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RESEARCH ARTICLE

Structural chromosome variations from Jinmai 47 and Jinmai 84 affected agronomic traits and drought tolerance of wheat

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Highlights

- Twelve structural chromosome variations were identified between Jinmai 47 and Jinmai 84, eight of which were significantly associated with key agronomic traits, including plant height, grain number per spike, and thousand-grain weight.
- Present/absent variations (PAVs) on chromosomes 2A, 6A, 1D, 2D and a CNV on 4B, were associated with drought tolerance coefficients, indicating their potential roles in regulating drought response in wheat.

Abstract

Structural variation is an important source of genetic variation in wheat and have been important in the evolution of the wheat's genome. Few studies have examined the relationship between structural variations and agronomy and drought tolerance. The present study identified structural chromosome variations (SCVs) in a doubled haploid (DH) population and backcross introgression lines (BC_5F_3) derived from Jinmai 47 and Jinmai 84 using fluorescence *in situ* hybridization (FISH). There are one simple translocation, 10 present/absent variations (PAVs), and one copy number variation (CNV) between Jinmai 47 and Jinmai 84, which distributed in 10 chromosomes. Eight SCVs were associated with 15 agronomic traits. A PAV recombination occurred on chromosome 2A, which was associated with grain number per spike (GNS). The 1BL/1RS translocation and PAV.2D were associated with significant reductions in plant height, deriving from the effects on LI2-LI4, LI2-LI4 and UI, respectively respectively. PAV.2D was also contributed to an increase of 3.13% for GNS, 1BL/1RS significantly increased spikelet number, grain length (GL), and grain thickness (GT). The effect of PAV.4A.1 on GL, PAV.6A on spike length (SL) and thousand-grain weight (TGW), PAV.6B on SL, GT and TGW were identified and verified. PAVs on chromosomes 2A, 6A, 1D, 2D, and a CNV on chromosome 4B were associated with the drought tolerance coefficients. Additive and interaction effects among SCVs were observed. Many previously cloned key genes and yield-related QTL were found in polymorphic regions of PAV.2B, PAV.2D, and CNV.4B. Altogether, this study confirmed the genetic effect of SCVs on agronomy and drought tolerance, and identification of these SCVs will facilitate genetic improvement of wheat through marker-assisted selection.

Keywords: wheat, structural chromosome variation, agronomy, drought tolerance, effect

1. Introduction

Wheat is one of the most important crops worldwide, and increasing grain yield is the main objective of wheat breeding (Shokat *et al.* 2020). Grain yield is a complex polygenic trait with several component traits, and/or other growth characteristics such as grain filling rate and grain number, and plant height also influence grain yield (Yang *et al.* 2019; Liu *et al*. 2024). Studies of the genetics of yield

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had focused on QTL mapping, association analysis, and gene-cloning of important agronomic traits. Since the early 1990s, about 1,000 QTLs were reported *via* interval mapping and about 2,000 marker-trait associations were identified using genome-wide association mapping. Grain yield-related QTLs have been found on all the 21 wheat chromosomes (Gupta *et al.* 2020). Many genes for yield-related traits have been identified, cloned, and characterized. For example, understanding of the genetic mechanisms of high yield has been advanced by map-based cloning of *TaCol-B5* (Zhang *et al.* 2022), *ZnF* (Song *et al.* 2023), and *GNI-A1* (Sakuma *et al.* 2019) and homology-based cloning of *TaGW2-6A* (Su *et al.* 2011), *TaAPO-A1* (Muqaddasi *et al.* 2019), and *TaGL3-5A* (Yang *et al.* 2019). In addition to the activity of QTL and genes, yield traits are also regulated at the transcription and translation levels, and through epigenetic modification and structural variation.

Structural variations (SVs) are common features of crop genomes, which involved in the origin, adaptation, and domestication of species (Trickett and Butlin 1994; Jin *et al.* 2023). SVs along with single nucleotide polymorphisms and epigenetic differences, are responsible for heritable phenotypic diversity (Yuan *et al.* 2021). SV was first discovered in *Drosophila* (Metz and Bridges 1917) and it has been important in the evolution of humans and other animals (Niebuhr 1978; Xia *et al.* 2023). Up to 70% of the flowering plants have undergone polyploidy events during evolution, which induces SVs, and promotes new phenotypic traits, thus providing variation for crop domestication and improvement (Thind *et al.* 2018). In recent years, crop genome and phenotypic variation has been studied in relation to SV (Liu *et al.* 2020; Hufford *et al.* 2021; Qin *et al.* 2021; Jin *et al.* 2023). SVs changed tomato gene dosage and expression levels resulting in modified fruit flavor, size, and production (Alonge *et al.* 2020). Chromosomal inversion played an important role in rice stress resistance (He *et al.* 2024) and a 13 Mb inversion locus (I*nv4m*) was associated with flowering time and highland adaptability (Crow *et al.* 2020). Wheat is a young allopolyploid species with a large genome, of which more than 85% is highly repetitive sequences (Wicker *et al.* 2018). Abundant SVs affecting growth, development and agronomic traits have occurred during evolution and domestication of wheat (Mahmoud *et al.* 2019; Walkowiak *et al.* 2020). Identification of more SVs and exploration of the genetic effect is a novel route that we take an insight into genetic of wheat yield.

SVs differ in size and complexity and hence different techniques have been used to characterize them in plant genomes. Methods to identify SVs include microscopic observation, array comparative genomic hybridization, and SNP array analysis, and DNA sequencing (Yuan *et al.* 2021). However, each approach has drawbacks. Microscopic observations are now mostly applied to confirm known SVs, due to low throughput and the limited resolution. Array comparative genomic hybridization cannot detect balanced SVs and copy number variation (CNV). The number of SVs detected using SNP array analysis depends on the density of SNPs in the target genome region. Moreover, SNP arrays cannot easily characterize repeat-rich and duplicated regions. With advances in DNA sequencing, SV has been mainly characterized at the nucleotide level, which plays an important role in the study of species diversity and genetic evolution. In wheat, many SVs related to agronomic traits have been identified using these techniques. For example, CNVs affect *Ppd-B1*, *Vrn-A1*, and *Rht-D1*, the key genes controlling photoperiod, vernalization, and dwarfism, respectively (Díaz *et al.* 2012; Li *et al.* 2012; Würschum *et al.* 2015); Soft Svevo wheat contains the 5DS/5BS translocation causing softer grain (Boehm *et al.* 2017). However, SV-trait studies in wheat are limited and fewer SVs were defined at the chromosome level related to agronomy. Additional work is needed to comprehensively decipher the association between structural chromosome variations (SCVs) and agronomic traits to support wheat improvement.

At present, molecular cytogenetic approaches are advantageous for identifying SCVs (Lang *et al.* 2019; Zou *et al.* 2021). A 3.2 Mb presence/absence variations (PAV) fragment under selection during maize domestication and adaptation was identified by using single-copy oligos fluorescence *in situ* hybridization (FISH) based on the genome assembly of maize inbreds B73 and Mo17 (Huang *et al.* 2021). Šimoníková *et al.* (2020) found that large differences in chromosome structure discriminated individual banana accessions using FISH. Also in wheat, 139 SCVs including translocations, pericentric inversions, PAVs, and CNVs were identified by FISH using an oligonucleotide multiplex probe (Zhao *et al.* 2022). Therefore, in the present study oligo-FISH was used to identify SCVs within a doubled haploid (DH) population and backcross introgression lines. SCVs in wheat related to plant height, grain number, grain weight, and their component traits and drought tolerance-responsive traits were comprehensively identified *via* association analysis. Results systematically enrich the understanding of the genetic mechanism of yield and drought resistance at the chromosomal variations level, and can also provide cytogenetic markers for molecular marker-assisted breeding of wheat.

2. Materials and methods

2.1. Plant materials

Three wheat germplasm sets were used in the present study: 1) a DH population (Panel-1) derived from Jinmai 47/Jinmai 84 with a total of 240 lines, 2) a collection of 281 wheat varieties (Panel-2), including 40 landraces and 241 modern cultivars from Shanxi Province, China (Zhao *et al.* 2022) and 3) 20 introgression lines (ILs) (Panel-3) derived from a backcross Jinmai 47/Jinmai 84 (BC_5F_3) population were used to verify the effects of PAV.2D. The female parent in Panel-1 was Jinmai 47, a high-yielding dryland variety with higher drought-tolerance, and the male parent was Jinmai 84, a high-yield variety adapted to irrigated conditions. They were approved by the Shanxi Variety Committee in 1995 and 2008, respectively.

