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(RESEARCH ARTICLE)

Agro-morphological, physiological and biochemical performances of two new durum wheat cultivars under salt stress conditions

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Abstract

Soil salinization requires developing genetically salt-tolerant and high-yielding genotypes, especially in semi-arid and arid regions. In this study, two new Tunisian durum wheat cultivars, *INRAT100* and *Dhahbi,* were tested for the first time in a semi-closed environment against salinity (0, 6 and 12 dS m-1). Dry weight of aerial and root parts, plant height, number of tillers per plant, spike and awn length, grain yield and its components, relative water content, relative membrane permeability, chlorophyll content, leaf chlorophyll fluorescence, i.e., initial fluorescence (F0) and the quantum yield of photosystem II (Fv/Fm), potassium (K+) and sodium (Na+) accumulation and the ratio K+/Na+ were assessed. Overall, most of growth and yield performance, physiological and biochemical attributes were affected by salinity. Notably, *INRAT100* was characterized by a higher K+ content, K+/Na+ ion selectivity, relative water content and Fv/Fm, grain yield and lower membrane permeability than that of *Dhahbi* under saline conditions. However, *Dhahbi* exhibited higher spike and awn length and a number of spikelets per spike. These findings suggest that INRAT100 is more tolerant to salinity than *Dhahbi* and might be recommended to farmers of marginal environments.

Keywords: Durum wheat; Yield-related traits; Physiological traits; Biochemical traits; Salinity

1. Introduction

Future agricultural crop productivity is jeopardized by soil salinization that affects 40% of soils in the Mediterranean basin [1]. In this area, the use of poor quality of water irrigation ranging from 1.5 to 15 dS m^{-1} and scarce winter rainfall contribute to further increase the salt problems, particularly in arid and semi-arid climates [2–4]. Durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.), associated with many Mediterranean traditional foods (e.g., pasta and couscous) in addition to a number of other semolina products (e.g., frike, bourghul, and unleavened breads), is a salt-sensitive glycophyte species [5, 6]. Since 6 dS m^{-1}, wheat growth and grain yield were reduced by 7.1% for each dS m^{-1} increase salinity of per and significant yield reduction occurs at 15 dS m^{-1} [7]. Environmental stress including salinity can cause about 50% of production losses [8].

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Salinity stress causes osmotic stress and ion imbalance, through increasing the assimilation of Na^+ ion. Higher concentration of Na^+ inhibits uptake of essential macronutrients such as potassium (K^+) and calcium (Ca^{2+}) from soil [9]. However, lower Na^+ and higher K^+ improve plant salinity tolerance [10] and were used as reliable criteria. Salinity also alters the ultrastructural cell components, disturbs the photosynthesis machinery, damages the membranous structure, increases the reactive oxygen species production, and reduces the enzymatic activity which limit the growth and yield of crops [11]. Hence, we need to develop genotypes with better tolerance to salinity stress and greater yield [12]. Since the twentieth century and to boost the productivity of Tunisian farmers, especially in rainfed environments, great efforts were made by the national breeding program, resulting in the registering of several durum wheat cultivars in the national catalogue (e.g., *Karim*, *Razzek*, *Om Rabiaa*, *Khiar*, *Nasr*, *Maali*, *Salim*, *INRAT100*, and *Dhahbi*) [13,14]. In particular, *Maali* exhibited the best and the most stable grain yield with the higher irrigation water salinities (6, 12 and 18 dS m^{-1}) [15–18]. However, the two new cultivars, *INRAT100* and *Dhahbi*, marketed to farmers in 2021, were never assessed against salinity stress. Therefore, the aim of the present study was to compare the potential of these cultivars based on agro-morphological, physiological, and biochemical responses under salt stress conditions.

2. Material and methods

2.1. Selected Durum Wheat Cultivars

Two durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) cultivars, *INRAT100* and *Dhahbi*, were used for this study. These two cultivars, released respectively in 2017 and 2018 and marketed to farmers in 2021, are characterized by dual resistance to septoria and powdery mildew.

2.2. Experimental Procedures

Sixty pots (25 cm diameter × 25 cm height, 5 kg of soil) were placed in a semi-closed environment at the National Agronomic Institute of Tunisia (36°49'52.5"N, 10°11'01.0"E) during the growing season 2020/21. A shelter was used to prevent the leaching of salt by rainwater. The minimum and the maximum air temperature and relative humidity recorded during the experiment ranged between 10-35°C and 57-70%, respectively. The soil was collected from the surface layer (0-20 cm) from an experimental farm of the National Agronomic Institute of Tunisia. The soil was characterized by a clay-loamy texture [19] (Table 1). Before pot installation, the collected soil was sieved (2 mm mesh size).

