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Screening of salt tolerance traits and the salt tolerance evaluation method in *Brassica napus* at the seed germination stage

Aldiyar Bakirov,[1] Yan Zhang,[1] Qi Zhang,[1] Shayakhmetova Altyn Seitahmetovna,[2] Xiaojuan Yu,[3] Yiji Shi,[1] Yu Xu,[1] Kai Wang,[1] Mengfan Qin,[1] Aixia Xu,[1] Zhen Huang[1]

[1]State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, China; [2]Agrotechnological Faculty, North Kazakhstan State University, Petropavlovsk, Kazakhstan; [3]Laboratory of Forest Microorganism, College of Forestry, Northwest A&F University, Yangling, China

Highlights

- *The salinity tolerance of 200 varieties of B. napus germplasms was investigated.*
- *B. napus is more vulnerable to saline conditions during the germination and early reproductive stages than the vegetative and flowering periods.*
- *Based on hierarchical cluster analysis, there was a wide variability of salinity tolerance among rapeseed germplasm.*
- *Low concentration of sodium chloride had a positive effect on shoot and root growth, germination and total weight in some B. napus seedlings.*
- *Total fresh weight can be utilized as the most efficient index for mass screening of salt tolerance in B. napus germplasms at the germination stage.*

Correspondence: Zhen Huang, State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, 712100, China.
Tel./Fax: +86.029.87082854. E-mail: huang_zhen.8@163.com

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Abstract

Soil salinity is one of the major abiotic stresses that negatively affect plant growth and agricultural productivity. For many crop species, the germination stage is one of the most sensitive stages to salinity stress. This study evaluated salt tolerance in 200 *Brassica napus* L. germplasms using hierarchical cluster analysis based on multiple morphological parameters, including germination rate, root length, fresh weight of root, shoot length, fresh weight of shoot, and total fresh weight. Membership function was used as a comprehensive index to select and evaluate salt tolerance of these germplasms, identifying 8 highly salt-tolerant germplasms, 40 salt-tolerant germplasms, 65 moderate salt-tolerant germplasms, 52 salt-sensitive germplasms, and 35 highly salt-sensitive germplasms lines. The responses of rapeseed germplasm to salt stress indicate differences in morphological parameters.

Furthermore, NaCl showed a positive effect on total fresh weight and biomass production of some germplasms at a concentration of 100 mmol L^{-1}. Since the correlation value of salt tolerance with total fresh weight was highest under 200 mmol L^{-1} NaCl, it can be considered the most reliable parameter to evaluate salt tolerance. Therefore, the findings of this study can be applied as an effective and reliable method for mass screening and evaluation of *Brassica napus* germplasm at the germination stage for breeding salt-tolerant rapeseed genotypes.

Introduction

Soil salinity is one of the main abiotic stresses of plants and a major limiting factor affecting the growth, function, and yield of crops. Soil salinisation affects nearly 6% of the entire land area, which is equivalent to approximately 1 billion hectares. Salinity is a significant environmental problem in agriculture, harming plant development and crop productivity in many countries. Worldwide in arid and semi-arid regions, 33% and 20% of irrigated and cultivated land are degraded and salt-affected (Ding *et al.*, 2010; Machado and Serralheiro, 2017; Rebey *et al.*, 2017; Chang *et al.*, 2018). The global saline area generally increases by 10% per year.

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With climate change, rainfall is rapidly reducing in some areas, and temperature gradually increases, leading to disappointing and dangerous changes in saline areas (Chen and Mueller, 2018; Arora, 2019). Therefore, salinisation can be viewed as a dynamic process that requires constant monitoring and evaluation by experts (Pankova *et al.*, 2017). Plants respond to salinity differently, which is reflected in changes in biomass accumulation rate, general productivity, and economic efficiency of various cultivars and in biochemical, morphological, and molecular changes of the plants (Taarit *et al.*, 2010; Liang *et al.*, 2018). Consequently, disorders appear in osmotic stress, ion toxicity, and nutrient deficiency, thereby disrupting plants' photosynthesis, respiration, and glucose synthesis (Ruiz-Lozano *et al.*, 2012; Torabi, 2014). One of the best ways to solve the soil salinisation problem is to screen for salt-tolerant genotypes and varieties (Mahmood, 2011; Ashraf *et al.*, 2012).

Salinity damage to crop development is related to the plant growth stage and the level of salinity (Chartzoulakis and Loupassaki, 1997). All plants begin their natural life by germination, which is one of the most critical and vulnerable stages that influence crop growth and development during the plant lifestyle. Generally, germination and early reproductive stages are considered more vulnerable to saline conditions than vegetative and flowering periods (Hamdy *et al.*, 1993; Misra and Dwivedi, 2004). It has been reported that salt stress significantly reduces the germination rate and establishment of seedlings in many plant species (Khan and Ungar, 1999; Guma *et al.*, 2010; Zivdar *et al.*, 2011), including the *Brassicaceae* family (Puppala *et al.*, 1999; Qasim *et al.*, 2003; Ashraf and McNeilly, 2004). Likewise, salt stress inhibits the ability of roots to absorb water, which in turn leads to the suppression of plant growth and a decrease in crop quality (Carpýcý *et al.*, 2009; Evelin *et al.*, 2009). Therefore, to ensure successful crop development and acceptable grain production, it is necessary to improve the salt tolerance of plants, starting from the germination stage.

Canola or Rapeseed (*Brassica napus L.*) is the second most significant salt-tolerance oil crop grown in many parts of the world, providing significant nutritional and economic value (Maas and Hoffman, 1977; Raymer, 2002). The most widespread ionic content of saline soils is NaCl (Cumming and Elliot, 1991). Na^+ and Cl^- are the two ions most commonly associated with plant toxicity because they are highly soluble in water, easily absorbed, and transported to the shoots in the transpiration stream (Niu *et al.*, 1995). A recent study found that in sensitive cultivars, the accumulation of Na^+ ions occurs more rapidly than in tolerant cultivars, leading to cell destruction and, ultimately, death of *B. napus* (Kanwal *et al.*, 2021). Some evaluation methods (Lee *et al.*, 2008; Carpýcý *et al.*, 2009; Guo-Wei *et al.*, 2011; Deng *et al.*, 2014) have been developed to screen salt tolerance in plant species. Different species have different criteria for evaluating salt tolerance at the germination stage. Several studies have been conducted to evaluate salt tolerance in *B. napus* during the germination stage (Bybordi and Tabatabaei, 2009; Wei-hua *et al.*, 2013; Kanwal *et al.*, 2021). However, these studies have only screened a few rapeseed germplasms and failed to use an effective screening indicator.

