Root Na\textsuperscript{+} Content Negatively Correlated to Salt Tolerance Determines the Salt Tolerance of Brassica napus L. Inbred Seedlings

Cheng-Feng Wang \textsuperscript{†}, Guo-Liang Han \textsuperscript{*\,†}, Zi-Qi Qiao, Yu-Xia Li, Zong-Ran Yang and Bao-Shan Wang \textsuperscript{*}

Abstract: Soil salinization is a major environmental stressor that reduces the growth and yield of crops. Maintaining the balance of ions under salinity is vital for plant salt tolerance; however, little is known about the correlation between the salt tolerance of crops and the ion contents of their roots and shoots. Here, we investigated the poorly understood salt-tolerance mechanisms, particularly regarding ion contents (particularly Na\textsuperscript{+}), in Brassica napus subsp. napus L., an agriculturally important species. Twenty B. napus inbred lines were randomly chosen from five salt-tolerance categories and treated with increasing concentrations of NaCl (0–200 mmol) for this work. We found that the root Na\textsuperscript{+} content is the most correlated limiting factor for the salt tolerance of B. napus; the higher the salt tolerance, the lower the root Na\textsuperscript{+} content. Correspondingly, the Ca\textsuperscript{2+} /Na\textsuperscript{+} and K\textsuperscript{+} /Na\textsuperscript{+} ratios of the roots were highly correlated with B. napus salt tolerance, indicating that the selective absorption ability of these ions by the roots and their translocation to the shoots play a pivotal role in this trait. These data provide a foundation for the further study of the molecular mechanisms underlying salt tolerance and for breeding salt-tolerant B. napus cultivars.

Keywords: Brassica napus L.; correlative analysis; growth; ion content; salt tolerance
Excess Na$^+$ is a major problem for plant cells as it induces a series of secondary stresses, such as deficiencies in nutrient minerals (particularly Ca$^{2+}$ and K$^+$) and oxidative stress [7]. Osmotic stress (the lowering of soil water potential) limits water uptake of plant cells which triggers a series of deleterious responses such as growth reduction, stomata closure, germination stagnation, and oxidative damage [20]. To survive in saline environment, plants have evolved different strategies such as salt exclusion, ion compartmentalization into vacuoles, and osmotic adjustment [20]. Recent work indicates that plant cells can sense Na$^+$ and regulate ion transport by the combination of glycosyl inositol phosphorylceramide (GIPC), Ca$^{2+}$ signaling, and Na$^+$ transport [21]. The efficiency of these regulatory pathways determines ion content of roots and shoots, ultimately determines the salt tolerance of plants.

It is vital to elucidate salt tolerance mechanisms for breeding reliable salt-tolerant crop cultivars that can grow on saline soils [22]. Salt tolerance and its underlying mechanisms vary extensively depending on plant species, growth, and developmental stages, salt compounds, and concentrations, and environmental factors. Many studies have examined the indicators of salt tolerance related to crop growth. Crops with a strong salt tolerance tend to have longer roots and greater fresh weights than those with weak salt tolerance [23,24]. Under salt stress, sustained crop growth and yields are generally considered the main evaluation criteria for determining crop salt tolerance and developing salt-tolerant cultivars [25–27]. In addition to growth and physiological indicators, salt-tolerance traits may be associated with the ion content of plants grown in the presence of salt [28]. There have been numerous reports detailing the balance of ions (Na$^+$, K$^+$, and Ca$^{2+}$, among others) related to plant salt tolerance [15,29,30]. In bread wheat, shoot ion-independent tolerance is considered an important trait for salt tolerance, favoring tissue expansion and tillering of plants before the shoots are affected by salt toxicity [31,32]. In general, the Na$^+$ content in roots and shoots of a non-halophyte will increase to varying degrees under salt stress, while the contents of K$^+$ and Ca$^{2+}$ will decrease to varying degrees [33–36]. These ionic parameters are often used as indicators of plant salt tolerance. However, to date, there is no unified conclusion about the correlation between ion content and crop salt tolerance, particularly in the roots and shoots of crop plants.

Brassica napus subsp. napus L. (rapeseed) is one of the most important oil crops in the world. Its seed oil has high nutritional value, being rich in fatty acids and vitamins, and is also widely used in industry [37–40]. B. napus is generally considered moderately salt tolerant, with different varieties showing substantial differences in salt tolerance [41]. Different indicators, such as growth, water content, membrane injury index, enzymatic activities, and ion contents, have been used to assess the salt tolerance of different B. napus varieties. The relative salt stress-induced reduction in the biomass of sensitive varieties was found to be significantly higher than that of the tolerant varieties [42], while the K$^+$ content and K$^+$–Na$^+$ selectivity were also important for salt tolerance [43]. Some reports suggest that plant salt tolerance depends mainly on the ability of the root system to limit the transport of Na$^+$ to the shoots [43–45].

Relatively few studies of salt tolerance have collected large numbers of samples for testing, despite the expectation that the salt tolerance of a particular species or different varieties could be evaluated more efficiently through the use of a larger amount of sample data. Recently, we evaluated the salt tolerance of 549 B. napus inbred lines and identified five categories of salt tolerance [46]. In the current work, a total of 20 B. napus inbred lines were randomly chosen from the five salt-tolerance categories, which were studied to reveal the underlying mechanisms with an emphasis on the correlation between ion content in the root and shoot and salt tolerance under salt (NaCl) stress. Our results indicate that root Na$^+$ content, highly negatively associated with salt tolerance, is the most reliable indicator for assessing salt tolerance in B. napus inbred seedlings.
2. Materials and Methods

2.1. Plant Materials

The seeds used in this experiment were harvested in May 2018 and stored at 6 °C. Four *B. napus* inbred lines were randomly selected from each of the five salt-tolerance categories [46] (high salt sensitivity, HSS; salt sensitivity, SS; moderate salt tolerance, MST; salt tolerance, ST; and high salt tolerance, HST) as biological replicates, for a total of 20 inbred lines. The following numbers of the seeds were used in our work. HSS: 557, 611, 668, 703; SS: 40, 74, 277, 323; MST: 39, 319, 388, 525; ST: 130, 344, 527, 667; HST: 137, 370, 460, 475.

2.2. Growth Conditions

Healthy and uniform *B. napus* seeds were presoaked in 8 mL of deionized water for 10 h, then sown on two layers of filter paper in 10 cm Petri dishes. In the morning of the first two days, the water in the dish was replaced with a fresh 8 mL of deionized water. From the third day onwards, the NaCl concentration was gradually increased by replacing the water with a salt solution every 12 h, increasing by 50 mmol (mM) each time to a final concentration of 50, 100, 150, or 200 mmol (mM). The NaCl was dissolved in 1/5 Hoagland solution (the pH of all solutions was adjusted to 6.0). Seeds cultured in 1/5 Hoagland solution were the control.

The seeds were cultured in a phytotron with a temperature of 28 ± 3/23 ± 3 °C (day/night), a light intensity of 600 µmol m⁻² s⁻¹ (14 h light/10 h dark), and a relative humidity of 70% [46]. Three biological replicates were performed.

2.3. Determination of the Growth Indicators

The following growth indicators were measured on the 9th day after sowing (Supplementary Materials S1). Shoot length (SL) and root length (RL): The aboveground part and the underground part (rhizome) of the seedlings were measured separately. After the leaves and roots were straightened out, a ruler was used to measure the lengths of 10 individuals for each biological repetition. Fresh weight of shoots (SFW), fresh weight of roots (RFW), and total fresh weight (TFW): The seedlings were rinsed with deionized water, wiped quickly with absorbent paper, divided into the shoots and roots, and weighed. TFW was sum of the SFW and RFW. Dry weight of shoots (SDW), dry weight of roots (RDW), and total dry weight (TDW): The shoots and roots were fixed at 105 °C for 15 min, dried at 80 °C until their weight was stable, then weighed separately. TDW was the sum of SDW and RDW. Dry weight to fresh weight of shoots ratio (S-DW/FW) and dry weight to fresh weight of roots ratio (R-DW/FW): These ratios were calculated as the dry weight over the fresh weight in the respective tissues. Based on these data, various relative values were also obtained (Supplementary Materials S1).

2.4. Na⁺, K⁺, and Ca²⁺ Analysis

The Na⁺, K⁺, and Ca²⁺ contents were measured according to Wang and Zhao [11], with some modifications. Each sample of dried fine-ground roots or shoots was incubated with 1 mL nitric acid, extracted in a boiling water bath for about 3 h, filtered, and diluted to 10 mL using ultrapure water. The ion contents were measured using an AP1500 flame photometer (Shanghai Aopu analytical instruments co., Ltd., Shanghai, China).

