Science in China Series C: Life Sciences

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The ploidy effects in plant gene expression: Progress, problems and prospects

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Polyploidy and haploid are widely employed in th e studies of genetics and evolution, and great progress has been made in these fields, inspiring the enthusiasm of scientists to explore the ploidy effects in gene expression. In this paper, we review th e gene expression and its regulation in polyploids, especially in autopolyploids. We summarize some limitations in previous reports on polyploidy gene expression and its regulation, especially the limitations in the research materials. We propose an idea to create homologous ploidy series with twin-seedlings and to employ high-throughput techniques to investigate the polyploidy transcriptome and its regulation.

ploidy, polyploidy, haploid, gene expression

Haploid rarely exists in nature as a species. Almost all the extant haploids are created by the techniques such as anther culture, parthenogenesis, etc. Genetically homologous diploid can be obtained by directly doubling haploid genome. Therefore, haploid is widely used to accelerate the progress of breeding program. Compared with the rare distribution of haploids, polyploidy is extremely common in nature. The evolution of all eukaryotes[1] and all or most extant angiosperms[2,3], including many important crops[4] such as rice[5−7], is considered to involve polyploidization. Polyploidization may be accompanied by some new useful phenotypes such as fertility, asexual reproduction, drought tolerance, disease and pest resistance, cell and organ size, flowering timing and biomass[8,9]. Therefore, polyploidy is also often used to breed new cultivars in breeding practice, such as in rice[10]. In short, both haploid and polyploidy are not only of theoretical importance in genetic and evolution studies, but also of practical importance in breeding, inspiring the great enthusiasms of scientists to explore the ploidy effects. Ploidy effects can be greatly reflected by the changes in gene expression. In this paper, we introduce recent achievements on gene expression of haploid and polyploidy, analyze the limitations in the previous studies and propose corresponding solutions.

1 Gene expression in haploid and polyploidy plants

There is only one set of chromosomes in haploid, and all the genes, no matter to be dominant or recessive, can express themselves at the RNA or phenotype level. Therefore, haploid is an important tool in genetic studies. However, we still do not know very well about the gene expression and regulation mechanism in haploids, with a certain exception in some species such as yeast[11,12], corn[13], potato[14], cabbage[15] and rice[16]. In contrast, gene expression in polyploidy has been actively studied

Received December 6, 2007; accepted December 18, 2007
doi: 10.1007/s11427-008-0039-3
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Supported by the National Natural Science Foundation of China (Grant No. 30771157) and the Changjiang Scholars and Innovative Research Team in University (Grant No. IRT0453)

in recent years[17]. Plant polyploidization can cause DNA sequence mutations such as chromosome rearrangements and gene losses (refer to the reviews by Pontes et al.[18]and Udall and Wendel[19]), DNA modification such as methylation[20,21], and a large amount of genome-wide changes in gene expression (refer to the reviews by Adams[17] and Chen et al.[22])

1.1 Gene expression in allopolyploid plants

The nonadditive expression of allopolyploids at the RNA or protein levels was extensively documented in *Arabidopsis*[23,24], cotton[25], *Senecio*[26], *Brassica napus*[27] and wheat[28,29]. During evolution, nonadditive expression might result in the subfunctionalization[30] of the duplicate gene in different organs or tissues[31]. Those modulations in gene expression could arise with the onset of polyploidization or within several generations after polyploid formation, and continue over the evolutionary process[32,33]. Genes in many function categories were found to alter their expression patterns in different cases. The expression of 60 out of 3072 transcripts was reproducibly altered in the allotetraploid wheat: 48 transcripts disappeared and 12 were activated. The silenced/lost genes included rRNA genes and genes involved in other function categories. The activated genes with a known function were all retroelements[28]. However, in synthetic or natural *Arabidopsis* auto/allotetraploids, the genes with novel expression patterns encoded proteins with all kinds of functions and with clustering on chromosomes[24]. In another study with *Arabidopsis* allotetraploids, transposons were underrepresented in the genes that display expression changes in the allotetraploids[23]. However, the author suggested that some other reasons might cause this underrepresentation, such as that many transposons were not included in the annotated genes for microarray analysis. Alternatively, the effects of genomic shock[34] may be "settled" in the selfing progeny (S_5). The percentages of genes in the hormonal regulation and cell defense and aging categories were 150%－175% of those in the same categories classified using all annotated genes in *Arabidopsis*[23], suggesting that these genes are particularly susceptible to expression changes in response to the perturbation resulting from intergenomic interactions in the allotetraploids. Many genes involved in the ethylene biosynthesis pathway were repressed in one or two allotetraploids, which may induce expression changes in ethylene-responsive genes involved in a wide range of developmental processes and fitness responses, including seed germination, leaf and flower senescence, fruit ripening, programmed cell death, and biotic and abiotic stress responses[35].

