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The potential of leaf chlorophyll content to screen bread-wheat genotypes in saline condition

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Abstract

Physiological traits, which are positively associated with yield under salt-stress conditions, can be useful selection criteria in screening for salt tolerance. We examined whether chlorophyll (Chl) content can be used as screening criterion in wheat. Our study involved 5 wheat genotypes under both saline and nonsaline field conditions as well as in a sand-culture experiment. Salt stress reduced significantly biomass, grain yield, total Chl and both Chl *a* and *b* in all genotypes. In the sand-culture experiment, Chl accumulation was higher in PF70354/BOW, Ghods, and H499.71A/JUP genotypes at nonsaline control, moderate, and high salt concentrations, respectively. In the field experiment, genotype H499.71A/JUP belonged to those with the highest Chl density. The SPAD (Soil Plant Analysis Development) meter readings were linearly related to Chl content both in the sand-culture and in the field experiment. However, salt stress affected the calibration of SPAD meter. Therefore, separate Chl–SPAD equations were suggested for saline and nonsaline conditions. The correlation coefficients between the grain yield and SPAD were positive and significant both in the sand culture and in the field experiment. These findings suggested that SPAD readings could be used as a tool for rapid assessment of relative Chl content in wheat genotypes. It could be used for the indirect selection of high-yielding genotypes of wheat under saline condition in sand-culture and field experiments.

Additional key words: biomass; NaCl; SPAD; *Triticum aestivum* L.

Introduction

Salinity is one of the most important environmental factors limiting wheat production in arid and semiarid regions of the world. It causes various physiological disturbances resulting from osmotic stress, ion toxicity, and imbalance of nutrient elements in the cytoplasm of the plant cells (Flowers *et al.* 1977, Muranaka *et al.* 2002).

Wheat is a moderately salt-tolerant plant (Maas and Grattan 1999) and it is widely grown on soils prone to salt accumulation. In response to salinity, considerable genotypic differences among wheat cultivars indicate that salt tolerance may be enhanced by breeding (Royo and Abio 2003). Conventional screening methods for salt tolerance are usually based on grain yield; they are expensive and time-consuming. An alternative approach is to identify the traits having a positive correlation with grain yield under salt-stress conditions.

Leaf Chl content is one of the important, physiological traits closely related to photosynthetic capacity (Kancheva and Mishev 2000, Muranaka *et al.* 2002, Arunyanark *et al.* 2008). Salinity affects Chl content in many crops by imposing adverse effects on Chl synthesis or accelerating its degradation, which reduces the photosynthetic capacity.

The ability to maintain Chl content under salt stress is suggested as a salt-resistance trait in wheat (Cuin *et al.* 2010). Hence, it can be used to select salt-tolerant genotypes in breeding programs (El-Hendawy *et al.* 2007, Din *et al.* 2008, Azizov and Khanisheva 2010). Besides, a positive correlation has been observed between grain yield and Chl in wheat (Reynolds *et al.* 2000, Gutiérrez-Rodriguez 2004). Changes in leaf Chl content and potassium content in shoot sap proved to be the best traits for screening in wheat breeding programs (Cuin *et*

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Abbreviations: AN – anthesis; BM – biomass; EC – electrical conductivity; ET – early tillering; GF – grain filling; GY – grain yield; Chl – chlorophyll; HS – high salt stress; LT – late tillering; MS – moderate salt stress; NSA – nonsaline conditions; SPAD – Soil Plant Analysis Development; TChl – total chlorophyll; S0 – 0 mM NaCl+$CaCl_2$, S100 – 100 mM NaCl+$CaCl_2$; S200 – 200 mM NaCl+$CaCl_2$.
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al. 2010). Data from various environments across Mexico, Iran, North Africa, and Asia showed that Chl content is the trait with high heritability in wheat, but it did not affect significantly wheat yield in all these environments (Lopes *et al.* 2012). Under stress conditions (salt, drought, heat), the correlation between the yield and Chl content becomes stronger (Reynolds *et al.* 2000, Arunyanark *et al.* 2008, Lopes *et al.* 2012). It suggests the critical role of Chl in growth performance under abiotic stresses.

Foliar Chl can be measured using portable Chl meters such as SPAD. Compared to traditional, destructive methods, this equipment saves time, space, and resource substantially (Netto *et al.* 2005, Tavakkoli *et al.* 2010). SPAD meter reading is applied as an index for the response of relative Chl content to different types of stresses, including moisture (Arunyanark *et al.* 2008), extreme temperatures (Balouchi 2010), and an excessive salt concentration in the plant root zone (Munns and James, 2003, Atlassi Pak *et al.* 2009, Akhtar *et al.* 2010; Cuin *et al.* 2010).

Calibration of SPAD output readings into units of leaf Chl concentration and interpretation of the relationship between these two parameters is not entirely straightforward (Markwell *et al.* 1995). Many studies have calibrated SPAD meter with extractable Chl contents; they have found generally that different equations should be employed for different plant species or cultivars (Giunta *et al.* 2002), growing conditions (Campbell *et al.* 1990), nutrient status, and genotypes (Munns and James 2003). However, to our knowledge, only a very little effort has been devoted to investigation of the relationship between leaf Chl content and SPAD readings in saline conditions in wheat.

Plant breeders prefer to evaluate their genetic material under controlled conditions, such as hydroponics, sand cultures, greenhouse, or growth chambers. Screening genotypes for salinity tolerance under such conditions is necessary to understand different mechanisms of salt tolerance among genotypes. However, genotypic differences observed under controlled conditions may not correspond to those observed under actual field conditions (El-Hendawy *et al.* 2009). Therefore, it is important to evaluate plants (with particular promise under controlled conditions) also under more variable, natural, field conditions to account for the possible genotypic differences.

Accordingly, we examined the performance of SPAD measurements as a screening criteria for salt tolerance in five different wheat genotypes under both controlled (sand culture) and field conditions.

Materials and methods

Sand-culture experiment included 5 wheat genotypes and 3 salinity levels of 0 (S0), 100 (S100), and 200 (S200) mM NaCl+$CaCl_2$ with three replications. It was carried out at the Agricultural Research Center, Zarghan, Fars Province, Iran between November 2009 and May 2010. The experiment was carried out under a rain-out shelter with an open-ended structure (28 m long and 8 m wide).

