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Salinity effect on seed germination in doubled haploid and parental barley genotypes (*Hordeum vulgare* L.)

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Abstract

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In cereals, resistance and tolerance to salinity are qualities that allow their cultivation in arid and semi-arid regions. Hence, we sought to select salt stress-resistant barley genotypes by applying sub-lethal NaCl concentrations and observing their effect on germination. This work was carried out using parent spring barley genotype (*Hordeum vulagre* L.) namely Tamelalt, and its doubled haploids progeny. Salt stress resistance was tested on seeds germination in Petri dishes provided with filter paper containing increasing concentrations of salt (0, 5, 10, and 15 $g.L^{-1}$) for 10 days at 25°C. The doubled haploids progeny derived from Tamelalt showed to be the most tolerant to salt stress with a germination rate under the most severe salt stress conditions (NaCl 15 $g.L^{-1}$), while the parent genotype Tamelalt was the least tolerant with a germination rate of only 26.67% under the same stress. Principal component analysis and hierarchical cluster analysis allowed the separation of DH lines and parent genotype. Two groups were identified. The DH lines were completely different from the parent genotype.

Keywords: barley; *Hordeum vulagre* L.; salinity; doubled haploid lines; germination

Introduction

Agricultural productivity around the world is subject to environmental constraints, particularly drought and salinity, because of their high impact and wide distribution. Plants are always exposed to different types of stresses (biotic and/or abiotic). Salt stress is one of the main abiotic constraints (Bartels & Sunkar, 2005; Chadli & Belkhodja, 2007) that can severely reduce crop growth and yield. 20% of cultivated land in the world, and 33% of irrigated land, are salt-affected and degraded (Machado & Serralheiro, 2017). The most important disturbances are recorded in the rhizosphere (Binzel & Reuveni, 2010) because of heavy accumulations of salts in irrigation water (Berthomieu et al., 2003).

Salinization in arid and semi-arid ecosystems results from the evaporation of water from the soil (Munns *et al.*, 2006). Tolerance to salinity is the desired potential in cereals in arid and semi-arid regions as Morocco. El-Goumi et al. (2017) and Genhua Niu et al. (2010) showed that the response of salt stress is genotype dependent. The salinity effects are manifested by osmolality (Bliss et al., 1986), ion toxicity (Hampson & Simpson, 1990), or combination of the two (Huang & Redmann, 1995). In vegetative plants, salt stress reduces cell turgor and affect rates of root and leaf elongation (Fricke et al., 2006). Also, the high intracellular concentrations of both Na^+ and Cl^- can disrupt the metabolism of dividing and expanding cells (Neumann, 1997).

Plants have the considerable genetic potential for environmental stress tolerance. In particular, the yield of cultivated plants has different degrees of sensitivity to salt and water stress (Al-Ashkar et al., 2019)100, and 200 mM NaCl. The tolerance for the presence of salts such as sodium chloride is a quality sought after in plants of agronomic interest in order to extend their cultivation in arid and semi-arid zones (Oubaidou et al., 2021). However, salt stress decreases daily germination, dry weight, leaf area, leaf water content, K^+ content, and increases proline and Na^+ levels (Abdi et al., 2016).

Biotechnologies provide new techniques to increase tolerance or resistance to salinity and drought and to reduce the vegetative cycle. (Hentour et al., 2020) concluded that anther culture under salt stress is an alternative methodology to create new varieties adapted to salt stress. (Al-Ashkar et al., 2019; Oubaidou et al., 2021)100, and 200 mM NaCl demonstrated that DH lines developed from an anther culture technique are more resistant to salinity than parent genotypes.

Germination is a vital stage in plant development. Given its importance, our study aims to investigate the effect of saline on the development of parent genotype and doubled haploids progeny.

Material and Methods

Parental genotype, Tamelalt P, and doubled haploids (DH) lines, Tamelalt DH1, Tamelalt DH 2, and Tamelalt DH3 obtained from anthers culture under salt stress at 5 $g.L^{-1}$ of NaCl were used to assess the effect of salinity on germination process of barley. Germination was carried out under different concentrations of sodium chloride 0 as a control, 5, 10 and 15 $g.L^{-1}$. For each genotype, 60 seeds were sterilized with commercial bleach (5%) for 10 min and washed 3 times with sterile distilled water.

Five seeds were put to germinate in Petri dishes containing three filter paper. In the control path, we added 5 ml of sterile distilled water (0 $g.L^{-1}$); in the saline stress path, we added 5 ml of saline solution containing 5, 10 or 15 $g.L^{-1}$ of NaCl. Then dishes were put in the darkness in an incubator adjusted at 25°C for 10 days. Three replicates were prepared for each treatment. The germination is identified by the exit of the radicle from the teguments of the sheath whose length is at least 2 mm.

