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COMPARISON OF TWO MOST COMMONLY USED SCREENING TECHNIQUES FOR SALT TOLERANCE IN CROP PLANTS

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Abstract

A comparison to test the consistencies and discrepancies of two most commonly used screening techniques (soil and hydroponic) for salt tolerance in crop plants were examined. In this regard, physiological responses of four sunflower genotypes i.e. Hysun-33, Hysun-39 (known moderately salt tolerant) S.28111 and SF0049 (unreported genotypes) growing in soil filled pots and hydroponics were observed. This study was carried out under greenhouse condition in complete randomize design. plants were treated with 75, 125 and 175 mM NaCl concentrations. Stress was applied to the plants in aliquot levels 30 DAS and plants were harvested 44 DAS for further analysis. Some photosynthetic traits; chlorophyll fluorescence, chlorophyll contents, stomatal conductance and relative water content were analysed. Plants grown in a pot experiment had higher PI_{abs}, stomatal conductance, photosynthetic pigments and hence higher plant fresh weight as compared to hydroponic plants. Despite differences in techniques, results revealed that both systems have shown almost similar trends in physiological traits of tested genotypes under saline environment. A significant correlation in performance index (PI_{abs}), RWC, stomatal conductance, proline contents and total carotenoids was found. Physiological assessment and their reliability of salt tolerance in crop plants using both experimental systems were discussed.

Key words: Hydroponic, Pot, Physiological performance, Salt stress, Screening, Sunflower.

Introduction

Salinity is a serious threat among the other abiotic stress to agricultural productivity. Salinity may cause 30% land loss within next fifteen years and up to 50% by the end of 2050 (Wang *et al*., 2003). This problem is also very serious in Pakistan because 10 million hector areas are affected by salinity (Hussain *et al*., 2013) out of which 60% is saline sodic. Low irrigation water has contributed a lot to salt accumulation in Pakistan compared to other countries. The growth and yield of crop are reduced when this salt accumulated in the soil. In this scenario, the choice becomes limited and the grower must go for screening the tolerant plants which are suitable for the saline conditions.

Sunflower (*Helianthus annuus* L.) is a 4th largest oilseed crop in the world. It is an important source of edible oil and as a source of food for animal consumption. Worldwide seed production is of 33.3 million tons that provide 8.5% of the total world volume (Saensee *et al*., 2012). Sunflower crop is considered as moderately salt tolerant. However, its growth and seed production are affected under slightly higher saline condition. The possible solution to the salinity is the selection of suitable genotypes of crop plants that utilize sufficiently high salt concentration and exhibit higher yield in the field. Screening of plant genotypes in the field is rather difficult, therefore no suitable sunflower genotypes have been recommended for the saline environments. Crops are often screened in nutrient solution by adding the appropriate amount of salt to develop the desired salt stress. Much of the work on developing screening criteria for the salinity tolerance in plants has been done using hydroponics or soil filled pots (Aslam *et al*., 1993; Munns *et al*., 2002; Munns & James, 2003; Genc *et al*., 2007; Klados & Tzortzakis, 2014). However, the lack of reliable screening technique and poor understanding of salinity and environmental interaction are still a great problem to find out the salt tolerant genotypes. Selection of salt tolerant genotypes appears as an arduous and desperate task. Therefore, it is important to find out the correlation between the most prevalent existing screening methods and develop a relatively quick, economical and non-invasive screening method that is useful for the assessment of salt tolerant genotypes. Several techniques have been used in past for rapid and effective screening against salt stress (Khalid *et al*., 2002; El-Hendawy *et al*., 2009) but there is need to evaluate the similarities and differences between these techniques. The objective of this investigation was to screen the sunflower genotypes under salt stress environments using pot and hydroponic systems. To achieve the objectives, some physiological and biochemical changes like; relative water contents (RWC), chlorophyll contents, chlorophyll fluorescence, stomatal conductance, and proline contents were examined and compared in both screening techniques under saline and non-saline environments.

Material and Methods

Plant material and experimental operation: The **e**xperiment was performed in green house located at stress physiology phenomic centre, department of Botany, University of Karachi. Four sunflower genotypes were screened for their tolerance to salt stress in two separate experiments providing identical environmental conditions. Seeds of *Helianthus annuus* (cv. S.28111, Hysun-33, Hysun-39, and SF0049) were collected from seed certification department government of Pakistan. S.28111 and SF0049 were new sunflower genotypes originated by Arysta Life Science and FMC Corporation respectively. Hysun-33 and Hysun-39 were known moderately stress tolerant. Seeds were surface sterilized with 3% of sodium hypo-chloride for five minutes. Seeds were germinated in Seedling tray at green house at a temperature of 26-28 ± 4ºC, 60-70% humidity and a photoperiod of 14/10 hours (day/night). Light intensity varied from 250-400 µmol photon m^{-2} s^{-1}.

Hydroponic experiment: One week after germination when third and fourth leaves began to appear, the seedlings of each sunflower cultivar were transplanted to 50 ml plastic tube (having holes at the bottom) with the help of foam wrapped at the junction of shoot and root, suspended 14 L iron tank. The seedlings were left for three days in ½ strength Hoagland's solution and renewed the cultural solution every fourth day. Three NaCl concentrations of 75mM, 125mM, and 175mM was used as the salinity treatments. Salt stress imposed in three increments while control plants were treated with only Hoagland's solution. Salinity treatments were initiated after 30 days of sowing and lasted about 2 weeks. The pH of solution was maintained to 6.5 throughout the experiment with HCl or KOH.

