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Development and identification of new synthetic *T. turgidum–T. monococcum* amphiploids

Hongyu Li[1], Xiaojuan Liu[1], Minghu Zhang[1], Zhen Feng[1], Dengcai Liu[1], Michael Ayliffe[2], Ming Hao[1], Shunzong Ning[1], Zhongwei Yuan[1], Zehong Yan[1], Xuejiao Chen[1] and Lianquan Zhang[1]*

[1]*Triticeae Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, Chengdu, Sichuan 611130, China and* [2]*CSIRO Agriculture, Box 1600, Clunies Ross Street, Canberra, ACT 2601, Australia*

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Abstract

Triticum monococcum ssp. *monococcum* has useful traits for bread wheat improvement. The synthesis of *Triticum turgidum–T. monococcum* amphiploids is an essential step for transferring genes from *T. monococcum* into bread wheat. In this study, 264 wide hybridization combinations were done by crossing 60 *T. turgidum* lines belonging to five subspecies with 83 *T. monococcum* accessions. Without embryo rescue and hormone treatment, from the 10,810 florets pollinated, 1983 seeds were obtained, with a mean crossability of 18.34% (range 0–89.29%). Many hybrid seeds (90.73%, 923/1017) could germinate and produce plants. A total of 56 new amphiploids ($AABBA^mA^m$) were produced by colchicine treatment of *T. turgidum* × *T. monococcum* F_1 hybrids. The chromosome constitution of amphiploids was characterized by fluorescence *in situ* hybridization using oligonucleotides probes with different chromosome and sub-chromosome specificities. Sodium dodecyl sulphate polyacrylamide gel electrophoresis analysis indicated that the *Glu-A1^m-b*, *Glu-A1^m-c*, *Glu-A1^m-d* and *Glu-A1^m-h* proteins of *T. monococcum* were expressed in some amphiploids. Despite resistance reduction in several cases, 45 out of 56 amphiploids exhibited resistance to the current predominant Chinese stripe rust races at both the seedling and adult plant stage. These novel amphiploids provide new germplasm for the potential improvement of bread wheat quality and stripe rust resistance.

Keywords: FISH, SDS-PAGE, stripe rust resistance, quality improvement

Introduction

Alien species are important resources for increasing the genetic diversity of bread wheat (*Triticum aestivum* L.) (Mujeeb-Kazi and Kimber, 1985). The cultivated einkorn *T. monococcum* L. subsp. *monococcum* ($2n = 2x = 14$, genome A^mA^m) is the first cultivated wheat. It is closely related to *Triticum urartu* Tumanian ex Gandilyan ($2n = 2x = 14$, A^uA^u) which is the A genome donor progenitor of hexaploid bread wheat (Dvorák *et al.*, 1993). *T. monococcum* ssp. *monococcum* has useful traits for bread wheat improvement, such as high-protein content (Tranquilli *et al.*, 2002), diverse *Glu-A1^{m}x* alleles (Li *et al.*, 2016, 2017), tolerance to cold stress (Aslan *et al.*, 2016), resistance to preharvest sprouting (Sodkiewicz, 2002) and high resistance to diseases (Mikhova, 1988; Hussien *et al.*, 1998; Chhuneja *et al.*, 2008; Rouse and Jin, 2011a, b; Schmolke *et al.*, 2012; Zaharieva and Monneveux, 2014). Moreover, its high tocol and carotenoid contents make it a promising source for functional food production (Brandolini *et al.*, 2008).

The application of cultivated einkorn in bread wheat breeding is greatly limited by its poor crossability and the

*Corresponding author. E-mail: zhanglianquan1977@126.com

sterile F_1 hybrids produced by its direct cross with bread wheat (The and Baker, 1975; Cox *et al.*, 1991; Plamenov *et al.*, 2009). Post-syngamic hybridization barriers resulting in embryo abortion and failure of endosperm development make direct transfer of useful genes from einkorn to bread wheat difficult (Bhagyalakshmi *et al.*, 2008). An alternative approach for introgressing traits from a diploid species into hexaploid wheat is to create amphiploids between diploid and tetraploid species which are then subsequently crossed with cultivated wheat (Dorofeev *et al.*, 1987).

