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SALINITY STRESS

Identification and Characterization of Salt Tolerance of Wheat Germplasm Using a Multivariable Screening Approach

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Abstract

Salinity is one of the major limitations to wheat production worldwide. This study was designed to evaluate the level of genetic variation among 150 internationally derived wheat genotypes for salinity tolerance at germination, seedling and adult plant stages, with the aim of identifying new genetic resources with desirable adaptation characteristics for breeding programmes and further genetic studies. In all the growth stages, genotype and salt treatment effects were observed. Salt stress caused 33 %, 51 % and 82 % reductions in germination vigor, seedling shoot dry matter and seed grain yield, respectively. The rate of root and shoot water loss due to salt stress exhibited significant negative correlation with shoot K^+, but not with shoot Na^+ and shoot K^+/Na^+ ratio. The genotypes showed a wide spectrum of response to salt stress across the growth stages; however, four genotypes, *Altay2000*, *14IWWYTIR-19* and *UZ-11CWA-8* (tolerant) and *Bobur* (sensitive), exhibited consistent responses to salinity across the three growth stages. The tolerant genotypes possessed better ability to maintain stable osmotic potential, low Na^+ accumulation, higher shoot K^+ concentrations, higher rates of PSII activity, maximal photochemical efficiency and lower non-photochemical quenching (NPQ), resulting in the significantly higher dry matter production observed under salt stress. The identified genotypes could be used as parents in breeding for new varieties with improved salt tolerance as well as in further genetic studies to uncover the genetic mechanisms governing salt stress response in wheat.

Introduction

The continuous salinization of arable land is a threat to global food security. Over 800 Mha of land are affected by salinity, which equates to more than 6 % of the world's total land area (FAO 2010) and affects more than 20 % of present-day agriculture (Mickelbart et al. 2015). Salinized soils extend over all the continents leading to annual losses of arable land to about 10 mha (Pessarakli and Szabolcs 1999). About 27.3 billion US dollars is spent annually to combat irrigation-induced salinity (Qadir et al. 2014). Salt stress, mainly due to accumulation of toxic Na^+ and Cl^- ions in plant tissues, causes osmotic and ionic stresses in plants. Wheat (*Triticum aestivum* L.) is one of most important crop plants worldwide with annual production of about 736 million metric tons (FAO 2015), but suffers significant grain yield losses due to soil salinity. Although, there are several strategies to increase wheat production in the salt-affected areas (such as leaching and drainage), the cultivation of tolerant genotypes is recognized as the most effective way to overcome this limitations. The prerequisite is the identification of wheat genotypes with proven wide adaptation under saline conditions. The cultivar, *Kharchia 65*, is one of the very few reputed donors of salt tolerance

(ST) in wheat and has been extensively used in breeding for ST cultivars globally (Chatrath et al. 2007). Thus, there is an urgent need to identify new sources of ST to broaden the gene base and to provide donor parents in locally adapted genetic backgrounds.

An imminent task is the efficient characterization of wheat plants for tolerance towards salt stress. The most valuable agronomical traits might serve as good surrogates to discriminate among genotypes under salt stress conditions. Munns and James (2003) consider biomass yield as a useful criterion because it permits the direct estimation of economic return under saline conditions. Moreover, it has been reported that shoot growth is more sensitive to salt stress than the root growth firstly, because the reduction in leaf area development relative to the root growth leads to a decrease in water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation of the salt concentration in the soil, and secondly, because the accumulation of Na^+ and/or Cl^- at toxic concentration levels affects the photosynthetic capacity resulting in less supply of carbohydrates to the young leaves, that further reduces the shoot growth rate (Munns and Tester 2008). The ST status of plants can be assessed as the percent biomass production in saline vs. control conditions (Genc et al. 2007) over a prolonged period of time. Selection of plants with high ST values would allow breeders to identify genotypes better adapted to the salinized arable lands. Screening for chlorophyll fluorescence characteristics has also gained increasingly interest in plant abiotic stress research. Salinity stress has negative impact on photosynthesis by inhibiting photosystem II (PSII) activity and destruction of chlorophyll pigments due to the accumulation of toxic ions. The relationship between the PSII operating efficiency and CO_2 assimilation in leaves allows fluorescence to be used to detect differences in the response of plants to environmental challenges and, consequently, to screen for tolerance to environmental stresses (Baker and Rosenqvist 2004).

Tolerance to salt stress is a complex biological phenomenon governed by several physiological and genetic factors, and it is growth stage specific (Haq et al. 2010). Little effort has been made so far to simultaneously characterize the wheat germplasm across different growth stages. Experiments carried out under controlled conditions were not exposed to those conditions that prevail in salt-affected soil such as spatial and temporal heterogeneity of soil chemical and physical properties, high diurnal temperature variations, low humidity and the presence of drought stress (Munns and James 2003). These could be one of the reasons why breeding for ST has not gained significant progress up till now. To meaningfully characterize the ST status of wheat genotypes, it is necessary to evaluate wheat response to salt stress across several developmental growth stages, with a view of identifying genotypes with desirable ST across all the growth stages. Access to new wheat genotypes with contrasting response to salt stress would allow for further characterization of the genetic mechanisms controlling ST in wheat.

The response of wheat to salt stress is genetically and physiologically controlled and may differ from one growth stage to another. Thus, a better understanding of these mechanisms and processes would help in the breeding programmes to enhance wheat production under salt stress. This study was designed to characterize salt tolerance in a set of winter and facultative wheat landraces, cultivars and elite breeding lines at the germination, seedling and mature plant field growth stages, with the aim to identify contrasting (salt-tolerant and salt-sensitive) genotypes for further genetic studies. The identified genotypes were evaluated for the effect of salinity on some key physiological traits including the cell membrane stability, osmotic potential, leaf chlorophyll fluorescence and dry matter production. The identified genotypes would be valuable resources for breeding programmes and scientific research towards better understanding of plant tolerance to salt stress.

Materials and Methods

Plant materials

A total of 150 winter and facultative wheat genotypes consist of advanced lines from the International Winter Wheat Improvement Program (IWWIP-Turkey/CIMMYT/ICARDA), cultivars from Turkey National Wheat Program (TNP) and cultivars from countries of the Central and Western Asia (CWA) region. To ensure that pure seeds were used and to minimize heterogeneity and contamination, multiplication step and cleaning were performed at the greenhouse of Crop Science and Resource conservation Institute (INRES), University of Bonn, Germany. The harvested seeds were then used for the ST evaluation at germination, seedling and mature growth stages.

Salt stress test

Salt-water flooding method as described by the Association of Official Seed Analysts (AOSA 2009) was adopted to evaluate the genotypes' germination ability under two salt types (NaCl and Na_2SO_4) and several concentrations: 100, 150, 200 mM for NaCl and 75, 100 mM for Na_2SO_4 plus control (without salt). Twenty-five seeds of each genotype, in three repetitions, were sown in 29 × 22.5 cm plastic transparent boxes containing blotting paper (ALBET Lab Science, Dassel, Germany) soaked in 75 ml of each salt treatment solution. Thereafter, the boxes were placed in a growth chamber with white fluorescent light (600 μmol m^{-2} s^{-1}; 14 h light/10 h dark) at 15 ± 1 °C, and relative humidity

of 65 ± 8 %. Ten days after sowing, the germination potentials of each genotype were determined with the scale from 0 to 9 as described by Mano et al. (1996).

