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Photosynthetic Behaviour and Mineral Nutrition of *Tamarix gallica* Cultivated Under Aluminum and NaCl Combined Stress

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Abstract: The lack of knowledge of plant tolerance and differential response to aluminum (Al) encouraged many researchers, in the last decade, to elucidate Al toxicity and tolerance mechanisms. The current study reported the impact of Al, a toxic element with negative effects on plant growth and development, in halophytic plant *Tamarix gallica.* Plants were subjected to different Al concentrations (0, 200, 500 and 800 µM) with or without NaCl (200 mM) supplementation. Growth, photosynthesis and mineral content were assessed. Al stress had a significant decrease on shoots' biomass production between 19 to 41%, and a little variation on chlorophyll content and photosynthetic efficiency (Fo, Fm, Fv fluorescence's and Fv/Fm). Furthermore, the Al-treatments did not affect significantly the content of potassium, calcium, and magnesium in different plant parts, whereas NaCl addition to the medium induced a decrease in these elements' concentrations. Our results have shown that *T. gallica* is able to accumulate the high levels of Al in shoots and roots, 6288 $\mu g.g^{-1}$ DW and 7834 $\mu g.g^{-1}$ DW respectively. It is considered as a hyperaccumulator plant of Al. In addition, Na^+ contents in shoots and roots exceed 23000 $\mu g.g^{-1}$ DW. Therefore, *T. gallica* presents a high tolerance at the same time to Al and NaCl phytotoxicity, so it is interesting to use in phytoremediation programs.

Keywords: Halophyte tolerance; combined stress; photosynthetic pigments; chlorophyll fluorescence; Al-accumulation; nutrients uptake

1 Introduction

Aluminum (Al) is considered as the most abundant metal and the third most abundant element in the earth's crust, but its availability depends on soil pH [1]. Al is also a beneficial element for plants at low concentrations but not required by all plants although promoting plant growth. Al toxicity is considered as the most widespread problem of ion toxicity stress in plants [2]. It is the most important factor constraining crop production on 67% of the total acid soil area in the world [3]. In soil, Al can be mobilized to aqueous form under highly acidic conditions [4]. For acidic pH of less than 4, the dominant speciation of aluminum corresponds to an only oxidation state (Al^{3+}), for a pH between 5 and 8, different form of aluminum hydroxide dominate. Al toxicity occurs only at soil pH values below 5.5 and is most severe in soils with low base saturation, poor in Ca and Mg [5]. Li and Johnson [6] indicated that Al solubility increases with soil depth when pH is less than 4.5.

Al phytotoxicity has been shown to trigger oxidative stress leading to cell membrane peroxidation, cellular structure damage, chromosome aberration and programmed cell death [7]. The inhibition of root growth and its development are considered as the primary effects of Al toxicity and used as a biomarker to estimate Al-sensitivity. Therefore, this leads to a decline in water and nutrient uptake by roots [8] by disturbing by disturbing the roots' absorption of some ions such as nitrate (NO_3^-), phosphate (PO_4^{3-}), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), thus impairing the transport of nutrients and the metabolic processes in shoots [9]. Furthermore, a reduction in dry mass could be induced as a result of a

decrease in the nutrient uptake and mineral deficiencies in shoots, resulting in root damage [10]. Additionally, Al exposure can affect the arrangement of the granum as well as the chloroplasts assembly and maintenance [9], as a consequence of cellular and ultrastructural alterations [5]. This variation can indirectly affect the photosynthetic activity by decreasing photosynthetic pigments contents [11]. Also, the trivalent cation Al^{3+} has been reported as damaging to the photosystem II (PSII) apparatus [11].

Additional to trace metal elements (TME), another stressor could also affect ecosystems like salinity, which is the major environmental factor that deteriorates the soil and decreases the crop productivity throughout the world [12]. However, halophyte species are naturally tolerant to salinity and are native to salty marginal areas. These species are able to develop different strategies to survive and complete their life cycles in such a constraint environments [13]. The tolerance plants to NaCl and/or TME may rely on some on physiological mechanisms such as (i) exclusion of excessive Na^+ or its compartmentation into vacuoles and upregulation of antioxidant defense genes and β-expansin proteins [14], (ii) synthesis of stress' phytohormones like jasmonic acid and salicylic acid [15], (iii) accumulation of proline for osmotic adjustment and increasing of the activity of several antioxidant enzymes [16] and (iv) apoplastic acidification, genes regulations, and synthesis of stress-responsive proteins [17].

Therefore, it would be interesting to identify among these halophytes species that tolerate high concentrations of TME in addition to their tolerance to salinity. Besides, the halophytes present several economic and ecological interests; it can be used for bioenergy, bioactive molecules, fodder, soil desalinization, landscaping.

T. gallica, also called salt cedars, is a halophytic shrub [18] and colonize coast, desert regions, and some saline depressions in Tunisia. These last areas are usually accumulation sites of industrial and urban effluents contaminated by TME [19]. In these sites, halophytes can tolerate a wide range of environmental stress conditions. Indeed, several recent works are interested in the screening of TME tolerant halophytes in saline conditions in order to valorize these species [20-23].

The objective of the present work is to study the physiological parameters and growth response to Al and NaCl of *T. gallica,* in order to better appreciate the tolerance of this species to the combined stress. Understanding the plant's response to Al stress in saline condition is crucial for valorizing the salty ecosystems and improving a high production in Al-contaminated soils, which could offer solutions for soil phytoremediation.

2 Material and Methods

2.1 Plant sampling and Experimental Setup

Young plants were obtained by cutting propagation: 5 cm long fragments of shoots were taken from mother plants taken from the natural habitat of this species (sabkha of Ariana in Tunisia), rinsed abundantly with distilled water, and placed for rooting in plastic pots (3 dm^3) containing a mixture of perlite/gravel (2:1, v/v) as a substrate. During a 6-week period of rooting, the cuttings were irrigated with tap water. Then, young rooted cuttings were regularly irrigated with a Hewitt nutritive solution [24] enriched with iron as complex EDTA-K-Fe and micronutrients as mixture of salts: $MnCl_2$; $CuSO_4$, 5 H_2O; $ZnSO_4$, 7H_2O; $(NH_4)_6$ Mo_7O_{24}, 4 H_2O; and H_3BO_3 and supplemented or not with NaCl (200 mM). After this pretreatment period, plants were divided into 8 groups of six plants. Control plants were regularly irrigated with the same nutritive solution and the seven others groups watered with Hewitt solution added with: a) Al 200 µM; b) Al 500 µM; c) Al 800 µM; d) NaCl 200 mM; e) Al 200 µM + NaCl 200 mM; f) Al 500 µM + NaCl 200 mM; g) Al 800 µM + NaCl 200 mM. The aluminum (in Al^{3+} form) is added from a pre-prepared concentrated aluminum chloride solution ($AlCl_3$), which is a powerful Lewis acid. The electrical conductivity (E.C.) and the pH of the nutritive solution averaged 1.7 $dS.m^{-1}$ and 7.22 respectively. The addition of Al^{3+} to the nutrient solution decreases the pH to values among 4.4 to 5.4 depending on the used doses. Experiments were performed in a greenhouse under semi-controlled conditions with a natural photoperiod, mean temperature (night-day) of 20-30°C, and relative humidity between 60 and 90%.

