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VARIATION IN CALLUS GROWTH AND *IN VITRO* REGENERATION AMONG CULTIVATED AND WILD WHEAT GENOTYPES UNDER INCREASING SALT STRESS CONDITIONS

I. Klay[1]*, L. Riahi[1], H. Slim-Amara[2] and A. Daaloul[2]

[1]Laboratory of Biotechnology and Bio-Geo Resources Valorization BVBGR-LR11ES31, University of Manouba, ISBST, 2020 Ariana, Tunisia
[2]Laboratory of Genetics and Cereal Breeding, National Agronomic Institute of Tunisia, University of Carthage, 1082 Tunis, Tunisia
*Corresponding author's email: imen.klay@gmail.com

ABSTRACT

Wheat is one of the most widely cultivated and important cereal crops globally, serving as a staple food for millions of people worldwide. However, wheat production is increasingly challenged by environmental stresses, particularly soil salinity. Developing salt-tolerant varieties is essential to enhance wheat yields in saline-prone regions, thereby ensuring food security and agricultural sustainability. This study evaluated the variations in salt stress tolerance among eight wheat genotypes, representing common wheat, durum wheat, and wild wheat, under *in vitro* culture conditions. Wheat calli, induced from immature embryos, were subjected to increasing NaCl concentrations in the culture media (0, 50, 100, 150 mM NaCl). Callogenesis rates, recorded after one month of *in vitro* culture during the induction phase, varied between 33% and 100%, with a significant effect of genotype. The *Aegilops* accessions showed the lowest callus weights at the end of the induction phase, while Vaga and Jenah Khotifa genotypes exhibited the highest biomass. Significant variations in callus growth and regeneration rates were observed among the studied genotypes under increasing salt stress levels. The obtained results indicated that the durum wheat variety Om Rabiaa, the common wheat variety Salambo, and the two wild accessions, especially MZ116, exhibited the highest salt stress tolerance potential among the studied wheat genotypes. Further investigations at transcriptomic and genomic levels are required to elucidate the molecular basis of their high tolerance to salt stress. These genotypes could be utilized to develop salt-tolerant cultivars, which is crucial in the context of global climate change, either through wheat breeding as donor parents or through genetic transformation strategies.

Keywords:*Triticum*, *Aegilops*, Salt stress, Callogenesis, Plant regeneration, Selection

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INTRODUCTION

Wheat (*Triticum* spp.) is a staple cereal crop in Europe, Western Asia, and Northern Africa, where it has been cultivated for thousands of years and remains essential for food security. It is a key component of the Mediterranean diet and is widely used in food products as a source of calories, proteins, minerals, dietary fiber, and bioactive compounds (Khalid *et al.*, 2023). With the global population steadily increasing, projected to reach 9 billion by 2050, the demand for wheat is expected to rise by about 60% (Malik *et al.*, 2021). However, wheat, like other major cereal crops, faces growing environmental stresses. Among these abiotic stresses, salinity is particularly detrimental, significantly reducing wheat yields and quality (Naeem *et al.*, 2022). Ongoing global climate changes, marked by periods of low rainfall and increasing temperatures, exacerbate the situation by increasing salt accumulation in soils (Al Maamory *et al.*, 2017). Salinity delays germination and causes ionic and osmotic stress, negatively impacting cellular and whole-plant levels. This leads to reduced germination rates, stunted growth, and lower grain yields (Ahmad *et al.*, 2015).

Improving wheat varieties is a continuous effort and a renewed challenge for plant breeders and biotechnologists. Wheat's salt tolerance relies on various mechanisms, including biochemical, physiological, anatomical, gene expression, and agronomic responses (Hussein *et al.*, 2023). Selecting wheat genotypes with superior salt stress tolerance is essential to develop more resilient wheat varieties that can thrive in regions affected by salinity. Local and improved wheat genotypes exhibit varying degrees of salt tolerance, and this variability is particularly valuable for identifying salt-tolerant

genotypes. Exploring wild wheat accessions as donors of important traits broadens the genetic diversity of cultivated wheat and provides genes for salt tolerance. Wild wheat genetic resources were employed in modern wheat breeding programs with the aim of enhancing tolerance to biotic and abiotic stresses (Miroshnichenko *et al.*, 2022). *Aegilops*, a close relative of wheat, has been recognized as a valuable genetic resource for improving various agronomic traits, including salt tolerance. Identifying promising *Aegilops* genotypes with high tolerance potential could provide valuable plant material for introducing these desirable traits into commercial wheat varieties through breeding programs or genetic engineering approaches (Klay *et al.*, 2019).

Advancements in tissue culture techniques have significantly improved crop selection and varietal creation, serving as a complement to classical plant breeding methods. The *in vitro* culture of plant cells, tissues, and organs allows the exploration of plant physiology and genetics and offers avenues for enhancing genetic diversity and plant improvement (Kacem *et al.*, 2017). These techniques have proven efficient in identifying tolerant genotypes under controlled conditions and facilitating the screening of tolerant lines derived from both conventional breeding programs and transgenic transformations (Benderradji *et al.*, 2012). The investigation of tolerance potential of crop genotypes at the cellular level is a prerequisite before developing salt-resistant lines to combat the adverse effects of soil salinity (Taratima *et al.*, 2022). Moreover, this approach can create additional variability that can be integrated into selection programs (Al-Khateeb *et al.*, 2020). In recent years, evaluating the salt stress tolerance potential of agronomic species under *in vitro* culture systems has regained interest. This method has been used to assess the variation of salt stress tolerance in several crops, including rice (Taratima *et al.*, 2022), triticale (Yazıcılar *et al.*, 2021), alfalfa (Yazıcılar and Bezirganoglu, 2023), grass pea (Khosravi *et al.*, 2022), and *Brassica* species (Shahbazi *et al.*, 2021), among others.

