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Physiological and Phytochemical Responses of *Matthiola incana* L. to Salinity Stress: Insights into Biochemical and Metabolic Adaptation

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Abstract

Matthiola incana L. is an ornamental plant with recognized phytochemical and antioxidant potential. However, limited information is available regarding its integrative physiological and metabolic responses to salinity stress. Therefore, this study investigated the effects of increasing salinity levels on growth performance, biochemical traits, antioxidant activity, and metabolite profiles of *M. incana*. **Methods:** Plants were exposed to four irrigation salinity levels (2, 4, 6, and 8 dS m^{-1}). Growth characteristics, photosynthetic pigments, soluble carbohydrates, proline accumulation, anthocyanin content, and DPPH radical scavenging activity were evaluated. Metabolic alterations induced by salinity stress were further analyzed using LC-MS and GC-MS techniques. **Results:** Moderate salinity (6 dS m^{-1}) significantly increased biomass and fresh weight by 11.58% and 38.99%, respectively, compared with the baseline salinity treatment (2 dS m^{-1}). Soluble carbohydrate and proline contents also increased markedly under moderate salinity conditions, indicating osmotic adjustment responses. Anthocyanin accumulation was highest at 4 dS m^{-1}, whereas DPPH radical

scavenging activity progressively increased with increasing salinity. In contrast, high salinity (8 dS m^{-1}) significantly reduced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents. Metabolomic analyses revealed salinity-induced changes in organic acids, phenolic compounds, flavonoids, and antioxidant-related metabolites, including scopoletin, hydroxycaffeic acid, ferulic acid, and ascorbic acid. **Conclusion:** The results demonstrate that *M. incana* exhibits physiological and metabolite-associated responses under moderate salinity conditions through osmotic regulation and enhanced antioxidant-related metabolism. These findings provide new insights into salinity-responsive biochemical metabolic in ornamental Brassicaceae species.

Keywords: Ornamental plant, Salt stress, Tolerance, Scavenging activity, LC-MS,

1. Introduction

Increasing soil salinity is one of the major environmental constraints affecting plant growth, physiological functions, and productivity worldwide [1]. The increasing salt made to soil and the source of means to irrigate (due to climate change, poor management of water, and a shortage of fresh water) will be one of the biggest barriers to sustainable plant agricultural systems going into the future [2]. The three major effects caused by salinity stress (osmotic stress, ion toxicities, and oxidative damage) all contribute to lower photosynthetic activity and disruption of nutrient homeostasis, and inhibit plant growth [3, 4].

Plants utilize a wide range of physiological and biochemical responses in order to help them deal with salinity stresses. The primary mechanism by which they do this involves osmotic adjustment through Compatible solutes (such as proline and soluble carbohydrates) to maintain cellular water and provide intra- and extrasellar stabilization of proteins/membranes [5]. Moreover, oxidative stress due to overproduction of reactive oxygen species (ROS) contributes to increased oxidative damage in cellular systems [6]. These physiological processes are largely the result of stress-induced changes in metabolic activity rather than providing any direct benefits of adaptation unless demonstrated scientifically.

Many studies have focused on agricultural crop responses to salinity, but research on ornamental plant responses is somewhat limited, despite more ornamental plants being used in urban and landscape settings, and more of these plants are receiving saline irrigation. Though widespread as ornamental plants, there is an apparent lack of research investigating the response of Brassicaceae species to salinity stress functionally and biochemically. Evidence also shows that most of these studies have only provided descriptive data, often looked at single traits, or did not consider how physiological and metabolite responses relate in a combined multi-tiered analysis.

The stock flower (*Matthiola incana* L.) is a prominent member of the Brassicaceae family known for its utility as an ornamental plant and for the

beauty of its flowers; However, while *M. incana* L. has many uses as a horticultural plant, there are very few studies detailing how plants in this family would respond to salinity stress through coordination of physiological and biochemical processes. In particular, the relationships between salinity-induced changes in photosynthetic pigments, osmolyte accumulation, antioxidant activity, and primary metabolites remain insufficiently characterized. Although salinity-induced changes in osmolyte accumulation, antioxidant activity, and photosynthetic performance have been extensively investigated in agricultural crops, comparable integrative studies in ornamental Brassicaceae species remain limited. Previous studies on ornamental plants under salinity stress have mainly focused on growth reduction and basic physiological responses, whereas coordinated analyses linking physiological traits with metabolite alterations are still scarce. In particular, little information is available regarding how salinity influences the interaction between osmotic regulation, antioxidant-related metabolites, and secondary metabolism in *Matthiola incana* L. To the best of our knowledge, no previous study has integrated physiological, biochemical, LC-MS and GC-MS analyses to investigate salinity-induced responses in *Matthiola incana*. Available studies on *M. incana* have mainly focused on horticultural performance and physiological responses, whereas comprehensive metabolomic characterization under salinity stress remains limited. Therefore, the present study aimed to investigate the effects of salinity stress

on growth, photosynthetic pigments, osmolyte accumulation, antioxidant activity, and metabolite profiles of *M. incana*.

2. Material and Methods

2.1. *M. incana* cultivation and salinity treatments

Seeds of the native species, *M. incana* L., were obtained from the Medicinal and Aromatic Plants Research Center, Faculty of Agriculture, University of Baghdad, Iraq and were taxonomically verified by Dr. Wurood Hantoosh Neamah, a medicinal plant specialist. Tap water commonly used for irrigation in the study region, with an electrical conductivity (EC) of approximately 2 dS m^{-1}, was used as the baseline irrigation water (control treatment, S2). The irrigation water with an EC of approximately 2 dS m−1 corresponds to the commonly available irrigation water used in the study region and was therefore considered the baseline control treatment. The salinity treatments were established to evaluate plant responses relative to prevailing local irrigation conditions rather than to a completely non-saline water source. Higher salinity treatments (S4, S6, and S8) were prepared by dissolving calculated amounts of NaCl into the same irrigation water source until the desired EC levels were achieved. The EC level of 2 dS m^{-1} reflects the commonly available irrigation water used in the study region and was therefore selected as the practical baseline control treatment under local cultivation conditions rather than a completely non-saline reference. The physicochemical properties and ionic composition of the irrigation water used

for each salinity treatment were analyzed prior to the experiment to ensure consistency among treatments. The measured parameters included pH, electrical conductivity (EC), and major ions including Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, and SO_4 The results are presented in Table 1. The experiment was conducted using a randomized complete block design (RCBD) with three blocks. Each salinity treatment included three biological replicates, and each replicate consisted of three plants grown in one pot. The pot was considered the experimental unit for statistical analysis. Mean values from the three biological replicates (n = 3) were used for ANOVA.

Seeds were sown in seedling trays filled with 100% peat moss substrate and grown for 30 days under controlled environmental conditions: day/night temperatures of 24 ± 2 °C and 18 ± 2 °C, respectively, a 14 h light/10 h dark photoperiod, light intensity of 250–300 µmol m^{-2} s^{-1}, and relative humidity of 60–70%. Irrigation was applied every other day using a standardized volume of saline solution sufficient to maintain the substrate near field capacity while avoiding waterlogging and leaching losses.

After 30 days, uniform seedlings were carefully selected and transferred to 25 cm diameter plastic pots filled with a soil-to-peat moss mixture at a ratio of 3:1 (v/v). Plants were acclimatized for one week under constant conditions. Afterwards, the different salinity treatments were started using 200 ml of each saline solution weekly per pot. Salt irrigation was applied continuously during the vegetative and flowering phases; no fresh water was added in

between salt irrigations. Irrigation scheduling was based on prevailing environmental conditions and the water requirements of the plants.

During the experimental period, plants were maintained under controlled environmental conditions (day temperature 24 ± 2 °C, night temperature 18 ± 2 °C, 14 h photoperiod, light intensity 250–300 µmol/m2/s, and relative humidity 60–70%). All pots were uniformly managed with regular irrigation, adequate drainage, and complete protection from natural rainfall throughout the experimental period.

2.2. Growth Traits

Growth measurements were recorded at the full flowering stage. Plants were harvested immediately after flowering assessments. Biomass refers to total dry biomass of the harvested plant material. Vegetative and floral growth traits were evaluated, including leaf number, flower number, fresh weight, and dry biomass.