2.2. Planting and phenotyping

In 2019–2020, the DH population was planted at Suobu Experimental Station of Linfen (S, 36°08´N, 111°52´E, altitude 450 m), and in 2020–2021 at Hancun Experimental

Station of Linfen (HC, 36°25′N, 111°67′E, altitude 450 m) and Yuncheng (YC, 34°35′N, 110°15′E, altitude 450 m) in Shanxi Province, China. Three experimental treatments were included at each planting (normal well-watered, NWW; once deficit-watered, ODW; twice deficit-watered, TDW). Monthly rainfall rates during the two trial years were recorded (Appendix A). The irrigation regimes were: NWW, irrigation at the overwintering, jointing, and filling stages. ODW, irrigation at the overwintering and jointing stages and TDW, irrigation at the overwintering stage. Twenty-one seeds were evenly sowed in 1.5 m rows per line spaced 0.3 m per plot. Field management practices were those commonly used in wheat production in the region. Lines in Panel-2 were grown at Linfen, in 2019–2020 and 2020–2021, and the field experiments were described in Zhao *et al.* (2022). And the ILs were grown at Linfen in 2021–2022.

Ten days before harvest, 10 plants were randomly chosen from the middle of each plot and 12 agronomic traits were recorded. Measurements were made for plant height (PH), spike length (SL), panilce exsertion (PE), the lengths of the uppermost (UI), second (LI2), third (LI3), fourth (LI4), and fifth (LI5) internodes from the top, spikelet number (SN), grain number per spike (GNS), basal sterility spikelets per spike (BSSS), and apical sterility spikelets per spike (ASSS). After harvest, grain length (GL), grain width (GW), grain thickness (GT), and thousand-grain weight (TGW) were measured. Drought tolerance coefficients (DTC) for traits in the DHs were calculated as described by Zhao *et al.* (2023).

2.3. Cytological analysis

Chromosome preparation and FISH analysis followed procedures described in Zhao *et al.* (2023). Probes used in the present study were modified versions of ONPM#7 (Chen *et al*. 2019). Probes pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, AFA-4, pSc119.2-1, 5S-1, and 5S-2 were modified with 6-carboxytetramethylrhodamine (TAMRA), and BSCL135-1, BSCL135-2, and $(GAA)_{10}$ were modified with 6-carboxyfluorescein (FAM). All oligonucleotides were synthesized and modified by General Biosystems (Chuzhou, China).

After hybridization, chromosomes were counterstained with DAPI mounting medium (H-1200, Vector, Burlingame, CA, USA) and observed with an Olympus BX60 microscope (Olympus Inc., Tokyo, Japan). Images were captured with a SPOT Cooled Color Digital (CCD) camera (DP80, Olympus Inc., Japan). Image analysis was conducted using Photoshop (Adobe Inc., San Jose,CA, USA). For karyotyping, 3–8 plants of each line were observed representing a total of 9–40 cells. Chromosomal variations caused by differences in heterochromatin were determined between parents and DHs.

2.4. Fst analysis and QTL analyses

The DH population was genotyped with a 15K SNP panel, which yielded 14,868 SNPs and used in the linkage analysis *via* QTL IciMapping 4.1 and visualized with JoinMap 4.0. Genetic map construction and linkage analysis of the DH population were described based on the pioneering work of Yang *et al.* (2022). The genetic differentiation (F_{st}) was calculated to explain population differentiation based on the presence/absence of SCV using Vcftools (v0.1.14) (Danecek *et al.* 2011). And the differentiation regions which of F_{st}>0.75 were regarded as the physical location for the corresponding SCV on the chromosome region.

QTLs were detected using WinQTLCart version 2.5 (https://brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm) based on the composite interval mapping method. QTLs were claimed to be significantly if above the 2.5 logarithms of odds (LOD) threshold. A QTL detected in three environments (including BLUP) was considered a stable QTL. QTLs either less than 1 cM apart or sharing common flanking markers were treated as a single locus and named according to McCouch *et al.* (1997).

2.5. Statistical analyses

SPSS 18.0 software (SPSS Inc., Chicago, Illinois, USA) was used to perform the Student's *t*-test and significant differences between phenotype values were determined using a threshold of P<0.05. Best linear unbiased prediction (BLUP) values of phenotypes were calculated using the lme4 package in R.

3. Results

3.1. Construction and phenotyping of the genetic population in wheat

Karyotypes of two widely-spread cultivars in Shanxi Province, Jinmai 47 (dryland cultivar) and Jinmai 84 (irrigated cultivar), were constructed with FISH analysis (Fig. 1-A and B). Jinmai 84 is wheat-rye 1BL/1RS translocation line, and 12 signal polymorphisms between the two karyotypes were examined, which distributed in ten chromosomes (1A, 2A, 4A, 6A, 1B, 2B, 4B, 6B, 1D, and 2D) (Fig. 1-C). In order to explore the genetic mechanism of agronomy and drought tolerance at the structural chromosome variations level, the two elite wheat cultivars were crossed as parents. A total of 240 lines of DH population derived from Jinmai 47/Jinmai 84 were constructed. Additionally, the resulting F_1 hybrids were backcrossed five times with Jinmai 84 and then self-crossed for three generations. A total of 20 backcrossed lines were screened, which of the karyotypes differ only in chromosome 2D, to verify the effects of PAV.2D.

Significant phenotypic differences were found between parents. The PH of Jinmai 47 was 14.17 cm greater than that of Jinmai 84 (P<0.05) (Table 1). The internode length was 1.44% longer for uppermost node of Jinmai 47 but was 35.74, 43.94, and 44.26% longer for that of Jinmai 84 from the 2nd node to the 4th node, respectively (P<0.05). The PE and SL of Jinmai 47 were 8.82 and 10.33% longer than that of Jinmai 84, respectively. Also Jinmai 47 had higher values than Jinmai 84 for traits related to grain number, but the opposite trends was observed for grain weight components. According to parents' characteristics and field performance, we performed field experiments with different irrigation regimes. Compared with parents, the DH populations showed abundant phenotypic variation for 16 agronomic traits in all environments, and transgressive

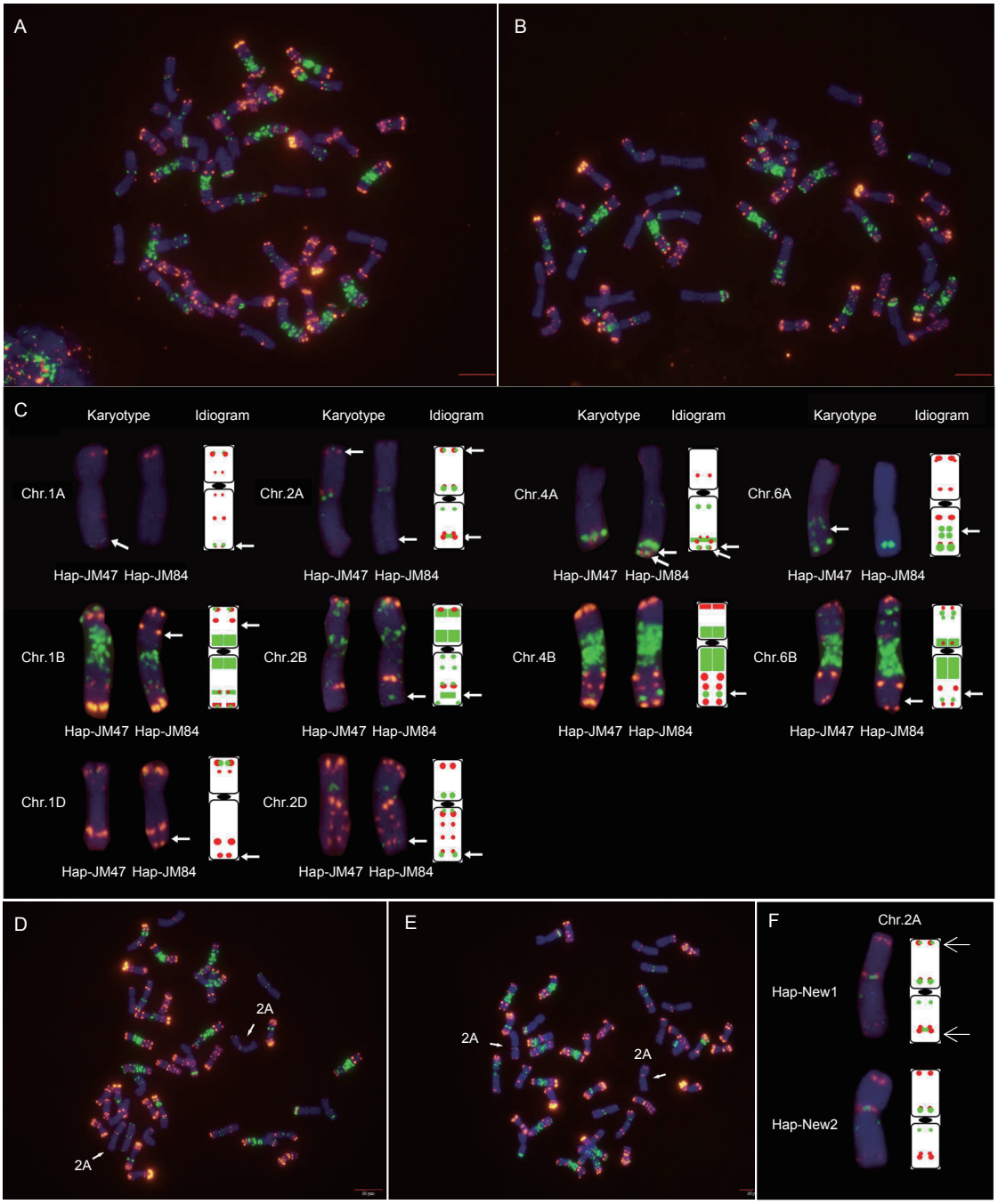


Fig. 1 Fluorescence *in situ* hybridization (FISH) karyotypes of Jinmai 47 and Jinmai 84. A, karyotypes of Jinmai 47. B, Karyotypes of Jinmai 84. C, karyotypes and Idiograms of polymorphic chromosomes between Jinmai 47 and Jinmai 84. Arrows indicate structural chromosome variations (SCVs) location. D–F, polymorphic types of 2A chromosomes among the doubled haploid (DH) population lines.

segregation was observed for all traits (Appendix B). The highest values of all traits were detected under NWW conditions, and the coefficient of variation of PE, BSSS, and ASSS were higher than other traits (Table 1). The highest heritability was observed for SL and GL, followed by the PH-related traits. BSSS had the lowest heritability.

