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Development and characterization of *Triticum turgidum–Aegilops umbellulata* amphidiploids

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Abstract

The U genome of *Aegilops umbellulata* is an important basic genome of genus *Aegilops*. Direct gene transfer from *Ae. umbellulata* into wheat is feasible but not easy. *Triticum turgidum–Ae. umbellulata* amphidiploids can act as bridges to circumvent obstacles involving direct gene transfer. Seven *T. turgidum–Ae. umbellulata* amphidiploids were produced via unreduced gametes for spontaneous doubling of chromosomes of triploid *T. turgidum–Ae. umbellulata* F_1 hybrid plants. Seven pairs of U chromosomes of *Ae. umbellulata* were distinguished by fluorescence *in situ* hybridization (FISH) probes pSc119.2/$(AAC)_5$ and pTa71. Polymorphic FISH signals were detected in three (1U, 6U and 7U) of seven U chromosomes of four *Ae. umbellulata* accessions. The chromosomes of the tetraploid wheat parents could be differentiated by probes pSc119.2 and pTa535, and identical FISH signals were observed among the three accessions. All the parental chromosomes of the amphidiploids could be precisely identified by probe combinations pSc119.2/pTa535 and pTa71/$(AAC)_5$. The *T. turgidum–Ae. umbellulata* amphidiploids possess valuable traits for wheat improvement, such as strong tillering ability, stripe rust resistance and seed size-related traits. These materials can be used as media in gene transfers from *Ae. umbellulata* into wheat.

Keywords: amphidiploids, FISH (fluorescent *in situ* hybridization), *T. turgidum* ssp., U genome, unreduced gametes

Introduction

Aegilops umbellulata is an important diploid donor species of several polyploid *Aegilops* that harbour U genomes. It harbours numerous desirable traits for hexaploid wheat improvement, such as resistance to leaf rust, stripe rust (Sears, 1956; Bansal *et al.*, 2017), and powdery mildew (Zhu *et al.*, 2006), tolerance to salt and drought stresses (Cakmak *et al.*, 1999), and high zinc and iron content (Wang *et al.*, 2011). Amphidiploids between *Ae. umbellulata* and tetraploid/hexaploid wheats have been used as the bridge for transferring genes from *Ae. umbellulata* into common wheat (Zaharieva *et al.*, 2003; Zhu *et al.*, 2006; Chhuneja *et al.*, 2008; Hadzhiivanova *et al.*, 2012).

Amphidiploids are developed by the whole genome doubling of a hybrid between species. The doubling is conventionally developed by colchicine treatment but is more conveniently achieved by relying on unreduced gametes. Unreduced gametes, a major route for the development of polyploids in nature (Storme and Geelen, 2013), have been reported in haploid plants of *T. turgidum* ssp. *durum* (Jauhar, 2003) and frequently occur in hybrids of *T. turgidum* with Triticeae species, such as *Ae. tauschii*, rye (Zhang *et al.*, 2010; Hao *et al.*, 2013, 2014) and *Ae. longissima* (Tiwari *et al.*, 2008). No report describing the production of *T. turgidum–Ae. umbellulata* amphidiploids using this approach has been published to date.

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Fluorescence *in situ* hybridization (FISH) has been used to identify parental chromosomes in amphidiploids, and when combined with genome *in situ* hybridization, can reveal alien introgressions. All 14 U chromosomes in diploid *Ae. umbellulata* and polyploid *Aegilops* species that harbour U genomes were differentiated by FISH using highly repetitive DNA sequences pSc119.2 and pAsl combined with 5S and 35S rDNA (Badaeva *et al.*, 1996; Kwiatek *et al.*, 2013). In another FISH assay, all 14 *Ae. umbellulata* chromosomes were identified using probes pSc119.2, pTa71 (the 45S rDNA clone from wheat) and $(CTT)_{10}$ repeats (Mirzaghaderi *et al.*, 2014).

The aims of the present study were as follows: (1) development of *T. turgidum–Ae. umbellulata* amphidiploids via unreduced gametes and identification of amphidiploids using FISH probes; (2) evaluation of the morphological characteristics and seed traits of the new amphidiploids. These newly developed amphidiploids are the basic germplasms that could be utilized in the manipulation of beneficial genes of *Ae. umbellulata*.

Materials and methods

Plant materials

Three *T. turgidum* L. ($2n = 4x = 28$, AABB) lines belonging to two subspecies, namely, ssp. *durum* cv. Langdon, and ssp. *dicoccum* PI 94668 and PI 349045 and four *Ae. umbellulata* Zhuk. ($2n = 2x = 14$, UU) accessions, PI 486259, PI 554395, PI 554413 and CIae 29, were used to generate *T. turgidum–Ae. umbellulata* amphidiploids. A wheat line, SY95-71, which has been previously shown to be highly susceptible to stripe rust, was used as spreader and susceptible control for stripe rust disease.

