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Effects of Four-Week Exposure to Salt Treatments on Germination and Growth of Two *Amaranthus* Species

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Abstract: Soil salinity represents one of the most restrictive environmental factors for agriculture worldwide. In the present study, the salt tolerance of two weeds of the genus *Amaranthus*, *A. albus* and *A. hybridus*, the latter cultivated as green vegetable in Africa, were analysed. Both species showed a remarkable salt tolerance phenotype during germination and vegetative growth. To evaluate the percentage and rate of germination, seeds were germinated in Petri dishes in a germination chamber under increasing concentrations up to 300 mM NaCl. Higher concentrations of salt ranging from 150 to 600 mM NaCl were applied for one month to plants grown in individual pots in the greenhouse. All seeds of *A. albus* germinated in the control and almost half of the seeds under 200 mM NaCl, but only 4% of the seeds under 250 mM NaCl. In *A. hybridus*, germination was considerably lower in all treatments and was completely prevented at 250 mM NaCl. The plant growth of both species was severely affected by high salt concentrations of 450 and 600 mM NaCl, but not under lower concentrations. At this stage of the biological cycle, *A. hybridus* showed a higher salt tolerance, as indicated by the smaller reduction in its growth parameters. The dry weight of leaves and roots of plants receiving 600 mM NaCl decreased in comparison to control: less than 60% in *A. hybridus* but more than 70% in *A. albus*. The salt tolerance of the two species contributes to their invasive potential, but on the other hand represents a useful trait when considering them as potential crops for the future.

Keywords: salt treatments; germination; growth; invasive weeds; edible species; salt tolerance

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1. Introduction

Soil salinity together with drought are the main drivers of yield reduction in many areas worldwide [1]. Secondary salinisation caused mainly by the use of saline groundwater and poor-quality wastewater for irrigation [2] is continuously increasing in the context of global warming. The rising temperatures and the increasing periods of drought will worsen this situation, especially in arid and semi-arid regions [3]. Soil salinity has an osmotic component, hindering water absorption by plants, as well as a direct effect due to the ion toxicity of Na^+ and Cl^- [4]. It also alters the nutritional balance of plants and affects the tilth and permeability of a soil [5]. Due to its impact on agriculture, soil salinity is routinely measured and monitored. As the measurement of total salt concentration in soil is complex, soil salinity is usually determined indirectly by measuring electrical conductivity (EC). This is a "surrogate measure of salinity" in which the salt content of the soil water extract is

determined by the ability of the extract to conduct electricity between two electrodes; the higher the salt concentration, the more current is conducted between the electrodes [6].

Most plants, including virtually all crops, are glycophytes unable to grow in saline soils, and only a small proportion of vascular terrestrial plants are halophytes. The 30 most widely used crops, which provide 90% of plant-based human nutrition, belong to the category of glycophytes [7]. As such, yield losses are registered in these crops even under low and moderate salinity, at EC of 4–8 dS m^{-1}, corresponding approximately to 40–80 mM NaCl [8,9]. Due to the increasing use of irrigation, imposed in many regions due to harshening climatic conditions triggered by global warming, by 2050 half of arable land will be affected by salinisation [10]. Predicting crop yields under a range of root zone salinity is useful for establishing management strategies to avoid the detrimental effects of soil salinity on crop production.

There is a continuum of salinity tolerance in plants, ranging from extremely susceptible, as are many crops, to tolerant to extreme concentrations of 2 M NaCl, reported in species of *Tecticornia* (family Amaranthaceae) from Australia [11]. The exact boundary or definition of halophytes is not clearly established. Generally, they are organisms with morphological and physiological traits that allow them to proliferate in saline soils [12,13] or, more specifically, plants that survive and complete their life cycle in environments where the salt concentration is 200 mM NaCl or higher [14]. Plant breeding programmes have helped to obtain new crop cultivars more resistant to salt. An alternative approach to address this challenge is the use of halophyte species that are tolerant to high levels of soil salinity as a source of food and forage production [15–18]. Over more than two decades, 26 species of halophyte have been cultivated in Australia, and their use is also continuously increasing worldwide for other purposes, such as desalinisation, phytoremediation of heavily metal-polluted areas, or as a new source of energy [19].

The Amaranthaceae (including the former family Chenopodiaceae) is the family with the highest proportion of salt tolerant species, since 44% of all halophytes belong to this family [20,21]. In addition to well-known halophytes, such as species of the genera *Salicornia*, *Sarcocornia*, *Arthrocnemum*, *Suaeda*, *Salsola* and many others, the Amaranthaeceae comprise taxa with marked salt tolerance even if they are not obligate halophytes. A well-known example of a salt tolerant crop of this family is quinoa (*Chenopodium quinoa* Willd.) which is also reported to be drought [22] and frost tolerant [23]. Quinoa is classified as a facultative halophyte [13] and tolerates salinities up to 40 dS m^{-1} and 400 mM NaCl [21,24,25]. Several species of *Amaranthus,* the type genus of the family, were also reported to be salt resistant during germination and early seedling development [26,27], some of them tolerating salinities up to 300 mM during vegetative growth [28]. The genus *Amaranthus* includes more than 100 accepted species [29], about 60 of American origin, and the rest native to Europe, Africa, Asia and Australia [30]. Several species of this genus, along with quinoa, are considered pseudocereals with high mineral contents and nutritional value [31]. Grain amaranths (*Amaranthus caudatus* L., *Amaranthus cruentus* L. and *Amaranthus hypochondriacus* L.) were cultivated for more than 8000 years in Central and South America, as they belong to the earliest known crops of the Aztecs [30,31]. Other amaranths, such as *Amaranthus tricolor* L, *Amaranthus blitum* L., *Amaranthus dubius* Mart. ex Thell., *Amaranthus cruentus* L. and *Amaranthus viridis* L., are consumed in tropical regions of Southeast Asia and Africa as boiled greens, having a similar taste to spinach [31,32]. Some *Amaranthus* species are also used as ornamental plants or have pharmaceutical properties [31]. Species of this genus are gaining popularity worldwide, as they are tolerant to heat, drought and salinity stress [33], and can also be considered as alternative crops in temperate regions [32]. On the other hand, many *Amaranthus* are noxious weeds, with a high invasive potential and some are catalogued among the worst weeds of the world. Having a C4 metabolic pathway, a high seed production and fast growth, they are efficient crop competitors for water, nutrients and light [34].

