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Genome-wide association analysis of root length traits in *Brassica napus* at germination stage under sodium carbonate stress

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Abstract Soil salinization has become one of the important factors affecting the sustainable development of agriculture. Among them, the soda-alkali soil composed of sodium carbonate (Na_2CO_3) has a more serious impact on crops. In this study, 175 *Brassica napus* accessions from different sources were used as materials, combined with Brassica 60 K SNP chip data, and the root length traits of *Brassica napus* during germination under Na_2CO_3 stress (0.15%) and control conditions were analyzed for genome-wide association (GWAS). GWAS analysis detected that 5 SNPs were significantly related to root length traits under stress conditions; a single SNP could explain 10.22–12.01% of the phenotypic variation. A total of 15 candidate genes related to Na_2CO_3 stress resistance were identified upstream and downstream of significant SNPs, including cation exchange protein genes (*CAX1*), members of the zinc finger protein family (*ZFHD1*), peroxidase family proteins (*POD*), and transcription factors (MYB family and WRKY family), etc. The expression analysis of 5 candidate genes in extreme phenotypic materials showed that *BnaA04g21850D* (*CAX1*) and *BnaA06g24040D* (*ACX5*) were induced by Na_2CO_3 stress in both materials; *BnaA06g31200D* (*ZFHD1*) and *BnaC02g37590D* (*MYB60*) were up-regulated expression in sensitive materials; *BnaA04g21990D* (*POD*) was up-regulated expression in alkali-tolerant materials, indicating that these candidate genes may be involved in the process of rapeseed response to Na_2CO_3 stress. This study can provide a reference for in-depth analysis of the molecular mechanism of Na_2CO_3 stress resistance in rapeseed.

Keywords *Brassica napus* · Sodium carbonate stress · GWAS · Germination stage · Candidate genes

Introduction

Sustainable development of world agriculture has been restricted by the growing problem of soil salinization severely. According to incomplete statistics, about 954 million hectares of soil in the world

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have been suffered varying degrees of salinization (Munns et al. 2008), and about 434 million hectares has composed of sodium carbonate (Na_2CO_3) and other alkaline salt types (Jin et al. 2008). Alkaline soil is characterized by high pH, high exchangeable sodium, dispersed physical properties and low water content (Vestin et al. 2006). Compared with salt (NaCl) stress, alkaline stress always caused much stronger inhibition of plant growth and development (He et al. 2018). Studies have shown that the degree of damage to plants from salt and alkali stress is in order of salt-alkali mixed stress > alkaline stress > salt stress (Wang 2015; Gong et al. 2016). Alkaline salts such as Na_2CO_3 in soda saline-alkaline soil can cause plants to suffer from ion poisoning and high pH stress, which seriously affects the normal growth of plants (Wang et al. 2015). At present, a series of advances have been made in the research on the mechanism of plant salt tolerance, and a large number of salt tolerance genetic sites and genes have been identified (Zhao et al. 2020). However, little attention has been paid on the mechanisms of plant adaptation to alkaline stress.

Rapeseed (*Brassica napus* L.; AACC, 2n = 38) is the third most important oil crop in the world. In recent years, with the rapid development of sequencing technology, the cost of developing single nucleotide polymorphism (SNP) markers has been greatly reduced. GWAS (Genome-wide association analysis) technology has been widely used in the genetic research of complex traits such as agronomy, quality and resistance traits of rapeseed (Li et al. 2014; Liu et al. 2016; Chen et al. 2018; Song et al. 2020; Tang et al. 2021). Among them, several genetic researches on the salt tolerance (NaCl) of rapeseed have been published. For example, 85 rapeseed inbred lines were used to investigate the salt tolerance traits of rapeseed seedlings and 62 QTLs were detected to be correlated in salt tolerance index, biological characteristics and ion balance significantly (Yong et al. 2015). In addition, according to the prediction of candidate genes and gene expression analysis, a possible salt tolerance candidate gene *TSN1* was obtained (Yong et al. 2015). Zhang et al. (2017) and He et al. (2017) used GWAS analysis on salt tolerance in the germination period of rapeseed. They identified 45 and 225 SNPs that were significantly related to salt tolerance during germination in the whole genome respectively, finding out 65 salt-tolerant candidate genes near these SNPs. In the early stage of our research, GWAS analysis was carried out on the salt tolerance of *Brassica napus* during the germination and seedling growth stages, and many SNPs that were significantly related to the salt tolerance at the germination and seedling stages were screened, and a few of them were found to be similar to the past research, which showed that the genetic mechanism of salt tolerance in rapeseed was extremely complicated (Wan et al. 2017, 2018). However, the current theoretical research on the resistance mechanism of *Brassica napus* to alkaline salt stress is limiting, while the molecular mechanism involved in the resistance of rapeseed to alkaline salt stress is still unclear.