Pot experiment: One week after germination when third and fourth leaves began to appear, the seedlings of each sunflower cultivars were transplanted into pots (15 × 18 cm) filled with 1.5 Kg of air dried soil. The soil type used for this trial was sandy loam. Five seeds per pot were sown and twelve days after emergence the pots were thinned to three plants each pot. Salinity treatments were initiated after 30 days of germination and lasted about 2 weeks. Control plants were irrigated with tape water and soil was kept humid (around 60-80% water holding capacity) during experiment. For salt stress, NaCl concentrations (75mM, 125mM 175mM) was achieved at aliquot levels and then soil was kept humid (60-80% water holding capacity) throughout experimental period. Soil moisture was measured with soil sensor, SDI-12 hydra probe II (Stevens water, USA). Experiments were arranged in randomized block design having four replicates of each treatment. To avoid the salt shock, salinity treatments were increased gradually in both experiments which may represent field environment. After two weeks of stress treatments, plant biomass, physiological measurements and biochemical quantification were investigated.

Chlorophyll fluorescence and stomatal conductance: The chlorophyll fluorescence and stomatal conductance (g_s) was recorded on the youngest fully expanded leaf between 9:00 AM – 11:00 AM using chlorophyll fluorescence meter (OS-30p+, Opti-Science, USA) and a Steady state diffusion porometer, Model SC-1 (Decagon devices) respectively. For chlorophyll fluorescence leaves were dark adapted using clips, after dark adaptation, the chlorophyll fluorescence parameters like; dark adapted quantum yield (Fv/Fm), which reflects the maximum photochemical efficiency of PSII, and photosynthetic performance index on absorption basis (PI_{abs}) were recorded (Maxwell & Johnson, 2000). Afterwards, plants were harvested and biomass production, relative water content, and some biochemical were quantified.

Photosynthetic pigments: 0.5g leaf samples were used to extract photosynthetic pigments in 10mlmethanol (96%) and centrifuge at 4000rpm for 10min. Chlorophyll ('a' and 'b') and carotenoid contents were determined (Lichtenthaler, 1987). The absorbance was read at 666, 653 and 470nm using spectrophotometer. The concentrations of photosynthetic pigments were calculated according to Lichtenthaler & Wellburn (1985):

$$Chl \text{ "a"} = 15.65\, A_{666} - 7.340\, A_{653}$$

$$Chl \text{ "b"} = 27.05\, A_{653} - 11.21\, A_{666}$$

$$\text{Carotenoids} = 1000\, A_{470} - 2.860\, Ca - 129.2\, \frac{Cb}{245}$$

where; A = Absorbance, Ca = Chlorophyll 'a' and Cb = Chlorophyll 'b'

Relative water content: Relative water contents were determined from youngest fully expanded leaf discs. Four small leaf discs were taken for each treatment and weight immediately after harvesting to obtained fresh weight (FW) and then each sample was placed in 90 mm air tight Petri-dish containing distilled water. The hydrated discs were taken out of the water after 4 hours and their surface was dried quickly and lightly with tissue paper and immediately weight to obtain their turgid weight (TW). Lastly, sample was dried for 24 h at 80^0C to determine dry weight (DW). RWC was calculated by the following formula with slight modification and expressed in percent (Smart & Bingham, 1974):

$$RWC = FW - \frac{DW}{TW} - DW \times 100$$

Proline analysis: Proline contents were estimated as per method described by Bates *et al.* (1973). 500mg leaves (fresh) were homogenized in 10ml of sulpho-salicylic acid (3% w/v) and extract was centrifuged @ 3500 rpm for 10 minutes. Then, supernatant (2ml) was mixed with acid ninhydrin (2ml) and glacial acetic acid (2ml). Samples were incubated in water bath for 1 hour at 95^0C. Afterwards reaction mixture was cooled and 4 ml of toluene was added at room temperature to the solution and shake for 20 seconds with vortex mixer. Chromophore that containing toluene was aspired and absorbance was read at 520 nm against toluene blank. Proline concentration on fresh weight basis was determined from standard curve and calculated as following:

Statistical analysis: Statistical analysis of the collected data was computed using Duncan's multiple range test ($p \leq 0.05$) and analysis of variance with the help of the personal computer software packages IBM SPSS Statistics (version 20). To test the differences among mean value Duncan's test were expressed on bar graph as alphabets. Correlation coefficients between pairs of physiological traits of salt treated sunflower plants were performed by Pearson's correlation analysis.

$$\mu moles\ proline\ g-1\ FW = (\mu g\ proline / ml\ x\ ml\ toulene) / 115.5\ \mu g / \mu mol) / (g\ sample) / 5$$

Results

Hydroponic experiment

Biomass: Salt stress noticeably suppresses the total fresh weights (TFW) of sunflower genotypes and they were significantly varied in response to 175 mM NaCl stress (Table 1). The total fresh weight was higher in S.28111 under salt stress as compared with other genotypes whereas Hysun-39 had lowest fresh weight under salt stress environment. The mean values showed that genotype S.28111 had highest fresh weight whereas Hysun has lowest varietal mean in hydroponic medium under control and treatments (Table 1). It is apparent that genotypes S.28111 and SF0049 had higher fresh weight. All the genotypes showed significant difference under control and saline environments. A greater reduction in biomass was observed in both Hysun genotypes, while S.28111 and SF0049 showed little reduction in biomass production.

Root/shoot ratio: In this experiment genotypes differed significantly for their root/shoot ratio. Genotype S.28111 had lower while SF0049 higher in root/shoot ratio than the other genotypes (Fig. 1). Root/shoot ratio increased with the intensity of salt concentrations in all genotypes, but a non-significant difference was detected only in Hysun-33 under 125 mM and 175 mM NaCl stress. Our results showed that SF0049 had maximum root/shoot ratio under salt stress.

RWC: A significant difference of salt stress was noted on relative water contents of all four sunflower genotypes (Fig. 1). The Hysun-33 differ significantly among the tested genotypes in saline condition. SF0049 had higher RWC in 175 mM NaCl as compared to other genotypes. The hydroponic mean value also showed that SF0049 has greater RWC as compared to all three genotypes and Hysun-33 had lowest hydroponic mean (Table 1).