Development of T. turgidum–Ae. umbellulata hybrids

All distant hybridization crosses used *T. turgidum* as the female parent and *Ae. umbellulata* as the male parent to produce *T. turgidum/Ae. umbellulata* F_1 hybrids. Emasculation and pollination were performed as described by Zhang *et al.* (2008). Briefly, the emasculated young spikes of *T. turgidum* were bagged for 2 d, then the pollen grains of *Ae. umbellulata* were pollinated to the pistils of *T. turgidum* thrice in 1 d to produce F_1 hybrid seeds. No embryo rescue and no hormone treatment were used on the pollinated young embryos. The hybrid seeds were germinated on Petri dishes lined with moist filter paper and then later transplanted to the field. The chromosomes of F_1 plants underwent spontaneous doubling to produce *T. turgidum–Ae. umbellulata* amphidiploids without using colchicine. All the fertile triploid F_1 plants were selfed to generate amphidiploids S_1 seeds. All the S_1 seeds were cytologically examined in terms of chromosome number in the root tip cells, and only seedlings with 42 chromosomes were transplanted to the field. The amphidiploid plants were selfed to obtain S_2 seeds. The seed sets of the F_1 hybrids were counted.

Assessment of morphological characteristics

All materials were planted at the Wenjiang experimental station of Sichuan Agricultural University for at least two consecutive years from 2012 to 2016. The agronomic traits, including tiller number, plant height, spike length and stripe rust resistance, were investigated in the field based on three to five amphidiploid plants for every *T. turgidum–Ae. umbellulata* cross and five from each parent. Stripe rust resistance of flag leaves was recorded as described elsewhere (Wellings and Bariana, 2004), when the entire spreader wheat line, SY95-71, was infected. Fifty randomly selected grains were assessed for six seed-related traits, which included seed length and width, aspect ratio, projection area, perimeter and grain weight, using an Epson Expression 11000XL scanner. The results were treated with Win SEEDLE™ 2012a (Regent Instruments, Canada).

FISH analysis

A total of 10 randomly selected seeds, five from each of the two amphidiploid plants, were germinated on Petri dishes lined with moist filter papers at 4°C for approximately 24 h, and then transferred to an incubator at a constant temperature of 23°C. The root tips were harvested when the roots reached 1–2 cm in length. The root tips were treated with nitrous oxide for 4 h, washed with 70% ethanol (Kato, 1999), digested in a cellulase/pectinase enzyme solution (4: 2) and the resulting suspension was dropped onto slides (Komuro *et al.*, 2013).

Four probes, namely, pSc119.2, pTa-535, pTa71 (Tang *et al.*, 2014) and $(AAC)_5$ (Cuadrado and Jouve, 2010), were used for FISH following the procedure described by Hao *et al.* (2011). Three probes [pSc119.2, pTa71 and $(AAC)_5$] were synthesized by Tsingke (Chengdu, China), whereas one probe (pTa-535) was synthesized by Sangon Biotech (Shanghai, China). DAPI (4', 6-diamidino-2-phenylindole) was applied onto the slides as counterstain, which was followed by chromosomal observation. After capturing FISH images, the coverslips of each slide were removed, and the slides were washed for the next FISH assay. Briefly, the slides were first washed with 70% ethanol for 5 min, followed by heating in boiling 2 × SSC buffer for 5 min to remove the probes. Then, the slides were washed with distilled water, briefly rinsed

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Table 1. Selfed seed set of F_1 hybrids of *T. turgidum* and *Ae. umbellulata*

Cross combination	Cross year	No. of F_1 seeds	No. of plants	No. of florets	No. of seed-setting	Selfed seed-set rate (%)[a]	Chromosome numbers in PMCs of F_1 hybrids
STU1 (PI 349045/CIae 29)	2012	3	3	2665	124	4.65	–[b]
STU2 (Langdon/PI 554395)	2013	6	5	3879	97	2.50	21
STU3 (PI 94668/PI 554395)	2013	1	1	2187	6	0.27	–
STU4 (PI 94668/CIae29)	2013	6	5	8381	53	0.63	21
STU5 (PI 94668/PI 554413)	2013	2	2	3441	3	0.09	–
STU6 (PI 349045/PI 428569)	2014	1	1	1062	4	0.38	–
STU7 (Langdon/PI 428569)	2014	16	15	2096	3	0.14	21

[a]Calculated as the percentage of selfed seed set over the total number of florets.
[b]Not checked.

with 70% ethanol and then air dried for next FISH assay (Komuro *et al.*, 2013).

