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Characterization of Interspecific Hybrids between Flowering Chinese Cabbage and Chinese Kale

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Abstract: Interspecific hybridization is considered to be an important driving force in the evolution, diversification, and formation of plant species. We selected one flowering Chinese cabbage variety and three Chinese kale varieties to make hybrids. Heterologous haploid offspring were obtained by embryo rescue and heterologous diploids were obtained by colchicine doubling. A total of 108 individuals of the F_2, F_3 and F_4 generations from three parental combinations were investigated for field traits and SSR (simple sequence repeats) markers. The results showed trait separation and the appearance and disappearance of SSR bands in the hybrids, showing significant differences among parental combinations and among the different generations. This proved that the phenotypes of the initial generations of allopolyploids were not stable. This study not only enriches the genetic resources available for breeding flowering Chinese cabbage and Chinese kale, but lays a theoretical foundation for exploring the segregation of traits in distant hybrids and in different generations.

Keywords: flowering Chinese cabbage; Chinese kale; synthetic *Brassica napus*; distant hybridization; trait separation

1. Introduction

Flowering Chinese cabbage (*Brassica rapa* var. parachinensis), a vegetable of the Brassicaceae family of Chinese origin, is a variant of Chinese cabbage that is still cultivated widely in southern China. The stalk is tender, easy to cook, and has a pleasant taste. Its growth period is short and the multiple cropping index is high, with only 40–56 days from germination to flowering and 80–90 days to seed maturation [1]. A related Brassica, Chinese kale (*Brassica oleracea* var. *alboglabra*), is widely distributed in southern China and southeast Asia [2]. Chinese kale is usually grown for its bolting stems as the most common edible part because they have a tender and crisp texture and good flavor. Chinese kale also has high nutritional value because of its high content of major antioxidants and putative anticarcinogenic compounds, including vitamin C, carotenoids, and glucosinolates [3,4].

Breeders have long used systematic breeding methods to breed flowering Chinese cabbage varieties, resulting in a narrow background of genetic resources [5–7]. In addition, the commercial production of Chinese kale mainly uses a conventionally-bred variety, and there are problems such as low yield, poor quality, and serious diseases and insect pests [8]. The above problems have caused the existing varieties to fail to meet market demand. Hybridization between different species, intergeneric crosses, and even distantly-related species can be used to combine different species or genus characteristics, to break through species boundaries, and to expand genetic variation to create new types of variation or new species [9–11]. The Japanese scholar, U Nagahara, first confirmed the origin of *B. napus* and proposed the "triangle of U", which was a theory of the evolution of several species of Brassica and their phylogenetic relationships. With the development of DNA sequencing

technology, it is now generally believed that *B. napus* was formed by hybridization and genome doubling of *B. rapa* (genome AA) and *B. oleracea* (CC) approximately 7500 years ago [12]. Flowering Chinese cabbage and Chinese kale are subspecies of *B. rapa* and *B. oleracea*, respectively. Hybridization between the two species readily produces offspring.

The hybridization of flowering Chinese cabbage and Chinese kale to form *B. napus* is a process of allopolyploidization. Synthetic allopolyploids show some abnormal and unstable mutations in cytology and phenotype, such as flowering time, plant height, and stalk diameter [13,14]. Such variation enriches the genetic background while also causing some drawbacks: in particular, stabilizing target traits takes a long time. This becomes the major obstacle in making use of artificial allopolyploid species and makes it difficult to develop new cultivars for field application [15,16].

Microsatellite sequences are highly susceptible to mutation and can be generated when a large number of mutations are induced by interspecific hybridization or polyploidization. The generation, deletion, and mutation of tandem repeats can change the structure of the genome, but such mutations can alleviate the genomic oscillation caused by polyploidization and be conducive to maintaining the stability and inheritance of the new polyploid genome [17–19].

Therefore, exploring the trait and sequence variation of offspring from selfing synthetic *B. napus* can help us understand the mechanism of phenotypic stability in allopolyploids while also generating important genetic resources for the breeding of flowering Chinese cabbage and Chinese kale.

The hybridization between flowering Chinese cabbage and Chinese kale has rarely been studied [13,18,20–22]. In the present study, one flowering Chinese cabbage and three Chinese kale varieties were selected for hybridization. Heterologous haploids were obtained by embryo rescue, and heterologous diploids were obtained by colchicine treatment. The offspring not only represent rich genetic resources, but might also serve as new vegetable-type rapeseed material that combines the advantages of the two species. Three generations of plants were investigated by analyzing field traits and SSR markers, thereby laying a theoretical foundation for exploring trait separation in the allopolyploidization process.

2. Materials and Methods

2.1. Plant Materials

Plant materials were provided by the Department of Chinese Cabbage, Institute of Vegetable and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China. We used the flowering Chinese cabbage inbred line 755, which is a multi-year breeding material, and three Chinese kale varieties, 719, 722, and 723 (Table 1).

Table 1. Flowering Chinese cabbage and Chinese kale parental materials.