There are two main methods for synthetic amphiploid production using *T. monococcum*. One method is by producing *Triticum timococcum* or synthetic *Triticum zhukovskyi* ($2n = 6x = 42$, $A^tA^tGGA^mA^m$) by crossing *Triticum timopheevii* and *T. monococcum* (Kostov, 1936; Cao *et al.*, 2000; Goncharov *et al.*, 2007). New *T. timococcum* lines were recently developed in order to introgress useful genes for conventional and organic wheat breeding (Mikó *et al.*, 2015). The second is by *Triticum turgidum–T. monococcum* amphiploid ($AABBA^mA^m$, $2n = 6x = 42$) production which combines useful einkorn genes with tetraploid *T. turgidum* wheat, usually *T. turgidum* ssp. *durum* (Dorofeev *et al.*, 1987; Gill *et al.*, 1988; Mujeeb-Kazi and Hettel, 1995; Watanabe *et al.*, 1997; Cakmak *et al.*, 1999; Megyeri *et al.*, 2011).

In the present study, we have developed 56 *T. turgidum–T. monococcum* amphiploids using 31 *T. turgidum* accessions from five subspecies. This article reports the development, molecular cytogenetic identification and the agronomic trait evaluation of these new synthetic *T. turgidum–T. monococcum* amphiploids.

Materials and methods

Plant materials

Sixty *T. turgidum* and 83 *T. monococcum* accessions with diverse geographic origins (Zhang *et al.*, 2008; Li *et al.*, 2016) were used in this study. Lines with PI or CItr prefixes were kindly provided by USDA-ARS, USA while AS lines were obtained from the Sichuan Agricultural University. These *T. turgidum* lines were derived from either subspecies *dicoccon* (26 lines), *durum* (three lines), *turanicum* (six lines), *turgidum* (24 lines) or *persicum* (one line) (Van Slageren, 1994). All 83 *T. monococcum* accessions used were *T. monococcum* ssp. *monococcum* (Zhang *et al.*, 2008; Li *et al.*, 2016).

Hybridization

Hybridization between these species was undertaken using *T. turgidum* as the female parent and *T. monococcum* as the male parent. Reciprocal crosses were not attempted since einkorn cytoplasm induces male sterility (The and Baker, 1975). Crosses were made under field conditions in the 2013–2014 crop season. Emasculation and pollination were done as previously described by Zhang *et al.* (2008). No embryo rescue or hormone treatment was applied for the production of F_1 seeds. The spikes were harvested and the number of seeds set per spike counted approximately 20 d after pollination. Crossability was expressed as the percentage of seed set per floret pollination for each line.

Chromosome doubling by colchicine treatment

F_1 seeds were germinated in Petri dishes and the root tips analysed cytologically prior to planting. Hybrid F_1 plants were chromosome doubled by colchicine treatment at the three-tiller stage according to Cao *et al.* (2000) and then transplanted in the field at the Wenjiang Experimental Station of Sichuan Agricultural University. Treated F_1 plants were self-fertilized and the seed set (percentage of selfed seed set per self-pollenated floret) for each plant calculated.

Cytological observation

Cytological observation on chromosome number in root-tip cells and chromosome pairing in pollen-mother cells (PMCs) were done as previously described by Zhang *et al.* (2007). For meiotic analysis, at least 50 PMCs were observed for each synthetic amphiploid. Univalent (I), bivalents (II), trivalents (III), quadrivalents (IV) and pentavalents (V) were counted and their average numbers were calculated.

Multicolour fluorescence *in situ* hybridization (FISH) was carried out according to Tang *et al.* (2014) and Zhao *et al.* (2016). For multicolour FISH, synthetic oligonucleotides Oligo-pSc119.2-1, Oligo-pTa71-2, Oligo-pTa535-1 and $(AAC)_5$ were used as probes to detect FISH signals in order to differentiate individual chromosomes of *T. turgidum* and *T. monococcum* in newly synthesized *T. turgidum–T. monococcum* amphiploids. Probe Oligo-pSc119.2-1 preferentially paints tandem repeats on B-genome chromosomes, Oligo-pTa71-2 is largely specific for the sub-terminal regions of 1BS and 6BS, Oligo-pTa535-1 preferentially paints tandem repeats on the A^m- and A-genome chromosomes, while $(AAC)_5$ is largely specific for the $6A^m$ chromosome (Megyeri *et al.*, 2012, 2017; Tang *et al.*, 2014; Zeng *et al.*, 2016). All probes were synthesized and labelled with FAM or Tamra (TSINGKE Biological Technology Company, Chengdu, China). Hybridization signals were observed using an Olympus BX-63 epifluorescence microscope and the images were photographed using a Photometric SenSys Olympus DP70 CCD camera (Olympus, Tokyo).

