

Assessment of Salt Tolerance in Pepper Using Chlorophyll Fluorescence and Mineral Compositions

Mohammad Reza ZARE BAVANI ¹ (✉)

Gholamali PEYVAST ²

Mahmoud GHASEMNEZHAD ²

Akbar FORGHANI ³

Summary

In this study, leaf chlorophyll fluorescence and mineral compositions was used to compare pepper (*Capsicum annuum* L.) cultivars response to salt stress. Twenty-six pepper cultivars were exposed to salt stress (100 mM NaCl) during two weeks. Thereafter, chlorophyll fluorescence components, stress tolerance index (STI), sodium, potassium and calcium content were measured. The results showed that a significant difference has been found among pepper cultivars for all studied characteristics. Reduced chlorophyll fluorescence parameters under salinity treatment were different between pepper cultivars. F_o/F_m , F_v/F_m was declined, with NaCl treatment in all cultivars. F_v/F_o , F_v/F_m , Φ_{exc} , Φ_{PSII} , ETR, q_p , K^+ , K^+/Na^+ and Ca^{++}/Na^+ were decreased but leaf Na^+ content was increased by salinity stress. A significant correlation was found between salt stress tolerance index and fluorescence characteristics such as F_o/F_m , F_v/F_o , F_v/F_m , F_v/F_m diminishing, Φ_{exc} , Φ_{PSII} , ETR, and q_p . Furthermore, there was a significant correlation between Na^+ , K^+ , K^+/Na^+ and Ca^{++}/Na^+ with salt stress tolerance index. Overall, chlorophyll fluorescence parameters followed by Na^+ , K^+ , K^+/Na^+ and Ca^{++}/Na^+ could be useful tool to screen salt tolerance pepper cultivars.

Key words

Ca^{++}/Na^+ , chlorophyll fluorescence, K^+/Na^+ , salinity, stress tolerance index

¹ Department of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran

✉ e-mail: mzarebavani@gmail.com

² Department of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran

³ Department of Soil Science, Faculty of Agriculture, University of Guilan, Rasht, Iran

Received: December 6, 2014 | Accepted: September 27, 2015

Introduction

Salt stress is one of the most important limiting factors for plant growth and agricultural productivity all over the world (Chaves et al., 2011). Salinity affects more than 6% of the arable land and about 30 to 50% of irrigated land worldwide (Chaves et al., 2011). The decline in availability of freshwater in arid and semi-arid areas has resulted in the increased use of bad quality water for irrigation of greenhouse crops that causes a salt stress that may be harmful for plant production (Lycoskoufis et al., 2005; Azuma et al., 2010).

Generally, the techniques used to evaluate plant tolerance to environmental stress from plant materials are based on methods that require sample destruction, a storage period and measurement in laboratory, resulting in an important delay in determining the crop status at certain moment. Chlorophyll fluorescence provides a rapid, non-invasive, non-destructive and accurate technique for evaluation of the plant tolerance to stress (Li et al., 2006). Measuring chlorophyll fluorescence is a sensitive method of assessing the efficiency of photosynthetic system II (PSII) and the changes in photosynthesis caused by environmental effects (Lichtenthaler, 1987). Ratio of the variable to the maximum components of fluorescence (F_v/F_m) is a measure of the capacity of PSII, which is sensitive to various environmental factors inducing different kinds of stress (Baker and Rosenqvist, 2004). Chlorophyll *a* fluorescence can give information on the ability of a plant to tolerate environmental stresses (Zribi et al., 2009). Chlorophyll fluorescence can also be useful in salinity-tolerance screening programs, because it detects effects of salt damage before visible signs of deterioration (Kaouther et al., 2012). There are some reports showing that chlorophyll fluorescence parameters could be useful to screen salt tolerance (Corney et al., 2003; Zribi et al., 2009; Bacarin et al., 2011; Mittal et al., 2012).

The nutritional status of plants with potassium (K) and calcium (Ca) has been regarded as characterization of salt tolerance in crop plants (Aktas et al., 2006). K^+/Na^+ and Ca^{++}/Na^+ ratios and tissue Na^+ concentration are used in screening crop plants for tolerance to salt stress (Munns and James, 2003). It was reported that salt tolerant cultivars of pepper had higher values of K^+/Na^+ and Ca^{++}/Na^+ ratios (Aktas et al., 2006; Zhani et al., 2012).

Pepper is one of the three most important Solanaceous vegetable crops in the world, which is generally considered as salt sensitive (Azuma et al., 2010). In greenhouse cultivation, all over the world, the lack of good quality water made producers to use saline underground water that causes severe reduction in crop growth and yield (Lycoskoufis et al., 2005). So, in arid and semiarid regions, salinity has a severe impact on the yield and quality of pepper (Del Amor et al., 2012). Therefore, the present study was carried out to determine whether chlorophyll fluorescence components can be used as potential physiological indicator for evaluating the salinity tolerance of pepper cultivars in seedling stage.