This study investigates the salt tolerance of *B. napus* germplasms at different salinity levels to develop a reliable and effective large-scale screening method for finding salt-tolerant germplasm for rapeseed breeding in saline soils. A total of 200 *B. napus* germplasms were evaluated. Based on correlation analysis, total fresh weight was considered the most accurate index to identify the salt tolerance level of *B. napus*. Our study can be used to improve breeding further and to gain new knowledge about the salt tolerance mechanisms of *B. napus*.

Materials and methods

Plant materials

Professor Huang Zhen kindly provided the seeds of 200 varieties of *B. napus* germplasms with different genetic backgrounds from the State Key Laboratory of Crop Stress Biology for Arid Areas of Northwest Agriculture and Forest University. After harvesting in 2019, the seeds were stored in a refrigerator at a temperature of 5°C.

Determination of morphological characteristics under salt stress

The seeds of each germplasm were surface sterilised with 70% ethanol for 5 min, thoroughly rinsed with purified water five times, and then placed in distilled water for 18 hours. Sterilised 120-mm Petri plates containing clean Whatman double-layer paper were divided into three equal sections, each section containing fifteen healthy uniformly shaped and sized seeds of *B. napus* germplasm lines. To save space, each Petry plate contained one concentration of sodium chloride (100 or 200 mmol L^{-1} NaCl or distilled water without NaCl as a control) with three different germplasm lines (every 15 seeds) of *B. napus*. The experiment was arranged in a complete randomised design (CRD) with three replicates for each experiment (Table S1). Under controlled conditions, the seeds were cultured in a germination chamber with a 16 h light/8 h dark period at light intensity 700 µmol $m^{-2}s^{-1}$ temperature cycle of 27±2°C:22±2°C (day:night) and 60-70% relative humidity (RH). The salt content of the Petri dishes was closely monitored and maintained during the experiments. When the radicle of the seed is at least 2 mm in length, it is considered germinated. To evaluate the salt tolerance of 200 rapeseed germplasms at the germination stage, fresh weight of root (FWR), fresh weight of shoot (FWS), shoot length (SL), and root length (RL) were measured on all 15 seeds peer trait individually after seven days.

The germination rate (GR) is calculated according to Eq. 1 (Lovato and Martins, 1997; Gharoobi *et al.*, 2012).

$$GR = \frac{GS7}{T} \times 100\% \quad (1)$$

where GS_7 is the number of seeds germinated after seven days; T is the number of total seeds.

The total fresh weight (TFW) was calculated as a sum of the FWS and FWR of one plant. For each morphological parameter, the salt tolerance index (STI) was also measured to capture the differences in salt tolerance among various rapeseed germplasms (Eq. 2).

$$STI_g = V_{gt}/V_{gc} \quad (2)$$

where STI_g is the STI of trait g, V_{gt} is the value of trait g observed in the NaCl-treatment plant, and V_{gc} is the value of the trait g observed in the control plant.

Evaluation of salt tolerance and hierarchical cluster analysis of morphological parameters

Salt tolerance in *B. napus* was evaluated using the fuzzy comprehensive evaluation method based on the membership function value (MFV) (Ding *et al.*, 2018). First, the MFV was determined using the following formula (Eq. 3):

$$Xi = \frac{X - Xmin}{Xmax - Xmin} \times 100\% \quad (3)$$

where X_i represents the membership function value of the STI in given rapeseed germplasm, X represents the measured value of STI, and X_{min} and X_{max} are the minimum and maximum values of STI monitored in all germplasms, respectively.

To rank the degree of resistance to salinity among rapeseed varieties from 0 to 1, the mean of MFV was calculated as the average of the membership function values of all traits for each cultivar. Hence, each germplasm has a unique mean MFV; the higher the mean MFV, the greater salt tolerance in that specific germplasm. The mean MFV of each germplasm was measured by averaging the membership function values of all traits: the germination rate, root length, fresh weight of root, shoot length, fresh weight of shoot, and total fresh weight (Chen *et al.*, 2012).

To classify germplasms, hierarchical cluster analysis based on the complete linkage method with the squared Euclidean distance analysis of the average means of MFVs traits was used in each group. The germplasms were divided into five independent salt tolerance groups: high salt-sensitive (HSS), salt-sensitive (SS), moderate salt tolerance (MST), salt tolerance (ST), and high salt tolerance (HST).

Statistical analysis

The data from the independent experiments are presented as mean ± standard deviation. Pearson correlations and standard error of means were statistically examined using IBM Corporation's Statistic Package for Social Science (SPSS) version 26 for Windows and ANOVA. Calculations and graphs were plotted using Origin 9.0 software (Origin Lab Corporation, Northampton, USA). The means of all growth parameters under different NaCl concentration considered significant: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; while non-significant (ns) (P>0.05).

Results

Morphological responses of *B. napus* germplasms to NaCl stress

The morphological parameters of each germplasm, including GR, RL, SL, FWR, FWS, and TFW, were measured at different NaCl concentrations (0, 100, and 200 mmol L^{-1}) (Table S2). The STIs of each germplasm under different growth parameters were also measured (Table S3) and shown in Figure 1. When the plants were treated with 200 mmol L^{-1} NaCl, the values of germination parameters of all examined seedlings were significantly (P<0.05) lower compared with those of 100 mmol L^{-1} NaCl (Figure S1). As shown in the data (Figure S1), the decrease in values of the physiological parameters was more pronounced with the application of the higher concentration of 200 mmol L^{-1} NaCl. Therefore, the effect of sodium chloride on salt tolerance parameters of *B. napus* germplasms can be evaluated using the STI values of different morphological traits.

