Effect of salinity on seed germination of different genotypes of durum wheat (*Triticum durum* Desf) and species related to wheat (*Aegilops geniculata* Roth.)

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Abstract:

In this study, our results show a depressive effect of salt on germination of different parameters, thus confirming other work, showed a similar effect of salt stress on the germination and growth of many plant species.

Seed germination under stress transfer in a medium without salt allowed us to conclude that the effect of salinity on germination stage result of the intervention of osmotic effects with the toxic effects of NaCl.

The evolution of growth (growth kinetics) over time can be translated into growth curve, it is useful to reduce to simple mathematical models to generate parameters facilitate the comparison, the speed of growth and the rate of cell proliferation are characters limit the length of the epicotyl and radicle and are affected by salt stress, therefore the use of growth kinetics according to the results of our study, the selection of tolerant genotypes is useful better than the other parameters classics.

Key words: germination, durum wheat, salt stress, Aegilops, growth kinetics

I : Introduction

The seed is a complex organ reserve, which allows the propagation of the species and the passage of unfavorable seasons. The seed consists of an embryo and tissue reserves vary greatly from one species to another. Regarding the seed of durum wheat caryopsis otherwise known, it is composed of draft embryonic roots and leaves which the cotyledon.

During the formation of a seed, the plant growth is stopped because the dry matter produced is primarily intended to develop reserves. During the maturation phase that follows, the starch content of the seed gradually increases and water content decreases considerably. The acquisition of desiccation tolerance, necessary after such a dehydration, is associated with the synthesis of specific proteins and the accumulation of sucrose and oligosaccharides protecting cellular structures [1].

When the water content is very low respiratory activity is extremely low, metabolism slows down. This life slowed or quiescent seed gives the ability to withstand long periods in extreme temperatures, drought, radiation. The transition between quiescence and metabolic recovery is ensured by the hormonal balance at the abscisic acid (ABA) and gibberellins (GA) and by the water potential cell.

Germination is a physiological stage which corresponds to the transition from the latent phase of life of the seed dry phase of seedling development. The germination process begins as soon as the dry seed is hydrated. The kinetics of water intake can characterize germination in three phases (Phase imbibition, germination phase in the strict sense, post germination growth phase [2]. But the study of the behavior of the grain in the field is essential to meet the challenges of adapting the plant to abiotic stresses and the growing demand of the world market in cereals. Germinative quality concerns including the ability to germinate grain more or less rapidly and resist stress environmental.

The first characteristic can have a direct impact on crop yields by increasing the rate of sprouted grain in a cereal crop. Grain resistance to environmental stress can reduce falls in yields caused by unfavorable weather conditions for this reason we This chapter will examine the effect of salinity on the germination of some varieties of durum wheat and wheat related species Aegilops.

II- Material and methods

II-1- Plant material

The choice of plant material is performed on a set of genotypes that have been studied by several team Biotechnology and Plant Breeding Genetics Laboratory of Biochemistry and Plant Biotechnology (GBBV) to chaabt arssas (University Mentouri Constantine). Grains genotypes selected for this work comes from ITGC-Elkhroub Constantine.

To study the response of seed germination of different genotypes used in progressive levels of salt stress, seven durum wheat genotypes and wild species (Aegilops) are selected (Table 1).

Table (1): Origine of genotypes

Genotypes		Origine
***Tritium durum* (Desf)**	Oued Zenati	Algeria : Guelma 1936
	Bidi 17	Algeria : constantine
	Hedba 03	Algeria
	Djenah-Khetifa	Tunisie
	Belikhe 2	Syrie
	Haurani(	Syrie
	Waha	ICARDA .I selection ITGC Khroub
***Aegilops geniculata* Roth**	**Aegilops geniculata**	Algeria

II -Conduct of test

a- germination Last

The grains are disinfected solution of sodium hypochlorite 0.5% and rinsed several times with distilled water, then put in germination in petri dishes, each box is lined with three layers of filter paper. The selected grains are deposited in each box three replicates per genotype and salt treatment.

b- Stress applied and measured parameters

Salt solutions are prepared based NaCl ranging from 5 g / l to 18 g / l NaCl with the control (without salt) (Table 2). We added 10 ml of distilled water for controls and 10 ml of saline for each concentration. Finally boxes dishes are trademarks in the dark for 15 days at room temperature, different parameter are measured daily throughout the germination stage (earliness of germination (in%), the rate of sprouted grains (in%), the rate of recovery after germination in distilled water (%), germination time (by day) divided between the first and the last grain sprouted, the length of the epicotyl, radicle length and germination rate%).

Table 2: The composition of the salt solution

NaCl Solution	T	N1	N2	N3	N4	N5	N6
g/L	0	5	10	15	16	17	18
Meq/l	0	85,47	171	256,41	273,5	290,59	307,69

III- Results

III-1- Rate of germination

The final rate of germination is shown in figure (1) for each treatment; measurements were obtained one week after the first germination. It should be noted that beyond this period, the grains remaining in the petri dishes showed no signs of germination throughout the duration of the test.

The analysis of variance with two factors (Annex) shows that there is a highly significant effect of salinity on the different varieties of durum wheat and related species as the wheat (Aegilops) with a different sensitivity to one variety to another vis-à-vis the salt stress.

The treatment of grain with water (control) induced a strong germination rates as close to 100%, however the changes resulting from the effect of salt concentrations, with a general observation that the 50% threshold is reached for all types of grains and in all conditions of the experiment.

According confidence interval (Annex), the genotypes were classified into three different groups, in Aegilops group away from the other group since it exhibits a high germination rate in high salt concentrations (N4, N5, N6), the second group includes varieties (Hedba, Oued Zenati Waha) and has an average germination in doses of salt, other varieties are grouped together in the last group and exhibit a low germination rate.

After the period of stress, ungerminated grains in different levels of salt stress are transferred to a medium without salt (distilled water), and then determines the number of sprouted grains in the new conditions (Fig. 2).

