The Transcriptional and Splicing Changes Caused by Hybridization Can Be Globally Recovered by Genome Doubling during Allopolyploidization

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Abstract

Polyploidization is a major driving force in plant evolution. Allopolyploidization, involving hybridization and genome doubling, can cause extensive transcriptome reprogramming which confers allopolyploids higher evolutionary potential than their diploid progenitors. To date, little is known about the interplay between hybridization and genome doubling in transcriptome reprogramming. Here, we performed genome-wide analyses of transcriptome reprogramming during allopolyploidization in wheat and brassica lineages. Our results indicated that hybridization-induced transcriptional and splicing changes of genes can be largely recovered to parental levels by genome doubling in allopolyploids. As transcriptome reprogramming is an important contributor to heterosis, our finding updates a longstanding theory that heterosis in interspecific hybrids can be permanently fixed through genome doubling. Our results also indicated that much of the transcriptome reprogramming in interspecific hybrids was not caused by the merging of two parental genomes, providing novel insights into the mechanisms underlying both heterosis and hybrid speciation.

Key words: **polyploidization, hybridization, genome duplication, plant evolution, transcriptome, splicing.**

Letter

Polyploidization is ubiquitous in the plant kingdom that almost all higher plant species have undergone one or multiple rounds of polyploidization during their evolution (Tang et al. 2010; Jiao et al. 2012; Alix et al. 2017). Allopolyploidization, involving interspecific hybridization and genome doubling, was suggested to occur more frequently in nature than autopolyploidization, to promote adaptive speciation (Abbott et al. 2013; Alix et al. 2017). Well-established allopolyploids often occur in environments where their diploid relatives are not found (Abbott et al. 2013; Alix et al. 2017). Recent studies have shown that allopolyploidization can induce extensive transcriptomic changes resulting in various phenotypic novelties, thus conferring higher evolutionary potential and plasticity in allopolyploids (Wang et al. 2006; Guo and Han 2014; Alix et al. 2017). The respective role of hybridization and genome doubling in composing the transcriptome reprogramming in allopolyploids is a basis toward understanding the mechanism underlying allopolyploidization but is still elusive (Qiu et al. 2020).

Though the transcriptional changes in allopolyploids compared with their progenitors have been frequently reported (Hegarty et al. 2008; Yoo et al. 2013; Zhao et al. 2013; Xu et al. 2014), very few studies have dissected the respective effects of hybridization and genome doubling during allopolyploidization. A previous study in *Senecio cambrensis* using "anonymous" cDNA microarrays found that expression changes in many floral genes which resulted from hybridization were attenuated after genome doubling (Hegarty et al. 2006). However, the inherent limitations of "anonymous" cDNA microarrays make it difficult to distinguish the expression of homoeologous genes from different subgenomes, thereby compromising their conclusion (Zhao et al. 2014). A recent RNA-Seq study in wheat reported that hybridization mainly caused the downregulation of genes from the D subgenome, which can be partially restored by genome doubling (Hao et al. 2017). However, very few genes in the A and B subgenomes were found to be affected during allopolyploidization (Hao et al. 2017). To date, little is known about the extent to which hybridization-induced transcriptional changes can be affected by genome doubling.

Splicing regulation is a key posttranscriptional regulatory mechanism in eukaryotes (Syed et al. 2012). Splicing variations of duplicated genes resulting from polyploidization can lead to phenotypic variations in polyploid plant species (Slotte et al. 2009; Simmonds et al. 2016). To the best of our knowledge, only a few studies have identified individual

genes showing splicing changes following polyploidization (Madlung et al. 2005; Terashima and Takumi 2009; Zhou et al. 2011; Saminathan et al. 2015). Some genome-wide studies have investigated the effect of polyploidization on splicing (Wang et al. 2019; Yu et al. 2020), but still, how hybridization and genome doubling affect gene splicing at whole-genome scale is poorly understood.

In this study, we reanalyzed publicly available RNA-Seq data sets and examined the transcriptome reprogramming during allopolyploidization in wheat and brassica lineages (Hao et al. 2017; Zhang et al. 2018). We found that both hybridization and genome doubling can induce genome-wide changes at gene transcriptional and splicing levels. Notably, a large proportion of hybridization-induced transcriptional and splicing changes in hybrids can be recovered to parental levels in allopolyploids after genome doubling. As transcriptome reprogramming is an important contributor to heterosis (Chen 2013), our results indicate that most of the heterosis from transcriptome reprogramming cannot be fixed in allopolyploids, which updates a longstanding theory "heterosis in interspecific hybrids can be permanently fixed through genome doubling" (Comai 2005; Chen 2010, 2013). Additionally, our results indicated that much of the transcriptome reprogramming in interspecific hybrids was not caused by the merging of two parental genomes, providing novel insights of the mechanisms underlying heterosis and hybrid speciation.