Only nine germplasms showed an STI of GR lower than 0.4 after the 100 mmol L^{-1} NaCl treatment. In contrast, 154 germplasms showed a high STI of GR (more than 0.6). After the 200 mmol L^{-1} NaCl treatment, 112 germplasms had an STI of GR less than 0.4. (Figure 1A). During the treatment with 100 mmol L^{-1} NaCl, most germplasms (154) had an STI of RL ranging from 0.4 to 1. However, after the treatment with 200 mmol L^{-1} NaCl, the STI of RL of the majority germplasm (189 germplasms) was reduced to less than 0.4 (Figure 1B). After the 100 mmol L^{-1} NaCl treatment, the STI of FWR of the main germplasms (103 germplasms) ranged from 0.4 to 1. Seven germplasms showed an STI of RFW greater than 1. Moreover, the number of germplasms with an STI of FWR less than 0.4 increased to 178 after treatment with 200 mmol L^{-1} NaCl (Figure 1C). Under the 100 mmol L^{-1} NaCl treatment, the STI of SL of 149 germplasms ranged from 0.6 to 1, with only 55 germplasms having an STI of SL less than 0.6 and five germplasms having an STI of SL greater than 1. After the 200 mmol L^{-1} NaCl treatment, 149 germplasms had an STI of SL less than 0.6 (Figure 1D). Moreover, under the NaCl stress, FWS and TFW generally changed similarly. After the treatment with 100 mmol L^{-1} NaCl, only a few germplasms (23 and 28 germplasms) had STIs of FWS and TFW less than 0.4. Conversely, 80 and 117 germplasms had high STI values (above 0.6). Moreover, with the treatment of 200 mmol L^{-1} NaCl, 83 and 42 germplasms showed STIs of FWS and TFW higher than 1. In contrast, 98 and 114 germplasms had STIs of FWS and TFW less than 0.4 (Figure 1E and F). All germplasms could properly germinate and develop without the salt treatment (Table S2). Under the 100 mmol L^{-1} NaCl, the average means of FWS, TFW, and GR decreased by

Table 1. Correlation coefficients between mean membership function value and salt tolerance index of each parameter of 200 *Brassica napus* germplasms at the germination stage under different levels of salt stresses (100 and 200 mmol L^{-1} NaCl).

	f	$STI_{GR\ 200}$	$STI_{FWS\ 100}$	$STI_{FWS\ 200}$	$STI_{FWR\ 100}$	$STI_{FWR\ 200}$	$STI_{SL\ 100}$	$STI_{SL\ 200}$	$STI_{RL\ 100}$	$STI_{RL\ 200}$	$STI_{TFW\ 100}$	$STI_{TFW\ 200}$
$STI_{GR\ 100}$	1											
$STI_{GR\ 200}$	0.678**	1										
$STI_{FWS\ 100}$	0.513**	0.501**	1									
$STI_{FWS\ 200}$	0.596**	0.842**	0.585**	1								
$ST_{IFWR\ 100}$	0.516**	0.485**	0.314**	0.417**	1							
$ST_{IFWR\ 200}$	0.495**	0.717**	0.363**	0.759**	0.499**	1						
$STI_{SL\ 100}$	0.533**	0.528**	0.633**	0.465**	0.545**	0.351**	1					
$STI_{SL\ 200}$	0.576**	0.811**	0.570**	0.905**	0.405**	0.720**	0.543**	1				
$STI_{RL\ 100}$	0.495**	0.598**	0.499**	0.561**	0.714**	0.534**	0.648**	0.519**	1			
$STI_{RL\ 200}$	0.475**	0.678**	0.319**	0.654**	0.477**	0.838**	0.354**	0.679**	0.597**	1		
$STI_{TFW\ 100}$	0.577**	0.569**	0.954**	0.631**	0.536**	0.456**	0.685**	0.613**	0.630**	0.417**	1	
$STI_{TFW\ 200}$	0.598**	0.852**	0.558**	0.985**	0.456**	0.822**	0.447**	0.890**	0.579**	0.714**	0.627**	1
Mean MFV	0.737**	0.889**	0.706**	0.911**	0.637**	0.802**	0.680**	0.889**	0.753**	0.757**	0.793**	0.922**

**Correlation is significant at the 0.01 level (2-tailed). MFV, mean membership function value; STI, salt tolerance index; STI_{FWR}, STI of fresh weight of root; STI_{FWS}, STI of fresh weight of shoot; STI_{TFW}, STI of total fresh weight; STI_{RL}, STI of root length; ST_{ISL}, STI of shoot length; STI_{GR}, STI of germination rate.

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9.11%, 22.12%, and 22.08%, respectively. However, after treatment with 200 mmol L^{-1} NaCl, the average means of FWS, TFW, and GR were decreased by 61.35%, 68.53%, and 60.73%, respectively. The average means of FWR and RL decreased more substantially by 54.76% and 62.91%, respectively, after the 100 mmol L^{-1} NaCl. In addition, the average means of FWR and RL decreased by 86.6% and 89.38%, respectively, after the treatment with 200 mmol L^{-1} NaCl. The SL was reduced by 31.12 % under the treatment of 100 mmol L^{-1} NaCl, and the average mean was lowered by 64.97 % under the treatment of 200 mmol L^{-1} NaCl.

Correlation analysis between salt tolerance index and different morphological traits under different salinity stresses

It was found that most morphological parameters were correlated with each other, and the correlation between different traits is shown in Table 1. According to the statistical data, STIs of TFW, RL, FWR, SL, and FWS showed a positive correlation under the 200 mmol L^{-1} NaCl treatment (Table 1). STIs of TFW and FWS had the highest correlation (0.985), followed by the correlation between the STIs of FWS and SL (0.905), whereas the lowest posi-