Proline: Salt stress substantially increased the free proline contents in leaf of sunflower genotypes. However, the genotypes studied in these experiments were differed significantly in their proline contents. Hysun-33 and Hysun-39 had considerably higher accumulation of proline contents whereas lowest in S.28111 in saline condition (Fig. 1). The proline contents in the youngest fully expanded leaf increase 5.92, 5.7, 5.8 and 7.44 folds in S.28111, Hysun-33, Hysun-39 and SF0049 respectively under 175 mM NaCl concentration compared to control.

Pigments: Salt stress significantly affected the concentrations of Chl 'a', Chl 'b' and carotenoids (Fig. 2). The order of total chlorophyll reduction in tested sunflower genotypes under 175 mM NaCl compared to control were varied, for instance maximum reduction in Hysun-33 > Hysun-39 > SF0049 > S.28111 has minimum. Reduction in chlorophyll was highest in Hysun-33 (46%) under severe salt stress as compared to S.28111 in which 22% reduction was observed. Carotenoid contents were significantly increased in all the tested sunflower Genotypes (Fig. 2). Lowest carotenoid contents were observed in Hysun-39 and highest found in Husun-33. The carotenoid contents in leaf varied over 2.33-fold among the four genotypes under sever salt stress, ranging from 0.36 µg mg^{-1} in Hysun-39 to 0.84 µg mg^{-1} FW in Hysun-33 (Table 1).

Chlorophyll fluorescence: There were significant difference among Genotypes in terms of photosynthetic performance index (PI_{abs}) and non-significant difference was found in maximum quantum yield (Fv/Fm ratio). PI_{abs} was significantly decreased in salt stress (Fig. 3). Decline in PI_{abs} was greater in Hysun-33 compare to other cultivars. There were small differences among the treatments in photochemical quenching (qP). A non-significant difference was found in moderate salt stress and control in all cultivars. Data showed significant difference between control and 175 mM NaCl stress in all Genotypes (Fig. 3).

Stomatal conductance: The studied genotypes significantly differ in stomatal conductance. Data showed that Genotype Hysun-33 and Hysun-39 had highest stomatal conductance in control (Fig. 3). The stomatal conductance in sunflower genotypes was significantly decreased with salt stress intensity. Hysun-33 and Hysun-39 were highly affected under severe salt stress whereas S.28111 and SF0049 had lower reduction to their respective controls. However, in 175mM NaCl concentration; 24, 31, 35 and 44% reductions were observed in S.28111, SF0049, Hysun-33 and Hysun-39 respectively.

Pot experiment

Biomass: Resistant plants protect their growth performance under saline environment. Salt stress decreased the whole plant fresh weight (TFW) and cause decline in dry weight in sunflower Genotypes (Table 1). Hysun-39 and SF0049 responded with decreased fresh weights while S.28111 and Hysun-33 had higher fresh weight under severe salt stress.

Root/shoot ratio: Root/shoot ratio is used to evaluate the stress avoidance potential (Bush, 1995). Cultivars differed significantly for root/shoot ratio (Fig. 1). Root/shoot ratio was higher in S.28111 while lower in SF0049. Root/shoot ratio increased with the imposition of salt stress in sunflower genotypes but Hysun-33 has showed non-significant difference under 125 mM and 175 mM NaCl stress. Results showed that overall SF0049 had maximum root/shoot ratio under salt stress. It is observed that sunflower respond to salt stress by increasing the proportion of assimilate diverted to growth and increased their root/soot ratio and the volume of soil available to the plant. Increase root to shoot ratio may be due to different sensitivities of root and shoot to ABA or may be of greater osmotic adjustments in roots compared to shoots under stressful environment (Samarah *et al.*, 2004).

Table 1. Effect of salinity on physiological parameters of four genotypes of sunflower (*Helianthus annuus* L.) in pot and hydroponic (HP) medium.

