Journal of Agriculture and Food Research 19 (2025) 101649

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Metabolomics of rapeseed (*Brassica napus* var. *oleifera* Del.) sprouts obtained with or without salinity from progeny seeds of mother plants grown in presence or absence of salinity

F. Sevi [a,1], B. Falcinelli [b,1], S. Frusciante [a], E. Fabene [a], G. Tosti [b], R. D'Amato [b], G. Diretto [a,**], P. Benincasa [b,*]

[a] Biotechnology and Agro-Industry Division, Biotechnology Laboratory, ENEA Casaccia Research Center, Via Anguillarese 301, S.M. di Galeria, 00123, (Rome), Italy
[b] Department of Agricultural, Food and E nvironmental Sciences, University of Perugia, Borgo XX Giugno, 74, 06124, Perugia, Italy

ARTICLE INFO

Keywords:
Germination
Salt
Intergeneration
Offspring
Epigenetics
Phytochemical

ABSTRACT

This work aimed at evaluating changes in the whole non volatile metabolome of rapeseed sprouts caused by imposing salt stress during the growth of mother plants where offspring seeds used for sprouting where taken from, or during the sprouting of offspring seeds, or during both phases. To this purpose, seeds harvested from mother plants grown with or without salinity (100 and 0 mM NaCl, respectively) were sprouted with or without salinity (50 and 0 mM NaCl) to obtain four treatments: 100-0, aimed at evaluating the intergenerational effect of stress applied to mother plants (i.e., stress memory); 0–50, aimed at evaluating the consequences of stress applied during sprouting (i.e., current stress); 100-50, aimed at evaluating the effect of combined salt stress (stress memory + current stress); 0-0, as a never stressed control. Overall, either the stress memory (100-0) or the current stress (0–50) boosted the concentrations of several phenolic compounds, fatty acids, cis-9-monoolein, while 100-0 also showed increased contents of spermidine, riboflavin, and chlorophyll *a*. Combining the two stress occurrences (i.e.,100-50) further triggered the over-accumulation of amino acids, organic acids, vitamins, carotenoids, chlorophylls and phylloquinone, as compared to 100-0 and 0–50 but, conversely, more phenolic compounds resulted down-regulated.

1. Introduction

In rapeseed (*Brassica napus* var *oleifera* Del.), salinity may strongly affect germination and seedling growth [1], as well as the phenolic content and antioxidant activity of sprouts, acting as an elicitor [2]. On the other hand, a body of literature hypothesized and often demonstrated that parental plants grown under salinity and/or water deficiency may generate progeny somehow primed to face the potential occurrence of salt stress and other kind of stresses, starting from the seed and seedling stage [3–6], probably due to epigenetic effects [7,8]. The stress tolerance of the progeny stands independent of the actual occurrence of the stress [9], and might involve the increased presence of protective compounds, as hypothesized by Galieni et al. [10]. In agreement with this hypothesis, Benincasa et al. [11] found that rapeseed sprouts obtained with distilled water from offspring seeds of plants grown under salinity showed greater phenolic content and antioxidant activity compared to sprouts from offspring seeds of non-stressed plants. Similar evidences were also obtained by Falcinelli et al. [12] for sprouts of wheat from offspring seeds of crop subjected to late nitrogen deficiency, confirming that, whatever the kind of stress occurring to mother plants, the progeny is somehow primed to face it by means of an increased phytochemical content. These Authors highlighted the potential relevance of their finding, since it could pave the way to new perspectives for obtaining high quality sprouts from plants grown under stressing conditions, imposed on purpose or naturally occurring, in view of the potential benefits given by antioxidant compounds on human health. To our knowledge, no study has investigated the effect of salinity applied both during parental plants growth and during sprouting of offspring seeds, and it might be supposed that these combined stresses might further boost the contents of phytochemicals and antioxidants as

* Corresponding author.
** Corresponding author.
E-mail addresses: gianfranco.diretto@enea.it (G. Diretto), paolo.benincasa@unipg.it (P. Benincasa).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jafr.2025.101649
Received 16 May 2024; Received in revised form 2 January 2025; Accepted 10 January 2025
Available online 11 January 2025
2666-1543/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

compared to the salinity applied to only parental plants or only sprouting; however, this hypothesis deserves to be investigated with an appropriate research carried out on purpose for its experimental validation. Moreover, another aspect of interest in the experiments of Falcinelli et al. [2] and Benincasa et al. [11] is that the higher antioxidant activity appeared not to be accounted for by only the increase in phenolic compounds. In this context, additional metabolites could come into play, which can be revealed only with a metabolomic ("global") approach rather than by the quantification of a limited number of compounds with antioxidant properties. Indeed, several molecules produced in the frame of plant cell metabolism are able to exert antioxidant activities: this group includes either primary and ubiquitous metabolites as amino acids (particularly tryptophan, methionine, histidine, lysine, cysteine, arginine and tyrosine, called antioxidative amino acids [13], organic acids [14] and water-soluble vitamins [15]; as well as secondary compounds, like different classes of isoprenoids (carotenoids, chlorophylls, tocochromanols, quinones, sterols [16]. In this context, previous studies have evidenced the high plasticity of plant cell facing different stressors, with a series of responses also including a strong increase in several antioxidants and, more in general, with a deep remodelling of cell metabolism [17]. In particular, salt-based stresses trigger serious oxidative condition with a constant increase of free oxygen radicals (as hydroxyl radicals, (OH•), singlet oxygen ($^1O_2$), superoxide radical, (O_2•-) (SOR), hydrogen peroxide (H_2O_2) and alkoxyl radical (RO)), which is counterbalanced by plants with a simultaneous accumulation of the antioxidant metabolic pool [18,19]. These findings offer great and innovative opportunities for human nutrition: in fact, all the compounds previously described, if improved in terms of their accumulation in edible sprouts, might lead to the generation of novel market products with beneficial traits for human health and, thus, for the consumers.

For these reasons and to fill the aforementioned gap, this work was aimed at analyzing the complete metabolome of sprouts obtained from off-spring seeds of plants grown in the absence or presence of salinity and germinated with or without salinity.

2. Materials and Methods

2.1. Growth of parental plants and seed production

Plants of the rapeseed cultivar Exagone (Monsanto), an F1 hybrid which was found to be highly tolerant to salinity [1], were grown from the seedling stage to final seed harvest in 20 L pots at a density of 3 plants per pot, to obtain the offspring seeds to be used for sprouting. Pots were periodically flushed with distilled water or NaCl solution 100 mM. Flushing was necessary in the salted treatment to avoid progressive salt concentration. A total of 4 pots per treatment were laid down outdoor, under full sunlight, in a completely randomized design. At flowering, cross-pollination among salt treatments was prevented by isolating treatments from each other with a bee-proof net. The pot substrate and the nutrient solution used for growing plants were the same used in Benincasa et al. [11] and detailed therein. The growth cycle of mother plants lasted around eight months, from mid October to mid June. Outdoor air temperature was not measured but it was in line with the normal trend of the location hosting the experiment, with cold winters (and several frosty mornings) and warm late springs. Rainfall could not affect salinity treatments since rainfall water infiltration was prevented by covering pot top surfaces.

2.2. Sprouting of offspring seeds

The offspring seeds obtained from the salted- and unsalted-grown mother plants (0 and 100, respectively) were then used for sprouting. Seeds from mother plants of treatments 0 and 100 were incubated on plastic trays with distilled water (0) or NaCl solution 50 mM (50), which was found by Falcinelli et al. [2] to be the highest NaCl concentration suitable for sprouting purposes in rapeseed (in fact, 100 mM NaCl had been found to severely depress sprout growth). Thus the four following treatments were obtained: 1) offspring seeds from mother plants grown without salinity (except for salts contained in the nutrient solution) and sprouted with distilled water (0-0); 2) offspring seeds from mother plants grown under salinity and sprouted with distilled water (100-0); 3) offspring seeds from mother plants grown without salinity (except for salts contained in the nutrient solution) and sprouted under salinity (0–50); 4) offspring seeds from mother plants grown under salinity and sprouted under salinity (100-50). Fig. 1 reports photos of one tray per treatment taken just before sprout harvest together with the description of treatments. Treatments were laid down according to a completely randomized block design with four replicates (trays). Each tray contained 2 g of seeds, corresponding to over 500 seeds. Sprouting was carried out as described in Falcinelli et al. [2] Briefly, in each tray, seeds were positioned on filter paper laid over cotton wetted with the solution (0 or 50 mMol) contained in the trays, to guarantee constant water availability while preventing anoxia. Distilled water was periodically added to trays to restore initial tray weight, assuming that weight change was mainly due to water evaporation. The trays were kept in a growth chamber at 18 °C in the dark. After germination, the trays were placed at a light/dark regime of 16/8 h with light intensity of 200 μmol photons m^{-2} s^{-1}. Air RH within the growth chamber ranged between 50 % and 80 % depending on the light regime, lower values when the light was switched on since they caused a moderate heating.

Rapeseed sprouts were collected after the complete development of cotyledons, occurred 7 days after sowing (DAS) in all treatments. The mean fresh and dry weights of an individual sprout were determined on 10 individuals per replicate. The remaining sprout biomass of each replicate of each treatment was weighed. Then replicates of each treatment were re-grouped two by two and stored at −80 °C. Prior to metabolomic analyses, samples were freeze-dried and ground to powder.

