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Exogenous Melatonin Enhances Salt Tolerance in Beetroot Through Physiological Mechanisms Independently of Membrane Glycerolipid Remodeling

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Abstract

Melatonin (Mel) is regarded as an eco-friendly substance that enhances plants' tolerance to abiotic stresses, including salt stress. Plant cell membranes are the primary perception site for the detection and initiation of expeditious responses to changing environmental factors such as salinity. It is important to understand how the plant cell membranes remodel lipids under salt stress conditions. The present study investigates comparative changes in basic physiological traits and membrane glycerolipid contents in the leaves of red and white beetroot (*Beta vulgaris* 'Ruby Queen' and 'Rodeo', respectively) cultivars grown hydroponically with Mel (50 µM) application and salt stress treatment (S, 250 mM NaCl), separately and in combination (S + Mel). The results show that Mel reduced salt stress-induced changes in pigment contents, chlorophyll fluorescence (*Fv/Fm*), electrolyte leakage, H_2O_2 levels, and stomatal conductance, but failed to lower malondialdehyde contents under salt stress. Moreover, Mel led to cultivar-specific salt stress-based changes in total polar lipids, glycolipids, and phospholipids and their molecular species. However, up-regulation of leaf expression of the lipid biosynthesis genes, *DGD1*, *DGD2*, *MGD*, *MGD2* and *SQD2*, was observed under S + Mel compared to salt stress alone. The present results, including cultivar specificity of changes in lipid classes and molecular species, suggest that exogenous Mel application causes positive changes in beetroot physiology through a mechanism likely to be largely independent of lipid metabolism.

Keywords Melatonin · Salinity stress · *Beta vulgaris* · Hydroponics · Lipidomics · Gene expression · Photosynthesis

Introduction

Salt stress is one of the most severe environmental factors limiting plant productivity, affecting 833 million hectares of agricultural land in over 100 countries (Ge et al. 2022; Devkota et al. 2022; Khan et al. 2024). The annual agricultural loss due to salinity stress worldwide has recently been reported at approximately 20–40% (Alam et al. 2023). Plants make the majority of their metabolic investments in extensive photosynthetic membranes-thylakoids by rearranging the structure of lipids, the most basic macromolecules of biological membranes, which play a vital role in all plant tissues (Liu et al. 2021). However, photosynthetic membrane lipid studies are still in their infancy, and the role of these lipids in providing lipid remodelling of photosynthetic membranes under abiotic stress conditions has yet to be investigated (Liu et al. 2021; Sah and Sofo 2024).

Beetroot or beet (*Beta vulgaris* L., fam: Chenopodiaceae) is a herbaceous flowering biennial or, rarely, perennial C3 plant, and one of the main vegetable crops, exhibiting wide distribution worldwide (Zayachkovskaya et al. 2021; Zhang et al. 2021; Stoica et al. 2025). According to recent data, the global distribution of beetroot is approximately 269.19 million tonnes (Stoica et al. 2025). Beetroots, either red/yellow or white, contain beneficial predominant nitrogen-containing bioactive pigment metabolites known as betalains

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(e.g. betanidin, isobetanin, and betanin) and yellow-orange betaxanthins (e.g. vulgaxanthin I and dopamine-betaxanthin). The major flavonoids in beetroots are mainly kaempferol and quercetin, while their phenolic acids are gallic, *p*-coumaric, and caffeic acids, and their mineral/ion nutrients are potassium (K, 356 mg/100 g), nitrate and nitrite (1379 mg/kg), which are particularly important among root vegetables. The essential tyrosine-derived pigments (betalains) of beetroots serve as natural food colorants (code E162) and possess potential antioxidant/radical scavenging properties. The plant and its products have therefore been considered in terms of their curative roles in numerous human and degenerative disorders, particularly respiratory and cardiovascular ailments, or other properties (e.g., anti-inflammatory, anti-cognitive, anti-cancer and anti-hepatitic activities) (Zayachkovskaya et al. 2021; Stoica et al. 2025).

Cultivated worldwide, sugar beet is the most important global source of table sugar and bioethanol. Sugar beet farming produced a yield of 269.19 million tonnes in 2021. Russia is the world's largest sugar beet producer, with total production of 45 million tonnes, followed by France, the USA and Germany, producing between 30 and 33 million tonnes, with a variation of 1 to 3 million tonnes (Zhang et al. 2021; Stoica et al. 2025). Türkiye is another of the world's leading sugar beet producers. According to Food and Agriculture Organization data for 2022, the total global sugar beet production area is estimated at 4,295,160 hectares (ha), and the total amount of production is 260,998,614 tons. Türkiye is estimated to occupy 6.39% (274,524 hectares) of the global sugar beet production area, and produces 7.28% (19 million tons) of the total yield (Semerci and Ural 2024). However, there are no satisfactory equivalent data available for red beetroots.

Despite the capacity of halophytes to adapt to environments with high concentrations of NaCl, they are not impervious to the adverse effects of salinity stress. Nonetheless, they are highly effective at maintaining redox balance, sustaining ion homeostasis, activating the antioxidant system for reactive oxygen species (ROS scavenging) and lipid remodelling under salt stress (Mann et al. 2023; Henschel et al. 2024; Su et al. 2025). Beetroots, whether red, yellow or white (sugar beet), have inherited salt stress tolerance traits over a lengthy period and are classified as salt-tolerant glycophytes or halophytes (Skorupa et al. 2019; Wang et al. 2024). Cultivated sugar beets have been shown to demonstrate a comparatively elevated degree of salt sensitivity during the processes of germination and early seedling growth when subjected to protracted exposure to elevated salt levels (Zhang et al. 2021; Wang et al. 2024).

Glycerolipids constitute the majority of plant membrane lipids. Monogalactosyl diacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), the only phospholipid evolved in plastids, form the plastidic bilayer membrane. MGDG and DGDG represent the most prevalent membrane lipids in green plant tissues, exerting a substantial influence on the structure of chloroplasts (and thylakoids) and the photosynthetic machinery. Extraplastidic plant cell membranes are fundamentally composed of six common phospholipid classes, including PG, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), as well as phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidic acid (PA). Research into phospholipids in plant stress studies has attracted considerable attention in recent years due to their important role in remodelling membrane rearrangements in response to adverse external factors including exposure to salt, drought, heavy metals, minerals, temperature and ultraviolet (UV) light (Chen et al. 2018; Han and Yang 2021; Guo et al. 2022). They also exhibit poorly understood responses to cope with the adverse effects of these stresses (Liu et al. 2021; Cook et al. 2021). The principal function of these lipids is to mediate proteolipid interactions and enhance the morphological plasticity of lipid bilayers. Research has demonstrated that salt stress can induce alterations in membrane lipid composition, resulting in a decline in membrane stability and fluidity, protein-lipid interactions, and membrane permeability for water, ions, and metabolites and other effects (Guo et al. 2022).

Melatonin (Mel; N-Acetyl-5-methoxytryptamine, $C_{13}H_{16}N_2O_2$) is a tryptophan-derived indolamine compound and a low-cost green chemical. It has been implicated in increasing the tolerance of plants to abiotic stresses including drought, water-deficit, salt, heavy metals, severe temperatures and UV radiation. The protective mechanisms of Mel against the abiotic stressors involve acting as a free radical scavenger and indirectly stimulating antioxidant enzyme activation. Both actions gear towards curtailing ROS toxicity, which is predominantly recognised as an antioxidant that potentially modulates scavenging of ROS (O_2^-, H_2O_2, $\cdot OH^-$, $\cdot O^-$, HOCl and O_3) and reactive nitrogen species (RNS; $\cdot NO$, $\cdot NO_2$, $\cdot$ONOO- and N_3) in plants (Chen et al. 2022; Zhang et al. 2023; Khan et al. 2024). Mel is considered an essential growth promoter or bioregulator in crop protection for inducing the production of antioxidant enzymes (SOD, POD, APX, CAT, DHAR, GST, GR, MDHAR and PPO, etc.) and antioxidant compounds (such as ASC, DHA, GSH, proline, flavonoid, carotenoid and phenolic compounds), promoting the accumulation of osmolytes (e.g., proline, soluble sugars and sugar alcohols, proteins and glycine betaine), helping maintain cell turgor and water retention, restoring plant metabolic activities, modulating signalling between transcriptomic cascade events and metabolites, maintaining membrane stability and

integrity reducing lipid peroxidation and limiting osmotic stress-induced cell damage (Florido et al. 2022; Guo et al. 2022; Chen et al. 2022; Zhang et al. 2023).

Studies have also indicated that different varieties of beetroot, either red/yellow or white, exhibit different resistances, tolerances and production yields under salt stress conditions. Previous studies involving exogenous Mel application in beetroots under salt stress have mainly focused on mitigating the adverse effects of the stress, increasing plant growth and yield, providing adequate water balance, regulating ion homeostasis, modulating antioxidant defence systems, improving photosynthetic activity, restoring redox homeostasis and controlling ROS levels and the up- and down-regulation of stress-related gene expressions (Wu et al. 2013; Zhang et al. 2021; Wang et al. 2017, 2024; Colak et al. 2024). However, studies investigating the effect of exogenous Mel on leaf lipids in salt stress-induced changes in membrane lipid profiling and at the gene transcriptional level in beetroots are scarce. The current study tests the hypotheses that Mel application enhances basic physiological traits under salt stress conditions, and that physiological changes in response to Mel under salt stress are correlated with changes in lipid metabolism and membrane composition. The results of the current study provide support for the application of Mel on salinised beetroot arable lands to increase sustainable crop productivity.

Materials and Methods

Plant material, Seedling growth, Salt Treatment and Melatonin Application

Common cultivated beetroot seeds, *Beta vulgaris* var. *crassa* 'Ruby Queen' (red, cyanic cultivar) were provided by the Anderson Seed and Garden (Utah, USA), whıle *Beta vulgaris* 'Rodeo' (white/sugar beet, acyanic cultivar) was obtained from Beta Ziraat ve Ticaret A.Ş. and the Aegean Agricultural Research Institute (Menemen, İzmir, Türkiye).

