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Identification of Durum Wheat Salt Tolerance Sources in Elite Tunisian Varieties and a Targeted FIGS Subset from ICARDA Gene Bank: Non-Destructive and Easy Way

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Abstract

The success of durum wheat breeding program for salt tolerance improvement depends on sources of tolerance, the screening method and the selection of target traits. In this study, we used morpho-physiological traits to elucidate the phenotypic and genetic variation in salinity tolerance of a 50 internationally derived durum wheat genotypes. Four Australian lines containing salt tolerance Nax genes from CSIRO (The Commonwealth Scientific and Industrial Research Organisation in Australia); six Tunisian old and new cultivars (Kerim, Khiar, Maali, Mahmoudi, Nasr and Selim) and forty ICARDA's gene bank landraces selected basing on FIGS Method (Focused Identification of Germplasm Strategy) were evaluated in semi controlled conditions at the INRAT Ariana experimental station. Significant genotypic variation and Pearson's correlations were found among the evaluated traits. The data were converted to salt tolerance indexes (STI) before statistical analysis.

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The high positive and significantly correlation of STI of grain yield and those of tillering (r=0.46), mean daily evapotranspiration (r=0.46), shoot dry weight (r=0.74), number of spikes per plant (r=0.74), spike length (r=0.30), thousand grain weight (r=0.36) and the chlorophyll content at 79 day after sowing (r=0.30) indicated that salt stress induces a high reduction in these parameters, leading to the reduction in grain yield. Therefore we can consider these parameters as the most relevant for salinity tolerance screening criterion in durum wheat breeding programs. Among the analysed genotypes the ICARDA's landrace IG-85714 from Greece showed better performances under salt stress. Among the analysed Tunisian varieties Maali and Nasr exhibited some level of tolerance. Approximately half of the analysed genotypes showed a moderate to high level of salt tolerance. These are the first sources for the salt tolerance in durum wheat identified in the ICARDA gene bank. This demonstrated that FIGS was effective for sampling large ex situ germplasm collections when seeking novel genetic sources of salt tolerance.

Keywords: traits; screening; salinity; durum wheat; landraces; gene bank.

1. Introduction

Salinity causes serious yield losses in wheat in many parts of the world. Among several abiotic environmental stresses, salinity is a major threat to the agricultural sustainability which adversely affects more than 800 million hectares of land worldwide that account for more than 6% of the global land mass [1]. Approximately 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity [2]. It has been estimated that more than 50% of the arable land would be salinized by the year 2050 [3]. For all important crops, average yields are only a fraction – somewhere between 20% and 50% of record yields; these losses are mostly due to drought and high soil salinity, environmental conditions which will worsen in many regions because of global climate change [2]. Salt stress affect almost all growth, development and yield components parameters resulting in a reduction in its yield [2, 4, 5, 6]. High salinity affects plants in several ways: osmotic stress [7, 8, 9, 10, 11], ion toxicity [12, 13] alteration in soluble carbohydrate content [14], nutritional disorders [8], oxidative stress [15], alteration of metabolic processes [16], membrane disorganization [17], reduction of cell division and expansion [9], changes in stomatal conductance [18, 19, 20], changes in photosynthetic assimilates or components such as enzymes, chlorophylls and carotenoids [21, 22]. Together, these effects reduce plant growth, development and survival.

Worldwide, extensive research is being carried out, to develop strategies to cope with abiotic stresses, through development of salt and drought tolerant varieties [23]. One major approach to generate salt-tolerant wheat varieties through breeding is to maximize the morpho-physiological genetic diversity between parental genotypes before intercrossing [24]. Many traditional landraces that can withstand high levels of salinity are good candidates for breeding salt-tolerant cultivars [25]. However, due to their undesirable agronomic traits, these landraces are not used. Until now, breeders have not fully succeeded in combing through huge gene bank collections to identify these useful genotypes. Moreover the morpho-physiological approach for screening salt tolerance of such collections of wheat genotypes can be costly, space- and time-consuming and labour-intensive. According to the ICARDA (International Center for Agricultural Research in the Dry Areas – cigar) the new 'FIGS' tool – the Focused Identification of Germplasm Strategy — allows gene bank managers and agricultural

researchers worldwide to screen large plant genetic resource collections more rapidly and accurately than was previously possible using traditional methods. FIGS combines both the development of a priori information based on the quantification of the trait-environment relationship and the use of this information to define a best bet subset of accessions with a higher probability of containing new variation for the sought after trait(s) [26]. The FIGS approach uses sophisticated algorithms that match plant traits with agro-climatic characteristics for more precise and rapid pinpointing of high-potential traits and genotypes.

The inherent subjectivity and the quantitative nature of salinity tolerance complicate the evaluation for salinity tolerance [25]. Selection on a quantitative trait with continuous polygenic variation based on several traits is likely to be more effective than selection based on single trait. Using a single specific physiological trait in salt tolerance screening is not sufficient, because no single process can account for the variation in plant response to salinity [27]. Identifying the multiple parameters associated with salt tolerance during different growth stages is important for evaluating wheat genotypes and improving their salt tolerance [4]. Besides the assessment of the reliability of physiological traits, however, it is also necessary to assess if they are quick, easy and economic techniques for screening [27].

The objective of this study was to examine the performance of specific agronomic and physiological traits as screening criteria for salt tolerance and to identify sources of tolerance in durum wheat landraces in the ICARDA gene bank using FIGS.

