

EVALUATION OF BARLEY (*HORDEUM VULGARE* L.) GERMPLASM FOR HIGH FORAGE PRODUCTION UNDER SALT STRESS

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Abstract

To explore high biomass producing salt tolerant cultivars of a potential forage crop barley (*Hordeum vulgare* L.), 30-day old plants of 105 different accessions from different origin were subjected to saline and non-saline (control) conditions for 45 days. Salinity stress (150 mM NaCl) markedly suppressed plant growth (shoot and/or root fresh and dry weights), chlorophyll pigments (*a* and *b*), internal CO₂ concentration, stomatal conductance, rate of transpiration and photosynthesis, while a considerable salt-induced increase was observed in all fluorescence related attributes including efficiency of photosystem-II (F_v/F_m), co-efficient of non-photochemical quenching (QN), photochemical quenching (QP), and non-photochemical quenching (NPQ) in all 105 accessions of barley. The response of all 105 barley accessions to salt stress varied significantly for all the morpho-physiological attributes determined in the present study. Overall, on the basis of shoot and root dry weights, accessions, 4050, 4053, 4056, 4163, 4228, 4229, 4244, 4245, 4290, 4414, 4415, 4427, 4452, Mahali, Jesto, 4165, 4229, 4249, 4405, 4409, 4426, 4456, and Giza 123 were found superior while accessions, 4245, 4158, 4166, 4246, 4406, 4423, 4441, 4442 4447, 4453 and 4458 inferior under saline conditions.

Introduction

Salinity is one of the key environmental factors that adversely influence crop productivity in several regions of the world (Pakniyat & Armion, 2007; Saleem *et al.*, 2011, 2012; Ashraf & Foolad, 2013; Shahbaz & Ashraf, 2013). Despite exploiting a variety of approaches to enhance crop productivity on salt affected soils, progress has been slow due to the complex mechanism of salt tolerance at physiological, biochemical and molecular levels (Ashraf & Akram, 2009; Akram & Ashraf, 2013; Iqbal & Ashraf, 2013). Studies on the potential in crops such as cauliflower (Batool *et al.*, 2013), sunflower (Akram *et al.*, 2012), wheat (Kanwal *et al.*, 2011), radish (Bano *et al.*, 2012), rice (Shahbaz & Zia, 2011) and cotton (Shaheen *et al.*, 2012) has been assessed using plant and cell characteristics including leaf fluorescence, gaseous exchange parameters, accumulation of osmo-protectants and ion uptake, hormonal regulation and antioxidant defense system metabolism to assess markers for better growth and yield. However, variation in salt tolerance has been observed from species to species and even from cultivar to cultivar.

Cereals are cultivated in more or less every region of the world and their growth and yield reduces due to adverse environmental cues (Shahbaz & Ashraf, 2013). Of common cereal crops, barley is generally categorized salt tolerant although cultivars show significant variation (Witzel *et al.*, 2009). Salt tolerant barley can be grown on salt affected soils to ensure forage for livestock being reared in arid and semi-arid regions (Dadshani *et al.*, 2004; Todd, 2006). The development of new improved salt-tolerant barley lines either through screening available germplasm or through genetic engineering could improve profits by fully utilizing all the saline area that is not currently under cultivation. The present study was carried-out to evaluate high biomass producing barley accessions under saline conditions. For this purpose, a large set of 105 accessions of barley was grown under saline conditions to determine their degree of

salinity tolerance using growth and photosynthesis related attributes as selection criteria.

Materials and Methods

To explore high biomass producing salt tolerant cultivars of a potential forage crop barley (*Hordeum vulgare* L.), 105 accessions of this crop were subjected to saline stress under greenhouse conditions in the net-house of the University of Agriculture Faisalabad (UAF), Pakistan during November, 2011 to April, 2012. The detail of the origins of all accessions of barley (*Hordeum vulgare* L.) is given in Table 1.

The plastic pots were filled with 8 kg well washed river sand. A two factor (salt stress stress and cultivars) factorial experiment was arranged in a completely randomized design (CRD) with three replications. Healthy seeds (50 g) of each accession were water-soaked for 8 h before sowing. Of these presoaked seeds, 10 were sown in each pot and after 15 days of seed germination, three seedlings were maintained in each pot. During the experimentation, the day/night temperature cycle was 25/10°C, relative humidity 60% and the day length 11-12 h. After 30 days of plant growth, salt stress treatment was applied. Two liters of each treatment: control (only Hoagland's nutrient solution) and salt stress (Hoagland's nutrient solution + 150 mM NaCl) were applied to each pot. The first salinity treatment was completed in three consecutive days with an increment of 50 mM NaCl per day to minimize the salt shock. Thereafter, the respective treatment solutions were applied twice a week to the plants grown under non-saline and saline conditions. After 45 days of salt stress treatments, two plants per pot were carefully harvested and washed with water. Shoots and roots were separated and fresh weights recorded. Then, the separated plant parts both shoots and roots were dried in an oven at 70°C for 72 h and dry weights recorded. In addition, the data for the following physiological variables were obtained:

Table 1. Barley (*Hordeum vulgare* L.) accessions and their origin.

