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Stomata and Xylem Vessels Traits Improved by Melatonin Application Contribute to Enhancing Salt Tolerance and Fatty Acid Composition of *Brassica napus* L. Plants

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Abstract: Salinity stress is a limiting factor for the growth and yield quality of rapeseed. The potentiality of melatonin (MT; 0, 25, 50, and 100 μM) application as a seed priming agent in mediating K^+/Na^+ homeostasis and preventing the salinity stress mediated oxidative damage and photosynthetic inhibition was studied in two rapeseed cultivars. We found that 50 μM MT treatment imparted a very prominent impact on growth, metabolism of antioxidants, photosynthesis, osmolytes, secondary metabolites, yield, and fatty acids composition. Days required for appearance of first flower and 50% flowering were decreased by MT application. Exogenous MT treatment effectively decreased the oxidative damage by significantly declining the generation of superoxide and hydrogen peroxide under saline and non-saline conditions, as reflected in lowered lipid peroxidation, heightened membrane stability, and up-regulation of antioxidant enzymes (catalase, superoxide dismutase, and ascorbate peroxidase). Furthermore, MT application enhanced the chlorophyll content, photosynthetic rate, relative water content, K^+/Na^+ homeostasis, soluble sugars, and proline content. Moreover, MT application obviously improved the oil quality of rapeseed cultivars by reducing glucosinolates, saturated fatty acids (palmitic and arachidic acids), and enhancing unsaturated fatty acids (linolenic and oleic acids except erucic acid were reduced). Yield related-traits such as silique traits, seed yield per plant, 1000 seeds weight, seed oil content, and yield biomass traits were enhanced by MT application. The anatomical analysis of leaf and stem showed that stomatal and xylem vessels traits are associated with sodium chloride tolerance, yield, and seed fatty acid composition. These results suggest the supportive role of MT on the quality and quantity of rapeseed oil yield.

Keywords: rapeseed; salinity stress; N-acetyl-5-methoxytryptamine; seed oil quality; antioxidant system; microstructure

1. Introduction

Salinity is known as one of the environmental stresses, and limits agricultural production and threatens land productivity, affecting food sustainability in many parts of the world. Globally, around 20% and 33% of cultivated and irrigated soil, respectively, is salt-influenced and degraded [1,2]. Salinity is an injurious stress for crop plants and reduces yield and quality. At the subcellular level, salinity stress induces oxidative stress, which has adverse effects on photosynthesis and chlorophyll fluorescence of plants [2,3]. Salinity inhibitory effects on plant growth and development are attributed to Na^+ and Cl^- ions phytotoxicity, low external osmotic potential, and nutrients deficiencies [2,4,5]. Ion phytotoxicity is caused by the substitution of K^+ by Na^+ in biochemical reactions and the loss of function of proteins, as Na^+ and Cl^- ions penetrate the hydration shells and interfere with the non-covalent interaction among the amino acids [6–8]. Salt-stressed plants often face disruptions in water relations and ion homeostasis owing to toxic ion accumulations. Plants have, therefore, many strategies/mechanisms to withstand salinity stress such as osmotic adjustment, Na^+ or Cl^- exclusion, and activation of the antioxidative system that protects sub-cellular structures from oxidative stress [2,4,7,9]. The reactive oxygen species (ROS) generation is increased during environmental stresses such as salinity. The excess generation of ROS damages proteins and nucleic acids and causes lipid peroxidation [10,11]. To alleviate the harmful impact of ROS, plant cells have developed defense systems containing enzymatic (superoxide dismutases (SODs), ascorbate peroxidase (APX), and catalase (CAT)) and non-enzymatic antioxidants (glutathione (GSH) and ascorbic acid (AsA)). Nector et al., [10] showed that superoxide can be converted to H_2O_2 by SODs. Next, H_2O_2 is scavenged into H_2O and O_2 by APX and/or CAT [12]. The oxidized ascorbate is reduced by the GSH that is generated by the oxidized glutathione (GSSG). The GSSG is then catalyzed by glutathione reductase (GR), resulting in nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) being sacrificed.

Rapeseed (*Brassica napus* L.) is one of the annual plants that belong to the *Brassicaceae* family. Globally, rapeseed is the second largest oil crop and its production was estimated at 71.50 million tons during 2016–2017 [13]. Although rapeseed is considered a semi salt-tolerant crop [14], salinity is an important global problem for rapeseed production [15]. Some rapeseed cultivars show high tolerance to salinity, while others are salt-sensitive [16,17]. Given the rising importance of rapeseed, it is of utmost importance to increase their tolerance against various environmental stresses, including salinity. Currently, genetic modifications and/or using some inducers such as plant hormones are the most important strategies for enhancing the plant performance under salinity stress. Hormonal treatment is commonly practiced to alleviate salt stress.

In this regard, plant hormones are an excellent choice for manipulating salinity stress. One of these plant hormones is melatonin (N-acetyl-5-methoxytryptamine), which is a pleiotropic molecule with multiple cellular and physiological functions in different organisms [18–21]. Melatonin (MT) is a naturally occurring compound in plants. MT may have the ability to regulate plant growth, development, and morphogenesis, and enhances crop production in different plant species [18–20,22]. MT was described as a stress tolerance inducer in many organisms because its natural synthesis is induced in plants exposed to biotic and abiotic stresses [21,23–27]. Previous reports showed that MT improved the resistance to multiple stresses such as drought, salinity, heavy metals, heat, pathogens, UV, radiations, chilling, herbicides, and nitrate stress [20,23,26–34]. MT has been reported to promote germination and early growth stages under NaCl stress in certain plants, for example, *Cucumis sativus* L. [35,36], *Gossypium hirsutum* L. [37], and *Cucumis melo* L. [29]. Germination, emergence, and development of rapeseed are negatively affected by NaCl stress [38,39], where they are critical factors affecting crop production [40]. The process of seed priming improves germination and enables seeds to overcome salinity stress and can improve plant establishment under stressful conditions [29,41,42]. Seed priming is a low cost technique and the easiest technique used for relief of the effects of the salinity issue [42,43]. Previous reports showed that exogenous treatment by MT is an appreciable way to improve stress tolerance in rapeseed at germination [44] and seedling stages [23], but the effective

doses of MT to promote rapeseed yield and its components under NaCl stress, as well as its role in improving water and nutrients transporting system, are not well documented. Therefore, this study was performed to test the hypothesis that MT application improves rapeseed yield under NaCl stress through enhancing ROS scavenging system, as well as morpho-physiological and anatomical traits.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Experimental Treatments

A pot experiment was conducted in the greenhouse of the College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China during 2018–2019. Agricultural soil was collected from the University Experimental Farm (depth 0–15 cm). The soil was air-dried and mixed daily until 9% of moisture content was reached. Air-dried soil was sieved through a sieve having 2 mm pores. Plastic pots (35 cm depth and 32 cm diameter) equally filled with 10 kg of air-dried soil. According to Soil Survey Staff [45], the soil texture of the used soil was silt clay loam. The soil had a pH of 6.78 (1:2.5, w/v, soil and water solution) and ECe 0.275 μS cm^{-1} (1:10, w/v, soil and water solution). The total organic matter, total N content, available P, and available K were 9.900, 0.122, 0.023, and 0.102 g kg^{-1} dry soil, respectively. Concentrations of Ca^{2+}, Mg^{2+}, K^+, and Na^+ cations were 6.05, 3.34, 4.83, and 0.52 g kg^{-1}, respectively. Cl^-, HCO_3^-, and SO_4^{2-} anions were 0.0055, 0.0637, and 0.222 g kg^{-1}, respectively [46]. All the pots were fertilized with urea, triple-superphosphate, and potassium sulfate fertilizers at rates of 0.20, 0.12, and 0.08 g kg^{-1} soil, respectively, which were incorporated into the soil before sowing. One month after sowing, plants were fertilized with urea at a rate of 0.20 g kg^{-1} soil, and the same amount of urea was applied after a month.

Rapeseed (*Brassica napus* L.) cultivars, Yangyoushuang2 and Xiangyouza553, were used as plant materials. Sterilized-seeds were primed in 25, 50, and 100 μM L^{-1} MT solutions for 8 h at 25 °C. Seeds primed in aerated water (hydro priming) used as a control, in which plants neither stressed with NaCl nor pretreated with MT (NsM0) or plants stressed with NaCl, but not pretreated with MT (SsM0). The volume of the priming solution was five times the weight of the seeds [47]. Seeds were washed thoroughly with distilled water and then re-dried near to the same original weight with forced air at 20 °C under shade. The MT was provided by Sigma Aldrich Company (M5250 SIGMA Melatonin powder >98% (TLC), China). On 9 October 2018, 10 seeds were sown in each plastic pot. The pots were randomly arranged in a greenhouse and rearranged several times during the growth period, and they were also isolated from raining. Thinning was carried out 14 days after sowing, leaving two plants in each pot. Pots were divided into two groups. One did not receive NaCl stress and the other group was irrigated with water containing 100 mM of NaCl. To maintain this salt concentration, the electrical conductivity (EC) was continuously monitored to avoid salt accumulation. Eighty-four pots were allocated for each group specified at 21 pots for each treatment. Eight treatments were formed in each experiment as follows: NsM0: non-NaCl stress + 0 μM MT, NsM25: non-NaCl stress + 25 μM MT, NsM50: non-NaCl stress + 50 μM MT, NsM100: non-NaCl stress + 100 μM MT, SsM0: 100 mM NaCl stress + 0 μM MT, SsM25: 100 mM NaCl stress + 25 μM MT, SsM50: 100 mM NaCl stress + 50 μM MT, and SsM100: 100 mM NaCl stress + 100 μM MT. Watering was carried out on regular intervals (3–7 days) throughout the experiment period. Irrigation of plants was supplemented until soil saturation and the excess water was allowed to drain out into the collection pans. The salinity treatments were maintained until the final harvest. The experimental layout was a completely randomized block design with three replicates (seven pots for each replicate) for each treatment. We selected 100 mM NaCl for the current study because the response of NaCl stress to different rapeseed cultivars varies considerably [17]. On the basis of our previous study [17], the highest level of NaCl (200 mM) resulted in a very prominent impact on growth, metabolism of antioxidants, photosynthesis, osmolytes, and secondary metabolites, especially for salt sensitive cultivars. While the moderate effect was generated by the level of 100 mM NaCl. In a preliminary study (unpublished), 25 μM, 50 μM, and 100 μM MT under normal or 100 mM NaCl conditions generated the greatest effects

on rapeseed germination and early growth stage. Therefore, these concentrations were selected for the main experiment for aiming of study role of MT in improving the yield and fatty acid composition of rapeseed cultivars. Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were assessed at the flowering stage (143 days after sowing). Samples were collected at the flowering stage (145 days after sowing) to assess the malondialdehyde (MDA) level, antioxidant enzyme activities, membrane stability index (MSI), and relative water content (RWC), as well as the concentrations of chlorophyll a, b, carotenoids, proline, total soluble sugars, Na^+, K^+, and Ca^{2+}.

