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Research article

Effect of salinity (NaCl) on plant growth, nutrient content, and glucosinolate hydrolysis products trends in rocket genotypes

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ABSTRACT

Salinity caused by NaCl is an abiotic stress inducing morphological and metabolic disorders. The impact of salinity (0, 65 and 130 mM NaCl) on morphological traits, elemental and volatiles composition of six rocket genotypes (G1-G6) was explored. A significant reduction of shoot biomass, plant height and leaf area as function of genotype and salinity level was observed. G5 was highly affected by NaCl: at 65 and 130 mM plants were 48.6% and 59.1% shortened compared with to control. The volatiles compositions was also analyzed. Glucosinolates increased under 65 mM, then decreased at 130 mM. In G1, glucosinolates start with 7.4 (control), raised to 21.50 (65 mM) and finally dropped to 4.34 (130 mM). This trend was observed also for erucin, the major rocket's isothiocyanate.

Rockets could be irrigated with saline water improving the health promoting compounds production. The evaluation of different genotype seems to be of great interest for future breeding programs.

1. Introduction

In the Mediterranean basin, water quality and amount appear to be critical factors limiting the development of agriculture (Jacobsen et al., 2012). It has been estimated that, in the next decades, agriculture will undergo water limitations due to the competition with municipal and industrial uses (Cosgrove and Loucks, 2015). To satisfy the increasing demand of water in agriculture, the use of marginal quality water, for example dual water, will become necessary. Desalinated seawater, which may contain a salt residue, is possibly used in several semi-arid regions for irrigation as well as sanitary uses. Other common source of saline water are represented by water wells in coastal areas (Custodio, 2010).

Salinity caused by NaCl is one of the most common abiotic stress affecting plant physiology (Fageria et al., 2012; Munns and Tester, 2008). Salt stress causes several plants disorders (nutrient ion imbalance, decrease in stomatal conductance, low photosynthetic activity, etc.) (Ivanova et al., 2015) morphological alteration (reduction in leaves number, plant size, roots length and fruit production), and secondary metabolites changes (signal molecules, hormones and oxidative compounds) (Munns and Tester, 2008). Therefore, the use of saline water for plant cultivation requires the identification specie-specific thresholds at which crops show sensitivity to salinity.

On the other hand, appropriate (controlled) levels of salinity have been found increasing many quality parameters of salt-tolerant horticultural crops (Rouphael et al., 2018a,b). Therefore, a comprehensive understanding of the effects that salinity may exert on produce yield and quality, especially on the production of secondary metabolites and on the sensory properties of edible vegetable organs, is necessary.

The human food intake is strongly related to the crop production that is affected, since the Ancient Times, by the salinity of irrigation water (Hanson et al., 2006). Among all, the secondary metabolites produced in plant as response to stress, glucosinolates (GLS) have been shown to increase, as a consequence of the salinity conditions, above the tolerance level in *Brassica* species (Pang et al., 2012). Different factors such as plant genotype, physiological stage and the salinity levels can influence the production of GLS (Martinez-Ballesta et al., 2013).

GLS are a class of sulfur-containing nutrient that confer the typical biting taste of many Brassicaceae, such as broccoli, Brussels sprout and rocket. Cruciferous vegetable belonged to Brassica genus differ each other for the GLS content and profiles (Maldini et al., 2017). This group

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Table 1
Description of rocket genotypes used for the experiment.

Code	Genotype	Origin	Distribution	Specific trait
G1	*Diplotax tenuifolia* (Dragon Tongue)	France	Mexfi Graines	Red-veined leaves
G2	*Diplotax tenuifolia* (var. Frastagliata)	Italy	Pagano Domenico e Figli	Jagged leaves
G3	*Diplotax tenuifolia* (var. Capriccio)	Italy	Four	Tangy indented leaves
G4	*Eruca sativa* (cropped)	Italy	Franco Raduazzo	Large leaves
G5	*Diplotax tenuifolia* (Foglia d'olivo)	Italy	Rossella Biagiotti	Long and narrow leaf with slightly serrated margins
G6	*Diplotax tenuifolia* (Piccante)	United Kindom	Premier seeds direct	Deep green, serrated foliage

of secondary metabolites are primarily produced by plant as part of the defense mechanisms against insect and animals (Mann et al., 2006). In addition several others factors such as the genotype, plant organ, age, environmental conditions and agricultural factors could alter the qualitative and quantitative production of GLS (Fahey et al., 2001).

More recently, interest has been shown on GLS potential role as phytochemicals in the human health maintenance (D'Antuono et al., 2009). When the plant tissue is disrupted (by chewing, cutting, or processing) GLS are hydrolyzed by myrosinase enzymes into a mixture of hydrolysis products including isothiocyanates (ITC). This class of chemicals induced several positive effects on human health through a wide range of mechanisms (Traka and Mithen, 2009). The increasing consumption of Brassicaceae has been associated with a significant reduction of cancer risk, this trend has been mainly related to the high consumption of Cruciferae (Higdon et al., 2007).

Rocket, is the common name for the wild (*Diplotax tenuifolia* L.) and cultivated (*Eruca sativa Mill.)* salad specie belonged to the Brassicaceae family, characterized by a bitter taste appreciated since the Roman age. The rocket consumption has been increasing in the last years because of the discovery of its richness in health promoting compounds. Vitamin C, GLS, flavonoids, carotenoids and phenols are the principal secondary metabolites found in this specie (Heimler et al., 2007). The amount of these compounds is strongly influenced by the environmental conditions. Nowadays rocket is largely produced in the Mediterranean region especially in Italy and Spain where the climatic conditions are favorable for this crop. Wild and cultivated rocket are mainly used for fresh consumption in salad and its distribution is favored by its cultivation needs. Rocket is an annual species that can be cultivated in winter, with new sprout in spring. Its cycle is short and it is very well adapted to harsh and poor growing conditions. In addition, in summer the long day-length and the higher temperature compared to the optimal maximum temperature (25 °C), induced a reduction of life cycle and an increase of growth rate (Hall et al., 2012). All these factors make wild and cultivated rocket a good candidate specie for a saline irrigation system.

The use of poor water quality, rich in NaCl, to cultivate fresh consumption vegetables (rocket) with high nutritional value for the human health, represent an interesting goal for the modern agriculture. In addition, the identification of the salinity level threshold, that still maintain or even promote the GLS hydrolysis products in rocket genotype, would give interesting prospects for the use of marginal agricultural areas.

The aim of this research was to explore the physiological and volatile organic compounds (VOCs) of wild and cultivated rocket species under saline irrigation. The study of VOCs profiles under stress induced by NaCl allowed identifying the species with the high content of ITC characterizing the wild and cultivated rocket. The evaluation of saline condition that can still ensure the nutrient value of the rocket represent an important goal of a sustainable agriculture.

2. Materials and methods

2.1. Plant material, treatments, and growth conditions

Seeds of *Eruca sativa Mill.* (cultivated rocket) and five wild genotypes of *Diplotax tenuifolia* L. (wild rocket) were used (Table 1). The experiment was performed in summer 2018. Rockets were grown in unheated glasshouse at the University of Sassari in Ottava, Sardinia, Italy (41°N, 8°E, 80 m a.s.l.). Climate data are summarized in Table S1 (Supporting information). Seeds were sown in polyethylene trays filled with peat moss. The germination was done in growth chamber at 24 °C, 18 h photoperiod, under cool white fluorescent light (300 µmol $m^{-2} s^{-1}$). At the stage of 4 leaves, young plants were moved in the greenhouse and transplanted in plastic pots (20 cm × 10.5 cm) filled up with soil (sandy-loam calcareous soil: gravel 16%; sand 58%; silt 27%; clay 15%; pH 7.4; total N 1.4 g/kg; organic C 12 g/kg; total limestone 560 g/kg).

Biometric parameters associated to plant growth and physiological status were measured in the six rocket genotypes (G1-G6) (Table 2) irrigated in absence (control, S0) and presence of NaCl (65 mM and 130 mM, respectively S65 and S130). The experimental design consisted in five replicates per each genotype and treatment arranged in a completely randomized scheme. Saline irrigation started 5 days after the plants were moved in the greenhouse. Plants were irrigated, twice per week, from 8 to 29 days after transplant, using 0.4 L per pot at each irrigation. The above irrigation scheduling induced a leaching fraction deemed appropriate for the maintenance, in the root zone, of soil moisture near to container capacity and salinity levels similar to those nominally reported for the saline treatments.

