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Research article

# Metabolomic insights into the mechanisms underlying tolerance to salinity in different halophytes

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ABSTRACT

Salinity is among the most detrimental and diffuse environmental stresses. Halophytes are plants that developed the ability to complete their life cycle under high salinity. In this work, a mass spectrometric metabolomic approach was applied to comparatively investigate the secondary metabolism processes involved in tolerance to salinity in three halophytes, namely *S. brachiata, S. maritima* and *S. portulacastrum*. Regarding osmolytes, the level of proline was increased with NaCl concentration in *S. portulacastrum* and roots of *S. maritima*, whereas glycine betaine and polyols were accumulated in *S. maritima* and *S. brachiata.*

Important differences between species were also found regarding oxidative stress balance. In *S. brachiata*, the amount of flavonoids and other phenolic compounds increased in presence of NaCl, whereas these metabolites were down regulated in *S. portulacastrum*, who accumulated carotenoids. Furthermore, distinct impairment of membrane lipids, hormones, alkaloids and terpenes was observed in our species under salinity. Finally, several other nitrogen containing compounds were involved in response to salinity, including amino acids, serotonin and polyamine conjugates.

In conclusion, metabolomics highlighted that the specific mechanism each species adopted to achieve acclimation to salinity differed in the three halophytes considered, although response osmotic stress and oxidative imbalance have been confirmed as the key processes underlying NaCl tolerance.

## 1. Introduction

World population is increasing rapidly to exceed nine billions by 2050 (Department of Economic and Social affairs of the United Nations, 2015). Hence, the global food production must increase substantially to ensure food security for the growing population. However, food production is seriously threatened by various environmental factors including salinity, drought, heat and cold, that cause significant yield loss in large areas (Nauš, 2010; Mantri et al., 2012). Among these factors, soil salinity is one of the major stresses adversely affecting plant growth and crop productivity, especially in arid and semi-arid regions. Of the current 230 million ha of irrigated land, 45 million ha (19.5%) have been already damaged by salt (FAO, 2016). In fact, salinity and pollution are major threats affecting soil and the ecosystem services (Rodriguez-Eugenio et al., 2018). Soil salinity affects plants in two ways: (i) high salt concentrations generate osmotic stress, thus hampering roots water uptake from soil, and (ii) high concentrations of salts inside the plant can have toxic effects (Hasegawa et al., 2000; Munns, 2002; Munns and Tester, 2008). Salts in rhyzosphere have an instant osmotic effect on cell growth and associated metabolism, whereas high concentrations of salts need some time to accumulate within plants to cause a subsequent ionic effect, leading to nutrient deficiency and oxidative stress (Chinnusamy et al., 2006). The detrimental effects of salinity on agricultural yield are significant, mainly because plants face delayed seed germination, slower growth, reduced tillering and perturbations in reproductive development as well as grain yield, quality and quantity (Hu and Schmidhalter, 2005; Munns and Tester, 2008; Horie et al., 2012; Nandal and Hooda, 2013). To survive against these stresses, plants respond and adapt with complex mechanisms that involve stress sensing, signal transduction, and the activation of a number of stress-related genes and metabolites. Understanding the metabolic changes in plants under various conditions, might help to identify new strategies to improve plant tolerance to stress rather than drive the selection of tolerant species or cultivars.

Halophytes are unique in their capability survive and complete their life cycle in a salt concentration of at least 200 mM NaCl (Flowers and

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Colmer, 2008), and to accumulate salts without detrimental effects. *Sesuvium portulacastrum* is an herbaceous facultative halophyte belonging to the family Aizoaceae (Lokhande et al., 2009). *Sesuvium* is one of the most known ‘salt accumulators’, as it accumulates high concentrations of salts in their cells and tissues and overcomes salt toxicity by developing succulence (Youssef, 2009). However, *Suaeda maritima* and *Salicornia brachiata* are a succulent halophyte belongs to *Chenopodiaceae*, and can accumulate considerable amounts of $Na^+$ in their shoots without having special salt secreting structures like salt glands or bladders. Both require an adequate concentration of NaCl for optimal growth and development (Reddy et al., 1993). Their growth was significantly reduced under 100–1000 mM NaCl, although no toxicity symptom appeared (Messeddi et al., 2001; Messedi et al., 2004). Moreover, some studies have reported that salinity induces the accumulation of low-molecular-weight organic compounds such as glycine betaine, proline, and sugars in the cytoplasm, that perform as osmolytes and osmoprotectors in these halophytes (Moghaieb et al., 2004). Besides osmolytes, plant secondary metabolites are important in the interaction with the surrounding environment. Salinity induces disturbance of secondary metabolites having an osmoregulatory role and often being involved in plant defense and stress acclimation (Zhao et al., 2005). Furthermore, these compounds are engaged in a variety physiological roles including ROS scavenging, enzyme activation, photoprotection and signal regulation (Zhao et al., 2005; Naoumkina et al., 2010; Arbona et al., 2013). With this regard, metabolomics is the understanding of metabolite networks, represented by the identification and quantification of metabolites present in an organisms during a given condition (Arbona et al., 2013), including those synthesized in response to environmental changes (Fiehn, 2002). Due to its holistic view on metabolites, metabolomics can provide a very informative picture on the biochemical processes underlying the plant response to either biotic or abiotic stresses (Bowne et al., 2011; Jorge et al., 2016).

A vaste literature has been already reported on plant metabolite profiling under salinity, in several individual model plants. However, a comparative assessment is still lacking, although it might be of interest since it might provide a more generalized picture of halophytes tolerance mechanisms towards salinity. Therefore, the aim of this study was to investigate and compare the impact of optimum and high NaCl levels on secondary metabolisms of *S. portulacastrum, S. maritima* and *S. brachiata* using metabolomics, to shed light onto the differential metabolite accumulation with their contrasting tolerance to adverse situations.

## 2. Materials and methods

### 2.1. Plant material and salt treatment

Seeds of *Salicornia brachiata, Suaeda maritima* and *Sesuvium portulacastrum* stem cuttings were collected from Pichavaram, Tamil Nadu India. The seedlings of *S. brachiata* and *S. maritima* were germinated in vermiculite filled pots kept in plastic trays and *S. portulacastrum* plants were propagated by making cuttings. The cuttings developed roots in about 7 d. The plants were transferred to hydroponics containing modified Hoagland's medium (Wang et al., 2007a) and maintained at 25 °C with a 16/8 h light/dark photoperiod conditions. The plants were acclimatized for 7 days and then treated with 200 mM and 500 mM NaCl for 14 days. The lower salinity level was chosen since euhalophytes have been defined as plants completing their life cycle in salt concentrations > 200 mM NaCl (Behr et al., 2017), whereas the highest concentration represented a stress condition even for halophytes (Lv et al., 2012; Yi et al., 2014). Harvesting (10 plants per treatment) was performed during day time and the tissues were immediately quenched in liquid nitrogen to deter all metabolic activity. Frozen leaves were crushed and ground to fine powder and stored at −80 °C for subsequent analyses.

### 2.2. Metabolomic analysis

Freeze dried leaf samples (0.50 g) were extracted in 5 mL of 0.1% HCOOH in 80% methanol using an Ultra-Turrax (Ika T-25, Staufen, Germany), filtered through a 0.22 μm cellulose membrane to avoid clogging during chromatographic separation, and transferred to an amber vial for analysis. The screening of plant metabolites was carried out using a liquid chromatograph coupled to a quadrupole-time-of-flight mass spectrometer (UHPLC/QTOF-MS). In more detail, a 1290 series liquid chromatograph system, equipped with a binary pump, degasser and a JetStream Electrospray ionization system, was interfaced to a G6550 iFunnel mass spectrometer (all from Agilent technologies Santa Clara, CA, USA). The instrument was operated as previously reported (Kumar et al., 2015). Briefly, the QTOF was run in SCAN mode (positive polarity, 100–1200 m/z range) and extended dynamic range mode. Chromatographic separation was achieved in reverse phase, using water and methanol as mobile phases on an Agilent Zorbax Eclipse-plus column (75 × 2.1 mm i.d., 1.8 μm). Formic acid (0.1%, *v/v*) and ammonium formate (5 mM) were used as mobile phase modifiers. The elution gradient started from 5% to 90% methanol within 35 min, injection volume was 3.5 μL and flow rate was 220 μL $min^{-1}$. Deconvolution, mass and retention time alignment, as well as filtering (mass accuracy < 5 ppm, single ion ID disabled) were carried out using the software Profinder B.06 (from Agilent Technologies). Post-acquisition processing was done according to Rouphael et al. (2016): compounds annotation was based on accurate mass, isotope spacing and isotope ratio, against the database exported from PlantCyc 9.5 (Plant Metabolic Network, http://www.plantcyc.org; accessed April 2017). Finally, compounds were filtered by frequency: those compounds that were not present in 100% of replications (N = 5) within at least one treatment were discarded.

The procedure adopted corresponded to an identification according to Level 2 (i.e., putatively annotated compounds) of COSMOS Metabolomics Standards Initiative (http://cosmos-fp7.eu/msi).

### 2.3. Proline assay

Proline was determined in the same extract produced for metabolomic analysis, using a liquid chromatograph coupled to a triple quadrupole tandem mass spectrometer (LC-MS/MS) (Salehi et al., 2018). A 1200 series liquid chromatograph, interfaced to a 6410A mass spectrometer via an electrospray ionization source (all from Agilent Technologies), was used. Extracts (3 μL) were separated on an Agilent Poroshell 120 EC-C18 chromatographic column (2.1 × 100 mm, 2.7 μm particle size) using a water-methanol binary gradient (from 70% methanol to 85% methanol in 6 min,) and a flow of 0.2 mL $min^{-1}$. Tandem MS target analysis was used for both identification and quantification, in positive ionization mode. The MS/MS transition used for was m/z 116 to 70, with collision energy of 18 V. Nitrogen (10 L $min^{-1}$, 275 °C) was used as gas in the electrospray, whereas capillary voltage was set to 4000 V. Proline was quantified by the external standard method using a calibration curve from freshly prepared solutions.

### 2.4. Statistical analysis

Each treatment was set in triplicate, in a completely randomized design. Interpretation of metabolomics was done in Mass Profiler Professional B.12.06 (Agilent technologies), as previously reported (Pretali et al., 2016). Compounds abundance was Log2 transformed and normalized at 75th percentile, then baselined against the median in control. An unsupervised hierarchical cluster analysis was then carried out using an “Euclidean” similarity measure and “Wards” as linkage rule. Differential metabolites were next gained using Volcano plot analysis, *i.e.* combining analysis of variance (moderated *t*-test, $P < 0.01$, Bonferroni multiple testing correction) with fold-change analysis (cut-off value = 5).

**Table 1**

Proline content in roots and leaves of *S. brachiata*, *S. portulacastrum* and *S. maritima* plants grown either under 0, 200 or 500 mM NaCl. Superscript letters denote homogenous classes as identified by ANOVA (Duncan post-hoc; Significance: n.s. = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

| | Salinity (NaCl) | Proline content (mg/100 g) | |
|---|---|---|---|
| | | Leaves | Roots |
| *S. brachiata* | 0 mM | $0.01^{a}$ | $0.04^{a}$ |
| | 200 mM | $0.02^{a}$ | $0.05^{ab}$ |
| | 500 mM | $0.04^{b}$ | $0.07^{b}$ |
| | Sig. | * | * |
| *S. portulacastrum* | 0 mM | $2.89^{a}$ | $3.61^{a}$ |
| | 200 mM | $5.11^{b}$ | $8.61^{b}$ |
| | 500 mM | $6.44^{c}$ | $13.61^{c}$ |
| | Sig. | *** | *** |
| *S. maritima* | 0 mM | $0.03^{a}$ | $2.04^{a}$ |
| | 200 mM | $0.03^{a}$ | $7.04^{b}$ |
| | 500 mM | $0.03^{a}$ | $12.04^{c}$ |
| | Sig. | n.s. | *** |

## 3. Results

### 3.1. Proline and other osmolytes

Looking at proline levels (Table 1), different strategies could be found to cope with osmotic stress, across the species considered. Indeed, LC-MS/MS target analysis revealed that a large increase in proline could be observed in both roots and leaves of *S. portulacastrum*, in a dose-dependent manner. For this species, a positive correlation existed between the proline content and salinity treatment. In this species, the level of proline was decreased at low salinity whereas a significant increase in proline was observed in both shoots and roots at high salinity. In more detail, proline levels increased from 2.89 to 6.44 mg $100\,g^{-1}$ and from 3.61 to 13.61 mg $100\,g^{-1}$ in leaves and roots, respectively. However, *S. maritima* showed proline accumulation in roots only (from 2.04 to 12.04 mg $100\,g^{-1}$), whereas no differences could be observed in leaves and roots from *S. brachiata*. Noteworthy, as will follow, UHPLC/QTOF-MS untargeted metabolomics pointed out that *S. maritima* and *S. brachiata* exhibited increases in glycine-betaine and polyols respectively, especially in shoots.

### 3.2. Metabolic response to salt treatments

In order to investigate the metabolic changes in *S. brachiata, S. maritima* and *S. portulacastrum* plants under salinity, a metabolomic approach based on UHPLC-QTOF mass spectrometry was applied. Although the post-acquisition filters reduced the number of IDs, more than 3600 compounds were annotated by UHPLC/QTOF-MS metabolomics in either shoots or roots from the halophytes under investigation. The list of compounds is provided as supplementary material together with their abundance and composite spectra.

