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Title

Pre-breeding of spontaneous Robertsonian translocations for density planting architecture by transferring *Agropyron cristatum* chromosome 1P into wheat

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Abstract

Agropyron cristatum, a wild relative of wheat, possesses many elite genes for enlarging the genetic diversity of wheat and improving wheat yield. Our previous study confirmed that the *A. cristatum* chromosome 1P carries alien genes that reduce plant height and leaf size. Here, we developed T1AL.1PS and T1AS.1PL Robertsonian translocations (RobTs) by using breakage-fusion mechanism based on wheat-*A. cristatum* 1P(1A) substitution line. Combining molecular markers and cytological analysis, we identified 16 spontaneous Robertsonian translocation lines from 911 F_2 individuals with a translocation frequency up to 1.7%. Fluorescence in situ hybridization (FISH) was applied to detect the fusion structures of the centromeres in wheat and *A. cristatum* chromosomes. Re-sequencing results indicated the junkpoint at the physical position of *Triticum aestivum* chr1A 212Mb and *A. cristatum* chr1P 230Mb. Genomic in situ hybridization (GISH) results of pollen mother cells showed that the produced translocation lines could form stable ring bivalent. The genetic analysis showed that introducing 1PS translocation fragment into wheat could increase the number of fertile tillers, grain number per spike and grain weight, and reduce flag leaf area of wheat without yield penalty. However, introducing 1PL translocation fragment into wheat reduce significantly flag leaf area and plant height with adverse effect on the yield components. Altogether, a high-efficient method for producing the spontaneous translocation lines by combining the molecular marker and cytogenetics technology were recommended. The pre-breeding of two spontaneous RobTs T1AL.1PS and T1AS.1PL pave the way for the wheat architecture improvement.

Key words: Wheat, *A. cristatum,* 1P, translocation, plant architecture

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

Data and materials supporting the current study can be obtained by contacting the corresponding authors.

Code availability

Not applicable

Author contributions

Bohui Han and Xiao Wang have contributed equally to this work.

LHL and JPZ conceived the research. BHH performed the research and wrote the paper. XW analysed the data. YYS, XLK, MZ and JWL collected the data, and HMH, SHZ, YQL, WHL, XMY, and XQL participated in the preparation of the reagents and materials in this study. All authors contributed to and approved the final manuscript.

Key message

We developed T1AL.1PS and T1AS.1PL Robertsonian translocations by using breakage-fusion mechanism based on wheat-*A. cristatum* 1P(1A) substitution line with smaller leaf area, shorter plant height and other excellent agronomic traits.

Introduction

Common wheat (*Triticum aestivum L*., 2n=6x=42, AABBDD) is the most widely cultivated crop and is one of the most important foods on Earth (IWGSC 2018; Li et al. 2019; Rahmatov et al. 2016). With the growth of population, wheat production must increase by more than 50% over current levels by 2050 to meet demand (Mottaleb et al. 2023). However, the long-term improvement of wheat varieties was mainly limited in the genetic recombination between regular varieties, which led to the exploitation and erosion of the genetic variation available and the decrease of species diversity (Qi et al. 2007; King et al. 2022). An effective means of increasing wheat yield is broading its genetic diversity by transferring the alien genes of wild relatives into wheat (Bohra et al. 2022; Zhang et al. 2015). *A. cristatum* (2n = 4x = 28, genomes PPPP), a tertiary gene pool of wheat improvement, provides a vast and untapped reservoir of genetic variation for agronomic traits of wheat. In addition to its resistance to biotic and abiotic stresses, *A. cristatum* carries many useful traits for improving wheat yield such as high floret numbers and tillers (Dong et al. 1992; Li et al. 1995). In our recently published study, we found that *A. cristatum* 1P addition line also reduce plant height and leaf area which were helpful for wheat plant architecture suitable for dense planting and increasing yield (Wang et al. 2022). But addition lines may carry more deleterious effects and doesn't stably inherited to offsprings. Hence, the development of translocation is an effective pathway for transferring and utilizing the alien genes from *A. cristatum* into wheat.

On account of translocation lines generally carry smaller alien segments, they are genetically more stable and less likely to have deleterious effects (Cai et al. 1998; Faris et al. 2008; Jiang et al. 1993). The use of substitution lines to induce the whole arm RobTs proved to be an effective way to produce translocation line. The whole arm translocation lines created by spontaneous translocation have better compensation effect and genetic balance. Alien chromosomes in whole arm translocation lines can compensate for the loss of homoeologous wheat chromosomes. Breakage-fusion mechanism induced by substitution line can produce spontaneous RobTs (Friebe et al. 2005). Centric misdivision followed by the fusion of the broken arms from different chromosomes results in whole-arm RobTs (Robertson 2005) which played a significant role in karyotype evolution (Friebe et al. 2005). It is reported that RobTs generally occurs between some homoeologous chromosomes, which has the advantages of good compensation and is easier to be used in wheat breeding. A successful case is the widespread application of T1BL.1RS in wheat breeding due to its remarkable yield potential and resistance to disease (Lukaszewski, 1993; Friebe et al. 1996; Rabanus-Wallace et al. 2021; Villareal et al. 1991). In addition,

Rahmatov et al. (2016) used breakage-fusion mechanism of univalent chromosomes to produce a new RobTs T2DS·2RL with stem rust resistance gene *Sr59* and the kernel protein content of translocation includes segment 2DS and 2RL has a significant improvement (Jagannath and Bhatia 1972; May 1978; Zeller and Koller 1981a). Ghazali et al. (2015) produced wheat–*Thinopyrum bessarabicum* whole arm RobTs T2E^{b}S.2BL. Li et al. (2020) created a spontaneous Robertsonian T4S^{l}S.4BL translocation chromosome carrying *Pm66* for powdery mildew resistance. Türkösi et al. (2018) developed a new T7BS.7HL RobTs conferring increased salt tolerance and (1,3;1,4)-β-D-glucan content. Liu et al. (2016) developed a set of 13 compensating wheat-*Ae. speltoides* RobTs covering the S genome of *Ae. speltoides* except for the long arm of chromosome 4S. Qi et al. (2021) identified T6DS.6PL and T6PS.6DL translocation lines from wheat-*Agropyron cristatum* 6P(6D) substitution line which could improve grain weight.

Previously, we reported that II-3-1c was identified as wheat-*A. cristatum* 1P (1A) disomic substitution line with hair glume and reduced plant height phenotypes (Pan et al. 2017). Recently, we proved that the introgression of *A. cristatum* chromosome 1P into wheat can improve plant architecture, including reducing plant height and leaf size of wheat (Wang et al. 2022). In this study, we reported development and identification of two types of heritable whole arm translocation lines T1AL.1PS and T1AS.1PL from substitution line II-3-1c and evaluated their genetic effects of the alien translocation segment through genetic segregation population. These RobTs provide us new germplasm resources for wheat architecture improvement.