Table 1 Physico-chemical properties of substrate

Composition	**Value**
Sand (%)	18.93
Loam (%)	57.99
Clay (%)	23.08
Ph	8.18
EC (dS m-1)	0.24
OM (%)	2.50
C (%)	1.45
Available P (ppm)	119.01
TN (%)	0.21
CaCO3 (%)	37.13
Bulk density	1.5

EC, electric conductivity; OM, organic matter; C, carbon; P, phosphorus; TN, total nitrogen; CaCO3, total limestone.

Ten seeds were sown on January 09, 2020, thinned to five plants after emergence for each pot. Randomised complete block design with five replicates per treatment ($n = 5$) was used to accommodate the two-way factorial experiment, with cultivars (*INRAT100* and *Dhahbi*) and level of salinity (0 [control], 6, and 12 dS m^{-1} [saline or brackish water]) as main factors. From the first day after sowing and throughout the experiment, irrigation was applied with tap water to reach 100% of water-holding capacity to exclude the water-deficit stress. Water losses were completed and estimated by the difference in weight of each pot between two successive days. To avoid heat stress effects and standardise the experiment conditions, the position of the blocks (05) was interchanged every two days, maintaining the position of pots within each block.

2.3. Plant Measurements

2.3.1. Agro-Morphological Parameters

The dry biomass of aerial (ADW, g) and root (RDW, g) parts, plant height (H, cm) and number of tillers per plant (T P^{-1}) were recorded at the flowering stage (Z65) [20] which is a key developmental stage of the plant [21]. At maturity (Z85), the number of spikes per plant (S P^{-1}), spike length (SL, cm), awn length (AL, cm), number of spikelets per spike (SK S^{-1}), and grain yield (GY, g pot^{-1}) were measured.

2.3.2. Relative Water Content

The relative water content (RWC, %) was measured at Z65 based on fresh, saturated, and dry weights as: [(FW - DW) / (SW - DW)] × 100 [22]. Briefly, the fresh flag leaves were cut into 2-3 mm pieces, weighed immediately to establish the fresh weight (FW). Then, samples were soaked in test tubes containing distilled water for 24 h at 4°C in the dark and the resultant saturated weight (SW) was measured. The dry weight (DW) of the leaves was determined after an oven drying period of 24 h at 72°C.

2.3.3. Relative Membrane Permeability

The relative membrane permeability (RWP, %) was calculated according to the following equation: $[(EC_1 - EC_0) / (EC_2 - EC_0)] \times 100$ [23]. Fresh leaves were excised at Z65 into equal pieces and transferred to test tubes containing 20 ml deionized distilled water. The test tubes were vortexed for 10 s and the solution was assayed for initial electrical conductivity (EC_0). These tubes were stored at 4°C for 24 h and then assayed for EC_1. The same samples were autoclaved at 121°C for 20 min to burst cell walls and liberate all electrolytes and then cooled down to 25°C to determine EC_2.

2.3.4. Chlorophyll and Chlorophyll A Fluorescence Parameters

A chlorophyll meter SPAD 502 Plus (Minolta, Japan) was used to estimate the chlorophyll content (Chl, SPAD value). At flowering stage (Z65), the 'SPAD value' was determined on flag leaves of five randomly selected plants per replicate. Four SPAD readings were taken per leaf and averaged to produce a single observation.

Chlorophyll *a* fluorescence measurements were also conducted at Z65 on flag leaves of five plants per pot, using a portable pulse-modulated fluorometer OSI 5P (modulating measure by ADC, BioScientifc Ltd). Leaf samples were clipped into a leaf clip (dark-adaptation cuvettes) and kept in darkness for 20 min. In the present investigation, the initial minimum fluorescence (F_0) and the maximum quantum yield of PS II photochemistry (F_v/F_m) in dark-adapted plants were considered:

- Initial fluorescence (F_0): the minimum value of the fluorescence that reflects the emission of molecules by the excited Chl*a* of the collecting antennas of photosystem II.
- Maximum fluorescence (F_m): the maximum value of the fluorescence which is obtained for the same measurement of light intensity.
- Variable fluorescence (F_v): the maximum capacity of photochemical saturation calculated as: $F_v = F_m - F_0$ [24].

