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IDENTIFICATION OF INDIVIDUAL *THINOPYRUM DISTICHUM* CHROMOSOMES IN D-TRITIPYRUM LINES AND DERIVATIVES

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ABSTRACT

D-tritipyrums (2n = 42; AABBJ^{d}J^d) are hexaploid hybrids with A and B genome chromosomes from *Triticum turgidum* L. var durum (2n = 28, AABB) and/or *Triticum aestivum* L. (2n = 42 = AABBDD) and a set of seven J^d chromosomes that derive from the J_1^d and J_2^d genomes of *Thinopyrum distichum* (Thunb.) Löve (2n = 28; $J_1^dJ_1^dJ_2^dJ_2^d$). A d-tritipyrum collection consisting of lines with diverse combinations of J^d chromosomes has been derived through reassortment of the J_1^d and J_2^d genome chromosomes, yet not all of these chromosome sets are genetically balanced and agronomically useful. Forty-seven d-tritipyrums and seven d-tritipyrum/wheat hybrid derivatives were therefore analyzed to identify the J^d genome chromosomes in each. Genome-wide sequence tags were acquired through genotyping by sequencing (GBS) and sorted with the aid of appropriate control genotypes to obtain a pool of *Th. distichum* associated sequence tags. Nine *Th.-distichum* single chromosome addition lines in hexaploid triticale were then used as reference genotypes to identify appropriate sequences for analyzing each homoeologous group within each line. Nineteen different J^d chromosome subsets were found among the lines. The "best" J^d genome was identified based on plant phenotype and fertility and included chromosomes $1J_2^d$, $2J_1^d$, $3J_2^d$, $4J_2^d$, $5J_1^d$, $6J_2^d$ and $7J_2^d$. Six phenotypically diverse d-tritipyrum lines with the selected genome will form the basis of a new breeding population and will be used as pivotal J^d genotypes to broaden its A, B and J^d genome variability through backcross-based introgression.

Keywords: *Triticum* X *Thinopyrum* hybrids - Genotyping by sequencing - allopolyploid development.

INTRODUCTION

Polyploid species of the genus *Thinopyrum* are believed to have originated through hybridization and polyploidization events involving three putative diploid progenitors; *Th. elongatum* Dewey (genomes E^{e}E^e; sometimes given as E = J^e), *Th. bessarabicum* (Savul & Rayss) Á. Löve (genomes E^{b}E^b; sometimes given as J = J^b) and a *Pseudoroegneria* Á. Löve species which contributed the StSt genomes (Arterburn *et al*., 2011). Another study (Pienaar, 1981) concluded that *Th. distichum* (Thunb.) Löve is a segmental allotetraploid with two similar genomes, which appeared to be variants of the J-genome (Pienaar *et al*., 1988) based on their close relationships with the genomes of *Th. elongatum* and *Th. sartorii* (Boiss. & Heidr.). Liu and Wang (1992) found that *Th. sartorii* is a segmental allotetraploid with genomes J^{b}J^{b}J^{e}J^e and believed that *Th. distichum* has similar genomes. Armstrong *et al*. (1992) showed that *Th. distichum* and *Th. junceiforme* (A. & D. Löve) A. Löve had similar C-band karyotypes. Pienaar (1990) suggested that the genomic formula of *Th. distichum* should be 2n=4x=$J_1^dJ_1^dJ_2^dJ_2^d$.

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A physical comparison of the *Th. bessarabicum* and wheat genomes by Grewal *et al*. (2018) revealed a reciprocal translocation between the long arms of 4J and 5J of *Th. bessarabicum*, which appeared to be the same as the 4A/5A translocation in wheat. Data obtained by Xu et al. (2020) suggested that this 4J/5J translocation does not occur in *Th. elongatum* and thus argued against a common origin for the 4J/5J and 4A/5A translocations. Grewal et al. (2018) found evidence of three further structural rearrangements among the *Th. bessarabicum* chromosomes; i.e. a possible reciprocal translocation involving the centromeric regions of chromosomes 2J and 5J; a translocation within the 6J long arm; and an inversion in the 7J short arm. The 2J/5J and 4J/5J translocations of *Th. bessarabicum* were not found in *Th. elongatum* (Xu et al., 2020).

Chromosome 5J of *Th. bessarabicum* was found to have a major effect on salt tolerance (Forster et al., 1988). Studies with *Th. elongatum* showed that tolerance of sudden salt stress was principally controlled by chromosomes $3J_1e$ and $5J_1e$ whereas tolerance of gradually imposed salt stress was principally controlled by chromosomes $3J_1e$, $4J_1e$ and $5J_1e$ (Zhong and Dvořák 1995). Comparison of *Th. distichum* X hexaploid triticale (*X Triticosecale* Wittmack; AABBRR)

secondary amphiploids led Marais *et al*. (2007) to suggest that chromosomes $2J_1^d$, $3J_1^d$, ($4J_1^d$; redesignated $1J_1^d$ by Marais *et al*., 2021) and $5J_1^d$ affected salt tolerance. The addition of only a single critical chromosome did not have a demonstrable effect on salt survivability of triticale whereas chromosomes $2J_1^d$ plus $3J_1^d$ were the only combination of two chromosomes at a time that produced a notable intermediate effect.

Recently, Marais *et al*. (2021) used genotyping by sequencing (GBS) to compare the synteny of eleven *Th. distichum* single chromosome additions in hexaploid triticale to common wheat and *Th. elongatum* chromosomes. This allowed for determining the homoeology of each of the eleven addition chromosomes; however, with the genomes being uncharacterized, the chromosomes had to be arbitrarily allocated to the J_1^d and J_2^d genomes. Their data revealed the likely presence of five translocations among the chromosomes, with the larger translocations being between $2J_2^d/4J_1^d$; $4J_1^d/5J_2^d$ and $1J_2^d/6J_2^d$ (likely reciprocal). Two smaller translocations probably occurred between $1J_1^d/4J_1^d$ and $4J_1^d/6J_1^d$. Thus, $4J_1^d$ appeared to be a substantially structurally modified chromosome.

An earlier study, King *et al*. (1997) named allohexaploid ($2n = 6x = 42$, $AABBJ^bJ^b$) hybrids of durum wheat and diploid *Th. bessarabicum*, "Tritipyrum". Such hybrids were called "b-" tritipyrums by Marais *et al*. (2014) to distinguish them from "d-" tritipyrums (2n = 42 = $AABBJ^dJ^d$). B-tritipyrums exhibit strong salt tolerance; have hard, brittle spikes that are non-free-threshing but lack in J^b genome variability required for breeding. D-tritipyrums have a single hybrid J^d genome that is a synthesis of seven chromosomes, each of which derives from either of the J_1^d or J_2^d genomes of tetraploid *Th. distichum*. Two d-tritipyrum "founder lines" (W1735 and W1736; 2n = 42) were developed by Marais et al. (2014) through pure line selection over several generations in F_2-derived progeny of the cross Calvin/*Th. distichum*//Calvin*2/ *Th. distichum* (provided by Dr. G. Fedak, Agriculture and Agrifood Canada, Ottawa). While both lines were salt tolerant, W1736 was very difficult to thresh due to its tough, brittle spikes. W1735 had non-brittle spikes, was solid-stemmed, easier to thresh (yet not comparable to wheat) and of semi-dwarf height.

To broaden d-tritipyrum J^d genome variability, W1735 was crossed with a secondary amphiploid: Inia-66 2*/*Th. distichum* (2n = 56; $AABBDDJ_1^dJ_2^d$) (Marais *et al*., 2014). The F_1 was then backcrossed to W1735 and 16 fertile backcross F_1 (2n = 7x ~49; $AABBDJ^dJ^d$) were obtained. From the sixteen lineages, 220 F_2 plants (2n = 41-43) were selected and used as basis to develop diverse inbred lines. The manner in which the d-tritipyrum population was developed allowed for numerous combinations of seven J^d chromosomes (re-arranged diploid genomes) to be formed from the 14 *Th. distichum* chromosomes. Due to their hybrid J^d genome, these secondary d-tritipyrums were very diverse with respect to plant height, flowering date, fertility, spikelets per spike, stem solidness, rachis brittleness, bract hardness, thresh-ability, kernel size and shape, re-growth after harvesting, waxiness of the plant surfaces, root development, salt tolerance, and perennial tendency (Marais *et al*., 2014).

It is well-documented by Pienaar (1981, 1983, 1990) and Pienaar *et al*. (1988) that moderately high allosyndetic pairing can occur between J_1^d and J_2^d chromosomes. In backcross F_1: *Triticum durum* *2/*Th. distichum*; genomes $AABBJ_1^dJ_2^d$, the J_1^d and J_2^d chromosomes produced an average of 4.8 bivalents per pollen mother cell. Similarly, in *Triticum aestivum* *2/*Th. distichum* F_1 hybrids (genomes $AABBDDJ_1^dJ_2^d$) 3.8 bivalents were formed among the J_1^d and J_2^d chromosomes. A low incidence of trivalents (0.12–0.18) and quadrivalents (0.08–0.19) indicated that the two *Thinopyrum* genomes could differ by at least two large reciprocal translocations. The high degree of homoeology of the J_1^d and J_2^d genomes was also borne out by data obtained with F_1 hybrids (genomes $RRJ_1^dJ_2^d$) of *Th distichum* and tetraploid rye (*Secale cereale* L.) (Marais and Marais, 2003). Frequent homoeologous recombination during d-tritipyrum line development would therefore have contributed significantly to the overall J^d genome diversity of the d-tritipyrum collection.