The plants were grown in large tanks (55 cm in diameter, 75 cm deep) filled with washed sand having an average sand density of 1.4 kg m^{-3}. Plants were irrigated with modified Hoagland's nutrient solution. The nutrient solution added to the local tap water consisted of 3 mM KNO_3, 2.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.17 mM KH_2PO_4, 1.5 mM $MgSO_4 \cdot 7H_2O$, 50 µM Fe as sodium ferric diethylenetriamine pentaacetate (NaFe-DTPA), 23 µM H_3BO_3, 5 µM $MnSO_4 \cdot H_2O$, 0.4 µM $ZnSO_4 \cdot 7H_2O$, 0.2 µM $CuSO_4 \cdot 7H_2O$, and 0.1 µM H_2MoO_4.

Five bread-wheat genotypes (*Triticum aestivum* L.) included two salt-tolerant breeding lines (H499.71A/JUP and PF70354/BOW), two salt tolerant local cultivars (Bam and Ghods), and a salt-sensitive breeding line (F78104); they were used in both sand-culture and field experiments.

Seeds of each genotype were sown in four rows per tank. The rows were spaced 12 cm apart. Sowing depth was approximately 2 cm. Seedlings were watered initially with tap water, then a half strength nutrient solution was introduced 2 d after emergence (DAE) and it increased to full strength at 3 DAE. NaCl (25 mM) and $CaCl_2$ (5:1 molar concentration) were added to the nutrient solutions twice a day to the final concentration of 100 and 200 mM. The stress was maintained until the final harvest.

Each irrigation event continued until the sand was saturated. The overflow irrigation returned to the reservoirs (1,000 l) through drainage by gravity. The plants were irrigated four times a day. Water utilized through the evapotranspiration process was replenished daily to maintain constant salinity in the nutrient solution. The nutrient solutions were changed every 8–12 d to avoid possible depletion of the nutrients.

To determine biomass (BM) and grain yield (GY), eight plants from each replicate were harvested. Plant material was then dried at 70°C for 48 h to determine the dry mass (DM). Data were averaged across the eight subsamples to determine biomass and grain yield per plant.

Field experiment was conducted between November 2009 and June 2010 at Agricultural Research Center (29º46'N, 52º44'E; 1,595 m a. s. l.). Total annual rainfall was 237 mm and mean monthly evaporation ranged from 12 mm in January to 413 mm in July (Fig. 1).

The plot size was 14 m^2 (2 m × 7 m) and the rows were spaced at 20 cm. The experiments were laid out in a complete block design with three replications, five wheat genotypes, and three water salinity levels for irrigation: 2.5 dS m^{-1} = nonsaline (NSA); 5.8 dS m^{-1} = moderate salt stress (MS); and 10.5 dS m^{-1} = high salt stress (HS). Electrical conductivity (EC) and ionic composition of irrigation water is shown in Table 1.

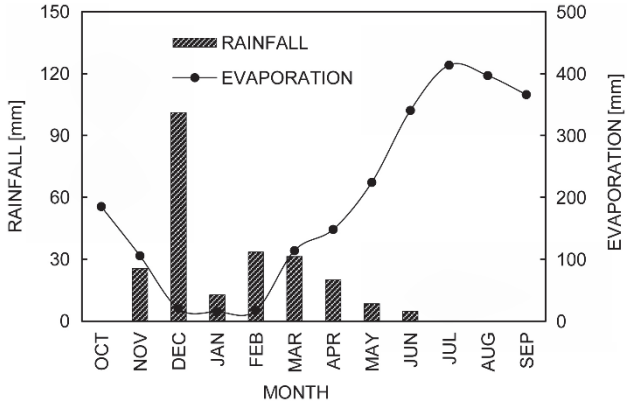

Fig. 1. Amount of monthly rainfall and evaporation in the study area in years 2009–2010 (Zarghan, Fars, Iran).

In the field experiment, the GY was evaluated by sampling 4 m^2 per plot.

SPAD Chl meter reading: Five plants from each tank were selected to record SPAD values at early tillering (ET), late tillering (LT), anthesis (AN), and grain filling (GF) stages. Three SPAD readings were taken from each side of the midrib of each leaf between 11:00 and 13:00 h using a *SPAD-502* (*Konica-Minolta*, Japan). The 6 readings per leaf were averaged to produce a single observation value for each leaf. An average SPAD value for each tank was derived from 30 observations (6 leaflets × 5 plants). The same procedure was performed in the field experiment. However, the number of samples increased to 17 plants for each plot.

Table 1. Chemical composition of irrigation water used in field experiment conducted in Zarghan, Fars, Iran.

Electrical conductivity of irrigation water	EC [dS m^{-1}]	Ca^{2+} [meq L^{-1}]	Mg^{2+}	Na^{+}	K^{+}	HCO_3^-	SO_4^{2-}	Cl^-
Nonsaline	2.2	6.2	6.1	7.3	0.20	1.5	13.0	5.4
Moderate salinity	5.8	8.2	15.5	34.7	0.11	3.2	5.6	48.6
High salinity	10.5	16.7	20.2	68.7	0.21	4.1	13.3	87.5

Leaf Chl determination: Chl was extracted from fresh leaf tissue with 80% (v/v) aqueous acetone. Absorbance of leaf extracts was measured at 645 nm and 663 nm (Lichtenthaler 1987) by spectrophotometer (*Spectronic GENESYS 6*, Madison, WI, USA). Leaf Chl concentration (Chl *a*, Chl *b*, and TChl) was calculated by the following equations (Arnon 1949) and finally the content was expressed on the leaf area basis [μg(Chl) cm^{-2}]:

$$\text{Chl } a = 12.7\, A_{663} - 2.69\, A_{645} \quad (1)$$

$$\text{Chl } b = 22.9\, A_{645} - 4.68\, A_{663} \quad (2)$$

$$\text{TChl} = 20.2\, A_{645} + 8.02\, A_{663} \quad (3)$$

where A_{645} and A_{663} were absorbance readings at wavelengths of 645 nm and 663 nm, respectively.

The chlorophyll concentration of the extract calculated from these equations was then converted to leaf chlorophyll density [μg(Chl) cm^{-2}(leaf area)].

Leaf Chl density was measured at LT and AN stages. These development stages were selected because they are critical for achieving high grain yield (Richards 2000).

Statistical analysis: The measured parameters were analyzed according to a randomized, complete block design. Analysis of variance (ANOVA) was performed to determine the significance of the differences between the responses of each genotype to salt stress. Mean separation among genotypes for each measurement was determined using a *Duncan*'s multiple range test. All the experimental data were analyzed using the *SAS software* (*SAS Institute*, Cary, NC, USA), and the difference between the treatments were considered significant at $P<0.05$.