The two species studied in this work have a dual consideration: they act as invasive weeds in many regions of the world, whereas in some other areas, they are locally cultivated

for human consumption as vegetables or as pseudocereal grains. *Amaranthus albus* L. originates from the southern United States and Mexico but it is naturalised in most countries in Europe, South America, North Africa, and a large part of Asia. In Europe, it is associated with anthropised environments, such as crops, wastelands, and roads, and only rarely appears in semi-natural habitats, such as polluted riverbanks [35]. *A. hybridus* L. is native to tropical and subtropical America but has expanded throughout the world, reaching a subcosmopolitan distribution. It is found in very altered, ruderal and agricultural environments, mostly irrigated crops, ditches, urban areas, etc., although it also appears in degraded riverbank communities [35]. The ranges of the two species also extend to regions of the world affected by soil salinisation (Figure 1). Both species are edible, consumed as green vegetables in Africa [36–39] or as pseudocereals, as their grains are rich in proteins, highly nutritive and consist of easily digested albumins and globulins [40,41]. *A. hybridus* is the most consumed leafy vegetable in Nigeria [37].

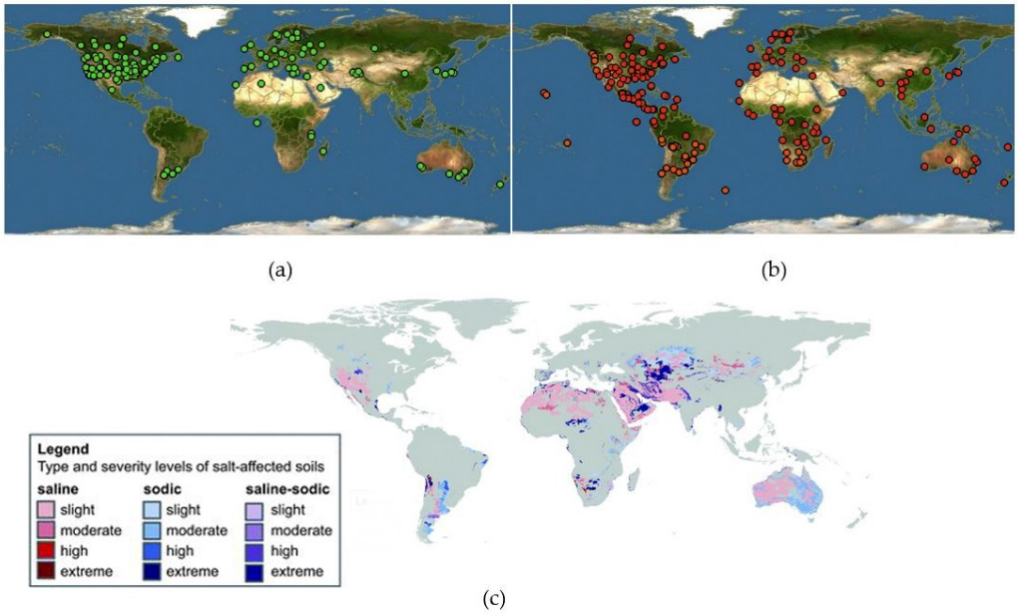


Figure 1. Global distribution of *Amaranthus albus* (**a**) and *A. hybridus* (**b**), modified from Discover Life (https://www.discoverlife.org/20/, accessed on 11 April 2022). Areas affected by salinity worldwide; occurrence of saline, sodic and saline-sodic soils (**c**) according to the Encyclopedia of the Environment (https://www.encyclopedie-environnement.org/en/zoom/land-salinization/, accessed on 11 April 2022).

The aim of this work was to compare the tolerance to salinity of *A. albus* and *A. hybridus*, collected from loquat fields they invade in the region of Valencia, Spain. For this purpose, we analysed (a) the effects of salt, in a range from 0 to 300 mM NaCl, on seed germination capacity and rate, and (b) the effects of salt concentrations, ranging from 0 to 600 mM NaCl, in aq. solution on growth parameters and on the accumulation of photosynthetic pigments (chlorophyll a, b and carotenoids) in these species.

2. Materials and Methods

2.1. Seed Germination

Seeds were germinated in standard 9 cm Petri dishes on filter paper moistened with 5 mL of water (control) or aqueous solutions of increasing NaCl concentration (50, 100, 150, 200, 250 and 300 mM NaCl). For each treatment, 100 seeds were sown in 5 Petri dishes (20 seeds per plate), each plate being considered a replicate. Plates were sealed with parafilm to avoid evaporation and were incubated in a growth chamber (model EGH1501HR from Equitec, Madrid, Spain) at 30 °C during 16 h light and 20 °C during 8 h darkness. The number of germinated seeds, measured as seeds with radicle emergence, was

registered every two days along a two-week period. Germination capacity was expressed as the percentage of germination and the germination rate as MGT (mean germination time) and calculated according to the formula:

$$MGT = \Sigma (D \times n)/N$$

where D represents the days from the beginning of the germination test, n is the number of seeds newly germinated on day D, and N is the total seeds germinated at the end of the experiment [42].

2.2. Plant Growth and Stress Treatments

Seedlings obtained from germinated seeds in the control treatment (standard Petri dishes with filter paper moistened with 5 mL of water) were transferred to 0.5 L pots (11 cm diameter) containing a substrate mixture of peat, perlite, and vermiculite (2:1:1), placed in plastic trays and watered twice a week with half-strength Hoagland solution [43]. The trays with the pots were maintained in a phytotron under long-day photoperiod (16 h of light and 8 h of darkness) conditions, with temperatures of 23 °C during the day and 17 °C at night. Relative humidity ranged between 50% and 80%. A total of seven plants per condition contained on a tray were watered twice a week for four weeks, those from the control treatment with half-strength Hoagland nutrient solution added to the trays (1.5 L per tray), and plants from the salt stress treatments with the same volume of the nutrient solution containing NaCl at 150, 300, 450 and 600 mM final concentrations. The trays were washed and dried before each irrigation, to avoid excessive accumulation of salts. After four weeks of treatments, plant material was collected, and the following growth parameters were determined: stem length increase, number of newly formed leaves, leaf area (measured by the program Digimizer v.4.6.1 software, MedCalc Software, Ostend, Belgium, 2005–2016), fresh and dry weight, and percentage of water content, calculated as:

$$WC\% = [(FW - DW)/FW] \times 100$$

2.3. Substrate Analysis

Substrate moisture was measured following the gravimetric method at the end of the treatments. A sample of each pot was weighed (SW), dried in an oven at 105 °C until reaching constant weight, and then weighed again (DSW). Soil water content was calculated as:

$$\text{Soil moisture (\%)} = [(SW - DSW)/SW] \times 100$$

The substrate's electrical conductivity ($EC_{1:5}$) was measured after four weeks of treatment. Samples were air-dried and then passed through a 2-mm sieve. A soil: water (1:5) suspension was prepared using deionised water at 20 °C and mixed for one hour at 600 rpm. Electric conductivity was determined using a Crison Conductivity meter 522 and expressed in dS m^{-1}.