The selective ion transport capacity was analyzed as described previously [47,48]. The followings were measured and analyzed (Supplementary Materials S2): Na⁺, K⁺, and Ca²⁺ contents (mmol·g⁻¹DW); K⁺/Na⁺ and Ca²⁺/Na⁺ ratios; ratio of the Na⁺ content in the shoot to that in the root (SNa); ratio of the K⁺ content in the shoot to that in the root (SK); ratio of the Ca²⁺ content in the shoot to that in the root (SCa); and the ratios of the K⁺/Na⁺ ratio in the shoot to that in the root (SK, Na) and the Ca²⁺/Na⁺ ratio in the shoot to that in the root (SCa, Na).
2.5. Statistical Analysis

The experiments performed here focused on the correlative analysis between growth parameters of root and shoot and their \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Ca}^{2+} \) parameters. Five salt concentrations (0, 50, 100, 150, and 200 mmol NaCl) and five salt tolerance levels (HSS, SS, MST, ST, and HST are treated as 1, 2, 3, 4, and 5, respectively) were quantified to construct a mathematical model for linear regression analysis. The correlation coefficients (\( R^2 \)) between the salt tolerances and ion contents of the roots and shoots of the \( B. \text{napus} \) lines belonging to the five salt-tolerance categories were analyzed from three aspects: (1) the correlation between the NaCl concentration and the individual ion content parameters in the roots and shoots of a particular salt-tolerance grade of \( B. \text{napus} \) inbred line (the y coordinate is the ion content parameter, and the x coordinate is the NaCl concentration); (2) the correlation between the salt-tolerance grade of \( B. \text{napus} \) inbred line and the individual ion content parameters in the roots and shoots of seedlings grown at a given NaCl concentration (the y coordinate is the ion content parameter, and the x coordinate is the salt-tolerance grade); (3) the correlation between growth index and the ion content parameters of all \( B. \text{napus} \) varieties under all treatments (the y coordinate is the ion content parameter, and the x coordinate is the growth indicator value).

Statistical and plotting analyses were performed using standard tools, such as Microsoft Excel 2010, GraphPad Prism 6.01, and SPSS 19.0 [49,50]. Specific mathematical tools were used, including the One-Way ANOVA and linear regression analysis. In the figures, different lowercase letters represent significant differences among the means (at a \( p < 0.05 \) level), as determined using Duncan's test. In the tables, asterisks (*) represent significant differences among the means at a \( p < 0.05 \) level or 0.01 level, respectively.

3. Results

3.1. Growth under Varying Degrees of NaCl Stress

Growth is one of the most reliable indicators for assessing plant salt tolerance. Various growth indicators in \( B. \text{napus} \) seedlings grown under varying degrees of NaCl stress were determined (Supplementary Materials S1). In general, the growth parameters of all inbred lines decreased with increasing NaCl concentrations, with the reduction of growth in the salt-sensitive inbred lines being greater than that of the salt-tolerant ones. The phenotypes of the \( B. \text{napus} \) seedlings were determined under different NaCl concentrations and salt-tolerance grades (Figure 1a). In particular, the total fresh weight was analyzed (Figure 1b), because this characteristic was previously shown to be the most correlated with \( B. \text{napus} \) salt tolerance [46]. No significant differences in phenotypes such as the total fresh weight were observed between the control and 50 mmol NaCl-treated seedlings; however, at 100 mmol NaCl, a small amount of necrosis was detected in the leaves. At 150 mmol NaCl, seedling growth was significantly damaged, while at 200 mmol NaCl, the damage was more serious and the leaves showed signs of wilting and yellowing, with salt-sensitive inbred lines showing more injury symptoms than salt-tolerant ones (Figure 1a). In all five salt-tolerant grades, the total fresh weight of seedlings decreased as the NaCl concentration increased, but for the inbred lines with higher salt tolerance, the total fresh weight of seedlings was significantly higher than those with lower salt tolerance (Figure 1b).
We therefore also calculated the relative values of plant growth indicators (Supplementary Materials 1) and performed a correlation analysis between the NaCl concentration of the growth medium and the relative value of each indicator (Figure 2). Our linear analysis indicated that both the relative values of SDW/SFW and RDW/RFW were positively correlated with the NaCl concentration (Figure 2i,j), whereas those of the other indicators were negatively correlated with the salt concentration (Figure 2a–h). In general, the *B. napus* inbred lines with lower salt tolerances showed greater changes in the relative values than those the lines with higher salt tolerances under different salt concentrations.

### 3.2. Correlation between the Relative Values of the Growth Indicators and NaCl Concentrations

Under NaCl stress, the relative value (treatment/control) represents the tolerance of a given genotype of a plant species: the higher the relative value, the higher the salt tolerance. We therefore also calculated the relative values of plant growth indicators (Supplementary Materials S1) and performed a correlation analysis between the NaCl concentration of the growth medium and the relative value of each indicator (Figure 2). Our linear analysis indicated that both the relative values of SDW/SFW and RDW/RFW were positively correlated with the NaCl concentration (Figure 2i,j), whereas those of the other indicators were negatively correlated with the salt concentration (Figure 2a–h). In general, the *B. napus* inbred lines with lower salt tolerances showed greater changes in the relative values than those the lines with higher salt tolerances under different salt concentrations.

### 3.3. Correlation between the Na*, Ca**, and K+ Content Parameters and the B. napus Salt Tolerance

Within each salt-tolerance grade, we found that the ion contents, absorption, and transport in the roots and shoots of the different varieties were related to the NaCl concentration in the growth medium. Among the five different salt-tolerance grades of the *B. napus* lines, those with moderate salt tolerance showed the strongest correlation between the ion content parameters and the NaCl concentration of the growth medium. The highest correlation coefficients were those of the shoot Na* content with the NaCl concentration ($R^2 = 0.94$ for highly salt-sensitive and highly salt-tolerant varieties), followed by those of the root Ca** content with the NaCl concentration (about 0.9–0.95), while the lowest correlation coefficients were those between $S_{K,Na}$ and the NaCl concentration (Table 1). There was no significant regularity in the $R^2$ values between the ion content parameters in different salt-tolerance grades of *B. napus* and the NaCl concentration of the growth medium. Together, the shoot Na* content was highly positively correlated with the NaCl concentration.

---

![Figure 1. Seedling growth phenotypes of five *B. napus* lines at five salt tolerance levels. (a) Phenotypes of the seedlings with high salt sensitivity (HSS, 557); salt sensitivity (SS, 323); moderate salt tolerance (MST, 525); salt tolerance (ST, 130); and high salt tolerance (HST, 475) at different NaCl concentrations. (b) Average total fresh weight of the *B. napus* lines when grown at different NaCl concentrations. Error bars indicate standard deviation ($n = 4$). Statistical significance was determined using a one-way ANOVA and Duncan’s multiple range test. Significant differences at $p < 0.05$ are represented by different letters above the bars. 557, 323, 525, 130, and 475, these numbers represent the number of the *B. napus* lines.](image-url)
Figure 2. Linear relationship between the NaCl concentration and the relative values of the various growth indicators. The indicators measured were the shoot fresh weight (SFW; (a)), root fresh weight (RFW; (b)), total fresh weight (TFW; (c)), shoot dry weight (SDW; (d)), root dry weight (RDW; (e)), total dry weight (TDW; (f)), shoot length (SL; (g)), root length (RL; (h)), ratio of dry weight to fresh weight of the shoots (S-DW/FW; (i)), and ratio of dry weight to fresh weight of the roots (R-DW/FW; (j)). Each point represents the mean of four replicates. HSS, high salt sensitivity; SS, salt sensitivity; MST, moderate salt tolerance; ST, salt tolerance; HST, high salt tolerance.
Table 1. Correlation coefficients between the shoot and root ion contents, absorption, and transport, and the NaCl concentrations in the *B. napus* inbred lines of five salt-tolerance categories.

|            | Shoot Na | Root Na | Shoot K | Root K | Shoot Ca | Root Ca |
|------------|----------|---------|---------|--------|----------|---------|
| HSS        | 0.94 **  | 0.85 ** | 0.71 ** | 0.89 **| 0.90 **  | 0.95 ** |
| SS         | 0.91 **  | 0.80 ** | 0.75 ** | 0.88 **| 0.88 **  | 0.93 ** |
| MST        | 0.95 **  | 0.85 ** | 0.72 ** | 0.89 **| 0.90 **  | 0.93 ** |
| ST         | 0.92 **  | 0.79 ** | 0.76 ** | 0.84 **| 0.89 **  | 0.90 ** |
| HST        | 0.94 **  | 0.71 ** | 0.56 ** | 0.91 **| 0.85 **  | 0.93 ** |

| K/Na       | Shoot K/Na | Root K/Na |
|------------|------------|-----------|
| HSS        | 0.70 **    | 0.78 **   | 0.90 **  |
| SS         | 0.68 **    | 0.76 **   | 0.90 **  |
| MST        | 0.74 **    | 0.81 **   | 0.94 **  |
| ST         | 0.72 **    | 0.81 **   | 0.94 **  |
| HST        | 0.67 **    | 0.86 **   | 0.92 **  |

| S_{Na}     | S_{K}     | S_{Ca}    |
|------------|-----------|-----------|
| HSS        | 0.85 **   | 0.49 **   | 0.30 *   |
| SS         | 0.86 **   | 0.70 **   | 0.30 *   |
| MST        | 0.88 **   | 0.71 **   | 0.64 **  |
| ST         | 0.90 **   | 0.60 **   | 0.31 *   |
| HST        | 0.80 **   | 0.69 **   | 0.41 **  |

*, ** = significant at 0.05 and 0.01 probability levels, respectively. HSS, high salt sensitivity; SS, salt sensitivity; MST, moderate salt tolerance; ST, salt tolerance; and HST, high salt tolerance.