3.2. Identification and distribution of SCVs in the DH population

Oligonucleotide probe multiplex was modified with 12 probes, which can identify SCVs at 230 loci (Zhao *et al.* 2022). Karyotyping of the DH population was performed. One simple translocation, 10 PAVs, and one CNVs were detected in DH population. Among them, 1BL/1RS translocation and CNV.4B derived from Jinmai 84, with the latter being represented as a pair of strong green signals located on the telomeric region of 4BL (Fig. 1-C). Ten PAVs mainly distributed on chromosomes 1A, 2A, 4A, 6A, 2B, 6B, 1D, and 2D. Of them, PAV.1A, PAV.2A.1, and PAV.6A, that are pairs of green signals respectively appeared on 1AL, 2AS, and 6AL, derived from Jinmai 47 (Fig. 1-C). PAV.2A.2, PAV.4A.1, PAV.2B, PAV.6B, and PAV.2D with pairs of green signals, and

PAV.4A.2 and PAV.1D with pairs of red signals, derived from Jinmai 84 (Fig. 1-C). Thus, aside from 1BL/1RS and PAV.6A, most variations were observed at chromosome extremities. All polymorphic chromosomes showed normal transmission in the DH population with polymorphisms conforming to a 1:1 segregation ratio (Appendix C).

3.3. SCV associated with agronomic traits

To identify SCV related to yield traits, we performed SCV association analyses for plant height, grain number, grain weight, and their related component traits in DH population. There were significant correlation between SCVs and 15 agronomic traits.

For the 1BL/1RS, it showed significantly decrease PH by 3.24 cm. Further analysis of PH component traits with 1BL/1RS, negative association were performed on LI2, LI3, and LI4 (Table 2). Moreover, a positive effect on SN was observed for 1BL/1RS, but had not effect on GNS due to the decreasing fertility of the spikelet, that 1BL/1RS increased ASSS and BSSS (Table 2). A similar tendency was observed for grain weight. 1BL/1RS showed positive effects on GL and GT, but no significant effect for TGW. The main reason may be the lower effect of 0.99 and 1.77% on GL and GT. CNV from chromosome 4B also showed significant negative effect on PH related traits, including LI2, LI3, and SL, with the phenotypic effect of 3.05–5.13% (Table 2).

Further analysis of the effect of 10 PAVs were performed. Except PAV.1A, PAV.2A.1, and PAV.2A.2, significant effect on 15 agronomic traits were detected from other PAVs. Firstly, PAV.1D had positive effects on PE and UI, with significantly increases of 15.59 and 5.14%, respectively, and negative effect on SN (Table 2). PAV.2B had significantly positive effect on PE of 0.82 cm (12.67%), TGW of 1.44 g (3.14%), GT of 0.03 mm (0.96%), and showing a decrease of 4.65% for GNS, 2.2% for SN. It can conclude the negative effect on GNS of PAV.2B mainly attributed to a decrease of spikelet numbers (Table 2). Then, PAVs on chromosome 4A were negatively associated with SL and GL, explained 2.11 and 0.82% of the phenotypic variance, respectively (Table 2). While PAV.6A had a positive effect on SL of 2.37%, and GL with negative effect. Besides, PAV.6A showed significant associations with LI4, LI5, ASSS, and TGW. The ASSS of lines with PAV.6A were significantly increased by 13.46%, and other three traits were reduced by PAV.6A. No significant effect on grain number related traits was found for PAV.6B. Six traits were influenced by PAV.6B, including PE, UI, TGW, and GT with positive effect and LI2 and GL with negative effect. The effect on PE was the largest, 26.29% (Table 2).

Notably that PAV.2D showed significant associations with 10 agronomic traits in DH population. For PH-related traits, PAV.2D was associated with not only SL and PE, but also UI, LI2, LI3, and LI4 with negative effect, resulting significantly decreased PH by 5.83 cm (Table 2). And the effect was larger than that of 1BL/1RS. Moreover, PAV.2D showing an increase of 1.43 on GNS mainly from increased spikelet density, and negative effects on TGW and GT (Table 2).

3.4. Recombination of SCVs and effect analysis

Except for chromosome 2A, the distribution of SCVs in DH population on other nine chromosomes were consistent with the types of Jinmai 47 and Jinmai 84. We found 67 lines had the 2A haplotype of Jinmai 47 (Hap-JM47, with PAV.2A.1) and 58 lines had 2A haplotype of Jinmai 84 (Hap-JM84, with PAV.2A.2) in DH population. Two new karyotypes of

Table 1 Phenotypic variation and heritability of traits for parental and doubled haploid (DH) population lines

Env.[1]	Trait	Plant height related traits[2]								Grain number[3]				Grain weight[4]			
		PH (cm)	SL (cm)	PE (cm)	UI (cm)	LI2 (cm)	LI3 (cm)	LI4 (cm)	LI5 (cm)	SN	BSSS	ASSS	GNS	GL (mm)	GW (mm)	GT (mm)	TGW (g)
Parents	Jinmai 47	88.51	10.10	6.69	22.48	22.33	14.25	10.43	5.37	20.24	0.95	0.68	53.40	6.57	3.79	3.12	43.31
	Jinmai 84	70.74	9.15	6.15	22.16	16.45	9.90	7.23	5.43	19.87	1.33	0.24	46.44	7.19	3.86	3.30	49.24
NWW	Mean	84.51	9.60	7.60	27.22	19.85	12.95	8.52	5.48	19.86	1.05	0.71	47.45	6.92	3.58	3.24	48.18
	Min.	61.20	7.11	–1.09	17.80	12.93	8.16	5.02	2.94	15.52	0.13	0.00	32.53	6.43	3.08	2.85	33.13
	Max.	107.81	11.86	18.81	38.20	28.89	17.72	13.59	9.85	22.08	2.20	3.27	66.55	7.64	3.98	3.63	58.33
	SD	10.64	0.78	3.22	3.67	3.04	2.20	1.76	1.30	0.97	0.35	0.67	6.56	0.22	0.14	0.16	4.54
	CV (%)	12.59	8.09	42.38	13.49	15.30	16.95	20.69	23.82	4.90	33.88	93.44	13.83	3.25	4.01	4.81	9.42
ODW	Mean	84.76	9.60	7.74	27.33	20.25	13.31	8.74	5.37	19.47	1.00	0.69	49.07	6.92	3.55	3.19	47.00
	Min.	62.63	7.66	–0.44	17.89	13.59	8.43	5.14	2.69	15.87	0.07	0.00	29.32	6.39	3.14	2.80	34.80
	Max.	110.13	12.37	20.27	39.07	27.05	17.66	12.15	8.57	21.83	1.93	2.93	65.96	7.51	3.98	3.71	56.90
	SD	9.95	0.78	3.17	3.59	3.01	2.25	1.68	1.14	1.01	0.31	0.51	5.95	0.23	0.11	0.15	4.19
	CV (%)	11.74	8.17	40.91	13.12	14.86	16.87	19.22	21.28	5.18	31.11	73.14	12.12	3.30	3.07	4.57	8.91
TDW	Mean	77.08	9.46	5.31	23.74	17.81	12.18	8.58	5.65	20.22	1.53	1.18	41.71	6.84	3.46	3.10	44.31
	Min.	55.03	7.55	–2.23	15.09	11.38	7.65	5.21	2.64	17.33	0.47	0.00	24.60	6.27	3.10	2.74	35.02
	Max.	103.10	11.80	16.68	34.41	25.47	18.25	12.89	9.33	23.07	2.73	4.20	60.07	7.54	3.83	3.54	53.60
	SD	11.02	0.79	2.98	3.54	3.22	2.60	1.96	1.42	1.00	0.43	0.75	6.32	0.24	0.12	0.14	3.62
	CV (%)	14.30	8.32	56.18	14.89	18.06	21.37	22.81	25.13	4.97	28.41	63.56	15.15	3.55	3.49	4.49	8.16
H^2		0.71	0.91	0.83	0.79	0.82	0.70	0.63	0.44	0.50	0.31	0.64	0.69	0.95	0.66	0.72	0.68

[1] Env., environment; NWW, normal well-watered; ODW, once deficit-watered; TDW, twice deficit-watered; H^2, broad-sense heritability.
[2] PH, plant height; SL, spike length; PE, panilce exsertion; UI, the lengths of the uppermost; LI2–LI5, the lengths of the second, third, fourth, and fifth internodes from the top.
[3] SN, spikelet number; BSSS, basal sterility spikelets per spike; ASSS, apical sterility spikelets per spike; GNS, grain number per spike.
[4] GL, grain length; GW, grain width; GT, grain thickness; TGW, thousand-grain weight.

