



Antioxidants responses of two halophytes under aluminium stress alone or combined with salt

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Authors' Contribution

Sghaier, D.B., carried out all the experiments, assured data analysis and prepared the manuscript.

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ABSTRACT

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Al stress is a serious environmental problem that affects plant physiology and biochemical processes and reduces productivity in the agricultural sector. This study is a comparative study between the antioxidant responses of two different halophyte species and focuses on two halophytes *Arthrocnemum indicum* and *Tamarix gallica*. Both halophytes species exposed to different concentrations of Al (200, 500, 800) μM alone or combined with NaCl (200 mM) and the effect of Al on oxidative damage and enzymatic and non-enzymatic antioxidant responses were determined. Our results demonstrated that *Tamarix gallica* was more tolerant to Al stress than *Arthrocnemum indicum* and this by a low level of MDA registered. In addition, *T. gallica* showed more marked antioxidant activities. However, salinity improves the tolerance of *A. indicum* by increased levels of glycine betaine and proline. Nevertheless, *T. gallica* is still more tolerant according to the tolerance index than *A. indicum* and this species is a favorable plant material to soils phytoremediation. *T. gallica* can be successfully used for phytostabilization of Al-contaminated saline soils.

Keywords: *Tamarix gallica*, *Arthrocnemum indicum*, Aluminium, combined stress, Oxidative stress, antioxidants responses

INTRODUCTION: Plants are exposed to various modifications of edaphic factors resulting from industrialization, urbanization and climatic factors leading to the alteration of the structure and nature of the soil (Mujeeb *et al.*, 2023). As a result, there is today acidification of soils (pH < 5.0) which are characterized by a strong Al^{3+} activity, the most toxic form of aluminium (Al) for plants (Guo *et al.*, 2018). Research on Al stress has been rapidly updated in recent years and a significant number of studies have been elucidated. Aluminium stress causes a series of morphological, physiological, biochemical and molecular changes in growing plants, reducing crop growth, development and yield (De Castro *et al.*, 2022). Over the past decades and therefore, many researchers have reported the mechanism of aluminium toxicity and tolerance in many plants (Nazari *et al.*, 2023). In fact, to tolerate the toxicity of Al^{3+} , the plant has developed different mechanisms of tolerance and detoxification (Sarkar *et al.*, 2020). In general, Al stress is one of the factors contributing to the imbalance in ROS production that induces oxidative stress (Darkó *et al.*, 2004). Bera *et al.* (2019) showed that Al induces oxidative stress by sensing the generation of O_2^- , H_2O_2 and $\cdot\text{OH}$ radicals that lead to lipid peroxidation, DNA damage and cell death in root tissues. To cope with this oxidative damage, plants activate the defence system which induces the activation of the antioxidant enzymes superoxide dismutase (SOD), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) (Joshi *et al.*, 2022). Another mechanism that plants use to survive is the production of compatible osmolytes such as proline, glycine betaine, and others that help control osmotic potential, cell turgor, cell homeostasis, and cell function (Hernández-Salinas *et al.*, 2022). It has also been reported that compatible osmolytes can scavenge ROS and other free radical compounds, thereby protecting the photosynthetic apparatus and preventing protein denaturation (Hurtado *et al.*, 2020). Several studies have been undertaken to confirm the effectiveness of halophytes to handle high concentrations of sodium chloride may be an increased tolerance to certain heavy metal ions. The renewed interest in the metal tolerance of halophytes in recent years mainly focuses on the possibility of using these plants for the phytoremediation of saline soils polluted by metals (Van Oosten and Maggio, 2015). Three salt adaptation strategies have been proposed: the first is salt exclusion where halophytes like *Rhizophora* sp. exclude excessive salt ions from the roots; second, salt excretions where halophytes readily take up salt ions from the root zone and excrete leaves using specialized salt glands, e.g. *Tamarix*; and the third is salt accumulation, in this halophyte rapidly absorbs salt ions and sequesters them in the vacuole, e.g. *Sesuvium portulacastrum* (Nikalje *et al.*, 2019). Studies have demonstrated that halophytes can endure heavy metals, for example, Cd, Pb, Zn, Cu, etc (Mujeeb *et al.*, 2023). Often, heavy metal pollution and soil salinization often occur together. In Tunisia, for example, there are different types of saline soils affected by heavy metals. The accumulation capacity of Halophytes could be the result of different mechanisms (Li *et al.*, 2020). The amount of salt in the soil affects metal accumulation in tissues and its presence can mitigate the effects of metal toxicity (Aljlil, 2017). In addition, halophyte plants can mitigate the effects