Based on the previous considerations, this study focused on the evaluation of the response of eight wheat genotypes representing durum wheat, common wheat, and wild wheat genotypes to increasing salt stress levels under *in vitro* culture system. After evaluating the variation in callus induction rates and biomass among the eight studied wheat genotypes, the primary objective was to assess the differences in their salt stress tolerance. This was achieved by analyzing the variations in callus growth and regeneration rates under various NaCl concentrations (0, 50, 100, 150 mM) in the culture medium. Understanding the behaviour of the studied genotypes after their exposure to various levels of salt stress holds significant importance for selecting parental lines with potential in the improvement of cultivated wheat varieties.

MATERIALS AND METHODS

Plant Material: This study evaluated the variation in salt stress tolerance induced by increasing NaCl concentrations (0, 50, 100, and 150 mM NaCl) among eight wheat genotypes under *in vitro* culture system. It involved eight wheat genotypes, including cultivated and wild accessions representing improved varieties, landraces, and wild accessions. The examined genetic resources comprised three durum wheat varieties (*Triticum turgidum* subsp. *durum*: Jenah Khotifa, Karim, Om Rabiaa), three common wheat varieties (*Triticum aestivum*: Salambo, Utique, Vaga), and two wild wheat accessions (*Aegilops geniculata*: MZ116, MZ144), originally provided by the International Maize and Wheat Improvement Center (CIMMYT). Detailed information regarding the characteristics of these durum, common, and wild wheat genotypes were listed in the study by Klay *et al.* (2019).

Immature embryos culture and callus induction: The response of eight wheat genotypes to salt stress induced by NaCl in the culture medium was evaluated using an *in vitro* culture protocol with immature embryos as initial explants. The wheat genotypes were planted in a greenhouse at the National Agronomic Institute of Tunisia, using agricultural soil from INAT as substrate. Immature embryos of the selected genotypes were collected 19 days after fertilization, which is considered the optimal stage for immature embryo culture. Immature seeds from the middle section of wheat spikes, which are more developed, were de-husked and sterilized through immersion in a 12% sodium hypochlorite solution for 10 minutes, followed by rinsing four times with sterile distilled water.

The extraction of immature embryos was achieved under laminar flow hood using sterilized forceps. The extracted embryos, with a size of approximately 1.5 mm, were transferred to Petri dishes, containing 30 mL of MS nutrient medium (Murashige and Skoog, 1962) modified and specified for wheat by Sears and Deckard (1982). The inoculation of the excised embryos was carried out scutellum facing upward and radicle axis inserted into the agar, with 10 embryos per Petri dish.Following inoculation, the test tubes were maintained in a growth room under a photoperiod of 16/8 hours and a temperature of 25±1°C.

Application of salt stress and *in vitro* regeneration: The protocol used to assess the *in vitro* response of wheat genotypes to salt stress consisted of four phases each lasting 30 days. The transition from one phase to another was achieved by transferring the calli to new Petri dishes to ensure the supply of nutrients and to adjust the concentration of the phytohormone 2,4-D, in the culture medium. The concentration of 2,4-D is gradually reduced during the four phases (Table 1). The development of

green microshoots from regenerable calli was achieved on the regeneration medium I and the development of roots on the regeneration medium II.

Sodium chloride (NaCl) was selected as the salt stress agent due to its prevalence as a major component in irrigation water and its significant role in soil structure degradation. To assess the response of various wheat genotypes to salt stress, three concentrations inducing selective pressure were chosen, alongside a control condition without NaCl (0, 50, 100, and 150 mM NaCl). These NaCl concentrations were applied at the Maintenance Phase I.

Acclimatization: After the root system developed, the regenerated plants were transferred to an *ex-vitro* culture condition. The regenerated seedlings were acclimatized in pots filled with sterile substrate (2/3 sand and 1/3 peat) after removing the remaining agar from the root system. The seedlings were covered with a polyethylene bag, which was gradually punctured and then removed after one week. Irrigation was performed daily during the first week using a diluted KNOP solution at a 1/10 ratio. The complete cycle, from induction to the acclimatization stage, took 150 days (Figure 1).

Studied parameters: The callus induction rates of the different genotypes were determined at the end of the induction phase. This parameter was calculated as the ratio of the total number of induced calli to the total number of inoculated embryos (Kacem *et al.*, 2017):

PI = (Total number of induced calli / Total number of inoculated embryos) x 100.

The average weight (g) of the obtained wheat calli was determined after each *in vitro* culture phase. The callus growth rates (CGR) under salt stress condition were determined as (Golkar *et al.*, 2017):

$$CGR\ (\%) = ((CW_f - CW_i) / CW_i) \times 100.$$

Where CW_i is the initial weight of the callus before salt application and CW_f is the weight of the callus after 30 days of treatment application.

The relative callus growth rates (RCGR) related to control were calculated as:

$$RCGR = ((GR_{treatment} - GR_{control}) / GR_{control}) \times 100.$$

Where $GR_{control}$ is the growth rate of control callus and $GR_{treatment}$ is the growth rate of callus under the salt treatment.

The percentages of regenerable calli, representing the percentage of calli that have developed at least one green microshoot, were calculated using the formula (Yadav *et al.*, 2020):

RC (%) = Number of calli with green microshoots / Number of calli inoculated on the medium x 100.

The number of regenerated microshoots per callus was determined at the end of the regeneration phase.

Data analysis: The analysis of variance for the studied parameters was conducted utilizing SAS software. This analysis employed the multivariate linear model (GLM Multivariate) to elucidate variations influenced by genotypes and NaCl treatments. Multiple mean comparisons and the establishment of rank classes to detect significant differences at a 5% level were carried out using the Duncan test.

RESULTS

Variation in callus induction rates and biomass: The ability of the studied genotypes to induce callus from immature embryos on a modified MS medium (Sears and Deckard, 1982) supplemented with 1 mg/L 2.4-D was evaluated. The obtained results showed that under 1 mg/L 2.4-D, the immature embryo culture succeed to induce callus with various aspects (Figure 2). Analysis of variance revealed a highly significant genotype effect on callus induction capacity of the studied germplasms. For durum wheat genotypes, the obtained results showed that callus induction rates vary between 72.96% (Jenah Khotifa) to 85.67% (Karim). Higher induction percentages ranging from 86.76% (Vaga) to 98.50% (Salambo) were observed for the common wheat genotypes. Concerning the wild wheat accessions, the lowest induction percentage was detected for the accession MZ144 (71.43%), while the MZ116 accession showed the highest rate of callus induction (86.39%). The comparison of means among the studied wheat genotypes allowed the establishment of three significantly different groups according to the Duncan test (Figure 2). The means callus induction rates for the three studied species revealed that common wheat have the highest potential to induce callus from immature embryos (93%) followed by durum wheat (81%) and then wild wheat genotypes (79%).