Results and Discussion

To investigate the effects of hybridization and genome doubling on transcriptome reprogramming during allopolyploidization, we reanalyzed the previously published RNA-Seq data sets of three interspecific crossing combinations in wheat and brassica lineages (Hao et al. 2017; Zhang et al. 2018) (fig. 1A, supplementary table 1 and supplementary figs. S1–S3, Supplementary Material online). For wheat combinations, two tetraploids of *Triticum turgidum* (AABB) were crossed with diploid *Aegilops tauschii* (DD) to produce triploid hybrids (ABD) whose genomes were then doubled to generate allohexaploid wheat (AABBDD) (fig. 1A) (Hao et al. 2017). Similarly, diploids *Brassica rapa* (A_rA_r) and *Brassica oleracea* (C_oC_o) were crossed to produce a hybrid (A_rC_o) which was used in the generation of allotetraploid brassica ($A_rA_rC_oC_o$) (fig. 1A) (Zhang et al. 2018). To examine the respective effects of hybridization and genome doubling on gene expression, significantly differentially expressed genes (DEGs) were identified by comparing hybrids with parents (Hybrid-vs-Parents) and allopolyploids with hybrids (Allopolyploid-vs-Hybrid), by applying the criteria "expression fold change $\geq$2 and false discovery rate $<$ 0.05" (supplementary tables 2–4, Supplementary Material online). We found that both hybridization and genome doubling can induce dramatic expression changes in thousands of genes (fig. 1B, supplementary fig. S4, Supplementary Material online). The combined effect of hybridization and genome doubling was further examined by comparing allopolyploids with parents (Allopolyploid-vs-Parents). Notably, for most comparisons (subgenomes A and B in wheat and subgenome A_r in brassica), far fewer DEGs were caused by the combined effect than those caused by hybridization or genome doubling alone (fig. 1B, supplementary fig. S4, Supplementary Material online). For example, for subgenome A in wheat combination 1, up to 4,759 and 4,735 DEGs were induced by hybridization and genome doubling, respectively, whereas only 1,849 DEGs were caused by their combined effect (fig. 1B). For subgenome D in wheat and subgenome C_o in brassica, the number of DEGs caused by the combined effect was still far fewer than the sum of DEGs caused by these two events alone (fig. 1B, supplementary fig. S4, Supplementary Material online).

To examine genome-wide splicing changes during allopolyploidization, the splicing efficiency of each intron was calculated (supplementary material and supplementary fig. S5, Supplementary Material online). Significantly differentially spliced introns (DSIs) were identified by applying the criteria "splicing efficiency change $\geq$ 20% and false discovery rate $<$ 0.05" (supplementary tables 5–7, Supplementary Material online) (Brooks et al. 2011). We found that both hybridization and genome doubling events can induce genome-wide splicing efficiency changes (fig. 1C, supplementary fig. S6, Supplementary Material online). Intriguingly, for most comparisons (subgenomes A and B in wheat and subgenome A_r in brassica), there were fewer DSIs caused by the combined effect of hybridization and genome doubling compared with those caused by either event alone (fig. 1C, supplementary fig. S6, Supplementary Material online). For example, for subgenome A in wheat combination 1, 803 and 604 DSIs were caused by hybridization and genome doubling, respectively, whereas only 423 DSIs were identified due to their combined effect (fig. 1C). For subgenome D in wheat and subgenome C_o in brassica, the DSIs caused by the combined effect were also far fewer than the total DSIs caused by these two events alone (fig. 1C, supplementary fig. S6, Supplementary Material online). Collectively, these results indicated that the combined effect of hybridization and genome doubling on gene expression and splicing is far less than the simple sum of their individual effects, which suggests an interplay between hybridization and genome doubling during allopolyploidization.

The relationship between hybridization and genome doubling was further examined by comparing gene expression and splicing changes induced by these two events (fig. 1D and E, supplementary figs. S7–S10, Supplementary Material online). Significantly negative correlations were observed between the expression fold changes induced by these two events for all the subgenomes in all three cross combinations (fig. 1D, supplementary figs. S7 and S8, Supplementary Material online). For example, the Pearson correlation coefficient (PCC) is -0.52 to -0.77 for different subgenomes of wheat combination 1 (fig. 1D). Furthermore, significantly negative correlations were also observed between the splicing efficiency changes caused by hybridization and genome doubling, as seen in wheat combination 1, which had a PCC of -0.65 to -0.72 (fig. 1E, supplementary figs. S9 and S10, Supplementary Material online). Taken together, these results indicate that genome doubling can cause global opposite