of metals in various manner through the retention of the supply of ions in structures preserved to accumulate salts, such as the cell wall, the vacuole or the trichome; use substances for the chelation of metals or by mechanisms of cross-tolerance of heavy metals with salinity (Mujeeb *et al.*, 2023). The formation of metal-chloride complexes in saline soils and root apoplast can either enhance or limit the bioavailability and root-thrust translocation of metal (Wali *et al.*, 2017). Ecophysiological research on salt and metal resistance of *Arthrocnemum indicum*, *Suaeda maritima* (Panda *et al.*, 2017), *Tamarix gallica*. Sghaier *et al.* (2019), revealed the phytoremediation capacity of halophytes. Therefore, halophytes could be considered a promising candidate for phytoremediation. Hence, establishing the different properties of species is required to establish careful species selection (Chen *et al.*, 2021). In fact, in the green remediation approach, it is necessary to analyze the different properties of species to determine the capacity for tolerance to contaminants and their behaviour with respect to oxidative stress; phytotolerance studies are required to identify metal tolerance in plant species and to figure out the negative impact of metals on the metabolic process in these species. Thus, the phytomanagement of polluted soils is promising, particularly in areas simultaneously altered by salinity.

OBJECTIVES: The objectives of this study were as follows: (1) compare the potential of two halophytes *A. indicum* and *T. gallica* to tolerate the negative effect of Al and the antioxidant mechanism involved to deal with this toxicity and its dynamics in relation to the presence of salinity under field conditions. (2) the potential of these to halophyte for the restauration of soil contaminated.

MATERIALS AND METHODS: Experimental plant material and growth condition: The plants were collected from the fallow of natural environments surrounding the roued sabkha. *Tamarix gallica* and *Arthrocnemum indicum* were propagated by stem cuttings (5 cm) which emerged in plastic pots filled with a mixture of perlite and gravel substrate (2:1; v/v) (Sghaier, 2023). Then, young rooted cuttings were provided with a Hewitt nutrient solution (Heath and Packer, 1968) supplemented with iron and micronutrients, three times a week for 3 months (Sghaier *et al.*, 2019). On completion of the incubation period, the nutrient set was divided into 8 treatments: control (0), 200, 500 and 800 μM alone or combined with NaCl (200 mM). The aluminium (in Al^{3+} form) is provided from a pre-prepared concentrated aluminium chloride solution (AlCl_3), which is a powerful Lewis acid. The electrical conductivity (E.C.) and the pH of the nutritive solution were around 1.7 $\text{dS}\cdot\text{m}^{-1}$ and 7.22 respectively. The addition of Al^{3+} to the nutrient solution declined the pH to values 4.4 to 5.4 depending on the used doses. Thus, the samplings were harvested, separated into shoots and roots and frozen into liquid nitrogen following transfer to - 80 C for further biochemical assay.

MDA content: The MDA content was determined according to Heath and Packer (1968), by using a mixture (20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA). The homogenate was extracted at 95°C for 30 min followed by centrifugation at 3000 g for 5 min at 4°C. Then, the absorbance reading was realized at 532 and 600 nm in a Shimadzu UV-1603 spectrophotometer.

Oxidative stress biomarkers: Leaves were collected and immediately frozen in liquid N₂ and stored at -80 °C. All enzymatic analyses were carried out at 4°C. For the extraction procedure, 500 mg of fresh leaves was added to 12 mL of sodium phosphate buffer (50 mM, pH 7.6) with 0.1 mM Na-EDTA. The mixture underwent centrifugation at 8923 rpm for 20 min, at 4°C, and the supernatant was undertaken for enzyme assays. Ascorbate peroxidase (APX) was realized as reported by [Tiryakioglu et al. \(2006\)](#) by recording the drop in absorbance upon oxidation of ascorbate at 290 nm. Guaiacol peroxidase (GPOX) was assayed according to [Bergmeyer et al. \(1974\)](#) by recording the rise in absorbance upon the formation of guaiacol oxidation products at 470 nm ($\epsilon = \frac{1}{4} 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$). Superoxide dismutase (SOD) activity was carried out by the method of [Marklund and Marklund \(1974\)](#) by noticing the decline of pyrogallol at 325 nm. Proteins were set according to [\(Bradford, 1976\)](#).

Osmo-compatible solutes: The free proline was determined by the addition of 3% aqueous sulfosalicylic acid, followed by centrifugation at 10,000 rpm for 15 min, and the proline was assessed by using ninhydrin as reported by [Bates et al. \(1973\)](#). GB was carried out according to [Grieve and Grattan \(1983\)](#). After dilution with 2 N H₂SO₄ followed by dissolving of the pellet (composed of periodide crystals) in 9 mL of 1,2-dichloroethane. The final step was the reading of the absorbance at 365 nm.

