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Appraisal of Salt Tolerance under Greenhouse Conditions of a *Cucurbitaceae* Genetic Repository of Potential Rootstocks and Scions

Giuseppe Carlo Modarelli [1], Youssef Rouphael [1], Stefania De Pascale [1], Gölgen Bahar Öztekin [2], Yüksel Tüzel [2], Francesco Orsini [3,*] and Giorgio Gianquinto [3]

1 Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; giuseppecarlo.modarelli@unina.it (G.C.M.); youssef.rouphael@unina.it (Y.R.); depascal@unina.it (S.D.P.)
2 Department of Horticulture, Faculty of Agriculture, Ege University, 35100 Bornova-Izmir, Turkey; golgen.oztekin@ege.edu.tr (G.B.Ö.); yuksel.tuzel@ege.edu.tr (Y.T.)
3 DISTAL—Department of Agricultural and Food Sciences, Alma Mater Studiorum—University of Bologna, 40127 Bologna, Italy; giorgio.gianquinto@unibo.it
* Correspondence: f.orsini@unibo.it

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Abstract: Soil salinization due to climate change and intensive use of water and soil is increasing exponentially. *Cucurbitaceae* species are cultivated worldwide and the identification of salinity tolerant genotypes to be used as rootstock or scion for securing yield stability in salt affected agricultural areas is a research priority. In the present greenhouse study, we assessed the response to salinity (0 mM a non-salt control and 150 mM NaCl dissolved in the nutrient solution) in the seedlings of 30 genotypes of cucurbits grown in a floating hydroponic system. The species tested included 16 genotypes of *Cucumis melo* L. (CM1-16), 6 *Citrullus vulgaris* Schrad. (CV1-6), 2 interspecific hybrids of *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch. (CMM-R1 and 2), 4 bottle gourd (*Lagenaria siceraria* (Molina) Standl. (LS1-4)), 1 *Cucurbita moschata* Duch. (CMO51-17), and 1 luffa *(Luffa cylindrica* Mill. (LC1)) species. Results highlighted different morphological and physiological traits between the species and genotypes and a different response to salt stress. We identified *C. maxima* × *C.moscata* interspecific hybrid CMM-R2, melon genotypes CM6, CM7, CM10, and CM16 together with watermelon genotypes CV2 and CV6 and bottle gourd LS4 as salt tolerant genotypes and possible candidates as salt resistant rootstock to be introduced in grafting programs.

Keywords: NaCl; *Citrullus vulgaris* Schrad; *Luffa cylindrica* Mill; *C. maxima* Duch. × *C. moschata* Duch.; seedlings; morpho-physiological traits; grafting

1. Introduction

Soil and water resource salinization is one of the main abiotic stress factors that reduce plant growth and crop productivity worldwide [1]. It has been estimated that the total land affected by salinization covers approximately 412 million ha and mainly occur in arid and semiarid regions of more than 100 countries in all continents [2]. Generally, a saline environment influences every aspect of crop physiology and growth by causing water deficits due to the low water potential in the root medium, plant toxic ions uptake (e.g., Na^+, Cl^-, SO_4^{2-}), reduction in the uptake/or transport to the shoot of K^+, Ca^{2+}, and Mg^{2+} [1]. Moreover, plants grown in saline environments experience reduction of photosynthetic capacity [3] and growth in terms of number of leaves, shoot length, decrease in photosynthetic pigment content due to the negative effect of Na solutes within plant cells, and a decrease in fresh and dry matter content [4].

Breeding programs to combine salt resistance traits from different germplasm are increasing among seed companies. However, they currently require complicated evaluation and result in delays in the release of new varieties in the market due to the complexity of the salinity traits [5].

Furthermore, plant responses to salinity derive from complex and multifaceted mechanisms [6] and salt tolerance traits involve several physiological and genetic features that overall limit the success of resistance and/or tolerance trait transfer into commercial varieties [1]. To reduce or avoid production losses due to salt stress, a sustainable and fast solution is grafting highly productive genotypes onto potentially salt tolerant rootstocks [1]. Vegetable grafting is a widely used technique in Japan, Korea, the Mediterranean basin, as well as in several European countries to avoid biotic (i.e., soilborne and foliar pathogens, weeds, and arthropods) or abiotic stressors like drought, flooding, heavy metals contamination, sub optimal temperatures, nutritional deficiencies, and salinity. The salt tolerance of grafted plants is influenced by both scion and rootstock [6]. Generally, the use of salt tolerant rootstocks allows the mitigation of the detrimental effects of salinity and guarantees stable yields during the growing cycle through specific morphological, biochemical, metabolic, and physiological mechanisms. Accordingly, under saline conditions, grafted plants tend to accumulate more biomass in the root system, thus allowing for mitigated salinity effects by increasing the root/shoot ratio [1,7–9]. Additionally, to cope with saline stress, grafted plants adopt strategies like salt exclusion in the shoot and retention of salt ions in the root system [1,7]. Besides these strategies, grafting promotes, at the cellular level, a better maintenance of potassium homeostasis, together with accumulation of compatible solutes and osmolytes in the cytosol, along with compartmentation of salt ions in the vacuole through the activation of the antioxidant defense system, and induction of hormones mediated changes in plant growth [1,7,10,11].

Cucurbitaceae species like melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), and watermelon (*Citrullus vulgaris* Schrad.) are generally considered salt sensitive or moderately sensitive crops, while being often cultivated in areas undergoing soil and water salinization [12,13]. In cucurbits, grafting commercial varieties into salt tolerant rootstocks was shown to reduce production losses by improving their photosynthetic capacity [1,3]. Watermelon is commonly grafted on bottle gourd (*Lagenaria siceraria* (Molina) Standl.), interspecific hybrids between *C. maxima* and *C. moschata*, and wild watermelon (*Citrullus* spp.) [14]. Cucumber is generally grafted on bottle gourd, luffa (*Luffa cylindrica* Mill.), *Cucurbita* interspecific hybrids, and *Cucumis* spp [15]. Finally, melon is generally grafted on interspecific hybrids between *C. maxima* Duch. and *C. moschata* Duch. and *Cucumis melo* L. rootstocks and in some cases on luffa [5]. However, their response to salinity, as rootstock vary between the genotypes, and completed screening to identify salt tolerant rootstock varieties to be adopted in commercial grafting program have not yet been carried out.

In the light of these observations, our aim was to evaluate the response to salinity stress in different melons (*Cucumis melo* L.), watermelon (*Citrullus vulgaris* Schrad.), interspecific hybrids of *C. maxima* Duch. × *C. moschata* Duch., bottle gourd (*Lagenaria siceraria* (Molina) Standl.), *Cucurbita moschata* Duch. *cv* Plovdivski 51–17, and luffa *(Luffa cylindrica* Mill.) *Cucurbitaceae* rootstock and scion genotypes, in terms of plant growth and photosynthetic pigments to identify salt tolerant genotypes to be used in commercial grafting programs.