Materials and methods

Plant material and treatments

The greenhouse experiment was conducted in 2013 at University of Guilan, Rasht, Iran. Seeds of twenty six pepper

Table 1. Names of pepper cultivars that were used in experiment

No.	Hybrid	Fruit type	Company
1	Ethem	Yellow-Long Conical	Petoseed
2	Dulce	Green- Jalapeno	Petoseed
3	Shanghai(SQ-Y)	Yellow- Bell	Petoseed
4	Luzon	Yellow- Bell	Bruinsma
5	PaxRGH	Yellow- Bell	Bruinsma
6	Paramo	Orange- Bell	Bruinsma
7	Lorca F1	Red- Bell	Bruinsma
8	Mentor	Red- Bell	Bruinsma
9	Snooker (root stock)	Green- Conical	Syngenta
10	Efests	Red- Conical	Nunhems
11	Semer kand	Red- Conical	Nunhems
12	SPADI	Red- Conical	Vilmorin
13	ACX 270	Red- Lamuyo	ABBOT and COBB
14	Exp. 10	Red- Lamuyo	Vilmorin
15	Tyson	Red- Lamuyo	Vilmorin
16	Daytona	Red- Lamuyo	Nunhems
17	Magic	Red- Lamuyo	Axia
18	Defender	Red- Lamuyo	Nunhems
19	Figaro	Red- Lamuyo	Vilmorin
20	Radin	Red- Lamuyo	Axia
21	ACX 248	Red- Lamuyo	ABBOT and COBB
22	Maral	Yellow- Lamuyo	Axia
23	Wanado	Red- Lamuyo	Axia
24	Octavio	Red- Lamuyo	Vilmorin
25	Sereno	Yellow- Lamuyo	Vilmorin
26	Exp. 4	Red- Lamuyo	Vilmorin

cultivars (Table 1) were surface sterilized for 10 min in sodium hypochloride (5%), then washed with deionized water and germinated for 12 days in perlite at 28°C in the incubator. Thereafter, seedlings were transferred to greenhouse with controlled environment at a temperature of 24±3°C and the relative humidity variation between 90% at night and 60% at midday. The seedlings were transplanted into 15 L black plastic containers containing aerated full nutrient solution consisted of macronutrients (4 mM N, 2 mM K, 0.25 mM P, 2 mM Ca, 1 mM Mg, and 1.88 mM S) and micronutrients (10 µmol B, 0.5 µmol Mn, 1µmol Zn, 100 µmol Fe, 0.2 µmol Cu and 0.02 µmol Mo). The solution was completely replaced every three days (Aktas et al., 2006). Salt stress treatments started when the pepper seedlings reached six to seven true leaf stage with a salty solution containing 100 mM NaCl for 14 days. The nutrient solution without NaCl was used as a control.

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence parameters including minimal fluorescence (F_0) and maximal fluorescence (F_m) of the youngest fully expanded leaves were measured 30 min after darkness adaptation of the leaves were measured using a pulse amplitude modulated fluorometer (Mini- PAM- 2000; Walz, Germany).. Steady-state yield of PSII fluorescence and fluorescence maximum (F'_m) were measured in the light adapted leaves.

The other fluorescence parameters were calculated using the below formulas:

- F_v/F_m (maximal photochemical yield of PSII in the dark-adapted state) = $(F_m - F_0)/F_m$;
- F_v/F_0 (the potential photosynthetic activity) = $(F_m - F_0)/F_0$;

- Φ PSII (effective quantum yield of PSII photochemistry) = $[F'm - (Fs)]/F'm$;
- Φ_{exc} (the efficiency of excitation energy capture by open PSII reaction centres) = $(F'm - F'o)/F'm$;
- photochemical quenching coefficient (qp) = $(F'm - Fs) / (F'm - F'o)$;
- non-photochemical quenching (NPQ) = $[(Fm - F'm)/F'm]$;
- ETR (the electron transport rate) = $yield \times PFD \times 0.5 \times 0.84$ (the standard factor 0.84 corresponds to the fraction of incident light absorbed by leaf);
- the physiological state of the photosynthetic apparatus (the quantum yield baseline) = Fo/Fm .

The salt tolerance index

The stress tolerance index was calculated using the Fernandez (1992) formula:

$$STI = (Y_s \times Y_p) / (Y_p)^2$$

Y_s = total dry weight in salt treatment, Y_p = total dry weight in control, Y_p = total dry weight of all cultivar in control.

Determination of ion contents

K^+ , Na^+ and Ca^{++} leaf content was analyzed by flame spectrophotometer.