Knowledge of the specific chromosomes contained within the different re-arranged J^d genomes of the d-tritipyrum collection will greatly facilitate ongoing development of the material. This study used genotyping by sequencing (GBS) in conjunction with previously identified hexaploid triticale – *Th. distichum* single chromosome addition lines (Marais *et al*., 2021), to identify the individual J^d genome chromosomes in 47 d-tritipyrum lines and seven derivatives that appeared to be chromosomally stable. The ultimate aim was to find a subset of the most promising lines that shares a single, re-arranged J^d genome. Such lines can provide a pivotal J^d genome in ongoing crosses and backcrosses to expand A, B and J^d genome diversity and improve the population. As needed, the remaining d-tritipyrum lines (not selected) may serve as donor germplasm with which to initiate backcrosses for continued introgression into the selected J^d genome.

MATERIALS AND METHODS

Plant material

Sixty-eight lines were used in the study and included 47 d-tritipyrum lines and seven selections from wheat/d-tritipyrum crosses. Also included were the parental genotypes *Th. distichum*, durum wheat cultivar Calvin, common wheat cultivar Inia-66, and two additional controls, i.e. hexaploid triticale cultivars Rex and Tobie. Nine single *Th. distichum* chromosome addition lines (for

$1J_1^d$, $2J_1^d$, $2J_2^d$, $3J_1^d$, $4J_1^d$, $4J_2^d$, $5J_1^d$, $6J_2^d$, and $7J_2^d$) in hexaploid triticale (Marais *et al*., 2021) were also among the entries. A single plant of each genotype was used for GBS and marker analyses. Seeds of the 54 d-tritipyrum lines/derivatives and the nine addition lines were germinated in petri dishes and root tips were cut, prepared and used for somatic chromosome number counts as outlined in Darlington and LaCour (1976). Single plants with the correct chromosome numbers (or closest to it) were transplanted to a greenhouse. At maturity, plant height (inches; base to tip of tallest spike), days to flower, and plant phenotype (visually scored on a 0 (poorest) to 5 (best) scale) were determined. A subset of 34 of the d-tritipyrum lines/derivatives was used for a separate assessment of salt tolerance.

Genotyping by sequencing

Duplicate leaf tissue samples were cut on each of the 68 plants for DNA extraction. Each of the 136 extracts was then used to initiate in duplicate the development of two-enzyme-based, complexity-reduced, barcoded libraries as described by Poland *et al*. (2012). Thus, four independently amplified libraries were obtained per entry (272 libraries in total) which were sequenced by Illumina (https://www.illumina.com). The sequence data were processed using the GBSv2 pipeline (https://bitbucket.org/tasseladmin/tassel-5-source/wiki/Tassel5GBSv2Pipeline). Only sequences with an exact match to a barcode followed by the expected sequence of five nucleotides remaining from a *Pst*I cut site were kept. GBSv2 was used to trim the sequences to 100 bp (removing the barcode but including the *Pst*I cut-site remnant), and the trimmed sequences were combined into tags. Tags were associated with individuals based on sequence barcodes following which the data were aligned. Pairwise alignment identified tags that were 100% identical across all or a subgroup of individuals via an in-house script in R3.6.2 language. This matrix of unique sequence tags associated with the individual genotypes was then processed as follows:

1. For each sequence tag, the total reads across replicates of a genotype were determined.
2. Sequence tags for which the total reads exceeded zero in one or more of the controls Inia 66, Calvin, Rex, and Tobie were nonspecific for *Th. distichum* and were removed from the data set.
3. Sequence tags with five or more total reads in *Th. distichum* were kept.
4. This remaining data set, consisting of primarily *Th. distichum*-specific sequence tags, was used for comparison of the addition and test lines to identify individual *Th. distichum* chromosomes.
5. Sequence tags that amplified in more than two addition chromosomes (= multi-group tags that amplified in more than one homoeologous group) were uninformative and were excluded from the dataset.
6. Sequences that amplified in only one addition chromosome (single tags) or two addition chromosomes (paired tags) were informative and their presence–absence patterns were analyzed across all lines.

Single tags included the following -

1. Highly diagnostic, homologue-specific tags that amplified only within one of the two homoeologous chromosome regions.
2. Tags that did not amplify (due to chance) in a second addition line, or went undetected as its homoeologue was not among the addition line controls. Such tags were amplified in both homoeologues among the test lines.

Paired tags were primarily amplified from homoeo-loci and included -

1. Homoeologue-specific tags that detected the presence of the two members of a specific homoeologous group.
2. Translocation-associated tags that occurred when the chromosome being analyzed carried a translocation from a non-homoeologue. The tag sequence that got amplified derived from the translocation donor chromosome and had synteny with the homoeologue of the translocation donor.

Tags that gave deviant presence-absence patterns: A small proportion of tags were -

1. Mis-grouped, multiple-target tags that escaped detection and prior removal and amplified in more than one homoeologous group or species.
2. Tags that occurred in chromosome regions that underwent homoeologous chromosome exchanges during line (or addition line) development produced additional small variations in the absence-presence patterns.

Marker analyses

Remnant DNA from the GBS analyses was used to also test the sequence-characterized amplified region (SCAR) markers UST-2, -3, -4, -5, -7, -14, -15 (Marais *et al*., 2007) on the panel of lines. DNA concentration was adjusted to ~10 ng μl^{-1} before PCR amplification and PCR products were visualized following agarose gel electrophoresis and staining with ethidium bromide. This was done to re-assess the usefulness of the markers for use within the current d-tritipyrum collection.

Salt tolerance trial

Thirty-four lines and two controls (Calvin and Inia 66) were tested for salt tolerance in a growth chamber. The trial was a randomized block with four replications. Each replication involved nine 6-inch plastic pots filled with approximately 900 ml of coarse sand. Four entries (a cluster of five plants per entry) were planted to the quadrants of a pot. A circle of 1mm plastic mesh was used to cover the holes in the bottom of each pot to prevent the sand from running out and to allow for proper drainage. The pots were raised three inches over a drip tray (on a plastic mesh) to further improve drainage. Seeds of each

entry were germinated in Petri dishes and healthy seedlings that were roughly in the same stage of growth were transplanted to a constant depth. Pots were placed in a growth chamber (16 hrs light: 8 hrs dark at 24°C) and watered on alternate days with 200 ml of standard nutrient solution (Miracle-Gro® Professional Peat-Lite® Special 20-10-20) prepared according to the manufacturer's (The Scotts Company, 1411 Scottslawn Road, Marysville, OH 43041) instructions. When most seedlings were in the two-leaf stage, salt treatment commenced. For 20 days the pots were watered with 0.025% NaCl added to the nutrient solution. Thereafter, the salt concentration was raised by 0.25% on every third day until 1.5% was reached. The highest concentration was maintained until the plants could be scored. Salt tolerance was rated on a scale of 1 to 10, with 1 being the most sensitive. The entries were compared within three maturity groups (early, intermediate and late). A visual score was assigned to each entry of a replication based on the average stress phenotype of the five plants within that rep X entry combination. Plants with the least discoloration and dieback from the leaf tips and the tip of the spike were considered to be the most tolerant.

RESULTS AND DISCUSSION

A total of 3,115,067 sequence tags were obtained of which 1,000,000 were analyzed. Tags that showed amplification in the durum wheat, common wheat and hexaploid triticale samples were removed and of the remaining tags those that amplified five or more times in *Th. distichum* were kept for analysis. The latter amounted to 95,771 tags that could be subdivided into: 39,500 tags that did not amplify in any of the nine addition lines; 2,043 tags that amplified in more than two addition lines; and 54,227 tags that amplified in one or two addition lines. The 54,227 potentially useful single and paired tags were subsequently organized into nine groups based on whether they amplified in a particular addition chromosome. A further 1,418 tags that amplified in the addition chromosomes did not amplify in any of the test lines and hence were not useful and were removed as well, leaving 52,809 tags. The eventual numbers of tags assigned to individual chromosome groups ranged from 2,181 ($4J_1^d$) to 8,527 ($7J_2^d$). The tags of each chromosome group were then analyzed to reveal patterns in presence/absence of amplification across the test lines and proved to be highly informative. Data obtained with the chromosome-specific (single) tags proved to be the most useful for determining the J^d chromosomes in each line. The shared (homoeologue-detecting tags) provided additional information about (a) the presence of chromatin from a particular pair of homoeologues, and (b) the likely presence of translocations. However, the amplification patterns associated with translocations were not fully revealed due to the un-availability of a complete set of chromosome addition lines. While the present set of nine control addition lines included at least one member of each homoeologous group, the second homoeologue was not available for five homoeologous groups.