Species	Genome Type	Code	Material Original	Name
Flowering Chinese cabbage	AA	755	Inbred line	flowering Chinese cabbage A
Chinese kale	CC	719	Inbred line	Cujie
Chinese kale	CC	722	Commercial variety	Sijicutiao Chinese kale
Chinese kale	CC	723	Commercial variety	Xianggangbaihuatian Chinese kale

2.2. Methods

2.2.1. Embryo Rescue and Colchicine Doubling

The four parental materials were grown in glasshouses. At the flowering stage, three crosses were pollinated separately: z1, 755 × 719; z2, 755 × 722; z3, 755 × 723. The flowering Chinese cabbage was the female parent, while the Chinese kale was the male parent. At 10–12 days after pollination, several ovaries from each combination were first treated with 75% absolute ethanol for 1 min, then with 8% NaClO for 15 min, and finally rinsed three times with sterile water. The ovules in the ovary were stripped with forceps and then inoculated into B5 medium. The inoculated ovules were cultured for

30–50 days at 25 ± 2 °C with illumination intensity of 2000 lx for 10 h day^{-1} [23,24]. When viable ovules had differentiated, rooted and developed into 5–6-leaf seedlings, heterogenous polyploids were obtained by treating the root of the seedlings with 5% colchicine for 24 h. The treated seedlings were then washed with water and planted in a glasshouse [25].

2.2.2. Ploidy Identification and Assessment of Plant Characteristics

Plant genome doubling after colchicine treatment was identified by flow cytometry (BD FACSCalibur™, BD Biosciences, San Jose, CA, USA). We took the young leaves of plants to do the experiment. The DNA content was estimated according to the protocol of Dolezel et al. [26]. The flowering Chinese cabbage parental DNA content (2C) was set as a reference. Its G1 peak was positioned on the abscissa (channel 190) by adjusting the instrument gain settings. The coefficient of variation of all samples was below 5% [27]. The successfully doubled plants were propagated to produce F_2, F_3, and F_4 generations by self-breeding. A total of 108 plants (12 plants for each genetic combination and generation) were planted in the field at the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. The planting date was 20 January 2017; the harvest date was 20 February 2017; the spacing within and between rows was 15 cm and 20 cm, respectively; and the number of plants per plot was 12. The field traits of flower color, flowering time, plant height, and stalk diameter were evaluated.

2.2.3. Genomic DNA Isolation and Molecular Analysis

Genomic DNA was isolated from the leaves of the parents and F_2, F_3, and F_4 hybrid plants using a modified CTAB method [28]. Nineteen SSR markers were randomly chosen from the Brassica Database [18] (Table S1) and used for PCR (Qingke, Beijing, China) amplification of genomic DNA. The PCR primers were purchased from Qingke Co., Beijing, China. The total volume of the reaction system was 5 µL. The PCR reaction mixtures contained: 1 µL of template DNA, 0.4 µL of forward and reverse primers (0.2 µL each), 2.5 µL 2 × super Taq mix (Qingke, Beijing, China), and 1.1 µL of water. The conditions for PCR amplification were 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; and finally 72 °C for 10 min. PCR products were detected by 8% non-denaturing polyacrylamide gel electrophoresis [18].

3. Results

3.1. Generation of Hybrid Offspring

The three combinations (z1, 755 × 719; z2, 755 × 722; z3, 755 × 723) produced respectively 70, 52, and 29 heterologous diploids after embryo rescue and 6, 7, and 8 allotetraploids after colchicine treatment (Table 2). Heterologous diploids were easily obtained in all three combinations. Heterogenous polyploids were identified by flow cytometry. The major peak of DNA fluorescence intensity for flowering Chinese cabbage was set at 190; the peak for Chinese kale was detected at 245, the peak for the heterologous diploids at 220, which was the average of the parents, and the peak for the allotetraploids at 435, which was twice the value of the heterologous diploids (Figure 1).

Table 2. Results of interspecific hybrids between flowering Chinese cabbage and Chinese kale.

Code	Cross Combination	Ovule Culture Number	Obtain Seedlings Number	Rate of Obtaining the Hybrids (%)	Colchicine Treatment Number	Doubled Number	Rate of Double (%)
z1	755 × 719	190	70	0.37	15	6	0.40
z2	755 × 722	140	52	0.37	15	7	0.47
z3	755 × 723	80	29	0.36	15	8	0.53