2.3.5. Ion Accumulation

To determine the flag leaf potassium (K^+) and sodium (Na^+) contents, the samples were recovered at Z65, dried at 65°C for 48h and finely ground before passing through a 2 mm sieve. Thereafter, 0.1 g of the obtained powder was calcined at 500°C for 3 h. For the extraction of mineral elements, 20 mL of nitric acid (1 N) was added and filtered. The amounts of exchangeable K^+ and Na^+ (mg g^{-1} dry weight) were then determined with a flame photometer [25].

2.4. Statistical Procedures

Analysis of variance (ANOVA) was performed using the GLM procedure to calculate the effects of salinity level and cultivar. A bivariate correlation procedure was used to calculate the Pearson correlation coefficients.

3. Results

3.1. Effect of Salinity on Agro-Morphological, Physiological and Biochemical Traits

Salinization of irrigation water reduced significantly ($p < 0.05$) the root dry weight (inhibition rate of 14.64 and 20.81% under 6 and 12 dS m^{-1}, respectively), plant height (0.60 and 8.73%), spike length (2.46 and 11.80%), grain yield (14.81 and 52.39%), chlorophyll content (15.20 and 22.43%), and F_v/F_m (0.83 and 1.66%) (Figure 1, 2, 3 and 4). On the other hand, the relative membrane permeability (increase rate of 48.72 and 336.00% under 6 and 12 dS m^{-1}, respectively) and Na^+ content (14.68 and 38.53%) were significantly increased under salt stress conditions. Nonetheless, a controversial result was obtained for the dry weight of aerial part, the ratio ADW/RDW, the awn length, the relative water content, F_0, K^+ content, and the ratio K^+/Na^+.

