

Research Article

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Genotypic variability for salt stress tolerance among wild and cultivated wheat germplasms at an early development stage

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Abstract: This study was conducted to evaluate the variability of salt tolerance potentials among nine wheat genotypes representing wild and cultivated species namely *Triticum turgidum* subsp. durum, *Triticum aestivum* and *Aegilops geniculata*. Ionomic and photosynthetic traits were used for the screening of the studied samples when faced with four salinity levels of NaCl (0, 50, 100 and 150 mM) under green house conditions at the seedling stage. The investigated genotypes exhibited different levels of salt stress tolerance. Ionomic and photosynthetic traits underline the distinctiveness of the common wheat varieties which highlighted particular performances under salt stress conditions and showed higher tolerance potentials among the studied genotypes. Interestingly, the Vaga variety showed more ability to maintain higher K^+/Na^+ ratios and Pq coefficients compared with the control conditions and stable Fv/F0 and Fv/Fm ratios. Stable behaviour was exhibited by wild *Aegilops* accessions while durum wheat varieties have been shown to be more sensitive to salt stress. Further investigations were required for the common wheat variety Vaga, which could be useful for successful breeding and biotechnological improvement strategies concerning wheat species.

Keywords: *Triticum*, *Aegilops*, Salt stress, ion contents, chlorophyll fluorescence, Varietal selection

1 Introduction

Wheat is among the oldest and the most cultivated cereal species worldwide and plays a major role in food security at the global scale (De Santis et al. 2018). As with other cereal crops, wheat germplasms face increasing impacts from abiotic stresses such as salinity, drought and high temperature, which severely affect plant growth, biomass and grain yields. Abiotic stresses are among the most significant factors, which limit crop growth and productivity. Tolerance to environmental stress factors presents generally complex quantitative traits mediated by large plant specific transcription factors and various physiological pathways (Riahi et al. 2013; Xu et al. 2015; Klay et al. 2018).

Nowadays, soil salinization is still increasing, mainly due to excessive irrigation and industrial pollution, and is among the main threats facing modern agriculture sustainability (Hamam and Negim 2014; Klay et al. 2014; Ben Romdhane et al. 2018). Salt stress is reported to be the main limiting environmental factor for plant growth and causes significant losses in crop yields, worldwide. The occurrence of salt stress is supposed to increase by around 25% by 2050 in vulnerable areas, which could adversely affect plant geographical distribution (Radanielson et al. 2018).

Wheat, among others glycophytes, is seriously affected by high salinity concentrations in soils. Developing new improved salt-tolerant wheat varieties is among the central focus of national and international breeders interested in this species. The increase of wheat productivity is required to meet increasing world consumption needs (Amiri et al. 2018). Genetic factors are considered to be among the more significant factors that define salt tolerance in plant species, in interaction with environmental conditions. Salinity affects growth, development and metabolic activity of plants, depending on their potential to tolerate NaCl content in the soils.

The investigation of the variability of salt tolerance ability among local genetic resources of wheat is of great

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interest for the selection of parental genotypes for further breeding and biotechnological improvement strategies. Based on previous reports, ionic and photosynthetic salt tolerance traits are considered as significant indicators for the screening of specific wheat germplasms under saline conditions (Hamam and Negim 2014). In this context, the present study investigates the variability of the salt stress tolerance ability among wild, landraces and modern varieties of wheat germplasms at an early developmental stage, based on ionic and photosynthetic traits. The main objectives of this study are the selection of specific genotypes for further genetic improvement programmes aiming to create new local wheat varieties with higher adaptation abilities to soil salinization in Tunisian agricultural lands.

2 Materials and methods

2.1 Plant material

The variability of salt stress response induced by different doses of NaCl (0, 50, 100 and 150 mM NaCl) at the juvenile seedling stage was addressed in this study, based on a sample of nine genotypes of wild and cultivated wheat, representing wild accessions, local landraces and modern varieties. The plant material, used in this experiment, corresponds to 3 durum wheats *Triticum turgidum* subsp. *durum* (Jenah Khotifa, Karim, Om Rabiaa), 3 common wheats *Triticum aestivum* (Salambo, Utique, Vaga) and 3 wild wheat accessions *Aegilops geniculata* (MZ116, MZ141, MZ144). The list of the different studied genotypes and their characteristics are shown in Table 1.