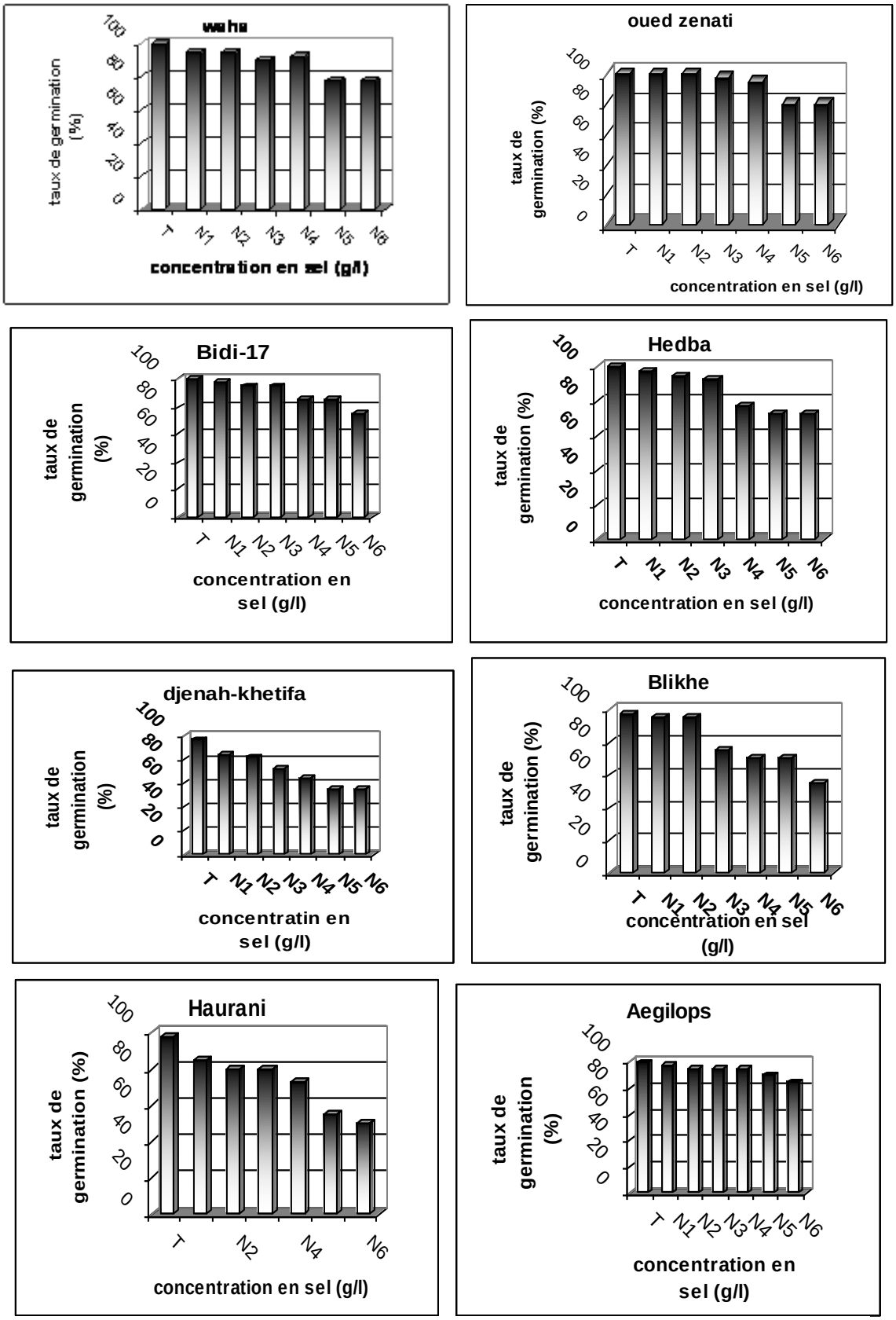


Fig.(1): Germination rate (%) of grains of different varieties of durum wheat and related species (Aegilops)

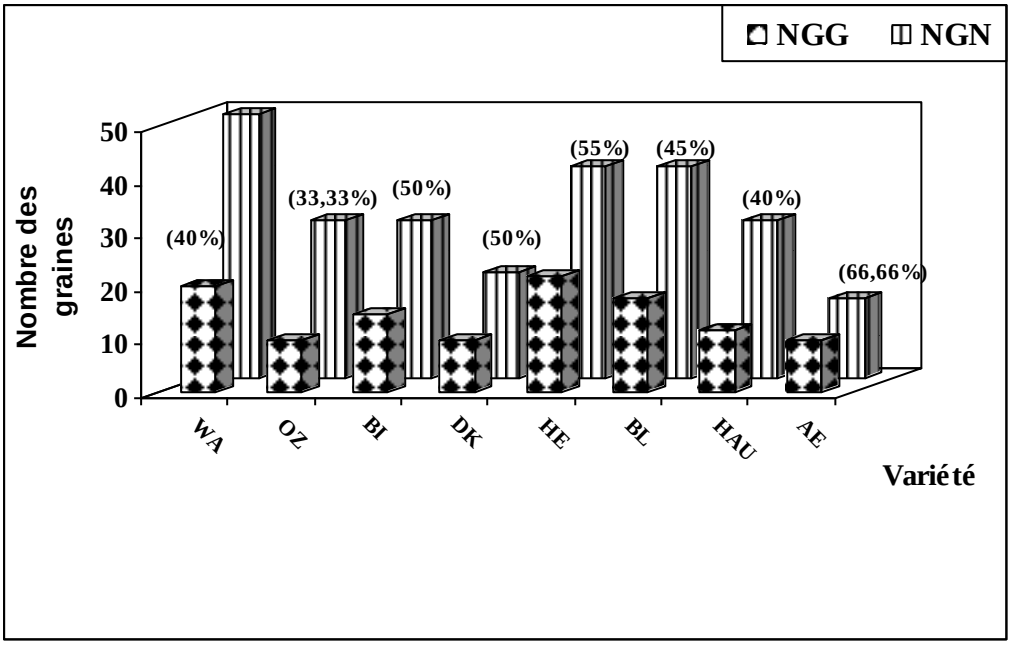


Fig. (2): Germination rate (%) of stressed grains are transferred to a medium without salt for different genotypes

Was observed according to Figure(5) that regardless of the genotype studied ungerminated grains in different stress levels are germinated in distilled water with a germination rate of a different genotype to another, the species Aegilops has a high rate (66.66%) as against Oeud Zenati presents the lowest rate (33.33%).

III-2-germination speed

Figure (3) shows the evolution of the speed of seed germination percentage. This parameter appears to vary with the treatment especially at high concentrations.

Overall, the grains stay in the water rapidly evolving whatever the variety with the observation of differences between varieties, according to analysis of variance (Annex), the variety (Blikhe) shows the high-speed 24, 22% against the Aegilops this down rate 19.83%.

The addition of saline at 5 g / l (N1) does not change the speed since it evolves with a rate close to that of the control observed for most varieties. At 10 g / l (N2) and 15 g / l (N3), salt causes a significant slowdown in the rate of germination compared to control and slow increases successively with increasing salt concentration (Figure 3).