In the present study, we performed GWAS to identify QTLs and candidate genes associated with root length under Na_2CO_3 stress at the germination stage with a panel of 175 rapeseed inbred lines. This research not only provides technical support for the identification of alkali-tolerant germplasm resources of *Brassica napus*, but also provides theoretical basis for the utilization of alkali-tolerant rapeseed resources and the cultivation of new varieties.

Materials and methods

Plant materials

A total of 175 *Brassica napus* accessions from different geographic origins were selected and successfully phenotyped during seed germination and early seedling growth stages under both normal and salt-stress conditions (supplementary Table S1). Five representative plants of each accession of the association panel were self-pollinated in the experimental field in Wuhan (114.35°E, 30.48°N) in 2019/2020 growing seasons.

Selection of an optimal Na_2CO_3 concentration

3 L of the Na_2CO_3 solutions (0.00%, 0.05%, 0.10%, 0.15%, 0.20% and 0.25%) were added to a box (36 × 24 × 6 cm) which was separated into 20 sections by nylon rope with two sheets of medical gauze on the top of the tray. Thirty healthy seeds from each *Brassica napus* accession were selected, surface-sterilized with 0.1% HgCl solution for 3 min, rinsed with distilled water for three times, and equally

distributed containing two sheets of medical gauze. The germinated seeds were counted on the seventh day after sowing. The seed germination experiment was performed in a green house (20 °C).

Na_2CO_3 stress tolerance evaluation

3 L of the 0.15% Na_2CO_3 solutions (treatment) or double-distilled water (control) were added into a box (36 × 24 × 6 cm) which was separated into 60 sections by nylon rope with two sheets of medical gauze on the top of the tray. Thirty healthy seeds from each *Brassica napus* accession were selected, and then equally placed into the tray sections. On the eighth day, 5 seedlings from each section were selected and the root length was accurately measured with ImageJ software. The seed germination experiment was three replications in a greenhouse (20 °C).

Phenotypic data analysis

Statistical analysis of all phenotypic data was performed with the software SPSS, version 18.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics was performed using the mean values of all phenotypic data from the traits of 175 rapeseed accessions.

Association mapping

The genotypes of 175 rapeseed accessions were identified using the Brassica 60 K Illumina® Infinium SNP array by Xu et al. (2016). In the early stage, the Brassica 60 K SNP chip was used to perform genotype analysis on 175 materials, and the markers with the minor allele frequency (MAF) less than 0.05 and SNP yield (call frequency) less than 80% were removed, and 19,176 high-quality SNP marker were finally obtained (Wan et al. 2017). By using General Linear Model (GLM) and Mixed Linear Model (MLM) in TASSEL5.0 software (Bradbury et al. 2007), combined with SNP chip genotype data, and Q value and kinship K value as covariates, we performed genome-wide association analysis to determine the interpretation rate (R^2) of associated sites and markers for phenotypic variation. This study used the Q model in GLM and the Q + K model in MLM as the most GWAS analysis model. When $P < 0.0001$ or $-\log_{10}P \geq 4.0$, SNP markers are considered to be significantly associated with traits (Wan et al. 2017; 2018; Zeng et al. 2021). R software was used to draw Quantile–Quantile scatter plot (QQ plot) and Manhattan plot (Manhattan plot) (Turner et al. 2014). Refering to the annotation information of the rapeseed genome (Chalhoub et al. 2014), candidate genes related to transcription factors, active oxygen scavenging and ion transporters were screened within 500 kb upstream and downstream of significant SNPs as described in previous studies (Wan et al. 2017).

Candidate genes expression analysis

According to the method above, germination stress treatment (Na_2CO_3 concentration of 0.10%) on the alkali-tolerant material (K) and sensitive material (G) were carried out, and the root tissues of the seedlings under normal and stress treatments were taken after 7 days. The extraction of total RNA was carried out according to the procedures of the Easystep Super Total RNA Extraction Kit. cDNA was synthesized by using RevertAid First Strand cDNA Synthesis Kit. By using SYBR Premix Ex Taq (Baosheng Bioengineering Co., Ltd., China), real-time fluorescence quantification was performed on the Bio-Rad CFX96 fluorescence quantitative PCR instrument (Bio-Rad, USA). BnActin was the internal reference gene, while the real-time fluorescence quantitative mix was Monad's MonAmp SYBR Green qPCR Mix9 (None ROX) (Monad Biotech Co., Ltd., Suzhou). Primer design was performed using Prier Premier 5 (Table 1).