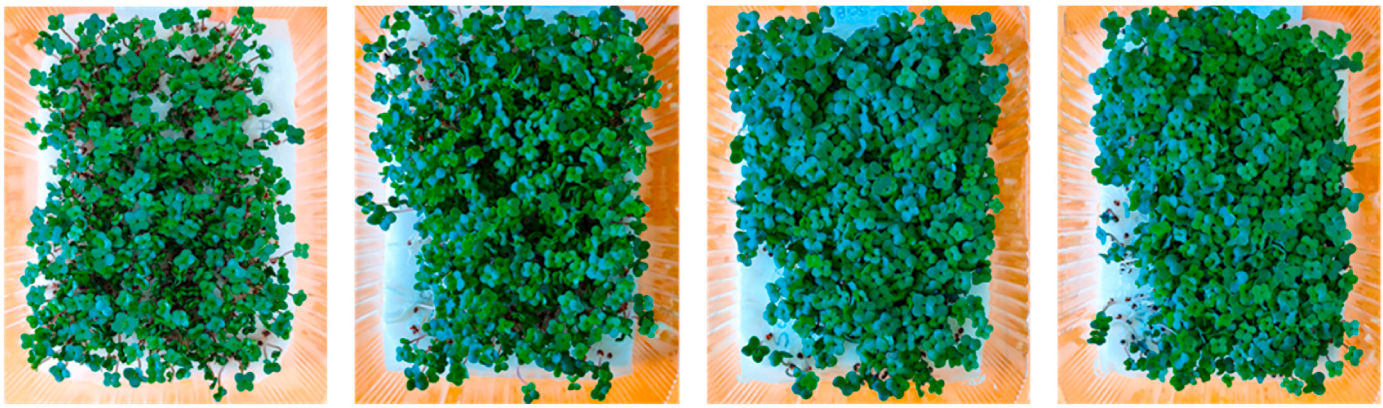
2.3. Germination test

A parallel germination test was carried out on three replicates of 100 seeds per each of the four treatments (0-0, 100-0, 0–50, 100-50). Germinated seeds were counted every 12 h for the first 2 days and then every day for other 5 days to determine total percent germination (G) and mean germination time (MGT) of germinated seeds.

2.4. Metabolomics analyses

LC-ESI-HRMS analyses of rapeseed semi-polar metabolome was performed as described in Diretto et al. [20] Semi-polar metabolites were extracted from 10 mg lyophilized, homogeneously ground sprout tissue with with 0.75 mL of cold 75 % (v/v) methanol, 0.1 % (v/v) formic acid, spiked with 10 μg/ml formononetin as internal standard. After shaking for 40' at 20Hz using a Mixer Mill 300 (Qiagen), samples were centrifuged for 15min at 20,000 g at 4 °C. Then, 0.6 mL of supernatant were removed and transferred to HPLC tubes. For each treatment, 2 independent biological replicates, consisting of 5 g of fresh sprouts each, were analyzed. For each biological replicate, at least 2 technical replicates were carried out. Liquid chromatography (LC) was carried out using a Phenomenex C18 Luna column (100 × 2.1 mm, 2.5 μm) and the mobile phase was composed by water-0.1 % formic acid (A) and acetonitrile-0.1 % formic acid (B). The gradient was: 95%A:5%B (1min), a linear gradient to 25%A:75%B over 40min, 2min isocratic, before going back to the initial LC conditions in 18min.

LC-APCI-HRMS analysis of the rapeseed non-polar metabolome was performed as previously described [21]. Non-polar metabolites were extracted from 5 mg of lyophilized, homogeneously ground sprout tissue was extracted using 1 mL of 25 % (v/v) methanol, 50 % (v/v) chloroform, 25 % (v/v) 50 mm-Tris-HCl, spiked with 50 μg/ml DL-α-tocopherol acetate as internal standard. After centrifugation, the



Treatment 0-0

Sprouts obtained from offspring seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted with distilled water (0 mM NaCl).
These sprouts allow to evaluate the **effect of never salt stress**

Treatment 100-0

Sprouts obtained from offspring seeds harvested in mother plants grown under salinity (100 mM NaCl) and sprouted with distilled water.
These sprouts allow to evaluate the **effect of salt stress memory** (i.e., the intergenerational effect of stress applied to mother plants)

Treatment 0-50

Sprouts obtained from offspring seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted under salinity (50 mM NaCl).
These sprouts allow to evaluate the **effect of current salt stress** (i.e., the stress applied directly during sprouting)

Treatment 100-50

Sprouts obtained from offspring seeds harvested in mother plants grown under salinity (100 mM NaCl) and sprouted under salinity (50 mM NaCl).
These sprouts allow to evaluate the **effect of combined salt stress (stress memory + current stress)**

Fig. 1. Photos and description of sprouts of the four experimental treatments.

organic hypophase was kept and the aqueous phase re-extracted with the same volume previously used of chloroform spiked with the internal standard. The pooled organic extracts were dried with a Speed Vac concentrator, and the residue was resuspended in ethyl acetate (100 μL). For each experimental group, 2 independent biological replicates, consisting of 5 g of fresh sprouts each, were analyzed; for each biological replicate, at least two technical replicates were carried out. LC separations were performed using a C30 reverse-phase column (100 × 3.0 mm; 3≤m, YMC Europe). The solvent systems were methanol (A); 75 % methanol (v/v), 25 % water (v/v) and 0.2 % ammonium acetate (B); tert-butyl-methyl ether (C). The gradient elution was as follows: 0–6 min 95 % A, 5 % B, and 0 % C; 1 min 80 % A, 5 % B, and 15 % C; 5 min 80 % A, 5 % B, and 15 % C; 20 min 30 % A, 5 % B, and 65 % C; 22 min 30 % A, 5 % B, and 65%C; 18 min 95 % A, 5 % B, and 0 % C.

5 μL of each sample were injected and a flow of 0.25 and 0.8 mL was used throughout the LC semi-polar and non polar runs, respectively. Mass spectrometry analysis was performed using a quadrupole-Orbitrap Q-exactive system (ThermoFisher scientific, USA), operating in positive/negative heated electrospray ionization (HESI) or atmospheric pressure chemical ionization (APCI) coupled to an Ultimate HPLC-DAD system (Thermo Fisher Scientific, Waltham, MA). For semi-polar metabolite analysis, mass spectrometer parameters were as follows: capillary and vaporizer temperatures 30 °C and 270 °C, respectively, discharge current 4.0 KV, probe heater temperature at 370 °C, S-lens RF level at 50 V. The acquisition was carried out in the 110/1600 *m/z* scan range, with the following parameters: resolution 70,000, microscan 1, AGC target 1e6, and maximum injection time 50. Full scan MS with data-dependent MS/MS fragmentation was used for metabolite identification. For non polar analysis, APCI parameters were as follows: nitrogen was used as sheath and auxiliary gas, set to 20 and 10 units, respectively. The vaporizer temperature was 300 °C, the capillary temperature was 250 °C, the discharge current was set to 5.5 μA, and S-lens RF level was set at 50. All solvents used were LC-MS grade quality (CHROMASOLV® from Sigma-Aldrich). Metabolites were quantified in a relative way by normalization on the internal standard (formononetin and DL-α-tocopherol acetate) amounts.

Targeted metabolite identification was performed by comparing chromatographic and spectral properties with authentic standards (if available) and reference spectra, in house database, literature data of rapeseed metabolites [22,23] and on the basis of the *m/z* accurate masses, as reported in the Pubchem database [24] for monoisotopic mass identification, or on the Metabolomics Fiehn Lab Mass Spectrometry Adduct Calculator [25] in the case of adduction detection.

2.5. Statistical analysis

Germination data were analyzed by one-way ANOVA according to a completely randomized design with three replicates, while growth data were analyzed by one-way ANOVA according to a randomized block design with four replicates. Means were compared by Fisher's Least Significant Difference (LSD) at p-value <0.05. For metabolomic data, pairwise Student t-test analysis was carried out for all the comparisons under study, at p-value <0.05. Finally, principal component analyses (PCAs), heatmaps and hierarchical clustering (HCL) were performed by using normalized data on the 0-0 treatment point.

3. Results

3.1. Seed yield from mother plants, seed germination and sprout growth

The seed yield from mother plants grown at 0 and 100 mM NaCl was 3.65 ± 0.12 and 2.59 ± 0.9 g per plant, respectively (data not shown). The different yield was mainly due to differences in seed number while mean individual seed weight (with a standard 10 % water content) was

not affected by salinity (3.77 ± 0.06 mg, on average over the two NaCl treatments).

Salinity treatments did not affect percent germination (G), which was close to 100 % in all treatments, while mean germination time (MGT) was slightly but significantly shorter for seeds harvested in plants grown under salinity, independent of the presence or absence of salinity in the germination substrate (i.e., treatments 100-0 and 100-50) (Table 1). Differences in sprout growth parameters were significant (Table 1), with individual fresh weight being higher in sprouts obtained in salted substrate (i.e., 0–50 and 100-50) and individual dry weight being higher in all salinity treatments (i.e., 100-0, 0–50 and 100-50) as compared to the unsalted control 0-0.