According to the table of the confidence interval was observed ranking of genotypes according to the overall speed of germination, Aegilops is always a group separate from other classifications with a speed of germination (18.65%) as against other durum wheat varieties ranged between 20.48% to 21.95% thus rates observed are analogous in the rate of germination in all varieties of durum wheat that are derived from the same species by the Aegilops this against a slower pace.

III-3-The duration of germination

According to the analysis of variance (Annex), the germination time is different from the other genotypes studied and within the same genotype differences were observed between the different levels of salt stress. Figure (4) shows that the duration of germination in the control medium is the same (2 days) for most genotypes except the variety Djenah khetifa and Aegilops (3 days), it continues until the third day in the middle of N1 from the two varieties Djenah khetif (4days) and Haurani (5 days), by cons, it spreads significantly until the 7th day in the treatment 18g / l (N6) for most varieties except the variety Haurani (5 days) and Aegilops (4 days). Germination time recorded in the table of confidence interval (Annex) distinguishes between genotypes and Aegilops Haurani a regular seed germination under salinity. This feature is not identified in other varieties of grains as germination time increased with salt concentration.

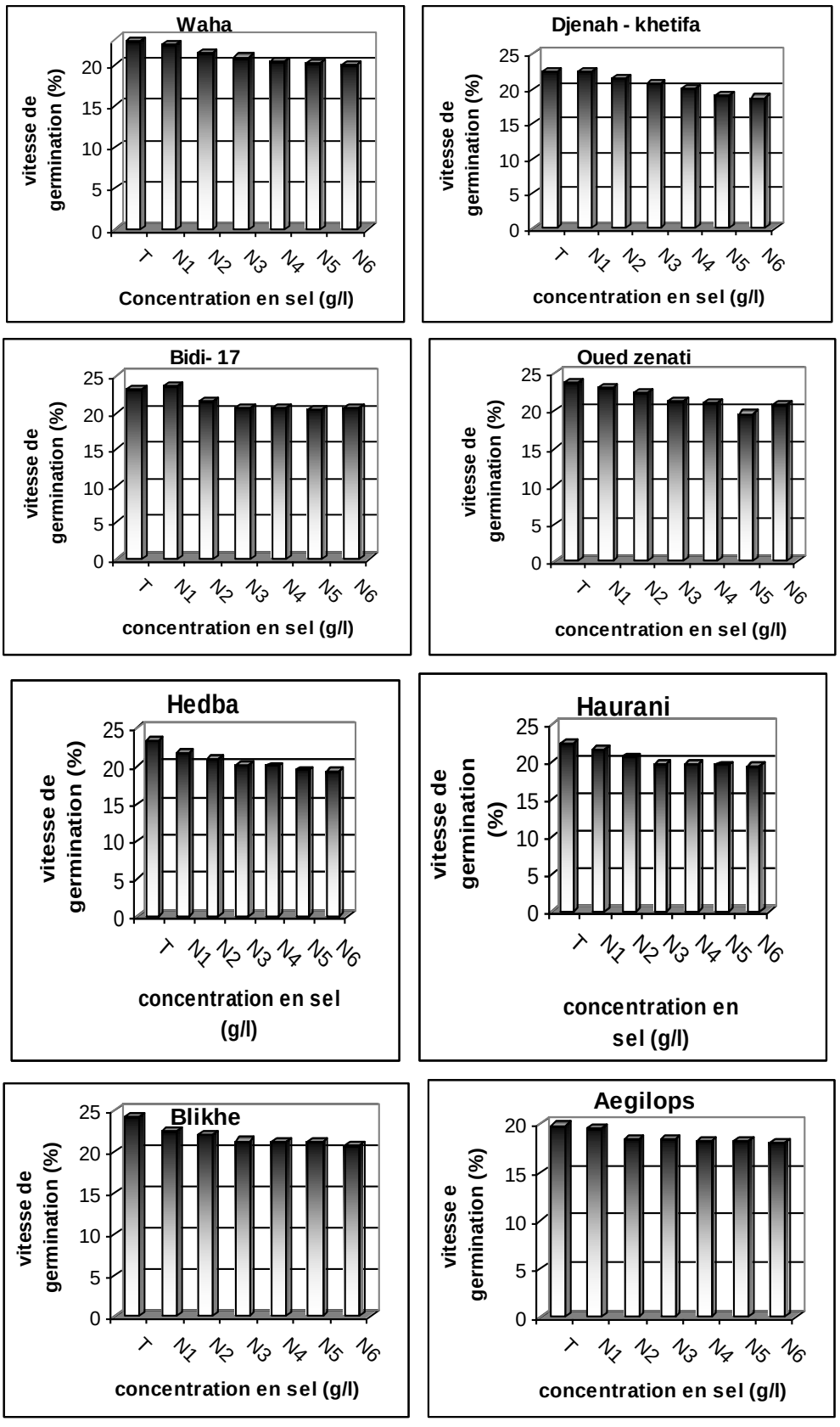


Fig. 3: Germination rate (%) of the grains of different genotypes according to the concentration of salt