Results and analysis

Different Na_2CO_3 concentrations of stress on the germination of rapeseed seeds

The results showed that when the concentration was lower (0.05%), there was no significant difference in root length under 0.05% Na_2CO_3 concentration; when the concentration was 0.10%, the root length growth and development were significantly different, while the difference between the materials was not significant; when the concentration reached 0.15%, the root length of rapeseed seedlings was severely inhibited, and the variation between materials was large; when the concentrations were 0.20% and 0.25%, the rapeseed seeds could not germinate (Figs. 1 and 2).

Table 1 qRT-PCR primers used in this study

Gene ID	Sequence of primer (5′-3′)	Amplification length (bp)
BnaA04g21850D	F: TCGCGATTTTGGCATTGACC	294
	R: TACTGCTGGCTCGTGAAAGAC	
BnaA04g21990D	F: TGGTGGCCAGTTCCTTTGGGA	153
	R: AAAGAGCGACAAGATCTGAGAGG	
BnaA04g22040D	F: AAGATAACACTACTTCTGGCTCGC	82
	R: GGTTCACTCCCACAATCTTCTTCA	
BnaA06g31200D	F: GCCGCGACCCCGATGACTCC	98
	R: GTGGTGAGGCTGGTACTCGATTGCA	
BnaC02g37590D	F: ATCGTCTTCCTCTGCTAATTCCAAT	194

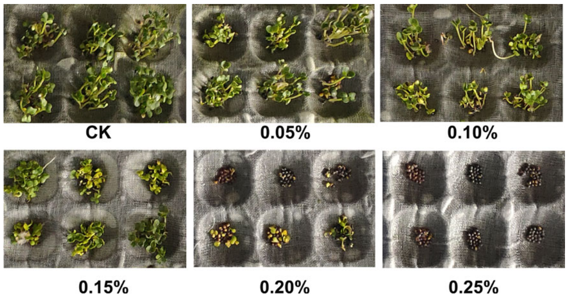

Fig. 1 The effect of different concentrations of Na_2CO_3 on the germination of rapeseed

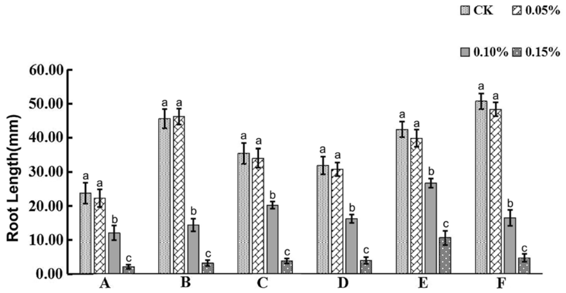

Fig. 2 The effect of different Na_2CO_3 concentrations on the root length of rapeseed seedlings during germination. Different lowercase letters indicate significant differences at the 0.05 level

Therefore, the Na_2CO_3 concentration of 0.15% could be used as the appropriate concentration to evaluate the alkali resistance of rapeseed during the germination period.

Phenotypic variation analysis of root length characters in ***Brassica napus*** under Na_2CO_3 stress

The extensive phenotype variation was observed for the root length under normal and Na_2CO_3 conditions (Tale 2). Comparing the coefficient of variation of root length traits of 175 rapeseed varieties under the control and treatment conditions, Na_2CO_3 stress had a wide range of phenotypic variation on the degree of inhibition of root growth of rapeseed germplasm with different genotype backgrounds (Table 2). The root length under the control condition showed a continuous normal distribution, and the root length under treatment conditions showed a partial normal distribution, indicating that the root length traits under the control and treatment conditions were controlled by polygenes, which conformed to the genetic characteristics of quantitative traits (Fig. 3).

GWAS results of root length traits

The QQ chart analysis results showed (Fig. 4a) that the root length (CRL) under the control had a larger false positive under the Q model, and the *P* value of the makers detected under the Q + K model was closer to the expected value, which could reduce false positives and make correlation analysis results more reliable. Root length under Na_2CO_3 stress (TRL) had the *P* values of the markers that detected to be close to the expected value under the Q model and the Q + K model. In summary, the Q + K model was selected under CRL, while the Q model and Q + K model were selected under TRL for correlation analysis.