The variation in biomass of the induced calli among the eight studied wheat genotypes was evaluated at the end of the induction phase after 30 days of immature embryos *in vitro* culture in NaCl free MS medium. The statistical analysis revealed a highly significant effect of genotype on the average weight of calli after 30 days of *in vitro* culture. The obtained results showed that the calli originated from the three *Aegilops* accessions during the initial culture phase showed the lowest growth rates as compared to durum and common wheat varieties (Figure 3). For this germplasm, the means values for callus weight of wild wheat accessions range between 0.023 g (MZ116) to 0.026 g (MZ144). For durum wheat, the highest calli weight was observed for the variety Jenah Khotifa (0.082 g) while the variety Vaga showed the highest callus weight (0.104 g) among common wheat genotypes and the eight studied genotypes. Comparable means of callus weights (g) were observed among Karim and Om Rabiaa durum wheat genotypes and between Salambo and Utique common wheat varieties (Figure 4).

Variation in calli growth rates under salt stress conditions: The variation in callus growth rates among the studied wheat genotypes was determined after one month of exposure to various salt stress levels. The analysis of variance revealed a highly significant effect of genotype, treatment, and genotype x treatment interaction on the callus growth rates of the wheat genotypes. In the absence of NaCl in the *in vitro* culture medium, the variety Salambo exhibited the highest growth rate (445%), while the lowest growth rates were observed for the Vaga (183%) and MZ144 (192%) genotypes (Figure 4).

The obtained results showed that the three applied concentrations of NaCl led to a decrease in callus growth rate compared to the control for the wheat genotypes Jenah Khotifa, Utique, and Vaga. Interestingly, a significant increase in callus growth over the control was observed for the genotypes Om Rabiaa and Salambo with the application of 50 and 100 mM NaCl. However, no significant variation was recorded at a concentration of 150 mM NaCl. The variety Karim exhibited unique behavior in response to salt stress, with a significant reduction in callus growth at 50 and 100 mM NaCl and a significant increase upon exposure to 150 mM NaCl. For the two wild wheat accessions, significant increases in callus growth over the control were noted for MZ116 at 150 mM NaCl and for MZ144 at 100 mM NaCl. Conversely, a significant reduction in callus growth compared to the control was observed for MZ116 at 100 mM NaCl and for MZ144 at 50 mM NaCl.

Variation in relative callus growth rates: The relative callus growth rate (RCGR) of the treated callus compared to the control under selective salt pressure was determined (Figure 5). The obtained findings showed a significant effect of both genotype and treatment on this parameter. The highest decreases in callus growth rates were recorded under 150 mM NaCl for Jenah Khotifa (62%) and Vaga (52%). Conversely, the highest increases in RCGR were observed for the Om Rabiaa genotype under 50 mM (77%) and 100 mM (51%) NaCl. Additionally, significant increases in callus growth over the control were noted for both Om Rabiaa and Salambo under 50 and 100 mM NaCl, ranging from 51% to 77% and 16% to 21%, respectively.

The *in vitro* culture of callus from the Karim variety under 50 and 100 mM NaCl resulted in a reduction in callus growth of approximately 38% compared to the control. However, at a concentration of 150 mM NaCl, there was a 27% increase in callus growth over the control. The two investigated wild wheat genotypes exhibited distinct responses to the three applied concentrations of NaCl. For MZ116, 50 mM NaCl concentration led to a 7% increase in callus growth, whereas it caused a 34% decrease in the callus growth of the MZ144 genotype. At the higher concentrations, the most significant positive effects were observed for MZ116 at 150 mM NaCl (increase of 27%) and for MZ144 at 100 mM NaCl (increase of 34%).

Variation in regenerable calli rates: The percentages of regenerable calli were determined for the various experimental conditions (Figure 6). Regenerable calli were characterized by a friable or compact appearance with a pale yellow or whitish colour. In contrast, non-regenerable calli generally exhibited an aqueous appearance followed by browning and necrosis. During the maintenance phase, the increase in the concentration of 2,4-D significantly reduced callus proliferation in favour of somatic embryogenesis and organogenesis (Figure 3). The regeneration percentage indicates the number of calli transferred to the regeneration medium that exhibit at least one microshoot. Statistical analyses of the percentage of regenerable calli at the end of the regeneration phase revealed a significant effect of genotype and treatment. In the absence of salt stress, significant intraspecific variation was observed for this parameter. For durum wheat, the percentage of regenerable calli ranged from 76% (Om Rabiaa) to 92% (Jenah Khotifa). Among common wheat genotypes, Utique showed the lowest percentage of regenerable calli (58%), while Salambo showed the highest rate (81%). For wild genotypes, the regenerable callus rates varied between 67% (MZ144) and 85% (MZ116).

The application of 50 mM NaCl resulted in a significant increase in the number of calli exhibiting microshoot development for the Utique genotype (73%). However, a significant decrease in the rate of regenerable calli compared to the control was recorded for the genotypes Karim, Vaga, and MZ144. No significant variation was observed for the remaining genotypes at this salt concentration. At 100 mM NaCl, no significant variation compared to the control was noted for the wheat genotypes Jenah Khotifa, Salambo, and MZ144. However, at this salt level, the percentage of regenerable calli decreased significantly for Karim, Utique, and Vaga, while it increased for Om Rabiaa and the wild accession MZ116. Under 150 mM NaCl, a significant increase in the regenerable calli rate over the control was observed only for the Salambo genotype, while MZ116 genotype showed a rate comparable to the control. A significant reduction in the regenerable calli rate was recorded for all the remaining wheat genotypes at this salt concentration.