Results

Production of F_1 hybrids and their selfed seed setting

Thirty-five hybrid seeds involving seven *T. turgidum/Ae. umbellulata* crosses were obtained from 2012 to 2014 (Table 1). Most of these germinated and produced 32 F_1 plants. All seven cross combinations were partially fertile. The seed setting number in F_1 hybrids from seven crosses varied from 3 to 124. The seed set rate of F_1 hybrid plants varied from 0.09 to 4.65%, with an average of 1.22%. The chromosome constitutions in the pollen mother cells (PMCs) of F_1 hybrid plants in three checked crosses STU2, STU4 and STU7 were 21 univalent (Table 1), suggesting that they were triploid hybrids with 21 chromosomes ($n = 21$).

Variations of morphological and seed-related traits between amphidiploids and their parents

The *T. turgidum–Ae. umbellulata* amphidiploids showed better morphological traits than either or both of their parents (Table 2). For example, the amphidiploid plants in four of the five *T. turgidum–Ae. umbellulata* crosses [STU1,

Table 2. Comparison of morphological characteristics of *T. turgidum–Ae. umbellulata* amphidiploids and their parents

Materials/generations	No. of tillers	Plant height (cm)	Spike length (cm)	Stripe rust resistance score	Year
T. t. ssp. *dicoccum* PI 349045	17.0 ± 4.8 c	98.1 ± 12.6 a	9.44 ± 1.24 a	2–3	2014
Amphidiploids STU1	25.4 ± 11.6 b	78.0 ± 9.9 b	8.20 ± 0.87 a	2–3	
Ae. umbellulata CIae 29	165.6 ± 30.4 a	33.1 ± 4.5 c	3.26 ± 0.28 b	2–3	
T. t. ssp. *durum* cv. Langdon	5.8 ± 1.0 c	102.4 ± 12.9 a	8.60 ± 0.80 b	4–5	2015
Amphidiploids STU2	20.0 ± 8.4 b	71.4 ± 14.1 b	10.75 ± 0.84 a	1–2	
Ae. umbellulata PI 554395	197.0 ± 12.3 a	30.7 ± 2.5 c	3.80 ± 0.25 c	1–2	
T. t. ssp. *dicoccum* PI 94668	15.0 ± 3.6 b	106.4 ± 9.0 a	9.81 ± 0.42 a	1–2	2015
Amphidiploids STU3	17.0 ± 5.7 b	78.9 ± 5.1 b	9.20 ± 0.89 a	1–2	
Ae. umbellulata PI 554395	197.0 ± 12.3a	30.7 ± 2.5 c	3.80 ± 0.25 b	1–2	
T. t. ssp. *dicoccum* PI 94668	15.0 ± 3.6 c	106.4 ± 9.0 a	9.81 ± 0.42 a	1–2	2015
Amphidiploids STU4	28.7 ± 9.1 b	72.5 ± 13.6 b	10.45 ± 2.07 a	2–3	
Ae. umbellulata CIae 29	120.0 ± 11.4 a	31.5 ± 2.2 c	3.39 ± 0.11 c	2–3	
T. t. ssp. *dicoccum* PI 94668	15.0 ± 3.6 c	106.4 ± 9.0 a	9.81 ± 0.42 a	1–2	2015
Amphidiploids STU5	55.0 ± 17.7 b	65.0 ± 5.8 b	9.17 ± 0.22 a	1–2	
Ae. umbellulata PI 554413	161.3 ± 15.4 a	32.6 ± 2.1 c	3.69 ± 0.19 b	1–2	

