

Genome-wide association mapping reveals genetic loci underlying phenotypic variation in early root vigour improvement by osmopriming in *Brassica napus* L.

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Genome-wide association mapping reveals genetic loci underlying phenotypic variation in early root vigour improvement by osmopriming in *Brassica napus* L.

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ABSTRACT

Background

Seed osmopriming is an effective pre-sowing treatment that enhances early seedling vigour and promotes robust root development in *Brassica napus* L., thereby contributing to improved establishment during the early growth stages. However, the genetic basis of variation in osmopriming responsiveness remains poorly understood. This study employed genome-wide association studies (GWAS) using 15,311 SNP markers in 228 spring-type *B. napus* accessions to uncover loci associated with root vigour response to polyethylene glycol (PEG-6000)-mediated osmopriming.

Results

PEG-6000 osmopriming significantly enhanced root vigour traits, increasing average radicle emergence (RE) from 72.7% to 84.4%, root length (RL) by 64.6% (18.2 mm to 29.9 mm), root growth rate (RGR) by 97.8% (1.37 mm/h to 2.71 mm/h), and vigour index (VI) by 88.6% (1355 to 2556) after 72 hours of sowing. Priming reduced trait variability and increased heritability (e.g., VI: 0.86 to 0.97), indicating enhanced genetic control. We also observed substantial phenotypic variation in relative response (e.g., 123.8% CV for RR-VI), which highlights the diversity of osmopriming responsiveness within the population. Our GWAS analysis identified 13 significant SNPs across seven chromosomes (A03, A04, A07, C01, C02, C03, C08), associated with relative responses in four root vigour traits (RR-RE, RR-RL, RR-RGR, and RR-VI). Candidate genes within these loci include regulators of osmotic stress adaptation, root development, cell wall remodelling, redox balance, and water transport. Notably, haplotypes *RE-Hap6* on chromosome A03 and *VI-Hap1* on chromosome C02 increased RR-RE and RR-VI by 44.7% and 164.9%, respectively.

Conclusion

Our study provides the first genomic dissection of osmopriming response in *B. napus*, revealing key loci and candidate genes underpinning priming-enhanced root vigour. These findings offer novel targets for marker-assisted breeding of stress-resilient rapeseed cultivars under climate-challenged environments.

Keywords Osmopriming, Early root vigour, *Brassica napus*, GWAS, Marker-assisted selection

1. BACKGROUND

Seedling establishment represents a critical bottleneck in crop productivity, particularly under challenging environmental conditions such as erratic rainfall, poor soil fertility, and physical soil constraints. In *Brassica napus* L. (oilseed rape), insufficient early root vigour undermines crop establishment, impairs nutrient uptake, and ultimately limits yield, issues exacerbated by soil compaction and nutrient deficiency [1, 2]. Improving early root development thus emerges as a key strategy to enhance stress resilience, optimize resource acquisition, and promote sustainable yield gains, especially in the face of mounting climate variability and the need to reduce agrochemical inputs [3, 4].

Seed osmopriming has emerged as a promising technique to enhance germination uniformity and strengthen early root vigour [5]. It enhances physiological preparedness, enabling improved tolerance and recovery from abiotic stresses such as drought and suboptimal thermal conditions. This pre-sowing treatment also optimizes metabolic efficiency, improving resource utilisation and maximising yield potential under variable environmental conditions [6]. Polyethylene glycol (PEG), a high-molecular-weight osmotic agent, is widely employed due to its inert properties and its ability to regulate water uptake without penetrating seed tissues [7]. By partially hydrating seeds in PEG solutions, pregerminative metabolic processes, including cell cycle activation, reserve mobilization, and hormonal signaling are initiated without radicle protrusion [8]. This initial metabolic induction primes seeds for rapid root growth upon sowing, thereby enhancing seedling establishment under stress conditions [9]. Osmopriming with PEG-6000 has been shown to enhance primary root length and seedling vigour in several crops under field or field-simulated conditions [10]. PEG-induced osmotic stress also modulate phytohormone signaling pathways, particularly those involving abscisic acid (ABA) and gibberellins (GA), which are critical for regulating germination [11]. In addition, osmopriming enhances the antioxidant defence system by stimulating reactive oxygen species (ROS) scavenging enzymes such as superoxide dismutase (SOD),

catalase (CAT), and peroxidase (POD), thereby mitigating oxidative damage during early seedling development [12, 13]. The resulting improvement in ROS homeostasis supports cellular integrity and accelerates radicle elongation under stress. Recent studies have also suggested that osmopriming may influence the expression of genes related to stress perception, hormonal balance, and energy metabolism, indicating a complex and coordinated molecular response [5, 14, 15, 16].

As one of the world's most widely cultivated oilseed crops, *Brassica napus* L., an allotetraploid species (AACC, $2n = 4X = 38$), is a vital source of vegetable oil, biofuels, and animal feed [17]. It exists in three major morphotypes depending on vernalization requirements: spring-type, semi-winter-type, and winter-type, each suited to different agro-climatic regions. Spring-type cultivars are primarily grown in Canada and northern Europe, winter-types are predominant in temperate regions such as western and central Europe, and semi-winter-types are extensively cultivated in China and parts of South Asia [17,18]. However, suboptimal seedling establishment remains a significant constraint, leading to yield losses due to uneven stands and poorly developed root systems [2]. While conventional breeding has focused on enhancing yield, flowering time and oil content, early root vigour has received comparatively little attention, partly due to difficulties in phenotyping and the complex nature of genotype-by-environment interactions [19]. Genome-wide association studies (GWAS) offer a powerful tool to dissect the genetic architecture of root traits, as demonstrated in rice [20] and soybean [21], however, the genetic mechanisms underlying phenotypic variation in osmopriming response remain poorly characterized in *B. napus*, limiting the effective integration of priming-responsive traits into elite cultivars. Identifying loci associated with priming responsiveness in root vigour traits could support marker-assisted selection for genotypes that benefit most from priming treatments, reducing the need for post-sowing interventions.

In this study, we employ GWAS to identify genetic loci associated with PEG-mediated osmopriming responses in spring-type *B. napus* by applying the Bayesian information and linkage disequilibrium iteratively nested keyway (BLINK) GWAS model, focusing on root vigour traits such as radicle emergence (RE, %), primary root length (RL, mm), root growth rate (RGR, mm/h), and vigour index (VI). We hypothesize that osmopriming modulates root vigour through genotype-specific mechanisms and that GWAS will uncover novel allelic variants governing relative response to osmopriming. Our findings aim

to provide foundational insights into the genetic basis of seed priming response, facilitating marker-assisted breeding for high-performance *B. napus* genotypes with improved establishment efficiency under suboptimal conditions.

2. MATERIALS AND METHODS

2.1 Plant material, osmopriming treatment and growth conditions

An association panel comprising 228 genetically diverse spring-type *B. napus* inbred accessions was employed in this study. These accessions, which originated from 16 different geographical regions were sourced from the gene bank materials of Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, IPK (Gatersleben, Germany) and the breeding materials of Deutsche Saatveredelung AG, DSV (Lippstadt, Germany) (Supplementary1, Table S1). Seeds of all inbred accessions underwent two generations of controlled self-pollination. The first generation was conducted in greenhouse conditions during the 2021/2022 season, where two seeds per accession were sown and self-pollinated individually. From one of the resulting self-pollinated plants per accession, eighteen seeds were sown in the field during the 2022/2023 growing season at the Deutsche Saatveredelung AG (DSV) breeding station in Asendorf, Germany, where they were self-pollinated again and harvested to produce the final seed stock.

For the osmopriming treatment, clean seeds were soaked in a PEG-6000 solution (osmotic potential -0.7 MPa) at a 1:2 seed-to-solution ratio for 24 hours at 25°C in darkness. The soaking was conducted in a sealed plastic container placed in a rotating drum while maintaining constant rotation to ensure proper mixing of the PEG-6000 solution with the seeds. This priming protocol was consistently employed in all subsequent analyses. Following priming, the seeds were transferred to a sieve and carefully washed several times for about 20 seconds with clean running tap water to remove the osmotic agent. After washing, the seeds were dried at $\sim 28^{\circ}\text{C}$ for 24 hours until they returned to their original weight, and subsequently either used directly for germination assay or stored under cold, low-humidity conditions for short-term preservation.

For the germination assay, seeds from each accession were placed in glass frame boxes. The boxes were lined with a layer of non-absorbent white foam, topped with two layers of filter paper. A blue filter at the top enhanced contrast, allowing for easier observation of root development. Before placing the seeds, the filter papers were thoroughly pre-soaked in distilled water to ensure consistent moisture levels throughout the germination process. This step helped create a uniform and optimal environment for seed hydration and germination. To ensure a fair comparison and minimize phenotyping bias, unprimed and primed seeds were placed side by side within the same glass frame, and different accessions were arranged together in a single frame. Each glass frame could hold four filter papers and seeds from six different accessions, with each filter paper containing three rows of seeds. Each row consisted of seeds from a single accession, ensuring an organized layout for consistent evaluation. The germination glass frames were securely closed and placed vertically in a dark chamber at a constant temperature of 20°C, where the seeds were allowed to germinate for 72 hours. All experiments were conducted at the Department of Seed Technology, Deutsche Saatveredelung AG Breeding Station, Asendorf, Germany.