2.2. Assessment of Growth Traits

Measurements of growth traits were performed at the flowering stage. Morphological parameters were measured using nine plants from each replicate. The number of leaves per plant was counted manually, and shoot and root lengths were measured using a meter scale. An LI-3000 portable area meter (LICOR, Lincoln, NE, USA) was used to measure total leaf area. Roots, leaves, and stem were placed in an oven at 105 °C for half an hour and then at 75 °C for 72 h.

2.3. Determination of Membrane Stability Index, Relative Water Content, Lipid Peroxidation, and Antioxidant Enzymes Activity

Cell membrane stability index (MSI) determination was done as described by Rady [48]. Relative water content (RWC) of rapeseed leaves was measured according to González & González-Vilar [49]. Fresh specimens of the second and third top fully expanded leaves were collected from the rapeseed plants and stored at −80 °C until analysis to determine the biochemical indices. Activities of catalase (CAT; EC 1.11.1.6), superoxide dismutase activities (SOD; EC 1.15.1.1), and ascorbate peroxidase (APX; EC 1.11.1.11), in addition MDA level, were assessed. Leaf sample (0.5 g) was homogenized in an ice-bath with 4.5 mL of 100 mM potassium phosphate buffer solution (pH 7.0) containing 1% polyvinylpyrrolidone (PVP) (w/v) and 0.05% Triton X-100 (v/v). For APX, the extraction buffer was supplemented with 2 mM ascorbic acid [50]. Homogenates were centrifuged at 15,000× g for 20 min at 4 °C. All biochemical indices of leaves were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (MEIMIAN, Jiangsu, China).

2.4. Determination of Ions, Proline, and Total Soluble Sugars Concentrations

For the quantitative analysis of Na^+, K^+, and Ca^{2+}, as well as proline and soluble sugars, the top fourth and fifth leaves of the rapeseed plants were dried at 70 °C for 3 days and were then ground into powder samples using tissue lyzer, MiLL MiXer MM300 (QIAGEN Inc., USA). Fifty mg of the powder was digested with 6 mL of 1 M HCl overnight and filtered through Whatman 45 mm paper. Ion contents were estimated using an FP6450 Flame Photometer (Shanghai instrument, China). Standard solutions of Na^+, K^+, and Ca^{2+} were used for calibration [51]. Proline concentration was assessed in the dried leaf samples using a rapid colourimetric method as previously described by Bates et al., [52]. The anthrone method was applied to determine the total soluble sugars [53].

2.5. Measurement of Chlorophyll Content, Photosynthetic Pigments and Gas Exchange Parameters

Chlorophyll measurement was carried out with soil and plant analyzer development (SPAD) value on the third top fully expanded leaf using a chlorophyll meter (Minolta, Japan). Nine readings per replicate with three replicates in each treatment were taken [54]. For photosynthetic pigments determination, five discs (each disc 1.00 cm diameter) of leaf tissue were homogenized with 25 mL acetone (80%) and then centrifuged. The samples were measured at absorbance values of 470, 645, and 662 nm (Perkin Elmer/Lambda 25) [55].

Gas-exchange parameters such as leaf net photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO_2 concentration (Ci), and stomatal conductance (gs) were assessed for photosynthetic

parameters using a portable photosynthetic system (LI-6400, United States). The data were taken between 09:00 and 11:00 using the third top leaf. The CO_2 concentration of the leaf chamber was 400 µmol mol^{-1}. The airflow speed was 500 µmol s^{-1}. The photosynthetically active radiation (PAR) was 1000 µmol m^{-2}s^{-1}. The air relative humidity was 75 ± 5% and leaf temperature was 24 ± 2 °C. The data were collected every 2–3 min with at least six replicates.

2.6. Assessment of Yield Traits

Assessment of yield traits was done at the end of the experiment; 21 plants were used from each treatment to measure plant height, root neck diameter, the number of effective branches, the total number of effective siliques, root biomass, shoot biomass, total siliques biomass, seed yield per plant, and 1000 seeds weight.

2.7. Assessment and Imaging of Green Siliques and Seeds Quality Traits

A flatbed scanner (BL-FS16-300) was used for imaging green siliques (pod containing seeds) and the seed of both rapeseed cultivars. The characteristics of the scanner system, including a plate-type charge-coupled device (CCD) scanner, are as follows: the optical resolution was 4800 × 9600 dpi, the maximum resolution was 19,200 dpi, and the range of scanning was 216 × 297 mm. Twelve green siliques were placed side by side on the glass panel of the scanner. Additionally, seeds were spread onto a specific glass-bottomed tray. The computer used in the experiment was Acer Aspire (Acer Inc., New Taipei City, Taiwan) C24-865-ACi5NT AIO Desktop, 23.8" Full HD, 8th Gen Intel Core i5-8250U, 12GB DDR4, 1TB HDD with 1G the flash memory and Windows 7 operating system. Image preprocessing and feature extraction were based on the Matlab R2008a integration platform (MathWorks, Natick, Massachusetts, USA).

2.8. Quantified and Qualified Determinations of Rapeseed Oil

The oil content of rapeseed was measured by a nuclear magnetic resonance instrument (NMR, mq-20, Bruker, Germany) [56]. The near-infrared reflectance spectroscopy (NIRS) method was performed using a near-infrared scanning monochromator (NIR System model 6500, Foss NIR Systems Inc., MD, USA) in the reflectance mode to determine the fatty acid composition of rapeseed oil. Intact seed samples (about 2 g) were placed in a standard ring cup and then scanned using standard methods [57].

2.9. Anatomical Traits and Analyses

For observation of stem and leaf anatomy, samples were taken at the beginning of the flowering stage. The fifth internode from the top of the stem with its leaf was fixed in formalin-acetic acid-alcohol (FAA) solution (containing 10 mL of formaldehyde + 50 mL of 95% (v/v) ethanol + 5 mL of glacial acetic acid + 35 mL of distilled water) for 72 h. Thereafter, the samples were dipped in 70% ethanol, cleared, and then dehydrated in tertiary butanol series, and embedded carefully in paraffin wax. Cross-sections, 20 µm thick, were cut by a rotary microtome (Zhejiang Jinhua Kedi Instrument Equipment Co., Ltd. China). The sections were sequentially placed two times in xylene for 20 min for each time, two times in anhydrous ethanol for 5 min, 75% alcohol for 5 min, and finally washed with tap water. For Safranin staining, a slice was put into the red dye solution for 1–2 h, washed by the fine stream of tap water, and then washed off the excess dye [58]. Decolourization was done by placing sections sequentially into 50%, 70%, and 80% gradient alcohols for 3–8 s. For solid green dyeing, the slices were stained for 30–60 s in solid green dyeing solution, and dehydrated in three cylinders of absolute ethanol. The sections were observed and documented using an upright optical microscope (Nikon Eclipse E100, Japan) equipped with a digital camera (Imaging system, Nikon DS-U3, Japan). Measurements were done using CaseViewer 2.3 software program (3DHISTECH Ltd.) for image analysis [59]. Assessment of stomata anatomical traits as described by Mohamed et al. [17].

2.10. Data Analysis

We used the Genstat 17th edition software package to detect significant differences [60]. Means were separated using the least significant difference (LSD) test at $p \leq 0.05$. The graphical presentation was carried out using Origin 8. We used R 3.5.1 to perform Pearson's correlation coefficient, the heatmap, and the principal components analysis.

3. Results

3.1. Melatonin Priming Improved Growth Traits of Rapeseed Cultivars

The results related to the impact of MT in the presence or absence of NaCl stress on growth traits of rapeseed plants are presented in Figure 1. Exposure of plants to NaCl stress obviously reduced all growth traits such as root length (RL), root neck diameter (RND), shoot length (SL), leaves number (LN), total leaves area (TLA), root dry weight (RDW), stem dry weight (SDW), and leaves dry weights (LDW). The mentioned growth traits were decreased under NaCl stress in Yangyoushuang2 by 5%, 22%, 46%, 7%, 16%, 23%, 36%, and 18%, respectively, while in Xiangyouza553, this reduction was 25%, 45%, 54%, 31%, 52%, 43%, 53%, and 49%, respectively, compared with the control. Under irrigation with saline (100 mM of NaCl) water, pre-treated seed with 25, 50, and 100 µM of MT recovered the stressed growth traits and improved growth traits compared with the NaCl-stressed control. MT treatment at 50 µM was more effective; it improved RL, RND, SL, LN, TLA, RDW, SDW, and LDW in Yangyoushuang2 by 9%, 9%, 11%, 4%, 7%, 31%, 14%, and 12%, respectively, while this improvement in Xiangyouza553 was 18%, 12%, 18%, 11%, 11%, 17%, 12%, and 19%, respectively, compared with SsM0 (NaCl stress + non-melatonin priming). However, the seeds treated with 25, 50, and 100 µM MT in the absence of NaCl stress stimulated the growth traits that were generally higher than that of the normal control (NsM0; no NaCl stress).
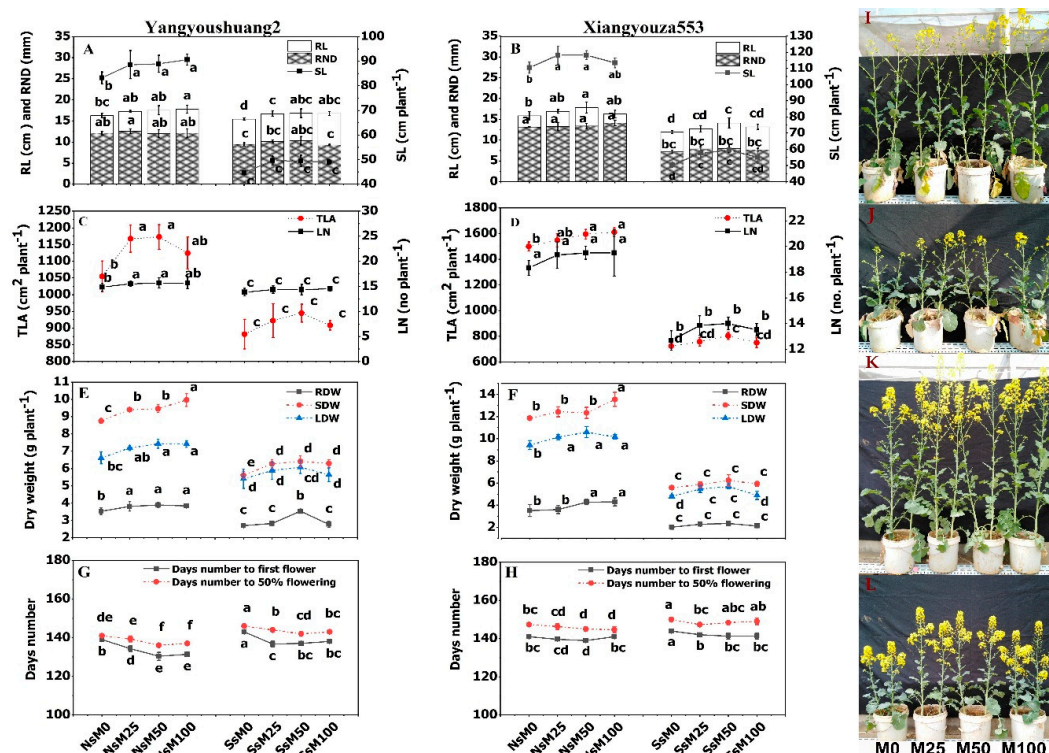