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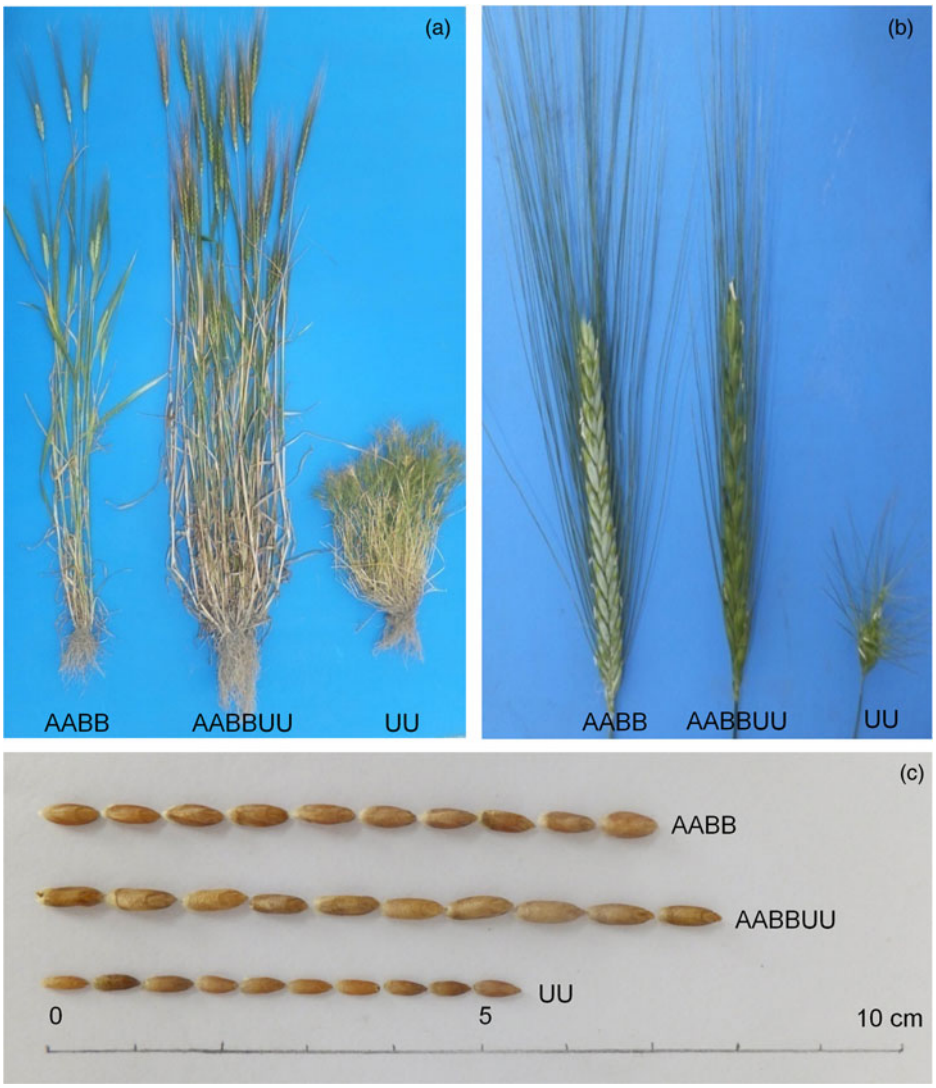

Fig. 1. Comparison of *T. turgidum* ssp. *Ae. umbellulata* hybrid plants STU4 with their female *T. turgidum* ssp. *dicoccum* PI 94668 (AABB) and male *Ae. umbellulata* CIae 29 (UU) parents in plants (a), spikes (b) and seeds (c).

STU2, STU4 (Fig. 1(a)) and STU5] showed more tillers than those of the tetraploid wheat parents. The amphidiploid plants (~60–80 cm) were taller than that of *Ae. umbellulata* (<35 cm) but shorter than the tetraploid wheat parents (~100 cm). The spike of amphidiploids were significantly longer than *Ae. umbellulata* parents but similar to [STU1, STU3, STU4 (Fig. 1(b)) and STU5] or a little longer (STU2) than those of the tetraploid wheat parent. All the amphidiploids showed similar stripe rust resistance as their *Ae. umbellulata* or tetraploid wheat parents. However, stripe rust resistance was observed in amphidiploids using Langdon, a stripe rust-susceptible tetraploid wheat, as the female parent.

The *T. turgidum–Ae. umbellulata* amphidiploids showed excellent seed size-related traits (Table 3). For example, the projection areas for amphidiploids STU2, STU5 and STU6; the grain lengths for amphidiploids STU2, STU3, STU4 (Fig. 1(c)) and STU6; the grain width for amphidiploids STU7; the ratios of grain length to grain width for amphidiploids STU4 (Fig. 1(c)) and STU6; the grain surface perimeters for amphidiploids STU5, STU6 and STU7; and the grain weights for amphidiploids STU2, STU5 and STU7 were, respectively, better than those of their parents.