Correlation between ion parameters of the *B. napus* varieties and their salt tolerances was shown in Table 2. Among the NaCl concentrations, the 200 mmol NaCl stress showed the highest correlation with the ion contents. Among the ion content parameters, root Ca^{2+}/Na^{+} showed the highest correlation with the salt tolerance of *B. napus* varieties. The highest correlation coefficients were between root Ca^{2+}/Na^{+} and the salt-tolerance grade of *B. napus* inbred lines (\(R^2 = 0.78\) for grown under 150 mmol NaCl stress, \(R^2 = 0.77\) for grown under 100 mmol NaCl stress), followed by those between the root Na^{+} content and the salt-tolerance grade of the *B. napus* inbred lines (\(R^2 = 0.75\) for grown under 200 mmol NaCl stress, \(R^2 = 0.74\) for grown under 150 mmol NaCl stress). The lowest correlation coefficients were those between the S_{Ca} and the salt-tolerance grade of *B. napus* inbred lines grown under NaCl stress (Table 2).

We also found that the ion contents were related to the growth indexes of *B. napus* inbred lines grown under different levels of NaCl stress (Table 3). In general, among the growth indexes, root length showed the highest correlation with the ion contents. Among the ion content parameters, the root Na^{+} content showed the highest negative correlation with the growth indexes. The highest correlation coefficient was found between root Ca^{2+}/Na^{+} and root length (\(R^2 = 0.78\)), followed by that between root Ca^{2+}/Na^{+} and root fresh weight (\(R^2 = 0.77\)), while the lowest correlation coefficients were those between S_{K,Na} and the growth indexes (Table 3). These results indicated that root growth including the fresh weight and elongation of *B. napus* inbred lines is highly positively correlated with the root Ca^{2+}/Na^{+} ratio under NaCl stress, which is a reliable indicator for evaluating the *B. napus* salt tolerance under salt stress.
Table 2. Correlation coefficients between the shoot and root ion contents, absorption, and transport, and different salt-tolerance grades of B. napus under various NaCl concentrations.

| Na   | Root | Shoot | Root | K   | Root | Shoot | Root |
|------|------|-------|------|-----|------|-------|------|
| 0    | 6.04 × 10⁻⁶ | 0.06 | 0.34 ** | 0.02 | 0.01 | 0.08 |
| 50   | 0.09 | 0.67 ** | 0.32 ** | 0.02 | 0.08 | 0.15 |
| 100  | 0.25 * | 0.61 ** | 0.56 ** | 0.10 | 0.35 ** | 0.20 * |
| 150  | 0.19 | 0.74 ** | 0.60 ** | 0.18 | 0.35 ** | 0.27 * |
| 200  | 0.25 * | 0.75 ** | 0.65 ** | 0.26 * | 0.44 ** | 0.33 ** |

| K/Na | Root | Shoot | Ca/Na | Root | S   | K, Na | S_{Ca, Na} |
|------|------|-------|-------|------|-----|-------|------------|
| 0    | 0.30 * | 0.10 | 1.54 × 10⁻³ | 0.10 | 0.11 | 0.07 |
| 50   | 0.31 * | 0.53 ** | 0.15 | 0.69 ** | 0.06 | 0.13 |
| 100  | 0.59 ** | 0.36 ** | 0.44 ** | 0.77 ** | 0.13 | 0.02 |
| 150  | 0.65 ** | 0.69 ** | 0.41 ** | 0.78 ** | 0.02 | 0.54 ** |
| 200  | 0.74 ** | 0.73 ** | 0.49 ** | 0.72 ** | 0.19 | 0.34 * |

| S_{Na} | S_K | S_Ca |
|-------|-----|------|
| 0    | 0.07 | 0.26 * | 4.28 × 10⁻³ |
| 50   | 0.24 * | 0.40 ** | 0.01 |
| 100  | 0.32 ** | 0.35 ** | 0.11 |
| 150  | 0.50 ** | 0.39 ** | 0.01 |
| 200  | 0.45 ** | 0.44 ** | 2.93 × 10⁻³ |

*, ** = significant at 0.05 and 0.01 probability levels, respectively.

Table 3. Correlation coefficients between the growth indexes and the ion content parameters of all B. napus varieties under all treatments.

| Na   | Root | Shoot | K   | Root | Shoot | Root | Ca   | Root |
|------|------|-------|-----|------|-------|------|------|------|
| TFW  | 0.53 ** | 0.71 ** | 0.64 ** | 0.49 ** | 0.55 ** | 0.50 ** |
| TDW  | 0.38 ** | 0.48 ** | 0.44 ** | 0.31 ** | 0.34 ** | 0.38 ** |
| SFW  | 0.38 ** | 0.58 ** | 0.55 ** | 0.34 ** | 0.40 ** | 0.36 ** |
| RFW  | 0.69 ** | 0.73 ** | 0.63 ** | 0.68 ** | 0.68 ** | 0.64 ** |
| SDW  | 0.19 ** | 0.27 ** | 0.25 ** | 0.14 ** | 0.15 ** | 0.20 ** |
| RFW  | 0.65 ** | 0.72 ** | 0.62 ** | 0.63 ** | 0.67 ** | 0.62 ** |
| SL   | 0.57 ** | 0.46 ** | 0.55 ** | 0.54 ** | 0.58 ** | 0.49 ** |
| SL   | 0.76 ** | 0.70 ** | 0.63 ** | 0.75 ** | 0.73 ** | 0.75 ** |
| S-DW/FW | 0.41 ** | 0.66 ** | 0.55 ** | 0.39 ** | 0.51 ** | 0.45 ** |
| R-DW/FW | 0.44 ** | 0.36 ** | 0.22 ** | 0.42 ** | 0.36 ** | 0.41 ** |

| K/Na | Root | Shoot | Ca/Na | Root | S   | K, Na | S_{Ca, Na} |
|------|------|-------|-------|------|-----|-------|------------|
| TFW  | 0.50 ** | 0.54 ** | 0.48 ** | 0.63 ** | 0.11 ** | 0.24 ** |
| TDW  | 0.31 ** | 0.34 ** | 0.30 ** | 0.42 ** | 0.11 ** | 0.14 ** |
| SFW  | 0.36 ** | 0.38 ** | 0.33 ** | 0.48 ** | 0.11 ** | 0.14 ** |
| RFW  | 0.62 ** | 0.71 ** | 0.64 ** | 0.77 ** | 0.07 ** | 0.41 ** |
| SDW  | 0.15 ** | 0.15 ** | 0.13 ** | 0.21 ** | 0.09 ** | 0.05 ** |
| RFW  | 0.58 ** | 0.68 ** | 0.60 ** | 0.76 ** | 0.07 ** | 0.34 ** |
| SL   | 0.57 ** | 0.54 ** | 0.58 ** | 0.55 ** | 0.16 ** | 0.49 ** |
| SL   | 0.64 ** | 0.72 ** | 0.68 ** | 0.78 ** | 0.07 ** | 0.47 ** |
| S-DW/FW | 0.36 ** | 0.42 ** | 0.36 ** | 0.53 ** | 0.06 * | 0.14 ** |
| R-DW/FW | 0.28 ** | 0.36 ** | 0.34 ** | 0.38 ** | 1.81 × 10⁻³ | 0.27 ** |

| S_{Na} | S_K | S_{Ca} |
|-------|-----|-------|
| TFW  | 0.24 ** | 0.08 ** | 0.11 ** |
| TDW  | 0.17 ** | 0.04 | 0.14 ** |
| SFW  | 0.13 ** | 0.02 | 0.08 ** |
| RFW  | 0.44 ** | 0.25 ** | 0.14 ** |
| SDW  | 0.07 ** | 2.19 × 10⁻³ | 0.10 ** |
| RFW  | 0.38 ** | 0.20 ** | 0.13 ** |
| SL   | 0.46 ** | 0.18 | 0.09 ** |
| RL   | 0.54 ** | 0.32 ** | 0.23 ** |
| S-DW/FW | 0.13 ** | 0.04 * | 0.10 ** |
| R-DW/FW | 0.33 ** | 0.31 ** | 0.14 ** |