Table 2 Stable structural chromosome variation (SCV) detected for wheat yield components in the doubled haploid (DH) population

Type	Chr.	Trait[1)]	Env.[2)]	Mean[3)]		*F*	*P*	%[4)]
				NC	SCV			
1BL/1RS	1B	PH	S1, S2, S3, HC2, HC3, YC1, YC2, BLUP	83.6860±9.23320	80.4441±10.7210	6.314	0.013*	–3.87
		LI2	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	20.0746±2.6541	18.5037±2.8376	19.633	0.000***	–7.83
		LI3	S1, S2, S3, HC1, HC2, YC1, YC2, YC3, BLUP	13.2394±1.8875	12.3725±2.2009	10.752	0.001**	–6.55
		LI4	S1, S2, S3, HC2, BLUP	8.8261±1.3933	8.3914±1.6883	4.746	0.030*	–4.93
		SN	S1, S3, HC1, HC2, HC3, YC2, BLUP	19.6491±0.6957	20.0591±0.7852	18.368	0.000***	2.09
		ASSS	S2, HC1, YC1, YC2, YC3, BLUP	0.7451±0.2955	0.9832±0.4018	27.49	0.000***	31.96
		BSSS	S1, S3, HC2, YC2, BLUP	1.1564±0.1639	1.2310±0.1679	12.135	0.001**	6.45
		GL	S1, S3, HC1, YC2, YC3, BLUP	6.8612±0.1915	6.9292±0.2067	6.989	0.009**	0.99
		GT	HC1, YC2, YC3	3.2323±0.1994	3.2895±0.2317	4.116	0.044*	1.77
PAV.1D	1D	PE	S1, S2, S3, HC2, HC3, YC1, YC3, BLUP	6.3865±2.7188	7.3819±2.9551	7.373	0.007**	15.59
		UI	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	25.4393±3.0657	26.7474±3.3660	9.906	0.002**	5.14
		SN	S2, HC3, YC1, YC2, BLUP	19.9762±0.8648	19.7251±0.6350	6.573	0.011*	–1.26
PAV.2A.1+2	2A	GNS	S2, S3, HC1	46.5503±9.3909	42.9655±7.0380	5.344	0.023*	–7.7
PAV.2B	2B	PE	S1, S2, S3, YC2, YC3, BLUP	6.4935±2.7761	7.3160±2.9366	4.973	0.027*	12.67
		SN	S2, S3, HC1, HC2, HC3, YC1, BLUP	20.0606±0.8252	19.6187±0.6237	21.549	0.000***	–2.2
		GNS	HC3, YC1, YC2, YC3, BLUP	47.1215±5.5757	44.9314±5.2554	9.751	0.002**	–4.65
		GT	S3, HC1, YC3, BLUP	3.1636±0.1224	3.1940±0.1152	3.903	0.049*	0.96
		TGW	S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	45.8031±3.2630	47.2436±3.0358	12.458	0.000***	3.14
PAV.2D	2D	PH	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	84.9344±9.8304	79.1041±9.5439	21.699	0.000***	–6.86
		PE	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	7.5957±2.9840	6.1362±2.5668	16.429	0.000***	–19.21
		UI	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	26.9753±3.2876	25.1661±3.0150	19.683	0.000***	–6.71
		LI2	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	20.1361±2.7174	18.4256±2.7325	23.631	0.000***	–8.49
		LI3	S1, S2, S3, HC1, HC2, YC1, YC2, YC3, BLUP	13.3139±2.0294	12.2868±2.0282	15.368	0.000***	–7.71
		LI4	S1, S2, S3, HC1, HC3, YC1, YC2, YC3, BLUP	8.9280±1.4973	8.2806±1.5566	10.785	0.001**	–7.25
		SL	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	9.7492±0.6583	9.3505±0.6204	23.259	0.000***	–4.09
		GNS	S3, HC3, BLUP	45.3876±4.9597	46.8103±5.9969	4.027	0.046*	3.13
		GT	S1, S2, S3, HC1, BLUP	3.1980±0.1082	3.1570±0.1279	7.202	0.008**	–1.28
		TGW	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, BLUP	47.1653±2.8311	45.7746±3.4773	11.593	0.001**	–2.95
PAV.4A.1+2	4A	SL	S1, S2, S3, HC1, YC1, BLUP	9.6740±0.6281	9.4697±0.6868	5.543	0.019*	–2.11
		GL	S1, S2, HC2, BLUP	6.9278±0.1720	6.8710±0.2179	4.700	0.031*	–0.82
CNV.4B	4B	LI2	S1, S3, HC2, HC3, YC2, YC3, BLUP	19.7758±2.7959	18.7610±2.8287	7.783	0.006**	–5.13
		LI3	S2, S3, HC3, YC1, YC3, BLUP	13.1088±2.0344	12.4753±2.1081	5.599	0.019*	–4.83
		SL	S1, S2, S3, HC1, HC2, YC1, YC2, YC3, BLUP	9.6926±0.6729	9.3974±0.6321	12.165	0.001**	–3.05
PAV.6A	6A	LI4	S2, HC1, YC1, YC2, BLUP	8.4119±1.5719	8.8931±1.5001	5.677	0.018*	–5.41
		LI5	S2, YC1, YC3, BLUP	5.3498±0.8698	5.6458±0.8119	7.142	0.008**	–5.24
		SL	S1, S3, HC1, YC1, YC2, YC3, BLUP	9.4243±0.6423	9.648±0.6748	6.672	0.010*	2.37
		ASSS	YC1, YC2, YC3, BLUP	0.9070±0.3866	0.7994±0.3393	4.995	0.026*	13.46
		GL	S1, HC2, YC1, YC3, BLUP	6.8678±0.2100	6.9323±0.1838	6.095	0.014*	–0.93
		TGW	S1, HC2, HC3	39.707±4.2232	41.0126±4.0060	5.700	0.018*	–3.18
PAV.6B	6B	PE	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	6.0434±2.5646	7.6323±2.9421	19.660	0.000***	26.29
		UI	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	25.2432±2.9748	26.8497±3.3617	15.209	0.000***	6.36
		LI2	S1, S3, YC2, BLUP	19.7356±2.8928	18.9167±2.7676	5.016	0.026*	–4.15
		GL	S1, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	6.9434±0.1915	6.8513±0.2012	13.105	0.000***	–1.33
		GT	S1, S2, S3, HC1, HC2, HC3, YC1, YC2, YC3, BLUP	3.1357±0.1121	3.2156±0.1140	29.860	0.000***	2.55
		TGW	HC3, YC3, BLUP	46.0486±2.9983	46.8776±3.3906	3.983	0.047*	1.8

[1)] PH, plant height; SL, spike length; PE, panilce exsertion; UI, the lengths of the uppermost; LI2–LI5, the lengths of the second, third, fourth, and fifth internodes from the top; SN, spikelet number; GNS, grain number per spike; BSSS, basal sterility spikelets per spike; ASSS, apical sterility spikelets per spike; GL, grain length; GT, grain thickness; TGW, thousand-grain weight.

[2)] Env., environment; S, Suobu Experimental Station of Linfen, Shanxi Province, China; HC, Hancun Experimental Station of Linfen; YC, Yuncheng, Shanxi Province. 1–3, normal well-watered (NWW), once deficit-watered (ODW), and twice deficit-watered (TDW), respectively; BLUP, best linear unbiased prediction.

[3)] NC, negative control, the lines without signal of structural chromosome variation.

[4)] % indicates the genetic effect of SCV, which was calculated by (SCV–NC)/NC×100. Treatment refers to containing SCV, and negative control means without SCV. Except PAV.2A.1+2 for grain number per spike (GNS), 1BL/1RS for grain thickness (GT), and PAV.6A for thousand-grain weight (TGW), the effect of other SCVs were the effect in BLUP treatment.

Values are mean±SD (n=3). *, **, and *** indicate the significant differences at the level of P<0.05, P<0.01, and P<0.001, respectively.

chromosome 2A present in the DH lines, including a type with signals of both PAV.2A.1 and PAV.2A.2 (Hap-New1) and a type without signals of them (Hap-New2) (Fig. 1-D–F), found in 58 and 57 lines, respectively (Appendix C). Previous research showed no significant difference on 16 agronomic traits with PAV.2A.1 or PAV.2A.2, but the lines with Hap-New1 (PAV.2A.1+2) had lower GNS than that lines with Hap-New2, indicating that interaction effect between PAV.2A.1 and PAV.2A.2 contributed to a decrease of GNS (Table 2).