Different statistic approaches were used to reduce data complexity and better point out differences. The unsupervised hierarchical cluster analysis evidenced three main clusters, based on species, thus indicating that the response was species-specific (Fig. 1). As far as concerns the organs within each species, salinity-triggered metabolomic changes involved both roots and leaves, in a dose-dependent manner. In more detail, separate sub-clusters could be found between control and the different NaCl dosages within each species. All replications within each treatment clustered together, indicating that the changes induced by salinity were hierarchically greater than biological and technical variability. Only for *S. portulacastrum* leaves a mixed cluster was observed, including replications from all salinity levels; this indicated that the treatments in this species had a slight effect on leaf metabolome. This unsupervised approach also suggested that, even the three species considered are relatively close; each of them adopted a distinct metabolic adaptation to salinity.

The following Volcano Plot analysis (unpaired *t*-test with $P < 0.001$ and fold-change $> 5$) allowed identifying 183 to 214 differential compounds from *S. brachiata* as a function of the NaCl dosage and the matrix considered. However, 60 to 95 and 67 to 104 differential compounds could be pointed out by Volcano Plot in *S. maritima* and *S. portulacastrum*, respectively. These numbers confirmed that NaCl salinity imposed a huge reprogramming of metabolic profile in shoots and roots of the species considered. The differential metabolites in leaves and roots, identified from Volcano analysis, are given in Tables 2–4, together with individual fold-changes and p-values. Metabolites were classified in biochemical classes, to facilitate the elucidation of response mechanisms to salinity in the species considered.

### 3.3. Flavonoid-related metabolites

The accumulation profile of different flavonoid-related compounds in leaves and roots of *S. brachiata, S. maritima* and *S. portulacastrum* when subjected to 200 mM and 500 mM NaCl could be identified. Distinctive patterns of flavonoid accumulation were observed between the species and also among stress treatments.

In *S. brachiata*, NaCl induced a clear and consistent up-regulation of several flavonoids in both leaves and roots, with some differences between mild and higher salt stress. Indeed, the levels of quercetin derivatives, kaempferol derivatives, anthocyanin derivatives including delphinidin, cyanidin and hesperitin showed a significant accumulation in shoots and roots, under both salt treatments. Moreover, the levels of flavones and peonidin were constitutively higher in *S. brachiata* at higher NaCl dosage. However, lower dosage induced the accumulation of phenolic compounds such as luteolin derivatives (luteolin 7-*O*-neohesperidoside, luteolin 7-*O*-gentiobioside). In turn, glycosylated anthocyanidins including pelargonidin 3-*O*-sophoroside and pelargonidin 3,7-di-*O*-β-D-glucoside, showed a significant accumulation in response to salt in both tissues.

The metabolomic change in *S. maritima* was significantly different; some flavonoids were down accumulated whereas some other an increase was observed at the lower salinity level in both shoots and roots. Hence, a clear trend could not be evidenced. Meanwhile, dihydroquercetin and 2-hydroxyeriodictyol levels were increased in both *S. brachiata* and *S. maritima* under salinity, while 2′,3,4,4′,6′-pentahydroxychalcone 4′-*O*-β-D-glucoside and 6C-glucosyl-2-hydroxynaringenin showed opposite trends in *S.brachiata* as compared to *S. maritima*. In roots, the levels of peonidin 3,5-diglucoside, derivatives of kaempferol, 2′-hydroxy 3,6,7,4′-tetramethylquercetagetin and 4′-methoxyisoflavone were down accumulated whereas, flavonol 3-*O*-galactosideand sakuranin were up accumulated in higher dosage.

Distinct results could be evidenced also in *S. portulacastrum*, where several flavonoids and phenolic compounds were down accumulated in both tissues under salinity. Phenolic metabolites such as biochanin-A, isorhamnetin 3-*O*-glucoside, rhamnetin, peonidin 3,5-diglucoside and derivatives of catechin were down accumulated at both dosages. In addition, the levels of quercetin, anthocyanin and kaempferol derivatives were reduced in leaves. However, hesperidin increased significantly its levels in both the treatments whereas flavanone-7-*O*-β-D-glucoside was up accumulated at higher NaCl dosage.

### 3.4. Phenylpropanoids and lignans

Phenolic compounds belonging to phenylpropanoids and lignans were altered in salt treated *S. brachiata* and *S. portulacastrum* plants, most of them being up accumulated. Phenylpropanoid including curcumin diglucoside, 1-*O*-feruloyl-β-D-glucose and 1-*O*-caffeoyl-β-D-glucose were increased in both tissues at the higher salinity level, whereas

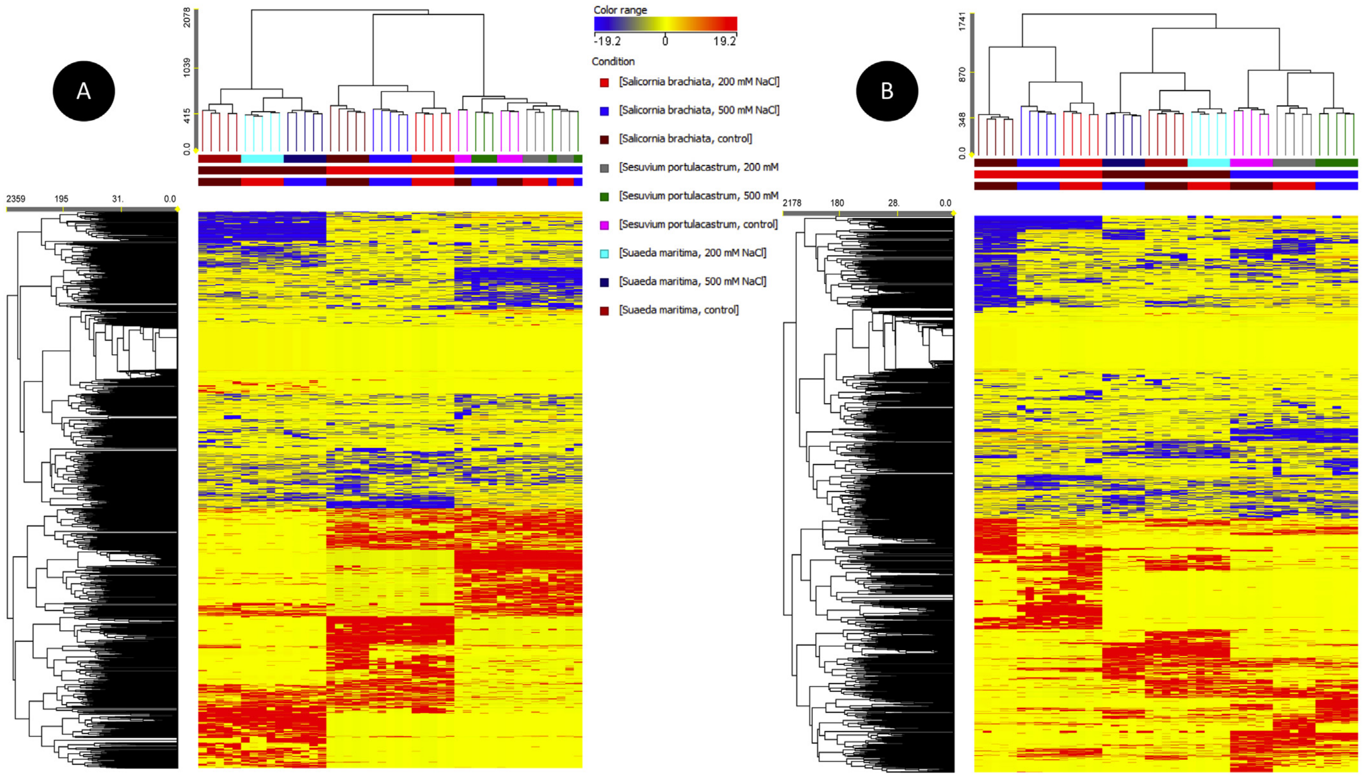

**Fig. 1.** Hierarchical cluster analysis produced from metabolites profile of leaves (A) and roots (B) from *S. maritima*, *S. brachiata* and *S. portulacastrum* grown either at 0, 200 and 500 mM NaCl. The unsupervised clusters were produced from the fold-change based heat-map (distance metric: Euclidean; linkage rule: Ward's) starting from untargeted metabolomic profiles.

caffeate was down accumulated in both tissues at the lower NaCl dosage. Lignans such as sesamolin, secoisolariciresinol, pinoresinol and matairesinol were up accumulated in both tissues of *S. brachiata* under the highest dosage. However, phenylpropanoids and lignans were marginally altered in *S. maritima*.

### 3.5. *Alkaloids and amine compounds*

The levels of several alkaloids were altered in *S. brachiata* under salinity, with the strongest up accumulation being recorded in leaves at the higher NaCl dosage. These trends were less evident at 200 mL NaCl, where some up and some down accumulations were found. On the other hand, the levels of several alkaloids (*e.g.*, deoxypumiloside, noscapine, chelirubine, and 2-descarboxy-betanidin) decreased in roots of *S. maritima*. Nonetheless, any significant impact on the accumulation of alkaloids-related compounds was observed in *S. maritima* shoots. In leaves of *S. portulacastrum* grown at 200 mM NaCl, cyclo-acetoacetyl-*L*-tryptophan, castanospermine, chelirubine were down accumulated whereas the levels of cyclo-dopa 5-*O*-glucoside, N-formyldemecolcine and colchicines increased.

Several dipeptides or amino acid related compounds could be recorded among differential compounds across species. Amino acids and dipeptides (*i.e.*, D-alanyl-D-serine, D-alanyl-D-alanine, L-alanyl-L-glutamate, glutamine, tyrosine, and cystine) in *S. brachiata* were found at reduced levels, but some compounds like 3-methoxytyramine, ornaline, *L*-homomethionine, tetrahomomethionine, 1-(3-aminopropyl)-4-aminobutanal, *L*-ethionine, feruloylagmatine, *N,N*-dihydroxy-L-isoleucine, N,N-dihydroxy-*L*-dihomomethionine, 4-(3-methylbut-2-enyl)-L-abrine, 5,10-methylenetetrahydropteroyl mono-*L*-glutamate were up accumulated. Among others, the levels of glycine betaine (i.e., an osmolyte), D-alanyl-D-serine, cystine, serotonin and threonine, 1-aminocyclopropane-1-carboxylate (precursor of ethylene), tricaffeoyl spermidine (a conjugated polyamine) were altered in *S. maritima*. In *S. portulacastrum*, the levels of *L*-cysteinyl-glycine, cystine, *N*5-formyl-*N*5-hydroxy-*L*-ornithine, 1-pyrroline-5-carboxylate were increases and *N*-benzoyl-*L*-glutamate, coumaroyl spermidine (a conjugated polyamine) were decreases across the treatments.

### 3.6. *Terpenes*

Terpenes were also involved in the response to salinity treatments, although with contrasting trends across species. Alteration in terpenes profile was evident in leaves and roots of *S. brachiata*. The most of sesquiterpenoids such as desoxyhemigossypol-6-methyl ether and heliespirone C, rather than costunolide and 15-hydroxysolavetivone were up accumulated under salinity, while the phytoalexin zealexin B1 was down accumulated under the lower NaCl dosage. Also the level of some triterpenoids (ursolate, 16-α-hydroxygypsogenate-28-β-D-glucoside) increased in salt stress conditions, together with diterpenoids (such as levopiramadiene-diol, abietadiene-related compounds, oleanolate and the phytoalexin oryzalexin). In roots of *S. brachiata*, the terpenoids taxol, oleanolate 3-β-D-glucuronoside-28-glucoside and glycyrrhetinate were up accumulated under salinity. A different trend was recorded in *S. maritima*, where several terpenes were accumulated in roots but unclear trends were observed in leaves.

In turn, the biosynthesis of triterpenoids including amyrins and betulinic acid was reduced in roots of *S. portulacastrum*. Like for *S. maritima*, unclear trends could be observed in leaves.

### 3.7. *Lipids*

Several oxidized forms of lipids (mainly hydroxy- or epoxy-derivatives, potentially acting as oxylipins), together with mono- and diglycerides, were up accumulated in leaves and roots of *S.brachiata* under both salt conditions. Among others, salinity altered the level of dihydroxyoctadeca-dienoates, HPODE, 3-oxo-2-(cis-2′-pentenyl)-

**Table 2**
Metabolites differentially accumulated in *Salicornia brachiata* leaves and root of plants grown under 200 or 500 mM NaCl salinity, as compared to control (no salinity). Compounds were selected through Volcano Plot analysis (unpaired *t*-test at $P < 0.01$ with Bonferroni multiple testing correction, and fold-change analysis with cut-off = 5). Missing values denote no significant differences.