Plant height, number of leaves, stress symptoms occurrence (yellow leaves for 50% of area) and appearance of flower were recorded twice a week. Leaf chlorophyll was indirectly measured by SPAD 502 Plus, Konica Minolta using the middle portion of 5 fully expanded leaves.

Leaves and root fresh weight (FW) and dry weight (DW) (after drying the plant tissue for 72 h at 60 °C), leaves area, number of leaves were measured at harvest during the destructive stage using a planimeter (LI-COR, model 3100 area meter). For these evaluations, five replicates per treatment combination were used.

Soil electrical conductivity (EC) was measured in association to each irrigation with deionized water (1:5 w/v) at 25 °C, using a Thermo Scientific Orion 3 Star conductimeter (Table S2, Supporting information).

2.2. Atomic absorption spectroscopy

Na, Mg, Ca and K in leaves and roots were determined after mineralization of 500 mg of plant tissues dry matter adding 8 mL of nitric acid (65%) and 2 ml of hydrogen peroxide (30%). Then the samples were subjected to microwave mineralization using a microwave digestion system. The resulting solution was then filled to a final volume of 50 ml. The mineral profile was obtained by flame atomic absorption spectroscopy (FAAS) using an atomic spectrophotometer equipped with a multi-element hollows cathode lamp and a deuterium background correction system (PerkinElmer AAnalist 200, Norwalk, CT, USA).

2.3. Headspace solid-phase microextraction (HS-SPME)/gas chromatography-mass spectrometry (GC-MS)

Gas chromatographic analyses were carried out as previously described in the literature (Aissani et al., 2015).

Table 2
Plant dry (DW) and fresh (FW) weight (g/pl), plant eight (cm/pl), and leaf mean area (LMA, cm^2/leaf) at the final destructive analysis. Rocket genotypes (G1-G6) were subjected to three treatments: S0, S65 and S130 (irrigation with water, 65 and 130 mM of NaCl respectively). In table are reported the average value ± standard deviation of five replicates.

Treatment	Genotype	Physiological traits under salinity irrigation							
		Shoot FW	Shoot DW (g/pl)	Root FW (g/pl)	Root DW (g/pl)	Root/Shoot	Total DW (g/pl)	Plant height (cm)	LMA (cm^2/leaf)
S0	G1	4.57 ± 2.40	0.54 ± 0.28	0.28 ± 0.10	0.05 ± 0.02	0.12 ± 0.05	0.59 ± 0.29	16.1 ± 2.7	4.34 ± 1.59
	G2	15.65 ± 6.42	1.84 ± 0.76	1.02 ± 0.47	0.14 ± 0.11	0.08 ± 0.04	1.99 ± 0.82	21.6 ± 3.0	7.78 ± 4.56
	G3	16.34 ± 4.64	1.92 ± 0.55	0.95 ± 0.84	0.18 ± 0.08	0.10 ± 0.05	2.10 ± 0.56	19.8 ± 3.6	8.13 ± 1.56
	G4	15.16 ± 4.39	1.78 ± 0.62	1.55 ± 0.60	0.29 ± 0.16	0.19 ± 0.14	2.07 ± 0.53	17.6 ± 3.5	16.17 ± 8.14
	G5	10.42 ± 3.11	1.23 ± 0.37	1.04 ± 0.28	0.18 ± 0.11	0.15 ± 0.07	1.41 ± 0.42	25.9 ± 3.9	5.84 ± 0.88
	G6	14.04 ± 4.92	1.65 ± 0.81	0.72 ± 0.27	0.13 ± 0.05	0.08 ± 0.02	1.79 ± 0.85	21.5 ± 5.1	8.75 ± 1.93
S65	G1	3.64 ± 2.04	0.43 ± 0.24	0.25 ± 0.27	0.05 ± 0.05	0.15 ± 0.16	0.48 ± 0.26	12.1 ± 1.5	3.95 ± 2.17
	G2	10.67 ± 4.17	1.26 ± 0.58	0.42 ± 0.13	0.09 ± 0.02	0.09 ± 0.06	1.35 ± 0.58	19.6 ± 0.5	8.23 ± 3.12
	G3	10.73 ± 2.13	1.26 ± 0.35	0.45 ± 0.29	0.11 ± 0.05	0.11 ± 0.06	1.25 ± 0.22	16.7 ± 1.8	5.52 ± 1.25
	G4	13.74 ± 6.23	1.62 ± 0.76	1.74 ± 0.77	0.33 ± 0.14	0.25 ± 0.19	1.94 ± 0.78	14.5 ± 2.8	17.82 ± 8.13
	G5	5.25 ± 2.18	0.62 ± 0.30	0.43 ± 0.26	0.06 ± 0.02	0.15 ± 0.15	0.68 ± 0.30	13.3 ± 1.1	5.67 ± 1.73
	G6	8.47 ± 2.11	1.00 ± 0.35	0.65 ± 0.15	0.12 ± 0.03	0.13 ± 0.04	1.16 ± 0.41	16.3 ± 2.6	6.17 ± 1.08
S130	G1	1.79 ± 0.71	0.21 ± 0.14	0.16 ± 0.10	0.03 ± 0.02	0.23 ± 0.14	0.23 ± 0.15	8.0 ± 1.2	3.12 ± 1.13
	G2	7.56 ± 2.03	0.89 ± 0.38	0.67 ± 0.24	0.13 ± 0.04	0.17 ± 0.12	1.02 ± 0.38	15.4 ± 1.9	6.31 ± 1.34
	G3	5.01 ± 0.71	0.59 ± 0.29	0.74 ± 0.26	0.14 ± 0.05	0.26 ± 0.13	0.73 ± 0.31	12.7 ± 1.0	5.48 ± 1.55
	G4	10.13 ± 3.54	1.19 ± 0.82	1.85 ± 1.33	0.35 ± 0.25	0.32 ± 0.10	1.54 ± 1.05	11.9 ± 3.4	17.80 ± 6.41
	G5	3.51 ± 1.17	0.41 ± 0.29	0.46 ± 0.17	0.09 ± 0.03	0.26 ± 0.11	0.50 ± 0.32	10.6 ± 0.8	5.10 ± 2.28
	G6	3.21 ± 1.82	0.38 ± 0.22	0.30 ± 0.12	0.06 ± 0.02	0.15 ± 0.12	0.51 ± 0.16	11.7 ± 3.6	5.08 ± 1.23
Probability level of significance (ANOVA)[x]									
Genotype (A)		< 0.001	< 0.001	< 0.001	< 0.001	0.0016	< 0.001	< 0.001	< 0.0001
Treatment (B)		< 0.001	< 0.001	n.s.	n.s.	< 0.001	< 0.001	< 0.001	< 0.0001
AxB		n.s.	0.002	n.s.	n.s.	n.s.	0.003	< 0.001	0.003.

[x] P value or not significant (n.s.) is reported for the statistical analysis performed through two-way ANOVA using genotype (A) and saline treatment (B) as main factors.

2.4. Statistical analysis

Collected data were analyzed by one-way or two-way ANOVA (depending on parameter type), using a GLM univariate procedure. Mean values were then separated by Tukey's (HSD). Principal component analysis (PCA) was performed to determine the degree of differentiation among the tested treatments to provide an overview of NaCl treatment effect on morphological, nutritional and volatiles factors. In order to show only the variable that effectively impact the variance, the following procedure was used for the PCA, i) only the dependent variables of the volatile fraction that showed significant differences (ANOVA) were used; ii) the chemical compound groups (aldheydes, ketones, glucosinolate derivatives and others) were used.

3. Results and discussion

3.1. Effects on plant growth

All genotypes showed a high variability in leaves, roots, total plant biomass and the biometric parameters evaluated (Table 2). In control condition, G3 presented the highest shoot DW and total plant DW, compared with the other tested genotypes, showing approximately a 3.5 folds higher value compared with the minimum shoot and total biomass (DW) observed in G1. Based on the root DW, root/shoot ratio and leaf area for each leaf, the best performing genotype was G4, with 5.8; 3.7 and 2.4 folds value of the two worst varieties G1 and G2 respectively. Significant differences were found among genotypes in terms of plant height: G5 (25.9 cm) ranked first, while G1 (16.1 cm) was the shortest variety.