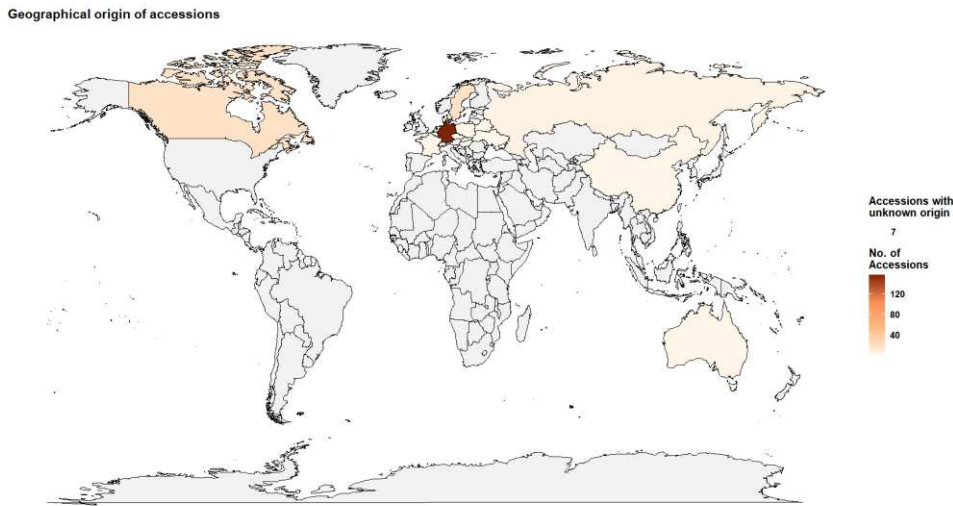


Figure 1. Geographic distribution of the 228 accessions analysed in this study. The provided scale indicates the number of accessions originating from each country.

2.2 Phenotypic trait measurements

Root trait measurements in germinating seedlings were assessed using the CropScore large-scale phenotyping system (<https://www.cropscore.com/home.html>). Seedlings were imaged hourly, and the CropScore software automatically quantified root traits. Each accession was evaluated based on multiple seedlings across two independent experimental runs under highly controlled conditions. A radicle was considered emerged when it reached 5 mm in length, as detected by the CropScore software. The assessed traits included radicle emergence (RE, %), root length (RL, mm), root growth rate (RGR, mm/h), and vigour index (VI), which was calculated using the formula: $VI = RE (\%) \times RL (mm)$ (Abdul-Baki & Anderson, 1973). RE, RL, and RGR were automatically tracked in real time, with data generated and downloaded 72 hours after sowing for further analysis upon experiment completion. The response to osmopriming treatment for each trait was evaluated using the relative response metric, which quantifies the genotypic performance following seed treatment compared to unprimed controls. The relative response, RR (%) was estimated using the following formula:

$$\text{Relative response, RR (\%)} = \frac{\text{Trait}_{\text{primed}} - \text{Trait}_{\text{unprimed}}}{\text{Trait}_{\text{unprimed}}} \times 100$$

2.3 DNA extraction, SNP genotyping and quality control

Genotypic data for 228 *Brassica napus* accessions was generated using the 19K *Brassica* Infinium SNP array (TraitGenetics, SGS INSTITUT FRESENIUS GmbH, Gatersleben, Germany), which comprises 18,566 single nucleotide polymorphisms (SNPs). This array was employed in strict accordance with the manufacturer's protocols. Genomic DNA was isolated from young leaves of eight seedlings per accession using an internally validated extraction protocol that utilized cetyltrimethylammonium bromide (CTAB) in combination with dichloromethane to optimize purity and yield. Subsequent genotyping was performed on the Illumina Infinium XT platform (Illumina Inc., San Diego, CA). It is noteworthy to state that a substantial proportion of the SNP markers incorporated in this array originate from the already established

Brassica 60K Illumina Infinium array [22], thereby ensuring comprehensive genomic coverage and facilitating robust comparative analyses. Following data acquisition, rigorous quality control measures were implemented. SNPs with a minor allele frequency (MAF) < 5% or a call rate below 90% were excluded from the dataset. This filtering process resulted in the retention of 15,311 high-quality SNP markers for downstream genome-wide association study (GWAS) analyses (Supplementary1, Table S2). Of these markers, 8,162 were attributed to the A-subgenome, while 7,149 corresponded to the C-subgenome, enabling detailed subgenomic investigations (Supplementary1, Table S3; Supplementary2, Fig. S1).

2.4 Population structure, principal component analysis & linkage disequilibrium (LD)

Population structure was evaluated using Bayesian clustering of multilocus allele frequencies, implemented in STRUCTURE software, v.2.3.4 [23]. The analysis employed the filtered SNP dataset, testing K values from K = 1 to K = 10 with five independent replicated runs for each K. Each run consisted of a 10,000-iteration burn-in period followed by 20,000 Markov chain Monte Carlo (MCMC) simulations. The optimal number of clusters (K) was identified using STRUCTURE HARVESTER by selecting the value associated with the highest ΔK [24, 25]. Individuals with ancestry coefficients > 50% were assigned to distinct subpopulations. Principal component analysis (PCA) was performed using the Trait Analysis by aSSociation, Evolution and Linkage (TASSEL v5.0) software [26], and the resulting components were visualized with scatterplots generated in R via the ggplot2 package. These principal components were incorporated as covariates in the genome-wide association study (GWAS) to adjust for population stratification. Kinship matrices were also computed in parallel using VanRaden's method with the GAPIT3 package in R. Linkage disequilibrium (LD) between SNP pairs was assessed as r^2 using also TASSEL v5.0. LD block sizes were determined for both A-subgenome and C-subgenome and the whole genome, with the r^2 threshold set to correspond to the observed LD decay. LD patterns around the GWAS peaks were generated and visualised using the LDheatmap package in R.

2.5 Genome-wide association analysis (GWAS), identification of candidate genes and haplotype analysis

Genome-wide association studies (GWAS) were performed using the GAPIT3 package in R, implementing the BLINK (Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway) model to leverage its superior statistical power and reduced false positive rate [27]. Population stratification was accounted for by incorporating the first three principal components (PCs) from principal component analysis (PCA) as fixed covariates. Additionally, a kinship matrix was estimated using the VanRaden method to provide an unbiased measure of genetic relatedness among individuals. Marker-trait associations were identified using a Bonferroni-adjusted significance threshold ($p \leq 3.29 \times 10^{-6}$, equivalent to $-\log_{10}(p) \geq 5.48$) and a false discovery rate (FDR) < 0.05 . Additionally, SNPs with suggestive significance ($p \leq 1 \times 10^{-4}$, equivalent to $-\log_{10}(p) \geq 4$) were evaluated for their potential biological relevance to osmopriming response, as they may represent secondary signals or contribute to polygenic adaptation. Candidate genes associated with significant SNPs were determined via a BLAST mapping strategy. The genomic regions surrounding these SNPs were aligned against the *Brassica napus* ‘Darmor-bzh’ reference genome using the BnIR JBrowser (<https://yanglab.hzau.edu.cn/BnIR/>), and homologous genes in *Arabidopsis thaliana* were identified through BLAST analysis [17, 28]. Based on the average linkage disequilibrium (LD) decay and r^2 values of each subgenome, candidate gene search regions were defined within a 200 kb window for A-subgenome (± 100 kb from peak SNPs) and 400 kb window for C-subgenome (± 200 kb from the peak SNPs). Genes within this window were functionally annotated for gene ontology terms using ShinyGO v0.82 (<https://bioinformatics.sdstate.edu/go/>), based on *Arabidopsis thaliana* gene IDs and the Araport11 genome database [29]. Haplotype analysis was conducted using phased genotypic data derived from GWAS-identified SNPs. Haplotype blocks were defined using the confidence interval method described by Gabriel *et al.* [30], as implemented in Haploview software (v4.2). To ensure robust comparisons, haplotypes represented by fewer than five individuals were excluded from downstream analysis.

2.6 Statistical Analysis

All statistical analyses were conducted using R (version 4.4.1). Descriptive statistics were generated via built-in functions. For the comparison of phenotypic data, we applied either the Student's t-test or Wilcoxon's test, based on whether the data met the assumptions of normality and homogeneity of variances or not. Pearson correlation coefficients among traits were determined with the *pairs.panel* function from the *psych* package. Principal component analysis was performed using the *prcomp* function from the *stats* package. Broad-sense heritability (H^2) was estimated with the formula $H^2 = \sigma^2G/(\sigma^2G + \sigma^2E/r)$, where σ^2G is the genetic variance, σ^2E is the error variance and r is the replication number, implemented via the *lme4* package using the *lmer* function. For the statistical evaluation of haplotype effects, the Kruskal-Wallis test followed by Dunn's post hoc test was applied since the assumptions of normality of residuals and homogeneity of variance were not met.

