Phenotyping of Salinity Tolerance in National Bio Resource Project-Wheat Core-Collection Lines

Mohammad Taheb Safi[1], Aziz Ahmad Osmani[2], Sayeed Qadir Danishiar[3]

[1, 2, 3]Departement of Biotechnology and Seed Technology, Agriculture Faculty, Kabul University, Jamal Mina, Kabul, 1006, Afghanistan
Corresponding author emailed: *taheb.safi2[at]gmail.com*
aziz.pahiz27[at]gmail.com
sqadir2014[at]gmail.com

Abstract: *Salinity and drought are major problems for wheat production in the world. More than 6% of the world's land is affected by salinity. Beside drought, salinity as well as affecting agricultural productivity in Afghanistan. Wheat (Triticum aestivum L.) is one of the major food crops in the Afghanistan. Elucidation of the morphological mechanisms involved in salinity tolerance is important. In this study, we used National BioResource Project (NBRP)-wheat core-collection, which consisted of 165 hexaploid wheat accessions, including landraces covering the geographical distribution and modern cultivars. In order to identify salt-tolerant accessions, we screened the core-collection based on the quantitative traits, including chlorophyll content (Chl), leaf number (LN), plant height (PH), and root length (RL) and we selected the Acc_ID 6x150 to 6x38 accessions as salt-tolerant. This study aims to recognize salt tolerant wheat genetic resources, which can then be used to accelerate wheat breeding for salt tolerance.*

Keywords: Wheat Core-Collection, Salinity Stress Tolerance, Hydroponic System, Quantitative Traits

1. Introduction

Soil salinity is a major abiotic stress that affecting more than 800 million hectares of land worldwide that accounts for more than 6% of the world's total land area [1]. Soil salinity beside drought is a major problem in many regions of Afghanistan [2]. Soil salinity can be determined by measuring the electrical conductivity (EC) of the soil saturation extract [3]. Soil is considered to be saline if the EC ≥ of 4 dS m-1 equivalent to about 40 mM NaCl, while the soils with EC's exceeding 15 dS m-1 are considered strongly saline [4]. Plants are affected by salinity in two ways, namely, osmotic stress and later causes ionic stress [1]. The former phase is caused by a high concentration of salt in soil that reduces capacity of plant roots to uptake water, which results in triggering a range of cellular and metabolic processes. Cell expansion, cell division, cell wall synthesis, protein synthesis, stomata conductance, and photosynthetic activity are all inhibited during the initial phase [1]. The later phase is associated with high concentration of salt within the plant itself can be toxic, which resulting in a prevention of many key physiological and biochemical processes such as nutrient uptake and assimilation. The destructive effects of soil salinity on the growth and development of crops include poor germination rates, reduced plant growth, low biomass, rare leaves number, less tillering and poor yields [5, 1].

Wheat (*Triticum spp.*) is the staple food crops that along with maize and rice provided two-thirds of global plant-derived food [6]. Wheat is grown in saline soil and its yield is seriously restricted by high salinity and other abiotic stresses [7]. Characterization and selection for improved salt tolerance in crops at seedling stages has been successfully used, for example, in rice [8], wheat [9], wheat-rye [10]. Selection based on combination of tolerance indices suggested by many researchers such as [10, 8, 11]. Furthermore, for selection based on a multiple salt tolerance index and combination of traits, principle component analysis is reported [10]. Characterization for chlorophyll

content (Chl) has also gained interest in plant abiotic stress research [12]. Chl under stress can be used as biochemical marker for selection salt tolerance genotypes [9]. Plant shoot growth is more sensitive to salt stress in contrast to the root growth [1]. The aim of this study was to recognize salt-tolerant genetic resources in hexaploid wheat using National Bioresource project (NBRP)-wheat core-collection lines.

2. Plant Materials and Methods

2.1. Plant materials

Plant materials used in this study were the collection of 165 accessions of National BioResource Project (NBRP)-Wheat hexaploid wheat core-collections [13, 14]. This core-collection includes165 wheat landraces and modern cultivars. Detailed information on accessions is available on the NBRP-Wheat database KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi/).