3.2. Global metabolic profiling

To infer the effect of the different salinity treatments, analyses of global metabolic profiling of either polar and non polar metabolomes were performed using a LC-HRMS platform and an ad hoc rapeseed targeted metabolomics database including, 109 (polar) and 60 (non polar) primary and secondary compounds, respectively (for more details, see Materials and Methods). Data were preliminarly expressed as fold with respect to the internal standard levels (Fold IS). First of all, heatmap visualization was used to identify the ions with the highest intensities in our experimental conditions (Fig. 2A–B): of interest, a large group of phenylpropanoids, glutamine, citric and pyruvic acids, and trehalose or isomers resulted the most abundant compounds in the polar metabolome (Fig. 2A), whereas α-tocopherol and α -tocopherol quinone, together with β-sitosterol, linoleic and linolenic acids were the non polar metabolites characterized by the highest Fold IS (Fig. 2B). Subsequently, we applied hierarchical clustering (HCL) to evaluate how the different treatments clustered each others and grouped basing on their similarity profiles: in both metabolomics fractions, 0-0 and 100-0 treatments clustered together; on the contrary, and notably, 0–50 and 100-50 points constituted a separate group in the case of non polar compounds (Fig. 2B), while the 100-50 treatment placed on the right side, resulting unclustered with any of the other conditions (Fig. 2A).

In addition, we also used principal component analyses (PCAs), applied either to treatments or metabolites (Fig. S1): whereas it was not possible to discriminate the formers, both at polar and non polar metabolome levels (Figs. S1A and S1B, respectively), PCAs according to the metabolites allowed for identifying total PPs (phenylpropanoids), Miquelianin, 6-Hydroxymethylherniarin, 1-O-Feruloyl quinic acid and sinapic acid (Fig. S1C), and total fatty acids, linolenic and linoleic acids, β -sitosterol and total sterols (Fig. S1D) as the polar and non polar compounds, respectively, mostly responsible for the total variance among the different treatments under study.

Subsequently, to better highlight salt-derived metabolic changes in salinity treatments, all data were subjected to normalization using alternative references. More in detail, the following pairwise comparisons were considered: i) 100-0 vs 0-0; ii) 0–50 vs 0-0; iii) 100-50 vs 0-0; iv) 100-50 vs 100-0; v) 100-50 vs 0–50. Then, all data were expressed, for each metabolite, as log2 adjusted fold with respect to the corresponding treatment, with blue and red shades colors to evidence, respectively, down- and up-accumulated polar and non polar compounds (Fig. 3A and B, respectively; Tables S2A and S2B, respectively). Specific alterations in polar and non polar metabolomes are depicted in sections 3.3 and 3.4, respectively.

3.3. Changes in polar metabolome

Amino acids were among the most affected compounds by salinity treatments. Indeed, this class, together with peptides and amines, were all reduced by salinity applied only during mother plants growth (100-0), except for spermidine, which increased by 7 times, and cysteine, which remained almost unaltered, as compared to the never salted control (0-0) (Fig. 3A and Tables S1A and S2A). On the contrary, the salinity applied only during sprouting (0–50 vs 0-0) caused slight variations, although statistically significant sometimes, with 2 down- and 7 over-accumulated metabolites. The combined application of salinity during both mother plant growth and sprouting (100-50 vs 0-0) caused a marked increase of several amino acids, especially arginine (9-fold), tryptophan (5-fold), phenylalanine (4-fold), tyrosine and leucine-isoleucine (about 3-fold). Likewise, but with a greater extent of variations, when compared to 100-0, 100-50 resulted in higher levels in most compounds, e.g. 83-fold for asparagine, 32-fold for glutamine, between 20- and 10-fold for tyrosine, treonine, aspartic acid, arginine, leucine-isoleucine, and tryptophan), and between 9- and 2-fold for many others, with the only decrease of spermidine (about 1/4). Similarly, in the comparison 100-50 vs 0–50, most of the metabolites increased, although with a less dramatic magnitude than in 100-50 vs 100-0 (e.g., 11-fold for arginine, from 4- to 2-fold for many others), and with the sole significant reduction of aspartic acid (about 1/2) (Fig. 3A and Tables S1A and S2A).

A similar effect of salinity treatments was observed for organic acids, most of which showed a marked decrease (from 0.5 to 0.05) in 100-0 vs 0-0 (except for benzoic acid) and slight variations in 0–50 vs 0-0, while 100-50 vs 0-0 displayed important increases for fumaric acid (almost 4-fold), pyruvic and citric acids (about 2-fold), and only the noticeable reduction of quinic acid (about ¼). When comparing 100-50 with 100-0, the extent of positive changes in several organic acids was more pronounced (30-fold for pyruvic acid, 15- to 10-fold for glutaric, fumaric and citric acids), with only quinic acid having a lower level (0.54). On the other hand, in the comparison 100-50 vs 0–50, 3 up- and 3 down-accumulated compounds were observed, with the greatest increase and decrease for fumaric acid (over 3-fold) and quinic acid (0.29), respectively (Fig. 3A and Tables S1A and S2A).

With respect to the aforementioned compounds, sugars were characterized by a different behaviour, with a general decrease in all salinity treatments with respect to the never salted control 0-0 (Fig. 3A and Tables S1A and S2A). Only 100-0 showed some significant increases (over 3-fold for sorbitol and around 2-fold for glucose and galactonate), but many others resulted dramatically reduced (even less than 1/10 of the content in 0-0), thus the overall content of total sugars was actually halved. The 0–50 displayed more limited variations except for the marked decrease of stechyose, raffinose and sorbitol. The treatment 100-50 showed a down-accumulation phenotype for most compounds, so

Table 1
Percent of seed germination (G), mean germination time (MGT), and individual fresh and dry weight of sprouts obtained for the four salinity treatments: 0-0 = never stressed control, i.e., sprouts obtained from offsprings seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted with distilled water (0 mM NaCl); 100-0 = sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted with distilled water (0 mM NaCl); 0–50 = sprouts obtained from offsprings seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted under salinity (50 mMol NaCl); 100-50 = sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted under salinity (50 mMol NaCl).

Treatments	G %	MGT (d)	Sprout fresh weight (mg)	Sprout dry weight (mg)
0–0	98	1.8	23	1.60
100–0	99	1.6	35	2.31
0–50	99	1.8	43	2.65
100–50	99	1.6	39	2.49
F test significance	-	**	*	*
LSD	-	*0.1*	*5.9*	*0.5*

** significant at $p \leq 0.01$; * significant at $p \leq 0.05$; *n.s.* not significant. The ANOVA was not performed for G since % data would have needed arcsin transformation, however differences were negligible and clearly not significant.

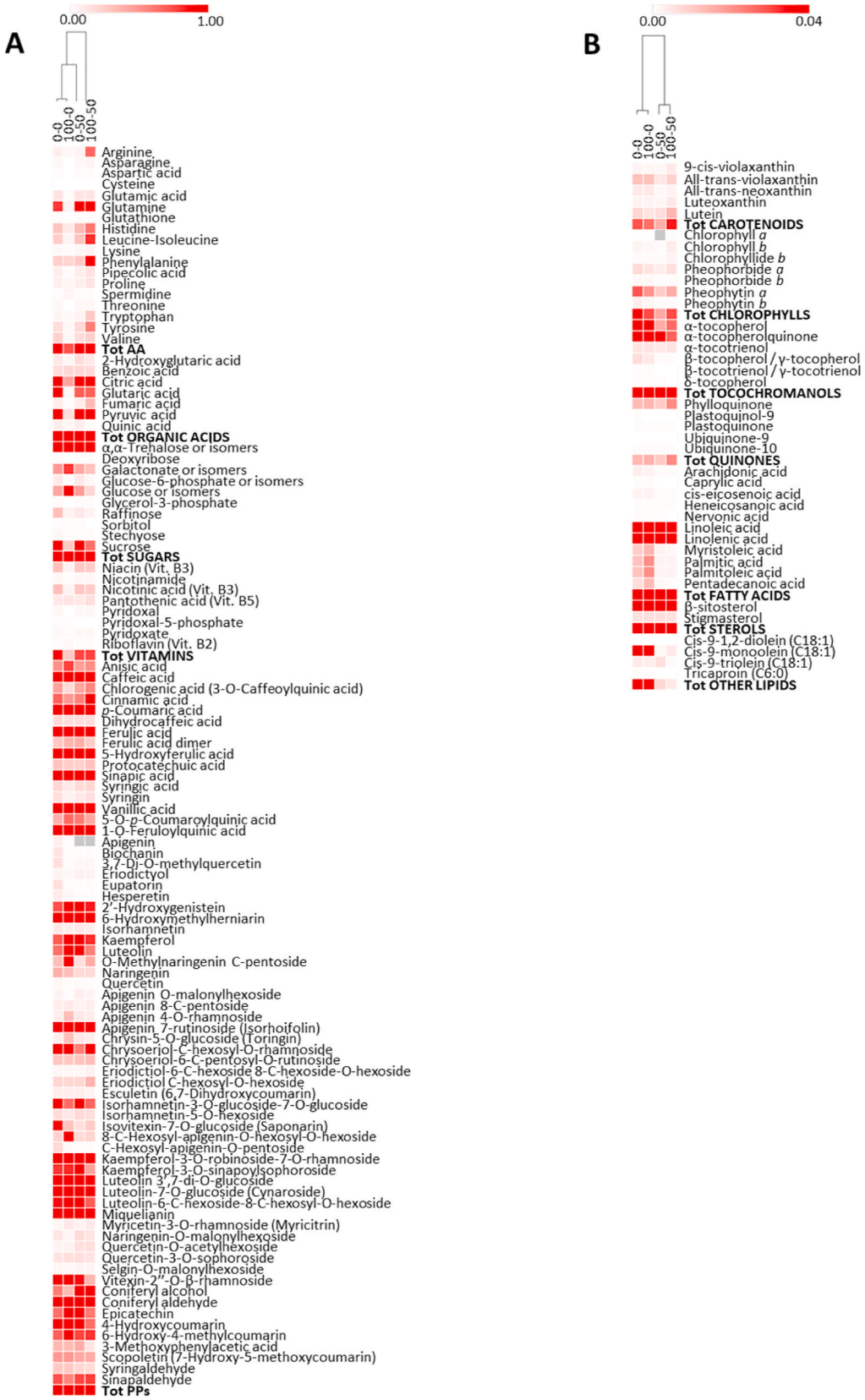


Fig. 2. Heatmap coupled to hierarchical clustering (HCL) of polar (A) and non polar (B) metabolomes in rapeseed sprouts of different salinity treatments, as compared to the concentration measured in the never salt stressed control (0-0), i.e., sprouts obtained from offsprings seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted with distilled water (0 mM NaCl). Salinity treatments: 100-0 = sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted with distilled water (0 mM NaCl); 0–50 = sprouts obtained from offsprings seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted under salinity (50 mMol NaCl); 100-50 = sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted under salinity (50 mMol NaCl). Data are expressed as fold with respect to the internal standard levels (Fold IS).