The correlation analysis between CRL and TRL was performed using the above model (Fig. 4b–d). As a result, 13 SNPs were identified, located on chromosomes A4, A6, C1, C2, C4, C6, C7 and C9, which can explain 10.22–12.01% genetic variation (Table 3). Among them, 8 SNPs were significantly associated with CRL, located on chromosomes A4, C1, C2, C4, C6, C7 and C9 (Table 3). Five SNPs were significantly associated with TRL, located on chromosomes A4, A6

Table 2 Descriptive statistics of root length traits under control and Na2CO3 stress conditions SD standard deviation, CV coefficient of variation, ** $P < 0.01$.

Trait	Range (mm)	Min (mm)	Max (mm)	Mean (mm)	SD	CV (%)
Root length (CK)	65.25	14.79	80.04	43.67**	11.76	26.92
Root length (Na_2CO_3)	16.63	0	16.63	3.63	2.62	72.53

$^{**}P < 0.01$

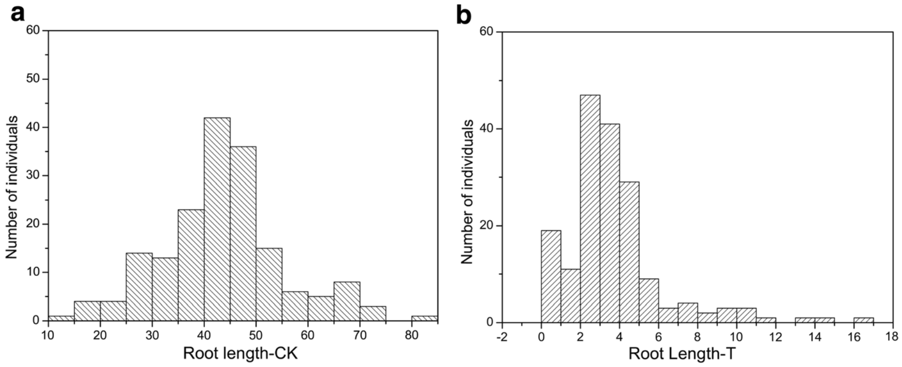

Fig. 3 Histogram of root length traits under control and Na_2CO_3 stress conditions

and C2 respectively. Three SNPs were detected by Q model and Q + K model at the same time (Table 3). The same or similar genetic SNPs were not identified under the control and stress conditions, indicating that there are large genetic differences in root length growth under different environments.

Candidate gene screening and expression verification

Brassica napus genes, which were the orthologous to *A. thaliana* stress tolerance-related genes located within 500 kb of significant SNPs, were regarded as possible candidate genes for Na_2CO_3 tolerance based on annotation information available in the *B. napus* reference genome (Chalhoub et al. 2014). Fifteen candidate genes were found within 500 k of the upstream and downstream SNPs significantly associated with TRL (Table 4), such as cation exchange protein gene *BnaA04g21850D* (*CAX1*), peroxidase superfamily proteins *BnaA04g21990*, *BnaA04g22000*, etc. The expression levels of some candidate genes in alkali-tolerant materials (K) and sensitive materials (G) were verified (Fig. 5). The results showed that *BnaA04g21850D* (*CAX1*) and *BnaA06g24040D* (*ACX5*) were induced by Na_2CO_3 stress in both materials; *BnaA06g31200D* (*ZFHD1*) and *BnaC02g37590D* (*MYB 60*) only up-regulated expression in sensitive materials; *BnaA04g21990D* (*POD*) only up-regulated expression in alkali-tolerant materials, indicating that these candidate genes may be involved in the process of rapeseed response to Na_2CO_3 stress.

Discussion

The root system is the direct organ of the plant to feel the soil environment, and the salt, alkali, acid, heavy metal and other unhealthy environments in the soil can have an adverse effect on the growth (Crawford 1990). Therefore, studying the response of roots to stress and analyzing the genetic rules of root traits are of great significance for plants to adapt to adverse environments. Previous studies have shown that rapeseed can tolerate higher concentrations of NaCl stress in both the germination and seedling stages. For example, the salt tolerance (NaCl) concentration of rapeseed germination was about 1.0%, and the half-lethal concentration was about 1.3% (Wan et al. 2018). The results of this study showed that the alkali salt (Na_2CO_3) stress had a more obvious inhibitory effect on rapeseed

