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Intergenomic Crossover Formation in Newly Synthesized Trigeneric Hybrids Involving Wheat, Rye and Barley †

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Abstract: Polyploidization, or whole genome duplication (WGD), has an important role in evolution and speciation. One of the biggest challenges faced by a new polyploid is meiosis, particularly, discriminating between multiple related chromosomes so that only homologs recombine to ensure regular chromosome segregation and fertility. Here we report the production of two new hybrids formed by the genomes of species from three different genera: a hybrid between *Aegilops taushii* (DD), *Hordeum chilense* ($H^{ch}H^{ch}$) and *Secale cereale* (RR) with the haploid genomic constitution $DH^{ch}R$ ($n = 7x = 21$); and a hybrid between *Triticum turgidum spp. durum* (AABB), *H. chilense* and *S. cereale* with the constitution $ABH^{ch}R$ ($n = 7x = 28$). We used genomic *in situ* hybridization to establish the chromosome composition of the new hybrids, and to study their meiotic behavior. Interestingly, there were multiple chromosome associations at metaphase I in both hybrids, indicating the presence of crossover formation between the different genomes. We tried to duplicate the genome of both hybrids to obtain the corresponding amphiploid, achieving success with the $ABH^{ch}R$ hybrid. Several amphiploids $AABBH^{ch}H^{ch}RR$ were obtained and characterized. These results indicate that recombination between the genera of three economically important crops is possible.

Keywords: *Triticum*; *Hordeum chilense*; *Secale cereale*; *Triticeae*; WGD (whole genome duplication); hybridization; meiosis; recombination; GISH

1. Introduction

Many of the world's most important crops, including wheat, rapeseed, sugarcane, and cotton, are recent allopolyploids. This is not a coincidence; allopolyploidy often show higher adaptability, can grow over larger geographical areas, and show better adaptation to the local environment than their diploid progenitors [1]. Despite their obvious advantages in adaptation, newly formed allopolyploids face the challenge of organizing two or more genomes that have evolved independently, within a single nucleus. Many will be the challenges, but probably, the biggest of all will be meiosis. Meiosis is the specialized cell division that generates haploid gametes for sexual reproduction. During meiosis, at least one crossover (CO) needs to be formed between every pair of homologs to ensure accurate chromosome segregation and balanced gametes. In newly formed allopolyploids, apart from the two identical homologs present in diploid species, there are also very similar chromosomes (homeologs), which will complicate the process of recognition and synapsis between homologs. Consequently, allopolyploidization is frequently accompanied by irregular meiosis, unbalanced gametes and sterility.

In this study we describe the production of two new intergeneric hybrids and one amphiploid formed by the genome of species from three different genera inside the *Triticeae* tribe. We then used genomic in situ hybridisation (GISH) to establish their chromosome composition and to study their meiotic behavior at metaphase I.

2. Materials and Methods

The plant material used in this study includes: the wild barley *Hordeum chilense* accession H7 ($2n = 2x = 14$; genome $H^{ch}H^{ch}$); *Aegilops tauschii* ($2n = 2x = 14$; genome DD); *Secale cereale* ($2n = 2x = 14$; genome RR); and ×*Tritordeum martinii* lines HT377 (with the translocation T1RS·1BL) and HT474 ($2n = 6x = 42$; genome $AABBH^{ch}H^{ch}$).

Genomic *in situ* hybridization (GISH) was performed as previously described [2]. Briefly, genomic DNA from *T. durum* (A and B genomes), *H. chilense* (H^{ch} genome) and *S. cereale* (R genome) were used as probes. *H. chilense* and *S. cereale* genomes were labeled with biotin-16-dUTP and digoxigenin-11-dUTP using the Biotin- or the DIG-nick translation mix, respectively (Sigma). Biotin-labeled probes were detected with Streptavidin-Cy3 or Streptavidin-Cy5; digoxigenin-labeled probes were detected with anti-digoxigenin-FITC (Sigma). *T. durum* genomic DNA was labeled by nick translation with tetramethyl-rhodamine-5-dUTP.

3. Results and Discussion

3.1. Production and Chromosome Constitution of the Trigeneric Hybrids and Amphyploids

3.1.1. Hybrid $H^{ch}DR$

H. chilense and *Ae. taushii* were duplicated after colchicine treatment. Tetraploid *H. chilense* was pollinated with tetraploid *Ae. Taushii,* and $H^{ch}H^{ch}DD$ hybrid plants were established after embryo rescue. Finally, *H. chilense* × *Ae. taushii* hybrids were pollinated with rye (*S. cereale*), and out of 200 florets pollinated, 2 adult hybrids $H^{ch}DR$ were recovered by embryo rescue. $H^{ch}DR$ hybrids showed vigorous vegetative growth and tillered profusely. They showed similar morphology to the female parent *H. chilense* × *Ae. squarrosa* hybridAll the florets were sterile.