Chromosome $1J_1^d$

Altogether, 4,322 tags were associated with addition chromosome $1J_1^d$. These included single tags that were either $1J_1^d$-specific (463 tags) or amplified from both $1J^d$ homoeologues (1,432 tags). This produced two major amplification patterns among the 54 test lines that are summarized in Figs. 1a and 1b, respectively. The patterns suggested that $1J_1^d$ occurred in 17 lines; $1J_2^d$ occurred in 29 lines and both chromosomes were absent from seven lines. Line 16M1008-3-1 showed reduced $1J^d$ amplification but regular $1J_1^d$-specific amplification. This line derived from crosses that involved a wheat parent. Marker UST15 was found to be amplified in $1J_1^d$ (addition line W1414; Marais *et al*., 2021). Here UST15 amplified the diagnostic band in all test lines except the seven that did not have a $1J^d$ chromosome suggesting that UST15 probably detects the same locus in both $1J_1^d$ and $1J_2^d$. UST15 also did not amplify the diagnostic band in line 16M1008-3-1 which would suggest that a $1J^d$ region that harbors the UST15 amplification site had been lost through an unknown structural change to this highly modified chromosome.

Marais *et al*. (2021) concluded that $1J_1^d$ is an extensively restructured chromosome that contains large chromatin regions that are of $4J^d$ and $6J^d$ origin. Here, 2,369 $1J_1^d$ paired tags co-amplified with non-homoeologous J^d chromosomes (Table 1) of which the vast majority (1,814) co-amplified with chromosome $4J_1^d$. It appears that a large amount of $4J_2^d$ chromatin had been translocated to $1J_1^d$ which then established significant homoeology between $1J_1^d$ and $4J_1^d$ (mechanism explained in Fig. 2). The predominant present-absent pattern produced by 1,758 of the 1,814 $1J_1^d$-$4J_1^d$ co-amplified tags was the same as the pattern produced by the $4J_1^d$-specific tags (the latter is shown in Fig. 6a), thus confirming the directionality of this translocation event.

The second most prominent among the co-amplifications was that of $1J_1^d$_$4J_2^d$ (284 tags; Table 1). The associated co-amplification patterns included 211 tags that amplified the $4J_2^d$-specific pattern shown in Figure 6b. Thus, in this smaller translocation event, $4J_1^d$ chromatin was apparently translocated to $1J_1^d$. The latter translocation was reported by Marais *et al*. (2021) who also detected a substantial translocation of $6J_2^d$ chromatin to $1J_1^d$ which established co-linearity between $1J_1^d$ and $6J_1^d$. The latter co-amplification could not be detected with the current data as addition chromosome $6J_1^d$ was not present among the controls. The more significant translocations that were detected in the present study and in the Marais *et al*. (2021) study are summarized in Figure 3.

Homoeologous group $2J^d$

A large number of tags amplified in $2J_1^d$ (7,618) and $2J_2^d$ (6,964). The results for the chromosome-specific $2J_1^d$ and

$2J_2^d$ tags are summarized in Figure 4 and suggest that the lines can be grouped into 30 lines that have $2J_1^d$; 19 lines with $2J_2^d$; three lines with both $2J_1^d$ and $2J_2^d$; one line (RIL-228) that appears to carry a translocation (possibly to a wheat chromosome) and one line (19M1-14-1) that has no associated $2J^d$ chromatin. The results also suggest that smaller variations in amplification occurred among the $2J^d$ chromosomes of individual lines that are probably ascribable to homoeologous crossovers that occurred earlier in their development when allosyndetic pairing structures involving homoeologues were formed. The three lines with both $2J_1^d$ and $2J_2^d$ (16M1005-B; 19M1-15-6 and 19M1-22-2) are related with 16M1005-B being one of the parents of the 19M1 cross. All three plants had 2n = 42 chromosomes. Since each of the sister lines 19M1-15-1 and 19M1-15-4 (Fig. 4) has $2J_2^d$ only (both 2n = 42), it appears that single copies of $2J_1^d$ and $2J_2^d$ occurred in 16M1005-B; 19M1-15-6 and 19M1-22-2 comparable to the situation in a heterozygote.

Of the total 14,582 tags, 3,676 co-amplified in an additional, non-homoeologous chromosome (Table 1). The most prominent of these associations was the co-amplification of 1,994 $2J_2^d$_$4J_2^d$ tags. This resulted from a prominent translocation of $4J_1^d$ chromatin to $2J_2^d$ (Marais *et al*., 2021; Fig. 3). A large number (1,809) of the latter co-amplification patterns were the same as produced by the $4J_2^d$-specific tags (Fig. 6b). Chromosome $2J_1^d$ also co-amplified 352 tags with $6J_2^d$, of which 170 tags produced a $2J_1^d$-specific pattern, suggesting that a translocation occurred from $2J_2^d$ to $6J_2^d$.

An evaluation of the SCAR markers showed that UST-2, UST-3 and UST-4 specifically detected $2J_1^d$. The three markers derive from unrelated, anonymous sequences on $2J_1^d$ that were amplified using RAPD primers OPK-17, OPE-16 and OPX-4, respectively.

Chromosome $3J_1^d$

Of the 6,859 tags amplified in $3J_1^d$; 5,149 were chromosome-specific whereas 1,058 amplified in both group $3J^d$ chromosomes (Fig. 5). Twenty-eight lines have $3J_1^d$; 23 lines appear to have $3J_2^d$; and three lines do not have a $3J^d$ chromosome. Of the latter three lines, RIL-159 has only 40 chromosomes (Table 1) and is likely to be a $3J^d$ nullisomic.

Apart from the co-amplification patterns already discussed, chromosome $3J_1^d$ co-amplified 13 to 139 tags with seven non-homoeologous chromosomes (Table 1), suggesting that this chromosome may be evolutionary well conserved. SCAR markers UST-5 and UST-7 detect the group 3L arms of wheat and were derived from the *Xpsr-931* RFLP locus sequence. Each marker detected the same *Th. distichum* polymorphism in both $3J_1^d$ and $3J_2^d$.

Homoeologous group $4J^d$

In total, 4,524 tags were amplified in chromosomes $4J_1^d$ (2,181) and $4J_2^d$ (2,343). The amplification patterns produced by 1,685 $4J_1^d$ chromosome-specific and 1,225 $4J_2^d$ chromosome-specific tags are shown in Figures 6a and 6b, respectively. Combined, 1,013 $4J^d$-detecting sequences occurred which are included in Figure 6a. The data suggested that 18 lines have $4J_1^d$ whereas 24 lines have $4J_2^d$. Seven lines (16M1005-8-11, 16M1008-B, and five selections from cross 19M1) do not have a $4J^d$ chromosome (believed to have 4D substituted for $4J^d$; unpublished results). RIL lines -193, -198, -228, -273 and 14M1088 appear to have $4J^d$ chromatin in their genomes, but they lack $4J_1^d$ and $4J_2^d$ per se. This means that the $4J^d$ chromatin that is detected could occur on a non-group $4J^d$ translocated chromosome such as $1J_1^d$ or $2J_2^d$ (Fig. 3). Since the group $4J^d$ chromosomes are substantially restructured, they were likely involved in multivalent pairing structures with non-homoeologues during d-tritipyrum line development. Failure to pair in meiosis or segregation from multivalent pairing structures could explain some of the observed anomalies. Apart from the absence of a $4J^d$ chromosome, the five lines show evidence of additional chromosomal instability: RIL-193 (2n =39) also lacks a $7J^d$ chromosome. RIL-228 (2n = 39) appears to have a translocation of $2J_1^d$ to a wheat chromosome. Line 14M1088 appears to be a 4D for $4J^d$ substitution (unpublished data). RIL-198 and RIL-273 have 2n = 42 chromosomes yet lack a $4J^d$ chromosome which would suggest either substitution or the presence of rearranged chromosomes.

It is clear from the above data that restructuring of $4J_1^d$ had a profound effect on the viability and chromosome stability of the hybrids. If the chromosome $4J^d$ results (Fig. 6) are compared with that of chromosome $1J^d$ (Fig. 1), it appears that 18 lines had the $1J_1^d$ plus $4J_1^d$ chromosome combination; 24 lines had $1J_2^d$ plus $4J_2^d$; five lines had $1J_2^d$ but lacked a $4J^d$ chromosome. No line had $1J_1^d$ plus $4J_2^d$. Thus, it would appear that the combinations of $1J_1^d$ plus $4J_1^d$ and $1J_2^d$ plus $4J_2^d$ provided for the best genetic compensation and viability among the d-tritipyrum lines.