The seedling stage screening was performed in a supported hydroponic system using the modified Hoagland solution as described by Tavakkoli et al. (2010). Four independent experiments designated E1, E2, E3 and E4, with three replications each, were conducted, in the greenhouse. In E1 (October–November, 2013) and E2 (February–March, 2014), the genotypes were screened with non-saline (control) and saline (100 mM NaCl) nutrient solution, while the solutions containing non-saline and saline (75 mM Na_2SO_4) were used to screen the genotypes during the E3 (April–May, 2014) and E4 (May–June, 2014) experiments. Supplementary Ca^{2+} as $CaCl_2$ was added to the saline nutrient solution in 20 : 1 molar ratio of NaCl or Na_2SO_4:$CaCl_2$ (Haq et al. 2010), to improve nutrient uptake and ameliorate the effects of salinity on the plant growth. In each experiment, comparisons were made between saline and non-saline conditions. The electrical conductivity EC values for control, 100 mM NaCl (+5.0 mM $CaCl_2$) and 75 mM Na_2SO_4 (+3.75 mM $CaCl_2$) solutions ranged as follows: 1.79–1.84, 11.89–12.54 and 12.44–13.68 dS m^{-1}, respectively.

A total of 156 cylindrical PVC tubes (4.5 cm diameter × 45 cm depth) were placed on each tub served by a separate tank containing 164 of nutrient solution at 75-min interval using EHEIM Universal-pump 1046 (EHEIM GmbH and Co, Deizisau, Germany). Prior to the transfer into the hydroponic system, seeds were exposed to 45 °C for 24 h to remove the inherent differential dormancy. The seeds were sown and germinated *in situ* in the tubes filled with Aquagran filter quartz, 2–3.15 mm (Euroquarz GmbH, Dorsten, Germany) with tap water. Three days after planting (DAP), salt treatments were introduced together with the nutrient solution. The salt application was carried out in an equal incremental basis for 3 days to avoid osmotic shock. The stress was continued for 22 days after the final salt stress level was reached. The nutrient solutions were changed every 7 days accompanied by adjustment of the pH to 5.5. Thereafter, the solution pH was monitored daily and adjusted to 6.0. The nutrient solution temperature varied from 14.1 to 21.7 °C. At harvest (28 DAP), plant shoots were cut off from the base and weighed to obtain the fresh shoot weight (FW). The harvested samples were dried at 55 °C for 10 days and weighed to obtain the dry shoot weight (DW). The relative shoot water loss (WL) due to salt stress was calculated on the basis of FW and DW in stress conditions (S) *vis-a-vis* the control conditions (C): $WL = [(FW_C - DW_C) - (FW_S - DW_S)]$.

The field trials were conducted under saline and non-saline soil conditions in three locations: Urgench (Uzbekistan) (41°32′60N and 60°37′60E, 91 m above sea level (masl) in 2011–2012; Karshi (Uzbekistan) (38°52′N and 65°48′E, 416 masl) in 2012–2013 and Dongying (China) (118°33′–119°20′E, 37°35′–38°12′N) in 2013–2014. The field layout for the trials in Uzbekistan was α-lattice design with three replications. Each plot measured 2 m^2 with different number of rows in different locations. In Dongying, seeds were sown in two rows (20 seeds per row) with plant spacing of 10 cm and the width is 1 m for each genotype. The soil chemical properties of all the field locations are presented in Table 1. At harvest, the seed grain yield (GY) was measured and recorded for both saline and non-saline fields.

Shoot Na^+ and K^+ concentration (%) determination

The 3rd leaf, stem and the remaining leaves (RLP) of each genotype were analysed for accumulated K^+ and Na^+ after 25 days of stress with 150 mM NaCl (+7.5 mM $CaCl_2$) in the hydroponics. Three replicates for each genotype were bulked and dried at 55 °C for 10 days. The concentrations of K^+ and Na^+ in the respective shoot parts were determined from 2-g grounded sample using atomic absorption spectrophotometer (type 2380; Perkin Elmer, Wellesley, MA, USA), and subsequently, the K^+/Na^+ ratios were calculated.

Salt tolerance estimation

The ST status of each genotype was determined for the measured traits across the growth stages as a ratio of trait mean value under salt stress to control condition (Genc et al. 2010). Thereafter, the 150 genotypes were ranked for each trait from the highest down to the lowest trait ST values. The overall ST ranking for each genotype was calculated as:

$$ST_{Overall} = \sum_{i}^{M} ST_{rankings}$$

where i is the ST estimates of genotypes for each measured traits and M is the number of measured traits across growth stages. Genotypes with extreme response to salt stress were identified as follows: tolerant (ST > 75th percentile) and sensitive (STg < 25th percentile).

Physiological analyses contrasting wheat genotypes

Two genotypes from each extreme were used to examine the effects of salt stress on some plant physiological and growth parameters such as leaf electrolyte leakage (EL), osmotic potential (ψ_π), chlorophyll a fluorescence (ChlF),

Table 1 Soil chemical properties of Karshi, Urgench and Dongying field locations

Soil chemical properties	Non-saline	Saline	Non-saline	Saline
	Karshi		Urgench	
Sodium concentration, dS m^{-1}	2.40–6.34	9.24–17.58	3.42–7.05	11.02–19.58
pH	7.67–8.00	7.59–7.81	6.76–8.03	7.54–7.83
Total dissolved solids (TDS), mg l^{-1}	1100–8400	2200–11 300	1200–1800	1400–10 500
Ca^{2+}, me l^{-1}	10.0–42.4	17.5–82.3	7.4–14.9	9.9–64.8
Mg^{2+}, me l^{-1}	4.9–22.2	7.4–30.4	2.5–0.5.0	2.5–40.1
Cl^-/SO_4^{2-}	0.14–1.55	0.16–0.58	0.20–2.13	0.07–1.48
Cl^-, me l^{-1}	n.a.	n.a.	2.9–13.8	3.9–66.1
Sodium absorption ratio (SAR)	n.a.	n.a.	0.95–5.62	0.48–13.82
Soil texture	Silty clay	Silty clay	Silty clay	Silty clay
	Dongying			
Sodium concentration, g kg^{-1}	1.9	4.3		
pH	7.58	8.06		
Organic, g kg^{-1}	17.86	9.96		
Phosphate, mg kg^{-1}	25.52	5.22		
Nitrate, mg kg^{-1}	72.02	34.04		
Potassium, mg kg^{-1}	258.04	693.15		
Water content, %	16.56	19.16		
Soil texture	Salic Fluvisols	Salic Fluvisols		

n.a. = not available (measured data were not consistent).

and shoot biomass production. The genotypes were grown under saline (150 mM NaCl) and non-saline conditions in the controlled conditions (Temperature: 20/15 °C; day length: 14 day/10 night hours) in the hydroponics.

