Discovery of common loci and candidate genes for controlling salt-alkali tolerance and yield-related traits in Brassica napus L.

Yan Zhang
Northwest A&F University: Northwest Agriculture and Forestry University
Qi Zhang
Northwest A&F University: Northwest Agriculture and Forestry University
Han Wang
Northwest A&F University: Northwest Agriculture and Forestry University
Shunxian Tao
Northwest A&F University: Northwest Agriculture and Forestry University
Hanming Cao
Northwest A&F University: Northwest Agriculture and Forestry University
Yiji Shi
Northwest A&F University: Northwest Agriculture and Forestry University
Aldiyar Bakirov
Northwest A&F University: Northwest Agriculture and Forestry University
Aixia Xu
Northwest A&F University: Northwest Agriculture and Forestry University
Zhen Huang (huang_zhen.8@163.com)
Northwest Agriculture and Forestry University https://orcid.org/0000-0001-6943-1990

Research Article

Keywords: Brassica napus L., QTL, SLAF-seq, salt-alkali, yield-related traits, candidate gene

Posted Date: July 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1778607/v1

Abstract

Rapeseed (*Brassica napus* L.) is one of the most important oil crops in the world. Its yield is decided by multiple yield-related traits, which is also susceptible to environmental factors. Many yield-related quantitative trait loci (QTLs) have been reported in *Brassica napus*; however, no studies have been conducted to investigate both salt-alkali tolerance and yield-related traits simultaneously. Here, specific-locus amplified fragment sequencing (SLAF-seq) technologies were utilized to map the QTLs for salt-alkali tolerance and yield-related traits. A total of 65 QTLs were identified, including 30 QTLs for salt-alkali tolerance traits and 35 QTLs for yield-related traits, accounting for 7.61–27.84% of the total phenotypic variations. Among these QTLs, 18 unique QTLs controlling two to four traits were identified by meta-analysis. Six novel and unique QTLs were detected for salt-alkali tolerance traits. By comparing these unique QTLs for salt-alkali tolerance traits with those previously reported QTLs for yield-related traits, seven co-localized chromosomal regions were identified on A09 and A10. Combining QTL mapping with transcriptome of two parents under salt and alkaline stresses, thirteen genes were identified as the candidates controlling both salt-alkali tolerance and yield. These findings provide useful information for future breeding of high-yield cultivars resistant to alkaline and salt stresses.

Key Message

Common loci and candidate genes for controlling salt-alkali tolerance and yield-related traits were identified in *Brassica napus* combining QTL mapping with transcriptome under salt and alkaline stresses.

Introduction

Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops in the world with the highest grain yield of rapeseed. With the rapidly growing demand for rapeseed oil, increasing the yield of oilseed crops is always a major breeding objective. In China, the self-sufficiency rate of vegetable oil is about 40%, which is far below the safety level of 60% (Wang 2007). Moreover, rapeseed planting areas have significantly decreased, and growth retardation and significant yield reductions of rapeseed have been observed with the increase of alkalinity and salinity of the rapeseed farmland (Machado and Serralheiro 2017). Therefore, it is urgent to improve environmental adaptation and yield of *Brassica napus* L.

Plant responses to salinity stress and agronomic traits such as seed yield and yield-associated traits (yield components and yield-related traits) are complicated quantitative traits that are controlled by numerous loci in *Brassica napus* (Paterson et al. 1988; Flowers 2004). Quantitative trait locus (QTL) mapping is one of the most common approaches for genetic analysis of quantitative traits. By traditional QTL mapping, QTLs for seed yield and yield-related traits have been identified in *Brassica napus* (Udall et al. 2006; Li et al. 2007; Mei et al. 2009; Cai et al. 2014). In contrast, QTLs associated with adaptation to salt stress are comparatively rare (Lang et al. 2017). Traditional genetic mapping methods need to create large-scale segregation populations and high-density genetic maps, which is time-consuming and costly. With the development of the next-generation sequencing (NGS) technology, a number of salt tolerance-related SNPs have been identified in *B. napus* using the 60k SNP array (Jian et al. 2014; Yong et al. 2015; Wei 2016; He et al. 2017; Hou et al. 2017; Wan et al. 2017; Zhang et al. 2017; Wassan et al. 2021; Zhang et al. 2022).

A high-density genetic linkage map is a valuable tool for QTL mapping. In *B. napus*, a number of genetic maps have been constructed based on restriction fragment length polymorphism (RFLP) markers (Ferreira et al. 1994;

Foisset et al. 1996), amplified fragment length polymorphisms (AFLPs) (Cai et al. 2008; He et al. 2008), simple sequence repeats (SSRs) (Piquemal et al. 2005; Xu et al. 2010) or a small amount of single nucleotide polymorphism (SNP) markers (Raman et al. 2014). However, the density of the genetic maps is relatively low due to the small number of available molecular markers. Construction of high-density genetic map requires a large number of molecular markers. Due to the variability of single nucleotide DNA sequence, SNPs are currently the markers of choice for high-density genetic mapping construction (Ganal et al. 2009). A large number of SNPs throughout the genome can be obtained by restriction-site associated DNA tag sequencing (RADseq), whole-genome resequencing (WGRS), genotyping-by-sequencing (GBS), and specific-locus amplified fragment sequencing (SLAF-seq) for high-density genetic map construction (Baird et al. 2008; Poland et al. 2012; Sun et al. 2013; Bowers et al. 2016). The SLAF-seq technology has been used to construct high-density genetic maps in various plant species, such as rice (Song et al. 2018), soybean (Li et al. 2017), sunflower (Zhou et al. 2018), and cucumber (Zhu et al. 2016). In addition, QTLs have been detected based on the SLAF-seq method, such as quality-related QTLs in peanut (Hu et al. 2018) and soybean (Zhou et al. 2018), P efficiency-related QTLs of soybean (Zhang et al. 2016), seed weight QTLs in *B. napus* (Geng et al. 2016), flowering time QTLs in *B. napus* (Xu et al. 2021) and salt tolerance QTLs in soybean (Do et al. 2018). However, no studies have been reported the simultaneous identification of QTLs for traits related to salt-alkali tolerance and yield using the SLAF-Seq technology in *Brassica napus*. Moreover, only a small number of candidate genes for yield-related traits have been cloned in rapeseed. *BnaC9.SMG7b*, from the major QTL for SS, *qSS.C9* (Zhang et al. 2012), functions as a positive regulator of seed number per silique in rapeseed by regulating the formation of functional female gametophytes (Li et al. 2015). The ribosome recycling factor (*BnRRF*) gene for seed weight has been identified based on whole-genome resequencing of 418 diverse rapeseed accessions (Hu et al. 2022). Some salt tolerance-related candidate genes have been identified by GWAS in *B. napus*, but the function of genes in *B. napus* in response to salt stress has not been elucidated.

In this study, we constructed a high-density genetic map of *B. napus* using the SLAF-seq technology. QTLs associated with salt-alkali tolerance and yield-related traits were identified. Combining QTL mapping and RNA-seq technologies, several potential candidate genes controlling both salt-alkali tolerance and yield have been identified. This study will lay a good foundation for understanding the genetic basis of rapeseed breeding for yield and salt-alkali tolerance traits.

Materials And Methods

Plant material and population construction

B. napus line 2205 (salt-tolerant) and line 1423 (salt-sensitive) were used as the parental lines to develop segregating populations. A cross was made between 2205 and 1423 to create F1. An $F_{7:8}$ population of 82 recombinant inbred lines (RILs) derived from the cross between 2205 and 1423 was used to construct the high-density genetic map and phenotypic evaluation. Two parents and their 82 individuals were planted in the greenhouse (16/8 h, 25/20°C). Two-week old leaf tissues of the two parents and RIL individuals were collected and frozen in liquid nitrogen before SLAF-seq analysis.

Phenotypic determination and statistical analysis

The alkaline and salt tolerance traits were evaluated at the seedling stage using a hydroponic system (Tocquin et al. 2003), with slight modification using large hydroponic containers (24 × 18 × 7 cm, L × W × H). In brief, thirty

healthy seeds from each of these 82 RIL lines and the parents were germinated in a plastic container with 0.25x modified Hoagland solution for six days. Ten similar seedlings per line were selected and individually transferred to the hydroponic system (0.25× for the first week, 0.5× for the second week) and grown for two weeks in a growth chamber (16/8 h, 25/20 °C). The seedlings were then treated with 200 mM NaCl for ten days and 75 mM $NaHCO_3$ for seven days in the four-five leaf stage, which were used to measure the morphological and physiological indexes, including salt tolerance rate (STR), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW) and chlorophyll content (SPAD). The grade standard of salt tolerance (STR) was determined as described previously (Ma et al. 2009). Shoots of individual seedlings were oven-dried at 105 °C for 30 min, and then dried further at 80◦C for 10 days to measure the SDW. The chlorophyll content (SPAD) was measured on a SPAD-502 plus (Konica Minolta, Japan). The units used were centimeters for length and grams for weight. For the yield-related traits, the 82 RILs were planted in the experimental field of Northwest Agriculture and Forestry University in Yangling, Shaanxi, China, in the winters of 2018, 2019 and 2020 and harvested in the springs of 2019, 2020 and 2021. During the maturity period, five plants per line were selected for yield-related trait determination in the springs of 2019, 2020 and 2021, respectively, including plant height (PH), length of main inflorescence (LMI), seeds per silique (SS), seed yield per plant (SYP), and number of siliques per plant (NSP). All phenotypic traits were evaluated using IBM SPSS version 20.0. Correlation coefficient and heatmap were obtained using the CloudTUTU platform (www.cloudtutu.com).

SLAF library construction and high-throughput sequencing

SLAF-seq was used to genotype 82 RIL individuals and their parents, as previously described with minor modifications (Sun et al. 2013). The *B. napus* reference genome, which has a size of 1.2 Gb (download link: http://www.genoscope.cns.fr/ brassica napus/data/), was used to perform a SLAF pilot experiment. The DNA of the RIL individuals and their parents was extracted from young leaf samples according to a modified CTAB method (Doyle 1990). First, the genomic DNA from each sample was digested at 37 °C with HaeIII and Hpy166II. Subsequently, fragments ranging from 364 to 414 base pairs (with indexes and adaptors) in size were gel-purified and diluted for pair-end sequencing (each end 125 bp) using an Illumina HiSeq 2500 system (Illumina, Inc; San Diego, CA, USA).

SLAF-seq data analysis and genotyping

The SLAF-seq data grouping and genotyping were performed using procedures described by Sun et al. (2013). Briefly, low-quality reads (quality score <20e) were filtered out and all SLAF pair-end reads were clustered based on sequence similarity by BLAT. Sequences with over 90% similarity were grouped into one SLAF locus. Minor allele frequency (MAF) evaluation was used to define alleles in each SLAF. Groups containing more than four tags were filtered out as repetitive SLAFs, and the SLAFs with 2-4 tags were identified as polymorphic SLAFs. High-quality SLAF markers for genetic mapping were filtered by the following criteria: (i) average sequence depths should be > 4-fold in each progeny and > 20-fold in the parents, (ii) markers with more than 10% missing data were filtered, and (iii) the chis-square test was performed to examine the segregation distortion.