Saline irrigation induced a significant reduction of shoot DW and total biomass in both NaCl concentrations. G4 shoot DW decreased from 1.74 g/pl (control) to 1.19 g/pl (S130) and from 0.54 g/pl to 0.21 g/pl in case of G1 shoot DW. A similar trend was observed for the total plant DW. In this case, the decrease of DW was less evident compared with the shoot. Shoot and total plant DW were significantly affected by the genotype and the treatments used.

The above data reflect the reduction of the plant height. As shown in Table 2, genotype and saline treatments induced significant reductions of plant size with an increasing effect from the S65 to S130, suggesting an optimum growth rate in control (S0) conditions. The plant height reduction varied among the genotypes: G2 suffer less the effect of the salinity, in fact at S65 and S130 plants were 9.3% and 26.9%, respectively, shorter compared to the control conditions; a stronger impact was registered in G5. In this case at S65 and S130 plants were 48.6% and 59.1% shortened compared with S0. The lower plant biomass and height, as a consequence of the salinity condition, have been reported in several plant species and these have been mainly associated to the osmotic stress and the ion toxicity that generate a repression of plant growth (Fageria et al., 2012; Munns and Tester, 2008).

Genotype and salinity significantly influenced leaf mean area (LMA). With the exception of G4, a general reduction of LMA was observed in all genotypes, from S0 to S130 treatments. The genotype that showed the highest reduction of this parameter was G6 with 29.5% and 41.9% reduction of LMA in S65 and S130, respectively. The reduction of LMA could be the plant physiological response to the saline conditions: under salt stress, plant, reduce the leaf area in order to prevent water loss and protect plant against water stress (Parida and Das, 2005).

Root growth showed a different trend compared to above ground organs and was not significantly affected by salinity. The genotype was the only significant factor determining a general small decrease of the root DW in the saline conditions. G4 root DW increased at S130 (0.35 g/pl), compared to the control (0.05 g/pl), contrary to all the other tested genotypes (Table 2). A similar trend was reported in lettuce treated with NaCl and Na_2SO_4 (Mahmoudi et al., 2010). Under these conditions, the authors observed equivalent root growth between untreated and treated plants. In the present study, the root/shoot ratio increased under saline irrigation. G2 showed in S0, S65 and S130 the lowest root/shoot values, while G4 had the highest value in all irrigation systems adopted. This data could be interpreted as a simultaneous reduction of the shoot versus root development as a consequence of the NaCl. The better performance observed in the case of G4 genotype would then reflect an enanched translocation factor of photoassimilates towards

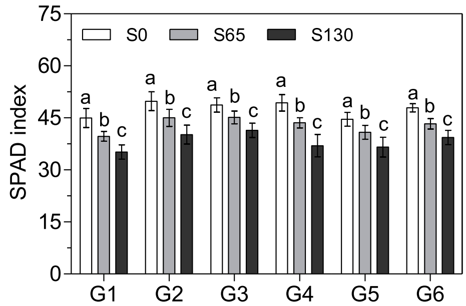

Fig. 1. SPAD chlorophyll measurements of rocket genotypes (G1-G6); the average value ± standard deviation of five measurement for treatment were performed twice a week. Plant were subjected to saline irrigations (S0 = control, S65 = 65 mM, S130 = 130 mM of NaCl). ANOVA analysis valuated the effect of the saline treatments. Post-hoc analysis was performed using Tukey's HSD test (P ≤ 0.05). Different letters above the bars indicated statistically significant difference among treatment at each genotype.

roots under salinity to counteract the typical detrimental effects of osmolytic substances the root zone.

In general, salinity irrigation showed a negative effect on the plant growth parameter considered. This trend could be caused by the consequent multiple disorder generate by the salinity in the plant physiological status. Reduction of photosynthetic rate, alteration of stomatal conductance, nutritional imbalance and other factors associated to this specific abiotic stress, are just some of the multiple conditions that are negatively affected by salinity (Fageria et al., 2012; Munns and Tester, 2008).

3.2. *Effects on salt-stressed leaf indicators*

The SPAD index was measured twice a week by non-destructive (i.e., optical) method to monitor the evolution of leaf chlorophyll in plants as a function of time and salinity treatments. The averaged values are reported, separately per each genotype, in Fig. 1. The SPAD index is representative of leaf chlorophylls due to its correlation with these pigments (Schepers et al., 1992). It has been used to evaluate plant response to biotic and abiotic stress or as indicators of plant nutritional status in different types of vegetable crops (Massa et al., 2018). Salinity stress is known to impair nutrition and induce senescence mechanisms in plants thus causing reduced chlorophyll content in leaves (Munns and Tester, 2008). Lower SPAD values have been associated to excess of Cl concentration in the root zone, and NaCl in general, in different plant species, including leafy vegetables (Lucini et al., 2015). In this work, the highest values were, on average, observed for the G2, G3 and G4, which significantly differed from the other genotypes (data not shown). Nevertheless, the G4 was the more sensitive for the reduction of SPAD index at increased salinity, compared to the control (−12% and −25% for S65 and S130, respectively). Contrarily G2, G3 and G6 appeared less sensitive to salinity. However, the presence of NaCl in the root zone led to a significant reduction of this parameter in all genotypes (Fig. 1) showing negative trends as a function of the time from transplant to harvest (data not shown) that was in agreement with previous works on rocket (Frezza et al., 2010).

The presence of stress conditions in plants were evaluated with the occurrence of chlorosis in 50% of leaves area. The number of chlorotic leaves and of days for the appearance were analyzed (Fig. 2a and b). Both genotype and treatment significantly influenced the yellowing symptoms (Table S3, Supporting information). Chlorosis was observed only for saline treatments with a high number of yellowed leaves in G1 and G5 at S130. The other genotypes showed a significant difference only between saline and not saline treatments. Based on the number of days for the appearance of chlorotic leaves, genotypes and treatments showed significant different trends (Table S3, Supporting information; Fig. 2b). In S0, no chlorotic symptoms were detected, while salinity

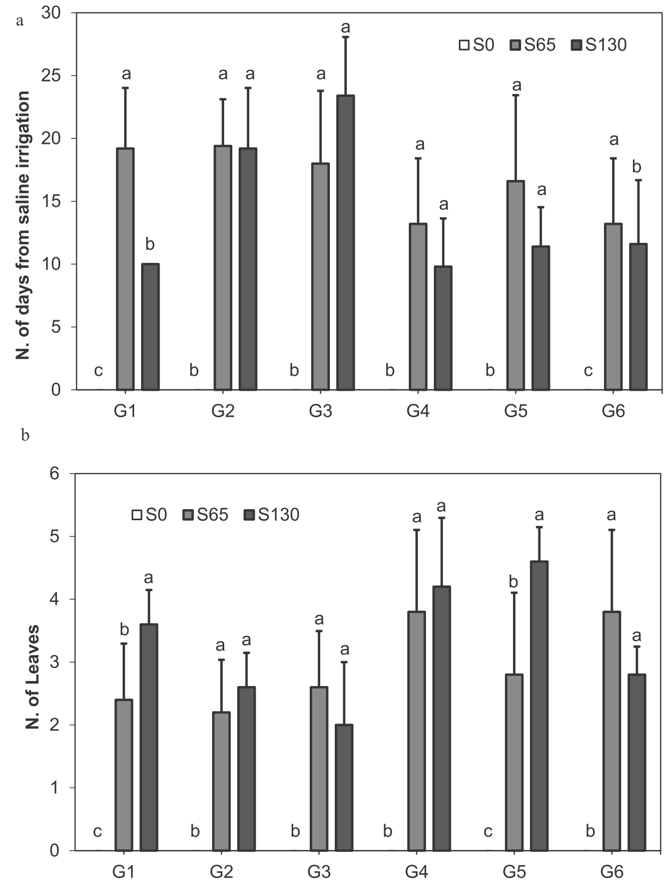

Fig. 2. Effect of different NaCl treatments on rocket genotypes (G1-G6). Salinity stress was evaluated as number of days, from saline irrigation, for the 50% leaf area with chlorosis (yellowing) (a) and as total number of yellowed leafs (b). Mean value ± standard deviation is reported. Bars with different letters are different according to ANOVA and Tukey's HSD test (P ≤ 0.05).

induced the presence of yellow leaves. In most genotypes, the S130 induced an early occurrence of yellowing in G1, G4, G5 and G6; while in G3 the S65 showed stress symptoms before the S130 treatment. In G2, the yellowing appeared almost in the same time for both saline treatments. The presence of yellowing has been associated to the reduction of chlorophyll due to the increased activity of the chlorophyll-degradating enzyme chlorophillase and to the toxic effect of ions that adversely affected the chlorophyll content in leaves (Munns and Tester, 2008; Parida and Das, 2005).

