J. Agric. Food. Tech., 5(4)1-7, 2015

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

Characteristics of seed, roots and coleoptile growth affected by the salt stress and the high temperature on two Tunisian landrace varieties of durum wheat (*Triticum durum*)

Ines JLASSI*, Yosra SLITI, Safia ELBOK Mohamed ELGAZZAH

Laboratory of Biodiversity, Biotechnology and Climate Changes, Faculty of Sciences of Tunis, University of Tunis El Manar, 2092, El Manar II, Tunis, Tunisia

Received: June 27, 2015
Accepted: August 20, 2015

ABSTRACT

Many papers concluded that roots and coleoptile growth of durum wheat are sensible to abiotic stress; drought and salt stress. So we studied the effect of salt stress on the growth of roots and coleoptile for two landrace varieties of durum wheat from the Centre of Tunisia: Biskri and Chetla. We affect 4 levels of concentrations of NaCl: 3.5, 7.1, 10.6 and 14.2 g/L with osmotic potentials of -0.3, -0.6, -0.9 and -1.2 MPa. We measured germination rate, mean germination time, length of coleoptile, length of roots, mean number of roots, weight of seeds germinated and water uptake. We found no significant difference between these measures for these two durum wheat varieties of the Centre of Tunisia.

KEY WORDS: NaCl, roots, coleoptile, water uptake, osmotic potential, durum wheat.

Abbreviations: LC: mean length of coleoptile; LR: mean length of root; NR: mean number of roots; OP: osmotic pressure; WU: water uptake.

1. INTRODUCTION

High concentrations of salts in the soil solution not only impair cell metabolism and photosynthesis but also impose an osmotic stress on cell water relations by increasing the toxicity of sodium in the cytosol **[1]**. Moreover, salt and water stresses are responsible for both inhibition or delayed seed germination and seedling establishment **[2]**. Under stress conditions, the germination of seeds is affected by creating an external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment **[3, 4]**.

The accumulation of soluble salts in soil leads to an increase in the osmotic pressure of the soil solution, which may limit the absorption of water by the seeds or plant roots. Salt damage to plants is attributed to reduction in water availability, toxicity or specific ions, and nutritional imbalance caused by such ions **[4, 5]**.

Salinisation is the scourge of intensive agriculture **[6]**. High concentration of salts has detrimental effects on the germination of seeds **[7,8]**, and plant growth. Many investigators have reported retardation of germination and growth of seedlings at high salinity. However plant species differ in their sensitivity or tolerance to salts **[9]**.

Generally, salinity leads to a reduction in plant growth. This reduction is a consequence of several physiological responses including modification of the ion balance, water status, mineral nutrition, stomatal behaviour, photosynthetic efficiency and carbon allocation and utilization **[10]**.

Many articles studied the effect of salt on the growth of durum wheat. But, they didn't study the effect of salt stress in juvenile stage of the landrace varieties of durum wheat in the Centre of Tunisia: Biskri and Chetla. The objective of this paper is to study the effect of four levels of concentrations of NaCl on the growth of germinated seed: germination percentage and mean germination time, the length of roots and coleoptiles, mean number of roots, and weight of the plant 7 days after of germination of two varieties of durum wheat (Biskri and Chetla) generally cultivated in the Centre of Tunisia.

2. METHODS

2.1. Plant material

We choose two ancient varieties of durum wheat. These varieties are known in the Centre of Tunisia; Kairouan as only few farmers still cultivate them: Biskri and Chetla. The climate of Kairouan is characterized by few rainfalls, salty irrigation water and high temperature. These two landrace varieties were characterized by a thousand kernel weight (TKW) correspond: TKW Biskri= 50.20 g and TKW Chetla= 49.20 g.