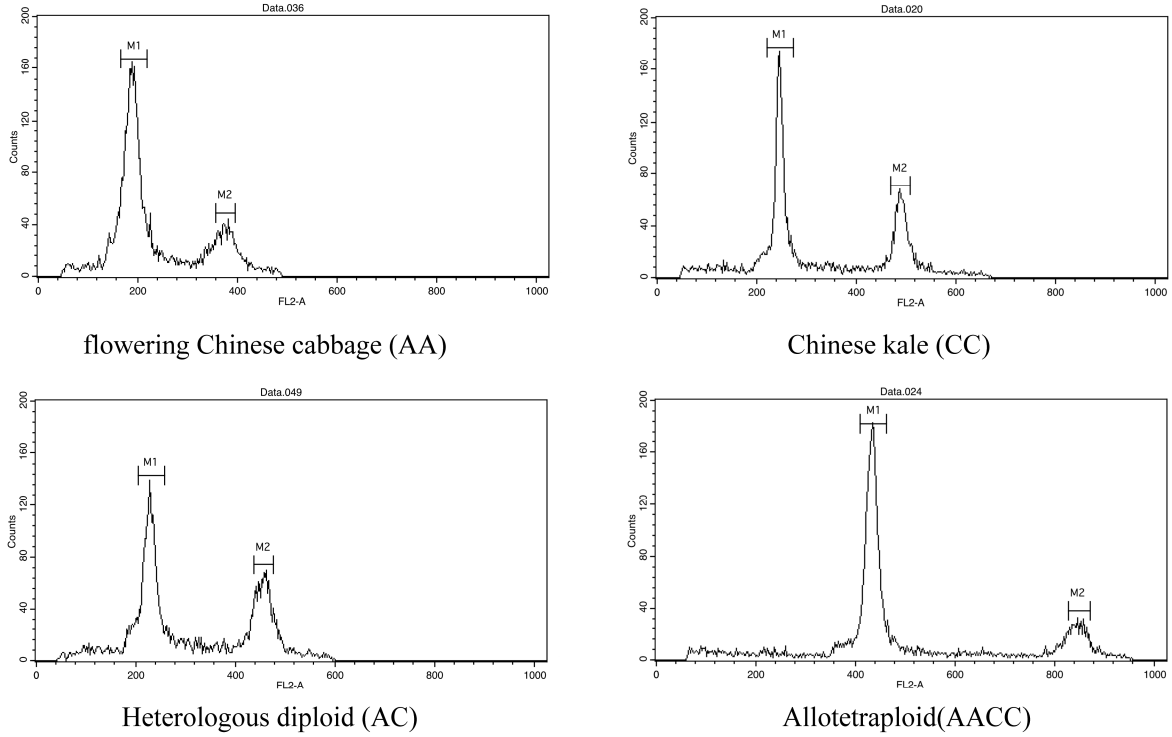

Figure 1. Ploidy identification by flow cytometry. The fluorescence signal represents nuclear DNA content; the peaks indicate interphase nuclei. Flowering Chinese cabbage (genome AA) was set to 190 as reference.

3.2. Morphological Characteristics

All hybrids had white flowers, indicating that white flowers were dominant over yellow flowers (Figure 2c), while the field performance of the hybrids was intermediate between that of the two parents (Figure 2a). However, the hybrids showed separation of characteristics in each combination: the leaf color and stem color of the offspring varied from the color of flowering Chinese cabbage to that of Chinese kale (Figure 2b). Comparing the edible part of the stalk, hybrids with wax-free surface powder were similar to flowering Chinese cabbage, while those with wax-containing powder were more similar to Chinese kale (Figure 2d). In addition, the values of flowering time, plant height, and stalk diameter varied among the 12 plants in each combination and generation. We used ANOVA to determine significant differences in the three traits among the hybrid combinations and among generations F_2 to F_4 (Figure 3). For plant height, the differences among all the three generations were significant (Figure 3a); for stalk diameter, F_3 differed significantly from F_2 and F_4; for flowering time, F_4 differed significantly from F_2 and F_3. The newly synthesized *B. napus* combinations had an unstable shape in the early generations, and there were differences in different traits among generations. For all three traits, z1 differed significantly from z2 and z3 (Figure 3b). The conclusions were supported through significant differences in data for three traits of 108 plants. Each generation (F_2, F_3, F_4) had 36 plants of data to support the analysis, and each combination ($_Z1$, $_Z2$, $_Z3$) was the same. These results showed that the performance of hybrid progeny depended on the respective Chinese kale parents.