2. Materials and methods

Fifty genotypes (Table 1) of durum wheat (*Triticum durum Desf.*) from Fifteen different countries (Algeria, Australia, Egypt, Ethiopia, Greece, India, Iran, Iraq, Jordan, Morocco, Oman, Syria, Tunisia, Turkey, Uzbekistan) were tested under salinity treatment and control: Four Australian lines containing salt tolerance Nax genes from CSIRO (The Commonwealth Scientific and Industrial Research Organisation in Australia); six Tunisian old and new cultivars (Kerim, Khiar, Maali, Mahmoudi, Nasr and Selim) and forty ICARDA's gene bank landraces selected basing on FIGS Method (Focused Identification of Germplasm Strategy). The FIGS subset was selected as described in [28]. Among the FIGS subsets 35 landraces were selected as putative salt tolerant and 5 were randomly selected basing on recorded passport data (Table 1).

The 50 genotypes (3 plants/tube) were grown under semi-controlled conditions in a rainout shelter during the 2013/2014 growing season in 12 litres tube (1m length x 0.125m diameter) filled by a mixture of ¼ of Peat Moss and ¾ loamy sand soil collected from the soil surface (0–15 cm) at the Ariana Experimental Station of INRAT. The soil was air-dried, ground, passed through a 5 mm mesh screen, and thoroughly mixed. The green house experimental conditions are the same as in [29]. The pots were placed on carts so as they could be moved under the shelter when it rains. Each group of tubes placed on a cart was surrounded with polystyrene to avoid temperature gradients between the tubes in the borders and those in the centre. The experiment was conducted in triplicate with a completely randomised design.

Table 1: Analysed genotypes, ICARDA code (IG), site code, variety or line name and type of subset (Salinity= putative salt tolerant, Random=random subset)

Order	ICARDA_ig, genotype number	Site_code or genotype name	Subset type	origin
1	IG-89017	ETH64:131	Random	Ethiopia
2	IG-96203	MAR87-1:31	Random	Morocco
3	IG-43330	OMN87:142	Random	Oman
4	IG-95853	SYR87-1:55	Random	Syria
5	IG-94651	TUN77:9	Random	Tunisia
6	IG-93977	DZA75:43	Salinity	Algeria
7	IG-93963	DZA75:43	Salinity	Algeria
8	IG-93978	DZA75:43	Salinity	Algeria
9	IG-93151	DZA75:95	Salinity	Algeria
10	IG-87457	EGY:12	Salinity	Egypt
11	IG-83479	EGY-S55-2	Salinity	Egypt
12	IG-83477	EGY-S55-1	Salinity	Egypt
13	IG-87438	EGY-S56	Salinity	Egypt
14	IG-83366	EGY-S57	Salinity	Egypt
15	IG-85847	ESP-S1603	Salinity	Spain
16	IG-85846	ESP-S1603	Salinity	Spain
17	IG-85020	ESP-S1946	Salinity	Spain
18	IG-85028	ESP-S1947	Salinity	Spain
19	IG-85714	GRC56:11	Salinity	Greece
20	IG-85715	GRC56:12	Salinity	Greece
21	IG-84830	IND47/48:45	Salinity	India
22	IG-84882	IND47/48:6	Salinity	India
23	IG-86075	IND-S413	Salinity	India
24	IG-85632	IRN-S235	Salinity	Iran
25	IG-85457	IRN-S406	Salinity	Iran
26	IG-83091	IRQ-S176	Salinity	Iraq
27	IG-96252	JOR83-2:46	Salinity	Jordan
28	IG-96367	MAR85:112	Salinity	Morocco
29	IG-95843	SYR87-1:49	Salinity	Syria
30	IG-95839	SYR87-1:49	Salinity	Syria
31	IG-96150	SYR88-2:2	Salinity	Syria
32	IG-84454	TUR48:255	Salinity	Turkey
33	IG-84776	TUR48:588	Salinity	Turkey
34	IG-82878	TUR48D:1	Salinity	Turkey
35	IG-82738	TUR48D:242	Salinity	Turkey
36	IG-82181	UZB:10	Salinity	Uzbekistan

37	IG-82233	UZB-S149	Salinity	Uzbekistan
38	IG-82553	ESP27:46	Salinity	Spain
39	IG-82635	IRN40:12	Salinity	Iran
40	IG-95836	SYR87-1:49	Salinity	Syria
41	var01	Mahmoudi	Random	Tunisia
42	var02	Nasr	Random	Tunisia
43	var03	Selim	Random	Tunisia
44	var04	Kerim	Random	Tunisia
45	Line01	NAX1_027	Random	Australia
46	Line02	NAX1_207	Random	Australia
47	Line03	NAX2_041	Random	Australia
48	Line04	NAX2_042	Random	Australia
49	var05	Khiar	Random	Tunisia
50	Var06	Maali	Random	Tunisia

Two treatments were used, a saline treatment (150 mM NaCl) and a control (no NaCl added). The salinity treatment was initiated at the three-leaf stage. For irrigation management and monitoring the field capacity and permanent wilting point were determined by using pressure plate (extractor) apparatus. The control of soil moisture was done by weighting the pots between two successive irrigations. Each pot was weighed before each irrigation event. The amount of irrigation water to be applied was determined by weighing the pots just before irrigation. The irrigation was done when reaching a decrease ½ of total available water (holding) capacity. The amount of water added is that to reach 80% of field capacity. Evapotranspiration volume (ET) between two consecutive irrigations was calculated by using the water balance method. The leaching fraction, the amount of drainage and rainfall was taken as zero since the pots were sheltered and not howled. The daily evapotranspiration (mm) was calculated by dividing the determined ET volume for the irrigation interval by soil surface area and the number of days between the irrigations [30]. Agro-physiological measurements were conducted at different growth stages. The height of the main shoot of each plant was measured with a ruler at 60, 90 and 120 DAS. Chlorophyll (Chl) content of different leaves (base, centre and flag leaves) was measured at 60, 90 and 120 DAS. In this protocol the rate of Chl was estimated per unit SPAD.