Accessions	Origin
4044, 4045, 4046, 4050, 4052, 4053, 4054, 4056, 4079, 4115, 4149, 4150, 4151, 4154, 4157, 4158, 4159, 4161, 4162, 4163, 4164, 4165, 4166, 4167, 4168, 4176, 4208, 4221, 4223, 4224, 4225, 4226, 4227, 4228, 4229, 4230, 4231, 4232, 4245, 4246, 4247, 4248, 4249, 4251, 4253, 4254, 4255, 4257, 4280, 4290	Pakistan
4405, 4406, 4407, 4408, 4409, 4410, 4411, 4412, 4413, 4414, 4415	Iran
4416, 4417, 4418, 4419	Cyprus
4420, 4421, 4422, 4423, 4424, 4425, 4426, 4427, 4428, 4430, 4434, 4435, 4436, 4437, 4438, 4439, 4440, 4441, 4442, 4443, 4444, 4446, 4447, 4448, 4449, 4450, 4451, 4452, 4453, 4454, 4456, 4457, 4458, 4459, 4460	Japan
Mahali, Giza-123, Giza-121, Sahrawi, Jesto	Saudi Arabia

Chlorophyll contents: The fresh leaves (500 mg) were extracted with 10 mL acetone (80% v/v), extract centrifuged at 8,000 x g for 10 min and the absorbance of the aliquot determined at 645 and 663 nm spectrophotometrically. Both chlorophyll *a* and *b* were calculated in mg g⁻¹ FW following Arnon (1949).

Photosynthetic attributes: Photosynthetic capacity (*A*), leaf internal CO₂ concentration (*C_i*), water-use efficiency (*A/E*), transpiration rate (*E*) and stomatal conductance (*g_s*) were recorded of all of the three replicates using a portable infrared gas analyzer (LCA-4; Analytical Development Company, Hoddesdon, England). The fully expanded youngest leaf from top of each plant was used for all these five measurements which were performed during full sunshine from 10.00 am to 12.00 noon. Following conditions were met during data recording: atmospheric pressure 99.9 kPa; ambient CO₂ concentration 352 μmol mol⁻¹; molar flow of air/unit leaf area 403.3 mmol m⁻² s⁻¹; PAR at leaf surface was maximum up to 1098 μmol m⁻² s⁻¹; water vapor pressure ranged from 7.0 to 8.8 mbar; temperature of leaf ranged from 34 to 39.1°C and ambient temperature from 31.2 to 35.9°C.

Chlorophyll fluorescence: Data for various chlorophyll fluorescence parameters were recorded following Strasser *et al.* (1995) using one plant from each of three replicates. Maximal quantum yield of PSII (*F_v/F_m*), photochemical quenching (*qP*), co-efficient of non-photochemical quenching (*qN*) and non-photochemical quenching (*NPQ*) were measured using a fluorescence meter (ADC BioScientific Ltd. Great Amwell Herts, UK).

Experimental design and statistical analysis: A two-way analysis of variance (ANOVA) of data for each parameter was computed using the MSTAT computer program (Anon., 1989).

Results

Application of 150 mM NaCl to the root growing medium induces prominent ($p \leq 0.001$) suppression in the shoot and root fresh and dry weights and shoot and root lengths of all 105 barley accessions (Figs. 1, 2) that has been confirmed using analysis of variance carried-out so far for determining the main effect (salt stress). The response of all 105 barley accessions to salt stress was identified as significant because the interaction term, accessions x salt stress was highly significant ($p \leq 0.001$). However, twelve accessions, 4050, 4053, 4056, 4167,

4248, 4290, 4415, 4436, 4451, 4452, Mahali and Jesto maintained their shoot fresh weight in the saline conditions. However, accessions, 4050, 4053, 4056, 4163, 4228, 4229, 4244, 4245, 4290, 4414, 4415, 4427, 4452 and Mahali excelled all accessions in terms of shoot dry weight under saline conditions. Of all barley accessions, 4221, 4223, 4224, 4414, 4418, 4436, 4447, 4453, and 4460 were the lowest while 4249, 4405, 4409, 4426, and Giza the highest in root fresh weight. Root dry weight was highest in accessions 4050, 4053, 4056, 4165, 4229, 4249, 4290, 4405, 4409, 4426, 4452, 4456, Jesto and Giza 123 under saline conditions. Maximum salt-induced reduction in root dry weight was observed in barley accessions 4245, 4246, 4406, 4441, 4447, and 4453, while all other barley accessions were found moderate in root dry weight under saline conditions (Fig. 2).