2.3. Biochemicals Traits

2.3.1. Photopigments Content of Leaves

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined in leaf tissues following the method described by Lichtenthaler [7] with slight modifications. Fresh leaf samples (0.5 g) were homogenized and extracted in 10 mL of 80% (v/v) acetone. The samples were incubated overnight at 4 °C in the dark to prevent pigment degradation. The extracts were then filtered, and the final volume of each filtrate was adjusted to 30

mL with 80% acetone. Absorbance of the clear supernatant was recorded at 663, 645, and 470 nm using a UV–Vis spectrophotometer against 80% acetone as blank. All measurements were performed in triplicate for each biological replicate (n = 3). Pigment concentrations were calculated using previously established equations (Akladious et al., 2015). Pigment contents were expressed on a fresh weight basis (mg g^{-1} FW) using the following equations:

Chlorophyll *a* (mg/g^{-1}) = $(10.3A_{663}\text{-}0.92\times A_{645}) \times V/1000\times W$ [1]

Chlorophyll *b* (mg/g^{-1}) = $(19.7A_{645}\text{-}3.87A_{663}) \times V/1000\times W$ [2]

Chlorophyll T = Chl *a* + Chl *b* [3]

Carotenoids (mg/g^{-1}) = $(4.2A_{470}$-0.026 Chl *a*- 0.426×Chl *b*) /1000× *W* [4]

Where: *V*= extraction volume (mL), *W*= sample weight (g)

2.3.2 Carbohydrates Content of Leaves

Leaf carbohydrate content was determined using the phenol–sulfuric acid method. Dried leaf samples (0.5 g) were homogenized with 70 mL of distilled water and heated in a water bath at 70 °C for 1 h [8]. The mixture was then cooled to room temperature and filtered. From the filtrate, 5 mL was diluted with 30 mL of distilled water. Subsequently, 1 mL of the diluted solution was transferred into a clean test tube and rapidly mixed with 5 mL of concentrated sulfuric acid and 1 mL of 5% (w/v) phenol solution. The mixture was vortexed for 30 s, then cooled to room temperature. Absorbance was measured at 490 nm using a UV–Vis spectrophotometer. Carbohydrate

concentration was determined using a glucose standard calibration curve and expressed on a dry weight basis (mg g^{-1} DW). The calibration curve was constructed using a series of glucose standards, and linearity was confirmed within the working range ($R^2 > 0.99$). The carbohydrates concentration was determined from the standard curve of glucose and calculated in dry weight following the equation:

$$\text{Carbohydrates} \quad (\text{mg/g}^{-1}) = \frac{(\text{Glucose concentration (mg mL-1)}) \times (\text{dilution factor}) \times (\text{total volum (mL)})}{\text{sample weight (g)}} \quad [5]$$

2.3.3. Proline Content of Leaves

Free proline content was determined according to the method described by Bates et al. [9] with slight modifications. Dried leaf samples (0.20 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid and filtered through filter paper. An aliquot of 2 mL filtrate was transferred into a test tube and mixed with 2 mL of acid ninhydrin reagent, prepared by dissolving 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid, followed by the addition of 2 mL glacial acetic acid. The reaction mixture was incubated in a boiling water bath (100 °C) for 1 h and then immediately cooled in an ice bath to terminate the reaction. Subsequently, 4 mL of toluene was added to each tube, and the mixture was vigorously vortexed to allow phase separation. The chromophore-containing toluene phase was collected and allowed to reach room temperature. Absorbance was measured at 520 nm using toluene as a blank with a UV–Vis spectrophotometer. Proline concentration was determined from a proline standard calibration curve and

expressed on a dry weight basis (µmol g^{-1} DW). The calibration curve showed high linearity within the working concentration range ($R^2 > 0.99$). Proline content was calculated using the following equation:

Proline (\mumol g^{-1}) = $\frac{C \times V}{115.5 \times W}$ [6]

Where: C = proline concentration from standard curve (µg mL^{-1}) , V = volume of toluene extract (mL), 115.5 = molecular weight of proline, W = sample weight (g)

2.3.4. Anthocyanin Content of Flowers

Shaded flower samples were air-dried and then ground into a very fine powder over room temperature. The total concentration of anthocyanins present was quantified by Sołovchenko et al [39] One gram of sample was mixed with the extraction solvent which consisted of a 15:10:0.15 volume mixture of methanol, distilled water and hydrochloric acid and 20 mL of chloroform. The sample (mixture) was shaken in darkness for two hours then placed into an incubator overnight at 4°C to allow the complete extraction of pigments. On the next day, the mixture was (centrifuged) at 7000 RPM for 15 minutes. The supernatant was carefully collected and stored in 15mL tubes at -30°C until analysis occurs. The absorbance of anthocyanins was spectrophotometrically measured at 530 nm and 657 nm with a UV-Vis spectrophotometer, and the content was calculated by using this formula Vankar and Srivastava, [10] and presented in A.U. g^{-1} DW using the following equation:

Total anthocyanin (mg g^{-1})= $\frac{(AA530-A657)\times V}{M}$ [7]

Where: A_{530} = absorbance at 530 nm, A_{657} = correction for chlorophyll 657 nm, V = extract volume (mL), M = sample weight (g)

2.3.5. Antioxidant Activity of Flowers Extract

Antioxidant activity was assessed for flower extracts by evaluating their ability to inhibit DPPH free radicals using the procedure as outlined by Williams et al et al. [11]. Briefly, 100 µL of each flower extract was diluted with 3.9 mL 0.004% (w/v) DPPH in methanol, mixed in a vortex mixer, and left to react at room temperature in the dark for 30 minutes. DPPH measurement at 515 nm after incubation using a UV-VIS spectrophotometer with methanol and DPPH solution without extract as controls. Each biological replicate was measured three times and mean values used to perform statistical analysis. DPPH radical scavenging activity calculated using this equation:

DPPH scavenging (%) = (A_C (control)-A_M (sample) /A_C (control) ×100

2.4. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis of Flower Extract

2.4.1. Sample preparation for LC-MS injection

Samples were prepared prior to analysis according to a specified standard operating procedure (SOP). The first step was to homogenize the samples by sonication for two minutes. Step two of the process involved mixing the samples together in a vortex for one minute. Step three involved separating

out any insoluble materials by centrifuging at 12,000 revolutions per minute (rpm) for ten minutes which provided a clear supernatant that could then be prepared for analysis by passing through a 0.22 µm filter with a syringe to remove any remaining particles. Final preparation before analysis of the samples was performed by injecting 5 µL of filtered sample into a liquid chromatography-mass spectrometry (LC-MS) system.

2.4.2. LC-MS instrumentation and analytical conditions

Approximately 0.5 g of dried flower powder was extracted with 10 mL of 80% methanol containing 0.1% formic acid. Samples were vortexed for 2 min, sonicated for 20 min at room temperature, and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was filtered through a 0.22 µm PTFE syringe filter prior to LC-MS analysis. The chromatography used for the analysis was completed using a Micromass Quattro Micro API Triple Quadrupole Mass Spectrometry (Waters Corporation, USA) with HPLC Waters Alliance 2695. The derivative was separated on a Eurospher C18 reverse-phase column (4.6 mm × 120 mm, particle size 5 µm) at a temperature of 35 °C. The mobile phase comprised of solvent A consisting of acetonitrile (with 0.1% formic acid; v/v) and solvent B which comprised of ultra-pure water (with 0.1% formic acid; v/v) was delivered at a constant flow rate of 0.3mL/min. The gradient procedure for the two mobile phases was as follows: 10%A (for the first 2mins); linearly increased to 70%A within an 8m period; sustained at

70% for 10mins; returned to original conditions (10%) at 23mins and allowed to re-equilibrate back to 10% until 26 mins.

The detection using mass spectrometry was performed using negative electrospray ionization (ESI-) mode under optimized conditions. The optimized conditions are 4.0 kV capillary voltage, 25 V cone voltage, 3 V extractor voltage and 0.1 V RF lens voltage. The source of gas used in this procedure was high purity nitrogen (grade 5.0) and it served as both nebuliser and desolvation gases supplied at 200 L/h. Ion source and desolvation temperatures were set to 120 °C and 350 °C respectively. Data collection and instrument operation was handled using MassLynx software (Waters Corporation).

The identification of metabolites has been verified through retention time and mass spectral matching using available databases. The LC-MS results were therefore interpreted as comparative semi-quantitative metabolite profiles based on relative peak areas (arbitrary units, AU). Throughout the course of testing, quality control (QC) samples, including blanks and replicate injections, were analyzed regularly to determine instrument performance stability and reproducibility of data.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Floral Extract

2.5.1. Sample preparation

The floral extract initially prepared for anthocyanin determination was additionally utilized for exploratory GC-MS profiling of volatile and semi-volatile metabolites. Since the extraction solvent system was primarily optimized for anthocyanin recovery, the GC-MS analysis was considered semi-qualitative and comparative in nature.

2.5.2. GC-MS Instrumentation and Conditions

Phytochemical identification was carried out using a GC (Agilent 7890B) with a Mass Spectrometer (5977A MSD) using Electron Ionization as a detection system, separated on an HP-5ms capillary column (5% phenyl 95% methyl siloxane) (30 m x 250 mm x 0.25 mm). The oven temperature program was: initial hold at 40°C for 5 minutes, ramped at 8°C/min to 300°C, held for 20 minutes. Helium was utilized as the carrier gas, flowing at 1 mL/min, while the purge flow was set at 3 mL/min. An injection volume of 0.5 µL was utilized for this experiment with pulsed-splitless injection at 290 degrees Celsius. The mass spectrometer was operated under electron ionization (EI) mode at 70 eV with a scan range of 44–750 m/z. The ion source temperature was maintained at 230 °C.

The spectra and retention indices were used to compare the mass spectrometry data to the authentic standards' mass spectrometry data and to spectral libraries, such as NIST 2014 and 2020 databases. The use of authentic standards along with a collection of mass spectral libraries significantly improved the accuracy with which the structure of volatile and

semi-volatile phytochemicals could be identified. The method of semi-quantitative analysis involved the use of authentic standards whenever feasible, while blank samples and replicate injections helped monitor equipment performance during the analysis and contribute to quality control of analytical methods.