(caption on next page)

Fig. 3. Heatmap of polar (A) and non polar (B) metabolomes in rapeseed sprouts of different salinity treatments, as compared to the corresponding reference. More in detail, the following pairwise comparisons were considered: i) sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted with distilled water (0 mM NaCl) (100-0) versus sprouts of the never salt stressed control (0-0), i.e., sprouts from seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted with distilled water (0 mM NaCl); ii) sprouts obtained from offsprings seeds harvested in mother plants grown without salinity (0 mM NaCl) and sprouted under salinity (50 mMol NaCl) (0–50) vs sprouts of 0-0; iii) sprouts obtained from offsprings seeds harvested in mother plants grown under salinity (100 mMol NaCl) and sprouted under salinity (50 mMol NaCl) (100-50) versus sprouts of 0-0; iv) sprouts of 100-50 versus sprouts of 100-0; v) sprouts of 100-50 versus sprouts of 0–50. Data are expressed, for each metabolite, as log2 adjusted fold with respect to the corresponding treatment. Blue and red shades refer to, respectively, down- and up-accumulated compounds. Asterisks identify statistically significant alterations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that the overall content was 1/3 of the 0-0 one. Finally, when compared to 100-0, the 100-50 exhibited 4 significantly over-accumulated compounds (in particular, about 8-, 6- and 4-fold for, respectively, deoxyribose, glucose 6-phosphate and glycerol 3-phosphate), and 3 significantly down-represented compounds (to be noticed, sorbitol was 1/100 and glucose was 1/5 of that recorded in 100-0). On the other hand, with respect to 0–50, the 100-50 was characterized by a significant down-accumulation for all compounds, with less than half overall content.

Most vitamins dramatically decreased in 100-0, being 1/3 of the total content observed in 0-0, with the only exception of riboflavin (VitB12), which increased by 4 times (Fig. 3A and Tables S1A and S2A). Moderate reductions were observed in most cases for 0–50 and 100-50, except for pyridoxal-5-phosphate (around 1/3 in both treatments). Of interest, in 100-50 vs 0-0, both pantothenic acid (VitB5) and riboflavin were highly over-accumulated (+41 % and +76 %, respectively). Comparing 100-50 versus 100-0, vitamins increased (up to 29-fold for pyridoxal and 11-fold for nicotinamine), whereas riboflavin was the only compound being down-represented (equal to 0.40). The same metabolite was characterized by a 2-fold increase in 100-50 vs 0–50.

Polyphenols were the most representative group in the polar metabolome, with 66 uniquevocally identified compounds (Fig. 3A and Tables S1A and S2A). Due to the great number of detected compounds and the variable alterations at compound-rather than sub class-level, it is not easy to draw general trends. However, and overall, by comparison with the control 0-0, total polyphenols increased by 29 % in 100-0 and 39 % in 0–50, and did not change actually in 100-50.

Considering the total number of differentially accumulated metabolites, 100-0 vs 0-0 showed 26 significantly over- and 21 down-accumulated compounds; 0–50 vs 0-0 displayed 28 over- and 14 down-represented metabolites, while 100-50 vs 0-0 was characterized by 11 and 23 over and down accumulated compounds, respectively (Fig. 3A and Tables S1A and S2A).

Of interest, all salinity treatments, as compared to 0-0, showed significant increases of miquelianin and 1-O-Feruloyl quinic acid, and significant decreases of apigenin, biochanin, eupatorin, hesperetin, naringenin, quercetin, isovitexin 7-O-glucoside (saponarin), and C-hexosyl-apigenin O-pentoside. Noticeable positive changes in 100-0 were those of 8-C-Hexosyl-apigenin O-hexosyl-O-hexoside (9-fold), O-Methylnaringenin C-pentoside (6-fold), apigenin 4-O-rhamnoside, chrysin 5-O-glucoside (Toringin), chrysoeriol C-hexosyl-O-rhamnoside (over 3-fold for all); in 0–50 those of miquelianin (8-fold) and quercetin O-acetylhexoside (3 fold); and, in 100-50, of miquelianin (6-fold), coniferyl alcohol (4-fold), cinnamic acid and quercetin O-acetylhexoside (about 3-fold for both). Additional, relevant reductions for all treatments were those of apigenin, biochanin, eupatorin, hesperetin, quercetin, isovitexin 7-O-glucoside (saponarin), and C-hexosyl-apigenin O-pentoside, which all showed values of less than 1/3 (even 1/30 or less, in some cases) of those recorded in 0-0. Furthermore, in 100-50, also vitexin 2″-O-beta-L-rhamnoside and 3-Methoxyphenylacetic acid were reduced to about 1/3 of those recorded in 0-0.

When comparing 100-50 to 100-0 and 0–50, total polyphenols decreased by 20 and 27 % respectively, with 17 significantly up- and 31-down represented compounds with respect to 100-0, and 15-up and 29 down-accumulated compounds with respect to 0–50. In both cases, 100-50 showed significant increases for 8 compounds (chlorogenic acid, cinnamic acid, syringic acid, syringin, vanillic acid, eriodictiol 6-C-hexoside 8-C-hexoside-O-hexoside, eriodictiol C-hexosyl-O-hexoside, coniferyl alcohol), whereas 18 compounds (ferulic acid, ferulic acid dimer, sinapic acid, 5-O-p-Coumaroylquinic acid, 6-Hydroxymethylherniarin, luteolin, apigenin 7-rutinoside, chrysin 5-O-glucoside, kaempferol-3-O-robinoside-7-O-rhamnoside, kaempferol-3-O-sinapoylsophoroside, luteolin 3′,7-di-O-glucoside, luteolin 7-O-glucoside, luteolin 6-C-hexoside 8-C-hexosyl-O-hexoside, vitexin 2″-O-beta-L-rhamnoside, coniferyl aldehyde, epicatechin, 4-Hydroxycoumarin, 3-Methoxyphenylacetic acid) resulted down-accumulated. Contrasting variations were also observed for eight compounds, four of which (anisic acid, biochanin, O-Methylnaringenin C-pentoside, chrysoeriol C-hexosyl-O-rhamnoside) displayed lower levels in the comparison 100-50 vs 100-0 and higher levels in 100-50 vs 0–50, while the other four (eriodictyol, apigenin O-malonylhexoside, isorhamnetin 5-O-hexoside, naringenin O-malonylhexoside) were found to be over-represented in the comparison 100-50 vs 100-0 and down-representedin 100-50 vs 0–50. In general, the variations observed in 100-50 vs 100-0 were greater than those observed in 100-50 vs 0–50 (Fig. 3A and Tables S1A and S2A).

3.4. Changes in non polar metabolome

The extent of changes in the non polar metabolome was more limited than in the polar metabolome and characterized by either potentially specific or stochastic alterations.

For instance, carotenoids were slightly reduced by salinity applied only during mother plants growth (100-0 vs 0-0), and a significant drop was recorded only for lutein. On the contrary, salinity applied only during sprouting (0–50 vs 0-0) caused more marked decreases, especially for all-trans-violaxanthin and all-trans-neoxanthin (down to 40 % and 28 %, respectively, of the content recorded in 0-0). Similarly, salinity applied to both mother plants and sprouting (100-50 vs 0-0) also reduced significantly all-trans-violaxanthin and all-trans-neoxanthin, but specifically boosted the contents of the other three (9-cis-violaxanthin, luteoxanthin and lutein) (Fig. 3B and Tables S1B and S2B). When normalized on 100-0 and 0–50, the 100-50 increased the overall content of carotenoids by 32 % and 115 %, with all compounds being over-accumulated, except for the decrease of all-trans-neoxanthin in the comparison 100-50 vs 100-0.

Chlorophylls and derived catabolites were characterized by a general reduction in 100-0 (vs 0-0), except for the 3-fold increase of chlorophyll *a*. In 0–50 (vs 0-0), the general metabolic down-representation regarded all compounds (including chlorophyll *a*) and was more marked. Notably, 100-50 (vs 0-0) displayed higher contents in chlorophyll *a* (20-fold) and in chlorophyll *b* and chlorophyllide b (around 2-fold), while all the other compounds decreased. In the comparison 100-50 vs 100-0, three compounds were positively affected (chlorophyll *a* by 6-fold, and chlorophyll *b* and chlorophyllide b by 3-fold) and two compounds resulted down-accumulated (pheophorbide b, by 1/2, and pheophytin b, by 2/3), although the overall chlorophyll content was unaltered. Finally, in 100-50 vs 0–50, most compounds increased significantly and the overall content was +79 %.