The seeds were selected based on a uniform size and then surface-sterilised for 10 min using sodium hypochlorite (NaClO, 5%, v/v). They were then washed five times in sterile water. The seeds were subsequently germinated in sterile Petri dishes moisturised with sterilised water, supplying optimal humidity. The plants were grown in a growth chamber with controlled temperature and light. The seeds germinated at a uniform size with similar morphological performance were next sown in plastic nursery pots (10 L x 20 W x 20 H), filled with vermiculite moistened with deionised water, in a greenhouse for one week. A completely randomised design with four biological replicates was developed for a growth system using a hydroponic nutrient solution (Hoagland solution, Hoagland and Arnon 1950). The beetroot seedlings subsequently reached a uniform height and were transferred to 0.5 L PVC pots containing 1/2 Hoagland nutrient solution, and culturing continued for approximately 12 days.

The Mel and salt dosages employed in the present research were established in the following four groups with four biological replicates: 0 mM (Control), NaCl (250 mM), Mel (50 µM), and NaCl (250 mM) + Mel (50 µM). Salinity treatment was applied incrementally, by adding 50 mM per day until the final desired salt concentration was reached in order to achieve the desired salinity concentration (250 mM) in a gradual manner, avoiding osmotic shock to the plants. The beetroot seedlings were grown under the following conditions: 25° C for 16 h in the light and 20° C for 8 h in the dark, 300 and 350 µmol·m^{-2}·s^{-1} light intensity, and 60–70% relative humidity. Mel was dissolved in a small amount of absolute ethanol, and the final required volume was adjusted using 1/2 Hoagland solution. The desired optimal Mel concentration was based on preliminary experiments and a recent previous study of ours (Colak et al. 2024) involving Mel at concentrations of 10, 25, 50 and 100 µM. In terms of NaCl concentrations, these were also based on the preliminary experiments and our recent study using the same beetroot cultivars at concentrations of 50, 150 and 250 mM to determine the optimal dosage for further experimentation (Colak et al. 2024) or on the basis of previous published research involving beetroots (Wu et al. 2013; Zhang et al. 2021; Liu et al. 2022; Wang et al. 2017, 2024). As the beetroot seedlings reached their third leaf stages after 12 days of treatment, they were harvested for further analysis (Fig. 1).

Measurement of Chlorophyll Fluorescence (*Fv/Fm*) and Stomatal Conductance (g_s)

Chlorophyll fluorescence (*Fv/Fm)*, a parameter that indicates the quantum efficiency of photosystem II, was determined using a plant efficiency analyser (Hansatech, UK). The *Fv/Fm* ratio for each treatment group of red and white beetroot was measured using fully expanded leaves (10 leaves) from each group. The leaves were adapted to a 20-minute dark period using leaf clips before measurement.

Determination of leaf stomatal conductance (g_s) was performed using a steady-state leaf porometer (SC-1, Decagon Devices, WA, USA) on fully expanded leaves ($n = 10$) between 11:00 and 14:00 h. The porometer was calibrated at 30-minute intervals throughout the entire experimental period.

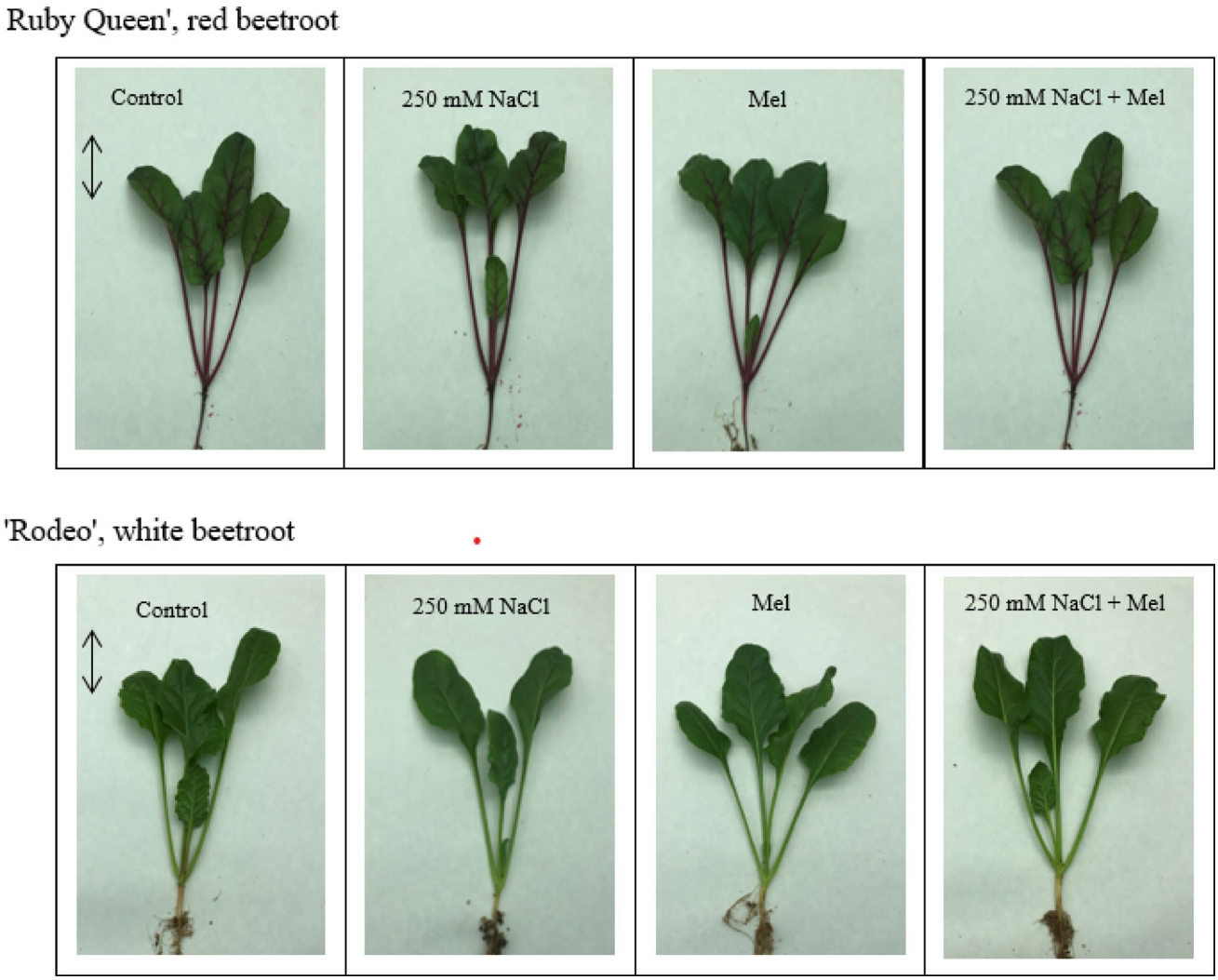


Fig. 1 Growth performance of red and white beetroot (*Beta vulgaris* L.) cultivars under the control condition and salt stress, in the presence of absence of exogenously applied melatonin (Mel). Mel; 50 µM. Scale bar: 3 cm

Measurement of Lipid Peroxidation (MDA) and H_2O_2 Content

Malondialdehyde (MDA) content was determined following the method described by Heath and Packer (1968) and published elsewhere, which is predicated on the thiobarbituric acid (TBARS) reaction. Briefly, 0.5 g fresh leaf sample was extracted using a solution of trichloroacetic acid (TCA; 0.1%). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4° C. The supernatant was then mixed with TCA and thiobarbituric acid (0.5% + 20% TCA). The reaction mixture was next incubated at 95° C for 30 min, and the absorbances at wavelengths of 532 and 600 nm were subsequently recorded. Results were expressed as nmol g^{-1} FW, calculated using an extinction coefficient of 155 mM cm^{-1} and an optical path length of 1 cm, i.e., $MDA = \Delta(OD532 - OD600)/1.56 \times 10^5$.

Determination of H_2O_2 was performed as described by Liu et al. (2014), as published elsewhere. A 5% (w/v) TCA solution in liquid nitrogen was used in the extraction. The homogenates were centrifuged at 10,000 rpm for 30 min at 4° C, and the resulting supernatant was used in the determination. The results were expressed as µmol 100 g^{-1} FW.

Lipid Extraction and Profiling

The extraction protocol was based on previously described methods published elsewhere (Welti et al. 2002; Xiao et al. 2010; Colak et al. 2025). Briefly, leaf discs of the studied beetroots ('Ruby Queen' and 'Rodeo') were treated with a 3 mL isopropanol solution containing 0.01% butylated hydroxytoluene (BHT) in a preheated 50 mL screw-capped glass tube at 75° C for 15 min to inactivate lipase. The lipids were then extracted five times with chloroform: methanol

(2:1, v/v), with 30 min of agitation each time. The extracts were next combined and washed once with 1 ml of 1 M potassium chloride (KCl) and once with 2 ml of deionised water. The organic solvent was then evaporated under nitrogen, and the lipid extract was dissolved in 1 ml of chloroform. The remaining plant tissues were dried overnight at 105° C and then weighed for lipid dry weight estimations. The total lipids were shipped on dry ice to the Kansas Lipidomics Research Center (Manhattan, Kansas, USA) for lipid content and composition determination by direct infusion sequential precursor and neutral loss scanning on an electrospray ionization triple quadrupole mass spectrometer (API 4000 QTrap; Applied Biosystems). Upon arrival, each sample was dissolved in 1 ml of chloroform, and 3–36 µl of each sample was used for polar lipid analysis. Molecular species of phospholipids and galactolipids were quantified in comparison to two internal standards of the same class as described previously (Xiao et al. 2010). Response factors were employed to correct for differences in phospholipid class quantifications between the internal standard mixture and the Avanti SPLASH LIPIDOMIX (Avanti Polar Lipids, Alabaster, AL, USA) to correct for differences in the response of the mass spectrometer to naturally occurring galactolipids in comparison to the saturated galactolipid internal standards. The SQDG was analysed on a Waters Xevo TQS mass spectrometer (Waters), using parameters employed in recent study published elsewhere (Colak et al. 2024) and quantified, without further correction, in comparison to the LPG 18:0 standard. Three biological replicates of each treatment for each beetroot cultivar were processed and analysed. The results were expressed as nmol mg^{-1} dry weight.

