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# Functional variation of potassium, sodium and chloride ions in selected salt-tolerant-calli from durum wheat (*Triticum durum* Desf.) mature embryo

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## Abstract
Mature embryos were used to establish callus cultures in MS modified medium. Cell lines calli were then submitted to increasing concentrations [0, 4, 8, 12, 16 g.L-1] of NaCl. Therefore growth, water and ions contents ($K^+$, $Na^+$ and $Cl^-$) were determined in both the control (unselected wild-type calli maintained in salt-free medium) and selected (tolerant and sensitive genotypes) calli. Selected salt-tolerant calli showed no growth reduction in comparison with the control when treated with 16 g.L-1 NaCl while a significant growth decrease was noted in salt-sensitive ones. Water content was significantly higher in salt-tolerant calli than in salt-sensitive ones. Both tolerant and sensitive calli accumulated less K+ than the control but K+ content of salt-tolerant calli remained higher than that of salt-sensitive ones. Accumulation of Na+ and Cl- was higher in salt-sensitive calli than in salt-tolerant ones with Na+ increase being the highest. The results indicated that Na+ especially but also Cl- exclusion play a key role in salt-tolerance in wheat calli lines. The comparison of K+/Na+ ratio allowed to classify wheat varieties Sebou, Anouar and Tarek as salt-tolerant and Marzak, Ourgh, Massa, Tomouh and Amjad as salt-sensitive ones.

*Keywords*: *Triticum durum*; salinity; callus; selection; salt tolerance; physiological traits

## Introduction
Salinity is an environmental challenge that severely limits plant growth and productivity worldwide [11; 17]. It has been estimated that salinity affected nearly 950 million ha of land in the world (Flowers, 2004) and approximately 20 mha of land deteriorates to zero production each year [16] mainly due to salinity. The progressive salinity of soil was estimated at around 20% of irrigated land [9]. Understanding the mechanisms that enable plants to adapt to salt stress is important to produce salt tolerant genotypes in order to exploit saline soils.
In vitro somatic cell and tissue culture technology has been used to study adaptive mechanisms of salt tolerance in several species [1; 4; 14]. Comparing the response of cultivars to salinity provides a convenient and useful tool for unveiling the basic mechanisms involved in salt tolerance. Moreover, studies at the cellular level are advantageous since they require relatively little space and shorter time for the selection, as well as the use of controlled environment [23].
Several studies have indicated that changes in the growth of plants subjected to salinity appear to be associated with the accumulation of toxic elements and/or osmotic adjustment and turgor maintenance against salts [20]. Plants in saline environment accumulate organic solutes such as sugars, amino acids and proteins to prevent water stress [23; 26] eventually to stabilize membranes and macromolecular structures. Accumulation of these compounds would be a metabolic adaptation observed in a number of stress-tolerant plants but not all higher plants accumulate the same substances. There are different processes of accumulation of ions in plants such as sodium ($Na^+$) potassium ($K^+$), calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) depending on the genotype and the variation in the external environment. Wyn and Gorham [28] reported that salt tolerance in wheat is related to poor absorption of $Na^+$ and $Cl^-$. Sabbah and Tal [24] have found that in in-vitro selected salt tolerant calli, the decrease in osmotic potential was not mainly correlated to electrolytes accumulation. Furthermore, Basu et al. [3] reported that $K^+$ was the first candidate to counteract the negative water potential of outside medium when exposed to salt stress.
In this study, Tissue culture is used to study the effect of NaCl stress on callus growth and the ionic solutes ($K^+$, $Na^+$ and $Cl^-$) accumulation in calli of eight wheat varieties with different levels of tolerance to salinity in order to identify the discriminate ions in wheat salinity tolerance.

## 2. Materials and methods

*2.1. Plant material*

Seeds of eight durum wheat (*Triticum durum* Desf.) varieties (Amjad, Anouar, Marzak, Massa, Ourgh, Sebou, Tarek and Tomouh), obtained from the National Institute of Agronomical Research (Morocco) were used in this experiment.