Leaf electrolyte leakage (EL) was performed following the procedure outlined by Apostolova et al. (2008), with slight modifications. Freshly harvested leaves (0.4 g) were placed in tubes, containing 50 ml distilled water and kept for 4 h in a shaking water bath at 30 °C for measuring the initial conductivity (EC1). The final electrolyte conductivity (EC2) was measured after boiling the leaf samples for 20 min, upon equilibration at 30 °C. The rate of EL per minutes (EL_R) for each of the identified genotype was calculated as:

$$EL_R = (EC2 - EC1)/(0.4 \times 20)$$

Leaf osmotic potential (ψ_π) was determined as outlined by Pérez-López et al. (2009). The four youngest leaves were detached from each genotype under non-saline and stress conditions and frozen in liquid nitrogen to break the cell walls. The samples were then thawed, and sap was extracted by squeezing with garlic press and microcentrifugation at 15 000 × g for 5 min. The ψ_π of the extracts was obtained using an OSMOMAT 3000 (Gonotec GmbH, Berlin, Germany). The ψ_π readings were taken from six different plants for each genotype.

Chlorophyll a fluorescence (ChlF) of the leaf samples of an 8-week-old wheat plants under saline and non-saline conditions was measured using the FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic). The OJIP parameters were analysed as follows: (i) fluorescence fast transients (Fo = fluorescence intensity at 50 μs, Fj = fluorescence intensity at J-step (at 2 ms), Fi = fluorescence intensity at i-step (at 60 ms), Fm = maximal fluorescence intensity, Fv = maximal variable fluorescence); (ii) PSII efficiencies (Fo/Fm = non-photochemical loss in PSII, Fv/Fo = efficiency of the water-splitting complex on the donor side of PSII, Fv/Fm = quantum yield of PSII, PI (ABS) = performance index on absorption); and (iii) specific energy fluxes (ABS/RCm = effective antenna size of an active reaction centre (RC), TRo/RC = maximal trapping rate of PSII, ETo/RCm = electron transport in an active RC, DIo/RC = effective dissipation in an active RC). A total of 24 data points were taken for each genotype. The light intensity reaching the leaf was 3000 mol (photons) m^{-2} s^{-1}, which was sufficient to generate maximal fluorescence.

Statistical analysis

Analysis of variance (ANOVA) was carried out for the trait values by adopting the restricted maximum-likelihood (REML) model using the GENSTAT 16 program to account for both spatial and temporal differences in the seedling and field screening experiments. The GENSTAT procedure was used to estimate the unbiased estimates of variance components due to genotypic (σ^2_g) and environment (σ^2_e)

effects (O'Neill (2010)). Thereafter, the heritability (h^2) estimates for the traits were calculated as described by O'Neill (2010) and Gitonga et al. (2014) using the equation: $h^2 = (\sigma_g^2)/[\sigma_g^2 + \sigma_e^2/r]$, where r is the number of replications of each genotype.

Results

Phenotypic analysis

Compared to control, all treatments with different salinity concentrations reduced seed germination significantly. These reductions amounted to 7, 19 and 33 % for 100, 150 and 200 mM NaCl, respectively, and 14 and 24 % for 75 and 100 mM Na_2SO_4, respectively (Fig. 1a). The interactions of salt treatment and genotypes were significant in all the stress concentrations applied, except for 100 mM NaCl. The effect induced by NaCl stress was stronger than Na_2SO_4, when equal elemental Na^+ concentrations were considered. Significant genotype-by-treatment interactions were also observed in all salt treatments applied, except for 100 mM NaCl. The h^2 estimates were 0.58 under 200 mM NaCl and 0.85 under 100 mM NaCl, while the coefficient of variation (CV) increased from 3 to 8 % with the increase in the salt concentrations. The genotypes responded similarly to salt stress of equal elemental sodium (Na^+), as indicated by their comparable values of h^2 and CVs (Table 2).

In DW, genotypes responded differently to salt stress as well as between the salt treatments across the four experiments at seedling stage (Table 2). Salt stress significantly decreased the DW by 51 % in E2, 50.6 % in E4, 39 % in E3 and 18.6 % in E1 (Fig. 1b). Significant genotypes × treatment interactions were observed in E2 and E3. The h^2 estimates of DW in response to salt stress varied from 0.42 in E1 to 0.73 in E2 and the observed CV of ≥15 %.

Highly significant ($P < 0.01$) differences among genotypes, salt treatment and their interactions were detected at all the four field trials. Salt stress caused the highest yield reduction in Dongying (82.8 %) and the lowest in Karshi (10.1 %). The CV ranged from 16.25 % (Karshi) to 71.6 % (Dongying), while the highest h^2 estimates were observed in Urgench with 0.76 (Table 2).

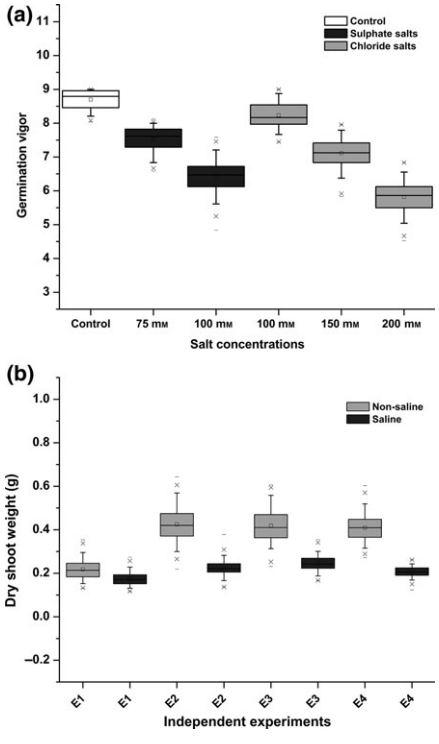

Fig. 1 Boxplot showing the effect of salt stress on germination vigour (a) and shoot dry mass (b) at germination and seedling stages, respectively. E1, E2, E3 and E4 are the four independent screening experiments conducted at the seedling stage in both control and salt stress conditions.

Correlations between ST estimates across growth stages

Significant positive and negative correlations occurred between some pairs of ST traits, based on genotype means, across the growth stages (Table 3). There were significant positive correlations between ST estimates at the germination, and the seedling growth stages, but no apparent significant trend was detected between ST traits for GY at the mature growth stage. Across the growth stages, the DW response to Na_2SO_4 salt increased with the decrease in the germination vigour in response to 100 mM Na_2SO_4, 150 mM NaCl and 200 mM NaCl salt stress. All the significant correlations observed between traits at germination and adult plant stages were negative. However, ST for DW estimated under NaCl salt stress showed negative and positive correlation with the ST for GY in Urgench and Dongying field trials, respectively.