Results

Root-zone salinity: In the sand-culture experiment, salinity was maintained uniform during the growth season, while the average soil EC of the field irrigated with saline water fluctuated with growing season (Fig. 2). The minimum EC of the fields was observed in May, when 70% of the total annual rainfall occurred. In the irrigation season, salts imported with the irrigation water were accumulated in the soil profile and increased the root-zone salinity level. Soil salinity at LT stage was 1.6, 5.2, and 10 dS m^{-1} in NS, MS, and HS fields, respectively. The EC increased to 2.1, 6.6, and 11.7 dS m^{-1} at GF stage.

Wheat BM and GY: Genotypes differed significantly in BM and GY at all salinity treatments in both the sand-culture and field experiments. In S0, BM values ranged from 41.2 to 50.3 g per plant; GY varied between 22.4 and 29.8 g per plant in different genotypes in the sand-culture experiment (Table 2). Significant reductions were observed in BM and GY under salt stress especially in the S200. High salt stress resulted in a reduction of BM and GY by 58 and 63%, respectively, compared with S0.

The interactions among genotypes and salinity levels were found in BM and GY during the sand-culture

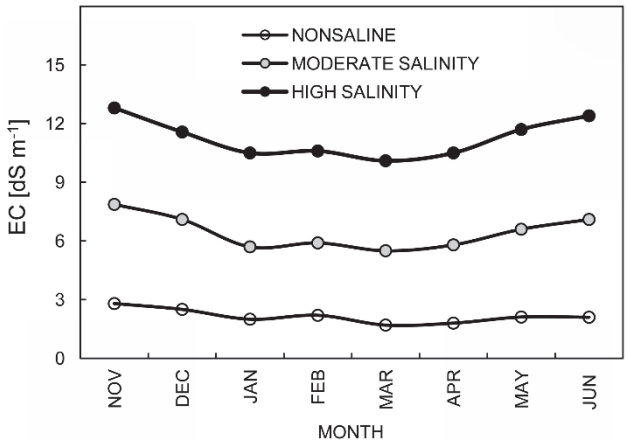

Fig. 2. Soil salinity (in term of electrical conductivity) changes during wheat growth season in the field experiment. EC – electrical conductivity.

experiment. For instance, Ghods and H499.71A/JUP produced an intermediate GY under the S0 condition, whereas they were superior under S100 and S200, respectively. PF70354/BOW showed contradictory results: although GY belonged to the highest one under S0, it fell among the low-yielding genotypes at both salinity levels. There were not genotype *vs*. salinity interaction effect in F78104 and Bam, therefore F78104 was a genotype with a low GY in both saline and nonsaline conditions. The cultivar Bam produced a medium GY under all salt levels.

Similarly to the sand-culture experiment, significant genotypic variation occurred also in BM and GY in the field experiment (Table 2). At NSA treatment, BM varied about 1.3-fold among the genotypes, ranging from 1.33 kg m^{-2} in F78104 to 1.75 kg m^{-2} in PF70354/BOW. There was also 1.4-fold variation in GY ranging from 0.61 to 0.85 kg m^{-2} among the genotypes.

Table 2. Biomass and grain yield of five wheat genotypes under different salinity levels in the sand-culture [g per plant] and field experiments. S – salinity; G – genotype. **– significant at the 1% level.

Experiment	Genotype	Biomass [kg m^{-2}]			Grain yield [kg m^{-2}]		
		Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity
Sand culture	Bam	45.5^{ab}	32.8^{ab}	21.7^{ab}	24.8^{bc}	16.8^{ab}	10.2^{a}
	H499.71A/JUP	47.1^{ab}	34.2^{ab}	23.7^{a}	27.5^{b}	18.4^{ab}	11.7^{a}
	Ghods	47.2^{ab}	36.8^{a}	21.2^{ab}	25.6^{b}	18.9^{a}	10.1^{a}
	PF70354/BOW	50.3^{a}	30.1^{b}	17.8^{b}	29.8^{a}	14.3^{bc}	$9.2a^{b}$
	F78104	41.2^{b}	20.2^{c}	12.6^{c}	22.4^{c}	11.2^{c}	6.6^{b}
	S	**			**		
	G	**			**		
	S × G	**			**		
Field	Bam	1.54^{b}	0.92^{a}	0.62^{a}	0.72^{b}	0.46^{a}	0.33^{a}
	H499.71A/JUP	1.67^{b}	0.98^{a}	0.61^{a}	0.69^{b}	0.46^{a}	0.30^{a}
	Ghods	1.47^{b}	1.00^{a}	0.61^{a}	0.71^{b}	0.43^{a}	0.30^{a}
	PF70354/BOW	1.75^{a}	0.94^{a}	0.70^{a}	0.85^{a}	0.40^{a}	0.29^{a}
	F78104	1.33^{c}	0.81^{b}	0.56^{b}	0.61^{b}	0.36^{b}	0.24^{b}
	S	**			**		
	G	**			**		
	S × G	**			**		

Ghods and PF70354/BOW had higher BM production than the other genotypes at MS and HS, respectively. The highest GY was also attributed to H499.71A/JUP at MS and Bam at HS. Slight differences among the genotypes were found in BM and GY, except for F78104, which had significantly lower BM and GY in the field experiment.

BM and GY varied in some of the studied genotypes depending on the treatment applied, showing again that there was the significant genotype *vs.* salinity interaction. The five genotypes grown in the field were divided into two distinct groups based on their BM and GY (Table 2). PF70354/BOW belonged to the highest-yielding genotypes and F78104 to the lowest-yielding ones. There was clearly the genotype *vs.* salinity interaction in Bam, H499.71A/JUP, and Ghods genotypes. They exhibited lower BM and GY under NS and therefore they fell into the lowest-yielding group, while they had the highest performance under salt stress and according to this they belonged to the highest-yielding group.

Total Chl content and effect of salinity: The *ANOVA* used for data obtained at LT and AN stages showed the significant effects of salinity and genotypes on leaf Chl density in both the sand-culture (Table 3) and field experiment (Table 4). Chl content was consistently higher in the control treatment than in the saline treatments. The imposition of salt stress resulted in a reductions of mean Chl content (pooled over genotypes and time of sampling) by 19.3 and 50% in the sand-culture experiment, and by 8.2 and 37.5% in the field experiment compared with the control treatments.

Table 3. Chlorophyll (Chl) *a*, Chl *b*, and total Chl density of five wheat genotypes under different salinity levels in the sand-culture experiment. S – salinity; G – genotype. ns – not significant; **– significant at the 1% level.