*, ** = significant at 0.05 and 0.01 probability levels, respectively. TFW, total fresh weight; TDW, total dry weight; SFW, fresh weight of shoots; RFW, fresh weight of roots; SDW, dry weight of shoots; RFW, dry weight of roots; SL, shoot length; RL, root length; S-DW/FW, dry weight to fresh weight of shoots ratio; R-DW/FW, dry weight to fresh weight of roots ratio.
The Na\(^{+}\), Ca\(^{2+}\), and K\(^{+}\) contents in the shoots and roots of the plants reflected their ion uptake, translocation, and compartmentalization, as well as their salt tolerance. The Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) contents; K\(^{+}\)/Na\(^{+}\) and Ca\(^{2+}\)/Na\(^{+}\) ratio; ratios of the Na\(^{+}\) content in the shoot to that in the root (S\(_{\text{Na}}\)), K\(^{+}\) content in the shoot to that in the root (S\(_{\text{K}}\)), and the Ca\(^{2+}\) content in the shoot to that in the root (S\(_{\text{Ca}}\)), and the ratios of the K\(^{+}\)/Na\(^{+}\) ratio in the shoot to that in the root (S\(_{\text{K}}$/S\(_{\text{Na}}\)) and the Ca\(^{2+}\)/Na\(^{+}\) ratio in the shoot to that in the root (S\(_{\text{Ca}}$/S\(_{\text{Na}}\)) are shown in Supplementary Materials S2. It can be seen that the correlations between S\(_{\text{K}}$/S\(_{\text{Na}}\), S\(_{\text{Ca}}$/S\(_{\text{Na}}\), and the indicators are not significant, or the correlation coefficients are low. In the three ratios of the shoot ion content to the root ion content, S\(_{\text{Na}}\) showed a higher correlation with the experimental indicators, followed by S\(_{\text{K}}\), while the correlations between S\(_{\text{Ca}}\) and the indicators were not significant, or the correlation coefficients were very low (Tables 1–3).

Take the linear relationships between the ion content and the NaCl concentrations for the highly salt-tolerant lines, the salt-tolerant grade with 200 mmol NaCl treatment, and the total fresh weight of the \(B.\ napus\) lines under different concentrations of NaCl shown in Figure 3 as an example. Both the Ca\(^{2+}\) and K\(^{+}\) contents in the roots and shoots gradually decreased with increasing NaCl concentrations, while the Na\(^{+}\) contents in the roots and shoots increased rapidly, particularly in the roots (Figure 3a). The higher the salt-tolerance grade of the \(B.\ napus\) inbred lines, the higher the Ca\(^{2+}\) and K\(^{+}\) contents and the lower the Na\(^{+}\) content (Figure 3b). Under the different NaCl concentrations, the higher the total fresh weight of the \(B.\ napus\) inbred lines (the higher the salt tolerance), the higher the Ca\(^{2+}\) and K\(^{+}\) contents, and the lower the Na\(^{+}\) content (Figure 3c).

Figure 3. Correlations between the ion content and the NaCl concentration, the grade of salt tolerance, or the growth indicator in \(B.\ napus\) seedlings. (a) Correlations between the different concentrations...
of NaCl (mmol) and Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\) contents in *Brassica napus* seedlings with high salt tolerance (HST). (b) Correlations between the salt tolerance category and the Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\) contents in *B. napus* seedlings grown at 200 mmol NaCl. (c) Correlations between the growth indicator total fresh weight (TFW) and the Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\) contents in *B. napus* seedlings grown under different NaCl concentrations. Salt tolerance category: 1, HSS, high salt sensitivity; 2, SS, salt sensitivity; 3, MST, moderate salt tolerance; 4, ST, salt tolerance; 5, HST, high salt tolerance.

4. Discussion

In the present study, a total of 20 *B. napus* inbred lines were randomly chosen from the five salt-tolerance categories described by Wu, et al. [46]. These lines were used to uncover the underlying mechanisms of salt tolerance, with an emphasis on its correlation between growth and ion parameters. This information would like to provide a basis for the further elucidation of the molecular mechanisms underlying *B. napus* salt tolerance, and for breeding salt-tolerant cultivars.

The salt tolerance of a plant is related to the ion balance of the plant itself. Under salt stress, the Na\(^{+}\) content of the plant increases. It is well known that salt stress inhibits plant growth, mainly due to the competition in transport between Na\(^{+}\) and other mineral elements such as K\(^{+}\) and Ca\(^{2+}\) [51–54]. This interferes with the plant’s ability to absorb other mineral elements, resulting in a deficiency of those elements, most commonly K\(^{+}\). At high Na\(^{+}\) concentrations, growth and Ca\(^{2+}\) uptake are reduced in sugarcane (*Saccharum* sp.), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) [52,55,56]. Even in the relatively salt-tolerant wild rapeseed (*Brassica campestris*), an increased soil Na\(^{+}\) concentration is accompanied by decreased Mg\(^{2+}\) and K\(^{+}\) uptake [57]. Some *B. napus* germplasms have higher Ca\(^{2+}\) and K\(^{+}\) contents, which may be related to their salt tolerance, because Ca\(^{2+}\) and K\(^{+}\) absorption by seedlings is considered to be particularly important in high-NaCl saline environments [58]. Na\(^{+}\) accumulation in the shoot tissues is regarded as one of the most useful phenotypes in screening for salinity tolerance [7]. Here, our results indicate that the ionic contents in *B. napus* seedlings were also affected by NaCl stress, and seedlings with poor salt tolerance have higher Na\(^{+}\) contents and lower K\(^{+}\) and Ca\(^{2+}\) contents. These changes in the abundances of the Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) ions consequently altered the other related ion parameters (K\(^{+}\)/Na\(^{+}\), Ca\(^{2+}\)/Na\(^{+}\), etc.) of the seedlings. By comparing the ion contents with the growth indicators of the five salt-tolerance grades of *B. napus* inbred lines, we found that the better the plant grows under salt stress (the higher salt tolerance), the higher the Ca\(^{2+}\) and K\(^{+}\) contents and the lower the Na\(^{+}\) content in the plant body (Supplementary Materials S2). In addition, the K\(^{+}\)/Na\(^{+}\) ratio is regarded as a reliable indicator of salt tolerance for many crops [59–62], and we showed that its highest value in our experiments is in the *B. napus* inbred lines with a higher salt tolerance (Supplementary Materials S2).

Many studies have been performed on the salt tolerance of plants [63–66], but few using so many inbred lines with different salt tolerances have been reported. Previous research has focused more on the analysis of the differences in the final physiological indicators, while we here focus on a correlative analysis of the changes in the physiological indicators in particular ion parameters with the salt tolerance of *B. napus* inbred lines under different NaCl concentrations. To date, evaluations of the salt resistance of crops using the most reliable indicators have not yet reached a clear conclusion; for example, some studies showed that the contents of Na\(^{+}\) and Ca\(^{2+}\) are more related to the yield of seeds than K\(^{+}\) [63], while others suggest that the contents of Na\(^{+}\) and K\(^{+}\) are more important than Ca\(^{2+}\) [67].