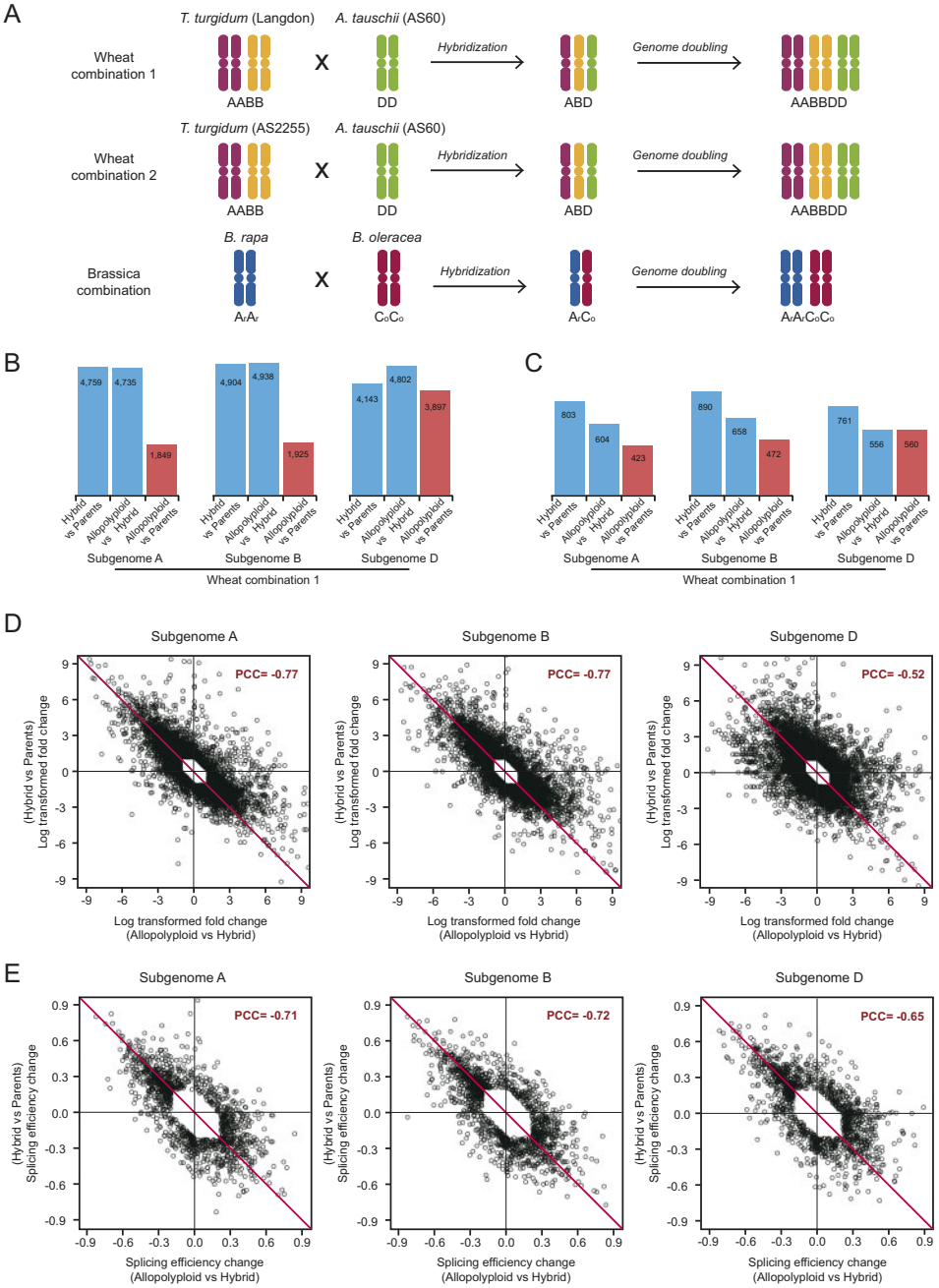

Fig. 1. Comparison of gene expression and splicing efficiency changes induced by hybridization and genome doubling during allopolyploidization. (*A*) Schematic representation of the synthesis of allopolyploid wheat and brassica. These hybrids and allopolyploids were produced in previous studies, which provided the data sets used in this study (Hao et al. 2017; Zhang et al. 2018). For wheat combinations, two tetraploids, *Triticum turgidum* (AABB) ssp. *durum* cv. Langdon (LDN) and ssp. *turgidum* accession AS2255, were crossed with diploid *Aegilops tauschii* ssp. *tauschii* accession AS60 (DD) to produce the two triploid hybrids (ABD). The resulting triploids were used to generate the allohexaploid wheat (AABBDD) through genome doubling. For the brassica combination, two diploids *Brassica rapa* (A_rA_r) and *Brassica oleracea* (C_oC_o) were crossed to produce the diploid hybrid (A_rC_o), which was then used to generate the allotetraploid brassica ($A_rA_rC_oC_o$) through genome doubling. (*B*) The number of DEGs caused by hybridization, genome doubling, and allopolyploidization in wheat combination 1. The numbers of DEGs caused by hybridization (Hybrid-vs-Parents, blue bars), genome doubling (Allopolyploid-vs-Hybrid, blue bars), and allopolyploidization (Allopolyploid-vs-Parents, red bars) are shown for each subgenome. (*C*) The number of DSIs caused by hybridization, genome doubling, and allopolyploidization in wheat combination 1. The numbers of DSIs caused by hybridization (Hybrid-vs-Parents, blue bars), genome doubling (Allopolyploid-vs-Hybrid, blue bars) and allopolyploidization (Allopolyploid-vs-Parents, red bars) are shown for each subgenome. (*D*) Significantly negative correlations between gene expression fold changes caused by hybridization and genome doubling in wheat combination 1. All DEGs identified from Hybrid-vs-Parents, Allopolyploid-vs-Hybrid, and Allopolyploid-vs-Parents were analyzed and plotted. The PCC of each comparison is shown (*P* values $<$ 2.2e-16 for all comparisons). (*E*) Significantly negative correlations between splicing efficiency changes caused by hybridization and genome doubling in wheat combination 1. All DSIs identified from Hybrid-vs-Parents, Allopolyploid-vs-Hybrid, and Allopolyploid-vs-Parents were analyzed and plotted. The PCC value of each comparison is shown (*P* values $<$ 2.2e-16 for all the comparisons).

effects compared with hybridization at both gene expression and splicing levels.