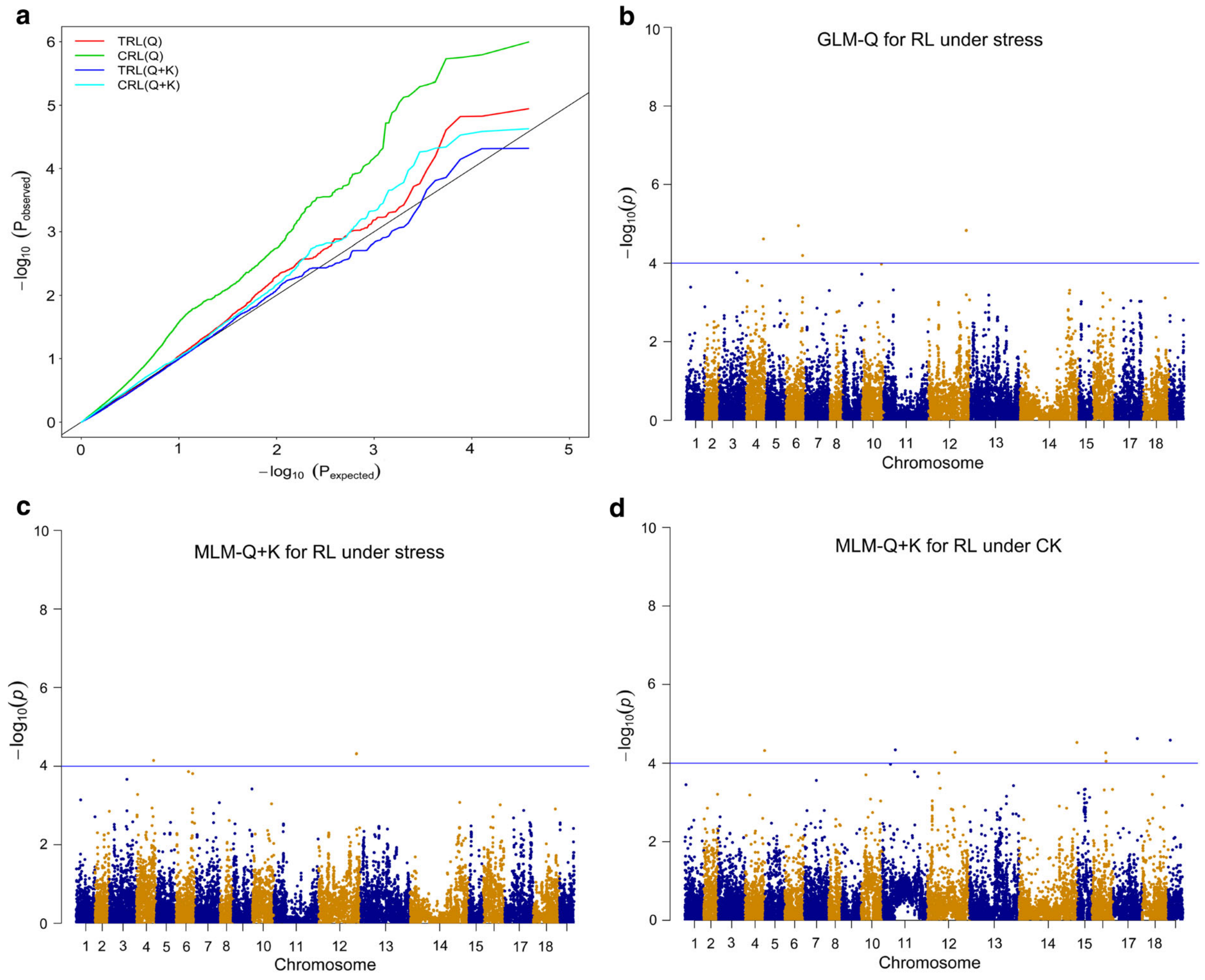

Fig. 4 Association mapping of three salt tolerance indices in the panel of 214 rapeseed inbred lines. **a** Quantile–Quantile plot of root length traits of rapeseed under control and treatment conditions; **b** GLM-Q model for root length traits of rapeseed under treatment conditions; **c** MLM-Q + K model for root length traits of rapeseed under treatment conditions; **d** MLM-Q + K model for root length traits of rapeseed under control conditions. The horizontal line depicts the significance threshold [$-\log_{10} P = 4.0$]

seed germination. The 0.10% Na_2CO_3 concentration could severely inhibit the germination of rapeseed seedlings while rapeseed cannot germinate under 0.20% Na_2CO_3 concentration. Comparing the stress of neutral salt (NaCl) and alkaline salt (Na_2CO_3), it was found that neutral salt had a stronger inhibitory effect on the growth of shoot parts, while alkaline salt had a stronger inhibitory effect on root growth. Compared with neutral salt, the excessive pH caused by alkaline salt would seriously inhibit the normal metabolic activities of the root system, and then affected the growth and development of the rapeseed root system. Therefore, enhancing the tolerance of rapeseed to alkaline environment was the key to improving the overall salt-alkali tolerance of rapeseed.