FISH identification of parental chromosomes

The *Ae. umbellulata* chromosomes were differentiated by the pSc119.2/$(AAC)_5$ and pTa71 probes (Fig. 2(a)–(d)). The pSc119.2 probe hybridized to the telomeric regions of mostly *Ae. umbellulata* chromosomes but pTa71 only showed significant signals on the short arms of 1U and

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Table 3. Seed traits of *T. turgidum–Ae. umbellulata* amphidiploids and their parents

Materials/generations	Grain projection area (mm^2)	Grain length (mm)	Grain width (mm)	Grain length/grain width	Grain surface perimeter (mm)	Grain wight (g/50 grains)
T. t. ssp. *durum* cv.Langdon[a]	6.16 ± 2.28**	6.29 ± 0.94*	1.53 ± 0.33**	4.39 ± 1.30	14.45 ± 6.33**	0.59 ± 0.01*
Amphidiploids STU2[a]	9.30 ± 5.17	6.92 ± 1.49	1.83 ± 0.64	4.06 ± 1.09	23.75 ± 14.51	0.83 ± 0.01
Ae. umbellulata PI 554395[a]	7.54 ± 2.66*	5.80 ± 0.85**	1.82 ± 0.42	3.32 ± 0.79**	21.64 ± 7.84	0.58 ± 0.01*
T. t. ssp. *dicoccum* PI 94668[a]	9.28 ± 2.25	6.80 ± 0.66**	1.99 ± 0.33**	3.47 ± 0.56	24.04 ± 8.43**	1.01 ± 0.01
Amphidiploids STU3[a]	7.90 ± 5.02	7.63 ± 1.34	1.59 ± 0.53	5.06 ± 1.23	15.80 ± 8.11	0.91 ± 0.01
Ae. umbellulata PI 554395[a]	7.54 ± 2.66	5.80 ± 0.85**	1.82 ± 0.42*	3.32 ± 0.79**	21.64 ± 7.84**	0.58 ± 0.02*
T. t. ssp. *dicoccum* PI 94668[a]	9.28 ± 2.25	6.80 ± 0.66**	1.99 ± 0.33	3.47 ± 0.56**	24.04 ± 8.43	1.01 ± 0.02*
Amphidiploids STU4[a]	10.48 ± 4.92	7.59 ± 1.28	1.93 ± 0.60	4.14 ± 0.89	26.67 ± 13.11	0.65 ± 0.03
Ae. umbellulata Clae 29[a]	5.57 ± 1.74**	5.02 ± 0.79**	1.60 ± 0.29**	3.17 ± 0.45**	15.63 ± 5.32	0.36 ± 0.01**
T. t. ssp. *dicoccum* PI 94668[a]	9.28 ± 2.25**	6.80 ± 0.66	1.99 ± 0.33	3.47 ± 0.56	24.04 ± 8.43**	1.01 ± 0.01*
Amphidiploids STU5[a]	12.44 ± 7.75	7.41 ± 2.13	2.18 ± 1.01	3.75 ± 1.10	35.01 ± 22.57	1.22 ± 0.01
Ae. umbellulata PI 554413[a]	7.88 ± 2.38**	6.36 ± 0.87**	1.78 ± 0.34**	3.65 ± 0.54	21.58 ± 7.34**	0.42 ± 0.02**
T. t. ssp. *dicoccum* PI 349045[b]	10.48 ± 2.42*	7.33 ± 0.77**	2.09 ± 0.34	3.58 ± 0.57**	27.80 ± 7.32**	0.91 ± 0.02
Amphidiploids STU6[b]	12.46 ± 5.60	8.41 ± 0.98	2.03 ± 0.82	4.43 ± 0.97	34.45 ± 17.62	0.92 ± 0.01
Ae. umbellulata PI 428569[b]	8.00 ± 3.35**	5.44 ± 1.17**	2.02 ± 0.47	2.72 ± 0.35**	23.28 ± 9.54**	0.56 ± 0.01**
T. t. ssp. *durum* cv. Langdon[b]	8.49 ± 1.90	6.54 ± 0.64	1.91 ± 0.30**	3.49 ± 0.46**	23.21 ± 5.90**	0.67 ± 0.01**
Amphidiploids STU7[b]	10.01 ± 3.42	6.59 ± 0.90	2.25 ± 0.33	2.96 ± 0.36	29.93 ± 12.56	1.17 ± 0.01
Ae. umbellulata PI 428569[b]	8.00 ± 3.35**	5.44 ± 1.17**	2.02 ± 0.47**	2.72 ± 0.35**	23.28 ± 9.54**	0.56 ± 0.02**

Note: a and b indicate the data from year 2014 and 2015, respectively.
The * and ** indicate the male or female parent showing significant differences from the amphidiploids at the 0.05 and 0.01 levels. No comparison was made between the two parents of each amphidiploid.

