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Identification of salt-tolerant cultivars and plant traits in wheat during germination and seedling emergence stages

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Abstract: In this study, we assessed the salt tolerance of 38 wheat cultivars from primary wheat cultivation regions in China using a membership function value (MFV) during the germination and seedling emergence stages. Based on salt tolerance assessment, three contrasting groups were classified, with 10 tolerant, 23 moderately tolerant and 5 sensitive cultivars under low salt stress, and 4 tolerant, 25 moderately tolerant and 9 sensitive cultivars under high salt stress and in addition to Na^+ and K^+ homeostasis regulation, nitrogen efficient transfer from seed to plant tissues denoted the significant positive correlation with salt tolerance, confirming the importance of nutrient spectra organisation. Salt-tolerant and moderately tolerant cultivars had lower trait network modularity than salt-sensitive cultivars, demonstrating that wheat with different salt tolerance uses alternative strategies to cope with salt stress. These results were important for germplasm evaluation and variety breeding of salt tolerance in wheat.

Keywords: salinity; *Triticum aestivum* L.; germination capacity; seedling growth; network analysis

Soil salinity is a critical global challenge to agricultural productivity and the primary concern for food security (Yang and Guo 2018). About 1 billion hectares of land are impacted by salinity, with more than 20% of the irrigated arable land (Qadir et al. 2014). The rise in groundwater level and climate change have exacerbated soil salinisation, presenting a major challenge to global food production (Zhu 2002). Salinisation is advancing at an alarming rate of roughly 3 hectares per min, driven by various biotic and abiotic factors (Shabala et al. 2014).

Wheat is the second most important cereal crop, feeding approximately 36% of the global population (FAO 2021). Although wheat is a moderately salt tolerance crop, it suffers significant yield loss of up to 60% under saline conditions, intensifying food insecurity (Munns and Tester 2008, Wang and Xia 2018). Enhancing salt tolerance in wheat is of significance for global food security. Therefore, it is necessary to understand wheat's response and adaptation mechanism to salt stress and to use salt-tolerant cultivars for wheat breeding and production in saline land. However, breeding for salinity tolerance in wheat remains challenging due to the complexity of the traits involved, encompassing morphological, physiological, and metabolic processes (Genc et al. 2007, Zhao et al. 2020). The scarcity of superior donor germplasm for salt resistance gene transfer, limita-

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tions in screening methods, and variations in trait contributions across development stages have hindered progress in the breeding programme (Flowers 2004, Ashraf and Akram 2009). Seed germination is a critical stage in the plant life cycle, especially for species inhabiting saline environments (Song et al. 2005). Rapid and synchronous germination is particularly crucial for agricultural output, especially when exposed to salt conditions. Therefore, the appropriate time and proportion of germination are crucial for the survival and propagation of seed plants (Mérai et al. 2018). Soil salinisation delays or reduces wheat germination rate, which impedes their subsequent growth and development. In irrigated agricultural production, salt moves upwards along with water evaporation, frequently causing salt accumulation in the topsoil (Huang et al. 2017). Therefore, the ability of seeds to maintain high germination rates and vigour under saline is crucial for their penetration of the salt-rich topsoil and successful development.

Salinity stress primarily disrupts ion homeostasis through the excessive accumulation of Na^+ and Cl^- ions, causing toxicity and osmotic stress. The structural similarity of Na^+ and K^+ enables Na^+ to substitute K^+ in key enzymatic reactions, disrupting physiological processes such as the Calvin cycle, glycolysis and starch synthesis (Wu et al. 2018). Additionally, Cl^- accumulation induces deficiencies in essential macronutrients like nitrogen and sulfur (Bazihizina et al. 2019). Salt stress decreases seed germination and seedling growth by affecting oxidative homeostasis, osmotic tolerance, and physiological metabolism (Zhao et al. 2020).

This study uses membership function value to assess the salt tolerance of 38 wheat cultivars from major wheat-growing regions in China during germination. The objectives are to (1) evaluate overall plant performance under saline conditions; (2) identify salt-tolerant cultivars to broaden the genetic base for wheat breeding, and (3) investigate agro-physiological traits associated with salt tolerance during seedling emergence to facilitate early screening of tolerant cultivars.

MATERIAL AND METHODS

Plant materials and experiment design. Thirty-eight wheat cultivars from major growing regions in China were collected for this study (Table 1). Seeds were surface-sterilised with 3% NaClO for 5 min and rinsed three times with distilled water. Twenty-five seeds of uniform size were sown in Petri dishes (15 cm diameter) containing 40 g of vermiculite. Three salt concentrations of 50 mmol/L NaCl (low salt, LS), 200 mmol/L NaCl (high salt, HS), and distilled water were used as the control for the seeds. The experiment was conducted in triplicates for each treatment. Petri dishes were covered with plastic lids to prevent water loss and incubated in a growth chamber at 25 °C, 75% relative humidity and 200 $\mu mol/m^2/s$ light intensity. Seed germination parameters were recorded from the 3rd to 7th day after sowing.

Germination traits collection. Germination rate (GR), germination potential (GP), germination index (GI) and germination vigour index (GVI) were calculated using the following formula:

$$GR = G_7/n \times 100\%, \; GP = G_3/n \times 100\%$$

where: G_3 and G_7 – number of seed germinating on 3rd and 7th day, respectively; n – total number of seeds used for germination.

$$GI = \sum_{n=1}^{7} = 1\frac{G_n}{n}, \; GVI - GI \times FW$$

where: G_n – number of seeds germinated on the nth day, and FW – total fresh weight of shoots and roots at the end of the experiment. Germination was defined as radicle protrusion through the seed coat, and coleoptile length exceeding 2 cm was recorded as germination for GP and GR, respectively.

Biomass accumulation and root morphology analysis. After harvest, the individuals were separated into shoots and roots to determine fresh weight and oven-dried at 80 °C until a constant weight for dry weight. Root morphology, including total root length, root volume, root diameter, and surface area, was assessed using scanned images with Win-Rhizo software (Regent Instruments Inc., Sainte Foy, Canada).

N, P, K^+ and Na^+ content determination. Plant nitrogen (N) and phosphorus (P) contents were determined *via* the Kjeldahl and the molybdate/ascorbic acid methods after H_2SO_4-H_2O_2 digestion, respectively. Potassium (K^+) and sodium (Na^+) contents were measured using an inductively coupled plasma-optical emission spectrometry instrument (ICP-OES; Pekin Elmer, Norwalk, USA) following HNO_3 digestion. The accuracy of the analyses was confirmed using a certified plant tissue standard (GBW07604, Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences). The Na^+/K^+ selection was calculated as follows:

$$Na^+/K^+_{\text{selecting}} = Na^+_{(\text{shoot/root})}/K^+_{(\text{shoot/root})}$$

where: $Na^+_{(\text{shoot/root})}$ – Na^+ ratio of shoots to roots and $K^+_{(\text{shoot/root})}$ – K^+ ratio of shoots to roots (Pitman 1984).