Three different measurements were performed at the base, the centre and apex of the leaf using a portable Minolta SPAD 502 Meter. Tiller number was recorded at 150 DAS. After harvesting, shoots were oven-dried at 70°C for 48 h to determine the dry weight (DW). The number of spikes/plant, the number of spikelets/spike, the grain number, the grain weight/spike and the 1000-grain weight were also determined at final harvest (150 DAS). The data were also converted to a salt tolerance index (STI) to allow comparisons among genotypes for salt sensitivity. STI was defined as the observation at salinity divided by the average of the controls [4]. This index reflects the reduction percentage of the trait. Analysis of variance (ANOVA) was performed using Statistica 5.0 v. '98 Edition.

3. Results

Salinity affected all of the agro-physiological parameters measured at different growth stages. Significant differences among genotypes were observed for majority of traits investigated (Tables 2, 3, 4, 5 and 6).

3.1. Growth and Development Traits

The values for tiller number, shoot dry weight and plant height at different stages for the salinity treatment varied significantly (Table 2) from those of the control. The flowering and heading dates were not significantly affected by salinity (Table 2). The salt-tolerance indexes (STI) of tiller number, plant height at 115 and 159 DAS, and heading date varied significantly among the analysed genotypes (Table 2).

Mean tiller number for some genotypes in the salinity treatment exceeded that of the control (Fig. 1). These genotypes (DZA93977, DZA93963, EGY87457, EGY83477, EGY-83366, ETH89017, GRC85714, IND84882, IRN82635, JOR96252, ESP85028, ESP82553, SYR95853, SYR95843, Maali, TUR82738, UZB82233) are originated from Algeria, Ethiopia, Egypt, Greece, India, Iran, Jordan, Spain, Syria, Tunisia, Turkey and Uzbekistan. For the rest of the analyzed genotypes the tiller number in the salinity treatment was lower by an average of 24.2% compared to that of the control.

Table 2: Variance Analysis of growth and development traits

Source of variation	df	Tiller	SDW	H68	H101	H115	H159	Flower	Heading
Trait									
Genotype (G)	49	2,83***	22,55***	199,72***	304,57***	775,18***	1348,59***	792,20***	1342,53***
Salinity (S)	1	15,48***	570,51***	157,61**	6275,98***	10977,92***	9836,5***	44,08	61,65
G x S	49	1,18	8,41	31,77	45,01	80,52	98,74	22,79	29,72
Error	200	1,03	9,39	53,36	75,18	76,2	81,92	25,55	25,99
STI									
Genotype (G)	49	0.17***	0.08	0,017	0.01	0,01**	0.014***	0.003	0.006**
Error	100	0.08	0.07	0.016	0.01	0.006	0,006	0.002	0.003

Note: Tiller = tiller number; SDW = shoot dry weight (g); H60 through H150 = plant height (cm) at 60 through 150 DAS, respectively; flower = flowering date, heading = heading date; RDW = root dry weight (g/plant). *,**, and *** indicate significance at 0.05, 0.01, and 0.001 levels, respectively.

The STIs of tiller number ranged from 0.43 (NAX1_207 from Australia) to 1.44 (GRC85714 from Greece). For tiller number, NAX1_207 was most affected by salinity and GRC85714 was least affected. For the STI of tiller number the Newman Keul classification revealed 14 significantly distinct classes of genotypes. The first class of genotypes having the lowest STI is composed of the line NAX1_207 (STI=0,43) and other twenty six varieties

belonging to both the first and second class. The last class of genotypes having the highest STI is composed of the variety GRC85714 (STI=1.44) and other sixteen varieties belonging to both the 13th and 14th class.

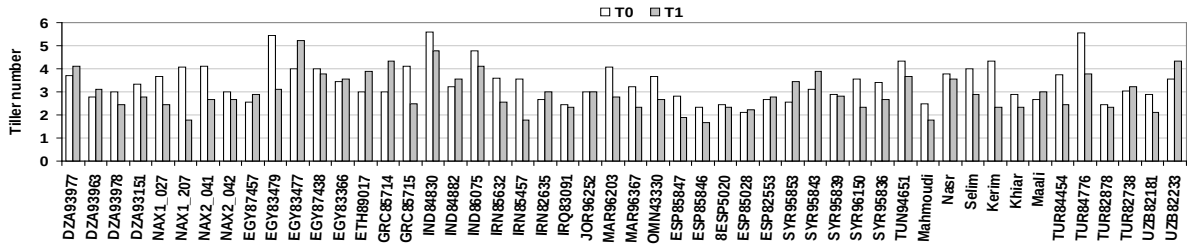

Figure 1: Effect of salinity treatment on tiller number (T1: salinity treatment, T0: control). Genotypes from ICARDA are illustrated by country code followed by the ICARDA genotype code (IG).

For some genotypes (DZA93977, EGY87457 , GRC85714, IND84882, SYR95853, UZB82233, Maali and Nasr) the mean shoot dry weight (SDW) was higher in salinity treatment compared to the control. These genotypes are originated from Algeria, Egypt, Greece India Syria, Tunisia and Uzbekistan. For the rest of the analyzed genotypes the mean SDW in the salinity treatment was reduced by 23,14% compared to the control. The STI of SDW ranged from 0.53 (TUR84454) to 1,27 (GRC85714). For SDW, TUR84454 was most affected by salinity and GRC85714 was least affected (Fig. 2). For the STI of tiller number the Newman Keul classification based on STI of shoot dry weight revealed 14 significantly distinct classes of genotypes. The first class of genotypes having the lowest STI is composed of the varieties TUR84454 (STI=0,43), TUR84776 (STI=0,43) and other forty genotypes belonging to both the first and second class. The last class of genotypes having the highest STI is composed of the variety GRC85714 (STI=1.44) and other seventeen genotypes belonging to both the 13th and 14th class.