Growth stage/ Genotype	Chl *a* density [μg cm^{-2}]			Chl *b* density [μg cm^{-2}]			Total Chl density [μg cm^{-2}]		
	Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity
Bam	23.4^{c}	23.2^{a}	13.4^{ab}	7.3^{c}	7.2^{a}	4.2^{ab}	30.7^{c}	30.4^{a}	17.7^{ab}
H499.71A/JUP	27.0^{b}	24.1^{a}	15.7^{a}	8.4^{b}	7.4^{a}	4.9^{a}	35.4^{b}	31.5^{a}	20.6^{a}
Ghods	23.0^{c}	23.4^{a}	14.9^{a}	7.4b^{c}	6.9^{a}	4.7^{a}	30.4^{c}	30.3^{a}	19.6^{a}
PF70354/BOW	31.0^{a}	22.7^{a}	10.4^{bc}	9.8^{a}	7.0^{a}	3.3^{bc}	40.8^{a}	29.7^{a}	13.8^{bc}
F78104	21.9^{c}	17.5^{b}	9.7^{c}	6.9^{c}	5.3^{b}	3.1^{c}	28.8^{c}	22.7^{b}	12.8^{c}
S	**			**			**		
G	**			**			**		
S × G	*			ns			*		
Bam	29.4^{b}	26.4^{ab}	17.6^{a}	10.1^{a}	8.9^{ab}	5.7^{b}	39.5^{b}	35.3^{ab}	23.3^{b}
H499.71A/JUP	38.6^{a}	24.8^{ab}	22.5^{a}	11.1^{a}	8.3^{ab}	7.2^{a}	49.7^{a}	33.1^{ab}	29.7^{a}
Ghods	40.3^{a}	29.5^{a}	18.6^{a}	11.3^{a}	9.8^{a}	6.0^{ab}	49.4^{a}	39.4^{a}	24.6^{ab}
PF70354/BOW	38.0^{a}	24.6^{ab}	12.7^{b}	11.3^{a}	8.2^{ab}	4.1^{c}	51.6^{a}	32.9^{ab}	16.8^{c}
F78104	30.2^{b}	19.5^{b}	11.7^{b}	8.2^{a}	6.7^{b}	3.8^{c}	38.4^{b}	26.2^{b}	15.4^{c}
S	**			**			**		
G	**			**			**		
S × G	*			ns			*		

The mean leaf Chl content in the sand-culture and field experiment were by 27.8 and 20.5% higher at AN than at LT, respectively. Increase in TChl was more pronounced in Ghods in nonsaline and moderate saline treatments of both experiments, whereas at high salinity level, H499.71A/JUP (in the sand-culture) and Bam and Ghods (in the field experiment) showed the greatest increase in TChl.

Table 4. Chlorophyll (Chl) *a*, Chl *b*, and total Chl density of five wheat genotypes under different salinity levels in the field experiment. S – salinity; G – genotype. ns – not significant; **– significant at the 1% level.

Growth stage/ Genotype	Chl *a* density [μg cm^{-2}]			Chl *b* density [μg cm^{-2}]			Total Chl density [μg cm^{-2}]		
	Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity
Late tillering									
Bam	20.9^{b}	19.7^{a}	12.8^{ab}	5.6^{a}	5.6^{a}	5.2^{a}	26.6^{b}	25.2^{a}	18.0^{a}
H499.71A/JUP	23.9^{a}	20.3^{a}	13.6^{a}	5.8^{a}	6.1^{a}	5.7^{a}	29.7^{a}	26.4^{a}	19.3^{a}
Ghods	21.5^{b}	20.5^{a}	12.5^{ab}	5.2^{a}	5.8^{a}	5.4^{a}	26.7^{b}	26.3^{a}	17.9^{a}
PF70354/BOW	21.5^{b}	17.4^{ab}	10.8^{ab}	5.9^{a}	5.8^{a}	5.0^{a}	27.4^{b}	23.2^{ab}	15.9^{ab}
F78104	16.4^{c}	13.6^{b}	9.3^{b}	4.7^{a}	4.9^{a}	4.3^{a}	21.1^{c}	18.5^{b}	13.6^{b}
S	**			**			**		
G	**			**			**		
S × G	ns			ns			ns		
Anthesis									
Bam	24.3^{bc}	24.0^{a}	15.4^{a}	7.2^{a}	6.3^{b}	6.2^{a}	31.4^{bc}	30.3^{a}	21.6^{a}
H499.71A/JUP	28.1^{a}	25.0^{a}	14.7^{ab}	7.5^{a}	7.0^{ab}	6.1^{a}	35.6^{a}	32.0^{a}	20.8^{a}
Ghods	25.7^{ab}	24.8^{a}	14.6^{ab}	8.1^{a}	8.3^{a}	6.5^{a}	33.8^{a}	33.1^{a}	21.1^{a}
PF70354/BOW	23.6^{bc}	21.6^{ab}	12.3^{ab}	7.3^{a}	7.1^{ab}	5.2^{a}	30.8^{bc}	28.7^{ab}	17.5^{ab}
F78104	21.9^{c}	18.0^{b}	10.6^{b}	6.4^{a}	6.3^{b}	5.1^{a}	28.2^{c}	24.3^{b}	15.7^{b}
S	**			**			**		
G	**			**			**		
S × G	ns			ns			ns		

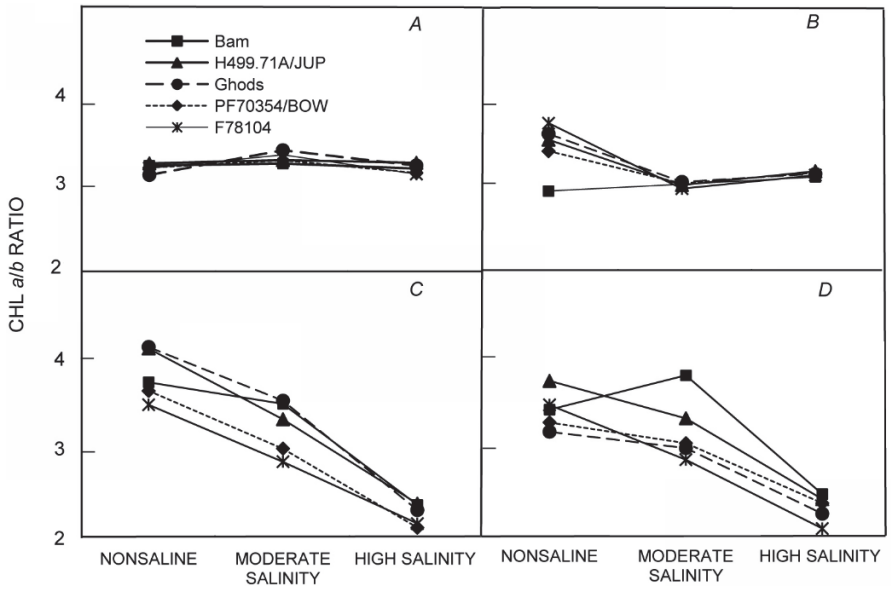

Fig. 3. Effect of different salinity levels on Chl *a/b* ratio. *A*: sand-culture experiment, late tillering stage; *B*: sand-culture experiment, anthesis stage; *C*: field experiment, late tillering stage; *D*: field experiment, anthesis stage. Chl – chlorophyll.