2.4. Photosynthetic Pigments

Chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Caro) were determined following a previously described method [44]. Fresh leaf material (0.05–0.10 g) was ground in the presence of liquid nitrogen. One ml of ice-cold 80% acetone was added to the sample, which was shaken overnight in the dark at 4 °C. After a 10 min centrifugation at 13,300× *g* and 4 °C, the supernatants were collected, and the absorbance was measured at 470, 646 and 663 nm. The following equations were used for the calculation of pigment concentrations [44]:

$$\text{Chl a } (\mu g/mL) = 12.21 \times (A_{663}) - 2.81 \times (A_{646})$$

$$\text{Chl b } (\mu g/mL) = 20.13 \times (A_{646}) - 5.03 \times (A_{663})$$

$$\text{Caro } (\mu g/mL) = (1000 \times A_{470} - 3.27 \times [\text{Chl a}] - 104 \times [\text{Chl b}])/227$$

Chlorophyll and carotenoid contents were finally expressed in mg g^{-1} FW.

2.5. Statistical Analysis

Data were analysed using Statgraphics Centurion XVI (Statgraphics Technologies, The Plains, VA, USA). A Levene test was applied to check whether analysis of variance (ANOVA) requirements were accomplished. Germination percentages were normalised by arcsine transformation prior to the analysis of variance. Significant differences between treatments were tested by one-way analysis of variance (ANOVA) at the 95% confidence level, and post hoc comparisons were made using Tukey's HSD test at $p < 0.05$. All mean values throughout the text are followed by SE. A multivariate analysis, including a Principal Component Analysis (PCA) and Pearson's Moment-Product Correlations, was performed with all parameters measured in the substrate and in the plants.

3. Results

3.1. Effects of Salt Stress on Seed Germination

All seeds tested germinated within two weeks under control conditions in *A. albus* (Figure 2a) but only 68% in *A. hybridus* (Figure 2b). A reduction down to 48% on the germination rate of *A. albus* seeds, however, was registered when salt was added to the moisture solution at 200 mM, and only 4% of the seeds germinated under 250 mM NaCl (Figure 3a). On the other hand, salinity produced a significant reduction of *A. hybridus* seeds down to 42% when 100 mM NaCl was added to the moisture solution. Higher salt concentrations of 150 and 200 mM resulted in a decrease in seed germination down to 22% and 6%, respectively, and germination was completely prevented in this species under 250 mM NaCl (Figure 3a). For both species, at 300 mM of NaCl solution applied to filter paper, no seed was able to germinate (Figure 2a,b).

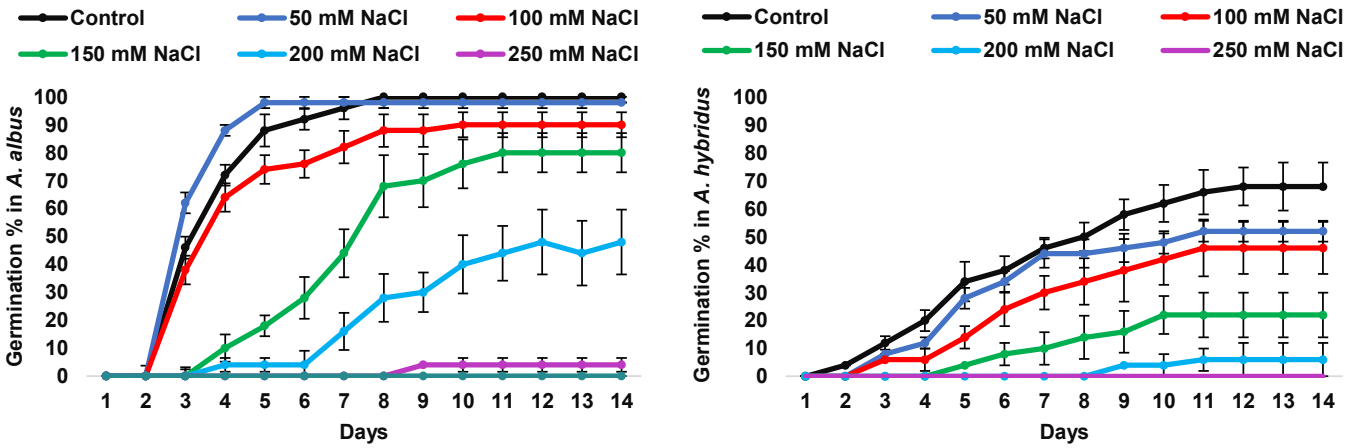
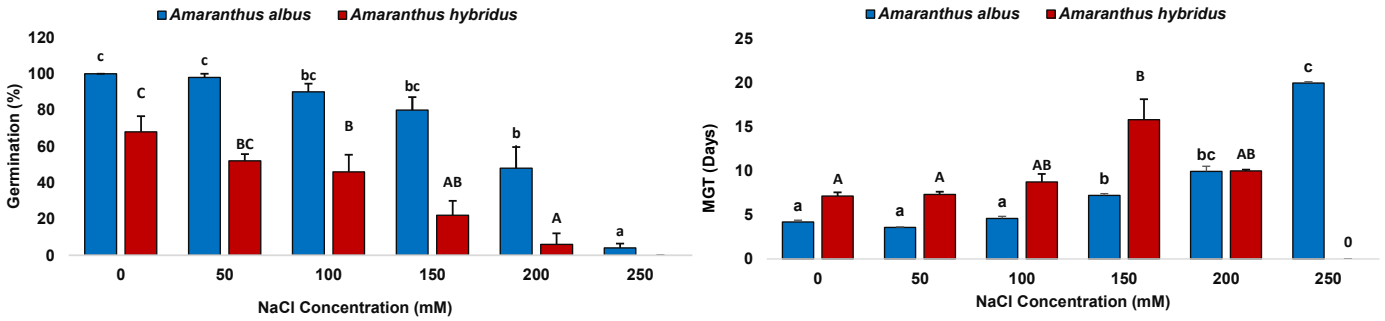


Figure 2. Germination percentage during 14 days in the presence of increasing concentrations of NaCl in *Amaranthus albus* (**a**) and *A. hybridus* (**b**).