*2.2. Callus establishment*

Callus cultures was induced from mature embryos following procedures outlined by Koutoua et al. [12] Murashige and Skoog [19] medium (MS) with vitamin B5 [8], containing 3.5 $mgL^{-1}$ 2,4-dichlorophenoxy acetic acid (2,4-D). The cultures were placed in a growth chamber at 28 ± 2 °C under dark condition. Calli obtained after 4 weeks on this MC medium, were thereafter transferred to media with different concentrations of NaCl.

*2.3. In vitro selection procedure*

In vitro selection of salt tolerant calli aimed to find and isolate cell clusters able to grow on a culture medium in the presence of increasing concentrations of salt (NaCl). 2400 portions of calli (300 calli per variety) of about 0.2 g fresh weight (equivalent to 0.012 g dry weight) were detached and transferred on the MC medium in the presence of a progressive salt stress (increasing concentrations of salt: 0, 4, 8, 12 and 16 $gL^{-1}$ NaCl), passing successively each 4 weeks on the next culture medium with gradually higher salt concentration for further proliferation and to initiate selection. Therefore, calli were incubated on the following MC medium enriched gradually with salt (M1: MC + 4 $gL^{-1}$ NaCl ; M2: MC + 8 $gL^{-1}$ NaCl ; M3: MC + 12 $gL^{-1}$ NaCl ; M4: MC + 16 $gL^{-1}$ NaCl). The check calli (600 portions of calli: 75 calli per variety) were subcultured on NaCl-free medium (0 $gL^{-1}$ NaCl) and were designated as non-selected callus line.

After 16 weeks, the identification of calli was carried according to ElYacoubi and Rochdi [7] who mentioned the following characteristics:

- salt-sensitive calli : very low-growing calli whose color turns brownish ;
- salt-tolerant calli : yellowish line with similar size as the check;
- control calli : yellowish non-selected line, maintained on salt-free medium.

After, the selected (salt-tolerant and salt-sensitive) calli were transferred to salt-free MC medium for 8 weeks for further proliferation (independence test). Then, they were cultured for 8 weeks on salt-selection-medium (MC + 16 $gL^{-1}$ NaCl) for stabilization (stability test). Meanwhile, the non-selected calli (control) were continuously cultivated on salt-free MC medium.

*2.4. Callus growth determination*

The fresh weight of the control (*c*) and the selected (*s*) calli (tolerant and sensitive calli) were determined at : 1) the end of the selection process (W0) corresponding to the end of the 16th week of progressive salt stress; and 2) the end of the stabilization test (WF) corresponding to after eight weeks of the independence test with 0 $gL^{-1}$ NaCl followed by eight weeks of stabilization at 16 $gL^{-1}$ NaCl for both types of selected calli. Thus, growth rate (GR) and relative growth rate (RGR) of fresh weight callus were calculated as:

$$GR = (WF-W0)/W0 \text{ and } RGR = 100xGR_{(s)}/GR_{(c)}$$

*2.5. Evaluation of the mechanisms of tolerance*

To explore some mechanisms involved in salt tolerance, growth (estimated by the fresh weight "FW" and dry "DW"), water content (WC) and accumulation of sodium ($Na^+$), potassium ($K^+$) and chloride ($Cl^-$) ions as well as K/Na ratio were determined in the different types of calli at the end of the stabilization test. Thus, 72 samples (8 varieties x 3 callus types x 3 calli) were analyzed.

*2.6. Ions measurement*

Calli were oven-dried at 80°C for 48 h and then finely ground. For Sodium and potassium determination, 200 mg of powder calli was recovered in silica crucibles and ignited in a muffle furnace at 500°C. $Na^+$ and $K^+$ estimation was done by using Flame photometer with the help of standard solutions of NaCl and KCl prepared from reagent grade salts. For Chloride determination, 100 mg of powder calli was digested in 5 ml nitric acid (100%). Cl- was measured by titration with silver nitrate according to Cotlove [5]. Ions concentrations are expressed in mg/g dried weight.

*2.7. Statistical analysis*

The experiment was conducted in a completely randomized design in factorial arrangement. For each experiment, 72 calli (3 samples for each of the 3 callus types and for each of the 8 varieties) were used. Variance analysis of all the studied traits was computed. Differences in mean values were tested and significance levels were obtained with LSD test at 5%.