TKW Biskri= 50.20 g ➔ weight of one seed Biskri= 0.0502 g

TKW Chetla= 49.20 g ➔ weight of one seed Chetla= 0.0492 g

* **Corresponding Author:** Ines JLASSI, Laboratory of Biodiversity, Biotechnology and Climate changes, Faculty of Sciences of Tunis, University of Tunis El Manar, 2092, El Manar II, Tunis, Tunisia. Tel.: +21622903789
E-mail address: inesjlassi@yahoo.fr

2.2. Salinity experiment

We examined the two landrace varieties of durum wheat at germination and early growth stages. Germination was studied for seven days using distilled water (0 MPa) and solutions with osmotic potentials of -0.3, -0.6, -0.9 and -1.2 MPa, prepared using 3.5, 7.1, 10.6 and 14.2 g/L NaCl **[11]**. The electrical conductivity (EC) values of NaCl solutions were 6.5, 12.7, 18.4 and 23.5 dS/m **[12]** respectively.

Germination tests were carried out in the dark growth chamber at 20°C ± 1°C. Seeds were considered germinated when the coleoptile were at least 1 mm long. The number of germinated seeds was recorded daily and the final germination percentage was calculated after 7 days. The germination rate was calculated using MAGUIRE's equation **[13]**: $M = (n1/t1 + n2/t2 + \ldots + n7/t7)/t$; where $n1$, $n2$,..., $n7$ represent the number of germinated seeds at times $t1$, $t2$, …, $t7$ (in days) **[14]**.

2.3. Germination tests

Three replicates of 25 seeds were placed in 9 cm Petri dishes containing filter papers with 5 mL test solutions. The Petri dishes with seeds were put in sealed plastic bags to avoid moisture loss. Based on the previous study **[15]**, seeds germinated at a temperature of 25°C in the dark in an incubator. Germination was scored when the coleoptile was 1 mm long. The numbers of germinated seeds were recorded every 24 hs for 6 days. At the end of each experiment, the final germination percentage and mean germination time (MGT) were calculated to evaluate seed germination characteristics.

In the salt stress treatments, filter papers and solutions of NaCl were renewed every 2 days to prevent accumulation of salts due to evaporation **[8]**.

2.4. Shoot and root measurements

Shoot length and root length were measured 6 days post incubation. Ten seedlings were selected randomly to measure shoot and root length. The number of roots was counted 6 days post incubation.

2.5. Seed water uptake

The water uptake of seeds necessary for germination was also determined. For this purpose, 3 g seeds were soaked in distilled water in a 5 mL beaker for 24 hs, the water uptake was expressed as the percentage increase in moisture content on fresh weight basis.

2.6. Statistical analyses

A completely randomized design was used in the experiments. All data were analyzed by Excel XTAT2013. Analysis of variance (ANOVA) was used to compare treatment effects and the significance of differences of the means using Duncan's multiple range tests ($p< 0.05$).

3. RESULTS

3.1. Germination

The two landraces are cultivated at 25°C for seven days. They grow in control condition and at four levels of salt stress (-0.3; -0.6; -0.9 and -1.2 MPa). Germination begins from the first 24h for Biskri at 0, -0.3 and -0.6 MPa with percentage respectively 8, 9.33 and 1.33%. However, for Chetla, germination begins from the first 24h at 0, -0.3 MPa with percentage of germination 9.33%. Germination begins after 48h at -0.9 an -1.2 MPa for Biskri and at -0.6, -0.9 and -1.2 MPa for Chetla with low percentages shown in **Table 1**. Germination decreases with the increase of osmotic pressure. We noted the highest percentage of germination for Chetla at -0.6 MPa in the fifth to the seventh day (100%) **(Figure 1)**. But for Biskri, the highest percentage was 98.67 % at -0.3 MPa **(Figure 2)**. At the highest level of salt, -1.2 MPa Bisrki and Chetla germinated with final important percentages: 76 % and 80 % respectively.

Table 1: Germination percentage of two landrace varieties of durum wheat under control conditions and at four levels of salt stress.