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| **LEAVES** | | | | |
| **Flavonoid related compounds** | | | | |
| Cirsimaritin/Ladanein | - | - | 6.12E-07 | +1.03 |
| Sakuranin | - | - | 1.88E-05 | +1.18 |
| Luteolin 7-O-neohesperidoside | 4.19E-11 | +4.96 | - | - |
| Luteolin 7-O-gentiobioside | 3.84E-14 | +5.14 | - | - |
| Tetramethyl myricetin | 5.03E-14 | +5.05 | - | - |
| Isovitexin 7-O-glucoside | 4.19E-11 | +4.96 | - | - |
| 6C-hexosyl chrysin | 5.85E-14 | +4.88 | - | - |
| Apigenin-7-O-gentiobioside | 4.19E-11 | +4.96 | - | - |
| Afrormosin-7-O-glucoside | 1.65E-15 | +4.94 | - | - |
| Dalpatein 7-O-β-D-apiofuranosyl -(1–6) - β-D-Glucopyranoside | 6.75E-15 | +4.84 | - | - |
| 2-hydroxyeriodictyol | 2.03E-13 | +4.80 | - | - |
| Taxifolin | 7.52E-14 | +5.03 | - | - |
| Rutin | 3.84E-14 | +5.71 | - | - |
| Pelargonidin 3,7-di-O β-D-glucoside/Pelargonidin 3-O-sophoroside | 4.19E-11 | +4.96 | - | - |
| Dihydroquercetin | 2.03E-13 | +4.80 | - | - |
| 6C-glucosyl-2-hydroxynaringenin | 3.93E-12 | +5.39 | - | - |
| **Phenylpropanoids and lignans** | | | | |
| Curcumin diglucoside | - | - | 1.10E-15 | +4.93 |
| 1-O-caffeoyl-β-D-glucose | - | - | 0 | +5.08 |
| 1-O-feruloyl-β-D-glucose | 1.12E-10 | +6.17 | - | - |
| Caffeate | 0 | −6.39 | - | - |
| 2,4-dihydroxycinnamate | 0 | −6.40 | - | - |
| Sesamolin | - | - | 8.46E-16 | −5.18 |
| Secoisolariciresinol | - | - | 7.65E-15 | +5.16 |
| Pinoresinol/Matairesinol | - | - | 3.00E-13 | +5.09 |
| **Alkaloids and amine compounds** | | | | |
| 6-*O*-methyl-N-deacetylisoipecoside aglycon | - | - | 7.47E-15 | +4.87 |
| Cinchoninone | - | - | 9.15E-16 | +5.09 |
| Strictosamide | - | - | 1.94E-15 | +4.94 |
| Solasodine | - | - | 2.30E-10 | −8.23 |
| 7,8-dihydroberberine | - | - | 1.97E-10 | −6.36 |
| Cyclo-acetoacetyl-*L*-tryptophan | 2.89E-11 | +4.79 | - | - |
| 3, 6-dihydronicotine | 1.38E-09 | +5.06 | - | - |
| Portulacaxanthin II | 6.14E-14 | +5.09 | - | - |
| 5-hydroxy-γ-coniceine | 1.37E-08 | −1.05 | - | - |
| Cyclo-dopa 5-*O*-glucoside | 1.44E-12 | −5.45 | 2.24E-12 | −5.34 |
| Harmol | 1.65E-15 | −5.67 | 1.19E-15 | −5.57 |
| Ricinine | 8.30E-15 | −5.99 | 1.01E-14 | −5.89 |
| Papaveroxine | 1.04E-14 | +4.85 | 6.38E-16 | +5.65 |
| Secoberbine | 5.39E-13 | +4.92 | - | - |
| D-alanyl-D-serine | 6.25E-09 | −1.75 | 8.46E-08 | −1.04 |
| D-alanyl-D-alanine | 1.79E-11 | −1.97 | 1.12E-10 | −1.52 |
| D-alanyl-D-Glutamate | 0 | −1.20 | 0 | −1.20 |
| Glutamine | - | - | 0 | −1.20 |
| Tyrosine | - | - | 5.28E-15 | +6.05 |
| 3-methoxytyramine | 8.12E-15 | +6.13 | 5.22E-16 | +6.14 |
| Ornaline | - | - | 1.71E-12 | +6.11 |
| L-homomethionine | - | - | 1.40E-14 | +4.88 |
| Tetrahomomethionine | - | - | 2.82E-15 | +5.00 |
| 1-(3-aminopropyl)-4-aminobutanal | - | - | 6.05E-12 | +5.44 |
| L-ethionine | - | - | 1.40E-14 | +4.88 |
| Feruloylagmatine | - | - | 3.27E-15 | +5.22 |
| N,N-dihydroxy-L-isoleucine | - | - | 4.34E-15 | +5.43 |
| **Terpenoids** | | | | |
| Desoxyhemigossypol-6-methylether | - | - | 6.68E-09 | +1.72 |
| Heliespirone C | - | - | 0 | +1.20 |
| Costunolide | 1.04E-14 | +4.74 | - | - |
| 15-hydroxysolavetivone | 6.89E-12 | +5.16 | - | - |
| Zealexin B1 | 3.85E-11 | −5.42 | - | - |
| Ursolate | - | - | 0 | +1.20 |
| 16-α-hydroxygypsogenate-28-β-D-glucoside | - | - | 1.51E-11 | +4.89 |
| Levopiramadiene-diol/Abieta-7,13-dien-18,18-diol/Palustradiene-diol/Oryzalexin D/E/F/S | 1.00E-14 | +5.89 | - | - |
| Cyclooctat-9-en-5,7-diol | 3.23E-16 | +5.62 | - | - |
| Oryzalexin B | 0 | +1.20 | 0 | +1.20 |
| Citronellate | 0 | +1.07 | - | - |
| Oleanolate | 9.84E-15 | +6.01 | - | - |

(*continued on next page*)

Table 2 (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Gypsogenate | 3.71E-12 | −2.17 | - | - |
| **Lipids** | | | | |
| Stearidonate | 8.30E-15 | +5.14 | 8.20E-12 | +5.28 |
| Coniferonate | 8.30E-15 | +5.14 | 8.20E-12 | +5.28 |
| A dihydroxyoctadeca-dienoate/HPODE | 7.46E-16 | +5.98 | 5.22E-16 | +5.97 |
| (9Z,12Z)-octadecadien-6-ynoate | 8.30E-15 | +5.14 | – | – |
| Omega hydroxycaprate | – | – | 8.20E-12 | +5.28 |
| 1-18:3-2-18:3-phosphatidylcholine | – | – | 4.34E-15 | +5.72 |
| 4-hydroxysphing-8-enine-20:1 ceramide | – | – | 1.11E-15 | +4.94 |
| 1-18:1-2-18:1 phosphatidate | – | – | 6.37E-09 | −6.31 |
| 2-trans-6-trans-farnesyl monophosphate | 7.99E-16 | +5.05 | - | - |
| (9S)-HPODE | – | – | 5.22E-16 | +5.97 |
| 2-C22:0-DCA-LPA | 7.02E-15 | +5.55 | 4.32E-15 | +5.26 |
| 1-16:0-2-lysophosphatidylcholine | 1.21E-15 | +7.09 | 9.15E-16 | +6.53 |
| (8Z,11Z,14Z)-icosatrienoate | 1.15E-14 | +5.89 | - | - |
| Colneleate/3-oxo-2-(cis-2′-pentenyl)-cyclopentane-1-octanoate/an epoxyoctadeca-dienoate | 3.23E-16 | +6.24 | - | - |
| Icosadienoate | 7.46E-16 | +5.62 | - | - |
| Phosphatidylglycerophosphate (1-octadecenoyl(9Z) 2-Palmitoyl) | 1.90E-14 | +5.41 | - | - |
| **Hormones** | | | | |
| Gibberellins A46 | – | – | 2.03E-12 | +5.25 |
| Gibberellins A25 | – | – | 4.71E-15 | +5.33 |
| Gibberellins A37/A97 | – | – | 7.65E-15 | +5.16 |
| Gibberellins A13/A17 | – | – | 2.03E-12 | +5.25 |
| Gibberellins A8 catabolite | – | – | 1.12E-13 | +5.31 |
| Gibberellin A7 | 0 | +1.20 | - | - |
| Dihydrozeatin-7/9-N-glucoside | - | - | 5.22E-16 | +4.95 |
| trans-zeatin-O-glucoside-7-N-glucoside | 5.62E-14 | +4.79 | - | - |
| Isopentenyladenine-9-N-glucoside | - | - | 0 | +1.20 |
| 3-O-β-D-glucoside digitoxigenin | 2.71E-14 | +5.69 | 5.28E-15 | +5.41 |
| 4α-carboxy-ergosta-7,24(24)-dien-3β-ol | – | – | 8.50E-16 | +5.69 |
| Lathosterol/5β-cholestan-3-one | 3.84E-14 | +5.33 | - | - |
| 24-alkyl sterol 2/3 | 1.49E-14 | +5.69 | - | - |
| 4α-carboxy,4β,14α-dimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol | 9.84E-15 | +6.01 | - | - |
| (24R)-cholest-5-ene-3β,7α,24-triol | 7.41E-14 | +5.13 | - | - |
| 4α-carboxy-stigmasta-7,24(24)-dien-3β-ol | 9.84E-15 | +6.01 | - | - |
| Indole-3-acetate | 2.04E-08 | −1.31 | - | - |
| Indole acetaldehyde | – | – | 2.56E-09 | −1.08 |
| 11-hydroxyjasmonate sulfate | 3.20E-13 | +4.89 | - | - |
| Jasmonoyl-phenylalanine | 2.20E-14 | +4.77 | 2.78E-09 | +5.05 |
| 4α-hydroxymethyl-5α-cholesta-7,24-dien-3β-ol | 1.49E-14 | +5.69 | - | - |
| Indole-3-butyryl-glucose | 1.21E-15 | −6.35 | 9.03E-16 | −6.25 |
| (22R,23R)-22,23-dihydroxy-campest-4-en-3-one | 8.12E-15 | +6.52 | - | - |
| Brassinolide | 0 | −2.16 | 0 | −1.74 |
| (22α)-hydroxy-campest-4-en-3-one | 1.49E-14 | +5.69 | - | - |
| 6-oxocampestanol | 1.80E-14 | +5.48 | - | - |
| (22R,23R)-22,23-dihydroxycampesterol/24-epicathasterone/3-dehydro-6-deoxoteasterone | 4.78E-15 | +6.00 | - | - |
| (22α)-hydroxy-campesterol/22-hydroxy-5α-ergostan-3-one | 1.80E-14 | +5.48 | - | - |
| (22R,23R)-28-homocastasterone 22-O-sulfate | – | – | 2.09E-13 | +4.93 |
| 6-deoxoteasterone | – | – | 2.63E-12 | +5.58 |
| (22R,23R)-22,23-dihydroxy-campest-4-en-3-one | 8.12E-15 | +6.52 | 9.14E-15 | +6.42 |
| **Carotenoids** | | | | |
| 18′-hydroxy-caroten-18-oate | 1.19E-13 | −5.97 | 1.96E-13 | −5.86 |
| Bixin | – | – | 1.27E-11 | −5.49 |
| **Carbohydrates** | | | | |
| 1/6-kestotriose/a Fagopyritol | – | – | 1.07E-14 | −5.38 |
| (1,4)-β-xylobiose | – | – | 0 | +5.25 |
| 2,4-diacetamido-2,4,6-trideoxy-α-D-mannopyranose | – | – | 3.15E-12 | +6.19 |
| β-L-arabinofuranosyl-(1,2)-β-L-arabinofuranose | – | – | 0 | +5.25 |
| D-glycero-D-manno-heptose 7-phosphate | 0 | +4.82 | - | - |
| D-sedoheptulose 7-phosphate | 0 | +4.82 | - | - |
| 2/3-didehydropyranose/ | 1.65E-15 | +5.31 | 3.70E-15 | +5.54 |
| D-hexose | 7.46E-16 | +6.20 | 3.70E-15 | +6.44 |
| Pentose | 2.58E-11 | +4.94 | - | - |
| **Others** | | | | |
| Vernoleate | – | – | 1.05E-14 | +6.70 |
| Soyasaponin III | 4.26E-09 | +4.89 | 5.22E-16 | +4.96 |
| 3-ureido-isobutyrate | – | – | 3.37E-13 | +5.10 |
| (3E)-2-oxohex-3-enedioate | – | – | 1.05E-14 | −6.70 |
| 5-deoxy-D-glucuronate | 1.90E-15 | −5.30 | 9.15E-16 | −5.50 |
| Allantoate | 1.31E-07 | −1.22 | - | - |
| 4-hydroxyphenylpyruvate | 0 | −6.39 | - | - |
| 11,13-icosadienoate | 7.46E-16 | +5.62 | - | - |

(*continued on next page*)