Methods

Plant materials

The development of RobTs was conducted on an F_2 population, derived from common wheat *cv*. Jimai22 and a wheat-*A. cristatum (L.)* 1P(1A) disomic substitution line II-3-1c (2n=40W+2P). Jimai22 is an elite commercial wheat cultivar with high yield, wide adaptability with the largest area in China. II-3-1c is a 1P(1A) disomic substitution line from the offspring of wheat-*A. cristatum* derivative II-3-1(Pan et al. 2017). Secondary segregating population of F_3 and F_4 were originated from the heterozygous individuals of 1PS and 1PL RobTs.

Strategy for producing RobTs

In order to produce compensating RobTs involving chromosomes 1A of wheat and 1P of *A.cristatum,* crosses were made between common wheat cv. Jimai22 and II-3-1c disomic substitution line. The F_1

plants were selfed and produced 911 F_2 seeds. The F_2 and F_3 progeny were genotyped by molecular markers for putative RobTs (Fig.1). These cytogenetic materials were developed and maintained at National Crop Genebank in Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.

Molecular markers analysis

In order to identify the translocated F_2 individual plants, we firstly use the two pairs of 1PS and two pairs of 1PL EST-STS markers specifc for the *A. cristatum* P genome chromosome arm in the wheat background to test the translocation lines or telosome lines (Table 1). The target F_2 positive individuals were grown F_3 family for further identification of EST-STS markers. According to the rate of positive plants in F_3 family, we selected those F_3 family for cytogenetics identification. Total genomic DNA was isolated from fresh leaf tissue using the CTAB protocol (Zhang et al. 2017). To distinguish the homologous, heterologous and negative plants, we developed the codominant markers including EST-STS markers and KASP markers (Table 1). These co-dominant markers were used for tracking the alien translocation fragment and genotyping in the genetic analysis.

SNP markers have been applied to high-throughput SNP genotyping arrays (Grewal et al. 2020) which are used to test $F_{3:4}$ and $F_{4:5}$ segregation population of 1PS translocation lines. Orthologous CDS sequences from diploid *A. cristatum* z1842 genome assemble and wheat cv. Chinese Spring reference genome assembly RefSeq v1.0 were aligned to identify putative interspecific SNPs. BLASTN was used for searching the interspecific SNP locus to design the KASP makers. The primer design procedures were followed by Grewal et al. (2020) with some slight modifications. The KASP markers primer sequences are showed in Table 1. Each KASP PCR is made up of 50ng of DNA template and 2.5μl of HiGeno 2× Probe Mix B (JasonGen Biotech Co., Ltd., Beijing, China) and 0.07μl SNP-Specific Primers. Thermal cycling conditions were 95°C for 10 min; followed by 10 cycles of touchdown PCR: 95°C for 20s, 61°C-55°C for 40s (drop 0.6°C per cycle); and finally 28-34 cycles of regular PCR: 95°C for 20s, 55°C for 40s.The fluorescence reading were performed on BMG LABTECH microplate reader. EST-STS markers of *A. cristatum* chromosome 1P were developed based on non-denaturing polyacrylamide gels according to the method of Zhang *et al.* 2017.

Cytogenetic analysis

To visualize P-genome chromatin in descendant of the 1P(1A) substitution line II-3-1C, genomic in situ hybridization (GISH) was operated in root tip cells using whole genome DNA from *A. cristatum*

labelled by nick translation with Texas Red-5-dCTP (PerkinElmer, Boston, MA), and use Fukuho genomic DNA as blocker(Han et al. 2015). Non-denaturing fluorescence in situ hybridization (ND-FISH) use oligonucleotide probes (oligo-pSc119.2-1 and oligo-pTa535-1) to identify which chromosome fragment of wheat was translocated on the 1P chromosome). The centromere was analysed by FISH use two centromeric sequences oligo-pAcCR1 and oligo-CCS1 as probes (Sun et al. 2021; Han et al. 2017). The root tips of all the materials in this study were excised when these roots grew to 1.5-2 cm and were disposed with nitrous oxide gas and 90% acetic acid (King et al. 2017; Sun et al. 2021).

In order to observe the meiotic behavior of the translocation lines we need to collect anthers containing pollen mother cells at the wheat booting stage. The anthers were disposed in Carnoy's Fluid (1:3 (v/v) acetic acid/ethanol) and save them in 70% ethanol for 48 hours (Megyeri et al. 2013). Anthers with appropriate stages were squashed in 45% acetic acid and viewed under phase contrast and dispose it on the surface of liquid nitrogen for 1min (Jauhar and Peterson 2006; Li et al. 2000). All slides were observed by Zeiss Axio Imager Z2 upright epifluorescence microscope (Carl Zeiss Ltd, Germany) and captured with a CCD camera. The period of fetch the root tip is of vital importance, should cut it off when it grow to 1.5-2cm and dispose with nitrous oxide gas and 90% acetic acid (King et al. 2017). The GISH and FISH were followed by previously described with some slight modifications (Fu et al. 2015; Han et al. 2006; Li et al. 2021; Sun et al. 2021).

Junction regions identification by using re-sequencing technology

To explore junction regions of segments in fine detail, we re-sequence the homozygous T1AL.1PS and T1AS.1PL translocation lines. Re-sequence data was used for characterising *A. cristatum* introgressions into wheat based on comparative mapping depths. By mapping raw reads of T1AL.1PS and T1AS.1PL translocation lines to a combined reference genome made up of the pseudomolecules of *A.cristatum* Accession No. z1842 (unpublished) and CS Refseq V1.0, we can get accurate translocation breakpoints according to the sequencing depth and the reads distribution. Genomic DNA was extracted using standard methods. The genomic DNA sample was fragmented by sonication to a size of ~350 bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adapters for Illumina sequencing with further PCR amplification. Subsequently, we used the Illumina Novaseq platform to generate 66.8Gb and 74.4Gb raw sequences with a 150bp read length. After sequence quality checking and filtering, we retained 62.6Gb and 69.8Gb high quality genomic data for sequence alignment. The remaining high quality paired-end reads were mapped to the CS reference genome using Burrows-

Wheeler Aligner software (Li et al. 2009) with the command 'mem -t 4 -k 32 -M'. In order to reduce mismatches generated by PCR amplification before sequencing, duplicated reads were removed using SAMtools (v0.1.1) (Li et al. 2009). Reads depth of each genome site was calculated by SAMtools (v0.1.1) (Li et al. 2009) with the command 'samtools depth'. Then average values of reads depth were estimated for 1-Mb sliding windows with a 1-Mb step size along each chromosome. Based on the average values, we create fit curve using the R package ggplot2 (Wickham, 2016).