2.2. Growth condition and stress treatment

The method is based on a hydroponics system in which salt is added to the Murashige and Skoog (MS) solution and the seedlings are grown. We used the method as described in [15]with minor modification. Salt treatment commence when seedlings established in hydroponics after 2-weeks of normal growth at the 3-leaves stages. Seeds are germinated in petri dishes and 7-days-old young seedlings were transferred to 10-L basin with 1/5 diluted MS medium and grown for 14 days. Seedlings at the three-leaf stage were exposed to 150 mM NaCl with 1/5 MS diluted medium for two weeks. The MS solution was renewed every seventh day to prevent nutrient depletion.

2.3. Phenotypic traits measurements

We measured four phenotypic traits including chlorophyll contents (Chl), leaf number (LN), plant height (PH) and root length (RL) from 165 accessions under control and salt

treatment. PH (cm) was measured from the longest leaf's tip to crown and RL (cm) was measured from crown to the longest root tip. All traits were measured at four time points (0-d, 3-d, 7-d and 14-d) over salt stress treatment period. In this study, we used a soil-plant analyses development (SPAD)-502 plus chlorophyll meter (SPAD models 502; Minolta corporation, Ltd., Osaka, Japan) to estimate leaf Chl. From each plant, second leaves were targeted for Chl measurements. The second leaves Chl were measured on three points.

2.4. Salt tolerance indices

Salt tolerance indices have been widely used to define the varieties or genotypes that show superior performance under stress and non-stress conditions in wheat[16,17,18,8]. In this study, five salt tolerance indices including of stress tolerances (TOL), stress susceptibility index (SSI), stress tolerance index (STI), mean productivity (MP), and geometric mean productivity (GMP) were used. According to [8], these salt tolerance indices were adjusted on Chl, LN, SL and RL under control and salt treatment conditions.

Salt tolerance indices were calculated as below:
TOL = Y_p – Y_s[16]. The genotypes with low values of this index are more stable in two different conditions.
SSI = $[1 - (Y_s / Y_p)] / [1 - (\bar{Y}_s / \bar{Y}_p)]$ [17]. The genotypes with SSI < 1 are more resistant to salinity stress.
STI = $(Y_p) \times (Y_s) / (\bar{y}_p)^2$[18]. The genotypes with high STI values will be tolerant to salinity stress.
MP = $(Y_s + Y_p) / 2$ [16]. The genotypes with high value of this index will be more desirable.
GMP = $\sqrt{(Y_s \times Y_p)}$[18]. The genotypes with high value of this index will be more desirable.
Where, Y_s and Y_p represent chlorophyll content for each genotype in salt stress and control conditions respectively, and $\bar{Y}_s$ and $\bar{Y}_p$ are mean chlorophyll content in salt stress and control conditions, respectively, for all genotypes.

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) of the means of the different treatments and the Pearson correlation coefficient between quantitative traits were implemented using software program SPSS Corp. v 25.0 for windows. Salt tolerance indices were calculated using the Excel tool package. The principal component analysis (PCA) was performed on the quantitative traits and Y_p, Y_s and other salinity tolerance indices including TOL, SSI, YSI, MP, and GMP using the software XLSTAT [20].

3. Results

3.1. Phenotyping in NBRP-wheat core-collection lines

To assess the behavior under salt stress, we subjected the 165 hexaploid wheat accessions of NBRP-wheat core-collection to a constant salt stress at 3-leaves stages for two weeks. Subsequently, we recorded four phenotypic traits including chlorophyll contents (Chl), leaf number (LN), plant height (PH) and root length (RL) from each accession under control and salt treatment at four time points: 0-d, 3-d, 7-d, and 14-days over stress-treated. A one-way analysis of