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Raw images were processed using Photoshop ver. 7.1 (Adobe Systems Incorporated, San Jose, CA, USA). Individual chromosomes of amphiploids were compared with the karyotypes of the previously published FISH patterns of *T. turgidum* (Zeng *et al.*, 2016) and *T. monococcum* (Megyeri *et al.*, 2012; Mikó *et al.*, 2015).

SDS-PAGE analysis

Seed protein extraction and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were undertaken as described by Yan *et al.* (2002). Detection of *Glu-A1^{m}x* proteins of *T. monococcum* was as described by Li *et al.* (2016). Bread wheat cultivars Chuanyu 12 (subunit 1, 7 + 8, 5 + 10), Longfumai 1 (2*, 7 + 8, 5 + 10) and Chinese Spring (null, 7 + 8, 2 + 12) were used as reference standards for comparing the electrophoretic mobility of HMW-GSs.

Stripe rust resistance evaluation

Field evaluation for stripe rust resistance was conducted both at seedling and adult plant stages at the Wenjiang Experimental Station of Sichuan Agricultural University in the 2015–2016 crop season. Lines were grown as individual plants spaced 10 cm apart in 2 m rows with 30 cm between rows. The highly rust-susceptible spreader variety SY95-71 was planted on each side of each experimental row. A stripe rust epidemic was initiated 6 weeks after planting by inoculating plants with urediniospores mixtures that included current predominant Chinese stripe rust races such as CYR32, CYR33 and CYR34. Rust isolates were provided by the Research Institute of Plant Protection, Gansu Academy of Agricultural Sciences. Stripe rust infection type (IT) on individual plants was recorded three times at 10 d intervals. Disease notes were taken when the flag leaves of the susceptible check SY95-71 were heavily infected. For each plant, the IT produced was estimated on a 1–9 scale (Wellings and Bariana, 2004) with the highest IT recorded used as the resistance type of the line. Plant ITs were divided into seven classes: highly resistant (1–2), resistant (3), moderately resistant (4), intermediate (5), moderately susceptible (6–7), susceptible (8) and highly susceptible (9).

Results

The crossability of T. turgidum *with* T. monococcum

Two hundred and sixty-four hybridization combinations were undertaken by crossing 60 *T. turgidum* lines, belonging to five subspecies, with 83 *T. monococcum* accessions (online Supplementary Tables S1–S3). From the 10,810 florets pollinated, 1983 seeds were obtained. The mean crossability of the 264 combinations was 18.34% and ranged from 0 to 89.29% depending upon the cross. Many (90.73%, 923/1017) of the hybrid seeds produced germinated and produce plants. Amongst the 264 *T. turgidum* × *T. monococcum* combinations, 34.47% failed to produce seeds and 6.44% had crossabilities <5%, while 9.47%, 9.09, 7.95, 7.95, 5.68 and 12.88% of combinations had crossabilities of 5–10, 10–15, 15–20, 20–25, 25–30 and 30–50%, respectively. One hundred and fifty-six combinations had crossabilities >5% and are listed in online Supplementary Table S1. A total of 6.06% of combinations had crossability frequencies >50% and all these latter highly compatible combinations were obtained from crosses between *T. turgidum* subspecies *turgidum* and *dicoccon* with *T. monococcum*. Of the five *T. turgidum* subspecies investigated, the *persicum* and *durum* subspecies exhibited highest crossability (>30%), while *dicoccon* had the lowest crossability with 11.90% (online Supplementary Table S2). Amongst the 83 *T. monococcum* accessions, PI352486, PI352484 and PI355517 showed the highest crossability (>50%).

Production of T. turgidum–T. monococcum *amphiploids*

Randomly selected hybrid seeds were germinated to produce F_1 plants. The F_1 seeds from 163 crosses germinated with the germination rate of 90.37% (913/1010) and produced vigorous F_1 plants (online Supplementary Table S4). Chromosome number of root-tip cells was used for hybrid confirmation with 21 chromosomes (triploid) present in hybrids (online Supplementary Fig. S1). Between one and five F_1 plants from each of the 163 crosses were chromosome doubled by colchicine treatment at the three-tiller stage. Treated plants from 70 of these crosses successfully generated selfed seed (S_1) (online Supplementary Table S4) although seed was viable from 56 synthetic amphiploids only. The chromosome number of root-tip cells with $2n = 42$ confirmed the success of chromosome doubling (online Supplementary Fig. S2). These 56 viable lines were produced from crosses involving 31 *T. turgidum* lines and 31 *T. monococcum* accessions (online Supplementary Table S4). Progeny from viable synthetic amphiploids grew vigorously (online Supplementary Fig. S3) and some of them produced more than 30 spikelets (Fig. 1).