	NaCl concentration	S.28111		Hysun-33		Hysun-39		SF0049		Salinity mean
		Pot	HP	Pot	HP	Pot	HP	Pot	HP	
PI_{abs}	Control	13.10	9.46	9.65	5.76	11.65	5.03	11.42	7.38	9.18
	75mM	11.57	8.76	7.24	5.06	11.30	4.88	9.57	7.23	8.20
	125mM	9.38	7.04	7.26	4.44	8.05	4.78	9.26	6.93	7.14
	175mM	8.76	5.70	5.92	3.21	7.43	4.06	8.25	6.04	6.17
	Pot & HP Mean	***10.70***	***7.74***	***7.52***	***4.62***	***9.61***	***4.69***	***9.62***	***6.89***	***7.67***
	Varietal mean	**9.22**		**6.07**		**7.15**		**8.26**		**7.67**
RWC (%)	Control	72.37	78.97	80.00	81.06	73.33	85.05	74.08	83.27	78.51
	75mM	66.47	76.02	65.89	74.95	63.07	74.61	63.08	76.07	70.02
	125mM	62.48	68.65	60.42	64.29	57.46	63.11	58.59	69.40	63.05
	175mM	56.47	61.84	46.82	55.81	49.40	58.81	53.69	64.09	55.87
	Pot & HP Mean	***64.45***	***71.37***	***63.28***	***69.03***	***60.82***	***70.39***	***62.36***	***73.21***	***66.86***
	Varietal mean	**67.91**		**66.15**		**65.60**		**67.78**		**66.86**
Stomatal conductance	Control	370.33	166.67	327.00	213.00	335.00	215.00	318.97	187.33	266.66
	75mM	305.33	147.33	240.33	192.67	290.67	165.00	262.30	161.67	220.66
	125mM	254.67	139.00	142.67	181.00	225.33	151.00	244.20	142.33	185.03
	175mM	235.00	126.00	105.37	137.20	180.67	121.33	206.80	130.00	155.30
	Pot & HP Mean	***291.33***	***144.75***	***203.84***	***180.97***	***257.92***	***163.08***	***258.07***	***155.33***	***206.91***
	Varietal mean	**218.04**		**192.40**		**210.50**		**206.70**		**206.91**
Total chlorophyll	Control	50.34	41.65	36.63	41.29	34.09	45.20	43.93	41.53	41.83
	75mM	50.13	37.03	36.21	38.63	33.59	39.00	43.95	39.78	39.79
	125mM	47.38	34.55	33.49	35.75	30.29	34.37	42.15	39.01	37.12
	175mM	42.96	32.66	29.51	22.21	30.06	29.71	37.77	31.96	32.10
	Pot & HP Mean	***47.70***	***36.47***	***33.96***	***34.47***	***32.01***	***37.07***	***41.95***	***38.07***	***37.71***
	Varietal mean	**42.09**		**34.21**		**34.54**		**40.01**		**37.71**
Carotenoid contents	Control	0.50	0.17	1.67	0.08	1.59	0.08	1.18	0.08	0.67
	75mM	0.62	0.40	1.70	0.15	1.64	0.12	1.18	0.22	0.75
	125mM	1.12	0.57	1.96	0.34	1.73	0.19	1.46	0.26	0.95
	175mM	1.21	0.71	1.96	0.84	1.78	0.36	1.62	0.65	1.14
	Pot & HP Mean	***0.86***	***0.46***	***1.82***	***0.35***	***1.68***	***0.19***	***1.36***	***0.30***	***0.88***
	Varietal mean	**0.66**		**1.09**		**0.94**		**0.83**		**0.88**
Total fresh weight	Control	3.34	2.54	3.09	2.27	2.85	2.36	2.80	2.15	2.68
	75mM	3.04	2.43	2.82	1.75	2.53	1.80	2.80	1.94	2.39
	125mM	2.44	2.17	2.56	1.45	2.38	1.39	2.28	1.82	2.06
	175mM	1.90	1.83	2.13	1.27	1.92	1.01	2.00	1.51	1.70
	Pot & HP Mean	***2.68***	***2.24***	***2.65***	***1.68***	***2.42***	***1.64***	***2.47***	***1.855***	***2.21***
	Varietal mean	**2.46**		**2.17**		**2.03**		**2.16**		**2.20**
Free proline	Control	3.20	1.61	2.91	2.59	2.38	2.07	3.07	1.49	2.41
	75mM	4.75	3.20	3.14	4.10	3.95	4.82	3.75	5.64	4.17
	125mM	6.25	5.56	4.87	10.81	5.43	7.55	4.15	7.92	6.57
	175mM	6.61	9.53	5.48	15.02	7.58	12.00	8.08	11.09	9.42
	Pot & HP Mean	***5.20***	***4.98***	***4.1***	***8.13***	***4.84***	***6.61***	***4.76***	***6.53***	***5.64***
	Varietal mean	**5.09**		**6.12**		**5.72**		**5.65**		**5.64**