3.5. SCV regulated drought tolerance of wheat

Functions of abiotic/biotic response to environmental stresses have been found in SVs from multiple plant species (Schiessl *et al.* 2019). In our previous research, a PAV on 7B was associated with drought tolerance (Zhao *et al.* 2023). To further verify the effect of SCV on abiotic stress tolerance, we performed associated analysis between SCVs and drought tolerance coefficient (DTC). Five SCVs were identified for DTC_PH (3), DTC_GNS (2), and DTC_TGW_(2) in more than three environments (Appendix D). PAV.2D was significantly associated with both the trait and its corresponding DTC value (Appendix D-a, b). As Osipova *et al.* (2020) reported in a 2D substitution line of Saratovskaya 29, chromosome 2D was involved in drought resistance regulation. The other four SCVs associated with DTC were PAV.2A.2, PAV.6A, CNV.4B, and PAV.1D. Association analysis showed CNV.4B significantly increased DTC_PH, whereas the lines with PAV.2A.2 had lower DTC_PH than NC (Appendix D-c, e). And PAV.6A and PAV.1D showed positive effect on DTC for GNS and TGW, respectively (Appendix D-d, f). Therefore, specific SCV are associated with drought tolerance of wheat.

3.6. Validation of the genetic effects of favored SCVs

To further validate the stable SCV identified in the DH population, the natural population from Shanxi Province (panel-2) was analyzed. Beside CNV.4B, PAV.1D, and PAV.2A.1+2, the effects of other SCVs were verified in panel-2. Firstly, 1BL/1RS was significantly negatively associated with PH and each internode length, and significantly increased SN, GL, and GT (Appendix E). These results were consistent with those from the DH population. Then for PAV.2B, PE showed significant differences among alleles in panel-2, but no significant differences on SN, GNS, TGW, and GT were observed (Appendix E). PAV.6B significantly increased GT and TGW. Significantly associated with SL, TGW, and GL for PAV.6A were consistent in both populations, showing increases in SL and TGW and a decrease in GL. Besides, positive PH-related traits were observed for PAV.6A, acting mainly on LI4 and LI5 in the DH population, and with significantly increases in PH, LI2, and LI3 in panel-2 (Appendix E; Table 1). PAVs on chromosome 4A had a positive effect on GL. It was previously found that PAV.4A.1 was significantly associated with GL in DHs (Zhao *et al.* 2022, 2023). It indicated that the effect of PAV.4A.1 had not affected by the interaction with PAV.4A.2.

Lastly, 11 agronomic traits were associated with PAV.2D in panel-2, and no significant effects for TGW were validated (Appendix E). The effects of PAV.2D for PH, PE, UI, LI2, LI3, LI4, and GNS were highly significant (P<0.05) in more than three environments, consistent with that of the DH population analysis. But the effect on GT was reversed, PAV.2D significantly increased GT by 2.51% (Appendix E). Since 1BL/1RS had a positive effect on grain yield, we performed an association analysis between PAV.2D and GT under different 1BL/1RS backgrounds in both DHs and panel-2. The same phenomenon was observed in the lines with 1BL/1RS in both panels, showing PAV.2D has negative effect on GT (Appendix F-a, b). However, PAV.2D was not associated with GT in DHs, and the positive effect presented in panel-2 under the backgrounds without 1BL/1RS (Appendix F-a, b). Perhaps the genetic background of natural population is complex and results need to be further verified. In summary, these results indicated that SCVs significantly associated with agronomic traits, and regulates plant growth and grain development.

3.7. Effect of PAV.2D further confirmed in introgression lines

PAV.2D showed negative effects on PH and length of internode in both the DH and natural populations. But its effect on GT was influenced by presence/absence of 1BL/1RS (Fig. 2-A and B). To further elucidate the genetic effects of PAV.2D, twenty BC_5F_3 introgression lines derived from Jinmai47/Jinmai84 were grouped based on PAV.2D, namely PAV.2D– and PAV.2D+ (Fig. 2-A and B). We found 20 ILs are 1BL/1RS translation line (Fig. 2-A and B). Comparisons of 16 agronomic traits were made between the PAV.2D– and PAV.2D+ groups. Results showed the internodes length of plants with PAV.2D were shorter than without it, and obvious differences occurred in PH-related traits (P<0.05) except for LI5 (Fig. 2-C–F). PAV.2D+ had significantly lower (P<0.05) values for PH, PE, UI, LI2, LI3, and LI4 than PAV.2D– (Fig. 2-E–G). PE, LI2, and LI3 showed large difference with 40.40, 26.73, and 33.19%, respectively. Additionally, PAV.2D significantly increased GNS by 13.93 and decreased GT (Fig. 2-H and J), while no significant difference occurred for TGW (Fig. 2-I). Overall, these results were consistent with those observed in the DH and natural populations, further confirming that PAV.2D has pleiotropic effects on plant height development and grain number-related traits.

3.8. Additive effects of the stable SCVs

Two stable SCVs for PH (1BL/1RS, PAV.2D), three stable SCVs for GNS (PAV.2A.1+2, PAV.2B, PAV.2D), and four SCVs for TGW (PAV.6A, PAV.2B, PAV.6B, PAV.2D) were detected in the DH population (Table 2). The additive effects of these SCVs on the corresponding traits were analyzed. The average corresponding trait values increased following with the increasing number of positive alleles (Fig. 3-A). Lines with favorable alleles at all the two SCVs had an average PH decrease of 8.50 cm *vs*. those with possessing contrasting alleles (Fig. 3-A; Appendix G). Lines with positive alleles on all three SCVs had significantly increased values for GNS (Fig. 3-A; Appendix G). The combination of positive alleles from PAV.6A, PAV.2B, PAV.6B, and PAV.2D had the largest effect resulting in a 9.48% increase in TGW (Fig. 3-A; Appendix G). There were also additive effects on their