Statistical analyses

The following parameters were calculated:

- Germination Percentage, %;
  GP = (number of germination seeds / number of total seeds) * 100;
- Length of roots, cm;
- Length of epicotyl, cm;
- Plant size, cm;
- Number of roots/plant;
- Reversibility of salt action: this parameter is used to determine the origin of the depressive effect of NaCl (osmotic or toxic). The seeds were placed to germinate in Petri dishes with 15 $g.L^{-1}$ of NaCl for four days. On the fourth day, the non-germinated seeds were rinsed three times with sterile distilled water to remove the unabsorbed salt. The dishes containing sterile distilled water were incubated for an additional four days;
- Relative water content (RWC): The water content is measured directly by weighing the weight of 5 plants, which determines a mass: $m_{w,}$ then weighing after drying for 24 hours in a ventilated oven at 60°C to evaporate the water, we measure a weight m_d.
  $RWC\% = (m_w – m_d) / m_d$

The results are subjected to analysis of the variance (ANOVA) and the averages are compared using the Duncan test at the 5% threshold. Cluster Analysis and Principal Component Analysis (PCA) performed based on a correlation matrix to reduce the dimensions of data space, and a biplot were drawn using R 4.1.0.

Result and Discussion

The results obtained after this study highlight the relation between genotype and NaCl concentration and show that the stressor (NaCl) influenced different studied parameters. Indeed, the analysis of variance revealed highly significant effects of genotype, salt stress, and interaction genotype x salt stress (Table 1). The mean performance of all traits showed highly significant differences between DH plants and parental varieties with the exceptions of NR and RWC (Table 2).

Germination Percentage (G %)

In parental genotype, Tamelalt P, the germination was affected by salinity compared to DH lines of that are most resistant. The depressive effect of salt stress was observed in Tamelalt P, while the germination percentage decreased with an increase in salinity (Figure 1).

Table 1. Variance analysis (ANOVA) of the parameters studied of barley genotypes in response to salt stress

Source of variation	df	Germination Percentage	Roots length	Epicotyls length	Plant size	Numbre of roots	Relative water content	df	Reversibility NaCl
Genotype	3	4044.44***	311.65***	120.81***	814.81***	1.28ns	890.47***	3	1033.96ns
NaCl	3	511.11**	333.88***	565.08***	1592.42***	2.95**	1277.81***	1	1.28ns
Genotype x NaCl	9	1138.27***	18.04***	12.02***	54.05***	4.18***	231.31***	3	1462.05ns

Table 2. Mean performance for each genotype across all salinity levels

Genotype	GP, %	LR, cm	LE, cm	PS, cm	NR	RSA, %	RWC, %
Tamelalt P	55.00 ± 24,31[a]	4.47 ± 3,55[a]	5.99 ± 6.03[a]	10.46 ± 9.33[a]	4.90 ± 2.05[a]	60.83 ± 26.54[ab]	76.16 ± 15.96[a]
Tamelalt DH1	97.22 ± 9.62[c]	12.15 ± 4.38[b]	10.12 ± 4.54[b]	22.26 ± 8.11[b]	5.40 ± 0.60[a]	72.00 ± 41.47[ab]	90.57 ± 3.95[b]
Tamelalt DH2	75.00 ± 17.32[b]	12.30 ± 4.09[b]	10.82 ± 4.57[bc]	23.12 ± 8.04bc	5.35 ± 0.59a	78.33 ± 20.41[ab]	88.07± 5.29[b]
Tamelalt DH3	88.33 ± 23.29[c]	12.62 ± 4.86[b]	11.43 ± 5.05[c]	24.05 ± 8.72[c]	5.45 ± 0.69[a]	100.00 ± 0.01[c]	89.40 ± 3.90[b]