Number of regenerated micro-shoots per callus: The variation in the number of regenerated microshoots per callus among the eight studied genotypes in response to the applied treatments was evaluated (Table 2). Statistical analysis of the number of shoots per callus at the end of the regeneration phase revealed a highly significant effect of both genotype and treatment. Under control conditions, the highest number of green shoots per callus was observed in the durum wheat variety Om Rabiaa

(2.37) and the common wheat variety Salambo (2.24). The lowest number of regenerated green shoots per callus was recorded for the genotypes Utique and Jenah Khotifa.

Under 50 mM NaCl treatment, the number of microshoots per callus increased significantly for the wheat genotypes Karim, Utique, and MZ116, with the highest level observed for the variety Karim. However, at this salt stress level, a significant decrease in this parameter was noted for the Salambo and Vaga genotypes. The application of 100 mM NaCl enhanced the microshoots regeneration in the genotypes Karim, Utique, Vaga, and MZ116 but led to a significant reduction in this parameter for Om Rabiaa and Salambo. The highest salt stress level (150 mM NaCl) increased the number of regenerated microshoots per callus in Jenah Khotifa, Vaga, and MZ144, while it decreased this parameter for Om Rabiaa, Salambo, and Utique genotypes.

Table 1. The concentration of 2,4-D at the different phases, each during one month of *in vitro* culture.

Culture phases	Concentration of 2,4-D (mg/L)
Induction	1
Maintenance I	0.75
Maintenance II	0.50
Regeneration I	0.25
Regeneration II	0

Table 2. Variation in the number of regenerated microshoots per callus among the studied wheat genotypes. Mean values followed by different letters are significantly different (P ≤ 0.05).

	Control	T1 (50 Mm NaCl)	T2 (100 Mm NaCl)	T3 (150 Mm NaCl)
Jenah Khotifa	0.45 ± 0.02 ^{b}	0.49 ± 0.02 ^{bc}	0.42 ± 0.02 ^{b}	0.54 ± 0.02 ^{c}
Karim	1.78 ± 0.08 ^{f}	4.66 ± 0.23 ^{i}	3.25 ± 0.16 ^{h}	2.00 ± 0.10 ^{fg}
Om Rabiaa	2.37 ± 0.11 ^{g}	2.59 ± 0.12 ^{g}	1.85 ± 0.09 ^{f}	1.72 ± 0.08 ^{ef}
Salambo	2.24 ± 0.11 ^{g}	1.13 ± 0.05 ^{d}	1.43± 0.07 ^{e}	1.50 ± 0.07 ^{e}
Utique	0.43 ± 0.02 ^{b}	0.54 ± 0.02 ^{c}	0.56 ± 0.02 ^{c}	0.29 ± 0.01 ^{a}
Vaga	1.41 ± 0.07 ^{e}	0.97 ± 0.04 ^{d}	1.94 ± 0.09 ^{f}	1.91 ± 0.09 ^{f}
MZ116	1.42 ± 0.07 ^{e}	2.67 ± 0.13 ^{g}	2.16 ± 0.10 ^{f}	1.68 ± 0.08 ^{ef}
MZ144	2.01 ± 0.10 ^{f}	2.12 ± 0.10 ^{f}	1.70 ± 0.08 ^{ef}	3.09 ± 0.15 ^{gh}

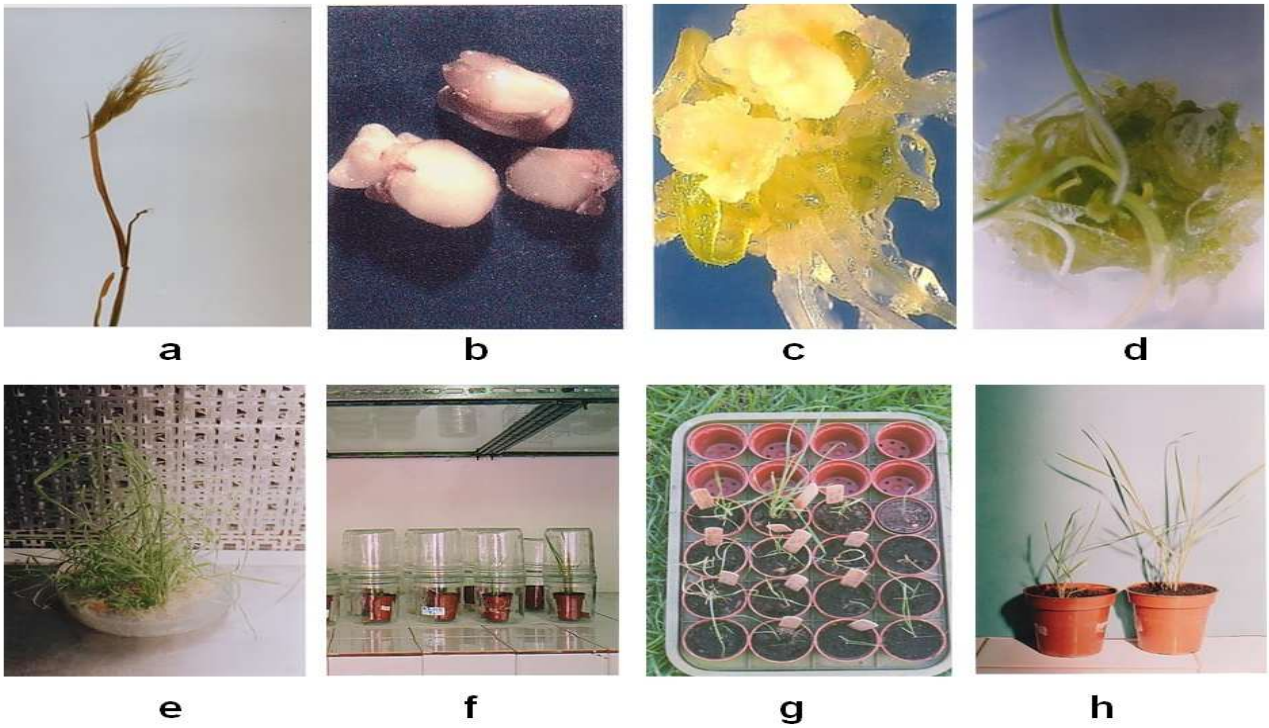

Fig. 1: The *in vitro* culture steps adopted in this study to evaluate callus induction and *in vitro* growth and plant regeneration (a; spike of wild wheat; b; immature embryo; c, d and e: callus growth and microshoots regeneration; f, g and h: acclimatization).

