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Salt stress effect on seed germination and some physiological traits in three Moroccan barley (*Hordeum vulgare* L.) cultivars.

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Abstract
This study was carried out in order to test the effect of different salinities concentrations on germination and several physiological traits in three Moroccan barleys cultivars including Arig 8, Asni and Tamelalt. Five different salinities concentrations were used (5, 10, 15 and 20 g/L) in the watering against a control (0g/L). The cultivars showed statistically significant (P<0.001) responses to salt stress. The increase of NaCl concentrations reduced significantly every measured traits, as the final germinating percent, roots and shoots lengths, fresh and dry biomass, relative water content and salt tolerance index, in all the cultivars. The salt tolerance index can be used as an effective criterion to choose tolerant genotypes. Based on this index, these studies led us to conclude Asni (with 42.75%) and Arig 8 (with 47.66%) to be salt-sensitive and moderate salt-sensitive cultivars, respectively, and Tamelalt (with 59.77%) to be a salt-tolerant cultivar; this assertion allowing the last one to be cultivated in saline soil.

Key-words: salinity, salt stress, barley, germination, relative water content, salt tolerance.

Abbreviations
MINEMWE: Ministry of Energy, Mines, Water and Environment.
USDA-ARS: United States Department of Agriculture – Agriculture Research Sources

1. Introduction
In Morocco, Barley is the second cereal after wheat, on the scale of production and food source. Unfortunately in this country, there is a considerable area of land concerned by salinity, as about 500.000 ha are affected (MINEMWE, 2010, http://www.minenv.gov.ma/PDFs/rdeem.pdf). Salinity is one of the major environmental factors that decrease crop productivity and threatens the global food balance. Some of the most serious soil salinity problems occur in arid and semi-arid regions of the world. Out these regions, salinity also affects agriculture in coastal areas and in areas affected by irrigation water of low quality [1]. A soil is qualified as "saline" when its electrical conductivity is about 4 dS/m (≈ 40 mM ≈ 2.38 g/L of NaCl) or higher values, with an approximate osmotic pressure of 0.2 MPa (USDA-ARS, 2008, http://www.ars.usda.gov/Services/docs.htm?docid=8908).
Germination and emergence in saline environment are criteria widely used for selecting salt tolerant genotypes [2, 3]. Germination and early stages of development should be confronted by higher salinity levels than the later stages, as the grown plants are more vigorous, and because germination and early seedlings growth take place in surface soil where there is a high salt accumulation due to evaporation and climbing of water by capillarity [4].
Several studies were made in order to determinate the morphological, physiological and biochemical traits associated with salt stress effects [2, 3, 5-9]. In cereals, germination, growth, retention of water in the plant and yield are negatively affected by salt stress [5, 9, 10], these traits have been shown in several species as barley [5, 6, 11-15], wheat [4, 7-9, 16-18], corn [19-21] and rice [22-24].
Cereals express a wide range of variation for salt tolerance; rice shows to be the most sensitive and barley the most tolerant [25, 26]. This variation can also be observed between different varieties in the same species.
Our study was made at aim to screen the degree of tolerance of three Moroccan barleys cultivars (*Hordeum vulgar* L.), according to different salt concentrations in the watering.

2. Materials and methods

2.1. Plant materials

The seeds of three local cultivars of barley named Arig 8, Asni and Tamelalt, were used. They were delivered by the gene bank center, INRA (National Institute for Agricultural Research) of Settat in Morocco.

2.2. Seeds Sterilization and salt stress application

Seeds were sterilized once by 70% ethanol for 1 min, then rinsed once for 10 min by 10% commercial bleach (final concentration 4.8% of sodium hypochlorite) for 10 min and washed extensively with sterile distilled water. Seeds germination was carried out in five conditions; four concentrations of NaCl (5, 10, 15, and 20 g/L), and distilled water alone (0 g/L of NaCl) being run as control. Each of the five treatments comprised five 55 mm diameter glass Petri dishes (i.e.: five duplicates), and five seeds were sown in each to germinate on Whatman's filter paper; these giving rise to a total number of 25 seeds per concentration value. Until the 10^{th} day, 1 mL of the appropriate solution was poured in each Petri dish. The Petri dishes were disposed in an incubator at 23±1 °C in dark conditions.