To determine how much hybridization-induced transcriptome reprogramming in hybrids can be recovered in allopolyploids after genome doubling, DEGs/DSIs identified in the hybrids were further classified into four groups (Supplementary Material online): DEGs/DSIs in hybrids being recovered to parental levels in allopolyploids (Group 1); DEGs/DSIs in hybrids being retained in allopolyploids (Group 2); DEGs/DSIs in hybrids being reinforced by genome doubling (Group 3); and others (Group 4) (fig. 2*A*–*D*). Most (89–96%) of the DEGs and DSIs can be classified into Groups 1–3 (fig. 2*A*–*D*). Notably, for A and B subgenomes in both wheat combinations, most hybridization-induced gene expression and splicing efficiency changes in hybrids can be recovered to parental levels after genome doubling (Group 1, 61–76% for DEGs and 54–75% for DSIs), whereas fewer were retained in allopolyploids (Group 2, 15–29% for DEGs and 20–35% for DSIs) (fig. 2*A*–*D*). For subgenome D in wheat, relatively more DEGs/DSIs in hybrids were retained in allopolyploids, but still, ~32% and ~39% of the DEGs and DSIs in hybrids were recovered to parental levels by genome doubling, respectively (fig. 2*A*–*D*). Likewise, in the brassica combination, more hybridization-induced DEGs (45–62%) and DSIs (49–63%) were recovered to parental levels compared with those which retained their hybridization-induced changes (31–42% for DEGs, 27–41% for DSIs) in the allotetraploid after genome doubling (fig. 2*A*–*D*). We also found that the DEGs and DSIs being recovered after genome doubling were distributed among all the chromosomes with no apparent preference, which indicated that this "recovery effect" is common among all the chromosomes (supplementary figs. S11 and S12, Supplementary Material online). In addition, most of the DEGs (51–70%) or DSIs (60–69%) caused by genome doubling were also found to be due to reversion to parental levels (supplementary fig. S13, Supplementary Material online). Conclusively, hybridization-induced transcriptome reprogramming in hybrids can be globally recovered in allopolyploids after genome doubling.

Having observed the recovery of hybridization-induced gene expression changes by genome doubling, we next attempted to determine the relative contribution of hybridization and genome doubling to the final transcriptome reprogramming in allopolyploids. The DEGs and DSIs identified from comparisons between allopolyploids and their parents were further grouped into three clusters according to the relative contribution of hybridization and genome doubling (Supplementary Material online): DEGs/DSIs mainly caused by hybridization (Cluster 1); DEGs/DSIs mainly caused by genome doubling (Cluster 2); and DEGs/DSIs significantly contributed to by both events (Cluster 3) (fig. 3*A* and *B*, supplementary figs. S14 and S15, Supplementary Material online). About 34–55% of DEGs and 31–51% of DSIs identified in allopolyploids were found to be mainly caused by hybridization, and comparable amounts of DEGs (21–45%) and DSIs (23–43%) were found to be mainly caused by genome doubling (fig. 3*A* and *B*, supplementary figs. S14 and S15, Supplementary Material online). Relatively fewer DEGs and DSIs were contributed to by both events with the same trend (fig. 3*A* and 3*B*, supplementary figs. S14 and S15, Supplementary Material online). These results suggested that both hybridization and genome doubling substantially and comparably contributed to transcriptome reprogramming in allopolyploids. In addition, the relative contribution of hybridization and genome doubling varied among different subgenomes and species. For example, in wheat combination 1, more DEGs were contributed to by hybridization in subgenomes A and B, whereas more DEGs were contributed to by genome doubling in subgenome D (fig. 3*A*).

It has long been considered that heterosis in interspecific hybrids can be permanently fixed through genome doubling to form allopolyploids (Comai 2005; Chen 2010, 2013). The fixation of heterosis can confer advantages to allopolyploids in adaptive evolution (Comai 2005; Chen 2010). However, little is known about how much heterosis in hybrids can be fixed in allopolyploids, or exactly what role genome doubling plays in the fixation of heterosis. We found that most of the transcriptome reprogramming which occurred in hybrids cannot be fixed in allopolyploids after genome doubling (fig. 2*A*–*D*). As transcriptome reprogramming is an important contributor to heterosis (Chen 2010, 2013), our results suggest that most of the heterosis resulting from transcriptome reprogramming in interspecific hybrids cannot be fixed in allopolyploids due to the "recovery effect" of genome doubling. Heterosis also arises from other factors, such as combination of different alleles (Chen 2013). Theoretically, heterotic phenotypes caused by other factors will not be affected by this "recovery effect." It is also possible that a small number of DEGs were due to homoeologous exchange, although it rarely occurred (Zhang et al. 2020). However, the distribution of DEGs/DSIs among all the chromosomes indicated that the impact of possible homoeologous exchange is very slight even if it occurred (supplementary figs. S11 and S12, Supplementary Material online).