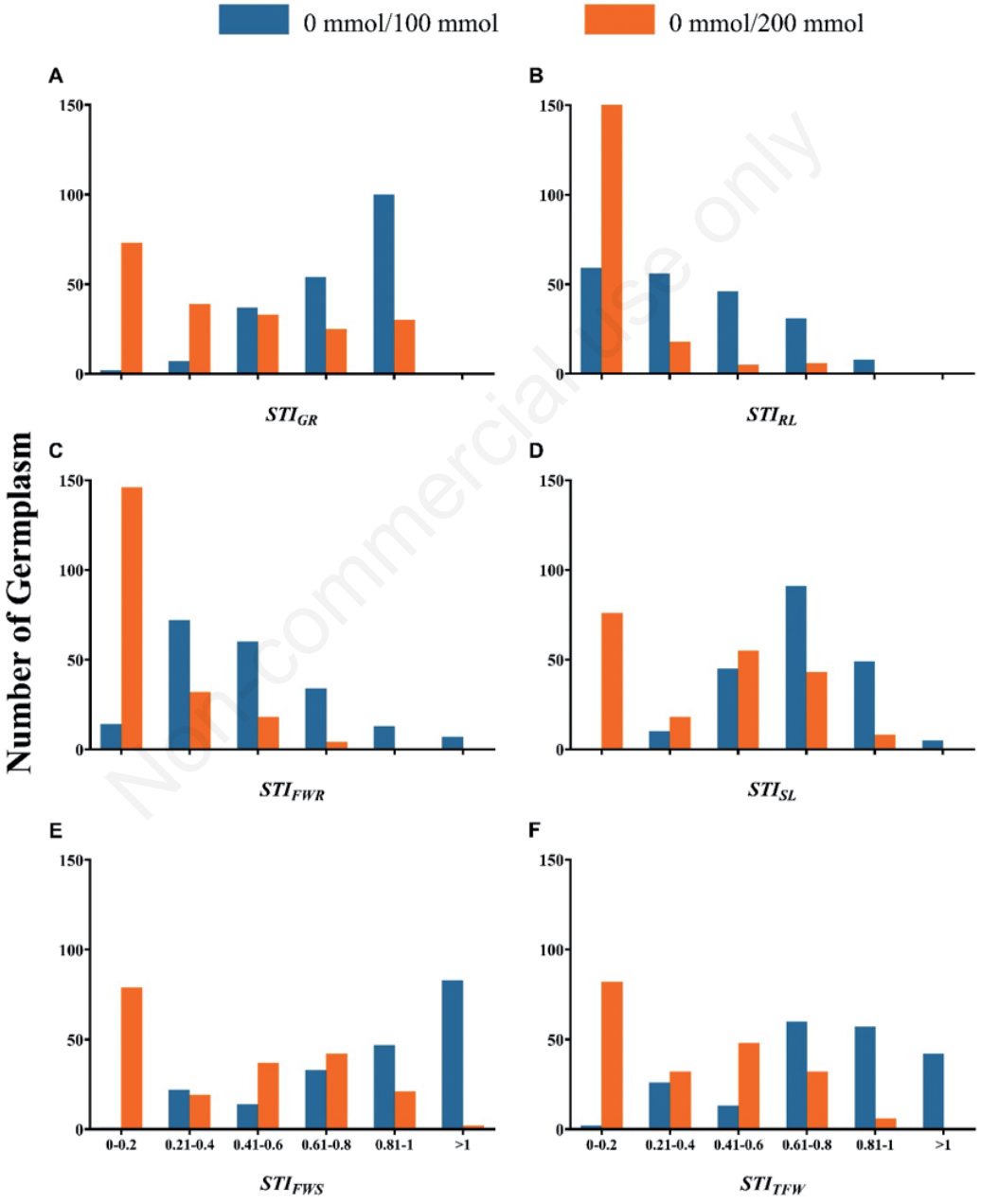

Figure 1. The 200 Rapeseed germplasms were classified according to the salt tolerance index (STI) of each parameter. A) STI of germination rate (STI_{GR}); B) STI of root length (STI_{RL}); C) STI of root fresh weight (STI_{RFW}); D) STI of shoot length (STI_{SL}); E) STI of shoot fresh weight (STI_{SFW}); F) STI of total fresh weight (STI_{TFW}).

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tive correlation (0.654) was between STIs of RL and FWS. The STI of each trait had a higher correlation coefficient under the 200 mmol L^{-1} NaCl treatment than that of 100 mmol L^{-1} NaCl treatment. The STIs of FWS and TFW under 100 mmol L^{-1} NaCl treatment had a high correlation coefficient (0.954).

Furthermore, after the treatment of 200 mmol L^{-1} NaCl, the STI of TFW showed the highest correlation (0.922), with a mean MFV. Moreover, the STIs of FWS, GR, and SL after the 200 mmol L^{-1} NaCl treatment were strongly correlated with the mean MFV (0.911, 0.889, and 0.889, respectively). Additionally, the correlation coefficient between the STIs of each parameter and the mean MFV decreased significantly under 100 mmol L-1 NaCl treatment.

The highest correlation was between the STI of TFW and the mean MFV (0.793) with the 100 mmol L^{-1} NaCl treatment, followed by the STI of RL (0.753). The correlation between the STI of FWR and the mean MFV and other parameters was also low.

According to a previous study (Chen *et al.*, 2012), the greater the average mean MFV, the stronger the salt tolerance in the plant. In this study, the STIs of TFW, FWS, SL, FWR, RL, and GR showed an obvious effect on the mean MFV; the higher the STI of each value, the larger the mean MFV. Therefore, to identify the most reliable morphological parameters that optimally reflect the salt resistance of individual rapeseed germplasm at the germination stage, a linear regression model between mean MFV and STI of all parameters was built (Figure 2).

The coefficient of determination was fairly high between the STIs of TFW, FWS, and the mean MFV (R^2=0.849 and 0.829, respectively) under the 200 mmol L^{-1} NaCl treatment (Figure 2F and D), whereas the coefficient of determination was relatively low (R^2=0.789, 0.789, and 0.642, respectively) between the mean MFV and STIs of GR, SL, and FWR. In addition, the minimum value (R^2=0.573) was observed between the STI of RL and the mean MFV. In contrast, the coefficient of determination under the 200 mmol L^{-1} NaCl treatment was higher than that under the 100 mmol L^{-1} NaCl treatment. As shown in Figure 2, under the 100 mmol L^{-1} NaCl treatment, the highest coefficient of determination was between the mean MFV and STI of TFW (R^2=0.628). The second highest was between the mean MFV and the STI of RL (R^2=0.566), followed by the mean MFV and STI of GR (R^2=0.542). Overall, since TFW showed the highest positive correlation under different NaCl treatments (100 and 200 mmol L^{-1} NaCl), it can be utilised as the most efficient index for mass screening of salt tolerance in *B. napus* germplasms at the germination stage.