Early flowering represents another symptom of plant stress response. Flowering percentage and number of days for the flowering were significantly affected by the genotype and the saline treatment used (Tables S3 and S4, Supporting information). Flowering percentage increased as response to the NaCl treatments. G4 was the only genotype without flowering, while the highest percentage of flowering was found in G1 (data not shown). Yet, the time for the flowering was deeply influenced by the NaCl treatment used. A general shortening of the flowering occurred in S130 for all genotype with the exception of G4 (data not shown).

3.3. *Effects on cation content in plant tissues*

Rocket showed differences in root and leaves elements content depending on genotype and salinity (Table 3). The increasing of salinity significantly affected the Na content in all genotypes with an increment of Na detected in roots and leaves corresponding to the increasing of the salinity levels. Na content in leaves increased roughly 0.45 in S65 and 1.8 in S130 folds compared with S0. At S130, G6 and G5 accumulated

Table 3
Leaf and root nutrient and non-nutrient (Na) cation content (g/kg) in rocket genotypes (G1-G6) subjected to three different saline treatments: S0 (Control) S65 and S130 (irrigation with 65 and 130 mM NaCl). In table are reported the average value ± standard deviation of five replicates.

Treatment	Genotype	Leaf					Root				
		Na	K	Na/K	Mg	Ca	Na	K	Na/K	Mg	Ca
S0	G1	25.35 ± 11.27	56.81 ± 21.10	0.50 ± 0.20	6.18 ± 0.26	43.01 ± 9.18	7.92 ± 0.04	32.55 ± 0.09	0.24 ± 0.00	3.18 ± 0.09	6.35 ± 0.07
	G2	20.27 ± 4.95	43.81 ± 18.64	0.54 ± 0.28	4.89 ± 0.67	48.79 ± 12.02	6.23 ± 0.06	29.18 ± 0.07	0.21 ± 0.00	3.31 ± 0.14	10.42 ± 0.09
	G3	22.73 ± 5.82	23.30 ± 8.75	1.19 ± 0.79	6.05 ± 1.22	61.84 ± 7.22	6.63 ± 0.06	33.92 ± 0.07	0.20 ± 0.00	2.67 ± 0.09	9.98 ± 0.04
	G4	21.04 ± 3.87	45.56 ± 14.02	0.50 ± 0.17	6.34 ± 1.33	56.24 ± 14.57	14.15 ± 0.06	24.95 ± 0.09	0.57 ± 0.00	2.59 ± 1.04	11.34 ± 0.05
	G5	20.88 ± 5.85	19.27 ± 7.43	1.29 ± 0.75	6.36 ± 2.05	49.16 ± 10.97	5.23 ± 0.01	21.46 ± 0.07	0.24 ± 0.00	3.54 ± 0.14	7.45 ± 0.04
	G6	23.00 ± 2.90	33.91 ± 18.03	1.15 ± 1.17	5.23 ± 0.48	45.22 ± 1.97	6.14 ± 0.06	37.00 ± 0.05	0.17 ± 0.00	3.82 ± 0.14	7.54 ± 0.07
S65	G1	33.36 ± 5.83	43.61 ± 6.05	0.77 ± 0.13	5.50 ± 0.68	40.38 ± 1.16	8.42 ± 0.08	30.93 ± 0.11	0.27 ± 0.00	2.85 ± 0.14	3.94 ± 0.87
	G2	29.00 ± 4.24	26.58 ± 6.65	0.92 ± 0.61	4.84 ± 0.74	33.80 ± 7.27	7.47 ± 0.05	29.23 ± 0.11	0.26 ± 0.00	2.90 ± 0.50	5.57 ± 0.42
	G3	35.40 ± 9.32	26.35 ± 6.69	1.37 ± 0.32	5.33 ± 1.32	40.69 ± 3.00	7.82 ± 0.06	30.27 ± 0.16	0.26 ± 0.00	3.27 ± 0.14	6.77 ± 0.20
	G4	24.57 ± 3.60	31.03 ± 8.19	0.84 ± 0.25	5.23 ± 0.88	37.47 ± 8.47	16.55 ± 0.06	23.69 ± 0.16	0.70 ± 0.01	1.62 ± 0.14	8.55 ± 0.45
	G5	40.14 ± 2.85	14.35 ± 8.79	4.19 ± 3.61	5.59 ± 0.40	40.28 ± 1.87	8.82 ± 0.06	21.01 ± 0.16	0.42 ± 0.00	3.07 ± 0.09	4.84 ± 0.83
	G6	29.96 ± 5.83	21.01 ± 12.54	1.82 ± 0.91	4.70 ± 0.37	30.44 ± 9.71	8.88 ± 0.11	33.36 ± 0.11	0.27 ± 0.00	3.75 ± 0.33	5.74 ± 1.73
S130	G1	43.29 ± 11.21	31.97 ± 15.97	1.47 ± 0.32	3.80 ± 0.63	15.84 ± 1.21	9.83 ± 0.07	29.51 ± 0.16	0.33 ± 0.00	2.47 ± 0.42	2.87 ± 0.20
	G2	45.26 ± 3.56	23.36 ± 9.46	2.19 ± 0.86	3.87 ± 1.83	15.06 ± 1.23	9.19 ± 0.07	29.28 ± 0.16	0.31 ± 0.00	1.68 ± 0.09	4.85 ± 0.58
	G3	56.48 ± 14.29	22.30 ± 10.87	2.15 ± 1.93	3.87 ± 0.58	20.89 ± 10.90	8.46 ± 0.05	29.71 ± 0.05	0.28 ± 0.00	2.28 ± 0.26	3.81 ± 0.52
	G4	46.43 ± 7.04	31.30 ± 12.32	1.68 ± 0.80	4.65 ± 1.02	15.25 ± 3.92	17.42 ± 0.09	21.79 ± 0.11	0.80 ± 0.01	1.14 ± 0.09	6.89 ± 2.28
	G5	57.05 ± 5.76	8.49 ± 2.89	7.48 ± 3.34	5.00 ± 0.75	20.14 ± 8.19	9.08 ± 0.05	18.23 ± 0.11	0.50 ± 0.00	2.43 ± 0.52	4.02 ± 0.20
	G6	39.70 ± 6.05	14.23 ± 2.99	2.82 ± 0.25	3.44 ± 1.20	14.02 ± 1.02	8.90 ± 0.09	25.03 ± 0.16	0.36 ± 0.00	3.43 ± 0.09	3.33 ± 0.20
Probability level of significance (ANOVA)[x]											
Genotype (A)		< 0.001	0.005	< 0.001	0.044	0.032	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment (B)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
AxB		< 0.001	n.s.	< 0.001	n.s.	n.s.	< 0.001	< 0.001	< 0.001	0.009	0.015

[x] P value or not significant (n.s.) is reported for the statistical analysis performed through two-way ANOVA using genotype (A) and saline treatment (B) as main factors.

the minor and the maximum level of Na in leaves. The genotype that ranked last for the accumulation of Na was, on average, G4 that also caused a significant interaction with values of Na content at S65 not significantly different from S0. Na content has a special role in leaves, which represent the edible part of rocket, due to its effect on the accumulation of other macrocations that, other than for plant nutrition, are key elements also in the human diet (Rouphael et al., 2018a,b). The identification of rocket genotype with different response in terms of Na accumulation could therefore have an interesting commercial value for breeding programs. The negative influence exerted by NaCl against total N concentration and N–NO_3 accumulation in leafy vegetables have been widely investigated by many authors (Rouphael et al., 2018a,b), while very heterogeneous responses have been reported for P depending on species and cropping system (Grattan and Grieve, 1999). In this work, we focused the attention on macrocations balance due to the strategic role that K, Ca and Mg play in contrasting Na-induced salinity. Nonetheless, several authors reported that the increasing level of NaCl induced the alteration of some cations, reporting as Na, Mg, Ca and K the most studied and affected (Rabie et al., 2005). G1 and G4 showed the highest values of K and Mg, compared with the other genotypes. However, all plant macrocations significantly decreases because of NaCl irrigations. Both leaves and roots showed the same trend. This is the obvious consequence of antagonistic mechanisms between Na and macrocations uptake into the plant, previously documented, which may vary as a function of plant species and cultivar, climate conditions and other environmental factors, and level of salinity (Munns and Tester, 2008; Grattan and Grieve, 1999). Indeed, the ability of plants, including *Eruca* genus, to maintain lower Na/K ratio and K homeostasis is a determinant factor to counteract the detrimental effects of salinity in higher plants (Shabala and Cuin, 2007). Interestingly, the G1, G2 and G4, which showed low values of Na/K in leaves, also underwent the lowest reductions in terms of total dry biomass (−25.5% control treatment S0, on average) at increased salinity compared with the other three genotypes (−43.1% control treatment S0, on average). Yet, G4 did not show early flowering at increasing salinity.