The experimental design was completely randomized with three independent replications and six plants for each replication. The data analysis was done using SPSS software 22.00. LSD test was used to compare between means and to determine significance between variables ($P \leq 0.05$).

Results and Discussion

Salt tolerance index

Salt tolerance index (STI) was significantly different among pepper cultivars under salt stress conditions (Table 3). The cultivars 'Paramo', 'Sereno' and 'Efefts' were more tolerant and 'PaxRGH', 'Exp. 10' and 'Mentor' were more sensitive cultivars to salt stress.

Previous study showed that STI is able to select cultivars with high yield potential and greater tolerance to stress. This indicator is based on the selection of cultivars with high yield in stress and non-stress condition (Fernandez, 1992).

Chlorophyll fluorescence (Photochemical Efficiency)

Salt stress resulted with declining of all fluorescence chlorophyll components except Fo , Fo' , Fo/Fm and NPQ (result not shown). Fo and Fo' were increased in 100 mM NaCl for all cultivars, but they were not significantly different from control plants. The observed Fm and Fm' did not significantly decrease in salt stress compared to control. Fo/Fm (the quantum yield baseline) increased significantly in 100mM NaCl for all cultivars. 'Sereno', 'Paramo' and 'Efefts' showed higher values, while 'PaxRGH', 'Exp. 10' and 'Magic' showed lower Fo/Fm than other cultivars.

The results showed that Fo/Fm ratio differed among pepper cultivars and correlated with STI. Normal values of Fo/Fm , as standard, were observed between 0.14 and 0.20 (Rohacek, 2002). The higher Fo/Fm indicates that the initial rate of reduction of the plastoquinone A (PQ_A) was higher than the rate of plastoquinone B (PQ_B) and the activity of photosystem I (PSI), when

plants were exposed to higher concentrations of NaCl (De Lucena et al., 2012). Rohacek (2002) suggested the increase relation Fo/Fm as stress indicator. Similar kind of results has been documented for *Brassica* species (Jamil et al., 2014).

In this study the higher values of Fv/Fo was found in 'Sereno', 'Paramo' and 'Efefts' cultivars and the lower values in 'PaxRGH', 'Exp. 10' and 'Magic' cultivars. The correlation between Fv/Fo and STI was completely dependent on pepper cultivars. Salinity reduced Fv/Fo and the more tolerance cultivars had the higher was Fv/Fo . In general, Fv/Fo ratio is a very sensitive index of the potential photosynthetic activity of plants under different environmental conditions. High salinity stress affects the efficiency of the photochemical process and the electron transport chain in PSII that resulted in decrease in Fv/Fo ratio (Li et al., 2010). The reduction of Fv/Fo in response to salt stress has been reported in *Acer* (Percival et al., 2003)

Salinity also significantly affected Fv/Fm ratio, which was measured in the dark adapted leaves of all pepper cultivars. In control plants, (Fv/Fm) ratio was in the range of 0.80 to 0.82 for all cultivars. In 100 mM NaCl, declines of approximately 6.65% to 19.96 % were found for 'Efefts' and 'PaxRGH' cultivars respectively. The degree of Fv/Fm declining was dependent on pepper cultivars. The lowest declining of Fv/Fm was observed in 'Efefts', followed by 'Paramo', 'SPADI' and 'Sereno' and the highest value was observed in 'PaxRGH', followed by 'Exp.10' and 'Magic' cultivars. In this study, it has been observed that salinity caused a significant reduction in the values of Fv/Fm (quantum yield of PSII), suggesting that salinity can induce perturbations in electron transport of PSII (Megdiche et al., 2008). In other hands, salt stress prevents the electron transfer from the primary acceptor, PQ_A to the secondary acceptor, PQ_B at the acceptor side of PSII that resulted in the decrease in Fv/Fm (Shu et al., 2012). The increase of NaCl in chloroplasts of plants causes the restriction of PSII and increases susceptibility to photodamage (Sudhir and Murthy 2004). The maximum photochemical efficiency (Fv/Fm) for a leaf in normal conditions varies between 0.75 and 0.85 and a reducing of this parameter shows photo-inhibitory damage (Kaouther et al., 2012). The effect of salinity stress on the maximum quantum yield of PSII (Fv/Fm) depends on the salt tolerance among the species or even among the genotypes (Lee et al., 2004; Jiang et al., 2006). Fv/Fm has been used vastly as a technique for early stress detection (Baker and Rosenqvist, 2004). Our results match with the studies in *Capsicum annuum* (Kaouther et al., 2012), *Solanum melongena* (Wu et al., 2012), *Lycopersicon esculentum* (Al-aghaby et al., 2005), *Cucumis sativus* (Shu et al., 2012), *Triticum aestivum* (Kanwal et al., 2011), *Brassica juncea* (Wani et al., 2013) and *Brassica* species (Jamil et al., 2014).