Tolerance index: The tolerance index is a measure of the plant's tolerance to ETM determined by comparing the biomass of plants subjected to a metal treatment with the control according to the following formula:

$$TI = \frac{\text{Biomass of treated plants}}{\text{Biomass of control plants}} \times 100$$

Statistical analysis: A one-way analysis of variance (ANOVA) or when parametric test assumptions were not satisfied Kruskal-Wallis test were used to compare the oxidative stress and the antioxidant responses towards aluminium on the plants in the presence and the absence of the salt. Depending on the type of test

(parametric or non-parametric), Bonferroni test or pairwise multiple comparisons were realized when significant differences were found ($\alpha = 0.05$ significance level). The statistical analysis was determined via Statistica software Statistica 12 (Statasoft). All the samples were analyzed in three replicates (n=3) and All values reported refer to mean \pm standard error. Data from oxidative stress, antioxidants responses (MDA, APX, GPX, SOD, GB and Pro) and Al treatment alone or combined with salt were correlated using Pearson correlation analysis and Principal Component Analysis (PCA) via R software.

RESULTS: Oxidative stress response: Figure 1A illustrated the MDA content after exposure to Al which increased significantly after exposure to 200 μM , 500 μM and 800 μM compared to the control group in *A. indicum*. Whereas, unvaried values were determined in *T. gallica*. The append of NaCl in the solution reduced the lipid peroxidation in *A. indicum* leaves, whereas, in *T. gallica*, the addition of salt did not alter the MDA level. *A. indicum* was more sensitive to Al than *T. gallica* and elevated values were recorded. Figure 1B revealed that after Al supply, the highest SOD activity was noted in *A. indicum* leaves treated with 800 μM with and without salt. Whereas, elevated SOD activity was noticeable in *T. gallica* at 500 μM Al and 800 μM combined with NaCl. Interestingly, the addition of NaCl alone to the medium enhanced SOD activity significantly ($p < 0.05$) in *A. indicum*, however, decreased activity was observed in *T. gallica* (figure 1B). Following the same pattern of response observed for SOD, in *A. indicum*, APX activity was unvaried in all treatment, while the highest APX activity revealed at 500 μM of Al alone in *T.gallica* (figure 1C). Regarding GPX activity, Al exposure did not alter the enzyme activity in both plants. Similarly, the NaCl had no statistical effect on the activity of this biomarker ($p > 0.05$) in *A. indicum* while a severe decrease was noted in *T. gallica*. Nevertheless, the negative effect of TME was reverted by the addition of NaCl; the studied enzymatic activity significantly improved by the additional supply of Al and NaCl ($p < 0.05$) while a continuous decrease was signalled in *T. gallica* (figure 1D).