2.3. Germination and emergence

The germinated seeds were numbered at the 1^{st}, 2^{nd}, 4^{th}, 6^{th} and the 10^{th} day (n^{th}) after seeding. Seeds were considered as being germinated when the radicle had protruded through the seed coat. Each day the germination percentage was calculated by the following formula:

Germination at n^{th} day (%) = 100 x (*number of germinated seeds at the n^{th} day* / *number of total seeds*)

Final germination (FG%) was presented through the mean value of total number of germinations in percent.
Five seedlings were chosed at random then shoot and root lengths (cm) were measured at the 10^{th} day.

2.4. Weight reduction percentage

The shoots and roots were separated and the fresh weights were measured; after being dried at 80 °C in Pasteur oven during 24 hours, the dry weights were immediately taken. According to each salt treatment, the fresh and dry weights rate, referred to the control, were calculated in percent, by the following equations:

Fresh weight (FW) percentage reduction:

$$\text{FWPR \%} = 100 \times [1 - (\textit{fresh weight}_{\textit{salt stress}} / \textit{fresh weight}_{\textit{control}})]$$

Dry weight (DW) percentage reduction:

$$\text{DWPR \%} = 100 \times [1 - (\textit{dry weight}_{\textit{salt stress}} / \textit{dry weight}_{\textit{control}})]$$

2.5.-Relative water content (RWC)

The water content respective to the fresh weight was calculated as described by Sumithra *et al.* [27]:

$$\text{RWC \%} = 100 \times [(FW - DW) / FW]$$

2.6. Salt Tolerance Index (STI)

It is quantified by the ratio, respectively to the control, of the total drys weight in salt stress, in percent, and calculated by the following equation:

$$\text{STI \%} = 100 \times (\textit{Total DW}_{\textit{salt stress}} / \textit{Total DW}_{\textit{control}})$$

2.7. Data treatment

The experiment was made as a completely randomized design (CRD) with five duplicates (n=5). The variances from each Petri dish comfort the data to be reliable. The data were statistically treated by Fisher's analysis of variance (ANOVA). The means values of cultivars, after NaCl treatments and their interactions were compared by the Least Squares Means Tukey's HSD (Honestly Significant Difference) test at 0.05 probability levels using the JMP SAS 10 Pro.

3. Results and discussion

Globally, the results revealed that several studied traits were significantly influenced by the salt stress levels, that is to say: the cultivars and the interaction cultivars x NaCl concentrations; while for the final germination percentage (FG%) and the water content in the shoots, in percent (RWC%), the interaction effect (cultivars x NaCl concentrations) was not significant, indicated that the responses of the tested set cultivars were not variable among NaCl treatments (Table 1).