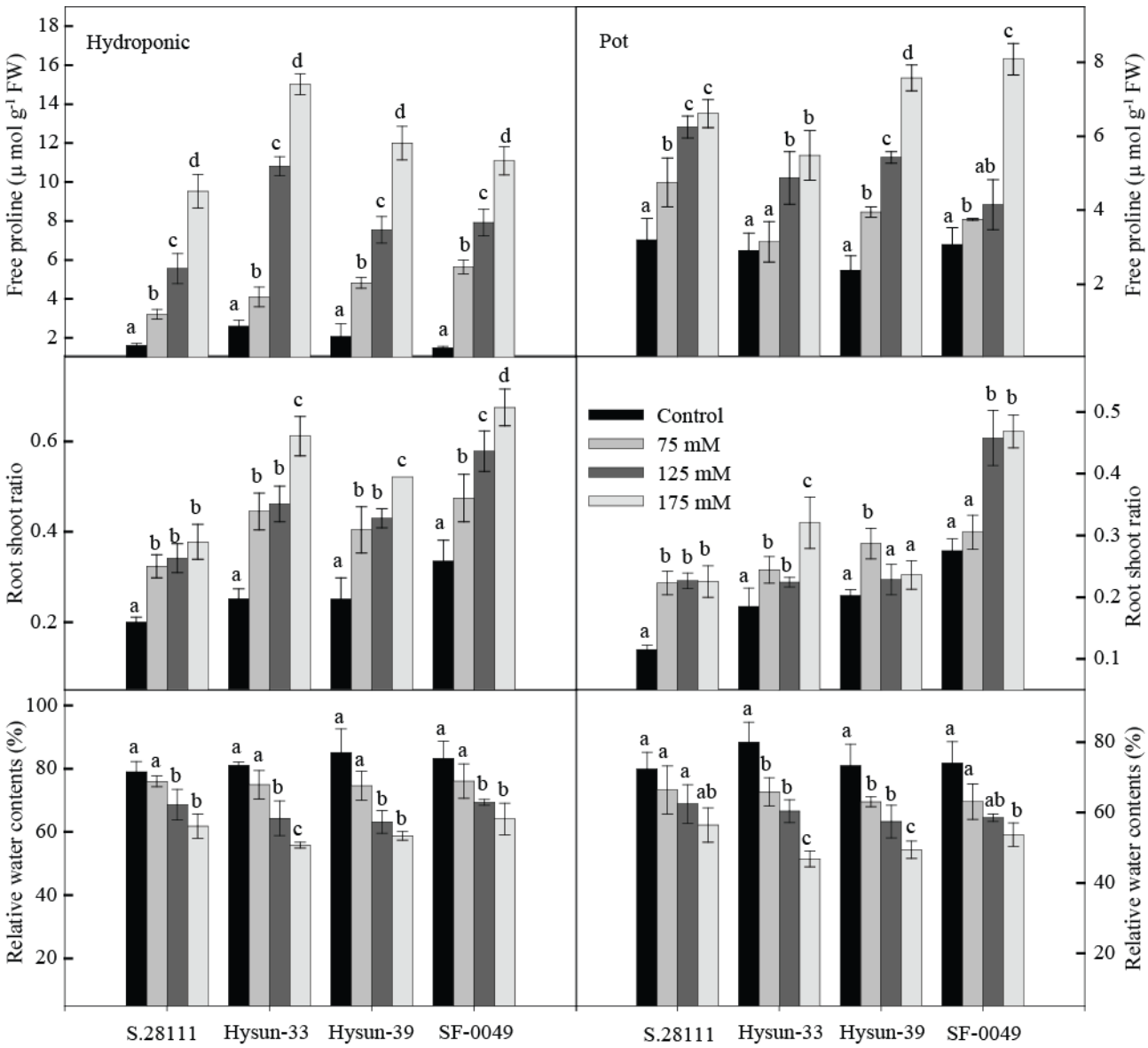

Fig. 1. Free proline contents, root/shoot ratio and relative water contents (RWC) of sunflower genotypes under salt stress environments in two different experiments. Vertical line on the bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at $p \leq 0.05$.

RWC: Relative water contents decreased significantly in all four genotypes due to salinity stress (Fig. 1). Cultivar Hysun-33 had highest RWC among the other cultivars in 175 mM salt concentration. In 75 and 125 mM NaCl concentrations there were no significant differences in RWC between genotypes. Hysun-33 and Husun-39 had significantly lower RWC than S.28111 and SF0049 under 175 mM NaCl concentration. It was lowest in Hsun-33 under salt stress. RWC was highest in S.28111 under severe salt condition.

Proline: Our results showed that salt stress significantly increased the proline contents (Fig. 1). Accumulation of proline contents in many plants species under salt and drought stress may be correlated with stress tolerance. The Pot experiment showed higher proline accumulation in SF0049 upon the exposure to 175 mM NaCl concentration. The increase in proline was more pronounced in intense salt stress (175 mM) than mild salt stress (75 mM).

Chlorophyll contents: A non-significant effect of mild and moderate salt stress (75 mM and 125 mM NaCl concentrations) was examined on the concentration of chlorophyll 'a' and 'b' in sunflower genotypes (Fig. 2). Decline in chlorophyll contents was significant under intense salt stress (175 mM NaCl) compared to control. The higher total chlorophyll contents were found in S.28111 and SF0049 whereas moderately tolerant Hysun-33 and Hysun-39 had lower chlorophyll contents under 175mM stress. However, the highest decline in total chlorophyll was observed in Hysun-33 (19%). Similarly, chlorophyll 'a' was also significantly reduced in both Hysun genotypes when they were exposed to 175 mM NaCl stress. However, genotypes S.28111 had higher chlorophyll 'a' contents than the other cultivars. Imposition of salt stress environment increased total carotenoids in tested genotypes, particularly in Hysun-33 (Fig. 2). Total carotenoid contents were lowest in S.28111 under control and salt stress as well.