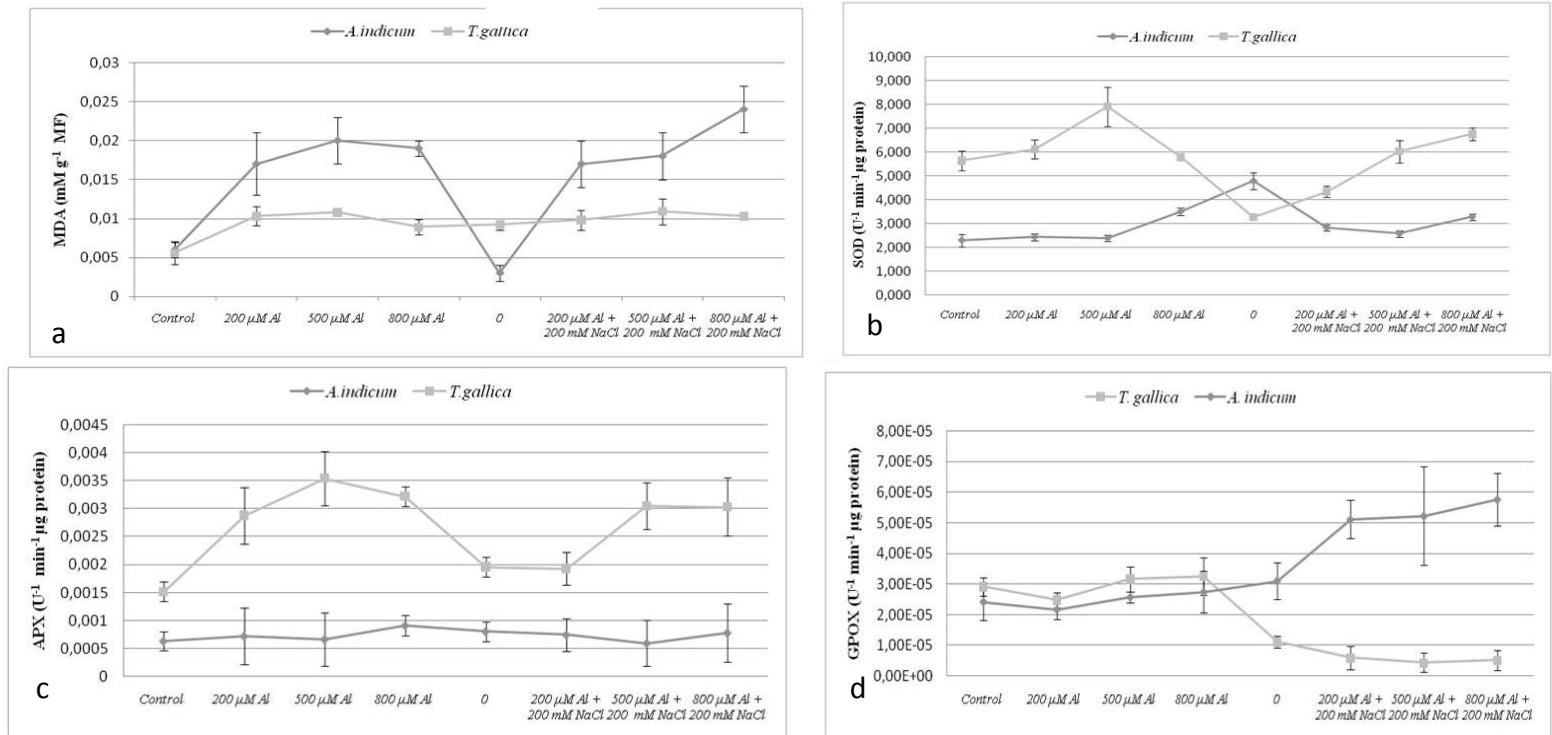


Figure 1: MDA content (a), enzymatic activity: SOD (b), APX (c) and GPX (d) in the leaves of *Arthrocnemum indicum* and *Tamrix gallica* under different concentrations of Al (0, 200, 500, 800 μM) in the absence or presence of 200 mM NaCl (values are means \pm SD, n = 3).

In *A. indicum*, the results obtained revealed a decline in the level of Pro at high concentrations of Al (500 and 800 μM). In contrast, *T. gallica* showed the presence of high levels of proline (Pro). This increase was amplified by salt supplementation to the medium in both plants (figure 2A). On the other hand, depending on the increasing concentrations of Al, there was no effect on the GB content in *T. gallica*. Similarly, the salt supplementation to the solution did not alter the contents of GB in *T. gallica*. While in *A. indicum*, GB content marked a rise at high doses of Al, but when this metal was provided simultaneously with NaCl, a significant rising in this parameter was recorded (figure 2B).

Tolerance index (Ti): Figure 3 confirmed the tolerance index in two halophytes, in the shoots, the highest tolerance index was found in *T. gallica* after exposure to increased Al concentration in the medium; however, when the combined stress was applied, *A.*

indicum demonstrated the highest tolerance index. In the roots, under low doses of Al alone or combined with salt, *A. indicum* showed an elevated Ti while under elevated Al concentrations alone or combined with salt, *T. gallica* presented an increased Ti.

Data correlation: To illustrate correlations among Al and antioxidant responses between different treatments, Pearson's coefficients were presented in a correlogram, as shown in figure 4. The obtained correlogram indicated that Al evinced a correlation with SOD, APX and Pro ($r = 0.57$; $r = 0.64$; $r = 0.66$ respectively; $p < 0.05$); the relationship between SOD and APX was conspicuous ($r = 0.85$; $p < 0.05$). Besides, this analysis indicated that the membrane lipoperoxidation, estimated by the Malondialdehyde (MDA), manifested a correlation with Pro ($r = 0.55$; $p < 0.05$). Concluding from these results, it is clear that to overcome the negative effects of Al, the plants activated the antioxidant process via SOD, APX and

Pro. Moreover, it is evident that SOD and APX coordinate together in order to reduce the deleterious effects of ROS. Finally, proline enters the relay to minimize the effects of lipid peroxidation (figure 4).