High-density genetic map construction and QTL Mapping

Based on the genotyping data of 82 RILs, marker loci were partitioned primarily into chromosomes using a modified logarithm of odds (MLOD) score values of >3 as the cutoff value. To ensure efficient construction of the high-density and high-quality map, the HighMap strategy was used to order the SLAF markers and correct

genotyping errors within the linkage groups (Liu et al. 2014). The genetic map was constructed by applying a multipoint method of maximum likelihood (Van Ooijen 2011) and genotyping errors were corrected with the SMOOTH algorithm (Van Os et al. 2005). A k-nearest neighbor algorithm was applied to impute missing genotypes (Huang et al. 2012). Finally, genetic map distances were estimated using the Kosambi mapping function (Kosambi 1943). QTLs were identified based on LOD scores at the significance level of 0.05 determined through 1,000 permutation tests using the CIM method with the WinQTLCart version 2.5 (Basten et al. 1997). As a result, a LOD score of 2.5 was used as the threshold to identify QTLs and these QTLs were termed identified QTLs (Burns et al. 2003). The 2-LOD intervals surrounding the QTL peak determined the QTL CIs. The QTLs with overlapping CIs for different traits were further integrated into one unique QTL by QTL meta-analysis using BioMercator 4.2 software (Goffinet and Gerber 2000), (Arcade et al. 2004). Referring to the nomenclature previously described (Udall et al. 2006), identified QTL were designated with an initial letter “q” followed by the abbreviation of the trait, the year or treatment, and the corresponding chromosome (e.g., *qPH19-C3*, *qSTRs-C6*, *qSTRa-A3*). Unique QTLs were designated with the initial letters “uq-" followed by the abbreviation of the trait and the corresponding chromosome (e.g., *uqPH-C3*). The linkage genetic map and QTLs were visualized using MapChart 2.2 (Voorrips 2002).

QTL comparison for all traits of present and previous QTLs

In order to further verify the reliability of the QTLs, QTL mapping studies co-localized with the present study were collected for alkaline and salt tolerance [9-18], yield-related traits including PH, LMI, SS, SYP and NSP (Udall et al. 2006; Jiaqin et al. 2009; Basunanda et al. 2010; Shi et al. 2011; Wang et al. 2016b; Zhao et al. 2016; Luo et al. 2017; Ye et al. 2017; Shen et al. 2018; Yang et al. 2018; Li et al. 2020; Hu et al. 2022) and flowering time (FT) (Long et al. 2007; Wei et al. 2014; Xu et al. 2015; Wang et al. 2016a; Wei et al. 2017; Li et al. 2018a; Li et al. 2018b; Jian et al. 2019; Wu et al. 2019; Xu et al. 2021) conducted on *B. napus.* QTL from previous studies and the present study were compared to the physical genomic regions of *B. napus*, “Darmor-bzh” (http://www.genoscope.cns.fr/brassicanapus/data/). If only one flanking marker can be aligned to the reference genome, we take a uniform area of 1 cM to delimit all QTLs.

RNA extraction, RNA-seq and data analysis

Total RNA was isolated from roots of line 2205 (salt-tolerant) and line 1423 (salt-sensitive) under salt treatments (3h and 24h after the treatments, ST) and control (0 h, CK) using TRizol (Invitrogen, Carlsbad, CA, United States) and purified using a Qiagen RNeasy kit (Qiagen, Germany). Each sample had three biological replicates. A total of 18 RNA samples were used to construct the cDNA library. Sequencing of each cDNA library was carried out on the Illumina HiSeq 6000 system. We used BWA to map clean reads to the *B. napus* genome (Darmor-bzh). Differential expression analysis of two samples was performed using the DESeq2 R package (Wang et al. 2009). Genes were considered to be differentially expressed genes (DEGs) with a $|\log2FC| \geq 1$ and an adjusted p-value <0.01. Venn diagram and gene-expression heat map were drawn using OmicShare tools (http://www.omicshare.com/tools). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to perform enrichment analysis of DEGs. GO and KEGG enrichment were performed using TBtools V1.076 software (Chen et al. 2020).

Quantitative real-time PCR analysis

Gene expressions were measured by RT-qPCR using the SYBR Green Master Mix (TaKaRa, Japan). The expressions of genes were calculated by the $2^{-\triangle\triangle Ct}$ method according to the method described by Vandesompele

et al., (2002) (Vandesompele et al. 2002) and the expression of actin2 was used as an internal control. Each assay was repeated three times independently, and the statistical significance was evaluated by Student's t test (significance, $P < 0.05$). Primers used for RT-qPCR are listed in Table S9.

Results

Phenotypic variation and correlation analysis

We measure six salt tolerance traits (STT), four alkaline tolerance traits (ATT) and five yield-related traits from the RIL lines and the two parents. All traits performed significant differences between line 1423 and line 2205 in all environments. Compared with other traits, PH showed the lowest coefficient of variance (CV), while SYP exhibited the highest CV (Table1). All traits exhibited continuous normal or near-normal distributions (Fig. S1). Correlation analysis among all traits was carried out. STR was usually described as an effective salt stress indicator. For STT, STR was significantly negatively correlated with all physiological and morphological indicators. For ATT, STR was only significantly negatively correlated with SPAD and RFW. SPAD was significantly positively correlated with RFW and RL (Fig. 1). A correlation was observed between salt tolerance traits and alkaline tolerance traits. For instance, aSPAD was significantly positively correlated with sRL, sRFW, sSFW, and sSDW. These results suggest that alkaline and salt tolerance in *B. napus* might be linked to these traits and different. Seed yield (SYP) showed significant positive correlations with all yield-associated traits across different years, and especially for NSP and SS with a correlation coefficient of 0.80 and 0.62, respectively. Interestingly, SYP was significantly positively correlated with sSPAD. However, sSPAD was significantly positively correlated with all salt-related traits. Taken together, these results demonstrate that there is a complex association between seed yield and other traits, either directly or indirectly, including salt and alkaline tolerance traits and yield-related traits.

Construction of the linkage map based on SLAF-Seq

In total, 33.35 Gb of raw reads consisting of 166.75 Mb paired-end reads were generated from Illumina sequencing of the SLAF libraries. The average percentage of Q30 bases (bases with a quality score of 30, indicating a 1% chance of an error and thus a 99% confidence level) was 94.83%, and the GC content was 36.43%. A total of 73.82 and 78.45 million reads were generated for the salt-tolerant parent 2205 and the salt-sensitive parent 1423, respectively, while 2.03 million reads were obtained for the 82 RILs. A total of 2,532,319 SNPs were detected, among which, 1,516,733 polymorphic SNPs were successfully sorted into eight segregation patterns (ab × cd, ef × eg, ab × cc, cc × ab, hk × hk, lm × ll, nn × np, and aa × bb), and 673,113 SNPs that belong to the aa × bb segregation pattern were used in linkage analysis. After a three-step filtering process (see Methods), 5,250 SNPs were used for the genetic map construction. In total, 4,159 SNP markers were grouped into 19 linkage groups (LGs) compared with the *B. napus* reference genome (Fig. S2). The total genetic distance was 1736.59 cM with a mean marker distance of 0.42 cM between adjacent markers. The lengths of LGs ranged from 46.14 cM (LG8) to 136.79 cM (LG9). The number of markers in each LG ranged from 84 to 327, with an average of 219 markers per LG (Table S1).

QTLs for salt-alkali tolerance and yield-related traits

A total of 65 QTLs were detected for salt-alkali tolerance and yield-related traits with a total phenotypic variance explained (PVE) of 7.61–27.84% and a LOD of 2.72–8.40 (Table S2 and Fig. 2). Notably, most QTLs for salt-alkali tolerance traits and each of QTL for SS and NSP showed a PVE of more than 10%. The QTLs for yield-related

traits were mainly distributed on chromosomes A01, A09, C08 and C09 (4-6 each), while the QTLs for salt-alkali tolerance traits mapped on A09, A10 and C04. By meta-analysis, 42 of 65 identified QTLs were integrated into 18 unique QTLs controlling two to four traits (Table S3). Six unique QTLs were identified for salt-alkali tolerance traits, of which four formed two adjacent QTL clusters with a genetic distance of 1cM on A10, and the other two controlling SDWs, SFWs and RFWs were located on A09 and C04, respectively, with an average PVE of 15%. Seven unique QTLs were identified for seed yield and yield-related traits. For example, QTL *uqA5* and *uqC6* for SYP and LMI, *uqC9-2* for SYP and NSP, *uqA9-2* for SYP, NSP, PH and LMI and *uqC3-2* for LMI and PH were identified with positive additive effects, which was in accordance with the significant positive correlations among these traits. Interestingly, five unique QTLs were detected for both salt-alkali tolerance and yield-related traits. For instance, QTL *qSYP19-A6-1* overlapped with QTL *qRFWa-A6* on A06. QTL *uqC8* controlling PH, LMI and RFW and *uqC9-1* controlling SS and RL showed consistent additive effects.

Comparative analysis of salt-alkali tolerance and yield-related traits of present and previous QTLs

Among the 18 QTLs for salt tolerance traits, ten salt-related QTLs contained 245 significantly associated SNPs identified previously, of which four were detected for the same traits (Table S4). Specifically, the QTLs *qSDWs-C3* and *uqC4* for SDW, *qRLs-C9* for RL, and *uqA10-3* for SFW included nine, one and two SNPs, respectively, indicating their high reliability. In a previous study, some QTLs were detected using the F2 of the same parents, among which five QTLs (*qSH12-a*, *qRDW19-c*, *qEC8-c*, *qSOD14-b* and *qLDW14-b*) and one QTL *qSH4-b* had overlapping CIs with the QTLs *qRFWs-C8* and *qRLs-C9* in the present study, respectively, indicating the complexity of salt tolerance traits. In addition, nine alkaline-related QTLs contained 163 significantly salt-associated SNPs identified previously, of which *qRLa-A10-1* and *qRLa-A10-2* for RL possessed five SNPs for the same traits. For yield-related traits, ten of 35 QTLs were colocalized with QTLs identified in the previous study (Table S5). One QTLs for SYP and NSP were identical to *qSYP21-A9* and *qNSP20-A1* in the present study, respectively. *qSS20-A1*, *qSS20-C1* and *qSS20-C9-1* (present study) were identical to 18 QTLs for SS. *qLMI19-C6* (present study) was identical to three QTLs identified previously. For PH, four QTLs (*qPH19-C8-1, qPH20-C8*, *qPH19-C4* and *qPH19-C8-2*) were identical to significantly PH-associated InDels identified by GWAS.

QTLs for salt-alkali tolerance traits co-localized with QTLs for yield-related traits

As shown in Table S3, six unique QTLs for salt-alkali tolerance traits were located on A09, A10 and C04, especially A10. To explore the connection between salt-alkali tolerance traits and yield-related traits, these six unique QTLs plus *qRLa-A10-1* and *qRLa-A10-2* identified in the current study were compared with the QTLs for yield-related traits previously identified. Since flowering time is significantly associated with plant height, it was added as a yield-related trait. We found that seven QTLs, composed of three identified QTLs and four unique QTLs, overlapped with the QTLs for the six yield-related traits previously identified except for *uqC4* (Table 2). *qRLa-A10-1* and *qRLa-A10-2* for RL overlapped with one SNP for SYP, one SNP for SS, one SNP for LMI, one QTL for LMI and one QTL for FT identified previously. *uqA10-1* and *uqA10-2* for SPAD and RFW overlapped with one QTL for SYP, one QTL for LMI, two QTLs for PH, one SNP for PH and twelve QTLs for FT identified previously. *qRFWs-A9* and *uqA10-3* for RFW, SDW and SFW overlapped with one QTL for SS, four QTLs for PH on A09, and one SNP for LMI, ten SNPs for FT and eleven QTLs for FT on A10 identified previously, respectively. Moreover, *uqA10-4* for STR and SDW overlapped with five QTLs for FT identified previously. Taken together, seven QTLs that co-control salt-alkali tolerance traits and yield-related traits were identified and named as cQTLs, suggesting that A09 and A10 are important loci for controlling both salt-alkali tolerance traits and yield-related traits.