## 3. Results

*3.1. Callus growth*

Salt stress affected the color and the proliferation of most cell clusters. However, some calli (3.9%) were classified as tolerant, since they kept yellowish appearance and had good growth, despite their presence in high salt concentration medium (16 $gL^{-1}$ NaCl). At the end of the progressive salt stress, average growth rate of stressed calli was 1.36 for tolerant and 0.55 for sensitive calli. These rates were lower than that of the control calli that had a rate of 1.56. Compared to the check, GR reductions (generally regarded as sensitivity index), were 13% for tolerant and 65% for sensitive calli. Therefore, for selected tolerant calli, this reduction remains largely below the salt tolerance classification threshold which is 50%, which confirms their tolerance to salinity (Tab. 1).

**Table 1:** Fresh weight (in g) of tolerant and sensitive durum wheat calli at the end of the progressive salinity test and the stability tests

| Calli type | Fresh weight at the end of the | | |
|---|---|---|---|
| | **Progressive test** | **Independence test** | **Stability test** |
| **Control** | 0.511 | 0.703 | 0.910 |
| **Tolerant** | 0.472 | 0.660 | 0.840 |
| **sensitive** | 0.309 | 0.377 | 0.383 |

Moreover, the significant difference observed between the RGR of the two types of selected calli proved that salt-tolerant ones had grown faster than salt-sensitive ones when they were cultured in the presence of NaCl (Tab. 2). After the independence test on salt-free MC medium for 2 months, the growth rate GR was statistically similar for both the control and salt-tolerant calli and it was lower for the sensitive calli (Tab. 1). Similarly, RGR was largely different between the two calli types (Tab. 2).

After the stability test on salt-free MC medium, the growth rate (GR) was statistically similar for both the check and salt-tolerant calli (around 0.78) and it was much lower (0.24) for the sensitive calli (Tab. 1). Therefore, salt effect resulted in a significant reduction ($P<0.05$) of the fresh and dry RGR of sensitive calli compared to those of tolerant ones (Tab. 2).

**Table 2:** Relative growth rate (RGR) of tolerant and sensitive durum wheat calli at the end of the independence and the stability tests based on fresh (FW) and dry weight (DW) of calli

| Trait | Initial calli weight considered as W0 | RGR at the end of the independence test | | RGR at the end of the stability test | |
|---|---|---|---|---|---|
| | | **Tolerant** | **Sensitive** | **Tolerant** | **Sensitive** |
| **FW** | : weight at the beginning of the progressive salt stress | 91.30 a | 27.40 b | 90.14 a | 26.00 b |
| **FW** | : weight at the end of the progressive salt stress | 108.10 a | 32.40 b | 101.7 a | 31.2 b |
| **DW** | | - | - | 98.7 a | 43.2 b |

*3.2. Water content*

Water content of calli decreased significantly with salt stress and callus type (Tab. 3). Thus, salt sensitive calli had the lowest water content followed by salt-tolerant calli. Indeed, water content passed from 90% of fresh weight in control calli to 78% and 42%, respectively in tolerant and sensitive calli (Tab. 4). Therefore, the type of callus kept water differently in presence of salt. However, differences were not significant between all eight varieties but they were significant for the interaction variety x callus type (Tab. 3).

*3.3. $K^+$ content*

$K^+$ concentration decreased under salt stress, it varied according to genotypes and type of calli (Tab. 3). Selected (tolerant and sensitive) calli lines accumulated significantly less $K^+$ than non-selected (control) callus

line. However, lower concentrations of accumulated $K^+$ were obtained in salt-sensitive calli (Tab. 5). As compared to the check, the reduction corresponded to 16.6 and 22.7% of $K^+$ content, respectively for salt tolerant and salt sensitive calli. $K^+$ content of salt-tolerant and salt-sensitive calli of Ourgh and Tarek genotypes are statistically identical. But, $K^+$ content of Tomouh genotype seems to be independent of the salinity because no significant difference was observed in the three type of calli. Furthermore, the comparison of K+ content revealed that under no salt stress, genotypes were clustered into four groups for control: Sebou (group 1) > Anouar = Tarek = Massa = Amjad (2) > Ourgh (3) ≥ Tomouth = Marzak (4). However with salt treatment, only three groups for salt tolerant calli were defined: Sebou = Anouar = Amjad (group 1) ≥ Tomouh = Tarek = Massa = Marzak (group 2) > Ourgh (group 3).