'Sereno', 'Paramo' and 'Maral' had significantly higher values of Φ_{exc} while 'Exp. 10', 'PaxRGH' and 'Tyson' lower values of Φ_{exc} compared with other cultivars. Φ_{exc} has been shown efficiency of excitation energy that reaches to reaction centers of PSII and the decrease in Φ_{exc} could be attributed to decrease in Fv/Fm or increase in NPQ of PSII (Zribi et al., 2009). In this study, Φ_{exc} was more affected by increasing salt stress in sensitive cultivars. These results are in agreement with the findings reported in tomato (Zribi et al., 2009) and in coastal plant species (Naumann et al., 2007).

Table 2. Correlations between chlorophyll fluorescence parameters, Na⁺, K⁺, K⁺/Na⁺, Ca⁺⁺/Na⁺ and stress tolerance index measured in pepper cultivars

	Fo/Fm	Fv/Fo	Fv/Fm	Fv/Fm diminishing	Φexc	ΦPSII	ETR	qp	Na ⁺	K ⁺	K ⁺ /Na ⁺	Ca ⁺⁺ /Na ⁺	STI
Fo/Fm	1												
Fv/Fo	-0.99**	1											
ΔF/Fm	-1.00**	0.99**	1										
Fv/Fm diminishing	0.99**	-0.98**	-0.99**	1									
Φexc	-0.84**	0.85**	0.84**	-0.81**	1								
ΦPSII	-0.66**	0.67**	0.66**	-0.65**	0.79**	1							
ETR	-0.66**	0.67**	0.66**	-0.65**	0.79**	1.00**	1						
qp	-0.53**	0.53**	0.53**	-0.53**	0.63**	0.97**	0.97**	1					
Na ⁺	0.82**	-0.80**	-0.82**	0.82**	-0.66**	-0.60**	-0.60**	-0.52**	1				
K ⁺	-0.67**	0.66**	0.67**	-0.66**	0.38	0.21	0.21	0.13	-0.65**	1			
K ⁺ /Na ⁺	-0.86**	0.86**	0.86**	-0.87**	0.66**	0.55**	0.55**	0.46*	-0.94**	0.79**	1		
Ca ⁺⁺ /Na ⁺	-0.88**	0.88**	0.88**	-0.88**	0.73**	0.64**	0.64**	0.54**	-0.95**	0.64**	0.97**	1	
STI	-0.86**	0.87**	0.86**	-0.87**	0.72**	0.65**	0.65**	0.56**	-0.86**	0.64**	0.92**	0.95**	1

*, ** Correlation is significant at the 0.05 and 0.01 level 2-tailed, respectively.

Table 3. Comparison of trait means of chlorophyll fluorescence parameters correlated with STI on pepper cultivars in salt stress