After three months of treatment, plants were harvest and divided into shoots and roots and rinsed three times in cold distilled water and blotted with filter paper. In order to eliminate trace elements adsorbed at the root surface, these organs were dipped, beforehand, in a cold solution of $CaCl_2$ during 5 min for removing ions adsorbed on the surface of roots. In order to estimate the water content (WC), the fresh weight (FW) was immediately determined, and the dry weight (DW) was measured after plant material desiccation in an oven at 60°C until constant weight.

Water content (WC) was calculated as:

WC= 100 * (FW - DW)/ FW

2.2 Pigment Profiling

Leaves used in pigment analysis were freeze-dried in the dark during 48 h, after which they were grinded in pure acetone with a glass rod. Further details on pigment analysis are described previously in [25].

In order to better evaluate the light harvesting and photo-protection mechanisms, the de-epoxidation state (DES) was calculated as described by [26]:

$$DES = \frac{[Anthera] + [Zea]}{[Viola] + [Anthera] + [\ Zea]}$$

2.3 Chlorophyll Fluorescence

The modulated chlorophyll fluorescence measurements were taken by FluorPen FP100 PAM (Photo System Instruments, Czech Republic) with detachable leaf-clip, which used for gentle fixing of a leaf sample. The method was described in further details in [25]. From this analysis several photochemical parameters were attained such as the performance index (PI) and all the energetic fluxes occurring in the PSII apparatus. The energetic fluxes inside the chloroplast could also be measured. In fact, the leaf was subjected to a determined amount of photosynthetic active radiation. These radiations were absorbed by chloroplasts PSII (ABS/RC or absorbed energy flux) that will trap a percentage of this flux (TR/RC or trapped energy flux). A percentage of this last flux will then be conducted to the electron transport chain (ETC) and used for energy conversion (ET/RC or transported energy flux). The remaining energy was dissipated in the form of heat and/or fluorescence (DI/RC or dissipated energy flux).

2.4 Elemental Analysis

Dry plant material was reduced to a fine powder in an agate mortar. Then, 50 mg of sample were digested in Teflon bombs using 3 ml of acid mixture composed with HNO_3:H_2SO_4:$HClO_4$ (10:1:0.5; v/v/v) during 2 h 30 min at 110°C. After that, the samples were taken into 50 ml of nitric acid 0.5%. Total concentrations of Na, K, Ca, Mg and Al were determined by atomic absorption spectrometry (Perkin Elmer PinAAcle 900T, USA). The blanks, used to set the zero atomic absorption spectrometer, were similarly processed as described above.

2.5 Statistical Analysis

All the samples were analyzed in six replicates and the mean values along with the standard deviation (±) are shown in bars in figures or in superscript in tables. The effects of treatments on the variability of the response parameters were assessed using regression analyses, two-way ANOVA. Statistical analyses were done with the Statistica 8 for Windows software. Tukey's HSD test ($p < 0.05$) was performed to define which specific mean pairs are significantly different.

3 Results

3.1 Growth and Water Status

Our results have shown that *T. gallica* was able to maintain a well growth on nutritive medium added with different Al concentrations. At the end of the experiment and under higher Al concentration treatments (500 and 800 µM), *T. gallica* presented a significantly decrease ($p < 0.05$) in shoots biomass (Tab. 1). Nevertheless, this drop did not exceed 41%. The production of dry biomass on medium supplemented with Al and NaCl decrease significantly ($p < 0.05$) with treatment compared to control (Tab. 1). The water content (WC) of the plants exposed to Al alone or combined with NaCl showed a uniform response (Tab. 1). The different treatments did not affect significantly ($p > 0.05$) the water content in roots and shoots of *T. gallica* plants.

Table 1: Dry weight (DW) and water content (WC) in *T. gallica* cultivated under combined stresses of Al and NaCl. Mean values of 6 repetitions ± SE. Different superscript letters represent statistically significant differences ($p < 0.05$)

	Shoots	
Treatments	**DW (mg)**	**WC %**
Control	$1295.35^{a} \pm 77.5$	$77.11^{a} \pm 0.7$
Al, 200 µM	$1052.80^{bc} \pm 39.9$	$74.57^{a} \pm 0.7$
Al, 500 µM	$836.40^{bc} \pm 68.4$	$77.12^{a} \pm 0.9$
Al, 800 µM	$772.70^{bc} \pm 70.4$	$76.98^{a} \pm 1.2$
NaCl, 200Mm	$994.90^{b} \pm 34.7$	$76.03^{a} \pm 2.0$
Al, 200 µM + NaCl, 200 mM	$905.83^{bc} \pm 40.5$	$76.10^{a} \pm 0.8$
Al, 500 µM + NaCl, 200 mM	$770.83^{b} \pm 28.1$	$79.23^{a} \pm 1.5$
Al, 800 µM + NaCl, 200 mM	$783.53^{b} \pm 45.8$	$80.90^{a} \pm 1.0$
	Roots	
	DW (mg)	**WC %**
Control	$489.99^{a} \pm 13.7$	$79.65^{a} \pm 1.2$
Al, 200 µM	$347.18^{bcd} \pm 19.1$	$80.14^{a} \pm 1.1$
Al, 500 µM	$348.93^{bd} \pm 14.5$	$82.89^{a} \pm 1.4$
Al, 800 µM	$395.37^{bd} \pm 18.1$	$82.74^{a} \pm 1.8$
NaCl, 200 mM	$368.14^{bc} \pm 17.6$	$79.43^{a} \pm 1.5$
Al, 200 µM + NaCl, 200 mM	$385.13^{bcd} \pm 22.0$	$81.70^{a} \pm 1.5$
Al, 500 µM + NaCl, 200 mM	$272.47^{bd} \pm 19.5$	$82.03^{a} \pm 0.9$
Al, 800 µM + NaCl, 200 mM	$297.05^{bd} \pm 33.2$	$82.24^{a} \pm 0.6$

3.2 Pigment Content

No significant alterations were observed in Chla and Chlb contents ($p > 0.05$). The test plants did not show any morphological or visible symptoms of toxicity. Levels of chlorophylls were not adversely affected in plants exposed neither to Al or/and to NaCl. In the same way, the levels of total chlorophylls and carotenoids did not demonstrate any variation in their concentrations, independently of the Al

concentration exposure (Tab. 2). In addition, the ratio of chlorophyll a/b (Tab. 2) and the level of chlorophyll a and b did not change significantly among treatments (Tab. 2). The same trend could be observed in NaCl exposed and control plants.