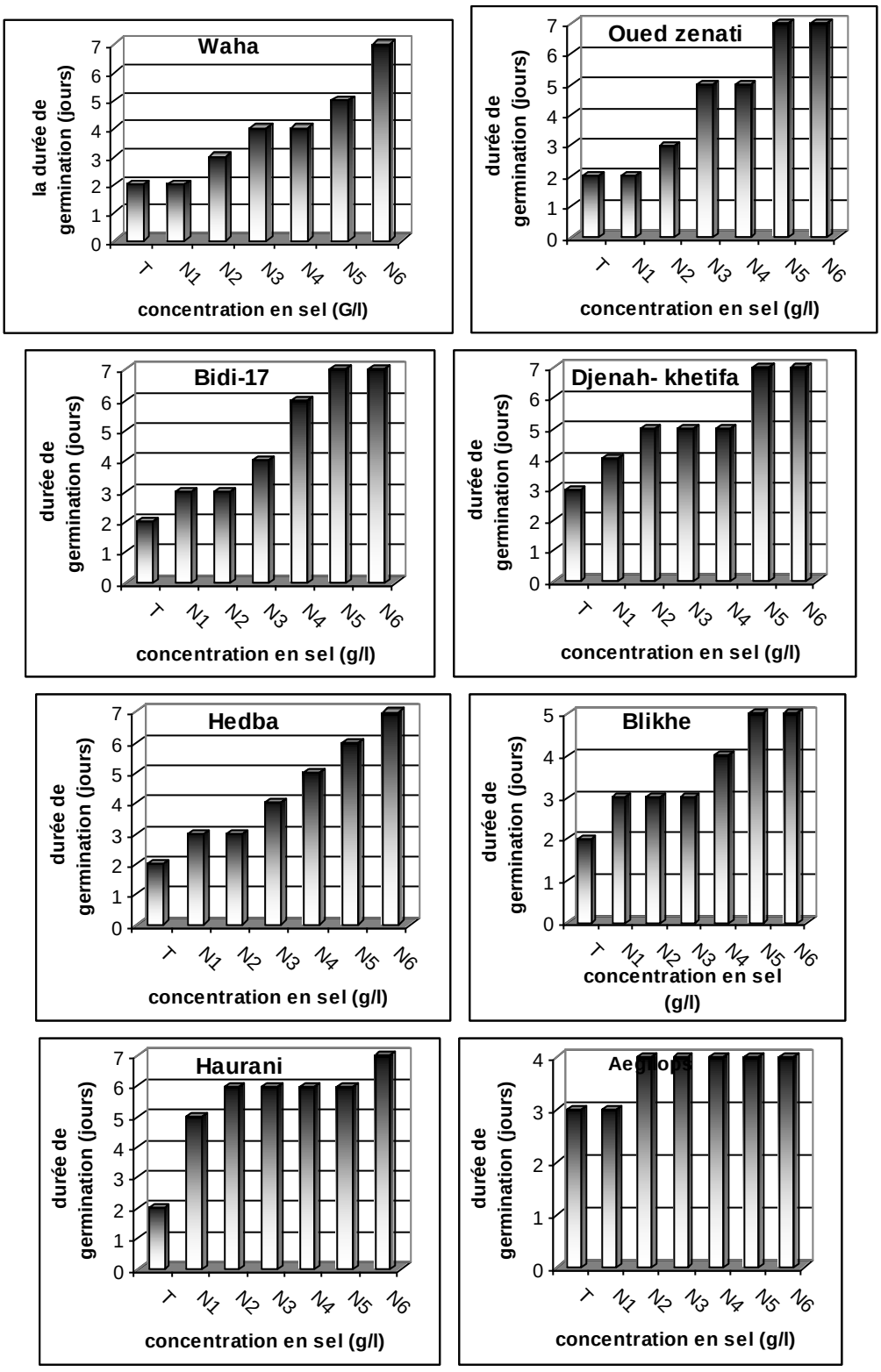


Fig.4: Effect of salt stress on germination time (days) grains of different genotypes

III-4- The length of the epicotyl

The elongation parameter of the aerial part, moderate salt stress does not seriously affect the length of the epicotyl genotypes tested (Figure 5). However, when the stress is severe, the discrimination between varieties is very clear. This discrimination is supported by an analysis of variance with two factors result is shown in (Annex). This analysis showed significant differences between the four treatments (control, 5, 10, 15 g / l). In addition, the test

length of the epicotyl (Figure 5), under severe stress, shows that Waha and Aegilops genotypes are significantly better in all genotypes (Annex), they are followed Blikhe varieties, Djenah - khetifa, Haurani, Hedba which constitute a homogeneous class different. However, the variety Bidi-17 is at the bottom of the ladder.

III-5-The length of the radicle

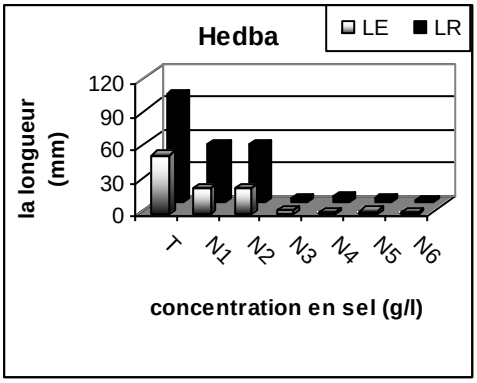
The result of the root system is illustrated in Figure (5). This figure shows that, under salt stress all varieties are affected by cons were observed between genotypes different behaviors vis-à-vis the different levels of stress such as Waha and Aegilops variety show in the severe stress (N4, N5, N6) by a small radicle against other genotypes in the radicle is almost canceled. This particular behavior is a form of tolerance to salt stress.

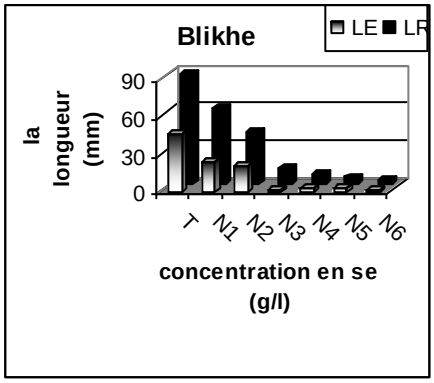
III-6- Growth kinetics

From Figure (6) it was observed that the growth kinetics of the epicotyl is sigmoidal form in all genotypes amidst witnesses, against the allure of this form is changed according to the level of treatment for variety, however, there are differences between genotypes for the degree of Repeat sigmoid shape in the different treatments, depending on the figure (6) it was found that Aegilops presents the sigmoid shape in all stress levels by the variety Waha guard against this form only for the control and treatment (N1) the variety has Haurani the sigmoid shape for the witness, level 1 (L1) and level 2 stress this particular behavior of different genotypes is correlated with the degree of resistance to salinity.

III-7- The correlation between the parameters studied

According to the correlation matrix (Annex) and regression graphs (Figure 7), it was found that there is a negative correlation between germination rate and germination time, when the speed is increased the duration tends to decrease and vice versa.


Hedba
LE
LR
la longueur (mm)
120
90
60
30
0
T
N1
N2
N3
N4
N5
N6
concentration en sel (g/l)


Blikhe
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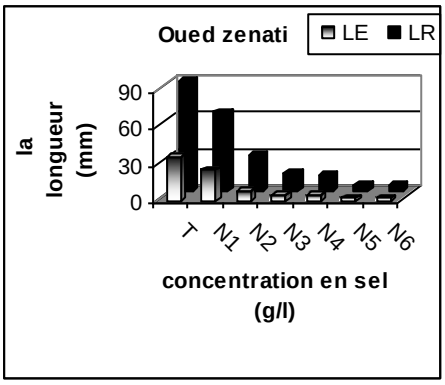
N6
concentration en se (g/l)


Oued zenati
LE
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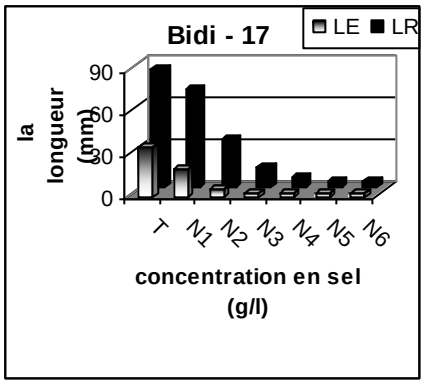
N6
concentration en sel (g/l)