Identification of differentially expressed genes by transcriptome sequencing

To investigate the potential molecular mechanism underlying the differences in salt tolerance between lines 2205 and 1423 and to explore salt-related candidate genes, RNA-seq analysis was performed using RNA extracted from the roots of the 1423 and 2205 plants under control conditions (mock treated) and salt treatments. We first tested the root length of line 1423 and line 2205 in response to the 200 mM NaCl treatment. Under salt stress, the root length of line 2205 is always higher than that of line 1423 and significant difference was observed at 3h and 24h between line 2205 and line 1423 (Fig. S3). Based on the results, we used the root tissues treated with NaCl for 3h and 24h in the RNA-seq experiment. After trimming off the adapter sequences and removing the low-quality reads, we obtained 484,217,730 and 467,967,990 clean reads for line 2205 and line 1423, respectively, with an average read length of 90 bp and a Q20 percentage (percentage of sequences with sequencing error rates lower than 1%) over 97%. In total, 433,216,368 (89.47 % of the clean reads) and 424,400,341(90.69% of the clean reads) reads were mapped to the *B. napus* reference genome for line 2205 and line 1423, respectively, and 93,990 transcripts were identified after comparison. Sequencing and assembly statistics are summarized in Table S6. Both Pearson's correlation and PCA showed a high correlation among the replicas, with the exception of sample T-3h1 (round markers in Fig. S4), which was identified as an outlier and excluded from downstream differentially expressed genes (DEGs) analysis (Fig. S4 and Table S7).

Global expression analysis showed that 15,274 DEGs named as S_SR were found in line 1423 and 12,845 DEGs named as T_SR were found in line 2205 after 3h and 24 h salt stresses (Fig. 3a, b). In total, 19,311 DEGs were identified under salt stress and named as "salt-inducible genes", among which, 6,466 and 4,037 genes showed unique differentially expression in lines 1423 and 2205, respectively. The NaCl treatment led to a less transcriptomic change in line 2205 compared with that in line 1423, indicating that line1423 is more sensitive to salt stress than line 2205 at the transcriptional level. A total of 12,815 DEGs were regulated at 0 h, 3 h and 24 h between lines 1423 and 2205 (Fig. 3c). To further screen salt tolerant genes, 2,909 genes that exhibited unique differential expression between lines 1423 and 2205 under normal conditions were removed, and the remaining 10,423 DEGs were named as V_SR. Among these three groups (S_SR, T_SR and V_SR), a total of 24,852 DEGs were identified and 4,882 DEGs were detected in all three groups, while 4,783 and 2,809 DEGs were only detected in S_SR and T_SR, respectively (Fig. 3d). The expression levels of eight randomly selected genes were measured by using RT-qPCR, which showed consistent results with RNA-seq, suggesting that the RNA-seq data were reliable (Fig. S5).

Functional classification of differentially expressed genes

In order to explore the salt tolerance mechanism of the two parents, three groups of the differentially expressed genes (DEGs), S_SR_ unique (4783), T_SR_ unique (2809) and STV_SR (4882), were functionally annotated using gene ontologies (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Fig. 4). GO enrichment analysis revealed that both T_SR_ unique (2809) and STV_SR (4882) DEGs were uniquely involved in secondary metabolic process and response to light stimulus (Fig. 4b, c), while the S_SR_ unique (4783) DEGs were uniquely involved in response to nitrogen compound, response to jasmonic acid and response to fatty acid (Fig. 4a). Moreover, DEGs identified in S_SR_ unique (4783) were enriched with significantly higher GO terms compared with those in T_SR_ unique (2809) and STV_SR (4882). KEGG enrichment analysis also provided similar enrichment results to the GO enrichment. Analysis of the KEGG pathways showed that metabolism pathways, especially for phenylpropanoid biosynthesis, flavonoid biosynthesis and carbohydrate metabolism were uniquely enriched among the T_SR_ unique (2809) and STV_SR (4882) DEGs (Fig. 4e, f), while plant hormone signal transduction, glutathione

metabolism, glycosaminoglycan binding proteins and amino acid metabolism were uniquely enriched among the S_SR_ unique (4783) DEGs (Fig. 4d). These results indicated that the two parents had different molecular mechanisms in response to salt stress, and line 1423 was more sensitive to salt stress and elicited more responses in the body. The similar enrichment results of the two groups (T_SR_ unique and STV_SR) also showed that most of the key salt tolerance related genes came from line 2205.

Candidate genes for controlling both salt-alkali tolerance and yield

To further reveal candidate genes controlling both salt-alkali tolerance and yield, these 24,852 DEGs were integrated with seven cQTLs and 1081 genes were found in these QTLs regions. A total of 390 DEGs response to salt stress were co-localized with the cQTLs (Fig. 5a). Since the cQTLs contained both alkaline and salt tolerance traits, we also detected the DEGs using the transcriptome data of the two parents under alkaline stress (unpublished). A total of 400 DEGs were located with the cQTLs in response to alkaline stress (Fig. 5b). Of these, 99 and 162 DEGs were not only "salt-inducible genes" (S_SR/T_SR), but also differentially expressed between lines 1423 and 2205 (V_SR), respectively. To further narrow down the number of candidate genes, the expression profiles of these 99 and 162 DEGs were performed (Fig. S6). The genes that were continuously induced in 1423 or 2205 and differentially expressed between the two parents were selected for further analysis, and, 28 (28S) and 75 (75A) genes were obtained respectively (Fig. 5c). The heat maps of the relative expression profiles are shown in Fig. 5d and 5e. A total of 100 genes were induced by salt or alkaline stress, of which three genes were in response to both salt and alkaline stress (Fig. 5c). Among these 100 genes, 85 were homologous to 83 *Arabidopsis* genes. GO and KEGG analyses were performed for these 83 *Arabidopsis* genes, and the result revealed that 64 DEGs were annotated to GO terms and 31 DEGs were mapped to biological pathways, especially the metabolism pathways (Table S8). Based on the functional annotation, 13 genes were screened as the candidate genes (Table 3), of which, four were involved in response to salt, cold and heat abiotic stresses and six genes were annotated with regulation of cell growth, pollen tube growth and gibberellin biosynthetic process. Notably, three genes (*BnaA10g16340D*, *BnaA10g20120D* and *BnaA10g25000D*) were annotated with both abiotic stress and plant organ development, which would serve as the most promising candidates.

Discussion

Rapeseed varieties with high stress resistance and high yield are highly desirable in the rapeseed oil industry. To make full use of rapeseed germplasm resources, it is extremely important to assess its genetic diversity and to detect QTLs associated with stress tolerance and yield-related traits. A high-density genetic map is essential for QTL mapping and identification of functional genes. The SLAF-seq strategy has been successfully applied to high-density genetic map construction and QTL mapping for many plants, including *B. napus* (Geng et al. 2016; Zhang et al. 2016; Do et al. 2018; Hu et al. 2018; Xu et al. 2021). In this study, by SLAF-seq, we constructed a high-density genetic map with 4,159 SNP markers using the RIL population derived from a cross between lines 2205 and 1423 (Fig. S2). The total genetic distance was 1736.59 cM with a mean SNP marker distance of 0.42 cM between adjacent markers (Table S1). In a previous study, a genetic map using the same parents has been generated based on SSR and AFLP markers using the F_2 populations (Lang et al. 2017). However, the map only includes 532 polymorphic markers and spans 1341.1 cM with an average marker interval of 2.52 cM, demonstrating that SLAF-seq is an efficient approach for acquiring abundant polymorphic markers to rapidly construct higher resolution genetic maps.

To our knowledge, a few studies have been reported on QTL mapping of salt-alkali tolerant related genes used linkage mapping in *B. napus*. Here, we identified QTLs for both salt and alkaline tolerance traits in *B. napus* using a high-density genetic map constructed by the SLAF-Seq technology and found that QTLs for salt tolerance and alkaline tolerance traits were very different. Most QTLs under salt stress were distributed on A09, A10 and C04, while QTLs under alkaline stress were only on A10. Although no overlapping regions were detected for the same trait under salt and alkaline stress, the QTLs for both were mainly distributed on A10, and the QTLs for RFW on A10 were relatively close (Table S2), suggesting that the two parents (lines 1423 and 2205) in our study have different mechanisms in response to salt and alkali stresses. Besides, the QTL (*qSTRa-A10*) for STRa and the QTL (*qSDWs-A10-2*) for SDWs with opposite additive effects were detected to overlap with each other on A10, which was consistent with the significant negative correlation between STRa and SDWs. Compared with the salt tolerant related QTLs from Lang et al. 2017, six co-associated QTLs were observed which were mainly distributed on C08. However, no QTL was detected for the same trait (Table S4), which may be due to differences of population types and environments for phenotypic investigation, suggesting that the population type has a great influence on QTL mapping and the salt tolerance traits are also greatly influenced by the environment. Plant response to salt stress is a complex physiological process, which causes changes in many related traits, such as fresh weight of plant and dry weight of plant (Yeo 1998). In this study, STR was significantly correlated with RL, RFW, SFW and SDW, suggesting that these indicators can reflect the salt tolerance of plants to some extent. QTLs *qSDWs-C3* and *uqC4* possessed nine significant SNPs for SDW, and *uqA10-3* possessed one significant SNPs for SFW identified previously. Similarly, six significant SNPs for RL were co-located with QTLs *qRLa-A10-1* and *qRLs-C9* (Table S4). These results revealed that these QTLs detected in the present study were stable and valid. We detected three uqQTLs (*uqA9-1, uqA10-3* and *uqC4*) controlling RFW, SFW and SDW simultaneously (Table S3). Notably, 17 SDW-related significant SNPs were co-located with QTL *uqA9-1*, but they were far from *uqA9-1* which is located at 3.76–4.99 Mb (Table S3), indicating that *uqA9-1* is a reliable and novel salt-related QTL associated with RFW, SFW and SDW. Interestingly, multiple QTLs for salt-alkali traits were identified on chromosome 10, especially, QTLs *uqA10-1*, *uqA10-2* and *uqA10-4* which were adjacent to each other with a PVE of more than 10%. The QTL *uqA10-4* was found to control both alkaline and salt tolerance traits. Moreover, these three QTLs contained seven salt-related significant SNPs for different traits identified previously, which further verified the reliability of the QTLs in the present study. These results suggest that the A10 chromosome is an important locus controlling alkaline and salt tolerance traits simultaneously.

Contrary to the alkaline and salt tolerance traits, QTL mapping of yield-related traits has been frequently reported in *B. napus*. In this study, 35 QTLs were obtained for yield-related traits, of which ten QTLs have been repeatedly reported by previous studies (Table S5). *qSS20-A1* and *qLMI19-C6* were repeatedly identified 3–15 times in at least two different experiments (Table S5), and six other identified QTLs (*qPH19-C4, qPH19-C8-2, qSS20-C1, qSS20-C9-1, qSYP21-A9 and qNSP20-A1)* were repeatedly detected only one time, all of which exhibit a PVE of more than 10% expect *qPH19-C4*. These results indicate that these QTLs are stable under different genetic backgrounds and may be useful in the molecular marker-assisted selection of breeding. It is worth noting that five QTLs (*qSYP21-A9, qSS20-C1, qSS20-C9-1, qPH19-C8-2*, *qLMI19-C6*) were repeatedly detected in previous studies, but they belonged to the pleiotropic QTL controlling two to three traits which exhibit a PVE of more than 15% in this study (Table S3). These QTLs will be highlighted as new pleiotropic QTLs. Besides these nine QTLs, the remaining QTLs identified in our study should be novel QTLs. Notably, QTL (*qPH21-C5*) for PH on C05 was detected for the first time.