Cultivar	Fo/Fm	Fv/Fo	Fv/Fm	Fv/Fm diminishing	Φexc	ΦPSII	ETR	Qp	Na ⁺	K ⁺	K ⁺ /Na ⁺	Ca ⁺⁺ /Na ⁺	STI
Ethem	0.31 b-f	2.25 a-f	0.69 a-f	14.66 a-e	0.49 e-h	0.19 d-g	24.08 d-g	0.39 e-g	14.25 b-d	37.90 a-e	2.66 jk	0.55 m-o	0.49 hi
Dulce	0.28 ef	2.54 a-d	0.72ab	12.47 c-e	0.52 c-h	0.28 a-f	34.77 a-f	0.53 a-f	9.96 h-j	38.85 a-d	3.90 de	0.83 c-f	1.02 c
Shanghai	0.32 a-e	2.15 c-f	0.68 b-f	15.81 a-d	0.52 c-h	0.31 a-c	39.17 a-c	0.60 ab	11.91 e-g	33.41 d-f	2.81 j	0.68 h-l	0.64 d-h
Luzon	0.32 a-e	2.17 c-f	0.68 b-f	15.17 a-e	0.51 d-h	0.25 a-g	31.46 a-g	0.49 a-g	19.07 a	31.58 f	1.66 o	0.44 pq	0.56 e-i
PaxRGH	0.35 a	1.86 f	0.65 f	19.96 a	0.48 gh	0.15 g	19.26 g	0.32 g	19.36 a	31.74 f	1.64 o	0.41 q	0.07 j
Paramo	0.27 f	2.72 a	0.73 a	9.87 e	0.58 ab	0.33 a	42.06 a	0.58 a-c	8.72 j	39.58 ab	4.54 a	0.96 a	1.36 a
Lorca F1	0.31 b-f	2.29 a-f	0.69 a-f	13.95 b-e	0.52 b-g	0.25 a-g	31.02 a-g	0.47 a-g	10.39 g-j	34.74 b-f	3.34 i	0.79 d-g	0.96 c
Mentor	0.32 a-e	2.14 c-f	0.68 b-f	15.90 a-d	0.49 f-h	0.20 a-g	24.69 d-g	0.41 c-g	14.54 bc	37.64 a-e	2.59 kl	0.58 l-n	0.45 i
Snooker	0.30 b-f	2.32 a-f	0.70 a-f	13.83 b-e	0.51 c-h	0.25 d-g	31.88 a-g	0.48 a-g	9.49 ij	38.29 a-e	4.04 cd	0.81 d-g	1.20 ab
Efests	0.27 f	2.72 ab	0.73 a	9.65 e	0.55 a-d	0.32 a-c	40.49 a-c	0.58 a-c	9.23 ij	41.72 a	4.52 a	0.92 a-c	1.28 a
Smerkand	0.30 b-f	2.38 a-e	0.70 a-f	13.27 b-e	0.52 c-h	0.24 a-g	30.43 a-g	0.46 a-g	10.66 g-i	39.20 a-c	3.68 f-h	0.73 g-j	0.73 de
SPADI	0.28f e	2.61 a-c	0.72 ab	10.08 e	0.55 a-e	0.35 a	44.05 a	0.64 a	9.20 ij	38.79 a-d	4.22 bc	0.89 a-d	1.23 ab
ACX 270	0.29 d-f	2.54 a-d	0.71 a-c	12.43 c-e	0.54 a-f	0.33 ab	41.03 ab	0.60 ab	8.85 ij	37.50 a-e	4.24 b	0.89 a-d	1.20 ab
Exp. 10	0.34 ab	1.98 ef	0.66 ef	18.42 ab	0.46 h	0.19 e-g	23.50 fg	0.41 c-g	17.76 a	32.80 ef	1.85 n	0.45 o-q	0.45 i
Tyson	0.31 b-f	2.29 a-f	0.69 a-f	13.70 b-e	0.49 f-h	0.22 b-g	27.32 b-g	0.45 b-g	10.64 g-i	33.54 d-f	3.15 i	0.74 f-i	0.75 d
Daytona	0.28 d-f	2.55 a-d	0.72 a-c	11.25 de	0.53 a-g	0.21 c-g	26.55 c-g	0.39 d-g	9.83 h	36.91 a-f	3.76 e-g	0.84 b-e	1.07 bc
Magic	0.33 a-c	2.01 ef	0.67 d-f	17.34 a-c	0.49 f-h	0.24 a-g	29.84 a-g	0.48 a-g	15.38 b	35.86 b-f	2.33 m	0.51 n-q	0.53 hi
Defender	0.30 b-f	2.33 a-f	0.70 a-f	14.70 a-e	0.56 a-d	0.32 a-c	40.10 a-c	0.57 a-d	12.66 d-f	33.67 c-f	2.66 jk	0.64 i-m	0.72 d-f
Figaro	0.30 b-f	2.39 a-e	0.70 a-f	13.16 b-e	0.53 a-g	0.24 a-g	29.93 a-g	0.44 b-g	10.64 g-i	37.74 a-e	3.55 h	0.77 e-h	0.76 d
Radin	0.31 a-f	2.22 b-f	0.69 a-f	14.98 a-e	0.51 d-h	0.18 fg	22.82 e-g	0.36 fg	13.93 b-d	38.68 a-d	2.78 jk	0.53 n-p	0.54 g-i
ACX 248	0.29 c-f	2.48 a-e	0.71 a-d	12.32 c-e	0.56 a-d	0.28 a-f	35.35 a-f	0.49 a-g	9.38 ij	36.02 b-f	3.84 ef	0.85 b-e	1.07 bc
Maral	0.28 d-f	2.54 a-d	0.72 a-c	12.20 c-e	0.57 a-c	0.32 a-c	40.10 a-c	0.56 a-e	13.71 b-e	33.55 d-f	2.45 lm	0.63 j-m	0.63 d-h
Wanado	0.31 a-f	2.25 a-f	0.69 a-f	14.64 a-e	0.53 a-g	0.33 ab	41.52 ab	0.61 ab	11.59 f-h	32.83 ef	2.83 j	0.69 h-k	0.71 d-g
Octavio	0.33 a-d	2.06 d-f	0.67 c-f	16.65 a-d	0.49 f-h	0.24 a-g	30.40 a-g	0.49 a-g	12.99 c-f	35.36 b-f	2.72 jk	0.60 k-n	0.55 e-i
Sereno	0.27 f	2.74 a	0.73 a	10.17 e	0.58 a	0.30 a-d	37.83 a-d	0.52 a-f	9.02 ij	39.65 ab	4.39 ab	0.94 ab	1.29 a
Exp. 4	0.29 c-f	2.43 a-e	0.71 a-d	11.85 c-e	0.53 a-g	0.30 a-e	37.36 j-e	0.55 a-e	10.37 g-j	36.97 a-f	3.56 gh	0.71 g-j	0.72 de