2. Materials and Methods

2.1. Growth Conditions, Plant Material, and Salinity Treatments

The experiment was carried out in a polyethylene covered double span nursery greenhouse at the Department of Horticulture, Faculty of Agriculture, Ege University (38°27′16.2′′ N, 27°13′17.8′′ E) in Bornova, Izmir Turkey during the spring 2014 growing season.

Plant material included 16 melon varieties (*Cucumis melo* L.), 6 watermelons (*Citrullus vulgaris* Schrad.), 2 interspecific hybrids of *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch., 4 bottle gourd (*Lagenaria siceraria* (Molina) Standl.) varieties, 1 luffa (*Luffa cylindrica* Mill.) that were obtained

from the Aegean Agricultural Research Institute Department of Biodiversity and Genetic Resources (Menemen, Izmir-Turkey) and 1 *Cucurbita moschata* Duch. *cv* Plovdivski 51–17 obtained from the "Maritsa" Vegetables Crops Research Institute (Plovdiv, Bulgaria) (Table 1). Two salt treatments were considered, by applying 0 (non-saline control) and 150 mM of sodium chloride (NaCl) dissolved in the nutrient solution. Eighty seeds per genotype were sown in polystyrene trays in a mixture of peat and perlite (75:25 *v:v*) on 5 May 2014 and placed in a germination room for three days (temperature of 24 °C, 60% RH) and then placed in an unheated nursery greenhouse (mean daily temperature of 28 °C) for two weeks. Seedlings were fertigated with commercial fertilizer twice per day until the start of the experiment.

On 22 May 2014, forty seedlings of each species/genotypes were divided per each salinity treatment and placed in separate floating hydroponic systems. The nutrient solution was composed as follows: nitrate 13.14 mM, phosphorus 0.94 mM, potassium 5.83 mM, calcium 3.79 mM, iron 35.8 µM, boron 37 µM, copper 1.6 µM, molybdenum 0.5 µM. The electrical conductivity (EC) of the nutrient solution was 2.0 dS m^{-1}. The nutrient solution was replaced every two days and aerated with air pumps. In the saline treatment (150 mM NaCl), salinity stress was induced by progressively dissolving 50 mM of NaCl every two days in the nutrient solution until the final concentration of 150 mM was achieved within one week.

Table 1. List of the tested species and the relative genotypes used in this study.

Species	Genotype		Species	Genotype	
	Original Code	Working Code		Original Code	Working Code
Cucumis melo L.	TR31586	CM1	*Citrullus vulgaris* Schrad.	TR40374	CV1
	TR40563	CM2		TR64141	CV2
	TR43722	CM3		TR43211	CV3
	TR45883	CM4		TR66066	CV4
	TR47822	CM5		TR43342	CV5
	TR48527	CM6		TR80748	CV6
	TR48611	CM7	*Lagenaria sicerania* (Molina) Standl.	Macis	LS1
	TR49583	CM8		TR62066	LS2
	TR51531	CM9		TR79616	LS3
	TR51763	CM10		TR82049	LS4
	TR61583	CM11	*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.	Nun 9075	CMM-R1
	TR61626	CM12		RS841	CMM-R2
	TR61851	CM13	*Cucurbita moschata* Duch.	Plovdivski 51-17	CMO 51-17
	Kirkagac	CM14	*Luffa cylindrica* Mill.		LC1
	Arava	CM15			
	Cesme	CM16			

2.2. Plant Growth Measurements

Plant growth was measured when salinity treatment reached its final concentration of 150 mM (12 days after the start of the NaCl treatment, at 29 days after sowing, DAS) on 3 plants per *Genotype* × *NaCl concentration* combination measuring the number of leaves, the shoot diameter, recorded with an electronic caliper, shoot and root length with a ruler. Shoot, root, and leaf fresh weight (FW) were recorded with an electronic balance and dry weight (DW) was recorded after drying the samples for 48 h at 70 °C. Dry matter (DM) was calculated as percentage of DW/FW.

2.3. Photosynthetic Pigments Determination

Chlorophyll *a*, *b* and carotenoid contents were determined at 29 DAS on 3 plants per each *Genotype* × *NaCl concentration* combination by grinding 250 mg of leaf tissue from fully expanded leaves with quartz crystals in 30 mL of acetone (80% in vol). Leaf extracts were read at 450, 645, and 663 nm with a

Varian Carry 100UV-Vis spectrophotometer (Varian Inc, Palo Alto, CA, USA), pigments content was calculated according to Arnon [16] and values were expressed as mg g^{-1} FW.

2.4. Electrolyte Leakage Analysis

Electrolyte leakage (i.e., membrane permeability) was measured at 29 DAS collecting three leaf disks (10 mm diameter each) from fully expanded leaves on 3 plants per *Genotype* × *NaCl concentration* combination and immersed in 50 mL of distilled water. EC was measured immediately (1 min -) (EC_1) and after 60 min of shaking (EC_{60}). The samples were then autoclaved (121 °C) for 25 min, and total conductivity (EC_T) of bathing solution was measured after cooling. Electrolyte leakage was calculated and expressed as a percentage (%) according to Blum and Ebercon [17] using the following Equation:

$$\text{Electrolyte leakage (\%)} = (EC_{60} - EC_1)/EC_T.$$

2.5. Statistical Analysis

A completely randomized design was adopted for the experiment. The experiment was conducted on a total of 40 plants per *Genotype* × *NaCl concentration* combination and each analysis was carried out on at least 3 plants per *Genotype* × *NaCl concentration* randomly selected. Data were analyzed by ANOVA using SPSS 25 software package (www.ibm.com/software/analytics/spss) and means were compared using Duncan post hoc test ($p \leq 0.05$). The cluster heatmap was generated using the ClustVis online software [18] using Euclidean distance as the similarity measure and hierarchical clustering with complete linkage on the genotype percent variation, $\log_{(x+1)}$ transformed, between 150 and 0 mM of NaCl on all the analyzed parameters. The principal component analysis (PCA) was conducted using the Primer 6 software package (PRIMER-e, Albany, New Zealand) on the percentage variation of all the morphological and physiological analyzed parameters to highlight differences between genotypes to the increase of NaCl concentration in the nutrient solution. Five components were extracted by the PCA analysis. The PCA output includes treatment component scores as well as variable loadings.