The study of chlorophyll degradation products shows that Zeaxanthin, Lutein and *β*-Carotene did not reveal any variation of their concentration in plants cultivated under different Al treatments. Contrarily, Pheophytin, Violaxanthin and Antheraxanthin showed a significant variation of their contents in leaves of plants cultivated at 800 µM Al combined with salt (Tab. 3).

Table 2: Chlorophylls (Chl) and carotenoids (Carot) contents (mg.g^{-1} FW), and pigments ratios in *T. gallica* cultivated under combined stresses of Al and NaCl. Mean values of 6 repetitions ± SE. Different superscript letters represent statistically significant differences ($p < 0.05$)

Treatments	Chl a (mg.g^{-1} FW)	Chl b (mg.g^{-1} FW)	Total Chl (mg.g^{-1} FW)	Total Carot. (mg.g^{-1} FW)	Chl a/b	Tot. Carot/ Tot. Chl
Control	81.49^{a} ± 2.8	67.54^{a} ±1.4	151.95^{a} ± 4.1	60.55^{a} ± 3.1	1.21^{a} ± 0.02	0.40^{a} ± 0.02
Al, 200 µM	87.88^{a} ± 3.6	77.94^{a} ± 6.7	165.83^{a} ± 13.1	58.93^{a} ± 9.3	1.18^{a} ± 0.09	0.39^{a} ± 0.08
Al, 500 µM	85.08^{a} ± 2.7	70.09^{a} ± 5.1	155.18^{a} ± 7.7	64.87^{a} ± 4.3	1.23^{a} ± 0.05	0.43^{a} ± 0.05
Al, 800 µM	82.71^{a} ± 1.7	65.56^{a} ± 2.6	148.20^{a} ± 2.4	67.63^{a} ± 2.6	1.27^{a} ± 0.04	0.46^{a} ± 0.03
NaCl, 200 mM	88.94^{a} ± 5.8	70.54^{a} ± 8.3	162.42^{a} ± 14.0	69.33^{a} ± 5.5	1.30^{a} ± 0.06	0.45^{a} ± 0.06
Al, 200 µM + NaCl, 200 mM	82.95^{a} ± 1.3	66.31^{a} ± 1.8	149.26^{a} ± 2.0	67.37^{a} ± 2.6	1.26^{a} ± 0.03	0.45^{a} ± 0.02
Al, 500 µM + NaCl, 200 mM	91.31^{a} ± 5.3	72.93^{a} ± 6.8	164.14^{a} ± 11.9	71.46^{a} ± 1.7	1.27^{a} ± 0.04	0.45^{a} ± 0.04
Al, 800 µM + NaCl, 200 mM	93.68^{a} ± 3.1	72.50^{a} ± 3.3	166.18^{a} ± 6.3	69.94^{a} ± 1.6	1.30^{a} ± 0.02	0.43^{a} ± 0.03

Table 3: Chlorophyll degradation products and carotenoids contents (µg.g^{-1} FW) in *T. gallica* exposed to increasing Al levels. Pheo: pheophytin, Viola: violaxanthin, Anthera: antheraxanthin, Zea: zeaxanthin, *β*Car: *β*-carotene, Lut: lutein. Mean values of 6 repetitions ± SE. Different superscript letters represent statistically significant differences ($p < 0.05$)

Treatments	Pheo	Viola	Anthera	Zea	*β*Car	Lut
Control	51.0^{a} ± 3.7	1.4^{a} ± 0.5	3.1^{a} ± 0.7	28.5^{a} ± 3.1	12.0^{a} ± 0.3	6.7^{a} ± 1.4
Al, 200 µM	53.5^{a} ± 2.8	3.0^{b} ± 1.3	3.9^{a} ± 1.9	26.4^{a} ± 9.5	12.8^{a} ± 1.6	5.5^{a} ± 1.8
Al, 500 µM	52.6^{a} ± 1.5	2.5^{a} ± 0.3	5.3^{a} ± 1.1	30.2^{a} ± 3.1	12.4^{a} ± 0.6	7.4^{a} ± 1.0
Al, 800 µM	52.1^{a} ± 3.4	2.0^{a} ± 0.7	4.0^{a} ± 1.1	30.9^{a} ± 2.4	12.1^{a} ± 0.6	7.7^{a} ± 1.1
NaCl, 200 mM	55.9^{a} ± 6.9	4.5^{b} ± 0.9	6.1^{b} ± 1.3	30.9^{a} ± 3.2	13.6^{a} ± 3.3	8.1^{a} ± 0.9
Al, 200 µM + NaCl, 200 mM	52.1^{b} ± 1.1	3.5^{a} ± 0.6	3.9^{a} ± 1.4	30.9^{a} ± 1.6	12.1^{a} ± 0.2	7.6^{a} ± 0.8
Al, 500 µM + NaCl, 200 mM	55.7^{a} ± 4.0	3.4^{a} ± 1.1	3.5^{a} ± 1.7	33.1^{a} ± 1.1	13.5^{a} ± 1.3	9.2^{a} ± 1.3
Al, 800 µM + NaCl, 200 mM	58.8^{b} ± 4.1	2.9^{b} ± 0.3	3.1^{a} ± 1.0	33.7^{a} ± 0.6	14.1^{a} ± 1.3	9.1^{a} ± 0.7

Further, depending to the xanthophyll concentrations, another evident signal of environmental stress is the xanthophyll cycle functioning, as revealed by the DES index. This index did not show significant variations among treatments ($p > 0.05$) (Fig. 1).