Table 1 The list of all primers used in the study

Gene	GeneBank ID	Primers
β-Tubulin	XM_010696832.2	Forward primer: 5'-CTGGC ACCATGGATTCTCTTA-3' Reverse primer: 5'-ATGTCC CTTAGCCCAGTTATTC-3'
18S_rRNA	AB008450	Forward primer: 5'-TCGGG CTTATCCCTTTGTATTT-3' Reverse primer: 5'-ACCAC CTGAACCTGCTAATG-3'
Actin	HQ656028.1	Forward primer: 5'-GTCT CCTGTCGTCTCTTTGT TT-3' Reverse primer: 5'-GCTCCT AAACCCTTTCCATCTC-3'
MGD	XM_019248096.1	Forward primer: 5'-GCATA CACCTTGGCCCTTTA-3' Reverse primer: 5'-ACGAG GAGCTGTCCCATAATA-3'
MGD2	XM_010689544.2	Forward primer: 5'-GGCG GTCGGAGAAAGATATT AG-3' Reverse primer: 5'-ACTG ACATCACCATCTCACA AA-3'
DGD1	XM_010677928.1	Forward primer: 5'-TAGCC AGTGTCCTTCAGAGTA-3' Reverse primer: 5'-TTTCCT GTCGGATGGGTTATG-3'
DGD2	XM_010673521.2	Forward primer: 5'-CTTC CTTGTCCTCCTCCAATA TG-3' Reverse primer: 5'-CCAAAT GCGGTTCGAGTAAAC-3'
SQD2	XM_010679807.2	Forward primer: 5'-CGCC AAGAGATGGAGAAGTA TG-3' Reverse primer: 5'-TCTTCC GCCAGAACCAAATAG-3'

Transcriptional Profiling of Lipid Biogenesis Genes

The expression of lipid biogenesis genes was determined by means of the RT-qPCR method. Beetroot lipid biogenesis genes encoding *MGD*, *DGD* and *SQD* were identified based on genome homology between plants. All plant MGD, DGG and SQD protein sequences were therefore obtained from the Uniprot database (https://www.uniprot.org/). These protein sequences were then scanned for homologs in the genome of *Beta vulgaris* ssp. *vulgaris* using the tblastn (https://blast.ncbi.nlm.nih.gov) algorithm (Altchul et al. 1990). For total RNA isolation, five equal-sized leaf discs from each experimental group were harvested, placed into 2 ml Eppendorf tubes, and immediately fixed in liquid nitrogen. The total RNA was extracted according to the TRIzol method (Akgul and Aydinoglu 2025). The cDNAs were synthesised with a SuperScript III First-Strand Synthesis Kit (Invitrogen) using the accompanying instructions. The primers were designed by SCiTools Oligo-Analyzer 3.1 (https://eu.idtdna.com) tool (Table 1). The designed primers were checked for melting temperature (Tm) at 58–61° C, hairpin formation at 50° C with $\Delta G > -3$ kcal/mol, and self- and heterodimer formation at 50° C with $\Delta G > -6$ kcal/mol and specificity using the BLASTn algorithm (Altschul et al. 1990). The expression level of the genes was determined using a RT-qPCR device (StepOne™ Real-Time System, Applied Biosystems) following the protocol supplied with Ampigene QPCR probe Mix (Enzo, 12012104). The relative transcript amount was determined according to the delta-delta Ct (2–ΔΔCt) method (Pfaffl 2001). Normalisation was performed with the beetroot β-tubulin, 18S_rRNA, and Actin genes (Table 1). The data represented an average of three biological and two technical replicates.

Statistical Analysis

The pigment contents, electrolyte leakage, membrane stability index and MDA and H_2O_2 content results represented

the measurements of four biological and technical repeats (mean ± SD, $n = 6$) and the lipid results are shown as three biological repeats ($n = 3$). One-way ANOVA and the multiple range test (IBM SPSS Statistics for Windows, version 22.0, Armonk, New York, USA, 2013) were employed to evaluate significant differences ($p < 0.05$) among the means. Principal component analysis (PCA) was performed to evaluate the variations in all measured physiological traits and lipid molecular species across the salt treatment and Mel application groups in comparison to the control groups.

Results

Changes in the external morphology of the beetroot leaves were observed and recorded under each treatment: control, Mel-applied (Mel), salt treated (S), and salt and Mel combined (S + Mel) (Fig. 1). Salt stress alone reduced the number of leaves to three mature leaves in the red and two in the white beetroot seedlings, while the control plants and the Mel-treated plants had four leaves. The Mel-treated red beetroot plants also appeared to have a fifth leaf at an early stage in the combined treatment (S + Mel) (Fig. 1).

Melatonin Application Under Salt Stress Increased Leaf Pigment Content and Fluorescence (*Fv/Fm*) in the Beetroots

Compared to the control plants, the applied salt stress (S; 250 mM NaCl) significantly ($p < 0.05$) reduced chl *a* and total chl in leaves in the red ('Ruby Queen') and the white ('Rodeo') beetroots. However, the pigment contents were significantly ($p < 0.05$) enhanced by the combined treatment (S + Mel) in 'Ruby Queen' (101 and 23%, respectively) and in 'Rodeo' (75 and 70%, respectively), relative to the salt-stressed plants (Fig. 2a). Mel application alone also induced notable increases in both contents compared to the control group. Similarly, the decrease in leaf carotenoid content was significantly suppressed ($p < 0.05$) by the salt treatment alone, but the content was increased by both Mel applications (23% in 'Ruby Queen' and 68% in 'Rodeo') compared to the control and the combined treatment (52% in 'Ruby Queen' and 43% in 'Rodeo') groups in comparison to salt stress alone. The *Fv/Fm* ratio was also enhanced by addition of Mel under both control and salt stress conditions (Fig. 2c). Likewise, the salt stress-induced decreases in stomatal conductance (g_s) in the red and the white beetroots were notably enhanced by Mel application (10% in 'Ruby Queen' and 14% in 'Rodeo') (Fig. 2d). These findings indicate that the negative responses of leaf photosynthetic efficiency and gs of the beetroots grown under salt stress were alleviated by the addition of Mel (S + Mel).

Fig. 2 Chlorophyll (**a**) and carotenoid (**b**) contents, *Fv/Fm* (**c**), stomatal conductance (**d**), electrolyte leakage (**e**), and membrane stability index (**f**) values of red ('Ruby Queen') and white ('Rodeo') beet cultivars grown under control conditions, salt stress (S; 250 mM NaCl) melatonin application (Mel; 50 µM) and salt stress with Mel application (S + Mel). Different lowercase letters above the columns indicate significant differences between the treatments according to a one-way ANOVA at $p < 0.05$. Each cultivar was compared individually. The same letters indicate no difference. Bars indicate ± SD. The four treatments were compared separately for each cultivar. Each column represents the mean of at least 15 seedlings. FW: fresh weight

Melatonin Application Boosted Leaf Electrolyte Leakage (EL) and Membrane Stability Index (MSI) in the Beetroots Under Salt Stress

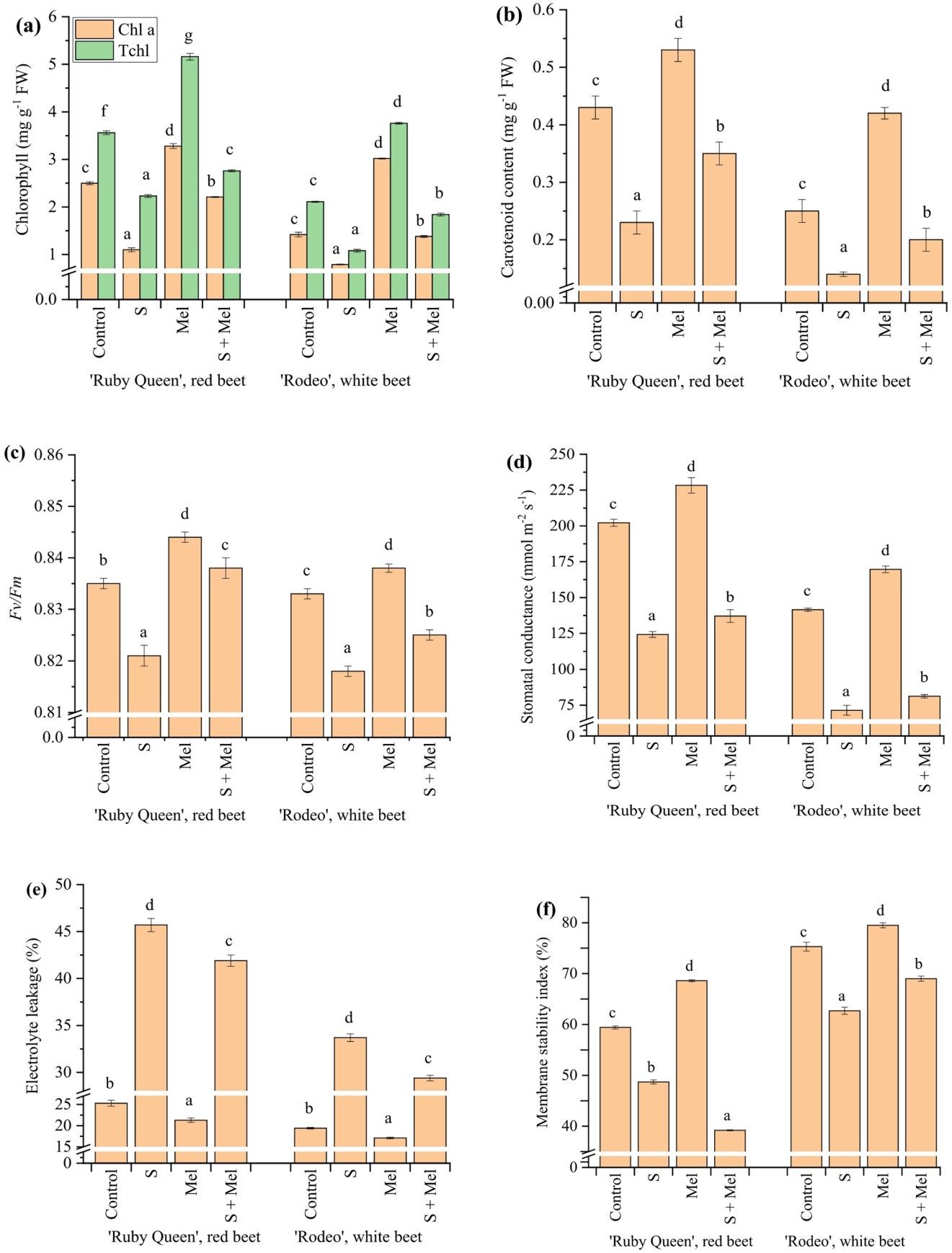
Compared to control values, salt stress alone significantly augmented EL (%)in both beetroot cultivars. The addition of Mel (S + Mel) led to decreases in EL in both cultivars (Fig. 2e). In white beetroot leaves, a salt stress-induced decrease in the membrane stability index (MSI, %) value was increased (10%) by exogenous Mel application. However, the red beetroot cultivar did not exhibit this effect, and a further decrease in MSI was in fact observed in the combined treatment (S + Mel) compared to salt stress alone (Fig. 2f). Taken together, these data show that Mel application reversed the adverse effects of salt stress-induced alterations by reducing oxidative damage and EL, but effects of Mel on ESI differed between the two cultivars for plants under salt stress (S + Mel).