Total Chl variability among genotypes: In the sand-culture experiment, comparison of the genotypes demonstrated that PF70354/BOW contained the highest TChl in S0 at both LT and AN stages (Table 3). At S100, H499.71A/JUP at LT and Ghods at AN stage showed the maximum Chl. At S200, H499.71A/JUP exhibited the highest TChl compared with other genotypes, whereas F78104 showed the lowest TChl at all salinity levels and at both growth stages.

The genotype *vs.* salinity interaction affected significantly TChl. The interaction was most clearly observed in PF70354/BOW, which in the control treatment had the highest TChl, whereas under saline conditions, it showed below average values at both growth stages. Bam had an average TChl at S0 condition; however, it had the least variation between the control and salinity treatments (40%) in comparison with the other genotypes (45–62%) at both growth stages.

In the field experiment, H499.71A/JUP had the highest TChl at LT, while at AN stage, H499.71A/JUP, Ghods, and PF70354/BOW had higher TChl compared with the other genotypes in NS condition (Table 4). At saline condition, Bam, Ghods, and H499.71A/JUP showed the higher TChl compared to other genotypes. These results indicated that genotypes responded differently to salt stress conditions because salinity did not decline Chl density of the genotypes at the same rate. For instance, Chl decreased by 4, 2, and 10% at MS, and by 31, 38, and 42% at HS in Bam, Ghods, and H499.71A/JUP respectively, at AN stage.

Chl *a/b* ratio: In the sand-culture experiment, the reduction in Chl *a* and Chl *b* was approximately the same in response to both salinity levels at LT. There was a 0–27% reduction in Chl *a* and 1–29% reduction in Chl *b* across different genotypes under S100 (Table 3). Similarly, S200 reduced both Chl *a* and *b* by 48% (average over genotypes) in comparison with S0. Therefore, Chl *a/b* ratio remained unchanged with increasing salinity at LT stage (Fig. 3*A*).

At AN stage, S100 reduced Chl *a* and Chl *b* by 29 and 19%, respectively (Table 3), while at S200, both Chls showed almost the same reductions (52 and 48% in Chl *a* and Chl *b*, respectively). As illustrated in Fig. 3*B*, at AN stage, with the exception of Bam, salinity significantly reduced Chl *a/b* ratio in all genotypes (from 11% in PF70354/BOW to 21% in F78104) with a proportionately higher decrease in Chl *a* than in Chl *b*.

Although there was a 10% variation among genotypes grown in the sand-culture in the Chl *a/b* ratio under S0 treatment, the variation was negligible under salinity treatments.

In the field experiment, Chl *a* was reduced more significantly than Chl *b* in response to salt stress at both growth stages (Table 4). The Chl *a* declined by 6% and 19% at MS and by 39% and 50% at HS in Bam and PF70354/BOW, respectively, at LT stage. In contrast to the changes in Chl *a* at LT stage, Chl *b* remained fairly constant in all genotypes and at both salinity levels.

Data obtained from the field experiment indicated that the loss of Chl *b* induced by salinity was lesser in comparison to loss of Chl *a*, resulting in the decline of the Chl *a/b* ratio. In all genotypes, Chl *a/b* ratio tended to decrease in saline-treated plants at both growth stages (Fig. 3*C,D*). At LT stage, the MS induced a 6–19% decrease in Chl *a/b* ratio in different genotypes. Further decrease in Chl *a/b* ratio (37–44%) was observed in the HS treatment. At AN stage, reductions in Chl *a/b* ratio were relatively lower than those at LT. Of all the genotypes, F78104 exhibited the least Chl *a/b* ratio; Bam and H499.71A/JUP had the highest Chl *a/b* ratio.

SPAD: Regardless to salinity treatments, distinct differences appeared among genotypes in SPAD readings (Table 5). In both sand culture and field experiments, H499.71A/JUP showed either the highest or second-highest SPAD value whereas, F78104 demonstrated the lowest one.

At S200, differences among the genotypes in SPAD values were higher, especially in the sand-culture experiment, where the SPAD values divided the five genotypes into three or four distinct *Duncan*'s groups at most growth stages in comparison to two or three groups at S100. This indicated that SPAD measurements could detect variations among genotypes more efficiently under saline condition than in control conditions.

SPAD readings tended to increase over the growing season from ET to AN stage. In the sand-culture experiment, SPAD values reached a maximum of 58.4, 56.5, and 53.1 (averaged over the genotypes) at S0, S100, and S200, respectively. The corresponding values of SPAD readings for the field experiment were 53.3, 51.8, and 50.6. At GF stage, a significant decrease in SPAD readings was observed (from 3.5 to 6.8 units) in the sand-culture experiment; however, SPAD readings declined insignificantly (from 0.71 to 2.5 units) at the field experiment.

Calibration of the *Minolta* SPAD Chl meter: A highly significant positive correlation was observed between SPAD readings and TChl density among the genotypes (Table 6). Linear regression models were adequate in explaining the SPAD-TChl relationships with significant correlation coefficient values (53.6–9.7%).

The results indicated that the time of sampling did not alter the relationships between Chl content and SPAD readings. However, salinity treatments and experimental condition influenced the SPAD values (Table 6). Significant differences were found between the calibration models obtained at different salinity treatments. Line slope and interceptions (constants) were responsible for the significant differences between the regression models. The slope of the regression models were greater for control treatment whereas equations for saline condition had a fairly gentle slope suggesting that a smaller change in Chl content caused a greater change in SPAD readings. This implies that SPAD reading was more sensitive to variations of Chl content in saline condition.

Table 5. SPAD value of wheat genotypes at four growth stages in sand-culture and field experiments.