Root tips metaphase spreads were analyzed by multicolour GISH to verify the genome constitution of the $H^{ch}DR$ hybrid (Figure 1a). The three recovered hybrids had 21 chromosomes, comprising 7 chromosomes from *H. chilense* (magenta), 7 chromosomes from rye (green) and 7 chromosomes from *Ae. taushii* (grey), confirming that they are true trigeneric hybrid ($n = 3x = 21$).

3.1.2. Hybrid $ABH^{ch}R$

To produce the trigeneric hybrid $ABH^{ch}R$, we used hexaploid ×*Tritordeum martinii* lines HT377 (with the translocation T1RS·1BL) and HT474. Tritordeum was crossed as female parent with rye as male parent. The $ABH^{ch}R$ hybrids all showed similar morphology to the tritordeum female parent, with an erect vegetative development and a smaller number of tillers than the $DH^{ch}R$. This hybrid displayed a morphology more characteristic of a crop and not the one of a wild species as the $H^{ch}DR$ does. All the florets were sterile.

Root tips metaphase spreads were analyzed by GISH to verify the genome constitution of the $ABH^{ch}R$ hybrids obtained (Figure 1b). All recovered hybrids possessed 28 chromosomes, containing 14 chromosomes from the AB genome (grey), 7 from *H. chilense* (magenta) and 7 from rye (green). This confirmed that they were true trigeneric hybrid ($n = 4x = 28$; $ABH^{ch}R$).

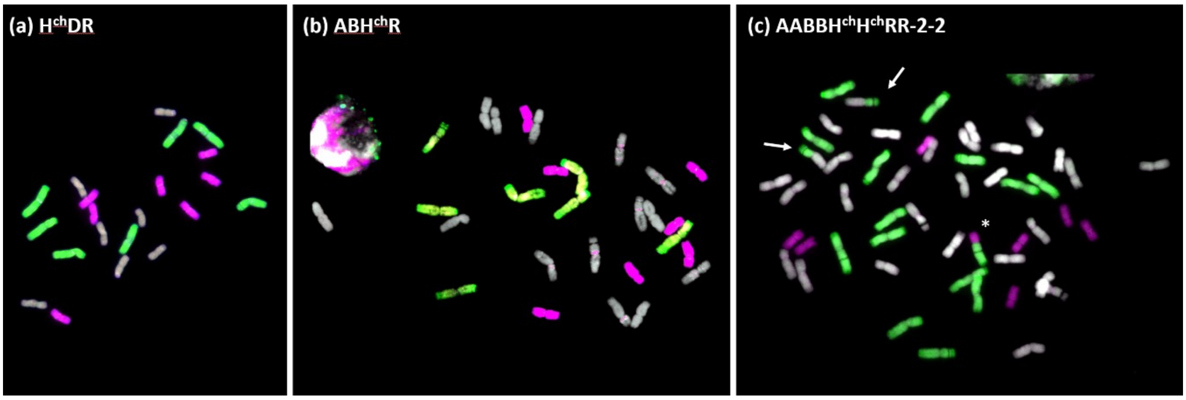

Figure 1. Root-tip metaphases of the trigeneric hybrids and amphiploid analyzed by GISH. (**a**) Hybrid $H^{ch}DR$ (n = 3x = 21) showing 7 chromosomes of *H. chilense* (magenta), 7 of rye (green) and 7 of *Ae. taushii* (grey). (**b**) Hybrid $ABH^{ch}R$ (n = 4x = 28) using tritordeum HT474, with 14 chromosomes from durum wheat (grey), 7 from *H. chilense* (magenta) and 7 from rye (green). (**c**) Aneuploid amphyploid $AABBH^{ch}H^{ch}RR$-2-2 with 51 chromosomes. The 2 translocations T1RS·1BL are indicated by an arrow. Another centromeric translocation between *H. chilense* and rye is marked with an asterisk.