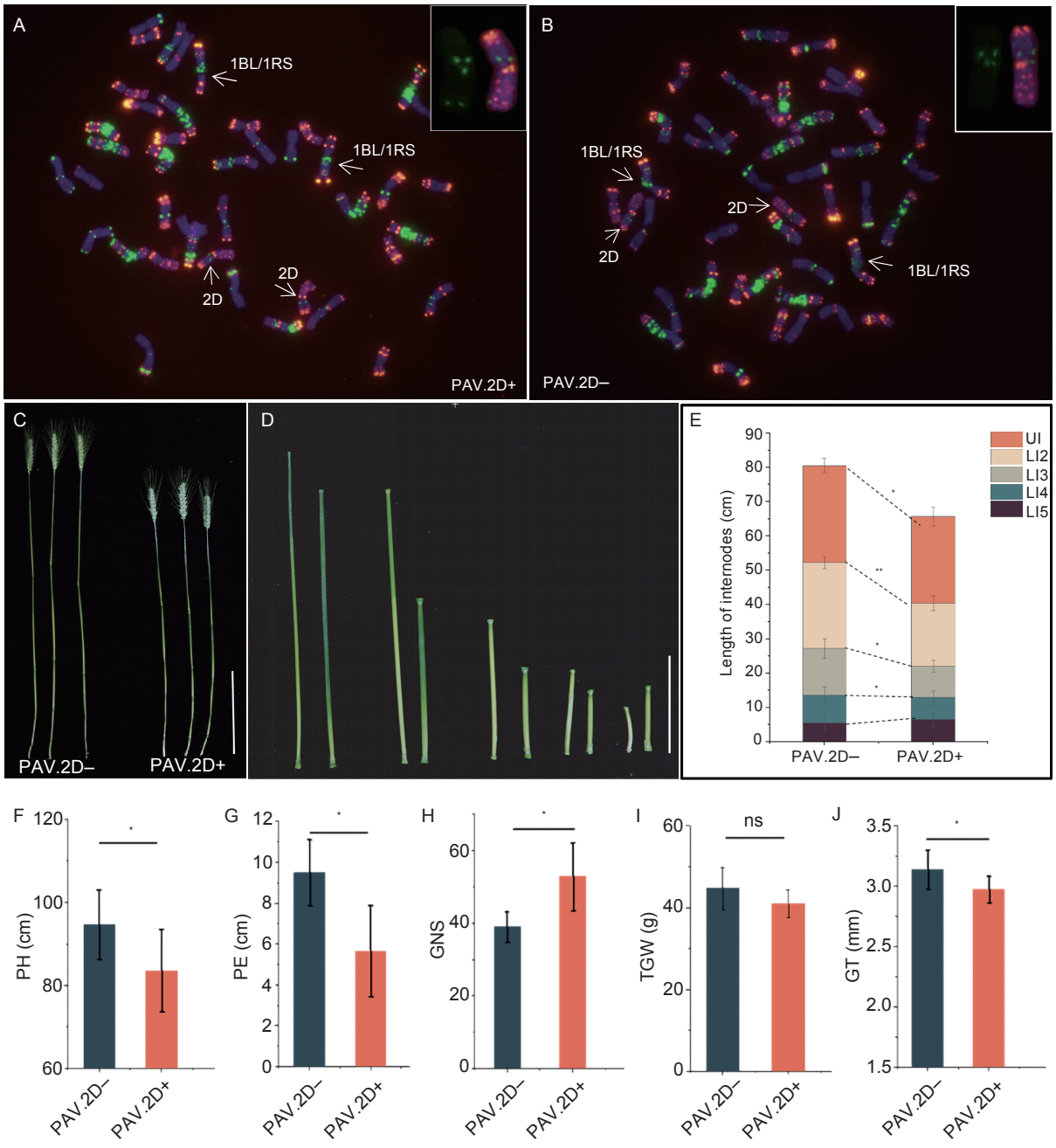


Fig. 2 Karyotype and agronomic traits in introgression lines. A and B, oligonucleotide multiplex *in situ* hybridization (FISH) karyotype of introgression lines (ILs). C, stem of ILs derived of *PAV.2D*, bar=20 cm. D and E, length of panilce exsertion and internodes of ILs at mature stage. Bar=10 cm. F–J, phenotypic differences between lines with allelic variations for *PAV.2D*. UI, the lengths of the uppermost; LI2–LI5, second, third, fourth, and fifth internodes from the top. PH, plant height; PE, panilce exsertion; GNS, grain number per spike; TGW, thousand-grain weight; GT, grain thickness. Bars mean SD (n=3). * and **, significant differences at P<0.05 and P<0.01 by the t-test, respectively; ns indicates no significant difference.

component traits (Appendix G).

The effect of 1BL/1RS and PAV.2D for PH and LI2–LI4 were validated in panel-2, and their additive effects were analyzed (Fig. 3-B). The combination of 1BL/1RS and PAV.2D had the largest effect on PH and on LI2–LI4. Compared with lines lacking positive alleles for decreasing PH, the positive allele from PAV.2D significantly decreased PH by 18.29%, which was higher than that for the single positive alleles of 1BL/1RS (15.20%). Also, the effects of PAV.2D on LI2, LI3, and LI4 were greater than that of 1BL/1RS. Additionally, lines with both 1BL/1RS and PAV.2D positive alleles significantly decreased PH (20.14%, 21.75 cm), LI3 (25.75%, 4.63 cm), and LI4 (30.35%, 4.10 cm) more than that did 1BL/1RS alone for PH (15.30%, 16.53 cm), LI3 (17.87%, 3.20 cm), and LI4 (19.39%, 2.62 cm) (Fig. 3-B). However, the combined effects of them did not differ significantly from the effect of PAV.2D alone (Fig. 3-B). It indicated that the positive allele of PAV.2D from Jinmai 84 had the larger effect on PH. These results validated the effectiveness of accumulation beneficial SCV for trait improvement.

3.9. Chromosomal mapping of SCV

To understand the effects of SCVs on genome diversity, genetic differentiation was performed using genotype data for 10 chromosomes in the DHs, which were categorized based on the presence/absence of SCVs. FISH polymorphism signals of 1A, 2A, 4A, 2B, 4B, and 2D showed clear population differentiation in the genome, of which SNP differentiation regions corresponded to FISH polymorphic signal chromosome sites (Appendix H). The highest Fst values on chromosome 2A occurred at 5.26–23.49 Mb and 631.69–705.41 Mb. Other SCVs segments with SNP differentiation were concentrated to 579.08–582.96 Mb (1A), 720.79–733.80 Mb (4A), 682.24–750.99 Mb (2B), 530.01–639.96 Mb (4B) and 599.09–609.76 Mb (2D), respectively (Appendix H). The absence of such differentiation for chromosomes 6B and 1D, may be due to only a small segment differentiating the two polymorphic signals and a low marker density in the polymorphic region.

3.10. QTL analysis on SCV polymorphic segment

A total of 26 QTL for PH, PE, SL, UI, LI2, LI3, LI4, TGW, and GT were detected on polymorphic segments of five chromosomes (2A, 4A, 2B, 4B, and 2D) with 12 stable QTLs were identified (Appendix I). Those stable QTLs were found on chromosomes 2B, 2D, 4B, and 4A (Appendix I). The QTL explained 1.79–19.23% of the phenotypic variation among different environments. Four stable QTLs were identified in more than three environments, *QTGW.saw.2B.1*, *QTGW.saw.2B.2*, *QPH.saw.2B*, and *QPE.saw.2B*, explaining 1.79–12.19% of the TGW, PH, and PE variance, respectively. The results for TGW and PE were consistent with the association analysis of PAV.2B in DH population (Table 2; Fig. 4-A). Additionally, the PAV.2B region is enriched for genes *TaSTT3b-2B* (774.952–774.959 Mb) related with grain weight (Zhu *et al.* 2022) and *TaARF12-2B* (766 Mb) (Li K *et al.* 2021) for plant height, spike length, and grain weight. QTL *QTKW.co-2B* was also identified in this region (Liu *et al.*

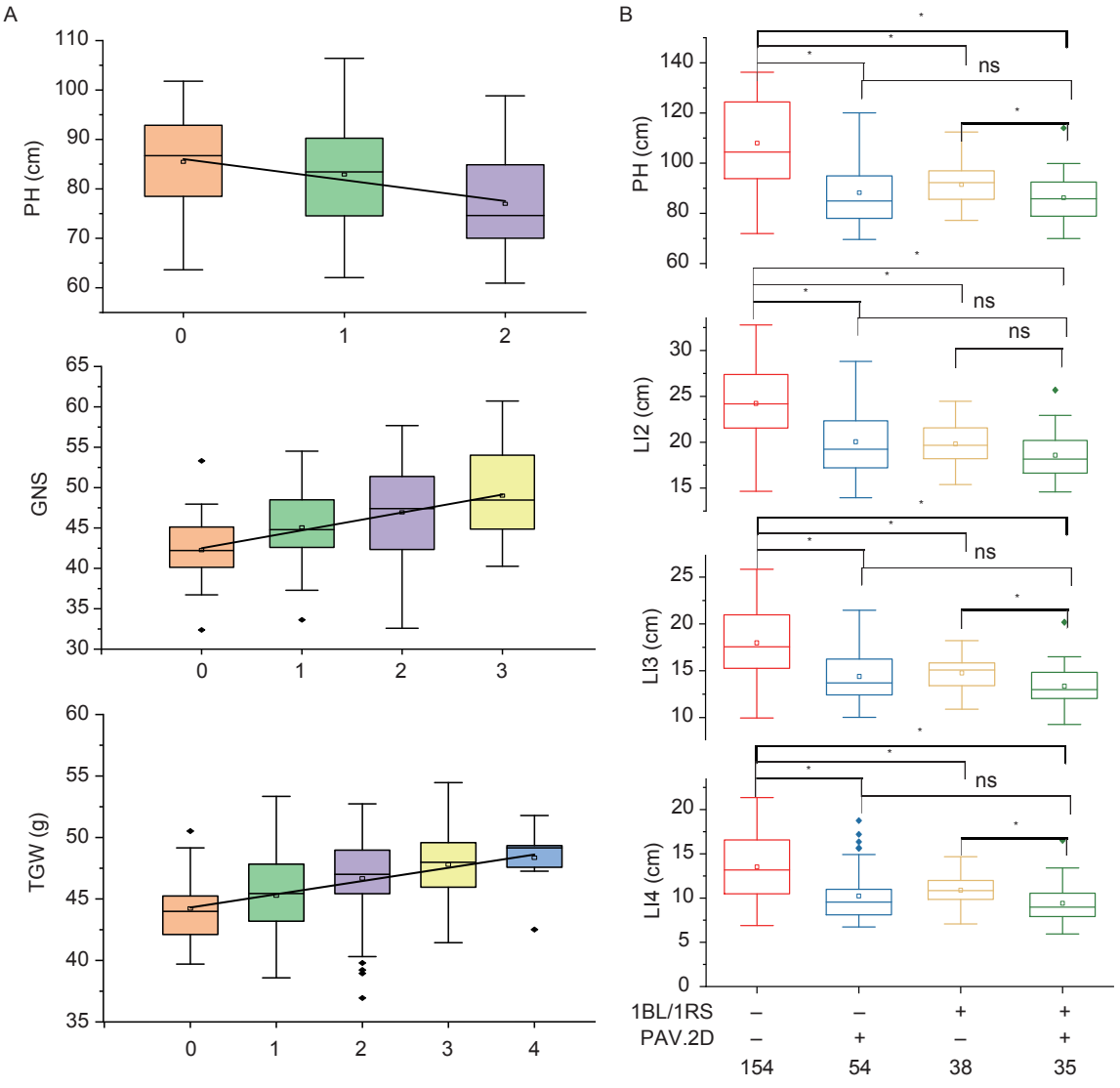


Fig. 3 Linear regressions between the number of structural chromosome variations (SCVs) and plant height (PH), grain number per spike (GNS), and thousand-grain weight (TGW) in the doubled haploid (DH) population (A) and additive effects of the SCVs for PH-related traits in the natural population (B). LI2–LI5, the lengths of the second, third, fourth, and fifth internodes from the top. Bars mean SD (n=3). *, significant difference at P<0.05 by the t-test; ns indicates no significant difference.