Table 1. The wheat cultivars used in this study

No.	Cultivar	Region	Source of cultivar
1	Jiemai19	Hebei	Elite variant of Jima32
2	Cangmai6004	Hebei	Xinong863/08950536//Shi5144
3	Chuanmai104	Sichuan	Chuanmai42/Chuannong16
4	Chuanmai66	Sichuan	99-1572/98-266//01-3570
5	Chuanyu21	Sichuan	Zhou88114/G159
6	Mianmai285	Sichuan	1275-1/99-1522
7	Chuanmai86	Sichuan	R4117/1572
8	Kechengmai4	Sichuan	37147/CD02-1574-3
9	Chuanmai83	Sichuan	07GH304/2962
10	Kechengmai6	Sichuan	CD-P35-1/98718//Chuanyu12
11	Chuanmai602	Sichuan	Guannong21/SW3243//Chuanmai42
12	Chuanmai1557	Sichuan	05126/Chuan05//Guan8
13	Neimai101	Sichuan	99-1572/M0501
14	Bainong418	Henan	Zhoumai18/Aikang58//Aikang58
15	Zhengmai168	Henan	Yumai13/90M434//Shi89-6021(Jimai38)
16	Zhongyu1211	Henan	Zhongyu12/Aikang58
17	Cunmai16	Henan	Zhoumai22/Zhoumai24//BainongAK58
18	Zhoumai32	Henan	Aikang58/Zhoumai24
19	Gaomai6	Henan	Zhoumai13/Bainong64//Zhoumai22
20	Jindi828	Henan	Zhoumai16/BainongAK58
21	Kaimai22	Henan	Zhoumai18/BainongAK58
22	Kelinmai969	Henan	Zhoumai16/Yanzhan4110//BainongAK58
23	Luomai26	Henan	Aikang58/Kaimai18
24	Luomai34	Henan	Zhoumai16/Aikang58
25	Luomai9908	Henan	Zhoumai13X/Bainong64
26	Pumai053	Henan	BainongAK58/Zhoumai18
27	Shengcaimai2	Henan	Hezhibuyu-7/Xingcai-3
28	Tunmai127	Henan	Aikang58/Zhoumai16
29	Xinkemai168	Henan	Aikang58/Zhoumai16//Luomai21
30	Xinmai32	Henan	Aikang58/Zhoumai22
31	Xinmai39	Henan	Han6172/Zhoumai18//Aikang58
32	Qimin7	Shandong	Aikang58/SN5843
33	Qimin8	Shandong	Shannong2149/Aikang58
34	Xinnong518	Anhui	Luomai21/Aikang58
35	Xumai36	Anhui	Huaimai18/Aikang58
36	Womai9	Anhui	Laizhou953/Aikang58
37	Longke1109	Anhui	Wanmai50/Aikang58
38	Guohong3	Anhui	Yangmai158/Aikang58

Salt tolerance evaluation. Membership function values (MFV) were used to evaluate wheat plant performance under a saline environment. Salt tolerance coefficients were calculated as the ratio of trait values under saline treatment and controlled treatment. Salt tolerance of MFV was calculated to the comprehensive evaluation value (D) and divided into three grades according to the average value ($\bar{D}$) and standard deviation (SD) of MFV when $D > \bar{D} + 1SD$ indicated salt tolerant, $\bar{D} - 1SD < D \leq \bar{D} + 1SD$

indicated moderate salt tolerant, D ≤ $\bar{D}$ – 1SD indicated salt sensitive by the method of Chen et al. (2012).

Plant traits network estimation. To explore connections among traits, the precision matrix and confidence interval for trait interaction were calculated using methods outlined by Flores-Moreno et al. (2019) and Kleyer et al. (2019). A threshold of $|r| > 0.4$ indicated pairwise correlations that were significant at $P < 0.05$. An adjacency matrix A = $[a_{i,j}]$ with $a_{i,j}$ ε [0, 1] was established by setting the correlation above the threshold to 1 and the relationship below the threshold to 0. We derived the precision matrix for all wheat cultivars and then for different salt tolerance types, respectively, under LS and HS treatments. Three common metrics were used to characterise the difference in the connection among plant traits, including modularity, edge density, and degree, according to Alon's (2003) method.

Statistical analysis. All experimental data were presented as means ± standard deviation from three replicates. The differences between treatments were analysed using SPSS 27.0 (SPSS Inc., Chicago, USA) with statistical significance determined by *P*-values less than 0.05 according to Duncan's multiple range tests.