3.4. HS-SPME GC/MS analysis

The volatiles data from rocket are reported in Table 4. Several classes of compounds were identified in the whole composition. Considering the origin of the chemicals, two main groups of compounds can be defined: i) the first deriving from the activity of thioglucoside glucohydrolase, commonly known as myrosinase, which catalyze the hydrolysis of GLS (Halkier and Gershenzon, 2006) and ii) the second mainly deriving from lipids involved in the lipoxygenase pathway and shikimic derivatives, which include aldheydes, alcohols, ketones, esters etc.

Forty-three compounds were identified. Among the whole composition, the class of aldheydes was the most represented followed by the class of ketones. Among aldheydes, the main compounds were hexanal (1.8–9.4%) and one isomer (retention index = 1527) of 2,4-heptadienal (3.0–9.8%). Among ketones, the main compound detected was 3,5-octadien-2-one which accounted in the sample G3 (S0 treatment) for 11.2%. Our results confirmed previous literature data on *Eruca sativa* volatiles, since almost totality of compounds identified were previously identified (Raffo et al., 2018). Since the main thioglucosides of *Eruca sativa* is 4-methylthiobutyl glucosinolate (glucoerucin) (Possenti et al., 2017), the major products of glucosinolate hydrolysis, were the 4 methylthio-butyl ITC (erucin) and 5-methylthio-pentane nitrile, both of them deriving from glucoerucin. Erucin amount ranged between 0.1% in G3 (S0) to 17.4% of S65-G1 (S65), pentanitrile 5-methyl thio was absent in the G3/G5 (S0) and in G2/G3 (S130) genotypes, while it was highly produced in G6 (S130) (6.4%). The occurrence of nitrile compounds deriving from glucosinolates reveals the presence of a protein, namely epithiospecifier protein, which promotes, during the hydrolysis of the parent glucosinolate, the formation of nitrile group in spite of ITC group (Macleod and Rossiter, 1985). The presence of epthiospecifier protein activity is quite common and several authors reported the presence of glucosinolate hydrolysis products in nitrile form (Raffo et al., 2018). By contrast, some other authors neglected the presence of any nitrile compounds deriving by the hydrolysis of GLS of *Eruca sativa* (Aissani et al., 2015). Arora et al. (2014), linked the variability of glucosinolate hydrolysis products to the extraction method, which could affect the stability and solubility of the parent glucosinolate in the extraction media. Our data neglected the presence of 4 mercapto butyl isothiocyanate, anyway its detection in a Wax column is hard to get, as reported by Raffo et al. (2018) since it results as a very broad peak and its presence in our samples could not be excluded.

The 43 identified compounds were divided in 4 principal classes (aldheydes, ketones, glucosinolate derivatives and others). Two-way ANOVA revealed significant different production volatiles group's base on genotype and the saline treatment adopted (Table 5).

3.5. Glucosinolate hydrolysis products

Glucosinolate hydrolysis products showed a variation trend along the three treatments in all genotypes (Fig. 3). In the samples under S65 treatment the sum of GLS hydrolysis products increased considerably, compared to the control, while its value decreased for all six cultivars when the plants were treated at S130 (Fig. 3). All genotypes, except G4 and G5, showed significant difference between S65 and both control or S130. By looking the erucin amount, the major ITC detected in the samples, the same GLS trend was observed for samples G1, G3, G5 and G6 (Fig. 4). For G2 and G4 S65 treatment, differently from other cultivars, the ratio between erucin and pentanitrile methyl thio is < 1, indicating that in these samples the epithiospecifier protein plays a key role, and that in these samples glucoerucin is the major glucosinolate derivative. These two genotypes did not show any statistical difference among the tested treatment (Fig. 4).

Among the studies on the effects of salinity on secondary metabolites production, some authors have shown, in agreement with our results, an increased biosynthesis glucosinolate when salt concentration was within the threshold of tolerance of some plants belonging to the family of Brassicaceae (Martinez-Ballesta et al., 2013). In addition, our data were supported by (Lopez-Berenguer et al., 2008), who found that the production of glucosinolates in broccoli decreased under irrigation with high salinity level (80 mM) as shown in our experiments in which rocket plants were stressed with NaCl solution 130 mM (S130). However, our results are partially in disagreement with a recent study conducted by (Cocetta et al., 2018), which did not find an increasing of GLS in rocket stressed by NaCl. These differences could be associated to the genotype used, the growing medium, the source of stress and the different methodology of stress application.

3.6. Principal component analysis

Principal components analysis was performed with a smaller number (16) of linear combinations of all considered variables, which significantly represented genotype performance under saline stress. Three main components were found having eigenvalues greater than 1.0, which in total accounted for about 80% of data variability (Table S5, Supporting information). However, a clear picture of genotypes response to saline irrigation was achieved by the association with the first two components of the PCA that accounted for 42% and 26%, respectively, of the total variation of the investigated plant performance indicators (Fig. 5 and Table S5, Supporting information). As shown in Fig. 5a, although partially overlapped, three groups could be clearly identified according the different irrigation conditions (S0, S65 and S130). The loading plot (Fig. 5b) shows the variables according their contribution on the discrimination. The highest values of PC1 are represented by Na and by the ratio between Na and K meaning that, as expected, those variables are characteristic in the S130 and (less) in

Table 4
Volatile compounds detected by SPME-GM/MS on different genotypes for each treatment. Results are expressed as the average of three independent replicates of percent of the total area of TIC signal (S0 = control, S65 = 65 mM, S130 = 130 mM of NaCl).

