinity on Photosynthesis in Maize Probed by Prompt Fluorescence, Delayed Fluorescence and P700 Signals. *Exp. Bot.,* **140:** 56-64.
18. Kainer, D., Lanfear, R., Foley, W. J. and Külheim, C. 2015. Genomic Approaches to Selection in Outcrossing Perennials: Focus on Essential Oil Crops. *Theor. Appl. Genet.*, **128(12)**: 2351-2365.
19. Kalaji, M. and Guo, P. 2008. Chlorophyll Fluorescence: A Useful Tool in Barley Plant Breeding Programs. *Photochem. Res. Progress.,* **29:** 439-463.
20. Maia, S. S., da Silva, R. C., Oliveira, F. D. A. D., Silva, O. M. D. P., Silva, A. C. D. and Candido, W. D. S. 2017. Responses of Basil Cultivars to Irrigation Water Salinity. *Rev. Bras. Eng. Ag. Amb.,* **21(1)**: 44-49.
21. Mehta, P., Jajoo, A., Mathur, S. andBharti, S. 2010. Chlorophyll a Fluorescence Study Revealing Effects of High Salt Stress on Photosystem II in Wheat Leaves. *Plant Physiol. Bioch.,* **48(1)**: 16-20.

22. Minhas, P. S. 1996. Saline Water Management for Irrigation in India. *Agric. Water Manage.*, **30:** 1-24.
23. Morales, M. R.and Simon, J. E.1996. New Basil Selections with Compact Inflorescences for the Ornamental Market. *Progress in New Crops.*, In:" *Progress in new crops*" (Ed) : J. Janick, ASHS Press, Arlington, PP. 543-546.
24. Moghaddam, M., Omidbiagi, R. and Naghavi, M. R. 2011. Evaluation of Genetic Diversity among Iranian Accessions of *Ocimum* spp. Using AFLP Markers. *Biochem. Syst. Ecol.,* **39(4-6)**: 619-626.
25. Moghaddam, M. and Mehdizadeh, L. 2015. Variability of Total Phenolic, Flavonoid and Rosmarinic Acid Content among Iranian Basil Accessions. *LWT,* **63(1):** 535-540.
26. Mosadegh, H., Trivellini, A., Ferrante, A., Lucchesini, M. Vernieri, P. and Mensuali, A. 2018. Applications of UV-B Lighting to Enhance Phenolic Accumulation of Sweet Basil. *Sci. Horti.,* **229:** 107-116.
27. Munns, R. and Tester, M. 2008. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.,* **59:** 651-681.
28. Oukarroum, A., Schansker, G. and Strasser, R. J. 2009. Drought Stress Effects on Photosystem I Content and Photosystem II Thermotolerance Analyzed Using Chl a Fluorescence Kinetics in Barley Varieties Differing in Their Drought Tolerance. *Plant Physiol.,* **137(2):** 188-199.
29. Paton, A. 1992. A Synopsis of *Ocimum* L. (*Labiatae*) in Africa. *Kew Bull.,* **47(3):** 403-435.
30. Paton, A. Harley, R. and Harley, M. 1999. *Ocimum*: An Overview of Classification and Relationships In: "*Basil*". (Eds.): Holm, Y and Hiltunen, R., CRC Press., Hardwood Academic, Amsterdam, PP. 11-46.
31. Pereira, W. E., de Siqueira, D. L., Martínez, C. A. and Puiatti, M. 2000. Gas Exchange and Chlorophyll Fluorescence in Four Citrus Rootstocks under Aluminium Stress. *Plant Physiol.,* **157(5):** 513-520.
32. Potashev, K., Sharonova, N. and Breus, I. 2014. The Use of Cluster Analysis for Plant Grouping by Their Tolerance to Soil Contamination with Hydrocarbons at the Germination Stage. *Sci. Total Environ.,* **485-486**: 71-82.
33. Pushpangadan, P. and George, V. 2012. **4-Basil**. In: "*Handbook of Herbs and Spices"*, (Ed.): Peter, K. V. Second Edition, Woodhead Publishing. PP. 55-72
34. Ramin, A. A. 2006. Effects of Salinity and Temperature on Germination and Seedling Establishment of Sweet Basil (*Ocimum basilicum* L.). *IJHSMP,* **11(4):** 81-90.
35. Rouphael, Y., Petropoulos, S. A., Cardarelli, M. and Colla, G. 2018. Salinity as Eustressor for Enhancing Quality of Vegetables. *Sci. Hortic.,* **234**: 361-369.
36. Scagel, C. F., Lee, J. and Mitchell, J. N. 2019. Salinity from NaCl Changes the Nutrient and Polyphenolic Composition of Basil Leaves. *Ind. Crops Prod.,* **127:** 119-128.
37. Shao, M., Liu, W., Zha, L., Zhou, C., Zhang, Y. and Li, B. 2020. Differential Effects of High Light duration on Growth, Nutritional Quality, and Oxidative Stress of Hydroponic Lettuce under Red and Blue LED Irradiation. *Sci. Horti.,* **268:** 109366.
38. Schreiber, U., Bilger, W. and Neubauer, C. 1995. *Chlorophyll Fluorescence as a Nonintrusive Indicator for Rapid Assessment of In Vivo Photosynthesis*. Ecophysiology Photosynthesis, Springer Study Edition Book Series, **100**: 49-70
39. Shannon, M. C. and Grieve, C. M. 1998. Tolerance of Vegetable Crops toSalinity. *Sci. Hortic*., **78(1-4):** 5-38.
40. Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F. and Hao, Z. 1999. Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb. *Perspectives on New Crops and New Uses,* **16:** 499-505.
41. Singh, A. 2015. Poor Quality Water Utilization for Agricultural Production: An Environmental Perspective. *Land Use Policy,* **43:** 259-262.
42. Singh, S., Lal, R. K., Maurya, R. and Chanotiya, C. S. 2018. Genetic Diversity and Chemotype Selection in Genus *Ocimum*. *J. Appl. Res. Med. Aroma*., **9:** 19-25.
43. Tester, M. and Davenport, R. 2003. Na^+ Tolerance and Na^+ Transport in Higher Plants. *Ann. Bot.,* **91(5)**: 503-527.
44. Zhu, J. K. 2001. Plant Salt Tolerance. *Trends Plant Sci*., **6(2):** 66-71.
45. Zushi, K. and Matsuzoe, N. 2017. Using of Chlorophyll a Fuorescence OJIP Transients for Sensing Salt Stress in the Leaves and Fruits of Tomato. *Sci. Horti.,* **219:** 216-221.