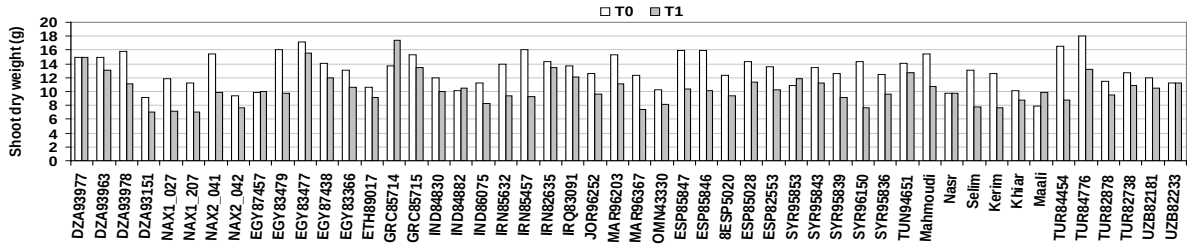

Figure 2: Effect of salinity treatment on shoot dry weight (T1: salinity treatment, T0: control).

Mean plant height in the salinity treatment was reduced by 2.4%, 10.33%, 12.19%, and 11.48%, respectively, at 68, 101, 115, and 159 DAS compared to the control. At 68 DAS, the STI of plant height ranged from 0.81 (UZB82181) to 1.14 (GRC85714). At 101 DAS, the STI of plant height ranged from 0.78 (DZA93977) to 1.06 (GRC85714). At 115 DAS, the STI of plant height ranged from 0.73 (EGY83477) to 1.12 (GRC85714). At 159 DAS, the STI of plant height ranged from 0.75 (TUR82738) to 1.05 (Nasr). The IG-85714 had the highest STI from 68 DAS to 115 DAS. At 159 DAS the IG-85714 has also a high STI (0.94). This genotype originating from Greece seems to have the least affected plant growth (Plant height and SDW) under salinity stress.

The flowering date was in some cases earlier in the salinity treatment and later in other cases, compared to the control. The flowering date in average was earlier (Australian line NAX2_041) or later (GRC85714) by a maximum of 7 days. The heading date also was in some cases anticipated in the salinity treatment and delayed in other cases compared to the control. The heading date in average was anticipated by a maximum of 9 days (ESP85020) and was delayed for a maximum of 8 days (DZA93977) in salinity treatment compared to the control.

The values for root dry weight (RDW), root volume (RV) and root surface (RS) for the salinity treatment varied significantly from those of the control (Table 3).

Table 3: Variance Analysis of root dry weight (RDW); root volume (RV) and root surface (RS)

Source of variation	df	RDW	RV	RS
Trait				
Genotype (G)	49	0,38***	31,26***	2,6'E+10***
Salinity (S)	1	3,82***	308,22***	2,6'E+11***
G x S	49	0,09**	9,61	1,1'E+10***
Error	200	0,05	7,68	5,5'E+09
STI of the trait				
Genotype (G)	49	0,26***	0,26***	0,65***
Error	100	0,08	0,1	0,13

*,**,***_/significant at 0.05, 0.01 and 0.001 levels, respectively

The mean root dry weight (RDW) of some genotypes (Line Nax1_207, Line Nax2_041, EGY83479, EGY83477, JOR96252, TUN94651, Nasr, Maali, TUR82878) in the salinity treatment exceeded that of the control (Fig. 3). For the rest of the analysed varieties the root dry weight in the salinity treatment was reduced by 32% compared to the control.

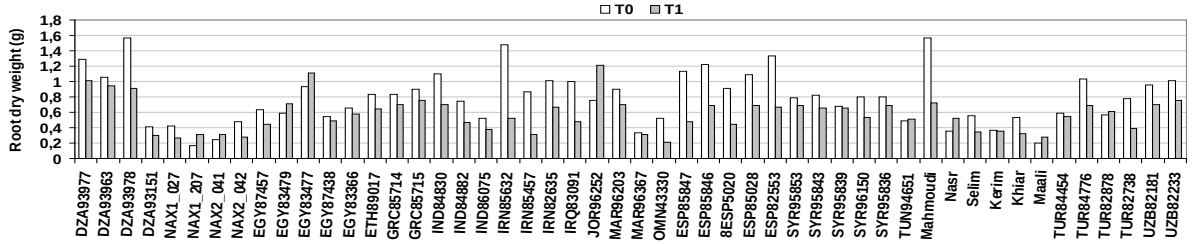

Figure 3: Effect of salinity treatment on root dry weight (T1: salinity treatment, T0: control)

The STI of RDW ranged from 0.35 (IRN85632) to 1.84 (Line NAX1_207). For RDW the IRN85632 was the most affected by salinity and the Line NAX1_207 was the least affected.

For the STI of root dry weight the Newman keuls classification revealed fourteen significantly distinct classes of

genotypes. The class of genotypes having the lowest STI is composed of the varieties IRN85632 (STI=0,35), IRN85457 (STI=0,36) and other 28 varieties belonging to either the first and the second class. The last class of genotypes having the highest STI is composed of the Tunisian variety Nasr (STI=1,47) and the line Nax1_207 (STI=1,84).

For the STI of root volume the Newman Keuls classification revealed 15 significantly distinct classes of genotypes. The first class of genotypes having the lowest STI is composed of the variety ESP85847 (STI=0,33) and other six varieties belonging to both the first and second class. The last class of genotypes having the highest STI is composed of the verity SYR95836 and other six varieties belonging to both the 15th and the 14th class.

For the STI of root surface the Newman Keul classification revealed 15 significantly distinct classes of genotypes. The first class of genotypes having the lowest STI is composed of the variety SYR96150 (STI=0,18), the variety UZB82233 (STI=0,24) and other thirty varieties belonging to both the first and second class. The last class of genotypes having the highest STI is composed of the line NAX1_207 (STI=2,4), the Tunisian variety Nasr (STI=1,87) and the line NAX2_041 (STI=1,83).