It was observed for both species that the time seeds need to germinate under different salt concentrations measured by the mean germination time (MGT) was significantly higher at 150 mM NaCl when compared to control conditions, though this increase was clearly more pronounced in *A. hybridus*. At 250 mM NaCl, only the seeds of *A. albus* germinated, and the rate of germination reached the highest value (Figure 3b).

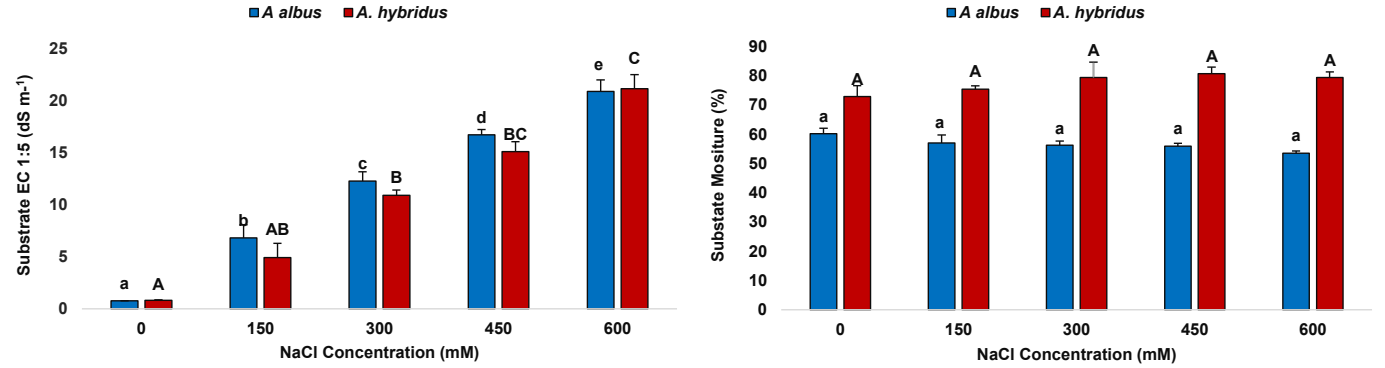


(**a**) (**b**)

Figure 3. Final germination percentages after 14 days (**a**) and mean germination time (MGT) (**b**) in *Amaranthus albus* and *A. hybridus*, as indicated in each case. Means ± SE, $n = 5$. Same letters indicate homogeneous groups between treatments for each species, according to the Tukey test ($\alpha = 0.05$). Lower-case letters were used for *A. albus* and capital letters for *A. hybridus*.

3.2. Substrate Analysis

Substrate electrical conductivity ($EC_{1:5}$) and moisture were analysed after one month of treatment when plant material was sampled at the end of the experiment. $EC_{1:5}$ increased significantly with higher NaCl concentrations for both species and reached values higher than 20 dS m^{-1} when water solution was supplemented with 600 mM NaCl (Figure 4a). In contrast, there were non-significant differences in moisture levels between the control and the substrates irrigated with different salt concentrations in the two species (Figure 4b). Interestingly, registered substrate moisture was higher in *A. hybridus* than that of *A. albus* for all salt concentrations tested (Figure 4b).



(**a**) (**b**)

Figure 4. Electric conductivity ($EC_{1:5}$) (**a**) and substrate moisture (**b**) measured after one month treatment with the indicated NaCl concentrations. Means ± SE, $n = 7$. Same letters indicate homogeneous groups between treatments for each species, according to the Tukey test ($\alpha = 0.05$). Lower-case letters were used for *A. albus* and capital letters for *A. hybridus*.

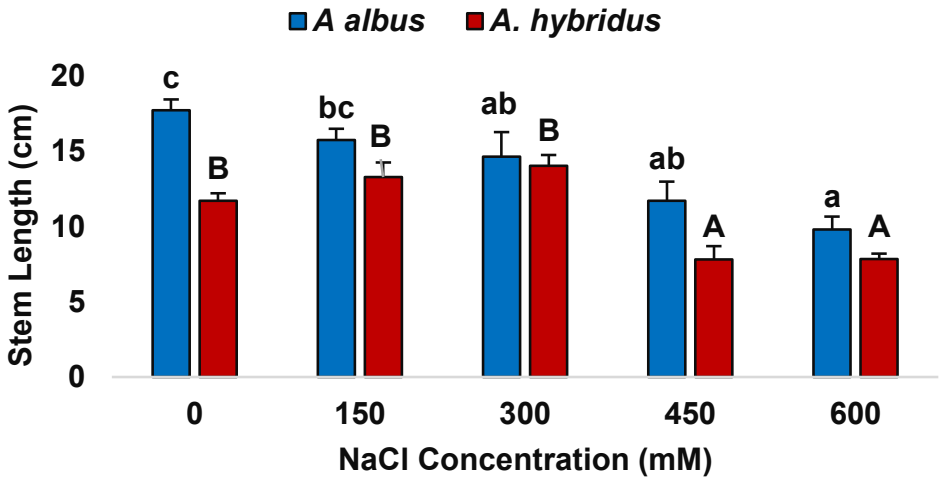
3.3. Effects of Salinity on Plant Growth

Although growth was inhibited by saline treatments in both species, these responded somewhat differently, as shown by the two-way ANOVA (Table 1). The effect of the factor "Species" was significant in all growth parameters except root weight, and the effect of the factor "Treatment" was also significant in all growth parameters except leaf water content. The interaction of the two factors ("Species × Treatment") was significant for the following parameters: leaf number, fresh weight of leaves and water content of leaves.

Table 1. Factorial ANOVA (F values) considering the effect of Species (A) and Treatment (B) and their interaction (A × B) on growth parameters and photosynthetic pigments in *Amaranthus albus* and *A. hybridus*. Asterisks indicate the degree of significance: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$, ns = not significant.