Bidi - 17
LE
LR
la longueur (mm)
90
60
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N1
N2
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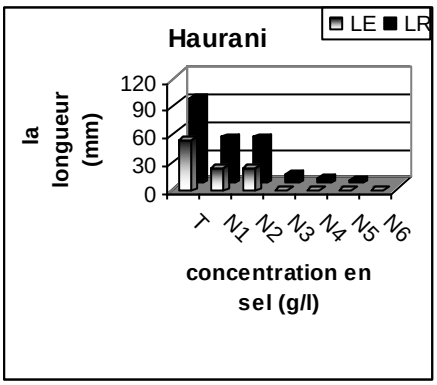
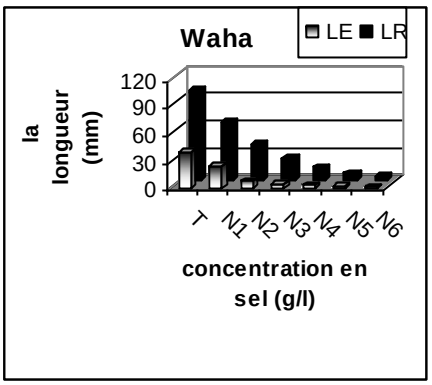
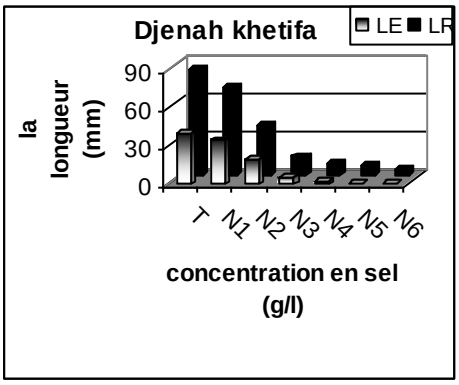
N4
N5
N6
concentration en sel (g/l)


Haurani
LE
LR
la longueur (mm)
120
90
60
30
0
T
N1
N2
N3
N4
N5
N6
concentration en sel (g/l)


Waha
LE
LR
la longueur (mm)
120
90
60
30
0
T
N1
N2
N3
N4
N5
N6
concentration en sel (g/l)


Djenah khetifa
LE
LR
la longueur (mm)
90
60
30
0
T
N1
N2
N3
N4
N5
N6
concentration en sel (g/l)

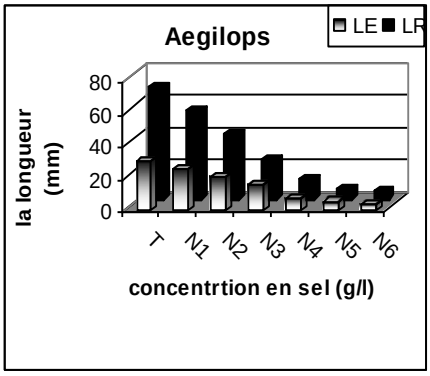

Aegilops
LE
LR
la longueur (mm)
80
60
40
20
0
T
N1
N2
N3
N4
N5
N6
concentrtion en sel (g/l)

Fig.5: Variation of the length of the epicotyl (LE) and radicle (LR) sprouted grains of different genotypes depending on the salt concentration

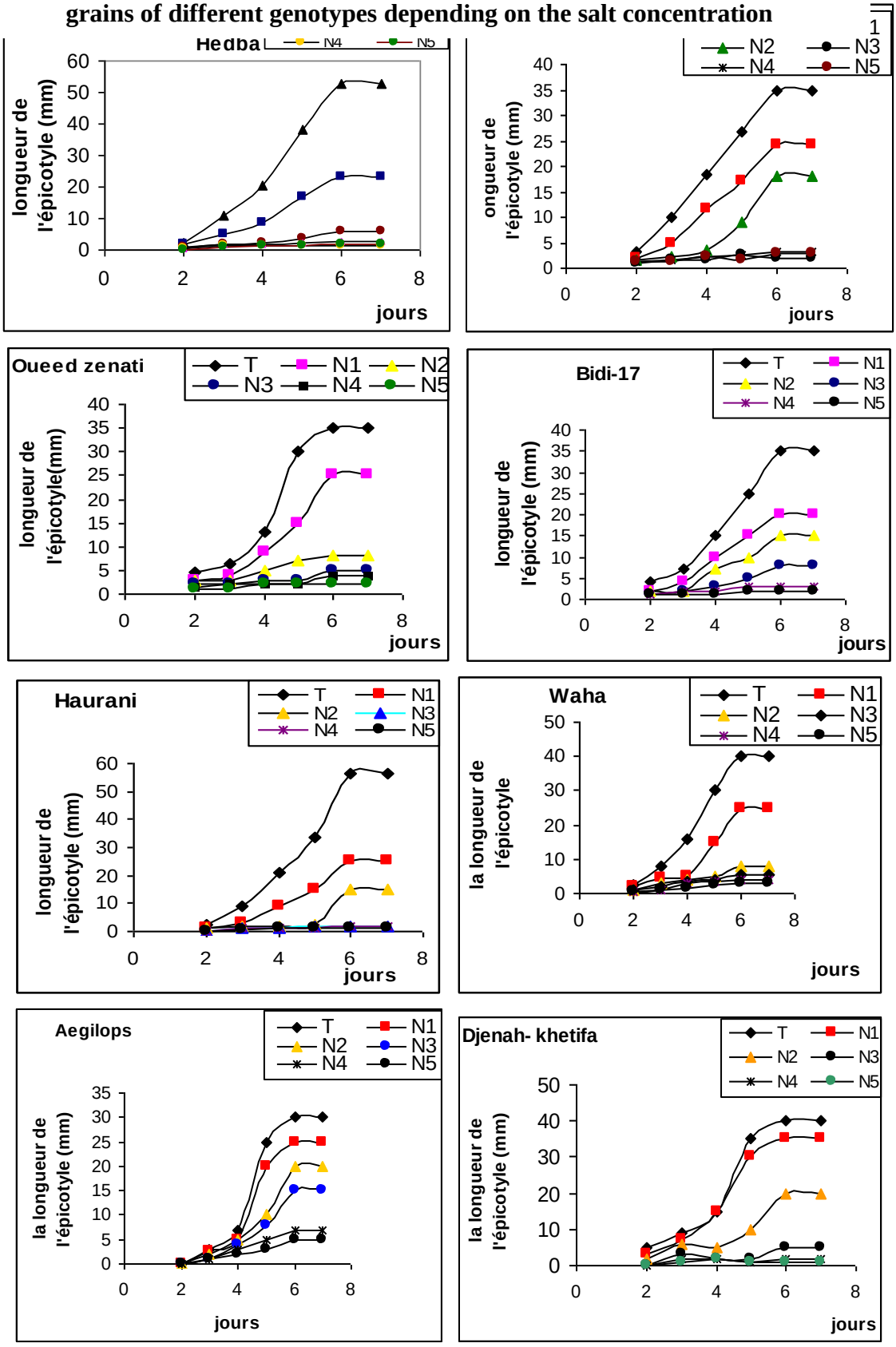
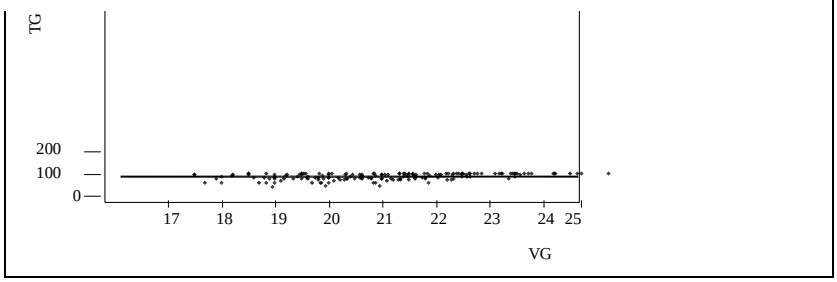
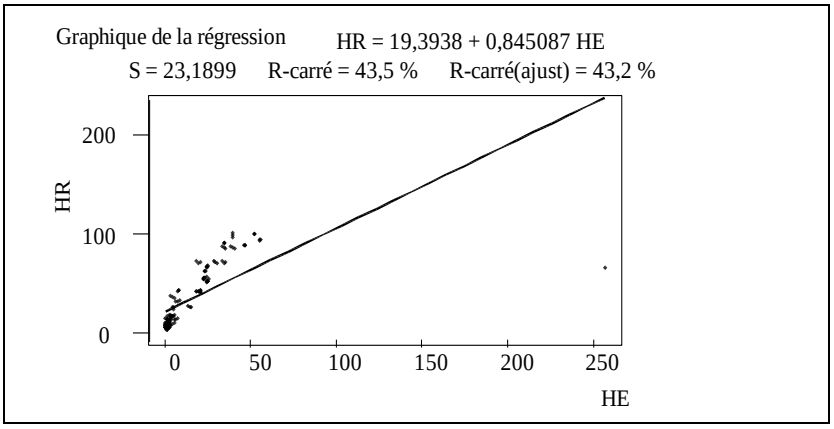