Figure 1. Effect of seed priming using 0 µM (M0), 25 µM (M25), 50 µM (M50) and 100 µM melatonin (M100) on root length (RL), root neck diameter (RND), shoot length (SL), number of leaves (NL), total leaves area (TLA), root dry weight (RDW), stem dry weight (SDW), leaves dry weight (LDW), and days required to first flower (DNFF) and 50% flowering (DN50F) of two rapeseed cultivars irrigated

with saline water. Ns: no NaCl stress, Ss: salt (NaCl) stress. (**A**) RL, RND and SL of Yangyoushuang2; (**B**) RL, RND and SL of Xiangyouza553; (**C**) TLA and LN of Yangyoushuang2; (**D**) TLA and LN of Xiangyouza553; (**E**) RDW, SDW and LDW of Yangyoushuang2; (**F**) RDW, SDW and LDW of Xiangyouza553; (**G**) DNFF and DN50F of Yangyoushuang2; (**H**) DNFF and DN50F of Xiangyouza553. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments ($p \leq 0.05$). The symptoms of plants at the flowering stage: (**I**) Xiangyouza553-Ns; (**J**) Xiangyouza553-Ss; (**K**) Yangyoushuang2-Ns; (**L**) Yangyoushuang2-Ss. Bar = 35 cm.

The appearance date of the first flower and 50% flowering in rapeseed plants was affected by the NaCl stress (Figure 1G,H). The days required for appearance of first flower (DNFF) and 50% flowering (DN50F) were increased from 139 and 141 days to 143 and 146 days for Yangyoushuang2 under NaCl stress conditions; similarly, in Xiangyouza553, the days required for appearance of first flower and 50% flowering were increased from 141 and 147 to 144 and 150 days under NaCl stress, respectively. MT application at all concentrations decreased the number of days required for appearance of first flower and 50% flowering under NaCl stress and the values were significantly ($p \leq 0.05$) lower compared with NaCl alone particularly at lower MT concentrations. The same trend was also observed under the application of MT at the three concentrations in the absence of NaCl stress.

3.2. Melatonin Priming Improved Antioxidant Enzymes Activities and Decreased Malondialdehyde

The activities of CAT, SOD, APX, and MDA level of NaCl-stressed plants were significantly increased for Yangyoushuang2 by up to 64%, 93%, 106%, and 41%, respectively, and 28%, 47%, 32%, and 111%, respectively, for Xiangyouza553 (Figure 2A–D). However, seed priming with MT further improved the activities of antioxidant enzymes in the absence or presence of NaCl stress. The application of 50 μM MT under NaCl stress had a maximum increase of CAT, SOD, and APX in Yangyoushuang2 and SOD and APX in Xiangyouza553. The maximum increase of CAT in Xiangyouza553 was observed by the application of 25 μM MT under NaCl stress condition. MT application at all three concentrations in presence of NaCl stress significantly decreased the MDA content compared with the NaCl-stressed control and the maximum decrease was observed by the application of 50 μM MT in Yangyoushuang2 and by 25 μM MT in Xiangyouza553.
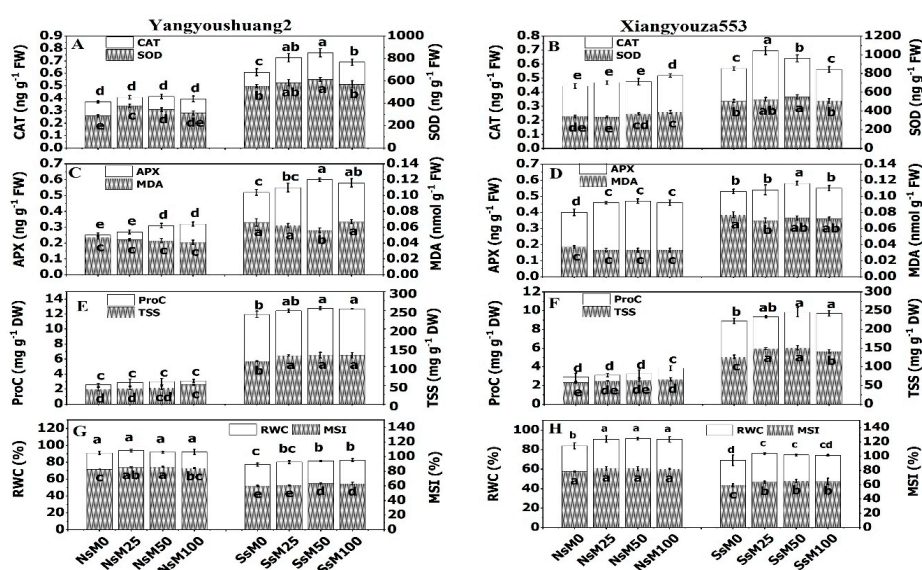

Figure 2. Response of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), malondialdehyde level (MDA), relative water content (RWC), membrane stability index (MSI), proline content (ProC), and total soluble sugars (TSS) of *B. napus* L. cultivars to seed priming with 0 μM (M0), 25 μM (M25), 50 μM (M50), and 100 μM melatonin (M100) under non-NaCl salt stress (Ns) and

NaCl salt stress (Ss). (**A**) CAT and SOD of Yangyoushuang2; (**B**) CAT and SOD of Xiangyouza553; (**C**) APX and MDA of Yangyoushuang2; (**D**) APX and MDA of Xiangyouza553; (**E**) ProC and TSS of Yangyoushuang2; (**F**) ProC and TSS of Xiangyouza553; (**G**) RWC and MSI of Yangyoushuang2; (**H**) RWC and MSI of Xiangyouza553. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments ($p < 0.05$).

3.3. Melatonin Priming and Accumulation of Osmolytes under NaCl Stress

Irrigation with saline water significantly ($p \leq 0.05$) increased the content of total soluble sugars (TSS) and proline (ProC) in Yangyoushuang2 by 2.90- and 4.70-fold, and by 2.20- and 3.10-fold, respectively, in Xiangyouza553, compared with unstressed plants (Figure 2E–F). However, MT treatment significantly enhanced ProC and TSS ($p \leq 0.05$) and the most effective level was 50 μM MT in general for both cultivars. The highest level of MT (100 μM) was found to be more effective to improve TSS and ProC levels in both cultivars under normal conditions.

3.4. Melatonin Priming Improved MSI and RWC

Data of membrane stability in terms of MSI and RWC of rapeseed plants grown under the effect of MT are shown in Figure 2G–H. The NaCl stress led to a significant decrease in RWC and MSI by 15% and 28% in Yangyoushuang2, and 18% and 25% in Xiangyouza553, respectively, compared with non-stressed controls. Priming seeds with 25, 50, and 100 μM MT improved the RWC and MSI in the presence of NaCl stress. Moreover, 50 μM or 100 μM of MT treatment showed a maximum increase of RWC and MSI for Yangyoushuang2, however, 25 μM or 50 μM of MT performed better under NaCl stress conditions for Xiangyouza553.

3.5. Melatonin Priming Improved K^+/Na^+ and Ca^{2+}/Na^+ Ratio

In this study, uptake of Na^+, K^+, and Ca^{2+} in the leaves of *B. napus* L. cultivars pre-treated with different levels of MT and grown under two levels of NaCl stress was also examined. According to the results, NaCl stress significantly ($p \leq 0.05$) increased the Na^+ level in leaves of both cultivars (Yangyoushuang2 by 3.1-fold and Xiangyouza553 by 4.5-fold). The results also showed a significant ($p \leq 0.05$) increase in uptake of Ca^{2+} and K^+ under NaCl stress in leaves of Yangyoushuang2 by 46% and 38%, and by 65% and 5%, respectively, in Xiangyouza553 (Figure 3A,B). It was observed that MT pre-treatment decreased the uptake of Na^+ ions under NaCl stress conditions. Further, 25 μM of MT was the most effective for decreasing the uptake of Na^+ in Xiangyouza553, while no significant ($p \leq 0.05$) decrease was observed between different levels of MT application for Yangyoushuang2. The maximum increase in K^+ content was observed by the treatment of 50 μM MT in Xiangyouza553. K^+/Na^+ and Ca^{2+}/Na^+ ratios were significantly decreased by 56% and 53% in Yangyoushuang2 and 77% and 63% in Xiangyouza553, respectively (Figure 3C,D). Seed priming with MT alleviated the stress by improving these parameters and the maximum increase was observed at 25 μM and 50 μM of MT treatment in Xiangyouza553; however, no difference was observed for Yangyoushuang2.