Melatonin Application Under Salt Stress Lowered Hydrogen Peroxide Levels but Increased Malondialdehyde Content in the Beetroots

Salt stress raised hydrogen peroxide and MDA levels compared to the control plants. The induction of the stress indicators was cultivar-specific, in that the increase in H_2O_2 content (Fig. 3a) was significantly higher in salt-stressed cyanic/red beetroot (85%) than in salt-stressed white/acyanic beetroot (47%). Mel application induced marked decreases in H_2O_2 content in the salt stress-treated plants. Treatment of the beetroot seedlings with NaCl significantly increased MDA levels (Fig. 3b), particularly in the white beetroot (58%). However, the addition of Mel (S + Mel) failed to reduce MDA levels.

Melatonin Application Did not Uniformly Reverse the Effects of Salt Stress on Membrane Glycerolipids in the Beetroots

Table 2 summarises the total contents of each lipid class in leaves of red/betalainic ('Ruby Queen', red beetroot) and non-betalainic ('Rodeo', white beetroot) cultivars under salt


(a)
Chl a
Tchl
Chlorophyll (mg g-1 FW)
(b)
Carotenoid content (mg g-1 FW)
(c)
Fv/Fm
(d)
Stomatal conductance (mmol m-2 s-1)
(e)
Electrolyte leakage (%)
(f)
Membrane stability index (%)
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet

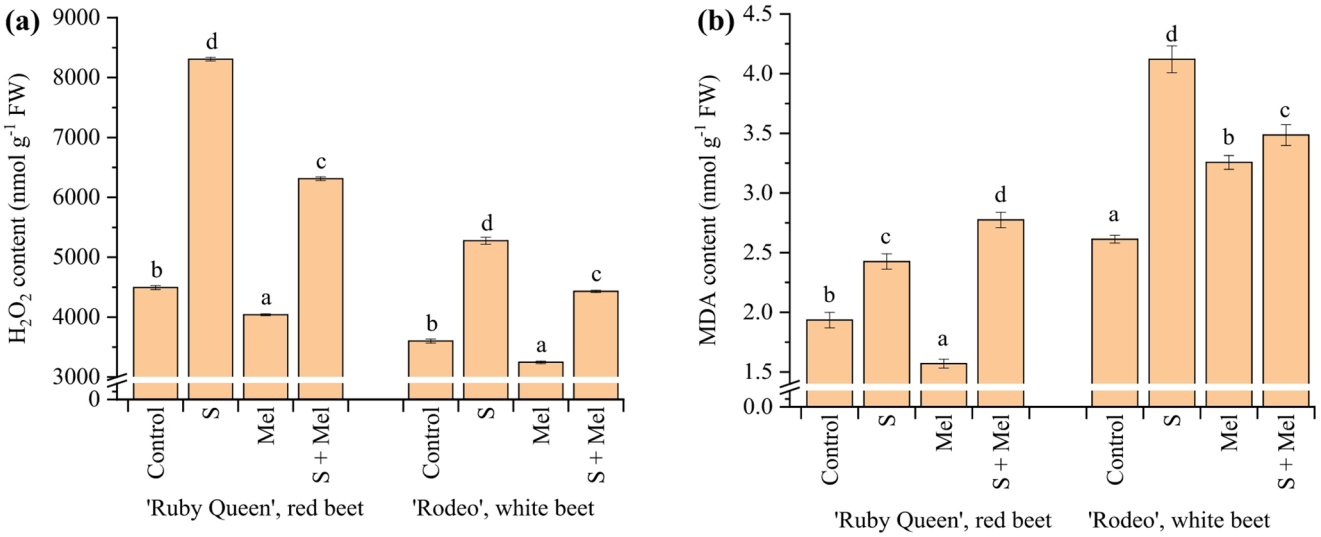


Fig. 3 Hydrogen peroxide (H_2O_2, a) and malondialdehyde (MDA, b) contents in leaves in red ('Ruby Queen') and white ('Rodeo') beet cultivars grown under control conditions, salt stress (S; 250 mM NaCl) melatonin application (Mel; 50 µM) and salt stress with Mel application (S + Mel). Different lowercase letters above the columns indicate significant differences between the treatments according to a one-way ANOVA at $p < 0.05$. Each cultivar was compared individually. The same letters indicate no difference. Bars indicate ± SD. The four treatments were compared separately for each cultivar. Each column represents the mean of at least 15 seedlings. FW: fresh weight

stress. As the table shows, MGDG and DGDG were dominant in the leaves, followed by PG and PC, being relatively high in leaves from the red beetroot. Total polar lipid content decreased significantly in both salt-stressed beetroots in comparison to the control plants (Table 2). The content was significantly reduced by the addition of Mel alone in the red beetroot and at an insignificant level in the white beetroot in comparison to the control seedlings. In terms of combined treatment (S + Mel), total polar lipid content was unchanged in the red beetroot and was reduced by the Mel supplement, compared to S (Table 2). PG and PC were the highest phospholipids in the beetroots. Compared to the control plants, PC content in both cultivars was unchanged by the salt treatment, whereas PG levels dropped in the red beet, but not in the white. The addition of Mel to the salt-treated beets did not restore PG levels in the red beet, and PC levels decreased in red beets in S + Mel compared to Mel application alone. The contents of other lipid analytes, PE, PI, PS, PA, LPG, LPC, LPE and SQDG, did not exhibit any patterns suggesting that Mel was capable of restoring decreases in levels due to salt stress (Table 2). Similarly, analysis of individual molecular species (Figs. 4, 5 and 6) did not reveal any changes that indicated that Mel significantly reduced decreases in lipid levels caused by salt stress.

Principal Component Analysis (PCA)

Data were analyzed using PCA based on Jolliffe's (2002) criterion. The bi-plots collectively highlighted close associations and strong correlations with the physiological traits and lipid molecular species as variables and with the treatments as observations (Fig. a and b, respectively). The principal components (PCs) obtained, PC1 and PC2, had eigenvalues greater than >1 and contributed to 100% of the entire accumulative variability among the physiological traits (Figs. 2 and 3). PC1 exhibited the greatest involvement in variability (99.73%), followed by PC2 (0.27%) (Fig. 8a). The first and second principal components contributed 100% in overall variances, which were plotted on PC1 (x-axis) and PC2 (y-axis) to determine the association amid different clusters (Fig. 8a). As shown in Fig. 8a, there were strong positive correlations between the treatments and the measured physiological traits of the beetroots (range; $r = 0.994–0.997$, $p < 0.05$, Fig. 8a). The H_2O_2 content of the red (RQ) and the white (R) beetroots resulted in a unique significant positive association with control (C), salt (S) treatment and Mel and the combined treatment (S + Mel) with their high score labels (Fig. 8a).

The PCA model was applied to all lipid data to determine the most important variables explaining the relationships between the lipid species and the salt and Mel treatments (Fig. 8b). Two PCs explaining 100% of the overall variance (73.98% and 24.76% for PC1 and PC2, respectively) divided the association into two quadrants, right upper and right below. The first principal component (PC1), which explains 73.98% of the overall variance, is clearly identified with the total MGMG, C36:6 MGDG and total polar lipid (TPL) contents closely associated and uniquely strong correlated with the treatments (range; $r = 0.992–0.999$, $p < 0.05$, Fig. 8b). PC1 clearly separates the red beetroot from the

Table 2 The total amount of lipid in red (cyanic) and white/sugar (non-cyanic) beetroot cultivars grown under salt stress and melatonin application conditions is shown. Values are means ± SD (nmol/mg dry weight; $n = 3$). Lipid amounts under various treatments were compared within each cultivar. a, b, c and d indicate significant differences ($p < 0.05$) between treatments. Mel: 50 µM