2.2 Experimental design and salt stress application

A field experiment, based on the nine investigated genotypes, was arranged in a randomized complete block design with three repetitions per treatment per genotype. Seeds of the nine studied genotypes were sown under greenhouse condition in plastic pots (35 cm in diameter) filled with sand, at a rate of 5 seeds per pot. The irrigation was carried out every 3 days, using tap water, during the germination phase. After the first leaf appearance, seedlings were irrigated with a nutritive solution of Hoagland (FAO 1984). Salt stress was applied 19 days after seeding emergence, using 4 salt treatments (0, 50, 100 and 150 mM NaCl), by adding the different concentrations of NaCl in the irrigation solution.

2.3 The ionic and photosynthetic traits

After three weeks of salt stress application (21 days), the analyses concerning ionic and photosynthetic traits were achieved as described by Radhouane (2013). The contents of the third leaf, in terms of Na⁺, K⁺ and Cl⁻ for each genotype, were investigated. For this purpose, the wheat foliar dry matter was ground to a fine powder using a ball mill. Extractions were achieved from 30 mg of plant material powder, using 50 ml of 0.5% HNO₃ at room temperature for 48 hours. The determination of sodium and potassium was carried out by emission flame photometry of the nitric extract. The contents are expressed as percentage of dry matter. The Cl⁻ anion content was assayed by coulometry methods, using a chloridometer (Buchler-cotlove).

For the measurement of the chlorophyll fluorescence parameters, a portable fluorescence induction monitor system (FIM 1500, Analytical Development Company Limited, ADC) was used, following the method described

Table 1: List of the studied wheat varieties and accessions along with their characteristics

Genotype	Species	Status	Origin and year of release
Jenah Khotifa	<i>Triticum turgidum</i> subsp. <i>durum</i>	Local landrace	Local Northern populations/1915
Karim	<i>Triticum turgidum</i> subsp. <i>durum</i>	Improved varieties	CIMMYT (1973)
Om Rabiaa	<i>Triticum turgidum</i> subsp. <i>durum</i>	Improved varieties	ICARDA (1987)
Salambo	<i>Triticum aestivum</i>	Improved varieties	CIMMYT/Tunisia (1970/1980)
Utique	<i>Triticum aestivum</i>	Improved varieties	CIMMYT/Tunisia (1989/1996)
Vaga	<i>Triticum aestivum</i>	Improved varieties	CIMMYT/Tunisia (1985 /1993)
MZ116	<i>Aegilops geniculata</i>	Wild accession	CIMMYT
MZ141	<i>Aegilops geniculata</i>	Wild accession	CIMMYT
MZ144	<i>Aegilops geniculata</i>	Wild accession	CIMMYT

by Djanaguiraman et al. (2006). This system automatically measured the initial fluorescence F_0 , the maximal fluorescence F_m , the variable fluorescence F_v . This allowed the determination of F_v/F_0 ratios, reflecting the efficiency of electron donation to the PSII using the formulae $F_v/F_0 = (F_m - F_0)/F_0$ and the maximum quantum yield of PSII as $F_v/F_m = (F_m - F_0)/F_m$. In addition, the photochemical quenching coefficients, Pq , were calculated following Jamil et al. (2007).