The different Newman Keuls classifications based on the STI of root dry weight, the root volume and the root surface were relatively similar. The STI of these parameters were highly and significantly correlated.

3.2. Evapotanspiration

The mean daily evapotranspiration was calculated for three consecutive periods. The first period (ETR1) is from sowing to the tillering stage. The second period (ETR2) is from the tillering stage to the flowering stage. The third period (ETR3) is from the flowering date to the maturity. In the first period the mean daily evapotranspiration (ETR1) was not significantly affected by salinity (Table 4). In the later periods the mean daily evapotranspiration (ETR2 and ETR3) was significantly affected by salinity (Table 4). The salt-tolerance indexes (STI) of the mean daily evapotranspiration of the second (ETR2) and third (ETR3) periods varied significantly among genotypes (Table 4).

For the STI of the mean daily evapotranspiration (ETR2) Newman Keul classification revealed three significantly distinct groups of genotypes. The first group of genotypes having the lowest STI is composed of the line NAX1_207 (STI=0.73) and other 47 genotypes belonging to both the first and second class. The third class of genotypes having the highest STI is composed of the line SYR95853 (STI=1.04) and other 46 genotypes belonging to both the second and third group.

For the STI of the mean daily evapotranspiration (ETR3) Newman Keul classification revealed three significantly distinct groups of genotypes. The first group of genotypes having the lowest STI is composed of 43 genotypes belonging to only the first group and other 6 genotypes belonging to both the first and second group. The Tunisian variety Nasr has the lowest STI (STI=0.42). The third group of genotypes having the highest STI is composed of the line NAX1_027 (STI=2.30) and the line NAX1_207 (STI=2.67).

Table 4: Variance Analysis of the mean daily evapotranspiration of the first (ETR1) second (ETR2) and third (ETR3) period. An example of a table

Source	Df	ETR1	ETR2	ETR3
Trait				
Genotype (G)	49	1,086	0,75	5,19
Salinity (S)	1	3,21	105,84***	15,31*
G x S	49	0,69	0,62	6,16*
Error	200	1,02	51.02	3,88
STI of the trait				
Génotype (G)	49	0,097	0,014***	0,56***
Error	100	0,075	0,006	0,14

*,**,***_/significant at 0.05, 0.01 and 0.001 levels, respectively

3.3. Chlorophyll content

The average Chlorophyll (Chl) content of flag leaves varied over time. At 79, 122 and 149 days after sowing it varies significantly in salinity treatment compared to the control (Table 5). The STI of chlorophyll content varied significantly at 79, 102, 122 and 136 days after sowing. The Chl content increased slowly at early vegetative stages reaching a maximum at advanced stages and fall down quickly before senescence. Compared to the control the average Chl content in salinity treatments increased by 3.1% and 0.7% at 79 and 102 DAS respectively. It decreased by 13%, 8.1% and 89% at 122, 136 and 149 DAS. The genotypes having a high increase of chlorophyll content in early stages had a rapid and high decrease of chlorophyll content at advanced stages.

Table 5: Variance analysis of physiological parameter (Chlorophyll content in SPAD unit) at 79, 102, 122, 136 and 149 DAS

Source	Df	SPAD79	SPAD102	SPAD122	SPAD136	SPAD149
Trait						
Genotype (G)	49	72,29***	138,35***	528,77***	451,38***	23,18**
Salinity (S)	1	107,11*	4,96	1305,19***	65,52	318,28***
G x S	49	23,34	34,18	130,67	78,17	23,12**
Error	200	20,62	36,62	97,5638885	90,7	14,11
STI of the trait						
Génotype (G)	49	0,031***	0,04**	0,24***	1,91*	0,88
Error	100	0,01	0,02	0,0712341	1,14	0,92

*,**,***_/significant at 0.05, 0.01 and 0.001 levels, respectively

3.4. Yield component parameters

Except for the spike length and grains per spike all the final harvest parameters varied significantly in salinity treatment compared to the control (Table 6).

Table 6: Variance analysis of yield component parameters

Source of variation	df	Spikes/plant	Spike length	Spike weight	spikelets/spike	Grains/spike	TGW	Grain yield
Trait								
Genotype (G)	49	1,66***	4,63***	0,71***	15,01***	116,44***	194,56***	1,74
Salinity (S)	1	7,94**	1,57	8,19***	264,18***	6,49	5921,17***	123,87***
G x S	49	0,951	0,53	0,27	7,73**	52,86	110,21	1,29
Error	200	0,8	0,67	0,31	4,66	53,93	86,43	1,3
ITS of the trait								
Genotype (G)	22	0,20**	0.02***	0,09	0,05***	0,01**	0,07*	0,08*
Error	46	0.11	0.01	0.05**	0,01	0.05	0,05	0,05

*,**,***_/significant at 0.05, 0.01 and 0.001 levels, respectively

Mean number of spikes per plant for all varieties in the salinity treatment was reduced by 12% compared to the control. The STI of the number of spikes per plant ranged from 0.36 (NAX1_207) to 1.6 (UZB82233). For this trait line NAX1_207 was the most affected by salinity and the UZB82233 was the least affected one. The number of spikes per plant of the variety UZB82233 and other 16 varieties (TUR82738, TUR84776, TUN94651, IND84882, SYR95843, IND84830, JOR96252, IRQ83091, ESP85028, DZA93963, IRN82635, EGY83477, SYR95853, ETH89017, GRC85714 and Maali) was higher in the salinity treatment compared to the control.