A significant ($p \leq 0.001$) reduction was observed in chlorophyll *a* and *b* pigments of all barley accessions under saline regime (Fig. 3). Of all barley accessions, 4246, 4248, 4257, 4426, 4428, 4446, 4449, 4452, 4457, 4458, and 4459 were found highest and 4044, 4054, 4159, 4161, 4166, 4167, 4221, 4226, 4231, 4411, 4442, 4443, Giza 123 and Sahrawi lowest in chlorophyll *a* contents. The response of all other barley accessions was moderate in chlorophyll *a* under salt stress. In terms of chlorophyll *b* contents, accessions 4248, 4257, 4422, 4426, 4434, and 4435 were the highest and 4167, 4226, 4228, 4231, 4248, 4410, 4411, 4413, 4441, 4442, 4447, 4448 and 4450 the lowest of all accessions under saline conditions (Fig. 3).

A marked ($p \leq 0.001$) salt-induced reduction was observed in photosynthetic and transpiration rates, stomatal conductance and sub-stomatal CO₂ concentration of all 105 barley accessions (Figs. 4, 5). The response of all barley accessions varied significantly ($p \leq 0.001$) under saline conditions in the all earlier mentioned gas-exchange characteristics. Of all barley accessions, the highest photosynthetic rate was observed in accessions 4150, 4158, 4227, 4163, 4245, 4444, 4458 followed by Jesto, while the lowest in accessions 4168, 4221, 4230 4442 and 4247 under saline conditions. Highest transpiration rate was observed in accessions 4151, 4163, 4415 and 4416, while the lowest in accessions 4221, 4224, 4448 and 4437 under saline conditions. Of all accessions, 4166, 4232, 4280, 4290, 4412, 4417 and 4428 were the highest, and 4247, 4228 and Jesto the lowest in stomatal conductance under saline conditions (Fig. 4). However, the response of all barley accessions slightly varied from each other in terms of sub-stomatal CO₂ concentration and *C_v/C_a* ratio under saline conditions (Fig. 5).

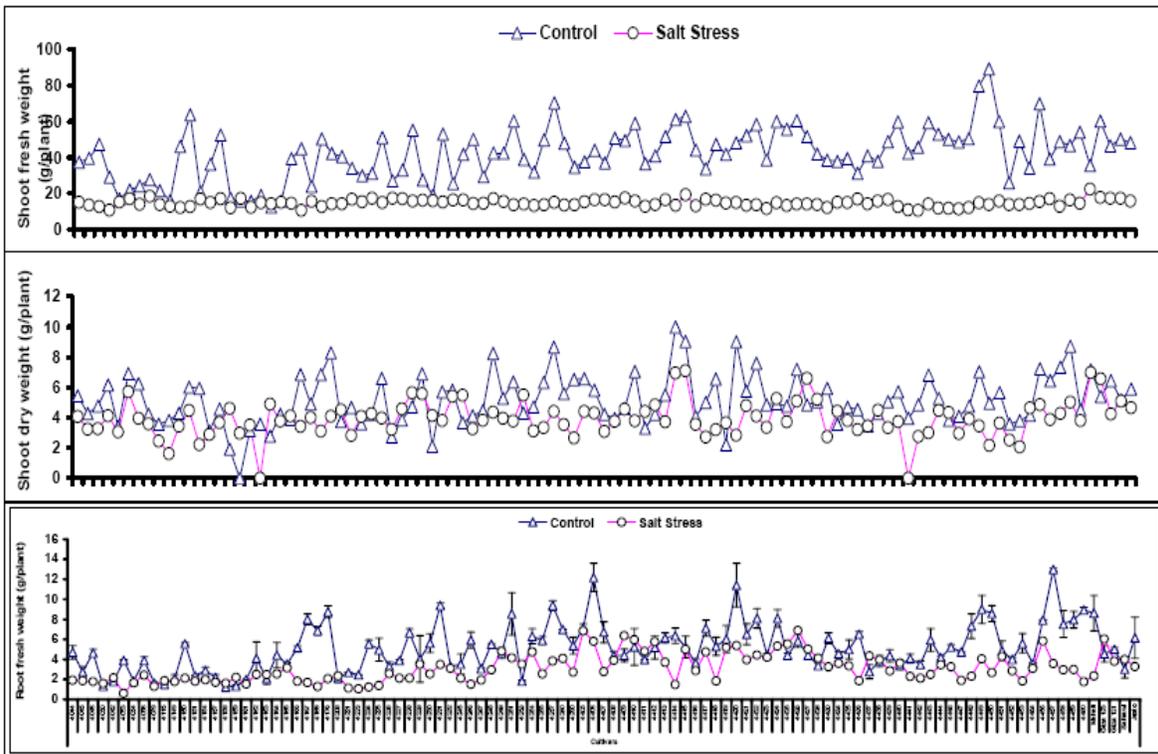


Fig. 1. Shoot fresh and dry weights and root fresh weight of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