Varieties	OP	D1(%)	D2(%)	D3(%)	D4(%)	D5(%)	D6(%)	D7(%)	Test F
	0	8.00	86.67	88.00	90.67	93.33	93.33	94.67	0.920
	-0.3	**9.33**	92.00	94.67	96.00	98.67	98.67	**98.67**	0.976
	-0.6	1.33	82.67	86.67	90.67	94.67	94.67	94.67	0.865
	-0.9	0.00	49.33	77.33	82.67	80.67	94.67	94.67	0.955
Biskri	-1.2	0.00	17.33	52.00	62.67	74.67	74.67	76.00	0.806
	0	**9.33**	96.00	96.00	96.00	96.00	97.33	97.33	
	-0.3	**9.33**	94.67	94.67	94.67	96.00	97.33	96.00	
	-0.6	0.00	86.67	92.00	97.33	100.00	100.00	**100.00**	
	-0.9	0.00	48.00	68.00	81.33	93.33	93.33	94.67	
Chetla	-1.2	0.00	12.00	32.00	46.67	80.00	80.00	80.00	

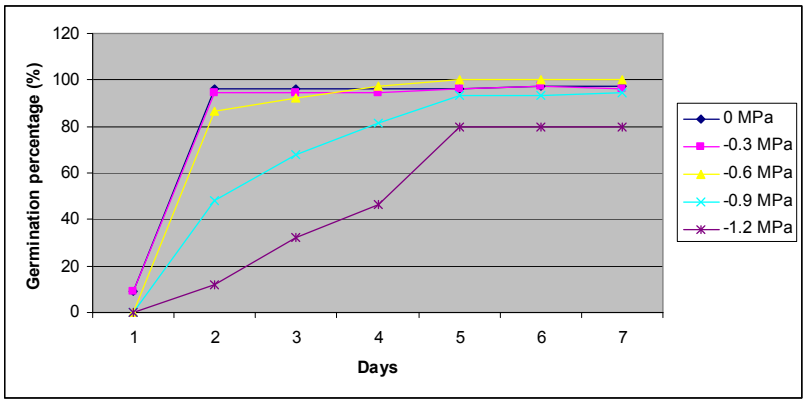

Figure 1: Germination percentage during 7 days of the genotype Chetla.

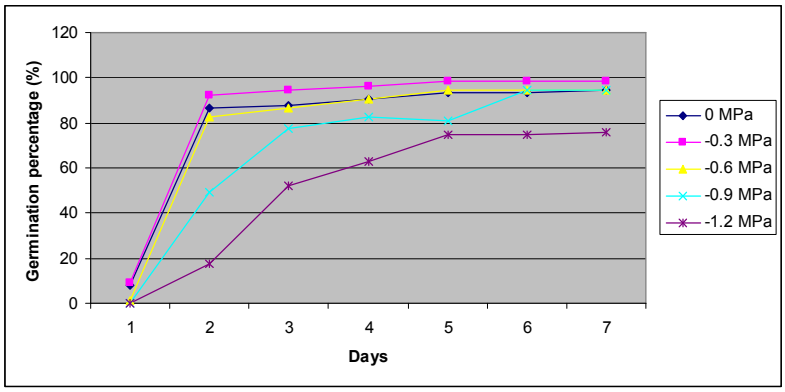

Figure 2: Germination percentage during 7 days of the genotype Biskri

There is no significant difference between Biskri and Chetla in germination percentage in all conditions **(Table 2)**.

Table 2: Statistical analysis with the test Duncan of differences between the modalities with interval of confidence 95%:

Contrast	Difference	Standard Difference	Value critique	Pr > Diff	alpha (Modified)	Significant
Biskri vs Chetla	0.000	0.000	2.048	1.000	0.050	No

3.2. Mean Germination Time (MGT)

The mean germination time increases with the increasing of salt stress **(Table 3)**. There is no significant difference between the values at 0, -0.3, -0.6 and -0.9 MPa for both varieties (for Biskri: 4.50, 4.49, 4.57 and 4.79 days; for Chetla: 4.45, 4.46, 4.58 and 4.84 days). At -1.2 MPa, the MGT was 5.02 days for Biskri and 5.29 days for Chetla.

Table 3: Mean Germination Time (MGT) of the two genotypes (Biskri and Chetla) under five conditions of germination.