Tocochromanols were mostly decreased, except for the substantial invariance of α-tocotrienol in all treatments and α-tocopherol-quinone in 100-0, however the reductions were slighter in 100-0 (around ½ of the content in 0-0), and much greater in 0–50 and 100-50 (in most cases,

from 1/5 to 1/10 or less of the content in 0-0) (Fig. 3B and Tables S1B and S2B). In the comparison 100-50 vs 100-0, most compounds displayed negative variations, except for α-tocotrienol, while 100-50 vs 0–50 did not exhibit significant variations except for the slight increase of α -tocotrienol and the strong reduction of α -tocopherol.

Among quinones, phylloquinone (vitamin K) was the most represented and it was not significantly affected in 100-0, decreased in 0–50 (−22 %), and increased in 100-50 (+63 %), as compared to 0-0 (Fig. 3B and Tables S1B and S2B). All the other quinones were markedly reduced in all treatments. Of interest, the total quinones followed the trend of the phylloquinone. Also, in the comparisons 100-50 vs both 100-0 and 0–50, phylloquinone was the only compound whose content was significantly higher, while the other compounds showed slight variations.

Finally, fatty acids in 100-0 showed some remarkable increases (from +50 % to +100 %, for caprylic, myristoleic, palmitic, palmitoleic, pentadecanoic) and some substantial invariances, while they all markedly decreased in 0–50 and 100-50 (from ½ to 1/5 or less), with the only exception of heneicosanoic acid, which displayed higher levels by over 5 times in 0–50 and did not change in 100-50. In the comparison 100-50 vs 100-0, fatty acids were markedly reduced (from ½ to 1/5 or less), whereas resulted unchanged in 100-50 vs 0–50, with the sole exception of heneicosanoic acid, which showed lower contents.

Sterols were rather invariable in all treatments, with the only stigmasterol significantly affected, being slightly increased in 100-0 (vs 0-0) and decreased in 100-50 vs 100-0.

Among other lipids, cis-9-monoolein (c18:1), which was the most represented, was characterized by higher contents (3-fold) in 100-0, whereas it almost disappeared in 0–50 and 100-50, with respect to 0-0. When comparing 100-50 with 100-0, cis-9-monoolein was reduced to nihil, while in 100-50 vs 0–50 this compound was increased, but the content was negligible anyway.

4. Discussion

Salinity stress and its effect on rapeseed seedling growth and physiology as well as on plant metabolic profiling have been largely investigated [19,26]. More in detail, and depending on salt concentration and duration, a severe impairment on plant metabolism and health status can be observed [27]. On the other side, moderate salt applications can prime plant tolerance and defence to multiple stresses, as well as boosting secondary metabolite accumulation, thus providing a useful tool for generating improved food products in terms of nutritional composition [10]. In this context, here we investigated the response of rapeseed sprouts to salinity either applied during the growth of the mother plant whose offspring seeds were used for sprouting, or applied directly during sprouting, or during both the mother plant growth and the sprouting of offspring seeds. To this aim, progeny sprouts of the different salinity treatments were subjected to high-resolution global metabolic profiling analyses of the two fractions (polar and non polar) composing the non volatile metabolome; more particularly, a targeted database of 109 (polar) and 60 (non polar) metabolites was constituded, by especially focusing on primary and secondary compounds with pro-nutritional properties. Therefore, the novelty of this work is that it allows to infer the influence of genetic- and environmental-based traits on plant metabolism at intergenerational level.

As far as the germination is concerned (Table 1), the lack of effect of salinity was expected, since 50 mMol NaCl is far below the concentration that may be critical for rapeseed, especially for the cultivar Exagone, which was found to be salt tolerant during germination [1]. However, as specified in Materials and Methods, 50 mMol NaCl had been proven to be the maximum concentration suitable for sprouting purposes, since higher salinity levels would impair sprout growth delaying harvest, reducing biomass yield and increasing the risk of mould occurrence [2]. The lower mean germination time for the seeds harvested in salt-stressed mother plants was also expected, based on previous experiments [11], and confirms that salinity may have intergenerational effects, so that the progeny comes out actually primed to face stressing conditions at least with regards to seed germination. It is worth to pinpoint that the stress imposed to mother plants was effective, since seed yield in mother plants grown with 100 mMol NaCl was just 71 % of the seed yield obtained in mother plants grown with 0 mMol NaCl (i.e., 2.59 vs. 3.65 g per plant, respectively).

The effect of salinity treatments on sprout growth parameters (Table 1) is not easy to interprete. Sprouts obtained in distilled water from seeds harvested in salt-stressed mother plants (i.e. 100-0) were expected to have greater fresh weights due to faster germination, involving earlier and greater water absorption and thus greater cell elongation or expansion. On the contrary, the highest fresh weights were recorded in treatments subjected to salinity during sprouting (i.e., especially 0–50), which was unexpected, given the lower osmotic potential in the substrate. We might assume that part of the higher fresh weight was accounted for by the NaCl ions adsorbed by sprouts, but this should have also caused some toxic effect, which was not observed actually and, moreover, this should have resulted in higher dry matter percent content, which, on the contrary, was low in 0–50 (as it can be calculated based on dry and fresh weights reported in Table 1). Paradoxally, the worst growth performance was observed in the never stressed control, which showed the lowest FW, although its dry matter percent content was high. It looks like NaCl 50 mM was not enough to impair growth and rather caused a sort of hormetic effect [10]. It has to be noticed, however, that 50 mM NaCl in the work of Falcinelli et al. [2] was the highest NaCl concentration that could be tolerated by sprouts to avoid impairment of growth, thus it should have been a level far beyond the level implying an hormetic effect. Also differences in photosysnthetic rate should not account for the differences in dry weights because at that growth stage (7 days after sowing), seedlings were still developing at the expense of seed reserves, and the contribution of photosynthesis to growth should have been negligible. Referring to data by Benincasa et al. [11] does not help, since in that work only two of the four treatments used here were included (0-0 and 100-0), and no salinity had been applied to the sprouting substrate.

Given the invariance in the germination percentage, the increased fresh and dry weight of individual sprouts observed in the three salinity treatments as compared to the never stressed control resulted in higher fresh and dry biomass yield of sprouts per tray at harvest.

Metabolomics analyses was exploited to reveal changes in a large diversified set of primary and secondary compounds. Overall, a large extent of metabolic variations was observed among treatments. However, it is noteworthy to underline that polar metabolome looked to be more responsive compared to the non polar one, although in both cases a series of alterations due to the occurrence of stochastic events or specific according to the metabolic class and the salinity treatment were recorded. Due to the complexity of the different metabolic responses, the overall changes of each class of polar and non polar metabolites in the five pairwise comparisons between salinity treatments have been schematically summarized in Fig. 4.

More in detail, 100-0, as compared to 0-0, displayed a decrease of amino acids (except for the increase of spermidine), organic acids, sugars (except for sorbitol and glucose), and vitamins (except for the increase of riboflavin), whereas phenolics were characterized by non univocal alterations of metabolites but the overall result was a moderate increase (Figg 3 and 4, Tables S1A and S2A). Among non polar metabolites, most of them decreased in 100-0 except for the marked increase of chlorophyll *a* and cis-9-monoolein and the moderate positive change in fatty acids (Figs. 3 and 4, Tables S1B and S2B). Discussing these evidences is not easy in lack of literature on the effect of salinity applied to mother plants. The only available reference is our previous work [11], where the sprouts obtained from offspring seeds of mother plants subjected to salt stress were found to show a moderate increase of total phenols and a marked increase of free phenolic acids and antioxidant activity. Results of the present study would confirm that the priming of offspring seeds against stress is likely due to the increase of some