2019). Segments QTL chromosome 2D showed the same phenomenon for PH related traits (Fig. 4-B; Appendix I), with this region enriched the plant height genes *TaARF12-2D* (Li *et al.* 2022) and grain weight gene *TaSTT3b-2D* (Zhu *et al.* 2022). No stable QTL for GNS and TGW were detected on the polymorphic segment of 2D, but the yield-related traits QTL *QKNS-2D-AN* (Shi *et al.* 2017) and *QTkw-2D.2* (Cui *et al.* 2014) occur on 2D, but *QGl.cau-2D.1* is a stable QTL for grain length (GL) on chromosome 2DS (Wang *et al*. 2024). Additionally, *Qdtc_SL.saw.2D*, *Qdtc_gns.saw.2D*, and *Qdtc_LI5.saw.2D* were co-localized with PAV.2D identified in the DH population (Appendix I). Thus, the polymorphic region of PAV.2D is enriched with functional genes. For the phenotypic effect of CNV.4B, two table QTLs, *QSL.saw.4B* and *QLI2.saw.4B* were identified for SL and LI2, explaining 3.84–15.88% and 4.42–6.11% of the phenotypic variance, respectively (Fig. 4-C). The genes *SVP3-4B/BM1-4B* for wheat spike, spikelet development/plant height (Li K *et al.* 2021) and *TaSRL1-4B* for plant height (Zhuang *et al.* 2021) are found in this region (Fig. 4-C). In the present study, we found PAV.4A.1+2 influenced GL, and no stable QTL were identified in the region of 720.79–733.80 Mb. *QSL.saw.4A* was identified in 672.5–684.0 Mb, explaining 4.49–8.07% of the phenotypic variance (Appendix I). In our previous research, the effect on SL was detected in PAV.4A.1 located within the 654.44–744.49 Mb with 90K SNP (Zhao *et al.* 2023). These results demonstrated the phenotypic effect of SCVs may be due to enrichment of specific genes within that region or nearby.

4. Discussion

4.1. Generation and effect mechanism of structural chromosome variation

The types of structural variation are abundant, including PAV, CNV, inversions, translocations, and more complex rearrangements, and widely distributed in plants, animals and microorganisms. Various cellular mechanisms can trigger generation of SV during meiotic or mitotic cell division. SV events are caused by recombination errors, like non-allelic homologous recombination (NAHR), DNA break repair errors, such as non-homologous end joining (NHEJ), or replication errors, including fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR) (Gabur *et al.* 2019). And changes in ploidy can also lead to generation of SV in plants. In plants, exogenous agents such as ionizing radiation or chemotherapy can invoke DNA double-strand breaks (DSBs), affecting DNA replication, and it also affect the cell division cycle and change the DNA methylation level, resulting in chromosomal variation. For example, a series of chromosome variants were constructed by using zebularine, a DNA methylation transferase inhibitor, in the octoploid triticale cultivar Jinghui 1 (Ma *et al.* 2016).

Large SV events can also be visualized directly at the chromosome level using molecular cytogenetic techniques such as FISH and genomic *in situ* hybridization (GISH). For example, Wu *et al.* (2024) performed FISH analyses on the mitotic metaphase spreads of homozygous early and late heading individuals, showing a large fragment of around 1–34 Mb is missing from the 2DS terminus in the *m406* mutant. Sequence recombination events in hybrid progeny can also be clearly detected by FISH analysis (Zou *et al.* 2021; Zhao *et al.* 2023). Additionally, at the present study, the 2A of some DH lines showed FISH signal patterns different from those of parents, perhaps because the recombination rate variation in the PAV.2A.1 and PAV.2A.2 differs or genes controlling recombination of homologous chromosomes during meiosis. Previous studies have indicated a correlation between crossover regions and gene-rich region in wheat (Gabur *et al.* 2019).

The spectrum of wheat genetic variation ranges from the single base pair to large chromosomal events. SCV is an important source of genetic variation that causes changes in linear structure at the chromosome level, leading to phenotypic changes. First, SCV results in the truncation and inactivation of genes. Classes of structural variations were linked to distinct DNA repair deficiencies, suggesting human cancerous etiology, such as including associations of the *CDK12* mutation with tandem duplications, *TP53* inactivation with inverted rearrangements and chromothripsis, and *BRCA2* inactivation with deletions (Quigley *et al.* 2018). Second, CNV and chromosome position exchange induced changes in gene expression. A higher copy number of the *Bot1* gene conferred boron tolerance in barley variety Sahara, and mRNA expression of this gene in Sahara were substantially higher than that in the variety Clipper (Sutton *et al.* 2007). In wheat, two copies of *Rht-D1b* in a tandem segmental duplication were three-fold more effective in reducing plant height than a single copy (Li *et al.* 2012). Crow *et al*. (2020) used two maize near-isogenic families containing a 13 Mb inversion locus *Inv4m* to detect the gene expression in nine tissues related to the inversion, that the inversion induces *cis*-acting gene expression and produced a variety of physiological effects. RT(2DL;4AL)-ter/2 in wheat induced substantial changes in gene expression primarily *via* trans-regulation, which increased in plant height and spike length compared with rev-WT (Lv *et al.* 2023). Third, SCV may be introduce or produce new genes. For example, in human cancers, SVs create fusion genes that encode novel oncogenic proteins. Gene fusion of *PVT1* with its neighboring gene *MYC* is most common in 8q24-amplified cancers (Jin *et al.* 2019). In wheat, the 1BL/1RS translocation is widely used in wheat breeding with 70% of the varieties of China developing in past two decades contain it. This translocation is valuable because 1RS contains genes for increased biomass (unpublished), root length development genes and drought tolerance genes (Gabay *et al.* 2023), and the disease resistance gene cluster (*Yr9*/*Pm8*/*Lr26*/*Sr31*) (Mago *et al.* 2005; Hsam and Zeller 2006; Han *et al.* 2020). In addition, SVs can also cause changes in the state of euchromatin and heterochromatin, thereby interfering with gene expression through epigenetics. Studies of homology dependent repair have determined that, following repair, DNA methyltransferases have been found to localize at DNA repair sites, specially CpG methylation, which will alter the expression of genes that promote tumorigenesis (Allen *et al.* 2017). In *Arabidopsis*, genome-wide DNA methylation is characterized by high methylation in heterochromatin,