2.4 Data analysis

All measurements were conducted in triplicates and results for each parameter were expressed as mean \pm SD. Variance analyses (at $P < 0.05$) were performed using the SAS software v. 9.1.3.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Variability of Na^+ , K^+ and Cl^- leaf contents

The third leaf of each genotype was analysed for accumulated Na^+ , K^+ and Cl^- content, in different stress conditions, with 0, 50, 100 and 150 mM NaCl. Under control conditions, leaf Na^+ contents varied between 0.16% (Utique) and 0.69% (Om Rabiaa) (Figure 1a). It is noted that durum wheat varieties (Jenah Khotifa, Karim, Om Rabiaa), *Aegilops* accessions (MZ116, MZ141, MZ144) and the two

common wheat varieties Salambo and Utique highlighted comparable behaviours concerning leaf Na^+ concentrations, under salt stress conditions. For these genotypes, Na^+ leaf contents increased significantly, compared with the control for the three salt stress treatments (50, 100 and 150 mM). However, the three salt stress treatments gave rise to a significant decrease in Na^+ leaf contents, compared with the control conditions, for the common wheat variety Vaga.

Concerning K^+ leaf contents, this parameter ranged from 1.23% (Vaga common wheat variety) to 2.43% (wild accession MZ116), under control conditions (Figure 1b). Under salt stress conditions, a highly significant decrease in K^+ leaf contents, compared with the control, was observed for the three durum wheat varieties Jenah Khotifa, Karim and Om Rabiaa. The three wild accessions, MZ116, MZ141 and MZ144, had decreased K^+ levels, compared with the control, which showed no significance under 50 mM of NaCl but was significant for the two salt treatments of 100 and 150 mM. For the three common wheat varieties, Salambo, Utique and Vaga, different

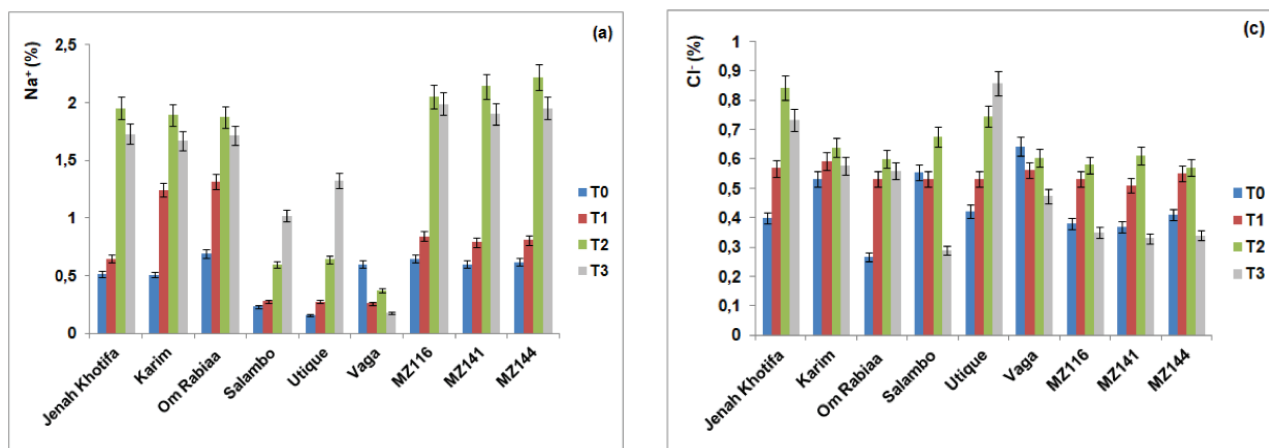


Figure 1: Variability of ions Na^+ (a), K^+ (b) and Cl^- (c) leaf contents (%) among the investigated durum wheat (Jenah Khotifa, Karim, Om Rabiaa) and common wheat varieties (Salambo, Utique, Vaga) and wild wheat accessions (MZ116, MZ141, MZ144) according to the four salt treatments (T0=0, T1= 50, T2=100 and T3=150 mM NaCl)

behaviours were observed (Figure 1b). Interestingly, the variety Vaga maintained a significantly higher K^+ leaf content than the control conditions for the three salt treatments of 50, 100 and 150 mM.