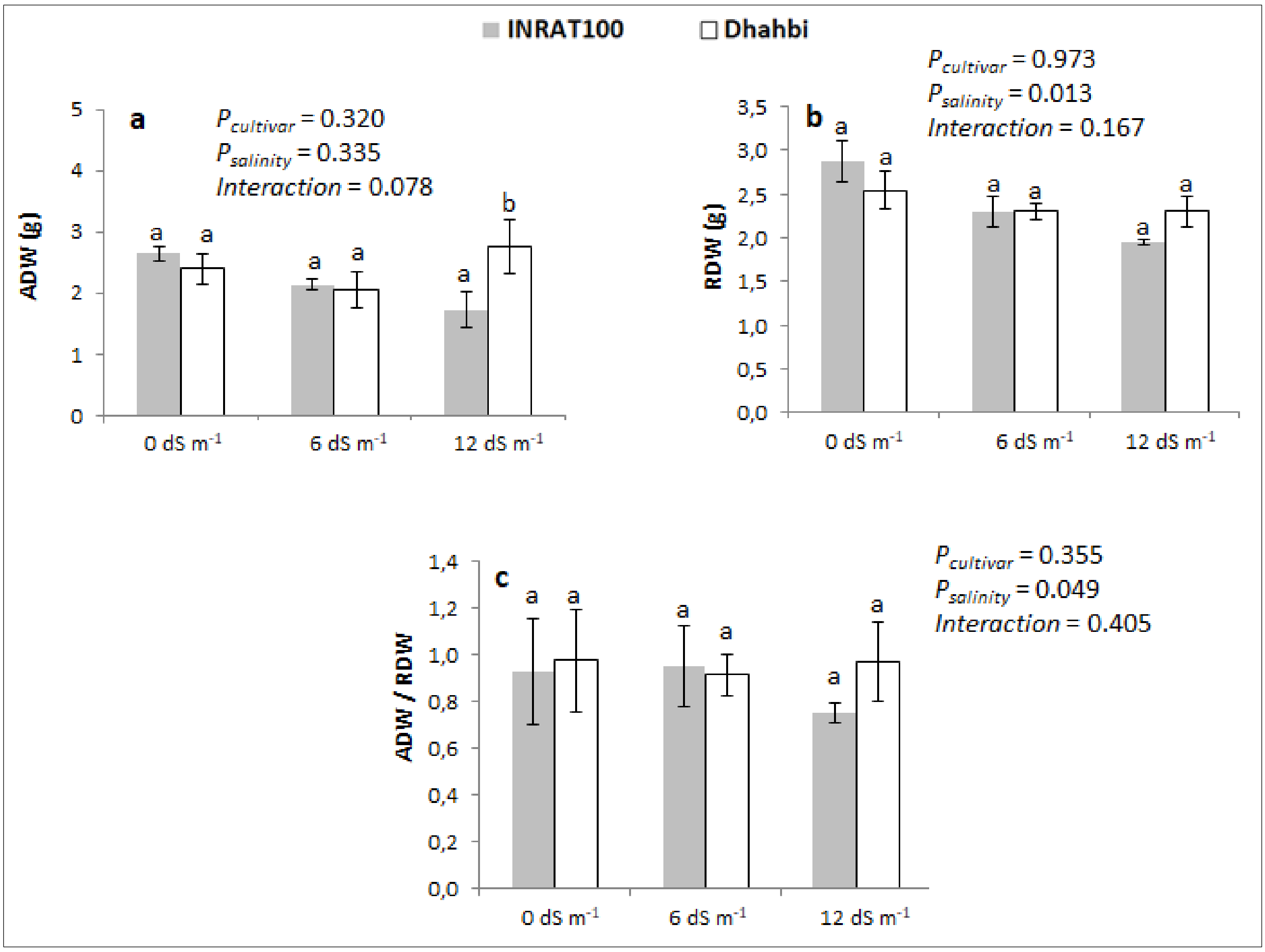

Figure 1 Salinity and cultivar effects on dry weight of aerial part (ADW, a), root dry weight (RDW, b) and the ratio (ADW/RDW, c)