1.2 Gene expression in the homologous ploidy series

Although gene expression of polyploids was actively studied and great progress has been made in this field[36,37], most of the studies were focused on allopolyploids. However, gene expression in allopolyploids is affected not only by ploidy effects but also by the merging between two different genomes. It is not easy to distinguish them from each other[22]. In the allopolyploids of *Senecio*[38], *Brassica napus*[27] and corn[39], the comparison studies between the hybrid and corresponding allopolyploid revealed that hybridization had a much greater effect on gene expression than genome doubling did, suggesting that much less sensitive loci of gene expression should exist in autopolyploids than in allopolyploids. This hypothesis was documented by gene expression in autopolyploids of yeast[11,12], *Arabidopsis*[23,24], potato[14] and cabbage[15]. In the yeast homologous ploidy series from haploid to tetraploid, only 17 genes were found to have different expression patterns. Of them, 10 were ploidy-induced and 7 were ploidy-repressed[11]. Ploidy-regulated genes had an unbiased distribution of locations in the yeast genome. They tended to have complex promoters; the average upstream intergenic space was longer than 1300 base pairs, whereas the genome-wide average intergenic space was 500 base pairs[11]. Recently, Storchová et al.[12] compared the transcriptome and protome between diploid and autotetraploid yeast. No locus with a distinct expression pattern was found. Compared with the diploid *Arabidopsis*, only 88 loci were revealed by gene chip technique to have a significant difference in gene expression of autotetraploids[23]. Using microarray, Stupar et al.[14] checked the transcriptomic variation of a synthetic autopolyploid series in potato (*Solanum phureja*) that includes one monoploid ($1x$) clone, two diploid ($2x$) clones, and one tetraploid ($4x$) clone. Among about 9000 genes designed in the cDNA microarray, statistically significant expression changes were observed among the ploidies for about 10% of the genes in both leaflet and root tip tissues. However, most changes were associated with the monoploid and were within the two-fold level (the most-common threshold used in microarray analysis). The co-altering genes in expression were limited.

Only a small portion of differentially expressed genes (about 1.4% of all genes on the array) overlapped in both leaflet and root tip (what expected at random was 1.11%)[14]. Gene ontology (GO) slim terms were assigned to all genes on the array for 27 molecular function and 27 structural component categories. In general, the ontology distribution of differentially expressed genes reflected the ontology distribution of the array for both molecular functions and structural components. However, there were some notable exceptions that some function ontologies such as for nucleic acid binding, structural molecule activity and cytosol and ribosomem, showed higher proportions of differentially expressed genes[14]. The proteomes, revealed by two-dimensional gel electrophoresis (2-DE) gels, also remained globally unchanged in a homologous ploidy series of haploid, diploid, and tetra- ploid *Brassica oleracea* cabbages[15]. Although some genes were proved to be critical to the survivals of auto- polyploids, they kept unchanged in expression during polyploidization[12]. Reviewing from the existing data on gene expression of different homologous ploidy series, only a little portion of the gene set was influenced by ploidy and the degree of influence was also limited. However, both the 4*x* and 1*x* plants were obviously different from its 2*x* parent in plant vigor, biomass and cell size[14]. Therefore, it induces us to speculate how close the relationship is between phenotype and gene expression in autopolyploids, if those ploidy-sensitive loci have a great contribution to phenotype variation, and what mechanism lies under those changes in gene expression.

2 Regulation mechanism in gene expression of haploid and polyploid

The growing amount of gene expression data in polyploidy research has stimulated scientists to speculate the regulation mechanisms driving those changes in gene expression[17,22,40]. However, to date, the experiment data to support their speculations are still very limited. From different points of view, different authors proposed their own explanations. Based on the relationship between gene expression and the chromosome or chromosome arm duplication, some scientists proposed a dosage effect or dosage compensation effect to understand ploidy effects in gene expression[13,41]. The cell size variation was also considered to cause the expression alteration in a yeast homologous ploidy series[11].