Three of six remaining co-amplifications that involved a group $4J^d$ chromosome and a non-homoeologue (Table 1) are in brief: (1) Chromosome $4J_1^d$ co-amplified with $5J_1^d$ (218 tags) confirming the presence of a translocation of $5J_2^d$ chromatin to $4J_1^d$ that was reported by Marais *et al*. (2021). It is possible that the 4J/5J translocation in *Th. distichum* is the same as the translocation reported by Grewal *et al*. (2018). (2) Another small translocation reported by Marais et al. (2021) transferred $6J_1^d$ chromatin to $4J_1^d$ and established $6J_2^d$_$4J_1^d$ homoeology; however, this study found only 22 such tags. (3) Co-amplification of $4J_2^d$_$6J_2^d$ (219 tags) occurred (Table 1) with 32 tags producing the $4J_2^d$-specific pattern, suggesting that a chromosome segment was transferred from $4J_1^d$ to $6J_2^d$ (Fig. 2).

Chromosomes $5J_1^d$, $6J_2^d$ and $7J_2^d$

The 6,622 $5J_1^d$ tags included 4,993 $5J_1^d$-specific tags and 1,140 $5J^d$ diagnostic tags that are shown in Figure 7. Forty lines had $5J_1^d$; seven lines did not have a $5J^d$ chromosome; whereas seven remaining lines had $5J_2^d$. Chromosome $5J_1^d$ co-amplified (261 tags) with $6J_2^d$ (Table 1) of which 182 tags produced the $5J_1^d$-specific amplification pattern among the test lines, suggesting that $5J_2^d$ chromatin had been translocated to $6J_2^d$. Chromosome $5J_1^d$ appears to be structurally better preserved which was also suggested by the results of Marais *et al*. (2021). SCAR marker UST-14 detects an anonymous sequence amplified on $5J_1^d$ by the RAPD primer OPV-7. The critical polymorphism amplified only in plants with $5J_1^d$ thus confirming its specificity.

Of the 7,373 $6J_2^d$ associated tags, 183 tags were $6J_2^d$-specific and 6,341 detected both $6J_1^d$ and $6J_2^d$. The amplification profiles (Fig. 8) suggested that 22 lines have $6J_1^d$, 25 lines have $6J_2^d$ and seven lines did not have a $6J^d$ chromosome. Chromosome $6J_2^d$ co-amplified 448 tags with $7J_2^d$ (Table 1) with the majority of tags producing the $7J_2^d$-specific amplification pattern which suggests that a chromosome segment was transferred from $7J_1^d$ to $6J_2^d$.

Among the 8,527 amplified $7J_2^d$ tags, 6,614 were $7J_2^d$-specific whereas 1,204 detected both $7J^d$ homoeologues (Fig. 9). The presence-absence patterns in the 54 lines revealed that 30 lines have $7J_2^d$; 16 lines have $7J_1^d$ and eight lines have neither of the $7J^d$ chromosomes.

Re-arranged J^d genomes

The *Th. distichum* chromosome translocations shown in Figure 3 do not include those that escaped detection because they are too small, or could not be detected due to incompleteness of the set of addition lines. Furthermore, some of the translocations shown in Fig. 3 could be the result of reciprocal exchanges of which the reciprocal products remain undetected. Considering the likely translocations, it appears that $4J_1^d$ and $1J_1^d$ have been modified the most while four additional chromosomes ($2J_2^d$, $4J_2^d$, $5J_2^d$ and $6J_2^d$) lost or received large chunks of non-homoeologous chromatin. Chromosome $6J_1^d$ was involved in a smaller translocation whereas chromosomes $3J_1^d$, $5J_1^d$, $7J_1^d$ and $7J_2^d$ appear to be the least modified. The status of chromosomes $1J_2^d$ and $3J_2^d$ is unknown. Marais et al. (2021) did BLAST comparisons of GBS sequence tags derived from eleven J^d addition chromosomes to the common wheat and *Th. elongatum* genomic data bases and concluded that $2J_1^d$, $3J_1^d$, $4J_2^d$, $5J_1^d$, $6J_1^d$, $7J_1^d$, and $7J_2^d$ aligned best with their homoeologues in the two reference genomes and may structurally be better preserved. However, their comparison did not consider the loss of chromatin through translocation, such as what happened in $4J_2^d$ (Fig. 2).

Theoretically, the two sets of *Th. distichum* chromosomes can be re-organized in $2^7 = 128$ different ways. If a single, genetically compensating J^d-genome is to be re-assembled from the 14 *Th. distichum* chromosomes, it seems that the best combinations would either be an assembly of the least modified chromosomes, or a compensating assembly that incorporates some/all of the highly modified (and inter-related) chromosomes. With both of chromosomes $4J_1^d$ and $4J_2^d$ apparently compromised by sizeable translocations to non-homoeologues, chromosome $4J^d$ may be problematic in any re-assembled J^d genome and may eventually require substitution by, or translocation to, a wheat chromosome.

The 44 d-tritipyrum lines (excluding three lines with 2n = 39 or 40) included 22 different combinations of J^d chromosomes (Table 2). The two most restructured chromosomes ($1J_1^d$ and $4J_1^d$) always occurred together in the same genomes (16 lines) whereas $1J_2^d$ and $4J_2^d$ (or a modified $4J^d$ chromosome) occurred together (27 lines). The two arrangements probably provided for the most complete compensation between the two chromosome groups. A similar tendency to associate was seen with regard to $3J_1^d$ and $6J_1^d$ (19 lines) versus $3J_2^d$ and $6J_2^d$ (19 lines); however, the two sets of associations ($1J^d$&$4J^d$ and $3J^d$&$6J^d$) appeared to occur independently from each other. Since a complete set of addition chromosomes was not available, the chromosome structural basis for the complementation cannot be explained. Three chromosome combinations ($1J_1^d$&$4J_2^d$; $1J_2^d$&$4J_1^d$ and $3J_2^d$&$6J_1^d$) appeared to result in low/no viability which reduced the potentially useful chromosome sets (= reorganized genomes) from 128 to 48. If the three recombined genomes (#12, #15 and #21 in Table 2) that each have a modified/substituted $4J^d$ chromosome are excluded, 19 of the 48 likely J^d genomes were present among the tested lines. The most frequently occurring genome (six lines) was genome #19. Possible reasons for the absence of the remaining genomes could be that they reduced viability and phenotypic appeal (the study focused on the better lines), or were not included by chance in the relatively small group of lines that was tested.

The most promising J^d genomes

The chromosome data of Table 2 were considered with agronomic data (plant height, earliness, plant phenotype and salt tolerance) to select a subset of ten d-tritipyrum lines, one 4D for $4J^d$ d-tritipyrum substitution line and one common wheat substitution line that has $2J_1^d$ and $3J_1^d$ in place of 2D and 3D, respectively (Table 3). The plant heights of the 43 d-tritipyrum lines ranged from 22 to 83 inches (average = 40); days to flowering ranged from 43 to 102 (average = 69); the phenotypic scores ranged from 0 to 4 (average = 1.8); and the salt tolerance scores of 26 lines ranged from 4.1 to 7.6 (average = 6.6). The eleven d-tritipyrum selections had non-brittle spikes with a moderately strong to tough rachis. With the exception of 17M1034, the lines were harder to thresh than wheat and comparable to triticale. The eleven d-tritipyrum selections had a base set of five J^d chromosomes in common ($1J_2^d$,

$2J_1^d$, $4J_2^d$, $5J_1^d$ and $7J_2^d$) and could be sub-divided based on the two remaining chromosomes (Table 3). The J^d genome of group A also included chromosomes $3J_2^d$ and $6J_2^d$. It is expected that the six group A genotypes will be fully cross compatible and produce hybrids with regular meioses. Group B line Matie differed from group A in having $3J_1^d$ instead of $3J_2^d$. Matie has a desirable semi-dwarf phenotype and early flowering and can be used in crosses with group A if the $3J^d$ homoeologues prove capable of regular meiotic pairing and crossover. In addition to having the better phenotypes, group A and B lines showed promising variation for salt tolerance (5.8-7.3), plant height and earliness and included three lines with very hard stems that were completely filled with pith over the full length of the stem. The three group C lines (Table 3) differed from group A in having the $3J_1^d$ and $6J_1^d$ chromosome combination and were characterized by varying degrees of aberrant flower development (upper spike) and tip sterility, resulting in poorer overall phenotypes. Of these, line 17M1034 had good salt tolerance, earliness and easier thresh-ability and can be used as donor line to incorporate these traits through backcrosses into group A. D-tritipyrum line 14M1088 has the same J^d genome as group C but with 4D substituted for $4J^d$. The latter line can be used for producing $4J_2^d$/4D translocation chromosomes if it proves necessary to restructure and replace $4J_2^d$. Continued development of the selected d-tritipyrum lines will also require broadening of the A and B genome variability through crosses with hexaploid wheat followed by backcrosses to the d-tritipyrum group A lines to restore the hybrid J^d genome. Finally, common wheat substitution line 19M1-15-4 can be evaluated as a potential source of salt tolerance genes that can be transferred to common wheat.