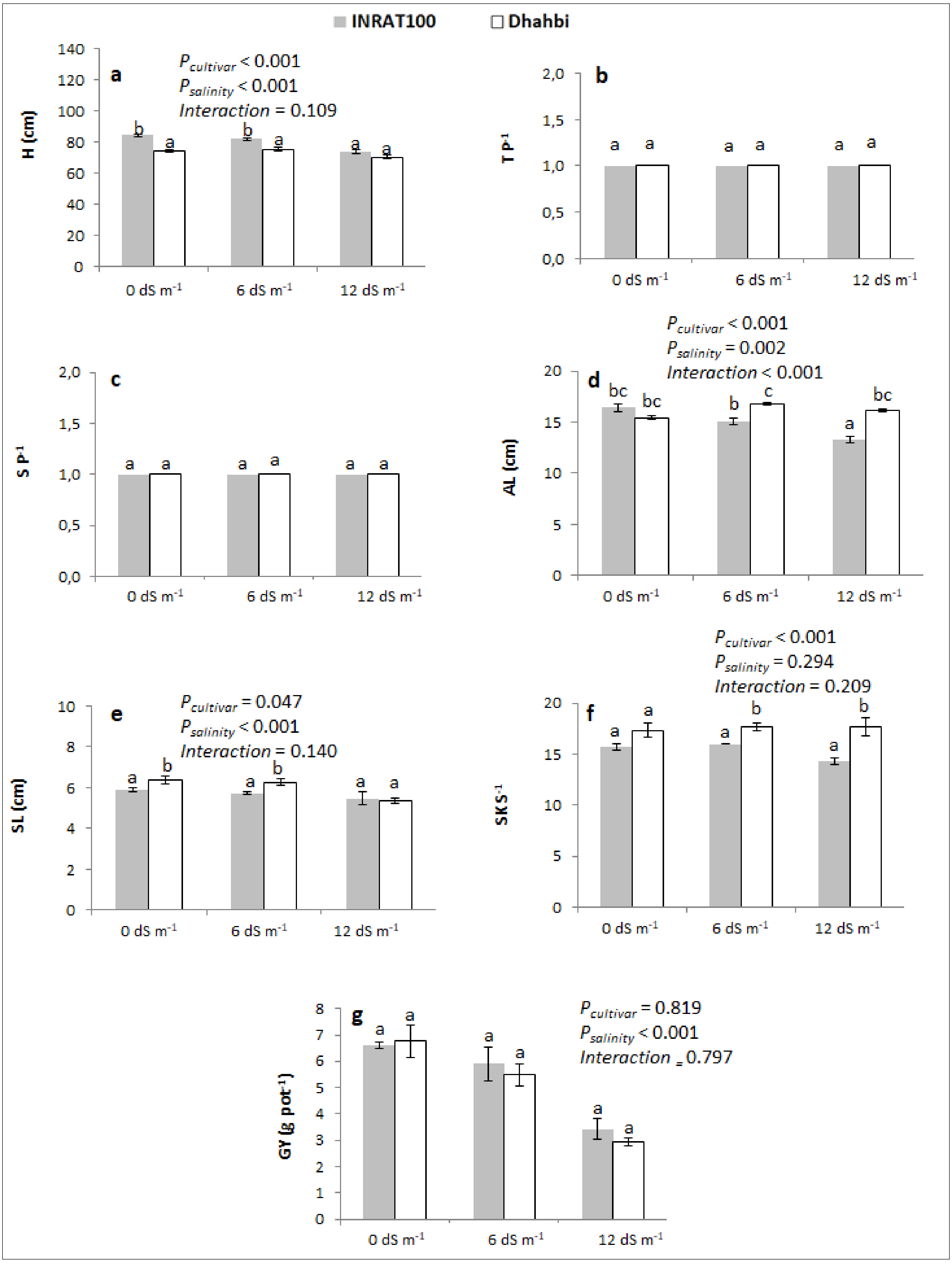

Figure 2 Salinity and cultivar effects on plant height (H, a), number of tiller per plant (T P^{-1}, b), number of spikes per plant (S P^{-1}, c), awn length (AL, d), spike length (SL, e), number of spikelets per spike (SK S^{-1}, f), and grain yield (GY, g)