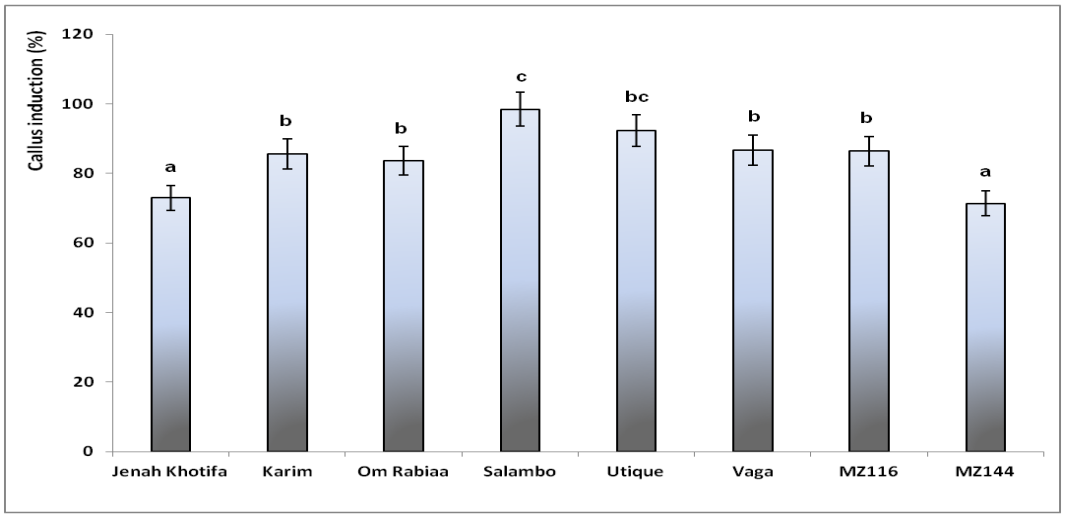

Fig. 2: Variation of callus induction rates among the studied wheat genotypes. Mean values followed by different letters are significantly different ($P \leq 0.05$).

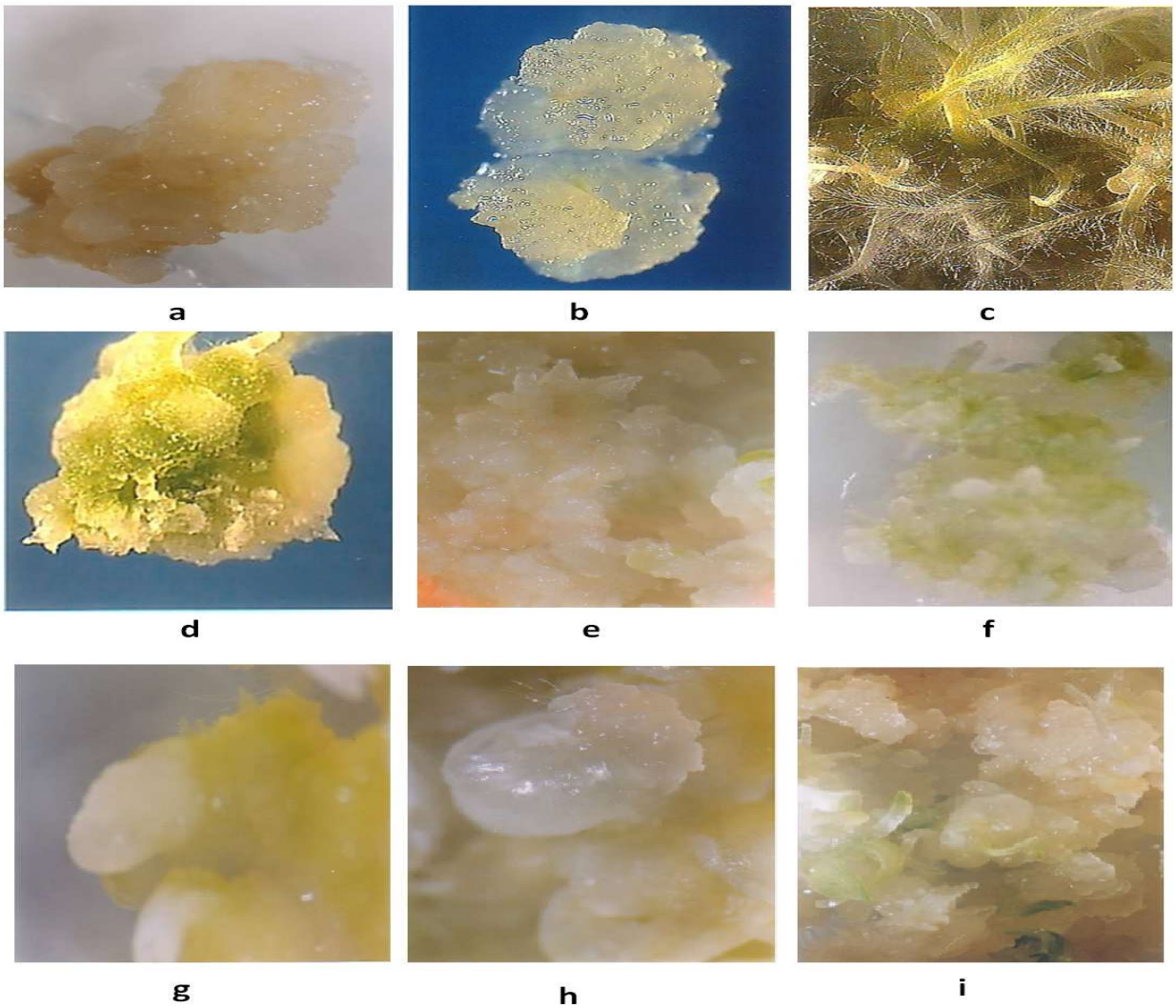

Fig. 3: Different aspects of calli obtained through culture of immature embryos in maintenance phase I (a: soft brown callus, b: soft and watery callus, c: rhizogenic callus), maintenance phase II (d: compact yellow-green callus, e: whitish callus, f: friable callus). Formation of somatic embryos (g, h, i).