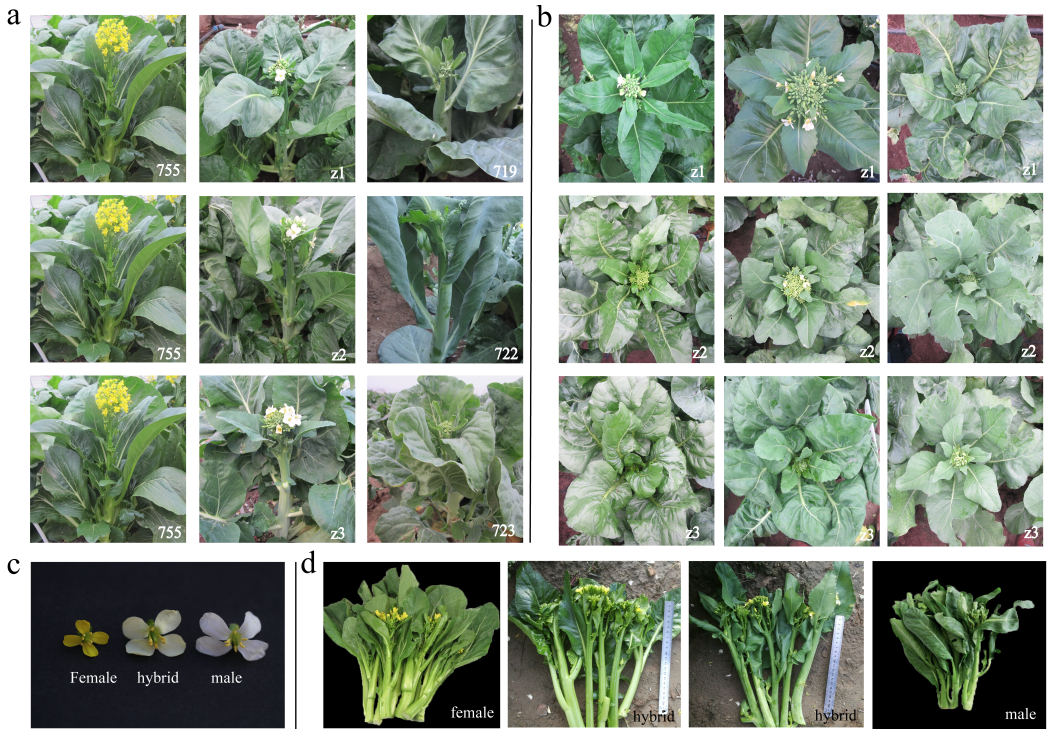

Figure 2. Morphology of parents and interspecific hybrids. (**a**) Morphology of parental and interspecific hybrid plants. (**b**) Leaf color of hybrid plants. (**c**) Flower color of parents and hybrid. (**d**) Stem (edible part) of parents and hybrids.

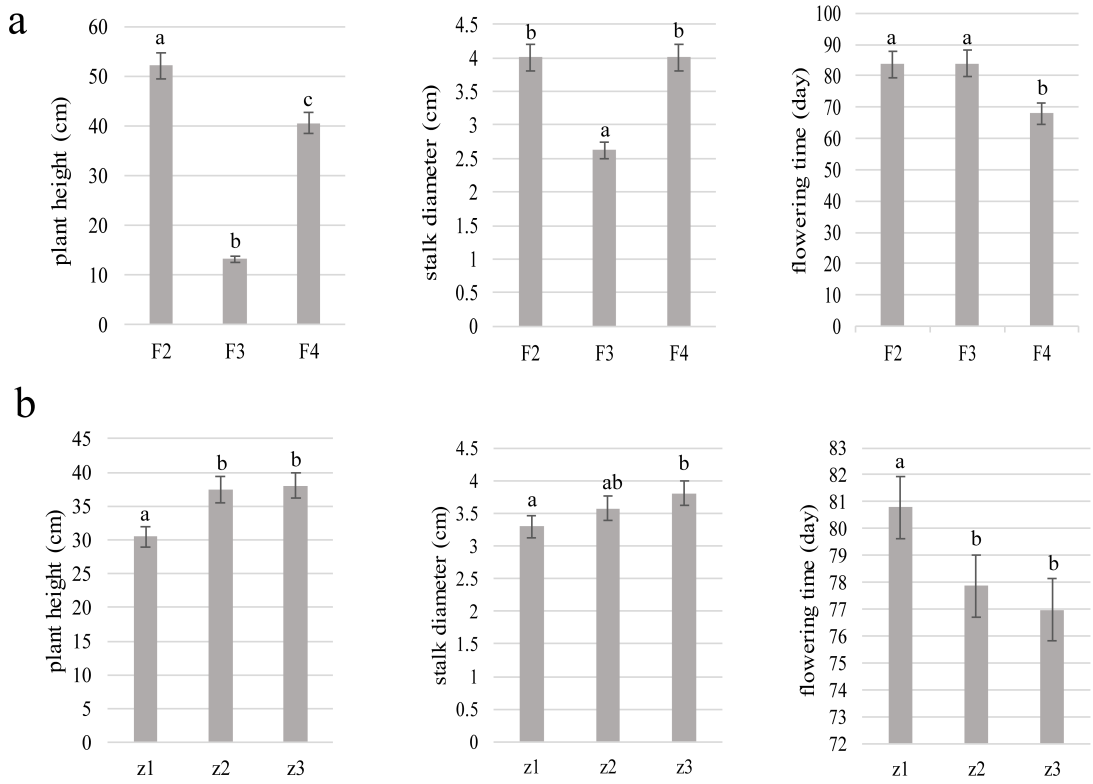

Figure 3. Significance analysis of morphological characteristics. (**a**) Significant difference analysis for three traits among different generations and (**b**) different genetic combinations. Different lowercase letters indicate significant differences ($\alpha = 0.05$). Error bars indicate standard error.

3.3. Molecular Characterization

Thirty SSR primer pairs (Table S1) were used to detect sequence variation among the hybrids and parents. Nineteen SSR markers were polymorphic between the parents and were analyzed in the hybrids. The 19 markers cover 8 chromosomes of genomic C, 9 chromosomes of genomic A, providing a good overview of variation in the plants. Novel bands were detected in the hybrids, while some parental bands were missing (Figure 4). The number lacking a band of the AA genome (AA_lack), lacking a band of the CC genome (CC_lack), and with novel bands that were not present in the parents (novel) were different in each of the 12 plants. We performed a significant difference analysis among the genetic combinations and three generations, F_2 to F_4 (Figure 5). There were differences among the combinations (Figure 5b): z1 differed significantly from z2 and z3, which was consistent with the results of the trait analysis (Figure 3b). It was speculated that genetic variation leads to the phenomenon of shape separation. However, there was no significant difference among the three generations (Figure 5c). This might have been because we used a relatively small number of SSR markers. In addition, AA_lack was significantly different from CC_lack and novel. The number of AA_lack and CC_lack hybrids was much higher than for novel (Figure 5a). The AA genome was much more variable than the CC genome and fewer new fragments appeared compared with the missing fragments. In summary, the conclusions were supported through significant differences in data for AA_lack and CC_lack numbers of 108 plants. Each generation (F_2, F_3, F_4) had 36 plants of data to support the analysis, and each combination ($_Z1$, $_Z2$, $_Z3$) was the same. These results showed that the genetic information of the early generations of the synthetic *B. napus* had changed. Genetic changes provide a molecular basis for trait variation.