**Table 3:** Variance analysis for GR, WC, $K^+$, $Na^+$, $Cl^-$ and K/Na in wheat calli

| | **Classification levels** | | | | |
|---|---|---|---|---|---|
| **Parameter** | Salinity | Variety | Callus type | variety x callus | salinity x callus |
| **GR** | 22.6** | 2.46 * | 113.2* | 0.69 * | 18.5* |
| **WC** | 31.2* | 2.25 ns | 47.0 ** | 18.9 * | 20.6* |
| **$K^+$** | 2.87* | 15.00 ** | 130.4 ** | 3.5 * | 3.3* |
| **$Na^+$** | 10.4* | 11.37 * | 127.3* | 4.8 * | 4.1* |
| **$Cl^-$** | 13.0* | 11.56* | 199.8* | 4.1* | 8.6* |
| **K/Na** | 3.6* | 1.24* | 1.93* | 1.30* | 1.4* |

*F-ratios are given for the main effects of the following levels of classification: salinity, variety, callus type and for interaction between these levels of classification (ns: not-significant; *: significant at 5%; **: significant at 1%).*

**Table 4:** Water content (WC) of wheat callus after treatment of selected calli with 16 $gL^{-1}$ NaCl

| | **Callus type** | | |
|---|---|---|---|
| **Wheat Variety** | **Control** | **Tolerant** | **Sensitive** |
| **Sebou** | 87.61 ± 1.7 a | 79.45 ± 1.5 b | 43.09 ± 1.2 c |
| **Anouar** | 91.82 ± 2.6 a | 81.99 ± 2.6 b | 42.55 ± 1.7 c |
| **Marzak** | 93.36 ± 1.3 a | 77.37± 1.6 b | 44.34 ± 1.8 c |
| **Ourgh** | 88.71 ± 1.9 a | 78.14 ± 1.8 b | 40.52 ± 1.4 c |
| **Tarek** | 89.54 ± 1.4 a | 76.84 ± 1.3 b | 38.62 ± 1.9 c |
| **Tomouh** | 90.71 ± 2.4 a | 80.00 ± 2.3 b | 45.91 ± 1.3 c |
| **Massa** | 89.43 ± 1.5 a | 73.05 ± 1.8 b | 39.82 ± 1.7 c |
| **Amjad** | 88.55 ± 2.2 a | 77.83 ± 1.4 b | 37.60 ± 1.4 c |
| WC average | 90,0 % | 78,0 % | 41,6 % |

*In row and column, values followed by a different letter are significantly different (LSD test at 5%).*

**Table 5:** $K^+$ content (in mg/g dried weight) of wheat callus after treatment of selected calli with 16 $gL^{-1}$ NaCl

| | **Callus type** | | |
|---|---|---|---|
| **Wheat Variety** | **Control** | **Tolerant** | **Sensitive** |
| **Sebou** | 17.31 ± 0.8 a | 13.96 ± 0.7 a | 12.22 ± 0.6 a |
| **Anouar** | 15.81 ± 0.5 b | 13.34 ± 0.5 a | 11.69 ± 0.3 a |
| **Marzak** | 12.93 ± 0.69 d | 12.00 ± 0.3 b | 10.71± 0.3 b |
| **Ourgh** | 13.69 ± 0.9 c | 10.20 ± 0.3 c | 10.54 ± 0.7 b |
| **Tarek** | 15.80 ± 0.5 b | 12.02 ± 0.3 b | 11.93 ± 0.8 a |
| **Tomouh** | 13.27 ± 0.8 cd | 12.65 ± 0.5 ab | 12.44 ± 0.5 a |
| **Massa** | 15.24 ± 0.7 b | 11.83 ± 0.2 b | 10.90 ± 0.0 b |
| **Amjad** | 15.13 ± 0.78 b | 13.43 ± 0.38 a | 11.72 ± 0.74 a |
| K+ average | 14,90 $mg.g^{-1}$ DW | 12,43 $mg.g^{-1}$ DW | 11,52 $mg.g^{-1}$ DW |

*In row and column, values followed by a different letter are significantly different (LSD test at 5%).*