In recent years, the GWAS were applied to identified abiotic stress-related genetic loci and candidate genes in rapeseed (He et al. 2017; Zhang et al. 2017; Wan et al. 2017, 2018), but the genetic mechanism of rapeseed to adapt to alkaline stress is still unclear. In this study, GWAS was used to successfully identify 5 SNPs that are significantly related to rapeseed root length under Na_2CO_3 stress. Currently, no previous genetic locus related to Na_2CO_3 stress resistance has been reported, thus, the genetic locus identified in this study can be considered

Table 3 SNP sites that are significantly associated with root length traits of *Brassica napus* during germination under normal conditions and Na_2CO_3 stress

Trait	SNP	Alleles	Chromosome	Position	$-Log_{10}^{P}$	R^2 (%)	Model
CRL	Bn-A04-p18409410	A/G	A04	18,481,814	4.32	11.17	Q + K
CRL	Bn-scaff_16056_1-p16322	T/C	C01	17,476,475	4.34	11.22	Q + K
CRL	Bn-scaff_20732_1-p213396	T/C	C02	32,100,830	4.27	11.04	Q + K
CRL	Bn-scaff_18903_1-p371596	T/C	C04	47,531,676	4.53	11.74	Q + K
CRL	Bn-scaff_15892_1-p1676940	T/G	C06	27,499,621	4.05	10.42	Q + K
CRL	Bn-scaff_15892_1-p1302640	T/C	C06	27,154,787	4.26	11	Q + K
CRL	Bn-scaff_16069_1-p3352312	T/C	C07	39,760,483	4.63	12.01	Q + K
CRL	Bn-scaff_20836_1-p196601	A/C	C09	12,807,036	4.58	11.89	Q + K
TRL	Bn-A04-p16303678	A/G	A04	16,484,408	4.61	11.17	Q
					4.14	11.17	Q + K
TRL	Bn-A06-p21933808	T/C	A06	20,956,378	4.19	10.22	Q
TRL	Bn-A06-p15210596	T/G	A06	16,683,528	4.95	10.17	Q
TRL	Bn-scaff_17109_4-p187218	A/G	C02	40,684,411	4.82	11.66	Q
					4.31	11.66	Q + K
TRL	Bn-scaff_17109_4-p169452	A/G	C02	40,688,812	4.83	11.67	Q
					4.32	11.67	Q + K

Table 4 Candidate genes for Na_2CO_3 stress resistance in *Brassica napus*

Rapeseed gene	Position	Homologs Arabidopsis	Gene annotation
BnaA04g21850D	A04: 16,605,802	*AT2G38170*	Cation exchanger 1 (*CAX1*)
BnaA04g21990D	A04: 16,673,254	*AT2G38380*	Peroxidase superfamily protein (POD)
BnaA04g22000D	A04: 16,678,423	*AT2G38380*	Peroxidase superfamily protein (POD)
BnaA04g22040D	A04: 16,687,875	*AT2G38470*	*WRKY33*
BnaA04g22070D	A04: 16,713,202	*AT2G15050*	Lipid transfer protein (*LTP*)
BnaA06g23250D	A04: 16,185,207	*AT1G05260*	Peroxidase superfamily protein (*POD*)
BnaA06g23260D	A04: 16,190,451	*AT2G35380*	Peroxidase superfamily protein (*POD*)
BnaA06g23270D	A06: 16,202,589	*AT2G41480*	Peroxidase superfamily protein (*POD*)
BnaA06g23750D	A06: 16,477,353	*AT4G26640*	*WRKY 20*
BnaA06g24040D	A06: 16,613,640	*AT2G35690*	Acyl-CoA oxidase 5 (*ACX5*)
BnaA06g31200D	A06: 20,964,387	*AT1G69600*	Zinc finger homeodomain 1 (*ZFHD1*)
BnaA06g31230D	A06: 21,005,875	*AT5G62470*	*MYB 96*
BnaC02g37290D	C02: 40,204,996	*AT1G24110*	Peroxidase superfamily protein (*POD*)
BnaC02g37590D	C02: 40,546,448	*AT1G08810*	*MYB 60*
BnaC02g38790D	C02: 41,781,108	*AT5G48870*	Superensitive to ABA and drought1 (*SAD1*)

as a new genetic locus. Interestingly, the SNP Bn-A04-p16303678 (located on A4 chromosome 16,484,408) identified in our study was 134 kb away from the SNP (located on A4 chromosome 16,618,792) related to root length under NaCl stress identified by He et al. (2017), suggesting that they are likely to be the same locus, indicating that rapeseed may have a certain commonality in responding to Na_2CO_3 stress and NaCl stress. Except for this site, no other locus was overlapped, revealing the difference in