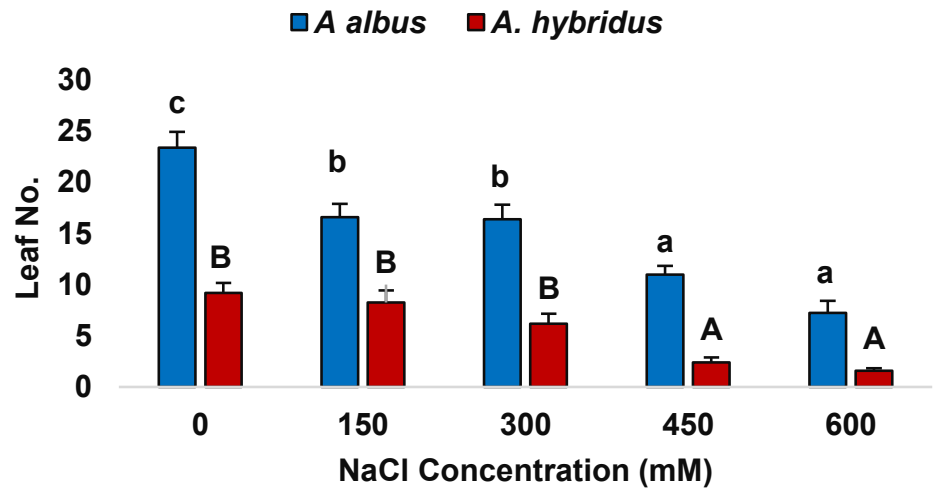
Variable	Factor A (Species)	Factor B (Treatment)	Interaction A × B
Stem Length	22.13 ***	16.65 ***	2.15 ^{ns}
Leaf no.	197.210 ***	40.45 ***	4.50 **
Leaf Area	12.10 **	3.73 *	2.25 ^{ns}
Leaf Dry Weight	65.40 ***	7.82 ***	4.41 **
Root Dry Weight	3.30 ^{ns}	5.29 ***	1.95 ^{ns}
Leaf Water Content	18.33 ***	0.9 ^{ns}	3.26 *
Root Water Content	7.45 ***	3.45 *	1.56 ^{ns}
Chlorophyll a	0.657 ^{ns}	3.68 *	3.59 *
Chlorophyll b	5.97 ^{ns}	1.06 ^{ns}	1.29 ^{ns}
Carotenoids	5.39 ^{ns}	10.97 ***	1.3 ^{ns}

For both species, the average stem length did not vary significantly under 150 mM NaCl treatment, nor did it vary for *A. hybridus* watered with 300 mM NaCl solution (Figure 5a). A stronger reduction was registered in plants under 450 and 600 mM NaCl treatments, reaching under the latter a reduction of 1.8-fold and 1.4-fold for *A. albus* and *A. hybridus*, respectively. Salt stress also caused a reduction in the number of leaves (Figure 5b), although *A. albus* was shown to be more susceptible, since it had already decreased significantly under the lowest concentration of salt. On the contrary, the effect of salt treatment on *A. hybridus* was only observed under 450 mM of NaCl and higher. Finally, leaf area did not vary significantly in *A. albus* and even slightly increased under 150 and 300 mM NaCl in *A. hybridus* (Figure 5c).

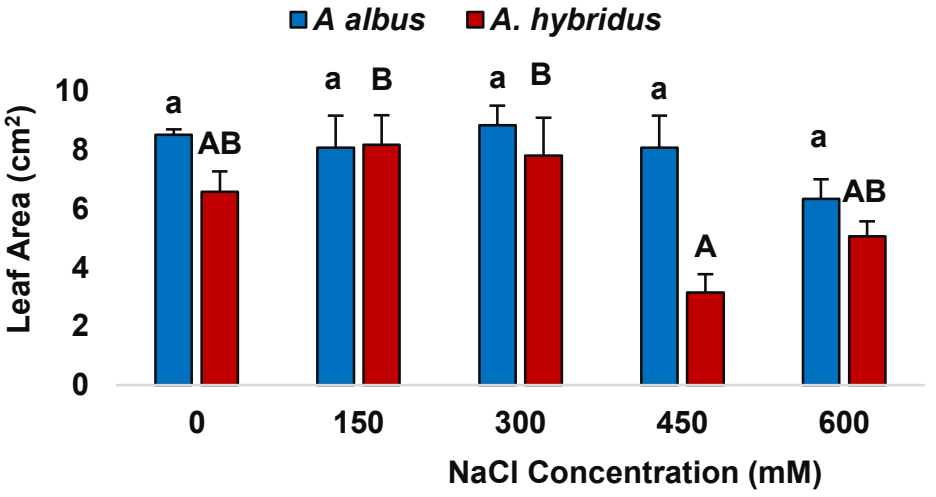


(a)

Figure 5. *Cont.*



(**b**)



(**c**)

Figure 5. Growth parameters for the two *Amaranthus* species after one month of treatments with different NaCl concentrations: stem length (**a**), number of leaves (**b**) and leaf area (**c**). Means ± SE, $n = 7$. Same letters indicate homogeneous groups between treatments for each species, according to the Tukey test ($\alpha = 0.05$). Lower-case letters were used for *A. albus* and capital letters for *A. hybridus*.

The dry weight (DW) of roots and leaves excised from plants watered with the lowest concentration of NaCl were not significantly altered, and specifically for *A. hybridus*, even a small increase, although not statistically significant, was registered for plants watered with solutions of 150 mM and 300 mM NaCl. The dry weight of leaves was significantly reduced in plants watered with 450 mM NaCl solution, and under 600 mM NaCl treatment, a drop of more than 70% for *A. albus* and less than 60% for *A. hybridus* was registered (Figure 6a). The DW of roots was only significantly reduced in the two species in the plants from the 600 mM NaCl treatment in a similar proportion to that of leaves (Figure 6b).

The water content of leaves (Figure 6c) showed only small fluctuations non related to the salt concentrations applied and the water content of roots did not show any variation (Figure 6d).