MGT	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	F
Biskri	4.50	4.49	4.57	**4.79**	**5.02**	0.994	0.417
Chetla	4.45	4.46	4.58	**4.84**	**5.29**		

3.3. Weight seedlings after 7 days

We measured the weight of seeds germinated and the weight of their roots after seven days of germination **(Table 4)**. The following table shows that the weight of seedling was between 0.079 and 0.142 g for Biskri and between 0.076 and 0.140 g for Chetla. The weight increases with the decrease of osmotic pressure. It is negatively correlated to the osmotic pressure (Pearson r=-0.626 for Biskri/osmotic pressure; Pearson r=-0.582 for Chetla/osmotic pressure). The highest weight of seedling was at -0.6 MPa: 0.142 g for Biskri and 0.140 g for Chetla. Biskri and Chetla have the maximum yield at -0.6 MPa. The difference of weight of seedling is not significant.

Table 4: Roots weight of germinated seeds after 7 days of germination, for the two Tunisian genotypes Biskri and Chetla.

Weight of roots (g)	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	Test F
Biskri	**0.079±0.016**	0.079±0.016	**0.142±0.041**	0.137±0.031	0.110±0.019	-0.626	
Chetla	**0.086±0.076**	0.076±0.018	**0.140±0.027**	0.127±0.033	0.110±0.015	-0.582	0.890
Moyenne	**0.082±0.046**	0.077±0.017	**0.141±0.034**	0.132±0.032	0.110±0.017	-0.611	

The weight after 7 days is nearly the triple of the weight of seed before germination for both varieties Biskri and Chetla (ratio FW/IW: 2.837 and 2.847 respectively) **(Table 5)**.

Table 5: Weight ratio between final weight (FW) and initial weight (IW) of roots under five conditions of germination for Tunisian genotypes (Biskri and Chetla).

ratio FW/IW	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa
Biskri	**1.585**	1.574	**2.837**	2.731	2.200
Chetla	**1.746**	1.553	**2.847**	2.596	2.250

3.4. Length of cleoptile (LC)

The length of coleoptile was measured for Biskri and Chetla after seven days of germination **(Table 6)**. It was between 3.38 and 1.24 cm for Biskri and between 3.83 and 1.22 cm for Chetla as presented in the following table. The highest measure of length of coleoptile was at -0.3 MPa for Biskri (3.38 cm) and at -0.6 MPa for Chetla (3.83 cm). Biskri is more tolerant to -0.3 MPa than Chetla. And Chetla is more tolerant to -0.6 MPa than Biskri. The length of coleoptile is correlated to the osmotic pressure. The growth of coleoptile of Biskri is more correlated to osmotic pressure than Chetla (Pearson r=0.883 for Biskri and 0.616 for Chetla).

Table 6: Statistical analysis for the parameter length of coleoptiles (LC) for two Tunisian genotypes (Biskri and Chetla): Pearson and Fisher tests.

LC (cm)	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	Test F
Biskri	3.16±0.99	**3.38±1.54**	3.02±1.71	2.40±1.56	1.24±0.65	0.883	0.784
Chetla	2.78±0.99	3.08±1.59	**3.83±1.45**	2.46±1.26	1.22±0.73	0.616	

3.5. Number of roots (NR)

We measured and represented in the **Table 7**, the number of roots (NR) of ten random germinated seeds after seven days of germination. We noted that the biggest number of roots was at -0.6 MPa for Biskri (5.57), however, for Chetla it was at 0 MPa (4.90). The difference between NR of both genotypes was non significant. The correlation between NR and the osmotic pressure for Chetla is more important than for Biskri (r=0.809 for Chetla and r=0.604 for Biskri).

Table 7: Statistical analysis for the parameter number of roots (NR) for two Tunisian genotypes (Biskri and Chetla): Pearson and Fisher tests.

NR	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	Test F
Biskri	4.80±0.61	4.10±0.92	**5.57±0.63**	3.73±0.94	3.23±1.36	0.604	0.734
Chetla	**4.90±0.92**	4.03±0.41	4.30±0.75	4.27±0.87	3.40±0.97	0.809	

3.6. Water Uptake (WU)

Water uptake (WU) was measured because it is an indicator of salt tolerance. It was decreasing with the increase of osmotic pressure (OP) **(Table 8)**. We found the highest value of WU at 0 MPa: 53.97 % for Biskri and 53.93 % for Chetla. WU represents no significant difference between Biskri and Chetla. As we applied the Pearson test, we found that for Chetla the correlation between WU and the osmotic is more important than for Biskri (P=0.932 for Chetla and P=0.628 for Biskri).