2.6. Statistical Analysis

Prior to conducting ANOVA, assumptions of normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively. Each salinity treatment consisted of three biological replicates (n = 3), and each replicate contained three plants per pot. Mean comparisons among treatments were performed using Tukey's honestly significant difference (HSD) post hoc test at $p \leq 0.05$ following one-way ANOVA. All reported means include their standard errors (SE) and sample sizes are clearly indicated.

Principal Component Analysis (PCA) was performed using R software (www.r-project.org), with the percentage of explained variance by each principal component explicitly reported to assess data dimensionality. Statistical validation of PCA results was conducted through permutation tests or scree plot analysis to ensure robustness. Pearson correlation analyses were conducted using the 'srplot' package (https://www.bioinformatics.com.cn/en), with significance levels (p-values) reported for all correlation coefficients.

3- Results and Discussion

3.1. Salinity-Induced Changes in Growth and Flowering Traits

Because the baseline treatment reflected the commonly used irrigation water in the study region (EC ≈ 2 dS m^{-1}), the results should be interpreted as plant responses to increasing salinity relative to local cultivation conditions rather than relative to a completely non-saline control. Biomass and fresh weight significantly increased under moderate salinity (6 dS m^{-1}) compared with the control treatment ($p \leq 0.05$), indicating a stimulatory response of *M. incana* to moderate salt exposure (Fig. 1a, b). Also, the results showed that 6 dS m^{-1}increased biomass and fresh weight by 11.58% and 38.99% compared to the control. The stimulatory effect observed at 6 dS m^{-1} may represent a hormetic response under moderate stress conditions. Alternatively, altered nutrient availability or experimental variability may also have contributed to the observed increase in biomass. Fig. 1c showed that the number of flowers per plant decreased with increasing stress, so that the highest at 4 dS m^{-1} and the lowest at 8 dS m^{-1} were 15.91 and 12 number, respectively. The highest number of leaves was observed at 2 dS m^{-1} and the lowest at 8 dS m^{-1}, 20.16 and 13.75 per plant, respectively (Fig. 1d). Salinity stress is known to influence plant growth by affecting water uptake and nutrient balance [11]. In one study, it was shown that salinity levels of 100–300 mM significantly reduced flower production in *Tagetes patula* [12], and high salinity levels reduced dry matter accumulation in *Thymus vulgaris* and *Thymus daenensis* [13]. Findings indicate that increasing salinity levels lead to reductions in

shoot and root growth, fresh and dry biomass, photosynthetic activity, and flowering parameters such as flower number and biomass [14, 15].

3.2. Photosynthetic Pigment Responses to Salinity Stress

The results showed that at 8 dS m^{-1}salinity, Chlorophyll a, Chlorophyll b and total chlorophyll decreased by 33.76%, 32.15% and 29.85%, respectively, compared to the control (2 dS m^{-1}) (Fig. 2a-c). The highest carotenoid content was observed at 2 and 4 dS m^{-1} with 6.8 and 6.98 mg/g wet weight, respectively, and the lowest at 8 dS m^{-1} with 3.23 mg/g wet weight (Fig. 2d). Salinity can affect pigment content by affecting ionic homeostasis and oxidative balance in plants [16]. A study showed that P. karka maintained photosynthetic pigments under moderate salinity (100 mM NaCl), while higher concentrations (300 mM) led to their reduction [17]. In sunflower, salinity stress negatively affected chloroplast structure and reduced pigment levels [18].

Salinity Stress Promotes Anthocyanin Content and DPPH Activity

The highest anthocyanin level was recorded at 4 dS m^{-1} (34.53 A.U. g^{-1} DW), while the lowest was observed in the control group (2 dS m^{-1}, tap water) (22.47 A.U. g^{-1} DW) (Fig. 3a). Also, salinity stress of 4, 6 and 8 dS m^{-1} showed an increase in DPPH scavenging by 26.34%, 27.63% and 30.53%, respectively, compared to the control (2 dS m^{-1}) (Fig. 3b). Salinity stress usually increases the production of ROS in plants, thereby stimulating the plant antioxidant system [40, 41]. For this reason, DPPH free radical

scavenging activity is often enhanced under salinity conditions, as the accumulation of antioxidant compounds such as phenols, flavonoids, and anthocyanins is enhanced [19]. Increased DPPH activity was observed in wheat genotypes under salinity conditions, especially during tillering [20]. Salinity levels have also been shown to increase antioxidant activity in lettuce compared to saline levels, especially with moderate water deficit, indicating that this may be a type of adaptation [21]. In contrast, the amount of dry matter in peppermint has decreased when tested in saline conditions, although the amount of anthocyanins have also increased under these conditions [22].

In the current study, antioxidant responses were primarily evaluated through non-enzymatic antioxidant activity (DPPH assay) and metabolite profiling of phenolic and flavonoid-related compounds. The experimental focus was directed toward phytochemical and metabolomic alterations associated with salinity stress rather than enzymatic antioxidant defense systems. Therefore, classical antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were not assessed in the present investigation. Nevertheless, the observed accumulation of antioxidant-related metabolites, including phenolic acids, flavonoids, coumarins, and ascorbic acid, strongly suggests activation of oxidative stress mitigation mechanisms under saline conditions. Future studies integrating enzymatic antioxidant assays and molecular analyses would provide a more comprehensive understanding of salinity tolerance mechanisms in *M. incana.*

Salinity-Driven Increase in Carbohydrate and Proline

According to the data collected, a maximum of 427.90% carbohydrate content was obtained at 6 dS m^{-1} (along with 39.34% proline) as compared with blank (2 dS m^{-1}, tap water) (Figs. 3c, 3d). Increased amounts of soluble carbohydrates help manage osmotic potential and create cellular and systemic stability, while proline is a major contributor to improving salinity tolerance by sustaining osmotic potential and integrity of membranes and ROS production [23, 24]. In study, similarly, increased proline levels under salinity have been observed in date palm [25], and rice [26], suggesting a conserved stress response mechanism. In some cases, total carbohydrate content in leaves and stems increases with salinity, as seen in tomato cv. Shirazy [27] as observed in horse gram [28], *Phaseolus vulgaris* [29].

the accumulation of both soluble carbohydrates and proline when exposed to moderate salinity may indicate that there are significant cellular adaptation mechanisms related to osmotic adjustment and maintaining cellular structure. Proline is well known to serve as an osmoprotectant, as well as being a molecular chaperone that helps stabilize membranes while scavenging for ROS (reactive oxygen species) and maintaining redox homeostasis when exposed to salt stress.

Increased soluble carbohydrate accumulation also contributes to osmotic balance and provide metabolic substrates necessary for stress-responsive pathways. Additionally, the presence of elevated concentrations of phenolic

acids, flavonoids, coumarins and compounds that are antioxidant-related, as determined by LC-MS analysis, indicates that the secondary metabolic pathways that are activated as a result of oxidative stress mitigation occur simultaneously. Some of these compounds include caffeic acid, ferulic acid, scopoletin, catechin and ascorbic acid, which are all known to play a role in the detoxification of ROS and are protective of cells during salinity exposure [23, 24].

These metabolic adjustments may be indicative of changes in metabolites associated with physiological responses to salinity. Lastly, the ongoing maintenance of biomass (as measured by dry weight) under moderate salinity exposure (6 dS m^{-1}) despite decreased pigment levels measured via photo-acoustic analysis suggests that *M. incana* may be capable of partially compensating for physiological stresses induced by salt through coordinated osmotic and antioxidant regulation. However, the integrated physiological and metabolomic data generated in the current study do not provide molecular validation for these observations[23]. Future transcriptomic and gene-expression studies are recommended to further elucidate the molecular basis of salinity tolerance in *M. incana*

Correlation (HEATMAP) and Principal component analysis (PCA)

Correlation analysis demonstrated strong positive associations between biomass accumulation, soluble carbohydrates, and proline content, suggesting coordinated osmotic adjustment mechanisms under moderate salinity conditions. Positive correlations between chlorophyll pigments and

leaf number indicate that maintenance of photosynthetic capacity contributed to vegetative performance. In contrast, DPPH activity showed negative associations with chlorophyll traits under high salinity, reflecting oxidative stress-induced metabolite profile alterations associated with salinity stress (Fig. 4a).

According to the heat map presented in Figure 4b, biomass was positively correlated with salinity level of 6 dS m^{-1}. Salinity treatments of 2 and 4 dS m^{-1} had a positive correlation with carotenoid content and a positive correlation with DPPH radical scavenging activity at all three levels of 4, 6 and 8 dS m^{-1}. Proline accumulation exhibited a positive correlation with a salinity treatment of 6 dS m^{-1}.