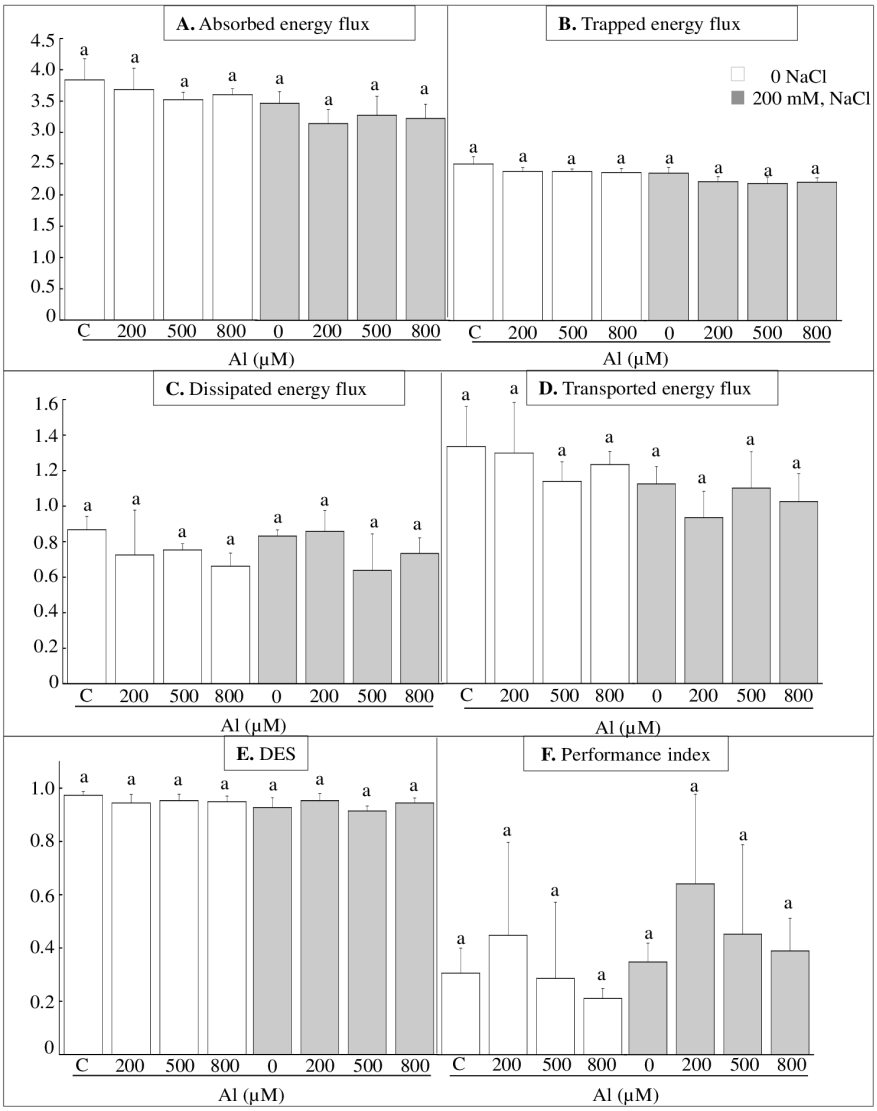

Figure 1: Energy fluxes (A, B, C, D, E) and performance index (F) in *T. gallica* cultivated under combined stresses with Al and NaCl. Mean values of 6 repetitions ± SE. Statistical significance at $p < 0.05$ between control and treated plants. Bars marked with the same letter are not significantly different at $p = 0.05$

3.3 Chlorophyll Fluorescence

Concerning the chlorophyll fluorescence parameters, Al exposure did not lead to significant alterations in PSII efficiencies, as reported above for the photosynthetic pigments. As shown in Tab. 2, all chlorophyll fluorescence parameters showed a slight difference between plants grown under different Al concentrations and with NaCl but not significantly. There were no significant variations detected on Fv/Fm values (Fig. 2).

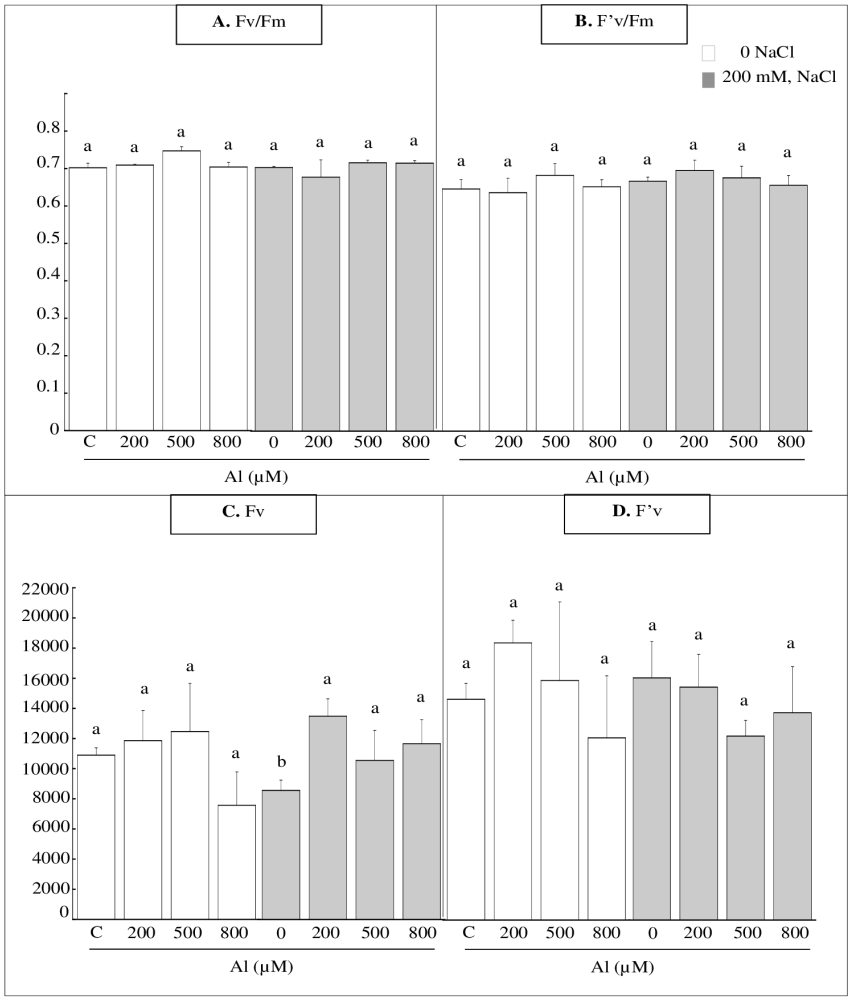

Figure 2: Variable fluorescence (A, B), PSII quantum yield (C, D) in light and dark-adapted leaves and non-photochemical quenching. Mean values of 6 repetitions ± SE. Statistical significance at $p < 0.05$ between control and treated plants. Bars marked with the same letter are not significantly different at $p = 0.05$

The values of light-adapted leaves variable fluorescence (F'v) and variable fluorescence on dark-adapted state (Fv) (Fig. 2) did not show any effect on the efficiency of operational PSII or maximum PSII quantum despite the lower fluctuation on F'v and Fv values. On the other hand, a different behaviour was detected on the Kautsky curves analysis, as observed on the photochemical phase (O-J) of the samples treated with Al (Fig. 3), showing lower fluorescence values in this phase while compared to the control. Considering the thermal phase (J-I-P), the same trend was observed, except in the plants cultivated in conditions of combined stresses (Fig. 3). Regarding the data relative to the energy transduction fluxes, the plants showed similar behaviour on absorbing, transporting, trapping and dissipating energy fluxes. However, no difference was detected among treatments (Fig. 1). This leaded to a uniform trend, in which concerns the performance index, an integrative variable. In order to estimate the plant vitality, the Performance Index (PI), could be used to sum all the processes within the JIP test. PI reflects the PSII energy transduction efficiency and gives more details about plant performance, especially under stress conditions. Consequently, if a stress affects any of these components, the effect will show up in the performance index. Our results show some fluctuation on PI but not significantly (Fig. 1(F)).