Fig.6: Effect of salinity on the growth kinetics of the epicotyl of sprouted grains of different genotypes





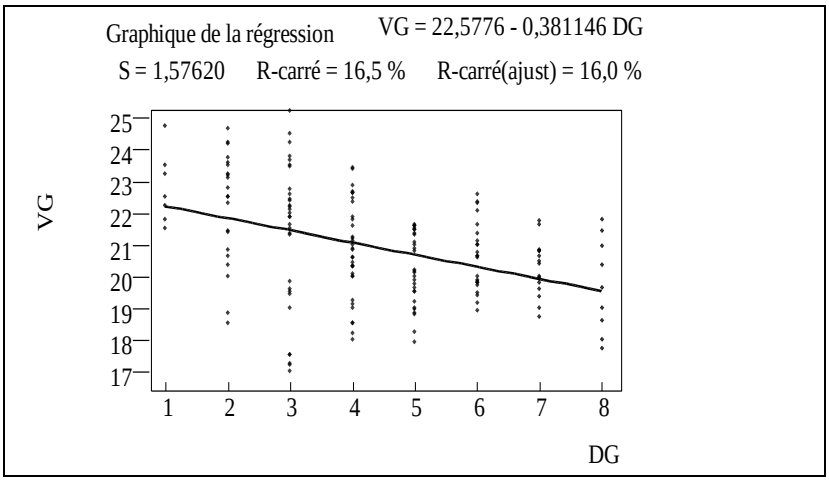


Fig.7: Regression graphs for the different parameters studied

to the length of the epicotyl and radicle the correlation coefficient is positive, thus increasing the length rhythm for both parameters. The length of the radicle is positively correlated with the speed of germination, no correlation

between germination rate and germination time and also between germination and speed of germination, this observation is indicated by a line parallel to the x-axis (Figure 7).

IV-Discussion

The effect of salinity on the germination of durum wheat is well studied by Azmi and Alam [3]. Generally salinity causes a decrease in water absorption of the seeds due to osmotic stress created by the high concentrations of NaCl in the seed media, but the effect of salt stress is not permanant, by that the salt unlike other substrates osmotic (PEG) can penetrate inside the seed tissues [4] and [5]. The accumulation of salts such as NaCl causes tissue toxicity and it prevents the release of radicle essential for the supply of water needed for growth [6]. For this reason it has been observed in our work that the length of the radicle and the epicotyl is affected significantly at high salt concentrations.

In our work, we have stressed grains transferred to a medium without salt are sprouted, but not in a comprehensive manner, for this reason it can be concluded that the effect of salt on seed germination of durum wheat is based majority of the osmotic effect because it lowers the osmotic potential of the germination medium (because of the high salt concentration), but we can say that at least some of the effects of salt stress was caused by ion toxicity.

Against by Redmann (1974) to the effect of salinity on germination only under the effect of ion toxicity without considering the osmotic effect, one that we found in this study the main factor in the decline of germination.

For germination as several authors [7] used this parameter as a criterion of selection for resistance to salinity since the tolerant variety gives a reasonable rate of germination in high concentrations .

In the final stage of germination, the inability of seeds to germinate seem to mean that with increasing salt concentration, the toxicity effect dominates due to the accumulation of Na^+ in the embryo, installing an osmotic inhibition [8]. This toxicity is directly influenced by the Na^+ [9] consistent with a mineral deficiency such as K^+ and the nature of the hormonal inhibition such as abscisic acid compound metabolized by the action of Na^+, owned identical to that of NaCl. This particular ABA inhibits the synthesis of enzymes (or nucleic acid) specific germination [10], limit the absorption of water and controls the regulation of the osmotic pressure cell [11] . These ionic alterations and hormonal assume that the embryo quiescence, expresses an inability to trigger metabolic functions in the presence of high concentrations of salts for use degradation products from reserves of the albumen. These metabolites, primarily carbohydrate compounds and nitrogen function as regulators of osmotic potential gold salt [12]. This operation marks the first embryonic events, following a water inlet, the mean transition from embryonic development to the germ and control of membrane and cellular mechanisms brought into play in germination.