**Table 2** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Coumaryl acetate | 1.79E-14 | +5.43 | 1.71E-09 | +5.69 |
| Phenylacetonitrile | 4.32E-15 | +5.78 | 2.69E-14 | +5.68 |
| 2,5-dihydroxybenzoate 5-O-β-D-glucoside | 3.63E-14 | +5.00 | 1.19E-15 | +5.22 |
| 9H-purine-2,6,8-triol/Urate | 8.06E-13 | −5.80 | 1.28E-12 | −5.69 |
| N-methylanthranilate | 1.69E-15 | +4.84 | 3.01E-13 | +4.97 |
| 1-guanidino-1-deoxy-cyllo-inositol 4-phosphate | – | – | 1.88E-13 | +5.06 |
| 2,3-dimethyl-6-phytyl-1,4-benzoquinol | 1.80E-14 | +5.50 | - | - |
| 3,4-dihydroxymandelonitrile β-D-glucoside | 1.02E-14 | +5.10 | - | - |
| 4-methoxy-3-indolylmethylamine | 8.30E-15 | +5.40 | - | - |
| Indol-3-ylmethyl-glutathione | 1.50E-11 | +5.00 | - | - |
| 4-hydroxybenzoyl-acetate | 0 | −6.40 | - | - |
| 7,8-dihydrofolate monoglutamate | – | – | 8.47E-16 | +5.11 |
| (R)-lipoate | – | – | 0 | +1.20 |
| N5-methyl-tetrahydropteroyl tri-L-glutamate | – | – | 1.45E-13 | +4.98 |
| Folate | – | – | 8.47E-16 | +5.11 |
| L-erythro-7,8-dihydrobiopterin | – | – | 4.54E-15 | −5.86 |
| Biopterin | – | – | 2.55E-14 | −5.68 |
| Coenzyme A | – | – | 1.35E-13 | −5.41 |
| Ayapin | 4.23E-14 | −5.80 | 6.29E-14 | −5.70 |
| 6-carboxy-5,6,7,8-tetrahydropterin | – | – | 3.76E-15 | +4.84 |
| 6-pyruvoyl tetrahydropterin | – | – | 2.55E-14 | −5.68 |
| 5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone | – | – | 1.61E-05 | −1.20 |
| 7,8-dihydromonapterin | 1.09E-13 | +5.20 | - | - |
| 1-(2-amino-7-methyl-4-oxo-7,8-dihydro-3H-pteridin-6-yl) ethyl-4-(β-D ribofuranosyl) aminobenzene 5′-phosphate | 4.19E-12 | +5.00 | - | - |
| 4α-hydroxy-tetrahydrobiopterin | 4.05E-15 | −5.90 | - | - |
| Prostaglandin F2α | 3.34E-15 | +5.10 | - | - |
| 5,6-dihydrothymine | 1.42E-06 | −1.50 | - | - |
| Esculetin | 0 | −1.20 | - | - |
| UDP-3-O-(3-hydroxymyristoyl)-α-D-glucosamine | 1.79E-13 | −5.63 | 2.90E-13 | −5.53 |
| Pyrimidin-2-one-ribonucleoside | - | – | 0 | +1.20 |
| Cytidine | – | – | 2.53E-11 | +5.18 |
| 5′,5‴-diadenosine tetraphosphate | 1.23E-15 | +5.30 | - | - |
| 5′,5‴-diadenosine hexaphosphate | 1.57E-13 | +4.80 | - | - |
| Guanosine | 1.83E-15 | +6.00 | - | - |
| Uracil | 0 | +1.20 | - | - |
| PreQ1 | 8.42E-14 | +5.70 | - | - |
| Indole-3-acetonitrile-glutatione conjugate | 9.81E-14 | +4.80 | - | - |
| Dhurrin | 3.63E-14 | +5.00 | 1.24E-15 | +5.22 |
| (R)-amygdalin | 4.48E-14 | +4.90 | 6.25E-16 | +5.37 |
| β/γ-tocopherol | 1.80E-14 | +5.50 | - | – |
| scyllo-inosose | 1.65E-15 | +5.30 | 1.05E-14 | +5.34 |
| 1-keto-D-chiro-inositol, D-myo-inositol (1,2,4,5,6)-pentakisphosphate | 2.23E-12 | +4.90 | | |
| Menaquinone-1 | 6.97E-09 | +1.74 | - | - |
| **ROOTS** | | | | |
| **Flavonoid related compounds** | | | | |
| Cirsimaritin/Ladanein | - | - | 1.08E-06 | +0.99 |
| Sakuranin | - | - | 1.12E-05 | +1.14 |
| Apigenin-7, 4′-dimethyl ether/Kievitone/Ononin/Orientin | - | - | 0 | +1.20 |
| Luteolin 7-O-neohesperidoside | 1.74E-08 | +4.96 | - | - |
| Luteolin 7-O-gentiobioside | 9.42E-12 | +5.71 | - | - |
| Tetramethyl myricetin | 1.35E-11 | +5.06 | - | - |
| Isovitexin 7-O-glucoside | 1.74E-08 | +4.97 | - | - |
| 6C-hexosyl chrysin | 1.63E-11 | +4.89 | - | - |
| Apigenin-7-O-gentiobioside | 1.74E-08 | +4.97 | - | - |
| Afrormosin-7-O-glucoside | 8.48E-14 | +4.94 | - | - |
| Dalpatein 7-O-β-D-apiofuranosyl -(1–6) - β-D-glucopyranoside | 8.24E-13 | +4.84 | - | - |
| 2-hydroxyeriodictyol | 6.50E-11 | +4.81 | - | - |
| Taxifolin | 2.15E-11 | +5.03 | - | - |
| Rutin | 9.42E-12 | +5.71 | - | - |
| Pelargonidin 3,7-di-O β-D-glucoside/Pelargonidin 3-O-sophoroside | 1.74E-08 | +4.97 | - | - |
| Dihydroquercetin | 6.50E-11 | +4.81 | - | - |
| 2′,3,4,4′,6′-pentahydroxychalcone4′-O-β-D-glucoside | 1.45E-09 | +5.40 | - | - |
| (-)-epicatechin/(+)-catechin/Leucopelargonidin | 0 | +4.48 | - | - |
| Hesperidin | 1.49E-11 | +4.64 | - | - |
| **Phenylpropanoids and lignans** | | | | |
| Curcumin diglucoside | 4.17E-11 | +4.59 | 4.56E-16 | +4.89 |
| 1-O-caffeoyl-β-D-glucose | - | - | 0 | +5.04 |
| 1-O-feruloyl-β-D-glucose | 4.72E-08 | +6.17 | - | - |
| Caffeate | 0 | −6.39 | - | - |

(*continued on next page*)

**Table 2** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Sesamolin | 9.14E-13 | +4.41 | 4.07E-16 | +5.14 |
| Secoisolariciresinol | - | - | 9.22E-15 | +5.12 |
| (Z)-2,4-dihydroxycinnamate | 0 | +6.39 | - | - |
| Pinoresinol/Matairesinol | - | - | 1.88E-13 | +5.05 |
| **Alkaloids and amine compounds** | | | | |
| 6-*O*-methyl-N-deacetylisoipecoside aglycon | - | - | 5.64E-15 | +4.83 |
| Cinchoninone | - | - | 2.20E-16 | +5.06 |
| Strictosamide | - | - | 5.73E-16 | +4.90 |
| Solasodine | - | - | 2.67E-10 | −8.27 |
| 7,8-dihydroberberine | - | - | 1.53E-10 | −6.40 |
| Indole-5, 6-quinone | - | - | 9.38E-10 | −0.80 |
| Senecionine | - | - | 5.11E-07 | −0.89 |
| Demecolcine/(S)-isoandrocymbine | 1.94E-10 | −5.06 | 6.99E-13 | −4.99 |
| Deacetylcolchicine | - | - | 7.17E-15 | +4.83 |
| Gramine | - | - | 1.77E-11 | +4.71 |
| Cyclo-dopa 5-*O*-glucoside | - | - | 2.98E-12 | −5.38 |
| Harmol | - | - | 2.20E-16 | −5.61 |
| Ricinine | - | - | 4.77E-15 | −5.93 |
| 5-hydroxy-γ-coniceine | 5.91E-06 | −1.05 | | |
| Papaveroxine | 1.83E-12 | +4.86 | 3.38E-16 | +5.61 |
| Secoberbine | 1.80E-10 | +4.92 | - | - |
| Aurachin B | 1.99E-12 | +5.08 | - | - |
| 6-O-methyl-N-deacetylisoipecoside | 2.42E-13 | +4.95 | - | - |
| Ginsenoside Ro | 3.81E-13 | +4.50 | - | - |
| Taxiphyllin | 0 | +5.01 | - | - |
| 2-naphthol glucoside | 0 | −1.20 | - | - |
| 5-methylthiopentyldesulfoglucosinolate | 2.49E-12 | −5.98 | - | - |
| D-alanyl-D-serine | 2.69E-06 | −1.76 | 9.25E-08 | −1.08 |
| D-alanyl-D-alanine | 7.13E-09 | −1.98 | 8.73E-11 | −1.56 |
| L-alanyl-L-Glutamate | 0 | −1.20 | 0 | −1.20 |
| Glutamine | - | - | 0 | −1.20 |
| Tyrosine | - | - | 4.75E-15 | −6.09 |
| 3-methoxytyramine | - | - | 1.71E-16 | +6.10 |
| L-homomethionine | - | - | 5.79E-15 | +4.84 |
| Tetrahomomethionine | - | - | 1.64E-15 | +4.96 |
| 1-(3-aminopropyl)-4-aminobutanal | - | - | 7.16E-12 | +5.40 |
| L-ethionine | - | - | 5.79E-15 | +4.84 |
| Feruloylagmatine | - | - | 2.18E-15 | +5.18 |
| N,N-dihydroxy-L-isoleucine | - | - | 3.61E-15 | +5.39 |
| N,N-dihydroxy-L-dihomomethionine | 6.75E-12 | +4.63 | - | - |
| 4-(3-methylbut-2-enyl)-L-abrine | 8.52E-11 | +4.56 | - | - |
| 5,10-methylenetetrahydropteroyl mono-L-glutamate | 6.14E-09 | +5.05 | - | - |
| **Terpenoids** | | | | |
| Taxol | - | - | 1.35E-14 | +4.56 |
| Oleanolate 3-β-D-glucuronoside-28-glucoside | - | - | 1.78E-14 | +5.23 |
| Glycyrrhetinate | - | - | 0 | −1.20 |
| 2,7-dihydroxycadalene | - | - | 9.72E-13 | −5.16 |
| Costunolide | 1.87E-12 | +4.75 | - | - |
| 15-hydroxysolavetivone | 2.61E-09 | +5.16 | - | - |
| Zealexin B1 | 1.57E-08 | −5.43 | - | - |
| Levopiramadiene-diol/Abieta-7,13-dien-18,18-diol/Palustradiene-diol/Oryzalexin D/E/F/S | 1.72E-12 | +5.90 | - | - |
| Cyclooctat-9-en-5,7-diol | 3.79E-15 | +5.62 | - | - |
| Oryzalexin B | 0 | +1.20 | - | - |
| Citronellate | 0 | +1.08 | - | - |
| Oleanolate | 1.57E-12 | +6.01 | - | - |
| Gypsogenate | 1.36E-09 | −2.18 | - | - |
| **Lipids** | | | | |
| Stearidonate | 1.25E-12 | +5.14 | 6.23E-12 | +5.24 |
| Coniferonate | 1.25E-12 | +5.14 | 6.23E-12 | +5.24 |
| A dihydroxyoctadeca-dienoate/HPODE | 1.79E-14 | +5.98 | 1.10E-16 | +5.97 |
| octadecadien-6-ynoate | 1.25E-12 | +5.14 | 6.23E-12 | +5.24 |
| Omega hydroxycaprate | – | – | 1.65E-15 | +5.68 |
| 1-18:3-2-18:3-phosphatidylcholine | – | – | 1.64E-15 | +5.75 |
| 4-hydroxysphing-8-enine-20:1 ceramide | - | - | 2.55E-16 | +4.90 |
| 1-18:1-2-18:1 phosphatidate | - | - | 6.24E-09 | −6.35 |
| 2-C22:0-DCA-LPA | - | - | 4.29E-15 | +5.22 |
| 1-16:0-2-lysophosphatidylcholine | - | - | 8.18E-16 | +6.49 |
| (8Z,11Z,14Z)-icosatrienoate | 2.13E-12 | +5.89 | - | - |
| Colneleate/3-oxo-2-(cis-2′-pentenyl)-cyclopentane-1-octanoate/a epoxyoctadeca-dienoate | 3.87E-15 | +6.24 | - | - |
| Icosadienoate | 1.86E-14 | +5.62 | - | - |
| Phosphatidylglycerophosphate (1-octadecenoyl(9Z) 2-palmitoyl) | 4.01E-12 | +5.41 | - | - |
| Linoleate | - | - | 4.43E-04 | +0.81 |

(*continued on next page*)