3.4 Nutrients Content

The shoots and the roots of plants showed variation with the increase of Al concentration in culture medium. The exception of this behaviour was observed in Ca contents, which decreased significantly ($p < 0.05$) at 800 µM Al. On the other hand, the salt induced a decrease in K and Mg shoots' content. In general, the addition of Al to the salty medium did not change the behaviour of the plants irrigated with saline solutions (Tab. 4). The effect of the combined treatment of Al and NaCl on nutrient contents was more pronounced than the Al-treatment alone, especially in shoots.

Compared with the control, our data showed that there were significant differences in Al content and accumulation in all plant parts among treatments (Tab. 4). The concentration of Al in both the roots and shoots of *T. gallica* increased with increasing Al medium concentration, with higher accumulations in the roots than in the shoots (Tab. 4). In fact, *T. gallica* accumulated 3280 $\mu g.g^{-1}$ DW in shoots and 5442 $\mu g.g^{-1}$ DW in roots. Moreover, when Al is combined with NaCl, there was a huge rise in the Al accumulation in the shoots and the roots compared to those supplied only by Al ($p < 0.05$). Indeed, *T. gallica* is able to accumulate 6288 $\mu g.g^{-1}$ DW in shoots and 7834 $\mu g.g^{-1}$ DW in roots. The Na contents in the shoots increased with increasing Na concentration in the nutrient solution. The Na accumulation was always higher in the shoots than in the roots (Tab. 4).

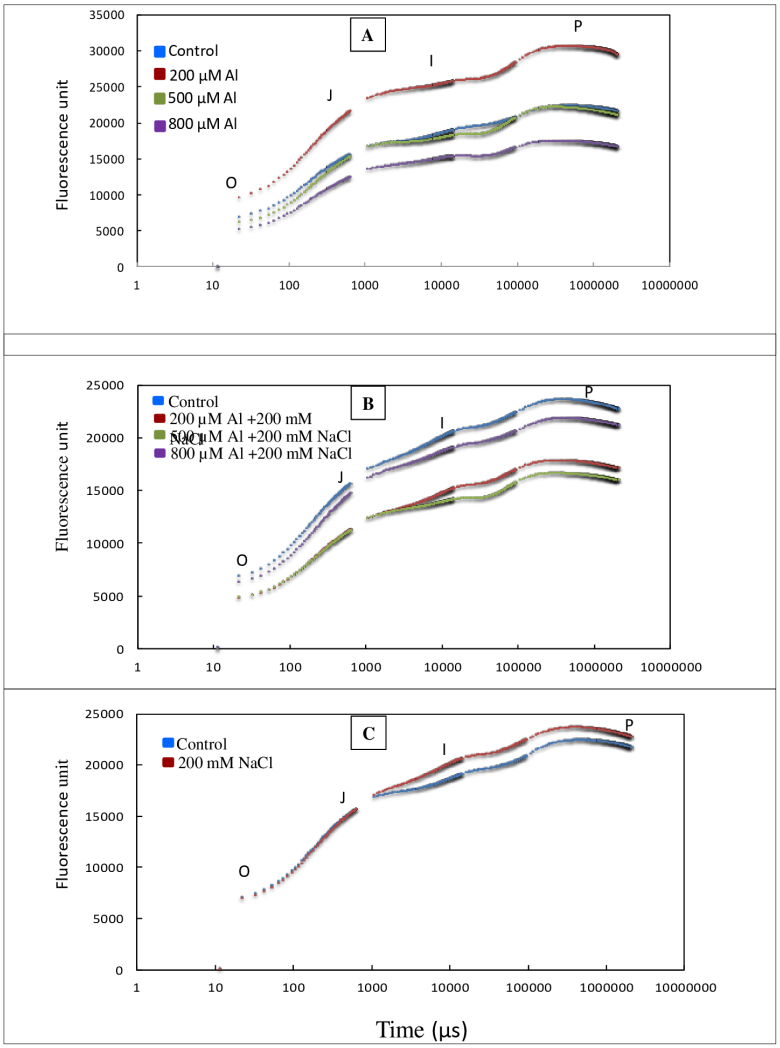

Figure 3: Average values of the Kautsky curves (A, B, C) in dark-adapted leaves of *T. gallica* cultivated under combined stresses with Al and/or NaCl. Mean values of 6 repetitions ± SE

Table 4: Potassium, Magnesium, Calcium, Sodium and Aluminum contents in *T. gallica* under combined stresses with Al and NaCl. Mean values of 6 repetitions ± SE. Statistical significance at $p < 0.05$ between control and treated plants. Numbers marked with the same letter are not significantly different at $p = 0.05$