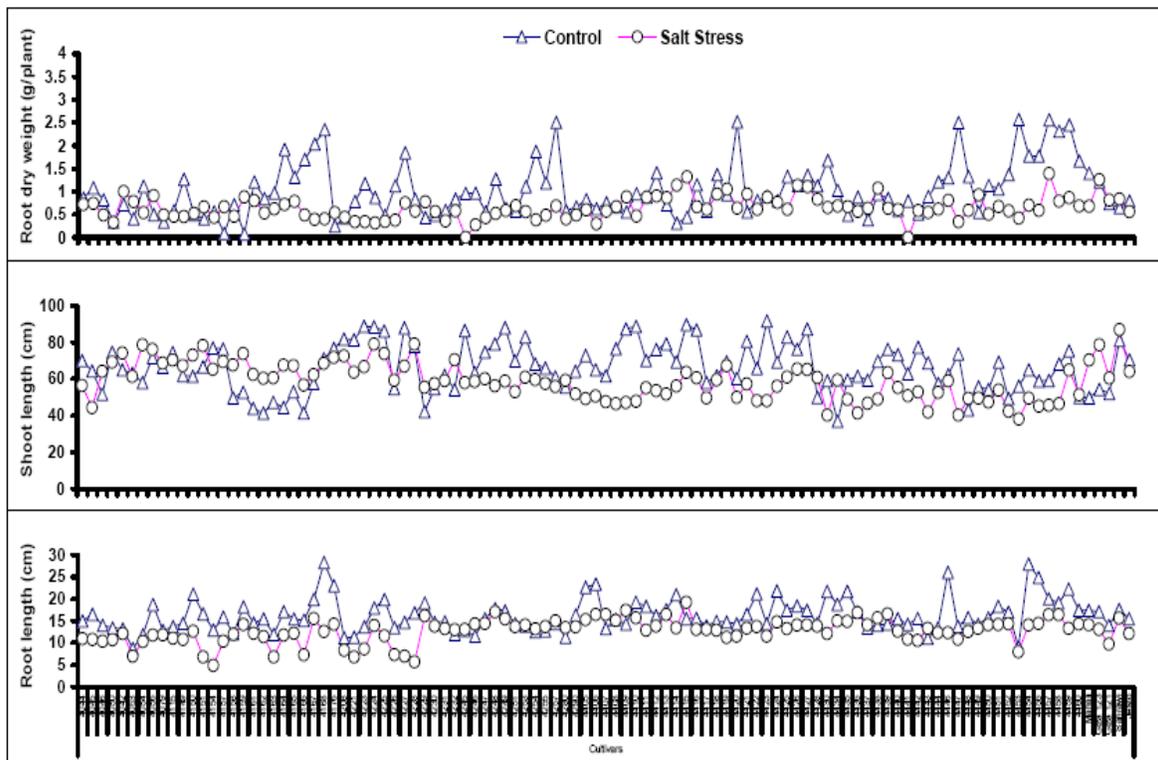


Fig. 2. Root dry weights and shoot and root lengths of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