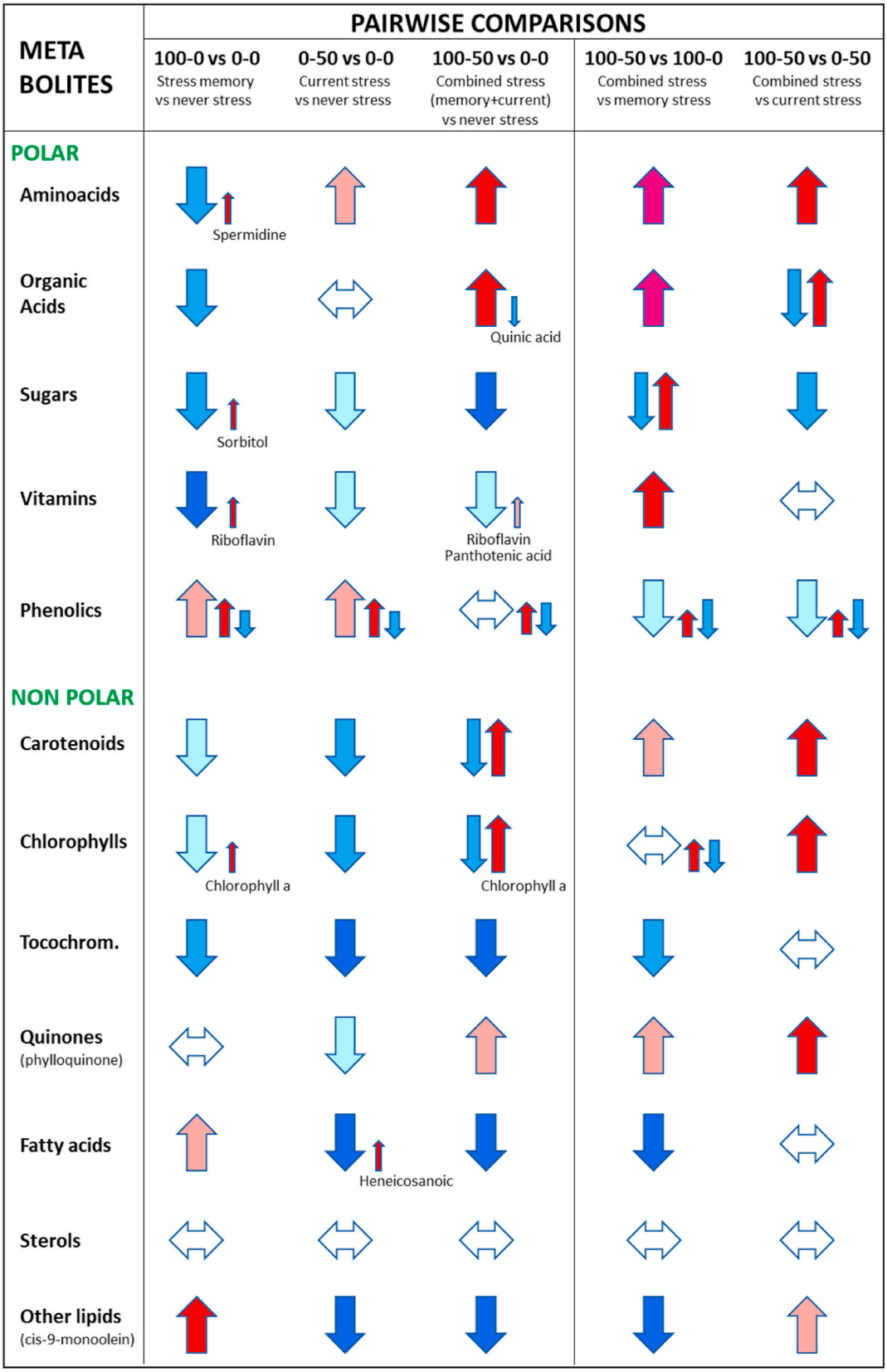


Fig. 4. A synthetic representation of main overall changes of polar and non polar metabolites in rapeseed sprouts for the five pairwise comparisons between salinity treatments (see Fig. 1 for the description of treatments). Upward arrows for each class of metabolites indicate overall increase (pink = moderate, red = marked, purple = very marked); downward arrows indicate overall decrease (light blue = moderate, blue = marked, deep blue = very marked); white double head arrows indicate substantial invariance; a couple of upward-red plus downward-blue arrows indicate not univocal marked variations of metabolites in that class; thin arrows indicate remarkable variations of specific metabolites in contrast with the general trend of that class of metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

phenolic compounds and, probably, to some other metabolites like spermidine, sorbitol and riboflavin. In detail, spermidine, is a polyamine [28], stimulated by high salt concentrations which is able to induce salinity tolerance in plants acting as scavengers of free radicals [19]. For this reason, it is included in osmoprotectants, i.e. the small, low-molecular-weight molecules that act as osmolytes, recognized to aid plants in extreme osmotic environments by balancing the osmotic difference between the cell's surroundings and the cytosol, stabilizing proteins and cell membranes, and preventingdehydration inside the cell [19]. Moreover, spermidine in *Calendula officinalis* was reported to facilitate reactive oxygen species (ROS) metabolism and photosynthesis, which subsequently improves plant growth and reduces the inhibitory effects of salt stress [29]. Also, sorbitol (i.e., sugar alcohol, or polyol) is an osmoprotectant, and it is reported to enhance salinity tolerance by improving antioxidant defence in many plant species [19]. Riboflavin is involved in plant resistance against biotic stress [30] and a recent work in rice seedlings found that its exogenous application would be useful to mitigate also the effect of salinity stress [31]. However, it has to be underlined that metabolic alterations can be partly or at a higher extent associated to species- or variety-specific effects, as also reviewed in Lagiotis et al. [5].

The treatment 0–50 showed, in addition to higher contents in many phenolic compounds, a moderate increase of several amino acids (Figg 3 and 4, Tables S1A and S2A), but more marked and generalized decreases in the non polar metabolome (Figg 3 and 4, Tables S1B and S2B). In this case, there is some literature on the metabolic changes caused by salinity applied directly during sprouting. In particular, Falcinelli et al. [2], working with the same rapeseed cultivar and sprouting conditions, found that sprouts obtained with 50 mM NaCl had a moderate increase in phenolic compounds (especially tannins and some phenolic acids) and antioxidant activity, which is in line with our results. Considering other studies on *Brassica* species, the effect of salinity on polyphenols is not always univocal. Yuan et al. [32] and Guo et al. [33] observed an increase in total phenolic content of radish and broccoli sprouts respectively only at high salt concentration. Hassini et al. [34] explained the decrease in total phenolic content of broccoli sprouts under salinity as a consequence of the different salt sensitivity and growth of plants. This is in agreement with previous observations of Falcinelli et al. [35], which reported different trends in total phenolic content of wheatgrass from two *Triticum* species. In particular, these authors found that polyphenols increased with increasing salinity in einkorn wheatgrass but not in emmer wheatgrass. This led to the hypothesis that other metabolites, in place or in addition to phenols, could come into play to explain the higher antioxidant activity of salted sprouts. In this perspective, the moderate increase we observed in the amino acid contents might play a role, or be the signal of the occurrence of a different metabolic mechanism to face salinity. Among non-polar metabolites, the reduction in chlorophyll and carotenoid content under salt conditions is widely reported in many plant species [36–38] and linked to the disturbance of photosyntetic process caused by salinity.

Coming to 100-50 (as compared to 0-0), we observed generalized and distinct increases in amino acids, together with most organic acids (Figs. 3 and 4, Tables S1A and S2A). Moreover, in this treatment we recorded a slight decrease in most vitamins, with the exception of riboflavin and panthotenic acid, which increased (Figg 3 and 4, Tables S1A and S2A), and a marked increase of some carotenoids and chlorophylls and of phylloquinone (Figg 3 and 4, Tables S1B and S2B). On the contrary, phenolic compounds showed more down-than up-accumulations (Figg 3 and 4, Tables S1A and S2A). Kambona et al. [6] highlighted that salt stress memory in plants has been mainly investigated at the somatic level (kept through mitosis in the same generation), while evidences of inter- and trans-generational effects are more limited. In any case, either the tolerance to current stress or the stress memory would involve an over-accumulation of the osmoprotectant proline, together with higher photosynthetic rate [6]. All these findings are partly matching our data, since also treatment 100-50 displayed higher levels in proline, and the over-accumulation of compounds of the photosynthetic apparatus (e.g. chlorophyll *a* and *b*, lutein). The role of proline in salt stress tolerance is well recognized [36,39]. Proline is also reported to protect RuBisCo activity and mitochondrial electron transport chain complex II [36].

Moreover, it is known that the response of plant tissues to an osmotic stress like that induced by drought or salinity may involve changes in both enzymatic and non enzymatic antioxidants, the latters including phenolic acids, flavonoids, ascorbate, tocopherols, carotenoids [19], and these metabolic changes can actually contribute in priming the offspring to stressful conditions [5].

In fact, as compared to the salinity stress applied to only mother plants (100-0) or only sprouting (0–50), the combined stress (i.e., applied to both mother plant and sprouting) (100-50) resulted in the boosting of amino acids, organic acids, vitamins, carotenoids, chlorophylls and phylloquinone, while some phenolic compounds decreased. The latter phenotype in the presence of salinity was unexpected, based on the literature and on the effect observed for 0–50. Since phenylalanine (the substrate for the production of phenylpropanoids, including phenolic compounds, in the shikimate pathway) [40,41] was highly present, it is hypothesizable that this molecule was recruited in the biosynthesis of compounds other than phenolics. It could be the case of coniferyl alcohol, which was highly present in 100-50. Indeed, coniferyl alcohol is a monolignol (lignin monomer) deriving from ferulic acid (thus a phenolic compound) necessary for lignin synthesis. It appears to act as a signal molecule to regulate the phenylpropanoid pathway and lignin synthesis. In particular, Guan et al. [42] reported that plants with a reduced coniferyl alcohol biosynthesis have higher phenylalanine ammonia-lyase (PAL) content. PAL is the enzyme which catalyzes the deamination of L-phenylalanine to form trans-cinnamic acid, and it is the key reaction considered as the link between primary and secondary metabolism [40]. Thus, any disturbance in its regulation and activity would interfere in the distribution of amino acids to produce primary or secondary metabolites. Guan et al. [42] observed a degradation of PAL after exogenous coniferyl alcohol application and speculated that it shifted the distribution of phenylalanine to protein synthesis. This might explain the higher level of amino acids observed in 100-50 treatment. The presence of coniferyl alcohol in treatments subjected to salt stress during sprouting (i.e. both 0–50 and 100-50), would suggest that lignin biosynthesis was taking place, and this might have played a role in increasing salt tolerance, since lignin regulates the osmotic balance of the cell by reducing the permeability of the cell wall and transpiration [43]. On the other hand, the high presence of coniferyl alcohol in 100-50 compared to 0–50 might be a stress memory consequence. In fact, it has been reported that an increased lignification in plants exposed to salt stress play an important role in plant adaptation against salinity, but the molecular functions and biosynthetic gene expression patterns involved are not fully understood, as it is a complex biological process involving multiple enzymes, and transcriptional and post-transcriptional regulations [44]. In any case, the stimulation of the lignin biosynthesis as a reaction to salinity, necessarily affects the enzymatic activity and therefore also the production of intermediate metabolites [45]. For example, cinnamic acid is necessary for the production of hydroxycinnamic acids, such as p-coumaric, ferulic and sinapic acids which lead to the formation of monolignols after polymerization with their alcohols (i.e. 4-coumaric alcohol, coniferyl alcohol and sinapyl alcohol, respectively) and then of lignin [40,46]. Vanillic and syringic acid are hydroxybenzoic acids and represent the constituents of lignin [40]. The benefits of these phenolic compounds for human health are well known and widely reviewed [46,47]. On the other hand, the increase of metabolites linked to lignin biosynthesis deserves to be considered. In fact, Shu et al. [43] reviewed the several biological activities attributed to lignin including antibacterial, antiviral and even antitumoral activity. More specifically, the single coniferyl alcohol was found to be useful in prevention or treatment of bile duct cancer (i.e., cholangiocarcinoma) [48].

