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Proceeding Paper

Salt Stress Effects on the Growth, Photosynthesis and Antioxidant Enzyme Activities in Maize (*Zea mays* L.) Cultivars †

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Abstract: Salt stress is considered one of the most damaging abiotic stresses for maize productivity. In this study, two-hybrid varieties (Sancia and Agrister) were cultivated under 0, 2, 4, and 6 g NaCl L^{-1}. Under 6 g NaCl L^{-1}, shoot length, dry weight, and chlorophyll content were higher for Agrister compared to Sancia. Moreover, an increase in proline and H_2O_2 was recorded for Sancia at 2 and 4 g·L^{-1} and for Agrister at 6 g·L^{-1}. Laterally, Agrister enhances the antioxidant enzymes catalase and peroxidase, while Sancia decreased peroxidase by 25% compared to the control. In conclusion, Agrister seems to be more tolerant than Sancia.

Keywords: salt stress; maize; physiological traits; antioxidants enzymes

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1. Introduction

In Tunisia, around 11.6% of the total land area is affected by salinity [1]. The high levels of NaCl affect plant development by inducing osmotic stress, stomatal closure, ionic toxicity, and nutritional imbalance, leading to reduced photosynthesis and the inhibition of protein synthesis and metabolic enzymes [2,3]. The hyperosmotic and hyperionic stresses caused by the high salinity induce oxidative stress by generating reactive oxygen species (ROS) within plant cells [4]. To alleviate ROS damage, plants induce non-enzymatic (phenolics and flavonoids) and enzymatic (SOD, GPX, CAT, and APX) antioxidants [2]. After wheat and rice, maize (*Zea mays* L.) ranks third among the most important cereal crop globally. It has been reported that soil salinity is a significant threats to maize production [5]. Although maize is considered moderately salt-sensitive [6], screening tolerant cultivars should be one of the main aims to achieve sustainable agriculture. In this context, the present research is conducted to evaluate salt stress tolerance in two contrasting cultivars, Agrister and Sancia, as well as to identify the best salt-tolerant cultivar to be grown on cultivated fields with salt concerns.

2. Materials and Methods

2.1. Plant Cultivation and Processing

The seeds of two simple hybrid cultivars (Agrister and Sancia) of maize were obtained from the Limagrain agricultural seed Corporation (Verneuil, France). The seeds were sown in a total of 40. The pots contained a mixture of standard peat/perlite (2:1, *v*/*v*) and were grown in a greenhouse with a relative humidity of 60–80% and a photoperiod of 14 h/day. A complete random design with five replicates was used. Plants were subjected to three

different levels of salt stress (2, 4, and 6 NaCl g·L^{-1}) by uniformly irrigating with 0.5 L of each salt solution every 2 days; control plants were irrigated with 0.5 L of fresh water.

2.2. Measurements and Sampling

The measurements of physiological traits occurred after 2 weeks of salt stress imposition in the recent fully expanded leaves. The total chlorophyll content was measured as described by [7] and expressed as g·mL^{-1}. The relative membrane permeability (RMP) was determined, based on electrolyte leakage of cells, as described by [8] and expressed in %. The content of total phenolic compounds was measured by the Folin–Ciocalteu method [9] and expressed as mg·g^{-1} FW. The content of hydrogen peroxide (H_2O_2) was measured according to [10] and expressed as mM. The guaiacol peroxidase was determined according to [11] and expressed as mmol·min^{-1}·mg^{-1} P. The catalase activity was determined according to [12] and was expressed as mmol.min^{-1}·mg^{-1} P. The content of proline was measured according to [13] and expressed as mg·g^{-1} FW. After 3 weeks of salt stress imposition, shoots were sampled for length measurements, and then were dried at 70 °C for dry weight measurements.

2.3. Statistical Analyses

The effects of salinity and cultivar and their interaction on the measured traits were determined through a two-factor (salinity × cultivar) analysis of variance (ANOVA) with RStudio.

3. Results

3.1. Effect of Salt Stress on Physiological and Morphological Traits

The results show that the salinity significantly affected all measured parameters, such as shoot length, biomass and RMP ($p < 0.001$), and chlorophyll content ($p < 0.05$); however, the interaction (S × V) affected only the chlorophyll content and RMP ($p < 0.001$) (Table 1). The 6 g NaCl L^{-1} induces, in Sancia, a reduction in dry matter, shoot length, and chlorophyll content, and an increase in RMP. Unlikely, we recorded in Agrister a slight decrease in shoot length and an increase in chlorophyll content associated with a maintained dry matter.

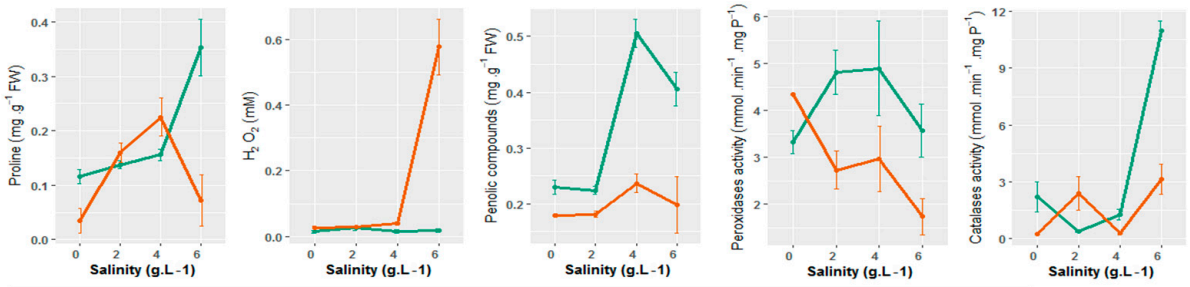
Table 1. Physiological changes in the maize cultivars Agrister and Sancia under salt stress; analysis of shoot length, biomass, chlorophyll content, and relative membrane permeability.