	'Ruby Queen', red beet (cyanic cv.)			
Lipid class	Control	250 mM NaCl	Mel	250 mM NaCl + Mel
DGDG	17.47 ± 0.69 d	16.42 ± 1.13 c	13.69 ± 0.66 a	14.78 ± 0.71 b
MGDG	70.26 ± 4.58 c	60.28 ± 6.94 b	54.35 ± 3.03 a	54.70 ± 3.67 a
SQDG**	1.64 ± 0.28 c	1.78 ± 0.25 c	1.38 ± 0.15 a	1.41 ± 0.13 b
PG	15.60 ± 0.23 c	13.02 ± 2.27 b	10.68 ± 1.53 a	13.82 ± 0.44 b
LysoPG	0.07 ± 0.02 b	0.04 ± 0.01 a	0.04 ± 0.02 a	0.04 ± 0.01 a
LysoPC	0.21 ± 0.01 c	0.12 ± 0.02 b	0.11 ± 0.03 a	0.08 ± 0.03 a
LysoPE	0.07 ± 0.02 b	0.04 ± 0.01 a	0.04 ± 0.02 a	0.03 ± 0.02 a
PC	17.65 ± 0.47 c	17.06 ± 2.12 c	12.50 ± 1.86 a	15.63 ± 1.90 b
PE	4.23 ± 0.58 b	3.20 ± 0.23 a	3.42 ± 0.53 a	3.53 ± 0.52 a
PI	3.09 ± 0.20 ab	2.98 ± 0.22 b	2.22 ± 0.15 a	2.82 ± 0.08 b
PS	0.56 ± 0.05 c	0.48 ± 0.06 b	0.38 ± 0.03 a	0.50 ± 0.03 c
PA	1.36 ± 0.11 d	1.21 ± 0.13 c	0.97 ± 0.08 a	1.17 ± 0.13 b
Total polar lipids	132.22 ± 6.71 b	116.65 ± 12.99 a	99.77 ± 7.33 a	108.52 ± 5.98 a
	'Rodeo', white beet (acyanic cv.)			
DGDG	14.84 ± 0.89 a	14.79 ± 1.90 a	15.03 ± 1.52 a	13.71 ± 0.45 a
MGDG	56.48 ± 2.84 c	50.82 ± 7.36 b	55.49 ± 8.01 c	43.52 ± 2.22 a
SQDG**	1.19 ± 0.16 a	1.21 ± 0.21 a	1.19 ± 0.17 a	1.14 ± 0.15 a
PG	11.86 ± 0.72 a	10.96 ± 1.92 a	11.94 ± 1.45 a	11.02 ± 0.81 a
LysoPG	0.02 ± 0.03 a	0.02 ± 0.10 a	0.02 ± 0.01a	0.02 ± 0.01 a
LysoPC	0.07 ± 0.01 a	0.07 ± 0.01 a	0.07 ± 0.02a	0.07 ± 0.01 a
LysoPE	0.04 ± 0.03 b	0.02 ± 0.02 a	0.04 ± 0.02 b	0.01 ± 0.02 a
PC	12.94 ± 0.24 a	12.10 ± 1.83 a	11.96 ± 0.97 a	12.98 ± 0.79 a
PE	4.01 ± 0.18 b	2.32 ± 0.57 a	4.05 ± 0.63 b	2.24 ± 0.19 a
PI	2.26 ± 0.15 b	1.94 ± 0.26 a	2.38 ± 0.23 c	1.81 ± 0.18 a
PS	0.51 ± 0.07 c	0.33 ± 0.05 b	0.49 ± 0.04 c	0.26 ± 0.03 a
PA	1.03 ± 0.09 b	0.92 ± 0.28 a	0.94 ± 0.12 a	1.09 ± 0.05 b
Total polar lipids	105.24 ± 4.34 c	95.49 ± 14.26 b	103.61 ± 12.49 c	87.87 ± 4.59 a

* The a, b, c, and d indicate significant differences ($p < 0.05$) among the trea tments

** The SQDG molar amounts are estimates based on comparison to a non-SQDG internal standard

white beetroot based on the lipid patterns. These findings indicated that the salt stress and Mel application exhibited a cultivar-specific effect on the values of the measured physiological traits and the lipid divergence. Principal component analysis reports the main significant contributors to overall variation in each differentiation axis (Fig. 8ab). The eigenvalues support describing all the variables from the measured physiological traits and lipid species that can be engaged. In this study, PC1 had eigenvalues > 1 and contributed 99.73% and 73.98% of total cumulative variability among different variables given in the figures (2, and 4–6). The PCA analysis in this study thus confirmed significant differences in the physiological traits and lipid species depending on the cultivar, the red/cyanic (betalainic) and the white/acyanic under salt stress and Mel application conditions. At the same time, it indicates some common features of the selected beetroot cultivars, owing to which the analyzed beetroots can be divided into more or less salt tolerant categories, with a cultivar-specific content of particular lipid species and measured physiological traits (e.g., H_2O_2 and MDA).

Melatonin-regulated Expressions of cultivar-specific Lipid Biogenesis Genes in the Beetroots Under Salt Stress

As shown in Fig. 7, the expression of the lipid biosynthesis genes was cultivar-specific. However, in the majority of cases the addition of Mel to plants under salt stress induced the expression of the analysed genes. Salt stress combined with Mel application (S + Mel) significantly upregulated the expressions of *DGD1* in the red beetroot, compared to D, while up*regulation was not statistically significant in the white beetroot treated with salt stress compared to the control group (Fig. 7a). *DGD2* expression downregulated in response to both salt stress and Mel application alone in both cultivars and was upregulated in both by the addition of Mel (S + Mel), compared to S (Fig. 7b). Salt stress and

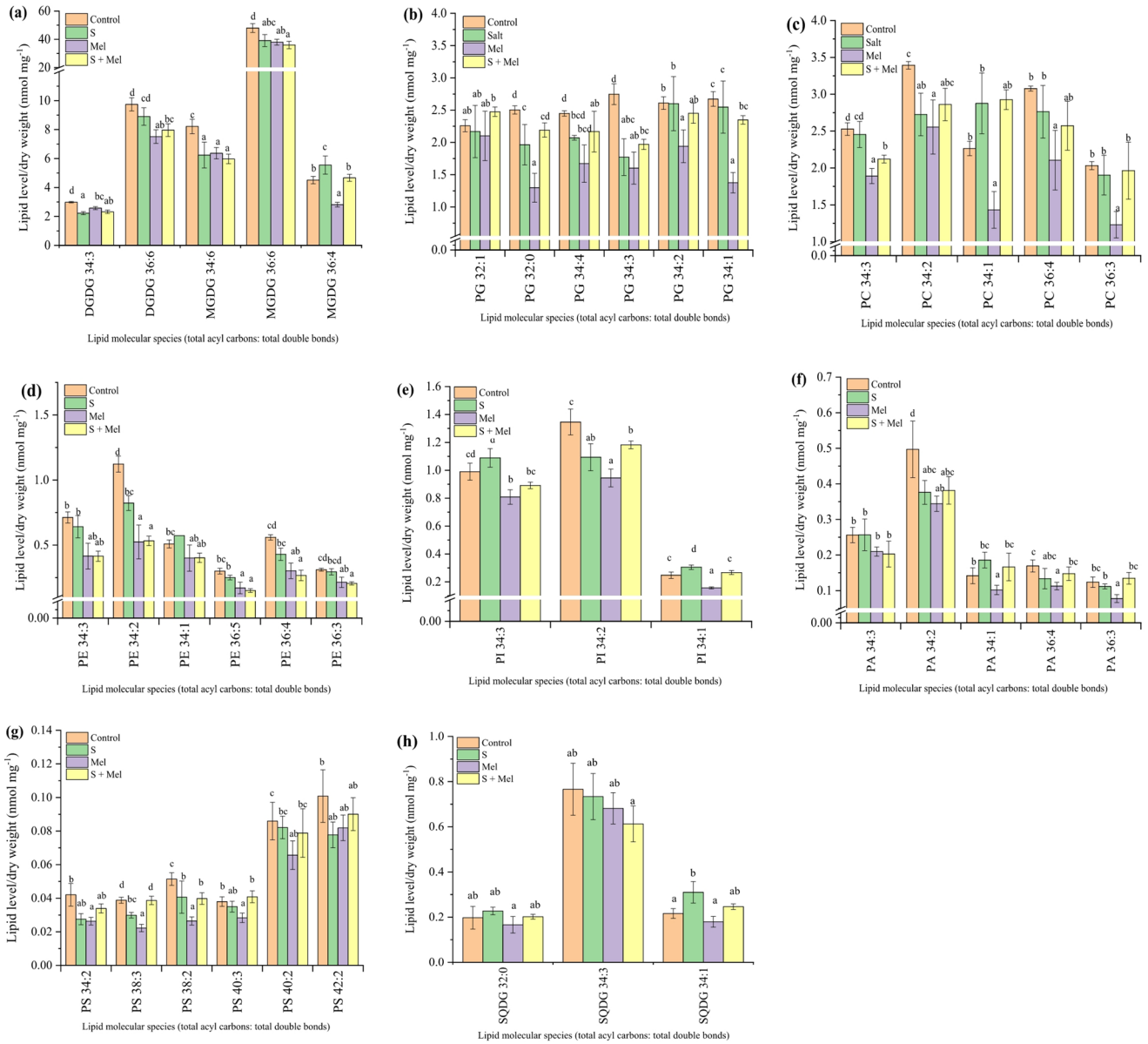


Fig. 4 Changes in molecular species of leaf lipids of red ('Ruby Queen') beet under control conditions, salt treatment (S; 250 mM NaCl), melatonin application (Mel; 50 µM), and salt treatment with Mel application (S + Mel). Changes in the molecular species of: DGDG and MGDG (**a**); PG (**b**); PC (**c**); PE (**d**); PI (**e**); PA (**f**); PS (**g**); sulfoquinovosyldiacylglycerol (SQDG, h). Values are means ± SD (nmol mg^{-1} dry weight; $n = 3$). The same letters indicate no difference ($p < 0.05$) among treatments, as calculated by one-way ANOVA. Bars indicate ± SD

Mel application alone significantly induced up-regulation of *MGD*, which was further induced by combined treatment in comparison to the control plants in the red beet (Fig. 7c). In contrast, in the white beet, relatively high up*regulation of *MGD* expression was achieved by the control plants. Significant down-regulation of *MGD* expression in salt-treated and Mel-treated beetroot was reversed by the combined treatment (S + Mel). *MGD2* was significantly up-regulated and down-regulated in white beet by the S and Mel treatments, although the combined treatments raised expression in both cultivars compared to salt stress alone (Fig. 7d). *SQD2* expression was also significantly up-regulated by combined treatment in both cultivars compared to its expression with salt treatment alone (Fig. 7e).