3. Results

3.1. Plant Growth and Biomass Production

The statistical analysis revealed intrinsic genotypic differences, and a genotypic specific response to the NaCl concentration in the nutrient solution in terms of number of leaves, shoot diameter, shoot length, and root length (Table 2). Compared to non-saline seedlings, under 150 mM NaCl, the number of leaves decreased significantly only in the melon genotype CM5 (−31%) while it did not vary significantly in the other genotypes (Figure 1a). Shoot diameter between the genotypes grown under 0 mM NaCl was higher in the interspecific hybrid CMM-R1 (on average 4.6 mm) and bottle gourd genotypes LS1, LS3, and LS4 (on average 4.66, 4.73, and 4.73 mm respectively), whereas bottle gourd LS2, watermelon CV3, and melon (CM9, CM10, CM14, and CM15) seedlings turned out to be thinner (Figure 1b). The genotype and the NaCl concentration significantly affected shoot diameter (Table 2); as a result, 150 mM of NaCl increased shoot diameter by 47% and 36% in CM10 and CV3 genotypes, respectively, while it decreased by −26% in CMM-R1 *C. maxima* × *C. moscata* hybrid and it did not vary in the other genotypes (Figure 1b). Shoot length was significantly affected by the genotypes (Table 2). Particularly, among seedlings grown in the absence of salt stress, melon genotypes (CM3, CM7, CM8, CM9, CM13, and CM16) produced the longest shoots, while the shortest were observed in bottle gourd LS2, watermelon (CV3, CV4, CV5, CV6) and the interspecific hybrids CMM-R1 and CMM-R2. (Figure 1c). A genotypic response to the NaCl concentration was also observed in shoot length (Table 2). Accordingly, compared to seedlings grown under 0 mM NaCl, shoot length in saline grown seedlings decreased significantly in all the tested genotypes except for the watermelon (CV3, CV4, and CV6), bottle gourd (LS1, LS2) genotypes, and the luffa (LC1) (Figure 1c). Seedling root length was significantly different among the genotypes. In fact, under 0 mM NaCl, melon CM5 developed the

longest root while melon CM16 and watermelon CV3 the shortest (Figure 1d). On the other hand, when 150 mM NaCl was applied, no significant differences in root length compared to control conditions were observed in the studied genotypes.

Table 2. Leaf number (*n* plant^{-1}), shoot and root length (cm), shoot diameter (mm), shoot and root dry weight (DW, g plant^{-1}), and dry matter content (DM, %) in seedlings of sixteen *Cucumis melo* L., six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. grown at 0 or 150 mM of NaCl. (1 week after the beginning of the treatment). Non significance or significance differences at $p \leq 0.05$, 0.01, or 0.001 are indicated as: ns, ** and *** respectively.

	Leaf Number	Shoot Length	Shoot Diameter	Root Length	Shoot DW	Root DW	Shoot DM	Root DM
Genotype (G)	**	***	**	**	**	**	**	**
Salt (NaCl)	**	**	ns	ns	**	ns	**	ns
G × NaCl	**	**	**	***	**	**	**	**

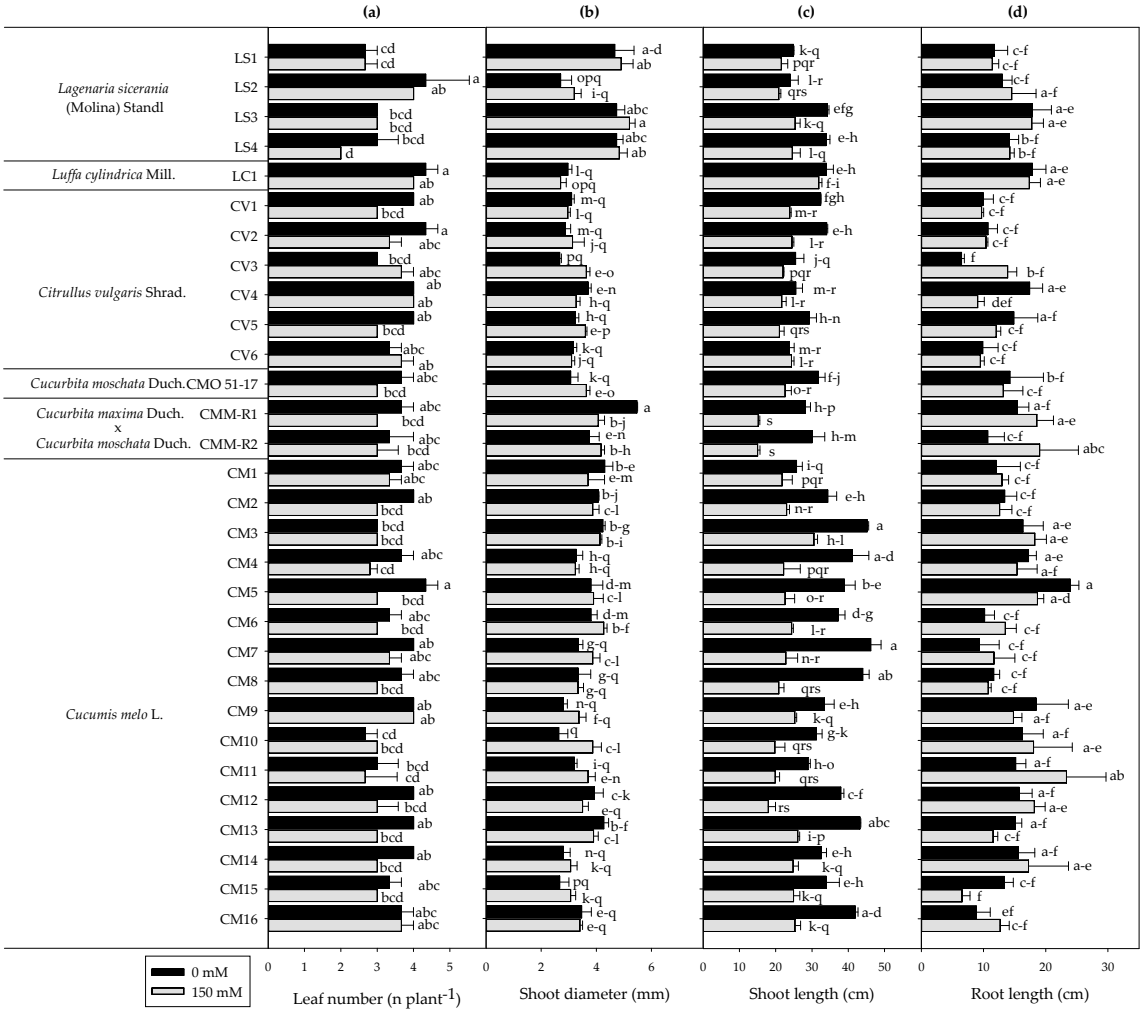

Figure 1. Plant growth, after one week of NaCl treatment, in term of number of leaves (**a**), shoot diameter (**b**), shoot length (**c**), and root length (**d**) in seedlings of sixteen *Cucumis melo* L, six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. Mean values ± standard errors; $n = 3$ followed by different letters within each parameter are significantly different based on Duncan post hoc ($p < 0.05$).