*3.4. $Na^+$ content*

Na+ content increased significantly in selected calli lines of all eight varieties. However, salt-sensitive calli accumulated the highest content of Na+ (Tab. 6). Furthermore, salt-tolerant and salt-sensitive calli had

respectively two and three times more Na+ than the check. Analysis of variance (Tab. 3) showed also a significant effect of genotype and type of callus. Indeed, with no salt treatment (check), genotypes were classified in four groups under no salt stress: Tomouth (group 1) ≥ Tarek = Marzak = Ourgh (group 2) ≥ Massa = Amjad = Anouar (group 3) > Sebou (group 4). But with salt treatment this grouping had changed. Salt tolerant calli were then clustered in three groups: Massa = Tomouh = Amjad = Marzak (group 1) > Tarek = Sebou = Anouar (group 2) > Ourgh (group 3).

**Table 6:** $Na^+$ content (in mg/g dried weight) of wheat callus after treatment of selected calli with 16 $gL^{-1}$ NaCl

| | Callus type | | |
|---|---|---|---|
| **Wheat Variety** | **Control** | **Tolerant** | **Sensitive** |
| **Sebou** | 7.88 ± 0.8 d | 32.65 ± 0.4 b | 37.67 ± 0.8 c |
| **Anouar** | 9.34 ± 0.1 c | 32.63 ± 1.2 b | 36.23 ± 0.8 c |
| **Marzak** | 10.82 ± 1.0 b | 36.18 ± 0.4 a | 49.20 ± 0.9 a |
| **Ourgh** | 10.12 ± 0.6 bc | 28.24 ± 0.7 c | 43.76 ± 1.0 b |
| **Tarek** | 11.70 ± 0.5 ab | 33.10 ± 0.8 b | 51.66 ± 0.9 a |
| **Tomouh** | 12.60 ± 0.3 a | 36.96 ± 0.9 a | 46.46 ± 1.8 b |
| **Massa** | 9.73 ± 0.3 c | 37.84 ± 0.6 a | 44.65 ± 0.6 b |
| **Amjad** | 9.41 ± 0.5 c | 36.38 ± 1.1 a | 47.62 ± 1.5 b |
| Na+ average | 10,20 $mg.g^{-1}$ DW | 34,25 $mg.g^{-1}$ DW | 44,66 $mg.g^{-1}$ DW |

*In row and column, values followed by a different letter are significantly different (LSD test at 5%).*

*3.5. $K^+/Na^+$ ratio*

$K^+/Na^+$ ratio decreased significantly under salt stress (Tab. 3). The reduction was higher in salt sensitive calli than in salt tolerant calli (Tab. 7). From 1.51 for the controls, the $K^+/Na^+$ ratio decreased to 0.36 for salt tolerant calli and to 0.26 for salt sensitive ones. Analysis of variance (Tab. 3) showed a significant effect of genotype and type of callus. Moreover, comparison of $K^+/Na^+$ ratio distinguished four groups for the controls : Sebou (group 1) > Anouar = Massa = Amjad (group 2) > Ourgh = Tarek = Marzak (group 3) > Tomouh (group 4) and only two groups for salt tolerant calli :Sebou = Anouar = Tarek (group 1) ≥ Ourgh = Amjad = Marzak = Tomouh = Massa (group 2).

**Table 7:** $K^+/Na^+$ ratio of wheat callus after treatment of selected calli with 16 $gL^{-1}$ NaCl

| | Callus type | | |
|---|---|---|---|
| **Wheat Variety** | **Control** | **Tolerant** | **Sensitive** |
| **Sebou** | 2.20 ± 0.10 a | 0.42 ± 0.03 a | 0.32 ± 0.01 a |
| **Anouar** | 1.69 ± 0.08 b | 0.41 ± 0.01 a | 0.31 ± 0.04 a |
| **Marzak** | 1.20 ± 0.09 c | 0.33 ± 0.01 b | 0.21 ± 0.01 b |
| **Ourgh** | 1.35 ± 0.03 c | 0.36 ± 0.02 ab | 0.24 ± 0.03 b |
| **Tarek** | 1.35 ± 0.08 c | 0.38 ± 0.01 a | 0.23 ± 0.02 b |
| **Tomouh** | 1.05 ± 0.04 d | 0.34 ± 0.03 b | 0.26 ± 0.02 ab |
| **Massa** | 1.59 ± 0.06 b | 0.31 ± 0.01 b | 0.24 ± 0.01 b |
| **Amjad** | 1.67 ± 0.08 b | 0.36 ± 0.03 ab | 0.24 ± 0.02 b |
| K/Na average | 1,51 | 0,36 | 0,26 |

