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Effect of Salt Stress on Germination and Biological Growth of 50 Genotypes of Durum Wheat (*Triticum durum* Desf)

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Abstract: This study aims to evaluate the physiological behaviour of 50 genotypes of durum wheat (*Triticum durum* Desf) in salt stress conditions at germination stage. In this context, a trial was conducted in a growth chamber with controlled temperature and photoperiod. The seeds are germinated in Petri dishes containing fresh water in one case and in the presence of NaCl (10 g/l) in another case for 8 days. The measurements were for the final germination, germination kinetics, the length of the shoot and epicotyl. The results show that salt reduces the germination capacity and speed of germination, as it affected the stem and root growth of the genotypes studied. The response of seeds to salt stress varies over time with this case, given that the final percentage of germination and seedling growth differed significantly between the species studied. The variety DZA75: 93,151 has been the most tolerant to salt stress with a germination rate of 81.33% then the variety ESP-85028 has shown the most sensitive to this stress with a germination rate of order of 0.66%.

Key words: Durum wheat, salt stress, NaCl, germination capacity, reduction index

INTRODUCTION

In arid and semi-arid regions, water is the main factor limiting the expansion and intensification of crops (Alem *et al*., 2002). Also in these regions the high variability of rainfall, combined with high evaporation favouring the accumulation of salts in the soil. This affects nearly 7% of the total area in the world (Munns *et al*., 2002). In Tunisia, saline soils cover about 10% of the total area of the country (Botia *et al*., 1998; Hachicha *et al*., 1994). The salts act on the germination of seeds by reducing their germination capacity (Bayuelo-Jiménez *et al*., 2002; Slama, 2004; Botia *et al*., 1998), so that by slowing its velocity, (Slama, 2004), it was demonstrated that the salinity inhibits germination by its osmotic effect (Karmous, 2007). It affects all the germination process following the decrease in water potential around seeds (Maas and Poss, 1989). However, the response of plant species to salt stress depends on the genotypes, salt concentration, culture conditions and plant growing stage (Mauromicale and Licandro, 2002). However, some authors suggest that the behaviour of some plants such as maize, faba beans and common beans at later stages does not show a clear reaction between resistance at germination stage and the later observed (Rajaskaran *et al*., 2000). Thus, this test may not be reliable for large-scale early discrimination of salt-tolerant species when their sensitivity remains unchanged with the stage of development (Mallek, 1989; Levigneron *et al*., 1995). The purpose of this study is to evaluate the effect of salt stress on germination behaviour and biological growth of 50 varieties of durum wheat.

MATERIALS AND METHODS

Plant material: The present study examined the seeds of fifty varieties of durum wheat, it comes to 4 Australian lineages, 6 Tunisian cultivars and 40 lines from ICARDA (International Center for Agricultural Research in the Dry Areas) (Table 1).

Experimental protocol: The germination test was conducted in a growth chamber at National Research Institute of agronomy of Tunis (INRAT). We tested the effect of sodium chloride (NaCl) on the germination ability and epicotyl's length and root of the 50 durum wheat genotypes. Two treatments: distilled water (control) and a solution of 10 g/l of NaCl (salt stress) were applied for each variety. For each treatment, 50 seeds are disinfected sodium hypochlorite 5% for 5 min, washed thoroughly with water, then rinsed with distilled water. They are then germinated in Petri dishes lined with filter paper. Petrie boxes were completely

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Table 1: Description of plant material and behaviour of different varieties with respect to salt stress (35 landrace supposed tolerant, sensitive supposed 5 and 10 not specified on the principle of FIGS)