Volatiles compounds	RI	S0						S65		
		G1	G2	G3	G4	G5	G6	G1	G2	G3
Aldheydes										
Propanal	803	0.9 ± 0.4	1.0 ± 0	1.6 ± 0.6	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.4	0.7 ± 0.4	1.2 ± 0.1	1.3 ± 0.7
Butanal	886	nd	nd	0.2 ± 0.3	nd	nd	0.3 ± 0.1	nd	nd	0.1 ± 0.2
Pentanal	991	0.2 ± 0.0	0.5 ± 0	0.7 ± 0	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0
2-butenal	1056	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0	0.6 ± 0	0.6 ± 0.1	0.8 ± 0.3	0.4 ± 0.2	0.6 ± 0.3	0.7 ± 0.5
Hexanal	1092	3.4 ± 0.3	5.7 ± 0.2	8.1 ± 1.8	7.7 ± 2.8	9.4 ± 2.6	5.8 ± 2.8	1.8 ± 0.3	4 ± 0	6.3 ± 0.9
2-pentenal	1148	1.2 ± 0.1	1.4 ± 0.2	1.5 ± 0	1.8 ± 0.2	1.2 ± 0.6	1.2 ± 0.2	0.8 ± 0.1	1.3 ± 0.3	1.6 ± 0.2
Heptanal	1192	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.3	nd	nd	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
2-hexenal	1239	4.4 ± 0.7	4.4 ± 0.6	3.4 ± 0.6	6.5 ± 1.4	4.0 ± 0.6	3.6 ± 1.1	3.9 ± 1.1	4 ± 0	3.2 ± 0.7
Octanal	1304	0.7 ± 0	0.7 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.7 ± 0.4	0.4 ± 0.3	0.4 ± 0.2	0.6 ± 0.3	0.6 ± 0.2
2-heptenal	1348	0.9 ± 0.3	1.1 ± 0.2	1.2 ± 0	1.1 ± 0.2	1.4 ± 0.3	1.0 ± 0.2	0.6 ± 0.2	0.9 ± 0	1.4 ± 0.3
Nonanal	1412	2.6 ± 2.5	3.2 ± 1.1	1.7 ± 0.8	2.4 ± 1.1	2.1 ± 0.6	2.8 ± 1.0	4.8 ± 2.4	2.6 ± 1.2	2.7 ± 1.4
2,4-hexadienal	1424	0.8 ± 0.1	0.3 ± 0	0.4 ± 0	0.2 ± 0.1	1.3 ± 0.6	0.7 ± 0.5	0.6 ± 0.2	0.2 ± 0	0.5 ± 0.4
2-octenal	1455	1.6 ± 0.2	2.3 ± 0.5	2.7 ± 0.6	2.7 ± 0.1	3.1 ± 0.2	2.2 ± 0.7	1.3 ± 0.3	2.0 ± 0.1	3.3 ± 0.8
2,4-heptadienal	1493	0.6 ± 0.4	1.6 ± 0.9	5.5 ± 0.1	5.3 ± 1.8	4.4 ± 1.7	3.5 ± 3.1	nd	2.6 ± 0.9	3.5 ± 1.0
7-octenal, 3,7-dimethyl	1501	0.4 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	nd	nd	5.2 ± 4.1	0.4 ± 0.2	nd	nd
Decanal	1521	nd	1.1 ± 0.5	0.8 ± 0.1	0.6 ± 0.4	0.6 ± 0.2	0.8 ± 0.2	1.7 ± 0.7	nd	0.5 ± 0.1
2,4-heptadienal	1527	5.1 ± 0.1	6.2 ± 2.3	6.7 ± 1.1	9.1 ± 0.3	7.2 ± 2.2	4.8 ± 0.9	3.0 ± 1.1	7.0 ± 1.4	7.5 ± 2.1
Benzaldheyde	1568	4.6 ± 0.8	3.6 ± 0.1	4.3 ± 1.4	4.2 ± 2.4	3.7 ± 0.4	3.8 ± 0.8	3.0 ± 0.1	3.6 ± 0.6	4.1 ± 0.4
2-decenal	1676	1.6 ± 1.0	0.6 ± 0.1	0.6 ± 0	1.0 ± 0.6	0.6 ± 0.1	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.2	0.5 ± 0
2,4-nonadienal	1740	0.5 ± 0	1.1 ± 0	1.1 ± 0.3	0.9 ± 0	1.3 ± 0	1.0 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	1.1 ± 0.3
4-ethyl-benzaldheyde	1758	nd	0.4 ± 0.2	0.4 ± 0	nd	nd	0.2 ± 0.1	nd	0.3 ± 0.1	nd
2-undecenal	1784	0.5 ± 0	0.5 ± 0.2	0.6 ± 0.3	0.8 ± 0.3	nd	nd	0.3 ± 0.1	0.5 ± 0.1	nd
Ketones										
1-penten-3-one	1033	0.3 ± 0	0.4 ± 0	0.3 ± 0	0.7 ± 0.4	nd	0.3 ± 0	0.2 ± 0.1	0.3 ± 0	0.6 ± 0.3
2,3-pentadione	1068	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.2 ± 0.1	nd	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
5-hepten-2-one-6-methyl	1354	3.2 ± 0.3	1.6 ± 1.1	2.1 ± 0.5	1.5 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	1.4 ± 0.4	2.4 ± 0.2	2.9 ± 2.4
3-octen-2-one	1430	nd	0.4 ± 0.2	0.6 ± 0.2	2.0 ± 0.4	0.7 ± 0.3	1.4 ± 0.8	nd	0.3 ± 0.1	nd
3,5-octadien-2-one	1549	4.4 ± 1.0	7.8 ± 3.5	11.2 ± 0.7	8.5 ± 2.5	10.3 ± 3	5.6 ± 0.9	3.1 ± 1.4	7.7 ± 2.2	7.3 ± 0.4
b-ionone	1988	5.8 ± 1.1	7.2 ± 0.1	6.5 ± 0.2	7.2 ± 1.2	6.6 ± 1.3	6.9 ± 0.7	3.2 ± 0.7	5.5 ± 1.7	5.9 ± 0.5
Glucosinolate derivatives										
methane isothiocyanate	1300	nd	nd	0.2 ± 0.1	nd	nd	0.1 ± 0	0	nd	0.2 ± 0.1
4-methylpentyl isothiocyanate	1572	0.2 ± 0	nd	nd	nd	nd	nd	0.3 ± 0	nd	nd
hexane 1-isothiocyanate	1624	0.1 ± 0	0 ± 0	nd	nd	nd	0.5 ± 0	0.2 ± 0	nd	nd
pentanitrile 5-methyl thio	1991	1.4 ± 0.5	0.7 ± 0.2	nd	0.8 ± 1.1	nd	0.8 ± 0.1	3.7 ± 1.3	6.1 ± 0.7	3.0 ± 2.7
Erucin	2213	5.7 ± 1.9	1.2 ± 0.1	0.1 ± 0	0.6 ± 0.5	0.2 ± 0	5.8 ± 0.9	17.4 ± 2.5	1.2 ± 0.9	6.7 ± 1.9
Others										
carbon sulfide	< 700	4.9 ± 0.2	2.8 ± 1.1	0.6 ± 0.1	0.9 ± 0.8	0.6 ± 0.3	2.9 ± 2.1	6.8 ± 2.0	3.0 ± 0.6	2.6 ± 1.6
carbon disulfide	735	0.5 ± 0	0.5 ± 0.4	0.4 ± 0.6	0 ± 0	0.7 ± 0	0.1 ± 0	0.9 ± 0.9	0.1 ± 0	nd
furan-2-ethyl	962	0.8 ± 0.3	0.8 ± 0.5	0.4 ± 0.1	0.6 ± 0.3	0.7 ± 0.4	0.6 ± 0	0.4 ± 0	0.8 ± 0.4	0.5 ± 0.2
tetrahydro thiophene	1125	nd	nd	0.3 ± 0.w1	nd	nd	nd	nd	nd	nd
Styrene	1277	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	nd	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	nd
hexanoic acid	1864	0.5 ± 0.2	1.7 ± 0.7	1.9 ± 0.5	1.2 ± 0	2.9 ± 1.6	1.7 ± 0.7	0.5 ± 0	2.0 ± 0.1	1.4 ± 1.2
1-penten-3-ol	1169	nd	0.4 ± 0.2	0.3 ± 0	0.6 ± 0.2	nd	0.2 ± 0.1	nd	0.3 ± 0	0.1 ± 0
1-pentanol	1215	nd	nd	nd	nd	nd	nd	nd	nd	0.9 ± 0.3
2-penten-1-ol	1329	nd	0.3 ± 0.1	0.3 ± 0	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	nd	nd	0.3 ± 0
cyclohexanol-2,6-dimethyl	1645	2.3 ± 0.8	3.6 ± 0.6	2.4 ± 0.7	2.7 ± 0.7	2.3 ± 0.6	2.3 ± 0.2	1.1 ± 0.1	1.8 ± 0.6	nd

(*continued on next page*)

Table 4 (*continued*)