**Table 2** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Behenate | - | - | 1.00E-05 | +0.93 |
| 1-18:2-2-18:3-phosphatidylcholine | - | - | 0 | +1.20 |
| 18-hydroxyoleate | - | - | 0 | −1.20 |
| 1-18:2-2-16:3-monogalactosyldiacylglycerol | - | - | 0 | −1.20 |
| Strictosamide | 1.34E-13 | +4.72 | - | - |
| Arachidoyl-CoA | 1.34E-11 | +4.72 | - | - |
| C22:0-DCA-CoA | 6.90E-09 | +4.64 | - | - |
| (9Z,12Z)-15,16-epoxyoctadeca-9,12-dienoate | 3.87E-15 | +6.24 | - | - |
| Phytanoyl-CoA | 1.34E-11 | +4.72 | - | - |
| 11,13-icosadienoate | 1.86E-14 | +5.62 | - | - |
| 3-oxo-behenoyl-CoA | 2.59E-11 | +5.14 | - | - |
| **Hormones** | | | | |
| Gibberellins A25 | - | - | 4.29E-15 | +5.30 |
| Gibberellins A37/A97 | - | - | 9.22E-15 | +5.12 |
| Gibberellins A13/A17/A46 | - | - | 1.51E-12 | +5.21 |
| Gibberellins A8 catabolite | - | - | 7.28E-14 | +5.27 |
| Gibberellins 44 (open lactone form) | - | - | 9.22E-15 | +5.12 |
| Gibberellin A7 | 0 | +1.20 | 0 | +1.20 |
| Gibberellin A12-aldehyde | - | - | 0 | +1.20 |
| 16 α 17-epoxy gibberellin A9/Gibberellin A4/A20/A51 | 1.15E-12 | +4.46 | - | - |
| Dihydrozeatin-N-glucoside | - | - | 1.10E-16 | +4.91 |
| trans-zeatin-O-glucoside-7-N-glucoside | 1.56E-11 | +4.79 | 8.07E-14 | +4.66 |
| trans-zeatin-O-glucoside/Isopentenyladenine-9-N-glucoside/Kinetin-N-glucoside | - | - | 0 | +1.20 |
| Benzyladenine-9-N-glucoside | - | - | 7.17E-15 | +4.63 |
| Isopentenyladenine-7-N-glucoside | 0 | −1.20 | 0 | −1.20 |
| Dihydrozeatin-9-N-glucoside-O-glucoside | 1.33E-12 | +4.47 | 1.10E-16 | +4.91 |
| 4α-carboxy-ergosta-7,24(24)-dien-3β-ol | 6.84E-14 | +5.56 | 1.72E-16 | +5.65 |
| Lathosterol/5β-cholestan-3-one/24-alkyl sterol 2/3 | 9.32E-12 | +5.33 | - | - |
| 4α-carboxy,4β,14α-dimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol | 1.57E-12 | +6.01 | - | - |
| (24R)-cholest-5-ene-3β,7α,24-triol | 2.10E-11 | +5.13 | - | - |
| 4α-carboxy-stigmasta-7,24(24)-dien-3β-ol | 1.57E-12 | +6.01 | - | - |
| Indole-3-acetate | 8.89E-06 | −1.31 | - | - |
| Indole acetaldehyde | - | - | 1.10E-09 | −1.12 |
| 11-hydroxyjasmonate sulfate | 1.04E-10 | +4.89 | - | - |
| Jasmonoyl-phenylalanine | 4.77E-12 | +4.77 | - | - |
| Indole-3-butyryl-glucose | 4.44E-14 | −6.35 | 6.41E-16 | −6.29 |
| (22R,23R)-28-homocastasterone 22-O-sulfate | - | - | 1.89E-13 | +4.89 |
| (22R,23R)-22,23-dihydroxy-campest-4-en-3-one | 1.12E-12 | +6.52 | 5.64E-15 | +6.46 |
| Brassinolide | 0 | −2.16 | - | - |
| (22α)-hydroxy-campest-4-en-3-one | 2.96E-12 | +5.69 | - | - |
| 6-oxocampestanol/22-hydroxy-5α-ergostan-3-one | 3.75E-12 | +5.48 | - | - |
| (22R,23R)-22,23-dihydroxycampesterol/24-epicathasterone | 5.59E-13 | +6.00 | - | - |
| (22α)-hydroxy-campesterol | 3.75E-12 | +5.48 | - | - |
| 3-dehydro-6-deoxoteasterone | 5.59E-13 | +6.00 | - | - |
| 6-deoxoteasterone | - | - | 3.22E-12 | +5.55 |
| **Carotenoids** | | | | |
| 18′-hydroxy-caroten-18-oate | 3.63E-11 | −5.97 | 1.88E-13 | −5.90 |
| Bixin | - | - | 1.31E-11 | −5.53 |
| 5,6-epoxy-3-hydroxy-12′-apo-β-caroten-12′-al | 0 | −1.20 | - | - |
| **Carbohydrates** | | | | |
| 1/6-kestotriose/Maltotriose/a Fagopyritol | - | - | 5.64E-15 | −5.42 |
| (1,4)-β-xylobiose | | - | 0 | +5.20 |
| 2,4-diacetamido-2,4,6-trideoxy-α-D-mannopyranose | - | - | 3.34E-12 | +6.23 |
| β-L-arabinofuranosyl-(1,2)-β-L-arabinofuranose | - | - | 0 | +5.20 |
| Furcatin | - | - | 4.62E-14 | +4.78 |
| 2-phenylethyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside | - | - | 1.28E-15 | +4.55 |
| D-sedoheptulose 7-phosphate | 0 | +4.82 | - | - |
| Benzyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside | 0 | +4.51 | - | - |
| UDP-2,3-bis[O-(3R)-3-hydroxymyristoyl]-α-D-glucosamine | 5.13E-10 | +4.63 | - | - |
| 2,3-didehydropyranose | 9.76E-14 | +5.31 | 1.65E-15 | +5.50 |
| D-hexose | 1.24E-14 | +6.20 | 1.65E-15 | +6.40 |
| **Others** | | | | |
| Vernoleate | - | - | 5.51E-15 | +6.66 |
| Coniferin | 1.83E-14 | +5.64 | - | - |
| 6 methylthiohexyldesulfoglucosinolate | 3.51E-13 | +5.79 | - | - |
| 2,5-dihydroxybenzoate 5-O-β-D-glucoside | 8.34E-12 | +5.00 | - | - |
| 3-ureido-isobutyrate | - | - | 2.21E-13 | +5.06 |
| (3E)-2-oxohex-3-enedioate/4-hydroxy-4-methyl-2-oxoglutarate | - | - | 2.60E-15 | −5.38 |
| 5-deoxy-D-glucuronate | - | - | 2.78E-16 | +5.46 |
| Allantoate | - | - | 6.58E-04 | −0.98 |
| 11,13-icosadienoate | 1.86E-14 | +5.62 | - | - |
| Coumaryl acetate | - | - | 2.04E-09 | +5.43 |

(*continued on next page*)

**Table 2** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Soyasaponin III | 1.83E-06 | + 4.89 | 1.10E-16 | + 4.92 |
| Phenylacetonitrile | - | - | 9.65E-04 | + 1.78 |
| Phenylacetonitrile oxide | - | - | 9.65E-04 | − 1.78 |
| 2,5-dihydroxybenzoate 5-O-β-D-glucoside | - | - | 1.22E-15 | + 5.18 |
| 9H-purine-2,6,8-triol/Urate | - | - | 1.78E-12 | − 5.73 |
| 1-guanidino-1-deoxy-cyllo-inositol 4-phosphate | - | - | 1.37E-13 | + 5.02 |
| Indol-3-ylmethylisothiocyanate-glutathione | - | - | 6.41E-14 | + 4.49 |
| Benzoate | - | - | 0 | + 1.20 |
| 8-oxoguanine | 1.48E-11 | + 4.39 | - | - |
| N-methylanthranilate | 1.02E-13 | + 4.84 | 2.66E-13 | + 4.93 |
| 7,8-dihydrofolate monoglutamate | - | - | 4.56E-16 | + 5.07 |
| (R)-lipoate | - | - | 0 | + 1.20 |
| Coenzyme A | - | - | 1.00E-13 | − 5.45 |
| 6-carboxy-5,6,7,8-tetrahydropterin | - | - | 1.93E-15 | + 4.80 |
| 1-(2-amino-7-methyl-4-oxo-7,8-dihydro-3H-pteridin-6-yl) ethyl-4-(β-D ribofuranosyl) aminobenzene 5′-phosphate | 1.55E-09 | + 4.96 | - | - |
| 4α-hydroxy-tetrahydrobiopterin | 4.31E-13 | − 5.89 | - | - |
| L-erythro-5,6,7,8-tetrahydrobiopterin | 5.14E-12 | + 5.72 | - | - |
| N5-methyl-tetrahydropteroyl tri-L-glutamate | - | - | 7.74E-14 | + 4.94 |
| L-erythro-7,8-dihydrobiopterin | - | - | 2.82E-15 | − 5.90 |
| Biopterin | - | - | 2.16E-14 | − 5.71 |
| Anthranil | 1.23E-09 | + 4.73 | - | - |
| 7,8-dihydromonapterin | 3.30E-11 | + 5.16 | - | - |
| Pyrimidin-2-one-ribonucleoside | - | - | 0 | + 1.20 |
| Cytidine | - | - | 2.08E-11 | + 5.14 |
| UDP-3-O-(3-hydroxymyristoyl)-α-D-glucosamine | 5.69E-11 | − 5.63 | 2.90E-13 | − 5.57 |
| 5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone | - | - | 9.06E-06 | + 1.24 |
| 5′,5‴-diadenosine tetraphosphate | 4.66E-14 | + 5.31 | - | - |
| 5′,5‴-diadenosine hexaphosphate | 4.89E-11 | + 4.81 | - | - |
| Guanosine | 1.14E-13 | + 5.97 | - | - |
| Uracil | 0 | + 1.20 | - | - |
| PreQ1 | 2.44E-11 | + 5.74 | 7.77E-14 | + 5.67 |
| D-gluconate/gulonate | 2.51E-11 | + 4.58 | - | - |
| phenylacetaldehyde oxime | 2.78E-14 | + 6.22 | - | - |
| 4-methylthiobutanaldoxime | 7.49E-09 | + 5.02 | - | - |
| 7-methylthioheptylhydroximate | 1.98E-09 | + 5.31 | - | - |
| 6-methylthiohexylhydroximate | 1.40E-10 | + 4.48 | - | - |
| 1-(3-aminopropyl)-4-aminobutanal | 8.15E-11 | + 5.95 | - | - |
| 2,2′-dihydroxybiphenyl | 4.07E-10 | + 4.25 | - | - |
| Salicortin | 4.42E-12 | + 4.56 | - | - |
| Coumaryl acetate | 3.61E-12 | + 5.43 | - | - |
| Anthranil | 1.23E-09 | + 4.73 | - | - |
| 2,3-dimethyl-6-phytyl-1,4-benzoquinol | 3.75E-12 | + 5.48 | - | - |
| Menaquinone-1 | 5.07E-04 | + 0.78 | 9.32E-09 | + 1.70 |
| 4-hydroxybenzoyl-acetate | 0 | − 6.39 | - | - |
| 4-hydroxyphenylpyruvate | 0 | − 6.39 | - | - |
| Indole-3-acetonitrile-glutatione conjugate | 2.95E-11 | + 4.85 | 2.20E-11 | + 5.32 |
| Dhurrin | 8.34E-12 | + 5.00 | 1.25E-15 | + 5.18 |
| Phenylacetonitrile | 4.88E-13 | + 5.78 | 1.69E-14 | + 5.64 |
| β/γ-tocopherol | 3.75E-12 | + 5.48 | - | - |
| scyllo-inosose | 9.76E-14 | + 5.31 | - | - |
| 1-keto-D-chiro-inositol, D-myo-inositol (1,2,4,5,6)-pentakisphosphate | 8.04E-10 | + 4.90 | - | - |

cyclopentane-1-octanoate, several epoxyoctadeca-dienoates.

In *S.maritima*, the alteration of profiles in membrane lipids and oxidized forms of lipids was much less evident and contrasting trends could be recorded. As an example, the level of HPODE, dihydroxyoctadeca-dienoates and 1-18:1-2-18:2-phosphatidylcholine were reduced whereas phosphatidylglycerophosphate (dioctadec-9-enoyl(Z)) was accumulated in both tissues. On the other hand, no impact on the accumulation of lipid-related compounds was observed in leaves of *S. portulacastrum*, whereas some lipids were accumulated in roots at the lower NaCl dosage.

### *3.8. Hormones*

*S. brachiata* showed a marked imbalance in hormone profile, with several gibberellins (*e.g.*, A25, A13/A17, A46) and cytokinins (mainly kinetin and zeatin glucosides), as well as brassinosteroids (hydroxycampest-4-en-3-one and 6-oxocampestanol, dihydroxycampesterol, cathasterone, brassinolide and deoxoteasterone derivatives), being up accumulated in both roots and leaves. The triggering of gibberellins by salinity was evident only at 500 mM NaCl.

Concurrently, a conjugated jasmonates (jasmonoyl-phenylalanine and 11-hydroxyjasmonate sulfate) were up accumulated whereas the auxin indole-3-acetate showed opposite trend.

In *S. maritima*, the imbalance of phytohormones was less evident and a specific response involving tuberonic acid (and therefore also jasmonate) was recorded under salinity. Under salinity, the level of dihydrozeatin and benzyladenine cytokinins was reduced whereas brassinolide increased. Gibberellins were also reduced in roots under salinity, although with a less marked trend, depending on NaCl dosage.

In *S. portulacastrum,* gibberellins (such as A8, A9, A6, A34-

**Table 3**

Metabolites differentially accumulated in *Sesuvium portulacastrum* leaves and root of plants grown under 200 and 500 mM NaCl salinity, as compared to control (no salinity). Compounds were selected through Volcano Plot analysis (unpaired *t*-test at $P < 0.01$ with Bonferroni multiple testing correction, and fold-change analysis with cut-off = 5). Missing values denote no significant differences.