The possible role of an increased concentration of spermidine, sorbitol and riboflavin in 100-50 has been discussed previously for 100-0. The treatment 100-50 also showed an increase of proline, whose role in salt tolerance has been discussed previously. In addition, eriodictiol 6-C-hexoside 8-C-hexoside-O-hexoside and the erodictiol C-hexoxyl-O-hexoside were detected only in 100-50. They are both flavonoids [49], but there is little information about their function in plant adaptation, except for the deterrent action of eriodictiol 6-C-hexoside 8-C-hexoside-O-hexoside in *Prunus mira* flowers against birds [49].

Considering the non polar metabolome, the higher presence of carotenoids and chlorophylls in 100-50, as compared to 100-0 and 0–50, could be a consequence of the increased concentration of abovesaid osmoprotectants and would be functional to the maintenance of photosynthetic activity. Hatzig et al. [3] working with the offspring of rapeseed subjected to a maternal drought stress found an alteration of metabolism which was addressed more towards growth promoting processes (i.e., carbon assimilation) than towards catabolic processes. This might be relevant, considering that lignin biosynthesis, which likely plays a role in salt tolerance, consumes about 30 % of the photosynthetically fixed carbon and is not reversible.

It has to be noticed that the increases in the concentrations of above said compounds was accompanied by an increase in the fresh and dry matter yield per tray as compared to the never stressed control. This further increases the advantage of applying salinity stress at any time (i.e., to mother plants or to seeds during sprouting or to both phases) in terms of phytochemical yield, which is the product between biomass yield and the metabolite concentration [2,10].

5. Conclusions

This is the first work investigating changes in the whole non volatile metabolome of rapeseed sprouts caused by either imposing a salt stress to mother plants (where the offspring seeds used for sprouting are taken from) or during sprouting or during both phases (mother plants growth and sprouting). Our results first confirm evidences from our previous works, in that either the intergenerational stress memory (i.e., derived to sprouts from the stress applied to mother plants) or the current stress (i.e., applied directly during sprouting) boost the concentrations of several phenolic compounds which likely play a role in the tolerance to salinity. In addition, some other compounds were increased such as fatty acids and cis-9-monoolein, and, limitedly to the case of salinity applied to mother plants, we recorded also increases in spermidine, riboflavin, and chlorophyll *a*. These findings are new, since previous works focused only on total polyphenols and some phenolic acids. However, the main outcome of this work is the effect of combining the stress memory and the current stress, which had never been studied before. Our results demonstrate that combining the two stress occurrences further boosted the concentrations of amino acids, organic acids, vitamins, carotenoids, chlorophylls and phylloquinone but, on the other hand, more phenolic compounds were down-regulated, as compared to the stress applied to only mother plants or only sprouting. Further research will have to investigate the reasons for these changes and the role of these compounds in the physiology of tolerance to combined salt stress occurrence. Evidences from this work suggest that combining salt stress memory (as it can be obtained from grain crops grown in saline soils) and current salt stress (as it can obtained by sprouting in saline substrate) can be exploited to increase the value of sprouts as functional foods, with widely recognized benefits for human health.

CRediT authorship contribution statement

F. Sevi: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **B. Falcinelli:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **S. Frusciante:** Formal analysis, Data curation. **E. Fabene:** Data curation. **G. Tosti:** Investigation, Data curation. **R. D'Amato:** Investigation. **G. Diretto:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis. **P. Benincasa:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Funding

This research was partially funded by the Project Ricerca di Base 2020 of the University of Perugia. Principal investigator: Paolo Benincasa. The funding source had no involvement in any phase of the research (i.e., conceptualization, methodology, formal analysis, data curation, writing and revising the manuscript).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge Mr. Silvano Locchi for his technical support in growing rapeseeds plants and sprouting seeds.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.101649.

Data availability

Data will be made available on request.

References

[1] P. Benincasa, R. Pace, M. Quinet, S. Lutts, Effect of salinity and priming on seedling growth in rapeseed (*Brassica napus* var *oleifera* Del.), Acta Sci. Agron. 35 (2013) 479–486, https://doi.org/10.3390/molecules22081377.
[2] B. Falcinelli, V. Sileoni, O. Marconi, G. Perretti, M. Quinet, S. Lutts, P. Benincasa, Germination under moderate salinity increases phenolic content and antioxidant activity in rapeseed (*Brassica napus* var *oleifera* Del.) sprouts, Molecules 22 (2017) 1377, https://doi.org/10.3390/molecules22081377.
[3] S.V. Hatzig, J.N. Nuppenau, R.J. Snowdon, S.V. Schießl, Drought stress has transgenerational effects on seeds and seedlings in winter oilseed rape (*Brassica napus* L.), BMC Plant Biol. 18 (2018) 297, https://doi.org/10.1186/s12870-018-1531-y.
[4] J. Guo, M. Du, H. Tian, B. Wang, Exposure to high salinity during seed development markedly enhances seedling emergence and fitness of the progeny of the extreme halophyte Suaeda salsa, Front. Plant Sci. 11 (2020) 1291, https://doi.org/10.3389/fpls.2020.01291.
[5] G. Lagiotis, P. Madesis, E. Stavridou, Echoes of a stressful past: abiotic stress memory in crop plants towards enhanced adaptation, Agriculture 13 (2023) 2090, https://doi.org/10.3390/agriculture13112090.
[6] C.M. Kambona, P.A. Koua, J. Léon, A. Ballvora, Intergenerational and transgenerational effects of drought stress on winter wheat (*Triticum aestivum* L.), Physiol. Plantarum 175 (2023) e13951, https://doi.org/10.1111/ppl.13951.
[7] A. Bilichak, I. Kovalchuk, Transgenerational response to stress in plants and its application for breeding, J. Exp. Bot. 67 (7) (2016) 2081–2092, https://doi.org/10.1093/jxb/erw066.
[8] J. Lämke, I. Bäurle, Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants, Genome Biol. 18 (2017) 124, https://doi.org/10.1186/s13059-017-1263-6.
[9] J. Molinier, G. Ries, C. Zipfel, B. Hohn, Transgeneration memory of stress in plants, Nature 442 (2006) 31, https://doi.org/10.1038/nature05022.
[10] A. Galieni, B. Falcinelli, F. Stagnari, A. Datti, P. Benincasa, Sprouts and microgreens: trends, opportunities, and horizons for novel research, Agronomy 10 (2020) 1424, https://doi.org/10.3390/agronomy10091424.
[11] P. Benincasa, E. Bravi, O. Marconi, S. Lutts, G. Tosti, B. Falcinelli, Transgenerational effects of salt stress imposed to rapeseed (*Brassica napus* var. *oleifera* Del.) plants involve greater phenolic content and antioxidant activity in the edible sprouts obtained from offspring seeds, Plants 10 (2021) 932, https://doi.org/10.3390/plants10050932.
[12] B. Falcinelli, A. Galieni, G. Tosti, F. Stagnari, F. Trasmundi, E. Oliva, P. Benincasa, Effect of wheat crop nitrogen fertilization schedule on the phenolic content and