Here, we approached this issue by taking into account the differences in the salt tolerance of the different inbred lines a single species and analyzed the relationships between the indicators and salt tolerance under various variables using linear regressions. First, we found that all salt-tolerance grades showed a high correlation of about $R^2 = 0.7$ with the salt concentration on average (Table 1); therefore, about 30% of the differences detected are related to the experimental materials and the external environment. Second, among the plant physiology parameters, higher NaCl concentrations are more strongly...
correlated with the ion contents, which is presumed to be because low concentrations of salt are not sufficient to cause significant differences in the ion contents and salt tolerances of different varieties of *B. napus* (Table 2). Third, root length is the growth parameter most strongly correlated with ion contents (Table 3). We speculate that this is related to the function of roots in selectively absorbing and transporting ions; that is, the longer the root, the better the plant grows and the more effectively it takes up and transports ions such as K⁺ and Ca²⁺ to the shoot. For Na⁺, a negative trend was found between root growth and salt tolerance. Using a comprehensive analysis comprising linear regression and significant difference tests, we found that, at the p < 0.01 level, the $R^2$ for the shoot Na⁺ content is 0.5 on average, and the $R^2$ for the root Na⁺ content is 0.57 on average; the $R^2$ for the shoot K⁺ content is 0.51 on average, and the $R^2$ for the root K⁺ content is 0.47 on average; the $R^2$ for the shoot Ca²⁺ content is 0.5 on average, and the $R^2$ for the root Ca²⁺ content is 0.48 on average. The correlation coefficients for the Ca⁺/Na⁺ and K⁺/Na⁺ ratios are also higher (about 0.4–0.5), while those for $S_{Ca, Na}$, $S_{K, Na}$, $S_{Na}$, $S_{K}$, and $S_{Ca}$ are lower (about 0.1–0.3). In conclusion, the root Na⁺ content is the most critical indicator of the salt tolerance of *B. napus*.

The root is the first organ of a plant exposed to a salty environment, and plays a momentous role in salt sensing [68,69] and signal transmission to the aerial tissues [70]. From observing plant growth and analyzing our initial data, we found that under salt stress, *B. napus* shoot growth decreases, root growth is poorer, plant growth reduces, and necrosis occurs. This supports that the root system was directly exposed to high concentrations of salt during the growth process, or because the root is more sensitive and thus more easily damaged than the shoot [71]. Later experiments found that salt (Na⁺) can be transported to the aerial parts of the salt-resistant plants, which may be to better protect the roots [72]. Studies have shown that halophytes are more efficient than non-halophytes in physiological regulation such as ions under salt stress [17,73]. In general, halophytes respond to salt stress signals faster, and are more efficient in maintaining membrane potential and voltage gating, which helps them maintain ion and osmotic homeostasis under stress [73,74]. This may also be the reason for the salt tolerance of salt-tolerant varieties. Specifically, in our experiment, the varieties with strong salt tolerance can transport salt ions to the above-ground part or deal with the absorption of salt ions more efficiently leading to less Na⁺ in the roots, while the varieties with weak salt tolerance have lower control efficiency leading to more Na⁺ in the roots.

Plant roots absorb water and inorganic salts from their environment and transport them upward to their aerial organs, which biosynthesize organics that are delivered to the underground parts of the plants. Excessive amounts of salt ions (mainly Na⁺) can damage plant tissues and negatively affect growth indicators, such as root length and fresh weight [75,76]. The elongation of the main root can thus reflect and be used to characterize plant salt tolerance [29]. At the cellular level, in order to shield the highly sensitive biosynthetic devices from harmful excess Na⁺, the concentration of Na⁺ in the cytoplasm must be kept at a relatively low level that can be tolerated by the intracellular machinery [77,78], especially within the aerial parts of the plants. Ion concentration in cytosol of plant cells under salt stress depends upon the import, transportation and deposition [43], which can be reflected in ion selectivity. Among the correlation coefficients between $S_{Ca, Na}$, $S_{K, Na}$, $S_{Na}$, $S_{K}$, $S_{Ca}$, and the various indicators, $S_{Na}$ also had the highest or higher correlation coefficient ($R^2 = 0.90$ for salt-sensitive varieties in Table 1, $R^2 = 0.50$ for *B. napus* inbred lines grown under 150 mmol NaCl stress in Table 2, $R^2 = 0.54$ for the root length of the *B. napus* inbred lines in Table 3).

The uptake, transportation, deposition, and efflux of Na⁺ in the plant roots determines the Na⁺ content and salt tolerance of a plant [79,80]. There are two main pathways by which Na⁺ enters the root xylem vessels from the soil: the apoplastic pathway and the symplastic pathway. There is mounting evidence to indicate that apoplastic transpiration plays a key role in the movement of Na⁺ into the shoots and the resulting salt tolerance, because the apoplastic barriers (Casparian bands and suberin lamellae) in the exodermis
and endodermis of roots can block the bypass flow of Na$^+$ and prevent it from entering the shoots [81–85]. In the case of the B. napus inbred lines, our results indicate that the root Na$^+$ content is highly negatively correlated with their salt tolerance. In addition, the K$^+$ and Ca$^{2+}$ contents are positively associated with their salt tolerance. These results suggest that root apoplastic barriers may partly participate in the salt tolerance of B. napus, which need to further study.

Membrane-bound translocating proteins facilitate ion transport across plant membranes. Many advances have been made in the study of ion transporters in plant salt tolerance. During exposure to salt stress, the salt overly sensitive (SOS) pathway is known to play a vital role in plants, and the exploration of salt stress perception, signal transduction pathways and regulatory mechanisms are all related to this [20,21,86,87]. The main components of this pathway are a plasma membrane Na$^+/$/H$^+$ antiporter (SOS1), a Ser/Thr protein kinase (SOS2), and a Ca$^{2+}$-binding protein (SOS3) [26,88,89]. The ion regulation ability of a plant determines the ion concentration in its tissues; the ability to balance ions between different tissues and cells is a pivotal mechanism for regulating plant salt tolerance [90–93]. It has been reported that the high-affinity K$^+$ transporters (HKTs) HKT1 and HKT2 are involved in the exclusion of Na$^+$ [94], and Zeeshan, et al. [95] have confirmed that they can recover Na$^+$ from the xylem vessels and limit the transport of Na$^+$ to the shoots. Peng, et al. [91] found that the activities of the Na$^+/$/H$^+$ antiporter and H$^+$-ATPase are pivotal factors that determine the ability of the plant to partition ions into its leaves. Salt stress can be related to the regulation of H$^+$-ATPase in the plasma membrane of the roots and leaves. H$^+$-ATPase, H$^+$-PPase, and the Na$^+/$/H$^+$ reverse transport proteins in the tonoplast can accelerate the absorption of K$^+$ and Ca$^{2+}$, the release of Na$^+$, and the accumulation of Na$^+$ in the vacuole, thus promoting the regional distribution of salt [96–100]. Most commonly, by isolating toxic amounts of Na$^+$ into the vacuoles through the action of the Na$^+/$/H$^+$ antiporters, the maintenance of cytosolic Na$^+$ at a tolerable concentration is achieved in non-halophytes [94,101]. Halophytes can even accumulate ions in vacuoles for osmotic adjustment to cope with saline habitats [17,102]. In the present research, significant differences were detected in the ion contents and selective absorption and transport in the B. napus inbred lines with different salt tolerances, which were somewhat correlated with their salt tolerance (Supplementary Materials S1 and S2). This suggests that membrane-bound translocating proteins in the roots, such as SOS1, Na$^+/$/H$^+$ exchanger 1 (NHX1), HKT1, HKT2, and H$^+$-ATPase also play a part in the uptake of Na$^+$, K$^+$, and Ca$^{2+}$ and their transport in B. napus under salt stress, which requires further exploration.

5. Conclusions

Under NaCl stress, the growth of rape seedlings was inhibited. Salt stress leads to an increase in Na$^+$ content and decrease of K$^+$, Ca$^{2+}$ content of the seedlings. We calculated the correlation coefficients of the growth parameters and ion parameters under different concentrations of NaCl (Supplementary Materials S1 and S2). We found that the salt tolerance of the B. napus has a linear relationship with salt concentrations. In addition, our results indicate that the physiological indicators of B. napus seedlings, such as the ion content and ion selective transport, changed slightly at low salt concentrations, while only small effects were detected for the inhibition of plant growth and no significant difference was found among different salt-tolerance categories. The physiological indicators changed more significantly with the increase of salt concentration, as did the significant differences among different salt-tolerance categories. Our analysis of the correlation coefficients indicated that the Na$^+$ content is the pivotal factor, particularly the root Na$^+$ content, for the salt tolerance of B. napus; the higher the salt tolerance, the lower the root Na$^+$ content. The Ca$^{2+}$/Na$^+$ and K$^+/$/Na$^+$ ratios of the roots were also highly correlated with salt tolerance in B. napus.
Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11070906/s1, Supplementary Materials S1: Absolute and relative values of the B. napus growth indexes; Supplementary Materials S2: Ion contents and related indexes for the B. napus lines in the different salt-tolerance grades under different degrees of NaCl stress.