3.1.3. Amphyploid $AABBH^{ch}H^{ch}RR$

Several trigeneric hybrids have been obtained in the last decades to be use as bridges in the transference of genes from wild species into wheat, with varying degrees of success [3–7]. However, apart from triticale and tritordeo, the production of the stable amphiploid is rare. Here, we treated the $H^{ch}DR$ and the $ABH^{ch}R$ hybrids with colchicine to induce whole genome duplication and obtain the corresponding amphiploids. Only the $ABH^{ch}R$ using tritordeum HT377 was duplicated. The chromosome number of the obtained amphiploids $AABBH^{ch}H^{ch}RR$ ranged from 49 to 54, none of them having the complete set of 56 chromosomes. Only 2 amphyploids produced progeny: $AABBH^{ch}H^{ch}RR$ -1 and $AABBH^{ch}H^{ch}RR$ -2. The morphology of the partial amphyploids was like $ABH^{ch}R$ hybrids but shorter. Each individual amphiploid was slightly different from each other due to the different chromosome composition. Unfortunately, all the florets were sterile.

We used the same combination of genomic GISH probes as with the $ABH^{ch}R$ hybrid to establish the genome constitution of the amphyploids. All individuals were aneuploids, with chromosome numbers ranging from 46 + 2 telosomic chromosomes (46 + 2 t) to 51 chromosomes (Figure 1c). *H. chilense* genome was the most affected, with only 7 chromosomes in some of the individuals. All amphyploids showed at least one T1RS·1BL translocation coming from tritordeum HT377. There were numerous telosomic chromosomes from all genomes.

3.2. Analysis of Meiotic Metaphase I Configuration in the Trigeneric Hybrid $H^{ch}DR$

In diploid species, only homologous chromosomes recombine during meiosis to ensure accurate chromosome segregation. However, no homologous chromosomes are present in a haploid hybrid. We used GISH to study the meiotic behavior of the three genome's chromosomes at metaphase I, using the same labelling conditions as for the somatic cells (Table 1). Surprisingly, 76.7% of the cells showed some chromosome associations. Most of these associations were rod bivalents between two chromosomes, but several ring bivalents and trivalents were also observed (Figure 2a–d). One might expect that most of these associations were between chromosomes belonging to the same genera; however, that was not the case, with the higher number of associations being observed between *Hordeum* and *Aegilops* (47.2 %) followed by *Aegilops* and *Secale* associations (29%). We could even detect several trivalents where chromosomes from the three genera were involved. These results highlight the potential use of this material to promote or stimulate recombination between genomes that would not normally recombine in a wild type situation. We are not able to demonstrate that all the associations observed are quiasmatic and produce a recombination event because this hybrid is sterile, and we cannot recover the results of these recombination events in the next generation. However, even if some of the more end-to-end associations could be nonchiasmatic, there are some very clear examples where the crossover structure is clearly observed between two pairs of chromosome (Figure 2a,d), confirming that there is recombination between the different genera.

Table 1. Number of chromosome associations observed in the trigeneric hybrid H^{ch}DR.

	No. and type of chromosome associations			**Total % of associations**
	Rod bivalent	**Ring bivalent**	**Trivalent**	
Hordeum-Hordeum	3	0	0	1.7
Aegilops-Aegilops	1	0	0	0.6
Secale-Secale	15	0	0	8.5
Hordeum-Aegilops	73	5	0	47.2
Hordeum-Secale	13	0	0	7.4
Aegilops-Secale	51	0	0	29.0
Hordeum-Aegilops-Secale	-	-	4	4.5
Hordeum-Aegilops-Aegilops	-	-	1	1.1
Total No.	156	10	10	

Analysis of Meiotic Metaphase I Configuration in the Trigeneric Hybrid ABH^{ch}R and Its Corresponding Amphiploid AABBH^{ch}H^{ch}RR.

A total of 100 meiotic metaphase I cells were analyzed, out of which 38 cells showed some chromosome association, frequently a single association (Table 2). Although associations were observed between all the different genomes (Figure 2e–h), the number was much lower than in H^{ch}DR hybrids, and moreover, only rod bivalent structures were detected. Rod bivalents have one association per bivalent instead of the two associations per trivalent and ring bivalent structures, which emphasizes the lower number of associations observed in the ABH^{ch}R hybrid compared with the H^{ch}DR. This could be explained due to the present of the *ph1* locus on the 5B genome. Due to its polyploid nature, polyploid wheat had to develop a mechanism to ensure than only homologous chromosome recombine, ensuring accurate chromosome segregation and fertility. Thus, wheat behaves as a diploid during meiosis, with every chromosome recombining only with its true homolog; a phenotypic behaviour which has been mostly attributed to *Ph1*, a dominant locus on chromosome 5B [8,9]. Moreover, not only the amount of associations is smaller, but also, contrary to the associations in H^{ch}DR, they are extremely distal and thus, it is possible that they are non-chiasmatic. Unfortunately, since ABH^{ch}R is also sterile, we cannot check the progeny to determine the output of the meiotic associations.