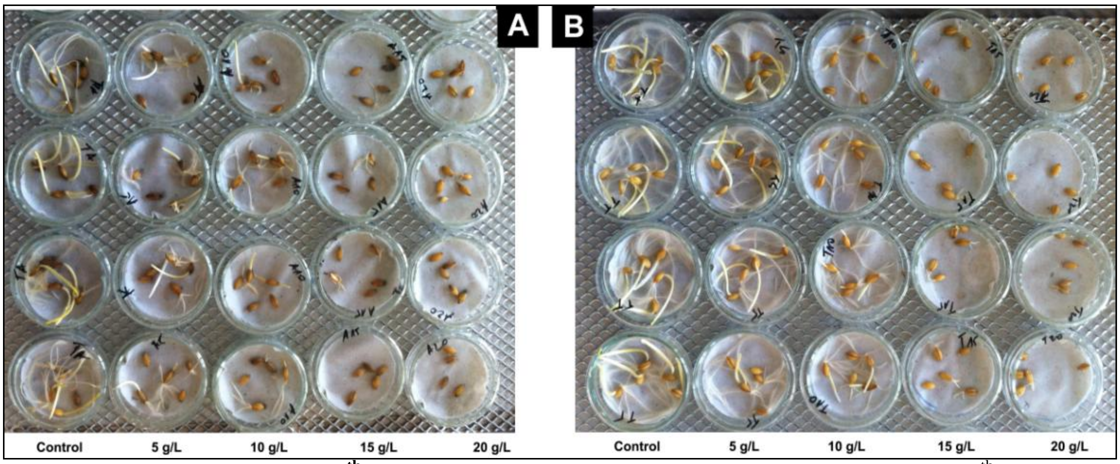
3.1. Germination and emergence

Final seeds germination and emergence were affected by salinity compared to the control (Picture 1 and 2). A decrease was expressed with the increase of NaCl concentrations (Figure 1). The sensitivity analysis showed a high significant difference in response to salt stress, between the cultivars (Table 1). Asni (35.24%) was more affected than Arig 8 (46.40%), and Tamelalt (58.80%), but Tamelalt and Arig 8 did not differ significantly (Table 2). Moreover, salinity showed significant difference between levels of NaCl concentration in the watering. The inhibitory effect on germination was clearly observed when comparing the effects of the highest NaCl concentrations to the controls (Table 2).

Table 1: Variance analysis (ANOVA) for the traits investigated for the three cultivars in response to salinity stress.

		Mean square				
Source of variation	**df**	**STI %**	**SL (cm)**	**Root FWPR %**	**Root DWPR %**	**Shoot RWC %**
Cultivars	2	1535.29 ***	14.19 ***	1885.88 ***	1203.93 ***	5.17 *
NaCl (g/L)	3	13090.98 ***	116.80 ***	4335.50 ***	4117.20 ***	14.39 ***
Cultivars x NaCl	6	109.65 ***	3.06 ***	208.16 ***	203.91 ***	1.55 ns
		FG %	**RL (cm)**	**Root RWC %**		
Cultivars	2	3472.41 **	46.44 ***	13.81***		
NaCl (g/L)	4	12799.28 ***	281.80 ***	8.56 ***		
Cultivars x NaCl	8	117.08 ns	5.003 ***	0.80 *		
		Shoot FWPR %	**Shoot DWPR %**			
Cultivars	2	2835.52 ***	2292.00 ***			
NaCl (g/L)	2	5115.54 ***	4016.97 ***			
Cultivars x NaCl	4	106.18 ***	109.34 ***			

*, **, ***: significant at 5%. 1% and 0.1% level, respectively; and ns: not significant.
FG, Final germination; RL, Roots length; SL, Shoots length; FWRP, Fresh weight reduction percentage; DWRP, Dry weight reduction percentage; RWC, Relative water content; STI, Salt tolerance index.



Picture 1: Germination of the seeds at the 6th day. Emergence of the shoot, and growth of the roots, at the 6th day, according to the salt concentration in the watering and free-salt control; **A**: Asni, Salt effects can already be detected from 5g/l in watering, compared with the control. **B**: Tamelalt, for this genotype, compared to the free-salt control, little effects are registered until 10g/l of salt.

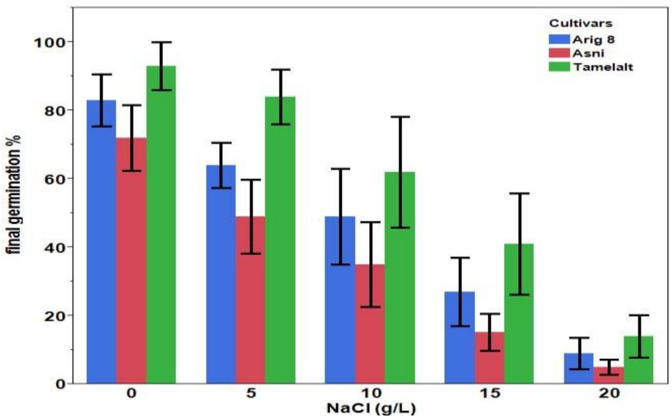


Figure 1: Effect of NaCl (5, 10, 15 and 20 g/L) and the control (0g/L NaCl), on final germination percentage for the three barley cultivars (Arig 8, Asni and Tamelalt), 10 days the seeds sowing. The data are expressed by the means±standard error.

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Table 2: means (± standard error) comparison of effects of cultivars, salinity stress levels and their interaction on the studied traits, according to least squares means tukey HSD Test.