Analysis of the shoot K^+ and Na^+ concentration

Highest K^+ accumulation was found in the stem and was significantly different from the amount in the 3rd leaf and/or RLP after 25 days of stress (Fig. 2). The K^+/Na^+ ratios in the 3rd leaf and stem were similar to each other and varied significantly from the K^+/Na^+ ratio in the RLP. The K^+ and Na^+ concentrations in the 3rd leaf, stem and RLP after 25 days of salt stress were positively correlated with each other. The shoot K^+/Na^+ ratio value was influenced stronger by the sodium than by potassium (Table 4). The shoot and root water losses due to the salt stresses applied were positively correlated with each other. Data indicated that the shoot K^+ was negatively correlated with root water loss,

Table 2 Analysis of ST traits at germination, seedling and maturity growth stages

Stage	Experiments	MS_G	MS_T	MS_{G*T}	CV_{ST}	h^2
	Germination score after 10 days of salt stress					
	100 mM NaCl	0.56**	48.61**	0.08^{ns}	2.87	0.85
	150 mM NaCl	0.55**	564.20**	0.20**	5.12	0.76
	200 mM NaCl	0.49**	1862.09**	0.36**	7.94	0.58
	75 mM Na_2SO_4	0.44**	307.59**	23.5**	4.23	0.8
Germination	100 mM Na_2SO_4	0.49**	1149.08**	0.40**	7.67	0.6
	Dry shoot weight (g per plant) after 25 days of salt stress					
	100 mM NaCl (E1)	716.74**	191.25**	91.01^{ns}	14.57	0.42
	100 mM NaCl (E2)	795.92**	3172.41**	357.04**	16.99	0.57
Seedling	75 mM Na_2SO_4 (E3)	583.50**	2104.01**	249.94**	14.74	0.63
	75 mM Na_2SO_4 (E4)	210.69*	1716.28**	125.23^{ns}	15.45	0.73
	Grain yield (t ha^{-1})					
	Urgench	1054.07**	494.71**	281.33**	23.07	0.76
Mature plants	Karshi	747.00**	188.77**	437.95**	16.25	0.57
	Dongying	217.13**	1791.53**	199.11*	71.6	0.23

Shown are as follows: MS – mean squares of 150 genotype (G) and treatment (T), CV – coefficient of variation and h^2 – heritability. All the experiments were replicated three times, and the number of stars indicates the significance level, *P < 0.05 and **P < 0.01.

shoot water loss (NaCl) and shoot water loss (Na_2SO_4); however, shoot Na^+ concentration and shoot K^+/Na^+ ratio did not correlate with the root/shoot water loss.

ST rankings of the germplasm

Based on the overall ST rankings (data not shown), 33, 39, 45 and 34 genotypes were considered as tolerant, moderately tolerant, moderately sensitive and sensitive to salt stress, respectively. The mean ST estimates ranged from 0.72 in tolerant genotypes to 0.63 in sensitive genotypes (Fig. 3a), while the overall mean was 0.67. The PC1 which accounted for 75.49 % of the observed variation in the cluster analysis plot clearly separated the 33 tolerant and 34 sensitive genotypes into two major groups (Fig. 3b). While tolerant genotypes showed higher capacity for K^+ uptake in the 3rd leaf and stem (in comparison with the population average) than the sensitive genotypes (Fig. 4a), the salt-sensitive genotypes had higher accumulated Na^+ than the salt-tolerant genotypes in the three shoot parts considered (Fig. 4b). These results translated to the significantly higher shoot K^+/Na^+ ratio observed in the tolerant genotypes compared to the sensitive ones (Fig. 4c). A total of 22 tolerant and 13 sensitive genotypes exhibited consistent response to salt stress in at least two growth stages (Table 5). Among them, three tolerant (Altay2000, *14IWWYTIR-19* and *UZ-11CWA-8)* and one sensitive (*Bobur)* genotypes were identified across the three growth stages.

Analysis of contrasting genotypes for membrane stability and osmotic potential

The data obtained from the measurements indicate that salt stress affected both the EL and $\psi\pi$ of the tolerant (*Altay2000* and *UZ-11CWA-8*) and sensitive (*UZ-11CWA-24* and *Bobur*) genotypes (Fig. 5). The amount of electrolytes leaked from the membranes of the sensitive genotypes was much higher than that observed in the

Table 3 Pearson correlation coefficients among ST estimates of the genotype mean across the three growth stages

Traits	1	2	3	4	5	6	7	8	9	10
$^1G_{75\ mM\ Na2SO4}$	1									
$^2G_{100\ mM\ Na2SO4}$	0.517**	1								
$^3G_{100\ mM\ NaCl}$	0.283**	0.188*	1							
$^4G_{150\ mM\ NaCl}$	0.495**	0.516**	0.426**	1						
$^5G_{200\ mM\ NaCl}$	0.563**	0.554**	0.242**	0.528**	1					
$^6DSW_{NaCl}$	−0.009	−0.013	0.04	0.038	0.006	1				
$^7DSW_{Na2SO4}$	−0.101	−0.163*	−0.024	−0.211**	−0.284**	0.171*	1			
$^8GY_{Urgench}$	0	−0.215**	−0.069	−0.071	−0.117	−0.178*	−0.081	1		
$^9GY_{Karshi}$	0.026	−0.025	0.015	0.027	−0.018	0.014	0.081	−0.071	1	
$^{10}GY_{Dongying}$	−0.245**	−0.455**	0.054	−0.026	−0.235**	0.214**	0.021	0.116	0.038	1

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed); G (germination score), DSW and GY are germination, seedling shoot dry weight and GY, respectively.

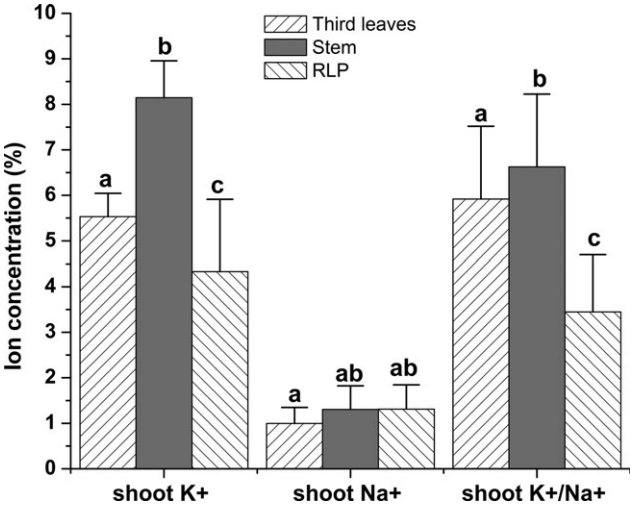

Fig. 2 Comparison of the amount of K^+, Na^+ accumulations (in %) and the K^+/Na^+ ratio in the shoot of the 150 genotypes after 25 days under salt stress. Letters on top of the error bars for each shoot parts indicate comparison of the means. Means with the same letter are not significantly different from each other.

tolerant genotypes after 8 weeks of salt stress (Fig. 5a). The rate of EL up to 11 % and 2 % due to salt stress was calculated for the sensitive and tolerant genotypes, respectively. Application of salt stress induced an increase in the osmotic potential of both tolerant and sensitive genotypes; however, the increase was highest in the sensitive genotypes (654 and 660 Osmol kg^{-1} for *UZ-11CWA-24* and *Bobur,* respectively) compared to the tolerant (610 and 575 Osmol kg^{-1} for *Altay2000* and *UZ-11CWA-8,* respectively) genotypes (Fig. 5b).