3.2. Plant Biomass Production

The statistical analysis revealed intrinsic genotypic differences, and a genotypic specific response to the NaCl concentration in terms of shoot and root dry weight and dry matter content (Table 2). Under non-saline conditions, melon genotypes CM12 and CM13, together with CMM-R1, produced heavier shoots while watermelon CV3 the lightest (Figure 2a). As compared to 0 mM NaCl, shoot dry weight between the genotypes was differently affected by 150 mM NaCl. A significant decrease in shoot dry weight was observed in melon (CM2, CM3, CM5, CM7, CM8, CM9, CM12, and CM13) as well as in CMM-R1, CMO 51–17, watermelon (CV1, CV2), and bottle gourd LS2, while it increased in CMM-R2, watermelon CV3, and bottle gourd LS1 and did not vary in the other genotypes (Figure 2a). In the absence of salinity, root dry weight was higher in watermelon CV4, followed by bottle gourd (LS3 and LS4), while the lowest root dry weights were recorded in melon genotypes (CM6, CM15, and CM16) (Figure 2b). When 150 mM NaCl was applied, root dry weight only decreased in CV4 (−79%), while it was not affected in the other genotypes (Figure 2b). Shoot dry matter content differed between the genotypes at both NaCl concentrations. Under 0 mM NaCl, the highest biomass accumulation was observed in watermelon CV6 while the lowest was associated with melon CM1 (Figure 2c). Furthermore, 150 mM (NaCl) resulted in significant increases in shoot dry matter in the bottle gourd genotypes (LS1, LS3, and LS4), together with watermelon (CV2, CV3, and CV5), melon CM1, and both the CMM-R1 and CMM.R1 interspecific hybrids (with the strongest accumulation registered in CMM-R2) (Figure 2c). Differences in root dry matter percentage were associated with genotypes and their interaction with salinity (Table 2). Moreover, under 0 mM NaCl, the highest root biomass content was observed in melon (CM2, CM3, and CM5), while the lowest in bottle gourd (LS1, LS3, and LS4) (Figure 2d). Compared to 0 mM NaCl, root dry matter content decreased in seedlings grown under 150 mM of NaCl of CMO 51–17 (−26%), melon CM3 (−8%), CM10 (−23%), CM11 (−20%), and the interspecific hybrids (CMM-R1: −18%) and (CMM-R2: −48%), while it significantly increased in melon (CM1, CM4, CM5, CM6, CM7, CM8, CM15, and CM16), together with watermelon (CV1, CV2, CV3, CV4, and CV5) (Figure 2d).

3.3. Photosynthetic Pigments

Intrinsic difference in photosynthetic pigments profile and content were observed between the genotypes (Table 3). Among non-salinized seedlings, chlorophyll (*a*, *b*, and total) and carotenoids content was higher in melon (CM11 and CM12), while the lowest concentration was observed in CM5. Compared to non-saline seedlings, chlorophyll *a* content significantly increased under salt stress in CMM-R1 and CMM-R2 (+47% and +61%), while it decreased in CMO 51–17 (−40%) and LC1 (−49%) and it did not vary in the other genotypes (Figure 3a). On the other hand, chlorophyll *b* content increased in CMM-R1 (+38%) and CMM-R2 (+64%) seedlings grown under saline conditions, while it decreased in LC1 (−51%), and was not affected by salinity in any of the other genotypes (Figure 3b). As compared to non-saline conditions, the total chlorophyll and carotenoid content increased under 150 mM NaCl in CMM-R1 (+44% and +52%, respectively), CMM-R2 (+62% and +54%), while they decreased in LC1 (−50% and −28%) and it did not vary for the other genotypes (Figure 3c,d).