3. RESULTS

3.1 Phenotypic variation in root vigour traits and relative response to osmopriming

To investigate phenotypic variation in root vigour traits and their response to osmopriming, we evaluated a genetically diverse association panel consisting of 228 spring-type *Brassica napus* inbred accessions collected from a broad range of geographical origins (Fig. 1). All accessions were treated with PEG-6000 solution, and the relative response of each accession was quantified in comparison to its respective unprimed control (Fig. 2a). Phenotypic assessments included measurements of radicle emergence (RE), root length (RL), root growth rate (RGR), and vigour index (VI) under both unprimed and primed conditions to characterize the extent of variation in seedling performance across the panel. We observed that osmopriming significantly enhanced all measured traits, supporting its effectiveness in improving early seedling establishment (Fig. 2b, Table 1). Mean RE increased from 72.65% (unprimed) to 84.38% (primed), representing a 16.1% absolute increase. RL showed a marked improvement of 64.6%, increasing from 18.15 ± 4.54 mm to 29.87 ± 4.61 mm. RGR nearly doubled from 1.37 to 2.71 mm/h (+97.8%), while VI increased from 1355 to 2556 units (+88.6%) (Fig. 2c–f, Table 1). These improvements collectively demonstrate the positive impact of osmopriming on root system development. Beyond mean trait enhancement, osmopriming significantly reduced phenotypic variability across the population. The coefficient of variation (CV) declined for all traits: RE from 25.8% to 16.8%, RL from 25.0% to 15.5%, RGR from 27.5% to 19.9%, and VI from 41.4% to 26.3%, indicating increased uniformity in response under priming. Heritability (H^2) estimates were generally high and increased under priming conditions for most traits, suggesting a stronger genetic contribution to trait expression in primed seedlings. Notably, H^2 for RE increased from 0.85 to 0.99, for RL from 0.78 to 0.93, and for VI from 0.86 to 0.97. RGR showed a slight reduction in heritability (from 0.83 to 0.75), potentially indicating a greater influence of non-additive or environmental factors on this dynamic trait. Crucially, the relative response (RR) metric further revealed substantial phenotypic variation in the osmopriming response itself (Table 1). Mean RR values were +26.6% for RE, +72.1% for RL, +113.7% for RGR, and +124.2% for VI. However, wide RR ranges across genotypes (e.g., RR-VI from –90.1% to +965.1%) indicate strong genotype-specific responses to priming. The large standard deviations and high coefficients of variation in RR values (e.g., 123.8% CV for RR-VI) underscore the diversity of osmopriming responsiveness within the population, pointing to

probable underlying genetic complexity. Together, these results demonstrate that PEG-mediated osmopriming not only improves root vigour traits and stabilizes phenotypic expression but also reveals substantial variation in relative response, highlighting the potential to genetically dissect and harness priming responsiveness for breeding more resilient seedling phenotypes.

267

Table 1: Descriptive statistics values for root vigour traits of unprimed and primed seedlings and their relative responses to osmopriming

Traits	Treatments	Mean	Min.	Max.	SD	CV		Kurtosis	Skewness	H ²
						(%)	Range			
RE (%)	Unprimed	72.65	14.71	100.00	18.71	25.76	85.30	0.26	-0.93	0.85
	Primed	84.38	8.82	100.00	14.15	16.77	91.18	4.85	-1.88	0.99
RL (mm)	Unprimed	18.15	8.96	30.39	4.54	25.03	21.43	-0.18	0.55	0.78
	Primed	29.87	14.20	39.44	4.61	15.45	25.24	0.36	-0.61	0.93
RGR (mm/h)	Unprimed	1.37	0.62	2.43	0.38	27.45	1.80	0.02	0.63	0.83
	Primed	2.71	1.05	3.97	0.54	19.90	2.92	-0.01	-0.27	0.75
VI	Unprimed	1355.06	183.22	2949.17	561.13	41.41	2765.94	-0.18	-0.12	0.86
	Primed	2556.02	141.79	3944.00	671.26	26.26	3802.21	0.33	-0.61	0.97
RR-RE (%)	-	26.56	-89.66	380.04	51.42	193.64	469.70	14.94	3.09	-
RR-RL (%)	-	72.08	-21.15	181.86	39.58	54.92	203.01	-0.32	0.33	-
RR-RGR (%)	-	113.67	-7.21	287.40	53.57	47.13	294.62	-0.11	0.36	-
RR-VI (%)	-	124.23	-90.14	965.07	123.84	99.69	1055.21	12.33	2.71	-

270

Min. = Minimum; Max. = Maximum; SD = Standard deviation; CV = Coefficient of variance; H² = Broadsense heritability

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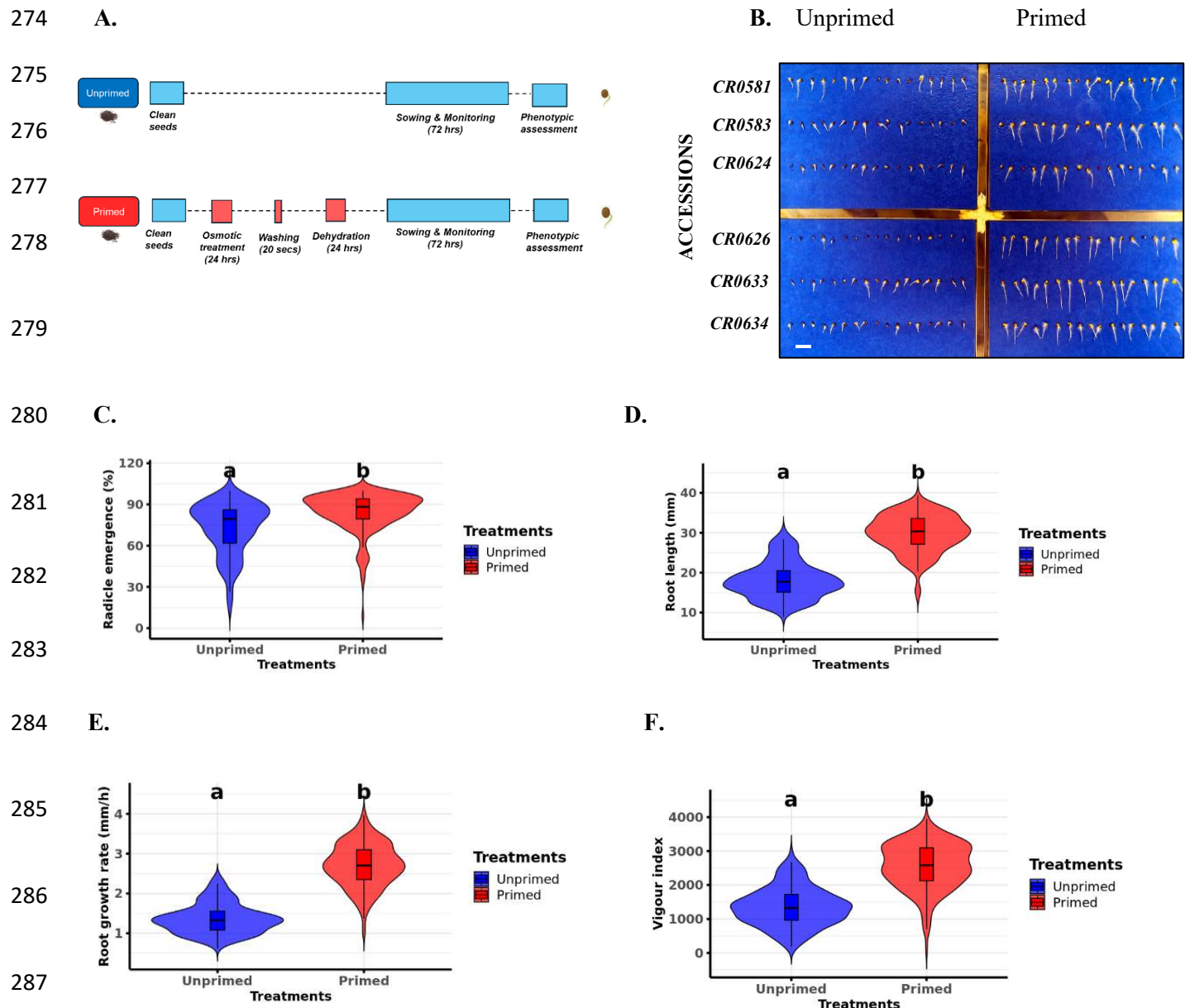


Figure 2. Effects of seed osmopriming on radicle development and root vigour across 228 accessions. (A.) Schematic diagram of unprimed and primed treatments; (B.) representative radicle images from six accessions under both treatments after 48 hours of sowing (scale bar = 25mm); (C.) radicle emergence (RE); (D.) root length (RL); (E.) root growth rate (RGR); and (F.) vigour index (VI), each shown as violin plots comparing unprimed and primed seeds across all accessions. Distinct letters displayed above the violin plots represent statistically significant differences ($P < 0.05$) between groups, determined by pairwise comparisons.