Chromosome observations in amphiploids

S_3 progeny from nine amphiploids were observed to contain around 40–42 chromosomes (Table 1). Plants from

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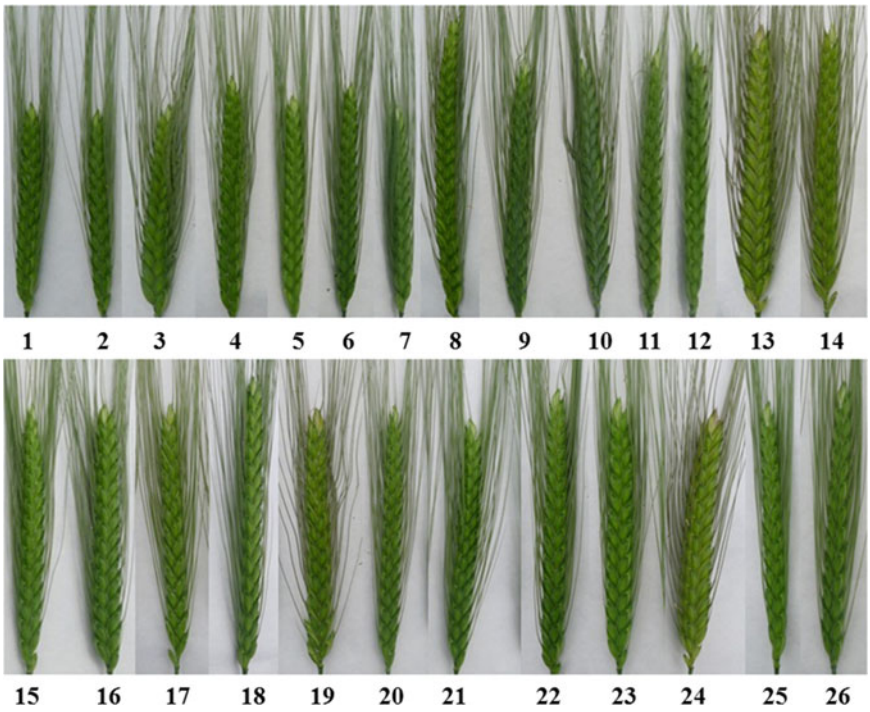

Fig. 1. Examples of spike morphology of amphiploids. (1) Syn-TAM-1, (2) Syn-TAM-2, (3) Syn-TAM-3, (4) Syn-TAM-4, (5) Syn-TAM-5, (6) Syn-TAM-6, (7) Syn-TAM-10, (8) Syn-TAM-11, (9) Syn-TAM-13, (10) Syn-TAM-14, (11) Syn-TAM-15, (12) Syn-TAM-25, (13) Syn-TAM-26, (14) Syn-TAM-27, (15) Syn-TAM-28, (16) Syn-TAM-29, (17) Syn-TAM-33, (18) Syn-TAM-35, (19) Syn-TAM-37, (20) Syn-TAM-38, (21) Syn-TAM-39, (22) Syn-TAM-41, (23) Syn-TAM-42, (24) Syn-TAM-43, (25) Syn-TAM-44, (26) Syn-TAM-46.

these nine lines that contained 42 chromosomes were used for multicolour FISH using probes Oligo-pSc119.2-1, Oligo-pTa71-2, Oligo-pTa535-1 and $(AAC)_5$ that are largely specific for the B genome, sub-terminal regions of 1BS and 6BS, A^m and A genomes, and chromosome $6A^m$, respectively. The red coloured Oligo-pSc119.2-1 probe gave strong signals on all the B genome chromosomes and weaker signals at the terminal end of the short or long arm on three A chromosomes (2A, 4A and 5A) (Fig. 2). The yellow coloured Oligo-pTa71-2 probe produced strong signals at the sub-terminal regions of chromosomes 1BS, 6BS (Fig. 2). The green coloured Oligo-pTa535-1 probe, which hybridized mainly to chromosomes of the A^m and A genomes (Fig. 2), could distinguish these two karyotypes with the inclusion of probe $(AAC)_5$ which identifies chromosome $6A^m$. The probe $(AAC)_5$ gave strong signals on chromosome $6A^m$ (Fig. 2(a)), which was different from signals on the other A^m chromosomes. The probe $(AAC)_5$ gave no signals on chromosome 6A of the examined tetraploid parent. Combining these four probes successfully discriminated the entire 42 chromosomes of synthetic *T. turgidum–T. monococcum* amphiploids (Fig. 2(a)).