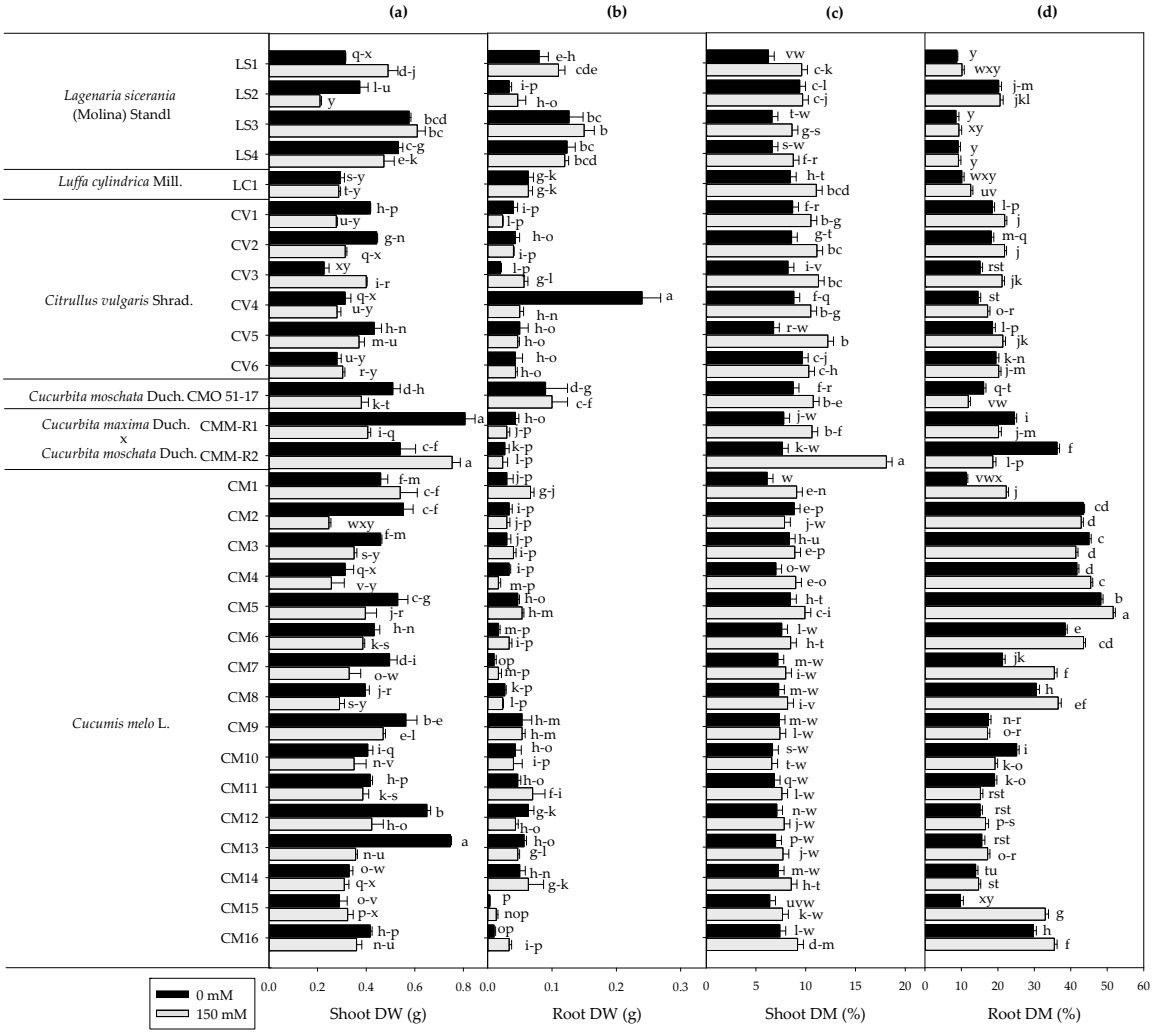

Figure 2. Plant biomass accumulation, after one week of NaCl treatment, in term of shoot dry weight (**a**), root dry weight (**b**), shoot dry matter content (**c**), and root dry matter content (**d**) in seedlings of sixteen *Cucumis melo* L., six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. Mean values ± standard errors; $n = 3$ followed by different letters within each parameter are significantly different based on Duncan post hoc ($p < 0.05$).

Table 3. Chlorophyll *a*, *b*, total carotenoids content, and electrolyte leakage in leaves of seedlings of sixteen *Cucumis melo* L., six *Citrullus vulgaris* Schrad, two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. grown at 0 or 150 mM of NaCl. (1 week after the beginning of the treatment). Non significance or significance differences at $p \leq 0.05$ or 0.01 are indicated as: ns and ** respectively.

	Chlorophyll *a* (mg g^{-1})	**Chlorophyll *b* (mg g^{-1})**	**Total Chlorophyll (mg g^{-1})**	**Carotenoids (mg g^{-1})**	**Electrolyte Leakage (%)**
Genotype (G)	**	**	**	**	**
Salt (NaCl)	ns	ns	ns	ns	**
G × NaCl	**	**	**	**	**

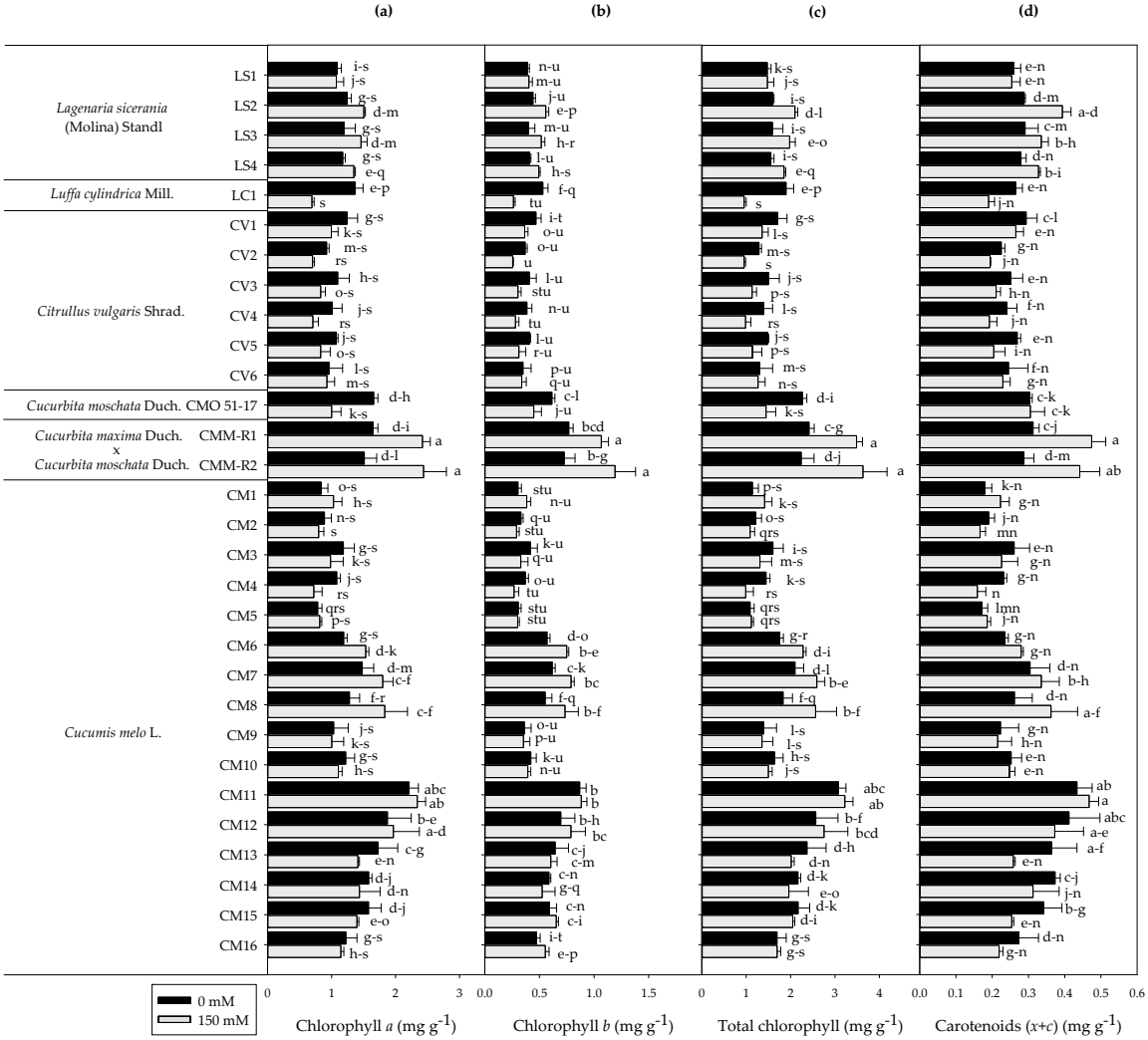

Figure 3. Photosynthetic pigment content, after one week of NaCl treatment, in term of chlorophyll *a* (**a**), chlorophyll *b* (**b**), total chlorophyll (**c**), and carotenoids (*x* + *n*) (**d**) in seedlings of sixteen *Cucumis melo* L., six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. Mean values ± standard errors; $n = 3$ followed by different letters within each parameter are significantly different based on Duncan post hoc ($p < 0.05$).

3.4. Electrolyte Leakage

Electrolyte leakage was significantly different between the genotypes (Table 3). Under 0 mM NaCl, CMM-R2, LS1, and CM2 showed the highest value of electrolyte leakage, while the lowest electrolyte leakage values were observed in CV4, LC1, LS4, and CM10 (Figure 4). Electrolyte leakage values were generally increased by salinity, although significant differences from control conditions were only observed in LS2 (+184%), CV13 (+243%), CM10 (+4662%), and CM13 (+509%) (Figure 4).