Principal component analysis: To depict the influence of Al stress on antioxidant machinery, a principal component analysis (PCA) was performed on the whole dataset (figure 5), distinguishing main components explaining distinct responses of plants treated with

applied stresses; the responses specific for stress-treated plants were divided into two components. The first component, PC1 (39.86% of the variance), was largely determined by enzymes activities APX and SOD (figure 5). The second component (PC2), which accounted for 30.05% of the variance, is mostly determined by the Pro, MDA and GB (figure 5).

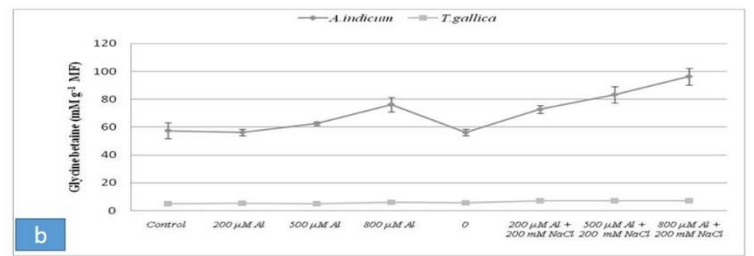
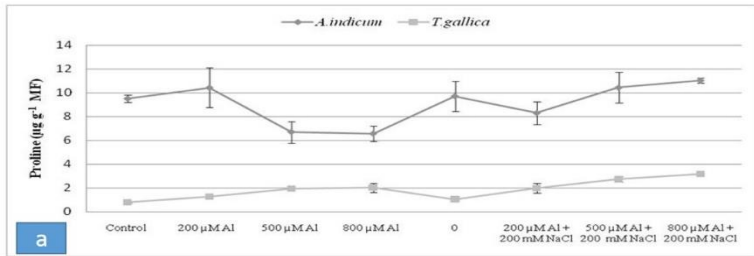


Figure 2: Proline (a) and glycine betaine (b) content in the leaves of *Arthrocnemum indicum* and *Tamrix gallica* under different concentrations of Al (0, 200, 500, 800 µM) in the absence or presence of 200 mM NaCl (values are means ± SD, n = 3).

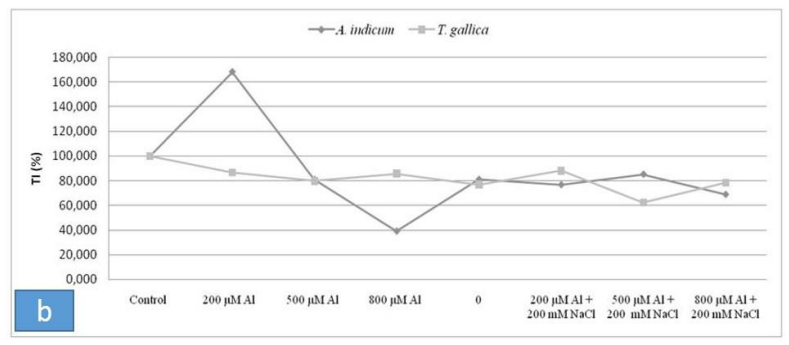
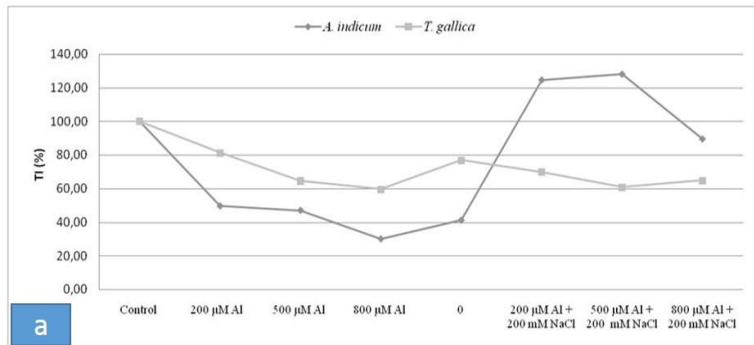


Figure 3: Tolerance index (TI) (a) in the leaves (b) in the roots of *Arthrocnemum indicum* and *Tamrix gallica* under different concentrations of Al (0, 200, 500, 800 µM) in the absence or presence of 200 mM NaCl.