Numbers with the same letters are not statistically different ($P < 0.05$)

The highest values of ΦPSII were observed in 'SPADI', 'Paramo' and 'Wanado' while the lowest value was in 'PaxRGH', 'Radin' and 'Exp. 10'. ΦPSII reflects electron transport rate, which is the lowest at 100 mM NaCl, indicating that the plants couldn't convert photon energy into chemical energy (Li et al., 2010). In our experiment ΦPSII was less affected in tolerant cultivars than in sensitive ones indicating better PSII functioning under the salt stress (Hanachi et al., 2014). ΦPSII (actual PSII efficiency) account efficiency of light use for electron transport by PSII and the principal factor assessing this efficiency is the ability of photosynthetic system to remove electrons from the quinone acceptors of PSII (photochemical quenching) (Zribi et al., 2009). ΦPSII

is good indicator for PSII activity and its regulation (Hanachi et al., 2014). Similar conclusions have been demonstrated for egg plants (Hanachi et al., 2014), coastal plant species (Naumann et al., 2007) and cucumber (Stepien and Klbus, 2006).

After exposure to salinity, electron transport rate (ETR) value was decreased significantly in all cultivars. 'SPADI', 'Paramo' and 'Wanado' showed higher, while 'PaxRGH', 'Radin' and 'Exp. 10' lower values of ETR compared with other cultivars. It was reported that the ETR decreases under salinity stress (Allakhverdiev et al., 2000; Moradi and Ismail, 2007; De Lucena et al., 2012). Salt stress increases the salt concentration in the cytosol and causes the decomposition of plastocyanin or cytochrome c553 complex

of PSI causing decline in the rate of electron transport mediated by PSI and PSII (Allakhverdiev and Murata, 2008). In the present study the results showed that ETR in all cultivars was decreased at the 100 mM NaCl concentration. This decrease was more pronounced in sensitive cultivars. Similar conclusions have been demonstrated for tomato (Zribi et al., 2009) and *Brassica* species (Jamil et al., 2014).

Comparison of the cultivars showed that 'SPADI' and 'Wanado' followed by 'ACX 270' had the highest values, while 'PaxRGH' followed by 'Radin' and 'Ethem' lowest values in qp under salinity stress. In contrast, NPQ was not affected significantly by the salt treatments. Our result shows that the treatment of pepper cultivars with 100 mM NaCl caused a decrease in the qp correlated with STI. The photochemical quenching (qp) represents the number of photons used by photochemical reactions per the absorbed photon numbers as well as the PSII ability to reduce the primary electron acceptor PQ_A under salinity (Hanachi et al., 2014). The qp can help to protect the photosynthetic apparatus by shifting electrons to O_2 under salinity stress (Ort and Baker, 2002). These results are in agreement with those reported by Zribi et al. (2009) and Jamil et al. (2014).

Determination of ion contents

The results showed that NaCl treatments significantly increased Na^+ content and decreased K^+ content, K^+/Na^+ and Ca^{++}/Na^+ ratio of pepper cultivars respectively (Table 3). 'Paramo', 'ACX 270' and 'Serenio' showed lower, while 'PaxRGH', 'Exp. 4' and 'Exp. 10' higher values of Na^+ as compared with other cultivars. The highest values of K^+ , K^+/Na^+ and Ca^{++}/Na^+ were observed in 'Paramo', 'Efestis' and 'Serenio'. 'PaxRGH', 'Exp. 4' and 'Exp. 10' showed the lowest values of K^+ , K^+/Na^+ and Ca^{++}/Na^+ among all cultivars. Low concentrations of Na^+ were observed in leaves of control plants (results not showed). NaCl treatment amplified Na^+ contents in leaves in the all cultivars and decreased K^+ (significantly) and Ca^{++} content (not significantly). It seems that the decrease in potassium and calcium content is due to an antagonistic effect between sodium with potassium and calcium. The achieved result was in agreement with the work of Chartzoulakis and Klapaki (2000), Lycoskoufis et al. (2005), Niu et al. (2010), and Zhani et al. (2012). In parallel with Na^+ accumulation a decline in K^+ content, the K^+/Na^+ and Ca^{++}/Na^+ ratio was observed in all pepper cultivars (Table 3). Aktas et al. (2006) showed that potassium and calcium content, K^+/Na^+ and Ca^{++}/Na^+ ratio decreased due to salinity in sensitive pepper cultivars more than in tolerant cultivars. Zhani et al. (2012) suggested that K^+/Na^+ ratio has a potential value as selection criterion for salt tolerance. According to these results, it was concluded that cultivars 'Paramo', 'Serenio' and 'Efestis' were the most salt stress tolerant due to its less Na^+ absorption, more K^+ accumulation and higher K^+/Na^+ and Ca^{++}/Na^+ ratio compared with the other studied cultivars.