Stem solidness in durum and bread wheat can provide protection against the wheat stem sawfly (*Cephus cinctus* Norton) (Biyiklioglu *et al*., 2018). Having solid stems may also benefit plants growing in dry environments where the water-holding capacity of the pith parenchyma could contribute to drought and heat tolerance (Saint Pierre *et al*., 2010). Fast-growing grasses such as tall wheatgrass (*Th. elongatum*), smooth bromegrass, tall fescue and switchgrass can furthermore be alternative sources of fiber for pulp and paper making, plywood production, etc. as wood resources become scarcer (Przybysz *et al*., 2018). In hexaploid wheat, stem solidness is a dominant trait primarily determined by the *Qss.msub-3BL* locus on chromosome 3BL, while additional minor genes were reported on chromosomes 1B, 3D, and 5D (Nilsen *et al*., 2016). In durum wheat, the solid stem trait was ascribed to *SSt1*, a single dominant gene that occurs in the same region as *Qss.msub-3BL* (Nilsen *et al*., 2016). Nilsen *et al*. (2020) suggested that copy number variation of the *TdDof* gene (which encodes a putative DNA binding protein and occurs inside the *SSt1* locus) regulates the degree of stem solidness. Hollow-stemmed wheat cultivars have a single copy of *TdDof*, whereas solid-stemmed cultivars carry multiple identical copies of the gene. Copy number-dependent expression of *TdDof* could be directly or indirectly involved with negative regulation of programmed cell death in the pith cells. Within the current d-tritipyrum collection it was not possible to associate complete stem solidness with any specific chromosome. Of the 43 d-tritipyrums listed in Table 2, 21 had $3J_1^d$ (of these 17 were hollow stemmed or nearly so and four were completely solid). The remaining 22 lines with $3J_2^d$ included 17 lines that were hollow stemmed or nearly so, and five were completely solid. Assuming that a major genetic determinant of the completely solid stem trait occurs on the group $3J^d$ chromosomes, it appears likely that in the wild species, only one of the $3J_1^d$ and $3J_2^d$ homoeologues may carry the trait locus. It also appears likely that during derivation of the d-tritipyrum lines, the two $3J^d$ chromosomes regularly paired and recombined in meiosis to introduce variation at the locus to both chromosomes.

Perennial growth is a highly complex adaptation that enables plants to enter a dormant period to avoid environmental stresses, usually cold or drought (Lammer *et al*., 2004). It not only requires plants to have the ability to preserve viable meristems during the adverse environmental conditions but also a biological life cycle that is tuned to that environment. Such adaptive abilities are believed to be largely quantitatively determined. Lammer *et al*. (2004) found that monosomic and disomic additions and substitutions of *Th. elongatum* chromosome 4E to annual Chinese Spring wheat caused the plants to be polycarpic. Plants with 4E were able to preserve viable axillary meristems past grain ripening and then initiate a second period of tiller outgrowth. This ability appeared to be a relatively simple genetic trait which was termed 'post-sexual cycle regrowth'. The gene involved (named *Pscr1)* was subsequently mapped to a short distal region of *Th. elongatum* arm 4ES (Abbasi *et al*., 2020). The latter authors pointed out that in addition to 4E, genes on other *Th. elongatum* chromosomes also appear to contribute to the ability for perennial growth. *Thinopyrum elongatum* is a bunch (caespitose) perennial grass whereas *Th. distichum* is a rhizomatous perennial. The majority of the selected d-tritipyrum lines regrew extensively from the lower nodes during and after ripening with W1735-13T having a particularly strong tendency for regrowth. Re-growth is initiated from the lower nodes rather than from the base of the plant. Interestingly, RIL-12 which has the group C J^d genome (= genome #14, Table 2), grew to a height of six feet and produced rhizomes having numerous short internodes with buds and roots developing from the nodes (Fig. 10). These traits could allow for the selection of plants with the ability to be perennial in an appropriate environment. In an environment that only supports seasonal growth, late season regrowth could be exploited to provide grazing to farm animals following a seed harvest.

Table 1. Numbers of paired tags that co-amplified in all possible combinations of the nine *Th. distichum* addition chromosomes. Only the eight stronger co-amplifications that involved non-homoeologues (underlined) were analyzed.

	Add $2J_1^d$	**Add $2J_2^d$**	**Add $3J_1^d$**	**Add $4J_1^d$**	**Add $4J_2^d$**	**Add $5J_1^d$**	**Add $6J_2^d$**	**Add $7J_2^d$**
Add $1J_1^d$	47	43	14	1,814[1]	284[1]	69	74	24
Add $2J_1^d$		801	139	32	51	142	352	119
Add $2J_2^d$			75	13	1,994[1]	94	119	72
Add $3J_1^d$				15	53	47	84	111
Add $4J_1^d$					66	218	22[1]	19
Add $4J_2^d$						99	219	61
Add $5J_1^d$							261	133
Add $6J_2^d$								448

[1] Marais *et al*. (2019) reported synteny between segments on non-homoeologous J^d chromosomes that resulted from translocations, four of which are also evident in the current data and are marked in the table. A fifth translocation from their study resulted in $1J_1^d$_$6J_1^d$ homoeology but was not detectable in the current dataset because a chromosome $6J_1^d$ addition line was not available.

Table 2. Re-arranged genomes that occurred among 43 d-tritipyrum lines. The J^d-genomes of 16 lines (light grey) included $1J_1^d$ and $4J_1^d$ which appeared to be the most extensively re-arranged of the fourteen *Thinopyrum distichum* chromosomes, whereas 24 lines (dark grey) had $1J_2^d$ and $4J_2^d$. Three lines had $1J_2^d$ in combination with a substituted/modified $4J^d$ chromosome.

J^d		**Chromosome group:**							**Entries with this pattern[1]**
Subset:		**1**	**2**	**3**	**4**	**5**	**6**	**7**	
1		$1J_1^d$	$2J_1^d$	$3J_1^d$	$4J_1^d$	$5J_1^d$	$6J_1^d$	$7J_1^d$	RIL-99; RIL-166; 14M1085; 14M1092
2		$1J_1^d$	$2J_1^d$	$3J_1^d$	$4J_1^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	RIL-30
3		$1J_1^d$	$2J_1^d$	$3J_1^d$	$4J_1^d$	$5J_2^d$	$6J_1^d$	$7J_2^d$	16M1008-3-1
4		$1J_1^d$	$2J_2^d$	$3J_1^d$	$4J_1^d$	$5J_2^d$	$6J_1^d$	$7J_1^d$	RIL-121
5		$1J_1^d$	$2J_2^d$	$3J_1^d$	$4J_1^d$	$5J_1^d$	$6J_1^d$	$7J_1^d$	RIL-190
6		$1J_1^d$	$2J_2^d$	$3J_1^d$	$4J_1^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	RIL-7
7		$1J_1^d$	$2J_2^d$	$3J_2^d$	$4J_1^d$	$5J_1^d$	$6J_2^d$	$7J_1^d$	W1736
8		$1J_1^d$	$2J_2^d$	$3J_2^d$	$4J_1^d$	$5J_2^d$	$6J_2^d$	$7J_2^d$	TDH-2
9		$1J_1^d$	$2J_1^d$	$3J_2^d$	$4J_1^d$	$5J_1^d$	$6J_2^d$	$7J_1^d$	RIL-232
10		$1J_1^d$	$2J_1^d$	$3J_2^d$	$4J_1^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	RIL-165
11		$1J_1^d$	$2J_1^d$	$3J_2^d$	$4J_1^d$	$5J_2^d$	$6J_2^d$	$7J_2^d$	RIL-97; 16M1001-2-5-2; 16M1001-2-5-3
12		$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J^d$ M[2]	$5J_1^d$	$6J_2^d$	$7J_1^d$	RIL-273
13		$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Matie
14		$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	RIL-12[5]; RIL-136[4,5]; RIL-189; TDH-1; 17M1034
15		$1J_2^d$	$2J_1^d$	$3J_1^d$	4D[3]	$5J_1^d$	$6J_1^d$	$7J_2^d$	14M1088
16		$1J_2^d$	$2J_2^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_1^d$	RIL-10[4]
17		$1J_2^d$	$2J_2^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	RIL-15[4]; RIL-307[4], TDH-9
18		$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_1^d$	RIL-89[4]
19		$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	W1735-13T[4]; RIL-54[4], RIL-251[4]; RIL-263; TDH-3; TDH-5
20		$1J_2^d$	$2J_2^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_1^d$	RIL-41; RIL-277; RIL-308
21		$1J_2^d$	$2J_2^d$	$3J_2^d$	$4J^d$ M[2]	$5J_1^d$	$6J_2^d$	$7J_1^d$	RIL-198
22		$1J_2^d$	$2J_2^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	RIL-36; RIL-46, RIL-283, TDH-7[4]

[1] Four of the 47 d-tritipyrum lines (RIL-159, RIL-193, RIL-228 and 16M1008-2) had 2n = 39 or 40 chromosomes and were excluded from the list.
[2] Not a regular $4J^d$ chromosome; has been structurally modified in an unknown manner.
[3] Has $4J^d$ replaced by 4D of wheat.
[4] Has completely solid stems.
[5] Produces rhizomes.