*In row and column, values followed by a different letter are significantly different (LSD test at 5%).*

*3.6. $Cl^-$ content*

$Cl^-$ content increased significantly in both selected types of calli. However, selected (tolerant and sensitive) calli accumulated different quantities of $Cl^-$ when exposed to NaCl (Tab.8). Indeed, this accumulation was lower in salt-tolerant calli than in salt-sensitive ones. From 40.1 mg/g dried weight in control, $Cl^-$ content increased to 61.5 mg/g dried weight in salt-tolerant calli (53.4% increase) while it reached 68.9 mg/g dried weight in salt-sensitive calli (71.8% increase). Moreover, the analysis of variance showed that the accumulation of $Cl^-$ in callus presents a significant effect of genotype and callus type (Tab. 3). The comparison of $Cl^-$ content revealed that the genotypes were regrouped in three groups under salt free conditions: Massa = Amjad (group 1) > Ourgh = Sebou (group 2) > Anouar = Marzak = Tarek = Tomouh

(group 3) and in five groups with salt-tolerant calli: Massa (group 1) > Amjad (group 2) > Tomouh = Ourgh (group 3) > Marzak = Anouar = Tarek (group 4) > Sebou (group 5).

**Table 8:** $Cl^-$ content (in mg/g dried weight) of wheat callus after treatment of selected calli with 16 $gL^{-1}$ NaCl

| Wheat Variety | Callus type | | |
|---|---|---|---|
| | **Control** | **Tolerant** | **Sensitive** |
| **Sebou** | 39.88 ± 1.9 b | 50.97 ± 2.0 e | 58.72 ± 2.0 d |
| **Anouar** | 36.56 ± 1.1 c | 56.14 ± 1.3 d | 60.20 ± 1.6 d |
| **Marzak** | 37.30 ± 1.7 c | 57.98 ± 1.1 d | 75.34 ± 2.9 b |
| **Ourgh** | 41.73 ± 1.0 b | 63.52 ± 2.3 c | 80.14 ± 2.3 a |
| **Tarek** | 35.08 ± 1.6 c | 55.03 ± 1.7 d | 73.86 ± 2.1 b |
| **Tomouh** | 37.30 ± 1.1 c | 64.26 ± 1.9 c | 56.13 ± 1.3 d |
| **Massa** | 47.27 ± 1.0 a | 74.97 ± 2.2 a | 68.89 ± 1.8 c |
| **Amjad** | 45.79 ± 1.3 a | 69.80 ± 2.0 b | 78.66 ± 2.1 a |
| $Cl^-$ average | 40,11 $mg.g^{-1}$ DW | 61,58 $mg.g^{-1}$ DW | 68,99 $mg.g^{-1}$ DW |

*In row and column, values followed by a different letter are significantly different (LSD test at 5%).*

## 4. Discussion

In vitro tissue culture provide a promising approach for breeding of plants subjected to abiotic stress conditions as salinity. Indeed, salt-tolerant cell lines have been developed in several plants such as rice [3], sunflower [1] and *Catharanthus roseus* [6]. The variability associated with cell and tissue culture has been termed 'somaclonal variation' by Larkin and Scowcroft [13].

We developed salt-tolerant wheat calli using in vitro selection techniques. Sustained growth of selected calli in NaCl medium indicated that the tissues were tolerant as reported in rice [3] and 'Troyer' Citrange [7]. The relative fresh weight growth of callus was higher with salt tolerant calli which showed almost the same rate as the check. On the other hand, sensitive calli had severely reduced calli's fresh weight under salt treatment as compared to the control. This reduction was significantly marked by the genotype for each callus type. These results indicated that salt-tolerant calli grew faster than salt-sensitive ones when they were cultured in the presence of NaCl. Similar results were also reported in wheat and other species [10; 18; 21].