Order origin	ICARDA	Site code	Origin	Subset	Dhe	Set type
1	89017	ETH64:131	ETH	0	135	random-set
2	96203	MAR87-1:31	MAR	0	119	random-set
3	43330	OMN87:142	OMN	0	132	random-set
4	95853	SYR87-1:55	SYR	0	149	random-set
5	94651	TUN77::9	TUN	0	132	random-set
6	93977	DZA75::43	DZA	1	135	salinity-set
7	93963	DZA75::43	DZA	1	137	salinity-set
8	93978	DZA75::43	DZA	1	138	salinity-set
9	93151	DZA75::95	DZA	1	139	salinity-set
10	87457	EGY::12	EGY	1	140	salinity-set
11	83479	EGY-S55	EGY	1	140	salinity-set
12	83477	EGY-S55	EGY	1	141	salinity-set
13	87438	EGY-S56	EGY	1	142	salinity-set
14	83366	EGY-S57	EGY	1	146	salinity-set
15	85847	ESP-S1603	ESP	1	140	salinity-set
16	85846	ESP-S1603	ESP	1	146	salinity-set
17	85020	ESP-S1946	ESP	1	147	salinity-set
18	85028	ESP-S1947	ESP	1	142	salinity-set
19	85714	GRC56::11	GRC	1	134	salinity-set
20	85715	GRC56::12	GRC	1	136	salinity-set
21	84830	IND47/48::45	IND	1	148	salinity-set
22	84882	IND47/48::6	IND	1	140	salinity-set
23	86075	IND-S413	IND	1	134	salinity-set
24	85632	IRN-S235	IRN	1	145	salinity-set
25	85457	IRN-S406	IRN	1	149	salinity-set
26	83091	IRQ-S176	IRQ	1	155	salinity-set
27	96252	JOR83-2::46	JOR	1	117	salinity-set
28	96367	MAR85:112	MAR	1	NA	salinity-set
29	95843	SYR87-1:49	SYR	1	132	salinity-set
30	95839	SYR87-1:49	SYR	1	149	salinity-set
31	96150	SYR88-2:2	SYR	1	132	salinity-set
32	84454	TUR48::255	TUR	1	141	salinity-set
33	84776	TUR48::588	TUR	1	145	salinity-set
34	82878	TUR48D:1	TUR	1	139	salinity-set
35	82738	TUR48D:242	TUR	1	141	salinity-set
36	82181	UZB::10	UZB	1	148	salinity-set
37	82233	UZB-S149	UZB	1	148	salinity-set
38	82553	ESP27::46	ESP	1	193	salinity-set
39	82635	IRN40::12	IRN	1	189	salinity-set
40	95836	SYR87-1:49	SYR	1	194	salinity-set
41	var01	Mahmoudi				Unknown
42	var02	Nasr				Unknown
43	var03	Selim				Unknown
44	var04	Kerim				Unknown
45	var05	NAX1-027				Unknown
46	var06	NAX1-207				Unknown
47	var07	NAX2-041				Unknown
48	var08	NAX2-042				Unknown
49	var09	Khiar				Unknown
50	var10	Maali				Unknown

randomized in a factorial design with two factors (variety and treatment) in three repetitions. The dishes are then placed in the dark in a growth chamber set at a temperature of 26±1°C for 8 days. Sprouted grains are counted daily to determine the germination capacity and follow the germination kinetics. Towards the end of the week we measured the epicotyls and ridicles length of different varieties at a rate of 10 seeds per box. Germination is indicated by the output of the radicle out of the integuments grain whose length is at least 2 mm as defined Como (Come *et al*., 1970).

Studied parameters:

1: Final germination rate: this parameter is expressed as the ratio of the number of germinated seeds on total number of seeds (Come *et al*., 1970)

2: Germination kinetics: the number of sprouted grains was counted daily until the 8th day of the experiment, to better understand the physiological significance of the germ behaviour of the studied varieties (Hajlaoui *et al*., 2007)

3: Length of the radicle and epicotyl: they were measured using a ruler to evaluate the plant growth with respect to the stress

From the obtained data we calculated the reduction index (IR) of germination rate (IRG), the index of length reducing of the radicle (IRR) and the epicotyl (IRE). The IR is defined by the following formula:

$$\text{IR} = \frac{\text{(the saline treatment medium-the average of the controls)}}{\text{the average of the controls}}$$

Statistical analysis: An analysis of Variance (ANOVA) with two factors (varieties and saline treatment) for various measured parameters was performed using Statistica program version 5. A comparison of the average according to the method of Newman and Keuls at the threshold 5% was carried out for parameters shown significant by ANOVA.

RESULTS

The results of the ANOVA of the indices of reduced of germination rate (WRI), radicle (IRR) and epicotyl (IRE) length are summarized in Table 2. These results show that the stress saline significantly affected ($p<0.05$) the germination capacity, root lets and epicotyl the length. Also, the varieties behave differently and significantly for these indices of reduction in various measured parameters.