Table 3. Information pertaining to the ten most promising d-tritipyrum lines and two useful hybrid derivatives (from crosses involving common wheat) that were selected based on plant phenotype and J^d genome similarity.

Group	Chr	Entry	Chromosome group							Stems[1]	Height	Days	Plant	Salt
	(2n)	number	1	2	3	4	5	6	7		(in)	flower	score[2]	tol.
A	42	W1735-13T	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Vs	32	81	4	7.3
A	42	RIL_54	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Vs	34	75	4	6.4
A	42	RIL_251	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Vs	37	91	2.5	6.1
A	42	RIL_263	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Sb	38	84	4	7.0
A	42	TDH-3	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Ho	41	85	4	6.6
A	42	TDH-5	$1J_2^d$	$2J_1^d$	$3J_2^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Ho	38	66	3	5.8
B	42	Matie	$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_2^d$	$7J_2^d$	Ho	33	63	4	6.0
C	42	RIL_189	$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	Ho	41	75	3	6.5
C	42	TDH-1	$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	Sb	42	78	1	6.9
C	42	17M1034	$1J_2^d$	$2J_1^d$	$3J_1^d$	$4J_2^d$	$5J_1^d$	$6J_1^d$	$7J_2^d$	Ho	35	43	3	6.9
d-tritipyrum substitution	42	14M1088	$1J_2^d$	$2J_1^d$	$3J_1^d$	[4D]	$5J_1^d$	$6J_1^d$	$7J_2^d$	Ho	41	52	1	7.0
Wheat substitution	42	19M1-15-4	1D	[$2J_1^d$]	[$3J_1^d$]	4D	5D	6D	7D	Ho	41	39	4	-

[1] Ho = hollow stems; Sb = Stems are solid to semi-solid at the base; Vs = Hard, solid stems filled with pith throughout
[2] Plants were visually scored for phenotypic appearance where 0 = very poor and 5 = very good, wheat-like appearance.

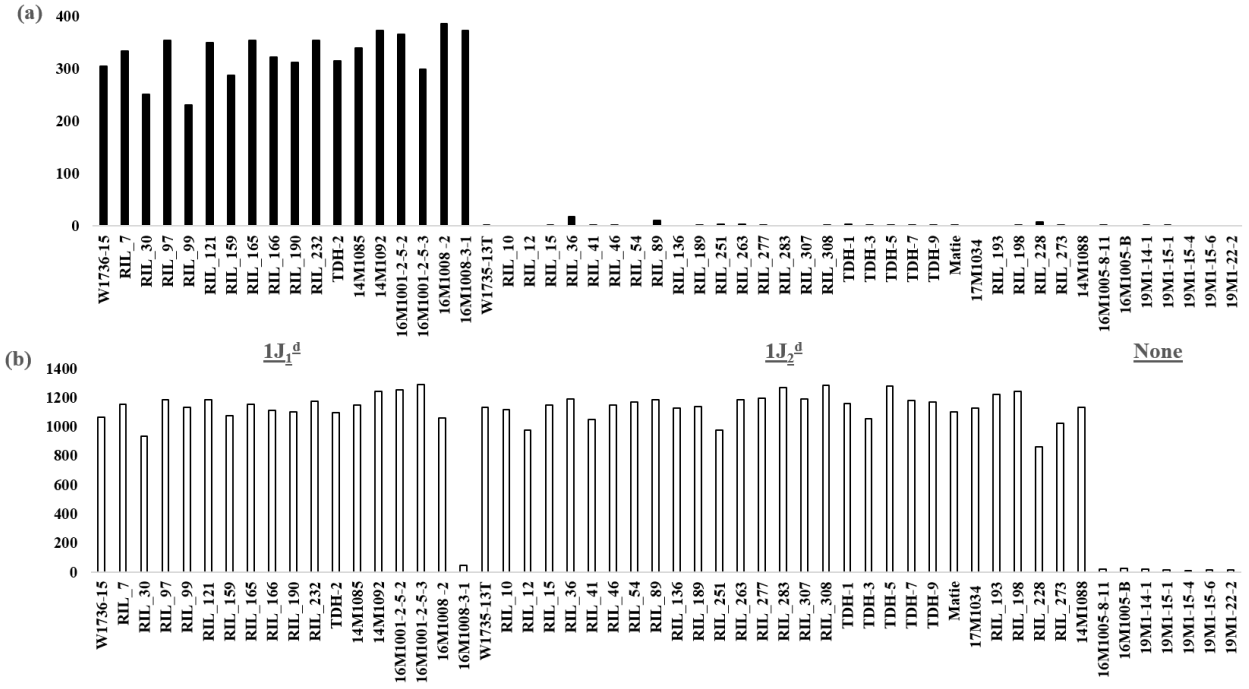

Fig. 1. Amplification of 1,895 $1J_1^d$ single copy tags in 54 d-tritipyrum lines and derivatives: (a) Of these tags, 463 amplified only in lines with $1J_1^d$. (b) Another 1,432 tags amplified in both $1J^d$ homoeologues. Eighteen lines had $1J_1^d$; 29 lines had $1J_2^d$ whereas seven lines had neither of the chromosomes.

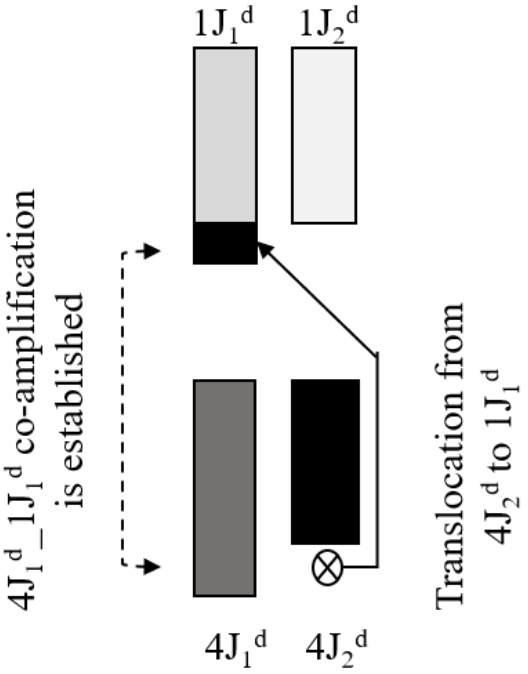

Fig. 2. In this example, a $4J_2^d$ chromosome segment was translocated to chromosome $1J_1^d$. This established translocation-associated $4J_1^d\_1J_1^d$ synteny. Amplification of tags that reside in the $4J^d$ homoeologous regions affected by the translocation were found to produce presence-absence patterns consistent with the homoeologous group $4J^d$ chromosomes, with the pattern of the un-translocated chromosome ($4J_1^d$) being the most prominent.

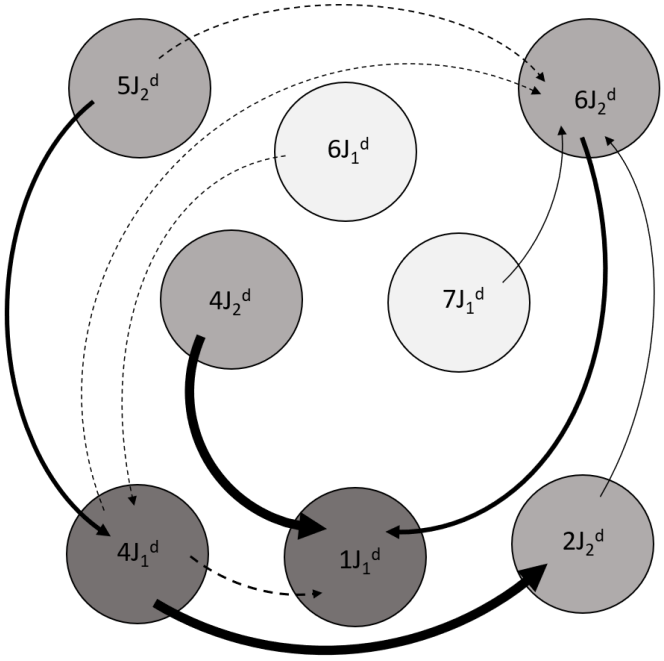

Fig. 3. A summary of possible *Thinopyrum distichum* translocations reported by Marais *et al*., (2021) and the present study. Arrows are used to show the origin and direction of each translocation with thicker arrows indicating larger translocated segments. Chromosomes (circles) filled with darker shades of grey appeared to be more extensively modified.