Volatiles compounds	S65			S130					
	G4	G5	G6	G1	G2	G3	G4	G5	G6
Aldheydes									
Propanal	1.6 ± 0.7	1.3 ± 0.3	1.1 ± 0.7	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	1.5 ± 0.4	1.1 ± 0.6	1.3 ± 0.1
Butanal	0.1 ± 0	nd	0.1 ± 0	nd	0.1 ± 0	0.1 ± 0	nd	0.8 ± 1.1	nd
Pentanal	0.6 ± 0.3	0.5 ± 0.4	0.5 ± 0.3	0.7 ± 0.3	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0	0.8 ± 0.3	0.6 ± 0.1
2-butenal	0.7 ± 0.2	3.1 ± 1.6	0.5 ± 0.3	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0	0.7 ± 0.4	0.4 ± 0.2
Hexanal	8.1 ± 4.4	6.8 ± 1.2	4.8 ± 2.0	8.7 ± 2.6	7.5 ± 0.9	5.2 ± 1.7	5.7 ± 0.8	8.3 ± 1.0	5.4 ± 0.1
2-pentenal	1.4 ± 0.4	1.2 ± 0.1	1.2 ± 0.6	1.6 ± 0.2	1.4 ± 0.3	1.6 ± 0.1	1.5 ± 0.2	1.7 ± 0.2	1.3 ± 0.1
Heptanal	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0	0.5 ± 0.2	0.3 ± 0.1
2-hexenal	5.0 ± 0.6	3.5 ± 1.2	3.1 ± 1.2	3.6 ± 0.4	3.8 ± 0.7	3.0 ± 0.4	5.9 ± 2.1	4.1 ± 1.0	3.1 ± 0.9
Octanal	0.3 ± 0.1	0.6 ± 0.4	0.5 ± 0.3	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.3	0.7 ± 0.3	0.8 ± 0.1	0.8 ± 0.4
2-heptenal	1.1 ± 0.1	1.1 ± 0.2	0.9 ± 0.4	0.9 ± 0.2	1.3 ± 0.4	1.0 ± 0	1.0 ± 0.3	1.4 ± 0.2	1.0 ± 0.3
Nonanal	0.8 ± 0.1	1.9 ± 1.0	3.0 ± 1.9	3.1 ± 1.0	2.3 ± 0.6	2.4 ± 0.1	3.4 ± 1.3	2.6 ± 1.0	3.2 ± 1.5
2,4-hexadienal	0.2 ± 0	0.4 ± 0.3	0.5 ± 0.4	1.1 ± 0	0.3 ± 0.1	0.3 ± 0	0.7 ± 0.9	0.6 ± 0.4	0.3 ± 0
2-octenal	2.4 ± 0.1	2.7 ± 0.3	1.9 ± 0.3	1.8 ± 0	2.9 ± 0.5	2.2 ± 0.4	2.3 ± 0.6	3.7 ± 0.3	2.5 ± 0.2
2,4-heptadienal	5.4 ± 0.2	1.1 ± 0.8	2.9 ± 1.9	3.8 ± 2.8	5.4 ± 2.7	4.4 ± 1.9	3.2 ± 0.9	1.2 ± 0.9	2.1 ± 0.1
7-octenal, 3,7-dimethyl	nd	0.3 ± 0.2	0.8 ± 0.5	0.7 ± 0	nd	nd	0.2 ± 0.1	1.3 ± 0.9	1.0 ± 0.5
Decanal	0.4 ± 0.1	0.8 ± 0.3	0.9 ± 0.4	1.1 ± 0.6	0.7 ± 0.3	0.7 ± 0	0.7 ± 0.2	0.8 ± 0.3	1.5 ± 0.7
2,4-heptadienal	9.0 ± 0.2	6.2 ± 0.7	5.6 ± 1.6	6.7 ± 2.0	6.1 ± 0.3	7.4 ± 2.2	9.8 ± 1.4	5.7 ± 2.3	6.3 ± 2.3
Benzaldheyde	3.0 ± 0.2	4.0 ± 0.2	3.6 ± 0.9	3.5 ± 1.7	3.6 ± 0.8	3.3 ± 0.3	4.8 ± 1.1	5.1 ± 0.1	4.1 ± 0.4
2-decenal	0.4 ± 0	0.7 ± 0.2	0.5 ± 0.2	nd	0.7 ± 0	0.8 ± 0	1.6 ± 1.1	1.0 ± 0.5	0.7 ± 0.1
2,4-nonadienal	0.7 ± 0.3	1.2 ± 0.2	0.9 ± 0.1	0.5 ± 0	1.0 ± 0.6	0.9 ± 0.2	1.1 ± 0.1	1.1 ± 0.3	0.9 ± 0
4-ethyl-benzaldheyde	0.3 ± 0.1	0.1 ± 0	0.2 ± 0.1	nd	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	0.1 ± 0	0.3 ± 0.1
2-undecenal	nd	0.8 ± 0.4	0.7 ± 0.3	0.5 ± 0	0.6 ± 0.1	0.6 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.6 ± 0.2
Ketones									
1-penten-3-one	0.4 ± 0	0.3 ± 0.2	0.2 ± 0	nd	0.3 ± 0.1	1.3 ± 0.4	0.6 ± 0.2	0.2 ± 0	0.3 ± 0.1
2,3-pentadione	0.2 ± 0	0.2 ± 0.1	0.1 ± 0	0.3 ± 0	0.2 ± 0	nd	0.3 ± 0.2	0.2 ± 0	0.1 ± 0
5-hepten-2-one-6-methyl	0.9 ± 0	2.3 ± 0.9	2.1 ± 0.4	2.4 ± 0.1	1.9 ± 0.7	1.6 ± 0.1	1.9 ± 0.2	2.7 ± 1.0	2.5 ± 1.2
3-octen-2-one	1.0 ± 0.1	0.3 ± 0.2	0.6 ± 0.4	nd	0.8 ± 0.6	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.7 ± 0.3
3,5-octadien-2-one	9.3 ± 1.3	8.5 ± 1.2	5.0 ± 1.1	8.0 ± 1.4	8.6 ± 0.5	9.4 ± 2.2	6.3 ± 3.3	10.0 ± 2.1	6.9 ± 0.2
b-ionone	6.6 ± 0.5	5.3 ± 1.9	6.4 ± 2.6	4.6 ± 2.9	7.9 ± 0.8	7.3 ± 0.1	4.4 ± 1.1	3.9 ± 1.4	5.3 ± 0.1
Glucosinolate derivatives									
methane isothiocyanate	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	nd	0.2 ± 0	nd	nd	nd
4-methylpentyl isothiocyanate	nd	0.1 ± 0	0.2 ± 0.2	0.3 ± 0	nd	nd	0.6 ± 0.1	nd	nd
hexane 1-isothiocyanate	nd	0.1 ± 0	0.4 ± 0.5	nd	nd	nd	nd	nd	0.1 ± 0.1
pentanitrile 5-methyl thio	2.6 ± 2.2	0.9 ± 0.3	4.3 ± 3.1	3.2 ± 3.0	nd	nd	0.7 ± 0	1.9 ± 0.7	6.4 ± 5.8
Erucin	0.2 ± 0	3.8 ± 3.3	14.8 ± 1.3	0.7 ± 0.3	0.4 ± 0	0.2 ± 0.1	0.6 ± 0	0.3 ± 0.1	1.6 ± 0.1
Others									
carbon sulfide	0.8 ± 0.5	1.6 ± 0	2.2 ± 1.2	1.2 ± 0.5	1.5 ± 1.0	1.5 ± 0.4	1.7 ± 1.0	1.1 ± 0.9	2.2 ± 0.5
carbon disulfide	Tr	nd	tr	0.1 ± 0	nd	nd	nd	nd	nd
furan-2-ethyl	0.9 ± 0.2	1.1 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.2	1.0 ± 0.3	1.1 ± 0.1	0.7 ± 0.5
tetrahydro thiophene	nd	0.1 ± 0	0.5 ± 0.3	nd	nd	nd	nd	nd	nd
Styrene	nd	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
hexanoic acid	1.1 ± 0.2	1.4 ± 0.9	1.2 ± 0.1	0.9 ± 0.3	2.1 ± 0.2	1.1 ± 0.9	0.7 ± 0.3	3.2 ± 2.6	1.1 ± 0.1
1-penten-3-ol	0.5 ± 0.1	0.1 ± 0	0.4 ± 0	0.3 ± 0.1	0.3 ± 0	0.4 ± 0.1	0.8 ± 0.4	0.2 ± 0.1	0.1 ± 0
1-pentanol	nd	0.2 ± 0.1	0.1 ± 0	0.2 ± 0.1	nd	0.2 ± 0.1	0.3 ± 0.1	nd	0.1 ± 0
2-penten-1-ol	0.4 ± 0	0.2 ± 0.1	0.2 ± 0.1	nd	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0	0.2 ± 0.1
cyclohexanol-2,6-dimethyl	2.7 ± 0.9	2.0 ± 0.9	1.7 ± 0.2	1.8 ± 0.6	2.8 ± 0.2	2.7 ± 0.3	1.3 ± 0.5	2.8 ± 0.6	2.0 ± 0.4

Table 5
Effect of saline treatments on the total amount of volatiles compounds (aldheydes, ketones, glucosinolate derivatives and others). The results of two-way ANOVA performed using the genotype (G1-G6) and the treatments (S0, S65, S130) as sources of variation was shown.