Salt tolerance evaluation

To rank the degree of resistance to salinity among rapeseed varieties, the MFVs of each individual trait and the mean MFV were determined (Table S4), and the distribution is presented in Figure 3. The higher the mean of MFV, the higher salt tolerance. The mean MFV ranges from 0.01 (N909) to 0.83 (N1131), with an average value and standard deviation of 0.38±0.2, respectively.

Grouping of rapeseed cultivars for salt tolerance was done by subjecting the data of the mean MFV of each parameter to hierarchical cluster (Euclidean distance) analysis. All rapeseed germplasms were clustered into five distinct groups at a 2.6 similarity coefficient level, shown in the dendrogram in Figure 4A. The hierarchical clustering results can be better understood by converting the dendrogram to a scatter plot (Oku, 2018). Gaussian blobs are built by bending the MFV variables on a two-dimensional plane using the Scikit-learn python library (Pedregosa *et al.*, 2011), while the hierarchical structure is preserved (Figure 4B).

The 200 *B. napus* germplasms were divided into five categories based on the results of a hierarchical cluster analysis, including 8 HST, 40 ST, 65 MST, 52 SS, and 35 HSS (Figure 5). The values of all morphological parameters germination rate (GR), shoot length (SL), root length (RL), fresh weight of shoot (FWS), fresh weight of root (FWR), and total fresh weight (TFW) between different salt tolerance groups presented in Figure 6.

To determine whether the total fresh weight provides an accurate estimate for classifying salt-tolerant genotypes, the total fresh weight parameters were measured in 100, 200 mmol L^{-1} NaCl and distilled water (0 mmol L^{-1} NaCl). Three germplasms were randomly chosen from each salt tolerance group and seedling phenotype to present germination changes in different salt tolerance groups (Figure 7A). The difference in total fresh weight between the salt-tolerant groups was compared. The results showed that the accumulation of total fresh weight with increasing salt concentration was relatively low and then sharply decreased under 200 mmol NaCl L^{-1} (Figure 7B). The total fresh weight values based on salt tolerance groups can be arranged in the following manner: HST < ST < MST < SS < HSS. However, some *B. napus* genotypes from the salt-resistant groups (HST and ST) showed an increase in total fresh weight and shoot fresh weight biomass production under the 100 mmol L^{-1} NaCl treatment compared with the control (0 mmol L^{-1} NaCl).

Discussion

Salinity tolerance remains a global problem for many crops, including *B. napus* (Puppala *et al.*, 1999; Ashraf and McNeilly, 2004). Due to the complexity of plant responses to environmental stresses, many crops have developed their mechanisms for salinity (Noreen and Ashraf, 2007; Maliro *et al.*, 2008; Sabir *et al.*, 2011). In particular, various genotypes have demonstrated statistically significant differences in their developmental characteristics when exposed to NaCl stress. Surprisingly, increasing salinity showed positive impacts on shoot and root growth, germination rate, total fresh weight, and biomass output in some germplasms of *B. napus* when treated with 100 mmol L^{-1} NaCl. This phenomenon can be partially explained because treatments with low NaCl concentrations positively affect germination because the accumulation of Na^+ and K^+ is optimal for metabolic activities, making these conditions more effective for germination and growth (Mahmoodzadeh, 2008). Studies have shown that *B. napus* is salt-tolerant to a certain extent (Maas and Hoffman, 1977). Furthermore, a previous study (Ahmad *et al.*, 2012) showed that shoot and root length, fresh biomass, and germination rate are positively impacted by applying 50 mmol L^{-1} of NaCl, which is consistent with the results in this study. The improved growth of *B. napus* genotypes at low-level NaCl concentrations may be due to better water absorption, leading to a faster increase in biomass output, which should be further verified in the future.

Since all crops begin their growth with seed germination, salinity can dramatically affect germination rates. In addition, most plants are more sensitive to salt stress during seed germination and seedling growth stages compared to the reproductive stage (Ashraf, 1994; Purty *et al.*, 2008). Therefore, when a plant is cultivated in saline soil, germination and seedling emergence of salt-sensitive germplasms are significantly reduced, resulting in low yields and reduced product quality (Bajji *et al.*, 2002; Cuartero *et al.*, 2006; Zivdar *et al.*, 2011).

Our study shows that NaCl stress had a variety of negative impacts on the germination and development of 200 *B. napus*

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germplasms (Figure S1). The mean MFV may be applied to evaluate crop salt tolerance; a higher mean MFV indicates stronger plant salt tolerance. To comprehensively evaluate the salt tolerance of *B. napus* germplasms, hierarchical cluster analysis with multiple parameters based on the furthest neighbour and the mean MFV was used (Figure 4A).

Among the 200 rapeseed germplasms, 8 germplasms were classified due to their high salt tolerance, 40 germplasms as salt-tolerant, 65 germplasms as moderately salt-tolerant, 52 germplasms as salt-sensitive, and 35 germplasms as highly salt-sensitive at the stage of germination (Figure 5). The maximum mean MFV was 0.835, suggesting that these germplasms were more salt-