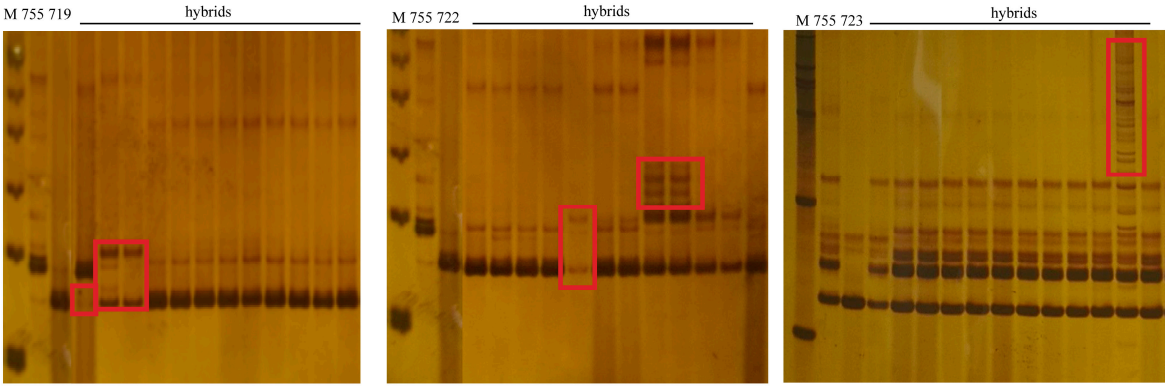

Figure 4. Molecular analysis of SSR markers in F_3 generation hybrids. The gels show PCR products generated using SSR primer pairs SSR-c08a09-1 or SSR-c07a06-1. M: DNA length marker; the codes on each gel indicate the parents and their hybrids; the red rectangles indicate specific novel bands or lack of parental bands.