Evaluation of agronomic traits

$F_{3:4}$ and $F_{4:5}$ family segregation population of heterozygous T1AL.1PS and T1AS.1PL translocation plants were planted in the experimental station of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Xinxiang, Henan Province during the 2020-2021 and 2021-2022 sowing seasons. The plant density was 30cm by 10cm with a 2m length. Leaf length and width of flag leaf, top second leaf, top third leaf, plant height, fertile tillering number, spike length, total spikelet number, kernel number per spikelet and grain number per spike were measured for each individual plant.

Results

The development of the translocation lines

In recent study, we have proved that the common wheat-*A.cristatum* 1P addition line carry genes that reduce the size of flag leaves. To promote the application of chromosome 1P in wheat enhancement, it is necessary to produce genetically stable translocation lines. In this study, we took substitution line 1P(1A) II-3-1c as a donor parent and got the F_2 segregation population by crossing substitution line II-3-1c with commercial wheat cv. Jimai22. We attempt got the spontaneous RobTs based on the breakage-fusion mechanism induced by substitution line. In order to identify high efficiently probable translocation lines in F_2 individuals, we screened 7 EST-STS markers and 2 KASP markers distributed on *A. cristatum* chromosome 1PS and 1PL for detecting rapidly the F_2 individuals (Table 2). As a result, a total of 911 F_2 plants were screened, 32 plants missing the short arm and 28 plants missing the long arm chromosome 1P specific markers were identified. To reduce the cytogenetics identification works, we continue tracking the segregation feature of F_3 family lines correspond to 60 F_2 plants by using molecular markers. In view of the transmission rate of translocation fragments is higher than 50%, we kept the positive ratio greater than 50% plants in F_3 family to do the GISH analysis for isolating translocation lines. Finally, we identified 9 T1AL.1PS and 7 T1AS.1PL spontaneous compensating wheat-*A. cristatum* RobTs. The frequency of recovered RobTs is 1.77%. The efficiency of identification has up to 26.7% in terms of the

positive plants after P-chromosome-specific molecular marker detection. Therefore, the study provides a highly efficient method for identifying the RobTs by combing the molecular marker detection and cytogenetics analysis.

Cytogenetic analyses of RobTs

Based on the marker results, 60 F_3 families were identified as potential wheat–*A. cristatum* RobTs. In order to identify the chromosome composition of the F_3 family, GISH analysis was employed for the cytogenetics detection for these plants missing short arm markers or long arm markers. The result showed that 9 F_3 family are short arm RobTs, 7 F_3 family are long arm RobTs and the remaining plants are telosomic addition lines (Table 2). The translocation lines identified by GISH analysis were listed in Table S1 and the agronomic traits of them were showed in Table S2. GISH/FISH analysis revealed that there are 42 chromosomes in the root tip somatic cells of these RobTs. The GISH/FISH pattern showed that 3 and 6 short arm translocation lines was homozygous and heterozygous for the compensating RobT T1AL.1PS, respectively (Fig.2a and Fig.2c) and 4 and 3 long arm translocation lines was homozygous and heterozygous for the compensating RobT T1AS.1PL, respectively (Fig. 2b and Fig. 2d).

To identify homologous chromosome pairing behavior in metaphase I of meiosis of RobT lines T1AL.1PS and T1AS.1PL, we sampled the pollen mother cells of young spikes and carried out GISH analysis. We found that 42 chromosomes paired as 21 bivalents at meiotic metaphase I and the translocation chromosomes formed a stable ring bivalent (Fig.2e and Fig.2f). The cytogenetics results suggested that the translocation chromosome arms of *A. cristatum* 1PS and 1PL can be stably inherited.

Whole genome sequencing uncovers the junction point of translocation

To reveal the junction point of translocation between *A. cristatum* chromosome 1P and wheat chromosome 1A, we conducted whole-genome sequencing (WGS) of RobT lines T1AL.1PS and T1AS.1PL, and have precisely pinpointed the borders of translocation. The wheat reference genome RefSeq v1.1 and the diploid *A. cristatum* assembly were integrated to form a combined reference genome and Illumina paired-end reads from the translocation lines were mapped to this mixed genome. With an average read depth of 10× Illumina paired-end reads, the macro-level visualisation of RobT lines T1AL.1PS and T1AS.1PL showed that the junction region occurred at the position of *Triticum aestivum* chromosome 1A 212.5Mb and *A. cristatum* chromosome 1P 230Mb (Fig.3 and Fig.S1), respectively. This study also confirms the balanced translocation event, gain of a chromosome 1PS or 1PL from *A. cristatum* and loss of a chromosome 1AS or 1AL.

Since the centromere position was located on 210.2-215.8M of Chr1A of the reference Chinese spring genome based on CENH3 ChIP-seq (IWGSC 2018; Coombes et al.2023), the junction of RobT lines T1AL.1PS and T1AS.1PL was the same region of centromere. Meanwhile, the centromere of *A. cristatum* chromosome 1P also predicted at the position of 230-238Mb (unpublished). Hence, the WGS data supported that the junction positions are located the region of centromere on wheat chromosome 1A and *A. cristatum*. To investigate the detailed structures of the centromeres in T1AS.1PL and T1AL.1PS translocation lines, we carried out FISH analysis using two centromeric sequences oligo-pAcCR1 from *A. cristatum* and oligo-CCS1 from wheat as probes (Wang et al. 2022). The results showed that the translocated chromosomes of all of T1AS.1PL and T1AL.1PS translocation lines contained fusion centromeres and approximately half of the centromere was derived from *A. cristatum* while the other half was derived from wheat (Fig.4). These results further proved that all of T1AL.1PS and T1AS.1PL translocation lines in this study are compensating RobT.

Agronomic performance evaluation of T1AL.1PS and T1AS.1PL

To evaluate the genetic effect of the translocation chromosome arms of 1PS and 1PL, the KASP and co-dominance EST-STS markers were developed to identify the genetic segregation population (Fig.5) and the agronomic traits of the homozygous positive plants and negative plants in segregation population were investigated. The identification results are shown in Table S3 and Fig.5. Compared with the negative plants, the flag leaf length of the T1AL.1PS plants reduced significantly, but the leaf width increased significantly. The similar effects were also observed in the top second leaves of T1AL.1PS plants (Fig.6). For the T1AS.1PL plants, the length and width of flag leaf had a significant decrease in two growing seasons, and the plant height also decreased significantly. These results suggest that *A. cristatum* 1PS and 1PL translocated fragments in RobT lines can bring the same plant architecture changes as that of addition line of *A. cristatum* 1PS and 1PL reported previously (Wang et al. 2022).