Principal component analysis (PCA) revealed a clear separation among salinity treatments based on physiological and biochemical traits. PC1 accounted for 58.5% of the total variance and was mainly associated with proline, carbohydrate accumulation, DPPH activity, and biomass-related traits, indicating that osmotic adjustment and antioxidant responses were major contributors to treatment differentiation under salinity stress. In contrast, chlorophyll-related traits showed negative loadings along PC1, reflecting pigment degradation under higher salinity levels. PC2 explained an additional 23.0% of the total variance and was associated primarily with flower-related traits and carotenoid content (Fig. 4c). The cumulative explained variance (81.5%) indicated strong reliability of the multivariate model.

LC-MS Profile of Flower Extract

According to metabolite analysis, salinity treatment appeared to affect the levels of organic acids, phenolic compounds, flavonoids, and metabolites related to antioxidant activity (Fig. 6; Table 2). Lactic acid was accumulated the most at the highest salinity (8 dS m^{-1}), while fumaric acid was increased in the moderate range (4 and 6 dS m^{-1}). The control treatment (2 dS m^{-1}) was the only treatment in which succinic acid was detected. Caffeic acid showed relatively higher abundance across all treatments but decreased with increasing salinity; however, hydroxycaffeic acid, ferulic acid and p-hydroxybenzoic acid tended to accumulate most under salinity conditions. Ascorbate was only found in the plants treated with salt. In addition, scopoletin, catechin, apiforol, and tetramethylscutellarein were all found to be increased in their levels as a result of salinity treatments, especially at 4 and 6 dS m^{-1}. In contrast, rutin, vitexin, kaempferol, and many of the phenolic glycoside compounds were only found in the control treatment (Table 2). The observed metabolite profile changes under salinity stress may reflect shifts in metabolite accumulation associated with stress adaptation.[30].

Salinity has been shown to increase adaptation responses via the alteration of metabolic carbon flux from growth-related pathways toward producing protective compounds that are important in regulating osmotic stress and reducing oxidative stress [31]. A prolonged period of salinity exposure in *Puccinelli tenuiflora* resulted in more flavonoids and phenolic amides produced, allowing for an improved ability of the plant to scavenge or

neutralise reactive oxygen species. However, organic acid accumulation did not increase [32]. Similarly, hyssop plants have also been reported to have an increase in total flavonoids and diosmin when exposed to increased salinity levels [33]. Canola has been shown to have higher amounts of fatty acids, amino acids, and flavonoids in salt-tolerant cultivars, leading to enhanced antioxidant enzyme activity and increased tolerance to salt stress [34].

GC-MS Profile of Flower Extract

According to GC–MS analysis (Fig. 5a–d), some compound classes were affected by changes in salinity. These include terpenoids, fatty acids, phenolics, nitrogen-containing compounds, and some sterols. Among terpenoids, bicyclo[3.1.1]heptane was mostly located in the higher and moderate salinity levels and eugenol was only detected in the control sample. The proportions of fatty acids (notably n-hexadecanoic acid and 9,12,15-octadecatrienoic acid) were high for all treatments, with minor differences due to salinity. Under salinity conditions, 4-vinylphenol and trans-p-coumaric acid are present at tended to decrease levels than in the control sample. The hydrazine derivatives had higher accumulation at 6 dS m^{-1} than at either 2 or 4 dS/m. With respect to sterol compounds (ɣ-sitosterol and Ergost-5-en-3-ol), their concentrations remained relatively constant across all salinity treatments (Table 2). The metabolic changes indicate that salinity stress triggers a coordinated change in plant metabolism where carbon is distributed away from specific routes of phenolic biosynthesis and towards routes that help the plant adapt to stressors [35]. This response may

contribute to increased production of protective and signaling molecules while protecting the stability of essential membrane constituents (for example, fatty acids and sterols), thereby maintaining cell integrity in a saline environment [36]. The presence of numerous distinct chemical compounds in the *Anethum graveolens* (dill) plant indicates that exposure to salinity and *Alhagi maurorum* extract causes major metabolic alterations to substances isolated from flower extracts, as seen by prominent changes noted in these compounds via GC-MS analyses [37]. The research discovered that the amount of NaCl has an impact on growth of Taif rose (*Rosa damascena*), but GC/MS results showed that some metabolites found in essential oils can be produced at their maximum level when salinity 500 ppm NaCl (moderate salinity), therefore, it is possible for salinity to promote the production of specific valuable metabolites until the plant reaches its maximal growth capacity [38].

A limitation of the present study is the absence of a known salt-tolerant reference genotype or species for comparative evaluation. Future studies integrating tolerant ornamental Brassicaceae species together with molecular and ion-homeostasis analyses would provide deeper insight into salinity adaptation mechanisms in *M. incana.* Also, Future metabolomic investigations integrating KEGG pathway enrichment analysis may provide deeper insight into salinity-responsive metabolic networks.

One limitation of the present study is that the extract used for GC-MS profiling was originally optimized for anthocyanin extraction rather than

comprehensive volatile metabolite recovery. Therefore, the detected GC-MS compounds should be interpreted as exploratory and semi-qualitative metabolite profiles rather than absolute representations of the complete floral metabolome. Future studies using metabolite-specific extraction procedures and derivatization protocols are recommended for more comprehensive GC-MS characterization.

Conclusion

The results demonstrated that *M. incana* exhibited differential physiological and metabolic responses under increasing salinity levels relative to the baseline irrigation condition (2 dS m^{-1}). Moderate salinity (particularly 6 dS m^{-1}) maintained or slightly enhanced some growth and biochemical traits, whereas higher salinity levels negatively affected photosynthetic pigments and several growth parameters. The present study demonstrated that *Matthiola incana* exhibits differential physiological and metabolic responses depending on salinity intensity. Moderate salinity levels, particularly 4–6 dS m^{-1}, promoted biomass accumulation, flower production, osmolyte accumulation, antioxidant activity, and the accumulation of several stress-related metabolites. In contrast, higher salinity levels (8 dS m^{-1}) negatively affected photosynthetic pigment content and some growth-related traits. The metabolite profiling results further suggested salinity-associated shifts in metabolite profiles associated with antioxidant defense and osmotic adjustment. Overall, the results suggest that *M. incana* may maintain acceptable growth and physiological performance under moderate salinity

conditions. Further studies involving ion-homeostasis measurements, tolerance indices and molecular analyses are required before definitive conclusions regarding salinity tolerance can be drawn.

Author contributions

Wurood Hantoosh Neamah and Fatimah Mahdi Jasim performed the experiment and contributed to data collection. Aqila Jumaah Hachim, Karima F. Abbas and Fatemeh Borna analyzed the data and wrote the primary manuscript. Heidar Meftahizade wrote the final manuscript. All authors read and approved the final version of the manuscript.

Declarations

Ethics approval and consent to participate Not applicable.

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Conflicts of interest

The author declares they have no financial interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article

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Table 1. The physicochemical properties and ionic composition of the irrigation water

Sample	PH	EC dS/m	Cl mg/L	SO4 mg/L	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	Hardness mg/L
1	6.48	2.08	549.83	11607.48	200.6	13	40.08	19.92	620
2	6.55	4.03	1029.68	8803.73	293.4	20.8	80.16	43.24	840
3	6.71	6.03	1659.49	17214.95	369.5	28.3	72.14	33.15	1220
4	6.75	8.01	2209.31	23102.80	435	34.7	120.24	60.12	1800

Table 2. Effect of four salinity stress levels on the peak area (AU) of major metabolites identified in *M. incana* using LC-MS analysis.

Compound Name	M/z	Chemical Class	MF	Peak Area (AU)			
				S2	S4	S6	S8
Lactic Acid	88.84	Hydroxy Carboxylic Acid	$C_3H_6O_3$	4121924	3308692	2649619	4403118
Fumaric Acid	114.90	Dicarboxylic Acid	$C_4H_4O_4$	2542696	3349872	3040644	2907605
Succinic acid	116.97	Dicarboxylic acid	$C_4H_6O_4$	327728	NA	NA	NA
Cinnamic Acid	147.02	Phenylpropanoid	$C_9H_8O_2$	2039492	1993653	1779345	1882368
Protocatechuic acid	153.04	Phenolic acid	$C_7H_6O_4$	52080	NA	NA	NA
Umbelliferon	161.04	Coumarin	$C_9H_6O_3$	280160	NA	NA	NA
Ascorbic Acid	174.95	Vitamin C (Antioxidant)	$C_6H_8O_6$	NA	1276517	1144498	1227594
p-Coumaric acid	163.04	Phenolic acid	$C_9H_8O_3$	513070	NA	NA	NA
Vanillic acid	167.00	Phenolic acid	$C_8H_8O_4$	50545	NA	NA	NA
Caffeic Acid	179.06	Phenolic Acid	$C_9H_8O_4$	2915725	1617223	1670997	1681359
Esculetin	177.03	Coumarin	$C_9H_6O_4$	137291	NA	100742	NA
Scopoletin	191.02	Coumarin	$C_{10}H_8O_4$	360915	910854	847920	718468
Sinapic acid	223.05	Phenolic acid	$C_{11}H_{12}O_5$	176932	NA	NA	NA
Ferulic Acid	193.03	Phenolic acid	$C_{10}H_{10}O_4$	NA	532209	307799	378277
Kaempferol	285.03	Flavonol	$C_{15}H_{10}O_6$	49901	NA	NA	NA
Apiforol	273.00	Flavan-3-ol	$C_{15}H_{14}O_6$	NA	477310	546084	415996
Eriodictyol	287.26	Flavanone	$C_{15}H_{12}O_6$	319254	412399	NA	255213