Among those speculations, DNA methylation and small RNA were relatively clearly documented to be involved in the ploidy regulation of gene expression. Evidence for the involvement of RNA-mediated gene regulation came from the synthesized wheat allopolyploids[42]. Reactivations of the transposons in the synthesized allopolyploids induced transcript readouts that were correlated with upregulation or downregulation of neighboring genes depending on whether the readout transcripts were in sense or anti-sense orientations[42]. Transcriptome analysis also indicated that there were a large amount of anti-sense and sense transcripts with unknown functions in *Arabidopsis*[43]. The role of DNA methylation in ploidy regulation of gene expression was first shown by *Arabidopsis* mutants. Hypermethylation of DNA cytosine has been shown to silence homeologs in *Arabidopsis* neopolyploids and in *A. suecica*. These experiments were done by using a methyltransferase inhibitor or by silencing a methyltransferase gene using RNA interference, causing reactivation of silenced genes[24,44]. Gene K7 in *Arabidopsis* allotetraploids was silenced. Further analysis revealed that CNG DNA methylation of K7 was less in allotetraploids than in the parents[45], suggesting that CNG DNA methylation might be the cause for K7 silencing. Isoschizomers were applied for detecting DNA methylation patterns in synthesized wheat allotetraploids. Transcript disappearance was associated with cytosine methylation alteration in the allotetraploid in 4 out of the 12 loci analyzed. Of those 4 loci, one altered its methylation pattern only after chromosome doubling, while the other 3 occurred in the F_1 hybrid as well as after chromosome doubling[28], suggesting both ploidy effects and distant hybridization could cause the alteration of DNA methylation pattern and then, influence gene expression. Another experiment further elucidated the role of genome merging in allopolyploid for DNA methylation and gene expression. Transcription factors TCP3 were expressed in natural autopolyploid *Cardaminopsis arenosa* but silenced in natural allopolyploid *A. thaliana*. Isoschizomers analysis showed that TCP3 was more heavily methylated in *A. thaliana* than in *C. arenosa*. After treating *A. thaliana* with aza-dC, *TCP3* was reactivated[44], suggesting genome merging results in DNA methylation and gene silencing. DNA methylation regulation for gene expression is associated with the chromatin structure. The hypermethylation can result in the contraction of chromatin, and the corresponding genes on it will become more

inactive. In fact, the methylation level is higher in heterochromatin than in euchromatin, and the gene transcription is also more active in heterochromatin[46].

3 The problems in the studies on ploidy effects of gene expression in plant

3.1 Limitations on research materials

According to the genome difference, polyploid can be classified as autopolyploidy (the doubling of a single genome) and allopolyploidy (the merger of two fully differentiated genomes). Both of them are common in nature[47] and are the mechanism for the formation of new plant species[48]. However, most of the studies on gene expression in plant polyploids were focused on allopolyploidy, which might result in the inaccuracy for understanding ploidy effect in gene expression. For example, because only a partial gene set overlapped between auto- and allotetraploid, Wang et al.[24] deduced that there should be different regulation mechanism in gene expression between auto- and allopolyploid. By comparison between diploid hybrid and corresponding allopolyploid, we can partially exclude the effect of genome merging on gene expression (e.g. in *Brassica napus* allopolyploid[8]). However, any phenotype in allopolyploid is seldom likely to be the simple sum of interspecific hybridization and genome doubling[23]. Therefore, a simple subtraction is not enough to exclude the interaction between them. Galitsk et al.[11] thought that, in the whole-genome expression analysis, one could parse the effects of ploidy effects from those of mating type by constructing isogenic sets of yeast strains that vary only in their ploidy. Guo et al.[13] also considered that only the homologous ploidy series with uniform genetic backgrounds could be used to precisely estimate ploidy effects in gene expression of corn polyploids.

In the previous studies on the ploidy effects in gene expression, most of them employed the synthesized haploids and polyploids while the natural polyploids were seldom used, except some recent polyploids such as *Spartina*[49], *Tragopogon*[50] and *Senecio*[38,51]. Most of the other polyploids have evolved for thousands of years, experienced several rounds of hybridization and genome doubling events and had ambiguous origin time and parent. It is very difficult to make a precise comparison between natural polyploid and its diploid parent[52]. Synthesized polyploid can perfectly make up this disadvantage because we clearly know its origin time and parents. However, the result from synthesized polyploid might be different from the natural one because of their different origin modes. For example, there were different loci with the altered expression pattern between synthesized and natural yeast polyploids[24], suggesting a different regulation mechanism between them. Furthermore, both colchicine and physical rays, which were widely used in polyploidy induction, tissue culture and plant regeneration, might introduce mutations into plant genome. Those mutations might modify the DNA structure, influence gene expression and eventually interference the accuracy of study on ploidy effects. For example, tissue culture can activate retrotransposon *Tos17*, and change the methylation status of its flank regions[53], DNA sequence and gene expression[54].

In addition, plants with odd ploidy were also rarely reported. However, *Arabidopsis* transgene was silenced in diploid and autotetraploid but reactivated in triploid[55]. Difference in gene expression also occurred between corn plants with odd and even ploidy[13]. Those cases suggested different mechanisms for gene expression and regulation in plants with odd and even ploidy.