DISCUSSIONS: Aluminium itself is not a transition element, but aluminium ions are able to induce electrostatic bonding presumably with an oxygen-donating ligand such as carboxylate and pectin of the cell wall and outer surface of the plasma membrane leading to the alteration of the cell wall properties which seems to be the basic mark of aluminium toxicity and could also play a role as a catalyst in the ROS production which further drive to oxidative stress in plants (Singh et al., 2017).

the fact that the preferendum growth and enhanced development of this specie were demonstrated at 200-400 Mm (Nisar et al., 2021). Previous studies have reported that MDA content was even lower in HM + NaCl-treated plants than in the controls (Pirzadah et al., 2019). This reinforces the hypothesis that salinity helped plants to cope with heavy metals (Zhou et al., 2019). Nevertheless, other authors reported that over-expression of OsPIN2, an auxin transporter gene would decrease the Al-activated generation of ROS and weaken lipid peroxidation (LPO) in rice roots (Singh et al., 2017). Further, the MDA content accumulation may be correlated with a global decrease or increase in total antioxidant activity; this suggests the physiological consequences of toxic element accumulation (Zhou et al., 2019). Under a stress state, the activation or the suppression of antioxidant enzymes to trap ROS could result in conjugation with each other (Abbas et al., 2018). Hence, plants must eliminate needless ROS compounds to escape damaging plant cells, which induces the activation of paths implicated in the regulation of antioxidant systems (Wu et al., 2016). The removal of reactive oxygen species is not an action of a single enzyme; however, it is mediated by the combined activities of a group of antioxidant enzymes (Wang et al., 2017). CAT, APX, and GPOX are considered the main enzymatic antioxidants, as they participate in the decomposition of H₂O₂ to water (Ali et al., 2021). These reactions have been signalled as being key points to reaching tolerance to Al stress in diverse plant species (Pontigo et al., 2017). This data indicated that *T. gallica* enhanced the activity of SOD under Al stress alone while under salt stress or mixed treatment there was a diminution in SOD synthesis, especially at low doses of Al. Contrarily, in *A. indicum* an augmentation in the studied enzyme was revealed at 800 µM of Al alone or combined with salt and was much higher under salt stress alone (figure 1B). Regarding APX activity, there was a rise in the activity of the studied enzyme in *T. gallica* after Al supply alone or added with salt and also in the presence of the salt alone in the medium whereas a stable activity was registered in *A. indicum* under all kind of treatments (figure 1C). Depending on our results (figure 4 & 5), the PCA analysis demonstrated that APX and SOD activities were correlated and their increased and also decreased simultaneously. In fact, it is well documented that SOD plays an important role as the primary line of defense, catalyzing the dismutation of the superoxide radical into hydrogen peroxide, which is subsequently neutralized by GOPX and APX through different substrates (Dhiman et al., 2021). The third tested enzyme was GPOX (figure 1D), our data presented an enhanced activity under Al stress combined with salt in *A. indicum*; contrary, a decrease was registered in *T. gallica*.

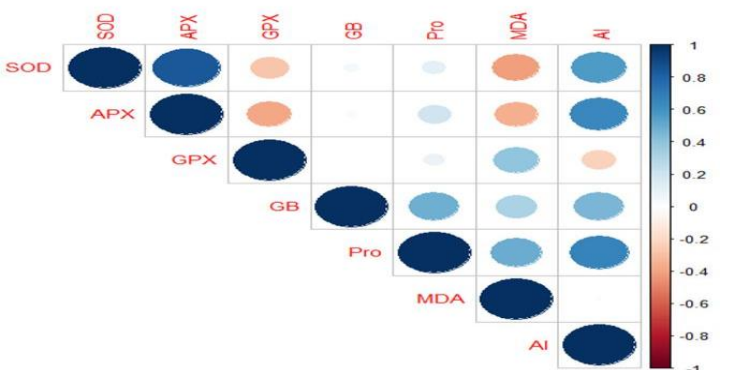


Figure 4: Correlation analysis between studied parameters (Al, MDA content, enzymatic activity: SOD, APX, GPX, Proline and glycine betaine content) in *Arthrocnemum indicum* and *Tamrix gallica*. Blue colored circles indicate positive correlations, while red colored circles indicate negative correlations.

Therefore, a drastic rise in oxidative stress signs has been revealed under Al stress (Gratão et al., 2019). In fact, after exposure to 90 days, an increase in MDA was propounded in two halophytes. *A. indicum* recorded higher lipid peroxidation product than *T. gallica* (figure 1A), showing that *A. indicum* exhibited oxidative damage more pronounced than *T. gallica*. This observation was coordinated as observed above in the TI (figure 3) indicating that under Al stress, *T. gallica* exhibited a higher TI than *A. indicum*. Our data suggest that this could be because *T. gallica* is a salt cedar and could excrete the Al and other toxic elements through these specials glands despite a strong accumulation of Al was observed in *T. gallica* than in *A. indicum* (unpublished paper) or low level of ROS scavenging enzymes producing. Surprisingly, in *A. indicum* MDA content was even lower after the addition of the salt alone in the medium. Similarly, the same behavior was observed in *A. indicum* under mixed stress but not significantly demonstrated, while, no effect was registered in *T. gallica* after salt supply or under combined stress. Tolerance Index pointed out that under combined stress *A. indicum* displayed elevated TI than *T. gallica* (figure 3). The diminution of MDA level and the increased TI in *A. indicum* could be explained by