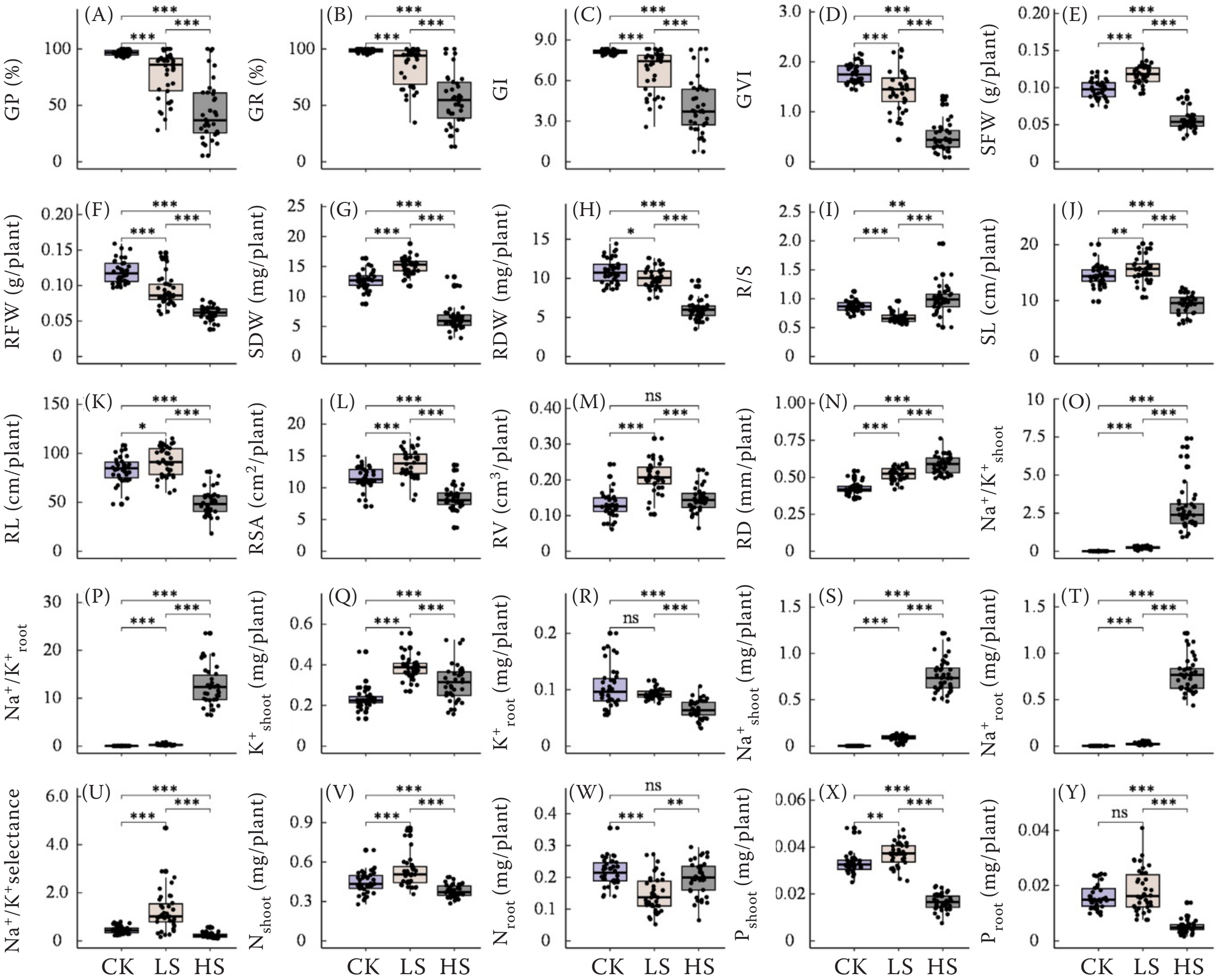

Figure 1. Plant traits among 38 wheat cultivars under control (CK), 50 and 200 mmol NaCl treatments. GP – germination potential; GR – germination rate; GI – germination index; GVI – germination vigor index; SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight; R/S – root to shoot ratio; SL – shoot length; RL – root lengt; RSA – root surface area; RV – root volume; RD – root diameter; Na^+/K^+_{shoot} – Na^+/K^+_{ratio} in shoot; Na^+/K^+_{root} – Na^+/K^+ ratio in root; K^+_{shoot} – K^+ content in shoot; K^+_{root} – K^+ content in root; Na^+_{shoot} – Na^+ content in shoot; Na^+_{root} – Na^+ content in root; N_{shoot} – total nitrogen content in shoot; N_{root} – total nitrogen content in root; P_{shoot} – total phosphorus content in shoot; P_{root} – total phosphorus content in root. Plant traits in wheat between salt treatments in this study was significantly different at **P* < 0.05, ***P* < 0.01 and *** *P* < 0.001 level; ns – not significant

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RESULTS

Phenotypic traits variation and correlation with salt tolerance coefficients. Salt stress profoundly affected germination parameters and root biomass across wheat cultivars (Figure 1). Compared to the control, wheat had a significant reduction of GP, GR, GI and GVI when exposed to salt stress (Figure 1A–D). The highest reduction in GP (94.5%), GR (86.3%), GI (90.9%) and GVI (95.5%) were recorded in a cultivar of Chuanmai1557 under HS treatment, while Xinkemai168 exhibited the highest reduction of GP (70.4%), GR (64.3%), GI (67.8%) and GVI (76.2%) under LS treatment. Conversely, the lowest reductions were observed in cultivars of Jiemai19 and Cangmai6004 under both LS and HS treatments.

Under LS treatment, shoot fresh and dry weights increased significantly, whereas root fresh and dry weights decreased. HS treatment, however, significantly decreased fresh and dry weights in both shoots and roots (Figure 1E–H). The greatest reductions in shoot and root dry weights were noted in Xinkemai168, while Cangmai6004 exhibited the smallest reductions compared to the control. On the other hand, the root-to-shoot ratio significantly decreased under LS treatment but increased under HS treatment (Figure 1I).

Salinity stress greatly affected root morphological traits. LS treatment significantly increased root length, surface area, volume, and diameter, but high salt stress reduced these traits except for root diameter (Figure 1K–N). The maximum (89.4%) and minimum (14.1%) root diameter increase were observed in Kaimai22 and Cangmai6004, respectively, compared with control. In addition, Na^+ content and Na^+/K^+ ratio in both shoots and roots increased significantly under LS and HS treatment. K^+ content increased in shoots under both treatments but decreased significantly in roots under HS treatment, as did N and P contents in shoots (Figure 1Q–Y). However, no significant difference in root N accumulation was noted between the control and HS treatment.

Under LS treatment, germination traits displayed strong positive inter-correlations and significant associations with other phenotypic traits (Table 2). GVI was positively correlated with shoot fresh weight, shoot dry weight, root dry weight, shoot Na^+ content, and the Na^+/K^+ ratio. Additionally, root morphological traits were strongly correlated (except for root diameter), while shoot P content exhibited significant positive correlations with both shoot and root fresh weights. Root N and P contents were positively associated with root diameter. Furthermore, the Na^+-K^+ selective rate significantly correlated with shoot N and P contents. Under HS treatment, germination traits maintained strong inter-correlations and were positively associated with shoot and root dry weights and shoot K^+ content (Table 3). Conversely, these traits were negatively correlated with shoot Na^+/K^+ ratio, shoot Na^+ content, and root P content. Shoot N content was negatively correlated with Na^+/K^+ ratios in both shoots and roots but positively associated with K^+ content. Root length displayed significant correlations with shoot K^+ content and Na^+/K^+ selective rate, while root diameter positively correlated with shoot P content.

Salt tolerant evaluation. At the germination stage, the D value for 38 wheat cultivars ranged from 0.39 to 0.68 for LS treatment and from 0.26 to 0.73 for HS treatment (Tables 4 and 5). The highest and lowest D values were noticed in Chuanmai83 and Longke1109 under LS treatment and in Zhongjiemai20 and Zhongyu1211 under HS treatment, respectively. Based on the results, all 38 wheat cultivars were grouped into three categories according to the comprehensive evaluation values of D (0.52 ± 0.07), that is, highly tolerant ($D > 0.59$), moderately tolerant ($0.45 < D \leq 0.59$) and sensitive ($D \leq 0.45$) to salinity under LS treatment. Similarly, sensitivity ($D \leq 0.35$), moderately tolerant ($0.35 < D \leq 0.57$) and tolerant ($D > 0.57$) to salinity was also recorded according to the D values of 0.46 ± 0.11 under HS treatment. It was worth noting that Kechengmai4, Zhongjiemai20, Cangmai6004 and Pumai053 showed consistently superior salt tolerance, whereas Chuanyu21, Cunmai16, Xinkemai168 and Luomai9908 were sensitive to salinity under either LS or HS treatment.