Table 2: Analysis of variance of reduction indices: The germination capacity, rootlets and epicotyl lengths

		----------------- Sum of squares means	-----------------	
	ddl	IR radical	IR epicotyl	IR germination
variety	49	0.1955^{***}	0.28332^{***}	0.16936^{***}
Erreur	100	0.02395	0.05292	0.01673

Germination capacity: Whatever the variety, stressed seed germination capacity is reduced compared to the control (Fig. 1). It is noteworthy that the variety ''DZA75: 93151'' has shown the most tolerant to salt stress with a germination rate of 81.33%. While the "ESP-85028" variety has demonstrated the most sensitive with a germination rate of about 0.66%. Observing the results of analysis of variance of the index reduction in germination (IRG) shows a significant difference between the different varieties. It allowed those varieties into four classes significantly ($p<0.05$) different (Fig. 1). The first class (A) is formed by the DZA75:93151 and EGY-87438 varieties with the germination rate reduction indices (IRG) indicating the lowest they have a very low germination capacity variable 10 g/l NaCl. The second class (B) groups IND-86075, EGY-83366, GRC56: 85715, EGY: 87457, ETH64: 89017 and IND47/48: 84.882 genotypes with relatively low IRG and can be there fore considered as moderately salt tolerant varieties during germination. The third class (C) groups EGY-83477 varieties Nasr, EGY-83479, ESP-85847, SYR87-1: 95853, UZB-82233, ESP-85020et DZA75: 93,977 who had relatively high values indicating that their germination capacity is moderately sensitive to salinity. The fourth class (D) gathers the remaining varieties characterized by high IRG and are the most sensitive for that character at the stage germination.

Kinetics of germination: This parameter allows monitoring germination over time, only in varieties with a higher or equal germination rate was 90%. These are variety ETH64: 89017, SYR87-1: 95853, EGY: 87457, EGY-83479, EGY-83477, EGY-87438, EGY-83366, ESP-85847, ESP-85020, GRC56: 85715, IND-86075, TUR48: 84776, TUR48D: 82878, ESP27: 82553. Mahmoudi, Nasr and Selim.

The evolution of germination over time (Fig. 3) shows that the germination rate varies depending on the variety and treatment. The curves relating to germination kinetics of the stressed grain are located below the control one. The first variety that has reached the maximum germination in the absence of salt is ETH64: 89017 (4 days). A significant increase in germination rates among varieties EGY-83366, GRC56: 85715, EGY: 87457, ESP-85847, EGY-83477 and 83479-EGY EGY-87438 is recorded and is, respectively about 87.33, 82.66, 76.66, 74, 74, 72.66 and 71.33%. On the contrary, at the remaining varieties the cumulative rate sprouted evolve at slower speed and has reached the maximum germination rate to the seventh day. In batches of grain soaked in saline solution, the cumulative rate sprouted after a slow evolution versus control in addition to a decrease in the rate of germination. In fact, the slopes of the germination kinetics curves of the stressed grain are less than those of the control. This indicates slower germination rate under the effect of sodium chloride. Different germination rates are recorded in varieties. According to Fig. 3, the highest cumulative rates towards the sixth day of sprouted grains are stored in varieties EGY-87438, GRC56: 85715 and ETH64: 89017 and are 70.66%, respectively; 60 and 58.66%. The germination kinetics curves of the varieties following a slow increase until the sixth day. They varied between 6.66 and 42.66%, respectively for Selim and EGY-83366. Between the sixth and seventh day, this rate increases to values between 10 and 66%, respectively in ESP27: EGY-82553 and 83366, stabilizing to the eighth day of observations.

Length of radical: The results of the root system development are indicated in Fig. 3, which shows that, all varieties except GRC56: 85715 (where root length was stimulated and reached 33.4% compared to the control) are significantly affected in salt stress

