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

Dhouha Belhaj Sghaier¹, Insaf Bankaji¹, Sylvia Pedro², Isabel Caçador² and Noomene Sleimi¹,*

¹LR. RME-Ressources, Matériaux et Ecosystèmes, Faculty of Sciences of Bizerte, University of Carthage, 7021 Jarzouna, Bizerte, Tunisie.

²MARE-Marine and Environmental Sciences Centre, Faculty of Sciences of the University of Lisbon, Portugal.

*Corresponding Author: Noomene Sleimi. Email: noomene.sleimi@gmail.com.

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 µg.g⁻¹ DW and 7834 µg.g⁻¹ DW respectively. It is considered as a hyperaccumulator plant of Al. In addition, Na⁺ contents in shoots and roots exceed 23000 µg.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 (Al³⁺), 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 (NO₃⁻), phosphate (PO₄³⁻), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), 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 grum 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 Al³⁺ 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 dm³) 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: MnCl₂; CuSO₄·5 H₂O; ZnSO₄·7H₂O; (NH₄)₆Mo₇O₂₄·4 H₂O; and H₃BO₃ 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 Al³⁺ form) is added from a pre-prepared concentrated aluminum chloride solution (AlCl₃), 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 Al³⁺ 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 harvested 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 CaCl₂ 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:

\[ WC = 100 \times \frac{FW - DW}{FW} \]

2.2 Pigment Profiling

Leaves used in pigment analysis were freeze-dried in the dark during 48 h, after which they were ground 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):

\[ DES = \frac{[\text{Antherax}] + [\text{Zeax}]}{[\text{Viola}] + [\text{Antherax}] + [\text{Zeax}]} \]

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 HNO₃:H₂SO₄:HClO₄ (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)

| Treatments                        | DW (mg)      | WC %        |
|----------------------------------|--------------|-------------|
| **Shoots**                       |              |             |
| Control                          | 1295.35a ± 77.5 | 77.11a ± 0.7 |
| Al, 200 µM                       | 1052.80bc ± 39.9 | 74.57a ± 0.7 |
| Al, 500 µM                       | 836.40bc ± 68.4 | 77.12a ± 0.9 |
| Al, 800 µM                       | 772.70bc ± 70.4 | 76.98a ± 1.2 |
| NaCl, 200 mM                     | 994.90b ± 34.7 | 76.03a ± 2.0 |
| Al, 200 µM + NaCl, 200 mM        | 905.83bc ± 40.5 | 76.10a ± 0.8 |
| Al, 500 µM + NaCl, 200 mM        | 770.83b ± 28.1 | 79.23a ± 1.5 |
| Al, 800 µM + NaCl, 200 mM        | 783.53b ± 45.8 | 80.90a ± 1.0 |
| **Roots**                        |              |             |
| Control                          | 489.99a ± 13.7 | 79.65a ± 1.2 |
| Al, 200 µM                       | 347.18bc± 19.1 | 80.14a ± 1.1 |
| Al, 500 µM                       | 348.93bc± 14.5 | 82.89a ± 1.4 |
| Al, 800 µM                       | 395.37bc± 18.1 | 82.74a ± 1.8 |
| NaCl, 200 mM                     | 368.14bc± 17.6 | 79.43a ± 1.5 |
| Al, 200 µM + NaCl, 200 mM        | 385.13bc± 22.0 | 81.70a ± 1.5 |
| Al, 500 µM + NaCl, 200 mM        | 272.47bc± 19.5 | 82.03a ± 0.9 |
| Al, 800 µM + NaCl, 200 mM        | 297.05bc± 33.2 | 82.24a ± 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±2.8         | 67.54±1.4         | 151.95±4.1            | 60.55±3.1                | 1.21±0.02| 0.40±0.02            |
| Al, 200 µM                  | 87.88±3.6         | 77.94±6.7         | 165.83±13.1           | 58.93±9.3                | 1.18±0.09| 0.39±0.08            |
| Al, 500 µM                  | 85.08±2.7         | 70.09±5.1         | 155.18±7.7            | 64.87±4.3                | 1.23±0.05| 0.43±0.05            |
| Al, 800 µM                  | 82.71±1.7         | 65.56±2.6         | 148.20±2.4            | 67.63±2.6                | 1.27±0.04| 0.46±0.03            |
| NaCl, 200 mM                | 88.94±5.8         | 70.54±8.3         | 162.42±14.0           | 69.33±5.5                | 1.30±0.06| 0.45±0.06            |
| Al, 200 µM + NaCl, 200 mM   | 82.95±1.3         | 66.31±1.8         | 149.26±2.0            | 67.37±2.6                | 1.26±0.03| 0.45±0.02            |
| Al, 500 µM + NaCl, 200 mM   | 91.31±5.3         | 72.93±6.8         | 164.14±11.9           | 71.46±1.7                | 1.27±0.04| 0.45±0.04            |
| Al, 800 µM + NaCl, 200 mM   | 93.68±3.1         | 72.50±3.3         | 166.18±6.3            | 69.94±1.6                | 1.30±0.02| 0.43±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, βCar: 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 | βCar | Lut |
|-----------------------------|------|-------|---------|-----|------|-----|
| Control                     | 51.0±3.7 | 1.4±0.5 | 3.1±0.7 | 28.5±3.1 | 12.0±0.3 | 6.7±1.4 |
| Al, 200 µM                  | 53.5±2.8 | 3.0±1.3 | 3.9±1.9 | 26.4±9.5 | 12.8±1.6 | 5.5±1.8 |
| Al, 500 µM                  | 52.6±1.5 | 2.5±0.3 | 5.3±1.1 | 30.2±3.1 | 12.4±0.6 | 7.4±1.0 |
| Al, 800 µM                  | 52.1±3.4 | 2.0±0.7 | 4.0±1.1 | 30.9±2.4 | 12.1±0.6 | 7.7±1.1 |
| NaCl, 200 mM                | 55.9±6.9 | 4.5±0.9 | 6.1±1.3 | 30.9±3.2 | 13.6±3.3 | 8.1±0.9 |
| Al, 200 µM + NaCl, 200 mM   | 52.1±1.1 | 3.5±0.6 | 3.9±1.4 | 30.9±1.6 | 12.1±0.2 | 7.6±0.8 |
| Al, 500 µM + NaCl, 200 mM   | 55.7±4.0 | 3.4±1.1 | 3.5±1.7 | 33.1±1.1 | 13.5±1.3 | 9.2±1.3 |
| Al, 800 µM + NaCl, 200 mM   | 58.8±4.1 | 2.9±0.3 | 3.1±1.0 | 33.7±0.6 | 14.1±1.3 | 9.1±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 $p = 0.05$](image)