Under LS treatment, only N content in shoots and roots significantly correlated positively with D value (Figure 2A–B). Under HS treatment, Na^+ content in shoots and Na^+/K^+ ratio in shoots or roots showed a negatively significant correlation with D value, whereas, such as K^+ content in shoots and roots, shoot and root dry weight, as well as Na^+/K^+ selectance positively correlated with D value (Figure 2C–J). In addition, the D value positively correlated with N content in shoots but not in roots.

Traits network analysis. Among all wheat genotypes, there was a high proportion of connections among traits across all salt-tolerant and moderately tolerant types and low modularity among traits (Figure 3). Under LS treatment, Na^+_{root} was the trait with the

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Table 2. Correlation analysis based on salt tolerance coefficient value under low salt treatment

	GP	GR	GI	GVI	SFW	RFW	SDW	RDW	R/S	SL	RL	RSA	RV	RD	Na^+/K^+_{shoot}	Na^+/K^+_{root}	K^+_{shoot}	K^+_{root}	Na^+_{shoot}	Na^+_{root}	Na^+/K^+ seletance	N_{shoot}	N_{root}	P_{shoot}
GR	0.94**																							
GI	0.99**	0.98**																						
GVI	0.88**	0.83**	0.87**																					
SFW	−0.03	−0.18	−0.09	0.25																				
RFW	**0.41***	**0.42****	**0.42****	**0.74****	0.15																			
SDW	0.17	0.18	0.17	**0.33***	**0.35***	0.28																		
RDW	0.25	0.23	0.25	**0.40***	0.22	**0.49****	**0.47****																	
R/S	0.14	0.09	0.13	0.18	0.01	0.30	−0.24	**0.72****																
SL	−0.07	−0.09	−0.08	−0.05	0.23	−0.03	0.02	0.15	0.18															
RL	0.13	0.14	0.13	0.25	0.22	0.25	−0.18	0.19	**0.37***	0.29														
RSA	−0.04	−0.10	−0.06	0.07	0.24	0.09	−0.31	0.07	**0.35***	0.13	**0.75****													
RV	0.00	−0.04	−0.02	0.09	0.13	0.18	**−0.36***	0.14	**0.48****	0.16	**0.75****	**0.81****												
RD	−0.01	0.03	0.00	0.00	−0.02	0.02	−0.21	−0.29	−0.13	−0.04	−0.19	−0.18	0.05											
Na^+/K^+_{shoot}	**0.43****	**0.34***	**0.40***	**0.39***	0.14	0.19	0.19	**0.39***	0.28	0.26	0.16	0.10	0.09	−0.20										
Na^+/K^+_{root}	0.02	−0.02	0.01	−0.04	−0.03	−0.07	−0.09	−0.09	−0.01	−0.28	−0.31	−0.19	−0.13	0.27	−0.15									
K^+_{shoot}	0.03	0.15	0.07	0.11	0.15	0.03	0.13	0.05	−0.03	−0.10	0.24	0.04	0.01	−0.07	**−0.41***	−0.03								
K^+_{root}	0.10	0.11	0.10	0.19	0.07	0.24	0.29	0.20	0.00	0.15	0.29	0.23	0.25	−0.05	**0.35***	**−0.52****	0.06							
Na^+_{shoot}	**0.47****	**0.42****	**0.46****	**0.53****	0.29	0.32	**0.37***	**0.47****	0.25	0.20	0.31	0.12	0.12	−0.26	**0.77****	−0.15	0.18	**0.50****						
Na^+_{root}	0.00	−0.06	−0.03	0.07	0.18	0.11	−0.05	0.01	0.06	−0.18	−0.17	−0.05	0.01	0.32	0.00	**0.82****	0.00	−0.15	0.07					
Na^+/K seletance	0.24	0.25	0.25	0.16	−0.09	0.04	0.13	0.06	−0.03	0.18	0.26	0.11	0.21	−0.11	**0.45****	**−0.53****	−0.21	**0.65****	0.31	**−0.52****				
N_{shoot}	−0.08	−0.06	−0.07	−0.05	−0.15	0.09	−0.14	−0.18	−0.11	−0.10	0.16	0.08	0.29	0.00	−0.21	−0.30	0.09	0.29	−0.12	−0.21	**0.32***			
N_{root}	0.09	0.08	0.09	0.08	−0.11	0.12	−0.28	−0.21	−0.04	−0.07	0.11	0.09	0.28	**0.34***	0.15	−0.12	−0.27	0.25	−0.02	0.03	0.32	**0.43****		
P_{shoot}	−0.13	−0.14	−0.14	0.17	**0.38***	**0.38***	0.29	0.21	0.03	−0.12	−0.01	0.06	0.01	0.05	−0.12	0.19	0.21	−0.06	0.15	0.25	**−0.42****	0.04	####	
P_{root}	0.09	0.10	0.10	0.08	−0.09	0.09	−0.05	−0.02	0.00	−0.07	0.04	−0.05	0.12	**0.33***	0.21	0.03	0.07	**0.38***	0.29	0.29	0.19	0.31	**0.39***	−0.02

GP – germination potential; GR – germination rate; GI – germination index; GVI – germination vigor index; SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight; R/S – root to shoot ratio; SL – shoot length; RL – root lengt; RSA – root surface area; RV – root volume; RD – root diameter; Na^+/K^+_{shoot} – Na^+/K^+_{ratio} in shoot; Na^+/K^+_{root} – Na^+/K^+ ratio in root; K^+_{shoot} – K^+ content in shoot; K^+ content in root – K^+_{root}; Na^+_{shoot} – Na^+ content in shoot; Na^+_{root} – Na^+ content in root; N_{shoot} – total nitrogen content in shoot; N_{root} – total nitrogen content in root; P_{shoot} – total phosphorus content in shoot; P_{root} – total phosphorus content in root; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table 3. Correlation analysis based on salt tolerance coefficient value under high salt treatment