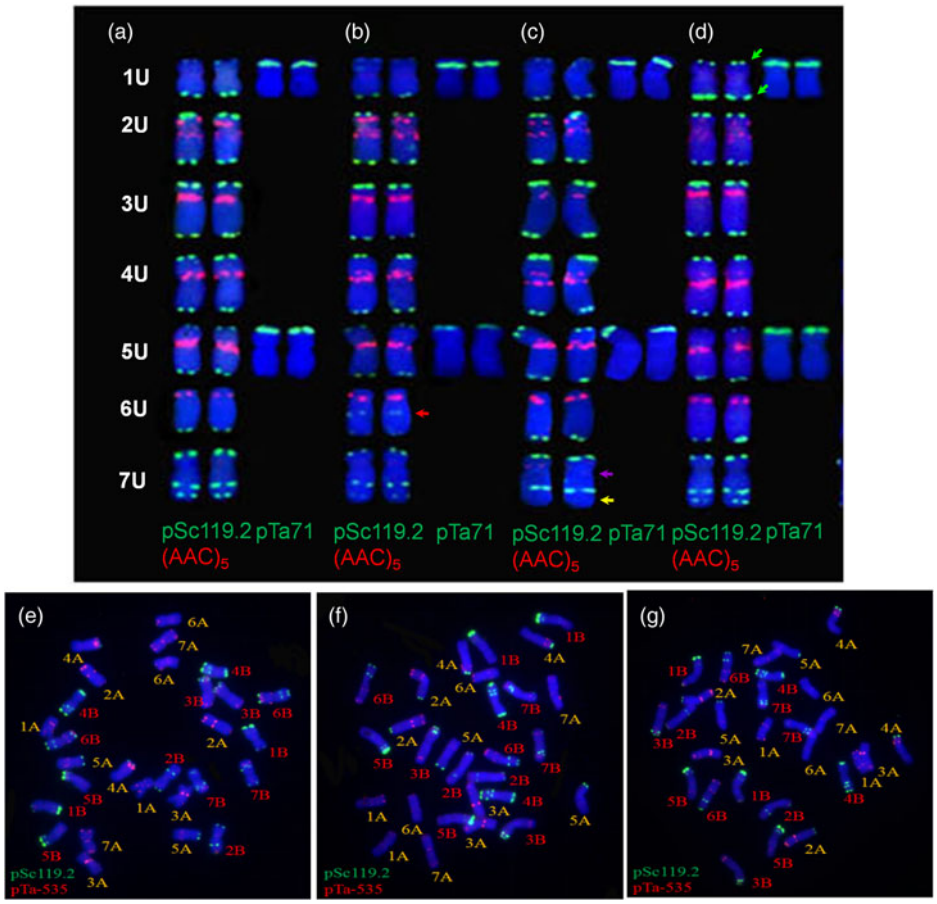

Fig. 2. Non-denaturing fluorescence *in situ* hybridization of the male *Ae. umbellulata* parents using probes pSc 119.2, $(AAC)_5$, and pTa 71 (a–d) and the female *T. turgidum* parents using probes pSc 119.2 and pTa-535 (e–g). (a) PI 428569; (b) PI 554413; (c) PI 554395; (d) CIae 29; (e) Langdon; (f) PI 94668; (g) PI 349045. The arrowheads in blue (d), red (b) and yellow (c) show the strong 1U pSc 119.2 signals on CIae 29, the additional 6U pSc 119.2 signals on PI554413, and the absence of pSc119.2 signals on PI 554395, respectively. The purple arrowhead (c) indicate the extra $(AAC)_5$ signals on PI 554395.

5U. Hybridization signals using the $(AAC)_5$ probe were observed on all U chromosomes except for 1U and 7U. Unlike the other U chromosomes, the hybridization signals of the $(AAC)_5$ probe on the 2U were localized near the centromeres, whereas those of the 3U were in the medial region of the short arms. Both the 4U and 5U showed strong $(AAC)_5$ signals on the centromeres but these had different signals for pTa71, with strong signals on the telomeres of the 5US and no signal for the 4U chromosomes. The 6U $(AAC)_5$ signals were on the interstitial sites of the short arms.

Four *Ae. umbellulata* accessions showed polymorphic FISH signals on the 6U, 7U and 1U chromosomes (Fig. 2 (a)–(d)). PI 554413 had two pairs of pSc119.2 signals on the 6UL chromosome instead of a pair of signals for the other three accessions (Fig. 2(b)). PI 554395 lost a pair of pSc119.2 signals near the telomeres of chromosome 7UL, and obtained a pair of weak $(AAC)_5$ signals on chromosome 7US compared with those in the other four accessions (Fig. 2(c)). The 1U pSc119.2 signals of CIae 29 were much stronger than the other three accessions (Fig. 2(d)). All the A and B chromosomes of tetraploid wheat could be differentiated by probes pSc119.2 and pTa-535 (Tang *et al.*, 2014) but no polymeric FISH signal was detected (Fig. 2 (e)–(g)).