Hybridization is both a common mechanism in plant speciation and one of the most important applications of genetics in crop breeding (Abbott et al. 2013; Huang et al. 2016). Recent hybridization events were found in nearly half of the world's crops and wild species during their evolution (Abbott et al. 2013; Alix et al. 2017). Recent studies demonstrated that hybridization can induce dramatic transcriptome reprogramming which can lead to phenotypic variations and serve as an important source of heterosis in hybrids (Chen 2013; Yoo et al. 2013). Transcriptome reprogramming in hybrids has typically been considered to be caused by the merger of two genomes or subsequent "genome shock" (Chen 2013). Our results suggested that a large proportion of hybridization-induced transcriptome reprogramming in interspecific hybrids (Group 1 in fig. 2*A* and *B*) was not attributed to genome merger, as it recovered to parental levels in allopolyploids possessing merged genomes. This proportion of transcriptome reprogramming in hybrids was probably due to other factors, such as a reduction of homologous chromosome sets in hybrids, as both parents and allopolyploids have two copies of homologous chromosome sets but hybrids only have one. Thus, our findings provide new insights into the

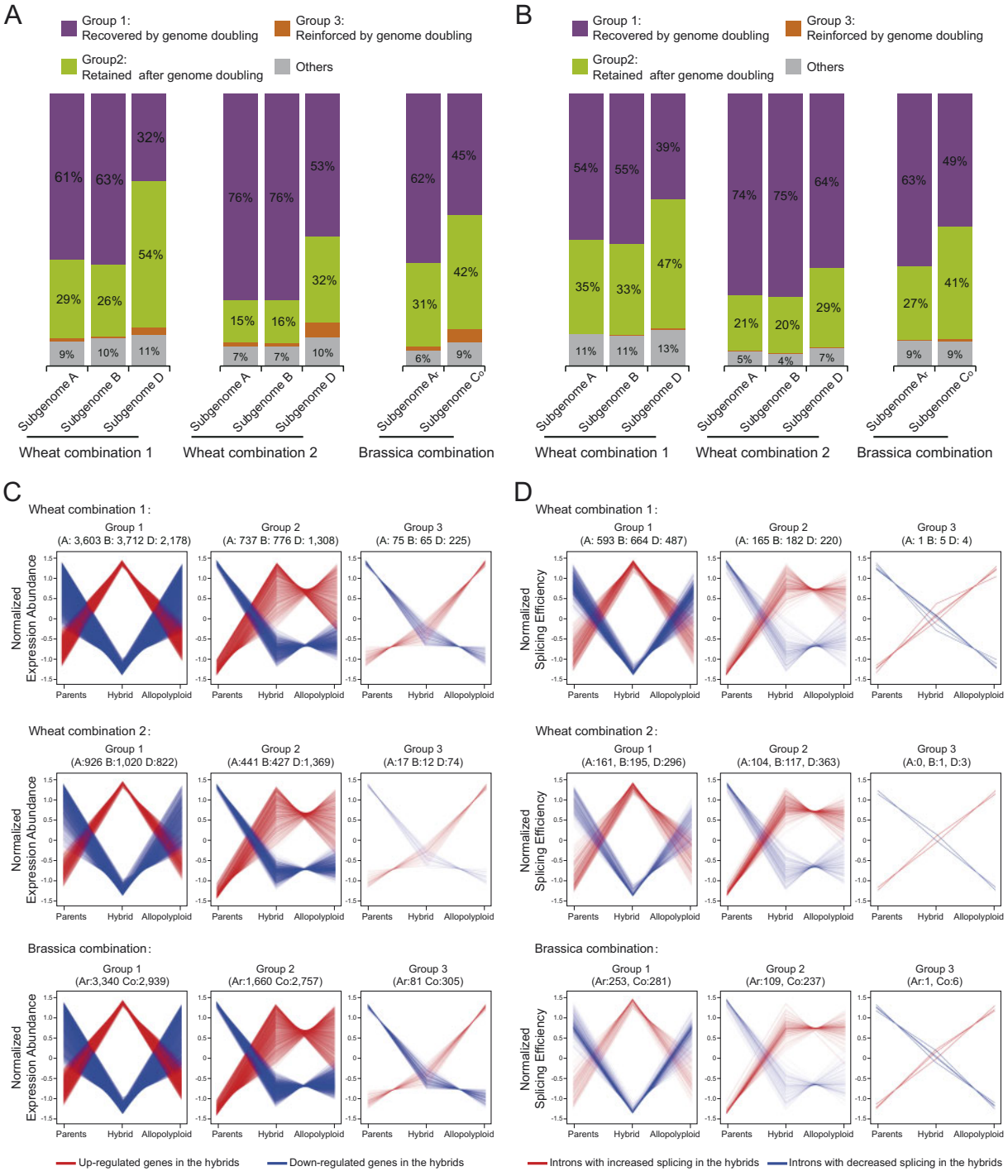

Fig. 2. Classification of DEGs and DSIs identified in hybrids when compared with their parents. (*A*) Classification of DEGs identified in the hybrids compared with their parents. These DEGs were classified into four groups: Altered expression in hybrids was recovered to parental levels by genome doubling (Group 1); altered expression in hybrids remained in allopolyploids after genome doubling (Group 2); hybridization-induced expression changes were reinforced by genome doubling (Group 3, genome doubling caused changes in the same direction as that caused by hybridization); and others (Group 4). (*B*) Classification of DSIs identified in hybrids compared with their parents. All DSIs were classified into four groups: altered splicing efficiency in hybrids recovered to parental levels by genome doubling (Group 1); altered splicing efficiency in hybrids remained in allopolyploids after genome doubling (Group 2); hybridization-induced splicing changes were reinforced by genome doubling (Group 3, genome doubling caused change in the same direction as that caused by hybridization); and others (Group 4). (*C*) The expression profiles of DEGs in Groups 1–3 from the classification analysis in (*A*). DEGs of the same group from all subgenomes were plotted together and each gene is represented by each single line. The numbers of DEGs in each subgenome are indicated. The upregulated and downregulated genes in hybrids are represented by red and blue lines, respectively. (*D*) The splicing efficiency profiles of DSIs in Groups 1–3 from the classification analysis in (*B*). DSIs of the same group from all subgenomes were plotted together and each intron is represented by each single line. The numbers of DSIs in each subgenome are indicated. The introns with increased splicing efficiency and decreased splicing efficiency in hybrids are represented by red and blue lines, respectively.

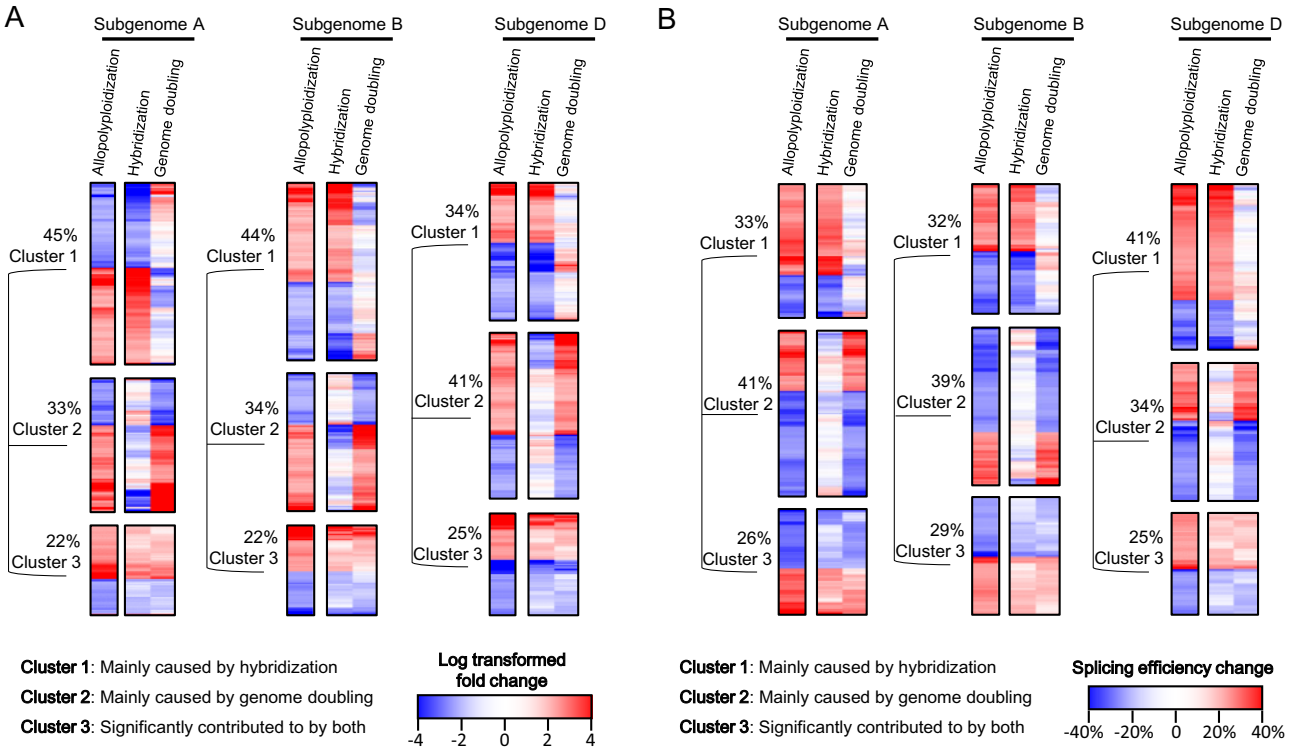

Fig. 3. Classification of DEGs and DSIs identified in allopolyploids when compared with their parents. (*A*) Classification of DEGs identified in allopolyploids compared with their parents in wheat combination 1. All DEGs can be classified into three clusters: expression changes mainly caused by hybridization (Cluster 1); expression changes mainly caused by genome doubling (Cluster 2); and expression changes significantly contributed to by both hybridization and genome doubling with the same trend (Cluster 3). Allopolyploidization: expression fold changes between allopolyploids and parents; Hybridization: expression fold changes between hybrids and parents; Genome doubling: expression fold changes between allopolyploids and hybrids. The expression fold changes are shown in heatmaps. Upregulated and downregulated genes are colored in red and blue, respectively. The color intensity reflects the magnitude of fold change. (*B*) Classification of DSIs identified in allopolyploids compared with their parents in wheat combination 1. All DSIs can be classified into three clusters: splicing efficiency changes mainly caused by hybridization (Cluster 1); splicing efficiency changes mainly caused by genome doubling (Cluster 2); and splicing efficiency changes significantly contributed to by both hybridization and genome doubling with the same trend (Cluster 3). The splicing efficiency changes are shown in heatmaps. Introns with increased and decreased splicing efficiency are colored in red and blue, respectively. The color intensity reflects the magnitude of splicing efficiency change.