Experiment	Genotype /Growth Stage	Salinity level Nonsaline				Moderate salinity				High salinity			
		Early tillering	Late tillering	Anthesis	Grain filling	Early tillering	Late tillering	Anthesis	Grain filling	Early tillering	Late tillering	Anthesis	Grain filling
Sand culture	Bam	47.38^{b}	55.40^{b}	58.47^{b}	52.00^{a}	46.77^{b}	45.70^{ab}	56.23^{b}	53.24^{a}	48.42^{a}	46.27^{bc}	52.10^{bc}	48.45^{ab}
	H499.71A/JUP	50.78^{a}	57.47^{a}	60.47^{a}	53.71^{a}	51.14^{a}	47.40^{a}	58.17^{b}	54.46^{a}	48.89^{a}	49.87^{a}	61.13^{a}	50.06^{a}
	Ghods	47.37^{b}	54.97^{b}	57.97^{b}	51.25^{a}	50.51^{a}	44.82^{ab}	62.30^{a}	54.99^{a}	47.71^{a}	48.90^{ab}	54.70^{b}	47.41^{bc}
	PF70354/BOW	50.15^{a}	58.50^{a}	61.50^{a}	53.31^{a}	47.78^{a}	46.97^{a}	55.10^{b}	52.68^{a}	47.90^{a}	44.27^{c}	48.70^{c}	47.84^{bc}
	F78104	44.86^{b}	50.57^{c}	53.55^{c}	47.64^{b}	45.82^{b}	41.97^{b}	50.67^{c}	49.19^{b}	42.75^{b}	41.03^{d}	48.91^{c}	46.33^{c}
Field	Bam	47.82^{a}	47.27^{c}	54.35^{a}	52.28^{a}	49.16^{a}	47.32^{b}	51.79^{a}	53.26^{a}	48.74^{a}	47.77^{ab}	53.43^{a}	53.25^{a}
	H499.71A/JUP	47.47^{a}	53.72^{a}	56.00^{a}	50.84^{a}	49.53^{a}	51.18^{a}	53.11^{a}	52.97^{a}	48.14^{a}	49.73^{a}	50.89^{ab}	50.93^{a}
	Ghods	46.82^{ab}	49.75^{b}	54.68^{a}	51.01^{a}	49.41^{a}	50.67^{a}	55.15^{a}	51.61^{a}	46.97^{a}	46.67a^{b}	52.66^{a}	52.75^{a}
	PF70354/BOW	48.96^{a}	50.22^{b}	52.77^{a}	53.32^{a}	47.25^{a}	46.80^{bc}	52.69^{a}	50.16^{a}	46.95^{a}	48.80^{b}	48.87^{bc}	48.35^{ab}
	F78104	44.21^{b}	47.36^{c}	48.69^{b}	47.19^{b}	43.93^{b}	43.82^{c}	46.51^{b}	44.75^{b}	43.30^{b}	41.43^{c}	47.07^{c}	44.08^{b}

Discussion

A critical aspect of improving plant salinity tolerance is to identify intraspecific differences in growth or physiological traits under salt stress (Tavakkoli *et al.* 2010). Significant and high genotype-*vs*.-environment interaction for the yield has been long recognized as the main reason for lack of consistency in genotypic performance across different environments (Cooper and Delacy 1994). Therefore, the identification of superior genotypes is possible only when genotypes have consistent performance in a wide range of environments (Basford *et al.* 1996). Therefore, the use of physiological traits as an indirect selection would be important in increasing the effectiveness of yield-based selection procedures.

Yield and yield-associated traits are complex quantitative traits as they are controlled by multiple genes and are highly influenced by environmental conditions (Shi *et al.* 2009), but the inheritance of Chl characteristics may be less complex. Leaf Chl concentration is recognized as a trait with the high heritability in wheat (Lopes *et al.* 2012). The results of the current study indicated that Chl content was more stable than BM and GY across different growth conditions (salinity and growth media). Chl content might be therefore a promising candidate as a selection criterion for the yield under salt stress.

Salt stress induced the significant reduction in the GY in both the sand-culture and field experiments (Table 2). Clear differences were observed among the genotypes in terms of the studied traits in the sand-culture experiment. However, these differences were small or undetectable in the field experiment. The *Duncan*'s grouping of the genotypes for the GY in the field experiment showed that just the most salt-sensitive genotype (F78104) differed significantly from the other genotypes. This was probably because the field is a complex and unpredictable environment (Munns and James 2003). Thus, screening large numbers of genotypes for salt tolerance in the field is difficult due to spatial heterogeneity of soil properties and seasonal fluctuations in precipitation. Therefore, the interaction effects of these factors might be so high that they mask the small genotypic variations.

The findings of this study also indicated that Chl *a*, Chl *b*, and TChl density were reduced with increasing salinity in the sand-culture (Table 3) and field experiments (Table 4) which was in good agreement with the findings of other studies showing the reduction of Chl under salt stress (Flowers *et al.* 1985, Reddy and Vora 1986, Tammam *et al.* 2008, Chookhampaeng 2011). Also, it has been shown that the tolerant cultivars maintain higher Chl content than the sensitive ones (Munir *et al.* 2013). However, the result of our study was inconsistent with the finding of Hassan (2004) who found that salt stress had a stimulation effect on Chl content in wheat genotypes. Tammam *et al.* (2008) also found that Chl content of wheat was stable up to 120 mM NaCl, but a significant reduction was observed at the concentration of 180 mM NaCl onward.

Salinity-induced reductions in Chl content may rise from the inhibitory effects of salt on Chl synthesis or the acceleration of Chl degradation (Reddy and Vora 1986). As stated by Chookhampaeng (2011), the production of activated oxygen species which can damage Chl is enhanced in response to salt stress.

Zhang *et al*. (2010) reported that salinity induced swelling of chloroplast thylakoids and caused destructions of the chloroplast envelope. Biosynthesis of Chl is also inhibited when Na^+ and/or Cl^- concentrate in the chloroplasts (Santos 2004, Azizov and Khanisheva 2010). Although Chl *a* and Chl *b* have approximately similar structure and the biosynthesis of both Chls follows basically the same route (Ge 2012), both fractions are adversely affected by salinity. Such structural alterations are responsible for declining Chl under saline conditions.

The results of the study indicated that Chl *a* and Chl *b* were highly correlated (data not shown). However, the variation among the genotypes for Chl *a* was larger than that for Chl *b*. It is evident from the results (Table 4) that the studied genotypes were statistically similar in terms of Chl *b*. As stated by Gonzalez *et al.* (2010), physiological traits to be used in the selection of high-yielding genotypes under stress must show intraspecific variability. Therefore, due to lack of variability concerning Chl *b*, we did not consider it as a suitable indicator for selecting the superior genotype.