3.2 Pearson's correlation and principal component analysis of relative response to osmopriming.

To examine the relationships among root vigour traits in response to osmopriming, we performed Pearson's correlation analysis on the relative responses of all four root vigour traits. The analysis revealed highly significant associations ($p < 0.001$) among the accessed root vigour traits (Fig. 3a). Notably, a strong positive correlation was observed in the osmopriming response between root length (RR-RL) and relative growth rate (RR-RGR) ($r = 0.94$, $p < 0.001$), suggesting that osmopriming-induced enhancements in root elongation are closely linked to overall seedling growth efficiency. Similarly, a robust correlation was detected between root emergence (RR-RE) and vigour index (RR-VI) ($r = 0.91$, $p < 0.001$), reinforcing the role of early root system establishment in determining seedling vigour. Moderately positive correlations were found between RR-RL and RR-VI ($r = 0.65$, $p < 0.001$) and between RR-RGR and RR-VI ($r = 0.61$, $p < 0.001$), indicating that while these traits are interrelated, additional independent factors may also contribute to vigour. In contrast, the weakest, yet still statistically significant, correlations were observed between RR-RE and RR-RL ($r = 0.32$, $p < 0.001$) and between RR-RE and RR-RGR ($r = 0.29$, $p < 0.001$), implying that early root emergence is less directly influenced by subsequent root elongation and growth rate under priming conditions. Collectively, these findings demonstrate that osmopriming differentially modulates key seedling traits, and that the observed correlations underscore the potential for targeted priming strategies to optimize multiple aspects of seedling performance, with implications for improving crop establishment under suboptimal conditions.

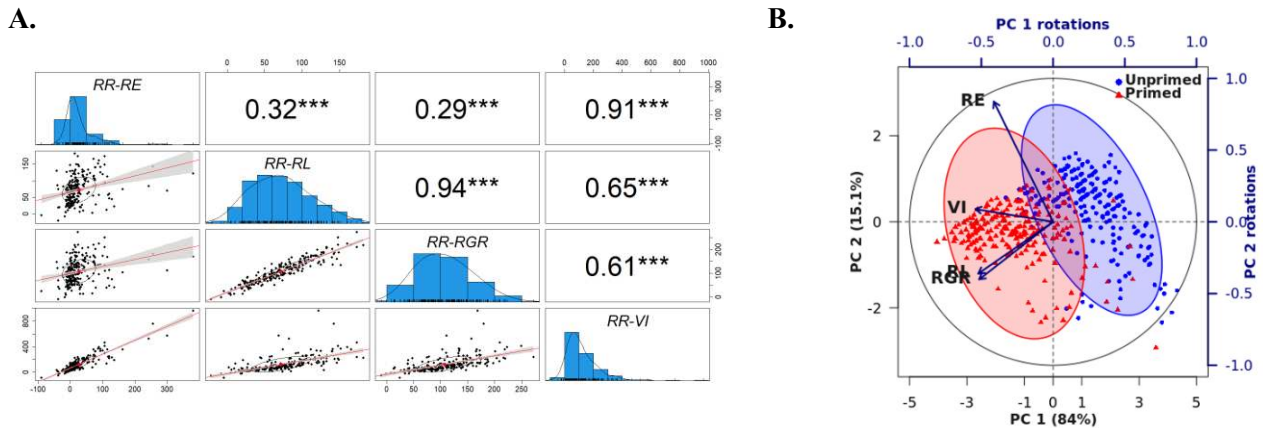


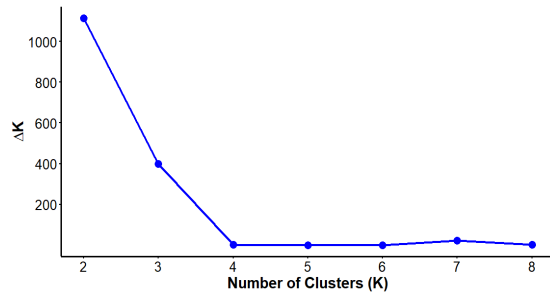
Figure 3. Phenotypic correlation and principal component analysis of relative response to osmopriming. (A.) Correlation analysis and frequency distributions of osmopriming response among four root vigour traits. The numbers in the boxes indicate the correlation coefficients (R-values) between the traits. The histograms represent the trend of distribution of each trait (B.) PCA biplot showing primed vs. unprimed seedlings based on four root vigour traits. RR-RE = relative response for radicle emergence; RR-RL = relative response for root length; RR-RGR = relative response for root growth rate; RR-VI = relative response for vigour index. *** represents significance at the $P < 0.001$ level

Principal component analysis (PCA) of root vigour traits revealed a clear and statistically robust separation between unprimed and primed seedlings, with the first two principal components explaining 99.1% of the total phenotypic variation (PC1 = 84.0% and PC2 = 15.1%) (Fig. 3b). The pronounced clustering of treatments along PC1, which accounted for the majority of variance, underscores osmopriming as the primary determinant of early root developmental plasticity. Strikingly, all measured traits exhibited strong positive loadings toward the primed cohort, demonstrating that PEG-induced priming uniformly enhanced root system vigour. The trajectory and magnitude of trait vectors further delineated hierarchical responses, with RL and RGR showing the strongest association with PC1, suggesting their pivotal role in priming-mediated phenotypic differentiation. These findings provide mechanistic insight into how osmopriming reshapes the multivariate phenotypic space, offering a quantitative framework for optimizing seed treatments to improve seedling establishment.

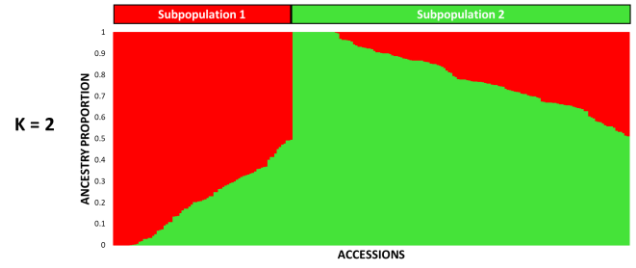
3.3 Population structure and linkage disequilibrium of the association panel

We evaluated the number of clusters (K) against the corresponding ΔK values to determine the optimal number of genetic subpopulations. While both $K = 2$ and $K = 3$ showed notable ΔK values, the highest peak was observed at $K = 2$, indicating that two subpopulations best represent the underlying genetic structure (Fig. 4a). STRUCTURE analysis classified the 228 *Brassica napus* accessions into two distinct groups: Subpopulation 1 and Subpopulation 2 based on the optimal clustering at $K = 2$ (Fig. 4b), however, results for $K = 3$ were also visualized to explore potential substructure (Supplementary2, Fig. S2). Subpopulation 1 comprised 79 genotypes, including 3 accessions from the IPK gene bank and 76 from DSV breeding lines. In contrast, subpopulation 2 consisted of 149 genotypes, of which 84 originated from the IPK gene bank and 65 from DSV breeding lines (Supplementary1, Table S4). Principal component analysis (PCA) based on genome-wide SNP markers revealed that the first and second components explained 10.04% and 3.23% of the total genetic variation, respectively, effectively capturing the genetic structure among the accessions (Fig. 4c; Supplementary2, Fig. S3). Additionally, nearest neighbour clustering using imputed SNP markers provided a clear separation between subpopulation 1 and subpopulation 2, reinforcing the population structure revealed by the previous analyses (Fig. 4d). Kinship-based hierarchical clustering further supported this subdivision, as visualized by a heatmap of the pairwise relatedness matrix (Supplementary2, Fig. S4). To assess patterns of linkage disequilibrium (LD), the squared correlation coefficient (r^2) was plotted against physical distance (Mb). As expected, LD decayed with increasing genetic distance across whole genome, consistent with recombination breaking down haplotype associations over time (Fig. 4e). Notably, the A- and C-subgenomes exhibited distinct LD profiles. The A-subgenome (8,162 SNPs) displayed a rapid LD decay, with a half-life of 0.08 Mb (~80 kb), indicating relatively weak haplotype persistence (Supplementary2, Fig. S5). In contrast, the C-subgenome (7,149 SNPs) exhibited stronger LD, with a half-life of 0.23 Mb (~230 kb), nearly three times slower decay than the A-subgenome (Supplementary2, Fig. S6). Genome-wide LD decay had an intermediate half-life of 0.13 Mb (~130 kb), reflecting the composite structure of *B. napus* subgenomes (Fig. 4e).

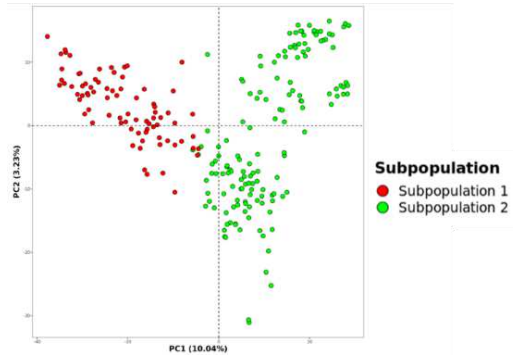
A.