3.6. Melatonin Priming Improved Photosynthetic Pigments and Gas Exchange Parameters

The NaCl stress improved total chlorophyll (SPAD), chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) contents of the two rapeseed cultivars. However, priming the rapeseed seeds with MT induced an additional increased SPAD, Chl a, Chl b, and Car contents, particularly under NaCl stress. Under NaCl stress conditions, 50 μM MT treatment successfully increased the content of Chl a, Chl b, and Cars by 16%, 7%, and 13% for Yangyoushuang2 and 16%, 11%, and 9% for Xiangyouza553, respectively (Figure 4). Under irrigation with saline (100 mM of NaCl) water, net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) were significantly decreased in Yangyoushuang2 by 14%, 42%, 32%, and 31%, and in Xiangyouza553 by 21%, 48%, 34%, and 39%, respectively (Figure 4). However, seed pre-treatment with MT at all concentrations

activated the above-mentioned attributes in the absence or presence of NaCl stress and greatly improved these photosynthesis-related parameters compared with those of NaCl-stressed control.
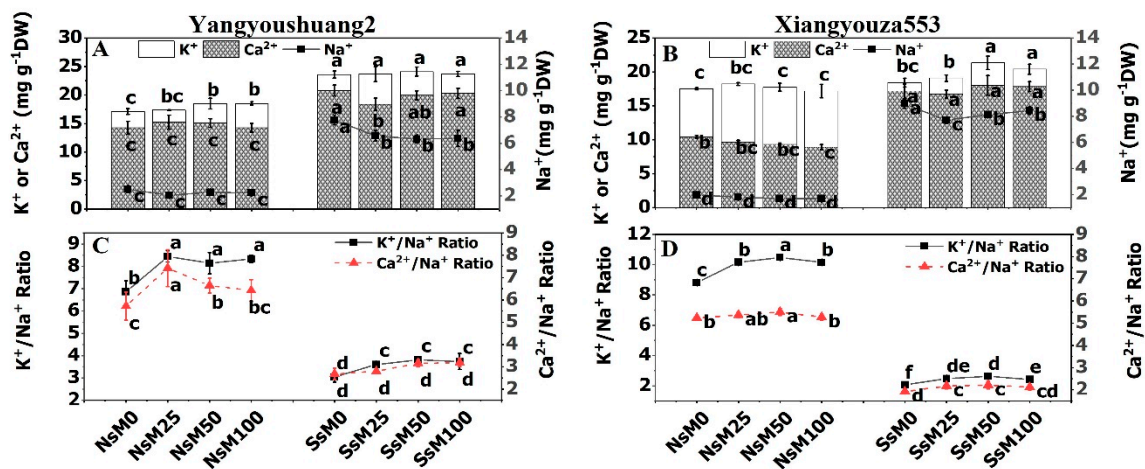

Figure 3. Response of Na^+, K^+, and Ca^{2+} concentrations, and K^+/Na^+ and Ca^{2+}/Na^+ ratio in *B. napus* L. cultivars to seed priming with 0 μM (M0), 25 μM (M25), 50 μM (M50), and 100 μM melatonin (M100) under non-NaCl salt stress (Ns) and NaCl salt stress (Ss). (**A**) Na^+, K^+ and Ca^{2+} concentrations in Yangyoushuang2; (**B**) Na^+, K^+ and Ca^{2+} concentrations in Xiangyouza553; (**C**) K^+/Na^+ and Ca^{2+}/Na^+ ratios in Yangyoushuang2; (**D**) K^+/Na^+ and Ca^{2+}/Na^+ ratio in Xiangyouza553. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments ($p \leq 0.05$).
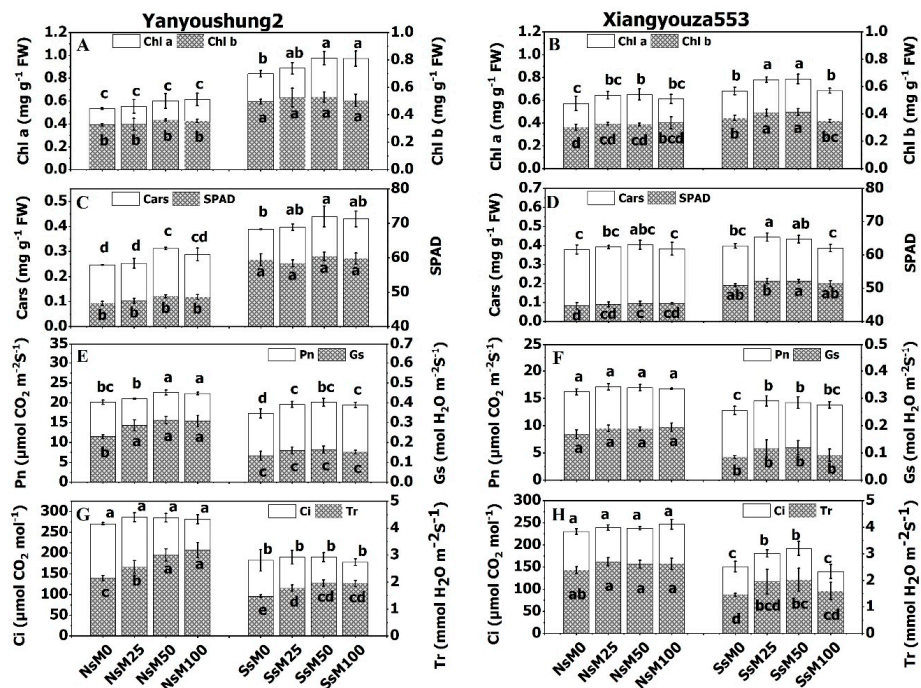

Figure 4. Response of two *B. napus* L. cultivars for chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Cars), soil and plant analyzer development (SPAD), net photosynthetic rate (Pn), stomatal conductance (Gs), intercellar CO_2 concentration (Ci), and transpiration rate (Tr) to seed priming with 0 μM (M0), 25 μM (M25), 50 μM (M50), and 100 μM melatonin (M100) under non-NaCl stress (Ns) and NaCl salt stress (Ss). (**A**) Chl a and Chl b of Yangyoushuang2; (**B**) Chl a and Chl b of Xiangyouza553; (**C**) Cars and SPAD of Yangyoushuang2; (**D**) Cars and SPAD of Xiangyouza553; (**E**) Pn and Gs of Yangyoushuang2; (**F**) Pn and Gs of Xiangyouza553; (**G**) Ci and Tr of Yangyoushuang2; (**H**) Ci and Tr of Xiangyouza53. Bars represent SD of three replicates. Different letters indicate significant differences among treatments ($p \leq 0.05$).

3.7. Melatonin Priming Improved Yield Traits and Fatty Acids Composition of Rapeseed Cultivars

In this study, the effect of NaCl stress and MT application on green siliques traits, seeds yield traits, seed quality traits, fatty acid composition, and yield biomass traits of *B. napus* cultivars was investigated. It was observed that NaCl stress decreased the total number of effective siliques (TES), the biomass of effective siliques (ESB), and green siliques traits (Figure 5A,B and Supplementary Table S1). The results showed an increase in green silique traits of *B. napus* cultivars with MT treatments under NaCl and non-NaCl stress compared with the non-pretreated control. The highest increase in most traits of green siliques was recorded with the treatment of 50 µM of MT under stress conditions, while, 50 µM or 100 µM of MT was best under non-stress conditions (Supplementary Table S1). The highest increase in dry siliques biomass was recorded with the treatment of 100 µM and 50 µM, under non-stress conditions, while the highest increase in dry siliques biomass was recorded with the treatment of 50 µM of MT under NaCl stress conditions. The same trend was observed for the total number of effective siliques, but no significant difference was observed among different concentrations of MT under the NaCl stress (Figure 5A,B). It was noticed that increasing the NaCl stress decreased the seed yield (SY) and 1000 seeds weight (1000 SW) in both *B. napus* cultivars. However, the application of MT increased the SY and 1000 SW. According to the results, the maximum increases in SY and 1000 SW were noticed at 100 µM followed by 50 µM of MT under non-stress conditions, while under NaCl stress, the maximum increase for SY and 1000 SW was noticed at 50 µM of MT (Figures 5C,D and 6).
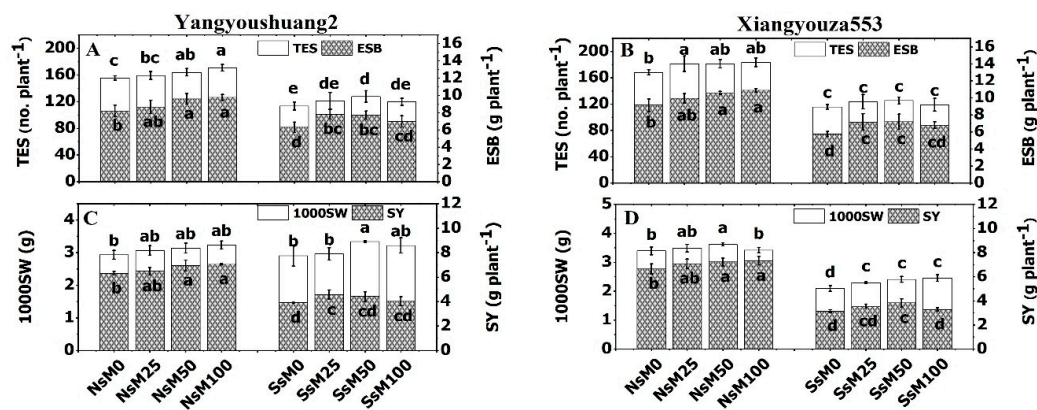

Figure 5. Effect of seed priming in 0 µM (M0), 25 µM (M25), 50 µM (M50), and 100 µM melatonin (M100) on total effective siliques number (TES), effective siliques biomass (ESB), 1000 seed weights (1000 SW), and seed yield per plant (SY) of two rapeseed cultivars irrigated with saline water. Ns: non-NaCl salt stress, Ss: NaCl Salt stress. (**A**) TES and ESB of Yangyoushuang2; (**B**) TES and ESB of Xiangyouza553; (**C**) 1000SW and SY of Yangyoushuang2; (**D**) 1000SW and SY of Xiangyouza553. Bars represent SD of three replicates. Different letters indicate significant differences among treatments ($p \leq 0.05$).
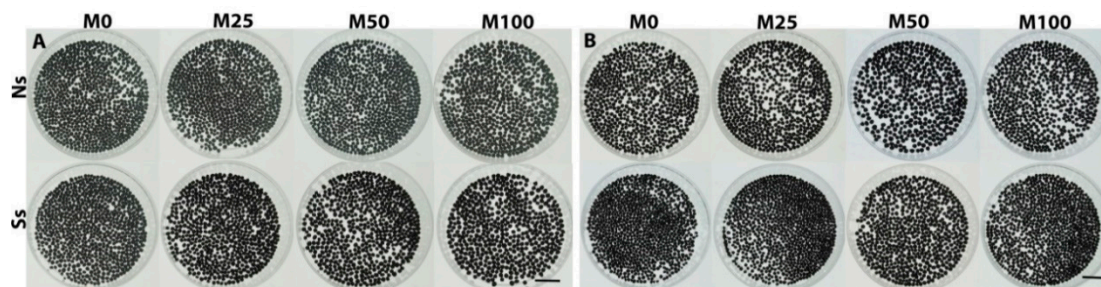

Figure 6. Photographs of two *B. napus* seeds affected by seed priming with 0 µM (M0), 25 µM (M25), 50 µM (M50), and 100 µM melatonin (M100) and salt stress (Ns: non-NaCl stress; Ss: 100 mM NaCl) using seed scanner. (**A**): Yangyoushuang2. (**B**): Xiangyouza553 cultivar. Bars = 1 cm.