Discussion

Under our experimental conditions, the applied salt stress adversely suppressed seedling growth in comparison to the control plants. However, Mel treatments (Mel and SS + Mel) significantly augmented the growth performance

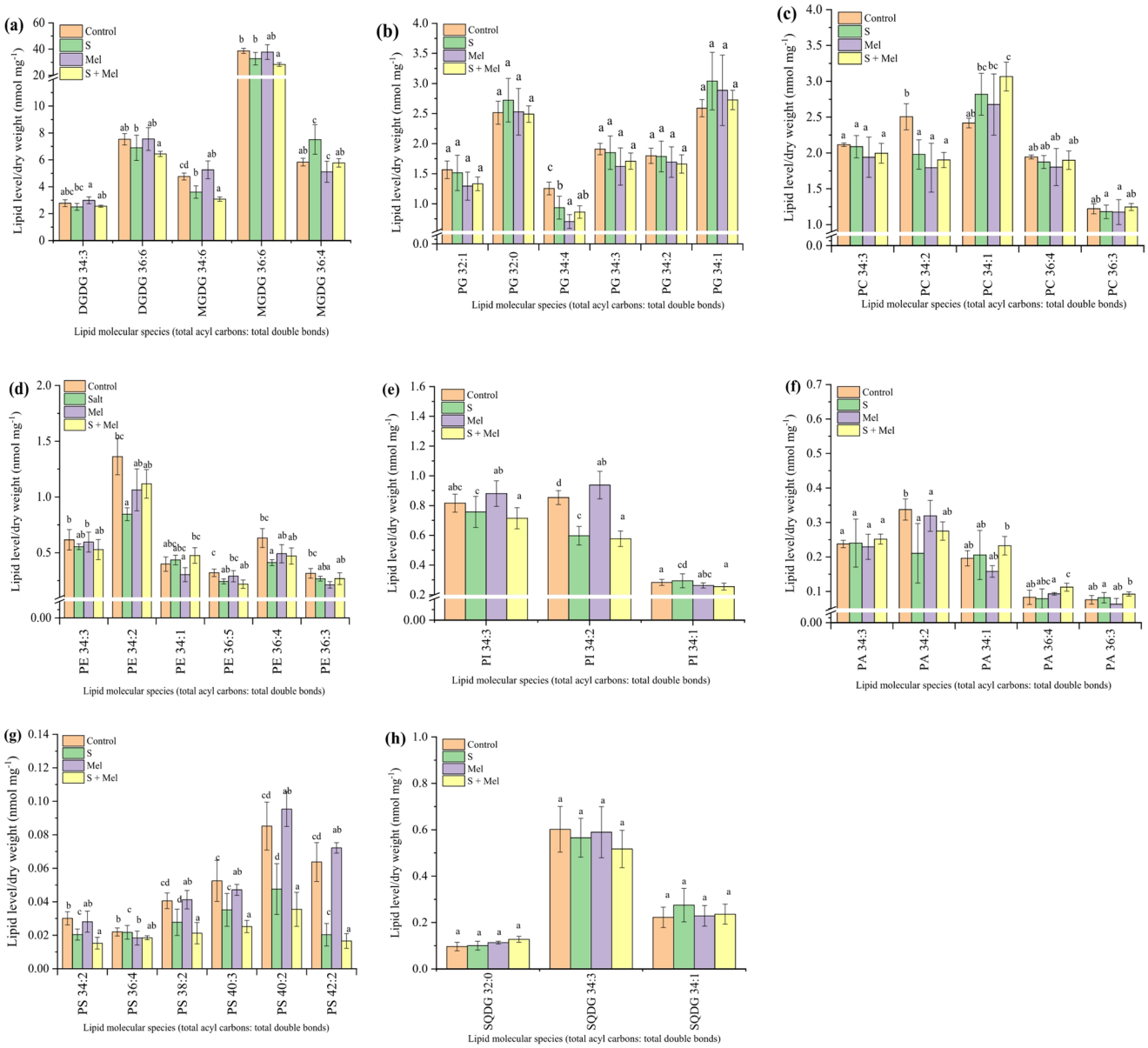


Fig. 5 Changes in molecular species of leaf lipids of white ('Rodeo') beet under control conditions, salt treatment (S; 250 mM NaCl), melatonin application (Mel; 50 µM), and salt treatment with Mel application (S + Mel). Changes in the molecular species of: DGDG and MGDG (**a**); PG (**b**); PC (**c**); PE (**d**); PI (**e**); PA (**f**); PS (**g**); sulfoquinovosyldiacylglycerol (SQDG, h). Values are means ± SD (nmol mg^{-1} dry weight; $n = 3$). The same letters indicate no difference ($p < 0.05$) among treatments, as calculated by one-way ANOVA. Bars indicate ± SD

of the beetroots under salt stress in comparison to the control and salt-stressed plants. This result was consistent with the results of Colak et al. (2024) on red ('Ruby Queen' and 'Scarlet Supreme') and white/sugar beets ('Rodeo' and 'Ansa') and the results of Zhang et al. (2021) on sugar beet 'KWS0143' with Mel application under salt stress (600 mM NaCl). These studies indicated that red beetroots (Colak et al. 2024), maintain good growth conditions under low or moderate salt concentrations.

Photosynthesis is also highly sensitive to salt stress. Decreases in chlorophyll content and the maximal efficiency of PSII (*Fv/Fm*) in the current plants are therefore inevitable (Liu et al. 2024). The *Fv/Fm* ratio is a highly important factor for evaluating photosynthetic performance. It is therefore considered the main physiological marker associated with PSII reaction centre damage or increased thermal dissipation of excitation energy (Jiang et al. 2020; Liu et al. 2024; Colak et al. 2025). Therefore, an increase in chlorophyll content promotes the capture and utilization of light energy by photosynthetic organs, providing a foundation for maintaining PSII and PSI activities (Liu et al. 2024). The results of the present study also revealed a substantial

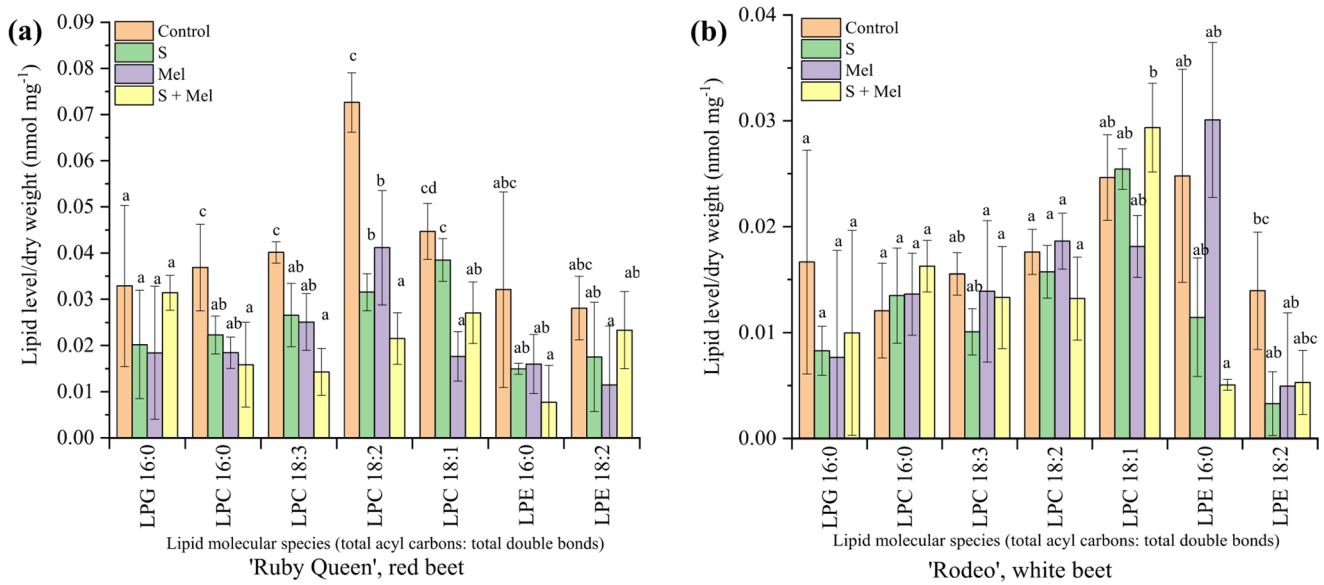


Fig. 6 Changes in molecular species of leaf lysolipids of red (**a**; 'Ruby Queen') (**a**) and white (**b**; 'Rodeo') beet cultivars under control conditions, salt treatment (S; 250 mM NaCl), melatonin application (Mel; 50 µM), and salt treatment with Mel application (S + Mel). Values are means ± SD (nmol mg^{-1} dry weight; $n = 3$). The same letters indicate no significant difference ($p < 0.05$) between treatments, as determined by one-way ANOVA. Bars indicate ± SD

cultivar-specific decrease in chlorophyll content and *Fv/Fm* in salt-stressed beetroot seedlings, particularly in the red beet. This adverse effect on the contents of chl and carotenoid pigments in both beetroots was alleviated by Mel supplementation in the salinised conditions. These results were in good accordance with study on sugar beet 'KWS0143' (Zhang et al. 2021), where the *Fv/Fm* and stomatal conductance (g_s) under salt stress (600 mM NaCl) decreased in the plant. Similar results were also noted in study of Wang et al. (2017) on sugar beet 'ST13092' demonstrating a notable dose-dependent decreases in chl content, photosynthetic rate, g_s and intercellular CO_2 concentrations in the plant under salinized condition (3–280 mM NaCl). The present experiment showed that Mel application may alleviate the salt stress hazards in photosynthetic machinery in the leaves of the beetroots, thereby improving downhill electron transfer in PSII.