Chl *a* decreased more significantly than Chl *b* in leaves exposed to salt stress, especially in the field condition. Therefore, the decrease in TChl resulted mainly from the destruction of Chl *a,* which is more sensitive to salinity than Chl *b*. The larger destruction of Chl *a* compared to Chl *b* was reflected in the decreasing Chl *a/b* ratio with increasing salinity (Fig. 3) which was in agreement with results found in peanut (Hajar *et al.* 1993) and radish (Munir *et al.* 2013). The reduction of Chl *a* by salt stress implied a lowered capacity for light harvesting by Chl *a*-binding core antenna proteins (PSI reaction centre). Therefore, plant cells make a large investment in Chl *a*/*b*-binding LHC (PSII reaction centre) (Johnson *et al.* 2000).

The Chl *a/b* ratio from data reported by Azizov and Khanisheva (2010) revealed that this ratio changed from –17% to +34% in the leaves exposed to salt stress in different wheat genotypes. Meanwhile, there was no relationship between Chl *a/b* ratio and salt tolerance of wheat genotypes which is similar to the results found in our study. Therefore, the Chl *a/b* ratio failed to be used as the indicator of salt tolerance.

Table 6. Relationship between total chlorophyll (Chl) density and SPAD readings at different salinity levels in sand-culture and field experiments (n = 15). Y – total chlorophyll density; X – SPAD readings. **– significant at the 1% level.

Experiment	Salinity level	Regression equation	R^2	Total Chl density		SPAD readings	
				Min	Max	Min	Max
Sand culture	Nonsaline	y = 1.8134 x – 63.668	0.536**	26.13	54.12	49.10	63.20
	Moderate salinity	y = 0.6008 x + 0.536	0.588**	22.07	44.94	41.00	64.17
	High salinity	y = 0.8956 x – 24.974	0.797**	10.23	34.80	38.02	63.10
Field	Nonsaline	y = 1.1012 x – 27.56	0.769**	20.21	36.60	46.32	57.22
	Moderate salinity	y = 1.1106 x – 28.614	0.739**	14.28	35.36	41.02	56.55
	High salinity	y = 0.7096 x – 16.441	0.738**	11.03	25.31	40.21	54.82

Table 7. Correlation coefficients between total chlorophyll density and grain yields and SPAD readings and grain yields at three salinity levels in sand-culture and field experiments (n = 15). ns – not significant,*,**– significant at the 5 and 1% level, respectively.

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Experiments	Growth stage	Total chlorophyll density			SPAD		
		Nonsaline	Moderate salinity	High salinity	Nonsaline	Moderate salinity	High salinity
Sand culture	Early tillering	ns	ns	ns	0.823**	0.814**	0.865**
	Late tillering	0.878**	0.897**	0.879**	0.621**	0.719**	0.784**
	Anthesis	0.756**	0.786**	0.864**	0.411	0.777**	0.747**
	Grain filling	ns	ns	ns	0.811**	0.845**	0.790**
Field	Early tillering	ns	ns	ns	0.851**	0.810**	0.886**
	Late tillering	0.638**	0.849**	0.792**	0.400	0.723**	0.705**
	Anthesis	0.430	0.834**	0.848**	0.478	0.614*	0.741**
	Grain filling	ns	ns	ns	0.863**	0.859**	0.879**

The relationships between the yield and Chl concentration in crop plants under drought stress have been the subject of several studies (Arunyanark *et al*. 2008, Gholamin and Khayatnezhad 2011). Among abiotic stresses, drought and salinity may induce similar responses in plants. Both stresses cause a decrease of the total soil water potential which influences the water activity inside the cell (Shalhevet and Hsiao 1986). However, only a limited number of studies documented the Chl concentration as a screening tool in saline condition. In this regard, Khan *et al.* (2009) and Azizov and Khanisheva (2010) showed that higher Chl content may provide an index for superior GY under saline conditions.

As discussed by Arunyanark *et al.* (2008), traits that are well associated with the yield are ideal to be used as selection criteria. Likewise, the results of our study demonstrated that Chl content was associated with the GY under salt stress (Table 7). The higher Chl content in tolerant cultivars may be associated with the ability to repair salt-dependent damage (Ghogdi *et al.* 2012) or to provide an efficient mechanism for uptake of essential elements for Chl formation from the saline soil. Therefore, we could conclude that genotypes having higher Chl content under salt stress should produce more grains than those having lower Chl.

According to Hill *et al.* (2013), when two characteristics have some kind of shared genetic basis, they tend to exhibit correlated responses under selection. High correlation between Chl and GY in our study suggested that genes for Chl trait are likely located in regions where genes for the GY exist. According to Zhang *et al.* (2009), Chl *a* and *b* are known to be controlled mostly by three loci on chromosome 5D in wheat. Similarly, Börner *et al.* (2002) indicated that many agronomic traits are located in a similar location on chromosome 5D. Since these two traits are genetically linked together, high correlation between Chl and GY could be therefore expected.

In this study, the close association between SPAD meter and leaf Chl content (Table 6) proved that SPAD meter could be used for rapid and cost-effective assessment of relative Chl in wheat. Linear equations were suggested to convert SPAD meter readings into Chl concentrations. These results were generally consistent with findings of most studies which quantified the relationship between SPAD readings and Chl concentration mostly through linear models (Cassol *et al.* 2008, Ruiz-Espinoza *et al.* 2010) compared to the nonlinear ones (Uddling *et al.* 2007).

Our results indicated that the growth environment altered the relationships between Chl contents and SPAD readings (Table 6). Generally, many factors affect Chl meter readings. Variety differences, stage of growth, plant diseases, nutrient deficiencies, and almost every type of plant stress can affect the ability of the plant to maintain Chl. Hence, meter readings must be calibrated for each field, soil, and environment as also other researchers suggest (Giunta *et al.* 2002, Arunyanark *et al.* 2008). Under saline condition in both experiments, GY and