	GP	GR	GI	GVI	SFW	RFW	SDW	RDW	R/S	SL	RL	RSA	RV	RD	Na^+/K^+_{shoot}	Na^+/K^+_{root}	K^+_{shoot}	K^+_{root}	Na^+_{shoot}	Na^+_{root}	Na^+/K^+ seletance	N_{shoot}	N_{root}	P_{shoot}
GR	0.92**																							
GI	0.98**	0.97**																						
GVI	0.92**	0.89**	0.93**																					
SFW	**0.34***	0.26	0.31	**0.60****																				
RFW	–0.02	0.06	0.01	0.22	**0.32***																			
SDW	0.53**	0.49**	0.53**	0.71**	0.66**	0.24																		
RDW	**0.46****	**0.43****	**0.46****	**0.49****	**0.37***	0.22	**0.53****																	
R/S	–0.14	–0.13	–0.14	**–0.33***	**–0.43****	–0.16	–0.71**	0.12																
SL	0.21	0.09	0.16	0.21	0.20	–0.05	0.14	0.24	0.00															
RL	0.20	0.21	0.21	0.15	–0.03	–0.19	0.09	0.17	–0.03	0.18														
RSA	0.07	0.07	0.07	0.05	–0.02	–0.22	0.04	0.02	–0.07	0.18	**0.76****													
RV	0.02	–0.05	–0.01	0.00	–0.02	–0.15	0.02	–0.01	–0.04	**0.38***	**0.65****	**0.71****												
RD	–0.21	–0.19	–0.20	–0.15	–0.03	0.17	0.08	–0.03	–0.18	–0.18	–0.24	–0.15	0.17											
Na^+/K^+_{shoot}	**–0.46****	**–0.53****	**–0.50****	**–0.49****	–0.17	–0.29	**–0.51****	**–0.41****	**0.38***	–0.02	–0.27	–0.20	–0.08	–0.02										
Na^+/K^+_{root}	**–0.35***	–0.31	**–0.34***	**–0.34***	–0.17	–0.11	–0.20	–0.20	0.01	–0.10	0.12	0.16	0.09	0.08	0.27									
K^+_{shoot}	**0.49****	**0.58****	**0.54****	**0.59****	0.32	0.19	**0.67****	**0.46****	**–0.36***	0.07	**0.34***	0.20	0.12	–0.06	**–0.79****	–0.23								
K^+_{root}	0.18	0.15	0.17	0.22	0.12	**0.37***	0.17	0.12	–0.09	0.04	–0.17	–0.05	0.08	0.09	–0.30	**–0.74****	0.17							
Na^+_{shoot}	**–0.62****	**–0.63****	**–0.64****	**–0.67****	**–0.36***	–0.27	**–0.53****	**–0.43****	0.31	–0.14	–0.22	–0.18	–0.19	–0.03	**0.82****	**0.37***	–0.56**	–0.37*						
Na^+_{root}	**–0.33***	–0.28	–0.31	–0.24	–0.03	0.32	–0.08	0.00	–0.05	–0.22	0.04	0.06	0.12	0.24	0.07	**0.54****	–0.18	0.01	0.21					
Na^+/K^+ seletance	–0.22	**–0.36***	–0.29	–0.31	–0.16	–0.11	**–0.46****	**–0.33***	**0.38***	0.03	**–0.43****	–0.29	–0.10	–0.06	**0.62****	**–0.44****	**–0.62****	**0.44****	**0.39***	–0.21				
N_{shoot}	0.27	0.24	0.26	0.23	0.04	0.07	0.27	**0.41***	–0.01	0.11	0.15	0.05	0.15	0.02	**–0.41***	**–0.41****	**0.41****	**0.49****	–0.32	–0.06	0.04			
N_{root}	–0.01	–0.07	–0.04	–0.03	–0.08	0.12	–0.01	–0.01	0.00	0.29	0.04	0.02	0.17	0.03	0.12	0.24	–0.11	–0.02	0.21	**0.43****	–0.03	0.09		
P_{shoot}	–0.10	–0.06	–0.09	–0.06	–0.18	0.13	0.15	0.08	–0.11	–0.05	–0.16	–0.09	–0.09	**0.40***	–0.14	0.30	0.23	–0.17	0.09	0.13	–0.31	0.10	0.28	
P_{root}	–0.42**	**–0.32***	**–0.39***	**–0.33***	–0.05	0.02	–0.24	–0.19	0.04	–0.24	0.09	0.14	–0.03	–0.07	0.14	0.22	–0.18	–0.15	0.31	0.29	–0.04	–0.19	0.02	–0.07

GP – germination potential; GR – germination rate; GI – germination index; GVI – germination vigor index; SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight; R/S – root to shoot ratio; SL – shoot length; RL – root lengt; RSA – root surface area; RV – root volume; RD – root diameter; Na^+/K^+_{shoot} – Na^+/K^+_{ratio} in shoot; Na^+/K^+_{root} – Na^+/K^+ ratio in root; K^+_{shoot} – K^+ content in shoot; K^+ content in root – K^+_{root}; Na^+_{shoot} – Na^+ content in shoot; Na^+_{root} – Na^+ content in root; N_{shoot} – total nitrogen content in shoot; N_{root} – total nitrogen content in root; P_{shoot} – total phosphorus content in shoot; P_{root} – total phosphorus content in root; *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$

Table 4. Ranking of wheat cultivars based on comprehensive evaluation of D value under low salt treatment

Cultivar	D value	Rank	Cultivar	D value	Rank
Chuanmai83	0.69	T	Chuanmai104	0.52	MT
Jiemai19	0.64	T	Shengcaimai2	0.52	MT
Neimai101	0.62	T	Zhongyu1211	0.52	MT
Kechengmai6	0.61	T	Xumai36	0.51	MT
Pumai053	0.61	T	Chuanmai66	0.50	MT
Xinmai32	0.61	T	Chuanmai86	0.50	MT
Cangmai6004	0.60	T	Gaomai6	0.50	MT
Kechengmai4	0.60	T	Mianmai285	0.49	MT
Bainong418	0.60	T	Xinmai39	0.49	MT
Kaimai22	0.60	T	Xinnong518	0.49	MT
Chuanmai602	0.58	MT	Qimin8	0.47	MT
Chuanmai1557	0.57	MT	Womai9	0.47	MT
Jindi828	0.57	MT	Luomai26	0.46	MT
Zhoumai32	0.55	MT	Zhengmai168	0.46	MT
Kelinmai969	0.54	MT	Luomai9908	0.45	S
Qimin7	0.54	MT	Cunmai16	0.42	S
Guohong3	0.53	MT	Chuanyu21	0.41	S
Luomai34	0.53	MT	Xinkemai168	0.41	S
Tunmai127	0.53	MT	Longke1109	0.40	S

T – salt tolerant type; MT – moderately tolerant type; S – sensitive type

highest connections to other traits for salt-tolerant cultivars, and SFW was more central to the network under HS treatment (Figure 4). The salt moderately tolerant and sensitive cultivars shared with the maximum *K* of GVI under LS treatment (Figure 4B–C). The maximum *K* of R/S ratio and GR were recorded in salt moderately tolerant and sensitive cultivars, respectively, under HS treatment (Figure 4E–F).