Table 8: Statistical analysis of water uptake parameter (WU) for two Tunisian genotypes (Biskri and Chetla): Pearson and Fisher tests.

WU%	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	Test F
Biskri	**53.97**	45.00	39.23	**47.67**	41.07	0.628	0.959
Chetla	**53.93**	**53.23**	47.53	40.90	42.47	0.932	

3.7. Length of principal root (LR)

We measured also the length of principal root for germinated seed **(Table 9)**. We noted that at 0 and -0.6 MPa, LR varied slightly from one genotype to the other: 3.69 cm Biskri, 3.65 cm for Chetla; 3.89 cm for Biskri and 3.88 cm for

Chetla, respectively at 0 MPa and -0.6 MPa. The root growth is complete at -0.6 MPa just like at 0 MPa. These genotypes tolerate the level of salt stress -0.6 MPa. There is no significant difference between these measures (F=0.969). It is clear that the LR and the osmotic pressure are correlated for both genotypes (P=0.808 for Biskri and P=0.843 for Chetla). Root coleoptile ratio increased under salt stress condition (Table 10) with a peak at -0.6 MPa and its value neared to root coleaoptile ratio in control condition (1.151 and 1.240 respectively).

Table 9: Statistical analysis of length of principal root parameter (LR) of two Tunisian genotypes (Biskri and Chetla): Pearson and Fisher tests.

LR (cm)	0 MPa	-0.3 MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Pearson	Test F
Biskri	**3.69±2.01**	3.31±1.71	**3.89±1.58**	2.76±1.69	1.05±0.72	0.808	0.969
Chetla	**3.65±2.01**	3.32±1.49	**3.88±1.71**	2.24±1.29	1.10±0.72	0.843	

Table 10: Statistical analysis of root coleoptile ratio of two Tunisian genotypes (Biskri and Chetla): Pearson and Fisher tests.

Omotic Pressure (MPa)	0	-0.3	-0.6	-0.9	-1.2	Pearson	F
Biskri	1.168	0.979	1.288	1.150	0.847		
Chetla	1.313	1.078	1.013	0.911	0.902	0.323	0.950
Biskri+Chetla	1.240	1.029	1.151	1.030	0.874		

3.8. Correlation between different parameters

In order to study how these parameters go, we applied the test of Pearson **(Table 11)**. The correlation was negative between Weight-LR (-0.088) and Weight-LC (-0.088). This means that when the roots and the coleoptile grow, the weight decreases. We also found high correlation (near to 1) between LR and LC. LR and LC grow simultanously. And finally, the correlation between NR-LR and NR-LC was 0.806 and 0.634 respectively. The correlation between NR-LR was higher than that between NR-LC.

Negative correlation was found between WU and weight (-0.766) also between WU and MGT (-0.740). We found also that MGT was negatively correlated to LC, NR, LR, WU and FG (final germination percentage). While a positive correlation was found between WU and LC, NR and LR (0.495, 0.511 and 0.582 respectively). FG was highly correlated to LC and LR (0.953 and 0.927 respectively).

Table 11: Pearson test of correlation between LC, NR, LR, Weight, WU, MGT and FG (final germination percentage) under the five germination conditions applied on the two Tunisian genotypes: Biskri and Chetla.

Pearson r	LC	NR	LR	Weight	WU	FG	MGT
LC	**1**						
NR	0.634	**1**					
LR	**0.935**	0.806	**1**				
Weight	-0.088	0.053	-0.088	**1**			
WU	0.495	0.511	0.582	-0.766	**1**		
FG	**0.953**	0.791	**0.927**	-0.058	0.530	**1**	
MGT	-0.949	-0.813	-0.954	0.367	-0.740	-0.919	**1**

4. DISCUSSION

Approaches used to screen plant material for salt tolerance include among others relative growth rate of shoots and roots **[16]**, photosynthesis activity **[17]**, rate of Na+ and K+ accumulation in the shoot and the roots, K+/Na+ discrimination **[18]** and crop yield **[19]**. Crop establishment comprises three processes which are germination, emergence and early seedling growth. These early growth stages are very sensitive to salt stress and could be used as criteria to screen for salt tolerance **[20]**.