p-Coumaroyl hexoside acid	325.12	Phenolic glycoside	$C_{15}H_{18}O_8$	481734	NA	NA	NA
5-Hydroxyferuloylmalate	341.02	Phenolic derivative	$C_{15}H_{16}O_9$	169185	NA	NA	NA
Catechin	288.97	Flavan-3-ol	$C_{15}H_{14}O_6$	NA	318787	338517	292235
Rhamnazin	329.11	Flavonoid (O-methylated)	$C_{17}H_{14}O_7$	607154	NA	NA	NA
p-Hydroxybenzoic Acid	136.98	Phenolic Acid	$C_7H_8O_3$	NA	225952	416511	166945
Curcumin	367.88	Polyphenol	$C_{21}H_{20}O_6$	398303	NA	NA	NA
Rosmarinic acid	358.94	Phenolic acid	$C_{18}H_{16}O_8$	68185	NA	NA	NA
1,2,2'-Triferuloylgentiobiose	869.16	Feruloylated Oligosaccharide	$\sim C_{48}H_{50}O_{24}$	NA	484293	521591	533483
Rutin	608.96	Flavonoid glycoside	$C_{27}H_{30}O_{16}$	709222	NA	NA	NA
Hydroxybenzoic acid-hexoside	299.65	Phenolic glycoside	$C_{13}H_{16}O_8$	216814	NA	NA	NA
Hydroxycaffeic Acid	195.04	Phenolic Acid	$C_9H_8O_5$	NA	1334554	181157 2	164434 4
Tetramethylscutellarein	341.05	Flavonoid	$C_{19}H_{18}O_6$	NA	694869	735997	708297
Quinic Acid	191.02	Cyclitol Carboxylic Acid	$C_7H_{12}O_6$	471622	910854	847920	718468
4-(Methylthio) but-3-enyl glucosinolate	417.94	Glucosinolate	$C_{12}H_{21}NO_5S_2$	350270	NA	NA	NA
Ferulic acid-dihexoside	355.04	Phenolic glycoside	$C_{24}H_{32}O_{15}$	492639	NA	NA	NA
Sinapoylhexoside	385.07	Phenolic glycoside	$C_{17}H_{22}O_{10}$	961559	NA	NA	NA
1-O-Sinapoyl-β-D-glucose	386.46	Phenolic glycoside	$C_{17}H_{22}O_{10}$	475396	NA	NA	NA
Hydroxyferuloyl-hexoside	371.07	Phenolic glycoside	$C_{17}H_{22}O_{11}$	888584	NA	NA	NA
Naringenin 7-O-glucoside	432.92	Flavanone glycoside	$C_{21}H_{22}O_{10}$	83573	NA	NA	NA
Sinapoylhydroxyferuloyl-dihexoside	431.00	Phenolic glycoside	$C_{34}H_{40}O_{20}$	1296170	NA	NA	NA
Vitexin	431.90	Flavone glycoside	$C_{21}H_{20}O_{10}$	77803	NA	NA	NA
3-Methoxynobiletin	449.17	Flavonoid	$C_{21}H_{22}O_8$	100413	NA	NA	NA
Dihydrokaempferol-hexoside	450.00	Flavonol glycoside	$C_{21}H_{22}O_{11}$	141254	NA	NA	NA
Myricetin 3-O-arabinoside	287.11	Flavonol glycoside	$C_{20}H_{18}O_{12}$	62228	NA	NA	NA
Dihydrokaempferol	100.92	Flavonol	$C_{15}H_{12}O_6$	53518	NA	NA	NA
Isovaleric acid	368.96	Fatty acid	$C_5H_{10}O_2$	344134	NA	NA	NA
Ferulic acid 4-O-glucuronide	341.09	Phenolic glucuronide	$C_{16}H_{18}O_{10}$	208431	NA	NA	NA
Kaempferol 3,7,4'-O-triglucoside	771.50	Flavonol glycoside	$C_{33}H_{40}O_{21}$	79506	NA	NA	NA

droxyphenylpropenes [6]-Gingerol	299.06	Phenolic compound	$C_{17}H_{26}O_4$	216814	NA	NA	NA

Abbreviation: S2 = Control (no salinity stress); S4–S8 = Increasing levels of salinity stress; MF = Molecular Formula; M/Z = Mass-to-Charge Ratio; AU = Arbitrary Units; NA = Not Available (compound not detected or data not acquired).

Peak area values are presented as relative abundance (arbitrary units, AU) for comparative semi-quantitative metabolite profiling among treatments. Also, LC-MS metabolite data are presented as relative peak areas (AU) obtained from exploratory semi-quantitative profiling and were used for comparative interpretation among treatments.

Table 3. GC-MS analysis of volatile and semi-volatile compounds in *M. incana* under different salinity stress levels

Metabolic Class	Key Compounds	Control (S2)	Low (S4)	Moderate (S6)	High (S8)
Terpenoids	Bicyclo[3.1.1]heptane	0.31	ND	0.35	0.32
	Eugenol	0.92	ND	ND	ND
Fatty Acids	n-Hexadecanoic acid	4.17	4.61	4.5	4.18
	9,12,15-Octadecatrienoic acid	9.8	9.91	9.07	9.89
	Pentadecanoic acid	0.91	ND	ND	ND
Phenolics	4-Vinylphenol	2.86	2.27	2.53	2.64
	trans-p-Coumaric acid	0.3	ND	ND	ND
Nitrogen Compounds	Hydrazine derivatives	0.56	ND	0.87	0.50
Sterols	γ-Sitosterol	5.57	5.46	5.54	5.59
	Ergost-5-en-3-ol	0.99	ND	1.03	1.03

ND = Not Detected (below detection threshold)

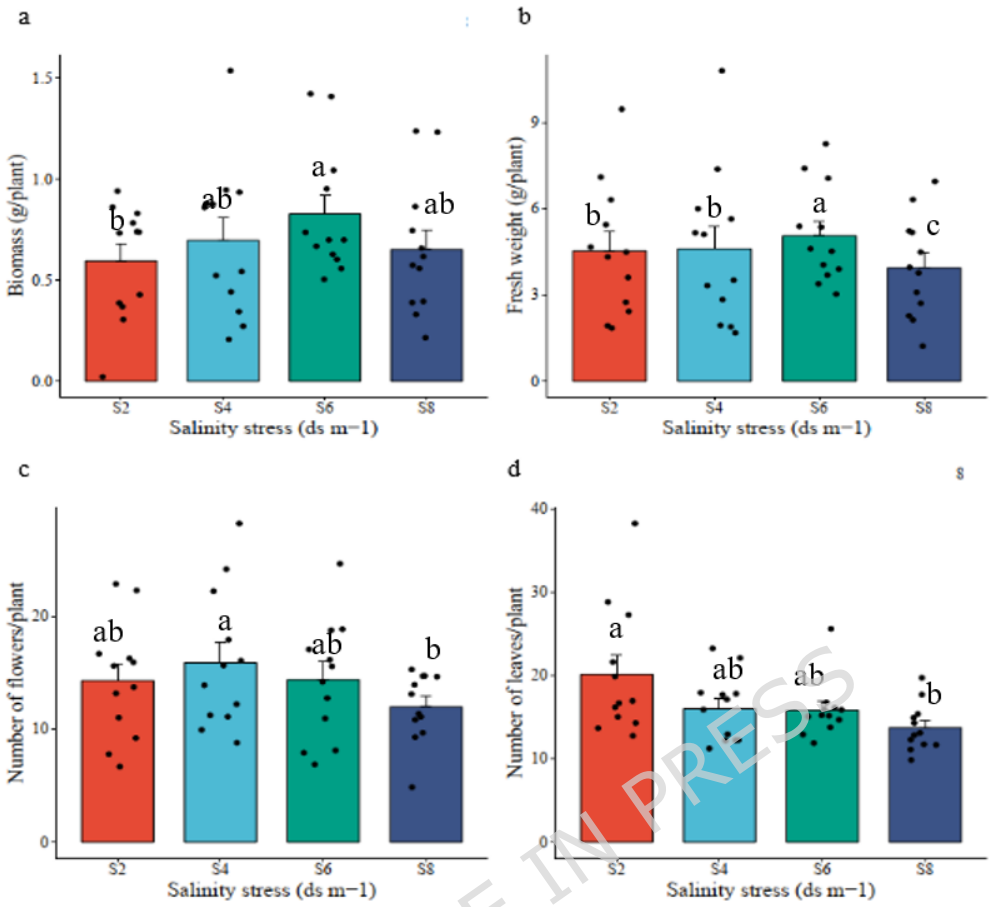


Fig. 1. Effect of salinity stress (2 (tap water), 4, 6 and 8 dS m^{-1}) levels on vegetative traits and flowers number of *Matthiola incana:* **(a)** biomass, **(b)** fresh weight, **(c)** number of flowers/plant and, **(d)** number of leave/plant. Data represent mean ± SE (n = 3). Different letters indicate significant differences among treatments based on the Tukey's HSD test ($p \leq 0.05$).

