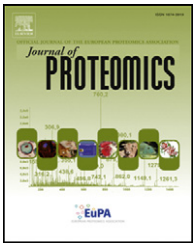
JOURNAL OF PROTEOMICS 112 (2015) 274–284

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



Comparative proteomic study on *Brassica* hexaploid and its parents provides new insights into the effects of polyploidization

Yanyue Shen[a], Yu Zhang[a], Jun Zou[b], Jinling Meng[b], Jianbo Wang[a,*]

[a]State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan 430072, China
[b]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 5 July 2014
Accepted 10 October 2014
Available online 18 October 2014

Keywords:
Polyploid
Proteome
iTRAQ
Gene expression
Expression level dominance
Brassica

ABSTRACT

Polyploidy has played an important role in promoting plant evolution through genomic merging and doubling. Although genomic and transcriptomic changes have been observed in polyploids, the effects of polyploidization on proteomic divergence are poorly understood. In this study, we reported quantitative analysis of proteomic changes in leaves of *Brassica* hexaploid and its parents using isobaric tags for relative and absolute quantitation (iTRAQ) coupled with mass spectrometry. A total of 2044 reproducible proteins were quantified by at least two unique peptides. We detected 452 proteins differentially expressed between *Brassica* hexaploid and its parents, and 100 proteins were non-additively expressed in *Brassica* hexaploid, which suggested a trend of non-additive protein regulation following genomic merger and doubling. Functional categories of cellular component biogenesis, immune system process, and response to stimulus, were significantly enriched in non-additive proteins, probably providing a driving force for variation and adaptation in allopolyploids. In particular, majority of the total 452 differentially expressed proteins showed expression level dominance of one parental expression, and there was an expression level dominance bias toward the tetraploid progenitor. In addition, the percentage of differentially expressed proteins that matched previously reported differentially genes were relatively low.

Biological significance
This study aimed to get new insights into the effects of polyploidization on proteomic divergence. Using iTRAQ LC–MS/MS technology, we identified 452 differentially expressed proteins between allopolyploid and its parents which involved in response to stimulus, multi-organism process, and immune system process, much more than previous studies using 2-DE coupled with mass spectrometry technology. Therefore, our manuscript represents the most comprehensive analysis of protein profiles in allopolyploid and its parents, which will lead to a better understanding of novelty and plasticity of the allopolyploid genomes.

* *Corresponding author*. Tel./fax: +86 27 68752213.
E-mail address: jbwang@whu.edu.cn (J. Wang).

http://dx.doi.org/10.1016/j.jprot.2014.10.006
1874-3919/

1. Introduction

Polyploidy is a wide spread and prominent process of angiosperm evolution through genomic merging and doubling. An autopolyploid combines multiple sets of the same genomes in their nucleus, including potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), and sugarcane (*Saccharum officinarum*), while an allopolyploid contains two or more different genomes, followed by chromosome doubling or fusion of unreduced gametes between species, such as wheat (*Triticum aestivum*), *Tragopogon mirus*, cotton (*Gossypium hirsutum*), *Arabidopsis suecica* and oilseed rape (*Brassica napus*) [1]. Heterozygosity and inter-genomic interactions contribute to phenotypic variation and growth vigor in allopolyploids [2,3], and they often grow more vigorously and have better fitness and superior traits than their progenitors, which have an advantage of natural selection and crop domestication [4].

In plants, it has been reported that allopolyploids undergo changes at the genetic level. These changes included DNA sequence elimination [5–7], chromosomal rearrangements [8], and transposon activation [9,10]. Alterations in gene expression upon allopolyploidization have also been found in cotton allopolyploids [11], *Tragopogon* allotetraploids [12], *Arabidopsis* allotetraploids [13], wheat allohexaploids [14] and *Brassica* allotetraploids [15]. Although genomic and transcriptomic changes have contributed greatly to our understanding of novelty and plasticity of the allopolyploid genomes, changes on the mRNA level do not necessarily correlate with changes on the protein level. Thus, studies are needed to investigate differential proteomes between polyploids and their parents, and determine their functional relations to polyploidization.