Author Contributions: Conceptualization, B.-S.W.; methodology, B.-S.W.; data curation, C.-F.W., Z.-Q.Q., Y.-X.L. and Z.-R.Y.; statistical analysis, C.-F.W., G.-L.H., Z.-Q.Q., Y.-X.L. and Z.-R.Y.; writing—original draft preparation, C.-F.W. and G.-L.H.; writing—review and editing, C.-F.W., G.-L.H. and B.-S.W.; funding acquisition, B.-S.W. and G.-L.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China: grant numbers 32002009, 31770288 and 31570251; Natural Science Foundation of Shandong Province: ZR2020QC031; China Postdoctoral Science Foundation: 2020M672114; and Key Technology Research and Development Program of Shandong: 2017CXCQ0313 and 2016GNC113012.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: We gratefully acknowledge Mao-Teng Li from Huazhong University of Science and Technology, Wuhan, China, for kindly providing the B. napus inbred lines with different genetic backgrounds.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Zhu, J.K. Plant salt tolerance. Trends Plant Sci. 2001, 6, 66–71. [CrossRef]
2. Zhang, J.F.; Zhang, X.D.; Zhou, J.X.; Liu, G.H.; Li, D.X. World resources of saline soil and main amelioration measures. Res. Soil Water Conserv. 2005, 12, 28–30.
3. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. Trends Plant Sci. 2014, 19, 371–379. [CrossRef] [PubMed]
4. Yuan, F.; Leng, B.; Wang, B. Progress in studying salt secretion from the salt glands in recrrotehalophytes: How do plants secrete salt? Front. Plant Sci. 2016, 7, 977. [CrossRef] [PubMed]
5. Singh, A. Soil salinization management for sustainable development: A review. J. Environ. Manag. 2021, 277, 111383. [CrossRef] [PubMed]
6. Kovda, V.A. Loss of productive land due to salinization. AMBIO A J. Hum. Environ. 1983, 12, 91–93.
7. Munns, R.; Tester, M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008, 59, 651–681. [CrossRef]
8. Ayyub, C.M.; Ali, M.; Shaheen, M.R.; Qadri, R.W.K.; Khan, I.; Jahangir, M.M.; Abbasi, K.Y.; Kamal, S.; Zain, M. Enhancing the salt tolerance potential of watermelon (Citrus lanatus) by exogenous application of salicylic acid. Am. J. Plant Sci. 2015, 6, 3267–3271. [CrossRef]
9. Shrivastava, P.; Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biol. Sci. 2015, 22, 123–131. [CrossRef]
10. Jamil, A.; Riaz, S.; Ashraf, M.; Foolad, M.R. Gene expression profiling of plants under salt stress. Crit. Rev. Plant Sci. 2011, 30, 435–458. [CrossRef]
11. Wang, B.S.; Zhao, K.F. Comparison of extractive methods of Na, K in wheat leaves. Plant Physiol. Commun. 1995, 31, 50–52.
12. Li, J.; Pu, L.; Han, M.; Zhu, M.; Zhang, R.; Xiang, Y. Soil salinization research in china: Advances and prospects. J. Geogr. Sci. 2014, 24, 943–960. [CrossRef]
13. Wang, J.; Huang, X.; Zhong, T.; Chen, Z. Review on sustainable utilization of salt-affected land. Acta Geogr. Sin. 2011, 66, 673–684.
14. Tarchoune, I.; Degl’Innocenti, E.; Kaddour, R.; Guidi, L.; Lachaâl, M.; Navarri-Izzo, F.; Ouergi, Z. Effects of NaCl or Na2SO4 salinity on plant growth, ion content and photosynthetic activity in Ocimum basilicum L. Acta Physiol. Plant. 2012, 34, 607–615. [CrossRef]
15. Wu, H. Plant salt tolerance and Na+ sensing and transport. Crop J. 2018, 6, 215–225. [CrossRef]
16. Fouilleux, E.; Bricas, N.; Alpha, A. ‘Feeding 9 billion people’: Global food security debates and the productionist trap. J. Eur. Public Policy 2017, 24, 1658–1677. [CrossRef]
17. Yuan, F.; Guo, J.; Shabala, S.; Wang, B. Reproductive physiology of halophytes: Current standing. Front. Plant Sci. 2019, 9, 1954. [CrossRef] [PubMed]
18. Han, G.; Yuan, F.; Guo, J.; Zhang, Y.; Sui, N.; Wang, B. Atsiz1 improves salt tolerance by maintaining ionic homeostasis and osmotic balance in Arabidopsis. Plant Sci. 2019, 285, 55–67. [CrossRef] [PubMed]
19. Freeman, J.L.; Bañuelos, G.S. Selection of salt and boron tolerant selenium hyperaccumulator stanleya pinnata genotypes and characterization of se phytoremediation from agricultural drainage sediments. Environ. Sci. Technol. 2011, 45, 9703–9710. [CrossRef]

20. Zhao, C.; Zhang, H.; Song, C.; Zhu, J.-K.; Shabala, S. Mechanisms of plant responses and adaptation to soil salinity. Innovation 2020, 1, 100017. [CrossRef] [PubMed]

21. Jiang, Z.; Zhou, X.; Tao, M.; Yuan, F.; Liu, L.; Wu, F.; Wu, X.; Xiang, Y.; Niu, Y.; Liu, F.; et al. Plant cell-surface GIPC sphingolipids sense salt to trigger Ca\(^{2+}\) influx. Nature 2019, 572, 341–346. [CrossRef] [PubMed]

22. Hanin, M.; Ebel, C.; Ngom, M.; Laplaze, L.; Masmoudi, K. New insights on plant salt tolerance mechanisms and their potential use for breeding. Front. Plant Sci. 2016, 7, 1787. [CrossRef] [PubMed]

23. Yang, Y.; Wu, Y.; Ma, L.; Yang, Z.; Dong, Q.; Li, Q.; Ni, X.; Kudla, J.; Song, C.; Guo, Y. The Ca\(^{2+}\) sensor SCaBP3/CBL7 modulates plasma membrane H\(^+\)-ATPase activity and promotes alkali tolerance in Arabidopsis. Plant Cell 2019, 31, 1367–1384. [CrossRef] [PubMed]

24. Munns, R.; Day, D.A.; Fricke, W.; Watt, M.; Arsova, B.; Barkla, B.J.; Bose, J.; Byrt, C.S.; Chen, Z.H.; Foster, K.J.; et al. Energy costs of salt tolerance in crop plants. New Phytol. 2020, 225, 1072–1090. [CrossRef] [PubMed]

25. Zeng, L.; Shannon, M.C. Salinity effects on seedling growth and yield components of rice. Crop Sci. 2000, 40, 996–1003. [CrossRef]

26. Zhou, Y.B.; Liu, C.; Tang, D.Y.; Yan, L.; Wang, D.; Yang, Y.Z.; Gui, J.S.; Zhao, X.Y.; Li, L.G.; Tang, X.D.; et al. The receptor-like cytoplasmic kinase STPK1 phosphorylates and activates CatC, thereby regulating H\(_2\)O\(_2\) homeostasis and improving salt tolerance in rice. Plant Cell 2018, 30, 1100–1118. [CrossRef] [PubMed]

27. Zhu, J.K. Cell signaling under salt, water and cold stresses. Curr. Opin. Plant Biol. 2001, 4, 401–406. [CrossRef]

28. Flowers, T.J. Improving crop salt tolerance. J. Exp. Bot. 2004, 55, 307–319. [CrossRef]

29. Liu, J.; Zhu, J.K. An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc. Natl. Acad. Sci. USA 1997, 94, 14960–14964. [CrossRef]

30. Jamil, M.; Lee, C.C.; Rehman, S.U.; Lee, D.B.; Ashraf, M.; Rha, E.S. Salt-tolerant Brassica species at germination and early seedling growth. Electron. J. Environ. Agric. Food Chem. 2005, 4, 970–976. [CrossRef]

31. Roy, S.J.; Negrão, S.; Tester, M. Salt resistant crop plants. Curr. Opin. Biotechnol. 2014, 26, 115–124. [CrossRef] [PubMed]

32. Asif, M.A.; Garcia, M.; Tilbrook, J.; Brien, C.; Dowling, K.; Berger, B.; Schilling, R.K.; Short, L.; Tittlermann, C.; Gillham, M.; et al. Identification of salt tolerance qtl in a wheat ril mapping population using destructive and non-destructive phenotyping. Funct. Plant Biol. 2021, 48, 131–140. [CrossRef] [PubMed]

33. Zheng, Q.-S.; Liu, H.-Y.; Long, X.-H.; Liu, Z.-P.; Niu, D.-D.; Gao, Y.-Y. Effects of salt stress on ionic absorption and distribution of rapeseed seedlings. Chin. J. Oil Crop. Sci. 2010, 32, 65–70.