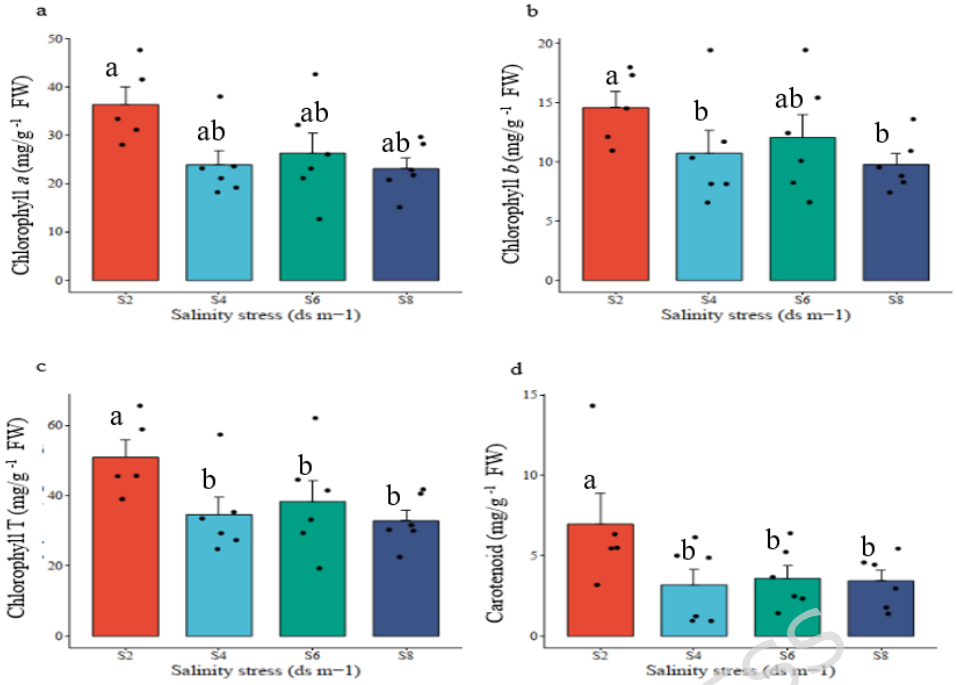


Fig. 2. Effect of salinity stress (2 (tap water), 4, 6 and 8 dS m^{-1}) levels on photopigments content in leaves of *Matthiola incana:* **(a)** chlorophyll *a*, **(b)** chlorophyll *b*, **(c)** chlorophyll T and, **(d)** carotenoids. Data represent mean ± SE (n = 3). Different letters indicate significant differences among treatments based on the Tukey's HSD test ($p \leq 0.05$).

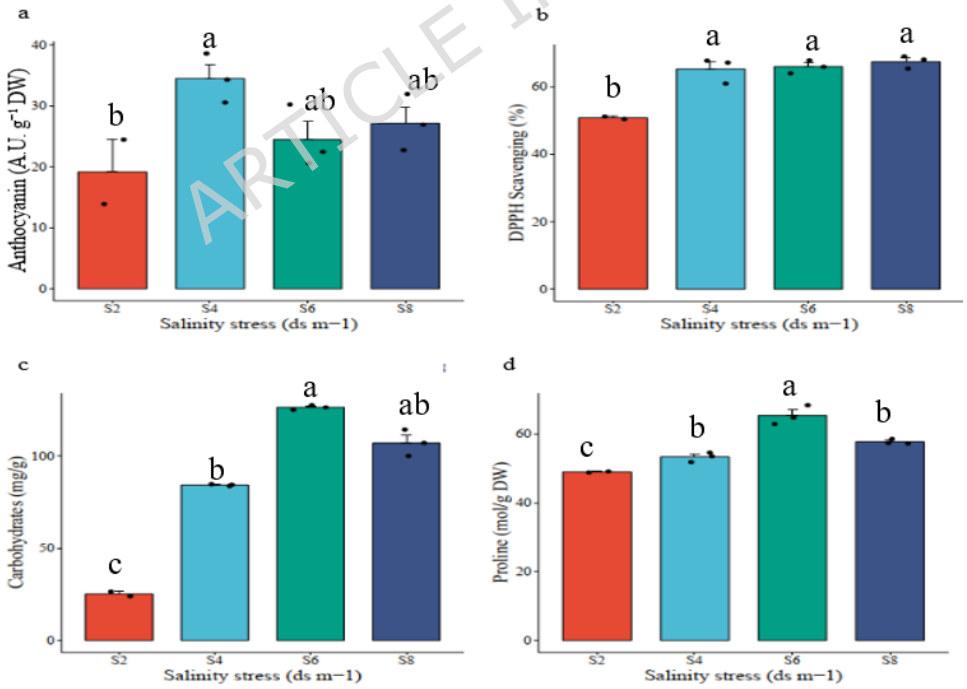


Fig. 3. Effect of salinity stress (2 (tap water), 4, 6 and 8 dS m^{-1}) levels on flower and leaf biochemical traits in *Matthiola incana:* **(a)** anthocyanin content, **(b)** DPPH scavenging, **(c)** carbohydrates and, **(d)** proline. Data represent mean ± SE (n = 3). Different letters indicate significant differences among treatments based on the Tukey's HSD test ($p \leq 0.05$).

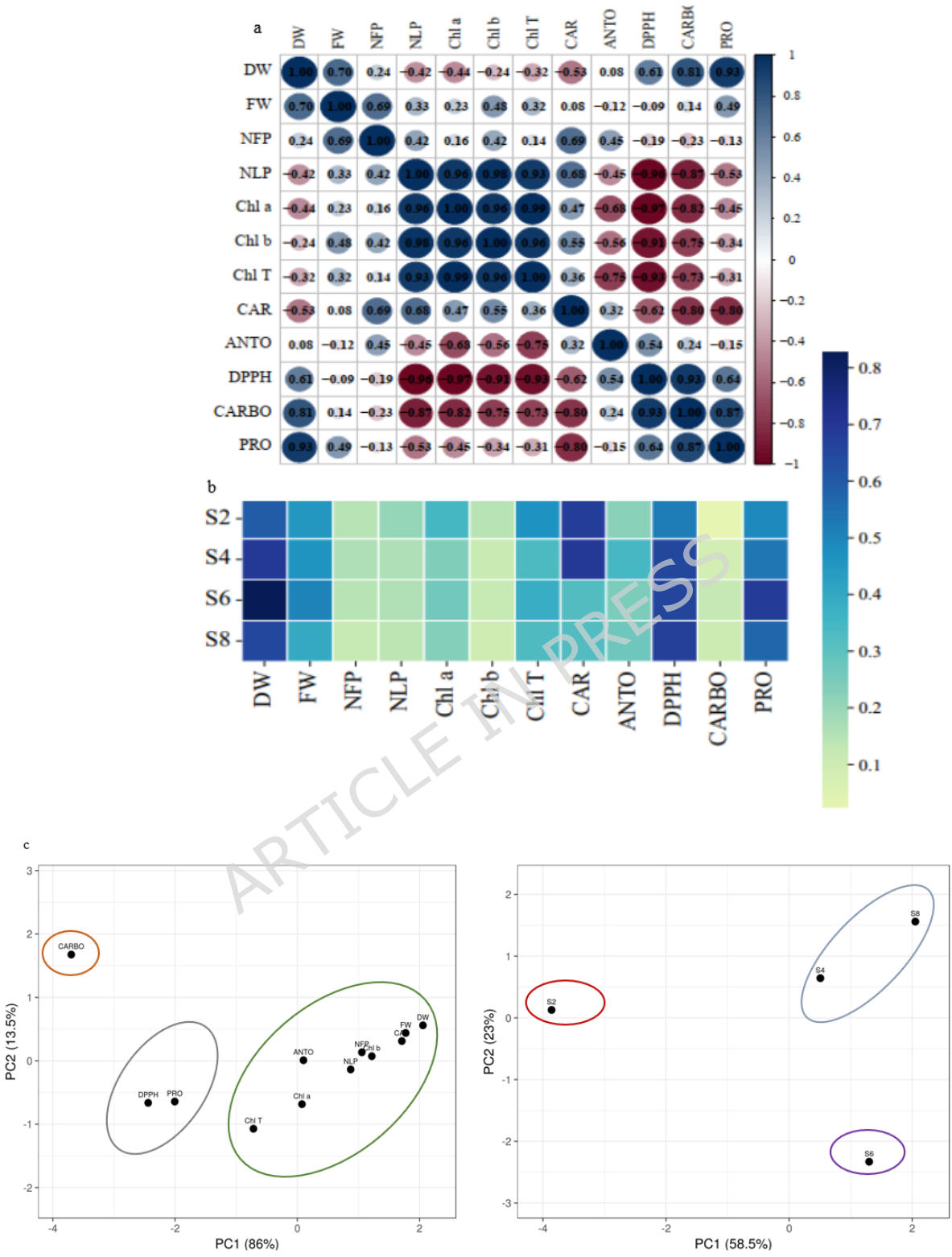
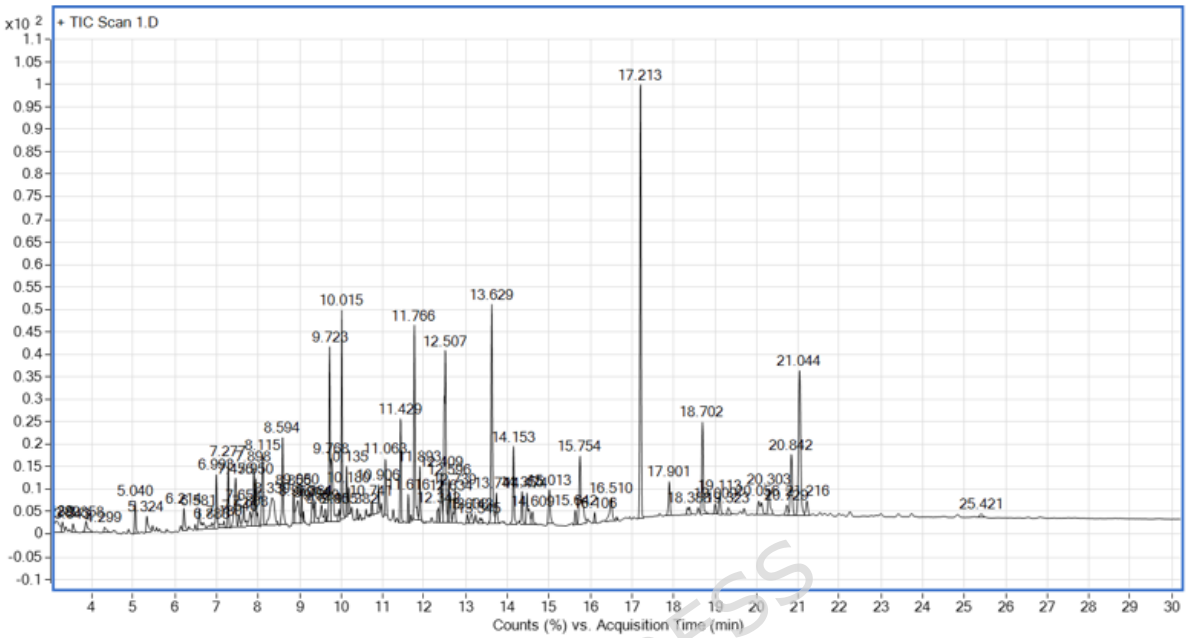