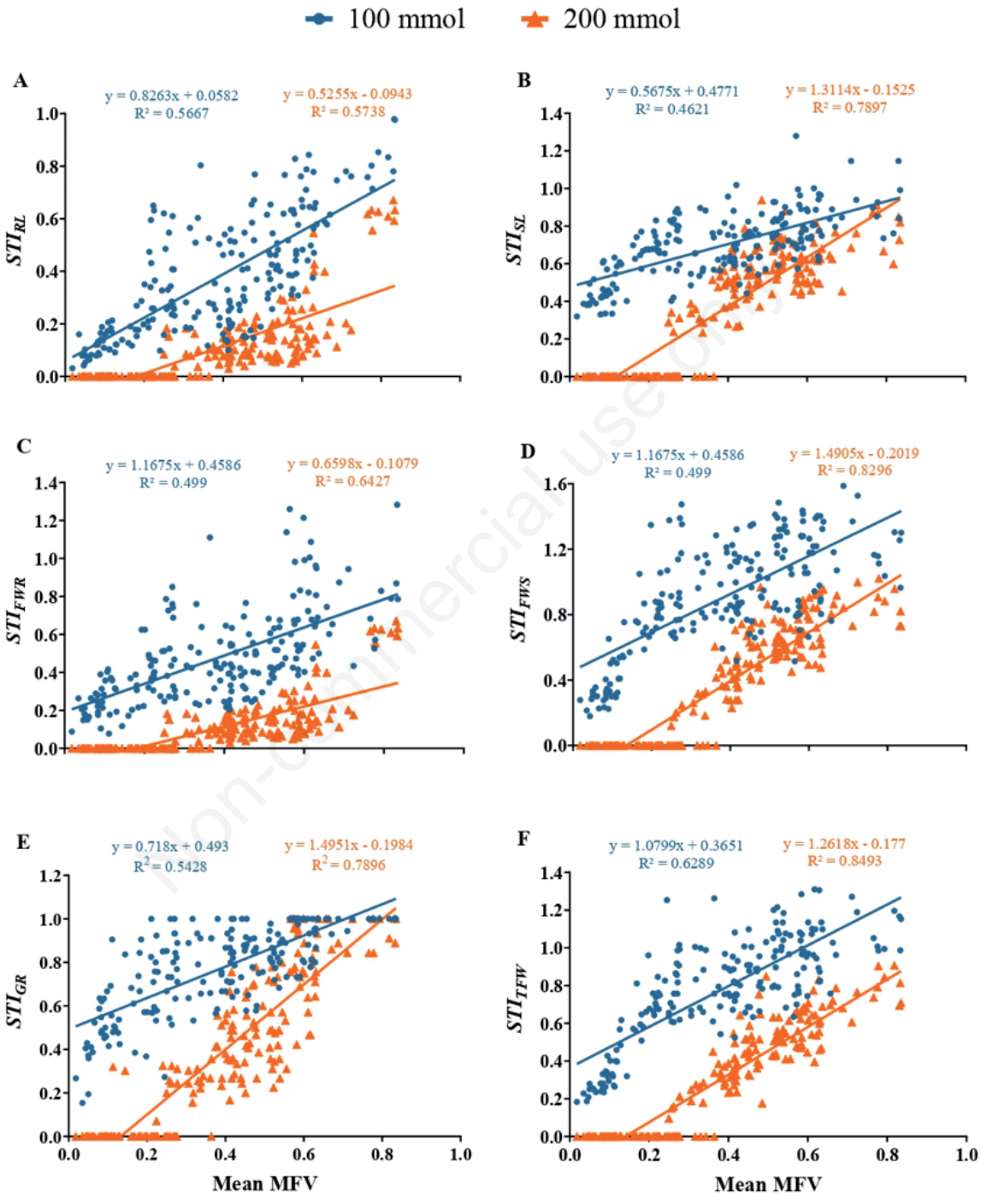

Figure 2. A linear correlation analysis between the mean membership function value (MFV) and salt tolerance index (STI) of each morphological parameter of individual *Brassica napus* germplasm under different salt stresses (100 and 200 mmol L^{-1} NaCl). A) mean MFV and STI of root length (STI_{RL}); B) mean MFV and STI of shoot length (STI_{SL}); C) mean MFV and STI of fresh weight of root (STI_{FWR}); D) mean MFV and STI of fresh weight of shoot part (STI_{FWS}); E) mean MFV and STI of germination rate (STI_{GR}); F) mean MFV and STI of total fresh weight (STI_{TFW}).

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tolerant when exposed to salinity during the seed germination stage. However, many germplasms showed mean MFVs close to zero, indicating that these germplasms were less salt-tolerant at the germination stage. Studies have shown that salt stress mainly harms seed germination and the emergence of seedlings (Parida and Das, 2005).

Salt stress significantly impacts *B. napus* growth and development, mainly through effects on germination, biochemical changes, and photosynthetic efficiency (Shah *et al.*, 2020). However, not all characteristics are equally suitable for salt tolerance screening. One of the reasons for the limited success of traditional salt tolerance breeding is the lack of acceptable and accurate crop selection criteria for assessing salt tolerance (Zeng *et al.*, 2002). Therefore, it is essential to identify the salt tolerance traits which can be used to determine salt tolerance in rapeseed at the germination stage accurately.

Correlation analysis and MFV were used to find the relationship between various morphological parameters under NaCl stress based on the STI values of GR, SL, RL, FWR, FWS, and TFW. According to the results of the correlation analysis, the STIs of TFW and FWS with the mean MFV under the 200 mmol L^{-1} NaCl treatment presented the highest correlation coefficients (0.922 and 0.911, respectively) as well as the highest coefficient of determination (R^2=0.849 and 0.829, respectively). The next highest correlation coefficient was 0.789, which is considered low and cannot be used for the reliable prediction of salt tolerance in rapeseed germplasms. Thus, STI of TFW corresponding regression models may be utilised to screen salt-tolerant rapeseed germplasms on a large scale during the germination stage.

One of the main goals proposed in this paper is to determine the germination potential of seeds under different levels of salinity stress, which could be achieved by screening and evaluating the salt tolerance traits of *B. napus*. Furthermore, the knowledge gained from this study can be used to screen rapeseed germplasms with different salt tolerance levels.

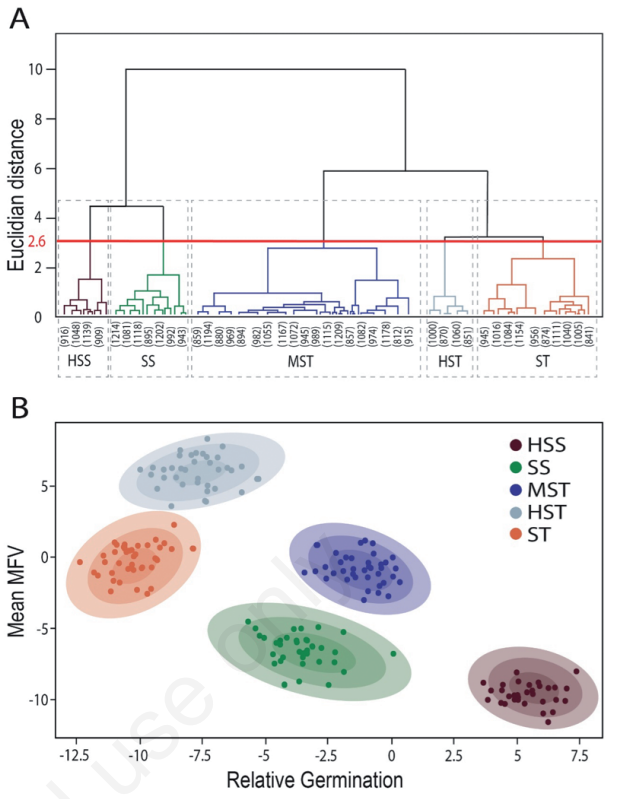

Figure 4. A) Hierarchical cluster analysis of 200 *Brassica napus* germplasms based on Furthest Neighbor method using multivariate parameters to determine the salt tolerance. High salt-sensitive (HSS), salt-sensitive (SS), moderate salt tolerance (MST), salt tolerance (ST), and high salt tolerance (HST); B) Gaussian blobs clustering result for the dataset.