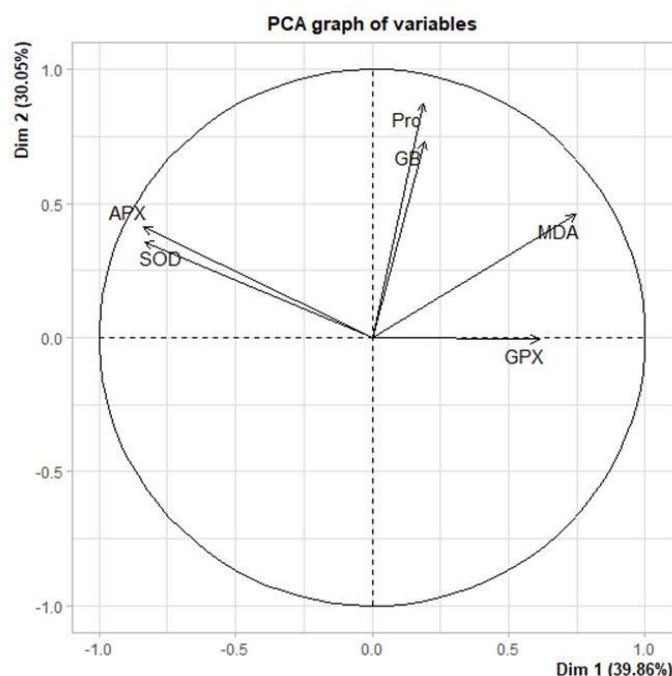
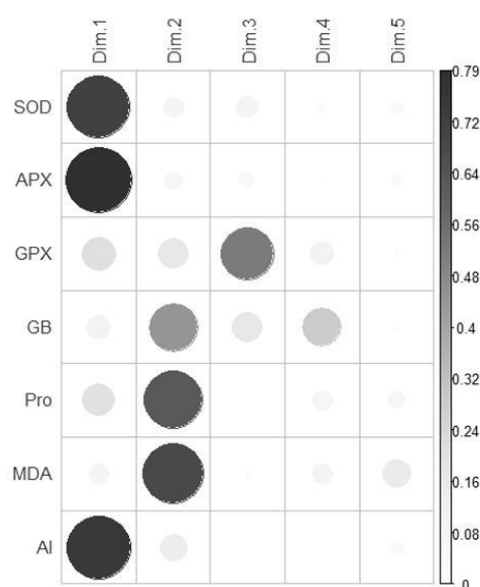


Figure 5: Principal component analysis (PCA) illustrations of oxidative stress and the antioxidant responses (enzymatic and non enzymatic) used under applied treatments. The first component (PC1) explains 39.86% of the inertia and the second component (PC2) represents 30.05% of the inertia.

Nevertheless, the Al and the salt stress have no impact on the GPOX activity in both halophytes. Hence, GPX activity is related not only to the plant species or its growth phase but also to the extent of toxicity (De Almeida *et al.*, 2015). The flexibility and the rapid response of the antioxidant enzymes to changing external conditions are primarily controlled by the redox state of the thiol groups in their amino acid sequences, the redox regulation of antioxidant plant defense is quite complex and requires both spatial and temporal coordination (Dvořák *et al.*, 2021). The higher activity of antioxidant enzymes in plants may indicate an intensification of their defence against oxidative damage (Phour and Sindhu, 2022). The concomitant increase in GPOX and APX activities can reduce the possible oxidative damage caused by Al and/or salt stress (Dhiman *et al.*, 2021). The higher Al concentration (400 μM Al) stimulated the activity of enzymes up to five folds mainly in the old leaves and roots in *Amaranthus blitoides* (Nazari *et al.*, 2023). Besides, mild oxidative stress at 50 μM Al was not able to induce the enzymatic antioxidants in the leaves (Pirzadah *et al.*, 2019). Conversely, (Hippler *et al.*, 2018) suggested that citrus plants grown in the presence of 20 μM Cu exhibited enhanced activities of CAT, APX and GPOX in roots and leaves. The authors explained this finding as being due to the dismutation of superoxide ions (O_2^-) to H_2O_2 by SOD and then the elimination of H_2O_2 by CAT and APX. Similarly, Ahmad *et al.* (2018) reported that 'PusaTarak' presented a lower extent of Al-induced oxidative damage than 'Pusa Vijay'. The authors suggested that the upregulated activities of cellular antioxidant systems (enzymatic and non-enzymatic components) better and more efficiently actively participate in the reduction of Al toxicity. Antioxidant enzymes are regulated at the level of transcription, which is mediated by diverse TFs that are rapidly activated by the redox perturbations in photosynthetic electron transport (Exposito-Rodriguez *et al.*, 2017). This finding was in agreement with our previous work demonstrating that the combined stress disturbed the photosynthesis pathway in *A.indicum* which was concomitant with increased GPOX activities. GPOX is involved in several roles in plants like redox homeostasis, catalysis of the reduction of H_2O_2 and the removal of LPO (Ahmad *et al.*, 2018).