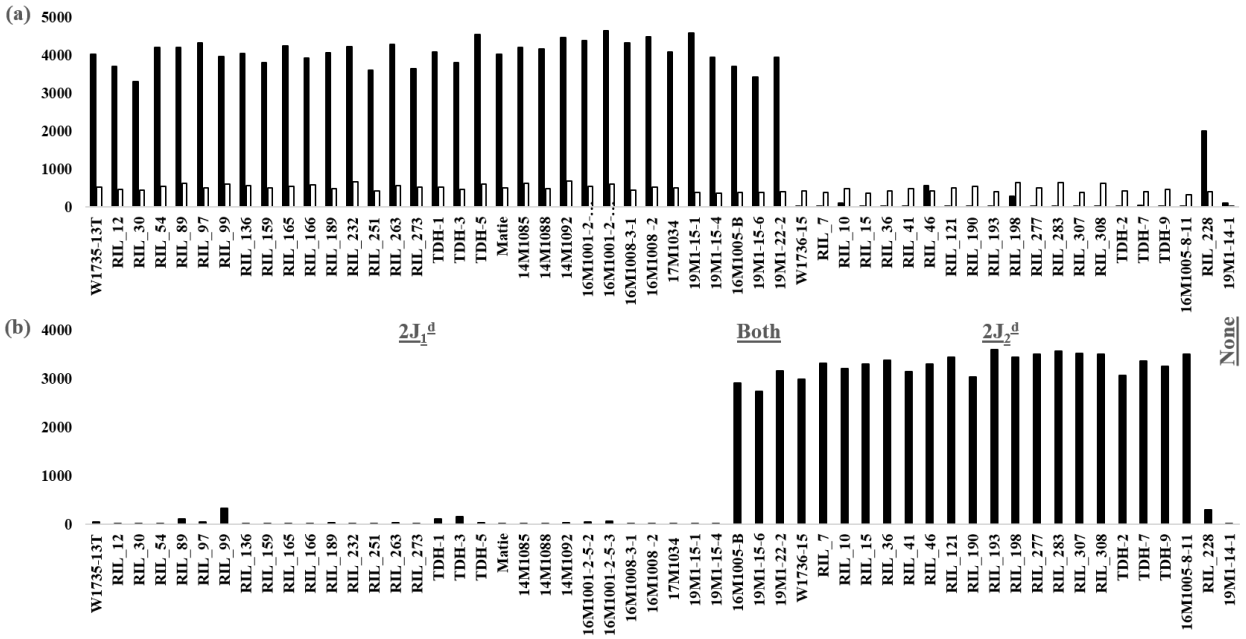

Fig. 4. Amplification of 9,922 $2J^d$ single and paired sequence tags in 54 d-tritipyrum lines and accessions: (a) A group of 5,080 chromosome-specific tags was associated with the presence of chromosome $2J_1^d$ (black bars) whereas 863 tags (white bars) appeared to amplify within both $2J_1^d$ and $2J_2^d$ regions. (b) 3,979 chromosome-specific tags were associated with $2J_2^d$. Thirty lines had $2J_1^d$; 19 lines had $2J_2^d$; three related lines (16M1005-B; 19M1-15-6 and 19M1-22-2) had both $2J_1^d$ and $2J_2^{d;}$ whereas one line (19M1-14-1) lacked a $2J^d$ chromosome. RIL-228 could have a smaller $2J^d$ translocated segment (possibly to wheat).

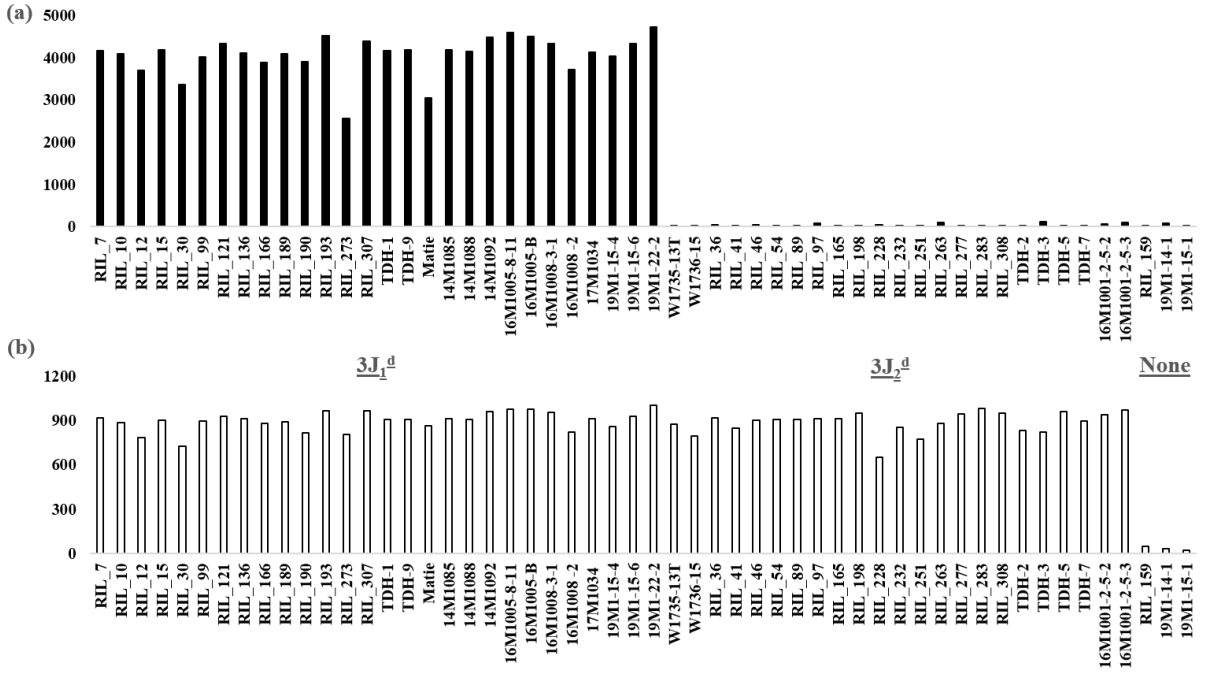

Fig. 5. Presence/absence of 6,207 single sequence tags that were associated with chromosome $3J_1^d$: (a) 5,149 tags detected $3J_1^d$ specifically, and (b), 1,058 tags detected both $3J^d$ homoeologues. Twenty-eight lines appeared to have $3J_1^d$; 23 had $3J_2^d$ and three lines (RIL-159, 19M1-14-1 and 19M1-15-1) did not have a $3J^d$ chromosome.

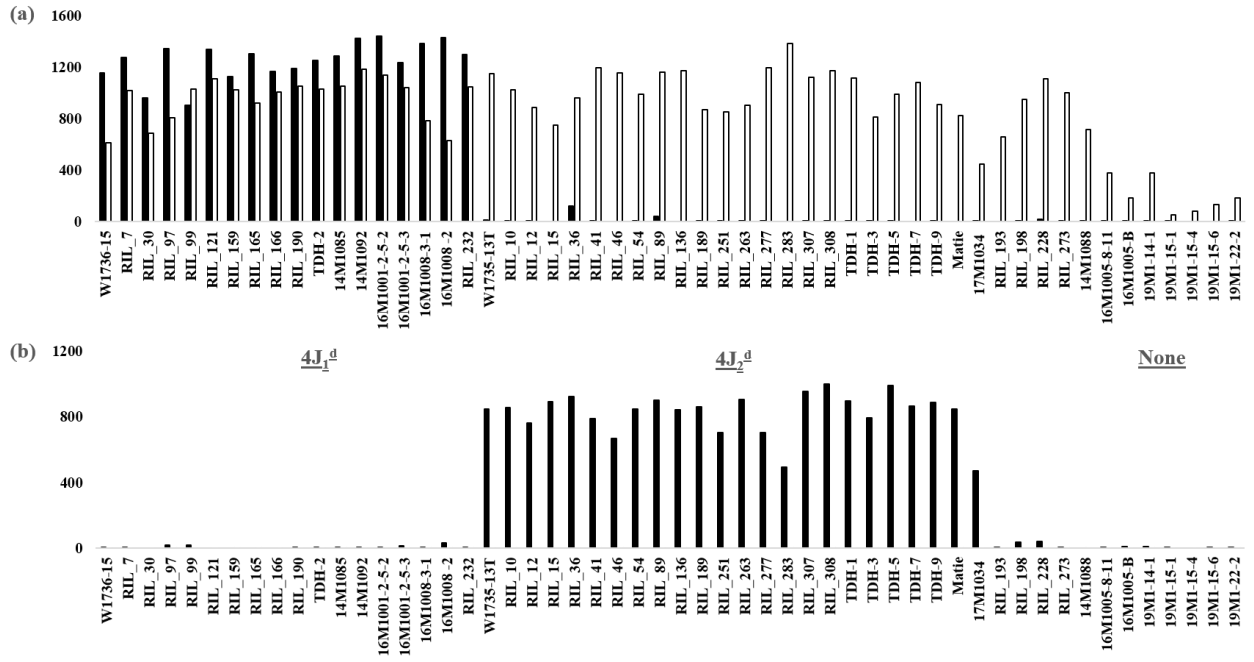

Fig. 6. Presence/absence of 3,923 *Th. distichum* group $4J^d$ single and shared sequence tags in 54 d-tritipyrum lines and accessions: (a) 1,685 tags amplified only from $4J_1^d$ regions (black bars) whereas 1,013 tags amplified in both $4J^d$ chromosomes (white bars). (b) 1,225 tags were chromosome $4J_2^d$–specific. The markers showed that 18 lines have $4J_1^d$; 23 lines have $4J_2^d$; five lines do not have either of $4J_1^d$ or $4J_2^d$ but have reduced amounts of $4J^d$ chromatin elsewhere in the genome (for example, on either or both of the $4J_1^d$ translocations to $1J_1^d$ or $2J_2^d$). Eight lines do not have a $4J^d$ chromosome.