	Shoots				
Treatments	K^+ (mg.g^{-1} DW)	Mg^{2+} (mg.g^{-1}DW)	Ca^{2+} (mg.g^{-1}DW)	Na^+ (mg.g^{-1} DW)	Al (mg.g^{-1} DW)
Control	$20.24^{ac} \pm 1.3$	$4.40^{abc} \pm 0.5$	$2.75^{ac} \pm 0.2$	$4.88^{a} \pm 0.4$	$0.80^{a} \pm 0.04$
Al, 200 µM	$20.17^{bc} \pm 1.3$	$5.13^{ab} \pm 0.5$	$3.17^{abc} \pm 0.2$	$4.84^{bc} \pm 0.2$	$1.49^{b} \pm 0.09$
Al, 500 µM	$19.81^{ac} \pm 1.3$	$5.08^{ac} \pm 0.2$	$3.10^{a} \pm 0.2$	$3.97^{a} \pm 0.2$	$2.54^{c} \pm 0.06$
Al, 800 µM	$20.77^{ac} \pm 1.2$	$5.81^{ac} \pm 0.3$	$2.62^{ac} \pm 0.1$	$5.81^{a} \pm 0.6$	$3.28^{d} \pm 0.03$
NaCl, 200 mM	$11.37^{ac} \pm 0.6$	$3.21^{ac} \pm 0.2$	$1.88^{ac} \pm 0.2$	$23.42^{a} \pm 1.3$	$0.32^{e} \pm 0.02$
Al, 200 µM + NaCl, 200 mM	$13.86^{b} \pm 0.8$	$4.13^{abc} \pm 0.4$	$2.35^{abc} \pm 0.3$	$28.20^{bc} \pm 1.9$	$0.61^{a} \pm 0.07$
Al, 500 µM + NaCl, 200 mM	$10.62^{bc} \pm 0.2$	$3.68^{abc} \pm 0.3$	$2.14^{abc} \pm 0.3$	$25.11^{bc} \pm 2.1$	$5.65^{f} \pm 0.07$
Al, 800 µM + NaCl, 200 mM	$8.07^{bc} \pm 0.6$	$2.88^{ab} \pm 0.1$	$1.48^{bc} \pm 0.1$	$21.69^{b} \pm 1.2$	$6.29^{g} \pm 0.19$
	Roots				
	K^+ (mg.g^{-1} DW)	Mg^{2+} (mg.g^{-1}DW)	Ca^{2+} (mg.g^{-1}DW)	Na^+ (mg.g^{-1} DW)	Al (mg.g^{-1} DW)
Control	$13.21^{ab} \pm 0.8$	$2.62^{a} \pm 0.2$	$2.74^{ac} \pm 0.1$	$2.86^{ac} \pm 0.1$	$1.02^{a} \pm 0.03$
Al, 200 µM	$12.72^{ab} \pm 0.2$	$2.68^{a} \pm 0.2$	$3.94^{ac} \pm 0.2$	$3.05^{b} \pm 0.1$	$2.16^{a} \pm 0.02$
Al, 500 µM	$14.08^{a} \pm 0.8$	$2.59^{a} \pm 0.2$	$3.33^{bd} \pm 0.6$	$2.84^{ac} \pm 0.3$	$3.20^{b} \pm 0.04$
Al, 800 µM	$14.37^{ab} \pm 1.0$	$2.86^{a} \pm 0.2$	$1.47^{ad} \pm 0.1$	$4.75^{ac} \pm 0.3$	$5.44^{c} \pm 0.06$
NaCl, 200 mM	$16.44^{ab} \pm 0.7$	$3.07^{a} \pm 0.2$	$1.90^{bc} \pm 0.1$	$18.00^{ac} \pm 0.8$	$0.44^{d} \pm 0.01$
Al, 200 µM + NaCl, 200 mM	$12.94^{ab} \pm 1.4$	$2.93^{a} \pm 0.1$	$1.53^{bc} \pm 0.1$	$20.42^{b} \pm 1.2$	$0.79^{a} \pm 0.12$
Al, 500 µM + NaCl, 200 mM	$12.01^{a} \pm 0.6$	$2.66^{a} \pm 0.1$	$1.46^{bc} \pm 0.1$	$21.82^{bc} \pm 1.1$	$6.47^{e} \pm 0.37$
Al, 800 µM + NaCl, 200 mM	$11.06^{a} \pm 0.8$	$2.29^{a} \pm 0.3$	$1.48^{bc} \pm 0.1$	$19.09^{b} \pm 0.7$	$7.83^{f} \pm 0.13$

4 Discussion

4.1 Biomass Production

In the present study, the growth of shoots and roots of *T. gallica* were affected by different concentrations of Al supplemented alone or in combination with NaCl, but this species is able to cope and to survive under combined stress. It was recognized that Al-toxicity threshold could be located between 320 and 530 µM [27], whereas in our data plants exposed to 800 µM Al did not show any visual toxicity in both above and belowground organs. Manousaki et al. [28] signaled that *Tamarix sp.* grew on polluted soils without showing visible signs of poisoning. Similarly to our results, Akaya and Takenaka [29] observed that no significant difference could be observed on water content among Al-treated groups. However, the increasing level of Al^{3+} activity in solution progressively decreased the growth of the shoot

and root of physic nut plants (*Jatropha curcas* L.), and at the two highest active Al^{3+} levels, plants showed morphological abnormalities typical of the toxicity caused by this metal [30]. To cope to alumic stress, some plants detoxified it by exudation of low molecular weight organic acids, which chelated Al in the rhizosphere, forming solid complexes with Al to prevent its uptake [31]. Also, Al complexation with specific metalloproteins such as calmodulin could form stable complex [32]. Others mechanisms were involved in tolerance to Al such as the TME excretion through salt glands. Metals could be excreted with salts on the leaf surface and it had been shown that the salt glands of *Tamarix sp.* were not selective [28].

In agreement with our study, Akaya and Takenaka [29] found that the chlorophyll contents of leaves were almost showing no significant variation in chlorophyll synthesis, in presence of Al and/or NaCl stress (Tab. 2), which might indicate an enhancement on the light harvesting efficiency as a stress counteractive measure. There are no evidence of membrane damage in the light-harvesting pigments (chlorophylls) and the light-protecting pigments (carotenoids). All these pigment characteristics were evidenced overlooking the photochemical process itself [33]. This can mean that there was no need for a rearrangement of the photosystem composition in order to avoid photoinhibition with an increase of total chlorophyll. Similarly, Chettri et al. [34] observed no effect on total chlorophyll, despite the high tissue metal contents suggesting that most of the metal cations were bound and rendered inert externally on the cell wall.

4.2 Chlorophyll Fluorescence

The photochemical efficiency of PSII value can be used as an indicator to measure the degree of tolerance of plants to environmental factors. In fact, a decrease in this ratio associated with a decrease in F_0, can indicate the presence of regulatory mechanisms acting in the antennae, while a decline in Fv/Fm accompanied with an increase in F_0, could present impairments accompanied with the inactivation of PSII [35]. In the present study, there were no changes in F_0, Fm, Fv/Fm, PI and all others chlorophyll fluorescence parameters despite the increasing in Al concentrations in culture medium, this can be considered as an important strategy of *T. gallica* to tolerate Al stress. This can indicate that there were no changes occurring in the energy transfer from the LHCII and preservation of PSII function and photosynthetic composition under Al and salinity exposure. It had been reported that rising on Al content of shoots significantly affected the photosynthetic activity [36] and might induce damage on chloroplast functioning [5].

Analysis of OJIP fluorescence can be applied to detect stress symptoms early. Comparing both the donor (J-I) with the acceptor (O-J) PSII sides, the former was more affected during Al treatment combined with NaCl exposure. This disorder of the structure and function alters the rate of oxygen evolution (OECs) and thus, increases the release of fluorescence quenching in the J-I phase [37]. Stressed plants dissipate more energy in order to overcome the accumulation of excessive ions reducing power, and prevent the photo-destruction of the photosynthetic apparatus [26]. One of the mechanisms of energy dissipation was the conversion of violaxanthin to zeaxanthin, through the xanthophyll cycle [26]. In this study, plants did not lose the excessive energy and showed a stable DES value and zeaxanthin concentrations. Other possible pathway to counteract the excessive energy accumulation was through heat dissipation [26]. It is considered as an internal protection mechanism of photosynthetic apparatus, as suggested by Konrad et al. [38] who reported that Al increased non-photochemical quenching (NPQ) and coefficient of non-photochemical quenching (qN).

Indeed, in previous studies, it has been reported that a high concentration of arsenic does not affect the different fluorescence parameters of chlorophyll in *T. gallica*. There is only an increase in energy dissipation fluxes, suggesting a mechanism of adaptation by this species to tolerate the excess of TME [25].