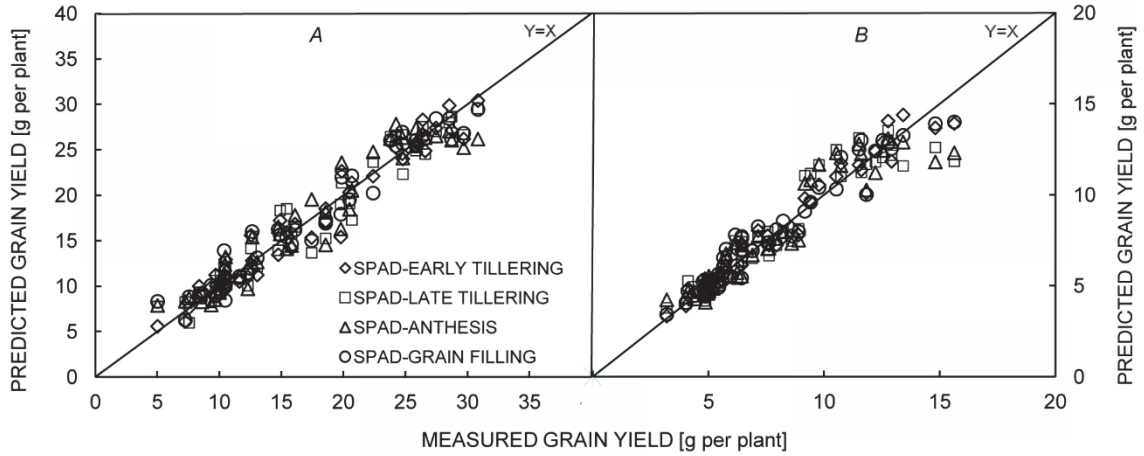

Fig. 4. Relationship between predicted grain yield computed from SPAD readings at four growth stages by linear regression model and measured grain yield in the sand-culture (*A*) and field (*B*) experiments.

Table 8. Correlation coefficient between SPAD readings of sand-culture and field experiments at different growth stages. *,**,***– significant at the 5,1 and 0.1% level, respectively.

Salinity level	Early tillering	Late tillering	Anthesis	Grain filling
Nonsaline (n = 15)	0.652**	0.529 *	0.731**	0.651**
Moderate salinity (n = 15)	0.574 *	0.508	0.675**	0.571*
High salinity (n = 15)	0.666**	0.655**	0.509	0.505
Pooled (n = 45)	0.636***	0.519***	0.571***	0.437**

SPAD reading showed correlated responses (Table 7). Simple regression models were devised for yields and SPAD values recorded at each growth stage; GYs were predicted using regression models. The measured GYs *vs*. the predicted grain yields were shown in Fig. 4. At all growth stages, SPAD predicted the GY with an acceptable accuracy, suggesting that SPAD can be measured at different times during the growth season due to its relationship with the GY; thus, it is possible to evaluate the wheat genotypes for their salinity tolerance at all growth stages.

In nonsaline condition, the relationship between SPAD and the GY was insignificant at AT stage in the sand-culture experiment as well as at LT and AT in the field experiment. Differences between the measured and predicted GY values were maximized at these two stages. However, the GY was still predicted with acceptable accuracy (data not shown).

This result also suggests that SPAD could be a reliable predictor of the GY regardless of temporal (time of SPAD reading) and spatial considerations (sand-culture and field experiments).

In our investigation, Chl content and SPAD readings had the highest values at the AT stage. This finding was consistent with the previous work of Ommen *et al.* (1999) who showed that the maximum Chl concentration occurred in the flag leaf of wheat at anthesis.

Growth stages affected leaf Chl content in some genotypes more than in the others; however, they had little effect on the overall ranking of genotypes (Tables 3, 4). Correlation coefficient analysis showed that SPAD values recorded at any growth stage significantly correlated with those at the other growth stages (data not shown). It confirmed a consistent selection of genotypes concerning the sampling time for Chl and SPAD under saline condition. Moreover, SPAD values measured at each stage in the sand-culture experiment positively correlated with their corresponding values in the field experiment (Table 8). Considering the fact that such association was not found for the biomass or GY, we could conclude that SPAD responses were consistent across different environments.

Most research concerning the salinity effect on crops has been conducted in controlled environments allowing scientists to better understand detailed responses to salinity and to diagnose mechanisms that plants use to manage salt stress (Läuchli and Grattan 2007). However, the extrapolation of results observed under controlled conditions may not correspond to those observed under field conditions (Shannon 1997). Under field condition, plants are exposed to spatial and temporal heterogeneity of soil salinity. As it was shown in Fig. 1, rainfall of *ca.* 240 mm occurred throughout the growing season; thus, the plants experienced periods of low to high salinity. Furthermore, in the field, additional plant traits, such as deep roots, are important (Munns and James 2003). Such factors can result in different rankings of genotypes between saline

field conditions and controlled environments. Therefore, it is vital that the plants that show particular promise under controlled conditions should be also evaluated under saline field conditions.

In evaluating the genetic merits of a genotype, it can be considered as thoroughly evaluated if the trial has been conducted in various environments (Saad and Rao 2001). In our study, wheat genotypes were evaluated concerning the SPAD value in two different environments (sand-culture and field) at four different growth stages. The results obtained using this indicator were consistent in both experiments. Thus, it is possible to use SPAD reading for the evaluation of wheat genotypes both in sand-culture and field conditions.

The effectiveness of SPAD meter as a screening criterion under abiotic stresses has been evaluated in a number of studies including moisture stress (Arunyanark *et al.* 2008), extreme temperatures (Balouchi 2010), and salt stress (Munns and James 2003, Atlassi Pak *et al.* 2009, Akhtar *et al.* 2010, Cuin *et al.* 2010). In the case of salinity stress, a close relationship was found between SPAD value and wheat leaf injury due to the salinity intensity (Munns and James 2003). The authors suggested that this simple and nondestructive Chl measurement could be a useful selection criterion to screen a large set of genotypes for salt tolerance. El-Hendawy *et al.* (2007) also found a significant positive relationship between SPAD values and the GY, indicating a variation among genotypes of wheat plant under saline conditions.

Consistent with the above findings, SPAD could be used to distinguish the salt-tolerant genotypes from the sensitive ones. However, salt stress is an important factor for calibration of SPAD meter. However, the data obtained from saline condition should not be pooled with those obtained in nonsaline condition; rather, separate equations should be fitted for saline and nonsaline conditions.

Conclusion: We concluded that salt stress significantly reduced biomass, GY, and the TChl content across the genotypes. However, salt-tolerant genotypes had higher yield and Chl content. Lower interaction effects of genotype *vs.* environment for Chl indicated that this characteristics was more stable than biomass and GY. A significant correlation between GY and Chl was found across salinity treatments, indicating that Chl content was a useful trait conferring salt tolerance in wheat. SPAD readings significantly correlated with the Chl content. However, salt stress affected the calibration of SPAD meter. In other words, separate Chl−SPAD equations should be fitted for saline and nonsaline conditions. This result suggests that SPAD reading could be used not only as a rapid and reasonably accurate tool for estimating the relative Chl status in wheat leaves but also for the indirect selection of high-yielding wheat genotypes under saline condition.

Although our investigation yielded useful findings regarding the effectiveness of Chl in wheat breeding program at saline condition, genetic factors associated with the Chl were left uninvestigated and should be a subject to future research.

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