S_3 plants with 42 chromosomes and analysed by FISH were also used for meiotic analysis of chromosome pairing in PMCs at metaphase I (Table 1, online Supplementary Fig. S4). Most of the 42 chromosomes paired as bivalents, while a low number of trivalents, quadrivalents and pentavalents were also observed. The presence of these multi-valents suggests that pairing between A^m and A chromosomes occurred, while pentavalents may be a consequence of chromosome rearrangements such as translocation.

SDS-PAGE analysis

S_3 seeds from 56 *T. turgidum–T. monococcum* amphiploids and their parents were used for SDS-PAGE analysis. The 31 *T. monococcum* parents of these amphiploids collectively expressed six *Glu-A1^{m}x* proteins (online Supplementary Table S5; Li *et al.*, 2016). Four of these *T. monococcum* proteins, *Glu-A1^m-b*, *Glu-A1^m-c*, *Glu-A1^m-d* and *Glu-A1^m-b*, were detected in numerous amphiploids (three, three, 31 and one line, respectively) (Fig. 3). However, this analysis was compromised by the co-migration of different *Glu-A1x* proteins present in *T. turgidum* and *T. monococcum*. Specifically *T. monococcum* *Glu-A1^m-c*, *Glu-A1^m-d*, *Glu-A1^m-e* and *Glu-A1^m-f* proteins had similar electrophoretic mobility to the *Glu-A1x*

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Table 1. Chromosome number distribution and chromosome pairing of *T. turgidum–T. monococcum* amphiploids

Accession no.	Combinations	No. of plants			Means of chromosome pairing configuration[a] ($2n = 42$)
		$2n = 40$	$2n = 41$	$2n = 42$	
Syn-TAM-3	AS2637 × CItr13961	0	0	8	4.27 rod II(0–8) + 14.53 ring II(10–19) + 0.90 I(0–3) + 1.03 III(0–4) + 0.10 IV(0–1)
Syn-TAM-10	PI154582 × PI307984	1	2	3	2.50 rod II(1–7) + 17.87 ring II(14–21) + 0.63 I(0–4) + 0.17 III(0–1) + 0.03 IV(0–1)
Syn-TAM-13	PI221401 × PI191098	1	0	4	2.47 rod II(0–6) + 17.13 ring II(13–21) + 0.5 I(0–3) + 0.5 III(0–3) + 0.20 IV(0–2)
Syn-TAM-24	PI184526 × CItr13962	1	1	4	1.87 rod II(0–4) + 18.53 ring II(16–21) + 0.47 I(0–3) + 0.20 III(0–2) + 0.03 IV(0–1)
Syn-TAM-26	AS2295 × PI352486	0	0	8	4.57 rod II(0–9) + 14.73 ring II(9–20) + 1.53 I(0–6) + 0.53 III(0–2) + 0.07 IV(0–2)
Syn-TAM-27	AS2295 × PI355517	3	2	9	4.67 rod II(1–10) + 14.87 ring II(9–19) + 2.23 I(0–6) + 0.23 III(0–2)
Syn-TAM-33	AS2305 × PI355517	3	0	9	3.53 rod II(0–9) + 15.13 ring II(11–21) + 1.13 I(0–4) + 1.13 III(0–4) + 0.03 IV(0–1)
Syn-TAM-37	AS2310 × CItr13961	2	2	6	2.57 rod II(0–7) + 16.53 ring II(9–21) + 0.97 I(0–8) + 0.57 III(0–2) + 0.20 IV(0–1) + 0.07 V(0–2)
Syn-TAM-43	AS2380 × CItr13963	1	2	5	2.40 rod II(0–6) + 17.33 ring II(13–21) + 0.23 I(0–2) + 0.50 III(0–2) + 0.20 IV(0–1)

[a]I, univalent; II, bivalent; III, trivalent; IV, quadrivalent; V, pentavalent.

proteins of *T. turgidum* parents of two, 11, three and two amphiploids, respectively. It was therefore not possible to distinguish the parental origin of *Glu-A1* proteins in these lines (online Supplementary Table S5).

Evaluation for stripe rust resistance

Field evaluation of stripe rust resistance showed that 80% of amphiploids (45/56), 74% of tetraploid parents (23/31) and 74% of diploid parents (21/31) were resistant (IT: 1–4) at the seedling stage to the mixed rust inoculum (online Supplementary Table S5). Amongst these plants at the adult stage, 89% (50), 65% (20) and 100% (31) of amphiploids, tetraploid parents and diploid parents were resistant, respectively (IT: 1–4). Forty-five amphiploids (80%) (Fig. 4), 19 tetraploid parents (61%) and 23 diploid parents (74%) were resistant to stripe rust disease at both the seedling and adult plant stages.