Chromosome identification of T. turgidum–Ae. umbellulata amphidiploids

The A, B and U chromosomes of *T. turgidum–Ae. umbellulata* amphidiploids could be clearly distinguished by probe combinations pSc119.2/pTa-535 and pTa71/$(AAC)_5$ (Fig. 3, and S1). After excluding the aneuploid plants at the seedling stage, all the *T. turgidum–Ae. umbellulata* amphidiploids showed identical chromosome compositions in all the examined seeds in both plants. For example, all the A, B and U chromosomes ($2n = 6x = 42$, AABBUU) from both

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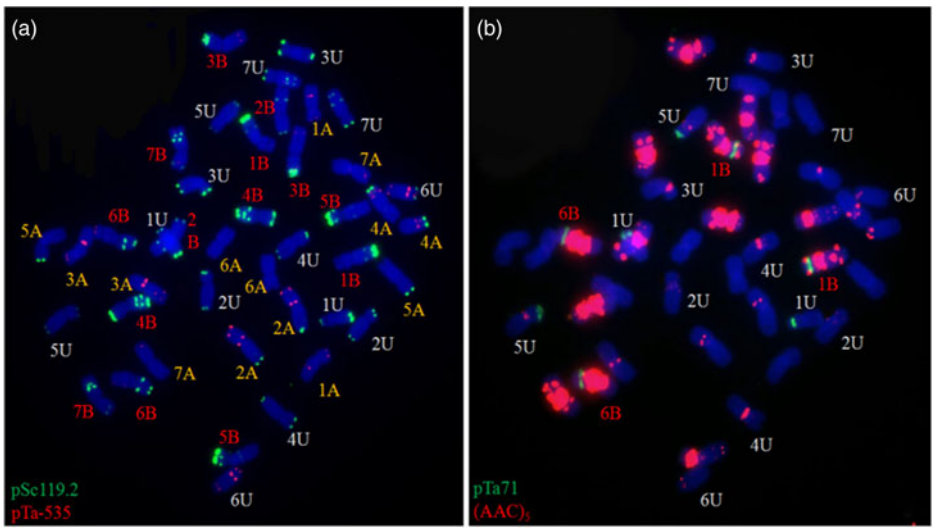

Fig. 3. Non-denaturing fluorescence *in situ* hybridization of *T. turgidum* ssp. *Ae. umbellulata* amphidiploids STU4 ($2n = 42 = 14$ A + 14 B + 14 U) using probe combinations of pSc 119.2 and pTa-535 (a), and pTa 71 and $(AAC)_5$ (b).

parents were identified in the amphidiploid plants of STU4 (Fig. 3). Although no Robersonian translocation was identified by FISH analysis, the probe signals on the 1U or 5U chromosome of some amphidiploids were different from their *Ae. umbellulata* parents. For example, the 1U pSC119.2 signals on the telomeres of PI 554395 were weakened or lost in the 1U of amphidiploids STU2 and STU3. Similarly, the 5U pSc119.2 signals on the telomeres of PI554413 and PI428569 were, respectively, stronger than those of their amphidiploids STU5, and STU6 and STU7 (Fig. S2).

Discussion

The U genome in diploid *Ae. umbellulata* and polyploidy *Aegilops* species was considered as one of the candidate donor that expands the genetic heterogeneity of wheat (Zhang *et al.*, 1998; Edae *et al.*, 2017). The amphidiploids of tetraploid/hexaploid wheat and *Ae. umbellulata* have been used as bridges for the direct transfer of genes from *Ae. umbellulata* into wheat (Chhuneja *et al.*, 2008). Unreduced gametes, which are regulated by a major quantitative trait on 3B in *T. turgidum* (Hao *et al.*, 2014), play important roles in the development of Triticeae allopolyploids. The formation of unreduced gametes has been observed in haploid plants of *T. durum* (Jauhar, 2003) and the F_1 hybrids of *T. turgidum* or *T. aestivum* with various diploid and polyploid *Aegilops* species other than *Ae. umbellulata* as far as the species out of genus *Aegilops* (e.g. *Dasypyrum villosum*) (Blanco *et al.*, 1987; Tiwari *et al.*, 2008). Here, we produced triploid *T. turgidum–Ae. umbellulata* F_1 hybrid seeds using three *T. turgidum* accessions belonging to two *T. turgidum* species, ssp. *durum* and *dicoccum* as female parents and four *Ae. umbellulata* accessions as male parents without any additional strategies such as hormone treatment of the young embryos. Most of these hybrid seeds germinated and eventually developed into seedlings. The selfed seed set (percentage of selfed seeds over total number of selfed florets) of F_1 hybrid plants could be ascribed to the formation of unreduced gametes, and therefore were considered as a good index for the formation of unreduced gametes (Zhang *et al.*, 2007). The seed set rates of seven *T. turgidum* subspecies (ssp. *dicoccon*, *turgidum*, *turanicum*, *dicoccoides*, *durum carthlicum* and *polonicum*) with *Ae. tauschii* F_1 hybrid plants in 115 crosses varied from 0 to 18.57%, with an average of 5.83% (Zhang *et al.*, 2010). However, the seed set rates of the two *T. turgidum* subspecies (ssp. *dicoccum* and *durum*) with *Ae. umbellulata* F_1 hybrid plants in seven crosses (with a mean of 1.22%, range: 0.09–4.65%) were lower than those of the seven *T. turgidum* subspecies with *Ae. tauschii* F_1 hybrid plants. The low selfed seed set in most *T. turgidum/Ae. umbellulata* crosses may be ascribed to low frequency of unreduced gametes formation like *T. durum/Ae. longissima* hybrids (Jauhar, 2007; Tiwari *et al.*, 2008), and low viability in the pollens of triploid F_1 hybrids. Unreduced gamete formation was also occurred in triploid *T. durum/Ae. longissima* hybrids but no selfed seed set, which resulted from complete male sterility (Vardi and Zohary, 1967).