34. Shang, X.F.; Dong, S.T.; Zheng, S.Y.; Wang, L.Y. Relationship between changes of Na\(^+\), K\(^+\), and Ca\(^{2+}\) contents during seed germination and salt tolerance in maize. Acta Agron. Sin. 2008, 34, 333–336. [CrossRef]

35. Akter, M.; Oue, H. Effect of saline irrigation on accumulation of Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) ions in rice plants. Agriculture 2018, 8, 164. [CrossRef]

36. Shang, F.; Sapkota, S.; Neupane, A.; Yu, J.; Wang, Y.; Zhou, K.; Lu, F.; Huang, R.; Zou, J. Effect of salt stress on growth and physiological parameters of sorghum genotypes at an early growth stage. Indian J. Exp. Biol. 2020, 58, 404–411.

37. Harwood, J.L.; Woodfield, H.K.; Chen, G.; Weselake, R.J. Chapter 6—Modification of oil crops to produce fatty acids for industrial applications. In Fatty Acids; Ahmad, M.U., Ed.; AOCS Press, Elsevier Inc.: Amsterdam, The Netherlands, 2017; pp. 187–236.

38. Zabair, M.; Maqbool, F.; Mehmoood, I.; Muzamml, S.; Waseem, M.; Imam, M.; Nadeem, H.U.; Azem, F.; Siddique, M.H. Chapter 4—Rapeseed oil. In Green Sustainable Process for Chemical and Environmental Engineering and Science; Inamuddin, Boddula, R., Asiri, A.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 41–55.

39. Sun, R. Economic/academic importance of Brassica rapa. In The Brassica Rapa Genome; Wang, X., Kole, C., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–15.

40. Neik, T.X.; Barbetti, M.J.; Batley, J. Current status and challenges in identifying disease resistance genes in Brassica napus. Front. Plant Sci. 2017, 8, 1–37. [CrossRef]

41. Ashraf, M.; Meineley, T. Salinity tolerance in brassica oilseeds. Crit. Rev. Plant Sci. 2004, 23, 157–174. [CrossRef]

42. Santangeli, M.; Capo, C.; Beninati, S.; Pietrini, F.; Forni, C. Gradual exposure to salinity improves tolerance to salt stress in Brassica napus L. Water 2019, 11, 1667. [CrossRef]

43. Rezaei, H.; Khosh Kohlgh Sima, N.A.; Malakouti, M.J.; Pessarakli, M. Salt tolerance of canola in relation to accumulation and xylem transportation of cations. J. Plant Nutr. 2006, 29, 1903–1917. [CrossRef]

44. Yahya, A. Salinity effects on growth and on uptake and distribution of sodium and some essential mineral nutrients in sesame. J. Plant Nutr. 1998, 21, 1439–1451. [CrossRef]

45. Yang, Z.; Li, J.-L.; Liu, L.-N.; Xie, Q.; Sun, N. Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet sorghum. Front. Plant Sci. 2020, 10, 1722. [CrossRef] [PubMed]

46. Wu, H.; Guo, J.; Wang, C.; Li, K.; Zhang, X.; Yang, Z.; Li, M.; Wang, B. An effective screening method and a reliable screening trait for salt tolerance of Brassica napus at the germination stage. Front. Plant Sci. 2019, 10, 530. [CrossRef] [PubMed]

47. Flower, T.J.; Yeo, A.R. Ion relations of salt tolerance. In Solute Transport in Plant Cells and Tissues; Baker, D.A., Hall, J.L., Eds.; John Wiley & Sons: New York, NY, USA, 1988; pp. 392–416.
48. Pitman, M.G. Transport across the root and shoot/root interactions. In *Salinity Tolerance in Plants*; Staples, R.C., Toenniessen, G.H., Eds.; John Wiley & Sons: New York, NY, USA, 1984; pp. 93–124.
49. Swift, M.L. Graphpad prism, data analysis, and scientific graphing. *J. Chem. Inf. Comput. Sci.* 1997, 37, 411–412. [CrossRef]
50. Han, G.; Wei, X.; Dong, X.; Wang, C.; Sui, N.; Guo, J.; Yuan, F.; Gong, Z.; Li, X.; Zhang, Y. Arabidopsis zinc finger protein1 acts downstream of GL2 to repress root hair initiation and elongation by directly suppressing bHLH genes. *Plant Cell* 2020, 32, 206–225. [CrossRef]
51. Nissen, P. Multiphasic uptake mechanisms in plants. *Int. Rev. Cytol. Surv. Cell Biol.* 1991, 126, 89–134.
52. Máser, P.; Gierth, M.; Schroeder, J.I. Molecular mechanisms of potassium and sodium uptake in plants. *Plant Soil* 2002, 247, 43–54. [CrossRef]
53. Kudo, N.; Sugino, T.; Oka, M.; Fujiyama, H. Sodium tolerance of plants in relation to ion balance and the absorption ability of microelements. *Soil Sci. Plant Nutr.* 2010, 56, 225–233. [CrossRef]
54. Ketehouli, T.; Idrice Carther, K.F.; Noman, M.; Wang, F.-W.; Li, X.-W.; Li, H.-Y. Adaptation of plants to salt stress: Characterization of Na⁺ and K⁺ transporters and role of cbl gene family in regulating salt stress response. *Agronomy* 2019, 9, 687. [CrossRef]
55. Errabli, T.; Gandonou, C.B.; Bouhdid, S.; Abrini, J.; Skali-Senhaji, N. Callus growth and ion composition in response to long-term NaCl-induced stress in two sugarcane (*Saccharum* sp.) cultivars. *Int. J. Biotechnol. Mol. Biol. Res.* 2017, 8, 1–9.
56. Zhang, J.-L.; Flowers, T.J.; Wang, S.-M. Mechanisms of sodium uptake by roots of higher plants. *Plant Soil* 2010, 326, 45–60. [CrossRef]
57. Wang, B.S. *Plant Biology under Stress*, 1st ed.; Higher Education Press: Beijing, China, 2010; p. 57.
58. Peng, Y.-H.; Zhu, Y.-F.; Mao, Y.-Q.; Wang, S.-M.; Su, W.-A.; Tang, Z.-C. Alkali grass resists salt stress through high [K⁺] and an endodermis barrier to Na⁺. *J. Exp. Bot.* 2004, 55, 939–949. [CrossRef] [PubMed]
59. Parida, A.K.; Das, A.B. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* 2005, 60, 324–349. [CrossRef]
60. Reddy, I.N.B.L.; Kim, S.M.; Kim, B.K.; Yoon, I.S.; Kwon, T.R. Identification of rice accessions associated with K⁺/Na⁺ ratio and salt tolerance based on physiological and molecular responses. *Rice Sci.* 2017, 24, 360–364. [CrossRef]
61. Tahal, R.; Mills, D.; Heimer, Y.; Tai, M. The relation between low K⁺/Na⁺ ratio and salt-tolerance in the wild tomato species *Lycopersicon pennellii*. *J. Plant Physiol.* 2000, 157, 59–64. [CrossRef]
62. Mansour, E.; Moustafa, E.S.A.; Desoky, E.-S.M.; Ali, M.M.A.; Yasin, M.A.T.; Attia, A.; Alsuaibani, N.; Tahir, M.U.; El-Hendawy, S. Multidimensional evaluation for detecting salt tolerance of bread wheat genotypes under actual saline field growing conditions. *Plants* 2020, 9, 1324. [CrossRef] [PubMed]
63. Abbaszadeh, F.; Rameeh, V.; Cherati, A. Salinity stress indices of seed yield and nutrient compositions in rapeseed (*Brassica napus* L.). *Int. J. Biol.* 2012, 4, 154. [CrossRef]
64. Long, W.H.; Pu, H.M.; Chen, S.; Zhang, J.F.; Qi, C.K.; Zhang, X.K. Evaluation for salt tolerance of three cultivated species of rapeseeds at germination stage. *J. Plant Genet. Resour.* 2014, 15, 32–37.
65. Liu, Y.; Wang, X.; Liu, G.; Sun, J.; Yao, Y.; Yang, Y. Ions absorption and accumulation in four typical salt-tolerant plants in coastal saline soil region. *Soils* 2017, 49, 782–788.
66. Hasanuzzaman, M.; Inafuku, M.; Nahar, K.; Fujita, M.; Oku, H. Nitric oxide regulates plant growth, physiology, antioxidant defense, and ion homeostasis to confer salt tolerance in the mangrove species, *Kandelia obovata*. *Antioxidants* 2021, 10, 611. [CrossRef]
67. Rameeh, V. Ions uptake, yield and yield attributes of rapeseed exposed to salinity stress. *J. Soil Sci. Plant Nutr.* 2012, 12, 851–861. [CrossRef]
68. Robbins, N.E.; Trontin, C.; Duan, L.; Dinneny, J.R. Beyond the barrier: Communication in the root through the endodermis. *Plant Physiol.* 2014, 166, 551–559. [CrossRef] [PubMed]
69. Muthert, L.W.F.; Izzo, L.G.; Zanten, M.V.; Aronne, G. Root tropisms: Investigations on earth and in space to unravel plant growth direction. *Front. Plant Sci.* 2020, 10, 1807. [CrossRef] [PubMed]
70. Choi, W.G.; Toyota, M.; Kim, S.H.; Hilleary, R.; Gilroy, S. Salt stress-induced Ca²⁺ waves are associated with rapid, long-distance root-to-shoot signalling in plants. *Proc. Natl. Acad. Sci. USA* 2014, 111, 6497–6502. [CrossRef]
71. Elsheikh, E.A.E.; Wood, M. Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). *J. Exp. Bot.* 1990, 41, 1263–1269. [CrossRef]
72. Wang, S.; Zheng, W.; Ren, J.; Zhang, C. Selectivity of various types of salt-resistant plants for K⁺ over Na⁺. *J. Arid. Environ.* 2002, 52, 457–472. [CrossRef]
73. Bose, J.; Rodrigo-Moreno, A.; Lai, D.; Xie, Y.; Shen, W.; Shabala, S. Rapid regulation of the plasma membrane H⁺-ATPase activity is essential to salinity tolerance in two halophyte species, *Atriplex lentiformis* and *Chenopodium quinoa*. *Ann. Bot.* 2015, 115, 481–494. [CrossRef] [PubMed]
74. Bose, J.; Rodrigo-Moreno, A.; Shabala, S. Ros homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* 2013, 65, 1241–1257. [CrossRef]
75. West, G.; Inzé, D.; Beemster, G.T.S. Cell cycle modulation in the response of the primary root of *Arabidopsis* to salt stress. *Plant Physiol.* 2004, 135, 1050–1058. [CrossRef]
76. Julkowska, M.M.; Koevoets, I.T.; Mol, S.; Hoefsloot, H.; Feron, R.; Tester, M.A.; Keurentjes, J.J.B.; Korte, A.; Haring, M.A.; Boer, G.J.D.; et al. Genetic components of root architecture remodeling in response to salt stress. *Plant Cell* 2017, 29, 3198–3213. [CrossRef]
77. Ottow, E.A.; Brinker, M.; Teichmann, T.; Fritz, E.; Kaiser, W.; Brosché, M.; Kangasjärvi, J.; Jiang, X.; Polle, A. *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf succulence under salt stress. *Plant Physiol.* 2005, 139, 1762–1772. [CrossRef] [PubMed]