Analysis of contrasting genotypes for leaf chlorophyll fluorescence

The pattern of fluorescence transients (Fo, Fj, Fi, Fm and Fv) varied among the genotypes under salt stress (Fig. 6a), but showed a similar trend under non-saline conditions. Salt stress significantly inhibited the fluorescence transients across all the OJIP phases, but the inhibition was more intense on the two sensitive genotypes. An increase in the *Fm/Fo* in tolerant genotypes (up to +2.95 % and +1.24 % for *Altay2000* and *UZ-11CWA-8*, respectively) and a decrease in sensitive ones (up to −3.0 % and −4.09 % for *UZ-11CWA-24* and *Bobur*, respectively) were observed after application of salt stress (Table 6). The *Fv/Fo* and Fv/Fm also showed similar trend between the two groups. The stress impact on the *PI(ABS)* was genotype dependent. It increased by 7.74 % in *Altay2000* but decreased by 2.67 %, 6.12 % and 8.67 % in *UZ-11CWA-8, UZ-11CWA-24* and *Bobur,* respectively. Salt stress also affected negatively all the energy fluxes, except ABS/RC and DIo/RC for Altay2000; however, the effect was more severe on the salt-sensitive genotypes (Table 6). The fix area estimates increased in all the genotype under salt stress (Fig. 6b), but the increase was much higher (up to +16 %) in tolerant genotypes than in sensitive genotypes (up to +8 %). The effects of salt stress on some of the physiological parameters described above resulted in the reduction of DW in both the tolerant and sensitive genotypes, although the reduction was much pronounced in the sensitive (79 % for *UZ-11CWA-24* and 76 % for *Bobur)* than in tolerant *(*21 % for *Altay2000* and 24 % for *UZ-11CWA-8)* ones (Fig. 7).

Discussion

Access to appropriate genetic diversity is critical to current and future breeding efforts to improve wheat yield in the areas affected by soil salinity. Considerable efforts have been made so far to identify salt-tolerant wheat genotypes, but with few studies reporting on the simultaneous evaluations of salinity tolerance in more than one growth stages. In the present study, 150 winter and facultative wheat germplasms were evaluated for ST at germination, seedling stage and mature plants grown under field conditions to identify genotypes that can be used in breeding and development of new wheat varieties with improved and desirable level of salt tolerance and for further genetic studies. The studied germplasm showed significant genetic variation for the traits measured across the growth stages. The germination vigour, dry shoot weight and grain yield were negatively affected by salt stress as already reported (Gomes-Filho et al. 2008, Munns and Tester 2008, Rasheed 2009). However, the variation in the plant growth and development in response to the applied salt stress provided an opportunity to identify genotypes with contrasting attributes under stress amongst the germplasm used. Salt-tolerant genotypes would differ from salt-sensitive ones by allowing optimal growth under saline conditions. The response to the applied salt stress could partly be attributed

Table 4 Correlation coefficients of the genotype mean of root and shoot water losses caused by salt stress conditions and the shoot accumulated K^+ and Na^+ after 25 days under salt stress

Traits	RWL_{NaCl}	RWL_{Na2SO4}	SWL_{NaCl}	SWL_{Na2SO4}	Shoot K^+	Shoot Na^+	Shoot K^+/Na^+ ratio
RWL_{NaCl}	1						
RWL_{Na2SO4}	0.348**	1					
SWL_{NaCl}	0.705**	0.317**	1				
SWL_{Na2SO4}	0.311**	0.650**	0.586**	1			
Shoot K^+	−0.099	−0.235**	−0.198*	−0.259**	1		
Shoot Na^+	0.111	0.036	0.004	−0.045	−0.015	1	
Shoot K^+/Na^+	−0.072	−0.089	−0.067	−0.046	0.393**	−0.817**	1

RWL and SWL are root and shoot water losses due to NaCl and Na_2SO_4 salt stress, respectively; **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

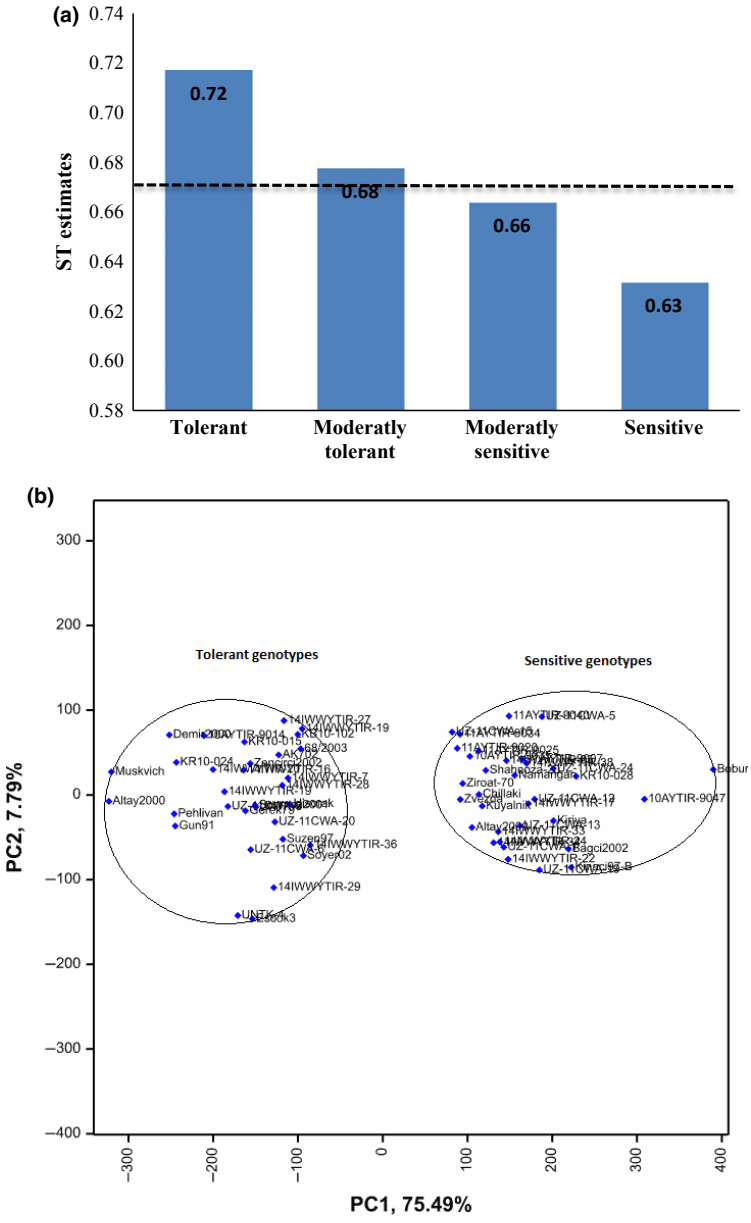

Fig. 3 Illustrated the representation of the studied genotypes based on the ST rankings. (a) ST status of all the 150 genotypes. The dotted line represents the average ST value of the entire population. (b) Scatter plot showing clustering of the the tolerant and sensitive genotypes based on the genotype variance–covariance matrix of their ST rankings across the three growth stages.