This study is the first to simultaneously map stress resistance and yield-related traits using a high-density genetic map in rapeseed. Correlation analysis revealed that there were certain correlations between salt-alkali tolerance and yield-related traits, which was also reflected in the genome positions and effects of the detected QTL. For example, *uqA6*, *uqC1, uqC3-1* and *uqC8* were identified to pleiotropically control both salt-alkali tolerance and yield-related traits (Table S3), which may be used as key QTLs for high stress resistance and yield-related traits. As mentioned above, the loci on the A09, A10 and C04 chromosomes were key loci for salt-alkali tolerance traits, especially A10 in our study. These key loci did not co-localize with QTLs for yield-related traits in this study, but seven QTLs co-localized with QTLs for yield-related traits have been reported previously, especially plant height and flowering time (Table 2). Such co-localization of salt-alkali tolerance and yield-related traits have not been reported in previous studies. To further explore the candidate genes controlling both salt-alkali tolerance and yield, thirteen candidate genes were identified by combining QTL mapping and RNA-seq analysis (Table 3). Among them, four genes (*BnaA09g07980D, BnaA10g14870D, BnaA10g19960D and BnaA10g25660D*) were annotated to response to salt, cold and heat stresses, but none was reported to function under salt stress except *BnaA10g19960D* which is homologous to *RAP2.6L* of *A. thaliana*. RAP2.6L overexpression enhances plant resistance to salt and drought (Krishnaswamy et al. 2011). Six genes (*BnaA09g09160D, BnaA09g08360D, BnaA09g08940D, BnaA09g08950D, BnaA10g25700D and BnaA10g26380D*) were related with cell growth, pollen tube growth and gibberellin biosynthetic process and homologous to *PILS5*, *CALS5*, *NAC036*, *BUP* and *CTR1* of *A. thaliana*. The mutant *cals5* exhibits severely reduced silique length and seed yield (Dong et al. 2005). *BnaA09g08940D* and *BnaA09g08950D* were homologous to *AT2G17040* (*ANAC036*) of *A. thaliana.* Overexpression of ANAC036 resulted in a semidwarf phenotype in *Arabidopsis* (Kato et al. 2010). Pollen development is a major factor that affects the yield of crops, and the pollen of the *bup* mutant is severely reduced (Hoedemaekers et al. 2015).The *ctr1-1* mutant performs a delayed flowering (Achard et al. 2007) and is more tolerant to salt and osmotic stress compared with the wild type during germination and post-germination development, especially when the stress level is high (Wang et al. 2007). Interestingly, three potential candidates (*BnaA10g16340D, BnaA10g20120D* and *BnaA10g25000D*), homologous to *CHR17*, *SWEET15/SAG29* and *DREB2* of *A. thaliana*, were identified that may regulate simultaneously salt resistance and yield of plants. Overexpression of StDREB2 enhances drought stress tolerance in cotton and *SlDREB2* mediates salt stress tolerance in tomato and *Arabidopsis* (Hichri et al. 2016; El-Esawi and Alayafi 2019). In addition, the expression of *DREB2* changes during pollen germination and pollen tube growth, and *DREB2*, as a downstream regulator of *ANAC019*, functions in flower development under drought stress in *Arabidopsis* (Wang et al. 2008; Sukiran et al. 2019). Although *chr17* single mutants not exhibit developmental defects, the *chr11-1 chr17-1* mutant plants are smaller with a much earlier flowering time, and the flowers fail to open, and are completely sterile compared with the wild-type (Li et al. 2012). Notably, *SWEET15/SAG29* exhibits opposing phenotypes in stress resistance and yield. The *sweet15* mutant exhibits retarded embryo development and reduced seed weight, while the overexpression of SAG29 exhibits accelerated senescence and the plants are hypersensitive to salt stress (Seo et al. 2011). Functional exploration of all the mentioned candidate genes has not been reported in *B. napus*. Further studies are needed to reveal the roles of these genes, which will be valuable for future breeding of high-yield cultivars resistant to salinity stress.

Conclusion

In conclusion, we identified 65 QTLs for salt-alkali tolerance and yield-related traits by SLAF-seq. A total of 18 unique QTLs controlling two to four traits were detected by meta-analysis, among which, six novel and unique

QTLs were identified for salt-alkali tolerance traits. Compared these unique QTLs with QTLs for the six yield-related traits previously identified, seven co-localized chromosomal regions were identified on A09 and A10. An integrated analysis of QTL mapping and RNA-seq identified several promising candidates controlling both salt-alkali tolerance and yield in these regions, which will lay a solid foundation for breeding of high-yield cultivars resistant to salt-alkali stress.

Declarations

Funding

The research was supported by the Key research and development projects of Yangling Seed-industry Innovation Center (Ylzy-yc2021-01).

Competing interests

The authors declare that they have no competing interests.

Author Contributions

ZH was responsible for designing and supervising this study; YZ performed data analysis and manuscript drafting. YZ, QZ, HW, ST and HC worked on phenotypic survey. YS, BA and AX provided assistance for data analysis and phenotypic survey. All authors have read and approved the content of the manuscript.

Data Availability

The datasets generated during the current study are available in the Sequence Read Archive (SRA) database of National Center for Biotechnology Information (NCBI) under accession PRJNA847790 and accession PRJNA847444.

References

1. Achard P, Baghour M, Chapple A, et al (2007) The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc Natl Acad Sci U S A 104:6484–6489. https://doi.org/10.1073/pnas.0610717104
2. Arcade A, Labourdette A, Falque M, et al (2004) BioMercator: Integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326. https://doi.org/10.1093/bioinformatics/bth230
3. Baird NA, Etter PD, Atwood TS, et al (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:1–7. https://doi.org/10.1371/journal.pone.0003376
4. Basten CJ, Weir BS, Zeng Z (1997) QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping. Raleigh, NC: North Carolina State University.
5. Basunanda P, Radoev M, Ecke W, et al (2010) Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (Brassica napus L.). Theor Appl Genet 120:271–281. https://doi.org/10.1007/s00122-009-1133-z
6. Bowers JE, Pearl SA, Burke JM (2016) Genetic Mapping of Millions of SNPs in Safflower (Carthamus tinctorius L.) via Whole-Genome Resequencing. G3 Genes, Genomes, Genet 6:2203–2211.

https://doi.org/10.1534/g3.115.026690

7. Burns MJ, Barnes SR, Bowman JG, et al (2003) QTL analysis of an intervarietal set of substitution lines in Brassica napus: (I) seed oil content and fatty acid composition. Heredity (Edinb) 90:39–48. https://doi.org/10.1038/sj.hdy.6800176
8. Cai CC, Tu JX, Fu TD, Chen BY (2008) The genetic basis of flowering time and photoperiod sensitivity in rapeseed Brassica napus L. Russ J Genet 44:326–333. https://doi.org/10.1134/s1022795408030137
9. Cai D, Xiao Y, Yang W, et al (2014) Association mapping of six yield-related traits in rapeseed(Brassica napus L.). Theor Appl Genet 127:85–96. https://doi.org/DOI 10.1007/s00122-013-2203-9
10. Chen C, Chen H, Zhang Y, et al (2020) TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Mol Plant 13:1194–1202. https://doi.org/10.1016/j.molp.2020.06.009
11. Do TD, Vuong TD, Dunn D, et al (2018) Mapping and confirmation of loci for salt tolerance in a novel soybean germplasm, Fiskeby III. Theor Appl Genet 131:513–524. https://doi.org/10.1007/s00122-017-3015-0
12. Dong X, Hong Z, Sivaramakrishnan M, et al (2005) Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. Plant J 42:315–328. https://doi.org/10.1111/j.1365-313X.2005.02379.x
13. Doyle JJ (1990) Isolation of Plant DNA from Fresh Tissue. Focus (Madison) 12:13–15
14. El-Esawi MA, Alayafı AA (2019) Overexpression of StDREB2 transcription factor enhances drought stress tolerance in cotton (Gossypium barbadense L.). Genes (Basel) 10:. https://doi.org/10.3390/genes10020142
15. Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of Brassica napus using doubled haploid lines. Theor Appl Genet 89:615–621. https://doi.org/10.1007/BF00222456
16. Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55:307–319. https://doi.org/10.1093/jxb/erh003
17. Foisset N, Delourme R, Barret P, et al (1996) Molecular-mapping analysis in Brassica napus using isozyme, RAPD and RFLF markers on a doubled-haploid progeny. Theor Appl Genet 93:1017–1025. https://doi.org/10.1007/BF00230119
18. Ganal MW, Altmann T, Röder MS (2009) SNP identification in crop plants. Curr Opin Plant Biol 12:211–217. https://doi.org/10.1016/j.pbi.2008.12.009
19. Geng X, Jiang C, Yang J, et al (2016) Rapid identification of candidate genes for seed weight using the SLAF-seq method in Brassica napus. PLoS One 11:1–14. https://doi.org/10.1371/journal.pone.0147580
20. Goffinet B, Gerber S (2000) Quantitative trait loci: A meta-analysis. Genetics 155:463–473. https://doi.org/10.1093/genetics/155.1.463
21. He J, Ke L, Hong D, et al (2008) Fine mapping of a recessive genic male sterility gene (Bnms3) in rapeseed (Brassica napus) with AFLP- and Arabidopsis-derived PCR markers. Theor Appl Genet 117:11–18. https://doi.org/10.1007/s00122-008-0747-x
22. He Y, Wu D, You J, Qian W (2017) Genome-wide association analysis of salt tolerance related traits in Brassica napus and candidate gene prediction. Sci Agric Sin 50:1189–1201. https://doi.org/10.3864/j.issn.0578-1752.2017.07.002
23. Hichri I, Muhovski Y, Clippe A, et al (2016) SlDREB2, a tomato dehydration-responsive element-binding 2 transcription factor, mediates salt stress tolerance in tomato and Arabidopsis. Plant Cell Environ 39:62–79. https://doi.org/10.1111/pce.12591