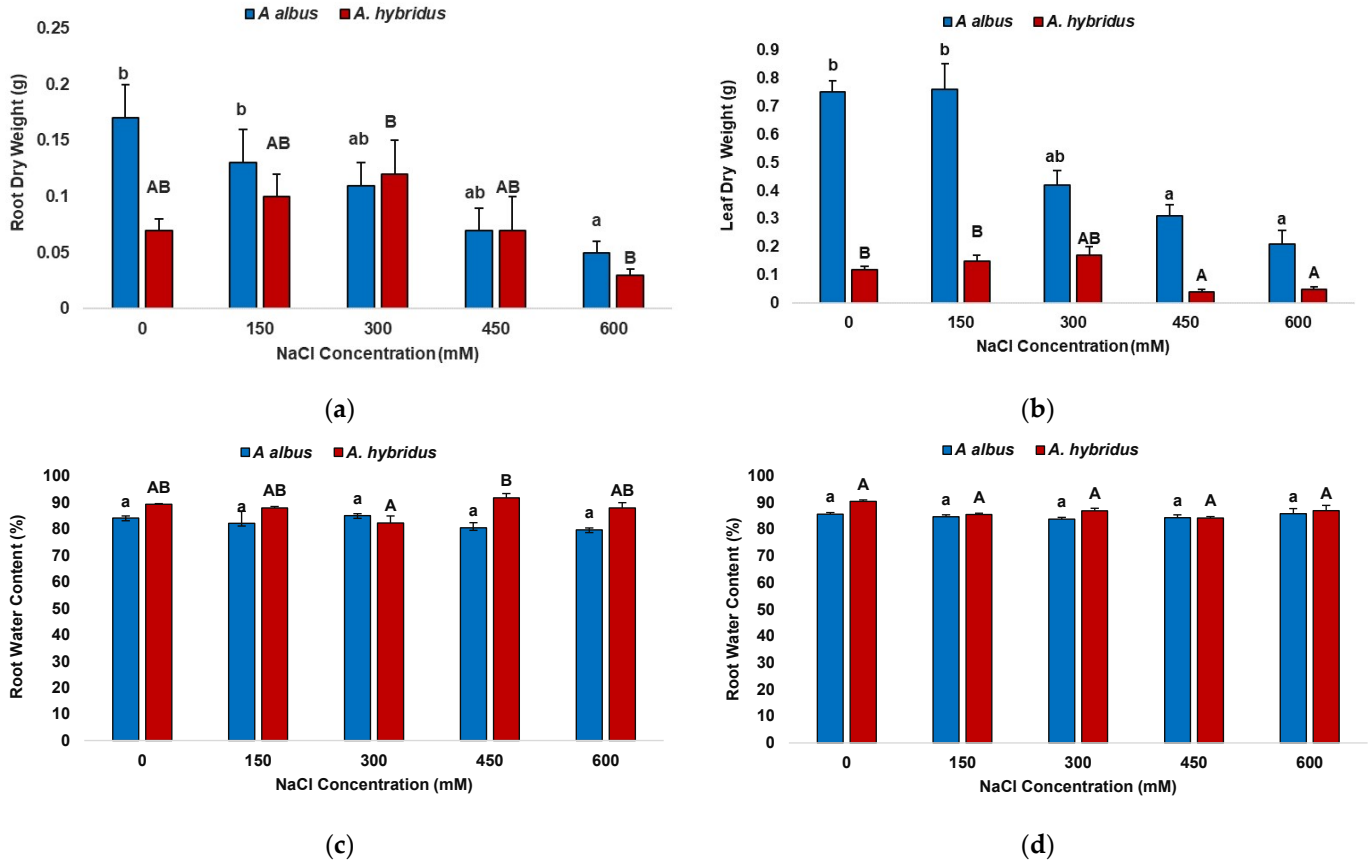
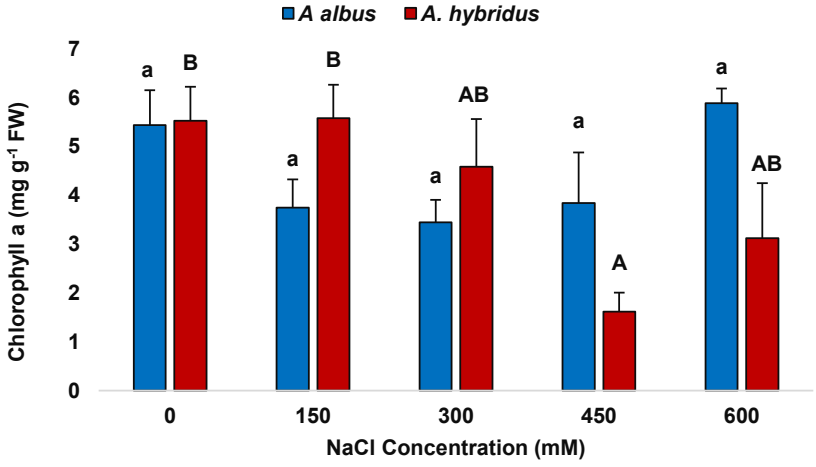


Figure 6. Dry weight of leaves (**a**) and roots (**b**), and water content of leaves (**c**) and roots (**d**) in the two *Amaranthus* species after one month of NaCl treatments. Means ± SE, $n = 7$. Same letters indicate homogeneous groups between treatments for each species, according to the Tukey test ($\alpha = 0.05$). Lower-case letters were used for *A. albus* and capital letters for *A. hybridus*.

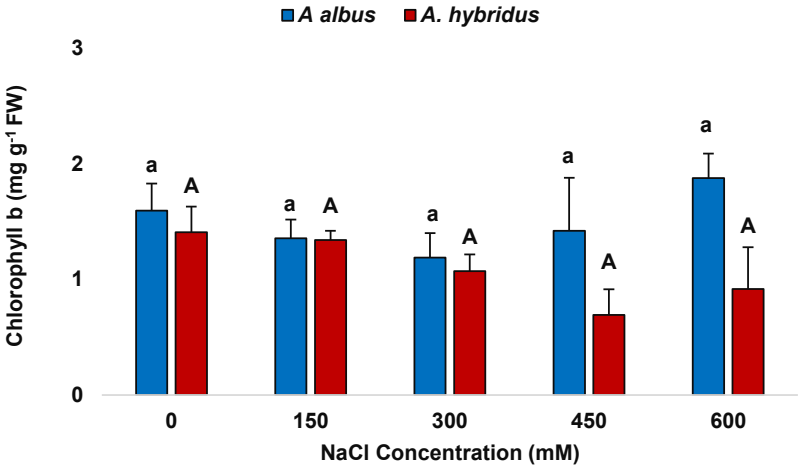
The contents of photosynthetic pigments in leaves were also determined. In *A. albus*, no significant differences were found between treatments in chlorophyll a and b concentrations. In *A. hybridus*, a significant reduction in chlorophyll a was found in plants subjected to 400 mM NaCl treatment, whereas chlorophyll b remained unchanged (Figure 7a,b). Total carotenoids decreased with increasing salt concentrations in the two species, but the reduction was significant only for *A. hybridus* plants watered with 450 and 600 mM NaCl solution (Figure 7c).

3.4. Multivariate Analysis of the Growth Parameters

Pearson moment correlations were performed for all measured parameters in plants of both species as well as the electroconductivity and moisture of their corresponding substrates (Figure 8). As expected, strong positive correlations were found between the growth parameters tested, such as stem length (SL), number of leaves (Lno), leaf area (LA), dry weight of leaves (DWL) and roots (DWR), whereas negative correlations were observed between these growth parameters and the EC of the substrate. Among the photosynthetic pigments, only carotenoids showed positive correlation with growth parameters, and a negative correlation with the EC of the substrate.