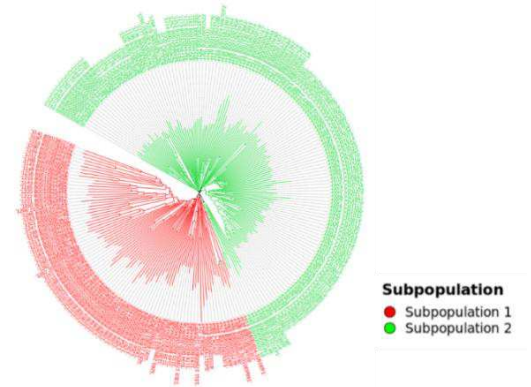
B.



C.



D.



E.

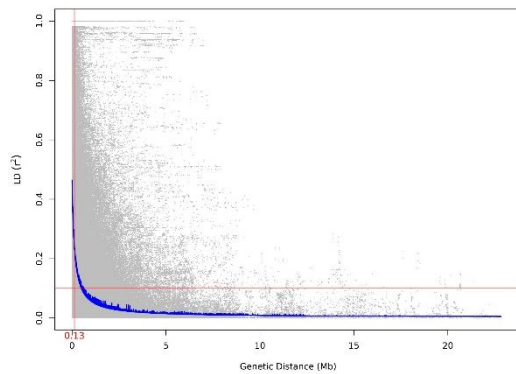


Figure 4. Population structure and linkage disequilibrium analysis. (A.) Delta K plot showing the number of subpopulations (B.) Subpopulations present in the association panel at $K=2$. (C.) 2D PCA plot elaborating subpopulations as identified by the PCA analysis (D.) NJ tree of accessions in the association panel (E.) Whole genome LD decay plot. The horizontal red line marks the genome-wide half-decay r^2 value ($r^2 = 0.1$). The blue curve illustrates the LD decay pattern modelled using a smoothing spline regression. The vertical red line at approximately 0.13 Mb (130 kb) shows the position where the decay curve meets the half-decay threshold at $r^2 = 0.2$.

3.4 Genome-wide marker-trait associations for response to osmopriming

We conducted genome-wide association studies (GWAS) to identify genomic regions significantly associated with the osmopriming response, using relative response metrics derived from four root vigour traits of unprimed and osmoprimed seedlings (i.e., RR-RE, RR-RL, RR-RGR & RR-VI). Each metric was analysed independently to capture trait-specific genetic associations linked to osmopriming. The BLINK fixed model was employed for its enhanced power in detecting associations while controlling for population structure [31]. Our analysis detected 13 SNPs associated with the osmopriming response across 7 chromosomes. 4 of these SNPs exceeded the genome-wide significance threshold ($-\log_{10} p \geq 5.48$), and 9 additional SNPs reached suggestive significance ($-\log_{10} p \geq 4$), mapping to intronic, exonic, and intergenic regions (Table 2). These associations revealed both trait-specific and pleiotropic genetic control of osmopriming response across the root vigour traits. For RR-RE, seven SNPs were identified on chromosomes A03, C02, and C03. Of these, three were located in close proximity on chromosome C02, therefore, we retained only the lead SNP from this cluster for downstream analysis to avoid redundancy. Three SNPs associated with RR-RL were localised to chromosomes A07, C01, and C08, respectively. A single significant SNP for RR-RGR was found on chromosome A04, while two SNPs linked to RR-VI were detected on chromosomes C02 and C03 (Fig. 5). The SNP *Bn-scaff_20942_1-p252525* on chromosome C02 and SNP *Bn-scaff_16002_1-p341408* on chromosome C03 exhibited pleiotropic genetic control, exhibiting significant associations in both RR-RE and RR-VI, suggesting a shared genetic mechanism influencing multiple priming-related traits (Fig. 5, Table 2). A total of 269 candidate genes resides within the LD intervals of the significant SNPs, implicating these regions in the genetic architecture of osmopriming response (Supplementary1, Table S5). Among these, 23 genes were prioritised as high-confidence candidates based on their physical linkage to lead SNPs within conserved LD blocks and functional relevance to osmotic stress adaptation, redox homeostasis, root development, and hormone-mediated drought responses. These candidates included homologs of *Arabidopsis* genes with established roles in stress-responsive root plasticity (Table 3).

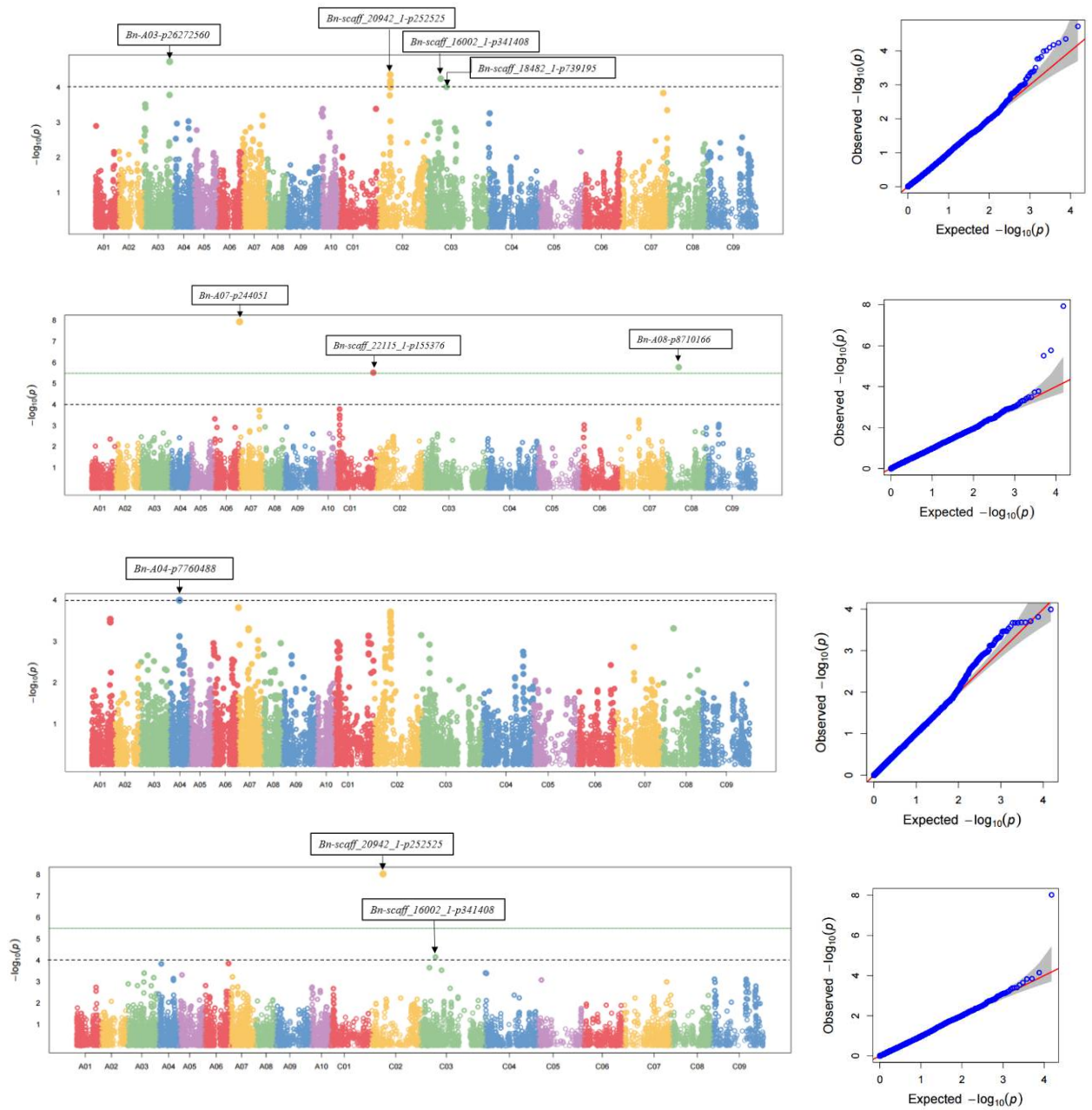


Figure 5. Genome-wide association studies for relative response to osmopriming. Manhattan and quantile–quantile plots for (A.) RR-RE (B.) RR-RL (C.) RR-RGR (D.) RR-VI. The green horizontal line represents the Bonferroni-adjusted significance threshold ($-\log_{10}P = 5.48$). The black dashed line represents the suggestive significance threshold ($-\log_{10}P = 4$).