variance (ANOVA) for quantitative traits showed significant differences $P<0.001$ between control and salt-treatment (Table 1). The obvious symptoms of salinity stress affects include reduce leaf area, necrosis and yellowish of old leaves, leaf rolling, subsequently leaf and seedling death over salinity period (Figure 1). Summary of the quantitative traits of the NBRP-wheat core-collection under control and salt stress is presented in (Table 2). Compared with their respective values in the control, the average values of Chl, LN, PH, and RL under salt stress treatment over 14-days were decreased by 58%, 32.6%, 12% and 27.8%, respectively, for the 165 wheat accessions (Figure 2). These suggested that salt stress treatment significantly affected all traits. The results of a Pearson correlation confession analysis among the quantitative traits in control and salt stress treatments showed positive correlations at 0.01 level between Chl salt with LN salt 0.39, PH control 0.28, PH salt 0.32 and RL salt 0.21 (Table 3).As mentioned above that correlation between Chl salt with other traits under salt treatment were positive. Therefore, we focused to select our salt tolerance accessions based on Chl under salt treatment.

Table 1: A one-way analysis of variance (ANOVA) of quantitative traits under control and salt stress

Trait	Source of variation	Sum of Squares	df	Mean Square	F	P-value
Chl	Between treatment	42555.3	1	42555.3	879.0	0.001
	Within genotype	15880.3	328	48.4		
	Total	58435.7	329			
LN	Between treatment	423.8	1	423.8	297.0	0.001
	Within genotype	468.0	328	1.4		
	Total	891.8	329			
PH	Between treatment	1771.7	1	1771.7	76.2	0.001
	Within genotype	7626.6	328	23.3		
	Total	9398.3	329			
RL	Between treatment	5192.7	1	5192.7	131.8	0.001
	Within genotype	12919.5	328	39.4		
	Total	18112.2	329			

Significant level = 0.001, df = degree of freedom, Chl = chlorophyll content, LN = leaves number, PH = plant height, RL = root length
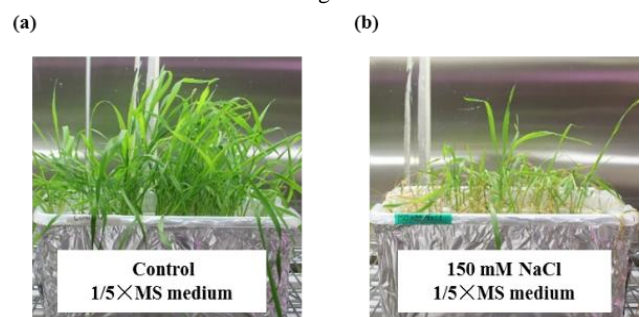

Figure 1: Evaluation of plant response against severe salinity stress

Salt stress was applied for 2-weeks. (a) and (b) indicate control and salt treatment (150 mM NaCl) respectively over 14-days.

Table 2: Summary of the quantitative traits of the NBRP-wheat core-collection lines under control and salt stress treatment conditions

Trait	Min.	Max.	Mean	S.D.
Chl control	33.3	50.0	39.2	2.6
Chl salt	4.2	35.5	16.5	9.5
LN control	4.3	11.7	6.9	1.4
LN salt	3.3	10.3	4.7	0.9
PH control	22.1	49.3	38.9	5.0
PH salt	21.8	45.3	34.3	4.7
RL control	10.7	56.0	28.5	7.3
RL salt	9.9	38.7	20.6	5.0

Seedlings at the three-leaf stage were exposed to 150 mM NaCl for two weeks. Data are mean of three replicates.

Min = minimum, Max = maximum, S.D.= standard division.

Chl = chlorophyll content, LN = leaf number, PH = plant height, RL = root length

3.2. Evaluation of traits using salt tolerance indices and principle component

To identify accessions which show wide response under control and stress conditions, we computed the quantitative data using the salt tolerance indices. Tolerance indices have been used by many investigators for assessment of wheat accessions under control and salt-stressed conditions. Based on the indices, accessions with the highest value for each of the criteria Ys, Yp, STI, MP, and GMP received a rank of 1, while for accessions with the lowest value for each of the indices TOL and SSI received a rank of 1 and such accessions considered as a salt-tolerance and other susceptible. Computing the SSI of Chl revealed that 68 accessions out of 165 accessions had SSI < 1. Based on the SSI of LN, 91 accession had SSI < 1, and estimation of the SSI of the PH and RL showed that 81 and 79 accessions out of 165 accessions had SSI < 1, respectively. The summary of salt tolerance based on Chl is presented in (Table 4). The estimated value of salt tolerance indices for Chl showed that the selection of salinity tolerant accessions based on a single criterion was contradictory. Because different indices introduce differner accessions as salt-tolerant.