### 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).
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 µg.g⁻¹ DW in shoots and 5442 µg.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 µg.g⁻¹ DW in shoots and 7834 µg.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](image_url)

**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 $p = 0.05$

| Treatments                          | Shoots | Roots |
|-------------------------------------|--------|-------|
|                                     | K⁺     | Mg²⁺  | Ca²⁺  | Na⁺   | Al     |
|                                     | (mg.g⁻¹ DW) | (mg.g⁻¹ DW) | (mg.g⁻¹ DW) | (mg.g⁻¹ DW) | (mg.g⁻¹ DW) |
| Control                            | 20.24±1.3 | 4.40±0.5 | 2.75±0.2 | 4.88±0.4 | 0.80±0.04 |
| Al, 200 µM                         | 20.17±1.3 | 5.13±0.5 | 3.17±0.2 | 4.84±0.2 | 1.49±0.09 |
| Al, 500 µM                         | 19.81±1.3 | 5.08±0.2 | 3.10±0.2 | 3.97±0.2 | 2.54±0.06 |
| Al, 800 µM                         | 20.77±1.2 | 5.81±0.3 | 2.62±0.1 | 5.81±0.6 | 3.28±0.03 |
| NaCl, 200 mM                       | 11.37±0.6 | 3.21±0.2 | 1.88±0.2 | 23.42±1.3 | 0.32±0.02 |
| Al, 200 µM + NaCl, 200 mM          | 13.86±0.8 | 4.13±0.4 | 2.35±0.3 | 28.20±1.9 | 0.61±0.07 |
| Al, 500 µM + NaCl, 200 mM          | 10.62±0.2 | 3.68±0.3 | 2.14±0.3 | 25.11±2.1 | 5.65±0.07 |
| Al, 800 µM + NaCl, 200 mM          | 8.07±0.6 | 2.88±0.1 | 1.48±0.1 | 21.69±1.2 | 6.29±0.19 |

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 Al³⁺ 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].

\( T. \textit{gallica} \) showed Na levels of 23419 µg.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, \( T. \textit{gallica} \) 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 \( T. \textit{gallica} \) and its capacity to accumulate amounts greater than 7834 µg.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 \( T. \textit{gallica} \) 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, \( T. \textit{gallica} \) can be classified as hyperaccumulator species, and it’s interesting to use in phytoremediation programs.

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