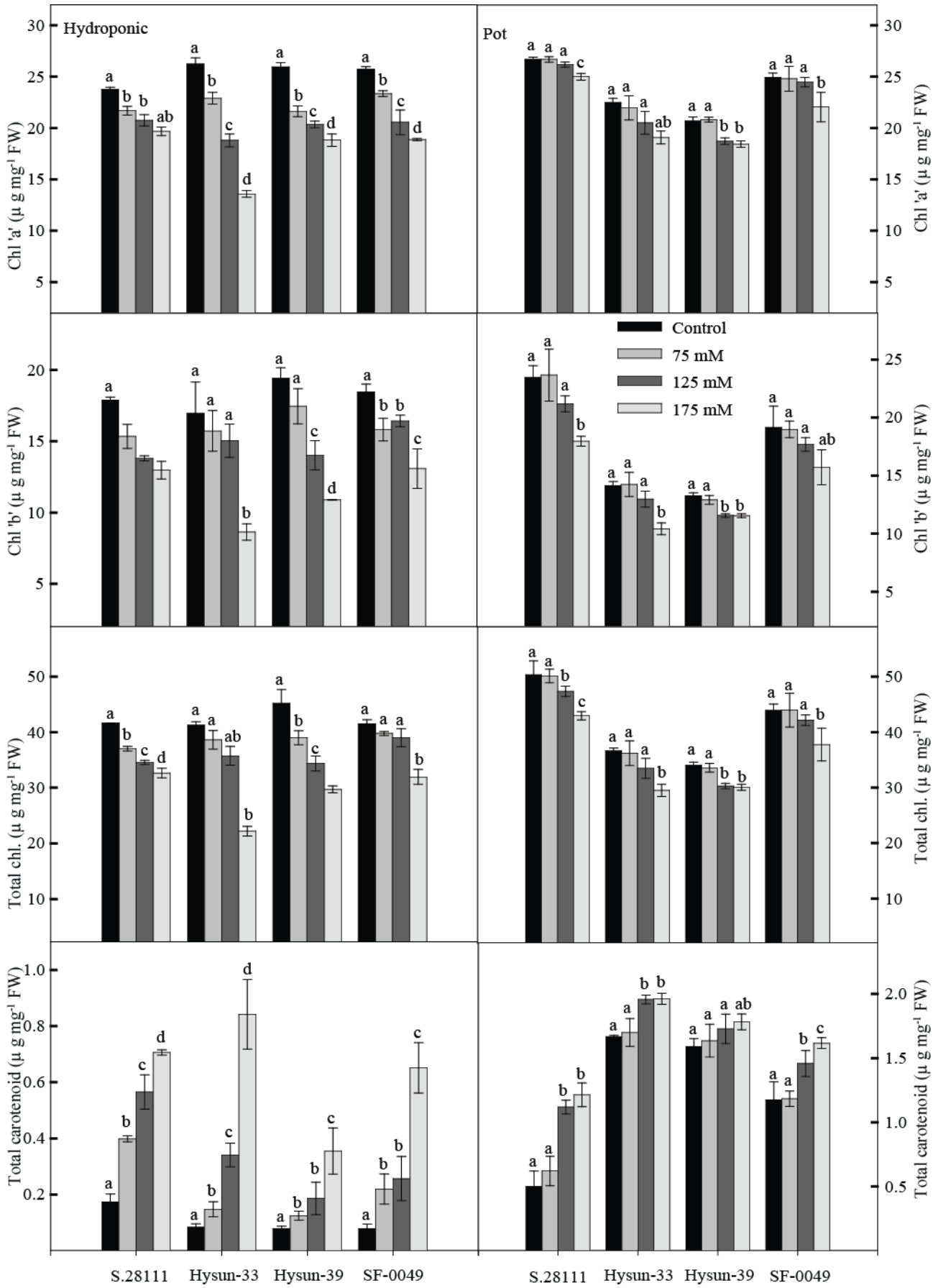

Fig. 2. Chlorophyll a, Chlorophyll b, total Chlorophyll and carotenoid contents of sunflower genotypes under salt stress environments in two different experiments. Vertical line on the bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at $p \leq 0.05$.

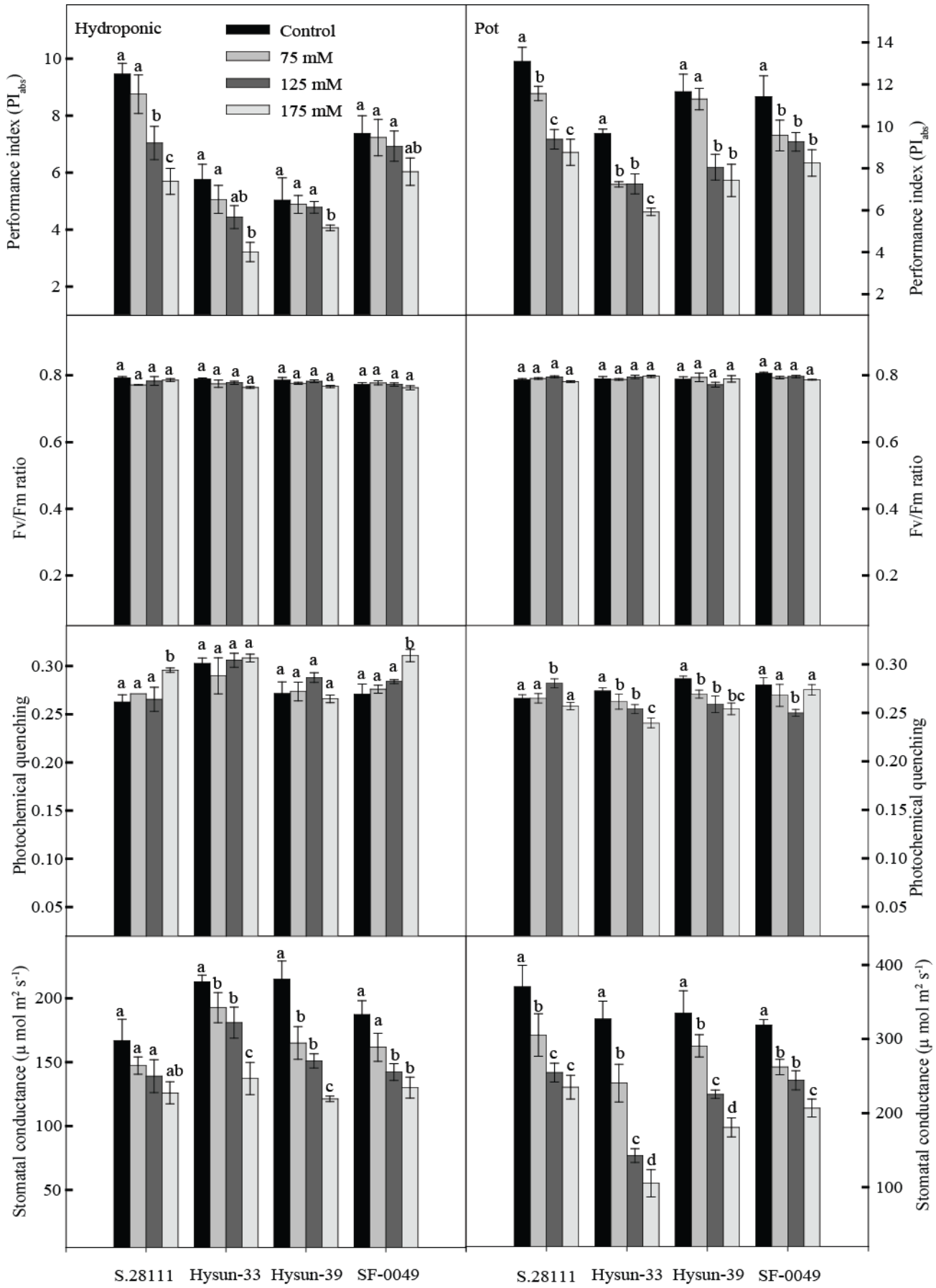

Fig. 3. Photosynthetic performance index (PI_{abs}), Dark adapted quantum yield (Fv/Fm ratio), photochemical quenching (qP) and stomatal conductance of sunflower genotypes under salt stress environments in two different experiments. Vertical line on the bar represents mean standard error (±). Similar alphabet on the error showed t-test non-significant at $p \leq 0.05$.