24. Hoedemaekers K, Derksen J, Hoogstrate SW, et al (2015) BURSTING POLLEN is required to organize the pollen germination plaque and pollen tube tip in Arabidopsis thaliana. New Phytol 206:255–267. https://doi.org/10.1111/nph.13200
25. Hou LT, Wang TY, Jian HJ, et al (2017) QTL mapping for seedling dry weight and fresh weight under salt stress and candidate genes analysis in Brassica napus L. Acta Agron Sin 43:179–189. https://doi.org/10.3724/SP.J.1006.2017.00179
26. Hu J, Chen B, Zhao J, et al (2022) Genomic selection and genetic architecture of agronomic traits during modern rapeseed breeding. Nat Genet 54:694–704. https://doi.org/10.1038/s41588-022-01055-6
27. Hu XH, Zhang SZ, Miao HR, et al (2018) High-density genetic map construction and identification of QTLs controlling oleic and linoleic acid in peanut using SLAF-seq and SSRs. Sci Rep 8:1–10. https://doi.org/10.1038/s41598-018-23873-7
28. Huang X, Zhao Y, Wei X, et al (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nat Genet 44:32–39. https://doi.org/10.1038/ng.1018
29. Jian H, Zhang A, Ma J, et al (2019) Joint QTL mapping and transcriptome sequencing analysis reveal candidate flowering time genes in Brassica napus L. BMC Genomics 20:1–14. https://doi.org/10.1186/s12864-018-5356-8
30. Jian HJ, Xiao Y, Li JN, et al (2014) QTL mapping for germination percentage under salinity and drought stresses in Brassica napus L. using a SNP genetic map. Acta Agron Sin 40:629–635. https://doi.org/10.3724/SP.J.1006.2014.00629
31. Jiaqin S, Ruiyuan L, Dan Q, et al (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in Brassica napus. Genetics 182:851–861. https://doi.org/10.1534/genetics.109.101642
32. Kato H, Motomura T, Komeda Y, et al (2010) Overexpression of the NAC transcription factor family gene ANAC036 results in a dwarf phenotype in Arabidopsis thaliana. J Plant Physiol 167:571–577. https://doi.org/10.1016/j.jplph.2009.11.004
33. Kosambi D (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175
34. Krishnaswamy S, Verma S, Rahman MH, Kav NNV (2011) Functional characterization of four APETALA2-family genes (RAP2.6, RAP2.6L, DREB19 and DREB26) in Arabidopsis. Plant Mol Biol 75:107–127. https://doi.org/10.1007/s11103-010-9711-7
35. Lang LN, Xu AX, Ding J, et al (2017) Quantitative Trait Locus Mapping of Salt Tolerance and Identification of Salt-Tolerant Genes in Brassica napus L. Front Plant Sci 8:1–13. https://doi.org/10.3389/fpls.2017.01000
36. Li B, Fan S, Yu F, et al (2017) High-resolution mapping of QTL for fatty acid composition in soybean using specifc-locus amplifed fragment sequencing. Theor Appl Genet 130:1467–1479. https://doi.org/10.1007/s00122-017-2902-8
37. Li B, Gao J, Chen J, et al (2020) Identification and fine mapping of a major locus controlling branching in Brassica napus. Theor Appl Genet 133:771–783. https://doi.org/10.1007/s00122-019-03506-x
38. Li B, Zhao W, Li D, et al (2018a) Genetic dissection of the mechanism of flowering time based on an environmentally stable and specific QTL in Brassica napus. Plant Sci 277:296–310. https://doi.org/10.1016/j.plantsci.2018.10.005
39. Li G, Zhang J, Li J, et al (2012) Imitation Switch chromatin remodeling factors and their interacting RINGLET proteins act together in controlling the plant vegetative phase in Arabidopsis. Plant J 72:261–270. https://doi.org/10.1111/j.1365-313X.2012.05074.x

40. Li R, Jeong K, Davis JT, et al (2018b) Integrated QTL and eQTL mapping provides insights and candidate genes for fatty acid composition, flowering time, and growth traits in a F2 population of a novel synthetic allopolyploid brassica napus. Front Plant Sci 871:1–20. https://doi.org/10.3389/fpls.2018.01632
41. Li S, Chen L, Zhang L, et al (2015) BnaC9.SMG7b functions as a positive regulator of the number of seeds per silique in Brassica napus by regulating the formation of functional female gametophytes. Plant Physiol 169:2744–2760. https://doi.org/10.1104/pp.15.01040
42. Li Y, Shen J, Wang T, et al (2007) QTL analysis of yield-related traits and their association with functional markers in Brassica napus L. Aust J Agric Res 58:759–766. https://doi.org/10.1071/AR06350
43. Liu D, Ma C, Hong W, et al (2014) Construction and analysis of high-density linkage map using high-throughput sequencing data. PLoS One 9:e98855. https://doi.org/10.1371/journal.pone.0098855
44. Long Y, Shi J, Qiu D, et al (2007) Flowering time quantitative trait loci analysis of oilseed Brassica in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433–2444. https://doi.org/10.1534/genetics.107.080705
45. Luo Z, Wang M, Long Y, et al (2017) Incorporating pleiotropic quantitative trait loci in dissection of complex traits: seed yield in rapeseed as an example. Theor Appl Genet 130:1569–1585. https://doi.org/10.1007/s00122-017-2911-7
46. Ma H, Wang R, Wang X, Ma H (2009) Identification and evaluation of salt tolerance of Jute germplasm during germination and seedling periods. J Plant Genet Resour 10:236–243. https://doi.org/10.13430/j.cnki.jpgr.2009.02.013.
47. Machado RMA, Serralheiro RP (2017) Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae 3:30. https://doi.org/10.3390/horticulturae3020030
48. Mei DS, Wang HZ, Hu Q, et al (2009) QTL analysis on plant height and flowering time in Brassica napus. Plant Breed 128:458–465. https://doi.org/10.1111/j.1439-0523.2008.01528.x
49. Paterson AH, Lander ES, Hewitt JD, et al (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726
50. Piquemal J, Cinquin E, Couton F, et al (2005) Construction of an oilseed rape (Brassica napus L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523. https://doi.org/10.1007/s00122-005-0080-6
51. Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253. https://doi.org/10.1371/journal.pone.0032253
52. Raman H, Raman R, Kilian A, et al (2014) Genome-wide delineation of natural variation for pod shatter resistance in Brassica napus. PLoS One 9:e101673. https://doi.org/10.1371/journal.pone.0101673
53. Seo PJ, Park JM, Kang SK, et al (2011) An Arabidopsis senescence-associated protein SAG29 regulates cell viability under high salinity. Planta 233:189–200. https://doi.org/10.1007/s00425-010-1293-8
54. Shen Y, Xiang Y, Xu E, et al (2018) Major co-localized QTL for plant height, branch initiation height, stem diameter, and flowering time in an alien introgression derived Brassica napus DH population. Front Plant Sci 9:1–13. https://doi.org/10.3389/fpls.2018.00390
55. Shi Y, Zhang X, Xu ZY, et al (2011) Influence of EARLI1-like genes on flowering time and lignin synthesis of Arabidopsis thaliana. Plant Biol 13:731–739. https://doi.org/10.1111/j.1438-8677.2010.00428.x
56. Song J, Li J, Sun J, et al (2018) Genome-wide association mapping for cold tolerance in a core collection of rice (Oryza sativa L.) landraces by using high-density single nucleotide polymorphism markers from specific-

locus amplified fragment sequencing. Front Plant Sci 9:1–15. https://doi.org/10.3389/fpls.2018.00875

57. Sukiran NL, Ma JC, Ma H, Su Z (2019) ANAC019 is required for recovery of reproductive development under drought stress in Arabidopsis. Plant Mol Biol 99:161–174. https://doi.org/10.1007/s11103-018-0810-1
58. Sun X, Liu D, Zhang X, et al (2013) SLAF-seq: An Efficient Method of Large-Scale De Novo SNP Discovery and Genotyping Using High-Throughput Sequencing. PLoS One 8:e58700. https://doi.org/10.1371/journal.pone.0058700
59. Tocquin P, Corbesier L, Havelange A, et al (2003) A novel high efficiency, low maintenance, hydroponic system for synchronous growth and flowering of Arabidopsis thaliana. BMC Plant Biol 3:2–11. https://doi.org/10.1186/1471-2229-3-2
60. Udall JA, Quijada PA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (Brassica napus L.): 2. Identification of alleles from unadapted germplasm. Theor Appl Genet 113:597–609. https://doi.org/10.1007/s00122-006-0324-0
61. Van Ooijen JW (2011) Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genet Res (Camb) 93:343–349. https://doi.org/10.1017/S0016672311000279
62. Van Os H, Stam P, Visser RGF, Van Eck HJ (2005) SMOOTH: A statistical method for successful removal of genotyping errors from high-density genetic linkage data. Theor Appl Genet 112:187–194. https://doi.org/10.1007/s00122-005-0124-y
63. Vandesompele J, Katleen De Preter, Filip Pattyn, et al (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:0034.1-0034.11. https://doi.org/10.1186/gb-2002-3-7-research0034
64. Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78. https://doi.org/10.1093/jhered/93.1.77
65. Wan H, Chen L, Guo J, et al (2017) Genome-wide association study reveals the genetic architecture underlying salt tolerance-related traits in rapeseed (Brassica napus L.). Front Plant Sci 8:1–15. https://doi.org/10.3389/fpls.2017.00593
66. Wang HZ (2007) Analysis and strategy for current domestic edible oil supply. Chinese J oil Crop Sci 29:347–349
67. Wang L, Feng Z, Wang X, et al (2009) DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics 26:136–138. https://doi.org/10.1093/bioinformatics/btp612
68. Wang N, Chen B, Xu K, et al (2016a) Association mapping of flowering time QTLs and insight into their contributions to rapeseed growth habits. Front Plant Sci 7:1–11. https://doi.org/10.3389/fpls.2016.00338
69. Wang X, Chen L, Wang A, et al (2016b) Quantitative trait loci analysis and genome-wide comparison for silique related traits in Brassica napus. BMC Plant Biol 16:1–15. https://doi.org/10.1186/s12870-016-0759-7
70. Wang Y, Liu C, Li K, et al (2007) Arabidopsis EIN2 modulates stress response through abscisic acid response pathway. Plant Mol Biol 64:633–644. https://doi.org/10.1007/s11103-007-9182-7
71. Wang Y, Zhang WZ, Song LF, et al (2008) Transcriptome analyses show changes in gene expression to accompany pollen germination and tube growth in arabidopsis. Plant Physiol 148:1201–1211. https://doi.org/10.1104/pp.108.126375
72. Wassan GM, Khanzada H, Zhou Q, et al (2021) Identification of genetic variation for salt tolerance in Brassica napus using genome-wide association mapping. Mol Genet Genomics 296:391–408. https://doi.org/10.1007/s00438-020-01749-8

73. Wei D, Cui Y, He Y, et al (2017) A genome-wide survey with different rapeseed ecotypes uncovers footprints of domestication and breeding. J Exp Bot 68:4791–4801. https://doi.org/10.1093/jxb/erx311
74. Wei D, Mei J, Fu Y, et al (2014) Quantitative trait loci analyses for resistance to Sclerotinia sclerotiorum and flowering time in Brassica napus. Mol Breed 34:1797–1804. https://doi.org/10.1007/s11032-014-0139-7
75. Wei Y (2016) Genome-wide association mapping of salt tolerance trait in the germination period in Brassica napus L. Huazhong agricultural university
76. Wu D, Liang Z, Yan T, et al (2019) Whole-Genome Resequencing of a Worldwide Collection of Rapeseed Accessions Reveals the Genetic Basis of Ecotype Divergence. Mol Plant 12:30–43. https://doi.org/10.1016/j.molp.2018.11.007
77. Xu J, Qian X, Wang X, et al (2010) Construction of an integrated genetic linkage map for the A genome of Brassica napus using SSR markers derived from sequenced BACs in B. rapa. BMC Genomics 11:594. https://doi.org/10.1186/1471-2164-11-594
78. Xu L, Hu K, Zhang Z, et al (2015) Genome-wide association study reveals the genetic architecture of flowering time in rapeseed (Brassica napus L.). DNA Res 23:43–52. https://doi.org/10.1093/dnares/dsv035
79. Xu Y, Zhang B, Ma N, et al (2021) Quantitative Trait Locus Mapping and Identification of Candidate Genes Controlling Flowering Time in Brassica napus L. Front Plant Sci 11:. https://doi.org/10.3389/fpls.2020.626205
80. Yang S, Chen S, Zhang K, et al (2018) A high-density genetic map of an allohexaploid brassica doubled haploid population reveals quantitative trait loci for pollen viability and fertility. Front Plant Sci 9:1–18. https://doi.org/10.3389/fpls.2018.01161
81. Ye J, Yang Y, Chen B, et al (2017) An integrated analysis of QTL mapping and RNA sequencing provides further insights and promising candidates for pod number variation in rapeseed (Brassica napus L.). BMC Genomics 18:1–14. https://doi.org/10.1186/s12864-016-3402-y
82. Yeo A (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. J Exp Bot 49:915–929. https://doi.org/10.1093/jxb/49.323.915
83. Yong H-Y, Wang C, Bancroft I, et al (2015) Identification of a gene controlling variation in the salt tolerance of rapeseed (Brassica napus L.). Planta 242:313–326. https://doi.org/10.1007/s00425-015-2310-8
84. Zhang D, Li H, Wang J, et al (2016) High-density genetic mapping identifies new major loci for tolerance to low phosphorus stress in soybean. Front Plant Sci 7:1–11. https://doi.org/10.3389/fpls.2016.00372
85. Zhang G, Zhou J, Peng Y, et al (2022) Genome-Wide Association Studies of Salt Tolerance at Seed Germination and Seedling Stages in Brassica napus. Front Plant Sci 12:1–13. https://doi.org/10.3389/fpls.2021.772708
86. Zhang L, Li S, Chen L, Yang G (2012) Identification and mapping of a major dominant quantitative trait locus controlling seeds per silique as a single Mendelian factor in Brassica napus L. Theor Appl Genet 125:695–705. https://doi.org/10.1007/s00122-012-1861-3
87. Zhang R, Deng W, Yang L, et al (2017) Genome-wide association study of root length and hypocotyl length at germination stage under saline conditions in brassica napus. Sci Agric Sin 50:15–27. https://doi.org/10.3864/j.issn.0578-1752.2017.01.002
88. Zhao W, Wang X, Wang H, et al (2016) Genome-wide identification of QTL for seed yield and yield-related traits and construction of a high-density consensus map for QTL comparison in Brassica napus. Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.00017