Bivariate Pearson correlations

We evaluated the correlation between chlorophyll fluorescence characteristics, leaf Na^+ , K^+ and Ca^{++} content and stress tolerance index (Table 2). Results showed a high correlation (≤ 0.6) between Fo/Fm, Fv/Fo, Fv/Fm, Fv/Fm diminishing, Φ_{exc} , Φ_{PSII} , ETR, qp, Na^+ , K^+ , K^+/Na^+ , Ca^{++}/Na^+ , and STI. Therefore, these characteristics could be considerable indicators for screening salt tolerance pepper cultivars.

Conclusion

To research the effect of the salinity stress on PSII, photochemistry was measured in twenty six pepper cultivars. In our study, the results showed that Fo/Fm, Fv/Fo, Fv/Fm, Φ_{exc} , Φ_{PSII} , ETR, and qp were affected significantly by salinity stress.

Chlorophyll fluorescence components at leaf scale can provide useful tools for non-destructive determination of plant tolerance under salt conditions. Based on the presented results, Fo/Fm, Fv/Fo, Fv/Fm, Fv/Fm diminishing, Φ_{exc} , Φ_{PSII} , ETR, and qp are sensitive indicators to salinity stress, thus are well suited for salinity stress detection. In addition, due to significant correlations observed between the STI and aforementioned traits, are good indicators for salinity tolerance screen. This parameter has a potential to be the effective and nondestructive tool to screen pepper cultivars for salt tolerance. In addition, 'Paramo' was tolerant and 'PaxRGH' susceptible among studied cultivars.

Abbreviations

Fo, Fm, Fv: minimum, maximum and variable fluorescence in dark-adapted state, Fo/Fm: the quantum yield baseline, Fv/Fo: the potential photosynthetic activity, Fv/Fm: maximal photochemical yield of PSII, Φ_{exc} : the efficiency of excitation energy capture by open PSII reaction centers, Φ_{PSII} : effective quantum yield of PSII photochemistry, ETR: the electron transport rate, qp: photochemical quenching coefficient, NPQ: non-photochemical quenching.

References

- Al-aghaby K., Zhu Z., Shi Q. (2005). Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress, *Journal of Plant Nutrition* 27 (12): 2101-2115
- Allakhverdiev S. I., Murata N. (2008). Salt stress inhibits photosystems II and I in cyanobacteria. *Photosynthesis Research* 98: 529-539.
- Allakhverdiev S. I., Sakamoto A., Nishiyama Y., Inaba M., Murata N. (2000). Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiology* 123: 1047-1056.
- Aktas H., Abak K., Cakmak I. (2006). Genetic variation in the response of pepper to salinity. *Scientia Horticulturae* 110: 260-266.
- Azuma R., N. I., Nakayama N., Suwa R., Nguyen N. T., Mayoral J. A. L., Esaka M., Fujiyama H., Sane H. (2010). Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). *Scientia Horticulturae* 125: 171-178.
- Bacarin M. A., Deuner S., da Silva F. S. P., Cassol D., Silva, D. M. (2011). Chlorophyll a fluorescence as indicative of the salt stress on *Brassica napus* L. *Brazilian Journal of Plant Physiology* 23(4): 245-253.
- Baker N. R., Rosenqvist E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* 55 (403): 1607-1621.
- Chartzoulakis K., Klapaki G. (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. *Scientia Horticulturae* 86(3): 247-260.
- Chaves M. M., Costa J. M., Saibo N. J. M. (2011). Recent advances in photosynthesis under drought and salinity. *Advances in Botanical Research* 57: 49-104.