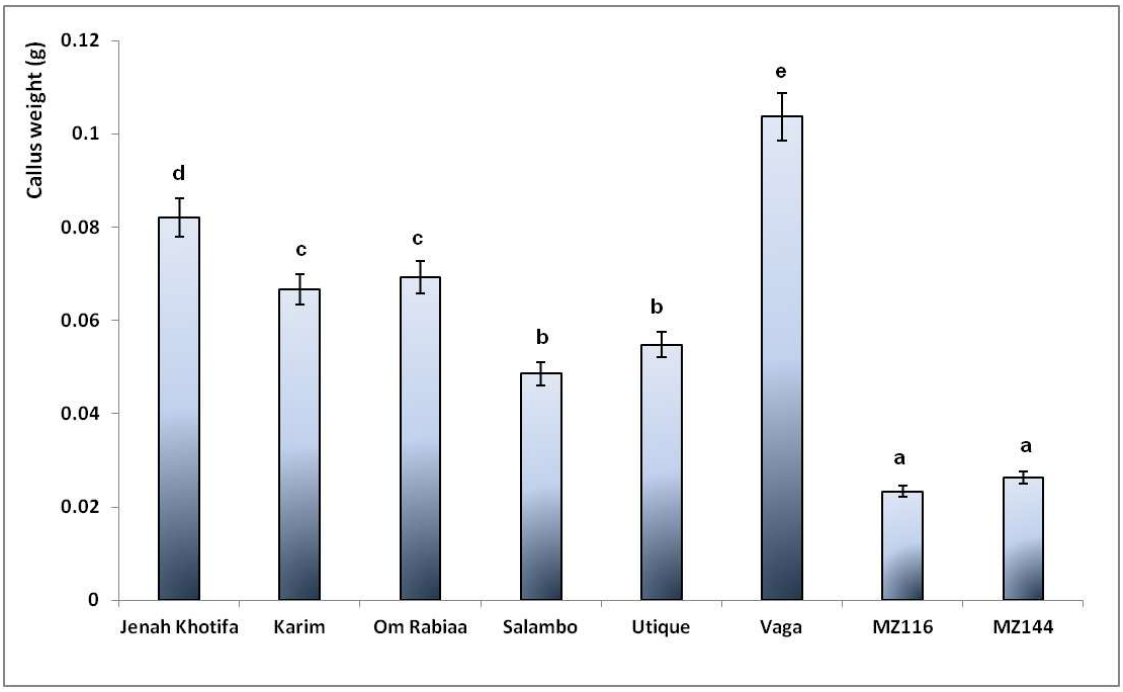

Fig. 4: Variation of calli average weight among the studied wheat genotypes at the end of the induction phase. Mean values followed by different letters are significantly different (P ≤ 0.05).

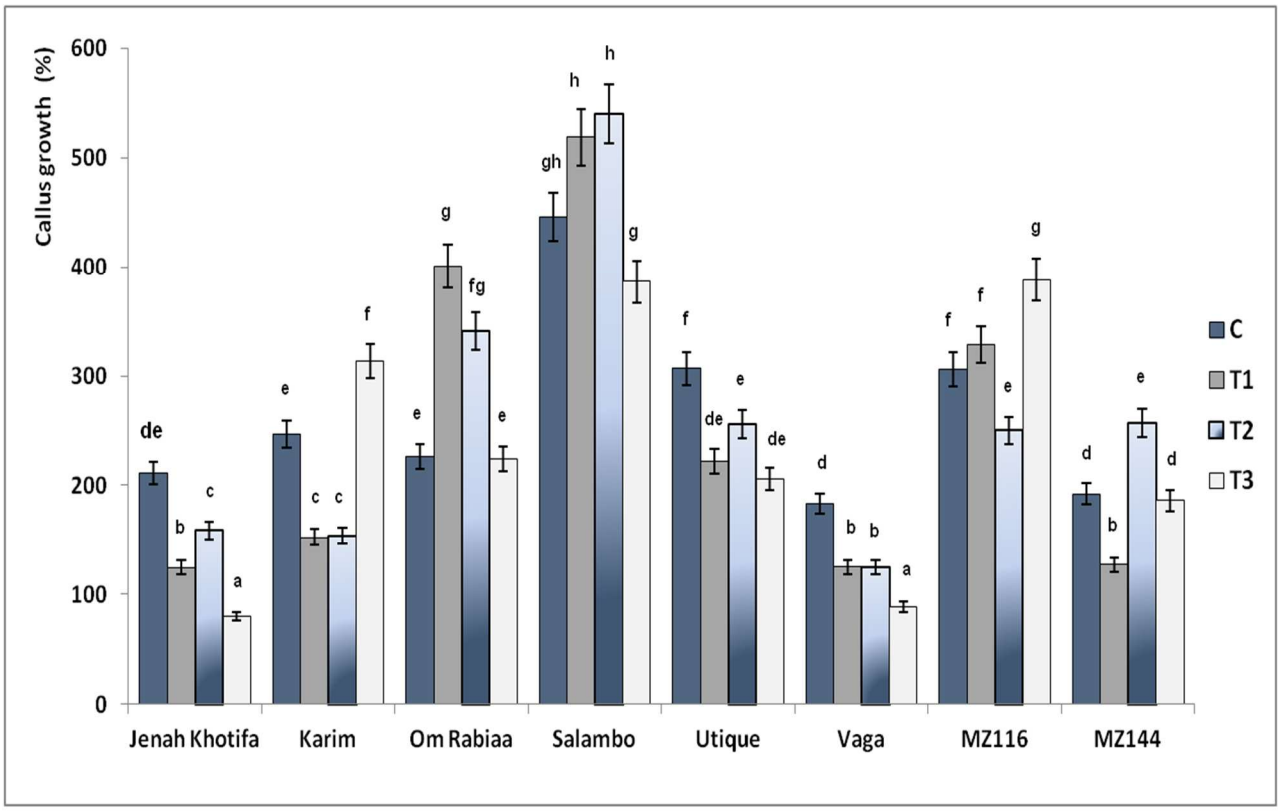

Fig. 5: Variation of calli growth rates among the studied genotypes under increasing NaCl concentrations (C: control, T1: 50 mM, T2: 100 mM, T3: 150 mM). Mean values followed by different letters are significantly different (P ≤ 0.05).