78. Dahuja, A.; Kumar, R.R.; Sakhare, A.; Watts, A.; Singh, B.; Goswami, S.; Sachdev, A.; Praveen, S. Role of atp-binding cassette transporters in maintaining plant homeostasis under abiotic and biotic stresses. *Physiol. Plant.* 2021, 171, 785–801. [CrossRef]

79. Chen, M.; Yang, Z.; Liu, J.; Zhu, T.; Wei, X.; Fan, H.; Wang, B. Adaptation mechanism of salt excluders under saline conditions and its applications. *Int. J. Mol. Sci.* 2018, 19, 3668. [CrossRef] [PubMed]

80. Xue, Z.; Zhao, S.; Gao, H.; Sun, S. The salt resistance of wild soybean (*Glycine soja* sieb. Et zucc. *Zydid 03262*) under NaCl stress is mainly determined by Na⁺ distribution in the plant. *Acta Physiol. Plant.* 2014, 36, 61–70. [CrossRef]

81. Krishnamurthy, P.; Ranathunge, K.; Franke, R.; Prakash, H.S.; Schreiber, L.; Mathew, M.K. The role of root apoplastic transport barriers in salt tolerance of rice (*Oryza sativa* L.). *Planta* 2009, 230, 119–134. [CrossRef]

82. Krishnamurthy, P.; Ranathunge, K.; Nayak, S.; Schreiber, L.; Mathew, M.K. Root apoplastic barriers block Na⁺ transport to shoots in rice (*Oryza sativa* L.). *J. Exp. Bot.* 2011, 62, 4215–4228. [CrossRef] [PubMed]

83. Ottow, E.A.; Brinker, M.; Teichmann, T.; Fritz, E.; Kaiser, W.; Brosché, M.; Kangasjärvi, J.; Jiang, X.; Polle, A. *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf succulence under salt stress. *Plant Physiol.* 2005, 139, 1762–1772. [CrossRef] [PubMed]

84. Zheng, Q.S.; Wang, R.L.; Liu, Y.L. Effects of Ca⁺⁺ on absorption and distribution of ions in salt-treated cotton seedlings. *Sci. Rep.* 2016, 6, 34548. [CrossRef] [PubMed]

85. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* 2002, 25, 239–250. [CrossRef] [PubMed]

86. Saddhe, A.A.; Mishra, A.K.; Kumar, K. Molecular insights into the role of plant transporters in salt stress response. *Physiol. Plant.* 2021, 173, 1481–1494. [CrossRef] [PubMed]

87. Tan, T.; Cai, J.; Zhan, E.; Yang, Y.; Zhao, J.; Guo, Y.; Zhou, H. Stability and localization of 14-3-3 proteins are involved in salt tolerance in *Arabidopsis*. *Plant Mol. Biol.* 2016, 92, 391–400. [CrossRef] [PubMed]

88. Guo, Y.; Halfter, U.; Ishitani, M.; Zhu, J.K. Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. *Plant Cell* 2001, 13, 1383–1400. [CrossRef] [PubMed]

89. Zhu, J.K. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* 2003, 6, 441–445. [CrossRef]

90. Zhong, D.; Yang, C.; Chong, J.; Li, C.; Kim, C.; Shi, D.; Wang, D. Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sierciana* during adaption to salt and alkali conditions. *Plant Soil* 2007, 294, 263–276. [CrossRef]

91. Chen, T.; Wang, W.; Xu, K.; Xu, Y.; Ji, D.; Chen, C.; Xie, C. K⁺ and Na⁺ transport contribute to K⁺/Na⁺ homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Res.* 2019, 48, 101526. [CrossRef]

92. Sen, A. Integrative expressional regulation of TaHKT2.1, TaNa⁺/H⁺ vacuolar antiporter, and TaSOS1 genes improve salt tolerance in *Triticum aestivum* L. *Int. J. Mol. Sci.* 2021, 22, 1762–1776. [CrossRef]

93. Chen, T.; Wang, W.; Xu, K.; Xu, Y.; Ji, D.; Chen, C.; Xie, C. K⁺ and Na⁺ transport contribute to K⁺/Na⁺ homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Res.* 2019, 48, 101526. [CrossRef]

94. Akkari, K.; Madhlo. Characterization of the Na⁺ delivery from roots to shoots in rice under saline stress: Excessive salt enhances apoplastic transport in rice plants. *Soil Sci. Plant Nutr.* 2002, 48, 371–378. [CrossRef]

95. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* 2002, 25, 239–250. [CrossRef] [PubMed]

96. Chen, T.; Wang, W.; Xu, K.; Xu, Y.; Ji, D.; Chen, C.; Xie, C. K⁺ and Na⁺ transport contribute to K⁺/Na⁺ homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Res.* 2019, 48, 101526. [CrossRef]

97. Apse, M.P.; Blumwald, E. Na⁺ transport in plants. *FEBS Lett.* 2007, 581, 2247–2254. [CrossRef] [PubMed]

98. Yang, C.; Chong, J.; Li, C.; Kim, C.; Shi, D.; Wang, D. Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sierciana* during adaptation to salt and alkali conditions. *Plant Soil* 2007, 294, 263–276. [CrossRef]

99. Chen, T.; Wang, W.; Xu, K.; Xu, Y.; Ji, D.; Chen, C.; Xie, C. K⁺ and Na⁺ transport contribute to K⁺/Na⁺ homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Res.* 2019, 48, 101526. [CrossRef]

100. Sen, A. Integrative expressional regulation of TaHKT2.1, TaNa⁺/H⁺ vacuolar antiporter, and TaSOS1 genes improve salt tolerance in *Triticum aestivum* L. *Int. J. Mol. Sci.* 2021, 22, 1762–1776. [CrossRef]

101. Blumwald, E.; Aharon, G.S.; Apse, M.P. Sodium transport in plant cells. *Biochim. Biophys. Acta* 2000, 1465, 140–151. [CrossRef]

102. Flowers, T.J.; Munns, R.; Colmer, T.D. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.* 2014, 115, 419–431. [CrossRef] [PubMed]