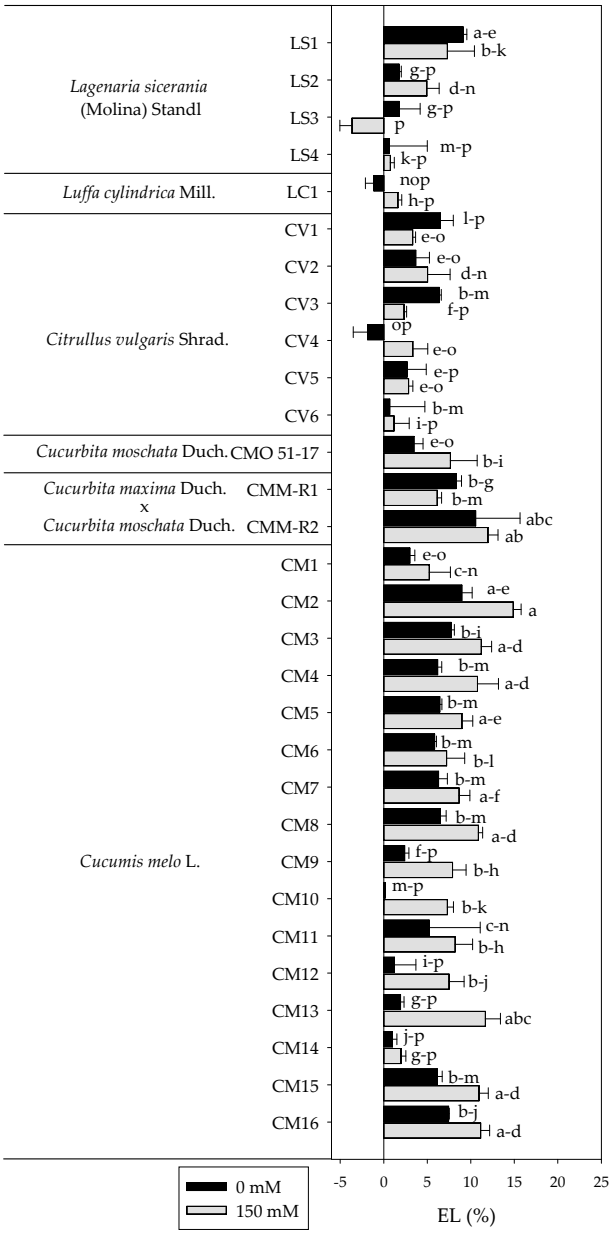

Figure 4. Electrolyte leakage (EL) in leaves, after one week of NaCl treatment, in seedlings of sixteen *Cucumis melo* L., six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. Mean values ± standard errors; $n = 3$ followed by different letters within each parameter are significantly different based on Duncan post hoc ($p < 0.05$).

3.5. Cluster Heat Map and Principal Component Analysis

To obtain a detailed overview and to better distinguish the morpho-physiological changes induced by salinity on the tested genotypes, a cluster heat map and a principal component analysis (PCA) were conducted considering the percentage variation between 150 and 0 mM NaCl for all the aforementioned measured parameters.

The cluster heatmap shows that the main clustering factor was the genotypes regardless of the species, highlighting common response traits to the increase of NaCl between the different genotypes (Figure 5). The genotypes formed two main clusters. The first was occupied by the watermelon genotype CV2 that clustered alone, mainly as a consequence of an increase in leaf number, root length,

shoot diameter, and shoot and root dry weight, together with a decrease in electrolyte leakage and photosynthetic pigment content (Figure 5). The second main cluster is split into two sub-groups. In order of distance, the first sub-group arranges bottle gourd LS2, LS3, and LS4 with melon CM8 and the interspecific hybrids CMM-R1 and CMM-R2, together into several further sub-sets characterized by similar positive variations in photosynthetic pigment content and negative variation in electrolyte leakage and root dry matter content (Figure 5). The second sub-group contains most of the genotypes and is organized into two further subsets, one of which has two divisions. The first subset is determined by similar variation in shoot and root dry matter content and electrolyte leakage grouping melon (CM1, CM6, and CM7, CM15 and CM16) and watermelon CV3 and bottle gourd LS1 genotypes together (Figure 5). Furthermore, the CM1 genotype showed the highest positive variation in shoot diameter, while CM6 and CM7 showed the highest increase in dry root weight (Figure 5). The decrease observed under saline conditions in photosynthetic pigments, root length, and dry weight, together with shoot length and diameter and leaf number accounted for the formation of the second subset. This is further subdivided into two divisions, the first of which contains watermelon (CV1, CV4, and CV5), melon (CM4, CM5, CM11, CM12, and CM13), and luffa (LC1) genotypes. Among these genotypes, LC1, CM4, and CV5 showed the highest decrease in photosynthetic pigment contents, shoot diameter and root length, and dry weight. A decrease in root dry weight was also observed in CM11, CM13, and watermelon CV1 (Figure 5). The second and last subdivision is explained mainly by a decrease in shoot and root dry matter and places, together melon (CM2, CM3, CM9, and CM10), watermelon CV6, and *C. moscata* CMO 51–17. Among the genotypes, melon (CM2, CM3, and CM10) showed the highest decrease in shoot dry matter, while melon CM3, the highest increase in electrolyte leakage and CV6 also showed the highest increase in shoot length (Figure 5).