The Cl^- leaf content, under control conditions, varied between 0.26% for Om Rabiaa and 0.64% for the Vaga variety (Figure 1c). Among the studied durum wheat varieties, the variety Karim maintained stable Cl^- leaf content, comparable to the control conditions, for the three salt stress treatments. However, Jenah Khotifa and Om Rabiaa highlighted a significant increase in Cl^- leaf concentration, compared with the control conditions, for the three NaCl concentrations. The three wild wheat accessions showed the same pattern of leaf Cl^- contents under the three treatments, with a significant increase compared with the control for 50 and 100 mM NaCl. Under 150 mM salt treatment condition, comparable levels to the control conditions were observed for the MZ116 and MZ141 wild accessions while a significant decrease was observed for MZ144. Different responses to salt stress were detected for common wheat varieties concerning Cl^- leaf concentration. The Utique variety presents a significant increase in Cl^- contents, compared with the control, for the three salt treatments while Salambo and Vaga showed a significant decrease in their Cl^- leaf contents, compared with the control conditions, under 150 mM NaCl (Figure 1c).

The variability of K^+/Na^+ ratios among the studied genotypes, cultivated under four salt conditions (0, 50, 100 and 150 mM NaCl), was undertaken. It is noted that K^+/Na^+ ratios decreased significantly, compared with the control, for all durum wheat varieties and wild accessions. Moreover, these two species showed comparable K^+/Na^+ levels, except under 50 mM NaCl condition (Figure 2). As for the previously studied ionic traits, common wheat varieties exhibited higher variability concerning their response under control and salt stressed conditions. It is noted that the Vaga variety presented a significant increase in K^+/Na^+ ratios, compared with the control condition, for the three salt treatments (50, 100 and 150 mM). The Salambo variety maintained K^+/Na^+ ratios, which were comparable to the control, under 50 mM NaCl. For Utique K^+/Na^+ ratios decreased significantly, compared with the control conditions, above the application of 50 mM NaCl (Figure 2).

The concentration levels of Na^+ , Cl^- and K^+ in plant tissues are considered to be significant indicators of salt stress tolerance (Iseki et al. 2017). Restricted levels of Na^+ and Cl^- uptake and high K^+ content levels are considered to be indicators of salt tolerance in plant species. Na^+ and Cl^- accumulation in plant aerial parts causes ionic stress establishment, which induces ion toxicity and negatively

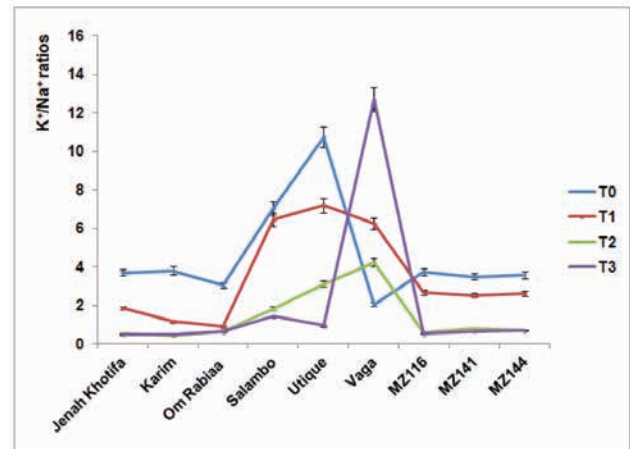


Figure 2: Effect of the four salt treatments (T0=0, T1=50, T2=100 and T3=150 mM NaCl) on the K^+/Na^+ ratios among the investigated durum wheat (Jenah Khotifa, Karim, Om Rabiaa) and common wheat varieties (Salambo, Utique, Vaga) and wild wheat accessions (MZ116, MZ141, MZ144)

affects growth and productivity (Tavakkoli et al. 2011). Indeed, excess Na^+ results in cytosolic K^+ efflux and deficiency, which causes severe damage to cellular homeostasis (Assaha et al. 2017).