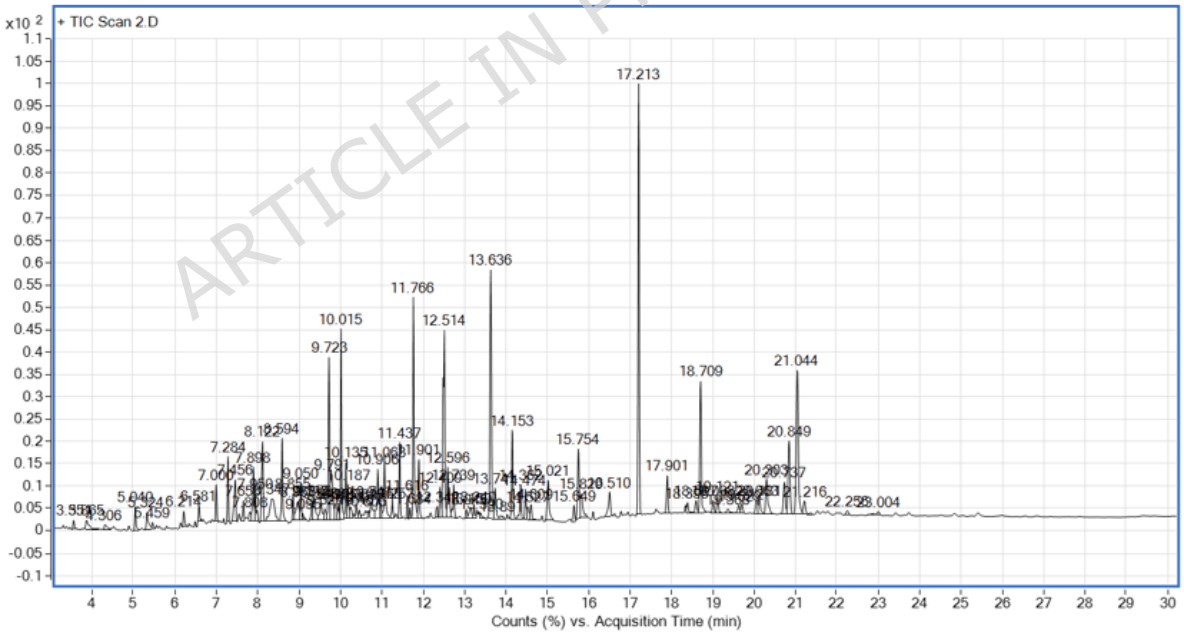
Fig. 4. (a,b) Hierarchical clustering analysis (HCA) of Pearson correlation coefficient (r) values between treatments and morphophysiological traits. **(c)** Principal component analysis (PCA). The tested variables included: CARBO: Carbohydrate; DPPH: Antioxidant activity; Chl a: Chlorophyll a; Ch b: Chlorophyll b; Chl T: Chlorophyll total; ANTO: anthocyanin; NLP:

Number of leaves/plants; NFP: Number of flowers/plants; CAR: Carotenoids; FW; Fresh weight; DW: Biomass. Irrigation solutions of 4, 6, and 8 dS m^{-1} (S4, S6, and S8)