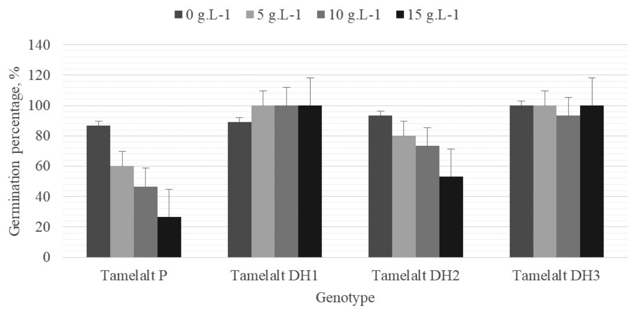

Fig. 1. Effect of salt stress in germination percentage (%) for each genotype

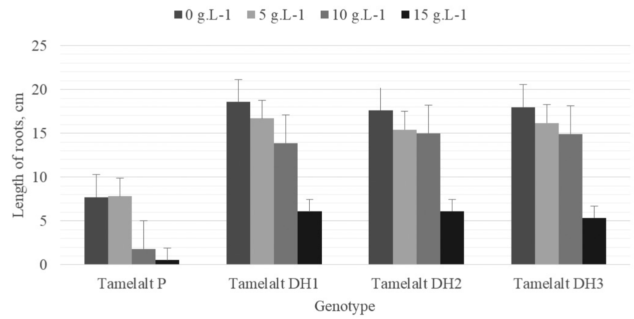

Fig. 2. Length of roots (cm) per NaCl concentration

Our results are reminiscent of those published by El-Goumi et al. (2014) and Djerah & Oudjehih (2015) in barley, Hmouni et al. (2017), and Mrani Alaoui et al. (2013) in wheat, Benidire et al. (2015) in broad bean (*Vicia faba)*, Ertan Yildirim (1994) in pepper, and Hajlaoui et al. (2007) in chickpea. Some studies have shown that the increase in salt concentration delays germination (Askri et al., 2007). However El-Tayeb (2005) reports that increasing of NaCl level reduced the germination percentage, the growth parameters (fresh and dry weight), potassium, calcium, phosphorus and insoluble sugars content in both shoots and roots of 15 day old seedlings.

Masmoudi et al. (2014) confirms that salt stress is harmful to germination and that NaCl is more toxic than KCl, MgSO4 and Na2SO4, they reports that calcium amendment by CaCl2 and CaSO4 reduced the inhibitory effect of salts.

Length of roots (LR)

After 10 days of germination at different concentrations of NaCl in Petri dishes, the length of the roots was measured (Figure 2). The results of the root system analysis showed that salinity exerts inhibitory effects on barley plant growth, which resulted in a decrease in roots length as a function of the increase in salinity. DH lines seemed more resistant to salt stress compared to their parent (Tamelalt P). It is important to note that the higher the NaCl concentration, the shorter the roots length. These results coincide with those obtained by Benidire et al. (2015); Djerah & Oudjehih (2015); Kadri et al. (2009); and Mrani Alaoui et al. (2013).

Length of epicotyl (LE)

The length of the epicotyl was measured at the end of the experiment. In the control conditions, the longest epicotyl was found in Tamelalt DH3 (17.78 cm). The results of foliar system showed that under moderate stress, the length of the epicotyls is slightly affected for DH lines (1, 2 and 3). However, the depressive effect of salt is very noticed from the concentration of 10 $g.L^{-1}$ for Tamelalt P (Figure 3).

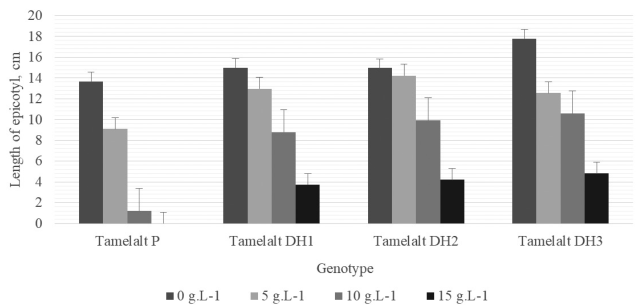

Fig. 3. Length of epicotyl (cm) per NaCl concentration

Our results are in agreement with those published by Benidire et al. (2015) which have also shown that salinity exerts an inhibitory effect on the growth of *V. faba* plant which results in a decrease in the length of the stem as a function of the increase of salinity in the medium. This effect has also been shown in barley and wheat (Djerah & Oudjehih, 2015; Mrani Alaoui et al., 2013). According to Munns (2002) the initial reduction in shoot growth is probably due to the hormonal signals generated by the roots.

Plant size (PS)

In control conditions (0 g.L^{-1}), Tamelalt DH3 recorded the longest plant size (root + shoot) (31.82cm). Compared to the control, the size of the plants decreased with increasing NaCl concentration. It is noteworthy that this reduction is important for Tamelalt P from the concentration of 10 g.L^{-1}. For the DH lines, the effect of salt is only apparent at the maximum concentration of NaCl (15 g.L^{-1}) (Figure 4). Djerah & Oudjehih (2015), reports that the impact of salt stress is manifested in the concentration of 6 g.L^{-1}.