Table 5. Ranking of wheat cultivars based on comprehensive evaluation of D value under high salt treatment

Cultivar	D value	Rank	Cultivar	D value	Rank
Jiemai19	0.73	T	Gaomai6	0.45	MT
Kechengmai4	0.73	T	Jindi828	0.45	MT
Cangmai6004	0.70	T	Xinmai39	0.44	MT
Pumai053	0.62	T	Xumai36	0.44	MT
Chuanmai602	0.57	MT	Zhoumai32	0.43	MT
Chuanmai86	0.56	MT	Qimin8	0.40	MT
Guohong3	0.54	MT	Chuanmai1557	0.38	MT
Kaimai22	0.52	MT	Chuanmai66	0.36	MT
Xinmai32	0.52	MT	Qimin7	0.36	MT
Luomai34	0.51	MT	Shengcaimai2	0.36	MT
Tunmai127	0.51	MT	Chuanyu21	0.35	S
Kechengmai6	0.50	MT	Cunmai16	0.35	S
Xinnong518	0.50	MT	Luomai9908	0.35	S
Luomai26	0.49	MT	Zhengmai168	0.35	S
Womai9	0.49	MT	Bainong418	0.34	S
Mianmai285	0.48	MT	Longke 1109	0.33	S
Chuanmai83	0.46	MT	Chuanmai104	0.32	S
Neimai101	0.46	MT	Xinkemai168	0.27	S
Kelinmai969	0.46	MT	Zhongyu1211	0.26	S

T – salt tolerant type; MT – moderately tolerant type; S – sensitive type

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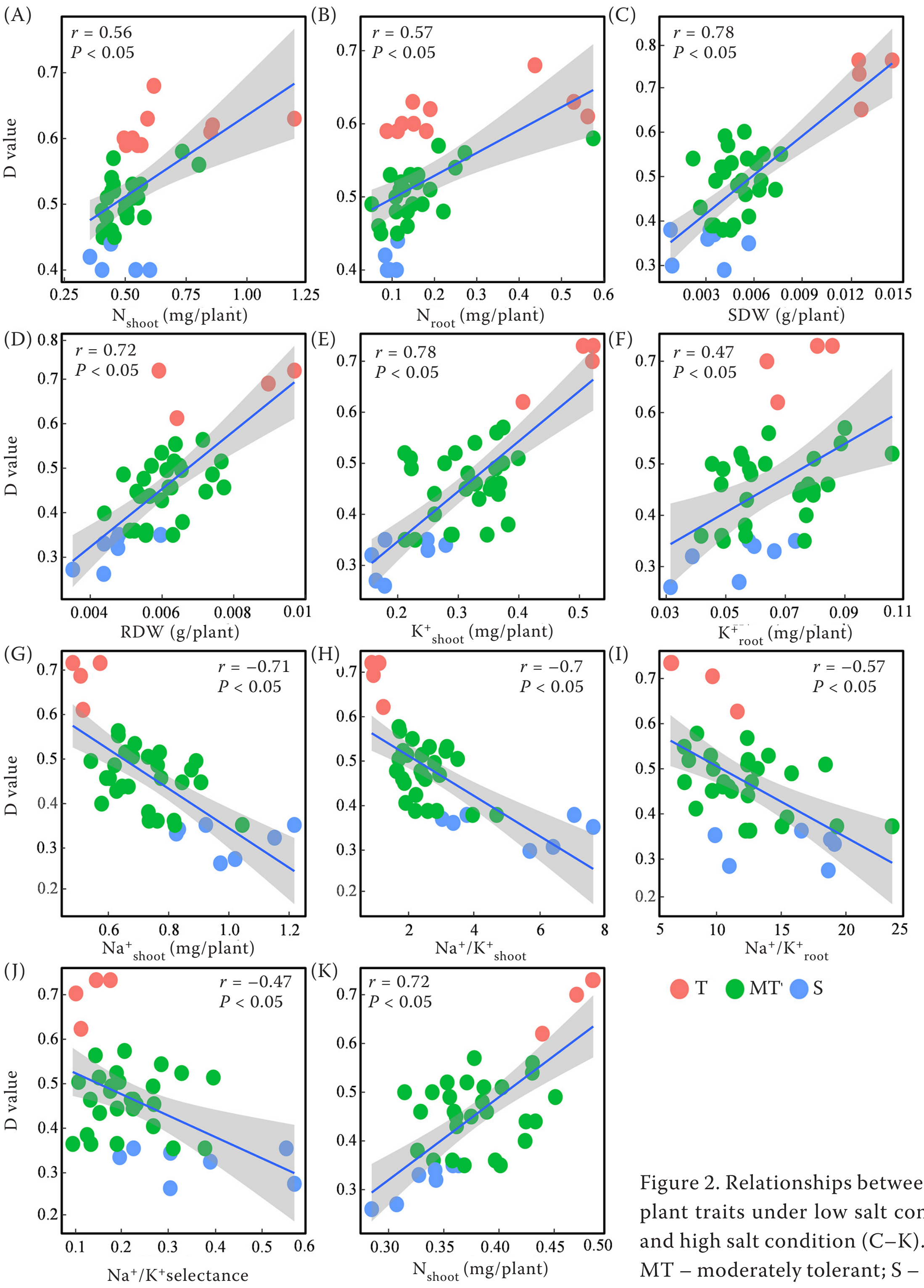

Figure 2. Relationships between D value and plant traits under low salt condition (A–B) and high salt condition (C–K). T – tolerant; MT – moderately tolerant; S – sensitive type

DISCUSSION

Plant response to salt stress is a complex process involving multiple organs, tissues and traits, alongside adjustments in physiological and biochemical processes to maintain growth and development (Zhou et al. 2024). Most crops, including wheat, are glycophytes susceptible to salinity, especially during the vegetative phases. Therefore, evaluating the response to salt stress during the seed germination stage is an important criterion for screening salt-tolerant germplasm in crops (Choudhary et al. 2021).