a

	AA_lack	CC_lack	novel
total number	18.11 ± 3.91 a	9.22 ± 2.80 b	2.55 ± 1.98 b

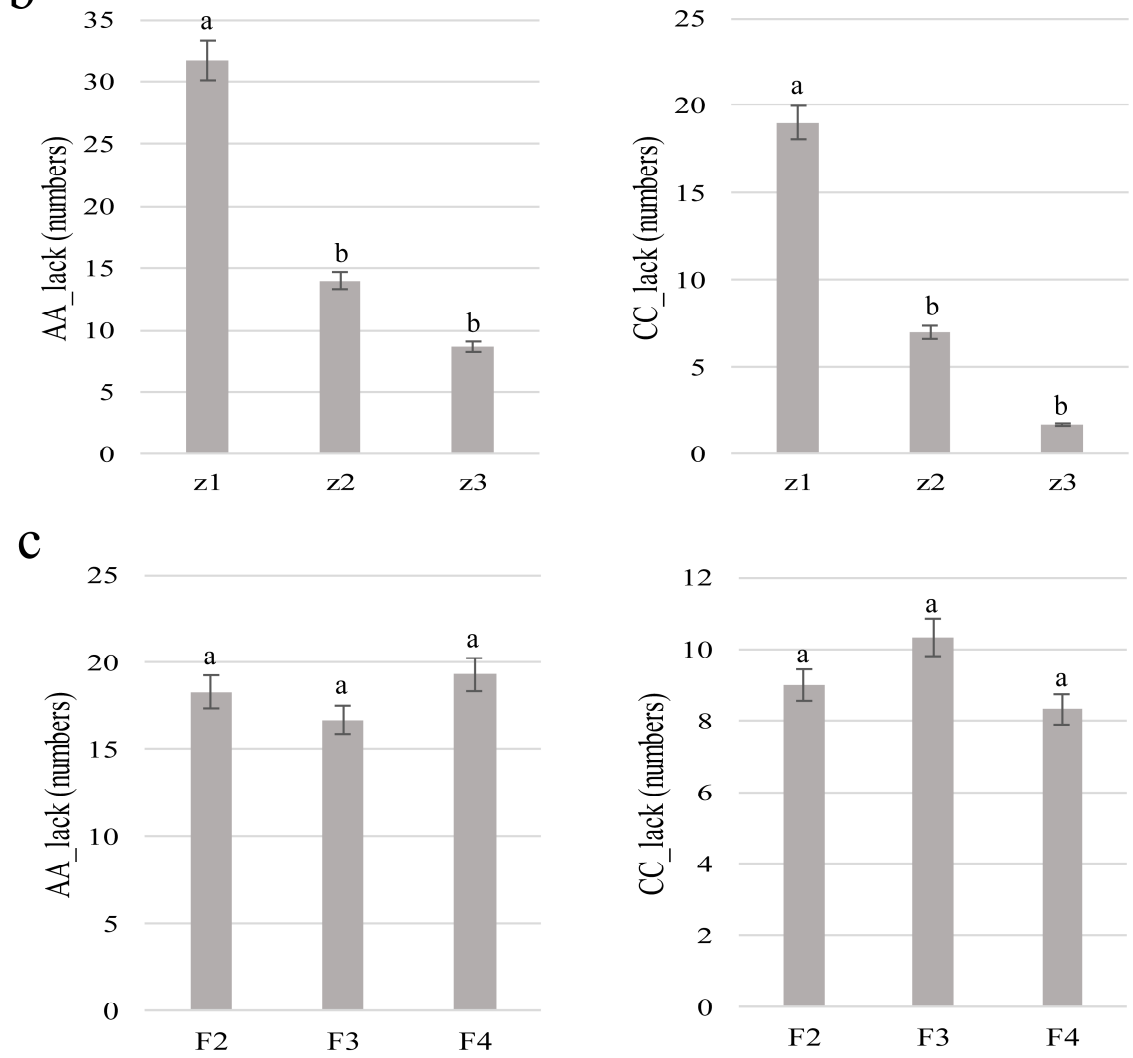

Figure 5. Significance analysis of molecular characterization using SSR markers. AA_lack means that number lacking a band of the AA genome; CC_lack means that number lacking a band of the CC genome; novel means the number with novel bands not present in the parents. (**a**) Significant difference analysis of the total number of AA_lack, CC_lack and novel bands; (**b**) significant difference analysis for the number of AA_lack and CC_lack bands in the different genetic combinations; (**c**) significant difference analysis for the number of AA_lack and CC_lack bands in different generations. Different lowercase letters indicate significant differences ($\alpha = 0.05$). Error bars indicate standard error.

4. Discussion

The synthesized *B. napus* F_1 is mostly a flourish grower and has a distinct hybrid advantage. By artificially synthesizing *B. napus* to introduce new germplasm resources, breeders have produced new *B. napus* materials with specific agronomic traits and quality traits such as disease resistance, cold resistance, low erucic acid, and high oil content [13,29,30]. In this study, flowering Chinese cabbage served as the female parent and three Chinese kale varieties served as the male parent. The hybrids showed significant variation in traits among the different genetic combinations and

different generations. Variant plants with epicuticular wax powder, short flowering time, and altered stalk diameters were found. Backcrosses between newly formed allopolyploid hybrids and the parents might broaden the genetic resources of flowering Chinese cabbage and Chinese kale. In addition, hybrids with desirable variation in agronomic traits could serve as breeding materials for *B. napus* by backcrossing. More importantly, as flowering Chinese cabbage and Chinese kale are known for their nutritional value and desirable taste [1,2], further nutritional analysis to compare the hybrid offspring with their parents will be required. Some hybrid offspring might have greater levels of some nutrients than their parents, which would provide powerful support for hybrids becoming commercially viable vegetables.

Previous studies have shown that synthetic *B. napus* has abundant variation, such as in flowering time, flower size, plant fresh weight, leaf shape and size, the surface wax layer, and other traits [13,18]. However, there have been few studies on the mechanism of variation in traits in the field between different genetic combinations and different generations [14]. In our investigation of the field characteristics of synthetic *B. napus*, traits were separated in different combinations and generations. Therefore, breeders could expand the population of self-bred offspring to screen for desirable variations in future breeding work. In addition, the traits of the three genetic combinations showed significant differences, indicating that the range of trait variation between combinations was limited by the parents. When configuring combinations, breeders should select parents with characteristics required for the target trait. In line with previous research conclusions, the traits showed significant differences among different generations, indicating that initial generations of synthetic *B. napus* were unstable and multi-generation self-crossing was necessary to obtain stable inbred lines for breeding. At last, expanding the number of populations could lead to more convincing conclusions, more hybrids, and more generations are waiting to be further studied.

Allopolyploid-induced variant DNA sequences are mainly concentrated in transposon sequences, random sequences, or repetitive sequences [31–33]. Through bioinformatics analysis, it was found that the microsatellite sequence density of the coding region in natural *B. napus* was higher than that of its diploid parent species, indicating that the SSRs in the gene coding region changed significantly during the polyploidization of *B. napus* [14,34–36]. In our study, novel and missing SSR sequences were detected in the hybrids. There were differences in the statistical results among the different combinations, which was consistent with the variation in the traits. However, there was no significant difference between generations, indicating that the difference in sequence changes caused by different fathers was significantly higher than that in the process of multiplication. In addition, the number of missing AA bands was significantly greater than the number of missing CC bands, which proved that the AA genome is more active than the CC genome when responding to whole-genome duplication [37]. This confirms that sequence changes in the early generations of synthetic *B. napus* and genetic changes in newly formed allotetraploids might provide a molecular basis for trait separation [38]. With further development of sequencing technology, it will be possible to explore the SSR variation mechanism across the whole genome and the effect of SSR variation on the traits.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/8/11/258/s1, Table S1: Characteristics of analyzed simple sequence repeat (SSR) markers.

Author Contributions: R.S. designed the experimental design and wrote the article. F.L., S.Z. (Shujiang Zhang), S.Z. (Shifan Zhang), and H.Z. contributed to the experimental work. Y.W. performed the experiment and wrote the article. All the authors read and approved the final manuscript.