89. Zhou F, Liu Y, Liang C, et al (2018) Construction of a high-density genetic linkage map and QTL mapping of oleic acid content and three agronomic traits in sunflower (Helianthus annuu L.) using Specific-locus amplified fragment sequencing (SLAF-seq). Breed Sci 68:596–605. https://doi.org/10.1270/jsbbs.18051
90. Zhu WY, Huang L, Chen L, et al (2016) A high-density genetic linkage map for cucumber (Cucumis sativus L.): Based on specific length amplified fragment (SLAF) sequencing and QTL analysis of fruit traits in cucumber. Front Plant Sci 7:1–11. https://doi.org/10.3389/fpls.2016.00437

Tables

Table 1
Phenotypic variation of the RILs and its parents

Traits	Treatments	Parents		Range	Mean ± SD	Variance	CV	Skewness	Kurtosis
		1423	2205						
STR	NaCl	4	1***	0.00–4.00	2.67 ± 0.82	0.68	0.31	-0.69	0.73
	NaHCO3	4	1***	0.00–5.00	2.38 ± 1.08	1.17	0.46	0.36	0.28
RL (cm)	NaCl	12.01 ± 1.14	17.50 ± 0.50***	9.35–24.17	15.89 ± 3.65	13.33	0.23	0.16	-0.86
	NaHCO3	11.43 ± 0.90	12.83 ± 0.68*	11.44–23.57	16.39 ± 2.61	6.82	0.16	0.78	0.44
SPAD	NaCl	25.54 ± 2.75	35.20 ± 2.85***	20.47–48.43	36.21 ± 5.45	29.71	0.15	-0.50	0.38
	NaHCO3	34.73 ± 1.86	43.50 ± 3.39*	17.55–56.40	38.35 ± 7.69	59.20	0.20	-0.31	-0.02
RFW (g)	NaCl	0.07 ± 0.02	0.26 ± 0.06***	0.05–0.44	0.22 ± 0.09	0.01	0.41	0.42	-0.29
	NaHCO3	0.10 ± 0.02	0.16 ± 0.01**	0.14–0.76	0.37 ± 0.13	0.02	0.36	0.88	0.96
SFW (g)	NaCl	0.56 ± 0.17	2.38 ± 0.43***	0.34–3.85	1.81 ± 0.82	0.66	0.45	0.57	-0.14
SDW (g)	NaCl	0.06 ± 0.02	0.20 ± 0.04***	0.05–0.38	0.18 ± 0.08	0.01	0.43	0.77	0.23
LMI (cm)	2019	76.25 ± 3.20	114.25 ± 7.18***	56.67–122.20	84.20 ± 11.89	141.44	0.14	0.38	1.26
	2020	44.00 ± 6.16	77.75 ± 11.09**	9.60–75.00	49.83 ± 13.13	172.30	0.26	-0.76	0.61
	2021	40.52 ± 6.64	68.38 ± 10.27**	22.60-69.75	46.69 ± 10.63	112.98	0.22	-0.18	-0.74
PH (cm)	2019	115.20 ± 2.59	164.50 ± 12.45***	87.67–176.60	136.77 ± 15.35	235.78	0.11	-0.19	0.85
	2020	150.00 ± 9.41	181.60 ± 19.48*	117.60-192.40	151.67 ± 14.82	219.75	0.10	-0.02	0.01
	2021	129.52 ± 3.54	174.50 ± 2.12***	81.00-174.50	122.05 ± 20.87	435.59	0.17	-0.10	-0.53

STR, salt tolerance rate; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RFW, root fresh weight; SPAD, chlorophyll content; PH, plant height; LMI, length of main inflorescence; SS, seeds per silique; SYP, seed yield per plant; NSP, number of siliques per plant. SD, standard deviation; CV, Coefficient of variation.

Traits	Treatments	Parents		Range	Mean ± SD	Variance	CV	Skewness	Kurtosis
		1423	2205						
SYP (g)	2019	17.65 ± 5.48	34.55 ± 10.90*	2.51–36.62	16.92 ± 7.30	53.30	0.43	0.43	-0.33
	2020	16.31 ± 4.77	34.55 ± 3.03*	3.45–37.38	16.41 ± 7.53	56.77	0.45	0.55	0.02
	2021	9.80 ± 2.06	36.84 ± 5.43***	3.01–36.84	13.45 ± 7.37	54.26	0.55	0.70	0.30
NSP	2020	138.39 ± 24.58	280.70 ± 46.67**	58.86-413.61	222.75 ± 78.24	6120.81	0.35	0.15	-0.34
	2021	135.07 ± 37.28	313.85 ± 17.58**	38.45-324.81	152.41 ± 64.50	4159.99	0.42	0.43	-0.33
SS	2020	22.5 ± 1.86	29.63 ± 3.35*	12.03–30.36	19.39 ± 4.15	17.24	0.21	0.18	-0.44
	2021	16.56 ± 2.30	26.50 ± 5.15**	6.80-30.15	21.15 ± 4.33	18.78	0.20	-0.84	1.72

STR, salt tolerance rate; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RFW, root fresh weight; SPAD, chlorophyll content; PH, plant height; LMI, length of main inflorescence; SS, seeds per silique; SYP, seed yield per plant; NSP, number of siliques per plant. SD, standard deviation; CV, Coefficient of variation.

Table 2
Co-localized QTLs for salt-alkali tolerance traits identified in this study and six yield-related traits reported previously

Present study			**Previous study**			
Chr	QTLs	Position (Mb)	Traits	QTLs	Position (Mb)	References
A09	*qRFWs-A9*	3.76–4.99	SS	*qPN028*	3.50–4.50	Shi et al. 2009
			PH	*qPH071*	3.50–4.50	Shi et al. 2009
			PH	*DHqPH50*	3.50–4.50	Shi et al. 2011
			PH	*qPH072*	3.58–4.58	Shi et al. 2009
			PH	*DHqPH51*	3.58–4.58	Shi et al. 2011
	qRLa-A10-1	11.37–12.29	SS		11.73	Luo et al. 2017
			LMI	*cqLMI-A10-2*	11.51–12.51	Zhao et al. 2016
	qRLa-A10-2	12.07-13.00	LMI		12.49	Hu et al. 2022
			SYP		12.6	Hu et al. 2022
			FT	*cqFT.A10-7*	12.44–12.8	Li et al. 2018a
	uqA10-1	13.00-13.41	LMI	*cqLMI-A10-3*	12.61–13.61	Zhao et al. 2016
			PH		13.09	Hu et al. 2022
			FT	*FTA10*	12.71–13.68	Xu et al. 2021
			FT	*dtf10a.1*	12.33–13.33	Quijada et al. 2006
			FT	*Long-qFT10-3*	1.936–13.359	Long et al. 2007
			FT	*Xu-BnaA10g18010D*	13.167–13.168	Xu et al. 2016
			FT	*Xu-BnaA10g18060D*	13.198–13.202	Xu et al. 2016
			FT	*Xu-BnaA10g18420D*	13.355–13.356	Xu et al. 2016
			FT	*Xu-BnaA10g18430D*	13.359–13.36	Xu et al. 2016
			FT		13.09	Xu et al., 2016
A10	*uqA10-2*	13.47–14.33	SYP	*hsy10.2*	13.60–14.60	Quijada et al. 2006
			PH		13.67–14.62	Hu et al. 2022

Present study		Previous study			
		PH	*hph10b.3*	13.07–14.07	Quijada et al. 2006
		FT	*QTL-A10*	13.34–14.49	Jian et al. 2019
		FT		13.38–15.19	Wang et al. 2016b
		FT	*qFT-A10-3*	13.38–14.38	Wang et al. 2016a
		FT	*hdtf10b.1*	13.17–14.17	Quijada et al. 2006
		FT	*dtf10b.3*	13.17–14.17	Quijada et al. 2006
uqA10-3	15.69–16.05	LMI		15.55	Hu et al. 2022
		FT		15.37–15.94	Li et al. 2018b
		FT	*Long-qFT10-6*	15.346–15.346	Long et al. 2007
		FT	*Wei-qFT11-4-A10*	13.713–16.058	Wei et al. 2014
		FT		15.33	Hu et al. 2022
		FT		15.35	Hu et al. 2022
		FT		15.81	Wei et al. 2017
		FT		15.81	Wei et al. 2017
		FT		15.81	Wei et al. 2017
		FT		15.8	Wu et al. 2019
		FT		15.94	Wu et al. 2019
uqA10-3/4	16.14–16.99	FT	*qFT115*	15.34–16.35	Shi et al. 2009
		FT	*qFT116*	15.34–16.35	Shi et al. 2009
		FT	*qFT117*	15.34–16.35	Shi et al. 2009
		FT	*RF2qFT52*	15.34–16.35	Shi et al. 2011
		FT	*RF2qFT53*	15.34–16.35	Shi et al. 2011

Table 3
Candidate genes for controlling both salt-alkali tolerance and yield

Gene ID in *B.napus*	Stress type	Homologous gene ID in *A. thaliana*	Gene name in *A. thaliana*	Function description in *A. thaliana*	GO annotation
BnaA09g07980D	a	*AT5G66400*	RAB18	Dehydrin protein family	Response to cold and water
BnaA10g14870D	a	*AT5G20630*	GER3	Germin-like protein	Response to cold
BnaA10g19960D	a	*AT5G13330*	RAP2.6L	ERF (ethylene response factor) subfamily B-4 of ERF/AP2 transcription factor family	Response to cold, salt stress; ethylene and salicylic acid
BnaA10g25660D	a	*AT5G04530*	KCS19	3-ketoacyl-CoA synthase family	Response to cold
BnaA09g09160D	a	*AT2G17500*	PILS5	Auxin efflux carrier family protein	Regulation of growth rate; plant organ formation
BnaA09g08360D	a	*AT2G13680*	CALS5	Glucan synthase-like2	Regulation of cell growth; pollen tube growth
BnaA09g08940D	a	*AT2G17040*	NAC036	NAC transcription factor family	Regulation of cell size; inflorescence development; reproductive shoot system development
BnaA09g08950D	s				
BnaA10g25700D	a	*AT5G04480*	BUP	Bursting pollen	Pollen tube development
BnaA10g26380D	a	*AT5G03730*	CTR1	RAF family of serine/threonine protein kinases	Gibberellin biosynthetic process; root and meristem development
BnaA10g16340D	a	*AT5G18620*	CHR17	Chromatin-remodeling complex ATPase	Response to heat; reproductive process
BnaA10g20120D	a	*AT5G13170*	SWEET15/SAG29	SWEET sucrose efflux transporter family proteins	Response to osmotic stress and salicylic acid; seed and fruit development

s" indicates gene was induced under salt (NaCl) treatment; "a" indicates gene was induced under alkaline ($NaHCO_3$) treatment; "a + s" indicates gene was induced under both salt (NaCl) and alkaline ($NaHCO_3$) treatment.