Traits		**Shoot Length (cm)**		**Shoot Dry Weight (g)**		**Chlorophyll Content (g/mL)**		**RMP (%)**	
Cultivar		**Agrister**	**Sancia**	**Agrister**	**Sancia**	**Agrister**	**Sancia**	**Agrister**	**Sancia**
Salinity	0 g·L^{-1}	34.66 ± 0.24 a	33.58 ± 0.42 a	13.56 ± 0.98 a	10.96 ± 0.61 a	39.68 ± 0.45 b	39.44 ± 0.75 a	86.82 ± 0.89 d	83.84 ± 0.32 d
	2 g·L^{-1}	30.83 ± 0.47 b	28.67 ± 0.62 b	10.34 ± 0.13 b	9.13 ± 0.08 b	41.45 ± 0.03 ab	40.33 ± 0.58 a	88.51 ± 0.19 c	92.74 ± 1.18 c
	4 g·L^{-1}	29.83 ± 0.94 b	27.10 ± 0.29 c	10.00 ± 0.33 b	8.77 ± 0.26 b	41.67 ± 1.55 a	37.46 ± 0.38 b	94.38 ± 0.73 b	96.56 ± 0.65 b
	6 g·L^{-1}	28.08 ± 0.51 c	26.00 ± 0.49 d	9.18 ± 0.20 b	7.69 ± 0.13 c	42.29 ± 0.17 a	36.46 ± 0.74 b	97.14 ± 0.18 a	98.90 ± 0.55 a
ANOVA	df								
Salinity	1	168,515 ***		47,962 ***		8817 *		564,379 ***	
Cultivar	3	24,402 ***		15,999 ***		48,628 ***		10,055 **	
S × C	3	2118 n.s.		1951 n.s.		30,741 ***		41,812 ***	

The symbols indicate statistical significance (n.s., $p > 0.1$, ** $p < 0.01$; *** $p < 0.001$). The sum square values with statistical significance are shown (n.s.: non-significant, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

3.2. Effect of Salt Stress on the Antioxidants Enzymes, H_2O_2, Proline, and Phenolic Compounds

The analysis of variance showed that all the traits were significantly affected by salinity, cultivar, and their interaction ($p < 0.001$). The 6 g NaCl L^{-1} stress induces a peak of H_2O_2 Sancia, but is maintained for Agrister. In addition, Agrister exhibited a higher phenolic content, peroxidases, and catalase activities than those of Sancia. Proline was high in Sancia at 2 and 4 g·L^{-1}, while it was higher in Agrister at 6 g·L^{-1} (Figure 1).



ANOVA	df	H_2O_2	Phenolic compounds	Catalase activity	Peroxidase activity	Proline
Salinity	1	0.33***	0.13***	159.66***	6.19***	0.07***
cultivar	3	0.133***	0.111***	28.711***	8.131***	0.027***
S×C	3	0.34***	0.05***	77.43***	9.68***	0.11***

Figure 1. The effect of salt stress (0, 2, 4, and 6 g·NaCl L^{-1}) on proline, H_2O_2, phenolic compounds, and peroxidase and catalase activities. The sum square values with statistical significance are shown (ns: non-significant, ***: $p < 0.001$).

4. Discussion

The applied salt stress affected Sancia development significantly compared to that of Agrister. This could be related to the accumulation of Na^+ in the roots, which reduces water absorption [14]. The reduction in total chlorophyll content is to be expected under stress conditions; its stability depends on membrane stability, which under saline conditions rarely remains intact [6]. Unlike Sancia, chlorophyll content and RMP were maintained in Agrister under the increasing salt stress, which shed light on its tolerance. Additionally, the higher level of proline in Agrister underlines the aptitude of the cultivar to minimize the salt effect by osmoregulation compared to Sancia. This is partially consistent with other studies on maize [3]. In response to salt stress, Agrister is able to mitigate the accumulation of H_2O_2 in plant cells by the biosynthesis of antioxidant metabolites (catalase, peroxidase, and phenolic compounds). Our results agree with those of [1,15], who suggested that CAT, peroxidases, and phenolic compounds were associated with salt stress tolerance. In conclusion, the hybrid Agrister was found to be more tolerant to salt stress than Sancia as indicated by the response of plant growth, chlorophyll content, and phenolic accumulation and their related antioxidant activity in the presence of NaCl.

Author Contributions: Z.K., O.M., M.B.J. and M.D.E.H. contributed equally to this work. Conceptualization, C.K.; methodology, Z.K. and A.A.; software, M.D.E.H.; data curation, O.M., writing—original draft preparation, Z.K. and M.B.J.; writing—review and editing, C.K., W.H. and M.B.J. All authors have read and agreed to the published version of the manuscript.

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