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| **LEAVES** | | | | |
| **Flavonoid related compounds** | | | | |
| Biochanin-A | - | - | 1.81E-11 | −5.22 |
| Isorhamnetin 3-*O*-glucoside | - | - | 3.71E-15 | −5.77 |
| Eupatolitin 3-glucoside | - | - | 4.12E-15 | −5.37 |
| Rhamnetin | - | - | 0 | −1.20 |
| Peonidin 3, 5-diglucoside | - | - | 5.22E-10 | −1.60 |
| Hesperidin | 8.34E-16 | +7.02 | 1.26E-15 | +7.26 |
| flavanone-7-O-β-D-glucoside | | | 1.28E-15 | +5.21 |
| Laricitrin/Patuletin/3,3′,4′,5,7- pentahydroxy-8-methoxyflavone | 0 | +1.00 | 0 | −5.04 |
| **Phenylpropanoids and lignans** | | | | |
| (+)-secoisolariciresinol diglucoside | - | - | 6.97E-15 | −5.64 |
| Curcumin | 8.72E-16 | +5.88 | - | - |
| 1-O-(4-coumaroyl)-β-D-glucose | 1.76E-12 | +1.44 | - | - |
| 1-O-caffeoyl-β-D-glucose | 6.17E-08 | −1.07 | - | - |
| **Alkaloids and amine compounds** | | | | |
| (S)-nandinine | - | - | 6.66E-15 | +5.27 |
| Dehydroscoulerine | - | - | 3.63E-14 | +5.58 |
| 2-descarboxy-betanidin | - | - | 4.81E-14 | +5.08 |
| 3′-*O*-demethyl-staurosporine | - | - | 5.40E-12 | +5.18 |
| Cyclo-acetoacetyl-*L*-tryptophan | 4.70E-15 | −5.39 | - | - |
| Castanospermine | 4.68E-12 | −5.73 | - | - |
| Chelirubine | 1.97E-16 | −5.66 | - | - |
| Cyclo-dopa 5-*O*-glucoside | 2.05E-14 | +6.07 | - | - |
| N-formyldemecolcine/Colchicines | 2.49E-15 | +5.34 | - | - |
| L-cysteinyl-glycine | 6.03E-16 | +5.56 | 6.65E-16 | +6.11 |
| Cystine | 9.29E-15 | +5.54 | 1.26E-15 | +5.60 |
| *N*5-formyl-*N*5-hydroxy-*L*-ornithine | 1.17E-12 | +5.77 | 6.65E-16 | +6.42 |
| N-benzoyl-L-glutamate | 1.78E-14 | −5.56 | 2.19E-14 | −5.62 |
| Coumaroyl spermidine | - | - | 9.07E-14 | −5.17 |
| **Terpenoids** | | | | |
| A hydroxy-β-amyrin/12,13 β -epoxy- β –amyrin/Betulinic acid | - | - | 1.10E-13 | −6.42 |
| Phytyl diphosphate | - | - | 3.17E-15 | +5.42 |
| Copal-8-ol-diphosphate | - | - | 1.52E-13 | +5.39 |
| hydroxytaxusin | 6.03E-16 | −5.47 | - | - |
| Heliocide B4 | 0 | +1.82 | - | - |
| **Hormones** | | | | |
| Gibberellin A8 | 6.44E-16 | −5.01 | - | - |
| Gibberellin A6/A34-catabolite | 0 | −1.20 | - | - |
| 16, 17-dihydro-16α, 17-dihydroxy gibberellin A9 | 3.73E-15 | −4.93 | - | - |
| A zeatin-*N*-glucoside | 2.49E-15 | −5.26 | - | - |
| 14-hydroxylanosterol/4 α -hydroxymethyl-stigmasta-7,24(24)-dien-3 β -ol | - | - | 1.10E-13 | −6.42 |
| **Carotenoids** | | | | |
| α/β -zeacarotene/neurosporene | - | - | 1.10E-13 | +5.54 |
| 9-cis-10′-apo-β-carotenal | 2.42E-11 | −5.20 | - | - |
| Norbixin | 5.33E-15 | +5.72 | - | - |
| **Carbohydrates** | | | | |
| D-mannitol/D-sorbitol | - | - | 8.83E-15 | +5.63 |
| Galactitol | - | - | 6.67E-05 | −1.70 |
| β-D-4-deoxy-gluc-4-enuronosyl-(1,3)-N-acetyl-D-galactosamine | - | - | 6.14E-15 | −5.27 |
| 1-O-malyl-β-D-glucose | 0 | −1.07 | 0 | −5.17 |
| 5-deoxy-D-glucuronate | - | - | 6.65E-16 | +7.29 |
| Agarobiose | 3.24E-13 | −5.41 | - | - |
| **Others** | | | | |
| 4-hydroxy-4-methyl-2-oxoglutarate/2-oxohex-3-enedioate | - | - | 6.65E-16 | −5.61 |
| 3-oxoadipate | 1.97E-16 | −5.31 | 6.69E-16 | −5.81 |
| Hexaric acid | 0 | −5.75 | 0 | −5.83 |
| Orotate | 2.68E-12 | −4.98 | - | - |
| 1-pyrroline-5-carboxylate | 2.12E-15 | +5.27 | - | - |
| 7,8-dihydropterin | 6.73E-16 | +6.82 | 1.70E-09 | +6.10 |
| Cichoriin | - | - | 2.97E-13 | +5.21 |
| (+)-marmesin | - | - | 9.55E-13 | +5.76 |
| Phycocyanobilin | - | - | 0 | −1.20 |
| Aminomethylpyrimidine | 0 | −4.62 | - | - |
| Coumarin | 0 | +1.20 | - | - |
| 5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone | 1.65E-08 | −1.15 | - | - |
| 3-butenylglucosinolate | - | - | 1.26E-15 | +5.62 |
| Hydroxyversicolorone | - | - | 8.83E-15 | +5.15 |
| Versicolorone | - | - | 1.92E-15 | −5.29 |

(*continued on next page*)

**Table 3** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Dicoumarol | - | - | 3.64E-15 | −4.97 |
| D, L-carnitine | 0 | +1.20 | 0 | +1.20 |
| Entacapone | - | - | 0 | +1.20 |
| Reduced riboflavin | - | - | 5.52E-06 | +1.02 |
| 1-guanidino-1-deoxy-scyllo-inositol 4-phosphate | 7.29E-08 | +1.01 | - | - |
| Pyridoxal 5′-phosphate | 8.72E-16 | −5.17 | 2.62E-15 | −5.23 |
| **ROOTS** | | | | |
| **Flavonoid related compounds** | | | | |
| Biochanin-A | - | - | 3.37E-11 | −5.24 |
| Isorhamnetin 3-*O*-glucoside | - | - | 5.71E-15 | −5.79 |
| Eupatolitin 3-glucoside | - | - | 6.79E-15 | −5.38 |
| Rhamnetin | - | - | 0 | −1.20 |
| Kaempferol-3-glucoside-7-rhamnoside/Luteolin 7-O-neohesperidoside | - | - | 5.71E-15 | +4.88 |
| 8-hydroxykaempferol | - | - | 0 | +1.20 |
| 2-hydroxynaringenin | - | - | 0 | +1.20 |
| Hesperidin | 1.42E-14 | +7.03 | 1.73E-15 | +7.24 |
| Flavanone-7-O-β-D-glucoside | - | - | 1.08E-14 | +5.19 |
| Laricitrin/Patuletin/3,3′,4′,5,7- pentahydroxy-8-methoxyflavone | 0 | +1.01 | 0 | −5.08 |
| dalcochinin | 2.03E-11 | +4.91 | - | - |
| **Phenylpropanoids and lignans** | | | | |
| (+)-secoisolariciresinol diglucoside | - | - | 1.33E-14 | −5.66 |
| Curcumin | 3.51E-14 | +5.88 | - | - |
| 1-O-(4-coumaroyl)-β-D-glucose | 2.72E-10 | +1.44 | - | - |
| 1-O-caffeoyl-β-D-glucose | 1.12E-05 | −1.07 | - | - |
| **Alkaloids and amine compounds** | | | | |
| (S)-nandinine | - | - | 8.20E-15 | +5.25 |
| Dehydroscoulerine | - | - | 2.49E-14 | +5.56 |
| 2-descarboxy-betanidin | - | - | 1.57E-13 | +5.10 |
| 3′-*O*-demethyl-staurosporine | - | - | 7.53E-12 | +5.20 |
| Castanospermine | 7.34E-10 | −5.73 | - | - |
| Chelirubine | 4.15E-16 | −5.67 | - | - |
| Cyclo-dopa 5-*O*-glucoside | 2.28E-12 | +6.07 | - | - |
| N-formyldemecolcine | 1.62E-13 | +5.34 | - | - |
| Colchicines | 1.62E-13 | +5.34 | - | - |
| L-cysteinyl-glycine | 7.84E-15 | +5.56 | 1.73E-15 | +6.09 |
| Cystine | 8.18E-13 | +5.54 | 2.03E-15 | +5.59 |
| *N*5-formyl-*N*5-hydroxy-*L*-ornithine | 1.78E-10 | +5.77 | 1.18E-15 | +6.40 |
| Coumaroyl spermidine | - | - | 1.64E-13 | −5.19 |
| 1-pyrroline-5-carboxylate | 1.11E-13 | +5.27 | - | - |
| **Terpenoids** | | | | |
| A hydroxy-β-amyrin/12,13β-epoxy-β-amyrin/Betulinic acid | - | - | 5.67E-14 | −6.44 |
| Phytyl diphosphate | - | - | 3.48E-15 | +5.40 |
| Copal-8-ol-diphosphate | - | - | 2.05E-13 | +5.37 |
| Medicagenate | 0 | +1.20 | - | - |
| 2 α-hydroxytaxusin | 7.36E-15 | −5.47 | - | - |
| Heliocide B4 | 0 | +1.82 | - | - |
| **Lipids** | | | | |
| (9R,10S)-dihydroxystearate | - | - | 0 | +1.20 |
| Cerotoyl-CoA | - | - | 4.27E-15 | +5.06 |
| trans-hexaprenyl diphosphate | - | - | 8.79E-14 | +4.78 |
| 1-oleyl-2-lyso-phosphatidate | - | - | 0 | +1.20 |
| Leukotriene A4 | 0 | +1.20 | - | - |
| **Hormones** | | | | |
| Gibberellin A8 | 9.66E-15 | −5.01 | - | - |
| Gibberellin A6 | 0 | −1.20 | - | - |
| Gibberellin A34-catabolite | 0 | −1.20 | - | - |
| 16, 17-dihydro-16α, 17-dihydroxy gibberellin A9 | 2.72E-13 | −4.93 | - | - |
| Gibberellin A51 | 0 | +0.97 | - | - |
| zeatin-*N*-glucoside | 1.58E-13 | −5.26 | - | - |
| 14-hydroxylanosterol/4α-hydroxymethyl,4β,14 α -dimethyl-9 β,19-cyclo-5 α -cholest-24-en-3 β -ol | - | - | 5.67E-14 | −6.44 |
| 4 α -formyl-4 β -methyl-5 α -cholesta-8,24-dien-3 β -ol | - | - | 0 | −1.20 |
| kinetin-7/9-N-glucoside | - | - | 0 | −2.83 |
| (+)-5-deoxystrigol | - | - | 4.52E-07 | +0.81 |
| 26-hydroxybrassinolide | - | - | 1.79E-08 | +0.80 |
| **Carotenoids** | | | | |
| α-zeacarotene | - | - | 6.22E-14 | +5.52 |
| Neurosporene/β-zeacarotene | - | - | 6.22E-14 | +5.52 |
| 9-cis-10′-apo-β-carotenal | 3.97E-09 | −5.20 | - | - |
| norbixin | 4.16E-13 | +5.72 | - | - |
| **Carbohydrates** | | | | |
| D-mannitol/D-sorbitol/L-iditol | - | - | 1.08E-14 | +5.61 |

(*continued on next page*)

**Table 3** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| Galactitol | - | - | 5.15E-05 | −1.72 |
| β-D-4-deoxy-gluc-4-enuronosyl-(1,3)-N-acetyl-D-galactosamine | – | – | 1.15E-14 | −5.29 |
| Agarobiose | 4.60E-11 | −5.41 | - | - |
| 1/6-kestotriose/Maltotriose/Fagopyritol A2/B2 | 1.31E-12 | −4.77 | 1.73E-14 | −4.85 |
| L-fuculose 1-phosphate | 1.94E-07 | −4.64 | 0 | −4.75 |
| Sucrose/Gentiobiose/Trehalose/D-cellobiose | - | - | 8.21E-07 | +0.78 |
| 2-phenylethyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside | - | - | 2.18E-07 | +0.85 |
| D-hexose | - | - | 2.93E-04 | +0.99 |
| 2,3-didehydropyranose | - | - | 6.65E-04 | +6.31 |
| 3-dehydro-D-glucose 6-phosphate | - | - | 1.39E-14 | −4.87 |
| 2-O-(4-deoxy-β-L-threo-hex-4-enopyranuronosyl)-α-L-rhamnopyranose | - | - | 0 | −1.20 |
| **Others** | | | | |
| 4-hydroxy-4-methyl-2-oxoglutarate/2-oxohex-3-enedioate | - | - | 1.73E-15 | −5.63 |
| 3-oxoadipate | - | - | 2.03E-15 | −5.38 |
| 1-O-malyl-β-D-glucose | 0 | +1.07 | 0 | −5.17 |
| 5-deoxy-D-glucuronate | 1.39E-11 | +5.07 | 1.18E-15 | +7.27 |
| Hexaric acid | 0 | −5.75 | - | - |
| Orotate | 4.19E-10 | −4.98 | 3.34E-12 | +5.06 |
| N-benzoyl-L-glutamate | 1.87E-12 | −5.57 | 1.35E-14 | −5.65 |
| 1-pyrroline-5-carboxylate | 1.11E-13 | +5.27 | - | - |
| 2,3-diphospho-D-glycerate | 9.42E-15 | −4.87 | - | - |
| aconitate | | | 1.08E-14 | −4.69 |
| N-(4-hydroxybenzoyl)-L-glutamate | 0 | +1.20 | - | - |
| 4-hydroxyphenylpyruvate | 0 | +1.20 | - | - |
| Cichoriin | - | - | 6.40E-13 | +5.19 |
| (+)-marmesin | - | - | 6.71E-13 | +5.75 |
| Phycocyanobilin/Protoporphyrin IX | - | - | 0 | −1.20 |
| Phytochromobilin | - | - | 4.99E-07 | −0.83 |
| (2R,3Z)-phycocyanobilin | - | - | 0 | +1.20 |
| 5,10-methenyl-THF/N5-methyl-tetrahydropteroyl mono-L-glutamate | - | - | 1.13E-07 | +0.88 |
| 7,8-dihydropterin | 1.08E-14 | +6.82 | 1.53E-09 | +6.09 |
| 6-formylpterin | - | - | 7.28E-12 | +4.82 |
| 6-hydroxymethyl-7,8-dihydropterin | 6.94E-12 | −4.78 | 3.27E-13 | −4.86 |
| 3-butenylglucosinolate | - | - | 2.03E-15 | +5.61 |
| Hydroxyversicolorone | - | - | 9.32E-15 | +5.13 |
| 15-demethoxyaclacinomycin T | - | - | 6.03E-15 | +5.15 |
| Versicolorone | - | - | 1.82E-15 | −5.30 |
| Aclacinomycin T | - | - | 5.51E-14 | −5.08 |
| 3-epihydroxy-2′-deoxymugineate | - | - | 0 | −1.20 |
| D, L-carnitine | - | - | 0 | +1.20 |
| Entacapone | - | - | 1.07E-13 | +5.55 |
| Reduced riboflavin | - | - | 1.10E-05 | +1.00 |
| β-tocopherol | - | - | 0 | +1.20 |
| Pyridoxal 5′-phosphate | 3.27E-14 | −5.17 | 3.17E-15 | −5.24 |
| Indole-3-yl-acetyl-myo-inositol L-arabinoside | 0 | −4.64 | 0 | −4.69 |
| D-myo-inositol 1,2-cyclic phosphate | 1.94E-07 | −4.64 | 0 | −4.75 |

catabolite, 16, 17-dihydro-16α, 17-dihydroxy gibberellin A9) and cytokinins (several zeatin glucosides) were down accumulated at lower dosage in both roots and leaves. However, contrasting trends could be observed for brassinosteroids changes between leaves and roots.