Gene ID in *B.napus*	Stress type	Homologous gene ID in *A. thaliana*	Gene name in *A. thaliana*	Function description in *A. thaliana*	GO annotation
BnaA10g25000D	s + a	*AT3G11020*	DREB2	Dehydration-responsive element-binding protein	Response to heat; pollen development

s" indicates gene was induced under salt (NaCl) treatment; "a" indicates gene was induced under alkaline ($NaHCO_3$) treatment; "a + s" indicates gene was induced under both salt (NaCl) and alkaline ($NaHCO_3$) treatment.

Figures

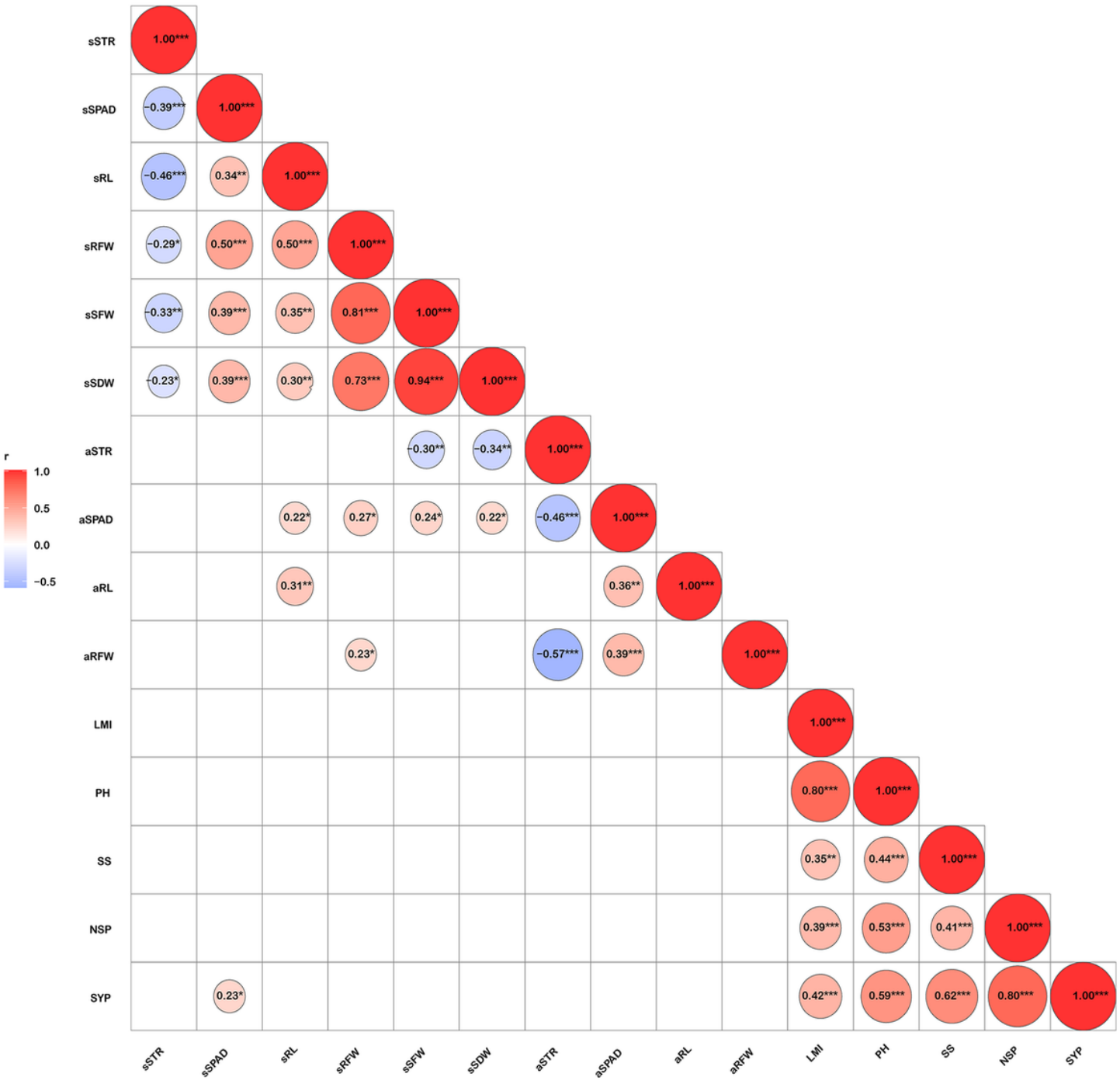

Figure 1

Correlation analysis of all traits for RIL populations. (*), (**), (***) significant levels of 0.5, 0.01 and 0.001 respectively

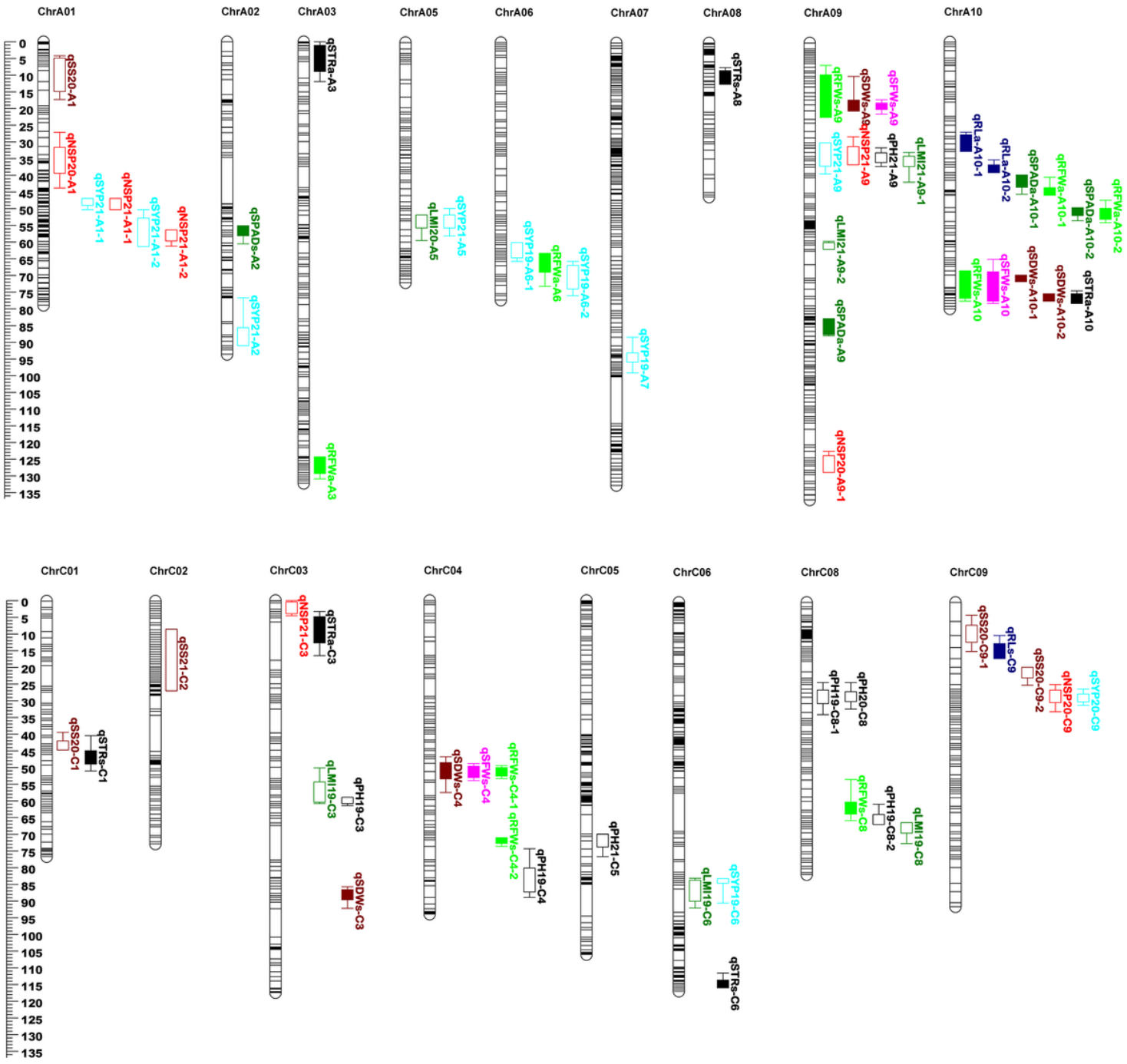

Figure 2

QTLs for all traits based on RIL populations. The SNP marker distributions were depicted on the 19 linkage groups based on their genetic positions in centiMorgans (cM). The QTLs are signed on the right of linkage groups. Different colors represent different traits; QTLs for alkaline and salt tolerance traits are solid, QTLs for yield-related

traits are hollow. The nomenclature of QTLs: “s” indicates salt (NaCl) treatment, “a” indicates alkaline (NaHCO3) treatment