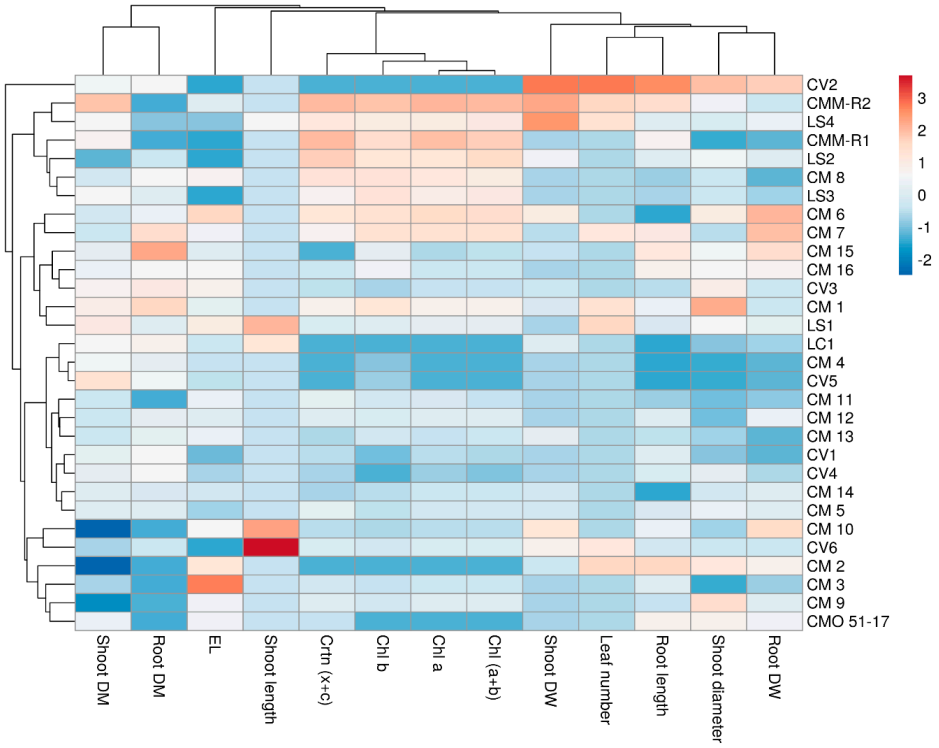

Figure 5. Cluster heat map analysis of the percentage variation to the increase of NaCl level of all the analyzed parameters in seedlings of sixteen *Cucumis melo* L, six *Citrullus vulgaris* Schrad., two *C. maxima* × *C. moschata*, four *Lagenaria siceraria* (Molina) Standl. different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. grown in a floating system in a nursery greenhouse. It was generated using the ClustVis online software [18] with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.

The PCA of the relative percentage variation of all the analyzed parameters in seedlings grown at 150 mM NaCl as compared with the control (0 mM NaCl) highlighted that the first three principal components (PCs) were related with eigenvalues higher than 1 and explained 64.5% of the total variance, with PC1, PC2, and PC3 accounting for 32.6%, 20.1%, and 11.9% respectively (data not shown). Both the species and the genotype contributed to the separation of PC1 and PC2, as highlighted in the PCA output, revealing common trait variations among genotypes regardless of the species (Figure 6). The melon genotypes CM3, CM4, CM5, CM11, CM13, and CM14, together with the watermelon genotypes CV1, CV3, and CV4, were concentrated in the upper right quadrant of the PCA output and positively correlated to an increase in electrolyte leakage (Figure 6). The watermelon genotypes CV2 and CV5, together with CMO 51–17, luffa LC1, bottle gourd genotype LS1, and melon genotypes CM2, CM10, and CM15 were concentrated in the negative lower right quadrant of PCA output and were correlated to an increase under saline conditions of shoot length and root dry matter content (Figure 6). The interspecific hybrid CMM-R1, together with melon CM8, CM9, CM12, and bottle gourd LS2 and LS3 were concentrated in the upper left quadrant of the PCA output (Figure 6) and together with the interspecific hybrid CMM-R2 (located in the lower left quadrant), were characterized with an increase in photosynthetic pigment content under saline conditions. In the left lower quadrant depicted genotypes CM1, CM6, CM7, and CM16, together with bottle gourd LS4 and watermelon CV6 and were characterized with increase in leaf number, shoot diameter, shoot dry weight, and dry biomass accumulation and root length under saline conditions (Figure 6).

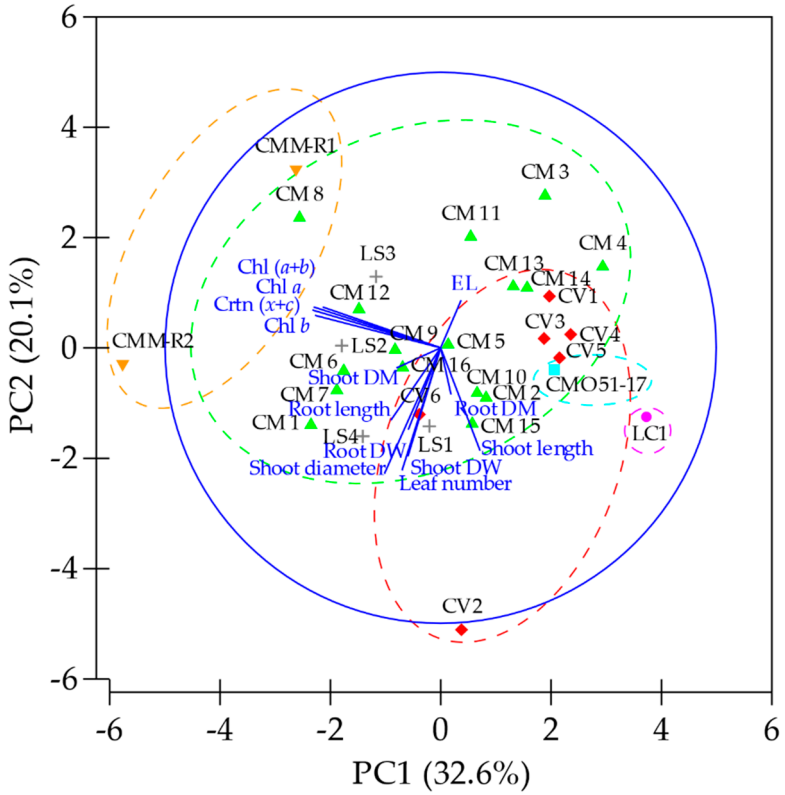

Figure 6. Principal component (PC) loading plot and scores of principal component analysis of the percentage of variation between 150 mM and 0 mM of NaCl of all the parameter analyzed in seedlings of sixteen *Cucumis melo* L. (CM), six *Citrullus vulgaris* Schrad (CV), two *C. maxima* × *C. moschata* (CMM-R), four *Lagenaria siceraria* (Molina) Standl. (LS) different genotypes, and *Cucurbita moschata* Duch. *cv* 51–17 and *Luffa cylindrica* Mill. grown in a floating system in a nursery greenhouse.

4. Discussion

It is well known that plants' response to different NaCl concentrations differs between species and even within genotypes of the same species. In this study, high variability in various morphological and physiological traits between the tested genotypes even from the same species were observed. Furthermore, different responses to NaCl were identified, confirming the high variability within the assessed germplasm collection.

According to Munns [19], a saline environment in the root zone triggers the activation of various mechanisms in plant physiology, morphology, and metabolism. This takes place in two main phases. Firstly, the presence of NaCl in the soil creates osmotic stress in the roots due to different solute concentration, causing the activation of multiple metabolic pathways that in the short term lead to stomata closure to reduce evapotranspiration and preserve water, and net photosynthesis reduction [1,20,21]. Morphological change primarily includes the growth inhibition of aerial organs like shoots and a reduction in the number of leaves. In the current experiment, 150 mM of NaCl significantly reduced the number of leaves only in melon (CM5), though a decreasing trend was observed in most genotypes. Shoot growth was significantly inhibited by salinity in most genotypes with the exception of watermelon (CV3, CV5, CV6) and luffa (LC1), which turned out to be more salt tolerant for this trait. The root system serves as an interphase between plant and soil, and its anatomy determines root performance [21]. Generally, root growth in a saline environment is inhibited less than the aerial organs [22]. In our study, root growth was not significantly affected by salt stress as the main factor, however, our data highlight a genotypic-specific response to salt concentration since root growth was promoted in salt-treated seedlings of CMM-R1, CMM-R2, and watermelon (CV3) and decreased in watermelon (CV6) and melon (CM14).