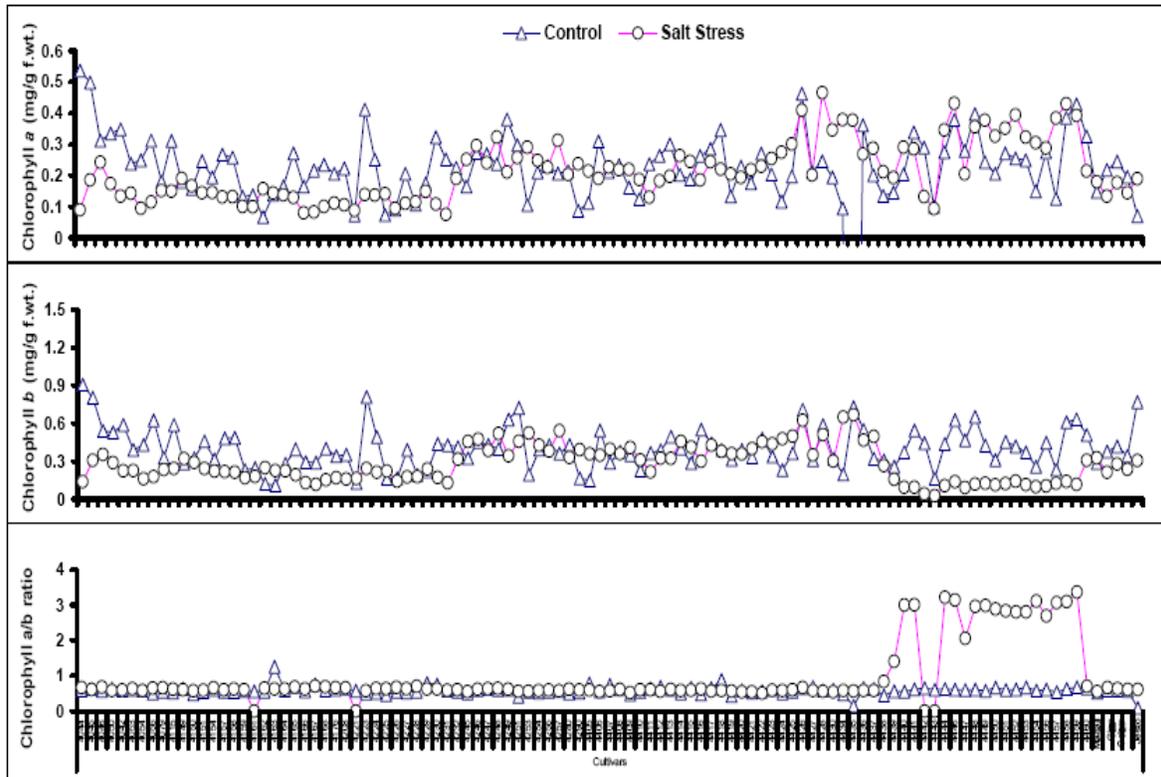


Fig. 3. Chlorophyll *a* and *b* contents and chlorophyll *a/b* ratio of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

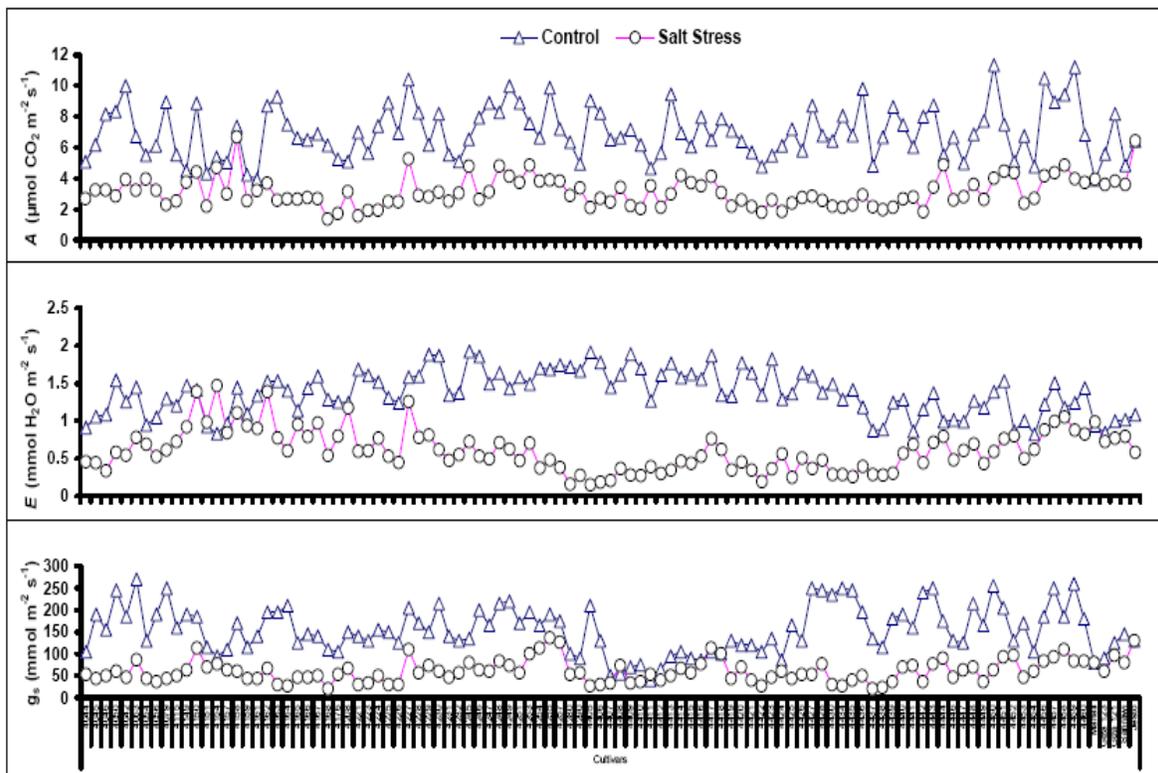


Fig. 4. Photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s) of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