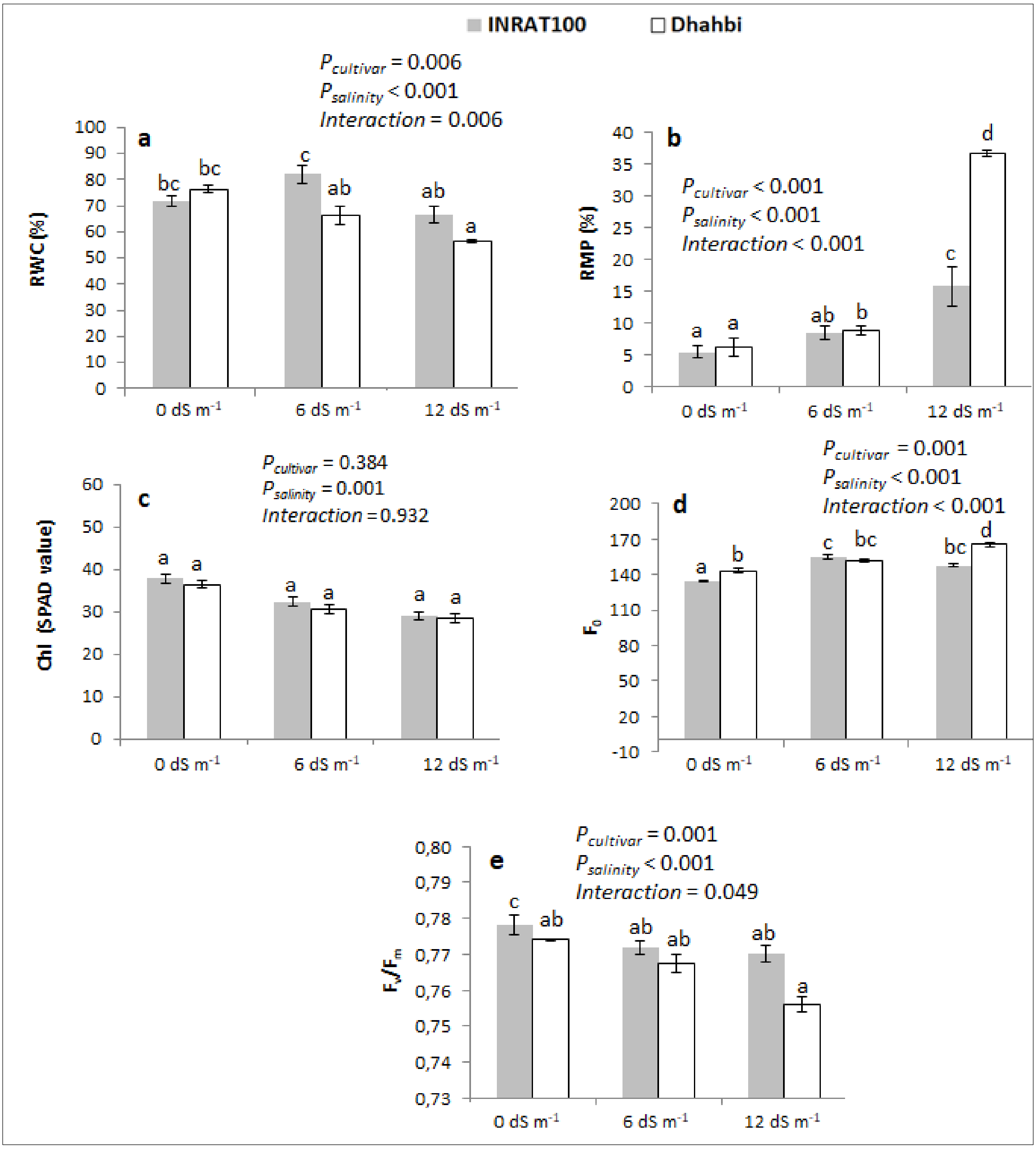

Figure 3 Salinity and cultivar effects on relative water content (RWC, a), relative water permeability (RWP, b), chlorophyll content (Chl, c), initial fluorescence (F0, d), and the theoretical quantum (Fv/Fm, e)