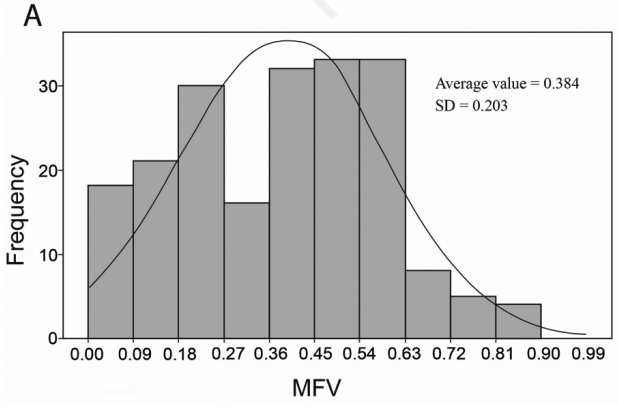

Figure 3. Distribution of membership function values (MFVs) among the 200 tested *Brassica napus* germplasms.

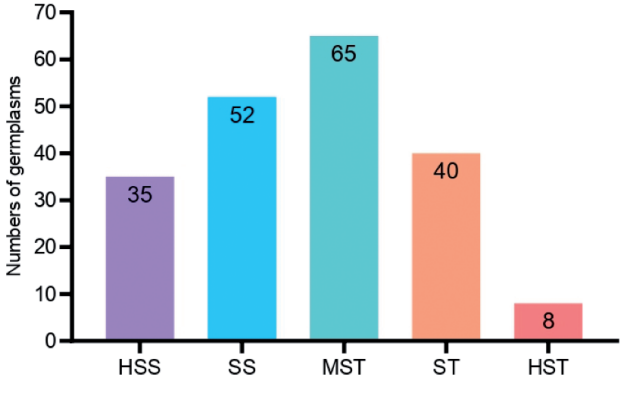

Figure 5. Classification of 200 *Brassica napus* germplasms with different levels of salt tolerance. The germplasms were classified into five groups, including high salt-sensitive (HSS), salt-sensitive (SS), moderate salt tolerance (MST), salt tolerance (ST), and high salt tolerance (HST).

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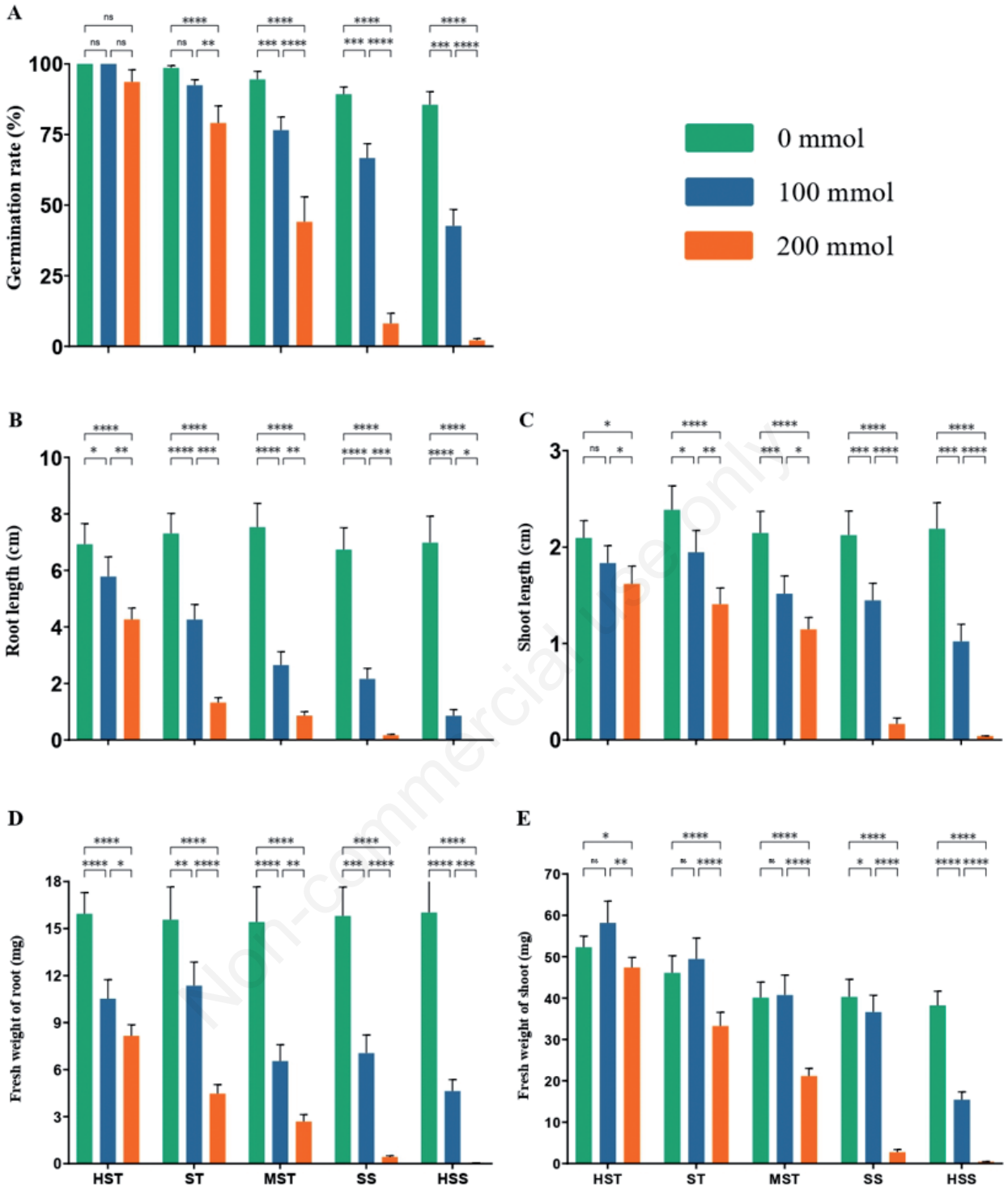

Figure 6. Effect of different salt stress treatments (0, 100, 200 mmol L^{-1} NaCl) on various salt tolerance groups of *Brassica napus* germplasms. A) Germination rate (%); B) root length (cm); C) shoot length (cm); D) fresh weight of root (mg); and E) fresh weight of shoot (mg). Results were expressed as means ± SD (n=3), significance values were presented as: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; while non-significant (ns) (P>0.05).