Parameter	Traits									
	FG %	RL (cm)	SL (cm)	Root FWRP %	Root DWPR %	Shoot FWRP %	Shoot DWRP %	Root RWC %	Shoot RWC %	STI %
Cultivars										
Arig 8	46.40±6.52 ab	5.95±0.85 b	5.29±0.58 b	55.56±3.16 b	50.41±2.87 b	41.77±4.97 b	37.50±4.679 b	89.28±0.21 b	83.99±0.34 ab	47.66±5.86 b
Asni	35.24±6.08 b	5.71±0.74 c	4.68±0.58 c	63.58±2.57 a	56.57±2.78 a	48.84±4.21 a	43.34±3.585 a	88.61±0.20 c	83.97±0.38 b	42.75±5.18 c
Tamelalt	58.80±7.45 a	7.83±1.03 a	6.35±0.58 a	44.25±4.65 c	41.15±4.56 c	22.29±3.29 c	19.61±2.958 c	90.09±0.10 a	84.86±0.22 a	59.77±6.71 a
NaCl (g/L)										
0	82.67±4.93 a	12.01±0.45 a	8.25±0.07 a	---	---	---	---	90.39±0.07 a	85.24±0.15 a	---
5	65.67±6.01 ab	9.10±0.66 b	6.75±0.36 b	33.79±4.24 d	29.86±3.76 d	18.43±2.61 c	17.21±2.6 c	89.69±0.21 b	84.98±0.29 a	79.08±2.77 a
10	48.67±8.17 bc	6.88±0.37 c	4.99±0.36 c	48.05±1.74 c	42.05±1.39 c	39.2±3.47 b	33.3±2.969 b	89.20±0.24 bc	83.60±0.46 b	63.82±2.31 b
15	27.73±6.39 cd	2.23±0.24 d	1.76±0.21 d	63.99±1.54 b	59.02±1.31 b	55.26±3.56 a	49.94±3.39 a	88.97±0.22 c	83.28±0.28 b	47.00±2.52 c
20	9.333±2.67 d	0.99±0.14 e	---	72.01±1.36 a	66.58±1.23 a	---	---	88.39±0.30 d	---	10.34±0.60 d
Cultivars x NaCl (g/L)										
0g/L NaCl										
Arig 8	83.0±7.68 ab	11.78±0.29 bc	8.30±0.10 a	---	---	---	---	90.35±0.02 a	84.99±0.20 a	---
Asni	72.0±9.57 abc	10.32±0.58 cd	8.14±0.09 ab	---	---	---	---	90.22±0.11 ab	85.43±0.33 a	---
Tamelalt	93.0±7.0 a	13.94±0.12 a	8.32±0.19 a	---	---	---	---	90.59±0.14 a	85.30±0.21 a	---
5g/L NaCl										
Arig 8	64.0±6.60 abcd	8.14±0.33 ef	6.86±0.13 bc	36.51±0.45 g	34.66±0.80 f	17.25±0.89 d	15.57±1.315 de	90.07±0.19 ab	84.69±0.19 ab	78.92±0.84 b
Asni	49.0±10.90 abcdef	6.88±0.69 fg	5.28±0.57 d	51.45±1.01 f	43.93±0.30 e	29.73±3.25 c	28.57±1.482 c	88.69±0.17 cde	85.05±0.83 a	67.07±1.01 c
Tamelalt	84.0±7.97 ab	12.28±0.25 ab	8.10±0.19 abc	13.40±2.00 h	10.99±1.79 g	8.308±1.54 e	7.495±3.249 e	90.32±0.13 a	85.19±0.34 a	91.24±2.36 a
10g/L NaCl										
Arig 8	49.0±14.0 abcdef	6.24±0.15 g	4.14±0.21 d	50.01±0.58 f	43.36±0.71 e	46.35±1.80 b	39.62±2.862 b	89.06±0.23 bcde	83.00±1.16 ab	59.32±2.05 d
Asni	35.0±12.35 bcdef	5.62±0.14 g	4.02±0.19 d	54.19±1.70 ef	45.84±2.28 e	49.64±0.76 b	41.14±2.163 b	88.43±0.35 def	83.01±0.38 ab	57.50±1.86 d
Tamelalt	62.0±16.17 abcde	8.78±0.15 de	6.80±0.10 c	39.96±1.35 g	36.94±2.03 f	21.63±2.30 d	19.14±2.056 d	90.12±0.23 ab	84.81±0.43 ab	74.64±1.99 b
15g/L NaCl										
Arig 8	27.0±9.95 cdef	1.908±0.33 hi	1.840±0.35 e	62.58±0.92 cd	56.76±0.59 cd	61.72±0.07 a	57.3±0.744 a	88.82±0.32 cde	83.28±0.10 ab	42.85±0.41 e
Asni	15.2±5.31 def	2.02±0.58 hi	1.284±0.23 e	71.36±0.45 b	65.62±0.48 ab	67.14±0.39 a	60.31±0.548 a	88.26±0.18 ef	82.41±0.33 b	38.19±0.51 e
Tamelalt	41.01±4.78 bcdef	2.76±0.20 h	2.160±0.43 e	58.02±0.92 de	54.66±0.63 d	36.93±1.74 c	32.2±0.844b c	89.82±0.23 abc	84.14±0.58 ab	59.96±0.77 d
20g/L NaCl										
Arig 8	9.0±4.58 f	0.64±0.07 i	---	73.12±0.72 ab	66.85±1.80 ab	---	---	88.10±0.55 ef	---	9.554±0.48 f
Asni	5.0±2.24 f	0.72±0.08 hi	---	77.30±0.99 a	70.87±1.15 a	---	---	87.45±0.11 f	---	8.229±0.36 f
Tamelalt	14.0±6.21 ef	1.38±0.19 hi	---	65.61±0.53 c	62.02±1.23 bc	---	---	89.62±0.11 abcd	---	13.24±0.28 f