Maintaining the ion homeostasis is cited to be among the most important traits, which define the salt stress tolerant of genotypes (Nguyen et al. 2013). High K^+/Na^+ ratio in plant tissues is considered to be a key for salt tolerance (Tavakkoli et al. 2011; Shabala and protosin 2014). The obtained ionic data highlighted the distinctiveness of the common wheat variety Vaga among the nine studied wheat genotypes. Interestingly, this variety exhibited remarkable behaviour under NaCl salt stress conditions and seems to be the most salt tolerant genotype followed by Salamdo then Utique. The *Aegilops* accessions showed lower intra-species variability, concerning the aptitude of the studied genotypes to tolerate high NaCl contents in the nutritive solution. However, these wild wheat accessions have shown higher salt stress tolerance than durum wheat genotypes, especially under 50 mM NaCl.

3.2 Chlorophyll fluorescence parameters

The effect of the four salt treatments on the efficiency of electron donation to the PSII reaction centre (F_v/F_o), the evolution of the maximum quantum efficiency of PSII (F_v/F_m) and the photochemical quenching coefficient (Pq) were evaluated after three weeks of salt stress application. These chlorophyll fluorescence parameters are considered to be effective and rapid non-destructive tools for salt tolerance screening in cereals species (El-Hendawy et al. 2017).

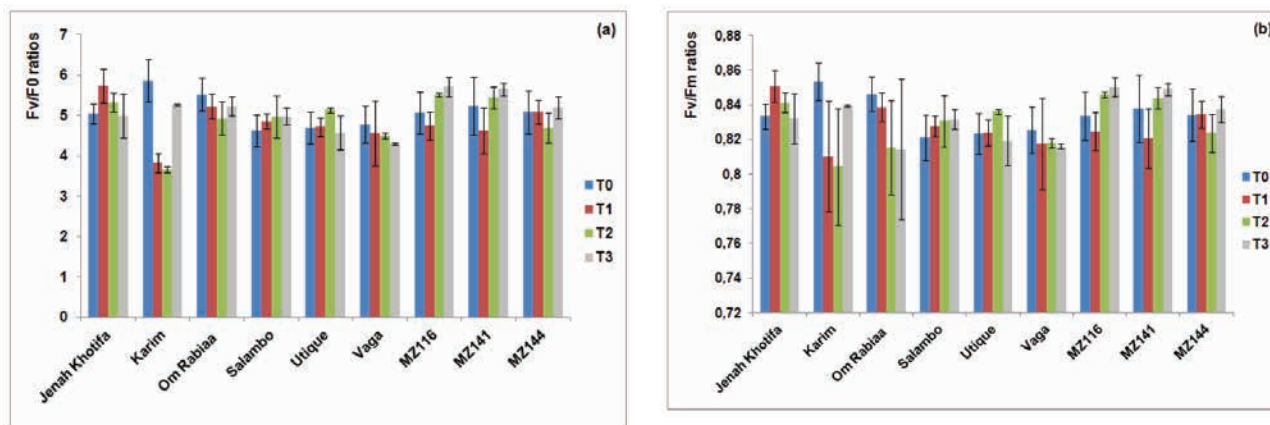


Figure 3: Variation of F_v/F_0 (a) and F_v/F_m (b) parameters among the investigated durum wheat (Jenah Khotifa, Karim, Om Rabiaa) and common wheat varieties (Salambo, Utique, Vaga) and wild wheat accessions (MZ116, MZ141, MZ144) according to the four salt treatments (T0=0, T1= 50, T2=100 and T3=150 mM NaCl)

In the absence of salt stress treatment, comparable F_v/F_0 mean values were obtained for the studied wheat genotypes (Figure 3a). The lowest F_v/F_0 ratio was observed for the Salambo wheat variety (4.61) while the durum wheat variety Karim exhibited the highest F_v/F_0 ratio (5.86). Except for the durum variety Karim, which presented a significant decrease in F_v/F_0 ratios for the salt stress treatments 50 and 100 mM NaCl, all the remaining wild accessions, common and durum wheat varieties exhibited no significant variability, compared with the control conditions, for the three applied salt treatments (Figure 3a). The F_v/F_0 ratio parameter is considered to reflect the potential of photosynthetic activity in stressed plant species. A reduction in F_v/F_0 ratios, in stressed genotypes, indicates the effect on the efficiency of the photosynthetic process and electron transport chain (de Melo et al. 2017).