Genotypes and callus types differ in intracellular ions accumulation. Both selected lines accumulated more $Cl^-$ and $Na^+$ ions than the control. However, in comparison with salt-sensitive calli, salt-tolerant calli accumulated less $Na^+$ and $Cl^-$ while accumulating more $K^+$. These results indicated that the lower reduction of K+ content and K+/Na+ ratio in salt tolerant genotypes than in salt-sensitive ones may be due to the accumulation of $Cl^-$ in the calli. This is in accordance with Javed's results [10]. The highest change of ions content after salt treatment was observed for $Na^+$ which tripled for tolerant calli and quadrupled for sensitive ones. Therefore, our results indicated that $Na^+$ toxicity may be the main part of salt effect on wheat calli. This confirms that Na+ exclusion mechanism which is an indicator of salt tolerance of whole plant was also expressed at cellular level contradicting the founding of Javed [10].

Our results indicated also that gradual adaptation of calli in response to culture in saline medium is directly related to the reduced uptake of $Na^+$ and $Cl^-$. Thus tolerance of selected calli may be due to their ability to limit tissue accumulation of $Cl^-$ and mainly $Na^+$. The excessive accumulation of $Na^+$ and $Cl^-$ known for their toxicity in cells is likely responsible for the reduced growth and even for sensitive callus necrosis [23]. The harmful effect of $Na^+$ depends on the accumulation place. Indeed, this accumulation is toxic in salt-sensitive calli because it is carried in cytoplasm but nontoxic for salt-tolerant calli since it takes place in vacuole [7]. In this case, the vacuolar sequestration would play an important role in water balance maintenance of calli by increasing the osmotic pressure in the cells. Moreover, Since $Na^+$ and $Cl^-$ content were appreciably high in salt tolerant calli compared to the control while the growth rate was similar for both calli types, it suggests a vacuolar sequestration of these ions, which limited the toxic effect of $Na^+$ and $Cl^-$ in cells while ensuring the maintenance of hydrous balance of selected calli by increasing osmotic pressure in salt-tolerant cells. Indeed, Orsini et al. [22] implied the ionic partitioning as means of tolerance to salinity.

$K^+$ plays a main role in osmotic adjustment during stress [27]. Indeed, salt-tolerant calli maintained higher $K^+$ concentration than salt-sensitive ones. However, with Ourgh and Tarek genotypes, there is no significant difference between the salt-tolerant calli and the sensitive ones in the capacity to accumulate $K^+$.

Moreover, $K^+$ content of Tomouh genotype seems to be independent of salt stress because no significant difference was observed between control, salt-tolerant and salt-sensitive calli. Therefore, $K^+$ content of calli cannot be considered as a strong trait for identifying salt tolerance calli.

To better understand the concepts of salt-tolerance and salt-sensitivity, some researchers used the ionic selectivity which is often expressed by $K^+/Na^+$ ratio as a trait to classify genotypes regarding their response to salinity and also to select tolerant cell line [2]. Maathuis and Amtmann [15] also suggested that the important element in salt tolerance is the ability to maintain a high level the $K^+/Na^+$ ratio in cytosol. Therefore, the most tolerant genotypes are those which generally maintain the highest $K^+/Na^+$ ratio [25]. The results obtained with the eight wheat genotypes showed that salt-tolerant calli had a high $K^+/Na^+$ ratio compared to salt sensitive ones. So in our case, the high $K^+/Na^+$ ratio found in Sebou, Anouar and Tarek genotypes seem to indicate that these three wheat genotypes are the most tolerant ones to salinity while Marzak, Ourgh, Massa, Tomouh and Amjad are the most sensitive ones.

## Conclusion

Salinity did not decrease growth parameters of salt tolerant calli even when they were cultivated under 16 $g.L^{-1}$ NaCl. Salt tolerance seemed to be related to the efficiency of a tissue to modulate the level of inorganic solutes in response to salt stress and the genotype is important in conferring this tolerance. Results of this study indicated that $Na^+$ and $Cl^-$ exclusion / compartmentalization is the main mechanism to counteract the negative effects of salt stress in wheat tolerant calli.

Salt tolerance found at callus level should be evaluated in regenerated plants from tolerant calli, in order to study the expression and the maintenance of this trait at the plant level. If salt tolerance is maintained in regenerated plants, this would be very helpful for plant breeding programs aiming to create salt tolerant varieties.

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