Proteomics is an important complement to genomics and transcriptomics because biological function is chiefly carried out by proteins and determination of protein abundance is mandatory to fully understand the function of a system. Indeed, the relationship between RNA transcripts and protein abundance is not direct because of post transcriptional regulation and post-translational modifications, which makes difficult to predict about the protein expression of polyploidy relative to their parents. Therefore, the application of proteomic approached to polyploidy systems will enhance our understanding of polyploidy evolution and adaptation. Up to now, only a few studies have examined proteomic changes in allopolyploids compared with their progenitors. These include studies on *G. hirsutum*[16], *A. suecica*[17], *B. napus*[18], and *T. mirus*[19]. However, most of these studies employed two-dimensional electrophoresis (2-DE) coupled with mass spectrometry, which has a limited power because of gel to gel variation and problems with protein quantification based on spot intensity. Isobaric tags for relative and absolute quantification (iTRAQ) is a suitable technology for detecting proteomic changes in allopolyploids due to the simultaneous identification of many more proteins and the high sensitivity for measuring proteomic changes in related species when coupled with MS analysis [20,21]. Therefore, the use of iTRAQ-based proteomic analysis in allopolyploids and their progenitors will provide valuable new insights into the effects of polyploidization at the proteome level [19].

Synthesized polyploids provide an attractive model system for studying the effects of polyploidization because the exact progenitors for the synthetic polyploids are known. The genus *Brassica* is commonly used as a model system to track early genomic and transcriptomic changes following polyploidization, including the synthesized *Brassica* allohexaploid generated by crossing *Brassica carinata* with *Brassica rapa* and chromosome doubling [22–25]. This synthesized *Brassica* hexaploid does not exist in nature, and its genome shows a higher level of heterozygosity and redundancy than natural tetraploids. Moreover, valuable traits such as drought and disease resistance from two cultivated species, *B. carinata* and *B. rapa*, are combined in the hexaploid itself, and new transgressive traits might be created and selected by breeding. Here, we investigated the effects of polyploidization on the proteomes of *Brassica* hexaploid using iTRAQ LC–MS/MS technology. Our study provide a general survey of protein regulation in response to changes in genome divergence (*B. rapa* vs *B. carinata*), genome polyploidization (*Brassica* hexaploid relative to its parents), and biological consequences of proteomic changes in these comparisons were predicted. In addition, we cataloged differences in the abundance of proteins and mRNAs by integrated high-resolution quantitative iTRAQ proteomics and profiling of gene expression using a previously reported RNA-seq data [23].

2. Materials and methods

2.1. Plant materials

Plant materials included *B. carinata* (BBCC, 234), *B. rapa* (AA, 2n = 20) and the fifth generation synthesized *Brassica* hexaploid (BBCCAA, 2n = 54). They were grown in soil at Hubei Academy of Agricultural Science, China. *Brassica* hexaploid used in this study were previously described by Tian et al. [22]. They were generated by crossing *B. carinata* (female parent) with *B. rapa* (male parent) and chromosome doubling, and were confirmed to be euploids (2n = 54) through chromosome identification. Leaves from three-month-old plants of *Brassica* hexaploid and its parents were collected for further use.

2.2. Protein extraction

Total proteins were isolated from young leaves of *B. rapa*, *B. carinata* and *Brassica* hexaploid separately, and each of them was composed of a pool of protein collected from several plants. To prepare peptides for iTRAQ, young leaves (3 g) of multiple plants from *B. rapa*, *B. carinata* and *Brassica* hexaploid, were ground into fine powder in liquid nitrogen. Two biological replicates were prepared for each sample. Samples were then resuspended in a lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris–HCl, pH 8.5) containing 1 mM PMSF and 2 mM EDTA (final concentration). After 5 min of vigorous vortex, 10 mM dithiothreitol (DTT) (final concentration) was added to the samples. The suspension was sonicated at 200 W for 15 min and then centrifuged at 4 °C, 30,000 *g* for 15 min. The supernatant was mixed well with chilled acetone (1:4, v/v) containing 10% (v/v) TCA and incubated at −20 °C overnight. The proteins in the supernatant were kept at −80 °C for further analysis.