The results of seed quality traits and fatty acid composition data are given in Figure 7 and Supplementary Table S2. It was noticed that increasing the NaCl stress significantly ($p \leq 0.05$) decreased the crude oil percentage (SOC), while crude protein percentage (SPC) increased in both *B. napus* cultivars (Figure 7A,B). The decrease in SOC owing to salinity stress treatment was 22% and 30%, while the increase in SPC owing to NaCl stress was 44% and 24% in Yangyoushuang2 and Xiangyouza553, respectively. However, the application of MT enhanced the SOC and SPC for both *B. napus* cultivars under NaCl or non-NaCl conditions. The highest increase in the SOC was recorded with 50 μM MT treatment for Yangyoushuang2 and Xiangyouza553; however, no significant difference was observed among 25 μM, 50 μM, and 100 μM MT for Xiangyouza553 under stress conditions (Figure 7A,B). The results showed that NaCl stress had a significant ($p \leq 0.01$) effect on seed moisture content, glucosinolate, and unsaturated and saturated fatty acids composition in both cultivars, except oleic acid in Xiangyouza553 cultivar, at $p \leq 0.05$ (Figure 7 and Supplementary Table S2). Glucosinolates, erucic, and saturated fatty acids (palmitic and arachidic acids) significantly ($p \leq 0.05$) decreased with the MT application, but the decrease in stearic acid was not significant. The interaction between MT and NaCl stress was non-significant for the seed quality traits and fatty acid composition traits, except arachidic acid in Xiangyouza553. MT pretreatment had a significant effect on the increase of linolenic and oleic acid in both cultivars, but the increase in linoleic acid was not significant ($p \leq 0.05$). In general, 50 μM or 100 μM of MT improved the oil quality and fatty acid composition traits under non-stress conditions, while 50 μM of MT was more effective under NaCl stress conditions for both cultivars (Figure 7 and Supplementary Table S2). Yield biomass traits were significantly decreased in the presence of NaCl stress such as plant height, root neck diameter, root biomass, and shoot biomass ($p \leq 0.01$) (Supplementary Table S3). MT application significantly enhanced the root neck diameter ($p \leq 0.01$) and shoot biomass ($p \leq 0.05$) in both cultivars, however, plant height was improved only in Xiangyouza553. The maximum increase was recorded at 50 μM of MT under NaCl stress. The interaction between MT and NaCl stress was not significant for the yield biomass traits, except the shoot biomass in Yangyoushuang2 (Supplementary Table S3). In the absence of NaCl stress, MT at 50 μM or 100 μM significantly ($p \leq 0.05$) improved plant height, root neck diameter, and shoot biomass compared with the non-NaCl stress control in both cultivars. Exogenous application of MT at all concentrations alleviated the deleterious effects of NaCl stress, particularly at 50 μM. In the presence of NaCl stress, MT at 50 μM had the highest increase in the plant height, root neck diameter, effective number of branches, root biomass, and shoot biomass in both cultivars, except root biomass and shoot biomass for Xiangyouza553.

3.8. Relationships

Pearson's correlation analysis was conducted to examine the relationship between observed parameters under the combination of MT and NaCl stress (Figure 8A). The results showed a significant positive correlation ($p \leq 0.05$) between SY, 1000 SW, SOC, linolenic acid with the growth, Pn, SPAD, ProC, K^+, K^+/Na^+, Ca^{2+}/Na^+, RWC, SOD, and CAT. Meanwhile, SPC, erucic acid, palmitic acid, MDA, and Na^+ had a negative correlation with above-mentioned traits. Besides this, the heat map showed a relation between interactive treatments and studied parameters (Figure 8B). The hierarchical analysis divided the different treatments into two main groups (NaCl stress (Ss) and non-NaCl stress (Ns)) and four sub-main groups (XYZ553-Ns, YYS2-Ns, XYZ553-Ss, and YYS2-Ss). Under NaCl stress, XYZ553-SsM25 and XYZ553-SsM50 clustered in the same group and showed higher performance compared with XYZ553-SsM0 and XYZ553-SsM100. Meanwhile, YYS2-SsM25, YYS2-SsM50, and YYS2-SsM100 were clustered in the same group that had higher performance compared with YYS2-SsM0. Under non-NaCl stress, YYS2-NsM100 and YYS2-NsM50 clustered in the same group that had better performance compared with YYS2-NsM25 or YYS2-NsM0. Meanwhile, XYZ553-NsM50, XYZ553-NsM25, and XYZ553-NsM100 clustered in the same group that had higher performance compared with XYZ553-NsM0. The overall results showed that using 50 μM MT alleviated the adverse effects of sodium chloride, as well as improved the growth and yield under

normal conditions. Interestingly, the application of salt stress increased DNFF and DN50F, palmitic acid, Na^+, Ca^{2+} concentration, MDA level, erucic acid, K^+, Chl a, Chl b, SPAD, oleic acid, Cars, APX, SPC, TSS, CAT, ProC, and SOD. While Gs, Ci, Pn, arachidic, RL, 1000 WS, glucosinolate, stearic, TLA, LDW, LN, RWC, SOC, Ca^{2+}/Na^+, K^+/Na^+ ratio, RDW, RND, SY, Tr, ESB, linolenic, SL, SDW, TES, MSI, and linoleic acid were decreased. Owing to high variation resulting from applied NaCl stress and normal condition in both cultivars, we carried out two principal component analyses (PCA) to show the effect of melatonin on all the parameters studied under normal condition and NaCl stress. The two components (Dim1 and Dim2) together explained 86.0% and 86.7% of data variability under normal and NaCl stress, respectively (Figure 8C,D). Under normal conditions, MT treatment at different concentrations resulted in an increasing trend in the variables of oleic, linoleic, SY, ESB, SPC, 1000 SW, RDW, SDW, LDW, TLA, LN, RND, CAT, APX, SOD, K^+/Na^+ ratio, TSS, ProC, Chl a, and Cars in Yangyoushuang2 in the following order: 50 μM > 100 μM > 25 μM MT (Figure 8C). Meanwhile, a cluster of linoleic, arachidic, SOC, RL, MSI, RWC, K^+, Ca^{2+}/Na^+ ratio, Ca^{+2}, SPAD, Chl b, Pn, Gs, and Ci was correlated with MT treatment in the following order: 100 μM > 50 μM > 25 μM in Xiangyouza553. Glucosinolate, palmitic, stearic, erucic, Na^+ level, and MDA were associated with the normal condition only (without MT). On the other hand, plants supplemented with MT under NaCl stress resulted in an increasing trend in the variables of 1000 SW, SY, SOC, linolenic, LDW, LN, RND, CAT, SOD, RWC, K^+, Ca^{2+}/Na^+, K^+/Na^+, ProC, Ci, Chl a, Chl b, and SPAD in Yangyoushuang2 in the following order: 50 μM > 25 μM > 100 μM MT (Figure 8D). Meanwhile, linoleic, oleic, SPC, ESB, TES, SDW, SL, APX, Tr, Cars, MSI, and TSS were associated with MT treatment in the following order: 50 μM > 25 μM in Xiangyouza553. The results of PCA showed that erucic, palmitic, steric, MDA, Na^+ level, DNFF, and DN50F were grouped in the cluster associated with NaCl stress only (without MT treatment).
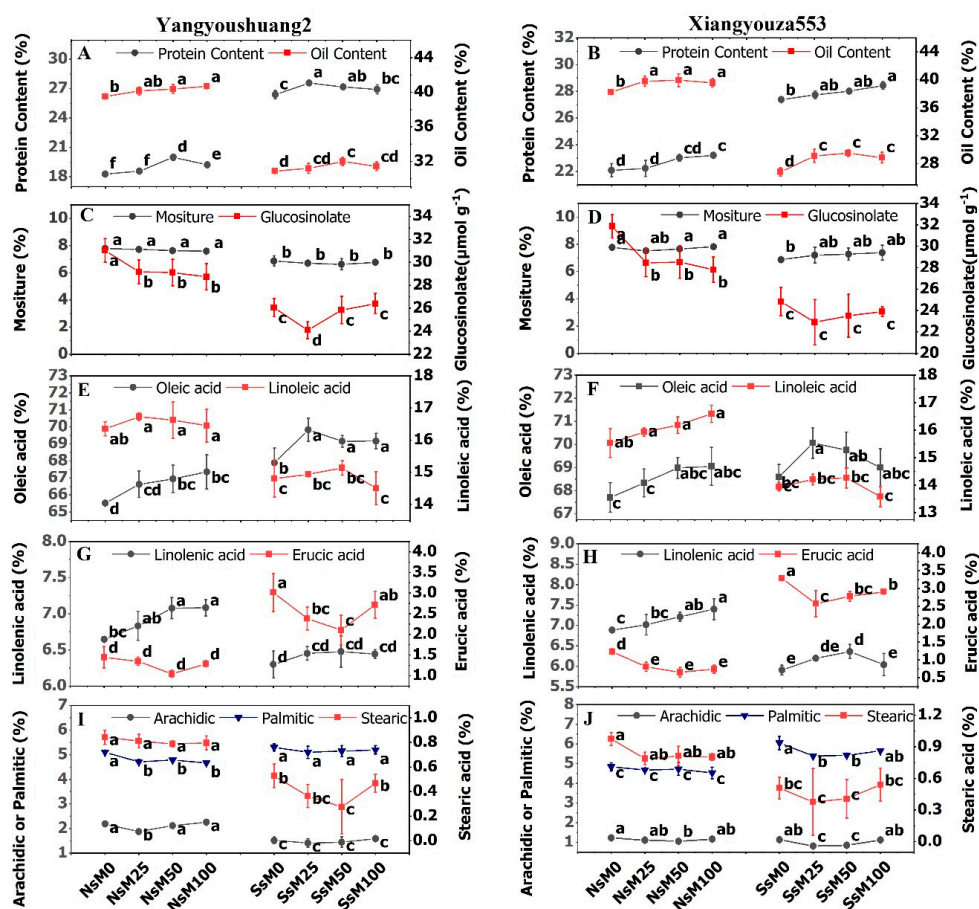