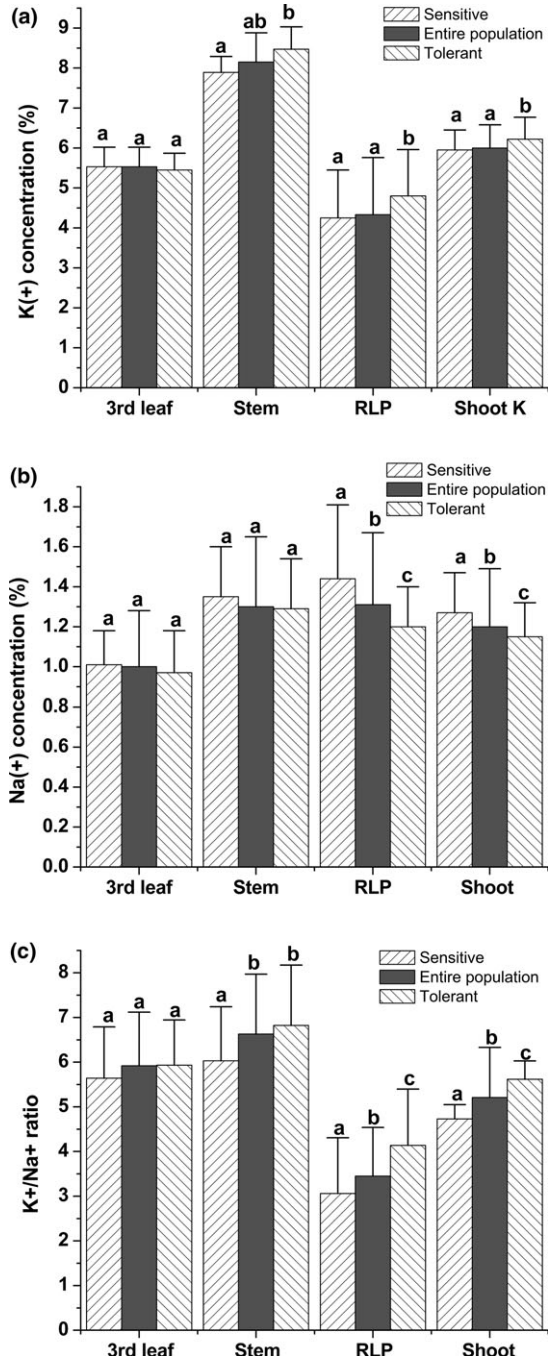

Fig. 4 Comparison of elemental constitution of different shoot parts of the studied genotypes. (a–c) show the concentration (%) of K^+, Na^+ and K^+/Na^+ ratio, respectively, for the 34 sensitive, entire studied population and 33 tolerant wheat genotypes after 25 days under salt stress. RLP is the ion concentration in the bulked leaves without the 3rd leaf, whereas the shoot is the mean ion estimates of the three shoot parts. Letters on top of the error bars for each shoot parts indicate comparison of the means. Means with the same letter are not significantly different from each other.

to inherent different genotype superiority due to the moderate-to-high heritability estimates in the studied germplasm set.

The ST estimates for each salt concentration at germination stage correlated positively with each other, suggesting similar mechanisms controlling salt tolerance at the germination stage. The within-growth stage correlation observed for ST traits at both germination and seedling stages in response to both NaCl and Na_2SO_4 applied stress provides evidence that both salt types are surrogate and can be used for the evaluation of wheat response to salt stress at the early seedling growth stage. Most of the ST estimates at germination stage were significant and negatively correlated with ST estimates at seedling stage. The mechanisms of salt stress response are highly growth stage specific and change during the plant life cycle (Walia et al. 2005).

Ion analysis revealed that the accumulated K^+ in the stem after salt stress was significantly higher than that accumulated in the 3rd leaf and RLP, but no significant difference was found between K^+ concentration in the 3rd leaf and RLP. This was in line with the findings in maize (Kobaissi et al. 2014) and barley (Booltink and Verhagen 1997). In contrast, there was no significant difference among the accumulated Na^+ in 3rd leaf, stem and RLP, although highest and lowest amounts were found in the stem and 3rd leaf, respectively. The high K^+ observed in the stem indicates that the ion is transported preferentially through the stem channels to other plant parts under salt stress conditions. The K^+ accumulation in the 3rd leaf, stem and RLP was positively correlated among each other, an indication that K^+ is mobile within the plant, and can be transported from the stem to the other shoot parts. The increase in the shoot K^+ was accompanied by a significant decline in the shoot Na^+, showing antagonism between K^+ and Na^+ (Elhamid et al. 2014). Antagonism exits between K^+ and Na^+ in the site of ion uptake due to direct competition of both ions for absorption in the plants (Epstein 1966).

The rate of root and shoot water loss due to salt stress correlated positively with each other, suggesting that shoot water loss is a direct consequent of the decreased water absorption capacity of root systems due to high osmotic potential exerted by salt stress around the plant rooting zone. The shoot K^+ concentrations increased with the decrease in the rate of root and shoot water loss, an indication that maintaining optimum K^+ status is favourable for water conservation in plant and would ultimately improve the plant growth and survival under salt stress. Reports have also indicated that sufficient K^+ status would contribute to greater water retention in plant tissues, due to its vital role in the osmotic adjustment and turgor regulation during stomatal movement that affects transpiration and

Table 5 Salt-tolerant and salt-sensitive genotypes identified based on the ST values in more than one growth stages

Entry name	Germination	Seedling	Mature field plant
	Tolerant		
Altay2000	✓	✓	✓
UZ-11CWA-8	✓	✓	✓
14IWWYTIR-19	✓	✓	✓
14IWWYTIR-10	✓	✓	
14IWWYTIR-20	✓	✓	
UZ-11CWA-17	✓	✓	
10AYTIR-9014	✓	✓	
Esaul	✓	✓	
KR10-015	✓	✓	
Demir2000		✓	✓
Gerek79		✓	✓
Esook3		✓	✓
Katia		✓	✓
14IWWYTIR-7		✓	✓
14IWWYTIR-8		✓	✓
14IWWYTIR-35		✓	✓
UZ-11CWA-5		✓	✓
UZ-11CWA-6		✓	✓
UZ-11CWA-11		✓	✓
14IWWYTIR-30	✓		✓
14IWWYTIR-38	✓		✓
169/2004	✓		✓
	Sensitive		
Bobur	✓	✓	✓
İzgi2001	✓	✓	
Konya2002	✓	✓	
UZ-11CWA-4	✓	✓	
10AYTIR-9047	✓	✓	
Oktyabrina	✓	✓	
14IWWYTIR-14		✓	✓
UZ-11CWA-13		✓	✓
UZ-11CWA-24		✓	✓
10AYTIR-9074		✓	✓
Turkmen-basy		✓	✓
Elomon		✓	✓
KR10-028	✓		✓

✓, indicates detected tolerant or sensitive genotypes in the corresponding stage.