To evaluate the agronomic traits of the RobT lines, we completed two years replicates phenotype investigation by using $F_{3:4}$ and $F_{4:5}$ family segregation population of heterozygous T1AL.1PS and T1AS.1PL translocation plants (Table S3). Among three yield component factors, the thousand grain weight, the number of fertile tiller and the grain number per spike increased significantly after the introduction of the translocated chromosome 1PS, which suggested that the introduction of 1PS may increase the yield component factor. Compared with the negative plants, the thousand grain weight, the number of fertile tiller and the grain number per spike of positive plants with T1AL.1PS increased

significantly by 3.5g, 3.1 and 7.4 respectively. But the introduction of the translocated chromosome 1PL showed the obvious negetive effect to grain weight. Our results indicated that 1PS translocation segment can be directly used in wheat breeding for elite plant architecture improvement, but 1PL translocation segment needs to overcome its linkage drag with the reduction of the grain weight (Fig.S2).

Discussion

During the domestication and artificial selection of crop, plant architectures suitable for density planting were continuous concerned. Growing evidence has shown that high-density planting is an effective measure for increasing crop yield per unit land area (Cao et al. 2022). Small leaves allow better light capture in canopy under high-density planting, thus enhancing photosynthesis efficiency and decreasing disease, and increasing the grain yield. Throughout the past half century, maize yields have increased in part because plants have been grown at increasing densities (Duvick 2005). In wheat, the ideal plant architecture of varieties with the high yield potential is expected to be small leaves, strong stems and large spikes for improving the light transmission and absorption efficiency under population competition in China (Ru et al. 2015). Wild ancestors are reservoirs of valuable traits, including diverse forms of high yield potential and resistance to both biotic and abiotic stresses, which remain crucial for adaptation of modern cultivars to future climates. Crop wild relatives have been used for decades in crop improvement for enhancing plant performance (Bohra et al. 2022). Tian et al. (2019) reported that introgressing the wild *UPA2* allele from teosinte into modern hybrids enhance high-density maize yields. Plant architecture improvement is a new direction in crop breeding (Wang et al. 2022), but the pre-breeding of plant architecture in wheat has rarely been reported. In the wheat pre-breeding of using wild relatives, the transfer of rye chromosome 1RS into wheat can provide substantive yield and stress tolerance benefits, and the famous T1BL.1RS translocations have been widely used in bread wheat breeding programs worldwide (Lukaszewski, 1990; Rabanus-Wallace et al. 2021). Here, we developed T1AL.1PS and T1AS.1PL Robersonian translocations by transferring alien chromosome 1P of *A. cristatum* into wheat. We found that introducing 1PS translocation fragment into wheat can increase the number of tillers, the grain number per spike and grain weight, and reduce the area of flag leaf of wheat without yield penalty. However, introducing 1PL translocation fragment into wheat reduce significantly the area of flag leaf, top second leaf, top third leaf and plant height, but the yield component factors TKW was reduced. These novel germplasms with good plant architecture from wild relative *A. cristatum* under the natural selection pave the way for plant architecture improvement adapting to the high-density

planting.

Many wheat-alien translocations have been produced for targeted chromosomes through centromere breakage-fusion mechanism (Ardalani et al. 2016; Li et al. 2000; Sears 1952). The hybrid centromere in translocation lines has a more stable structure and could transmit to the next generation (Friebe et al. 2005; Wang et al. 2017). According to statistics, most of existing wheat-related species translocation lines were gotten by spontaneous translocation (Li et al. 2000). However, the frequency of generate spontaneous translocation line is low and the high-throughput identification mothods are lacking. Many studies have been reported on the frequency of generate spontaneous translocation line between wild relatives and wheat. Friebe et al. (2005) reported that the frequency of RobTs observed in progenies derived from double-monosomic 1Ht/1A plants is 1%. A higher frequency of about 5% RobTs involving chromosome 2B of wheat and 2Eb of *Th. bessarabicum* was reported (Ghazali et al. 2015). The frequency of compensating RobTs were 0.83% among progenies of double monosomic wheat- *Ae. searsii* introgression plants (Liu et al. 2011). Two short-arm wheat-*Th. intermedium* RobT plants have been identified from 1152 F_2 plants, and two long arm wheat-*Th. intermedium* RobT plants were gotten from 840 F_2 plants(Liu et al. 2011). Liu et al. (2016) reported that the average frequency of recovered RobTs between wheat and *Aegilops speltoides* is 3.2 %, ranging from 0.7 % for chromosome 6S to 6.8 % for 5S. The frequency of group-7 compensating translocation chromosomes was 0.3-0.9% by crossing the monosomic stocks CSM7A, CSM7B and CSM7D and 7H addition line (Danilova et al. 2018). A compensating wheat-*Thinopyrum elongatum* RobTs with a positive effect on flour quality T1AS.1EL derived flour is better suited to bread-making than Chinese Spring- and Norin 61-derived flour and that this is because of its greater gluten diversity (Tanaka et al. 2017). In the present study, the frequency of the compensating RobTs recovery between *A. cristatum* 1P and wheat 1A is 1.76% and is up to 26.7% with the aid of specific molecular marker detection. The frequency is close to that of wheat-*Aegilops speltoides* RobTs 1A/1S (Liu et al. 2016). The frequency of this study means that about 300 progeny would be sufficient to isolate five compensating RoBTs by the breakage-fusion mechanism of univalent chromosomes. If molecular markers specific for wild relatives was used for assistance selection, the frequency of recovered RobTs is up to 26.7%. Hence, our study provide a effective universal strategy for recovery of compensating RobTs.

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Figure legends

Fig.1 Strategy for the production of RobTs.

Fig.2 GISH/FISH pattern of mitotic metaphase chromosomes homozygous for wheat-*A. cristatum* Robertsonian translocation. GISH pattern for the T1AL.1PS translocation line (a) and T1AS.1PL translocation line (b), FISH pattern for the T1AL.1PS translocation line (c) and T1AS.1PL translocation line (d). GISH pattern for the pollen mother cell in T1AL.1PS translocation line (e) and T1AS.1PL translocation line (f). *A. cristatum* genomic DNA was used as GISH probe (red), chromosomes were counterstained with DAPI (bule). Tam535 Cy5 (red) and Psc119.2 (green) were used as FISH probes, chromosomes were counterstained with DAPI (bule). *A. cristatum* chromosome segments are labeled by white arrows in the images. Scale bar=10nm.

Fig.3 Resequencing based whole genome uncovered the breakpoint of translocation. Combing Chinese spring wheat genome and diploid *A. cristatum* genome as mixed references, raw reads of homozygous RobTs were mapped on group 1 chromosomes. Gain of a large region of chromosome 1P from *A.cristatum* (light blue line) and loss of a large region on chromosome 1A of wheat (light blue line) in 1P.1A. Resequencing depth is about 10×reads per million versus 'Chinese Spring'. The re-sequencing result of T1AL.1PS(a) and T1AS.1PL(b).