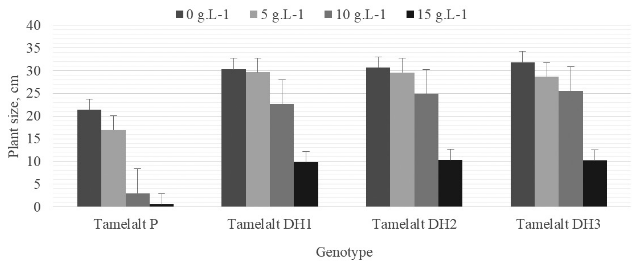

Fig. 4. The size of the plants by genotype as a function of NaCl concentration

Number of roots (NR)

The increase in NaCl concentration slightly affected rhizogenesis, which resulted in a reduction in the number of roots. The results suggested that salinity exerts inhibitory effect on roots growth of Tamelalt P. The average number of which varied from 6.5 to 2.6. However, for the DH lines, the salinity did not remarkably affect the development of the root system (Figure 5).

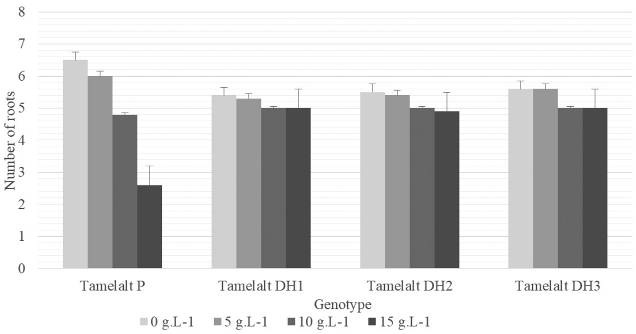

Fig. 5. Average number of roots as a function of NaCl concentration

Adjel et al. (2013), Djerah & Oudjehih (2015) and Nibau et al. (2008) highlights that salt stress causes a decrease in the number of roots. Bennaceur et al. (2001) showes that tolerant varieties can develop a root system to be able to withstand salinity.

Reversibility of salt action (RSA)

The reversibility of salt action was studied at a maximum concentration of 15 g.L^{-1} NaCl. We have shown that salt exerts at high doses (15 g.L^{-1}) a depressive effect on seeds germination. This inhibition can be osmotic or toxic, while the results of our study showed that the transfer of seeds in distilled water without salt is followed by an immediate resumption of germination mainly for Tamelalt P whose seed germination increases from 26.7% to 91.7% (Figure 6). Therefore, these results indicate a reversible effect of salt on the seed germination of barley genotypes.

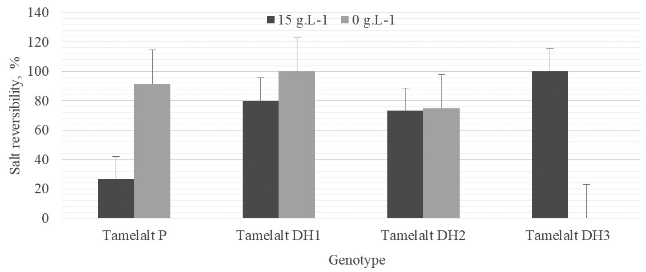

Fig. 6. Reversibility of salt action in 15 g.L^{-1} NaCl

The results of the reversibility of salt action have shown that NaCl has an osmotic effect. This reversible has been demonstrated in several studies. It is observed in legumes (Tsoata, 1995), in *Vicia faba* (Hajlaoui et al., 2007), in Citrus (Zekri, 1993), in barley (Bliss et al., 1986) and wheat (Mrani Alaoui et al., 2013).

Relative water content (RWC)

During the germination phase, NaCl caused a decrease in the relative water content by increasing NaCl concentration. NaCl at different concentrations adversely affected the relative water content regardless of genotype. DH lines recorded the highest water content in the presence of different salt concentrations compared to the parent genotype (Tamelalt P) (Figure 7).

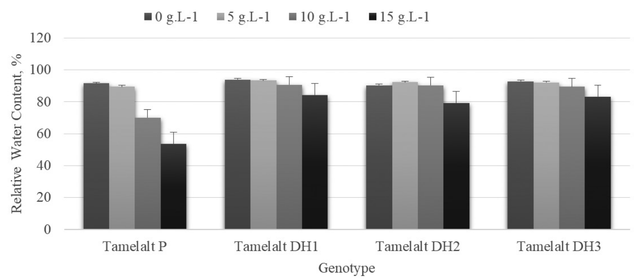

Fig. 7. Effect of salinity on relative water content (%)

El-Tayeb (2005) reports that increasing NaCl concentration decrease not only leaf relative water content but also the photosynthetic pigments (Chl *a, b* and carotenoids). Munns (2002) explains that salinity reduces the ability of plants to absorb water, which rapidly leads to a reduction in growth rate, as well as a series of metabolic changes. In Rice, Polash et *a*l., (2018) demonstrated that relative water content decreased significantly with the increasing of salinity to 100 mM NaCl during 7 days.