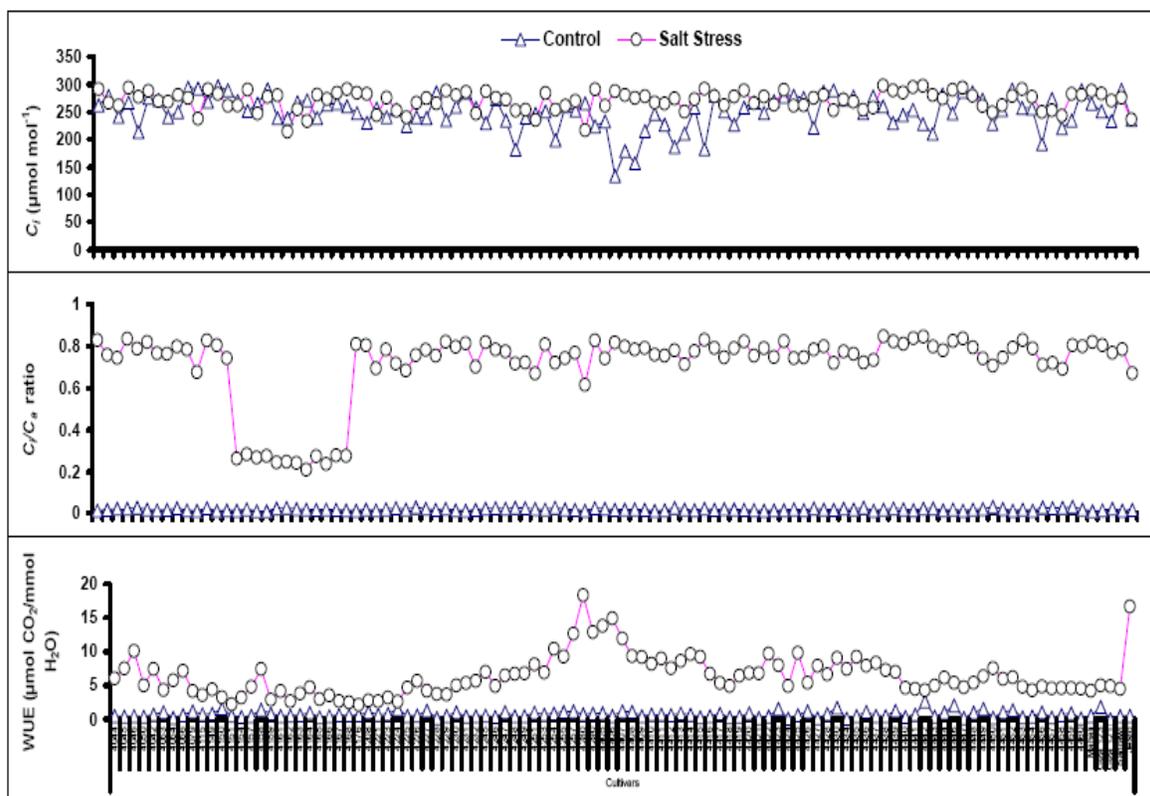


Fig. 5. Sub-stomatal CO₂ concentration, C_i/C_a ratio and water-use efficiency (WUE) of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

Table 2. Analyses of variance (mean squares) of data for growth, chlorophyll pigments, photosynthetic attributes, and leaf fluorescence of 105 accessions of barley (*Hordeum vulgare* L.) grown for 30 days under saline conditions.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Chlorophyll <i>a</i>
Cultivars (Cvs)	104	314.2***	7.178***	15.02***	0.624***	0.111***
Salinity (S)	1	120571.5***	233.5***	630.8***	21.403***	0.017ns
Cvs x S	104	329.7***	4.309***	8.100***	0.597***	0.125***
Error	420	33.81	1.168	1.996	0.076	0.0325
		Chlorophyll <i>b</i>	Chlorophyll <i>a/b</i> ratio	<i>A</i>	<i>E</i>	<i>g_s</i>
Cultivars (Cvs)	104	0.0704***	1.133***	6.766***	0.186***	5893.8***
Salinity (S)	1	2.363***	24.48***	2360.0***	92.077***	1422387.7***
Cvs x S	104	0.064***	1.075***	4.624***	0.297***	4745.2***
Error	420	0.0048	0.0422	0.793	0.0378	488.2
		C_i	C_i/C_a	WUE	<i>Fv/Fm</i>	<i>NPQ</i>
Cultivars (Cvs)	104	1856.9***	0.0209**	14.06***	0.028***	0.069***
Salinity (S)	1	66573.4***	80.95***	4995.5***	0.063**	0.451***
Cvs x S	104	1874.1***	0.021**	13.814***	0.035***	0.066***
Error	420	720.4	0.014	3.667	0.0077	0.0017
		<i>QN</i>	<i>QP</i>			
Cultivars (Cvs)	104	0.041***	10483.1ns			
Salinity (S)	1	0.019ns	4756581.5***			
Cvs x S	104	0.0401***	10460.3ns			
Error	420	0.0098	56018.4			