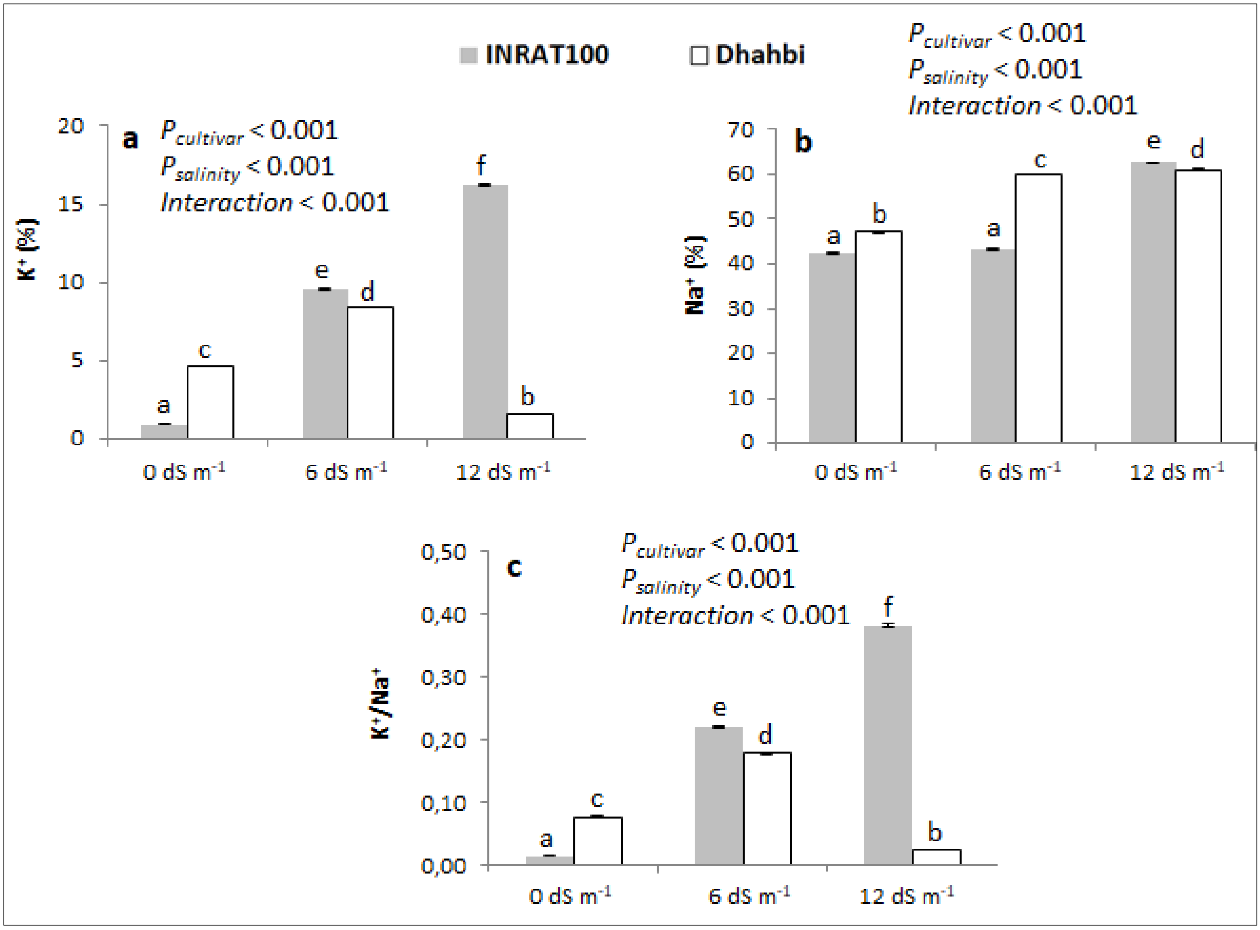

Figure 4 Salinity and cultivar effects on potassium (K^+, a) and sodium (Na^+, b) contents, and the ratio K^+/Na^+ (c)

3.2. Interactive Effect of Cultivar and Irrigation Water Salinity on Agro-Morphological, Physiological and Biochemical Traits

A genotypic variation ($p < 0.05$) was observed for plant height, awn and spike length, number of spikelets per spike, relative water content, membrane permeability, F_0, F_v/F_m, and ion accumulation (Figure 1, 2, 3 and 4). In particular, at 12 dS m^{-1}, *Dhahbi* showed a higher dry weight of aerial and root parts and the ratio ADW/RDW compared to *INRAT*100 that exhibited a better performamce under 0 and 6 dS m^{-1}. The number of tillers or spike per plant was the same for both cultivars. Under stressful conditions (6 and 12 dS m^{-1}), a higher awn length was recorded for *Dhahbi*. In addition, the spike length and the number of spikelets per spike have the tendency to be higher for *Dhahbi* cultivar. Regarding the grain yield, a slightly higher performance was obtained, however, for *INRAT100* compared to *Dhahbi*. At 6 and 12 dS m^{-1}, *INRAT100* showed also a higher relative water content, F_v/F_m and accumulated higher amount of K^+ than *Dhahbi*. Similar result was obtained for the ratio K^+/Na^+. Nonetheless, a controversial result was noted for chlorophyll content and F_0. Considering the relative membrane permeability, a clear prevalence was observed for *Dhahbi* at 12 dS m^{-1}.

3.3. Correlation between Agro-Morphological, Physiological and Biochemical Traits

Positive correlations ($p < 0.05$) between grain yield and plant height, chlorophyll content, F_v/F_m, relative water content, and spike length were recorded (Table 2). Otherwise, strong negative correlations ($p < 0.01$) among grain yield and F_0, Na^+ content, and membrane permeability were obtained. Na^+ content was negatively associated ($p < 0.01$) with plant height, chlorophyll content, F_v/F_m, and relative water content, and positively associated ($p < 0.01$) with membrane permeability.

Table 2 Relationship between agro-morphological, physiological and biochemical traits

	H	Chl	Fv/Fm	F0	RWC	SK S-1	AL	K+	Na+	K+/Na+	RMP	RDW	ADW	ADW/RDW	SL
Chl	0.43														
Fv/Fm	0.68**	0.49*													
F0	-0.50*	-0.65**	-0.78***												
RWC	0.64**	0.42	0.57**	-0.31											
SK S-1	-0.27	0.04	-0.37	0.41	-0.04										
AL	0.18	0.36	-0.13	0.06	-0.16	0.64**									
K+	-0.12	-0.51*	0.11	0.05	0.12	-0.51*	-0.73***								
Na+	-0.75***	-0.71***	-0.65**	0.52*	-0.73***	0.07	-0.18	0.40							
K+/Na+	-0.09	-0.52*	0.10	0.06	0.11	-0.55*	-0.74***	0.99***	0.39						
RMP	-0.65**	-0.56*	-0.86***	0.72***	-0.69**	0.19	-0.01	-0.17	0.61**	-0.15					
RDW	0.43	0.51*	0.44	-0.39	0.16	0.28	0.50*	-0.62**	-0.57**	-0.62**	-0.34				
ADW	0.13	0.19	-0.10	0.13	0.04	0.37	0.43	-0.66**	-0.23	-0.65**	0.22	0.19			
ADW/RDW	0.02	0.01	-0.09	0.01	0.05	-0.16	0.03	-0.11	-0.08	-0.12	0.02	-0.36	0.70**		
SL	0.21	0.52*	0.29	-0.28	0.23	0.52*	0.66**	-0.45	-0.43	-0.48*	-0.50*	0.54*	0.07	-0.21	
GY	0.58*	0.76***	0.66**	-0.67**	0.63**	0.15	0.35	-0.30	-0.80***	-0.33	-0.78***	0.44	0.13	0.14	0.53**