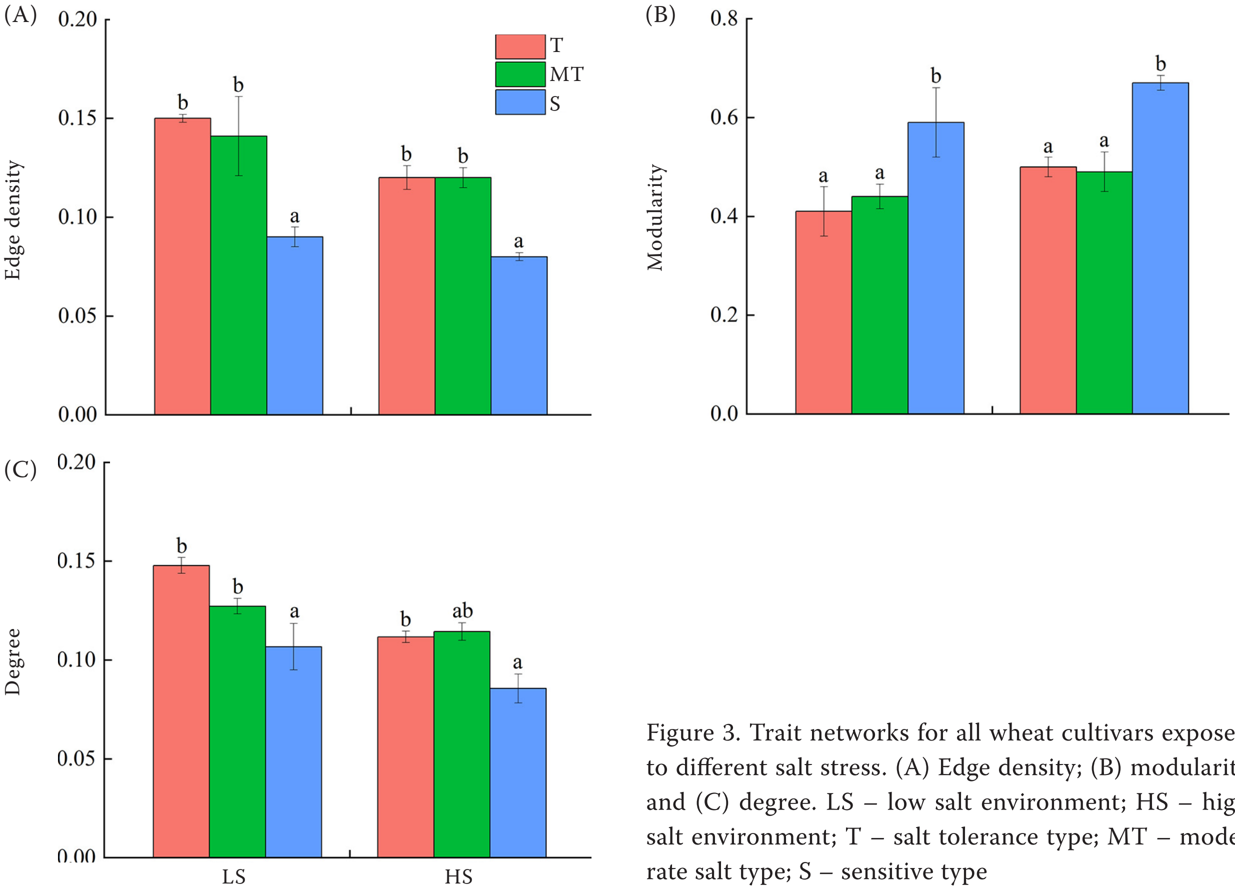

Figure 3. Trait networks for all wheat cultivars exposed to different salt stress. (A) Edge density; (B) modularity and (C) degree. LS – low salt environment; HS – high salt environment; T – salt tolerance type; MT – moderate salt type; S – sensitive type

Evaluation of salt tolerance in wheat

Wheat exhibits moderate salt tolerance, but significant genetic variability exists among cultivars. This study utilised the MFV method to integrate multiple observations, demonstrating varying salt tolerance among wheat cultivars. The germination stage is critical for establishing plants in a saline environment, making it an ideal phase for salt tolerance evaluation (Li et al. 2020, Rajabi et al. 2020). In this study, cultivars Jiemai19, Kechngmai4, Cangmai6004 and Pumai053 exhibited consistent salt tolerance, while Luomai9908, Cunmai16, Chuanyu21, Xinkemai168 and Longke1109 displayed sensitivity under both LS or HS conditions. These findings provide valuable resources for breeding programs that improve wheat's salinity resilience.

Physiological mechanisms of salt tolerance. Plants employ various salt tolerance mechanisms to acclimate exposure to salinity (Deinlein et al. 2014). Based on our analysis, the correlation between D value and plant traits was different under LS and HS treatment (Figure 2). This indicated that differential strategies potentially regulated wheat responses to different levels of salt stress. As is known, root growth strategies are dynamically changed in response to salinised conditions, by modulating root system architecture traits and directional root growth (Julkowska et al. 2017, Dinneny 2019). In the present study, most wheat cultivars exhibited biomass accumulation accretion and root morphology improvement under LS treatment (Figure 1). This may be because seed priming with NaCl can promote their growth and improve tissue tolerance under salt stress (Paul et al. 2023). However, HS treatment notably inhibited seed germination, biomass accumulation and root system development. This was linked to ionic imbalance and K^+ deficiency caused by impaired selectivity of root membrane due to excessive Na^+.

Highly salt-tolerant plants showed strong Na^+ rejection and preferential restriction because lower maintenance of Na^+ content, higher amounts of K^+ retention and vacuolar compartmentalisation are required to cope with a salinised environment (Chakraborty et al. 2019, Mohanty et al. 2023). K^+ is an important determinant of cell fate, with salt-induced cytosolic K^+ loss being causally linked to metabolic process interference, reactive oxygen spe-