Five amphiploid lines (Syn-TAM-12, Syn-TAM-17, Syn-TAM-22, Syn-TAM-51 and Syn-TAM-53), the tetraploid parent PI221401 and the eight diploid parents (CItr13961, CItr13962, CItr17652, CItr17653, CItr17662, PI265008, PI355521 and PI560726) were susceptible at the seedling stage but resistant at the adult plant stage (online Supplementary Table S5). These lines are potentially useful germplasm sources for incorporating adult plant resistance into breeding programmes.

Reduction of stripe rust resistance was observed for both seedling and adult plant resistance in some amphiploids (online Supplementary Table S5). At the seedling stage, the resistance from three *T. monococcum* lines (PI503874, PI518452 and PI560727) was completely lost in their amphiploid derivatives (Syn-TAM-8, Syn-TAM-9 and Syn-TAM-23), while the resistance from some lines was partially reduced in their amphiploid. Similar situations were also appeared at the adult plant stage. Some factors such as chromosome absence and suppression under the new amphiploid background could cause resistance loss or reduction.

Discussion

The success or failure of interspecific hybridization largely depends on crossability. Crossability is hence an important factor for developing amphiploids (Megyeri *et al.*, 2011). Our results demonstrate that crossability between *T. turgidum* ssp. *durum* and *T. monococcum* ssp. *monococcum* is affected by parental genotypes (The and Baker, 1975; Gul Kazi *et al.*, 2011). In this study, some *T. turgidum* and *T. monococcum* genotypes showed high crossability thereby enabling the successful development of new amphiploids, while crosses between other genotypes were unsuccessful.

Resistance suppression can be a problem when transferring resistance from a lower ploidy level (Kema *et al.*, 1995; Ma *et al.*, 1997; Knott, 2000; Ahmed *et al.*, 2014). In this study, stripe rust resistance from *T. monococcum* was probably suppressed in several *T. turgidum–T. monococcum*

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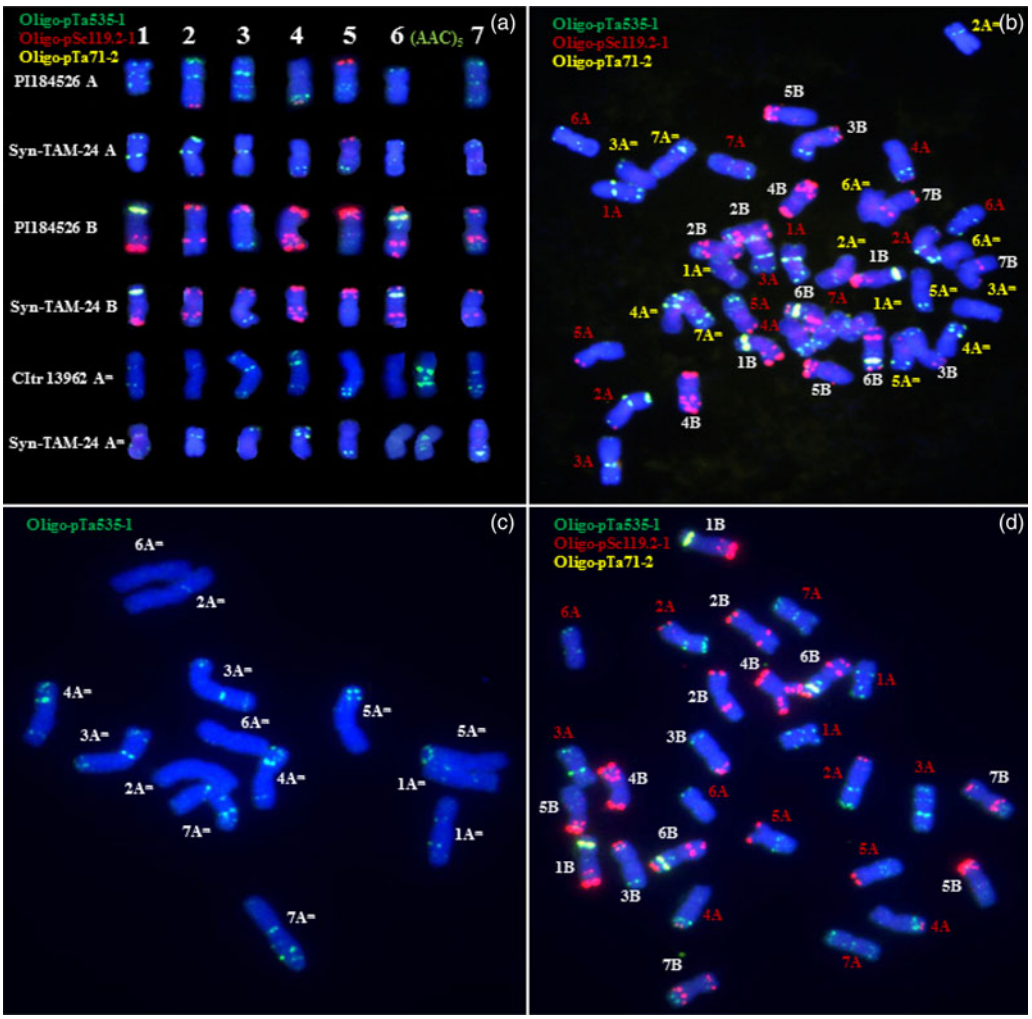