Figure 7. Effect of seed priming in 0 μM (M0), 25 μM (M25), 50 μM (M50), and 100 μM melatonin (M100) on seed protein content, oil content, moisture content, glucosinolate, and fatty acid composition

cultivars of two rapeseed irrigated with saline water. Ns: non-NaCl salt stress, Ss: NaCl salt stress. (**A**) protein and oil contents in Yangyoushuang2; (**B**) protein and oil contents in Xiangyouza553; (**C**) moisture and glucosinolate contents of Yangyoushuang2; (**D**) moisture and glucosinolate contents in Xiangyouza553; (**E**) oleic and linoleic contents in Yangyoushuang2; (**F**) oleic and linoleic contents Xiangyouza553; (**G**) linolenic and erucic contents in Yangyoushuang2; (**H**) linolenic and erucic contents in Xiangyouza53. (**I**) arachidic, palmitic, and stearic contents in Yangyoushuang2; (**J**) arachidic, palmitic, and stearic contents in Xiangyouza53. Bars represent SD of three replicates. Different letters indicate significant differences among treatments (*p* ≤ 0.05).
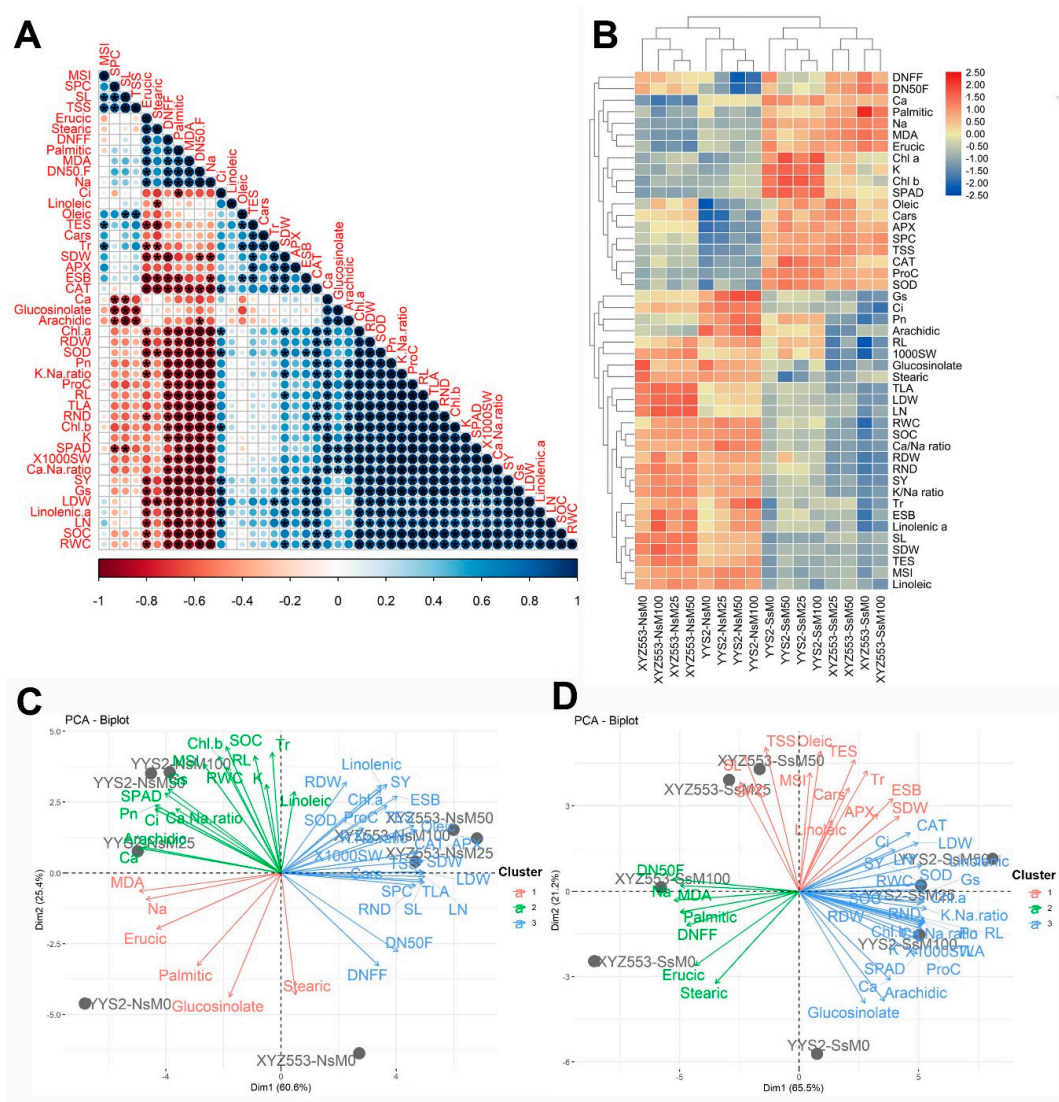

Figure 8. Relationship graphs. (**A**) Pearson's correlation among different studied parameters under NaCl stress. (**B**) Heat map shows a hierarchical clustering analysis between studied parameters and treatments. (**C,D**) Biplot of different studied parameters for each treatment (n = 3), showing the first two dimensions (Dim1 and Dim2) of the principal component analysis (PCA) model for both cultivars grown under normal and NaCl stress, respectively. The colours represent variations in the data. *: *p* ≤ 0.05. DNFF: days number to the first flower, DN50F: days number to 50% flowering, RL: root length, RND: root neck diameter, SL: shoot length, NL: number of leaves, TLA: total leaves area, RDW: root dry weight, SDW: stem dry weight, LDW: leaves dry weight, TES: total effective siliques number, ESB: effective siliques biomass, 1000 SW: 1000 seed weight, SY: seed yield per plant, SPC: seed

protein content, SOC: seed oil content, Chl a: chlorophyll a, Chl b: chlorophyll b, Cars: carotenoids, SPAD: total chlorophyll meter, Pn: net photosynthetic rate, Gs: stomatal conductance, Ci: intercellular CO_2 concentration, Tr: transpiration rate, RWC: relative water content, MSI: membrane stability index, ProC: proline content, TSS: total soluble sugars, CAT: catalase, SOD: superoxide dismutase, APX: ascorbate peroxidase, MDA: malondialdehyde. XYZ553: Xiangyouza553, YYS2: Yangyoushuang2, Ns: non-NaCl salt stress, Ss: NaCl salt stress. M0, M25, M50, and M100: the treatments by 0 μM, 25 μM, 50 μM, and 100 μM of melatonin, respectively.

3.9. Melatonin Priming Improved Performance of Stomata and Transporting System by Xylem Vessels

To have deep knowledge about the role of exogenous MT in improving rapeseed growth, yield-related traits, fatty acids composition, and sodium chloride tolerance, we further analysed the stomatal traits and cross-sections in stem and leaf of MT and non-MT treated plants. The anatomical observations were carried in the stem and leaf of normal control (NsM0), stress control (SsM0), non-NaCl stress + melatonin 50 μM (NsM50), and NaCl stress + melatonin 50 μM (SsM50). Data in Supplementary Table S4 showed that stomatal aperture, thickness of the upper epidermis, xylem vessel, height and width of midvein, area of the vascular bundle, and number of xylem vessels per vascular bundle were decreased, while the thickness of the blade, palisade, and spongy parenchyma were increased in response to NaCl stress. However, seed priming in 50 μM MT caused positive changes in the above-mentioned characteristics in the absence or presence of the NaCl stress and maintained the leaf anatomical characteristics at the status in which plants could overcome NaCl stress. We found that MT treatment has a high impact on the stomatal aperture and area of leaf xylem vessel, as shown in Figure 9. The diameter of the stem section decreased significantly for both Yangyoushuang2 and Xiangyouza553 by 5% and 25%, respectively, compared with the normal controls (Supplementary Table S4 and Figure 10). This was mainly because of the decrease in thickness of the pith diameter (7% and 27%), the average area of xylem vessel (19% and 33%), and the average area of the vascular bundle (6% and 27%), respectively. Seed priming with 50 μM MT significantly improved the stem anatomical characteristics such as area of xylem vessel, number of vascular bundles, number of xylem vessels per bundle, and area of vascular bundle, as well as the increase of thickness of cortex and pith that led to the increased diameter of stem section (Figure 10 and Supplementary Table S4). The rectangles in Figure 10 show MT treatments have a high impact on improving the thickness of stem xylem vessels.
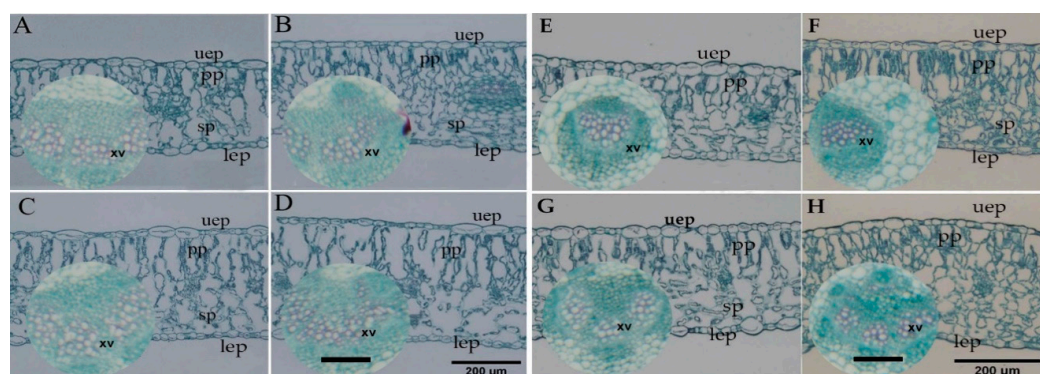