The aim of our work was to study the tolerance of two landrace varieties of Tunisian durum wheat (Biskri and Chetla) in control condition and four salt stress levels (-0.3; -0.6; -0.9 and -1.2 MPa) at juvenile stages in order to prepare for breeding new salt stress resistant varieties. Seed germination and seedling growth of wheat (*Triticum durum*), like in other crops, were negatively affected by salinity stress **[21]**. This study showed that salt stress inhibits coleoptile growth more than root growth. Similar results were found by **Foolad (1996)** on tomato **[22], Keiffer and Ungar (1997)[23]** and **Huang and Reddman (1995)** on barley **[24]**. The significant correlation coefficient found between coleoptile and roots under both low and high salt condition was also found for different wheat (*Triticum aestivum* L.) cultivars in Iran by Moud and Maghsoudi **[25]**.

Our wheat seedling responses were not different to salt stress though responses to different levels of salinity, this indicate that there is no genetic variation among our two landraces in terms of early seedling growth rate under salt stress condition. These results differ from the findings of Moud and Maghsoudi **[25]**.

Physiologically, many processes are affected by salinity, but reduced cell growth, leaf area, biomass and yield are the most important ones. Reduction in growth is a common phenomenon of salt stressed plants; this has also been

observed in cultured cells, tissues or organs on medium supplemented with NaCl **[26]**. Under salt stress condition elongation rate of coleoptile may decrease with low soil potential **[27]** and seedling may not be well established due to weak shoot and root growth. In fact, slower growth is an adaptive feature for plant survival under stress and the extent of salt tolerance often appears to be inversely related to growth rate **[26,28]**.

It is well documented that osmotic stress, ion toxicity and secondary stress (i.e. oxidation), are the three major damages to plants under salt stress **[29,30,31]**. High salt concentration in soil inhibits uptake of water and nutrients by plant roots due to osmotic stress **[32].** These conditions ultimately interact with several cellular components, including DNA, proteins, lipids, and pigments in plants, impeding the growth and development of a vast majority of crops **[26]**. We found that water uptake decreases with the increase of salt stress (osmotic pressure). Direct and excessive entrance of sodium ions into plant cells will cause ion toxicity and imbalance, restraining plant photosynthesis and metabolism **[32,33,34]**.

Fortunately plants have developed some mechanisms for salt stress adaptation or tolerance including tissue tolerance to osmotic stress, ion homeostasis and detoxification **[31,34]**. Plants respond to ionic stress by active exclusion of salt ions or by shunting salt ions into storage tissues in order to maintain cellular homeostasis. Osmotic stress arises because of the presence of salts (usually Na+) which affects a plant's ability to absorb water and thus limits water availability to plant tissues. The plant's response to osmotic stress is physiologically similar to the response to drought stress. Ultimately both responses lead to the accumulation of osmolytes and other compounds, often via regulation by abscisic acid (ABA) signalling. If the initial response to either ionic or osmotic stress is insufficient, plants have several mechanisms to limit salt damage, including developmental modifications and the production of hormones and anti-oxidative enzymes **[35]**.

Our two landrace varieties tolerated the osmotic stress and germinated even in high salt stress.

5. Conclusion

Phenotypic traits were sufficient to conclude that the two Tunisian landrace varieties Biskri and Chetla both tolerate and resist salt stress. Such study can give tough result and tough conclusion to select resistant varieties for culture or for breeding new variety more resistant to salt stress. During this study, all measures at -0.6 MPa were mostly near to those at control condition. That means that our varieties act at -0.6 MPa as at 0 MPa, with salt concentration 7.1 g/l. they have high performance at -0.6 MPa more than other stress condition.

Acknowledgement

We thank engineer Abdelkarim HAMMAMI and his personnel for allowing us to use his laboratory of soil analysis, in CRDA Kairouan. In addition, we thank Professor Salah JLASSI for proofreading this paper.

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