Chlorophyll fluorescence: Imposition of salt stress on tested sunflower genotypes didn't affect photochemical efficiency (PS II measured as Fv/Fm) in pot experiment. A very small difference in photochemical quenching was observed in tested sunflower cultivar when they were exposed to salt stress compared to control plants. Hysun-33 and SF0049 showed higher decline in qP under intense salt stress. S.28111 had lowest decline in qP under 175 mM NaCl concentration. Sunflower genotypes exhibited substantial decrease in performance index (PI_{abs}) in salt stress as compared to control. Severe salt stress (175 mM NaCl) significantly reduced the PI_{abs} in all the cultivars as compared to control and 125 mM NaCl concentration (Fig. 3). However, highest decreased in PI_{abs} was shown in Hysun-33. The reduction in PI_{abs} observed to be 33, 38, 36 and 27% under 175 mM NaCl stress in S.28111, Hysun-33, Hysun-39 and SF0049 genotypes respectively.

Stomatal conductance: The stomatal conductance of all the genotypes decreased continuously with increased salt concentrations i.e. 75 mM, 125 mM, 175 mM NaCl. The genotypes Hysun-33 and Hysun-39 had significantly greater stomatal conductance than S.28111 and SF0049. Compared to control, stomatal conductance was reduced to 68 and 46% in Hysun-33 and Hysun-39 under 175 mM concentration respectively. The lowest decline in terms of percentage was found in SF0049 (35%) whereas S.28111 had 37% decline.

Comparison of both systems: The consistencies and discrepancies between the two mediums were studied and their correlation is presented in Table 1. The Physiological attributes in both systems were consistent and significantly correlated. Regardless of the imposition of salinity to the same degrees, based on molar concentrations (75, 125, 175 mM) of NaCl solution using hydroponics and soil medium, some of the variations in salt tolerance among the sunflower genotypes was found among both systems. However, screening in both systems pot and hydroponic experiments identified two salt tolerant genotypes S.28111 and SF0049. Our results showed similar trends (decreased or increased) in tested parameters but the inconsistent results were found in photochemical quenching, between pot and hydroponic experiments. Among the physiological assessments, higher stomatal conductance and PI_{abs} was found in pot experiment. The mean hydroponic and pot value (Table 1) showed that relative water contents were lower in the sunflower plants grown in pot compared to hydroponics, the difference was almost 08% in general. Significant correlation was found between pot and hydroponic experiments among these parameters, i.e. total fresh weight, PI_{abs}, RWC, stomatal conductance, leaf area, proline contents and total carotenoids. The ranking of four sunflower genotypes grown in two different experiments based on their growth and physiology in hydroponics culture was correlated with their ranking based on their salinity tolerance at three NaCl concentrations in pot experiment. Our results from both experiments showed that Hysun-33 was most affected genotype under sever salt stress compared to other three genotypes. Varietal mean (Table 1) showed that S.28111 showed promising results among other three cultivars in this investigation.

Discussion

Comparative physiological screening of sunflower genotypes under saline and non-saline environments in two most commonly used, i.e. soil filled pot and hydroponic mediums were conducted. Results showed that there was a significant difference in plant biomass among the two experiments. The overall health of plants can be assessed through root/shoot ratio and is used to evaluate the stress avoidance of a plant (Bush, 1995). Our results showed that RWC was higher in hydroponic experiment compared to pot experiment. A maximum decline in RWC was observed in (175 mM NaCl) while minimum was noted for salt free control environment (Fig. 1). Reduction in water contents may affect the physiological processes and alter the metabolism. Lower water potential in leaves caused decline in photosynthetic activity (Iyengar & Reddy, 1996). Tolerant genotypes increase their water use efficiency in stressful environment. It is suggested that sunflower plants increase their water use efficiency by producing greater biomass in pot experiment compared to hydroponic plans. The proline accumulation was more in hydroponic plants. Hysun-33 had lowest proline contents (5.4 µmole g^{-1} FW) under 175 mM salinity in pot experiment and highest (15.02 µmole g^{-1} FW) in hydroponic medium. This indicated that proline had inconsistent results in terms of concentration/accumulation in both systems whereas the trend was found to be similar (Fig. 1). The accumulation of proline as a compatible osmolytes, has been observed as a basic tactic for the protection and survival of plant genotypes under abiotic stress (Smirnoff & Cumbes, 1989; Hare & Cress, 1997; Alia *et al.,* 2001). However, Proline concentration increased in all the cultivars in both screening methods indicating tolerance strategy of sunflower to alleviate the adverse effects of salinity.

Results declared that chlorophyll contents were higher in pot experiments under 175 mM NaCl concentration. In both experiments, S.28111 was found to be tolerant genotype in terms of chlorophyll contents under salinity stress whereas Hysun-33 had lowest tolerance among the tested genotypes (Table 1). It has been reported that photosynthetic pigments decreased in crop plants under stressful environments (Akram & Ashraf, 2011; Tayyab *et al.,* 2016: Jan *et al.,* 2016). Under salinity, the chlorophyll biosynthesis is much more affected than the breakdown of chlorophyll contents (Ashraf & Harris, 2013). Salt tolerant species can accumulate more chlorophyll contents than sensitive species (Alamgir & Ali, 1999). Hence, the accumulation of chlorophyll contents is proposed as the important biochemical indicator of salt tolerance in crop plants. Reduction in chlorophyll contents and increase in carotenoid contents plays an important role in photo-protection under salt stress (Ashraf & Harris, 2013). Salt stress significantly increased carotenoid contents in sunflower cultivars in salt treatments. The carotenoid contents were found to be higher in pot experiment (pot & HP mean, Table 1). Increased carotenoids under salt stress are necessary for photosynthesis, scavenging reactive oxygen species and play an important role as a forerunner in signalling during the growth and development of plant

(Davison *et al.,* 2002; Verma & Mishra, 2005). Carotenoid contents in leaf are of considerable attention for plant improvements i.e. breeding and genetic engineering (Li *et al.,* 2008) and it is suggested that carotenoids could be used as selection standards for salt stress tolerance among the plant genotypes.