Figure 9. Transections of two rapeseed leaf blade affected by the application of melatonin and NaCl stress at flowering stage. (**A–D**): Yangyoushuang2, (**E–H**): Xiangyouza553. (**A,E**): non-NaCl stress + 0 μM melatonin (control). (**B,F**): NaCl-stress + 0 μM melatonin. (**C,G**): non-NaCl stress + 50 μM melatonin. (**D,H**): NaCl stress + 50 μM melatonin. Uep: upper epidermis, pp: plastid parenchyma, sp: sponge parenchyma, lep: lower epidermis, xv; xylem vessels of midvein. Bars in the circle sections = 200 μm.
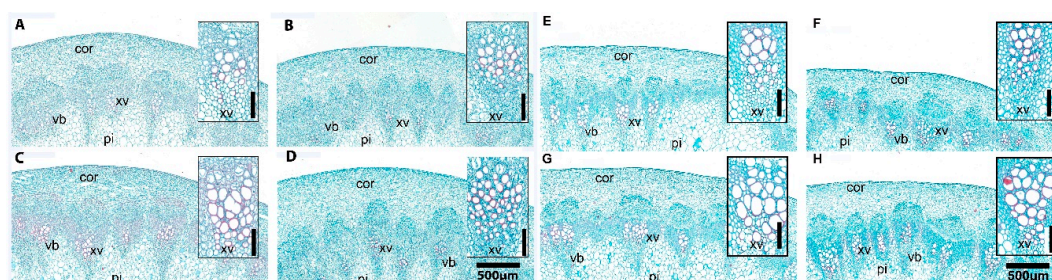
Figure 10. Transections of two rapeseed stem affected by the application of melatonin and salt stress at flowering stage. (**A–D**): Yangyoushuang2, (**E–H**): Xiangyouza553. (**A,E**): non-NaCl stress + 0 μM melatonin (control). (**B,F**): NaCl-stress + 0 μM melatonin. (**C,G**): non-NaCl stress + 50 μM melatonin. (**D,H**): NaCl stress + 50 μM melatonin. Cor: cortex, phv: phloem vessels, xv: xylem vessels, vb: vascular bundle, pi: pith. Bar in the small rectangle = 100 μm.

We further carried out Pearson's correlation among growth, yield, fatty acid composition, and anatomical traits to analyze the role of MT in improving previous traits. The stomatal aperture and area of leaf and stem xylem vessel were positively correlated with shoot dry weight ($p \leq 0.01$), seed yield ($p \leq 0.01$), linoleic acid content ($p \leq 0.01$), linolenic ($p \leq 0.01$), and seed oil content ($p \leq 0.05$). Meanwhile, they were negatively correlated with erucic acid and palmitic acid content at $p \leq 0.01$ (Figure 11). Overall, among all the anatomical traits, the stomatal aperture and area of the xylem vessel were highly correlated with growth, yield-related traits, and fatty acid composition under MT treatment.
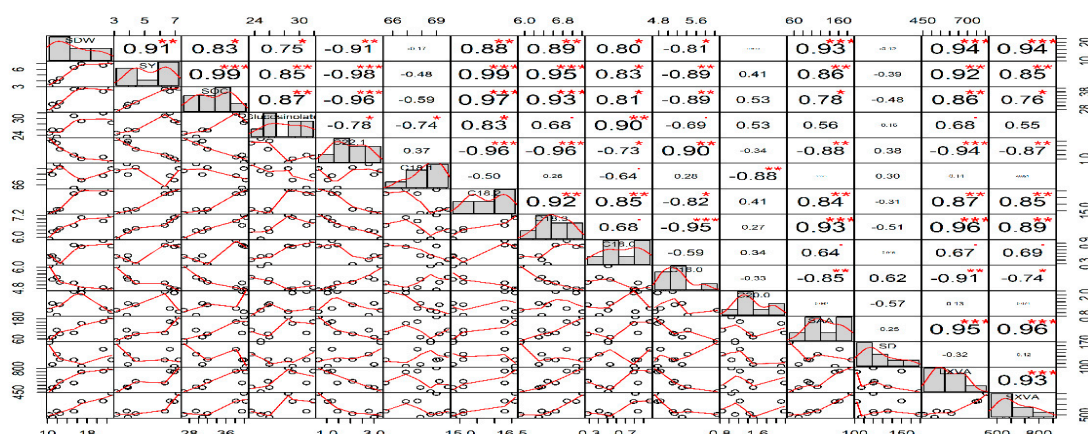

Figure 11. Pearson's correlation coefficients among growth, yield, fatty acid composition, and anatomical traits of two *B. napus* cultivars exposed to salinity stress (0 and 100 mM NaCl). Shoot dry weight (SDW); seed yield (SY); seed oil content (SOC); erucic (C22:1); oleic acid (C18:1); linoleic acid (C18:2); linolenic acid (C18:3); stearic (C18:0); palmitic (C16:0); arachidic (C20:0). Stomatal aperture area (SAA); stomata density (SD), leaf xylem vessel area (LXVA); stem xylem vessel area (SXVA). Asterisk "***","**","*" donate significant at *p*-values (0.001, 0.01, 0.05).

4. Discussion

Salinity stress is a global problem that severely threatens rapeseed production. Some studies showed that MT application can improve rapeseed tolerance to adverse conditions thanks to its pleiotropic functions [23,44], but the appropriate dose of MT for promoting rapeseed yield and fatty acid composition, as well as its role in improving the stomata and vascular system development, are not well documented. We found that the growth and yield of rapeseed plants were markedly reduced in plants under NaCl stress. However, exogenous MT application significantly alleviated those inhibitory effects; these benefits were more pronounced with 50 μM MT pretreatment (Figures 1 and 8B).

NaCl stress caused a significant reduction of the rapeseed root system, and aboveground biomass (Figure 1). These growth reductions could be attributed to the osmotic impact resulting from NaCl stress that enhances growth inhibitors, a decrease of growth promoters, and disturbance of the water balance of NaCl-stressed plants. These inhibitory effects of NaCl led to ionic imbalance, stomatal closure, reduction in photosynthesis, accumulation of toxic ions, disturbance in ionic homeostasis, and consequently inhibition of growth [9,21,61–64]. Seed priming with MT under NaCl stress effectively improved growth characteristics, and its role in improving the growth of unstressed rapeseed plants was observed (Figure 1). The best response was obtained with MT at 50 μM concentration. These data are in agreement with Zeng et al., [23], who found that exogenous MT could support root development and improve the biomass of rapeseed seedlings under salt stress, thereby alleviating the NaCl stress. The positive effect of MT on growth may be owing to modifying the concentration of toxic ions, photosynthetic efficiency, and increased mineral uptake in the stressed plants. Besides, MT was more effective in lowering the oxidative damage by modulating the anti-oxidative systems including enzymatic and non-enzymatic antioxidants (Figsures 1–4, and 8A). Moreover, MT has an important role in improving germination and early growth stages performance through enhancing compatible components, the activity of antioxidant enzymes, and lowering MDA, which led to a smaller decline of growth traits under NaCl stress [20,23,33]. Its role in modulating the composition of mineral elements and enhancing the activities of enzymes involved in nitrogen metabolism, as reported earlier, was also observed [27,28].

Plants continuously generate ROS as a product of various metabolic pathways, and under stress condition, ROS are overproduced, generating oxidative stress. Cell membrane damage is one of the adverse issues that occur in stressed plants and maintenance of its stability and integrity is closely linked to plant tolerance against stress [65]. This damage in membrane integrity is created owing to lipid peroxidation by the ROS acting on membrane lipids. We studied the effect of seed priming with MT on the accumulation of MDA, which is an indicator of oxidative damage to membranes. MT pre-treatment reduced the accumulation of MDA and improved both MSI and RWC, conferring a beneficial effect in ameliorating the oxidative stress caused by NaCl stress (Figure 2). This beneficial effect could be owing to the increase of SOD, CAT, and APX activities by MT treatment. A significantly negative correlation between MDA level with RWC and activities of antioxidant enzymes (SOD and CAT) was observed in our experiment (Figure 8A). This cellular protection effected by MT through antioxidant activation led to improvement of the MSI, which appeared to be related to the improvement in the growth and photosynthetic performance (Figure 12). According to some studies, MT application reduces oxidative damage caused by ROS through enhancing the activities of antioxidant enzymes, decreasing the accumulation of MDA, and regulating seed germination-related plant hormones [37,66–71]. This is because of the ability of MT to promote lipid and starch catabolism and protein synthesis associated with stress response [35,72].