	Aldheydes	Ketones	Glucosinolate derivatives	Others
Genotype	< 0.005	< 0.0001	0.0001	< 0.0001
Treatment	< 0.05	< 0.05	< 0.0001	< 0.05
Genotype × Treatment	n.s.	< 0.005	n.s.	< 0.01

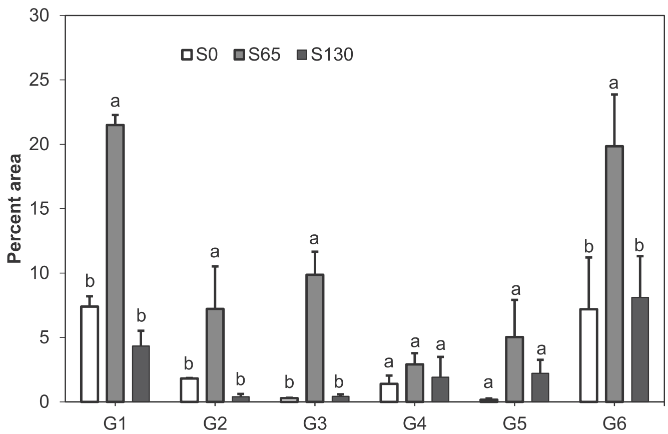

Fig. 3. Sum of glucosinolates hydrolysis products amount in different rocket genotypes (G1 to G6) and irrigation conditions. Results are expressed as mean ± standard error of three independent replicates of percent of the total area of TIC signal (S0 = control, S65 = 65 mM, S130 = 130 mM of NaCl). Post-hoc analysis was performed using Tukey's HSD test (P ≤ 0.05). Different letters above the bars indicated statistically significant difference among treatment at each genotype.

S65. By contrast, in the lower values of PC1 are located shoot dry weight, total dry weight and other biometric parameters suggesting that those variables mainly discriminate the control (S0) along the PC1. Unlike growth parameters, both Na and K were strongly characterized by the third component revealing the complex interaction between

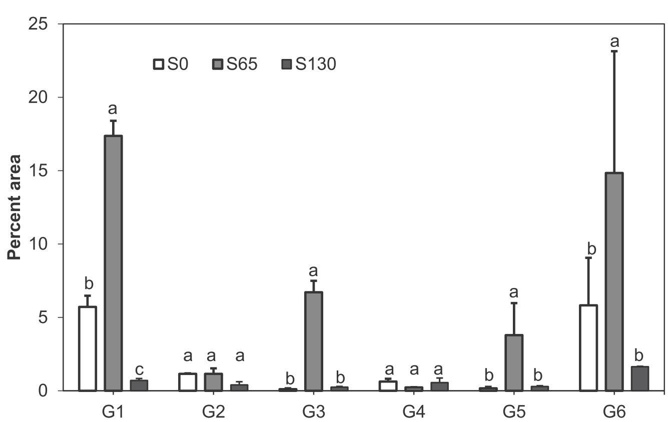

Fig. 4. Erucin amount in different rocket genotypes (G1 to G6) and irrigation conditions. Results are expressed as mean ± standard deviation of three independent replicates of percent of the total area of TIC signal (S0 = control, S65 = 65 mM, S130 = 130 mM of NaCl). Post-hoc analysis was performed using Tukey's HSD test (P ≤ 0.05). Different letters above the bars indicated statistically significant difference among treatment at each genotype.

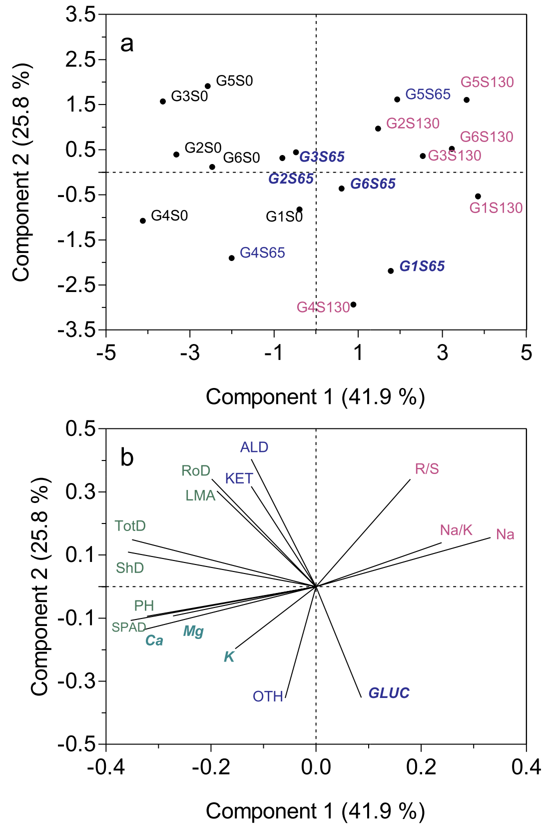

Fig. 5. Data scores (A) and variable loadings (B) obtained by PCA: leaf mean area (LMA); plant height (PH); root dry weight (RoD); root/shoot dry weight ratio (R/S); shoot dry weight (ShD); total dry weight (TotD); shoot calcium concentration (Ca); shoot magnesium concentration (Mg); shoot potassium concentration (K); shoot sodium concentration (Na); Na/K ratio (Na/K); SPAD index (SPAD); Aldheydes (ALD); Glucosinolate derivatives (GLUC); Ketones (KET); other volatile compounds (OTH). In Fig. 5a, black, blue and purple represent S0, S65, and S130 treatments, respectively, while combinations (genotype x treatment) in blue bold-italic stay for those genotypes that showed higher accumulation of glucosinolates at S65 (see Fig. 3). In Fig. 5b, green represents variables related to plant growth and tissue mineral content (bold-italic), blue represents volatile compounds, and purple represents variables positively correlated with saline stress. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plant nutrition and salinity (Table S5, Supporting information). However, Na accumulation was also negatively correlated with macrocations concentration in plant tissues (Fig. 5). Indeed, G2 and G4, which showed the best performance in terms of salinity tolerance, could be grouped and correlated with higher accumulation of macrocations, lower accumulation of Na and high biomass production. The latter relationship can be also verified for G3 and G6 that, however, suffered more the presence of salinity at high concentration compared with G1, G2 and G4. The loading plots also highlights a clear relationship between the genotypes G1, G2, G3, G6 irrigated with 65 mM NaCl (S65) and glucosinolates characterized by high value of PC1 in combination with low values of PC2.

Finally, the PCA was able to distinguish three main categories of genotypes: i) genotypes more productive in general (G2, G3, G4 and G5); ii) genotypes that accumulates higher mineral elements and show higher tolerance to salinity in terms of biomass production (G1, G2 and G4); iii) genotypes that show major affinity with the production of glucosinolates under medium (65 mM NaCl) saline stress (G1, G2, G3 and G6).

4. Conclusion

Physiological and biochemical plant parameters varied based on the genotype and treatment: growth parameters (root, shoot, height and LMA), SPAD index and mineral elements were significantly affected by genotype and the saline treatment. Interestingly, health promoting secondary metabolites, such as glucosinolates derivatives, significantly increased as the consequence of the moderate saline treatment, and deeply decrease with the highest level of NaCl treatment. PCA distinguished three genotypes (G1, G2; G4) that showed higher tolerance to salinity based on biomass production and cations accumulations, two of them (G1, G2) are also reported to increase the glucosinolate products under moderate salinity condition (65 mM NaCl). Yet, the variability observed in the response of six genotypes to salinity appears of great interest for future breading programs.

Author contributions

G.L.P. designed and performed the study, analyzed the chemical data and drafted the manuscript, P.P.U. performed, collected and analyze the chemical data, D.M. analyzed data and critical review of the article; S.M. designed and perform the research, analyzed the agronomic data, drafted the manuscript and critical review the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.05.012.

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