It was observed that salt stress markedly affected the stomatal conductance in both screening methods. At 175 mM NaCl treatment, the stomatal conductance was worst affected in all cultivars. Salt stress reduced the gas exchange attributes as already demonstrated by Noreen *et al*. (2012). High salt concentration can lead to K^+ deficiency due to ion exchange mechanism. This could be the reason for the disturbance in stomatal conductance under salt stress. The higher stomatal conductance was found in pot experiment compared to hydroponic experiment.

The PI_{abs} was higher in pot experiment as compared to hydroponic experiment. However, Lowest PI_{abs} was found in Hysun-33 and highest value found in S.28111 in both the systems. Our results showed that higher salt concentrations significantly decreased the value of PI_{abs} in both systems and also confirmed that PI_{abs} was sensitive parameter to detect the differences between different salt concentrations and among the cultivars. A significant correlation was found in PI_{abs} among the two systems (Table 2). Chlorophyll fluorescence was used to quantify the effect of abiotic stress on photosynthesis and could be an excellent indicator of salt stress (Strauss *et al.,* 2006; Mehta *et al.,* 2010; Habib *et al.,* 2013). The higher PI_{abs} in pot experiment might be related to higher absorption of CO_2 through leaf stomata. Fv/Fm ratio is most extensively used photosystem II (PS II) efficiency indicator and it has been used as an indicator for stress tolerance and sensitivity in crop plants (Penuelas & Boada, 2003; Viljevac *et al.,* 2013; Siddiqui *et al.,* 2015). Our data demonstrated that Fv/Fm ratio was not significantly affected in sunflower genotypes under saline environment. The inhibition in photosynthesis during salt stress may be due to the reduced leaf water potential. Similar findings were shown in some other crop plants (Jamil *et al.,* 2007; Shahbaz *et al.,* 2013). In this investigation, photochemical quenching (qP) were inconsistent and not significantly correlated in two experiments (Table 2) under salt stress environment. Earlier it was reported that qP was increased in saline environment and photosynthesis remained unaffected in lower salinity (Kurban *et al.,* 1999). Salinity is known to restrain numerous physiological processes by decreasing stomatal conductance and reducing chlorophyll contents in plants (Soussi *et al.,* 1998; Siddiqui *et al*., 2014). It is suggested that better PI_{abs} and stomatal conductance in pot experiments might be the reason of higher biomass production compared to hydroponic plants.

Table 2. correlation coefficients (r) between pairs of performance index (PI), Fv/Fm ratio, Relative water contents (RWC), Stomatal conductance (SC), Leaf area (LA), Total chlorophyll (T-Chl), Carotenoids (Car), Proline, photochemical quenching, root/shoot ratio (RSR), and total fresh weight (TFW) of salts stressed sunflower plants grown in two different environments.

		Traits from pot experiment									
		PI	**Fv/Fm**	**RWC**	**SC**	**LA**	**T-chl**	**Car**	**Proline**	**RSR**	**TFW**
Traits from Hydroponic experiment	PI	0.840**	0.500*	0.710**	0.275	0.842**	0.49	-0.741**	-0.785**	-0.754**	0.803**
	FvFm		-0.101	0.277	0.207	0.127	0.075	0.034	-0.125	0.150	0.192
	RWC			0.938**	0.827**	0.876**	0.557*	-0.495	-0.926**	-0.743**	0.843**
	SC				0.273	0.875**	0.306	-0.585*	-0.820**	-0.846**	0.844**
	LA					0.712**	0.675**	-0.582*	-0.773**	-0.432	0.604*
	T.Chl						-0.127	-0.348	-0.533*	-0.483	0.542*
	Car							0.500*	0.582*	0.585*	-0.621*
	Proline								0.757**	0.496	-0.689**
	RSR									0.838**	-0.596*
	TFW										0.866**

**Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

It has been reported that the response of plant's early developmental stage under saline condition affect the productivity in field condition (Munns & James, 2003; Willenborg *et al*., 2005). Thus, plants screened at seedling stage for salinity tolerance by using hydroponic and pots could show considerable salinity tolerance at lateral growth stages. At seedling growth stage, root/shoot ratio, chlorophyll contents, performance index, stomatal conductance can be good parameters for screening against saline environments. The tolerance or sensitivity may differ according to the type of species and cultural medium in which trial has been taken. In this experiment, both techniques showed almost similar trends in tested traits and can be used for the screening of sunflower cultivars for salinity tolerance. However, each technique has its own advantages and disadvantages. To manage the salt stress, plants often showed those physiological variations that aim to increase their water use efficiency which empowered plants to become tolerant. The study related to the understanding of the mechanisms of salt stress affects photosynthesis may provide useful tools to improve the growth of crop plants and future crop engineering. Results from this kind of studies can be a better source for the plant biologists and crop breeders

that are involved in the development of stress tolerant crop plants. Further molecular work is needed to evaluate the molecular events in salt stress of this screened material. Field study will verify the use of screening techniques and potential use of sunflower cultivars.

Screening at seedling stage in pot and hydroponic is a convenient and reliable method for the determination of differences with respect to salt stress tolerance because field screening is difficult where only limited number of genotypes can be handled. The sunflower genotypes showed almost similar trend in salt tolerance while using both screening methods. This indicates that both techniques are effective for the assessment of salt tolerant crop plants. The present study also suggested that PI_{abs}, RWC, Stomatal conductance, photosynthetic pigment concentration, and free proline were the reliable parameters for salinity tolerance related studies in both techniques. These parameters are quick and easy to measure. Further, field trials of selected plants under salt stress environments would be helpful to verify these screening techniques in both systems.

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