The spike weight for all varieties in the salinity treatment was reduced by 14% compared to the control. The STI of spike weight ranged from 0.42 (TUR84454) to 1.33 (UZB82181). For this trait the variety TUR84454 was the most affected by salinity and the UZB82181 was the least affected one. The spike weight of the variety IG-82181 and other eight varieties (MAR96367, EGY87457, Khiar, GRC85715, Line Nax2_041, Kerim, Maali, Line Nax1_207) was higher in the salinity treatment compared to the control.

The number of spikelets per spike for all varieties in the salinity treatment was reduced by 11% compared to the control. The STI spike weight ranged from 0.58 (DZA75:43) to 1.23 (Maali). For the number of spikes per plant the variety DZA75:43 was the most affected by salinity and the Maali variety was the least affected. The number of spikes per plant of the Tunisian variety Maali and for other 10 varieties (OMN43330, MAR96367, Line Nax1_207, Selim, UZB82181, Khiar, IND86075, DZA93151, IND84882, Nasr, Maali) was higher in the salinity treatment compared to the control.

The thousand grain weight for all varieties in the salinity treatment was reduced by 16% compared to the

control. The STI of spike weight ranged from 0.45 (TUR84454) to 1.18 (Nasr). For this trait the variety TUR84454 was the most affected by salinity. The thousand grain weight of the variety Nasr and five (Khiar, JOR96252, GRC85715, TUR82878, Nax1_207) other varieties was higher in the salinity treatment compared to the control.

The grain yield for all varieties in the salinity treatment was reduced by 27% compared to the control. The STI of grain yield ranged from 0.23 (TUR84454) to 1.11 (ESP85020 and GRC85714). For this trait the variety TUR84454 was the most affected by salinity. The grain yield of the varieties ESP85020 and GRC85714 was higher in the salinity treatment compared to the control (Fig. 4). For the STI of the grain yield the Newman Keul classification revealed 10 significantly distinct classes of genotypes. The first class of genotypes having the lowest STI is composed of the TUR84454 and other nine varieties belonging to both the first and second class. The last class of genotypes having the highest STI is composed of the variety GRC85714 (STI=1.44) and other twenty three genotypes belonging to both the 9th and 10th class.

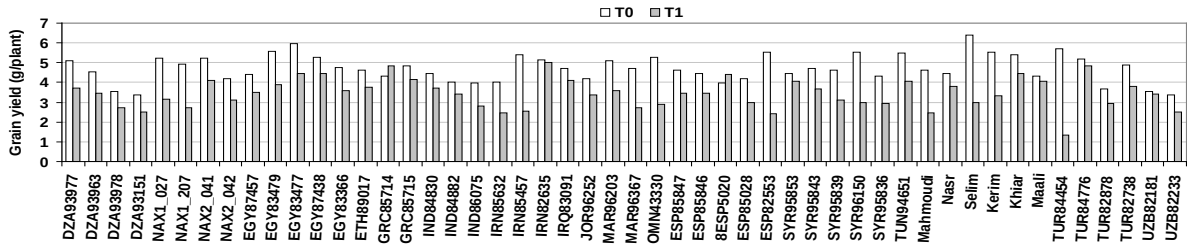

Figure 4: Effect of salinity treatment on grain yield (T1: salinity treatment, T0: control). Genotypes from ICARDA are illustrated by country code followed by the ICARDA genotype code (IG).

Correlation of Traits Related to Salinity Tolerance

To better understand the traits that best describe salinity tolerance, relationships among STI of all traits were analyzed. The Pearson correlation matrix (data not shown) of STIs showed different correlations among the analysed traits in response to salt stress in durum wheat genotypes.

All correlations presented hereafter are significant ($p<0.05$).

The STI of the grain yield was positive and highly correlated to the STI of tillering (r=0.46), the evapotranspiration ETR2 (r=0.46), the shoot dry weight (r=0.74), the number of spikes per plant (r=0.74), the spike length (r=0.30), thousand grain weight (r=0.36) and the chlorophyll content at 79 DAS (r=0.30); it was highly but negatively correlated to evapotranspiration ETR3 (r=0.34). The highest relationship was between the STI of the yield and those of shoot dry weight (Fig. 5) and the number of spikes per plant.

3.5. The STI of the shoot dry weight was positive and highly correlated to the STI of the spike length (r=0,43), number of spikes per plant (r=0,76), the tillering (r=0,76), the plant height at 68 DAS (r=0,52), the plant height at 102 DAS (r=0,38), chlorophyll content at 79 DAS (r=0,55), the evapotranspiration ETR2 (r=0,69); it was highly but negatively correlated to the STI of evapotranspiration ETR3 (r=-0,41).

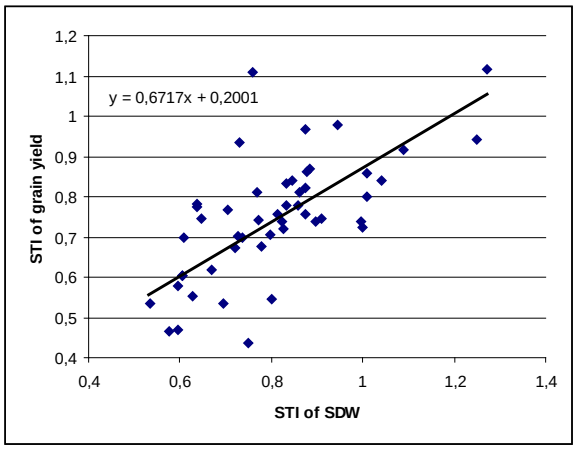

Figure 5: Relationship between STI of Shoot dry weight (SDW) and STI of grain yield