3.2 Limitations on research methods and contents

Northern blotting, RT-PCT etc. were firstly applied to investigate gene expression in haploid and polyploid (e.g. in corn[13]). However, those methods can only work with a small portion of loci in genome. In recent years, gene expression arrays were widely used to analyze the transcriptomes of haploid and polyploid. The most outstanding feature for microarray is its potential to screen thousands of loci upon genome. Nevertheless, there are still some limitations for microarray. For example, the design of microarray heavily depends on the known genome sequence. However, most of the plant genomes are not sequenced to date. All the probes were derived from the known or predicted expression sequences, which might result in a poor comprehensiveness. In addition, the accuracy in expression microarray can be influenced by PCR amplification, factors to interference with hybridization and base compositions in targeting sequence[56]. The experimental data are still very limited for gene expression ploidy regulation mechanisms such as DNA methylation and small RNA. Most of them only involved several loci on genome. Therefore, those data are not enough to fully understand the relationships between gene expression and DNA methylation/small

RNA at the genome level. However, the whole genome is involved during ploidy shift, which requires us to understand ploidy effects also at the whole genome level.

4 Prospects

4.1 The homologous ploidy series derived from twin-seedling is the ideal materials to study ploidy effects

Twin-seedling is common in nature. We created a homologous ploidy series with the same rice twin-seedling line[16,57]. SSR analysis showed that those haploids and polyploids have a uniform genetic background. Thus, they are an ideal system for the study on ploidy effects and have unique merits as follows. First, the ploidy series is spontaneously originated under natural conditions and thus, can partially imitate the natural ones. Second, the definition of diploid parent can facilitate the comparative study on ploidy effects, avoiding the disadvantages that no definite parent can be acquired in natural haploids and polyploids. Third, compared with their diploid parents, there is no base sequence mutation[16,57], avoiding its interference with the study on ploidy effects. The uniform base sequence among the ploidy series is due to the features of twin-seedling itself. The origination of haploid and polyploid is a mild process, which is free of inducing treatment or tissue culture and thus, avoids artificial introduction of mutations. After origination, the haploids and polyploids do not experience any meiotic or gametophytic developmental stages, and are not subject to selective forces. Therefore the pressure on genome is little to keep it from base sequence mutation[14]. The last reason is that the newly formed polyploid, such as *Brassica napus*[21], might be lack of genetic variation. Fourth, both the haploids and polyploids are derived from the same genetically homologous diploid line. Therefore, they formed a ploidy series, facilitating us to precisely analyze the change trends of ploidy effects with the ploidy increasing. Fifth, the ploidy series are in M_0 generation and thus, we can directly know what happened immediately after formation of haploid and polyploid, which is very important for understanding the establishment, adaptation and persistence of new polyploidy species[8]. Sixth, if the twin-seedling has a homologous genotype, we can create a homologous ploidy series, which can be used to directly know about ploidy effects. Seventh, odd ploidy plants, which are difficult to be synthesized, often emerge from twin-seedlings[16,57].

4.2 The potentials of throughput sequencing techniques for the study on transcriptome and its regulation of haploid and polyploid

Combining the methods of methylcytosine immunoprecipitation (mCIP) and single-chip tiling microarray, Zhang et al.[46] and Zilberman et al.[58] reported the first comprehensive DNA methylation map of an entire genome and analyzed the relationship between genome DNA methylation and transcriptome. The methods they used can also be applied in the study on transcriptome and its regulation of haploid and polyploid. However, because of the disadvantages with microarray, we recommended throughput sequencing to substitute for gene chip hybridization. A hundred million of DNA short fragments can be simultaneously detected with throughput sequencing technique. Furthermore, compared to microarray, there is no need to know the genome sequence in advance, and it is also free of interfering factors as in microarray analysis[56], ensuring the accuracy of analysis. Two high-throughput sequencing techniques are currently available: a high-throughput pyrosequencing approach developed by 454 Life Sciences[59] and a fluorescent nucleotide-based system developed by Solexa[60]. The 454 system can produce 400000 reads of over 100 bases per run. The Solexa system can produce 40 million reads of about 25－35 bases. Most ~30-mers can be unambiguously aligned to a reference genome sequence[61]. Therefore, the Solexa system has been successfully used to analyze posttranslational histone modifications in the human genome[62]. Furthermore, the Solexa technique platform can be commercially available. Therefore, we think that Solexa system is a better choice for the studies on the transcriptome, genome DNA methylation and small RNA of haploid and polyploid.

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