To recognize the superior salt tolerant accessions based on the combination of multiple salt tolerance indices, total rank of the all indices were estimated and based on this criterion the most desirable salt tolerant accessions were identified. In consideration to all indices, Acc_ID 6x150, 6x67, 6x113, 6x50, 6x95, 6x46, 6x18, 6x2, 6x136, 6x38, 6x22, 6x99, 6x65, 6x128, 6x31, and 6x55 were identified as the most salt-tolerant accessions.
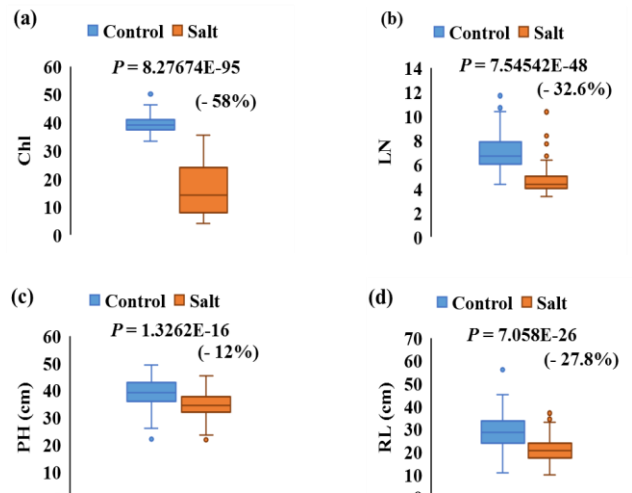

Figure 2: Effect of salt stress treatment on wheat at 14-days. Blue and orange boxes indicate control and salt treatment respectively. In each box and whisker plot, the center values are the medians. The bottom and top edges of the boxes indicate the twenty-fifth and seventy-fifth percentiles. Whiskers mark the range of the data, excluding outliers. Significant differences, t-test, $p < 0.05$ ($n = 165$).

Table 3: Pearson correlations coefficient among quantitative traits under control and salt stress treatment over 14 days of salt treatment

Traits	Chl control	Chl salt	LN control	LN salt	PH control	PH salt	RL control	RL salt
Chl control	1							
Chl salt	-0.060	1						
LN control	0.076	-0.015	1					
LN salt	-0.126	.391**	.427**	1				
PH control	0.048	.283**	0.103	-0.027	1			
PH salt	0.083	.319**	0.085	-0.006	.813**	1		
RL control	0.032	-0.005	.285**	0.143	.303**	.229**	1	
RL salt	0.004	.212**	.187*	.278**	.302**	.306**	.781**	1

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

Principal component analysis (PCA) is one of the most wildly used techniques to measure the relationship between the indices and accessions. Accordingly, selection based on a combination of multiple salt tolerance indices may provide useful criteria for improving salt tolerance of genetic resources.

To further confirm the estimated results of indices, PCA was constructed for Chl on salt tolerance indices under both control and salt-stressed conditions. The PCA biplot accounted for 99.7% of total variation based on the Chl data under control and salt stress conditions (Figure 3).
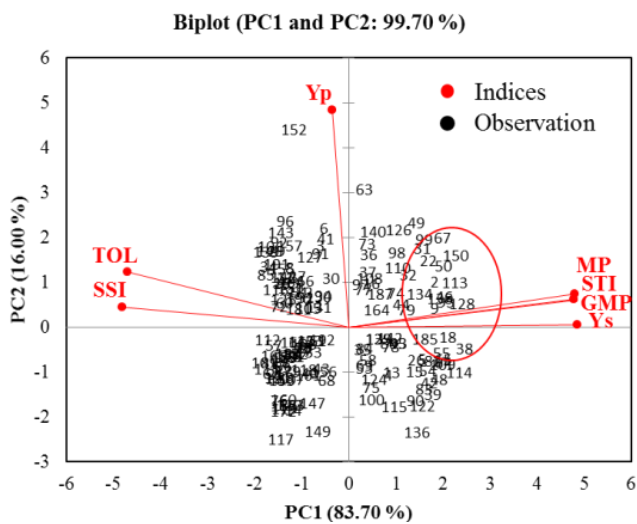
Figure 3: Principle components analysis indicates relationships between chlorophyll content control Yp and salt stress Ys, and salt tolerance indices. The observations marked inside red ellipse indicates salt tolerance accessions. (n = 165). TOL = stress tolerances, SSI = stress susceptibility index, STI = stress tolerance index, MP = mean productivity, GMP = geometric mean productivity.