The STI of the number of spikes per plant was significantly and highly correlated to the STI of the spike weight (r=-0,38), the tillering (r=0,85), the shoot dry weight (r=0,76), the plant height at 68 DAS (r=0,46), the chlorophyll content at 79 DAS (r=0,52), the evapotranspiration ETR2 (r=0,55), the evapotranspiration ETR3 (r=-0,48). The STI of the tillering was positive and highly correlated to the STI of the number of spikes per plant (r=0,85), the plant height at 68 DAS (r=0,47), the shoot dry weight (r=0,76), the chlorophyll content at 79 DAS (r=0,53) and the evapotranspiration ETR2 (r=0,64); it was highly but negatively correlated (r=-0,40) to the spike weight. The STI of the evapotranspiration ETR2 was positive and highly correlated to the STI of the number of spikes per plant (r=0,55), the chlorophyll content at 79 DAS (r=0,49), the spike length (r=0,32) and the plant height at 68 DAS (r=0,55); it was highly but negatively correlated (r=-0,47) to evapotranspiration ETR3. The STI of ETR3 was highly and negatively correlated to the STIs of the number of spikes per plant (r=-0,48), the tillering (r=-0.41), the plant height at 68 DAS (r=-0.32), the chlorophyll content SPAD at 79 DAS (r=-0.33) and the shoot dry weight (r=-0.41). The STI of spike weight was positive and highly correlated to the STIs of the thousand grain weight (r=0.53), the number of grains per spike (r=0,80), the number of spikelets per spike (r=0,56), the number of spikes per plant (r=-0.38), the plant height at 159 DAS (r=0.53) and the evapotranspiration ETR3. The STI of spike length was positive and highly correlated to the STIs of the spikelets per spike (r=0.59), the plant height at 159 DAS (r=0.49). The STI of grains per spike was positive and highly correlated to the STIs of the spikelets per spike (r=-0.61) and the plant height at 159 DAS (r=0.53). The STI of spikelets per spike was positive and highly correlated to the STIs of the plant height at 159 DAS (r=0.49). The STI of the number of spikes per plant was positive and highly correlated to the STIs of the plant height at 159 DAS (r=0.49) and the chlorophyll content at 79DAS (r=0.52). The STI of the root dry weight was positive and highly correlated to the STIs of the STIs of the root surface (r=0.69) and the root volume (r=0.74). The STI of the plant height at 68DAS was positive and highly correlated to the STI of the chlorophyll content at 79 DAS (r=0.53).

4. Discussion

In summary salt stress affected significantly the shoot dry weight, tiller number, plant height (68, 101, 115 and 159 DAS) root dry weight, root volume, root surface, evapotranspiration (ETR2 and ETR3), chlorophyll content

(79, 122 and 149 DAS) spikes per plant, spike weight, number of spikelets per spike, thousand grain weight and grain yield. Genotypes varied significantly for major shoot and root parameters, suggesting that salinity tolerance in durum wheat is controlled in both shoot and root. These results were consistent with our pervious results [5, 6, 24, 31] and with findings of [32], who reported that salinity-induced reduction in root surface area and changes in major root and shoot traits at the phytomer level in wheat.

The salt tolerance indexes varied significantly for tiller number, plant height (115 and 159 DAS), heading date, root dry weight, root volume, root surface, evapotranspiration (ETR2 and ETR3), chlorophyll content (79, 102, 122 and 136 DAS), number of spikes per plant, spike length, number of spikelets per spike, number of grains per spike, thousand grain weight and grain yield.

Undoubtedly, success of indirect selection for salinity tolerance using physiological attributes as markers depends on the strength of relationship of such markers with plant response to salinity [21]. In order to evaluate the association of morpho-physiological traits with the plant tolerance objective (grain yield) we analysed the correlations between STIs of the different measured parameters and those of the grain yield. The high positive and significantly correlation of STI of grain yield and those of tillering (r=0.46), evapotranspiration ETR2 (r=0.46), shoot dry weight (r=0.74), number of spikes per plant (r=0.74), spike length (r=0.30), thousand grain weight (r=0.36) and the chlorophyll content at 79 DAS (r=0.30) indicated that salt stress induces a high reduction in these parameters, leading to the reduction in grain yield. Therefore we can consider these parameters as the most relevant for salinity tolerance screening criterion in Tunisian durum wheat breeding programs. These agronomic and physiological traits have all been proposed as selection criteria for screening salt tolerance under controlled conditions [27, 33, 34, 35, 36, 37, 38].

The most affected traits related to grain yield under salt stress were the shoot dry weight and tiller number. Both of these parameters were highly correlated and correlated to number of spikes per plant and grain yield under salt stress. They appear to have a greater negative impact on grain yield than any other yield component.

Total dry weight is frequently considered as an indicator of salinity tolerance [3, 39, 40, 41]. 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 [1].

The number of tillers per plant is also an important yield parameter under salinity because it determines the grain bearing panicles [42]. Salt-tolerant cultivars always show relatively lower rate of reduction in total tillers and spike-bearing tillers than salt-sensitive ones, resulting in their higher grain yield [4].

The reduction in tiller number and shoot dry weight is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behaviour and photosynthetic rate. Photosynthesis, the most fundamental and intricate physiological process in all green plants, is also

severely affected in all its phases by salinity [43]. Photosynthesis is considered as one of the potential, physiological, selection criteria for stress tolerance [21]. The accumulation of Chlorophyll has been proposed as one of the potential biochemical indicators of salt tolerance in wheat [44, 45]. Because photosynthesis, can be measured by a non-destructive, rapid and easy technique using SPAD (Soil Plant Analysis Development) meter, this physiological traits may be important to be used as screening criteria [27, 46]. The previous studies showed that the SPAD meter readings were linearly correlated with chlorophyll content and maximum net photosynthesis rate in wheat [18, 46]. In this study, the Chlorophyll content increased slowly at early vegetative stages reaching a maximum at advanced stages and fall down quickly at senescence. This reveals that senescence was enhanced by salinity as reported in our previous studies [5, 24, 31]. The genotypes having a high increase of chlorophyll content in early stages had a rapid and high decrease of chlorophyll content at advanced stages. Significant genotypic variation in SPAD values and highly correlations with yield-components under salt conditions were observed. However, the genotypic variation and the correlations with yield-component parameters were greater at 79DAS than at the other dates.