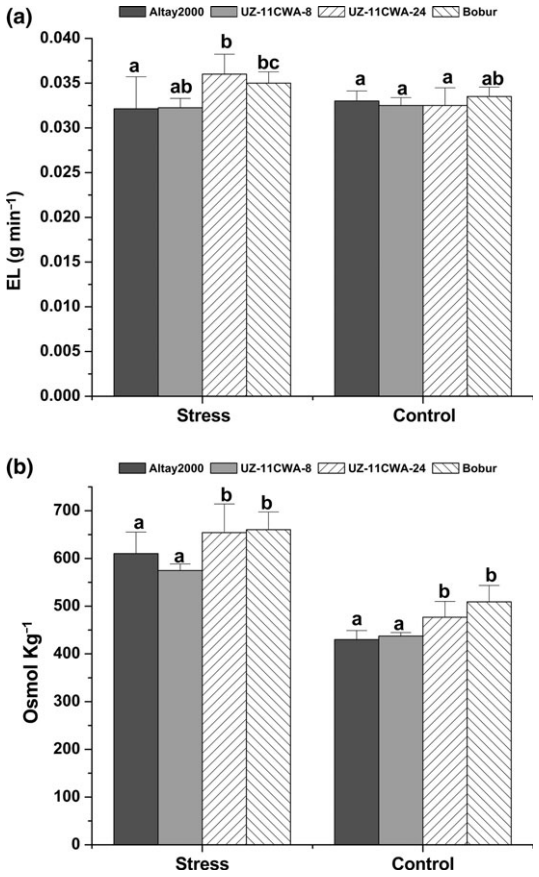

Fig. 5 Rate of release of electrolytes into deionized water per-min intervals (a) and osmotic potentials (b) for the leaf segments of the contrasting ST genotypes: tolerant *Altay2000* and *UZ-11CWA-8 and* sensitive *UZ-11CWA-24* and *Bobur* under salt stress and control conditions. Letters on top of the error bars indicate comparison of the genotype means under control and salt stress conditions. Means with the same letter are not significantly different from each other.

photosynthetic rates and xylem hydraulic conductance (Guo et al. 2007, Tuna et al. 2010, Wang et al. 2013, Sá et al. 2014).

Some of the genotypes analysed in this study have been previously reported to be resilient to different abiotic and biotic stresses. Four genotypes with high ST estimates have been shown to be resistant to different stresses: Gerek-*79* and *Altay-2000* to drought-, salt- and cold-resistant genotypes (Mutlu et al. 2009, Kara and Kara 2010, Akfirat and Uncuoglu 2013), *Katia* to zinc and drought tolerance (ICARDA, 2005) and *Demir2000* to lodging, cold, stripe and leaf rust resistant (Mazid et al. 2009). However, the salt stress-sensitive genotype *Bobur* is susceptible to stripe rust at seedling and mature stages (Ziyaev et al. 2013). These findings may suggest cross-tolerance among these stress factors in wheat. Mantri et al. (2010) reported that plant responses to fungal infection (*Ascochyta blight*) are similar to high salinity stress.

Among the genotypes identified in this study showing contrasting response to salt stress (Table 5), *Atlay2000*, *14IWWYTIR-19* and *UZ-11CWA-8* were tolerant, while *Bobur* was sensitive, across the three growth stages. These genotypes could serve as additional sources of ST for exploitation in breeding programmes and genetic studies. The ionomics revealed that the tolerant genotypes had lower shoot Na^+ and higher shoot K^+ concentration than the sensitive ones. Salt-tolerant crops are characterized with higher affinity of K^+ over Na^+ uptake (Teakle and Tyerman 2010, Kausar et al. 2014). The significantly higher shoot K^+/

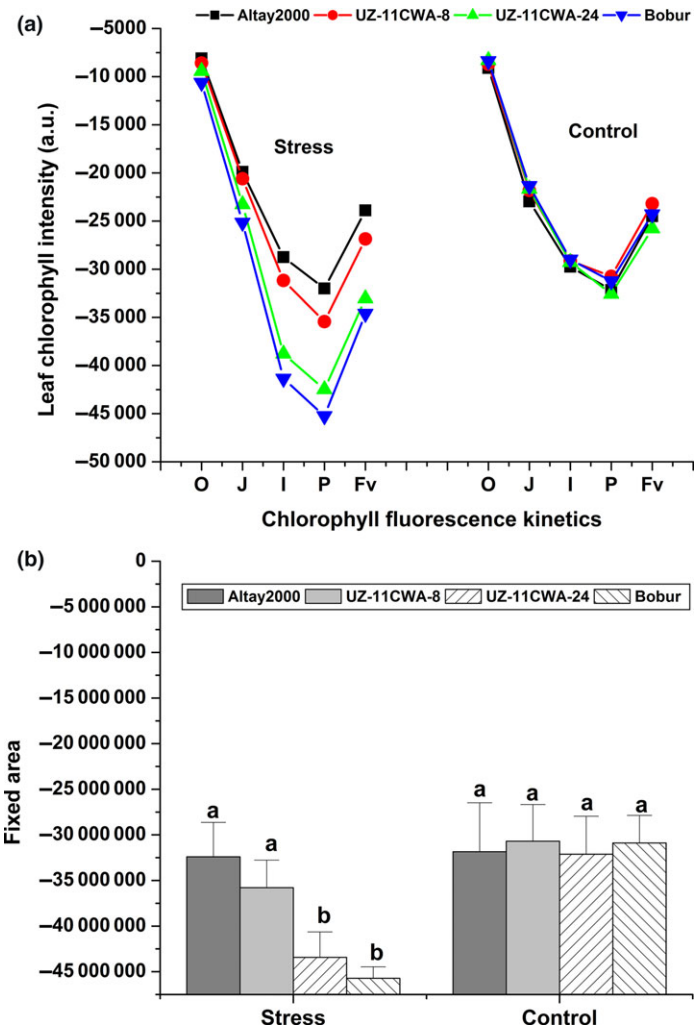

Fig. 6 Effect of salt stress on the chlorophyll a fluorescence and OJIP test parameters of light-adapted leaves of two tolerant (Altay2000, UZ-11CWA-8) and two sensitive wheat genotypes (UZ-11CWA-24) identified in this study. (a) Chlorophyll *a* fluorescence kinetics curve (Fo = fluorescence intensity at 50 μs; Fj = fluorescence intensity at J-step (at 2 ms); Fi = fluorescence intensity at i-step (at 60 ms); Fm = maximal fluorescence intensity; Fv = maximal variable fluorescence). (b) Fix area representing the area above the chlorophyll fluorescence curve between Fo and Fm (size of the plastoquinone pool). Letters on the error bars indicate comparison of the genotype means under control and salt stress conditions. Means with the same letter are not significantly different from each other.

Na^+ ratio compared to the sensitive ones is a consequence of the high shoot K^+ and low shoot Na^+ concentration. Optimum K^+/Na^+ ratio plays a vital role in maintaining an ideal osmotic and membrane potential for cell volume regulation in plant under salt stress and, has contributed to increase salt tolerance in wheat (El-Hendawy et al. 2009). Thus, the difference in ST among the two extreme genotypes could be attributed to their K^+/Na^+ discrimination ability associated with the machinery of water flow in plant under salt stress. The presented data showed increased levels of EL in sensitive genotypes caused by salt stress, whereas the EL was low in the tolerant genotypes. This suggests a negative impact of the salt stress on the cell membrane integrity. Salt stress would increase reactive oxygen species that often results in programmed cell death in plant (Demidchik et al. 2014). The rate of EL which measures the amount of membranes leaked over a given time period due to membrane injury can be considered useful screening protocol for discriminating among wheat genotypes for ST. Salt stress induced an increase in the leaf osmotic potential in both groups, but the impact was less in *Atlay2000* and *UZ-11CWA*, which could be attributed to efficient osmotic adjustment in the tolerant genotypes due to the higher shoot K^+/Na^+ ratio.