ns = Non-significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

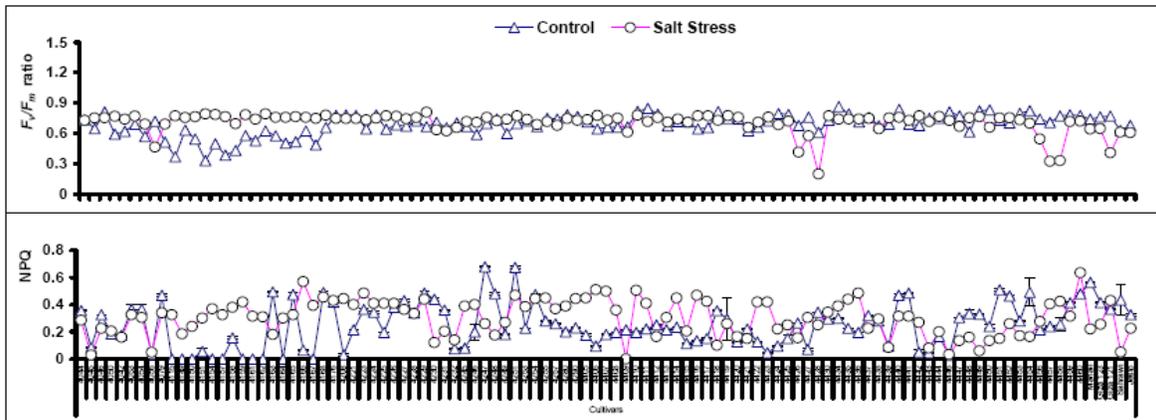


Fig. 6. Efficiency of photosystem-II (F_v/F_m), non-photochemical quenching (NPQ) and electron transport rate (ETR) of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

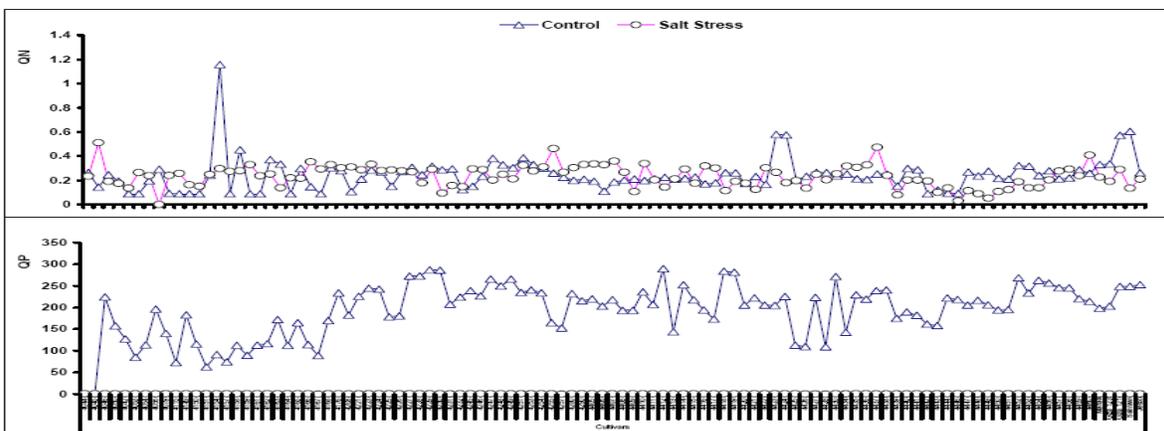


Fig. 7. QN and QP of 105 accessions of barley (*Hordeum vulgare* L.) grown under control and saline (150 mM NaCl) conditions.

In the present study, data recorded for different chlorophyll fluorescence attributes showed that imposition of salt stress significantly increased all fluorescence related attributes including the photochemical quenching (QP), efficiency of photosystem-II (F_v/F_m), and non-photochemical quenching (NPQ), while co-efficient of non-photochemical quenching (QN) remained same in all barley accessions (Table 2; Figs. 6, 7). Of all accessions, F_v/F_m was maximum in accessions 4162, 4229, 4251, 4406, and 4430, while minimal in accessions 4056, 4426, 4428, 4457, 4458 and Sahrawi. In addition, NPQ was highest in accessions 4159, 4166, 4223, 4229, 4251, 4406, 4436 and 4460, while lowest in accessions 4045, 4056, 4409, 4418, 4439, 4443, 4446, 4449 and Sahrawi under saline conditions. However, QP remained same in all barley accessions under saline and non-saline conditions.

Discussion

Soil and/or water salinity is one of the major environmental constraints affecting crop yield and productivity (Essa & Dawood, 2001; Saleem *et al.*, 2011, 2012; Akram & Ashraf, 2013; Batool *et al.*, 2013; Ashraf & Harris, 2013; Shahbaz & Ashraf, 2013). Different scientists have used different physiological/biochemical

attributes as useful selection criteria to assess inter-cultivar variation for salt tolerance (Ulfat *et al.*, 2007; Aghaei *et al.*, 2008; Sankar *et al.*, 2011). In the present study, 150 mM NaCl induced considerable reduction in shoot and root fresh and dry weights of all barley accessions but the accessions differed significantly. A number of studies have shown significant intra-and/or inter-specific genetic variation for different traits with differential salinity tolerance ability which provides a good opportunity for selecting tolerant varieties with enhanced yield or forage under saline regimes through different selection techniques (Ashraf & Akram, 2009; Sabir *et al.*, 2009; Munns *et al.*, 2010; Noreen *et al.*, 2012). In the present study, of all barley accessions 4050, 4053, 4050, 4163, 4228, 4229, 4244, 4245, 4290, 4414, 4415, 4427, 4452, Mahali, 4165, 4229, 4249, 4405, 4409, 4426, 4456, Jesto and Giza 123 were found superior while accessions, 4245, 4158, 4166, 4246, 4406, 4423, 4441, 4442 4447, 4453 and 4458 inferior in shoot and root dry weights under saline conditions.