In our study, greater toxic ion content was observed in leaves of NaCl-stressed plants; however, MT pre-treatment exhibited a beneficial effect by decreasing the toxic ion accumulation and mitigating K^+ level in leaves of rapeseed plants. High levels of NaCl led to a reduction in the internal levels of K^+ owing to the competition between Na^+ and K^+ ions [63,73]. Plant tolerance to NaCl stress is closely linked to the ability to maintain a suitable K^+/Na^+ ratio [7,64,74]. Salinity caused a decrease in the K^+/Na^+ and Ca^{2+}/Na^+ ratio in leaves in our experiments (Figure 4). Exogenous MT improved the ratio values, compared with NaCl-stressed plants without MT pretreatment. Improved ion homeostasis is related to an increase in K^+ and a decrease in Na^+ levels that may be related to the upregulation of several target genes, such as *NHX1* and *SOS2* in rapeseed seedlings [24,44], and *MdNHX1* and *MdAKT1* transcript levels in *Malus hupehensis* seedlings [68], which was highly upregulated by melatonin treatment under conditions of NaCl stress. Chen et al. [6] showed that exogenous MT treatment in bermudagrass significantly induced the transcripts of K^+ transporting genes expression under both normal and low K^+ stress conditions, which increases the accumulation of K^+ level under low K^+ stress. The same role was observed by Zhang et al. [27] in cucumber under nitrate stress and by Liang

et al. [28] in apple plants under moderate drought stress. Ca^{2+} signalling plays a critical role in plant biotic and abiotic stress responses [75]. MT treatment improved the Ca^{2+}/Na^+ ratio under NaCl stress in Xiangyouza553 cultivar. This improved ratio may be owing to the involvement of Ca^{2+} signalling in melatonin-triggered salinity tolerance [75]. In our study, NaCl stress caused a significant increase in proline and total soluble sugars contents in rapeseed plants (Figure 2) and MT treated-plants had higher proline and soluble sugars concentration. This could be attributed to the prevention of proline degradation owing to MT action as an antioxidant [76]. A positive correlation between K^+ level, K^+/Na^+, Ca^{2+}/Na^+ ratios, and proline concentration with growth, seed yield, and seed oil content photosynthetic performance under NaCl stress was observed (Figure 8A). Accumulation of proline and soluble sugars and improved K^+ level, K^+/Na^+, and Ca^{2+}/Na^+ ratio under NaCl stress provide cellular protection by maintaining a balance between the osmotic cytosol intensity, and that of the cell vacuole and external medium that maintain cell turgor [61,77,78]. This cellular protection effect by MT led to improvement of the MSI and RWC, which appeared to be related to better water absorption capacity (Figure 12).
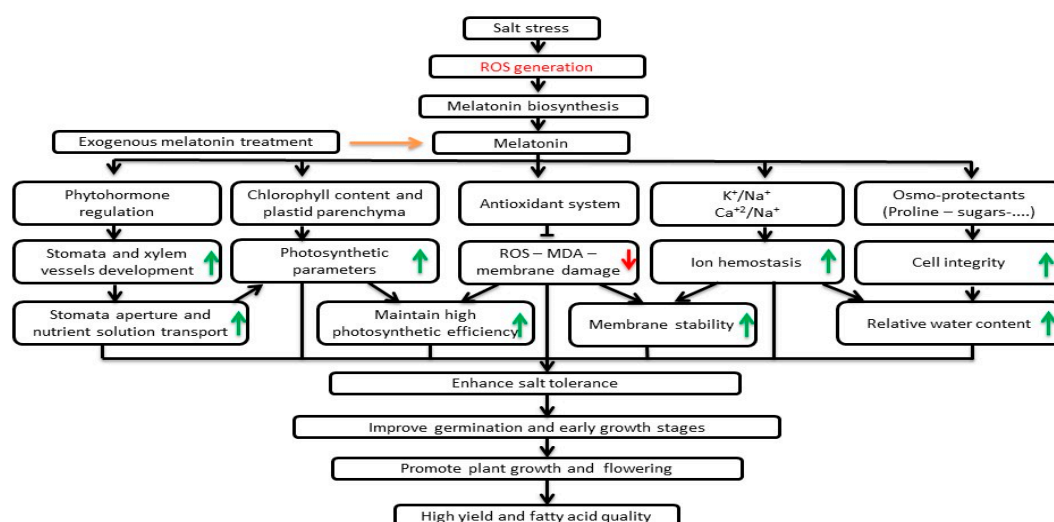

Figure 12. Schematic diagram of mechanisms of melatonin to improve the NaCl stress tolerance, yield, and fatty acid quality of rapeseed plants. ROS; reactive oxygen species, MDA; malondialdehyde.

Under NaCl stress, gas exchange parameters (net photosynthetic rates, stomatal conductance, intercellular CO_2 concentration, and transpiration rate) were obviously reduced. However, using MT apparently improved these photosynthetic parameters (Figure 4). Reduction in gas exchange parameters measured in stressed plants is associated with the presence of ions at toxic levels that dehydrate mesophyll cells, inhibit enzymes involved in carbohydrate metabolism, and stomatal limitations [11,17,28,79]. Faseela et al. [80] reported that NaCl stress resulted in a high reduction of maximum quantum yield of electron transport and the electron transport from the PSII donor side to PSII reaction center in rice seedlings. Our previous study showed that NaCl stress had a critical variable impact on chlorophyll content among rapeseed cultivars at the seedling stage [17]. MT application improved photosynthetic gas exchange parameters possibly because of the protective effect of MT against chlorophyll degradation, resulting in an increased leaf chlorophyll content, promoting photosynthetic capacity, delaying leaf senescence, enhancing the expression of genes encoding antioxidant enzymes, and significantly improving the activities of ROS scavenging enzymes, thereby attenuating the oxidative damage [22,71,81–85]. The correlation analysis showed that the increase of photosynthetic gas exchange parameters by MT application under NaCl stress may be owing to its effectiveness in increasing the activity of the enzymatic defense systems by SOD and CAT, reducing the MDA level, improving the K^+/Na^+ and Ca^{2+}/Na^+ ratio, and promoting a high level of osmotic metabolites (such as proline and soluble sugars). This mechanism has a role in maintaining

cell turgor, optimizing water absorption, and increasing the chlorophyll content, thereby maintaining RWC, increasing CO_2 availability, and optimizing the photosynthetic process (Figures 8A and 12).

Sodium chloride stress caused significant reductions of the rapeseed yield-related traits; however, seed protein content was increased (Figures 5 and 6). This yield reduction could be attributed to the accumulation of toxic ions, disturbance in ionic homeostasis, stomatal closure, reduction in photosynthesis, and consequently inhibition of growth due to NaCl toxicity [9,61,62,86]. The oil quality of rapeseed is associated with fatty acid composition; fundamentally, the quantity of linoleic, oleic, and linolenic acids. Our data revealed that NaCl stress altered the fatty acid composition. The content of glucosinolates, polyunsaturated fatty acids (linoleic and linolenic), stearic, and arachidic acids decreased, whereas that of monounsaturated ones (erucic and oleic) and palmitic increased under NaCl stress. Previous studies also reported that fatty acid composition was influenced by stress conditions [87]. However, the effect of MT application on fatty acid composition has not been documented in rapeseed plants. Drought and high-temperature stress during the stage of seed filling in rapeseed crop altered the oil fatty acid composition by increasing oleic and palmitic acid contents and decreasing linoleic, linolenic, and stearic acid contents [88,89]. A decrease in linoleic and linolenic acid contents and an increase in oleic acid content have been attributed to the enzymes involved in biosynthesis and the conversion of fatty acids in several cellular compartments [88]. The MT application of 50 μM improved seed and oil yield and oil quality of rapeseed cultivars to be healthier compared with control under NaCl stress by decreasing glucosinolates, erucic, and saturated fatty acids (palmitic and arachidic acids) and enhancing unsaturated fatty acids (linolenic and oleic acid) for both rapeseed cultivars. Under the combination of MT and NaCl treatments, a positive correlation was found between seed yield, seed oil content, 1000 seed weight, arachidic acid, and unsaturated fatty acid (linolenic acid) with the growth traits, SOD, CAT, chlorophyll content, photosynthetic traits, K^+/Na^+, proline level, and RWC. Meanwhile, they were significantly negatively correlated with erucic acid, palmitic acid, MDA, Na^+ level, and the number of days required for the appearance of first flower and 50% flowering (Figure 8A). Our results revealed that MT application can improve NaCl tolerance, yield, and oil fatty acid composition, which was evidenced by the close association of these treatments under normal and NaCl conditions with the growth, ion homeostasis, metabolism of antioxidants, photosynthesis, osmolytes, secondary metabolites, and water relations in the PCA (Figure 8C,D).

Exposure of rapeseed plants to NaCl stress reduced stomatal aperture and area of xylem vessel, height and width of midvein, and area of the vascular bundle, which could be owing to the inhibition of cellular expansion and elongation (Figures 9 and 10 and Supplementary Table S4). The closed stomata and small xylem vessels cause a significant reduction in the translocation of water and nutrients from the root system [90–92]. In another way, MT improved the leaf and stem anatomical characteristics particularly stomatal aperture and area of xylem vessel. MT-priming strengthened the cell wall and maintained cell expansion in response to stress, and its role for improving the stomata anatomical traits and protecting the chloroplast structure was observed [72,81]. This improvement in stomatal aperture and area of xylem vessel may be owing to the role of MT for activation of phytohormones, which control the activity of pro-cambial of vascular tissues and maintenance of cell turgor pressure. Person's correlation showed that stomatal aperture and area of xylem vessel were highly associated with growth, yield-related traits, and fatty acid composition under MT treatment (Figure 11). These results indicated great importance of MT in improving translocation of water and nutrients from the root system into active parts of the plant through improving stomatal and xylem vessel performance (Figure 12). Overall, our results provide new insights into the role of MT in improving oil fatty acid composition through improving stomata and transporting system by xylem vessels for enhancing rapeseed salinity tolerance.

5. Conclusions

In summary, melatonin (MT) plays an important role in enhancing plant tolerance to many stress conditions. Our results revealed that MT application can improve oil quality traits of the seeds by

improving the linoleic, linolenic, and oleic acid, and by decreasing glucosinolates, erucic, and saturated fatty acids. Our results provide new insights into the role of MT improving stomata and transporting system by xylem vessels for improving yield, oil fatty acid composition, and enhancing rapeseed salinity tolerance. Therefore, we recommend using MT that can serve as an efficient pretreatment for the improvement of physiological, metabolic processes, quality of oil, and tissues' anatomical traits, which may improve tolerance to NaCl stress in rapeseed. However, further investigations of identifying and cloning of MT regulated genes of rapeseed are crucial to improve NaCl tolerance in rapeseed plants.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1186/s1. Table S1. Changes of green silique traits of two *B. napus* cultivars under two levels of sodium chloride stress and seed priming with different levels of melatonin. Table S2. ANOVA analysis based on moisture content, glucosinolate, erucic acid, and fatty acid composition traits in two *B. napus* cultivars at different levels of seed priming with melatonin under two levels of sodium chloride stress. Table S3. Changes of yield biomass traits in two *B. napus* cultivars under two levels of sodium chloride stress and seed priming with different levels of melatonin. Table S4. Responses leaf and stem anatomical features in two *B. napus* L. cultivars with melatonin seed priming and sodium chloride stress conditions.

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