4.3 Mineral Uptake

The current study showed that the addition of Al alone affects slightly the K, Ca and Mg contents in shoots and roots. In this line, Akaya and Takenaka [29] found that the leaf mineral content of the seedlings was not influenced by the Al concentration in the medium. In fact, the effect of Al on nutrient

uptake depended on the concentration of Al, the time of exposure, plant species and also by the capacity of the plant to preserve its cationic balance.

On the other hand, the combined stress (Al + NaCl) decreased significantly the K, Ca and Mg contents in plant tissues, compared to the control and to plants stressed with Al only. The presence of salt in the rooting environment had been shown to affect plant metabolism by affecting ion uptake [39]. Sleimi, Guerfali, and Bankaji [16] signaled that higher doses of salt (≥ 200 mM) induce a decrease in shoots potassium content in *Plantago maritima.* The mineral status of the plants could be affected by saline condition under the effects of a complex network of interactions, showing a reduction of nutrient uptake and/or transport from roots to shoots. This decrease could be associated with a Na/K ratio inducing a competitive inhibition of the absorption process [40]. Similarly, De Vos et al. [41] observed that at 200 mM NaCl, the concentrations of K, Ca and Mg were reduced as compared to the control in *Cochlearia officinalis*. Furthermore, one of the proposed mechanisms explaining the decrease in the uptake of macronutrients (Ca, Mg) was the competition for the common binding sites. Taking into consideration, some of the structural functions Ca plays could be compromised due to the presence of large amounts of Na which may replace electrostatically bound Ca in cell walls and cell membranes [42].

T. gallica showed Na levels of 23419 $\mu g.g^{-1}$ DW in saline conditions and can reach 28199 in case of combined stress while keeping a good growth, which confirms its halophytic character.

4.4 Aluminum tolerance

Aluminum was accumulated in considerable amounts in plant tissues and the presence of NaCl into the medium induced an increase on Al uptake and accumulation (Tab. 4). Looking deeper, *T. gallica* presented a high Al accumulation in shoots; which could be due to the large vacuoles, which facilitated the storage of metals. The higher leaf biomass proportion compared to total plant biomass could be another reason to facilitate metal uptake by diffusion [43]. Al is suggested to be transported via the xylem transport system into the leaves, which show the highest Al levels. Radial transport via ray parenchyma to bark tissue is also likely given the high Al concentrations in the bark tissue [44].

Furthermore, salinity was known to increase the bioavailability of trace metal elements especially for mobile ones [28]. Additionally, our solution irrigation's have a pH between 4.4 to 5.4 which would increase the solubility of Al. In fact, Al toxicity occurs at soil pH values below 5.5 [5].

The performance of *T. gallica* and its capacity to accumulate amounts greater than 7834 $\mu g.g^{-1}$ DW, allow us to classify it among the hyperaccumulator species of Al. Indeed, Jansen et al. [45] signaled that hyperaccumulators store the aluminum in their aboveground tissues in quantities above 1000 ppm

5 Conclusion

In summary, our study shown that *T. gallica* is able to cope and to survive in presence of high external concentrations of Al and/or NaCl, confirming its halophytic character. This TME does not disrupt photosynthetic parameters and plants are able to maintain a proper nutrient uptake. In addition, the presence of NaCl, in culture medium, induced an increase of large amount of Al-accumulation in shoots and roots. Therefore, *T. gallica* can be classified as hyperaccumulator species, and it's interesting to use in phytoremediation programs.

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References

1. Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., Martínez-Estévez, M. (2017). Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science, 8,* 1767.
2. Barcelo, J., Poschenrieder, C. H. (2002). Fast root growth responses, root exudates and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: a review. *Environmental and Experimental Botany, 48,* 75-92.
3. Eswaran, H., Reich, P., Beinroth, F. (1997). Global distribution of soils with acidity. In: A. Z. Moniz AMCF, R. E. Schaffert, N. K. Fageria, C. A. Rosolem and H. Cantarella (eds.), *Plant-Soil Interactions at Low pH. Brazilian Soil Science Society*, pp. 159-164. Beckley West Virginia, USA.
4. De Wit, H. A., Mulder, J., Nygaard, P. H., Aamlid, D. (2001). Testing the aluminum toxicity hypothesis: a field manipulation experiment in mature spruce forest in Norway. *Water Air and Soil Pollution, 130,* 995-1000.
5. Vitorello, V. A., Capaldi, F. R. C., Stefanuto, V. A. (2005). Recent Aluminum toxicity in plants advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology, 17,* 129-143.
6. Li, W., Johnson, C. E. (2016). Relationships among pH, aluminum solubility and aluminum complexation with organic matter in acid forest soils of the Northeastern United States. *Geoderma, 271,* 234-242.
7. Achary, V. M., Jena, S., Panda, K. K., Panda, B. B. (2008). Aluminum induced oxidative stress and DNA damage in root cells of *Allium cepa* L. *Ecotoxicology and Environmental Safety, 70,* 300-310.
8. Nicoloso, F. T., Tabaldi, L. A., Cargnelutti, D., Goncalves, J. F., Pereira, L. B. (2009). Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Chemosphere, 76,* 1402-1409.
9. Mihailovic, N., Drazic, G., Vucinic, Z. (2008). Effect of aluminum on photosynthetic performance in Al-sensitive and Al-tolerant maize inbred lines. *Photosynthetica, 46,* 476-480.
10. Taylor, G. J. (1988). The physiology of aluminum phytotoxicity. In: H. Sigel, M. Dekker eds., *Metal ions in biological systems: aluminum and its role in biology*, pp. 123-163. New York.
11. Jiang, H. X., Chen, L. S., Zheng, J. G., Han, S., Tang, N. et al. (2008). Aluminum induced effects on photosystem II photochemistry in citrus leaves assessed by the chlorophyll a fluorescence transient. *Tree Physiology, 28,* 1863-1871.
12. Hasegawa, P. M. (2013). Sodium (Na^+) Homeostasis and salt tolerance of plants. *Environmental and Experimental Botany, 92,* 19-31.
13. Eid, M. A. (2011). Halophytic plants for phytoremediation of heavy metals contaminated soil. *Journal of American Science, 7(8),* 377-382.
14. Farooq, M., Hussain, M., Wakeel, A., Kadambot, H., Siddique, M. (2015). Salt stress in maize: effects, resistance mechanisms, and management. *Agronomy for Sustainable Development, 35,* 461-481.
15. Bankaji, I., Sleimi, N., López-Climent, M. F., Perez-Clemente, R. M., Gomez-Cadenas, A. (2014). Effect of combined abiotic stresses on growth, trace element accumulation and phytohormone regulation in two halophytic species. *Journal of Plant Growth Regulation, 33,* 632-643.
16. Sleimi, N., Guerfali, S., Bankaji, I. (2015). Biochemical indicators of salt stress in *Plantago maritima*: implications for environmental stress assessment. *Ecological Indicators, 48,* 570-577.
17. Hussain, M., Ahmad, S., Hussain, S., Lal, R., Ul-Allah, S. et al. (2018). Rice in saline soils: physiology, biochemistry, genetics, and management. *Advances in Agronomy, 148,* 231-287.
18. Harrouni, M. C., Daoud, S., Koyro, H. W. (2003). Effect of seawater irrigation on biomass production and ion composition of seven halophytic species in Marocco. In: H. Lieth, M. Mochtchenko eds., *Tasks for vegetation science (Cash Crop Halophytes)*, vol. 38, pp. 59-70. Kluwer Academic Pub., Netherland.
19. Yoshida, M. (2013). Mobility of contaminated heavy metals and metalloids in sediment caused by recent industrial activities: cases in Algeria and Tunisia. *Proceedings of the 16th International Conference on Heavy Metals in the Environment, 1, 33002.*
20. Milić, D., Luković, J., Ninkov, J., Zeremski-Škorić, T., Zorić, L. et al. (2012). Heavy metal content in halophytic plants from inland and maritime saline areas. *Central European Journal of Biology, 7(2),* 307-317.