Principal Component Analysis (PCA) and Cluster Analysis

Principal component analyses were conducted for all measured traits, parental genotype and DH lines. PCA resulted in a clear separation between DH lines (Tamelalt DH1, Tamelalt DH2, and Tamelalt DH3) and parent genotype (Tamelalt P) based on trait combinations. In this experiment, the essential information was expressed in PCA1 and PCA2 that explained 97% of the total variability. The first principal component (PC1) explained 79.8% of the variation, followed by the second principal component (PC2), representing 17.2% of the variation (Figure 8). PCA2 had a positive correlation with all traits. PCA1 showed higher values for RSA, G % and RWC. The results showed that RWC is positively correlated with PS, LE, and LR. The positive correlation was also found between NR, PS and LE. The G % was not associated with any of the variables tested. RSA is negatively correlated with NR and LE. Wang et al. (2020) reported that based on the results of principal component analysis (PCA), germination percentage, plant size can be selected as representative indicators for saline tolerance evaluation during seed germination.

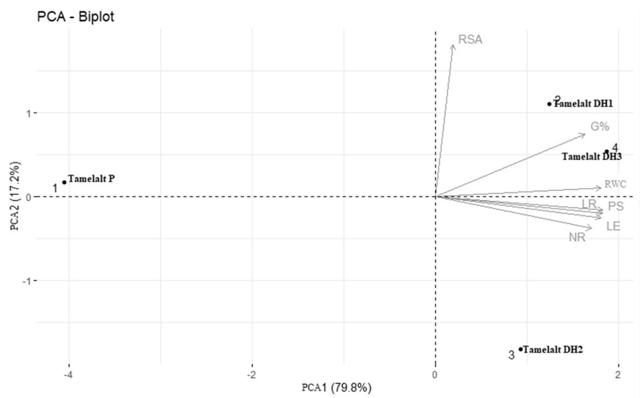

Fig. 8. Principal components analysis of 4 barley genotypes. Biplot vectors are trait factor loadings for PC1 and PC2 of 6 measured traits. Parent (P), doubled haploid (DH), number of roots (RN), Length of epicotyl (LE), Length of roots (LR), Plant size (PS), Germination Percentage (G%), Reversibility of salt action (RSA), Relative water content (RWC)

Hierarchical clustering showed that the parental genotype Tamelalt P don't belong to the same category as its DH progeny and separated the genotypes into three groups. The first cluster consisted of Tamelalt P. The second group was made up of DH lines in two sub-clusters. The first sub-cluster included Tamelalt DH2 and the second one consister of Tamelalt DH1 and Tamelalt DH3 (Figure 9).

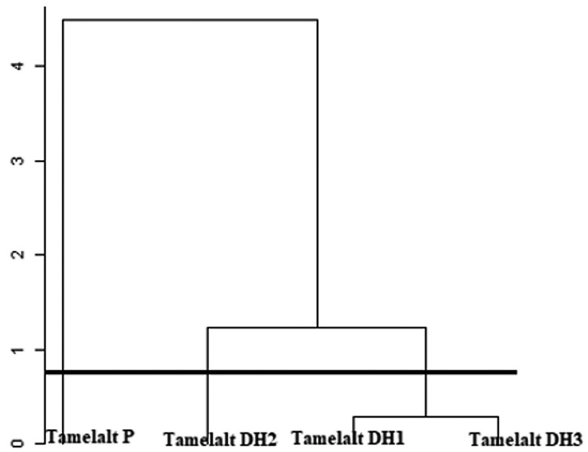

Fig. 9. Dendrogram showing clustering of 4 barley genotypes based on the Euclidean distance. DH: doubled haploid

Conclusion

Barley's response to salt stress varies depending on the genotype, the NaCl concentration of the germination medium, and the parameter under study. Salt stress marked a decrease in all parameters studied from the concentration of 10 $g.L^{-1}$, and the pattern of this decrease varies according to the genotypes. The results show that Tamelalt DH is more tolerant to salinity compared to their parent variety (Tamelalt). NaCl revealed an osmotic effect because of the reversibility of its action in all genotypes. The results reported in this study suggest that barley is more or less tolerant to salinity at the germination stage. This study should be complemented by similar field experiments to confirm the true tolerance of studied genotypes.

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