According to this analysis, strong positive correlations were found between the Ys, STI, MP and GMP indicating that they are the same in ranking of accessions. This group of indices were negatively associated with TOL and SSI. No relation was found between Chl under stress (Ys) and control (Yp) conditions, as indicated by the angle between these two indices was above 90 degrees. The high salt-tolerant accessions were marked inside red circle on PCA on positive dimension. These results suggested that selection for salt tolerance accessions based on the combination of all quantitative traits under salt stress and control conditions is the more effective way.

Table 4: List of 15 high ranked salt tolerance NBRP-wheat accessions based on salt tolerance indices that calculated for chlorophyll content stress over 14-days salt treatment

Acc_ID	Yp	Rank	Ys	Rank	TOL	Rank	STI	Rank	MP	Rank	GMP	Rank	SSI	Rank	Total Rank	Overall Rank
6x150	42.0	27	35.5	1	6.5	19	1.0	1	56.5	1	38.6	1	0.3	15	65	1
6x67	43.0	13	33.6	9	9.4	29	0.9	2	55.1	2	38.0	2	0.4	28	85	2
6x113	39.9	60	34.3	3	5.5	13	0.9	4	54.3	3	37.0	4	0.2	11	98	3
6x50	41.8	29	33.3	10	8.5	27	0.9	3	54.3	4	37.3	3	0.4	26	102	4
6x95	39.3	68	34.3	2	5.0	9	0.9	5	54.0	5	36.7	5	0.2	9	103	5
6x46	39.7	63	34.0	6	5.7	14	0.9	6	53.8	6	36.7	6	0.2	13	114	6
6x18	38.7	87	34.2	4	4.5	6	0.9	9	53.5	7	36.3	9	0.2	5	127	7
6x2	40.0	52	33.2	13	6.8	23	0.9	7	53.2	9	36.4	7	0.3	19	130	8
6x138	39.7	64	33.3	12	6.3	16	0.9	8	53.2	8	36.4	8	0.3	17	133	9
6x38	37.7	113	34.0	5	3.7	1	0.8	10	52.8	10	35.8	10	0.2	1	150	10
6x22	41.7	31	30.7	22	11.1	31	0.8	11	51.5	17	35.8	11	0.5	31	154	11
6x99	43.0	14	29.7	26	13.3	36	0.8	13	51.2	19	35.7	13	0.5	34	155	12
6x9	39.0	75	32.7	16	6.3	17	0.8	14	52.2	15	35.7	14	0.3	18	169	13
6x65	37.7	114	33.7	8	4.0	4	0.8	15	52.5	11	35.6	15	0.2	4	171	14
6x128	39.5	67	32.3	18	7.2	24	0.8	12	52.1	16	35.7	12	0.3	22	171	15

Acc_ID = accessions ID, Yp = Chlorophyll content control, Ys = Chlorophyll content salt stress, TOL = stress tolerances, SSI = stress susceptibility index, STI = stress tolerance index, MP = mean productivity, GMP = geometric mean productivity