Table 2. Number of chromosome associations observed in the trigeneric hybrid ABH^{ch}R.

	Rod bivalent	**Total % of associations**
Triticum-Triticum	4	8.5
Hordeum-Hordeum	5	11.9
Secale-Secale	4	8.5
Triticum-Hordeum	15	35.7
Triticum-Secale	10	23.8
Hordeum-Secale	4	8.5
Total No.	42	

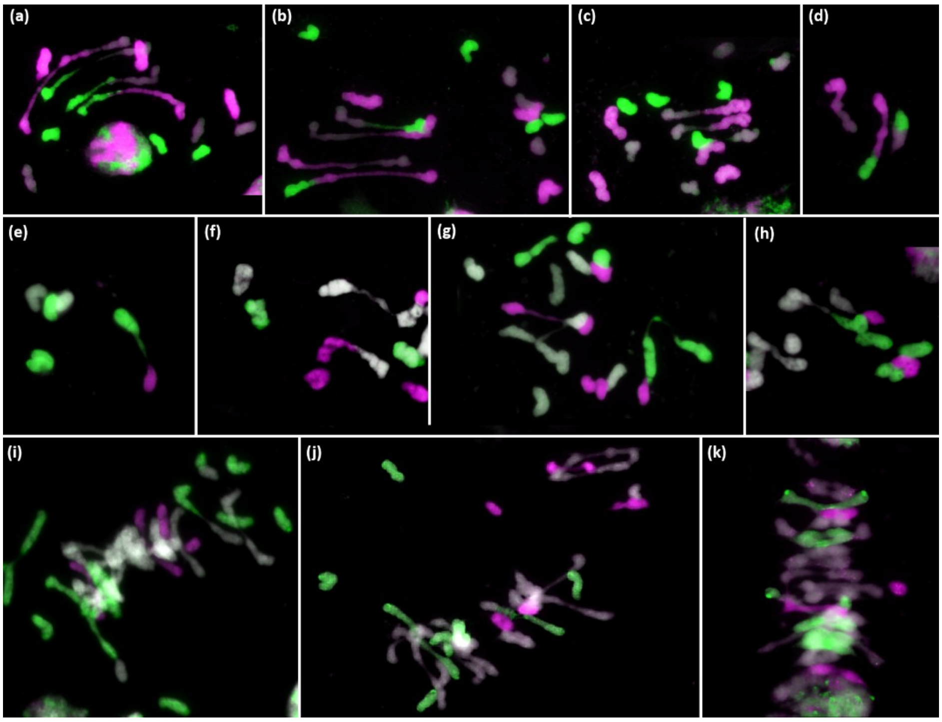

Figure 2. GISH of meiotic metaphase I configuration in the trigeneric hybrids DH^{ch}R (**a**–**d**), ABH^{ch}R (**e**–**h**) and aneuploid amphiploid AABBH^{ch}H^{ch}RR (**i**–**k**). *H. chilense* is shown in magenta and rye in green. In DH^{ch}R, *Ae. Taushii* is shown in grey. In ABH^{ch}R and AABBH^{ch}H^{ch}RR, durum wheat is shown in grey.

Finally, we analyzed the meiotic behaviour of two of the amphiploids: the AABBH^{ch}H^{ch}RR -1-1 and AABBH^{ch}H^{ch}RR -2-1 (Figure 2i–k). A total of 125 and 112 meyocites were analyzed from each genotype respectively. Interestingly, despite being aneuploids and presenting multiple chromosome reorganizations, the presence of associations between the different genera was only anecdotical, as shown in Figure 2i. There were multiple rod and ring bivalents as expected in an amphyploid, but also trivalents and quatrivalent were present (Figure 2j) due to their aneuploidy. No difference in terms of associations was observed between AABBH^{ch}H^{ch}RR -1-1 and AABBH^{ch}H^{ch}RR -2-1.

4. Conclusions

Due to their economic importance, *Triticeae* species such wheat, rye and barley have been bred intensively in the past hundreds of years resulting in massive improvements in yield and quality, but also in a huge decrease in their genetic diversity. Thus, the incorporation of genetic variability from related and wild species into cultivated ones is a priority, but also a challenge, due to the presence of reproductive barriers that hinder genetic transfer among them. The results presented here demonstrate that recombination between three cultivated species as distant as wheat, rye and barley is possible. The next challenge will be to recover these recombination events so they can be transferred into crops.

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