### *3.9. Carbohydrates*

The levels of several carbohydrates was significantly altered in *S. brachiata* roots in response to salt stress conditions. The content of oligosaccharides such as 1/6-kestotriose/maltotriose/fagopyritol, benzyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside and β-L-arabinofuranosyl-(1,2)-β-L-arabinofuranose was altered under salinity.

In *S.maritima*, the changes in carbohydrate levels was rather clear, with the content of several oligosaccharides (e.g., agarobiose, agarotriose neoagarotetraose) being up accumulated in both tissues. Several other carbohydrates were altered by salinity, although they presented contrasting trends as a function of the dosage and the plant organ considered.

Interestingly, the alteration of carbohydrates in *S. portulacastrum* was moderate, even though an increase in the osmolytes sugar alcohols (D-mannitol/D-sorbitol/L-iditol) could be observed. Among others, 1-kestotriose, L-fuculose 1-phosphate and maltotriose were down accumulated in roots at both dosages whereas maltose, trehalose, melibiose, sucrose, mannobiose, a D-hexose were altered only at the higher NaCl dosage.

### *3.10. Others*

Several other compounds, variably ascribable to different biochemical classes, were altered under either 200 mM or 500 mM NaCl across the species considered.

Like for previous classes, *S. brachiata* underwent the wider metabolic reprogramming under NaCl conditions. It is interesting to note that tetrahydrobiopterin and folate cofactors (*e.g.*, 7,8-dihydrofolate monoglutamate, N5-methyl–tetrahydropteroyl tri-L-glutamate, folate L-erythro-7,8-dihydrobiopterin and biopterin) were induced by salinity in *S.brachiata*, possibly indicating that one-carbon metabolism might be involved in response to NaCl. Furthermore, also purine and pyrimidine derivatives were well represented among compounds altered by NaCl in both roots and leaves. The level of acid sugars (D-gluconate, D-mannonate, L-idonate, aldehydo-L-galactonate, L-gulonate) was increased under NaCl conditions.

**Table 4**

Metabolites differentially accumulated in *Suaeda maritima* leaves and root of plants grown under 200 or 500 mM NaCl salinity, as compared to control (no salinity). Compounds were selected through Volcano Plot analysis (unpaired *t*-test at $P < 0.01$ with Bonferroni multiple testing correction, and fold-change analysis with cut-off = 5). Missing values denote no significant differences.

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| **LEAVES** | | | | |
| **Flavonoid related compounds** | | | | |
| Dihydroquercetin | 9.85E-15 | +5.78 | 5.98E-16 | +5.87 |
| 2′,3,4,4′,6′-pentahydroxychalcone 4′-O-β-D-glucoside/6C-glucosyl-2-hydroxynaringein | - | - | 5.66E-16 | +5.51 |
| Epicatechin-3-O-gallate | - | - | 0 | −1.20 |
| 3, 7, 4′-trimethylquercetin/Nevadensin/8-hydroxy-salvigenin | - | - | 6.97E-16 | −5.21 |
| **Alkaloids and amine compounds** | | | | |
| Deoxypumiloside | - | - | 1.98E-15 | −5.04 |
| Noscapine | - | - | 3.67E-14 | −5.08 |
| Chelirubine | - | - | 4.70E-16 | −5.23 |
| 2-descarboxy-betanidin | - | - | 2.10E-15 | −5.08 |
| Glycine betaine | 0 | +3.20 | 0 | +3.60 |
| D-alanyl-D-serine | - | - | 4.15E-08 | +1.18 |
| Cystine | 3.60E-10 | +5.20 | 4.12E-16 | +6.04 |
| Serotonin | 1.86E-14 | +5.35 | 7.58E-10 | +5.36 |
| Threonine | - | - | 1.98E-15 | −5.35 |
| 1-aminocyclopropane-1-carboxylate | - | - | 2.10E-13 | −5.36 |
| L-ethionine | - | - | 3.01E-15 | −5.03 |
| Tricaffeoyl spermidine | - | - | 1.73E-15 | −4.90 |
| 4-coumaroyl-3-hydroxyagmatine | - | - | 3.24E-13 | −5.19 |
| **Terpenoids** | | | | |
| p-cymene | - | - | 1.17E-14 | −5.14 |
| Monodeglucosyl des-acyl avenacin A | - | - | 4.43E-16 | +5.32 |
| Oryzalexin C | 0 | +1.20 | - | - |
| Camptothecin | 4.60E-15 | +5.58 | - | - |
| **Lipids** | | | | |
| A dihydroxyoctadeca-12,15-dienoate/HPODE | - | - | 4.43E-16 | −5.76 |
| 1-18:1-2-18:2-phosphatidylcholine | - | - | 4.46E-16 | −5.88 |
| Phosphatidylglycerophosphate (dioctadec-9-enoyl(Z)) | - | - | 5.26E-16 | +5.24 |
| Leukotriene B4 | - | - | 0 | −1.20 |
| (9Z)-12,13-dihydroxyoctadeca-9-enoate | 4.60E-15 | +5.81 | - | - |
| Stearidonate | 0 | −1.20 | - | - |
| **Hormones** | | | | |
| Tuberonic acid glucoside | 9.85E-15 | −5.27 | 7.90E-16 | −5.20 |
| Dihydrozeatin/N6-dimethylallyladenine | - | - | 4.12E-16 | −5.81 |
| Benzyladenine | - | - | 1.36E-15 | −5.38 |
| Jasmonoyl-1-aminocyclopropane-1 carboxylate | - | - | 1.07E-13 | −5.24 |
| Brassinolide | 0 | +1.20 | - | - |
| **Carbohydrates** | | | | |
| Agarobiose | - | - | 5.91E-16 | +5.52 |
| Neoagarotetraose | - | - | 1.18E-15 | +4.94 |
| Benzyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside | - | - | 4.12E-16 | −6.09 |
| **Others** | | | | |
| 3-oxo-palmitate | 0 | +1.20 | 0 | +1.20 |
| Hexaric acid | 0 | +1.20 | 0 | +1.20 |
| 8-methylthiooctylhydroximate | 7.67E-15 | +5.25 | 4.43E-16 | +5.41 |
| Glucarolactone | 4.60E-15 | +5.20 | 5.98E-11 | +5.11 |
| (2E,4E)-6-(2-aminophenyl)-2-hydroxy-6-oxohexa-2,4-dienoate | 0 | −1.20 | 0 | −4.96 |
| α, γ-9Z-octadecenedioate | - | - | 1.25E-14 | +6.63 |
| D-galactarate/D-glucarate | - | - | 7.72E-16 | +6.01 |
| D-threo-isocitrate | - | - | 2.55E-11 | +5.90 |
| Aurachin B | - | - | 8.28E-15 | +5.56 |
| 2-oxindole-3-acetyl-β-D-glucose | - | - | 1.71E-15 | +5.27 |
| 2′-deoxyadenosine | - | - | 9.48E-16 | +5.50 |
| 5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone | - | - | 1.90E-08 | −1.16 |
| Benzyl acetate | - | - | 1.20E-08 | −1.26 |
| 6-hydroxypseudooxynicotine | - | - | 4.43E-16 | −5.48 |
| Indolylmethylthiohydroximate | - | - | 1.01E-15 | −5.11 |
| 4-hydroxy-4-methyl-2-oxoglutarate/oxohex-3-enedioate | - | - | 5.91E-16 | −5.45 |
| 3-epihydroxymugineate | - | - | 4.65E-15 | −5.15 |
| (-)-phaseollidin | - | - | 2.53E-15 | −5.32 |
| 15-demethoxy-epslon-rhodomycinone | - | - | 3.99E-14 | −5.22 |
| 4-methoxy-3-indolylmethylamine | 1.86E-14 | −5.36 | - | - |
| Homoglutathione | - | - | 0 | −1.20 |
| 3-oxoadipate | - | - | 1.83E-15 | −5.05 |
| 5-hydroxy-1,4-naphthoquinone | 0 | −1.20 | - | - |
| **ROOTS** | | | | |

(*continued on next page*)

**Table 4** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| **Flavonoid related compounds** | | | | |
| Dihydroquercetin/2-hydroxyeriodictyol | 1.48E-13 | +5.78 | 5.37E-16 | +5.90 |
| Epicatechin-3-O-gallate | - | - | 5.08E-16 | +5.22 |
| 3, 7, 4′-trimethylquercetin/Nevadensin/8-hydroxy-salvigenin | - | - | 7.51E-16 | +5.20 |
| Peonidin 3,5-diglucoside | - | - | 1.87E-13 | −4.80 |
| 2′-hydroxy3,6,7,4′-tetramethyl quercetagetin | - | - | 4.05E-14 | −5.22 |
| 4′-methoxyisoflavone | - | - | 3.98E-15 | −4.50 |
| Flavonol 3-O-galactoside | - | - | 7.36E-07 | +0.74 |
| Sakuranin | - | - | 1.67E-08 | +0.80 |
| **Alkaloids and amine compounds** | | | | |
| Deoxypumiloside | - | - | 2.01E-15 | −5.04 |
| Noscapine | - | - | 4.89E-14 | −5.08 |
| Chelirubine | - | - | 5.08E-16 | −5.23 |
| 2-descarboxy-betanidin | - | - | 2.32E-15 | −5.07 |
| Solanine | - | - | 1.18E-15 | −4.80 |
| Chanoclavine-I | - | - | 6.56E-11 | −0.85 |
| Betaxanthin | - | - | 8.65E-09 | +0.82 |
| Glycine betaine | 0 | +1.20 | - | - |
| D-alanyl-D-serine | 1.14E-04 | +0.84 | 5.55E-08 | +1.18 |
| Cystine | 1.91E-08 | +5.20 | 2.53E-16 | +6.05 |
| Serotonin | 4.46E-13 | +5.35 | 8.18E-10 | +5.36 |
| Threonine | - | - | 2.48E-15 | +5.34 |
| L-ethionine | - | - | 3.93E-15 | +5.02 |
| Tricaffeoyl spermidine | - | - | 2.00E-15 | +4.89 |
| 4-coumaroyl-3-hydroxyagmatine | - | - | 4.23E-13 | +5.18 |
| **Terpenoids** | | | | |
| p-cymene | - | - | 1.30E-14 | −5.13 |
| Monodeglucosyl des-acyl avenacin A | - | - | 3.98E-16 | +5.32 |
| Des-acyl avenacin A | 6.68E-13 | +4.88 | 2.62E-15 | −4.79 |
| Gypsogenin-28-β-D-glucoside | 8.35E-13 | +4.80 | - | - |
| Oryzalexin C | 0 | +1.20 | - | - |
| Camptothecin | 4.14E-14 | +5.57 | - | - |
| Citronellol | - | - | 1.46E-12 | +4.79 |
| **Lipids** | | | | |
| HPODE/dihydroxyoctadeca-dienoate | - | - | 5.08E-16 | −5.76 |
| 1-18:1-2-18:2-phosphatidylcholine | - | - | 5.11E-16 | −5.56 |
| Phosphatidylglycerophosphate (dioctadec-9-enoyl(Z)) | - | - | 5.33E-16 | +5.86 |
| 9,10–12,13-diepoxyoctadecanoate | - | - | 5.93E-09 | +0.72 |
| Phosphatidylglycerophosphate (1-octadecenoyl(9Z) 2 palmitoyl) | - | - | 5.60E-16 | +4.90 |
| 4-hydroxysphing-8-enine-20:1 ceramide | - | - | 1.06E-14 | +5.11 |
| 1-18:2-2-18:1-phosphatidylcholine | - | - | 1.48E-08 | −0.78 |
| 1-16:0-2-18:2-diacylglycerol-trimethylhomoserine | - | - | 2.10E-05 | −0.92 |
| 1-16:0-2-18:3-diacylglycerol-trimethylhomoserine | - | - | 1.39E-05 | −0.97 |
| (9Z)-12,13-dihydroxyoctadeca-9-enoate | 9.05E-15 | +5.80 | - | - |
| 1-16:0-2-18:3-digalactosyldiacylglycerol | 3.46E-13 | +4.90 | - | - |
| **Hormones** | | | | |
| 16, 17-dihydro-16α, 17-dihydroxy gibberellin A9 | 1.84E-10 | −4.80 | 1.24E-12 | −4.72 |
| Gibberellin A46/A51 | 0 | −1.20 | – | – |
| Brassinolide | 0 | −1.40 | - | - |
| Dihydrozeatin | - | - | 3.79E-16 | −5.80 |
| N6-dimethylallyladenine | - | - | 3.94E-16 | −5.81 |
| Benzyladenine | - | - | 1.69E-15 | −5.37 |
| 2-oxindole-3-acetyl-β-D-glucose | 4.03E-13 | +4.80 | 1.82E-15 | +5.27 |
| 1-aminocyclopropane-1-carboxylate | - | - | 2.18E-13 | +5.35 |
| Tuberonic acid glucoside | - | - | 1.00E-15 | −5.19 |
| Jasmonoyl-1-aminocyclopropane-1 carboxylate | - | - | 1.31E-13 | −5.23 |
| **Carbohydrates** | | | | |
| Agarobiose | - | - | 5.08E-16 | +5.51 |
| Neoagarotetraose | - | - | 1.61E-15 | +4.93 |
| Benzyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside | - | - | 3.98E-16 | −6.09 |
| Agarotriose/Raffinose | - | - | 2.05E-15 | +5.04 |
| D-hexose | - | - | 3.92E-09 | +0.71 |
| 1-deoxy-D-xylulose | - | - | 2.12E-11 | −4.86 |
| p-nitrophenyl-β-D-xylobioside | - | - | 2.71E-14 | −4.73 |
| 2-O-(4-deoxy-β-L-threo-hex-4-enopyranuronosyl)-α-L-rhamnopyranose | - | - | 1.04E-15 | −4.62 |
| 3-deoxy-D-imanno-octulosonate | 3.34E-09 | +4.80 | - | - |
| (1,4)-β-xylobiose | 0 | +0.90 | - | - |
| **Others** | | | | |
| α, γ-9Z-octadecenedioate | - | - | 1.15E-14 | +6.64 |
| D-glucarate/D-galactarate | - | - | 9.63E-16 | +6.01 |
| D-threo-isocitrate | - | - | 2.12E-11 | +5.90 |
| Aurachin B | - | - | 1.02E-14 | +5.56 |
| 2′-deoxyadenosine | - | - | 1.12E-15 | +5.50 |