4. Discussion

4.1. Assessment of salt tolerance in NBRP-wheat core-collection lines

The hexaploid 165 NBRP-wheat accessions that collected from various geographical regions were evaluated at seedling stage under control and 150 mM NaCl solution in order to study the phenotypic and genetic variation within the core-collection and to recognize the potential salt-tolerant wheat accessions. Quantitative traits showed significant differences $P<0.001$ between control and salt-treatment. Accumulation of high concentration of salt in cell walls or cytoplasm when the vacuoles can no longer sequester incoming salts that causes leaves injury, leaves yellowish, leaves death and later leads to plant death [1]. In our cause, the visual symptoms of salinity stress affects include reduced leaf area, leaf necrosis and yellowish, leaf tip death, leaf rolling and subsequently seedling death over salinity stress (Figure 1). Compared with their respective values in the control, the average values of Chl, LN, PH,

and RL, under salt stress treatment over 14-days were decreased by 58%, 32.6%, 12% and 27.8%, respectively for the whole accessions (Figure 2). Among these, Chl was extremely effected, while, LN and RL were moderately and PH was slightly effected by salt stress treatment. Furthermore, salt tolerance accessions maintained relatively high Chl in contrast to susceptible accessions. These results are in agreement with [9], who reported that decrease in Chl under salinity stress is observed more in salt sensitive genotypes in comparison to tolerance cultivars.

According to the Pearson correlation confession analysis (Table 3) of the quantitative traits in control and salt stress treatments, there were positive correlations between Chl salt with LN salt, PH control, PH salt and RL salt. A significant negative correlation between Chl control and other traits in control condition, and the correlation coefficient values were lower and negative with traits under salt-stress condition. The Pearson correlation confession analysis suggested that all traits, except for Chl control, exhibited salt-stress response. As discussed above that correlation

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between Chl salt with other traits under salt treatment were positive. Therefore, selection based on Chl criterion under salt stress will lead us to towards high salt-tolerant accessions [21, 22].

4.2. Screening of salt tolerant lines

Salt tolerance indices have been widely used by many investigators for the assessment of wheat genotypes under control and salt-stressed conditions [9, 11]. An accession with the highest value for each of the criteria Ys, Yp, STI, GMP, and MP received a rank of 1, while for accessions with the lowest value for each of the indices SSI, and TOL received a rank of 1 and considered as a salt-tolerance accessions [23,11]. Computed indices suggested that selection for salt tolerance accession based on the Chl under salt stress is the more effective way. SSI is successfully used for identifying superior rice genotype at germination stage under salt stress [24], for selecting salt tolerant wheat) [23], and for identifying resistant genotypes to stresses [17]. Hence, to recognize the superior salt tolerant accessions based on the combination of multiple salt tolerance indices, total rank of the all indices were estimated and based on this criterion, 20 salt tolerance wheat accessions out of 165 were identified (Table 4). Results were in agreement with [10] who screened a set of wheat-rye by using principal component analysis for resistant line under drought and [11], who evaluated 10 accessions of wheat genotypes under salt stress.

For selection based on a multiple salt tolerance index, PCA is one of the most wildly used techniques to measure the relationship between the indices and accessions [10]. To further confirm the estimated results of indices, PCA was constructed for Chl on salt tolerance indices. According to PCA biplot (Figure 3), strong positive correlations were found between the Ys, STI, MP and GMP indicating that they are the same in ranking of the selected accessions. The same result was reported [10].

5. Conclusion

There were apparent effects of the salt treatment that caused the leaf scorch in all wheat accessions. A one-way analysis of variance (ANOVA) for quantitative traits showed significant differences ($P<0.001$) between control and salt-treatment. The data suggested that salt stress treatment significantly affected all traits. Among these, Chl was extremely effected, while, LN and RL were moderately and PH was slightly affected by salt stress treatment. Moreover, salt tolerant accessions showed less leaf damage in contrast to susceptible wheat, as indicated by their Chl content. Furthermore, salt tolerance accessions maintained relatively high Chl in contrast to susceptible accessions. We could identify a total of 15 out of 165 accessions of NBRP-wheat core-collection as salt tolerance with preference of Acc_ID6x150 to 6x38 as the candidates of salt-tolerant. The salt tolerant accessions were examined three times and the same results were yielded. Altogether, these results disclosed the genetic diversity within NBRP-wheat core collection based on the phenotypic parameter measurements. Identification and selection of potential salt tolerant wheat accessions are useful to improve salt tolerance in breeding programs.

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