Fig. 2. Examples of FISH identification using four synthetic oligonucleotides probes of Oligo-pSc119.2-1 (red), Oligo-pTa535-1 (green), Oligo-pTa71-2 (yellow) and $(AAC)_5$ (green). FISH karyotypes of A, B, A^m genomes in Syn-TAM-24 and its parents (a), a cell of Syn-TAM-24 (b), *T. monococcum* ssp. *monococcum* CItr13962 (c), and *T. turgidum* ssp. *turanicum* PI184526 (d).

amphiploids both at seedling and adult plant stages. However, 50 amphiploids exhibited adult plant resistance to the current predominant Chinese stripe rust races and 45 were resistant at both the seedling and adult plant stages, with some lines carry resistance from both *T. turgidum* and *T. monococcum*. These novel amphiploids are promising genetic resources for introducing new wheat stripe rust resistance into breeding programmes.

HMW-GSs are components of the glutenin polymer and play a key role in determining the unique visco-elastic properties of wheat dough (Payne 1987). *T. monococcum* ssp. *monococcum* is considered a valuable resource for wheat bread-making quality improvement (Tranquilli *et al.*, 2002). Variation at the *Glu-A1x* locus in common wheat is rare, however, diverse *Glu-A1^{m}x* alleles are present in *T. monococcum* ssp. *monococcum* (Li *et al.*, 2016, 2017). In this study, *Glu-A1^m-b*, *Glu-A1^m-c*, *Glu-A1^m-d* and *Glu-A1^m-h* proteins were detected in amphiploid plants that could potentially further improve wheat quality.

Chromosome pairing and recombination between A^m and A genomes is essential for transferring genes from *T. turgidum–T. monococcum* amphiploids into bread wheat. Meiosis of PMCs in hybrids between *T. turgidum* ssp. *dicoccum* and *T. monococcum* was described by Mather (1936) and a maximum of seven configurations found. Meiotic analysis from three *T. aestivum/T. monococcum* hybrids showed on average five bivalents and 0.16 trivalents per cell (Cox *et al.*, 1991). In our study, multi-valent chromosome pairing was also observed at meiosis in amphiploids. These studies suggest that chromosome pairing does occur between A^m and A chromosomes, enabling amphiploids to be used as a 'bridge' to transfer useful genes from *T. monococcum* into bread wheat.

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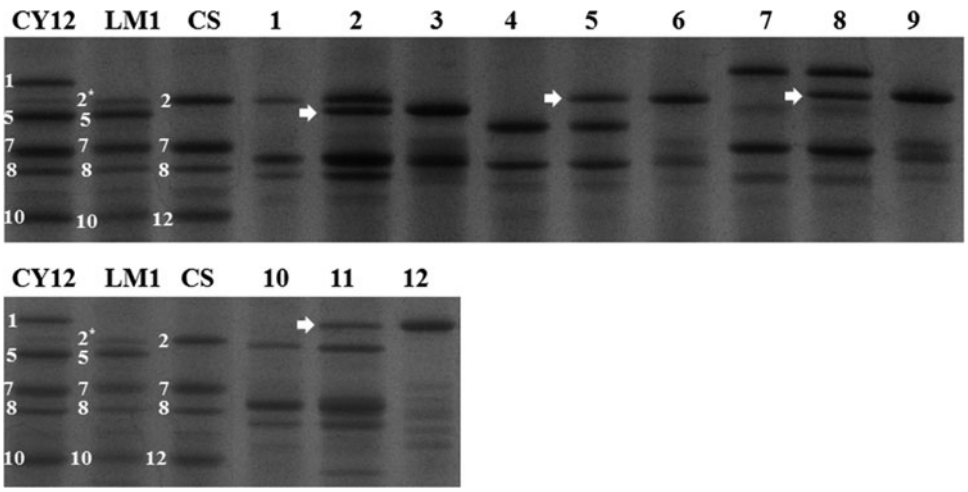