For each treatment, a same letter links genotypes with not significantly different means at $P = 0.05$.
FG, Final germination; RL, Roots length; SL, Shoots length; FWRP, Fresh weight reduction percentage; DWRP, Dry weight reduction percentage; RWC, Relative water content; STI, Salt tolerance index.

For all the cultivars, roots and shoots lengths decreased as NaCl concentrations were increased (Picture 2, Figure 2); moreover, for both roots and shoots the maximum lengths value were expressed in the control conditions and the smallest with the highest NaCl concentration. Under salt stress conditions, the highest mean lengths value for roots (7.83 cm) and shoots (6.35 cm) were showed by Tamelalt (Table 2). The shoots were more affected than the roots and a clear inhibition of the shoots growth was showed with 20 g/L NaCl concentration (Figure 2B).

Picture 2: Comparison of Asni and Tamelalt at the 10th day of germination. Shoot and roots development for a single unit of Asni and Tamelalt, out of the 25 analyzed, at the 10th day, according to the salt concentration for the watering, and free-salt control. A clear slowing down of the germination can be observed with the increase of salt in the watering, for the two genotypes.

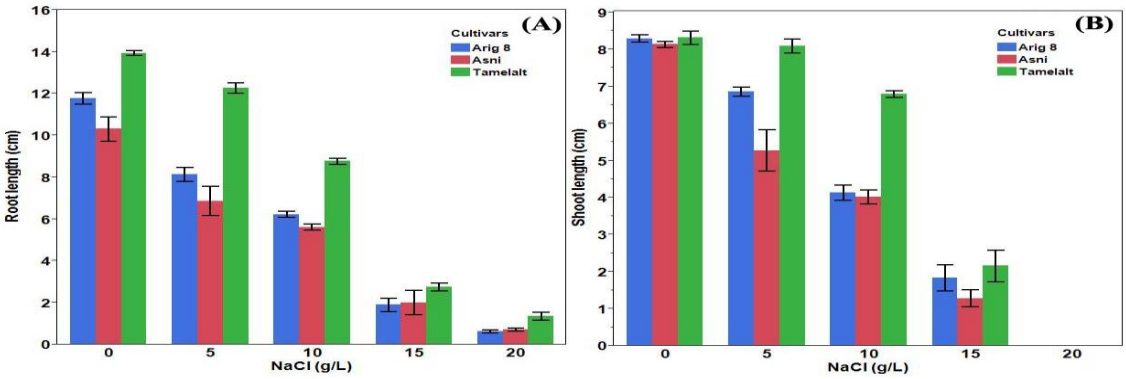

Figure 2: Effect of NaCl (0, 5, 10, 15 and 20 g/L) on the lengths of the roots (A) and the shoots (B) for the three barley cultivars (Arig 8, Asni and Tamelalt) after 10 days of the seeds sowing. The data are presented through the means±standard error.