Fig.4 Centromere structures in T1AL.1PS translocation chromosomes (a) and T1AS.1PL translocation chromosomes (b). Oligo-pAcCR1 (green) and oligo-CCS1 (red) were used as centromere probes. Chromosomes were counterstained with DAPI (bule).

Fig.5 Genotype calling of the KASP marker of T1AL.1PS (a) and amplification patterns of EST-STS marker in T1AS.1PL (b). **a** Red dots correspond to negative plants (W:W), green to heterozygosis plants (W:P), blue to homozygosis plants (P:P) and black non-template controls (NTC). **b** Marker represent DNA marker; Z559 is *A. cristatum* cultivar; JM22 is common wheat cultivar; II-3-1c is substitution line plants; 66501-6 is homozygosis plants; 66706-1 is heterozygosis plants; 66701-3 is homozygosis short arm translocation line plants and 66691-3 is heterozygosis short arm translocation line plants.

Fig.6 Investigation of leaves trait in wheat-*A. cristatum* 1P translocation lines (a). Schematic representation of flag leaves in 1PS and 1PL (b).

Tables

Table 1 Markers used for tracing *A. cristatum* 1P chromosome in the wheat background

Primer name	Marker type	Position	Primer sequence	Allelic effects
209189-FAM	KASP	1PS	GAAGGTGACCAAGTTCATGCTTGTCC GGGAAGGCCTAGTG	Dominant
209189-HEX	KASP	1PS	GAAGGTCGGAGTCAACGGATTTGTCC GGGAAGGCCTAGTC	Dominant
209189-COM	KASP	1PS	CAGAACAGGAGCATCATGACGG	Dominant
P131676-FAM	KASP	1PS	GAAGGTGACCAAGTTCATGCTGGATC CAATAGAATTACAACTGGACAG	Co-dominant
W131676-HEX	KASP	1PS	GAAGGTCGGAGTCAACGGATTGGATC CAATAGAATTACAACTGGACAC	Co-dominant
131676-COM	KASP	1PS	GCTACCATGAACACAGATGATAGAAC CT	Co-dominant
AgC23256F	EST-STS	1PS	GTACCACATCTGAACACAAATTGC	Dominant
AgC23256R	EST-STS	1PS	GATAGCGAGGTGCTTGACAAA	Dominant
AgC8189F	EST-STS	1PS	GGAAAGCCTACATCCACATGTAC	Dominant
AgC8189R	EST-STS	1PS	CGATGTCACTGCCGTCAGC	Dominant
AgC6184F	EST-STS	1PL	CATTTTGTAGTGTTGCCCGTT	Dominant
AgC6184R	EST-STS	1PL	TTGGCGTAGAGCGAGAAACA	Dominant
AgC2142F	EST-STS	1PL	GGATGGGATTGGGTCTGATT	Dominant
AgC2142R	EST-STS	1PL	CGTCTTTTGCGTTCCAGTTC	Dominant
HIT4_2F	EST-STS	1PL	CTGCAGTCGAGGTACATCAACAG	Co-dominant
HIT4_2R	EST-STS	1PL	CCAAGCTCATCTACCCAGCTTC	Co-dominant
LEA14_2F	EST-STS	1PL	ACCGCCGGCGAGTTCAAG	Co-dominant
LEA14_2R	EST-STS	1PL	CACGCGGATGCAGAGCAAG	Co-dominant

Table 2 Molecular marker and cytogenetics identification results of progenies derived from 1P(1A) substitution line and common wheat cv. Jimai22

Target P chromosome	Number of plants	Ratio
F_2 population individuals	911	100%
Plants missing short arm marker	32	3.5%
Plants missing long arm marker	28	3.1%
Plants with a short arm RobTs (GISH)	9	1%
Plants with a long arm RobTs (GISH)	7	0.77%
Plants with a complete P chromosome	3	0.32%
Frequency of RobT recovery (%)	16	1.77%