Salinity stress leads to a decrease in plant biomass, mainly in the epigean organs and this is ascribed to a decrease in CO_2 fixation by photosynthesis due a reduction in stomatal conductance, together with nutrient disorders caused by the toxic action of Na^+ and Cl^- ions inhibiting Ca^{2+} and K^+ uptake [1,21–23].Under conditions of 150 mM NaCl, shoot dry weight was negatively affected only in melon (CM2, CM5, CM7, CM8, CM12, and CM13), *C. moscata* (CMO 51–17), watermelon (CV1 and CV2), and in the interspecific hybrid CMM-R1, the latter was the most salt sensitive compared with the other genotypes. In particular, watermelon (CV3), the interspecific hybrid CMM-R2, and bottle gourd (LS1) significantly increased shoot dry weight in response to salinity. On the other hand, shoot dry matter accumulation increased in saline conditions in bottle gourd (LS1, LS3, LS4) and in watermelon (CV2, CV3, and CV5) and melon (CM1) and both the interspecific hybrids CMM-R1 and CMM-R2. Many salinity tolerance traits in grafted plants are associated with the root system. A salt tolerant rootstock genotype alleviates the deleterious shoot growth inhibition, allocating more biomass to the root system, increasing the root surface able to uptake water and nutrients, resulting in a higher growth rate and biomass accumulation [24,25]. Accordingly, with 150 mM NaCl, root dry weight did not decrease in any of the genotypes except for watermelon (CV5), and root dry matter accumulation was significantly enhanced in melon (CM1, CM4, CM5, CM6, CM7, CM8, CM15, and CM16) as well as watermelon (CV1, CV2, CV3, CV4, and CV5), while it decreased in melon (CM3, CM10, and CM11) and CMM-R1 and CMM-R2. Several studies have reported the growth depressing effect of salt on melon [26–28], cucumber [3,29], mini-watermelon [30], tomato [31], and lettuce [32] grown hydroponically under greenhouse conditions. The second phase of the salt stress starts with the accumulation of sodium and chloride ions in the leaves and their degrading action on cell membranes and chloroplast membranes. This degrades the tonoplast together with chlorophyll molecules because of chlorophyllase enzyme [33] as well as the interference of salt ions with pigment-protein complexes which accelerate leaf senescence and abscission [34]. Contradictory information is available in literature on salt stress related to chlorophyll contents among the same species and to others. In fact, studies on melon *cv.* Parnon and *cv.* "Tempo F1", under saline conditions, reported a decrease in chlorophyll content [35,36] in line with studies on pumpkin [37], cucumber [38], tomato [31], canola and wheat [39,40]. Conversely, other

authors observed an increase of photosynthetic pigments in different melon genotypes [41–43] in line with studies on other species like hot pepper [44], sunflower [45], and sesame [46].

Sanoubar and collaborators in 2016 [47] indicated that chlorophyll concentration in stressed tissues can be construed as an index of tissue tolerance to NaCl. Accordingly, our results indicate a good tolerance in most of the genotypes, in particular in the interspecific hybrids CMM-R1 and CMM-R2, where photosynthetic pigments significantly increased under saline condition. Contrarily, in luffa, the decreasing chlorophyll content in response to salinity could be associated with greater sensitivity to salt stress [21]. Adaptation mechanisms induced by salt stress generally influence the light harvesting complex due to a faster decrease of chlorophyll *a* content as compared to chlorophyll *b*, which leads to an increase in the chlorophyll *a*/*b* ratio [34]. In our experiment, the chlorophyll *a*/*b* ratio was modified under saline condition only in CMO 51–17 and watermelon (CV1) where chlorophyll *a* decreased and chlorophyll *b* did not vary. Different hypotheses have been formulated to explain the increase in photosynthetic pigments in response to salinity. The first considers the morphological modifications associated with the decrease of leaf area due the smaller cell size, resulting in an enhancement of photosynthetic pigments concentration [45,46]. A second hypothesis, proposed by Garcia-Valenzuela and collaborators [48], associated the increase of photosynthetic pigments to the faster response that is generally observed in the biosynthesis of pigments as compared to the cell growth rate [48]. To date, however, most the credited hypothesis relates the increase observed in photosynthetic pigments to a short term acclimatization response to salinity and that a prolonged exposure would in any case be detrimental [49]. The ability to isolate or exclude sodium and chloride is a key factor to consider as a salt tolerance trait [27,28]. The longer the salt stress is prolonged, the higher the accumulation of sodium and chloride inside the plant is. To cope with this osmotic disorder, plants exclude uptake of these compounds primarily through root exclusion, and subsequently through compartmentation, by isolating salt in vacuoles. However, the higher the ability to isolate (salt) in the vacuole is, the less the cell membrane is damaged. Moreover, if the concentration increases inside the cytosol, plants start to synthesize different organic solutes to maintain the osmotic turgor and to reduce the deleterious effect of salt due to cell membrane degradation and the increase of reactive oxygen species (ROS) [19,21,50]. In our experiment, electrolyte leakage increased dramatically only in LS2, CV4, CM10, and CM13, revealing a high index of internal damage due to the presence of sodium and chloride inside the leaf cells of these genotypes which must be considered a sensitive response to salt stress.

5. Conclusions

Salinity stress leads to a decrease in plant growth with more pronounced deleterious effect in the shoot rather than the roots. Similar physiological and morphological salt adaptation traits were observed in different genotypes from different species. In light of the above consideration, we identified *C. maxima* × *C. moscata* interspecific hybrid CMM-R2, melon genotypes (CM6, CM7, CM10, and CM16), together with watermelon (CV2 and CV6) and bottle gourd (LS4) as salt tolerant genotypes and possible candidates as salt resistant rootstock to be introduced in grafting programs. Contrarily, luffa and melon (CM1, CM2, CM3, CM4, CM5 CM8, CM9, CM11, CM12, CM13, CM14, and CM15), watermelon (CV1, CV3, CV4, and CV5), together with CMO 51–17 and bottle gourd LS1 proved salt sensitive. In conclusion, this study provides information to growers, scientists, extension specialists, and breeders on the behavior of the tested genotypes. Further research on these genotypes is needed to clarify their performance in saline environments in the long term and their compatibility with commercial grafted varieties.

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