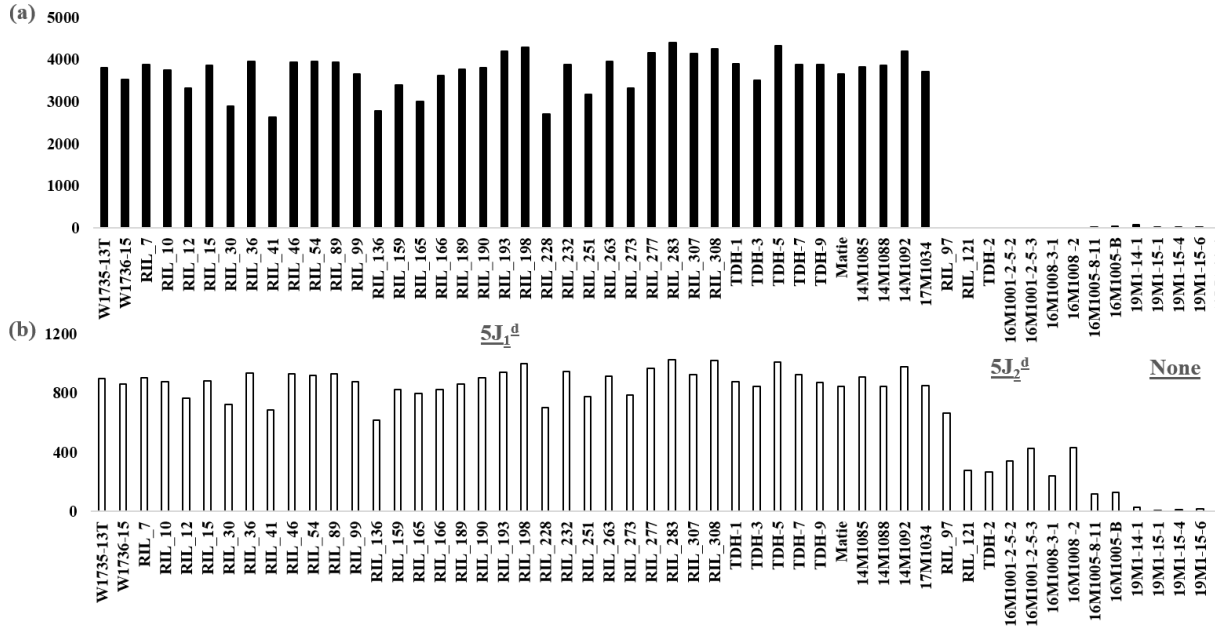

Fig. 7. Amplification of (a) 4,993 $5J_1^d$–specific sequence tags and (b) 1,140 sequence tags that occur in both $5J^d$ homoeologues in 54 test lines. Forty lines have $5J_1^d$, seven lines appear to have the $5J_2^d$ homoeologue and another seven lines completely lack a $5J^d$ chromosome.

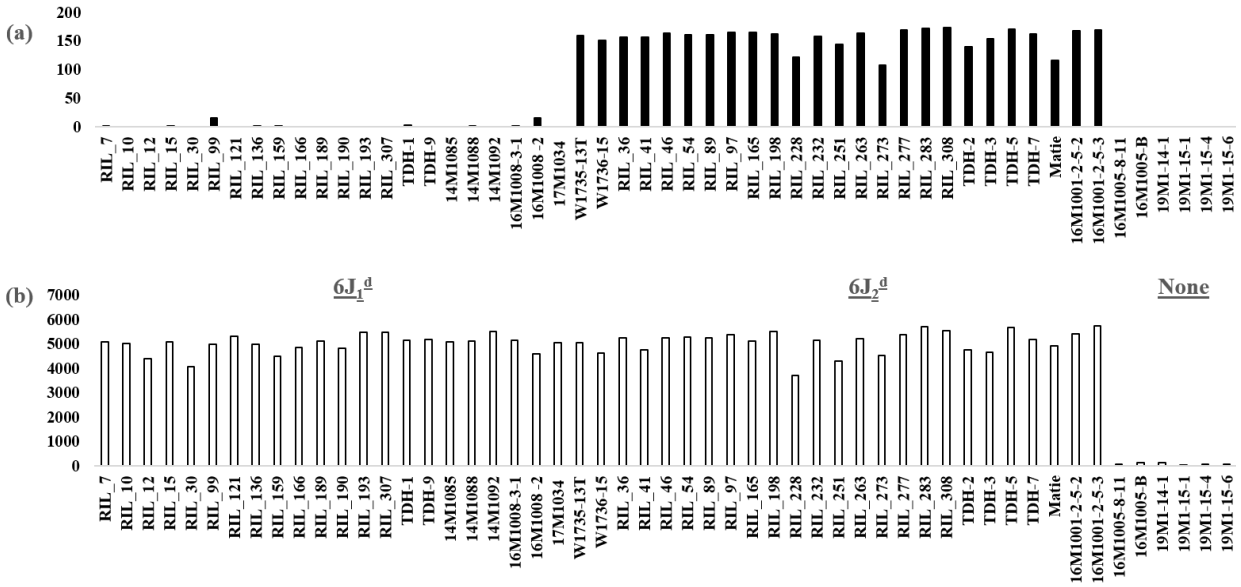

Fig. 8. A total of 7,373 single and paired sequence tags were associated with $6J_2^d$. Of these, (a) 183 were $6J_2^d$-specific tags and (b) 6,341 detected both $6J_1^d$ and $6J_2^d$. Twenty-two lines appear to have $6J_1^d$; 25 lines appear to have $6J_2^d$ and seven lines lack a $6J^d$ chromosome.

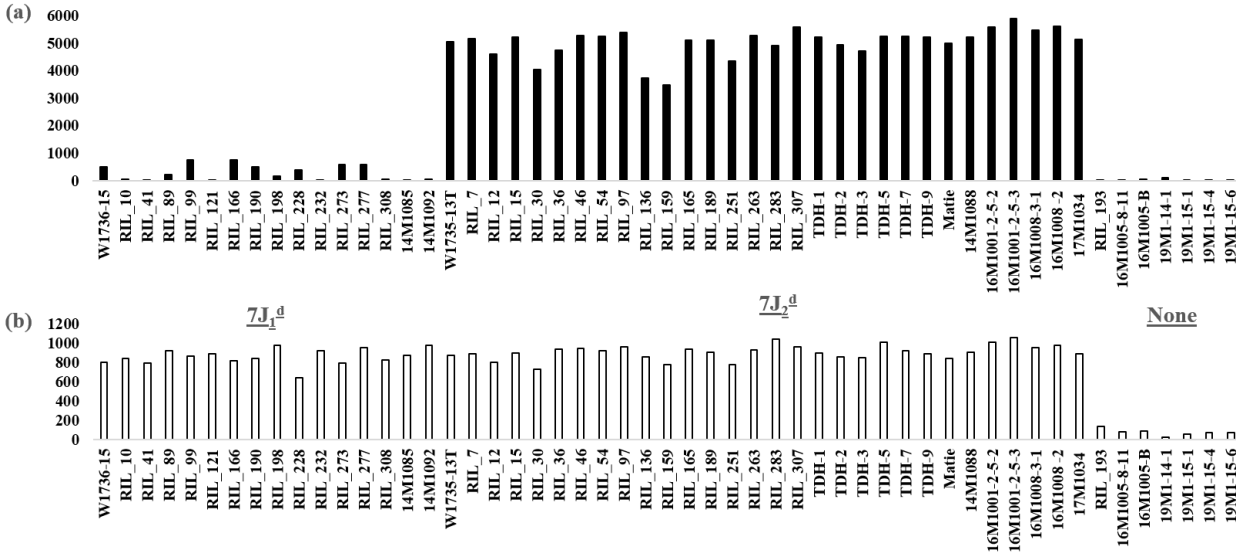

Fig. 9. Amplification patterns that were associated with 7,818 sequence tags amplified in addition chromosome $7J_2^d$. (a) The 6,614 $7J_2^d$–specific sequence tags and (b) 1,204 $7J^d$ homoeologous group tags suggested that 30 lines had $7J_2^d$; 16 lines had $7J_1^d$ and eight lines have neither of the $7J^d$ chromosomes.

Fig. 10. A rhizome produced by d-tritipyrum RIL-12.

CONCLUSION

Genotyping by sequencing methodology made it possible to determine and compare different J^d chromosome combinations within a population of highly diverse d-tritipyrum lines and arrive at a consensus genome that is aligned with the best plant phenotypes. Exclusive use of the chosen J^d genome is expected to result in regular meiotic chromosome pairing and Mendelian segregation in future hybrid progenies which will greatly facilitate ongoing attempts to develop the hybrids through crossbreeding and selection.

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