In addition, the course shows that the germination rate of the seeds to germinate is even slower than the salinity of the medium is increased. Most authors admit the existence of this relationship in halophyte and glycophytes and conclude that the duration of the germination process is greatly affected, we found the same result is shown in the correlation matrix between the germination rate and germination time ($r = - 0.75$).

The evolution of growth (growth kinetics) over time can be translated into growth curve, it is useful to reduce to simple mathematical models [13] to generate parameters facilitate comparison, the growth rate and the rate of cell proliferation are characters limit the length of the epicotyl and radicle and are affected by salt stress, therefore the use of growth kinetics according to the results of our study for the selection of tolerant genotypes is useful better than other conventional parameters.

Relationship with germination salinity remains a very complex phenomenon, however, except for the characterization we have to establish some parameters from classic because of the possible involvement of other factors such as embryo dormancy [14] enhanced by the presence of salts, which is also not excluded.

The first result on the germination is not sufficient to create a hierarchy of plant material studied behavior vis-à-vis the salinity. Tolerance to salt stress remains the result of many functional adaptive mechanisms during the life of the plant. It is recognized by some authors that this tolerance changes over time. For [15] varies in the same direction during germination and plant growth but [15] found that the sensitivity of plants such as peas and bean salinity is greater during the development phase, and becomes weaker when germination with high salt content Tolerance is greater during the adult phase.

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V- Conclusion

In this study, our results show a depressive effect of salt on germination of different parameters, thus confirming other work, showed a similar effect of salt stress on the germination and growth of many plant species.

Seed germination under stress transfer in a medium without salt allowed us to conclude that the effect of salinity on germination stage result of the intervention of osmotic effects with the toxic effects of NaCl.

The classification of genotypes according to their degree of resistance to salinity showed that stress tolerant variety keeps decreasing low for most of the parameters studied.

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VII-Annex

Two-factor ANOVA controlled TG (germination rate) based on STRESS (salt stress) and (Variety) VAR

Analysis of variance for TG

Source	DL	SC	CM	F	P
STRESS	6	62763	10460	2,28	0,041
VAR	7	58588	8370	1,82	0,089
Interaction	42	186993	4452	0,97	0,531
Erreur	112	513840	4588		
Total	167	822183			

Confidence interval 95%

```
STRESS       Moyenne   -------+---------+---------+---------+----
N1               131                        (---------*--------)
N2                92              (--------*--------)
N3                87            (--------*--------)
N4                82          (--------*--------)
N5                73      (--------*--------)
N6                67    (--------*---------)
T                 99                (--------*--------)
                       -------+---------+---------+---------+----
                              60        90       120       150
```

Confidence interval 95%

```
VAR          Moyenne        ------+---------+---------+---------+-----
AEGILOPS          97                     (---------*---------)
BIDI              90                   (---------*---------)
BLIKHE            80              (---------*--------)
DJENAH K          73            (--------*---------)
HAURANI           73            (--------*---------)
HEDBA             87                 (---------*---------)
OUED ZEN          93                    (---------*---------)
WAHA              89                 (---------*---------)
                       ---- --+---------+---------+---------+-----
                            60        90       120       150
```

ANOVA two factors were controlled: VG (germination speed) depending STRESS (salt stress) VAR (Variety)

Analysis of variance for VG

Source	DL	SC	CM	F	P
STRESS	6	195,092	32,515	32,75	0,000
VAR	7	157,799	22,543	22,71	0,000
Interaction	42	29,920	0,712	0,72	0,889
Erreur	112	111,187	0,993		
Total	167	493,998			

Confidence interval 95%

```
STRESS       Moyenne   ------+---------+---------+---------+-----
N1             22,15                          (----*---)
N2             21,16                   (---*---)
N3             20,40          (---*---)
N4             20,07       (---*---)
N5             19,88     (---*---)
N6             19,91     (---*---)
T              22,78                                  (---*---)
                       ------+---------+---------+---------+-----
                            20,00     21,00     22,00     23,00
```

Confidence interval 95%

```
VAR          Moyenne   --------+---------+---------+---------+---
AEGILOPS       18,65   (----*---)
```

```
BIDI           21,54                                    (---*----)
BLIKHE         21,95                                        (---*----)
DJENAH K       20,73                              (---*----)
HAURANI        20,48                           (---*---)
HEDBA          21,03                                (---*----)
OUED ZEN       21,73                                       (---*----)
WAHA           21,16                                (----*---)
                        --------+---------+---------+---------+---
                               19,00     20,00     21,00     22,00
```

ANOVA two factors were controlled: DG (germination time) depending STRESS (salt stress) VAR (Variety)

Analysis of variance for DG

Source	DL	SC	CM	F	P
STRESS	6	308,79	51,46	51,46	0,000
VAR	7	63,43	9,06	9,06	0,000
Interaction	42	77,40	1,84	1,84	0,006
Erreur	112	112,00	1,00		
Total	167	561,62			

Confidence interval 95%

```
STRESS       Moyenne   -----+---------+---------+---------+------
N1              3,17          (--*---)
N2              3,75               (--*---)
N3              4,37                    (--*---)
N4              4,87                        (---*--)
N5              5,87                                (--*--)
N6              6,37                                    (--*--)
T               2,25   (---*--)
                       -----+---------+---------+---------+------
                            2,40      3,60      4,80      6,00
```

Confidence interval 95%

```
VAR          Moyenne   ------+---------+---------+---------+-----
AEGILOPS        3,71      (-----*-----)
BIDI            4,57                (-----*-----)
BLIKHE          3,57    (-----*-----)
DJENAH K        5,14                          (-----*------)
HAURANI         5,38                              (-----*-----)
HEDBA           4,29             (-----*-----)
OUED ZEN        4,52                (------*-----)
WAHA            3,86         (-----*-----)
                       ------+---------+---------+---------+-----
                             3,50      4,20      4,90      5,60
```