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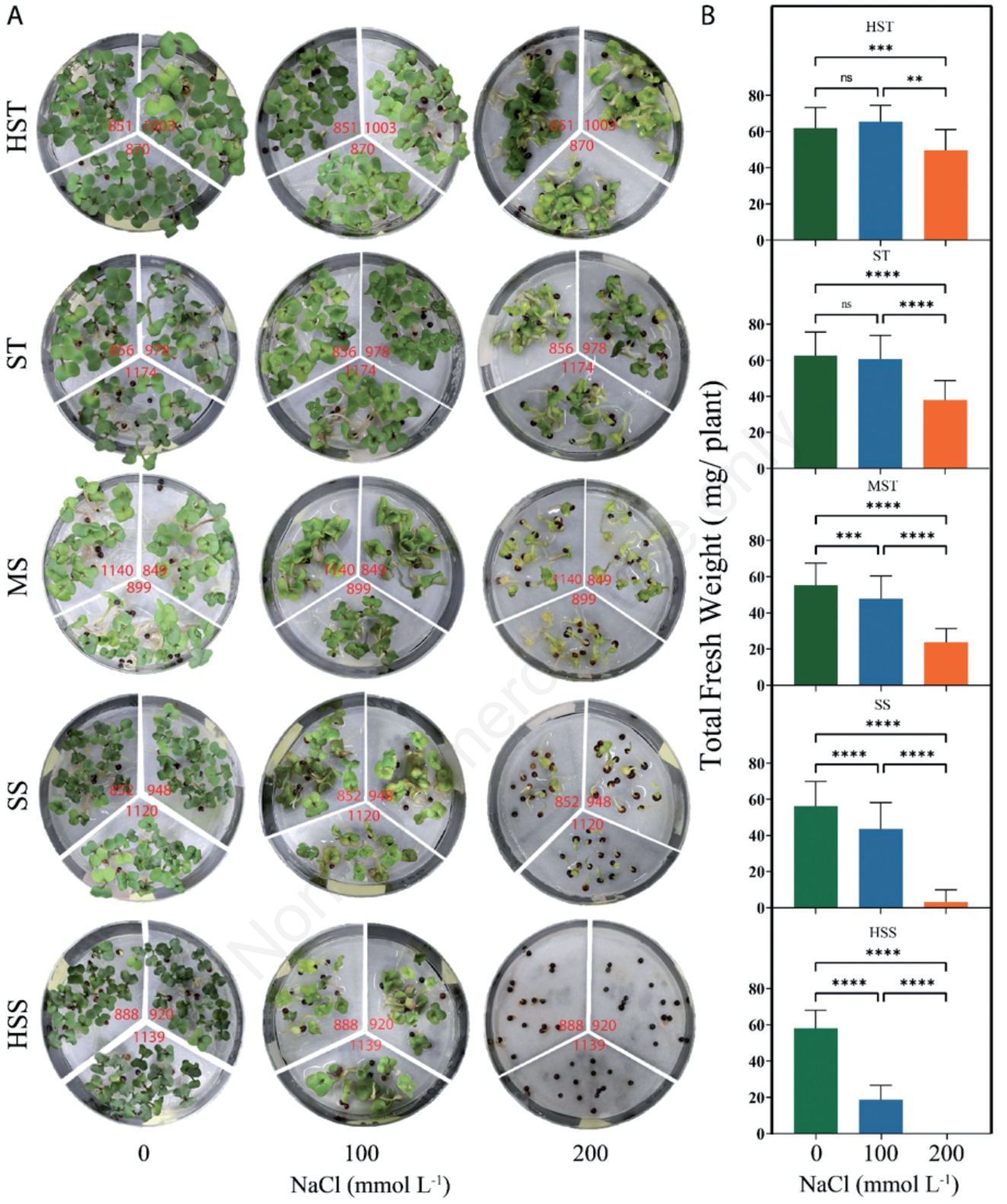

Figure 7. A) Phenotypes, the numbers in petri plates (in red) represent the varieties number of *Brassica napus* germplasms; B) total fresh weight of various salt tolerance groups of *Brassica napus* germplasms at seven days after sowing under different salt treatment (0, 100 and 200 mmol L^{-1} NaCl). Results were expressed as means ±SD (n=3), significance values were presented as: **P<0.01; ***P<0.001; ****P<0.0001; while non-significant (ns) (P>0.05).

Conclusions

Using hierarchical cluster analysis based on membership function values, 200 *B. napus* germplasms were evaluated at the germination stage, which could be classified into five salt tolerance groups, including 52 SS, 35 HSS, 40 ST, 65 MST, and 8 HST. To demonstrate the difference in salt tolerance in various germplasms, correlation analysis was performed between the mean MFV and STI of each trait. STI of TFW had the strongest correlation with rapeseed salt tolerance under 200 mmol L^{-1} NaCl treatment at the germination stage. More specifically, the NaCl treatment at the concentration of 100 mmol L^{-1} showed a positive, stimulatory effect on some morphological parameters, indicating that this treatment might lead to an optimum concentration of Na^+ for cell metabolic activities and processes. Furthermore, our study suggests that the correlation coefficient was higher under the 200 mmol L^{-1} NaCl treatment than that under the 100 mmol L^{-1} NaCl treatment. These findings have practical and theoretical significance for evaluating the salt tolerance of rapeseed.

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