(*continued on next page*)

**Table 4** (*continued*)

| Compound | P value (corr.) | Log fold-change | P value (corr.) | Log fold-change |
|---|---|---|---|---|
| | 200 mM | | 500 mM | |
| 5-(hydroxymethyl)-2-methyl-4(1H)-pyrimidinone | - | - | 3.06E-08 | −1.15 |
| Benzyl acetate | - | - | 1.67E-08 | −1.26 |
| Indolylmethylthiohydroximate | - | - | 1.00E-15 | −5.10 |
| 4-hydroxy-4-methyl-2-oxoglutarate/2-oxohex-3-enedioate | - | - | 5.37E-16 | −5.45 |
| Aklanonate | - | - | 4.05E-14 | −5.22 |
| 3-epihydroxymugineate | - | - | 5.60E-15 | −5.14 |
| (-)-phaseollidin | - | - | 3.57E-15 | −5.32 |
| Cichoriin | - | - | 3.92E-15 | +5.32 |
| 7,8-dihydrofolate monoglutamate | - | - | 5.77E-08 | +0.75 |
| Mycothiol | - | - | 1.69E-15 | +5.16 |
| Isocitrate | - | - | 2.12E-11 | +5.90 |
| Ciceritol | - | - | 3.93E-15 | +5.08 |
| 6-methylthiohexanonitrile oxide | - | - | 5.37E-16 | +4.97 |
| 7-hydroxychlorophyllide | - | - | 1.81E-14 | +4.78 |
| (-)-medicarpin | - | - | 3.98E-15 | −4.53 |
| trans-cinnamate | - | - | 2.50E-15 | −4.85 |
| Indole-3-acetonitrile-γ-glutamylcysteine conjugate | - | - | 1.61E-15 | −4.70 |
| S-hydroxymethylglutathione | - | - | 4.45E-13 | −4.63 |
| Coproporphyrin III | - | - | 6.81E-13 | −4.72 |
| 5,6-dihydrothymine | - | - | 2.16E-13 | +4.59 |
| 1-O-malyl-β-D-glucose | 0 | +1.20 | - | - |
| 8-methylthiooctylhydroximate | 8.44E-14 | +5.20 | - | - |
| Glucarolactone | 2.94E-14 | +5.20 | - | - |
| 4-methoxy-3-indolylmethylamine | 4.46E-13 | −5.40 | - | - |
| Homoglutathione | - | - | 3.98E-16 | −5.59 |
| 3-oxoadipate | - | - | 1.48E-15 | −5.05 |
| (R)-3,4-dihydroxymandelonitrile | 0 | +1.20 | - | - |
| Methoxyanigorufone | 0 | −1.00 | - | - |
| Bergapten | 0 | −1.20 | - | - |

In *S. portulacastrum*, the clearer trends were observed for carotenoids and pterins, both increasing under NaCl salinity.

The levels of conjugated polyamines were altered in all the three species considered, even though the specific compound differed from species to species: coumaroyl spermidine decreased in *S. portulacastrum*, feroloylagmatine increased in *S. brachiata* and tricaffeoyl spermidine decreased in *S. maritima*.

## 4. Discussion

Environmental stress in plant science could be defined as any change in growth condition(s) that alters or disrupts plant metabolic homeostasis and requires an adjustment of cellular metabolic pathways, in a process that is commonly referred to as acclimation (Mittler, 2006; Suzuki and Mittler, 2006). Because plants cannot escape from frequently varying environmental and seasonal conditions that negatively affect their growth and development, their survival depends mainly on the initiation of highly complex adaptive responses. However, this response can involve a variety of metabolisms, often interconnected one to each other, thus representing a challenging area of investigation. Therefore, a metabolomics approach is helpful to achieve a better understanding of the plant system under saline environment. On this basis, we conducted a study focusing on the changes in metabolite profile of *S. brachiata, S. maritima* and *S. portulacastrum* triggered by sodium chloride (0, 200, 500 mM). Facultative halophytes are interesting model plants to study salt stress tolerance mechanisms because of their ability to grow in both saline and non-saline conditions. Indeed, these species might provide useful insights into salt tolerance mechanisms, in the light of strategies to improve salt tolerance in crops. In our experiment, different halophytes were considered, aimed to better include the different mechanisms underlying acclimation to salinity. Notably, wide very distinctive responses to salinity could be observed between *S. portulacastrum*, *S. brachiata* and *S. maritima*.

Salinity is well known to induce an osmotic stress, which generates an oxidative imbalance as secondary stress (Gratão et al., 2005; Munns, 2002). The accumulation of compatible solutes acting as osmolytes, low molecular weight chaperons of hydrophilic nature, plays roles in detoxification of reactive oxygen species and stabilization of proteins conformation (Muchate et al., 2016). At membrane level, osmolytes help in replacement of water at the surface of membrane proteins (Kumari and Parida, 2018). Although osmotic adjustment was present in all species and tissues, different osmolytes were accumulated under salinity among the three species investigated. Our result indicates that the level of proline was increased in *S. portulacastrum* with increasing concentration of NaCl, whereas glycine betaine and polyols were increased in *S. maritima* and *S. brachiata.* These results are in agreement with previous findings for *S. portulacastrum* and *S. maritima* (Moghaieb et al., 2004; Muchate et al., 2016).

Literature also suggests that an increase in osmolytes correlates to a decrease in ROS production, thus improving tolerance to salt stress (Behr et al., 2017; Matysik et al., 2002; Szabados and Savoure, 2010). Besides osmotic adjustment compounds, we could identify several compounds involved in oxidative stress balance, with important differences between species subjected to low and highs salinity. In *S. brachiata*, the amount of flavonoids and other phenolic compounds increased in presence of NaCl in the substrate, whereas these metabolites were down accumulated in *S. portulacastrum*. Indeed, flavonoids are one of the most important classes of plant phenolics, being powerful antioxidants (Agati et al., 2012; Brunetti et al., 2013) likely acting to counteract the imbalance in oxidative status induced by salinity. In general, the level of salt stress had a significant impact on the accumulation of flavonoids and other phenolics in our plants, with a bell-shaped trend across NaCl concentrations. It might be postulated that the accumulation of these compounds, produced to lower oxidative damage caused by ROS (Modrianský and Gabrielová, 2009; Sandoval-Acuna et al., 2014; Slimestad and Verheul, 2009), might have been hampered by metabolic disruption under the highest salinity level (500 mM). Nonetheless, carotenoids, another class of important antioxidant compounds, were found up-regulated in *S. portulacastrum* under salinity conditions. These latter, are reported to be triggered by different osmotic stresses such as salinity, nanoparticles or drought (Abid et al., 2018; Lucini et al., 2015; Salehi et al., 2018). Carotenoids accumulation in plants under salinity might play a direct role as antioxidants, but they are also involved in the photosynthetic machinery (Abid et al., 2018). Carotenoids exhibit antioxidant activity in plastids by ending the chain reaction in lipoperoxidation, by scavenging singlet oxygen and preventing its formation by reacting with chlorophyll (Das and Roychoudhury, 2014). In *S. portulacastrum*, the increased levels of carotenoids were observed together with a down accumulation of phenolics. Overall, our result imply that the level of antioxidant compounds was species specific and salinity level related, even though this mechanism of plant adaptation to salinity was common to the three halophytes considered. Notably, halophytes have been proposed as a good source of antioxidant compounds (Oueslati et al., 2012).

Alterations in fatty acid profile and rearrangement of membrane lipids can be considered an important metabolisms for achieving salt tolerance in plants (López-Pérez et al., 2009), since they are oriented to modify plasma membrane composition under stress conditions. The accumulation of oxidized forms (mainly hydroxy- or epoxy-derivatives) of lipids, together with mono- and diglycerides increase under salinity, might be explained by the need to maintain membranes integrity (Tisi et al., 2008). However, membrane lipids and oxidized forms of lipids were both down accumulated in *S. maritima* under salinity, whereas no impairments could be observed in *S. portulacastrum*. Hence, like for previous classes of metabolites, the changes in lipids profile were species-specific.

Terpenes may also contribute to relieving damages by abiotic stresses, including high salt in plants. In our species, several alkaloids and terpenes were altered under salinity. Metabolites from these classes are reported as stress-related compounds (Arbona et al., 2013; Lucini et al., 2015). Several examples suggest the signaling role for terpenoid phytoalexins (Pretali et al., 2016; Ryu and Cho, 2015; Schmelz et al., 2014). Furthermore, they are involved in the interaction with different transcription factors related to apoptosis, cell cycle arrest, DNA repair processes in response to oxidative stress caused by various abiotic factors (Kaur et al., 2015).

Regarding hormone profiles, several gibberellins and cytokinins were up accumulated in *S. brachiata*. Cytokinins play influential roles in many plant growth and developmental processes and are considered as master regulators during plant growth and development. The alteration of endogenous levels of cytokinins and gibberellins in response to stress confirms their involvement in abiotic stress including drought and salinity (Clipson et al., 1988; Ryu and Cho, 2015). Recently, experiments have been performed to investigate the role of gibberellins in osmotic stress response in Arabidopsis thaliana seedlings (Wani et al., 2016). The induced increase in ABA level is reported to be an anti-transpirant strategy for water conservation, that further helps in maintaining cell turgor by promoting the synthesis of osmoprotectants and antioxidants under both salinity and drought conditions (Kumari and Parida, 2018). In *S. brachiata*, brassinosteroids appeared to be impaired under salinity. These hormones are implicated in a wide range of physiological and biochemical responses in plants, and they have been found to mediate plant response under abiotic and biotic stress (Ali, 2017). Furthermore, a specific response that involved tuberonic acid and therefore jasmonate was also identified in *S. maritima*. Jasmonate is a well-known plant stress hormone, whose involvement is generally not surprising (Ahmad et al., 2016). Indeed, the increased of jasmonate has also been reported in tomato, *A. thaliana* and *T. halophila* under salt stress conditions (Kumari and Parida, 2018).

A complex and diverse alteration of the levels in nitrogen-containing compounds was observed under salinity, as a function of the species considered. The positive effects of amines accumulation in the plant cell have been associated with the maintenance of membrane integrity, stabilization of enzymes and alleviation of oxidative stress following accumulation of $Na^+$ and $Cl^-$ in different organs (Afzal et al., 2009; Behr et al., 2017; Roy et al., 2005; Roychoudhury et al., 2011; Tisi et al., 2008; Wang et al., 2007b; Yiu et al., 2009). Interestingly, in *S. maritima*, serotonin was induced by salinity; this compound is actually known for its role in plants abiotic stress tolerance (Kaur et al., 2015).

Even the impairment of nitrogen-containing compounds was species-related, a substantial agreement could be observed between 200 and 500 mM NaCl within a species, with the main biochemical classes showing the same trend in alteration. Several amino acids were found at reduced levels in *S. brachiata* under salinity, while amino acids derivatives were accumulated. These latter might actually act as compatible osmolytes, rather than the corresponding canonical amino acids. Furthermore, polyamine conjugates (*i.e.*, tricaffeoyl spermidine and coumaroyl spermidine, in *S. maritima* and *S. portulacastrum* respectively) were involved in salt stress response. These compounds are reported to be acylated for regulatory purposes or for recruitment into secondary metabolic pathways, and are implicated in a wide range of developmental processes in plants, such as cell division and responses to environmental stresses (Luo et al., 2009; Rouphael et al., 2016).

Concluding, as expected the tolerance of the species considered against salinity involved the two main toxicity effects related to this condition, namely the cope with osmotic stress by accumulating compatible solutes, and the contrast to oxidative imbalance. However, when moving at molecular level, metabolomics highlighted that the specific mechanism each species adopted to achieve these common goals differed in the three halophytes considered. Several compounds have been identified as critical players in salt stress mitigation across the species considered, some of them being well known, some other being novel. A deeper understanding of these complex interplay of plant metabolites might help crop breeding in selecting those varieties that better cope with salt stress.

## Declarations of interest

None.

## CRediT authorship contribution statement

**Jenifer Joseph Benjamin:** Formal analysis, Data curation, Writing - original draft. **Luigi Lucini:** Conceptualization, Data curation, Writing – review & editing. **Saranya Jothiramshekar:** Formal analysis. **Ajay Parida:** Conceptualization, Writing – review & editing.

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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.2018.11.006.

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