(**a**)



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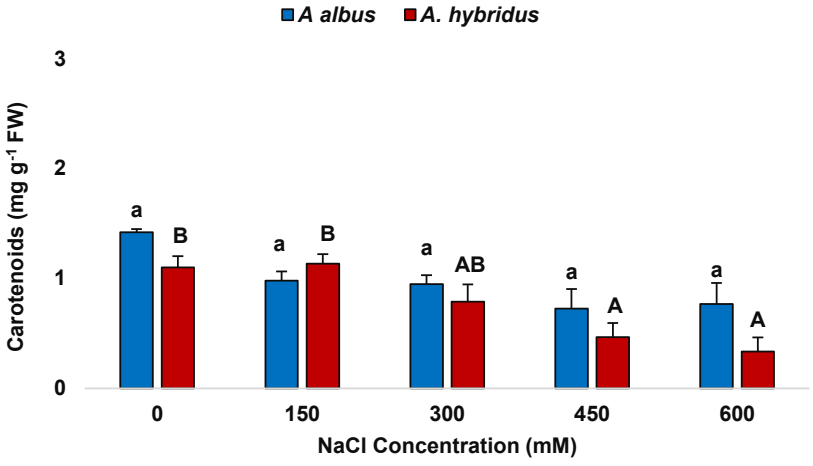

(**c**)

Figure 7. Concentration of chlorophyll a (**a**), chlorophyll b (**b**) and carotenoids (**c**) in the two *Amaranthus* species after one month of NaCl treatments. Means ± SE, $n = 7$. Same letters indicate homogeneous groups between treatments for each species, according to the Tukey test ($\alpha = 0.05$). Lower-case letters were used for *A. albus* and capital letters for *A. hybridus*.

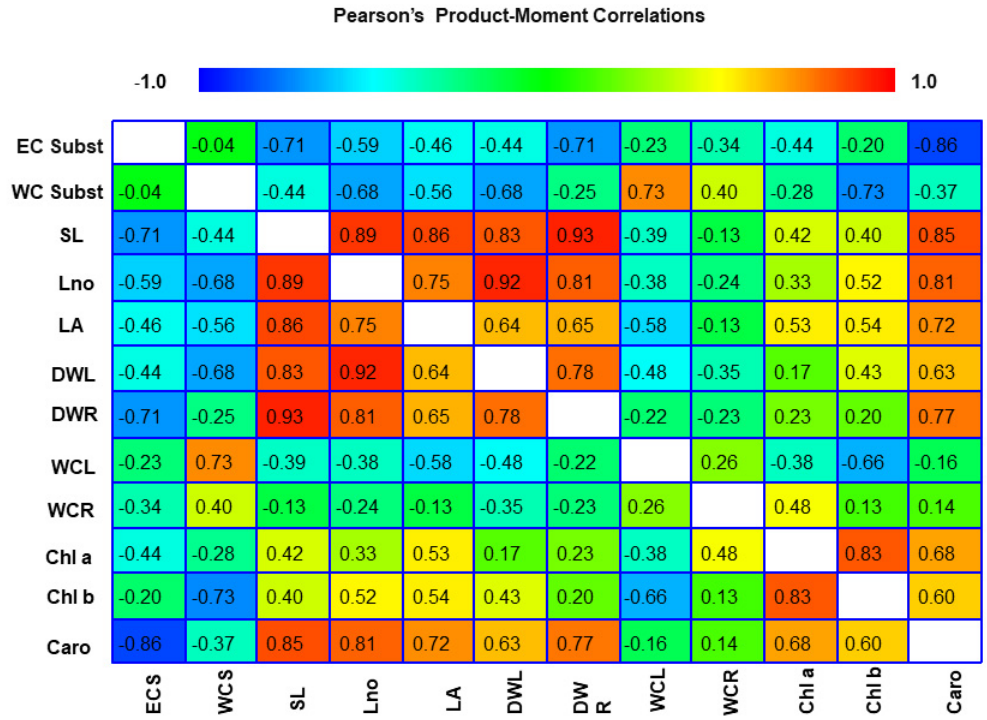

Figure 8. Pearson's correlations between electrical conductivity of the substrate (EC subst), substrate water content (EC subst), stem length (SL), number of leaves (Lno), leaf area (LA), fresh weight of leaves (FWL) and roots (FWR), water content of leaves (WCL) and roots (WCR), chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Caro).

All parameters analysed were subjected to a Principal Component Analysis (PCA). Two components with eigen values higher than one were found (Figure 9). The first component, which explains 59.78% of the total variability, correlates positively with plant growth parameters and negatively with substrate EC, while the second component, which explains an additional 21.56% of the variability, also correlates negatively with EC, but positively with substrate moisture. Except for leaf and root water content, which were closely related to substrate moisture, and separated along the second component, all other growth parameters fall on the positive side of the PCA1 axis. The PCA revealed a good separation of the two species. In *A. albus,* all scores fell on the positive side of PCA1, while *A. hybridus*'s scores fell on the negative side, except for the 100 mM NaCl treatment. The PCA2 axis separated the scores of *A. hybridus* better than those of *A. albus*, with control, 150 and 300 mM on its positive dimension and 450 and 600 mM on the negative side.

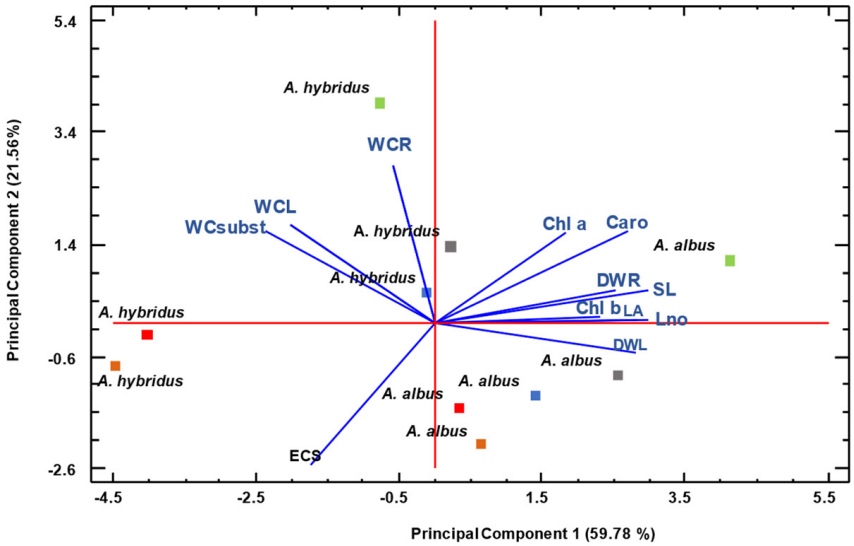

Figure 9. Principal Component Analysis (PCA) performed with mean values of substrate parameters (EC and water content) and parameters measured in plants: number of leaves (Lno), stem length (SL), leaf area (LA), dry weight of roots (DWR) and leaves (DWL), water content of roots (WCR) and leaves (WCL), chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Caro). Colours used for the treatments: control in green, 150 mM NaCl in grey, 300 mM NaCl in blue, 450 mM NaCl in red and 600 mM NaCl in brown.