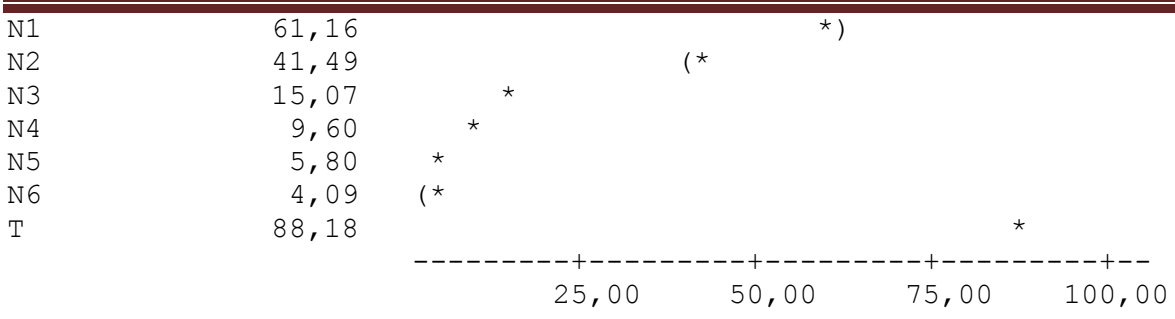
Two-factor ANOVA controlled HR (Height of the radicle) according to variety and salt stress (Var)

ANOVA for HR

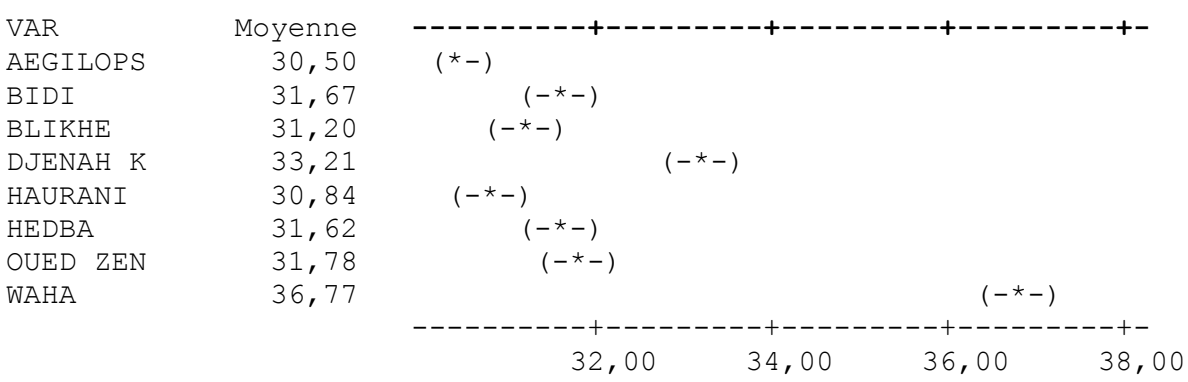
Source	DL	SC	CM	F	P
STRESS	6	152408,6	25401,4	3,4E+04	0,000
VAR	7	597,9	85,4	115,92	0,000
Interaction	42	4886,1	116,3	157,89	0,000
Erreur	112	82,5	0,7		
Total	167	157975,0			

Confidence interval 95%

```
STRESS       Moyenne   ---------+---------+---------+---------+--
```



Confidence interval 95%

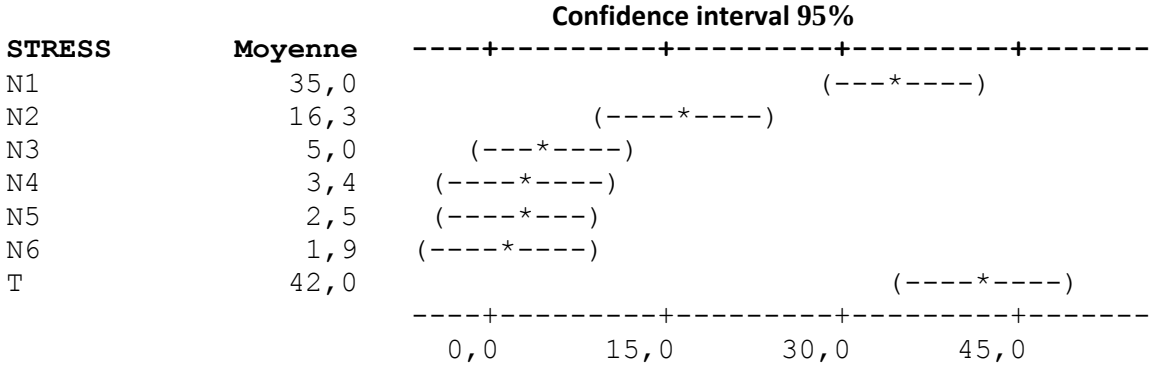


Two-factor ANOVA verified: HE (height epicotyl) versus stress (salt) (Variety) VAR

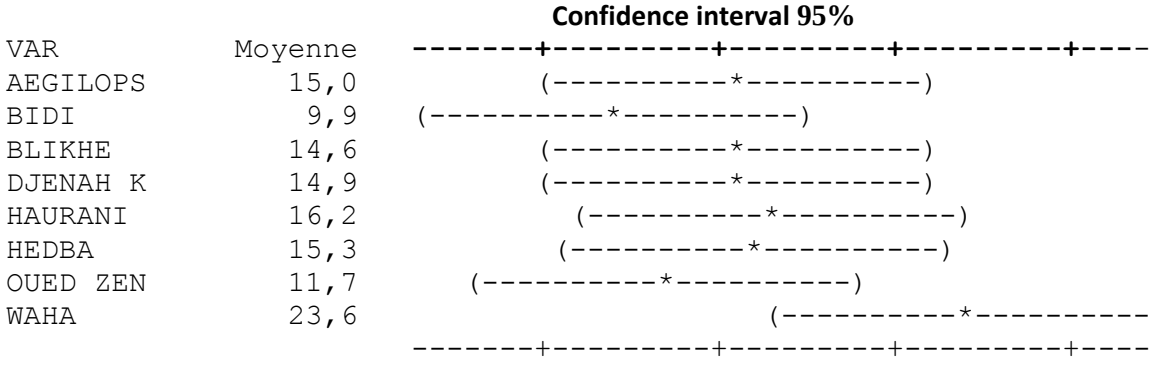
Analysis of variance for HE

Source	DL	SC	CM	F	P
STRESS	6	40786	6798	21,24	0,000
VAR	7	2379	340	1,06	0,393
Interaction	42	17186	409	1,28	0,156
Erreur	112	35851	320		
Total	167	96202			

Confidence interval 95%



Confidence interval 95%



7,0 14,0 21,0 28,0

Correlations HR (Height radicle) HE (height epicotyl) TG (germination rate) VG (germination speed) DG (germination time)

	HR	HE	TG	VG
HE	0,659			
TG	0,184	0,118		
VG	0,640	0,444	0,040	
DG	-0,693	-0,494	-0,149	-0,406