The chlorophyll fluorescence transients (*Fo*, *Fj*, *Fi*, *Fm* and *Fv*) in both tolerant and sensitive genotypes declined (Fig. 6a) under saline conditions, but the sensitive genotypes were more severely affected. The decrease in Fo due to salt stress indicates an increased thermal dissipation (Guidi et al. 2002, Bussotti et al. 2011), while the decrease in Fv may be attributed to the pigment losses due to salt injury. Salinity stress reduces photosynthesis by inhibiting photosystem II complex (PSII) at both acceptor [QA] and donor side (oxygen evolving complex OEC) and destruction of chlorophyll pigments by accumulation of toxic ions (Chen and Murata 2011). However, the higher fluorescence transients observed in the tolerant genotypes can be attributed to higher number of deactivating PSII and PSI associated with increase in the excitation energy (increased energy trapping capacity of PSII) and decrease in the

Table 6 Effect of salt stress on the energy fluxes of two salt-tolerant (in asterisk) and two salt-sensitive wheat genotypes

Energy fluxes	Genotypes	Control	Stress	Effect of salt (%)
Fm/Fo	Altay2000*	−4.46	−4.33	+2.95
	UZ-11CWA-8*	−4.44	−4.38	+1.24
	UZ-11CWA-24	−4.37	−4.50	−3.01
	Bobur	−4.40	−4.58	−4.09
Fv/Fo	Altay2000*	−3.46	−3.33	+3.80
	UZ-11CWA-8*	−3.44	−3.38	+1.61
	UZ-11CWA-24	−3.37	−3.50	−3.90
	Bobur	−3.40	−3.58	−5.30
Fv/Fm	Altay2000*	−0.77	−0.77	+0.88
	UZ-11CWA-8*	−0.77	−0.77	+0.38
	UZ-11CWA-24	−0.77	−0.78	−0.89
	Bobur	−0.77	−0.78	−1.19
PI(ABS)	Altay2000*	−1.55	−1.43	+7.47
	UZ-11CWA-8*	−1.50	−1.54	−2.66
	UZ-11CWA-24	−1.46	−1.55	−6.12
	Bobur	−1.50	−1.63	−8.67
ABS/RC	Altay2000*	−2.94	−2.95	+0.49
	UZ-11CWA-8*	−2.99	−2.92	−2.34
	UZ-11CWA-24	−3.12	−2.97	−4.83
	Bobur	−3.13	−2.94	−6.23
TRo/RC	Altay2000*	−2.28	−2.27	+0.34
	UZ-11CWA-8*	−2.31	−2.25	+2.70
	UZ-11CWA-24	−2.41	−2.31	−3.98
	Bobur	−2.42	−2.29	−5.04
ETo/RC	Altay2000*	−1.28	−1.26	+1.31
	UZ-11CWA-8*	−1.31	−1.28	+2.15
	UZ-11CWA-24	−1.38	−1.31	+5.15
	Bobur	−1.40	−1.31	+6.3
DIo/RC	Altay2000*	−0.66	−0.69	−3.33
	UZ-11CWA-8*	−0.67	−0.67	−1.09
	UZ-11CWA-24	−0.71	−0.66	+7.59
	Bobur	−0.72	−0.64	+10.25

Fm/Fo, non-photochemical loss in PSII; Fv/Fo, efficiency of the water-splitting complex; Fv/Fm, maximum quantum yield of PSII; PI(ABS), performance index; ABS/RC, effective antenna size of an active reaction centre (RC); TRo/RC, maximal trapping rate of PSII; ETo/RC, electron transport in an active RC; DIo/RC, effective dissipation in an active RC.

photochemical quenching coefficient (Krause and Weis 1991, Guidi et al. 2002). Baker (2008) suggested the use of fluorescence induction parameters to detect metabolic perturbations by abiotic stresses. Under salt stress, the Fv/Fm, Fo/Fm and Fv/Fo declined in tolerant genotype and increased in the sensitive genotypes, suggesting different mechanisms are controlling these physiological traits in wheat, making them useful parameters for distinguishing salt stress tolerant from sensitive genotypes. The PI(ABS) was also affected by salt stress (increased by +7.47% in Atlay2000 and decreased by −2.66%, −6.12% and −8.67% in UZ-11CWA-8, UZ-11CWA-24 and Bobur, respectively), but no noticeable pattern was observed between the tolerant and sensitive genotypes and could be considered genotype specific. The fix area was twice higher in the tolerant genotypes compared to the sensitive ones. Salt stress also affected the energy fluxes (including *ABS/RC*, *TRo/RC*, *ETo/RC* and *DIo/RC*) of the contrasting wheat genotypes the genotypes, but the effect was more severe on the sensitive genotypes. From these results, it can be anticipated that salt stress reduced energy absorption, energy trapping efficiency and conversion of excitation energy into electron flow by damaging oxygen evolving complex, over reduction of QA resulting in occurrence of chronic photoinhibition.

In conclusion, the ST index can be utilized to discriminate against genotypes response to salt stress in wheat. The identified contrasting wheat genotypes clearly showed differential physiological responses mechanisms to salt stress. The tolerant genotypes (*Atlay2000* and *UZ-11CWA-8)* exhibited higher shoot K^+/Na^+ ratio, higher membrane stability, lower osmotic potential and higher rates of PSII photochemical activities than sensitive (*UZ-11CWA-24* and *Bobur*) genotypes which resulted in the significantly higher dry matter observed under salt stress condition. These parameters might be routinely used to screen for salt tolerance in plants, and the identified genotypes could be considered for inclusion in wheat breeding programme and in future genetic studies for salt tolerance.

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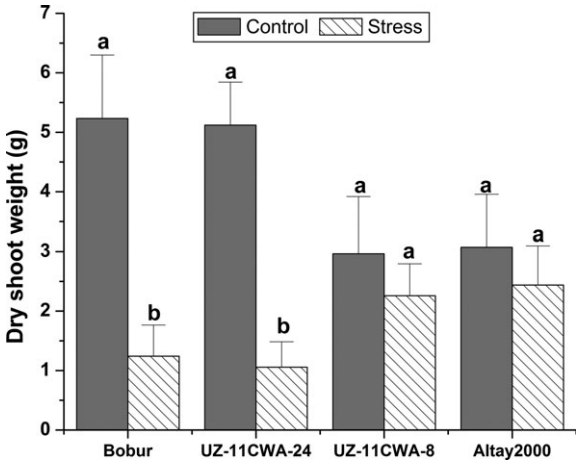

Fig. 7 Salt stress intensity (SI) on the sensitive (*UZ-11CWA-24* and *Bobur*) and tolerant (*Altay2000* and *UZ-11CWA-8)* wheat genotypes grown for 6 weeks in hydroponics under 100 mM NaCl stress SI calculated as: SI = 100[1- ($DW_{stress}/DW_{control}$)] using 14 plants for each genotype. Letters on top of the error bars for each genotype indicate comparison of the means under control and salt stress conditions. Means with the same letter are not significantly different from each other.

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