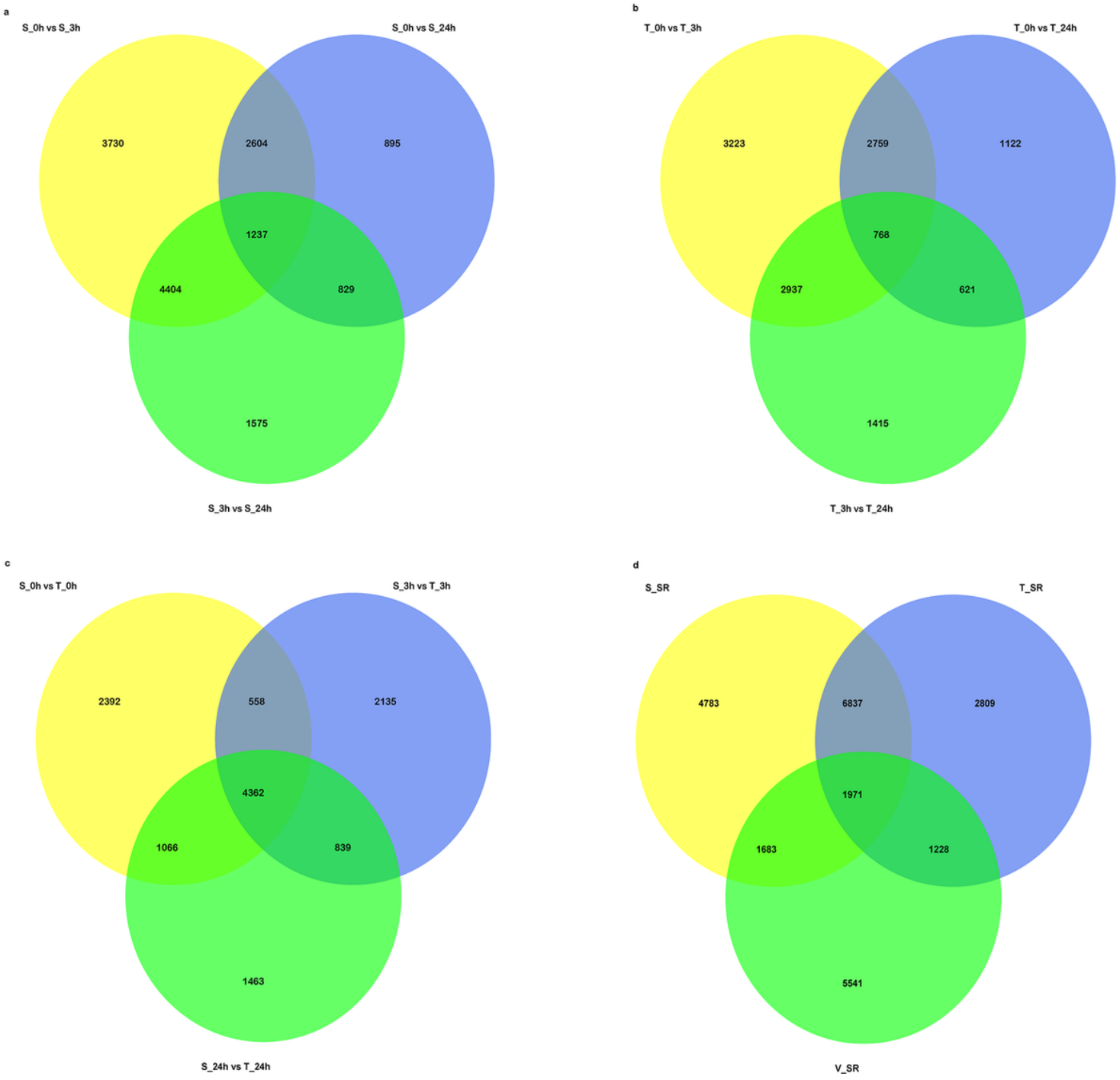

Figure 3

Venn diagram showing the number of differentially expressed genes. (a) Differentially expressed genes in line 1423 under salt stress. (b) Differentially expressed genes in line 2205 under salt stress. (c) Differentially expressed genes between lines 1423 and 2205 at 0h, 3h and 24h. (d) The Venn diagram showing the overlaps of differentially expressed genes of S_SR, T_SR and V_SR. S, sensitive salt variety 1423; T, salt resistant variety 2205;

S_SR, differentially expressed genes in line 1423 response to salt stress; T_SR differentially expressed genes in line 2205 response to salt stress; V_SR, differentially expressed genes between lines 1423 and 2205 at 3h and 24h

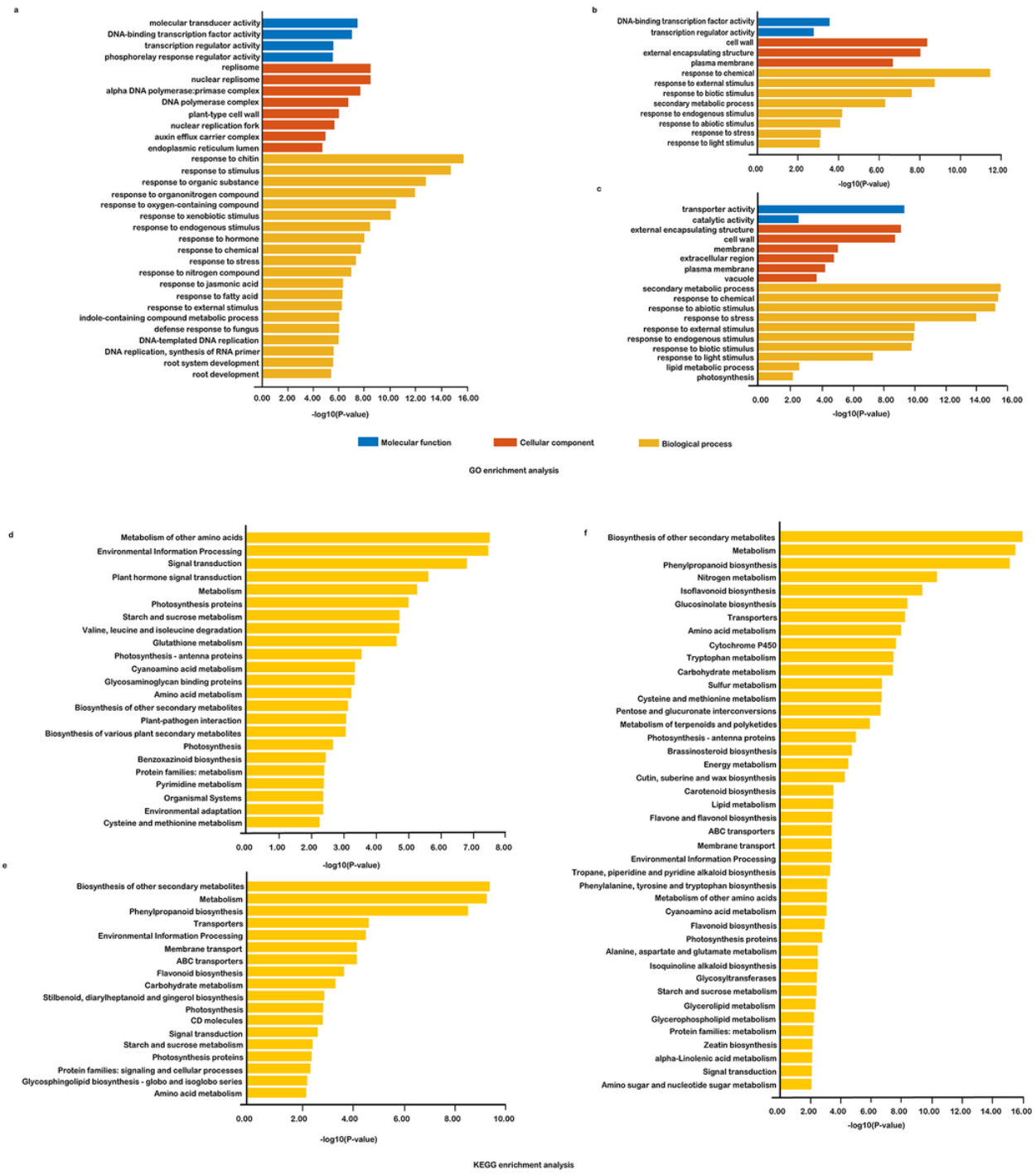

Figure 4

GO and KEGG pathway enrichment analysis for the DEGs. (a, b, c) GO enrichment analysis for the identified 4783 DEGs (a), 2809 DEGs (b) and 4882 DEGs (c). (d, e, f) KEGG pathway enrichment analysis for the identified 4783 DEGs (d), 2809 DEGs (e) and 4882 DEGs (f)

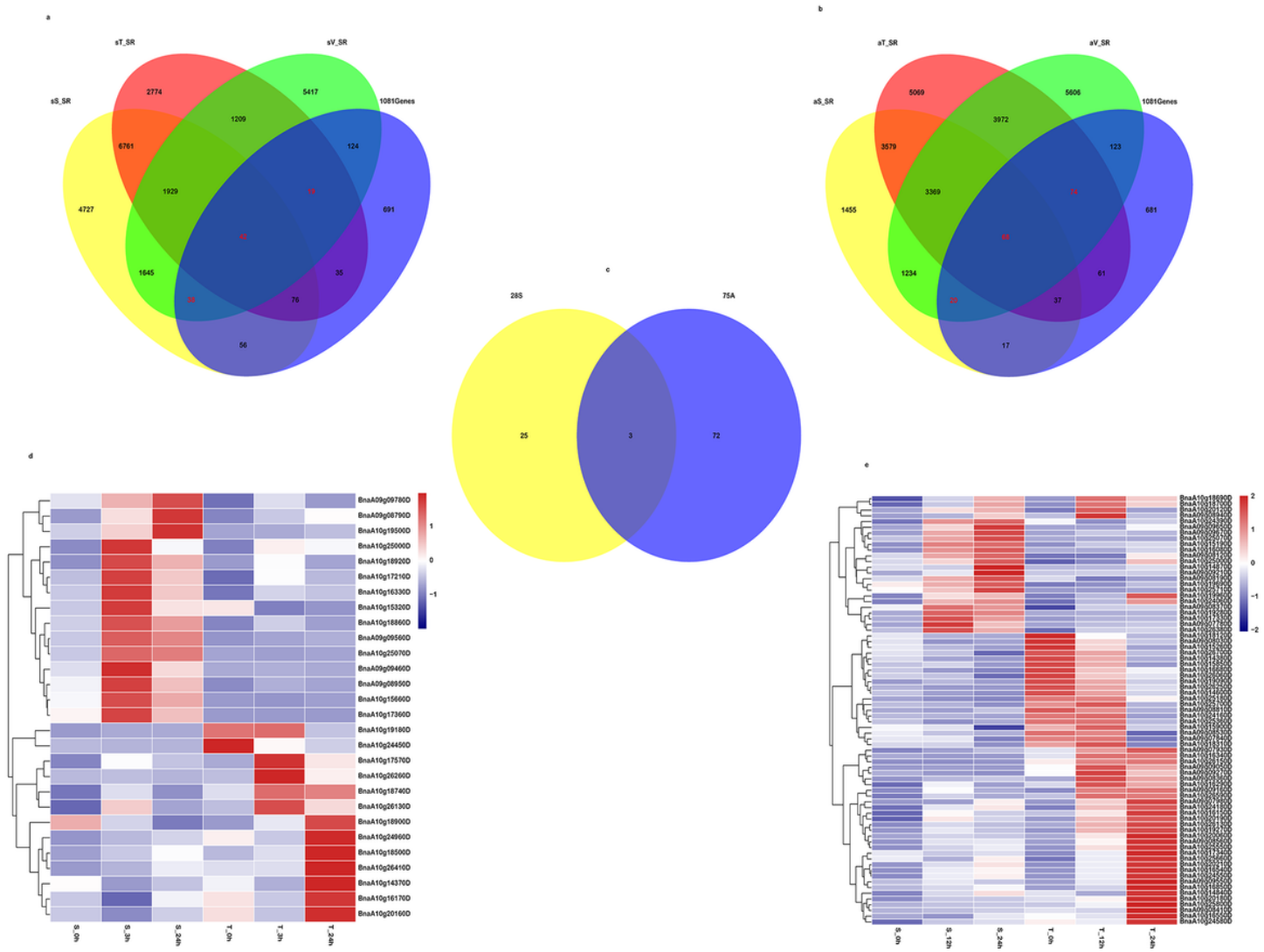

Figure 5

Screening and expression analysis of differentially expressed genes response to salt and alkaline stress within the cQTLs. (a, b) Differentially expressed genes response to salt (a) and alkaline (b) stress within the unique QTLs. (c) The Venn diagram showing the overlaps of differentially expressed genes of s36 and a61. (d, e) Heat maps of relative expression profiles for differentially expressed genes response to salt (d) and alkaline (e) stress within the cQTLs. “s” indicates salt (NaCl) stress, “a” indicates alkaline (NaHCO3) stress

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile1.docx
- Supplementaryfile2.xlsx