Using the selfed seed set of partial fertile triploid F_1 plants, we obtained complete amphidiploids from seven combinations, and all parental chromosomes were verified by FISH. The results of the present study suggest that the union of

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unreduced gametes, which was responsible for the spontaneous doubling of chromosomes of interspecific hybrids via first-division restitution (FDR) and/or single-division meiosis (SDM) that often occurs in *T. turgidum/Ae. tauschii* and *T. turgidum* ssp. *durum/Ae. longissima* triploid hybrids (Tiwari *et al*., 2008; Zhang *et al*., 2010), were also functional to produce *T. turgidum–Ae. umbellulata* amphidiploids. Whether the FDR, SDM or both of them were responsible for the development of *T. turgidum–Ae. umbellulata* amphidiploids needs to be further investigated.

The *T. turgidum–Ae. umbellulata* amphidiploids possess some desirable traits for genetic improvement of wheat. For example, the *T. turgidum–Ae. umbellulata* amphidiploids had more tillers than those of their female tetraploid wheat parents. Similarly, the *T. turgidum–Ae. longissima* amphidiploids (Tiwari *et al*., 2008) also had strong tillering characteristics like *T. turgidum–Ae. umbellulata* amphidiploids. The *T. turgidum–Ae. umbellulata* amphidiploids (Table 3) also showed better seed size-associated traits (grain projection areas, grain length, grain surface perimeters, grain length, grain width, grain length/width and 50-grain weight) than those of their parents. This phenomenon is also reflected in the amphidiploid seeds of *T. turgidum–Ae. longissima* (Tiwari *et al*., 2008). Furthermore, some *T. turgidum–Ae. umbellulata* crosses (e.g. STU2) exhibited better stripe rust resistance than the tetraploid parent Langdon and was highly similar to the *Ae. umbellulata* parents, suggesting that the stripe resistance genes/traits of *Ae. umbellulata* were expressed in the *T. turgidum–Ae. umbellulata* amphidiploids. In our previous investigation, the high-molecular weight glutenin subunits of *Ae. umbellulata* were also expressed in *T. turgidum–Ae. umbellulata* amphidiploids (Dai *et al*., 2015). Therefore, the *T. turgidum–Ae. umbellulata* amphidiploids can serve as intermediate bridges for gene transfer of valuable genes/traits of *Ae. umbellulata* such as disease resistance, strong tillering ability, large and long seeds, and other special high-molecular weight glutenin subunits into wheat.

In summary, seven *T. turgidum–Ae. umbellulata* amphidiploids were produced by the unreduced gametes of *T. turgidum* and *Ae. umbellulata* triploid F_1 hybrids. The *T. turgidum–Ae. umbellulata* amphidiploids possess some valuable traits, such as multiple tillers, stripe rust resistance, as well as excellent seed size-related traits for wheat improvement. All the parental chromosomes in the amphidiploids could be clearly identified by FISH probe combinations of pSc119.2/pTa535 and pTa71/$(AAC)_5$. Furthermore, four *Ae. umbellulata* parents showed polymorphic FISH loci on chromosomes 1U, 6U and 7U. These newly developed amphidiploids are valuable for introducing important *Ae. umbellulata* genes/traits to wheat.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262118000254.

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