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Characterization of interspecific hybrids between flowering Chinese cabbage and broccoli

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ABSTRACT

sInterspecific hybridization is widely observed within diverse eukaryotic taxa and is considered an important driver of genome evolution. We selected one flowering Chinese cabbage variety and one broccoli variety for hybridization. Heterologous haploid offspring were obtained by embryo rescue, and heterologous diploids were obtained by colchicine-induced chromosomal doubling. The field traits and simple sequence repeat markers of 124 F2 plants were investigated. We also analyzed the nutritional components of the parental and 10 progeny F2 plants. The parental traits were separated in the hybrids, with traits tending to shift from those of flowering Chinese cabbage to those of broccoli, and trait values showed normal distributions. Simple sequence repeat patterns varied, with the number of missing bands being significantly greater than that of novel bands in hybrids. Additionally, there were higher levels of some nutritional components in the hybrids compared with in the parents. Thus, the phenotypes of the early formed allopolyploids were unstable and accompanied by dramatic changes in the genome. Hybrids showed new traits and high levels of nutritional components. This study not only increased the genetic resources available for flowering Chinese cabbage but also laid a theoretical foundation for exploring trait segregation in early formed allopolyploids.

1. Introduction

The Brassica vegetable flowering Chinese cabbage (*Brassica rapa* L. ssp. *chinensis* var. *utilis* Tsen. et Lee) is a variant, in which the bud is the edible portion, of a subspecies of *B. rapa* that originated from China and is still cultivated widely in Guangdong, China (Li et al., 2011). The stalk is tender and is known for its pleasant taste. Its growth period is short, and the multiple cropping index is high, with only 40–56 d from germination to flowering and 80–90 d to seed maturation(Peng et al., 2015). Broccoli (*Brassica oleracea* L. var. italica), a variant, in which the bud as the edible portion, of a subspecies of *B. oleracea*. It has high contents of organosulfur compounds that increase the activity levels of enzymes involved in the detoxification of carcinogens and other foreign compounds(Chaudhary et al., 2014).The inflorescences of broccoli are consumed worldwide and are recognized for their anticarcinogenic properties(Bachiega et al., 2016).

However, flowering Chinese cabbage is mainly produced by long-term breeding from easy bolting materials of Chinese cabbage vegetables, resulting in the genetic resources having a narrow background. Additionally, promotional varieties cannot meet market demand in terms of resistance and quality (Zhang and Liu, 2010; Yan, 2014). Distant hybridization can synthesize parental advantages or create heterosis, promoting intergenic exchanges and the formation of new species(Cicin, 1954).The use of interspecific hybridization can enrich the genetic back ground and the cultivation of types with ideal agronomical traits. Distant hybridization is an important manner of creating genetic improvements(Whitney et al., 2010; Chen et al., 2018). In addition, based on the "U-triangle" (1935) theory and Brassica genome sequencing data, *Brassica napus* was generated from a cross between *B.rapa* and *B.oleracea* approximately ~7500 years ago(Chalhoub et al., 2014). Broccoli and Chinese cabbage are subspecies of *B. rapa* and *B. oleracea*, respectively. Hybridization between the two species readily produces offspring. Broccoli's high nutritional qualities also make it a good male parent.

Hybridization between flowering Chinese cabbage and broccoli could result in heteropolyploids. Early formed *B. napus* has abundant variability in many characteristics, such as flower size, flowering time, waxy layer characteristics and leaf shape and size (Gaeta et al., 2007; He et al., 2017). However, stabilizing target traits takes a long time, making it difficult to develop new cultivars for field application. Thus,

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this presents the main obstacle in using artificial heteropolyploid species(Mestiri et al., 2010; Tian et al., 2010). In addition, the mutation and disappearance of large numbers of repetitive DNA sequences are very common among synthetic polyploid species (Tang et al., 2008; Zhang et al., 2016). Distant hybridization and polyploidization are important factors in inducing tandem repeat variation and in promoting sequence evolution (Tang et al., 2009). Therefore, exploring the trait and sequence variation of selfing offspring of synthetic *B. napus* can help us understand the mechanism of phenotypic stability in allopolyploids and provides important genetic resources for the breeding of flowering Chinese cabbage.

There have been few reports on the hybridization between flowering Chinese cabbage and broccoli(Zhang et al., 2004; Gaeta et al., 2007; Karim et al., 2014; Zhang et al., 2016; Shen et al., 2017). Here, one flowering Chinese cabbage variety and one broccoli variety were selected for hybridization. Heterologous haploids were obtained by embryo rescue, and the heterologous diploids were obtained by colchicine-induced chromosomal doubling. The offspring could serve as new vegetable-type rapeseed materials that combine the advantages of the two species. In addition, the field traits and simple sequence repeat (SSR) markers of 124 F2 plants were investigated, and the nutritional components of parental and 10 F2 plants were analyzed. This research laid a theoretical foundation for exploring trait separation in the allopolyploid polyploidization process.