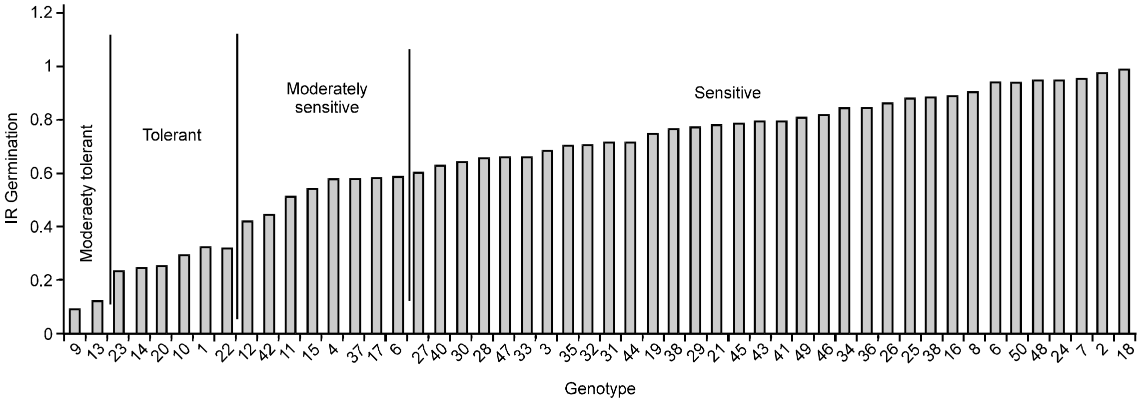
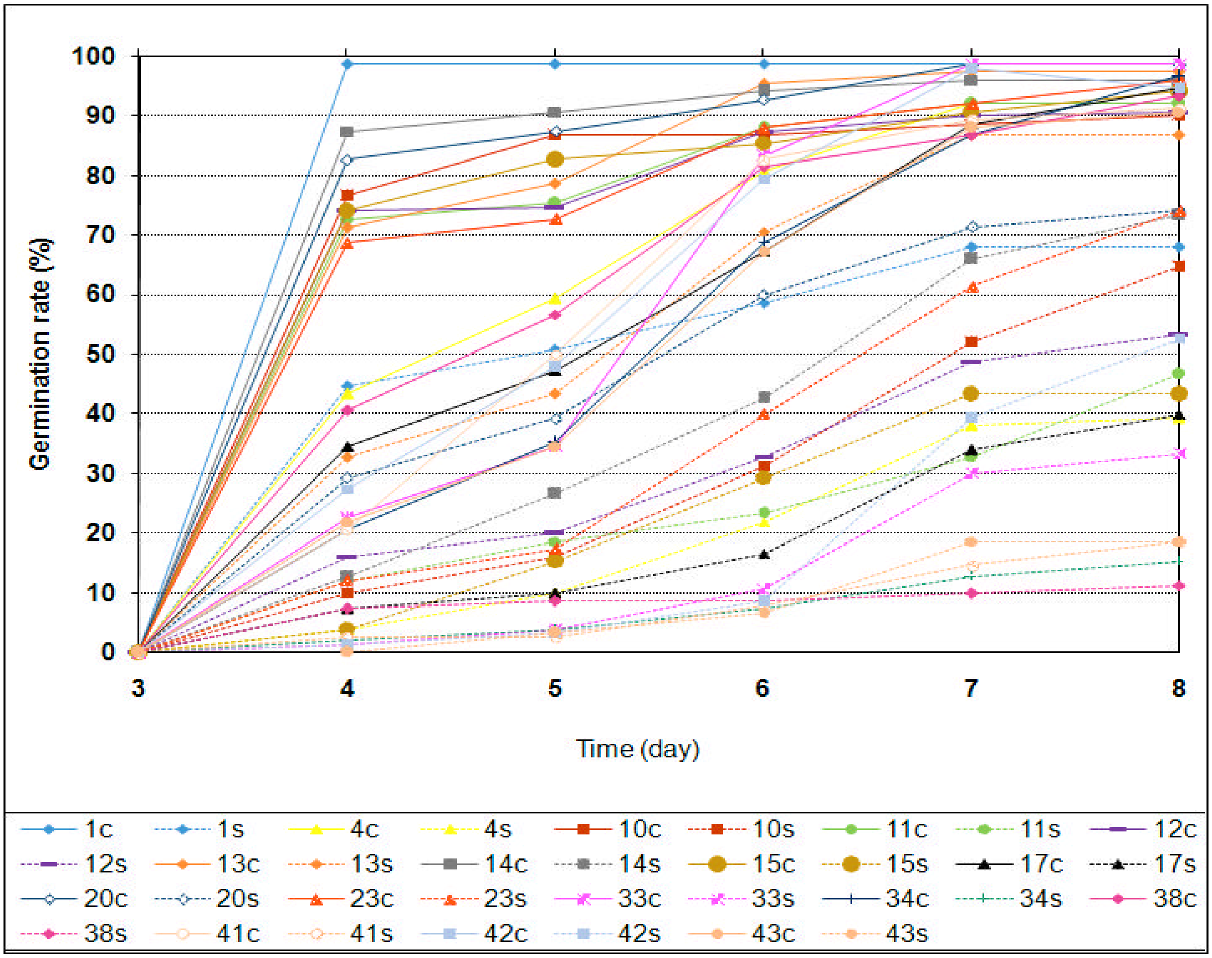