The F_v/F_m parameter vary in the same way as the F_v/F_0 ratios among the studied genotypes. Indeed, the highest F_v/F_m ratio (0.85) was recorded for the Karim variety and the lowest value was obtained for the Salambo common wheat variety (0.82). Based on the obtained results, the variability of F_v/F_m ratios, under the three applied salt treatments (50, 100 and 150 mM NaCl), was not significant, compared with the control conditions, for all the nine studied genotypes (Figure 3b). The F_v/F_m ratio generally reflects the efficiency of plant leaf photosynthesis. The increases in F_v/F_m values, under salt stress conditions, traduce an increase in photochemical conversion efficiency of PSII. The F_v/F_m ratio is the most widely used parameter to characterise the maximum quantum yield of PSII photochemistry (Stirbet et al. 2018). The decrease in F_v/F_m ratio reflects photo-inhibitory damage of the PSII reaction centres, caused by the applied salt stress, which is accompanied by chloroplast ultra structural damage (Yamane et al. 2008; Khoshbakht and Asghareei 2015).

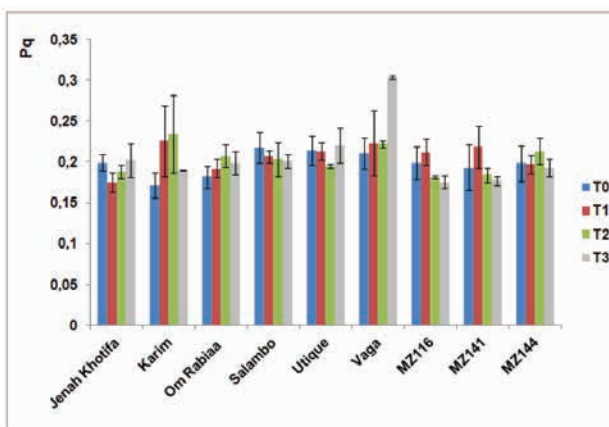


Figure 4: Variation of the photochemical quenching coefficients (Pq) among the investigated durum wheat (Jenah Khotifa, Karim, Om Rabiaa) and common wheat varieties (Salambo, Utique, Vaga) and wild wheat accessions (MZ116, MZ141, MZ144) according to the four salt treatments (T0=0, T1= 50, T2=100 and T3=150 mM NaCl)

In the absence of salt stress, the photochemical quenching coefficients did not show a significant variability among the studied genotypes, with a mean value of 0.19. Except for the Vaga common wheat variety, for which the photochemical quenching coefficient values increase significantly compared with the control conditions for the 150 mM NaCl salt treatment (0.30), all the remaining genotypes showed no significant variability, compared with the control conditions, concerning this parameter (Figure 4). The photochemical quenching coefficient measures the fraction of PSII centres in the open state (Terletskaia et al. 2017). The reduction of the quenching coefficient, under increasing salt stress conditions, present a negative impact on photosynthetic rate and PSII efficiency and may be explained as a consequence of stomatal closure (Jamil et al. 2007).

4 Conclusions

The results suggest that ionomic and photosynthetic traits underline the distinctiveness of the common wheat varieties Vaga, Utique and Salambo, which highlighted performances, under salt stress conditions, at the seedling stage and showed higher potential for salt stress tolerance among the nine studied wheat genotypes. Interestingly, the Vaga variety presented remarkable behaviour and exhibited the ability to maintain a suitable potassium diet and to limit the accumulation of sodium in young leaves with stable chlorophyll fluorescence parameters. This common wheat variety could serve as a genetic resource for further genetic improvement strategies concerning wheat salt tolerance.

Conflict of interest: Authors state no conflict of interest.

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