Fig. 3. SDS-PAGE profiles of HMW-GSs in some amphiploids and their parents. (1) AS2310, (2) Syn-TAM-38, (3) PI355517 (*Glu-A1^m-b*), (4) AS2637, (5) Syn-TAM-3, (6) CItr13961 (*Glu-A1^m-c*), (7) PI94670, (8) Syn-TAM-8, (9) PI503874 (*Glu-A1^m-d*), (10) AS2334, (11) Syn-TAM-56, (12) PI355521 (*Glu-A1^m-h*), bread wheat CY12, cv. Chuanyu 12 (1, 7 + 8, 5 + 10); LM1, cv. Longfumai 1 (2*, 7 + 8, 5 + 10); and CS, cv. Chinese Spring (7 + 8, 2 + 12). The *Glu-A1^{m}x* alleles expressed in amphiploids were indicated by white arrows.

FISH was an effective tool for identifying chromosomes from the A and B genomes of *T. turgidum* and A^m genome of *T. monococcum* ssp. *monococcum* (Megyeri *et al.*, 2012, 2017). In this study, the combination of oligonucleotide probes Oligo-pSc119.2-1, Oligo-pTa71-2, Oligo-pTa535-1 and $(AAC)_5$ successfully differentiated individual chromosomes originating from *T. turgidum* and *T. monococcum* ssp. *monococcum* in newly synthesized *T. turgidum–T. monococcum* amphiploids. These probes can be further used as cytological markers in future breeding with these *T. turgidum–T. monococcum* amphiploids.

In conclusion, we have produced new *T. turgidum–T. monococcum* amphiploids that are potentially valuable resources for wheat improvement. Ongoing work will select those *T. turgidum–T. monococcum* amphiploids lines with useful traits and then introduce these traits into bread wheat followed by backcrossing. It is envisaged that these new traits will make a significant contribution to future wheat improvement.

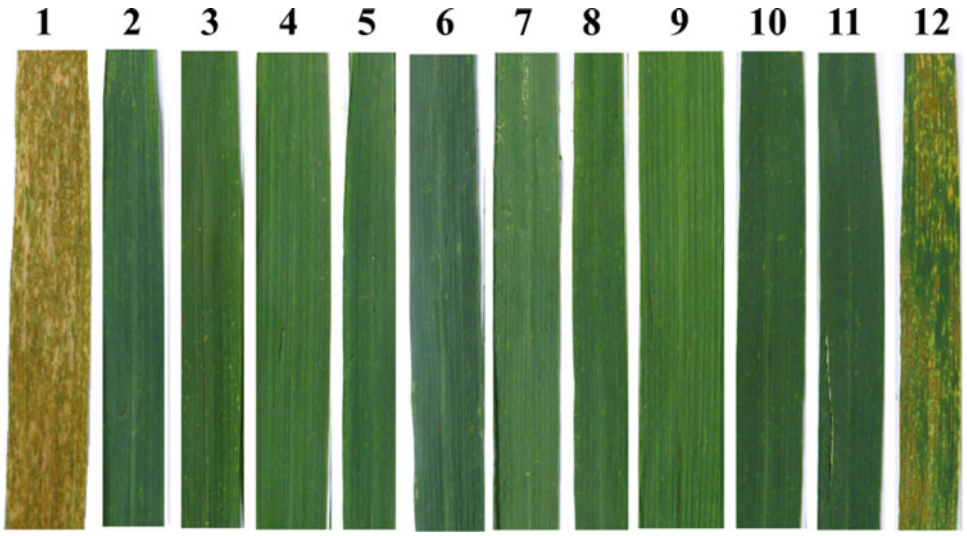

Fig. 4. Stripe rust resistance at the adult stage of some amphiploids. (1) The bread wheat check SY95-71, (2) Syn-TAM-1, (3) Syn-TAM-2, (4) Syn-TAM-3, (5) Syn-TAM-4, (6) Syn-TAM-5, (7) Syn-TAM-6, (8) Syn-TAM-10, (9) Syn-TAM-11, (10) Syn-TAM-13, (11) Syn-TAM-14, (12) Syn-TAM-15.

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Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262118000175.

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