Fig. 1: Final germination Reduction (IRG) of different wheat varieties under salt stress (10 g/l NaCl)



· C = contol (0 g NaCl.L^{-1}; S = 10 g NaCl.L^{-1}

Fig. 2: Effect of NaCl (10 g/l) on the evolution of different genotypes germination over time

conditions. The analysis of variance of the index of rootlets length reduction (IRR), shows significant differences between the different genotypes tested (Table 2) and can be classified into 4 homogeneous classes. The first class (A) is formed by the variety GRC56: 85,715 which has a very low IRR, this variety is

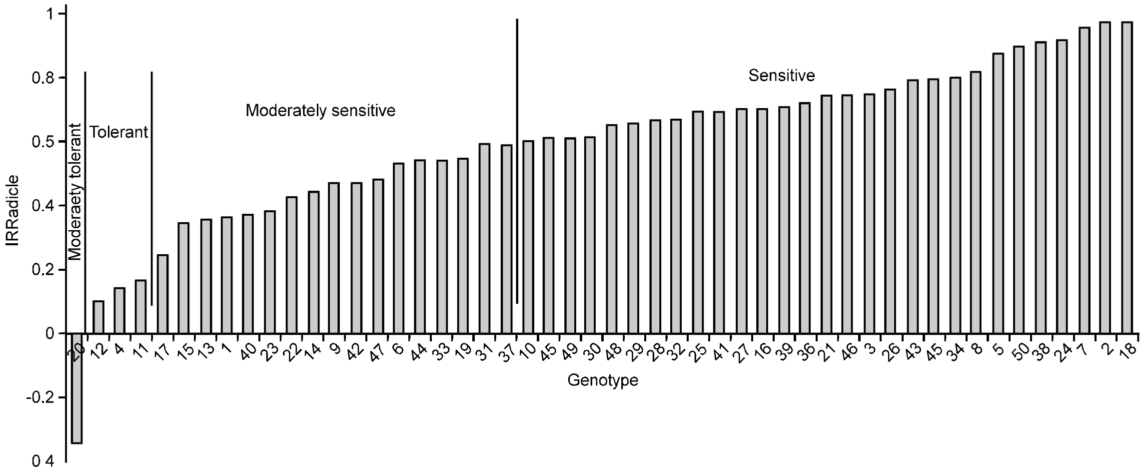


Fig. 3: Index of reducing the length of the radicle (IRR) of different wheat varieties under salt stress (10 g/l) of NaCl

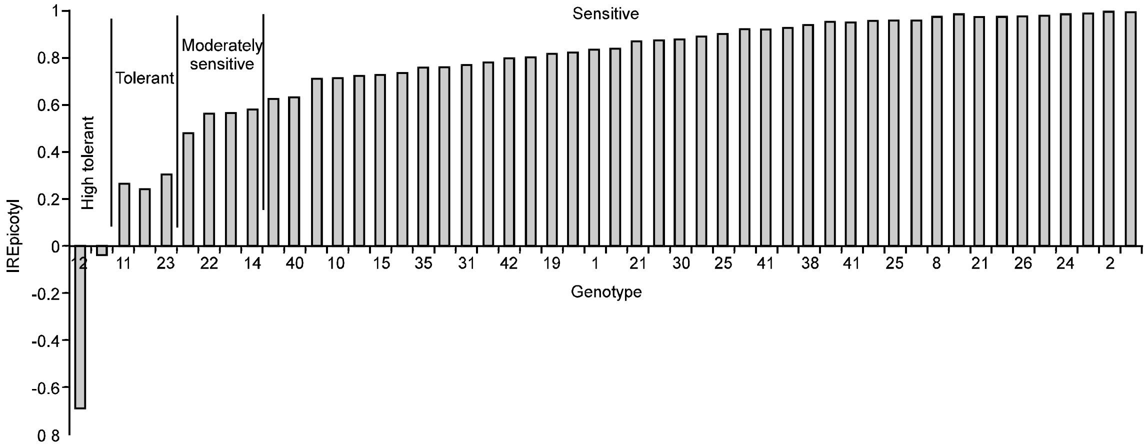


Fig. 4: Length reduction ratio of the epicotyl of different wheat varieties under salt stress (10 g/l) of NaCl

very salt tolerant for this character. The second class (B) is composed of the varieties EGY-83477, SYR87-1: EGY-95853 and 83479 with low IRR and can be therefore considered as moderately salt tolerant varieties. The third class (C) includes varieties ESP-85020, ESP-85847, EGY-87438, ETH64: 89017, EGY-83366, DZA75: 93151, Nasr NAX2-041, DZA75: 93977, Kerim TUR48: 84776, GRC56: 85714, SYR88-2: 96150, 82233 and UZB-EGY: 87457 with relatively high IRR and thus show themselves as moderately salt-sensitive varieties. The remaining varieties form a group (D) characterized by high IRR, these varieties are most sensitive to this character germination stage.