3.2. Weight percentage reduction

The results obtained in Table 1 point out that fresh and dry weights were affected by salt stress in all the cultivars (Figure 3, Figure 4). However Tamelalt was less affected than Arig 8 and Asni especially for shoots weights (Table 2).

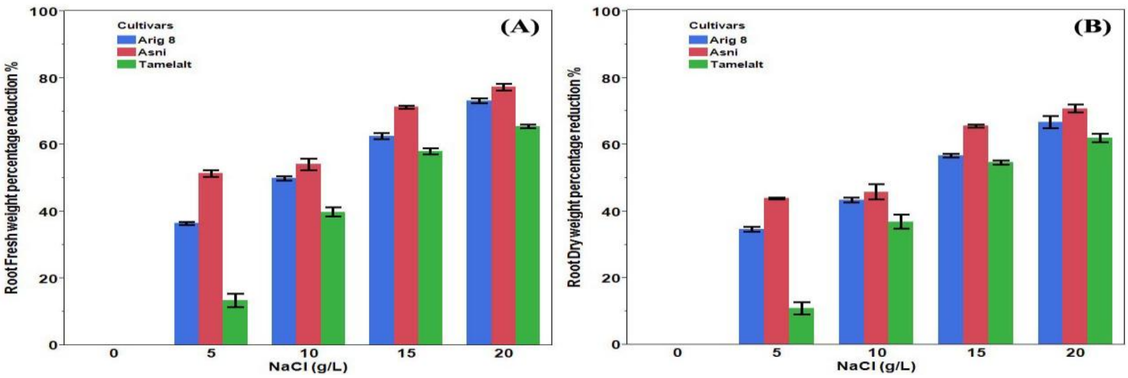

Figure 3: Roots fresh (A) and dry (B) weight reduction, in percent, according to NaCl treatment (0, 5, 10, 15 and 20 g/L) for the three barley cultivars (Arig 8, Asni and Tamelalt) after 10 days of the seeds sowing. The data are expressed by the means±standard error.

Roots fresh weights were significantly reduced under salt stress (Figure 3A). Tamelalt with a percentage reduction of 44.25% was more tolerant than Arig 8 (with 55.56%) and Asni (63.58%) (Table 2). However, there was a strong decrease in roots fresh weights with the highest NaCl level (20 g/L) compared to the control, with a reduction of 72.01% (all cultivars combined). In the same way, in roots dry weights, percentage reduction was significant among cultivars and between NaCl levels (Table 2) (Figure 3B).

The fresh and dry weight percentage reduction was lower in the shoots than in the roots (Figure 4). The fresh percentage reduction in the shoots was important for Asni (48.84%) and Arig 8 (41.77%) compared to Tamelalt (22.29%), this difference in reduction was also significant according to NaCl levels. With 15 g/L of NaCl level, the highest value of percentage reduction of 55.26% was shown, compared to 5 g/L NaCl level which showed the lowest reduction of the means of 18.43% (Table 2) (Figure 4A). The same observations could be made for dry weights percentage reductions (Figure 4B). The differences were significant between the cultivars, and Tamelalt showed the lowest mean reduction (19.61%) whereas Arig 8 and Asni showed highest values, with 37.50% and 43.34% respectively. The 15 g/L NaCl level revealed the highest reduction of 49.94% compared to 5 g/L NaCl level which showed the lowest value of 17.21% (Table 2).