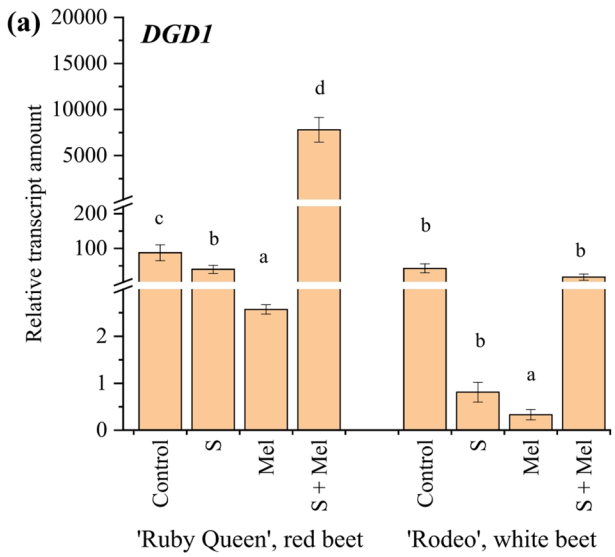
Electrolyte leakage (EL) and membrane stability index (MSI) are widely used stress indicator tests for measuring plant stress tolerance in response to stress-induced injuries (Demidchik et al. 2014; Sanwal et al. 2022). An increase in salt stress induced-increase in EL and the concurrent distribution of Na^+ within cells and organs, leading to toxic effects on membrane permeability and ion efflux (Demidchik et al. 2014; Ge et al. 2022). In the current study, the applied salt stress notably induced cultivar-specific increases in EL values, particularly in the red beetroot ('Ruby Queen'). However, this increment was reduced in the combined treatment (S + Mel) in the current beetroots (Fig. 2e). In agreement, similar results were also shown in studies on sugar beets (Zhang et al. 2021; Wang et al. 2024). Relative water loss from plant cells has a deleterious impact on membrane structure and function (Sanwal et al. 2022), while the stress caused a reduction in MSI values. Previous studies also noted the positive effect of Mel in terms of improving MSI (Helal et al. 2024). Consistent with previous studies by Zhang et al. (2021) and Wang et al. (2024), both the red and white beetroots in the present study exhibited significant improvements in MSI and concomitant decreases in lipid peroxidation.

The applied salt stress also led to cultivar-specific notable elevation in H_2O_2 (e.g., red beetroot) and MDA (e.g., white beetroot) contents. Mel-treated beetroots exhibited a decrease in H_2O_2 and MDA contents compared to the levels observed in plants under salt stress, and combined treatment (S + Mel), respectively. This is consistent with findings reported for cotton grown under salt stress and Mel application (Jiang et al. 2021). Mel directly scavenges H_2O_2 and enhances the activities of antioxidant enzymes (Colak et al. 2024) to detoxify H_2O_2 indirectly. This is probably due to Mel, as an electron donor, reacting directly with H_2O_2, hydroxyl radicals (·OH) and related molecules (Colak et al. 2024). The current results suggest that the applied salt stress led to insignificant decreases in total polar lipid contents of the beetroots. Such a decrease in salt stress conditions has been attributed to promoted lipid degradation and elevated lipid peroxidation (Yu et al. 2018, 2021; Ge et al. 2022; Henschel et al. 2024). Mel thus improves the beetroot seedlings' ability to scavenge ROS and reduces membrane lipid peroxidation damage to cells.

Salt stress led to species-specific alterations in membrane integrity and permeability during remodelling. In this context, halophytes increase non-bilayer forming and negatively charged lipids, in particular PE and PS, to minimise membrane damage, reduce plasma membrane depolarisation and maintain K^+/Na^+ balance (Su et al. 2025). Salt stress has been reported to induce lipolysis and lipid peroxidation and reduce lipid biosynthesis, thus lowering total membrane lipid contents in salt-sensitive species. However, salt-tolerant species increase lipid homeostasis, also increasing total lipid content. Salt stress also increases the saturation of fatty acids in sensitive species, resulting in more rigid membranes. However, halophytes exhibit a high proportion of unsaturated fatty acids, thus maintaining membrane fluidity. In addition, salt-tolerant species increase the contents and proportions of specific lipid groups, such as phospholipids. For instance, PC, PE, PI and PS contents are increased in these species, with high PC/PE ratios also being related to increased salt tolerance (Henschel et al. 2024). In agreement, in the current beetroots, salt stress induced an increase in the PC/PE ratio in the red (5.3) and the white (5.2) cultivars. However, Mel application alone led to cultivar-specific decreases in that ratios (3.6 and 2.9, respectively). Noteworthily, combined treatment (S + Mel) particularly increased the ratio in white beetroot (5.8). A higher PC/PE ratio has also been reported to contribute to the formation of the bilayer phase and the maintenance of membrane fluidity (Bian et al. 2023). The present study revealed that the salt stress-induced loss in the levels of PC species (e.g., 34:1–4) in leaves of the red beetroot increased, while the levels of PE species (34:1–6 were subsequently lowered. In contrast, salt stress-induced decreases in the levels of both PC and PE species in the white beetroot were non-significantly promoted. Studies have shown that PC maintains membrane fluidity and PE-rigidified membranes sustain membrane stability under salt stress (Bian et al. 2023; Henschel et al. 2024; Su et al. 2025). The current results suggest that both cultivars tended to increase the content of saturated or unsaturated PC lipids, even if this was not statistically significant, in order to maintain membrane fluidity and stability under salt stress. In addition, salt stress supplemented by Mel application (S + Mel) induced PC or PE unsaturations in the white beetroot.

MGDG and DGDG are a crucial component of the PSII lipid structure on thylakoid membranes, comprising ~ 80% of this, and are essential for photosynthesis (Liu et al. 2021; Shimojima and Ohta 2022). In the present study, the applied salt stress resulted in a pronounced diminution of MGDG, DGDG, SQDG and PG contents, except for an insignificant increase in PG levels in the white beetroot. These findings are consistent with previous reports of salt-stress-induced declines in MGDG and DGDG levels in other plant species, such as *Thellungiella halophila*, *Sulla carnosa*, and *S. coronaria* (Liu et al. 2021), and sorghum (Ge et al. 2022), in which drastic declines in lipid classes were reported. PG, the major phospholipid in chloroplast envelopes, is the principal plastidic phospholipid and is essential for maintaining the structure and function of chloroplasts and thylakoid membranes of the photosystem complexes. It plays an important role in plant salt tolerance (Liu et al. 2021; Bian et al. 2023; Su et al. 2025). A significant increase in PG content has been shown to confer a survival advantage, associated with less damage to the thylakoid membrane and better photosynthetic capacity under salt stress (Henschel et al. 2024; Su et al. 2025). In the present case, PG species exhibited significant cultivar-specific changes, either salt stress and Mel application alone or the combined treatment (S + Mel). This phenomenon may indicate that the thylakoid membrane stability was likely to be maintained through increasing/reducing the PG contents to enhance survival rate in these salt-tolerant beetroots under salt stress and Mel application treatments. It has been reported that the biosynthesis of MGDG and DGDG is essential for stress tolerance in plants (Henschel et al. 2024; Su et al. 2025). Research has demonstrated that a decline in the level of DGDG and MGDG leads to a decrease in chlorophyll content and photochemical efficiency (Su et al. 2025). In the present beetroots, insignificant salt stress- or Mel application-induced changes in the lipid species can be attributed to some extent to the different salt tolerances of these two beetroots.

Significant enhancement was obtained in the gene expressions of the key enzymes of lipid biosynthesis (*DGD1*, *DGD2*, *MGD*, *MGD2* and *SQD2*) investigated in the present beetroots (Fig. 8), although this was not fully reflected in the lipid levels. The relative transcript levels of *DGD1*, *DGD2*, *MGD*, *MGD2* and *SQD2* in both beet cultivars were significantly down-regulated by salt stress and Mel applications alone (see Fig. 8). In contrast, a significant up-regulation occurred when salt stress was combined with Mel application. The observed discrepancies between the treatments and lipid levels, or to some extent gene expressions, may be attributable to the difference in salt stresss responses of the current beetroot cultivars managed by a good capacity of K^+ and Ca^{2+} maintenance and an efficient ROS scavenging capacity supplemented by the presence of betalains and phenolics (e.g., phenolic acids) (Colak et al. 2024). Interestingly, we observed significant differences in basic physiological parameters, consistent with recently published findings (osmotic potential, minerals, betalains, and phenolic acids) (Colak et al. 2024). However, further research is required to elucidate the mechanisms by which these specific lipid species modify the physical properties of the membrane during Mel-applied remodelling, and how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DGD1
Relative transcript amount
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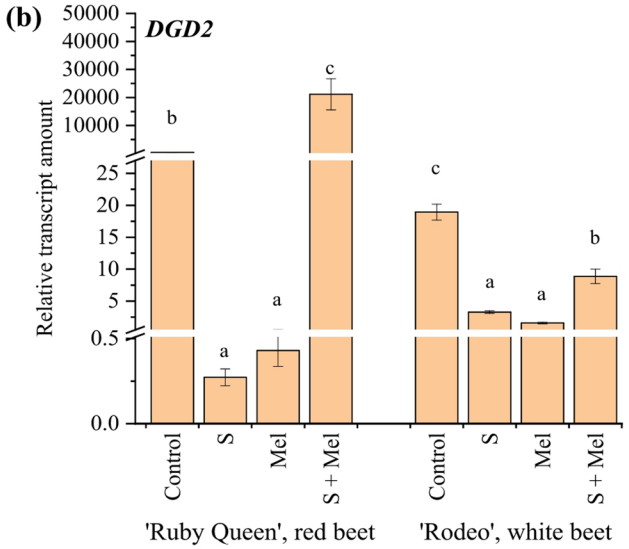
15000
10000
5000
200
100
2
1
0
c
b
a
d
b
b
a
b
Control
S
Mel
S + Mel
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet


(b)
DGD2
Relative transcript amount
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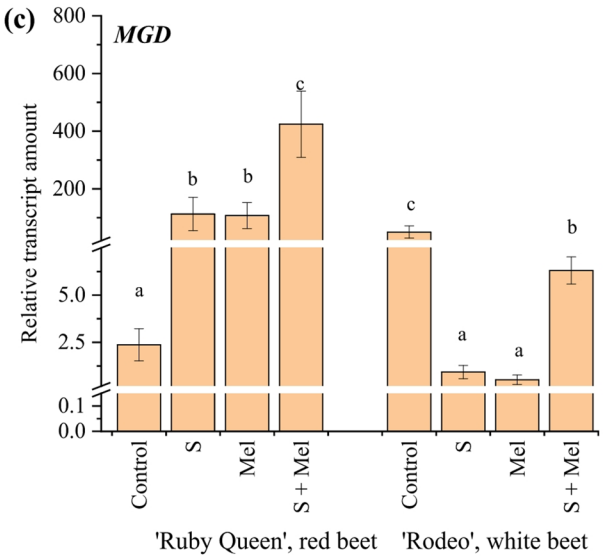
30000
20000
10000
25
20
15
10
5
0.5
0.0
b
a
a
c
c
a
a
b
Control
S
Mel
S + Mel
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet


(c)
MGD
Relative transcript amount
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600
400
200
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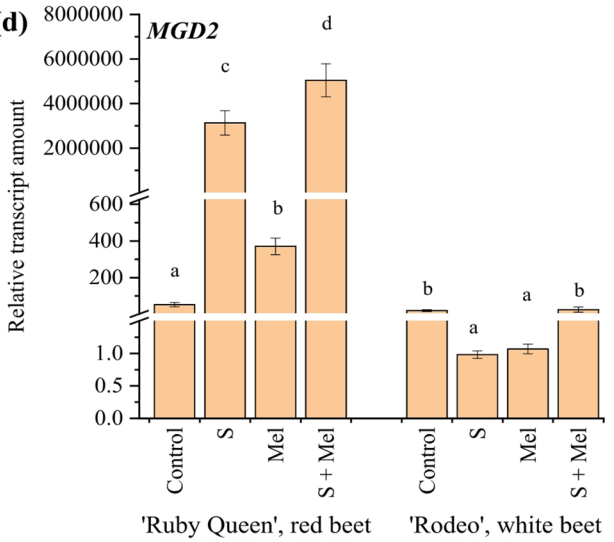
2.5
0.1
0.0
a
b
b
c
c
a
a
b
Control
S
Mel
S + Mel
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet


(d)
MGD2
Relative transcript amount
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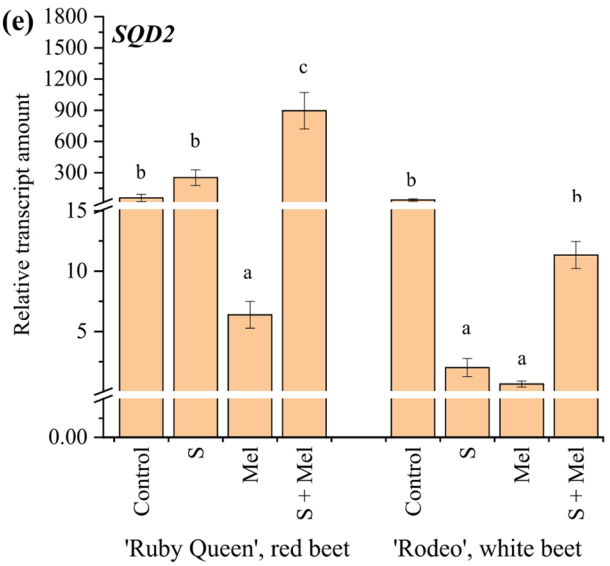
6000000
4000000
2000000
600
400
200
1.0
0.5
0.0
a
c
b
d
b
a
a
b
Control
S
Mel
S + Mel
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet


(e)
SQD2
Relative transcript amount
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1500
1200
900
600
300
15
10
5
0.00
b
b
a
c
b
a
a
b
Control
S
Mel
S + Mel
Control
S
Mel
S + Mel
'Ruby Queen', red beet
'Rodeo', white beet

Fig. 7 Expressions of galactolipids (DGDG and MGDG) and sulfoquinovosyldiacylglycerol (SQDG) genes in the leaves of red (*Beta vulgaris* 'Ruby Queen') and white (*B. vulgaris* 'Rodeo') beetroot cultivars under salt stress (S, 250 mM) and melatonin application (Mel, 50 µM). (**a**) *DGD1*; *digalactosyldiacylglycerolsynthase1* (**b**) *DGD2; digalactosyldiacylglycerolsynthase2*, (**c**) *MGD*; *monogalactosyldiacylglycerol synthase* and (**d**) *MGD2*; *monogalactosyldiacylglycerol synthase2* and (**e**) *SQD2sulfoquinovosyl diacylglycerol synthase2*. Values are means ± SD ($n = 3$). Different letters indicate significant differences at $p < 0.05$

they facilitate the beetroots' tolerance to salt under conditions of Mel application.

Plants cope with salt stress by limiting the uptake of Na^+ and Cl^- into the roots, sequestering Na^+ and Cl^- into vacuoles and enhancing Na^+ and Cl^- exclusion from shoots and leaves. Plants are also classified as halophytes or glycophytes, depending on their levels of tolerance to salt, this being either retained or excluded in shoots and roots (Liu et al. 2024). Halophytes are primarily attributable to their adaptation to saline environments rather than to the exclusion of salts. Conversely, glycophytes have been observed to exclude Na^+ and Cl^- from their shoots, whilst retaining a greater quantity of toxic ions in their roots. Consequently, the accumulation of toxic ions into vacuoles can facilitate the osmotic regulation of salt-tolerant plants in the example of beetroots (Colak et al. 2024). The regulation of Na^+ uptake into the plant cells is predominantly facilitated by the high-affinity K^+ transporter (HKT1) and non-selective cation channels (NCSSs). The chloride ions (Cl^-) enter cells primarily via chloride channels (CLCs), specifically the slow anion channel-associated 1 homolog 3 (SLAC1/SLAH). Ion antiporters, including the Na^+/H^+ antiporter (NHX1) and the salt overly sensitive protein (SOS1), play a pivotal role in the regulation of cytosolic Na^+ levels. These antiporters function either by extruding Na^+ from the cytoplasm or by sequestering it into vacuoles. K^+ is imperative for the regulation of cell osmotic potential, the activation of enzymes, the mitigation of oxidative stress and the protection of plants from abiotic stress-induced damage. Halophytes have been observed to selectively absorb and transport K^+ into their aerial parts via specific K^+ transporters (AKT1 and HAK5), thereby contributing to the maintenance of a high K^+/Na^+ ratio within the leaves (Liu et al. 2024; Colak et al. 2024). Mel application has been reported to provide a good K^+/Na^+ homeostasis when combined with salt stress. Consistent with findings reported by Shabala and Pottosin (2014) and Yu et al. (2018), the salt stress-triggered H_2O_2 or MDA contents in the studies led to an enhancement of activity of a range of K^+ permeable channels in perturbation of intracellular K^+ homeostasis. This good capacity of salt stress + Mel-driven K^+ maintenance can be partially ascribed to Mel-induced ROS scavenging and subsequent deactivation of ROS-dependent K^+ permeable channels, consistent with previous findings by Yu et al. (2018) in sweet potato and recent findings by Colak et al. (2024) in four beetroot cultivars. Colak et al. (2024) noted a cultivar-specific enhancement in K^+ and Ca^{2+} concentrations when salt stress was combined with Mel application. Current findings indicated that salt stress alone and its combination with Mel is not capable of altering the lipids of the studied beetroot cultivars at significant levels, leading to apparent lipid remodelling. These two treatments are also not intended to induce lipid remodelling that might result in excessive membrane stability or in the formation of lamellar phases within the bilayer.

Conclusion

Current results may represent potential mechanisms by which Mel mitigates damage to red and white beetroot caused by salt stress. However, salt stress and Mel application, either alone, or in combination, caused alterations in levels of lipid molecular species that were mostly insignificant. Future studies should further investigate the role of exogenous Mel application under salt stress conditions by comparing other salt-tolerant red and white beetroot cultivars already under cultivation. The present study also supports other above researches by showing that red beetroot cultivars are more tolerant to salt stress than white/sugar beets due to their high betacyanin contents and high Na^+ retention in their tissues. Based on the current results, the white beetroot produces salt stress responses earlier, inducing the production and activation of antioxidant defence components, and eliminating ROS, with an abrupt increase in stress indicators, rather than leading to lipid remodelling. Future studies are now needed to further expand on lipid remodelling research with other red and white beetroot cultivars under salt stress and Mel application conditions, including under field conditions field conditions. This will shed light on the membrane lipidomics of exogenously applied hormone-like chemical agents during salt stress tolerance, in terms of halophytes and glycolphytes.

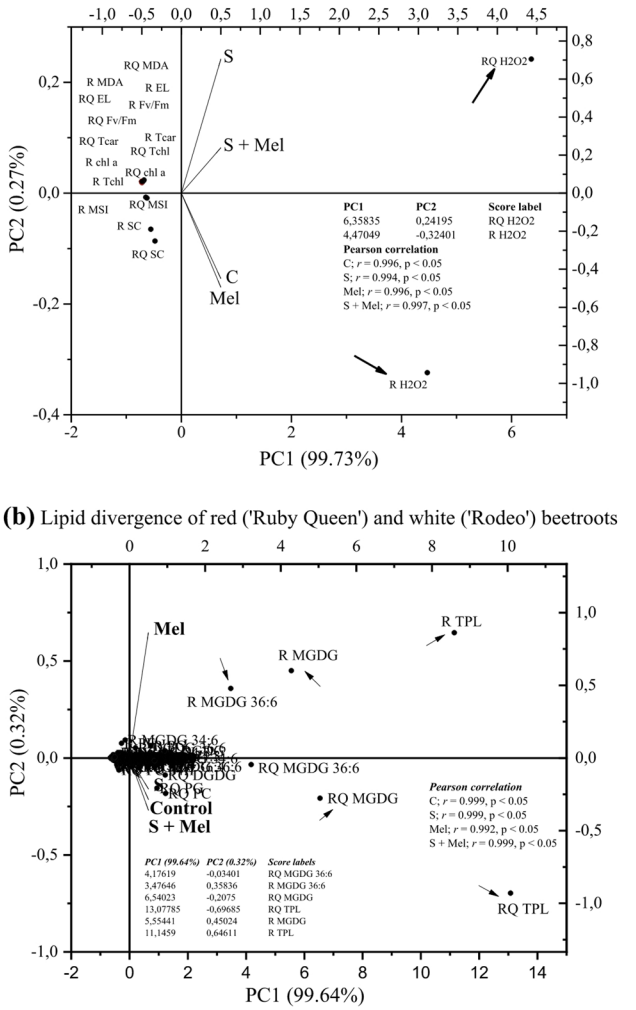

Fig. 8 Bi-plots of the principal component analysis (PCA) illustrating the clustering patterns of (**a**) physiological traits and (**b**) lipid divergence of red (RQ; 'Ruby Queen') and white (R; 'Rodeo') beetroots under salt stres (S; 250 mM NaCl) treatment and melatonin (Mel; 50 µM) application

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Data Availability Data sharing is not applicable to this article as all newly created data are already contained within this paper.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that no conflict of interest applies.

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