a



b



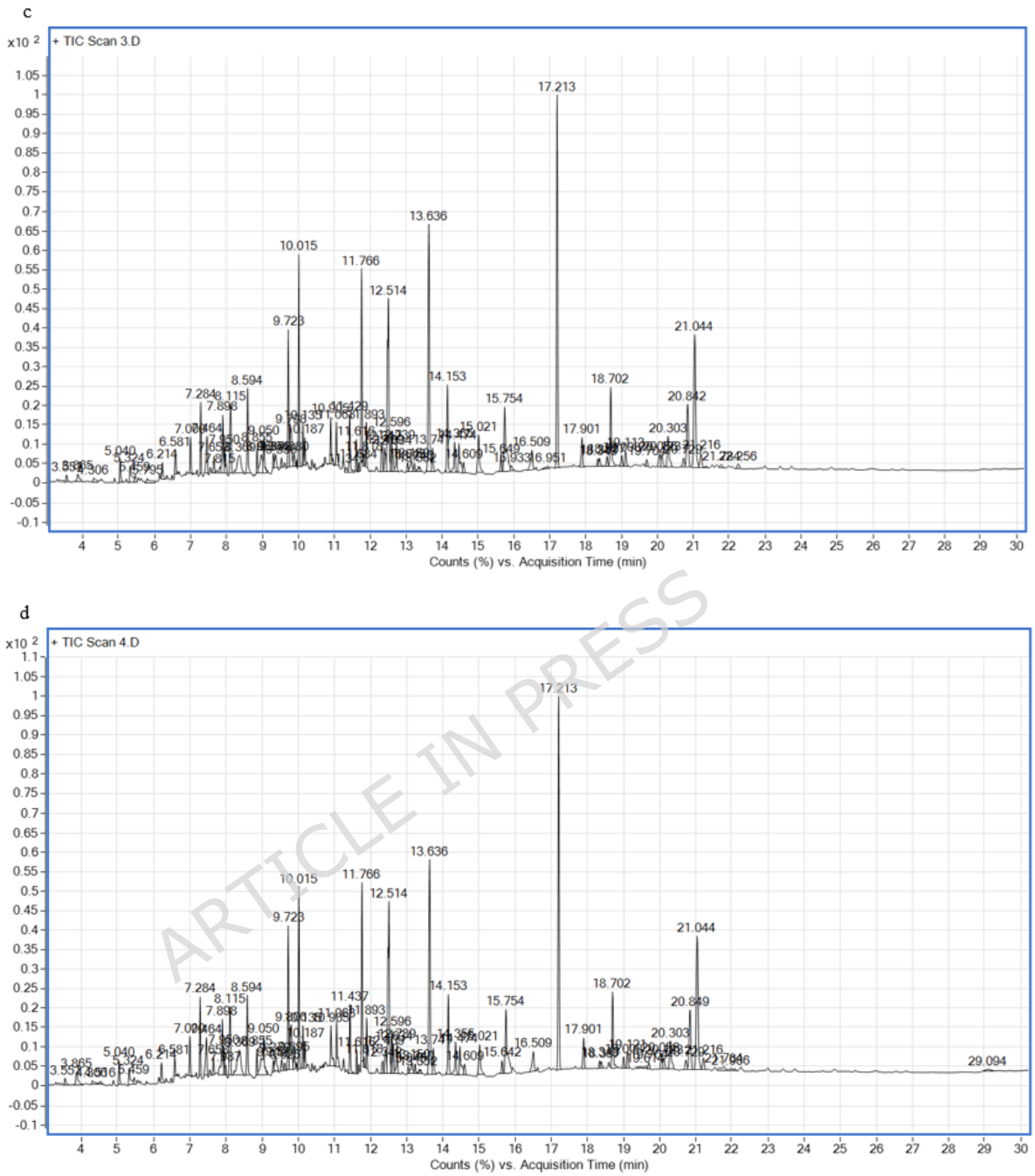
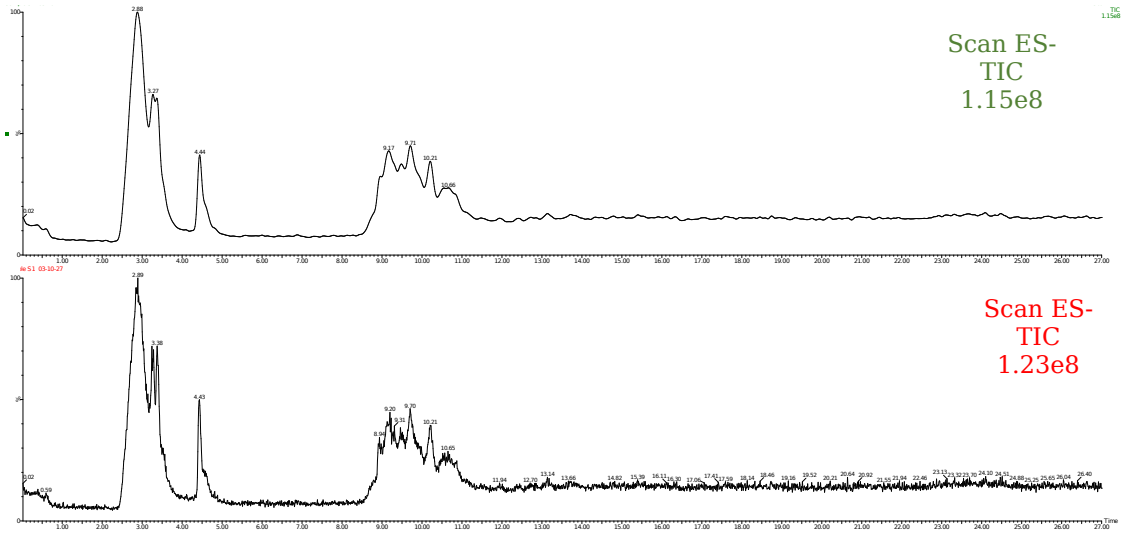
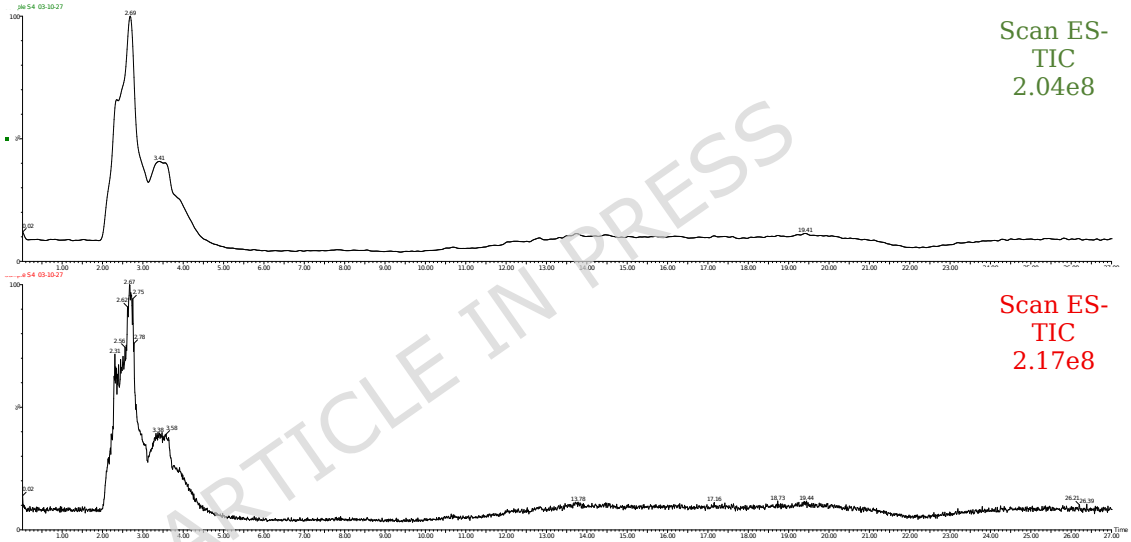


Fig. 5. GC-MS chromograph of volatile and semi-volatile compounds in *M. incana* under **(a)** (2 (tap water), **(b)** 4 dS m^{-1}, **(c)** 6 dS m^{-1}, **(d)** 8 dS m^{-1} levels

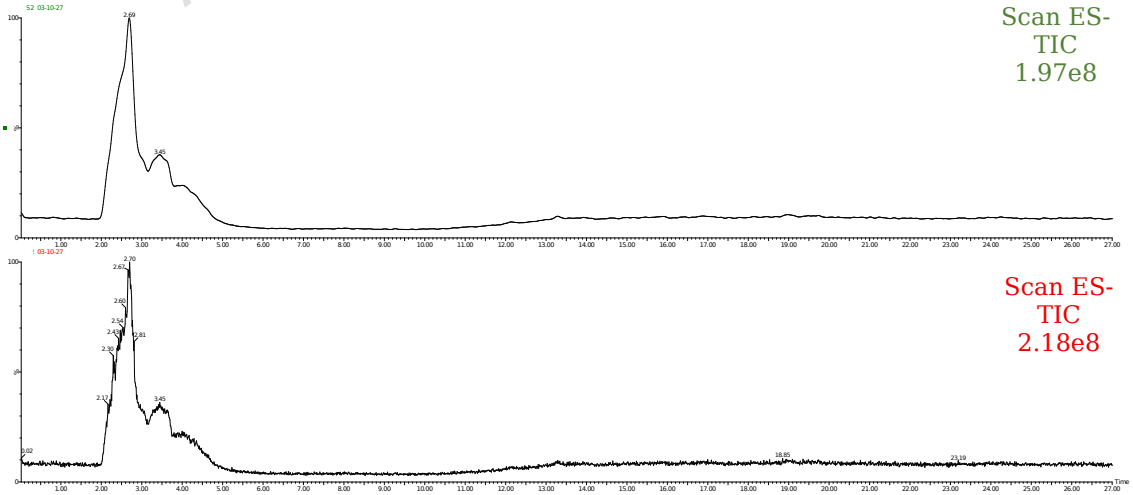
a)



b)



c)



d)

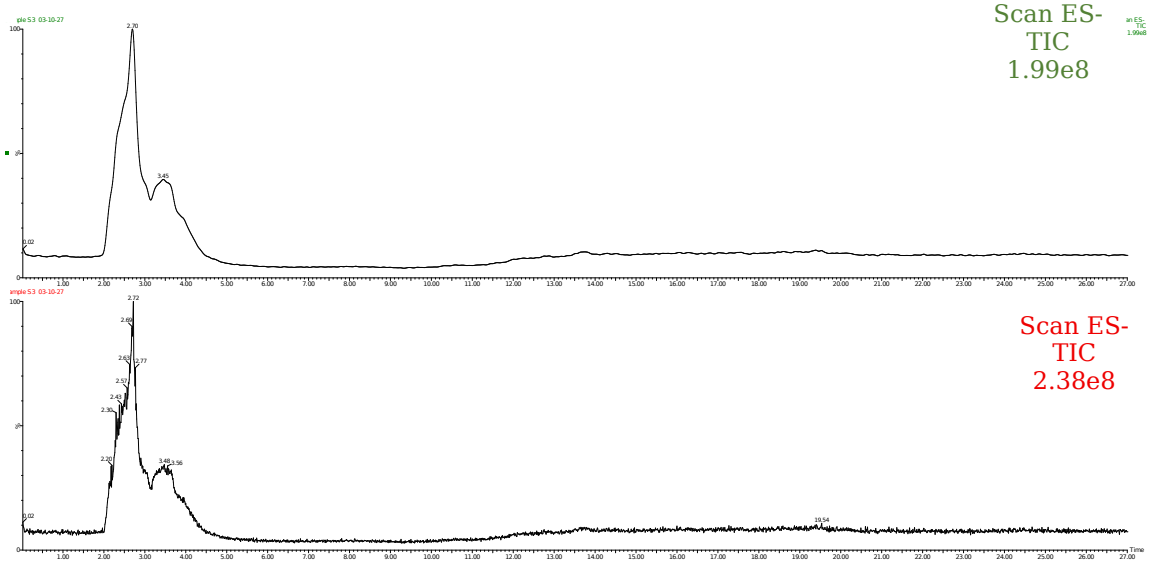


Fig. 6. Total Ion Chromatograms (TIC) of major metabolites identified in *Matthiola incana* under four salinity stress levels: S2 (a), S4 (b), S6 (c), S8 (d) obtained by LC-MS in negative electrospray ionization (ESI-) mode, with retention times (min) shown above major peaks.