mechanism underlying heterosis and hybrid speciation. In addition, several previous studies have demonstrated that hybridization-induced DNA methylation alterations can also be recovered in allopolyploids after genome doubling (Beaulieu et al. 2009; Hegarty et al. 2011; Xu et al. 2012; Qi et al. 2018). Together with our findings at gene transcriptional and splicing levels, this "recovery effect" of genome doubling implies a novel gene expression regulatory mechanism which is worth further investigation.

Materials and Methods

All RNA-sequencing data sets were published by previous studies and downloaded from the NCBI SRA database (supplementary table 1, Supplementary Material online, https://www.ncbi.nlm.nih.gov/sra/, last accessed February 20, 2021) (Hao et al. 2017; Zhang et al. 2018). For wheat species, the youngest fully expanded leaves at development stage 5 were collected from the parental, hybrid, and allohexaploid plants for RNA-sequencing (Large 1954; Hao et al. 2017). For brassica species, the young and fully expanded leaves at third-leaf stage were collected for RNA-sequencing (Zhang et al. 2018). For detailed methods, please see the Supplementary Material online.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

Acknowledgments

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Author Contributions

Z.L., Q.S., and Z.N. conceived the research; Z.L. and J.Q. designed the analyses; Z.L., J.Q., R.M., and H.L. performed

the data analysis; J.Q. and Z.L. wrote the manuscript with input from all authors.

Data Availability

All raw RNA-Seq data sets were downloaded from the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra, last accessed February 20, 2021). Data sets for wheat cross combination 1 were under accession numbers: SRR3406932, SRR3474182, SRR3474190, SRR3474194, SRR3474195, SRR3474196 and SRR3474198. Data sets for wheat cross combination 2 were under accession numbers: SRR3474176, SRR3474179, SRR3474185, SRR3474187, SRR3474199 and SRR3474201. Data sets for the brassica cross combination were under accession numbers: SRR3584243, SRR3584242, SRR5482051, SRR5481714, SRR5481715, SRR5481777, SRR5481776, SRR5481713 and SRR5481712.

References

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJ, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R, et al. 2013. Hybridization and speciation. *J Evol Biol*. 26(2):229–246.

Alix K, Gerard PR, Schwarzacher T, Heslop-Harrison JSP. 2017. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Ann Bot*. 120(2):183–194.

Beaulieu J, Jean M, Belzile F. 2009. The allotetraploid *Arabidopsis thaliana-Arabidopsis lyrata* subsp. *petraea* as an alternative model system for the study of polyploidy in plants. *Mol Genet Genomics*. 281(4):421–435.

Brooks AN, Yang L, Duff MO, Hansen KD, Park JW, Dudoit S, Brenner SE, Graveley BR. 2011. Conservation of an RNA regulatory map between *Drosophila* and mammals. *Genome Res*. 21(2):193–202.

Chen ZJ. 2010. Molecular mechanisms of polyploidy and hybrid vigor. *Trends Plant Sci*. 15(2):57–71.

Chen ZJ. 2013. Genomic and epigenetic insights into the molecular bases of heterosis. *Nat Rev Genet*. 14(7):471–482.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nat Rev Genet*. 6(11):836–846.

Guo X, Han F. 2014. Asymmetric epigenetic modification and elimination of rDNA sequences by polyploidization in wheat. *Plant Cell*. 26(11):4311–4327.

Hao M, Li A, Shi T, Luo J, Zhang L, Zhang X, Ning S, Yuan Z, Zeng D, Kong X, et al. 2017. The abundance of homoeologue transcripts is disrupted by hybridization and is partially restored by genome doubling in synthetic hexaploid wheat. *BMC Genomics* 18(1):149.

Hegarty MJ, Barker GL, Brennan AC, Edwards KJ, Abbott RJ, Hiscock SJ. 2008. Changes to gene expression associated with hybrid speciation in plants: further insights from transcriptomic studies in Senecio. *Philos Trans R Soc B*. 363(1506):3055–3069.

Hegarty MJ, Barker GL, Wilson ID, Abbott RJ, Edwards KJ, Hiscock SJ. 2006. Transcriptome shock after interspecific hybridization in senecio is ameliorated by genome duplication. *Curr Biol*. 16(16):1652–1659.

Hegarty MJ, Batstone T, Barker GL, Edwards KJ, Abbott RJ, Hiscock SJ. 2011. Nonadditive changes to cytosine methylation as a consequence of hybridization and genome duplication in *Senecio* (Asteraceae). *Mol Ecol*. 20(1):105–113.

Huang XH, Yang SH, Gong JY, Zhao Q, Feng Q, Zhan QL, Zhao Y, Li WJ, Cheng BY, Xia JH, et al. 2016. Genomic architecture of heterosis for yield traits in rice. *Nature* 537(7622):629–633.