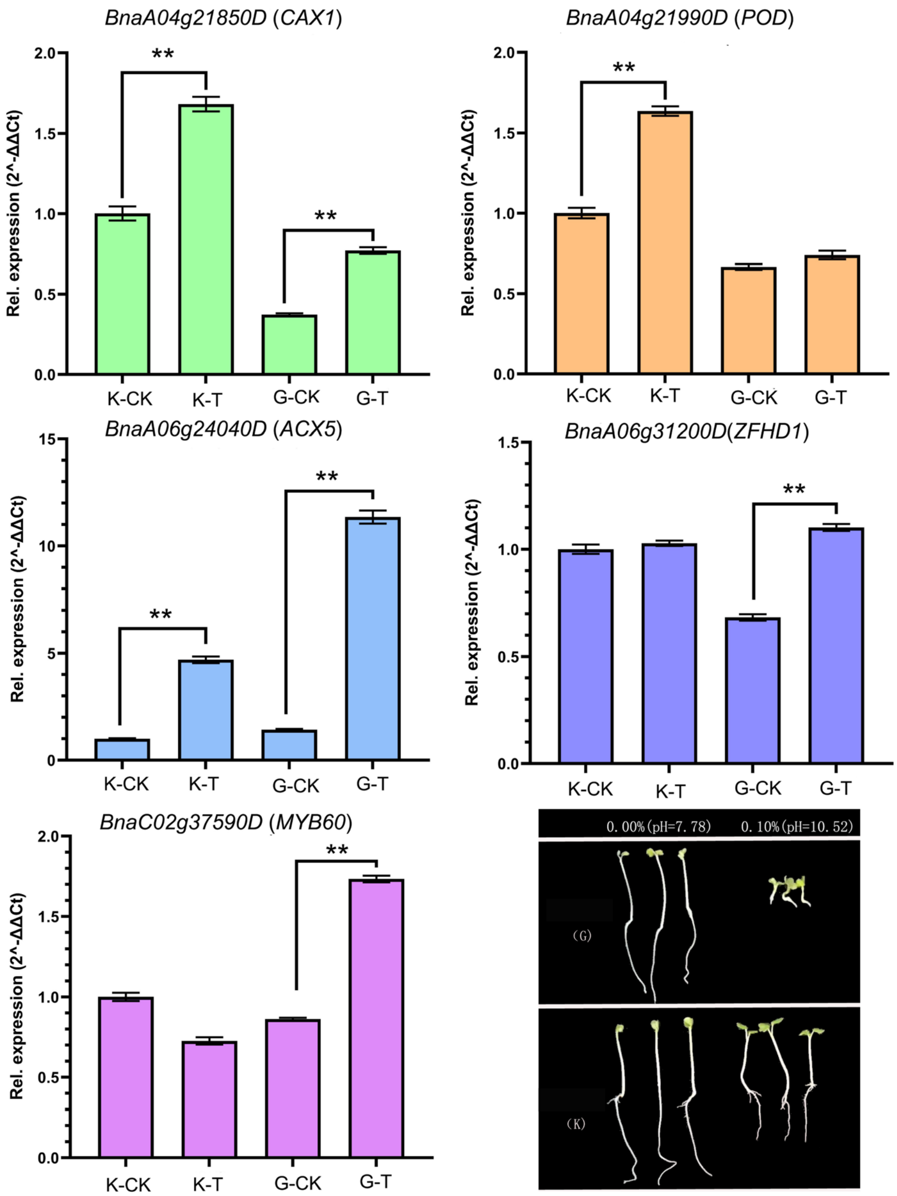

Fig. 5 Verification of expression levels of some candidate genes. K: Alkali-tolerant rapeseed, G: Sensitive rapeseed, CK: the control treatment, T: the stress treatment

salt tolerance and alkali tolerance of rapeseed in the genetic level.

High salt ion concentration and pH are some of the main characteristics of saline-alkali soil (Vestin et al. 2006). Plants growing in saline-alkali soil were subjected to osmotic stress, that seriously affected plant growth and development, and even lead to its death (He et al. 2018; Ma et al. 2020). In order to cope with salt-alkali stress, plants have evolved a variety of mitigation strategies, such as synthesizing osmotic regulators, improving the antioxidant capacity of enzymes and selective absorption of ions (Zhu et al. 1998; Ashraf and Foolad 2005; Zhang et al. 2016). Some resistance genes have also been identified one after another, such as the key gene of proline synthesis *P5CS* (Funck et al. 2020), the ion transporter family gene *OsHKT* (Chuamnakthong et al. 2019), transcription factors such as MYBs (He et al. 2018) and so on.