21. Han, R., Quinet, M., André, E., van Elteren, J. T., Destrebecq, F. et al. (2013). Accumulation and distribution of Zn in the shoots and reproductive structures of the halophyte plant species *Kosteletzkya virginica* as a function of salinity. *Planta, 238,* 441-457.
22. Liang, L., Liu, W., Sun, Y., Huo, X., Li, S. et al. (2017). Phytoremediation of heavy metal contaminated saline soils using halophytes: current progress and future perspectives. *Environmental Reviews, 25,* 269-281.
23. Bankaji, I., Pérez-Clemente, R. M., Caçador, I., Sleimi, N. (2019). Accumulation potential of *Atriplex halimus* to zinc and lead combined with NaCl: effects on physiological parameters and antioxidant enzymes activities. *South African Journal of Botany, 123,* 51-61.
24. Hewitt, E. J. (1966). *Sand and water culture methods used in the study of plant nutrition*. Technical Communication N°22 of the Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstore, Kent.
25. Sghaier, D. B., Duarte, B., Bankaji, I., Caçador, I., Sleimi, N. (2015). Growth, chlorophyll fluorescence and mineral nutrition in the halophyte *Tamarix gallica* cultivated in combined stress conditions: Arsenic and NaCl. *Journal of Photochemistry and Photobiology (B) Biology, 149,* 204-221.
26. Duarte, B., Santos, D., Marquez, J. C., Caçador, I. (2013). Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback: implications for resilience in climate change. *Plant Physiology and Biochemistry, 67,* 178-188.
27. Li, C., Xu, H., Xu, J., Chun, X., Ni, D. (2011). Effects of aluminum on ultrastructure and antioxidant activity in leaves of tea plant. *Acta Physiologiae Plantarum, 33,* 973-978.
28. Manousaki, E., Kadukova, J., Papadantonakis, N., Kalogerakis, N. (2008). Phytoextraction and phytoexcretion of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non-saline and saline soils. *Environmental Research, 106,* 326-332.
29. Akaya, M., Takenaka, C. (2001). Effects of aluminum stress on photosynthesis of *Quercus glauca* Thumb. *Plant and Soil, 237,* 137-146.
30. Steiner, F., Zoz, T., Soares, A., Junior, P., Castagnara, D. D. et al. (2012). Effects of aluminum on plant growth and nutrient uptake in young physic nut plants. *Ciências Agrárias, 33(5),* 1779-1788.
31. Kochian, L. V., Hoekenga, O. A., Pinéros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annual Review of Plant Biology, 55,* 459-493.
32. Dalović, I. G., Maksimović, I. V., Kastori, R. R., Jelić, M. Ž. (2010). Mechanisms of adaptation of small grains to soil acidity. *Proceedings for Natural Sciences/Matica Srpska (Novi Sad), 118,* 107-120.
33. Zinnert, J., Nelson, J., Hoffman, A. (2012). Effects of salinity on physiological responses and photochemical reflectance index in two co-occurring coastal shrubs. *Plant and Soil, 354,* 45-55.
34. Chettri, M. K., Cook, C. M., Vardaka, E., Sawidis, T., Lanaras T. (1998). The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. *Environmental and Experimental Botany, 39(1),* 1-10.
35. Franklin, L. A., Levasseur, G., Osmond, C. B., Henley, W. J., Ramus, J. (1992). Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta, 186,* 399-408.
36. Ohtagaki, T., Miwa, M., Izuta, T., Totsuka, T. (1996). Photosynthetic response of Japanese Cedar seedlings grown in brown forest soil acidified by adding H_2SO_4 solution. *Journal of Japan Society for Atmospheric Environment, 31,* 11-19.
37. Panda, S. K., Baluska, F., Matsumoto, H. (2009). Aluminum stress signaling in plants. *Plant Signaling & Behavior, 4,* 592-597.
38. Konrad, M. L. F., Da Silva, J. A. B., Furlani, P. R., Machado, E. C. (2005). Gas exchange and chlorophyll fluorescence in six coffee cultivars under aluminum stress. *Bragantia, 64,* 339-347.
39. Alam, S. M. (1999). Nutrient uptake by plants under stress conditions. In: M. Pessarakli ed., *Handbook of plant and crop stress*, pp. 285-314.
40. Díaz-López, L., Gimeno, V., Lidón, V., Simón, I., Martínez, V. et al. (2012). The tolerance of *Jatropha curcas* seedlings to NaCl: an ecophysiological analysis. *Plant Physiology and Biochemistry, 54,* 34-42.
41. De Vos, A. C., Broekman, R., De Almeida Guerra, C. C., Rijsselberghe, M. V., Rozema. J. (2013). Developing and testing new halophyte crops: a case study of salt tolerance of two species of the *Brassicaceae*, *Diplotaxis tenuifolia* and *Cochlearia officinali*. *Environmental and Experimental Botany, 92,* 154-164.

42. Maathuis, F. J. M., Ahmad, I., Patishtan, J. (2014). Regulation of Na^+ fluxes in plants. *Frontiers in Plant Science, 5,* 467.

43. Fritioff, A., Kautsky, L., Greger, M. (2005). Influence of temperature and salinity on heavy metal uptake by submersed plants. *Environmental Pollution, 133,* 265-274.

44. Schmitt, M., Watanabe, T., Jansen, S. (2016). The effects of aluminium on plant growth in a temperate and deciduous aluminium accumulating species. *AoB Plants,* 8.

45. Jansen, S., Broadley, M. R., Robbrecht, E., Smets, E. (2002). Aluminum hyperaccumulation in angiosperms: a review of if phylogenetic significance. *The Botanical Review, 68,* 235-269.