4. Discussion

Seed germination represents the most susceptible stage in the biological cycle of plants, which is severely affected by adverse environmental conditions. Salinity drastically reduces or completely hinders seed germination in crops [7]. The osmotic component of the salt stress induces a decrease in the water uptake by dry seeds, negatively affecting the process of imbibition necessary for germination. Salinity is also associated with the toxicity of Na^+ and Cl^-, which, in excess, disrupt nucleic acid and protein metabolism, energy production and respiration, promoting the accumulation of excess reactive oxygen species (ROS), which damage macromolecules and cellular structures [45]. The process of germination is regulated by plant hormones, such as gibberellin (GA) and abscisic acid (ABA) [46]. Whereas ABA promotes seed dormancy and inhibits germination, GA has an opposite effect, stimulating germination by disrupting dormancy [7]. Under saline conditions, the balance of these plant hormones is also altered, triggering additional negative effects in seed germination.

In halophytes, or salt tolerant plants, the maximum salt tolerance for seed germination to occur has been reported as varying from 1.7 to 0.26 M NaCl depending on the halophyte species and other environment conditions [7]. However, the optimal seed germination of halophytes species occurs mostly in the absence of stress [47], and in the natural environments is usually produced after periods of intense rainfalls, when soil salinity is alleviated [48]. Amaranthaceae includes taxa that were reported as the most salt tolerant at the germination stage. Germination under NaCl concentrations above 1 M was reported in several genera, such as *Salicornia* [49], *Suaeda* [50] and *Sarcocornia* [51]. In quinoa, a strong inhibition of germination was found under 300–500 mM NaCl conditions, but in some cultivars no effect on germination was observed upon 300 mM NaCl treatment as compared to control conditions [21]. Amaranths were also reported as tolerant to low and moderate salinity during germination, although there is a large variability, sometimes even among genotypes within the same species. The seed germination of the noxious weed *A. retroflexus* from China was higher upon 150 mM NaCl treatment than under control conditions [52], whereas for seeds collected from Queensland (Australia), the germination percentage dropped by 50% at only 100 mM NaCl conditions [53]. Moreover, large varia-

tions in germination percentages under salt were reported in cultivars of edible *A. cruentus* from Benin [26].

Our findings on *A. albus* and *A. hybridus* are supported by previous reports, which indicated *A. albus* to be a more tolerant species during germination than other species [54]. In our experimental conditions, the germination percentage of *A. albus* was not significantly affected by the lowest concentration of salt; it reduced by 10% at 100 mM and it dropped to 48% at 200 mM NaCl. Similarly, seeds of this species, collected in a natural environment in Anatolia (Turkey), did not show any reduction in germination percentage up to 150 mM NaCl, and a reduction of only 20% under 200 mM NaCl [54]. In *A. hybridus*, salinity treatment resulted in a reduction of 14% at 100 mM NaCl, of 42% at 150 and 62% at 200 mM NaCl compared to control. Seeds of the same species from Nigeria, however, showed a reduction in germination of more than 50% at 150 mM NaCl [55].

Salinity affects not only the germination capacity, but also the mean germination time (MGT). The MGT increased significantly in both species starting at the concentration of 150 mM NaCl, especially in *A. hybridus*. This delay in germination is related to the effect of salt on enzymatic activity and to the reduction in water uptake by imbibition, which is essential during germination [7]. Hydrolytic enzymes play an important role in germination, such as α-amylase, which metabolises endosperm starch into sugars necessary for the growth of the embryo [56]. The activity of this enzyme decreases under salt stress, triggering a reduction in the translocation of sugars necessary for the embryo and a change in the osmotic potential, which further decreases water uptake [7,57,58].

Amaranth species are considered as salt tolerant during all stages of their biological cycles [28]. The high salt tolerance of amaranth is associated with a low basal stomatal conductance due to a low stomatal density and aperture, which contributes to the avoidance of leaf water loss under salt stress. This fact, together with their C4 metabolism and other anatomical adaptations, such as a well-developed root system, contribute to the tolerance of multiple types of environmental stress, including salinity and drought [34]. Due to their ability to grow under harsh environmental conditions, grain amaranth species are even regarded, together with quinoa, as pseudocereals of the future [32,59]. The two species we investigated in this work survived as long as one month in salt concentration up to 600 mM NaCl, and a significant reduction in their growth was registered only for the 450 and 600 mM NaCl treatments. Contrary to the results of germination percentages and time of germination, which indicated that *A. albus* is more salt tolerant, during vegetative growth, this species was more susceptible to salinity than *A. hybridus*, as shown by all the growth parameters analysed. Leaf dry weight, which is one of the most reliable parameters when assessing salt tolerance [60], showed a more pronounced reduction in *A. albus* than in *A. hybridus* upon salt treatment. In the latter, photosynthetic pigments did not vary significantly under stress, whereas in *A. albus*, carotenoids suffered a significant reduction. Previous data indicated that the growth of *A. hybridus* seedlings or young plants is stimulated by low or moderate concentrations of salt [55], and some authors even consider this species a halophyte [61]. On the other hand, *A. albus* was reported as highly competitive in saline soils [62].

Multivariate analysis indicated that the growth parameters that correlated best with EC and substrate moisture were leaf and root dry weight and leaf water content, followed by stem length and number of leaves, but not leaf area and root water content. Therefore, the latter parameters are not suitable as indicators of salt tolerance in the two species analysed. Of the photosynthetic pigments analysed, the best negative correlation with substrate EC was that of carotenoids, which may be used as biochemical markers for stress in the two species.

In conclusion, both species appear to tolerate moderate NaCl concentrations, although their performance under salinity was different. When comparing the two species, *A. albus* was found to be slightly more tolerant during germination, while *A. hybridus* was found to be more tolerant during vegetative growth. Their spread as invasive species in many regions is facilitated by their remarkable salt tolerance, which allows them to cope with

harsh environmental conditions. Moreover, since both species, especially *A. hybridus*, are appreciated as green vegetables in different parts of the world, they may represent an interesting crop for saline agriculture.

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