Fig.1

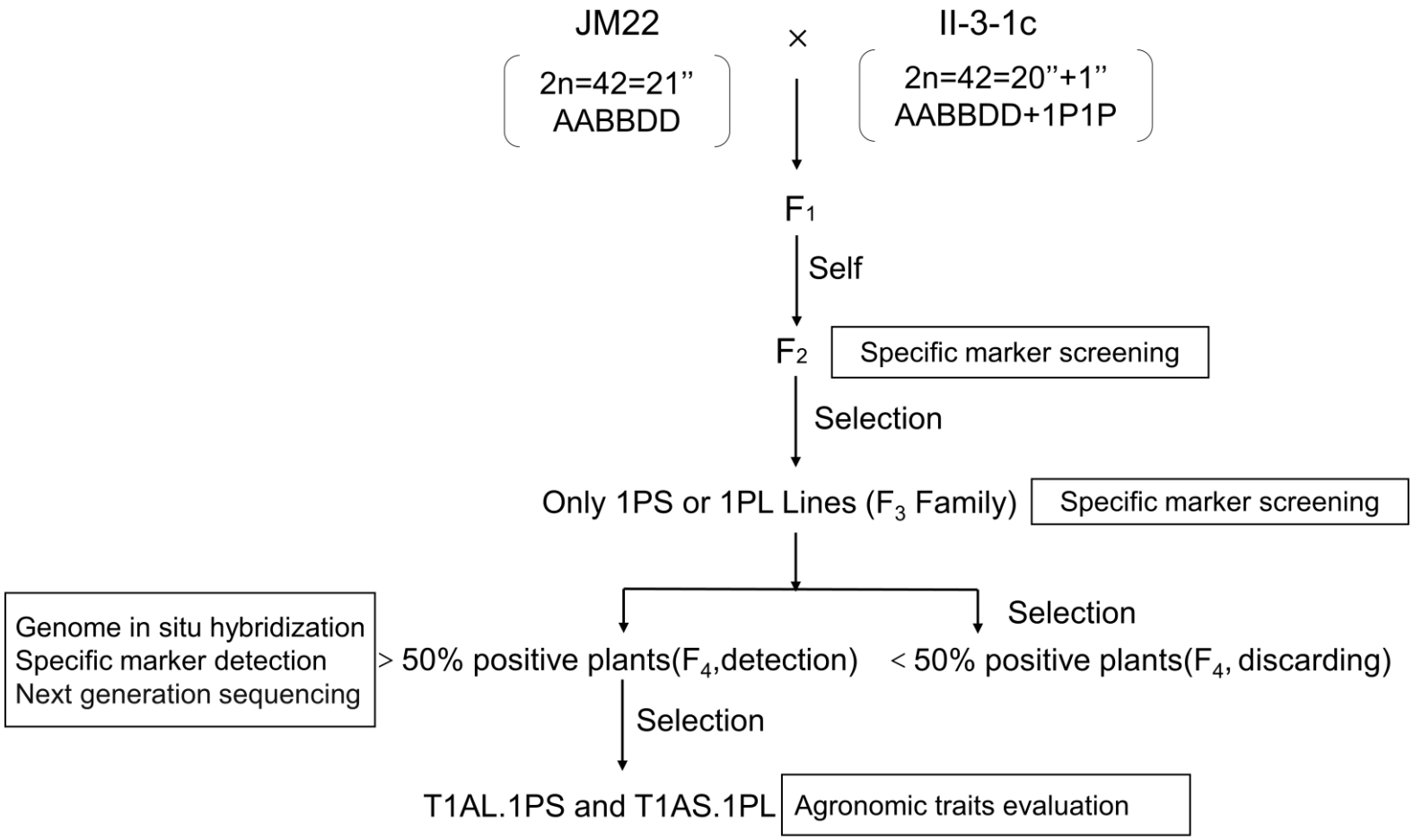

JM22
2n=42=21”
AABBDD
×
II-3-1c
2n=42=20”+1”
AABBDD+1P1P
F1
Self
F2
Specific marker screening
Selection
Only 1PS or 1PL Lines (F3 Family)
Specific marker screening
Selection
Genome in situ hybridization
Specific marker detection
Next generation sequencing
> 50% positive plants(F4,detection)
< 50% positive plants(F4, discarding)
Selection
T1AL.1PS and T1AS.1PL
Agronomic traits evaluation

Fig.2

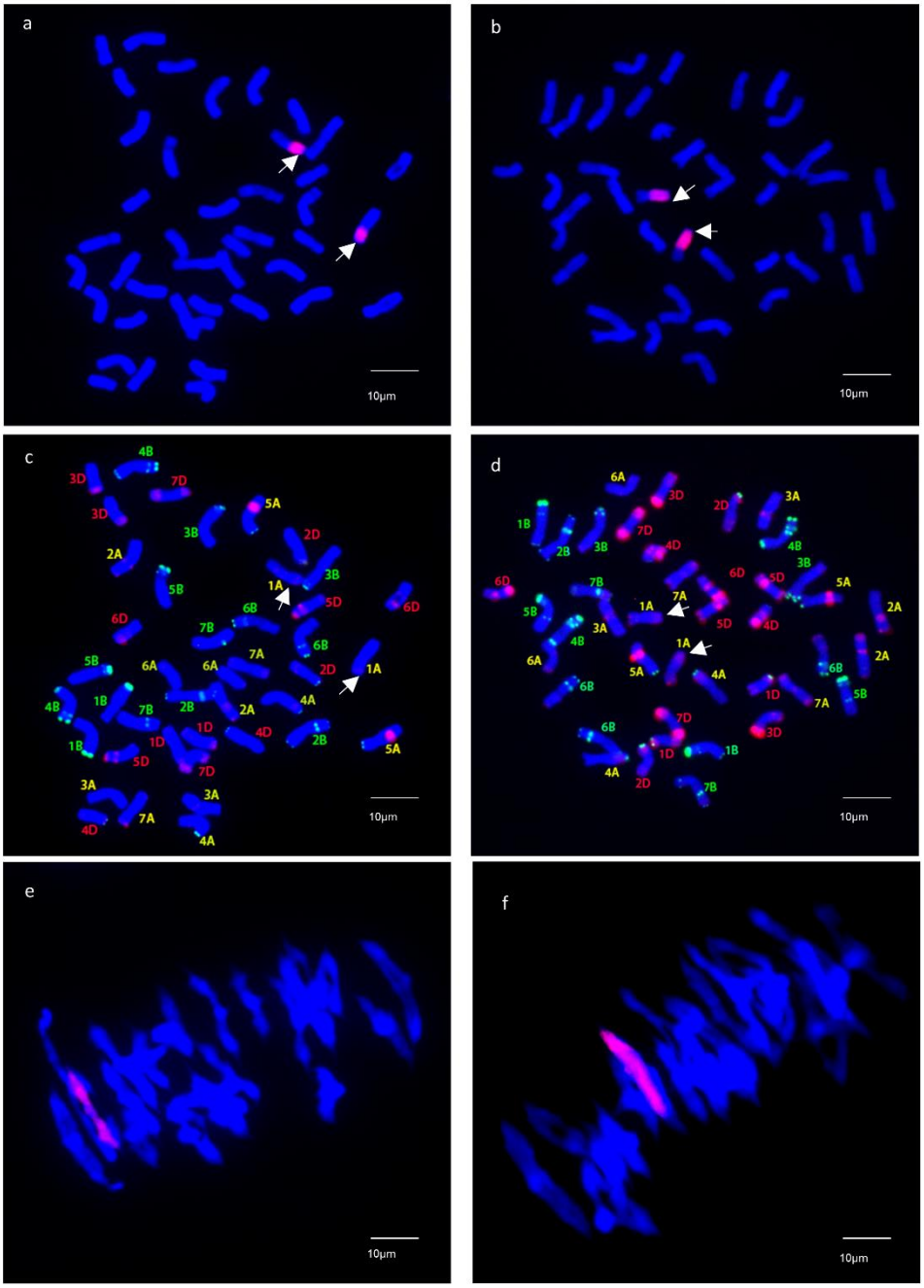

a
10μm
b
10μm
c
4B
3D
7D
5A
3D
3B
2D
2A
5B
1A
3B
6B
5D
6D
6D
7B
7A
6B
5B
6A
6A
2D
1A
4B
1B
7B
2B
2A
4A
1D
1D
4D
2B
1B
5D
5A
7D
3A
3A
7A
4D
4A
10μm
d
6A
3D
2D
3A
1B
7D
2B
3B
4D
4B
3B
6D
6D
5D
5A
7B
7A
5B
1A
2A
3A
5D
4D
4B
1A
6A
5A
4A
2A
6B
6B
1D
7A
5B
7D
6B
3D
1D
1B
4A
2D
7B
10μm
e
10μm
f
10μm