H, plant height; Chl, chlorophyll content; F_v/F_m, theoretical quantum; F_0, initial fluorescence; RWC, relative water content; SK S^{-1}, number of spikelets per spike; AL, awn length; K^+, potassium; Na^+, sodium; RWP, relative water permeability; RDW, root dry weight; ADW, dry weight of aerial part; SL, spike length; GY, grain yield.

4. Discussion

Irrigation with saline water (6 and 12 dS m^{-1}) has negatively affected the chlorophyll content and slightly reduced the theoretical quantum (F_v/F_m), while the initial fluorescence (F_0) was increased. In our context, the chlorophyll accumulation might be reduced in three ways by: (i) inhibiting chlorophyll biosynthesis, (ii) stimulation of chlorophyll degradation, (iii) or both processes [26]. The slight reduction of F_v/F_m suggests that the reaction centers incorporated in the membranes, in particular those of PSII, are not yet damaged or slightly damaged and that the chain of resonance energy transfers from antenna molecules to the reaction centers is still maintained [27]. However, the relative leaf water content was reduced under salt stress. It is already known that salt stress induces osmotic stress, which reduces the ability of the roots to draw water from the soil [28] and subsequently the turgidity of plant cells [29].

Furthermore, salinity induced an increase in membrane permeability, indicating a peroxidation of membrane lipids due to oxidative stress and loss of ion selectivity [30]. The membrane permeability was negatively correlated with chlorophyll content, F_v/F_m and water content. In fact, the degree of cell membrane injury could affect the distribution in photosynthetic rate and the stratiform structure of the chloroplasts [31]. Alteration in physiological and biochemical processes contributed subsequently to the decrease in final grain yield confirmed by a positive relationship between grain yield and chlorophyll content, F_v/F_m, relative water content, etc.

Salt-induced stress (6 and 12 dS m^{-1}) reduced the height of the vegetation, the length of spikes, and the grain yield and its components (number of spikes per plant and number of spikelets per spike). Several studies reported a depressive effect of salinity on growth (e.g., biomass and plant height), fertility and productivity of durum wheat [32–35]. Furthermore, our results revealed that the awn length was slightly affected by salinity. In previous study, awn length was positively associated with grain yield under water deficit conditions [36]. Towfiq and Noori [37] reported that awns increase the photosynthetic area of the ear by 36-59% and increase the grain yield by 10-16%. Morphologically, salt stress might induce a significant reduction in dry weight of the awns but not their length as claimed by Khamssi and Najaphy [38] for water stress.

Considering the two cultivars, *Dhahbi* was characterized by spikes and awns longer and a number of spikelets per spike higher than that of *INRAT100* under saline conditions. Nonetheless, *INRAT100* showed a higher grain yield indicating than this trait is not necessarily correlated with its components.

Contrary to *Dhahbi*, the K^+ content and K^+/Na^+ ion selectivity have increased more than Na^+ in *INRAT100* under the two applied saline constraints. The interaction between the relative concentrations of K^+ and Na^+ has been considered a major strategy and key factor in determining salt tolerance in plants [39]. In general, a high K^+ concentration increases the osmotic potential that leads to water entry from the external environment for the maintenance of cell turgor and the development of biochemical processes [40]. Indeed, *INRAT100*, which accumulated more K^+ ions, had higher relative water content and F_v/F_m, and lower membrane permeability than that of *Dhahbi*. Based on these findings, we can deduce that the *INRAT100* cultivar is a salt-excluder genotype (regulation of HKT gene expression) [41, 42] and more tolerant to salinity than *Dhahbi*.

5. Conclusion

In this study, comparison of two new durum wheat cultivars under salt regimes showed that *INRAT100* performed better in term of grain yield compared to *Dhahbi*, depicted by higher relative water content, F_v/F_m, preferential K^+ uptake and translocation to leaves and lower membrane permeability. Thus, these physiological and biochemical parameters might considered as crucial traits for salinity tolerance. In fact, correlation study under stress conditions revealed that tolerance is a phenotypic expression of a set of complex physiological, biochemical, and morphological properties that may interact with one another.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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