Length of the epicotyl: The evaluation of salt stress effect on the elongation of the aerial part proves that salt stress has seriously affected the epicotyl length of tested wheat varieties with the exception of varieties GRC56: 85715 EGY-83477 and where salt stress stimulated stem elongation to reach 69.19% in GRC56: 85715 and 3.3% for EGY-83477 compared to control (Fig. 4). The analysis of variance of the index of reduction the epicotyl length (IRE) (Table 2), shows significant differences between the different genotypes tested and can be classified into 4 homogeneous classes. The first class (A) is formed by GRC56 varieties: EGY-85715 and 83477 that are significantly the best of all varieties having the lowest IRE. They are considered very tolerant for that character. They are followed by varieties EGY-83479, EGY-87438 and IND-86075 which constitute a second class (B) with relatively low IRE and can be therefore considered as moderately salt tolerant varieties. The third class (C) groups SYR87-1 varieties: 95853, IND47/48: 84882, DZA75: EGY-93977 and 83366 with relatively high IRE and thus show as varieties moderately sensitive to salt. The remaining varieties gathered in a group (D) characterized by high IRE. These varieties showed the most sensitive at this stage and for that character.

DISCUSSION

The study of the effect of the rate of 10 (g/l) of sodium chloride on the germination of durum wheat seeds shows a variable effect of salt stress on germination and growth of aerial and underground portion different studied wheat varieties dice the first age. The study of Benaceur *et al.* (2001); Mrani *et al.* (2013); Allah, 1991; Garcia-Legaz *et al.* (1993), reported a similar effect of salt stress on the growth of durum and other plant species (Ungar, 1982; Mauromicale and Licandro, 2002) also asserts that the seed germination of halophytes in saline medium is variable and specific. Indeed, the study shows that NaCl reduces the germination capacity and slows down the speed of germination of different varieties studied. This confirms the results of Slama (2004) who showed that the salt also affects the germination by slowing down its speed. The same results were found in chickpea (Casting, 1979; Hajlaoui *et al.*, 2007). Comparing the indices cuts mean germination rate, these results allowed us to classify the varieties studied in three significantly different groups. The first group consists of DZA75: EGY-93151 and 87438, which are the most salt tolerant varieties. The second group consists of mid-tolerant varieties namely IND-86075, EGY-83366, GRC56: 85715, EGY: 87457, ETH64: 89017 and IND47/48: 84882. The third group includes EGY-83477 varieties Nasr, EGY-83479, ESP-85847, SYR87-1: 95853, UZB-82233, ESP-85020 and DZA75: 93977 and are considered moderately sensitive to salt stress. The fourth group is composed of the remains varieties which are the most sensitive to salt stress on germination stage. Regarding the growth of the radicle and epicotyl, we see a negative impact of salt on their growth depending on the variety criteria; it confirms the work of Allah (1991); Guerrier (1984), who showed that in case of severe stress growth stems, leaves and roots were significantly decreased. It is also reported that among the variety ‘GRC56: 85715’ length of radicle and epicotyl are stimulated by the salt, the particular behaviour of this variety can be considered as an early form of salt stress tolerance, as it was reported in the work of Benaceur *et al.* (2001).

The inhibitory effect on the rate and the speed of germination and growth of the epicotyl and rootlet of soaked seeds in saline water could be explained by a decrease in the osmotic potential of the medium after the addition of the salt as it had been shown by Slama (2004) and Karmous (2007) which affects the absorption of water by the seed and induces excessive accumulation of Na^+ and Cl^- (Hajlaoui *et al.*, 2007) and a K^+ deficiency (Guerrier, 1984) in the embryo. This ionic imbalance make embryos incapable trigger metabolic functions in the presence of high concentrations of NaCl for the use of degradation products from reserves endosperm.

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