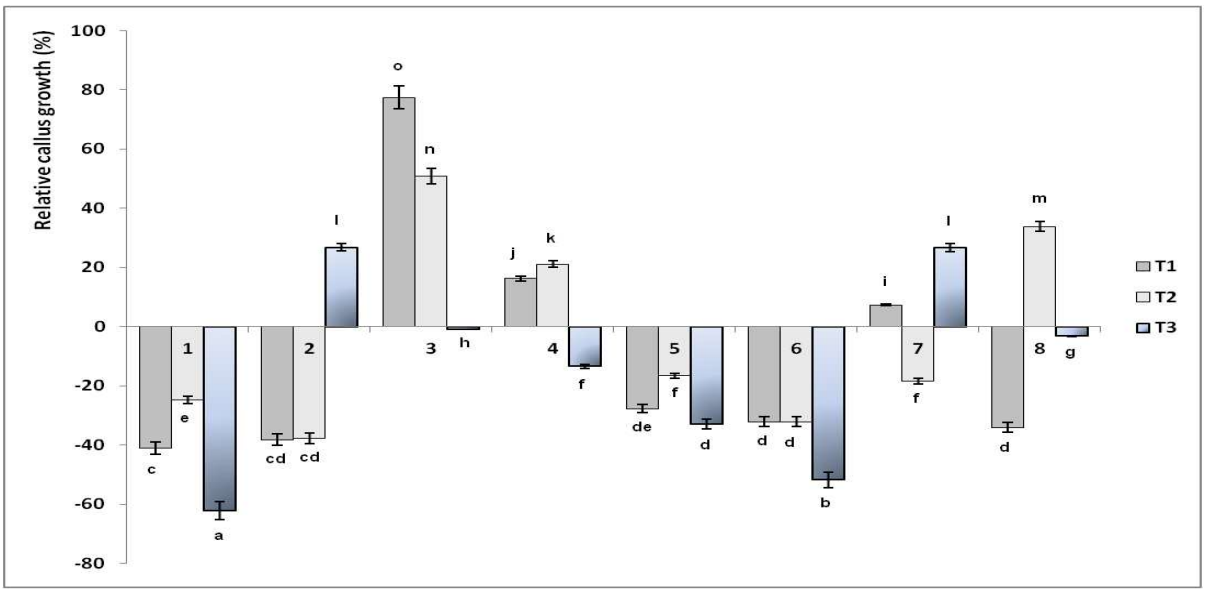

Fig. 6: Variation of relative calli growth rates among the studied genotypes under increasing salt stress induced by NaCl. (C: control, T1: 50 mM, T2: 100 mM, T3: 150 mM). 1, 2, …….. 8: wheat genotypes codes (Table 1). Mean values followed by different letters are significantly different (P ≤ 0.05).

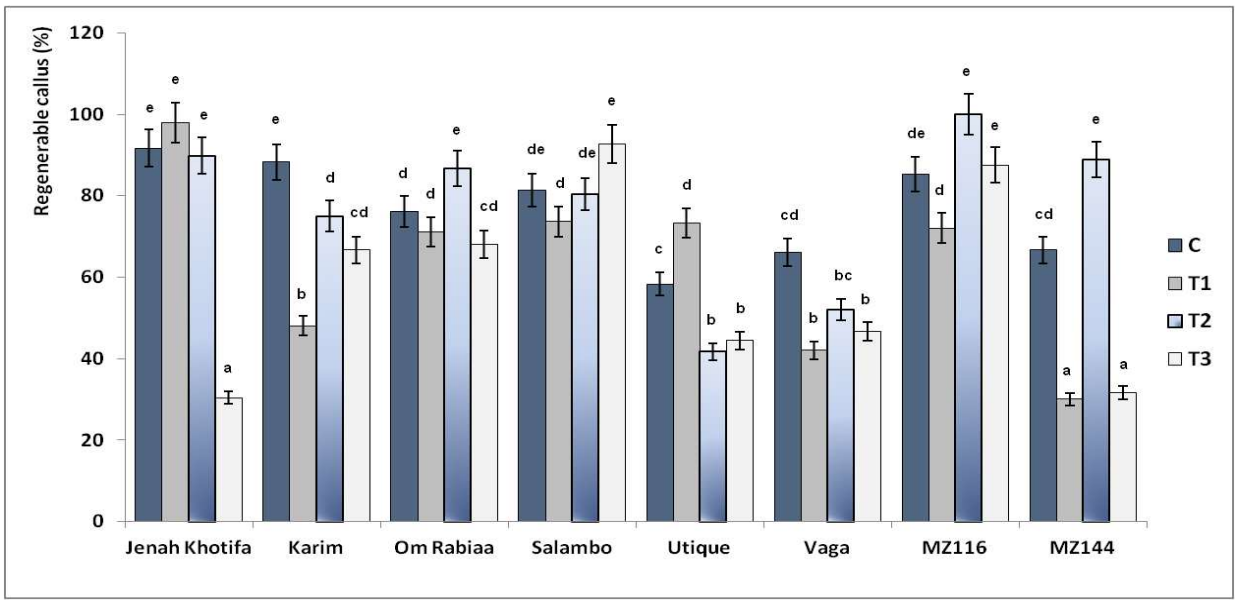

Fig. 7: Variation of regenerable calli rates under various NaCl concentrations (C: control, T1: 50 mM, T2: 100 mM, T3: 150 mM). Mean values followed by different letters are significantly different (P≤ 0.05).