[DOR: 20.1001.1.16807073.2022.24.1.6.6]

تنوع طبیعی تحمل به شوری در توده‌های ریحان ایرانی براساس فلئورسانس کلروفیل، ویژگی‌های مورفولوژیکی و رشد

س. شیراحمدی، م. اثنی عشری، س. علی‌نیایی فرد، و غ. عباس اکبری

چکیده

به منظور انتخاب توده‌های شاخص گیاه ریحان در تحمل به تنش شوری، ۱۵ توده از نقاط مختلف ایران براساس ویژگی‌های مورفولوژیکی و رشد و شاخص‌های فلورسانس کلروفیل ارزیابی شدند. دو سطح شوری شامل (شاهد، بدون نمک) و کلرید سدیم با غلظت ۴۰ میلی مولار (در محلول غذایی هوگلند با هدایت الکتریکی۵/۵ دسی زیمنس بر متر) استفاده گردید. تجزیه خوشه‌ای به روش میانگین k جهت شناسایی و توزیع توده‌ها انجام شد. براساس نتایج حاصل از پژوهش، توده‌ها به سه خوشه تحت تنش شوری طبقه‌بندی شدند. شوری اثر معنی داری در سطح احتمال یک درصد بر پارامترهای مورفولوژیکی و رشد در تمام توده‌ها نشان داد. در مقایسه با شاهد، کلرید سدیم، ارتفاع و تعداد برگ در توده‌های خاش، زابل و ارومیه را با درجه اختلاف بالاتری از سایر توده‌ها کاهش داد. اثر کلرید سدیم و توده با کاهش فراتر از ۴۵ درصد وزن تر برگ، اثر توده را در شرایط شوری تائید نمود. زیست توده گیاه نیز از غلظت کلرید سدیم تاثیر پذیرفت. دامنه کاهش زیست توده کل از ۱۹ درصد تا ۴۳ درصد وابسته به نوع توده متغیر بود. تیمار شوری فعالیت کمپلکس فتوسیستم II را تحت تاثیر قرار داد که از داده‌های فلورسانس کلروفیل قابل اثبات بود. شاخص حداکثر کاریی کوانتومی فتوسیستم II و شاخص عملکرد (PI)، مهم‌ترین ویژگی های تاثیرپذیر از شوری بودند. در مجموع ارزیابی داده های مورفولوژیکی و فیزیولوژیکی، توده اردبیل به عنوان توده برتر در شرایط شوری انتخاب شد. بنابراین، انتخاب توده برای مطالعه مکانیسم تحمل به شوری در گیاه ریحان حائز اهمیت است.

[DOR: 20.1001.1.16807073.2022.24.1.6.6]