449

450

451 **Table 2:** Marker trait associations detected for response to osmopriming in four root related traits using
452 the BLINK GWAS model

SNP_id	Traits	Alleles	Chromosomes	Position (bp)	MAF	Genomic location	Number of candidate genes within LD window	P- value	Effect size	PVE (%)
Bn-A03- p26272560	RR- RE	A/G	A03	24660653	0.37	BnaA03g47930D (Exon 12)	40	1.88E- 05	18.67	-
Bn-A04- p7760488	RR- RGR	T/C	A04	9078490	0.4	Intergenic	30	1.02E- 04	15.01	-
Bn-A07- p244051	RR- RL	T/C	A07	206928	0.1	BnaA07g00200D (Intron 8)	26	1.17E- 08	21.42	18.82
Bn- scaff_22115_1- p155376	RR- RL	A/G	C01	35743833	0.1	BnaC01g36460D (Exon 4)	34	1.69E- 06	17.94	25.66
Bn- scaff_20942_1- p252525	RR- RE RR- VI	A/G	C02	11015784	0.34	Intergenic	30	4.43E- 05 9.61E- 09	17.94 48.69	- 15.43
Bn- scaff_16002_1- p341408	RR- RE RR- VI	A/G	C03	13967249	0.07	BnaC03g24850D (Exon 5)	36	5.77E- 05 7.16E- 05	27.74 52.56	- -
Bn- scaff_18482_1- p739195	RR- RE	T/C	C03	19626762	0.08	BnaC03g31900D (Intron 1)	60	9.84E- 05	26.91	-
Bn-A08- p8710166	RR- RL	T/C	C08	12464832	0.1	BnaC08g08380D (Intron 3)	13	3.08E- 06	19.03	12.21

453

454 MAF = Minor allele frequency; bp = base pair; PVE = Phenotypic variance explained

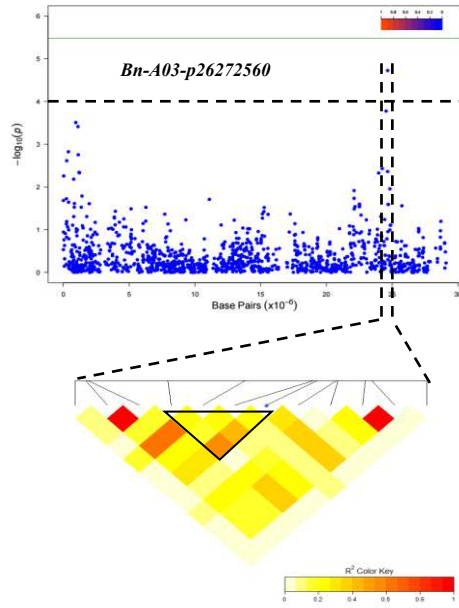
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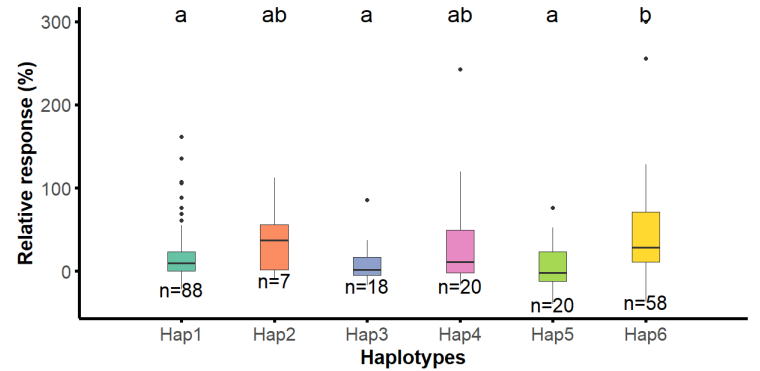
3.5 Identification of favourable haplotypes associated with osmopriming response

A key genomic region on chromosome A03, defined by the lead SNP *Bn-A03-p26272560*, was found to be in strong linkage disequilibrium (LD) with three adjacent SNPs, collectively forming a haplotype block spanning approximately 121 kb region. Haplotype analysis of this block identified six predominant haplotypes within the association panel, namely: *RE-Hap1* (TGGA), *RE-Hap2* (-GGA), *RE-Hap3* (TATA), *RE-Hap4* (TATG), *RE-Hap5* (TAGA), and *RE-Hap6* (CATG), each exhibiting distinct responses to osmopriming. Among these, *RE-Hap6* (CATG) demonstrated the most substantial priming-induced enhancement, with a mean increase of 44.70%, significantly surpassing *RE-Hap1* (TGGA, 18.96%), *RE-Hap3* (TATA, 9.21%), and *RE-Hap5* (TAGA, 5.88%) (Fig. 6), implicating it as a favourable haplotype for enhanced root performance upon priming. Similarly, on chromosome C02, the significant SNP *Bn-scaff_20942_1-p252525* was in strong LD with four nearby SNPs, forming a second haplotype block spanning approximately 123 kb region. We identified four major haplotypes in this region: *VI-Hap1* (GTAA), *VI-Hap2* (GCTAA), *VI-Hap3* (ACTGG), and *VI-Hap4* (ACCGG), which also exhibited differential responses to osmopriming. Strikingly, *VI-Hap1* (GTAA) displayed the most pronounced response, with an average priming-induced increase of 164.94%, considerably higher than *VI-Hap3* (96.79%) and *VI-Hap4* (81.53%) (Fig. 7). These findings highlight both *RE-Hap6* on A03 and *VI-Hap1* on C02 as favourable haplotypes associated with superior osmopriming responsiveness, representing valuable molecular targets for marker-assisted selection aimed at enhancing drought resilience in *Brassica napus* breeding programs.

A.



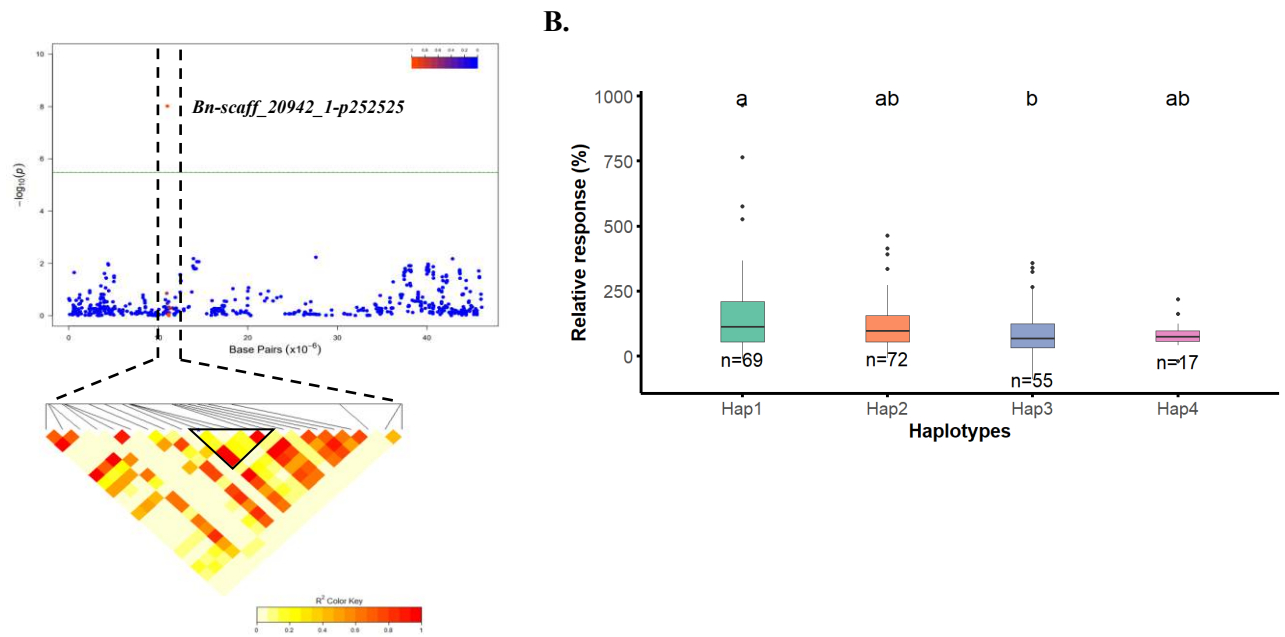
B.



C.

Position (bp)	24539574	24602386	24657477	24660653	Frequency	Average relative response (%)
Haplotypes						
Hap1	T	G	G	A	88	18.96
Hap2	-	G	G	A	7	36.28
Hap3	T	A	T	A	18	9.21
Hap4	T	A	T	G	20	36.36
Hap5	T	A	G	A	20	5.88
Hap6	C	A	T	G	58	44.70

Figure 6. GWAS results and haplotype analysis for *SNP Bn-A03-p26272560* associated with RR-RE. (A.) A Manhattan plot illustrating RR-RE is shown. The green and black horizontal lines mark the Bonferroni & suggestive significance threshold, respectively. Linkage disequilibrium (LD) among SNPs on chromosome A03 is displayed below, based on pairwise r^2 values, which are color-coded according to the provided scale. (B.) Phenotypic differences in osmopriming response (RR-RE) between the identified haplotypes (C.) Haplotype analysis of *SNP Bn-A03-p26272560* (in orange) and three other linked SNPs. Six haplotypes (Hap1, Hap2, Hap3, Hap4, Hap5 and Hap6) were identified in 211 accessions.



Position (bp)	11015784	11019856	11021936	11131769	11139601	Frequency	Average relative response (%)
Haplotypes							
Hap1	G	T	T	A	A	69	164.94
Hap2	G	C	T	A	A	72	121.26
Hap3	A	C	T	G	G	55	96.79
Hap4	A	C	C	G	G	17	81.53

Figure 7. GWAS results and haplotype analysis for *SNP Bn-scaff_20942_1-p252525* associated with RR-RE & RR-VI. (A.) A Manhattan plot illustrating RR-VI is shown. The green and black horizontal lines mark the Bonferroni & suggestive significance threshold, respectively. Linkage disequilibrium (LD) among SNPs on chromosome C02 is displayed below based on pairwise r^2 values, which are color-coded according to the provided scale. (B.) Phenotypic differences in osmopriming response (RR-VI) between the identified haplotypes (C.) Haplotype analysis of *SNP Bn-scaff_20942_1-p252525* (in orange) and four other linked SNPs. Four haplotypes (Hap1, Hap2, Hap3 and Hap4) were identified in 213 accessions.