Additionally, we noticed that the activities of SOD and APX were highest in *T. gallica* than *A. indicum*. This result allowed us to determine that in *A. indicum*, there were other defense processes to face the lethal effect of Al and the combined stress. Prolonged subjection to environmental stress provoked the generation of various osmolytes like various amino acids and their derivatives and sugars, preventing the plant from stress. Heavy metals and NaCl may trigger the synthesis of quaternary ammonium compounds such as glycinebetaine (GB) and proline (Pro) in different halophytic plant species. It has long been known that halophytes have a superior ability to accumulate large amounts of these compounds (Hayat *et al.*, 2021). Also, a high level of resistance to pollutants in *Suaeda maritima* was mainly related to the synthesis and the accumulation of proline and soluble sugars in the roots, stems and leaves (Lutts

and Lefèvre, 2015). The synthesis of GB was noted in *A. indicum* at high Al concentrations, salt addition and also under the combined stress while a stable content was revealed in *T.gallica* (figure 2B). It is reported that *Arthrocnemum* species are characterized by their ability of GB production under harsh environmental condition (Nisar *et al.*, 2021). Conversely, an augmentation in Pro synthesis was registered in *T. gallica* (figure 1A). Regarding the second PCA component Pro and GB are coordinated together in both halophytes to manage the oxidative stress (figure 4 & 5). Several reports revealed that aluminium toxicity induced the production of osmolyte proline which might be due to stress-induced ROS generation, as proline plays an important role in the detoxification of ROS (Chung *et al.*, 2020). Al induced a high accumulation of proline, which leads to an elevated osmotic adjustment (Hurtado *et al.*, 2020), improved water content (Alzahrani *et al.*, 2018), and enhanced C and N storage (Hurtado *et al.*, 2020). In addition, there were few productions of oxidative stress, chlorophyll damage and biomass in Al-tolerant plants that exhibited high proline levels (Ahmad *et al.*, 2018). Proline has been believed to be with a key role in environmental stress defense. Also, it protects the structure of proteins and stabilizes the cellular redox balance (Shakeri *et al.*, 2019). In addition, a high accumulation of proline was linked with increased activity and transcription of antioxidant enzymes (Saed-Moucheshi *et al.*, 2014). The over-synthesis of proline in plants exposed to harmful conditions has been thoughtful as an adaptive response leading to the reduction of the cellular osmotic potential and function as a stress signal (Rady *et al.*, 2020). Pirzadah *et al.* (2019) revealed the boost in proline content in Al-resistant and Al-sensitive *Fagopyrum* species, *F. tataricum* and *F. Kashmirianum*, respectively, at 300 μM Al stress. Chowra *et al.* (2017) also showed that aluminium stress induced the production of proline and GABA in black gram plant. Bera *et al.* (2019) showed that the synthesis of glycine betaine resulted from the oxidative stress caused by Al in the rice variety. In fact, the accumulation of glycine betaine sustains the membrane integrity and the prevention of other cellular structures.

CONCLUSIONS Our results demonstrated that the combined application of salinity and Al resulted in a more pronounced reduction in oxidative stress than their applications alone. A comparison of the two halophytes revealed that *T. gallica* exhibited higher tolerance index values and antioxidant enzyme activities, suggesting that was more tolerant to Al stress than *A. indicum*. However, the salt addition ameliorated, in *A. indicum*, the antioxidant defense mechanisms and boosted the production of osmolytes like proline and glycine betaine, which play active roles in ensuring plant survival. Based on the values of MDA and TI, *T. gallica* can be successfully used for phytostabilization of Al-contaminated saline soils.

CONFLICT OF INTEREST: All the authors mentioned in this paper declared that they have no conflict of interest regarding this paper.

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