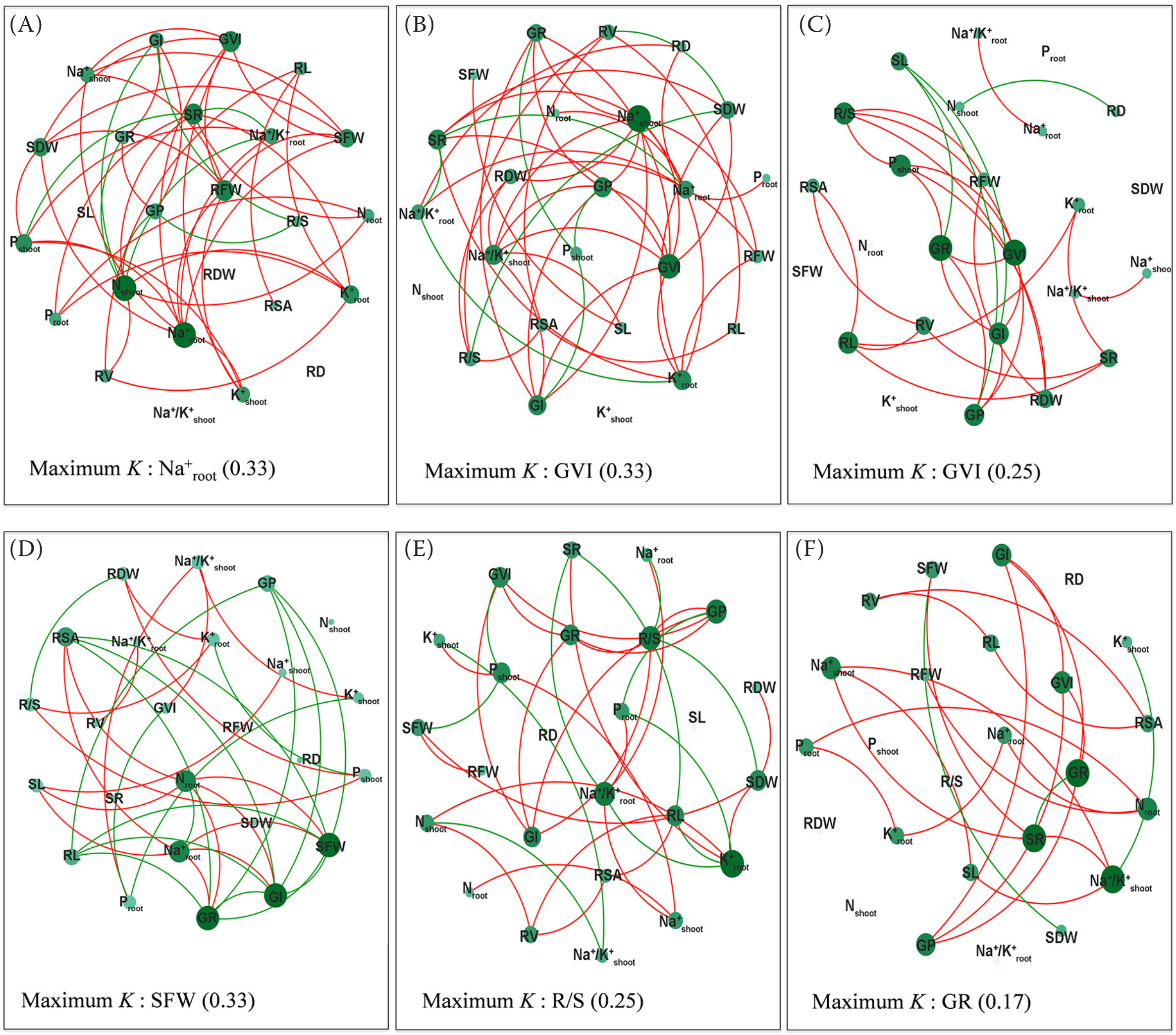

Figure 4. Trait networks for all wheat cultivars with different types of salt tolerance were exposed to different salt stresses. Green and red edges – negative and positive correlations, respectively; node size – degree; K – connectivity among plant traits; A and D – trait network for salt-tolerance cultivars in low and high salt treatment; B and E – trait network for moderate salt-tolerance cultivars in low and high salt treatment; C and F – trait network for salt-sensitive cultivars in low and high salt treatment

cies formation and cell damage (Shabala and Cuin 2008). In this study, K^+ accumulation in shoots or roots had a significantly positive correlation with D value, indicating that increased K^+ accumulation in tissues to ensure the K^+ metabolic requirement can enhance the salt tolerance in wheat. Moreover, Na^+ accumulation in shoots exhibited a significantly negative correlation with the D value (Figure 2). This was likely because Na^+ exclusion from the shoot effectively enhanced plant salt tolerance (Davenport et al. 1997, Munns et al. 2006). This is especially true for wheat. In addition, Na^+/K^+ selectance can reflect Na^+ and K^+ acquisition preference and regulate Na^+/K^+ homeostasis in plants (Hasegawa et al. 2000). Our findings revealed that wheat cultivars with salt tolerance had lower Na^+/K^+ selectance, which indicated more dominance in K^+ selectivity to decrease Na^+/K^+ ration and improve the salt tolerance.

On the other hand, it is beneficial to enhance the synthesis of compatible solutes of N-containing compounds, such as amino acids, amides and betaines, with the increase of N content in tissues, which is of central importance for plant survival and growth under salt stress (Lauchli and Luttge 2002). The compatible solutes accumulate in the cytosol, decreasing cytoplasmic water potential and acting as osmoprotectants to overcome osmotic stress. This study's shoot and root N accumulation positively correlated with the D value under LS treatment. Only shoot N accumulation scaled positively with D value under HS treatment, indicating higher N content in plant tissues contributed to improved salinity tolerance in wheat during the germination stage. In other words, the N efficient mobilisation establishment from seed to seedling improved salt tolerance and seed germination under salinity exposure, where the N metabolism process cannot also be ignored.

Traits and their relationships vary within different salt-tolerant wheat. Plant traits are not independent, and the coordination of traits generally exists (Flores-Moreno et al. 2019). Highly connected plant traits are expected due to biological and selection processes that favour the efficient use and acquisition of resources within and across plant tissues to cope with changing environments (Reich et al. 2014). To our knowledge, edge density variation in trait networks has been linked to a compromise between connection efficiency and cost, and higher modularity of plant traits confers an advantage under variable conditions and will also decouple the functional modules, ensuring their independence from each other (Alon et al. 2003). In this study, across different genotypes, we found that wheat cultivars with salt tolerance possessed a significantly higher proportion of connectedness across traits and lower modularity compared to wheat cultivars with salt-sensitive type, indicating enhanced network connectivity and a competitive advantage in acquiring and utilising resources for salt-tolerant cultivars. Furthermore, the reduced degree of modularity fosters more effective connections between functional groups, leading to consistent functional division that maximises the potential of various traits in response to environmental conditions. Within wheat cultivars, modularity was greater in salt-sensitive types than in salt-tolerant or moderate-tolerant types under LS or HS treatment, indicating that modules in the traits network in salt-sensitive types were more independent. This suggested less coordination between trait modules was true for salt-sensitive types in response to salt stress, leading to a looser overall network. Consequently, it is necessary to strengthen the coordination of plant functional traits to improve the salt tolerance of wheat.

High centrality, characterised by a high number of connections to other traits in a network, is likely influential in regulating key functions or involving regulating multiple functions. Na^+ and K^+ balance maintenance is an important mechanism of salt tolerance in growing plant tissues (Tester and Davenport 2003, Zhao et al. 2023). In our study, the root Na^+ content was the trait with the most connection to other traits across salt-tolerant cultivars under LS treatment, but SFW was a central trait under HS treatment. Moreover, GVI and GR were central traits within salt-sensitive cultivars under LS and HS treatment, respectively. This indicated that trait centrality was labile within wheat genotypes across stress conditions. The variation of trait centrality may reflect the selectivity, heritability, and scale dependence of biophysical forces acting on traits and some functional relevance of traits in the trait network (Flores-Moreno et al. 2019). When faced with severe salt stress, water loss reduction and water use efficiency improvement can allow wheat plants to cope with salt stress effectively; the acclimation to low salt stress tended to depend more on the ion balance regulation in plant tissues.

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