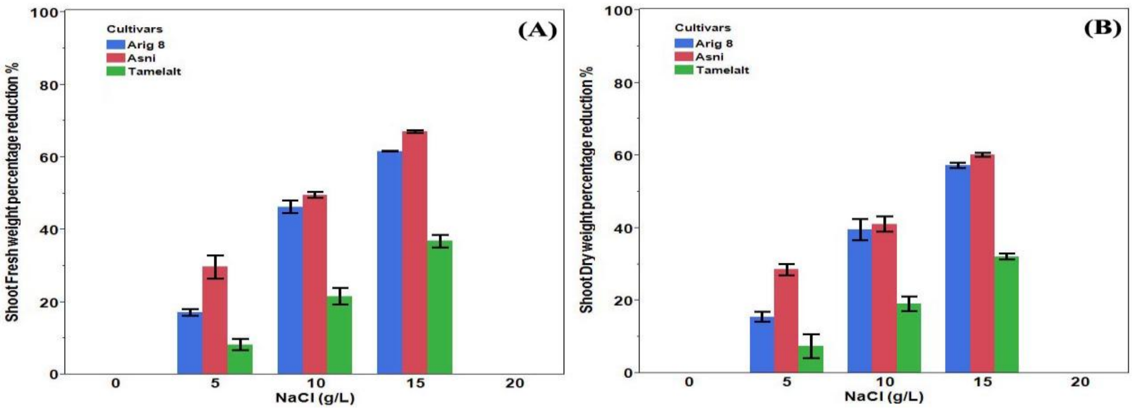

Figure 4: Shoots fresh (A) and dry (B) weight reduction percentage according to NaCl treatment and the three barley cultivars (Arig 8, Asni and Tamelalt) after 10 days of the seeds sowing. Data are expressed by the means±standard error.

3.3. Relative water content

Increasing salinity caused the diminution of water content in the roots and the shoots (Figure 5A, 5B). Significant differences of relative water content were shown in the roots and the shoots among cultivars at different NaCl levels (Table 1). For all cultivars, the reduction of relative water content was the most noticeable with the highest NaCl level, where Asni and Arig 8, regarded as salt-sensitive cultivars (Table 2). Considering the cultivars, the highest means of relative water content in the roots and the shoots, recorded for the control, were found for Tamelalt, with 90.09% and 84.86%, respectively, and the lowest recorded with high NaCl levels were for Asni (87.45% and 82.41%) (Table 2). In salt stress, the highest means of relative water content were observed for the control, with 90.39% for the roots and 85.24% for the shoots, while the lowest values were recorded with 20 g/L of NaCl level giving 88.39% in the roots and with 15 g/L NaCl level giving 83.28% in the shoots (Table 2).

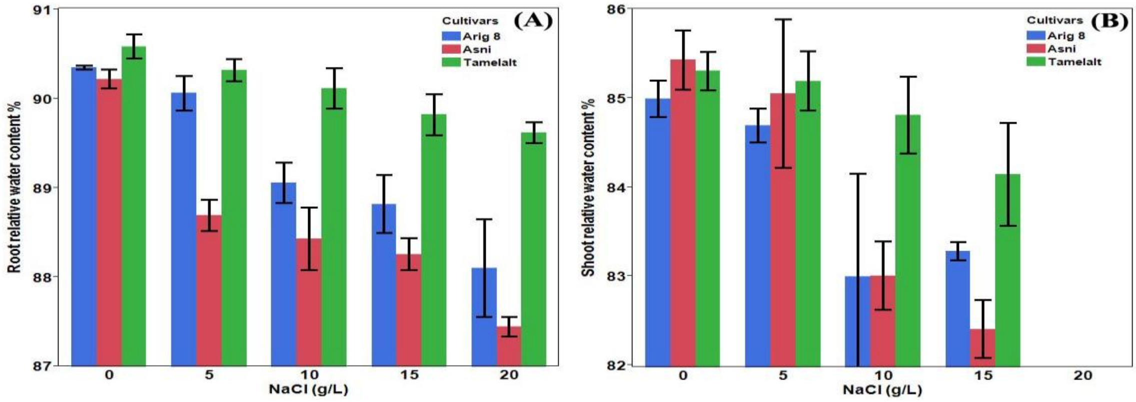

Figure 5: effect of NaCl treatment (0, 5, 10, 15 and 20 g/L) on relative water content percentage in the roots (A) and the shoots (B) for the three barley cultivars (Arig 8, Asni and Tamelalt) after 10 days of the seeds sowing. Data are expressed by the means±standard error.

3.4. Salt Tolerance Index

Salt tolerance Index decreased with the increase of salt stress (Figure 6).The ANOVA made on salt tolerance index data showed significant differences ($P < 0.001$) between the cultivars, NaCl levels and their interaction (Table 1). Tamelalt showed the highest salt tolerance index (59.77%) while Arig 8 and Asni showed the lowest (47.66% and 42.75% respectively). The effect of salt stress on this index was outstanding mainly with 20 g/L NaCl, which showed the lowest index value (10.34%) (Table 2).