Fig.3

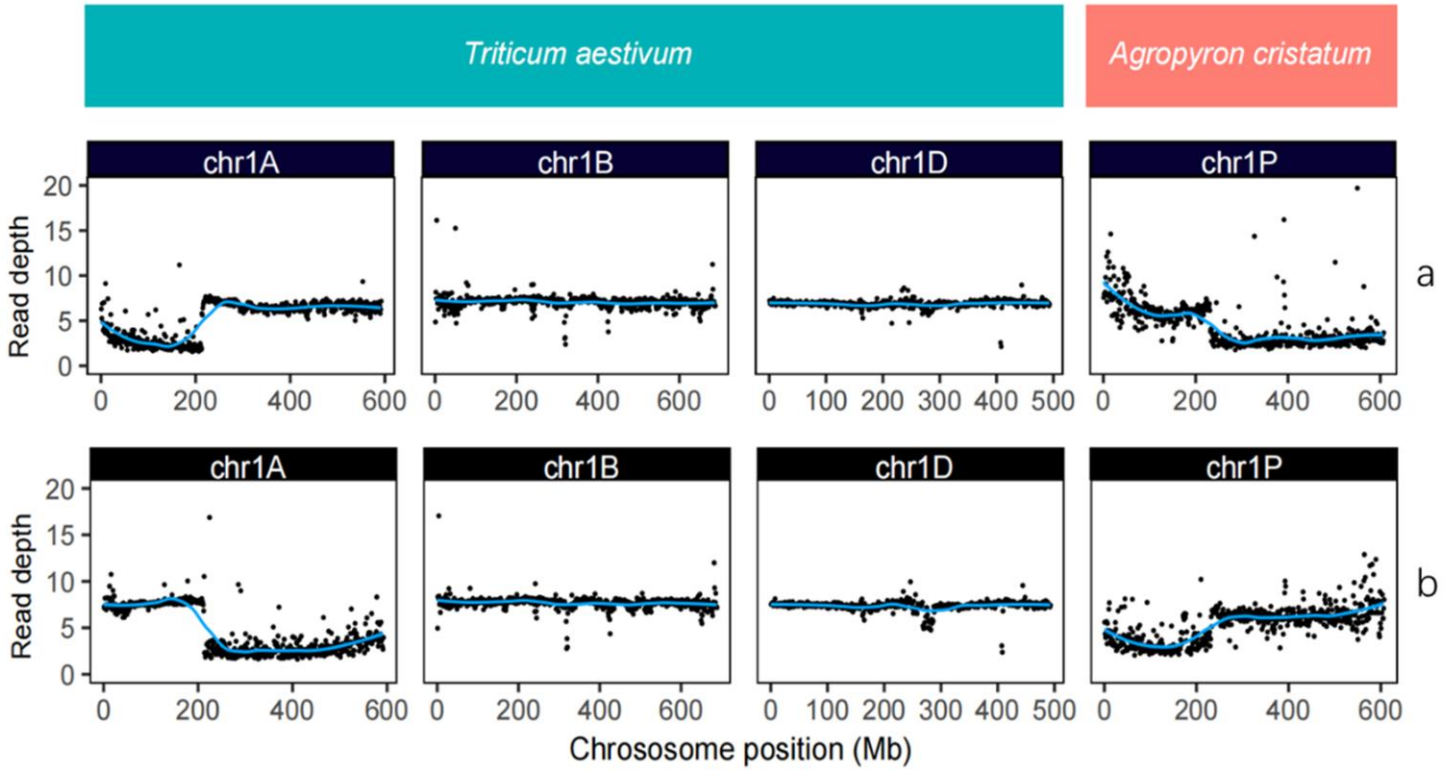

Triticum aestivum
Agropyron cristatum
chr1A
chr1B
chr1D
chr1P
Read depth
Chrososome position (Mb)
a
b

Fig.4

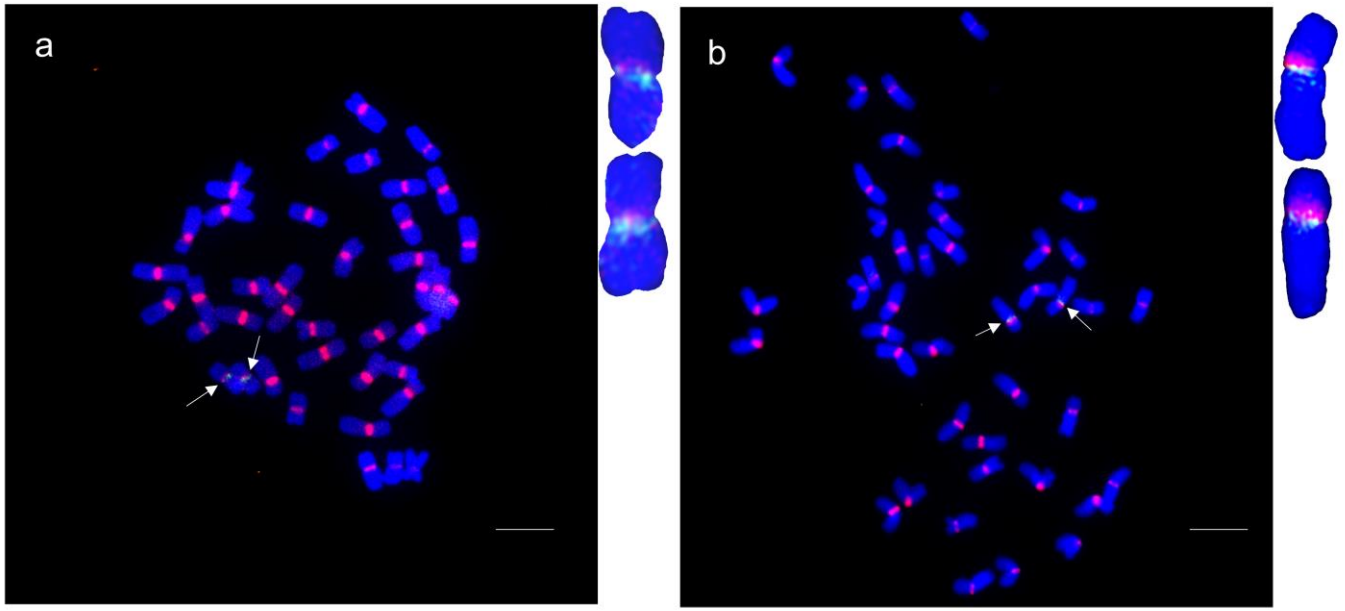
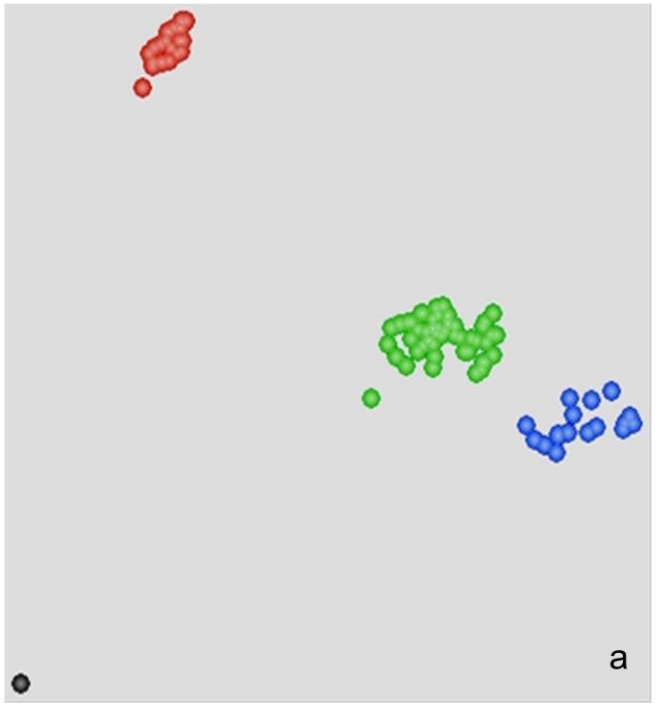