In this study, 15 candidate genes were screened near significant SNPs. Among these candidate genes, several transcription factors were screened, including WRKY family (*WRKY20* and *WRKY33*), MYB family (*MYB60* and *MYB96*) and *ZFHD1*. Studies have shown that these transcription factors are all induced and expressed by adversity stress and participate in the process of regulating the response of plants to environmental stress (Tran et al. 2005). The results of this study showed that both *MYB60* and *ZFHD1* were only up-regulated in sensitive material, but there was no significant difference in their expression levels in resistant materials. This difference in expression levels may be one of the reasons for the difference in alkali tolerance in rapeseed. In addition, candidate genes also include *CAX1*, *LTP*, *ACX5*, *SAD1* and *POD*. *CAX1* can encode a bubbly Ca^{2+} transporter, which plays an important role in the process of plant roots dealing with salt damage (Navarro-León et al. 2020). The results of qRT-PCR showed that *CAX1* was induced by Na_2CO_3 stress in both resistant and sensitive rapeseeds, indicating that this gene had a certain function in regulating the resistance of rapeseed to Na_2CO_3 stress. Non-specific lipid transporter (nsLTP) is an important type of small alkaline secreted protein in plants, which plays an important role in plant resistance to adversity and stress (Lindorff-Larsen and Winther 2001). Overexpression of a pepper lipid transfer protein gene *CaLTP1* in *Arabidopsis* can significantly enhance the salt tolerance of the plant (Jung et al. 2005). Under salt stress conditions, the root length of the over-expression line is significantly longer than that of the wild type (Jülke and Ludwig-Müller 2016). It is likely to also participate in the process of regulating the resistance of rapeseed roots to Na_2CO_3 stress. Jasmonic acids (JAs) are a new type of plant growth regulators that are widely present in higher plants. They play the important role in regulating plant growth and development, photosynthetic characteristics, and stress resistance (Susanne 2002). *ACX5* encodes acyl-CoA oxidase, which is involved in the biosynthesis of jasmonate (Schilmiller and Howe 2007). The results of this study showed that *ACX5* was up-regulated when resistant rapeseed and sensitive rapeseed were subjected to Na_2CO_3 stress, indicating that the JA metabolic pathway may play a certain role in the Na_2CO_3 rapeseed response to stress. *SAD1* can encode a polypeptide of Sm-like snRNP protein. Previous studies have shown that compared with wild-type *Arabidopsis*, *SAD1* mutants are more sensitive to ABA and salt stress, and are importantly reflected in the growth and development of roots. This study was based on rapeseed root traits (Xiong et al. 2001; Seifert et al. 2014). Besides, among the candidate genes, there were several peroxidase superfamily protein genes, which were distributed near significant SNPs on A4, A6 and C2 chromosomes. qRT-PCR showed that under Na_2CO_3 stress, the expression of this gene was upregulated in alkali tolerant rapeseed, but not in sensitive rapeseed. The difference in expression of this gene might be one of the reasons for the difference in alkali tolerance.

Brassica napus has become an ideal material for the development and utilization of saline-alkali land due to its high biological yield, strong salt-tolerant ability, and wide range of suitable planting areas. With the further in-depth study of salt and alkali tolerance in *Brassica napus*, using conventional hybridization, molecular marker-assisted selection, gene editing and other methods, excellent genes can be transferred into existing high-quality rapeseed varieties or genetic editing technology. The key genes for salt tolerance were improved directionally to create rapeseed materials with stronger salt and alkali tolerance, and new varieties with high yield, high quality and high resistance were cultivated.

Conclusion

GWAS identified a total of 5 genetic locus that are significantly related to root length traits under Na_2CO_3 stress conditions, and 15 candidate genes were screened near SNP sites, and expression levels of some candidate genes were verified. These candidate genes may be regulating rapeseed. Na_2CO_3 plays an important role in resistance to stress.

Author Contributions Conceived and designed the experiments: HW, CZ, JS and JW. Performed the experiments and analyzed the data: JC, HZ and JT. Data acquisition:JC, HZ, CL, JR, JT, and JL. Wrote the paper: JC. Contributed to writing the manuscript: HZ, CL, JT and HW. Revised the manuscript: HW, CZ and JS.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments shown in the manuscripts submitted for publication comply with the current laws of the country in which they were performed.

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