- Corney H. J., Sasse J. M., Ades P. K. (2003). Assessment of salt tolerance in eucalypts using chlorophyll fluorescence attributes. *New Forests* 26: 233–246.
- De Lucena C. C., De Siqueira D. L., Martinez H. E. P., Cecon P. R. (2012). Salt stress change chlorophyll fluorescence in mango. *Revista Brasileira de Fruticultura* 34(4): 1245-1255.
- Del Amor F. M., Cuadra-Crespo P. (2012). Plant growth-promoting bacteria as a tool to improve salinity tolerance in sweet pepper. *Functional Plant Biology* 39: 82–90.
- Fernandez, G. C. J. (1992). Effective selection criteria for assessing plant stress tolerance. In: Kuo, C.G. (ed.) *Adaptation of food crops to temperature and water stress*. Proc. Int. Symp., Taipei, Taiwan. 13- 18 Aug. 1992. Publ. no. 93-410. Asian Vegetable Res. and Dev. Center, Shanhua, Taiwan. P 257-270,
- Hanachi S., Van Labeke M. C., Mehouchi T. (2014). Application of chlorophyll fluorescence to screen eggplant (*Solanum melongena* L.) cultivars for salt tolerance. *Photosynthetica* 52 (1): 57-62.
- Jamil M., Rehman S. U., Rha E. S. (2014). Response of growth, PSII photochemistry and chlorophyll content to salt stress in four *Brassica* species. *Life Science Journal* 11(3): 139-145.
- Jiang Q., Roche D., Monaco T., Hole D. (2006). Stomatal conductance is a key parameter to assess limitations to photosynthesis and growth potential in barley genotypes. *Plant Biology* 8(4): 515-521.
- Kanwal H., Ashraf M., Shahbaz M. (2011). Assessment of salt tolerance of some newly developed and candidate wheat (*Triticum aestivum* L) cultivars using gas exchange and chlorophyll fluorescence attributes. *Pakistanian journal of Botany* 43: 2693–2699.
- Kaouther Z., Mariem B. F., Fardaous M., Cherif H. (2012). Impact of salt stress (NaCl) on growth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (*Capsicum frutescens* L.). *Journal of Stress Physiology and Biochemistry* 8(4): 236-252.
- Lee G., Carrow R. N., Duncan R. R. (2004). Photosynthetic responses to salinity stress of halophytic seashore paspalum ecotypes. *Plant Science* 166: 1417–1425.
- Li R., Guo P., Baum M., Grande S., Ceccarelli S. (2006). Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agricultural Science in China* 5: 751-757.
- Li G., Wanb S., Zhoua J., Yanga Z., Qina P. (2010). Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Industrial Crops and Products* 31: 13–19.
- Lichtenthaler H. K. (1987). Chlorophyll fluorescence during the autumnal chlorophyll breakdown. *Journal of Plant Physiology* 131:101–110.
- Lycoskoufis I. H., Savvas D., Mavrogianopoulos G. (2005). Growth, gas exchange and nutrient status in pepper (*Capsicum annum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. *Scientia Horticulturae* 106: 147–161.
- Megdiche W., Hessini K., Gharbi F., Jaleel C. A., Ksouri R., Abdelly C. (2008). Photosynthesis and photosystem-2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. *Photosynthetica* 46: 410–419.
- Mittal S., Kumari N., Sharma V. (2012). Differential response of salt stress on *Brassica juncea*: Photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. *Plant Physiology and Biochemistry* 54: 17-26.
- Moradi F., Ismail A. M. (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS scavenging system to salt stress during seedling and reproductive stages in rice. *Annals of Botany* 99: 1161-1173.
- Munns R., James R. A. (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil* 253(1): 201-218.
- Naumann J., Young D., Anderson J. (2007). Linking leaf optical properties to physiological responses for stress detection in coastal plant species. *Physiologia Plantarum* 131: 422-433.
- Niu G., Rodriguez D. S., Call E., Bosland P. W., Ulery A., Acosta E. (2010). Responses of eight chile peppers to saline water irrigation. *Scientia Horticulturae* 126(2): 215-222.
- Ort D. R., Baker N. B. (2002). A photoprotective role for O₂ as an alternative electron sink in photosynthesis? *Current Opinion in Plant Biology* 5: 193–198.
- Percival G. C., Fraser G. A., Oxenham G. (2003). Foliar Salt Tolerance of *Acer* genotypes using chlorophyll fluorescence. *Journal of Arboriculture* 29(2): 61-65.
- Rohacek K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* 40 (1): 13-29.
- Shu S., Guo S. R., Sun J., Yuan L. Y. (2012). Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. *Physiologia Plantarum* 146: 285–296.
- Stepien P., Klbus G. (2006). Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biologia Plantarum* 50: 610–616.
- Sudhir P., Murthy S. D. S. (2004). Effects of salt stress on basic processes of photosynthesis. *Photosynthetica*, 42: 481-486.
- Wani A. S., Ahmad A., Hayat S., Fariduddin Q. (2013). Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of *Brassica juncea*. *Saudi Journal of Biological Sciences* 20: 183–193.
- Wu X. X., Ding H. D., Zhu Z. W., Yang S. J., Zha D. S. (2012). Effects of 24-epibrassinolide on photosynthesis of eggplant (*Solanum melongena* L.) seedlings under salt stress. *African Journal of Biotechnology* 11 (35): 8665–8671.
- Zhani K., Elouer M. A., Aloui H., Hannachi C. (2012). Selection of a salt tolerant Tunisian cultivar of chili pepper (*Capsicum frutescens*). *EurAsian Journal of BioSciences* 6: 47- 59.
- Zribi L., Fatma G., Fatma R., Salwa R., Hassan N., Nejib R. M. (2009). Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato *Solanum lycopersicum* (variety Rio Grande). *Scientia Horticulturae* 120: 367–372.