In summary, factors promoting tiller number, shoot dry weight and Photosynthesis, are of critical importance to crop yield in a saline environment. Among the traits evaluated for salt stress response, the grain yield was significantly correlated to shoot traits, but not to root traits, suggesting that salinity tolerance is more likely controlled in the shoot [25].

The Newman Keuil classification based on different trait STI showed different ranking of genotypes in response to salinity stress, indicating wide natural phenotypic variation among the 50 durum wheat genotypes. Varietal differences showed that it is natural for varieties to be superior in one trait and inferior in others. These results are in accordance with previous results obtained in rice [25] and wheat [4].

A table of standardised STI data (data not shown) was used to easily identify exceptional extreme Z-scores or SD-score (standard deviation scores). Our aim was to identify genotypes with extreme traits values under salt stress. Different extreme values were recorded within the dataset. Different genotypes showed high mean Z-score. The genotype GRC85714 had the highest mean Z-score (1.32) followed by two Tunisian varieties Nasr (1.01) and Maali (0.74). The GRC85714 has a high Z-score of grain yield (z=2.29), number of grains per spike (z=2.37), tillering (z=2.32), shoot dry weight (z=2.8), plant height (Z-scores of 2.18, 2.92 and 3.54 at 68, 101 and 115DAS respectively) chlorophyll content (Z-scores of 2.21, 2.15 and 2.87 at 79, 122 and 136 DAS respectively) and evapotranspiration (ETR2). Compared to the analysed genotypes GRC85714 showed less reduction in yield, biomass, chlorophyll content and evapotranspiration under salt stress indicating better performances under these conditions. The GRC85714 has a medium Z-score of the number of grains per spike (Z=1.48), the booting date (Z=1.21), the flowering date (Z=1.78) and chlorophyll content at 102 DAS (Z=1.12). Among the analysed Tunisian varieties Maali and Nasr exhibited some level of tolerance. The Nasr variety has high Z-scores of 1000 grain weight (Z=12.21) root surface (Z=2.27), root dry weight (Z=2.32), plant height at 159 DAS (Z=2.34), booting date (Z=2.49), heading date (Z=3.06) and evapotranspiration ETR2 (Z=2.29). It has a medium Z-scores of the spike length (Z=1.63), grains per spike (Z=1.36), spikelts per spike (Z=1.54), shoot dry weight (Z=1.23), root volume (Z=1.21), plant height at 115 DAS (Z=1.59), heading date (Z=1.41) and chlorophyll content at 102JAS (Z=1.04). The Maali variety has high Z-scores of spike weight (Z=2), spike

length (Z=2.18) the number of spikelts per spike (Z=2.67), the shoot dry weight (Z=2.66) and the root dry weight (Z=2). It has a medium Z-scores of the grain yield, the number of grains per spike, the root volume (Z=1.53), the chlorophyll content at 79DAS (Z=1.07) and 102 DAS (Z=1.2), the evapotranspiration ETR2 (Z=1.02) and ETR3 (Z=1.24). Among the analysed genotypes the GRC85714 showed the best performances under salt stress followed by the two Tunisian varieties NASR and Maali. The GRC85714 had a high salt tolerance for the most relevant salt tolerance traits. Therefore, GRC85714 can be used as novel sources of salinity tolerance. The genotype ESP85020 showed also good performances of grain yield under salinity conditions but it could not be considered as potentially promising genotype for salinity tolerance because it has not good performances under salt stress for other traits. For this genotype we did not expect a stability of the yield under salt stress in other environmental conditions. On the other hand in the luck of commercial Tunisian varieties adapted for salt tolerance the Nasr and Maali varieties could be used under moderate salt stress. These results were consistent with our pervious results [5, 31]. Approximately half of the analysed genotypes showed a mean Z-score greater than 1 showing a moderate to high level of salt tolerance. These genotypes have at least one salt tolerance related trait with high Z-score. These are the first sources for the salt tolerance in durum wheat identified in the ICARDA gene bank. This demonstrated that FIGS was effective for sampling large ex situ germplasm collections when seeking novel genetic sources of salt tolerance. Additionally it can be an effective tool to enhance the discovery and deployment of new genes for abiotic stress. This method has successfully identified traits Related to Drought Adaptation in Vicia faba [47], Genetic Resources wheat germplasm with resistance to Sunn pest [48], stem rust resistance [26] and Russian wheat aphid [28].

5. Conclusion

In Tunisia, durum wheat breeding programs have been successful in breeding high yielding varieties. However, these varieties have not been evaluated for salt tolerance. Here, we evaluated the morphological and physiological responses of 50 diverse wheat genotypes that included Tunisian durum wheat varieties, international FIGS selection landraces and two Australian (CSIRO) wheat lines containing salt tolerance genes (Nax). The more related salt tolerance traits were identified and used for screening and classification objective.

The factors promoting tiller number, shoot dry weight and Photosynthesis, are of critical importance to crop yield in a saline environment. Among the analysed genotypes the ICARDA's landrace IG-85714 from Greece showed better performances under salt stress. Among the analysed Tunisian varieties Maali and Nasr exhibited some level of tolerance. Approximately half of the analysed genotypes showed a moderate to high level of salt tolerance. These genotypes showed at least one salt tolerance related trait. These are the first sources for the salt tolerance in durum wheat identified in the ICARDA gene bank. This demonstrated that FIGS was effective for sampling large ex situ germplasm collections when seeking novel genetic sources of salt tolerance.

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