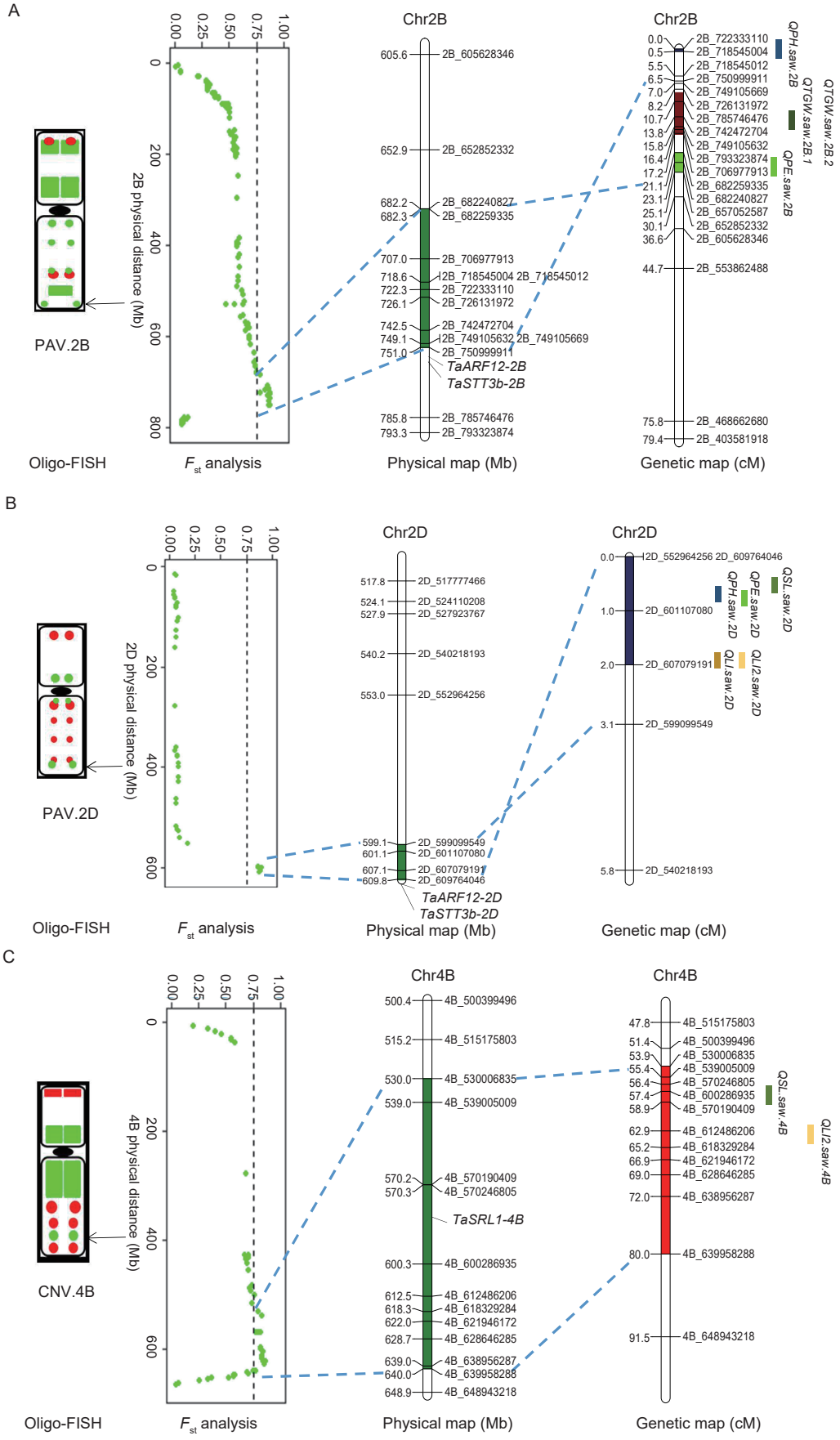


Fig. 4 Stable quantitative trait loci (QTLs) detected for agronomic traits on differential segments of chromosomes 2B, 2D, and 4B. FISH, fluorescence *in situ* hybridization.

causing genes in heterochromatin to be often in a repressed and inactive state, and these genes are mostly related to stress resistance to adaptive traits (Arora *et al.* 2022). Whether SCV also acts on phenotypic variation through other means remains to be further explored.

The present study found that SCV affects agronomy and has a regulatory effect on wheat drought resistance. Further analysis of QTL in these SCV polymorphic loci detected QTL/genes controlling the corresponding traits (Fig. 3; Appendix I). We infer that the genetic effect of these SCVs may involve the loss of genes. The pleiotropic and interaction effects present among SCVs (Table 1; Appendix F) may be due to change in gene expression, but this hypothesis must be verified. Further work will combine resequencing and transcriptome analysis to determine the SCV segment location and regulatory mechanism.

4.2. Phenotype diversity contributed by plant SVs

SV is a type of genetic variation identified through the comparison of genome structures which often have direct and significant associations with phenotypic variations (Zhang X *et al.* 2018). For example, a SV in *POP2* improved lint percentage of cotton by 15.23% (Jin *et al.* 2023). SVs in tomato affected fruit size and flavor by altering gene expression (Alonge *et al.* 2020). In rice, CNV at the *GL7* locus was associated with grain length (Wang *et al.* 2015). Montenegro *et al.* (2017) used 18 wheat cultivars to identify PAVs associated with important agronomic traits, including response to environmental stress and defense response. Many SCVs have been identified and conducted genetic diversity analysis in wheat (Huang *et al.* 2018; Hu *et al.* 2022), but few studies have been conducted to characterize the role of SVs in shaping yield composition.

Previous research detected 139 SCVs at 230 loci using oligo-FISH, 29 of which were significantly related to agronomic traits (Zhao *et al.* 2022). These demonstrated SCV was involved in the regulation of wheat agronomic traits. Eight SCVs identified in the present study *via* the DH population were significantly associated with 15 yield-traits, with positive and negative effects ranged from 0.59 to 31.96% and effects on each component of yield traits were analyzed. For example, we found that 1BL/1RS and PAV.2D can significantly reduce PH, the latter effect was larger. Subsequent analysis of the components of PH revealed that LI2, LI3, and LI4 were reduced, accounting for most of their effect on plant height. 1BL/1RS can significantly increase SN, GL, and GT, consistent with previous studies that found 1BL/1RS had a positive effect on grain yield traits (Villareal *et al.* 1995; Howell *et al.* 2014). PAV.2A.1 was not significantly related to 16 agronomic traits using the DH populations. However, Mg2A-3 (PAV.2A.1) significantly reduce TGW and GT in DH populations from Linfen 5064/Jinmai 47 in a previous study (Zhao *et al.* 2022), possibly due to an interaction effect with 1BL/1RS. This idea is supported by the finding that PAV.2A.1 also reduces GT significantly in the absence of 1BL/1RS in the background in DHs from Jinmai 47/Jinmai 84 (Fig. 2-C). In summary, this study confirmed the genetic effect and potential utility of SCV for modern molecular wheat breeding.

Multiple genes/SCVs control the same trait with additive effects. A 1.2-kb PAV and S35 in *UB3* were significantly associated with kernel row number, and the favorable haplotype of 1.2 kb-PAV-S35 was selected during maize improvement (Liu *et al.* 2015). In wheat, additive genetic interaction was detected in the action of *TaGW2-B1* and *-D1* for grain weight, and the increase in TGW was the highest in *gw2kn-b1d1* (10.40–13.14%), intermediate in *gw2kn-b1* (5.73–12.04%), and relatively low in *gw2kn-d1* (1.18–8.74%) (Zhang Y *et al.* 2018). In the present study, lines both with 1BL/1RS and PAV.2D had significantly reduced PH compared to each SCV alone and to NC. The GNS of genotypes with the superior alleles of PAV.2A.1+2, PAV.2B and PAV.2D increased by 6.74, and lines containing the four superior allelic SCV variants showed a 9.48% increase in TGW (Appendix E). Thus, increasing the numbers of favorable SCVs alleles can enhance agronomic traits. Favorable SCVs with additive effects could be used in marker-assisted selection in breeding programs. Furthermore, in plant breeding, manipulation of a single gene typically results in changes to several phenotypic traits. Overexpression of H^+-ATPase results in enhanced light-induced stomatal opening, photosynthesis, and plant growth in *Arabidopsis* (Wang *et al.* 2014). Plants with favorable traits such as disease resistance, good grain quality, and high yield can also carry unwanted characteristics such as excessive height, and late heading and maturity. Such multiple effects of SCVs were also observed in the present study (Table 2; Appendix G). For example, PAV.2B had opposing effect on TGW and GNS (Appendix G). Thus, when seeking to improve a single trait through breeding effects on other agronomic traits must be considered.

4.3. SCV significantly affect drought tolerance

Wheat is grown in regions of the world that are most susceptible to drought (Mao *et al.* 2022). More frequent drought events due to global climate change in already arid growing regions pose an ongoing threat to wheat production (Hickey *et al.* 2019), which had led to greater focus on drought-tolerance in wheat. However, as a complex trait, research of drought resistance has focused primarily on changes in agronomic traits and different drought resistance indicators (Baidyussen *et al.* 2024). Current studies have mostly sought to mine QTL/genes through linkage and association analysis at the nucleic acid level (Li X R *et al.* 2021; Guo *et al.* 2023), such as *TaPYL1-1B*, *TaNAC071-A* (Mao *et al.* 2022), and *TaMPK3* (Liu *et al.* 2022). Various SVs have been produced during wheat evolution and domestication, and these can affect adaptation to biological and abiotic stresses. Mahmoud *et al.* (2020) found SVs significantly affect glyphosate tolerance in maize and PAV was observed in 23–51% of the genes within confidence intervals of a QTL for *Verticillium longisporum* resistance in *Brassica napus* (Gabur *et al.* 2020). Lines containing a paracentric inversion in *Arabidopsis thaliana* have strong fecundity under drought stress (Fransz *et al.* 2016). A *de novo* genome assembly of *Solanum sitiens* revealed three inversions associated with drought tolerance (Molitor *et al.* 2021) and in grape 26 CNVs containing genes were involved

in pathways of relevance in the response to abiotic stresses and drought (Catacchio *et al.* 2019). Our previous study found that the PAV variation on chromosome 7B was related to wheat drought resistance (Zhao *et al.* 2023). These results indicated that SCV is involved in the regulation of drought resistance. Thus, by using different drought stress treatments, the present study found that PAV.2A.2, PAV.6A, CNV.4B, and PAV.1D are involved in the regulation of drought tolerance coefficient. Among them, PAV.6A and CNV.4B showed a positive effect, while PAV.1D and PAV.2A.2 had a negative effect. These results enrich knowledge of SCVs related to drought resistance and also provide a new perspective on the genetic characteristics of drought resistance.

5. Conclusion

One simple translocation, 10 present/absent variations, and one copy number variation were detected between Jinmai 47 and Jinmai 84, which distributed in 10 chromosomes. Eight SCVs were associated with agronomic traits of wheat. We confirmed the effect of PAV.4A.1 on grain length, PAV.6A on spike length and TGW, PAV.6B on spike length, GT, and TGW, and 1BL/1RS on PH, SN, GL, GT, and PAV.2D on PH, GNS, GT. Also PAVs on chromosomes 2A, 6A, 1D, 2D, as well as CNV on chromosome 4B were associated with the drought tolerance. Many previously cloned key genes and related QTL were found in polymorphic regions of PAV.2B, PAV.2D, and CNV.4B. Those identified SCVs will facilitate genetic improvement of wheat through marker-assisted selection.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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