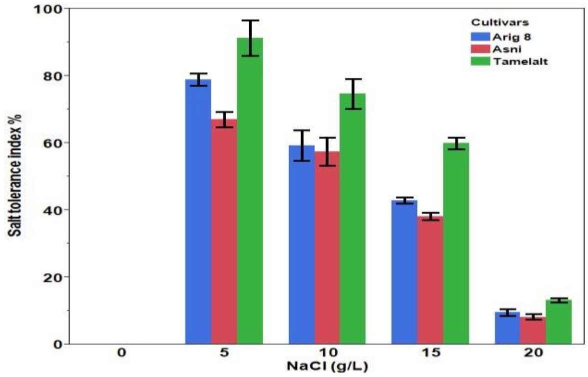

Figure 6: Salt tolerance index as a function of the NaCl treatment (0, 5, 10, 15 and 20 g/L) and the three barley cultivars (Arig 8, Asni and Tamelalt) after 10 days of the seeds sowing. Data are expressed by the means±standard error.

In this study, the evident salt stress effect on germination, emergence, biomass weight and relative water content was detected. The increase of salt concentration had a negative effect on germination for which the rate decreased; this result has also been reported by several authors [10, 5, 24]. This decrease might be caused by the high osmotic pressure of the solutions slowing down the intake of necessary water for germination and by the toxic effect of high salt concentration on embryo [28]. Sayar *et al.* [29] reported that high salt concentration inhibits the mobilization of the seed reserves and the growth of embryonic axis.

By the same way the roots and the shoots lengths decreased, when salt concentration was increased. These results, in concordance with those of Naseer *et al.* [12], El madidi *et al.* [5] and Khodarahmpour *et al.* [21] showed that the shoots were more affected than the roots. At first, the salt stress induces rapid osmotic changes which affect the roots growth within a few minutes, and therefore the disruption of the shoots development [25]; likewise, toxic ions spread toward the leaves and accumulated there; this can lead to metabolic dysfunction and toxicity [30]. Furthermore, Albacete *et al.* [31] reported that salt stress affects the hormonal equilibrium (cytokinins/auxins) which causes trouble in shoot growth, impairment, and changes in biomass partitioning.

The fresh and dry mass decreased largely by the effect of salt stress; this decrease was also related to cultivars performances and it was important in the salt-sensitive cultivars (Asni and Arig 8). This decrease in biomass was obtained by Chen *et al.* [32].The decrease in dry biomass maybe caused by the increase of Cl^- concentration in the tissue [33].

The water content was significantly reduced by the increase of NaCl concentration; similar results were reported too by Chen *et al.* [34], Chutipaijit *et al.* [35] and Munns *et al.* [36]. This reducing was also associated to the salt-tolerance potentialities of the cultivars; so, a slight decrease water content was shown in Tamelalt considered as a salt-tolerance cultivar, while the highest decrease in Asni leads to regard it as a salt-sensitive cultivar. The increased solute content of the cells in the salt-treated plants causes more water to be taken up than in the control leaves, resulting in an apparent low RWC in the salt treatment. [36].

As cited by Abbas *et al.* [24], "salt tolerance index" is considered to be a reliable criterion for salt tolerance. Our results showed obviously that salt tolerance index was negatively affected by salt stress in all cultivars, which coincide with Abbas *et al.* [24] findings, and Tamelalt showed a higher salt tolerance index than Arig 8 and Asni.

4. Conclusion

It is clear that all the studied traits were negatively affected by the increase of salt concentration. Germination and emergence were affected in the early stage of plant grouwth; therefore this impact can cause damage in crops production. The different responses between the cultivars can be used to assess salt-tolerance potentialities in cultivars, and this work shows that the salt tolerance index can be used as salt tolerance criterion to choose tolerant cultivars in cereals. Tamelalt was showed to be a salt-tolerant cultivar; while Arig 8 and Asni were revealed to be (unveiled) moderate salt-sensitive and salt-sensitive cultivars, respectively. Therefore, the farmers should be advised to use Tamelalt cultivar to be cultivated in salty soils.

Acknowledgements
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