4. DISCUSSION

Root vigour during early seedling establishment is a cornerstone of drought resilience, as rapid root elongation and lateral branching enable seedlings to access soil moisture and nutrients before abiotic stressors intensify [32, 33]. In *Brassica napus* L., weak root systems exacerbate vulnerability to soil compaction and water deficits, leading to uneven stands and yield losses [34]. Seed osmopriming with PEG-6000 mitigates these challenges by synchronizing germination and amplifying root growth kinetics, effectively "pre-arming" seedlings to withstand suboptimal conditions [5, 35].

Our study provides the first genome-wide association of seed osmopriming responses in *B. napus*, integrating phenotypic and genomic insights to elucidate mechanisms underlying priming-enhanced root vigour. Across the 228 accessions analysed, we observed substantial variation in osmopriming responses for radicle emergence (RR-RE), root length (RR-RL), root growth rate (RR-RGR), and vigour index (RR-VI), with some genotypes showing minimal gains while others exhibited dramatic improvements (Fig. 2, Supplementary1, Table S1). Furthermore, osmopriming significantly enhanced RE, RL, RGR and VI, with mean increases of 16.1%, 64.6%, 97.8%, and 88.6%, respectively. These gains reflect improved early root system development, which is critical for rapid seedling establishment and are consistent with previous findings that osmopriming stimulates metabolic reactivation and accelerates root system growth during early seedling development [35, 36]. The reduction in coefficients of variation and the increase in broad-sense heritability for primed traits indicate that osmopriming not only stabilizes phenotypic expression but also magnifies underlying genetic signals, thereby offering breeders a clearer pathway to select for genotypes with superior priming responsiveness [15, 37].

Population structure analysis of 228 spring-type *Brassica napus* L. accessions revealed two distinct genetic subpopulations, supported by STRUCTURE analysis, principal component analysis (PCA), and nearest neighbour clustering. The highest ΔK at $K = 2$ highlighted this bipartite subdivision, underscoring the robustness of the SNP dataset and the genetic differentiation within the panel. Subpopulation 1 predominantly comprised mostly DSV breeding lines, suggesting a narrower genetic base likely shaped by intense selection and breeding, while Subpopulation 2 was characterized by the presence of both IPK gene bank accessions and DSV breeding materials, representing broader genetic diversity [23, 38].

Linkage disequilibrium (LD) decay patterns varied markedly between the A- and C-subgenomes. The A-subgenome displayed rapid LD decay (~80 kb half-life), indicative of higher recombination rates or larger effective population sizes, whereas the C-subgenome exhibited slower LD decay (~230 kb), possibly reflecting stronger selective sweeps, reduced recombination, or historical bottlenecks [39]. The genome-wide LD decay (~130 kb) reflects the composite structure of *B. napus* allopolyploid genome [17]. These differences have practical implications for genome-wide association studies (GWAS) and marker-assisted selection; the faster LD decay in the A-subgenome requires higher marker density for effective QTL detection, while the slower decay in the C-subgenome allows targeting broader haplotype blocks [40]. Overall, the elucidation of population structure and LD dynamics provides a framework to improve breeding strategies and germplasm utilization by exploiting the unique genetic potentials of each subgenome. Such insights contribute to more precise genetic mapping and accelerated crop improvement in *B. napus*.

We also identified key candidate genes underlying significant SNPs associated with osmopriming responsiveness, revealing key regulatory components in root development, hormonal signaling, redox homeostasis, osmotic stress adaptation, and cell wall dynamics (Fig. 8, Table 3).

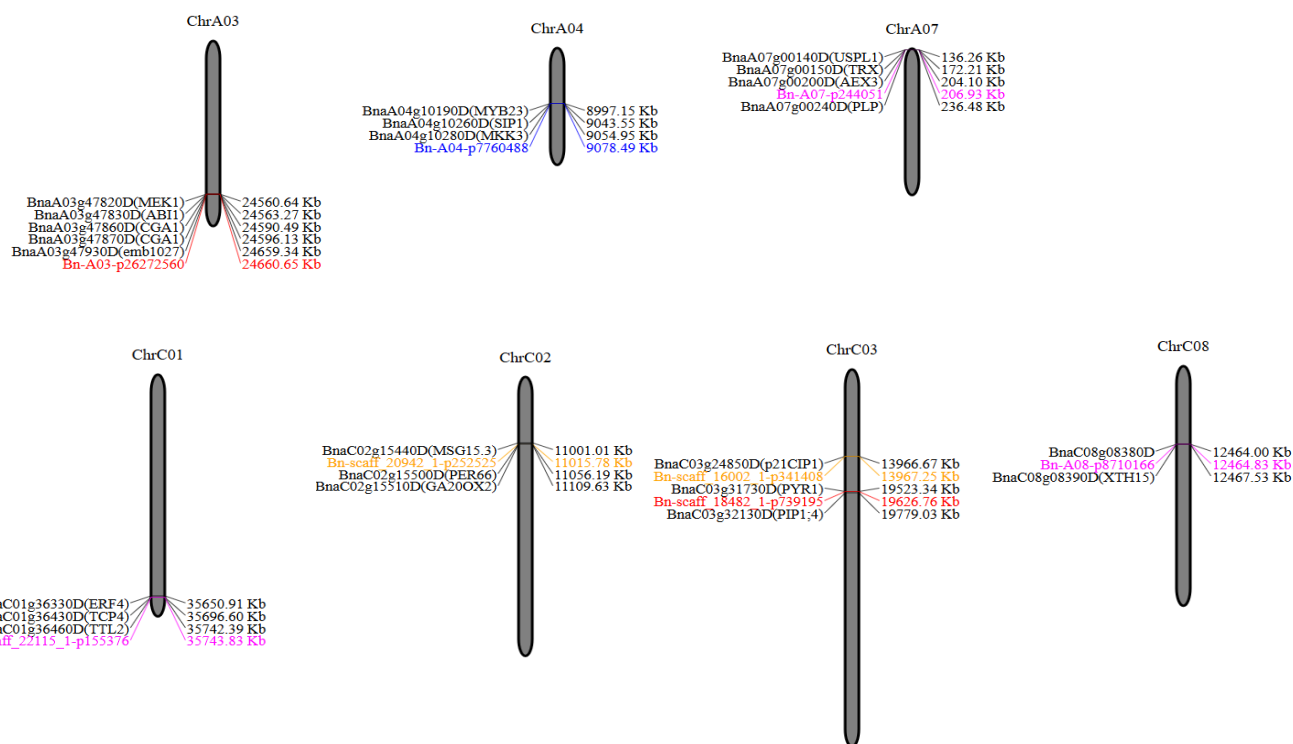


Figure 8. The distribution pattern of key candidate genes and their corresponding SNPs associated with osmopriming response (RR-RE = red, RR-RL = magenta, RR-RGR = blue, RR-RE & RR-VI = orange). Abbreviations of the orthologous genes in *Arabidopsis thaliana* are indicated in brackets following the candidate gene names. Numbers represent the relative distances in the genome in Kb

Among the significant associations identified in our GWAS, the SNP *Bn-A03-p26272560* was located near the gene ABI1 (BnaA03g47830D), a member of the clade A PP2C phosphatase family known to function as a negative regulator of abscisic acid (ABA) signaling [41]. This association suggests a genetic basis for the regulation of seed germination traits through ABA-mediated pathways. ABI1 is a well-characterized modulator of ABA responses, particularly during early seedling development, where it contributes to the suppression of premature germination and the activation of abiotic stress responses [41, 42]. Recent findings have demonstrated the functional significance of this pathway in seed priming, revealing that the chemical inhibition of ABA signaling via a receptor antagonist markedly enhances seed germination under high-temperature stress and significantly boosts the effectiveness of osmopriming in lettuce [43]. Furthermore, emb1027 (BnaA03g47930D) which contains the SNP *Bn-A03-p26272560* in its exonic region and encodes an arginyl-tRNA synthetase, suggests a potential role in metabolic priming through enhanced mitochondrial translation, aligning with reports linking ATP biosynthesis to successful seed imbibition [8, 44]. In proximity, CGA1 (BnaA03g47860D/BnaA03g47870D), a cytokinin-responsive transcription factor, may enhance abiotic stress adaptation and chloroplast development post-priming [45]. The SNP *Bn-scaff_18482_1-p739195*, associated with RR-RE, is linked to PIP1;4 (BnaC03g32130D), encoding the plasma membrane intrinsic protein, which plays a critical role in water transport and cell expansion during seedling development. This is consistent with a previous study highlighting PIP1;4's involvement in facilitating efficient water uptake, a key process enhanced by osmopriming to improve seed germination and early growth under stress conditions [12]. Similarly, *Bn-A04-p7760488*, associated with RR-RGR, is linked to BnaA04g10260D, homologous to seed imbibition 1-like (SIP1), a gene implicated in sugar transport, drought tolerance and regulating water uptake during seed imbibition [46]. The identification of SIP1 within a priming-responsive locus underscores the importance of tightly controlled water absorption during the rehydration phase, which is crucial for activating metabolic pathways that lead to improved seedling root vigour. Together, these findings emphasize that osmopriming enhances seed performance by modulating genes critical for water balance and cellular expansion, further integrating physiological and molecular mechanisms underpinning priming-induced stress tolerance. The pleiotropic SNP *Bn-scaff_20942_1-p252525*, associated with both radicle emergence (RR-RE) and vigour index (RR-VI), exemplifies hormonal crosstalk in priming response. The presence of GA20ox2 (BnaC02g15510D)