Fig.5


a

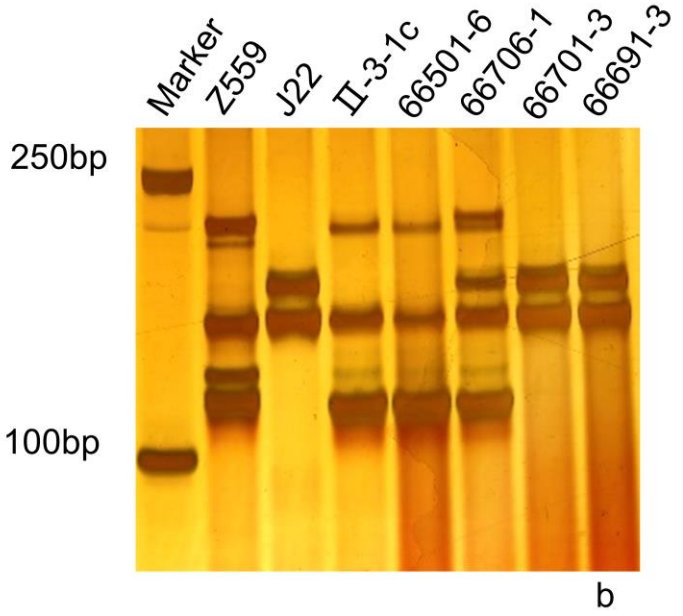
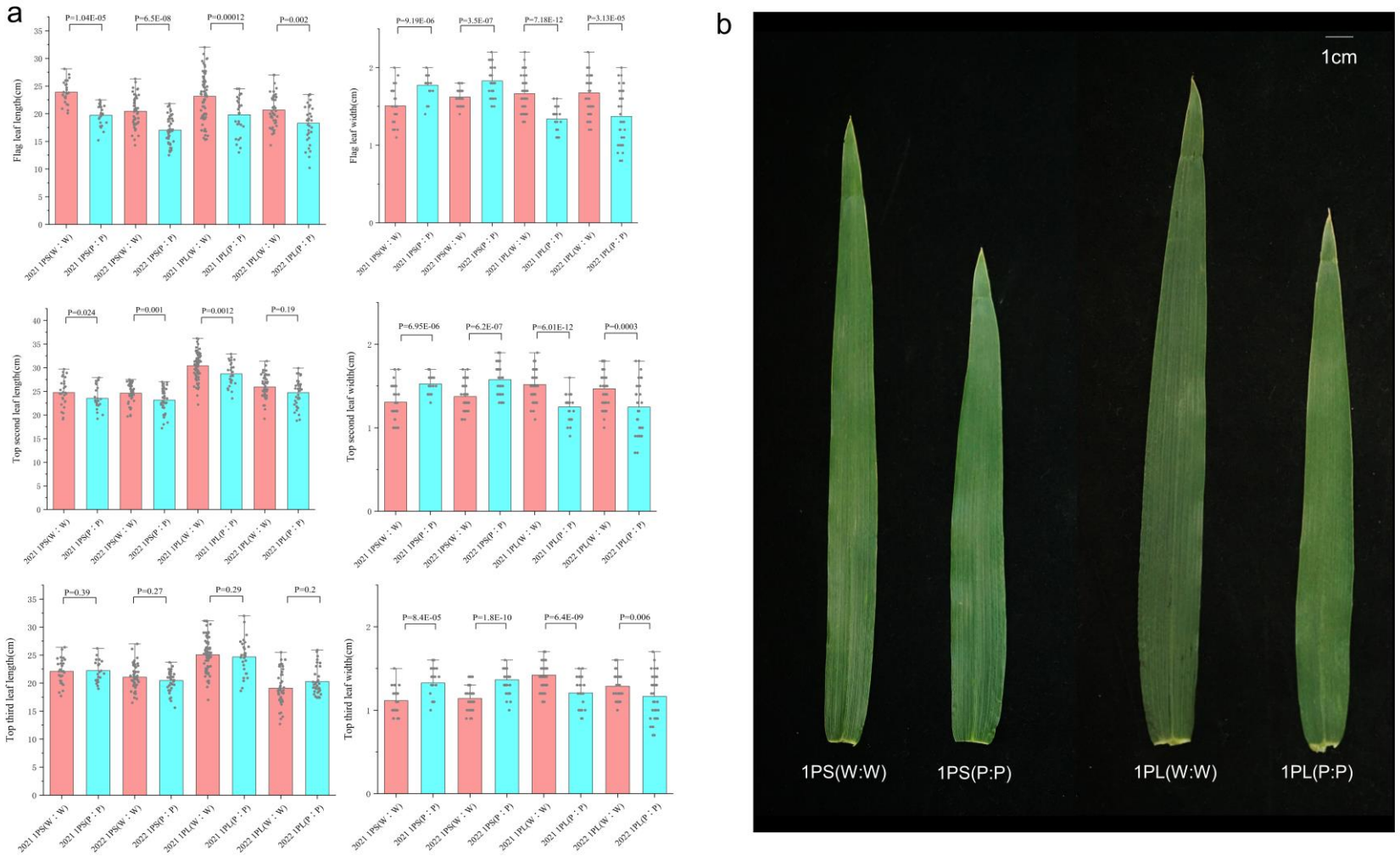

Marker
Z559
J22
Д-3-1c
66501-6
66706-1
66701-3
66691-3
250bp
100bp
b

Fig.6


a
Flag leaf length(cm)
P=1.04E-05
P=6.5E-08
P=0.00012
P=0.002
Flag leaf width(cm)
P=9.19E-06
P=3.5E-07
P=7.18E-12
P=3.13E-05
Top second leaf length(cm)
P=0.024
P=0.001
P=0.0012
P=0.19
Top second leaf width(cm)
P=6.95E-06
P=6.2E-07
P=6.01E-12
P=0.0003
Top third leaf length(cm)
P=0.39
P=0.27
P=0.29
P=0.2
Top third leaf width(cm)
P=8.4E-05
P=1.8E-10
P=6.4E-09
P=0.006
2021 1PS(W : W)
2021 1PS(P : P)
2022 1PS(W : W)
2022 1PS(P : P)
2021 1PL(W : W)
2021 1PL(P : P)
2022 1PL(W : W)
2022 1PL(P : P)
b
1cm
1PS(W:W)
1PS(P:P)
1PL(W:W)
1PL(P:P)

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