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References

1. Peng, Y.; Shi, D.; Zhang, T.; Li, X.; Fu, T.; Xu, Y.; Wan, Z. Development and utilization of an efficient cytoplasmic male sterile system for Cai-xin (*Brassica rapa* L.). *Sci. Hortic.* **2015**, *190*, 36–42. [CrossRef]
2. Sun, B.; Yan, H.; Liu, N.; Wei, J.; Wang, Q. Effect of 1-MCP treatment on postharvest quality characters, antioxidants and glucosinolates of Chinese kale. *Food Chem.* **2012**, *131*, 519–526. [CrossRef]
3. Deng, M.; Qian, H.; Chen, L.; Sun, B.; Chang, J.; Miao, H.; Cai, C.; Wang, Q. Influence of pre-harvest red light irradiation on main phytochemicals and antioxidant activity of Chinese kale sprouts. *Food Chem.* **2017**, *222*, 1–5. [CrossRef] [PubMed]
4. Sun, B.; Liu, N.; Zhao, Y.; Yan, H.; Wang, Q. Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. *Food Chem.* **2011**, *124*, 941–947. [CrossRef]
5. Li, G.G.; Zhang, H.; Huang, H.D.; Qiao, Y.C.; Zheng, Y.S. Research progress on flowering Chinese cabbage breeding in Guangdong province. *Chin. Veg.* **2010**, *20*, 9–14.
6. Yan, R.L. *The Origin and Variety Selection and Cultivation of Flowering Chinses Cabbage*; China Agriculture Press: Beijing, China, 2014.
7. Zhang, H.H.; Liu, Z.Z. Market demand and breeding status of flowering Chinese cabbage. *Chin. Veg.* **2010**, *3*, 10–12.
8. Qin, Y.G.; Yang, C.Q.; Cao, B.H.; Chen, G.J.; Lei, J.J. Advances in Research on Genetic Breeding and Biotechnology of Chinese Kale. *Chin. Agric. Sci. Bull.* **2009**, *25*, 296–299.
9. Cicin, N.V. *Distant Hybridization in Plants*; Moskva: Moscow, Russia, 1954.
10. Chen, J.; Luo, M.; Li, S.; Tao, M.; Ye, X.; Duan, W.; Zhang, C.; Qin, Q.; Xiao, J.; Liu, S. A comparative study of distant hybridization in plants and animals. *Sci. Chin. Life Sci.* **2018**, *61*, 285–309. [CrossRef] [PubMed]
11. Whitney, K.D.; Ahern, J.R.; Campbell, L.G.; Albert, L.P.; King, M.S. Patterns of hybridization in plants. *Perspect. Plant Ecol. Evol. Systemat.* **2010**, *12*, 175–182. [CrossRef]
12. Chalhoub, B.; Denoeud, F.; Liu, S.; Parkin, I.A.P.; Tang, H.; Wang, X.; Chiquet, J.; Belcram, H.; Tong, C.; Wincker, P.; et al. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* **2014**, *345*, 950–953. [CrossRef] [PubMed]
13. Gaeta, R.T.; Pires, J.C.; Iniguez-Luy, F.; Leon, E.; Osborn, T.C. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *Plant Cell* **2007**, *19*, 3403–3417. [CrossRef] [PubMed]
14. He, L.Q.; Tang, R.H.; Jiang, J.; Xiong, F.Q.; Huang, Z.P.; Wu, H.N.; Gao, Z.K.; Zhong, R.C.; He, X.H.; Han, Z.Q. Rapid gene expression change in a novel synthesized allopolyploid population of cultivated peanut × Arachis doigoi cross by cDNA-SCoT and HFO-TAG technique. *J. Integr. Agric.* **2017**, *16*, 1093–1102. [CrossRef]
15. Mestiri, I.; Chague, V.; Tanguy, A.M.; Huneau, C.; Huteau, V.; Belcram, H.; Coriton, O.; Chalhoub, B.; Jahier, J. Newly synthesized wheat allohexaploids display progenitor-dependent meiotic stability and aneuploidy but structural genomic additivity. *New Phytol.* **2010**, *186*, 86–101. [CrossRef] [PubMed]
16. Tian, E.; Jiang, Y.; Chen, L.; Zou, J.; Liu, F.; Meng, J. Synthesis of a Brassica trigenomic allohexaploid (*B. carinata* × *B. rapa*) de novo and its stability in subsequent generations. *Theor. Appl. Genet.* **2010**, *121*, 1431–1440. [CrossRef] [PubMed]
17. Tang, J.; Baldwin, S.J.; Jacobs, J.M.; Linden, C.G.; Voorrips, R.E.; Leunissen, J.A.; Eck, H.; Vosman, B. Large-scale identification of polymorphic microsatellites using an in silico approach. *BMC Bioinform.* **2008**, *9*, 374. [CrossRef] [PubMed]
18. Zhang, X.; Liu, T.; Li, X.; Duan, M.; Wang, J.; Qiu, Y.; Wang, H.; Song, J.; Shen, D. Interspecific hybridization, polyploidization, and backcross of *Brassica oleracea* var. alboglabra with *B. rapa* var. purpurea morphologically recapitulate the evolution of Brassica vegetables. *Sci. Rep.* **2016**, *6*, 18618. [CrossRef] [PubMed]
19. Tang, Z.; Fu, S.; Ren, Z.; Zou, Y. Rapid evolution of simple sequence repeat induced by allopolyploidization. *J. Mol. Evol.* **2009**, *69*, 217–228. [CrossRef] [PubMed]
20. Karim, M.M.; Siddika, A.; Tonu, N.N.; Hossain, D.M.; Meah, M.B.; Kawanabe, T.; Fujimoto, R.; Okazaki, K. Production of high yield short duration Brassica napus by interspecific hybridization between *B. oleracea* and *B. rapa*. *Breed. Sci.* **2014**, *63*, 495–502. [CrossRef] [PubMed]
21. Shen, Y.; Sun, S.; Hua, S.; Shen, E.; Ye, C.Y.; Cai, D.; Timko, M.P.; Zhu, Q.H.; Fan, L. Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid *Brassica napus* uncovers key roles for small RNAs. *Plant J.* **2017**, *91*, 874–893. [CrossRef] [PubMed]