indicates that gibberellin-driven root elongation may counter ABA-mediated growth suppression [47], aligning with Nakaune *et al.* (2012) who found the upregulation of GA20ox genes in NaCl-primed tomato seeds [48]. Co-mapped PER66 (BnaC02g15500D) and MSG15.3 (BnaC02g15440D) further underscore redox regulation as a key component of priming efficacy [35]. These roles are reinforced by GRXS12 (BnaA07g00150D), a thioredoxin superfamily protein contributing to redox buffering during seed germination, and TTL2 (BnaC01g36460D) which had significant SNP *Bn-scaff_22115_1-p155376* on its exonic region is known to stabilize proteins under oxidative stress [49]. The SNP *Bn-A08-p8710166* on chromosome C08, associated with RR-RL, harbors XTH15 (BnaC08g08390D) within its linkage disequilibrium window. Consistent with the oxidative remodeling hypothesis, XTH15 mediates xyloglucan modification, facilitating cell wall loosening and promoting root elongation under osmotic stress [50]. This gene has also been previously implicated in seed priming responses, underscoring its pivotal role in osmopriming-induced root growth enhancement [51]. The association of USPL1 (BnaA07g00140D) and DENN domain proteins (BnaA07g00200D) with osmopriming-linked loci suggests novel mechanisms in cell wall signaling and vesicular trafficking during seed rehydration. Additional structural and signaling elements, including TCP4 (BnaC01g36430D), ERF4 (BnaC01g36330D), and PLP-dependent enzymes (BnaA07g00240D), underscore the intricate regulatory networks underpinning transcriptional and metabolic priming. Notably, ERF family transcription factors like ERF4 are known to integrate multiple hormonal and stress signals, such as ethylene, jasmonate, ABA, salicylic acid, and salt stress, to modulate downstream genes involved in cell wall remodeling and germination, as demonstrated by ERF-mediated regulation of cell wall-modifying enzymes in tomato seeds (e.g., SIERF2 inducing SIMAN2). This suggests the multifaceted role of these factors in coordinating priming responses through hormone-responsive gene expression [52]. Our haplotype analysis revealed two key haplotype blocks associated with differential osmopriming responses, with the chromosome A03 block (lead SNP *Bn-A03-p26272560*) containing six haplotypes where *RE-Hap6* (CATG) showed the strongest priming-induced enhancement, while the chromosome C02 block (lead SNP *Bn-scaff_20942_1-p252525*) contained four haplotypes with *VI-Hap1* (GTAA) exhibiting the most pronounced response. The marked performance differences between haplotypes, particularly *RE-Hap6* on chromosome A03 and *VI-Hap1* on chromosome C02, highlight their potential as robust functional markers for marker-assisted

selection to improve drought resilience through enhanced seedling vigour in breeding programs. Further studies are needed to validate these associations across diverse genetic backgrounds and elucidate their precise regulatory mechanisms through functional genomics approaches [53].

Table 3: Putative candidate genes associated with osmopriming response in root vigour traits

SNP_id	Traits	Chr	Candidate genes	Arabidopsis homologue	chrA03:24563270 - 24566288	Annotation	Reported in papers for seed priming
Bn-A03- p26272560	RR-RE	A03	BnaA03g47830D	AT4G26080	chrA03:24560638 - 24563048	ABA INSENSITIVE 1 (ABI1); Protein phosphatase 2C	[43, 51]
	RR-RE	A03	BnaA03g47820D	AT4G26070	chrA03:24659340 - 24663144	MAP kinase/ ERK kinase 1 (MEK1)	[51]
	RR-RE	A03	BnaA03g47930D	AT4G26300	chrA03:24590485 - 24592208	Embryo defective 1027 (emb1027); ArginyltRNA synthetase, class Ic	
	RR-RE	A03	BnaA03g47860D	AT4G26150	chrA03:24596130 - 24597812	Cytokininresponsive gata factor 1 (CGA1)	
	RR-RE	A03	BnaA03g47870D	AT4G26150	chrA04:8997151 - 8999910	Cytokininresponsive gata factor 1 (CGA1)	
Bn-A04- p7760488	RR- RGR	A04	BnaA04g10190D	AT5G40330	chrA04:9043551 - 9047080	Myb domain protein 23 (MYB23)	
	RR- RGR	A04	BnaA04g10260D	AT5G40390	chrA04:9054950 - 9057786	Seed imbibition 1-like (SIP1)	[46]
	RR- RGR	A04	BnaA04g10280D	AT5G40440	chrA07:136262 - 140175	Mitogenactivated protein kinase kinase 3 (MKK3)	
Bn-A07-p244051						unknown seed protein like 1 (USPL1); BURP	
	RR-RL	A07	BnaA07g00140D	AT1G49320	chrA07:172207 - 173454	Thioredoxin superfamily protein (GRXS12)	
	RR-RL	A07	BnaA07g00150D	AT2G20270	chrA07:236484 - 239481	Pyridoxal phosphate (PLP)dependent transferases superfamily protein	
	RR-RL	A07	BnaA07g00240D	AT2G20340	chrA07:204101 - 209217	DENN (AEX3) domaincontaining protein	
	RR-RL	A07	BnaA07g00200D	AT2G20320	chrC01:35742394 - 35745260		
Bn- scaff_22115_1- p155376	RR-RL	C01	BnaC01g36460D	AT3G14950	chrC01:35650908 - 35651657	Tetratricopetiderepeat thioredoxinlike 2 (TTL2)	
	RR-RL	C01	BnaC01g36330D	AT3G15210	chrC01:35696597 - 35697693	Ethylene responsive element binding factor 4 (ERF4)	[52]
	RR-RL	C01	BnaC01g36430D	AT3G15030	chrC02:11109631 - 11111035	TCP family transcription factor 4 (TCP4)	
Bn- scaff_20942_1- p252525	RR- RE, RR-VI	C02	BnaC02g15510D	AT5G51810	chrC02:11056194 - 11057360	Gibberellin 20 oxidase 2 (GA20ox2)	[48]
	RR- RE, RR-VI	C02	BnaC02g15500D	AT5G51890	chrC02:11001007 - 11003773	Peroxidase superfamily protein (PER66)	
	RR- RE, RR-VI	C02	BnaC02g15440D	AT5G51950	chrC03:13966672 - 13968097	Glucosemethanolcholine (GMC) oxidoreductase family protein (MSG15.3)	[35]

Bn-scaff_16002_1-p341408	RR-RE, RR-VI C03	BnaC03g24850D	AT2G44510	chrC03:19523341 - 19524503	CDK inhibitor P21 binding protein	
Bn-scaff_18482_1-p739195	RR-RE C03	BnaC03g31730D	AT4G01026	chrC03:19779031 - 19780668	Abscisic acid receptor (PYR1)	
					Plasma membrane intrinsic protein	
	RR-RE C03	BnaC03g32130D	AT4G00430	chrC08:12467531 - 12468650	1;4 (PIP1;4);	[12]
					Xyloglucan	
Bn-A08-p8710166	RR-RL C08	BnaC08g08390D	AT4G14130	chrC08:12464000 - 12466009	endotransglucosylase/hydrolase 15 (XTH15)	[48]
	RR-RL C08	BnaC08g08380D	-	chrA03:24563270 - 24566288	-	

5. CONCLUSION

Notably, the enhancement of early root vigour traits through priming appears to be driven by coordinated genetic networks, underscoring the importance of specific allelic variants in regulating stress-adaptive developmental responses. In this study, we identified several such allelic variants in association with the seed osmopriming response, providing new insights into the genetic basis of priming-induced phenotypic plasticity. Functional annotation revealed that key components of this response include signaling pathways involving response to abscisic acid, kinase activities, cell growth, as well as genes involved in redox regulation, water uptake, and cell wall remodelling, which collectively contribute to the modulation of root development under stress conditions (Supplementary1, Table S6; Supplementary2, Fig. S8). By integrating SNP- and haplotype-level analyses, this work establishes a strong foundation for pinpointing functional variants and advancing the development of climate-resilient cultivars through genomics-assisted breeding.

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883 **Data Availability**

884 All data generated or analysed during this study are included in this published article [and its
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886

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924 **Ethics declaration**

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