In the present study, a marked reduction was observed in chlorophyll *a* and *b* contents of all barley accessions at high saline regimes. Of all barley accessions, 4246, 4248, 4257, 4426, 4428, 4446, 4449, 4452, 4457, 4458, and 4459 were found highest and 4044,

4054, 4159, 4161, 4166, 4167, 4221, 4226, 4231, 4411, 4442, 4443, Giza 123 and Sahrawi lowest in chlorophyll *a* contents. In terms of chlorophyll *b* contents, accessions 4248, 4257, 4422, 4426, 4434, and 4435 were the highest and 4167, 4226, 4228, 4231, 4248, 4410, 4411, 4413, 4441, 4442, 4447, 4448 and 4450 the lowest of all accessions under saline conditions. These results are analogous to those observed in 18 different accessions of proso millet by Sabir *et al.*, (2009). They found that salt stress significantly decreased leaf chlorophyll *a* and *b* contents. The decrease in chlorophyll contents in barley accessions can be interrelated with salt-induced enhanced activities of some key enzymes involved in chlorophyll degradation (Hernandez & Almansa, 2002; Ashraf & Harris, 2013), and/or disorder of proteins and chloroplast structure (Yang *et al.*, 2011), or process of chlorophyll biosynthesis (Akram & Ashraf, 2013).

It has already been well established that toxic level of salt causes a marked reduction in different gas exchange characteristics including photosynthetic and transpiration rates as well as stomatal conductance e.g., sunflower (Akram & Ashraf, 2011), rice (Tiwari *et al.*, 1997), wheat (Kanwal *et al.*, 2011), sorghum (Netondo *et al.*, 2004), safflower (Siddiqi *et al.*, 2009), cauliflower (Batool *et al.*, 2013), pea (Noreen *et al.*, 2012), and okra (Saleem *et al.*, 2011) under saline conditions. But, analogous to the results of the present study on barley, the degree of salt-induced reduction in different gas exchange characteristics varied significantly in different cultivars depending upon developmental stage of photosynthesizing tissues, nature/characteristics of cultivar, chlorophyll pigments, factors involved in stomatal/non-stomatal regulation which ultimately impair photosynthetic rate (Dubey, 2005; Sabir *et al.*, 2011; Saleem *et al.*, 2011; Ashraf & Harris, 2013).

Leaf fluorescence is one of the potential indicators for appraising salinity tolerance in plants as has already been observed by a number of researchers (Maxwell & Johnson, 2000; Zribi *et al.*, 2009; Mehta *et al.*, 2010; Saleem *et al.*, 2011). In the present study, data recorded for different chlorophyll fluorescence attributes showed that imposition of salt stress significantly increased all fluorescence related attributes including the efficiency of photosystem-II (F_v/F_m), non-photochemical quenching (NPQ), and photochemical quenching (QP), while co-efficient of non-photochemical quenching (QN) remained unchanged in all barley accessions. Of all accessions, F_v/F_m was maximal in accessions 4162, 4229, 4251, 4406, and 4430, while minimal in accessions 4056, 4426, 4428, 4457, 4458 and Sahrawi. Furthermore, NPQ was highest in accessions 4159, 4166, 4223, 4229, 4251, 4406, 4436 and 4460, while lowest in accessions 4045, 4056, 4409, 4418, 4439, 4443, 4446, 4449 and Sahrawi under saline conditions. These findings are in contrast to what recently Saleem *et al.*, (2011) have reported in two okra cultivars. Similarly, Mehta *et al.*, (2010) also reported a non-significant effect of salt stress on F_v/F_m in wheat and Zribi *et al.* (2009) in tomato on qP , NPQ , qN and F_v/F_m . However, parallel to our results, in a study with different cultivars of wheat, Kanwal *et al.*, (2011) found a significant salt-induced increase in leaf maximum chlorophyll fluorescence (F_m), maximum fluorescence at steady state (F_{ms}), and photochemical fluorescence quenching (qP).

In conclusion, salinity stress (150 mM NaCl) markedly suppressed plant growth (shoot and/or root fresh and dry weights), chlorophyll pigments (*a* and *b*), internal CO₂ concentration, stomatal conductance, rate of transpiration and photosynthesis, while a considerable salt-induced increase was observed in all fluorescence related attributes including F_v/F_m , QN, QP, and NPQ in all 105 accessions of barley. The response of all 105 barley accessions to salt stress varied significantly for all the morpho-physiological attributes determined in the present study. Overall, on the basis of shoot and root dry weights, accessions, 4050, 4053, 4056, 4163, 4228, 4229, 4244, 4245, 4290, 4414, 4415, 4427, 4452, Mahali, Jesto, 4165, 4229, 4249, 4405, 4409, 4426, 4456, and Giza 123 were found superior while accessions, 4245, 4158, 4166, 4246, 4406, 4423, 4441, 4442 4447, 4453 and 4458 inferior under saline conditions.

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