2. Materials and methods

2.1. Plant materials

The commercial variety of flowering Chinese cabbage, Sijiuhuangcaixin, was the female parent, and the commercial variety of broccoli, Zhongqingerhao, was the male parent. The plant materials were provided by the Cabbage Laboratory of the Institute of Vegetable and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China.

2.2. Embryo rescue and colchicine-induced doubling

We planted parental materials in glasshouses. The ovaries of plants at 12–14 d after pollination were treated with 75% ethanol for 30 s, sterilized with 7% NaClO for 15 min, and finally washed with sterile water three times. The ovules in the ovary were stripped with tweezers and inoculated into MS medium. The inoculated ovules were cultured at 25 ± 2 °C with 10 h light d^{-1} at an intensity of 2000 lx for 35–55 d (Sharma et al., 1996; Ayotte et al., 1987). The seedlings of the live ovules were grown until four or five leaves appeared after differentiation and rooting. Then, the roots of seedlings were soaked in 2% colchicine for 48 h to obtain heterogeneous polyploids before they were planted in a greenhouse (Zhao et al., 1996).

2.3. Determining ploidy and characteristics

The root tips of parents and hybrids were cut, incubated in saturated *p*-dichlorobenzene for 2.5 h at room temperature, and then fixed in Carnoy's solution (methanol: acetic acid = 3:1) for 24–48 h. They were stored in 70% ethanol at 4°C. From the root, 1–2 mm of tip was cut and placed in a 0.075-mol/L KCl hypotonic solution for 30 min and then rinsed with distilled water three times. Tips were digested with a mixture of 2.5% cellulase and 2.5% pectinase at 37 °C for 55 min. The enzyme solution was removed by washing with distilled water at 4 °C for 30 min. Finally, the root tip was placed on a slide, the fixative as added prior to flame drying, and the sample was observed under a phase-contrast microscope. Slides showing chromosomal dispersion and dissociation were stained with DAPI and photographed under a fluorescence microscope(Lou et al., 2017).

The morphological traits recorded included plant height, leaf length and width, flower color, flowering time, flower size, stalk diameter, pistil size, petiole length, number of branches, leaf color and leaf margin(Gaeta et al., 2007).

2.4. Genomic DNA isolation and PCR analysis

Genomic DNA was isolated from leaves of the parents and 124 F2 hybrids using a modified CTAB method(Kidwell and Osborn, 1992). Sixteen SSR markers were randomly chosen from the Brassica Database (Supplementary Table 1) and used for PCR amplification. The PCR primers were purchased from Qingke Co, Beijing, China. The amplifications were performed in 5-μl volumes containing 4–8 ng of template DNA, 0.4 μl of forward and reverse primers (0.2 μl each), 2.5 μl 2× super Taq mix, and water to make up the volume. The thermal cycling conditions were 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; and, finally, 72 °C for 10 min. The PCR products were separated on an 8% polyacrylamide gel by electrophoresis and visualized with silver staining (Zhang et al., 2016).

2.5. Nutrient measurement

Nutrient elements were measured from parents and 10 representative individual plants. The sampling sites were the edible parts: flower buds and young stems. The nutrient elements included protein, dry matter, sugar, cellulose, Ca, Cu, Mn, P and Zn. The experiment was performed at the Vegetable Quality Supervision and Testing Center of the Ministry of Agriculture, Beijing, China.

3. Results

3.1. Obtaining hybrid offspring

Haploid (AC) hybridization between flowering Chinese cabbage and broccoli was performed by embryo rescue. Then, allopolyploids (AACC) were obtained by colchicine-induced chromosomal doubling. All heterogeneous polyploids were identified by the number of apical chromosomes, with 10 hybrids each having 38 chromosomes (Fig. 3b). Seeds were collected by allopolyploid (AACC) bud self-pollination. The 124 F2 and 6 parental (three each) plants were grown in the greenhouse of the Chinese Academy of Agricultural Sciences' Institute of Vegetables and Flowers (Beijing, China).

3.2. Morphological characteristics

The comprehensive performance of hybrids was between that of the two parents (Fig. 1a).The averages of most F2 traits were between those of the parents (Fig. 2a). However, the hybrids showed a separation of characteristics (Fig. 1).The wax powder content gradually increased (Fig. 1b). Offspring had single branches, two branches or multiple branches. Some offspring had stunted growth (Fig. 1c). The colors of leaves and buds gradual changed from yellow to dark green (Fig. 1d).The edges of the leaves gradual progressed from a blunt to a complex saw pattern (Supplementary Table 2).Thus, traits tended to shift from those of the flowering Chinese cabbage to those of broccoli. In addition, hybrids showed new traits, such as yellow leaves and buds. The values of flowering time, plant height, upsetting, flower size, pistil size, leaf size and petiole length varied among the 124 F2 plants. All trait values were divided into 10 levels, and the number of plants belonging to each level was determined. The trait values showed a normal distribution (Fig. 2b). A small number of individual plants were highly variable.

3.3. Molecular characterization

Sixteen SSR primer pairs (Supplementary Table 1) were used to detect the sequence variation of hybrids and parents. Novel bands existed in the hybrids, while some parental bands were missing