DISCUSSION

Tissue culture approach has occurred as an effective method to underline the salt tolerance potential of plant genetic resources at cellular and juvenile development stage (Sahu *et al.*, 2023). *In vitro* selection using callus culture was reported as highly reproducible technique and a powerful tool for the selection of salt tolerant genotypes at the cellular level. The use of callus culture in selecting salt-tolerant cell lines has contributed effectively towards the regeneration NaCl-tolerant lines in various plant species (Shahbazi *et al.*, 2021).

The capacity to induce callus from immature embryos on a modified MS medium for cereals was studied across the eight examined genotypes. The obtained results revealed significant callus induction rates with the auxin 2,4-D, showing considerable variation among the genotypes, ranging from 71% to 92%. There was higher variation in callus proliferation efficiency, as represented by callus biomass, depending on the wheat

genotype and species. The wild *Aegilops* accessions exhibited lower callus weights compared to cultivated wheat. Callus initiation and growth are of great interest in cereal tissue culture (Pour *et al.*, 2020). Induction marks the initial phase of *in vitro* culture, revealing the ability of immature embryos to undergo callogenesis. This finding aligns with previous reports indicating that immature zygotic embryos are commonly utilized explants for callus induction in wheat due to their high totipotency. Additionally, the auxin 2,4-D is recognized as the most suitable phytohormone for callus induction in wheat (Khokhar *et al.*, 2016). The significant impact of genotype on both callus induction and growth has been previously observed in rice (Htwe *et al.*, 2011) and wheat (Senhaji *et al.*, 2021). A notable variation in callus induction rates among cereal species and genotypes has been documented (Xu *et al.*, 2022).

Callus growth and microshoot regeneration efficiency are significant parameters that define the salt tolerance potential of agronomic crops (Rai *et al.*, 2011). The variation in callus growth rates among the studied genotypes was assessed during the maintenance phase by applying increasing NaCl concentrations. Salts that induce stress in plants exist in various forms and concentrations, including chlorides, sulfates, nitrates, borates, carbonates, and bicarbonates. NaCl is particularly known for its high toxic effect, largely due to its very high solubility. Excessive amounts of Na and Cl in the roots zone increase the osmotic potential, leading to physiological drought by hindering plants from adequately utilizing water in the soil, thereby slowing down growth processes (Yildirim *et al.*, 2023).

The obtained findings revealed a significant variation in callus growth based on genotype, species, and treatment. Genotypes such as Jenah Khotifa, Utique, and Vaga exhibited the highest susceptibility to the applied concentrations, experiencing decreased callus growth under salt stress conditions, while the remaining genotypes were less affected. Interestingly, the presence of NaCl in the culture medium enhanced callus growth for these wheat genotypes, with variations based on the applied concentration. Notably, the varieties Om Rabiaa, Salambo, and the two wild accessions showed greater resistance to salt stress based on the callus growth parameter. The stimulation of growth in the presence of salt can be considered as an indicator of salt tolerance (Farooq *et al*., 2017). According to Hannachi *et al.* (2021), the calli lines that were developed on medium up to 120 mM NaCl had a selective advantage. The absence of significant decrease in calli growth indicated high tolerance of this genotype to salinity stress (Shahbazi *et al.*, 2021). Generally, salt stress induces inhibition of callus growth, possibly due to induced water deficit and/or toxicity associated with excessive ion uptake, particularly Na^+ and Cl^-. Salinity stress represents a form of negative osmotic stress, leading to reduced water availability in callus cells, which in turn consumes energy and consequently reduces callus growth rates (Ghane *et al.*, 2014).

The regenerable callus rates and the number of regenerated microshoots per callus exhibit significant variation according to the studied genotype under both normal and salt stress conditions. Callus viability and regeneration rates of salt-treated callus are known to be influenced by genotype factors (Haque *et al.*, 2017). The regenerable callus rates corroborate previous findings, indicating that Om Rabiaa, Salambo, and the accession MZ116 exhibit the highest tolerance potential to salt stress among the studied genotypes. Callus growth and regeneration capacity are commonly used to assess the salt tolerance of agronomic crops (Htwe *et al.*, 2011; Mashkina *et al.*, 2021). It's noted that *in vitro*-regenerated plants potentially have fewer deleterious genetic issues, as the regeneration step eliminates non-vigorous genotypes engendered by genetic mutations. *In vitro* culture of plant cells, tissues, or organs on a medium containing selective stress agents offers the opportunity to select and regenerate plants with desirable characteristics, and it could be highly effective in improving agronomic crop yields and quality (Baklouti *et al.*, 2022).

This study confirmed previous findings regarding the salt stress tolerance potential of the studied wheat genotypes. Notably, wild accessions, particularly accession MZ116, and the variety Salambo were identified as the most resistant at the germination stage compared to the other tested genotypes (Klay *et al.*, 2014). Furthermore, ionomic and photosynthetic traits highlighted considerable salt stress potential exhibited by the two wild *Aegilops* accessions and the common wheat variety Salambo under increasing salt stress conditions at the seedling stage. In contrast, durum wheat varieties have been shown to be more sensitive to salt stress (Klay *et al.*, 2019).

Conclusion: The obtained findings revealed significant variations in the studied parameters among the genotypes at both interspecific and intraspecific levels. The overall results showed that the durum wheat variety Om Rabiaa, the common wheat variety Salambo, and the wild accession MZ116 exhibited considerable salt stress tolerance potential and could be utilized in the creation of new salt-tolerant wheat varieties. The observed variation in salt stress tolerance among wheat genotypes at the cellular level is highly significant for the development of new salt-tolerant varieties and hybrids. This is particularly valuable for the selection of contrasting wheat genotypes for salinity tolerance, which can be further exploited to understand the genetic factors responsible for salt tolerance or to develop improved varieties with enhanced stress tolerance. This can be achieved either conventionally through interspecific

breeding or through genetic transformation tools. Further experimentations could involve field trials to validate the salt tolerance and detailed molecular characterization to identify key genes involved in salt stress tolerance of these wheat genotypes.

Conflict of Interest: The authors declare no conflicts of interest

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