antioxidant activity of sprouts and wheatgrass obtained from offspring grains, Plants 11 (15) (2022) 2042, https://doi.org/10.3390/plants11152042.
[13] N. Xu, G. Chen, H. Liu, Antioxidative categorization of twenty amino acids based on experimental evaluation, Molecules 22 (12) (2017) 2066, https://doi.org/10.3390/molecules22122066.
[14] Q. Liu, G.-Y. Tang, C.-N. Zhao, R.-Y. Gan, H.-B. Li, Antioxidant activities, phenolic profiles, and organic acid contents of fruit vinegars, Antioxidants 8 (4) (2019) 78, https://doi.org/10.3390/antiox8040078.
[15] M.A. Asensi-Fabado, S. Munné-Bosch, Vitamins in plants: occurrence, biosynthesis and antioxidant function, Trends Plant Sci. 15 (10) (2010) 582–592, https://doi.org/10.1016/j.tplants.2010.07.003.
[16] S. Pollastri, I. Baccelli, F. Loreto, Isoprene: an antioxidant itself or a molecule with multiple regulatory functions in plants? Antioxidants 10 (5) (2021) 684, https://doi.org/10.3390/antiox10050684.
[17] M. Fujita, M. Hasanuzzaman, Approaches to enhancing antioxidant defense in plants, Antioxidants 11 (5) (2022) 925, https://doi.org/10.3390/antiox11050925.
[18] U. Sarker, S. Oba, The response of salinity stress-induced *A. tricolor* to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants, Front. Plant Sci. 11 (2020) 559876, https://doi.org/10.3389/fpls.2020.559876.
[19] M. Singh, J. Kumar, S. Singh, V.P. Singh, S.M. Prasad, Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review, Rev. Environ. Sci. Biotechnol. 14 (2015) 407–426, https://doi.org/10.1007/s11157-015-9372-8.
[20] G. Diretto, X. Jin, T. Capell, C. Zhu, L. Gomez-Gomez, Differential accumulation of pelargonidin glycosides in petals at three different developmental stages of the orange-flowered gentian (*Gentiana lutea* L. var. Aurantiaca), PLoS One 14 (2) (2019) e0212062, https://doi.org/10.1371/journal.pone.0212062.
[21] J.L. Rambla, A. Trapero-Mozos, G. Diretto, A. Rubio-Moraga, A. Granell, L. Gómez-Gómez, O. Ahrazem, Gene-metabolite networks of volatile metabolism in Airen and Tempranillo grape cultivars revealed a distinct mechanism of aroma bouquet production, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.01619.
[22] A. Iwase, K. Mita, D.S. Favero, N. Mitsuda, R. Sasaki, M. Kobayshi, Y. Takebayashi, M. Kojima, M. Kusano, A. Oikawa, H. Sakakibara, K. Saito, J. Imamura, K. Sugimoto, WIND1 induces dynamic metabolomic reprogramming during regeneration in *Brassica napus*, Dev. Biol. 442 (1) (2018) 40–52, https://doi.org/10.1016/j.ydbio.2018.07.006.
[23] B.B. Misra, Cataloging the *Brassica napus* seed metabolome, Cogent Food Agric. 2 (1) (2016), https://doi.org/10.1080/23311932.2016.1254420.
[24] Pubchem database, http://pubchem.ncbi.nlm.nih.gov (most recent access date: 2/04/2024).
[25] Metabolomics Fiehn Lab Mass Spectrometry Adduct Calculator, http://fiehnlab.ucdavis.edu/staff/kind/Metabolomics/MS-Adduct-Calculator/(most recent access date: 2/04/2024).
[26] L. Ambrosino, C. Colantuono, G. Diretto, A. Fiore, M.L. Chiusano, Bioinformatics resources for plant abiotic stress responses: state of the art and opportunities in the fast evolving -omics era, Plants 9 (5) (2020) 591, https://doi.org/10.3390/plants9050591.
[27] E. Dell'Aversana, K. Hessini, S. Ferchichi, G.M. Fusco, P. Woodrow, L.F. Ciarmiello, C. Abdelly, P. Carillo, Salinity duration differently modulates physiological parameters and metabolites profile in roots of two contrasting barley genotypes, Plants 10 (2) (2021) 307, https://doi.org/10.3390/plants10020307.
[28] J. Qiao, W. Cai, K. Wang, E. Haubruge, J. Dong, H.R. El-Seedi, X. Xu, H. Zhang, New insights into identification, distribution, and health benefits of polyamines and their derivatives, J. Agric. Food Chem. 72 (10) (2024) 5089–5106, https://doi.org/10.1021/acs.jafc.3c08556.
[29] F. Baniasadi, V.R. Saffari, A.A.M. Moud, Physiological and growth responses of *Calendula officinalis* L. plants to the interaction effects of polyamines and salt stress, Sci. Hortic. 234 (2018) 312–317, https://doi.org/10.1016/j.scienta.2018.02.069.
[30] H. Boubakri, M. Gargouri, A. Mliki, F. Brini, J. Chong, M. Jbara, Vitamins for enhancing plant resistance, Planta 244 (2016) 529–543, https://doi.org/10.1007/s00425-016-2552-0.
[31] K. Jiadkong, A.N. Fauzia, N. Yamaguchi, A. Ueda, Exogenous riboflavin (vitamin B2) application enhances salinity tolerance through the activation of its biosynthesis in rice seedlings under salinity stress, Plant Sci. 339 (2024) 111929, https://doi.org/10.1016/j.plantsci.2023.111929.
[32] G. Yuan, X. Wang, R. Guo, Q. Wang, Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts, Food Chem. 121 (4) (2010) 1014–1019, https://doi.org/10.1016/j.foodchem.2010.01.040.
[33] R.F. Guo, G.F. Yuan, Q.M. Wang, Effect of NaCl treatments on glucosinolate metabolism in broccoli sprouts, J. Zhejiang Univ. - Sci. B 14 (2013) 124–131, https://doi.org/10.1631/jzus.B1200096.
[34] I. Hassini, M.C. Martinez-Ballesta, N. Boughanmi, D.A. Moreno, M. Carvajal, Improvement of broccoli sprouts (*Brassica oleracea* L. var. *italica*) growth and quality by KCl seed priming and methyl jasmonate under salinity stress, Sci. Hortic. 226 (2017) 141–151, https://doi.org/10.1016/j.scienta.2017.08.030.
[35] B. Falcinelli, P. Benincasa, I. Calzuola, L. Gigliarelli, S. Lutts, V. Marsili, Phenolic content and antioxidant activity in raw and denatured aqueous extracts from sprouts and wheatgrass of einkorn and emmer obtained under salinity, Molecules 22 (2017) 2132, https://doi.org/10.3390/molecules22122132.
[36] A. El Moukhtari, C. Cabassa-Hourton, M. Farissi, A. Savouré, How does proline treatment promote salt stress tolerance during crop plant development? Front. Plant Sci. 11 (2020) 1127, https://doi.org/10.3389/fpls.2020.01127.
[37] A. Hameed, M.Z. Ahmed, T. Hussain, I. Aziz, N. Ahmad, B. Gul, B.L. Nielsen, Effects of salinity stress on chloroplast structure and function, Cells 10 (8) (2021) 2023, https://doi.org/10.3390/cells10082023.
[38] M. Hasanuzzaman, M.R.H. Raihan, A.A.C. Masud, K. Rahman, F. Nowroz, M. Rahman, M. Fujita, Regulation of reactive oxygen species and antioxidant defense in plants under salinity, Int. J. Mol. Sci. 22 (17) (2021) 9326, https://doi.org/10.3390/ijms22179326.
[39] F. Zulfiqar, M. Ashraf, Proline alleviates abiotic stress induced oxidative stress in plants, J. Plant Growth Regul. 42 (8) (2023) 4629–4651, https://doi.org/10.1007/s00344-022-10839-3.
[40] M.E. Saltveit, Synthesis and metabolism of phenolic compounds, in: L.A. de la Rosa, E. Alvarez-Parrilla, G.A. González-Aguilar (Eds.), Fruit and Vegetable Phytochemicals: Chemistry and Human Health, John Wiley & Sons, Inc., Publication, New York, USA, 2017, pp. 115–124, https://doi.org/10.1002/9781119158042.ch5.
[41] C.I. Vong, T. Rathinasabapathy, M. Moncada, S. Komarnytsky, All polyphenols are not created equal: exploring the diversity of phenolic metabolites, J. Agric. Food Chem. 70 (7) (2022) 2077–2091, https://doi.org/10.1021/acs.jafc.1c07179.
[42] M. Guan, C. Li, X. Shan, F. Chen, S. Wang, R.A. Dixon, Q. Zhao, Dual mechanisms of coniferyl alcohol in phenylpropanoid pathway regulation, Front. Plant Sci. 13 (2022) 896540, https://doi.org/10.3389/fpls.2022.896540.
[43] F. Shu, B. Jiang, Y. Yuan, M. Li, W. Wu, Y. Jin, H. Xiao, Biological activities and emerging roles of lignin and lignin-based products— A review, Biomacromolecules 22 (12) (2021) 4905–4918, https://doi.org/10.1021/acs.biomac.1c00805.
[44] S. Zhao, Q. Zhang, M. Liu, H. Zhou, C. Ma, P. Wang, Regulation of plant responses to salt stress, Int. J. Mol. Sci. 22 (9) (2021) 4609, https://doi.org/10.3390/ijms22094609.
[45] X. Han, Y. Zhao, Y. Chen, J. Xu, C. Jiang, X. Wang, J. Zhang, Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants, For. Res. 2 (1) (2022), https://doi.org/10.48130/FR-2022-0009.
[46] H.R. El-Seedi, A.M. El-Said, S.A. Khalifa, U. Goransson, L. Bohlin, A.K. Borg-Karlson, R. Verpoorte, Biosynthesis, natural sources, dietary intake, pharmacokinetic properties, and biological activities of hydroxycinnamic acids, J. Agric. Food Chem. 60 (44) (2012) 10877–10895, https://doi.org/10.1021/jf301807g.
[47] D. Carregosa, R. Carecho, I. Figueira, C.N. Santos, Low-molecular weight metabolites from polyphenols as effectors for attenuating neuroinflammation, J. Agric. Food Chem. 68 (7) (2019) 1790–1807, https://doi.org/10.1021/acs.jafc.9b02155.
[48] B. Promraksa, P. Katrun, J. Phetcharaburanin, Y. Kittirat, N. Namwat, A. Techasen, W. Loilome, Metabolic changes of cholangiocarcinoma cells in response to coniferyl alcohol treatment, Biomolecules 11 (3) (2021) 476, https://doi.org/10.3390/biom11030476.
[49] S. Zhang, H. Ying, G. Pingcuo, S. Wang, F. Zhao, Y. Cui, X. Zeng, Identification of potential metabolites mediating bird's selective feeding on *Prunus mira* flowers, BioMed Res. Int. 2019 (2019), https://doi.org/10.1155/2019/1395480.