Jiao Y, Leebens-Mack J, Ayyampalayam S, Bowers JE, McKain MR, McNeal J, Rolf M, Ruzicka DR, Wafula E, Wickett NJ, et al. 2012. A genome triplication associated with early diversification of the core eudicots. *Genome Biol*. 13(1):R3.

Large EC. 1954. Growth stages in cereals. Illustration of the Feekes scale. *Plant Pathol*. 3(4):128–129.

Madlung A, Tyagi AP, Watson B, Jiang HM, Kagochi T, Doerge RW, Martienssen R, Comai L. 2005. Genomic changes in synthetic *Arabidopsis* polyploids. *Plant J*. 41(2):221–230.

Qi X, Wang H, Song A, Jiang J, Chen S, Chen F. 2018. Genomic and transcriptomic alterations following intergeneric hybridization and polyploidization in the Chrysanthemum nankingense x Tanacetum vulgare hybrid and allopolyploid (Asteraceae). *Hortic Res*. 5(1):5.

Qiu T, Liu Z, Liu B. 2020. The effects of hybridization and genome doubling in plant evolution via allopolyploidy. *Mol Biol Rep*. 47(7):5549–5558.

Saminathan T, Nimmakayala P, Manohar S, Malkaram S, Almeida A, Cantrell R, Tomason Y, Abburi L, Rahman MA, Vajja VG, et al. 2015. Differential gene expression and alternative splicing between diploid and tetraploid watermelon. *J Exp Bot*. 66(5):1369–1385.

Simmonds J, Scott P, Brinton J, Mestre TC, Bush M, del Blanco A, Dubcovsky J, Uauy C. 2016. A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains. *Theor Appl Genet*. 129(6):1099–1112.

Slotte T, Huang HR, Holm K, Ceplitis A, St Onge KR, Chen J, Lagercrantz U, Lascoux M. 2009. Splicing variation at a FLOWERING LOCUS C homeolog is associated with flowering time variation in the tetraploid *Capsella bursa-pastoris*. *Genetics* 183(1):337–345.

Syed NH, Kalyna M, Marquez Y, Barta A, Brown JW. 2012. Alternative splicing in plants–coming of age. *Trends Plant Sci*. 17(10):616–623.

Tang H, Bowers JE, Wang X, Paterson AH. 2010. Angiosperm genome comparisons reveal early polyploidy in the monocot lineage. *Proc Natl Acad Sci U S A*. 107(1):472–477.

Terashima A, Takumi S. 2009. Allopolyploidization reduces alternative splicing efficiency for transcripts of the wheat DREB2 homolog, WDREB2. *Genome* 52(1):100–105.

Wang J, Tian L, Lee HS, Wei NE, Jiang H, Watson B, Madlung A, Osborn TC, Doerge RW, Comai L, et al. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. *Genetics* 172(1):507–517.

Wang R, Liu H, Liu Z, Zou J, Meng J, Wang J. 2019. Genome-wide analysis of alternative splicing divergences between *Brassica* hexaploid and its parents. *Planta* 250(2):603–628.

Xu C, Bai Y, Lin X, Zhao N, Hu L, Gong Z, Wendel JF, Liu B. 2014. Genome-wide disruption of gene expression in allopolyploids but not hybrids of rice subspecies. *Mol Biol Evol*. 31(5):1066–1076.

Xu Y, Zhao Q, Mei S, Wang J. 2012. Genomic and transcriptomic alterations following hybridisation and genome doubling in trigenomic allohexaploid *Brassica carinata* x *Brassica rapa*. *Plant Biol (Stuttg)*. 14(5):734–744.

Yoo MJ, Szadkowski E, Wendel JF. 2013. Homoeolog expression bias and expression level dominance in allopolyploid cotton. *Heredity (Edinb)*. 110(2):171–180.

Yu K, Feng M, Yang G, Sun L, Qin Z, Cao J, Wen J, Li H, Zhou Y, Chen X, et al. 2020. Changes in alternative splicing in response to domestication and polyploidization in wheat. *Plant Physiol*. 184(4):1955–1968.

Zhang DW, Pan Q, Tan C, Liu LL, Ge XH, Li ZY, Yan ML. 2018. Homoeolog expression is modulated differently by different subgenomes in *Brassica napus* hybrids and allotetraploids. *Plant Mol Biol Rep*. 36(3):387–398.

Zhang Z, Gou X, Xun H, Bian Y, Ma X, Li J, Li N, Gong L, Feldman M, Liu B, et al. 2020. Homoeologous exchanges occur through intragenic recombination generating novel transcripts and proteins in wheat and other polyploids. *Proc Natl Acad Sci U S A*. 117(25):14561–14571.

Zhao Q, Zou J, Meng J, Mei S, Wang J. 2013. Tracing the transcriptomic changes in synthetic Trigenomic allohexaploids of *Brassica* using an RNA-Seq approach. *PLoS One* 8(7):e68883.

Zhao S, Fung-Leung WP, Bittner A, Ngo K, Liu X. 2014. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. *PLoS One* 9(1):e78644.

Zhou RC, Moshgabadi N, Adams KL. 2011. Extensive changes to alternative splicing patterns following allopolyploidy in natural and resynthesized polyploids. *Proc Natl Acad Sci U S A*. 108(38):16122–16127.