22. Zhang, G.Q.; Tang, G.X.; Song, W.J.; Zhou, W.J. Resynthesizing Brassica napus from interspecific hybridization between *Brassica rapa* and *B. oleracea* through ovary culture. *Euphytica* **2004**, *140*, 181–187. [CrossRef]
23. Ayotte, R.; Harney, P.M.; Machado, V.S. The transfer of triazine resistance from *Brassica napus* L. to *B. oleracea* L. I. Production of F1 hybrids through embryo rescue. *Euphytica* **1987**, *36*, 615–624. [CrossRef]
24. Sharma, D.R.; Kaur, R.; Kumar, K. Embryo rescue in plants—A review. *Euphytica* **1996**, *89*, 325–337. [CrossRef]
25. Zhao, J.; Simmonds, D.H.; Newcomb, W. High frequency production of doubled haploid plants of *Brassica napus* cv. Topas derived from colchicine-induced microspore embryogenesis without heat shock. *Plant Cell Rep.* **1996**, *15*, 668–671. [CrossRef] [PubMed]
26. Dolezel, J.; Greilhuber, J.; Suda, J. Estimation of nuclear DNA content in plants using flow cytometry. *Nat. Protoc.* **2007**, *2*, 2233–2244. [CrossRef] [PubMed]
27. Yu, H.L.; Fang, Z.Y.; Liu, Y.M.; Yang, L.M.; Zhuang, M.; Lv, H.H.; Li, Z.S.; Han, F.Q.; Liu, X.P.; Zhang, Y.Y. Development of a novel allele-specific Rfo marker and creation of Ogura CMS fertility-restored interspecific hybrids in Brassica oleracea. *Theor. Appl. Genet.* **2016**, *129*, 1625–1637. [CrossRef] [PubMed]
28. Kidwell, K.K.; Osborn, T.C. Simple plant DNA isolation procedures. In *Plant Genomes: Methods for Genetic and Physical Mapping*; Beckmann, J.S., Osborn, T.C., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 1992; pp. 1–13. [CrossRef]
29. Ozminkowski, R.H.; Jourdan, P. Comparing the resynthesis of *Brassica napus* L. by interspecific somatic and sexual hybridization. I. Producing and identifying hybrids. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 808–815.
30. Eickermann, M.; Ulber, B.; Vidal, S. Resynthesized lines and cultivars of Brassica napus L. provide sources of resistance to the cabbage stem weevil (*Ceutorhynchus pallidactylus* (Mrsh.)). *Bull. Entomol. Res.* **2011**, *101*, 287–294. [CrossRef] [PubMed]
31. Gemayel, R.; Vinces, M.D.; Legendre, M.; Verstrepen, K.J. Variable tandem repeats accelerate evolution of coding and regulatory sequences. *Ann. Rev. Genet.* **2010**, *44*, 445–477. [CrossRef] [PubMed]
32. Yaakov, B.; Kashkush, K. Massive alterations of the methylation patterns around DNA transposons in the first four generations of a newly formed wheat allohexaploid. *Genome* **2011**, *54*, 42–49. [CrossRef] [PubMed]
33. Zhang, L.; Zuo, K.; Zhang, F.; Cao, Y.; Wang, J.; Zhang, Y.; Sun, X.; Tang, K. Conservation of noncoding microsatellites in plants: Implication for gene regulation. *BMC Genom.* **2006**, *7*, 323. [CrossRef]
34. Grover, A.; Sharma, P.C. Is spatial occurrence of microsatellites in the genome a determinant of their function and dynamics contributing to genome evolution? *Curr. Sci.* **2011**, *100*, 859–869.
35. Yotoko, K.S.; Dornelas, M.C.; Togni, P.D.; Fonseca, T.C.; Salzano, F.M.; Bonatto, S.L.; Freitas, L.B. Does variation in genome sizes reflect adaptive or neutral processes? New clues from Passiflora. *PLoS ONE* **2011**, *6*, e18212. [CrossRef] [PubMed]
36. Raskina, O.; Barber, J.C.; Nevo, E.; Belyayev, A. Repetitive DNA and chromosomal rearrangements: Speciation-related events in plant genomes. *Cytogenet. Genome Res.* **2008**, *120*, 351–357. [CrossRef] [PubMed]
37. Nicolas, S.D.; Leflon, M.; Liu, Z.; Eber, F.; Chelysheva, L.; Coriton, O.; Chèvre, A.M.; Jenczewski, E. Chromosome 'speed dating' during meiosis of polyploid Brassica hybrids and haploids. *Cytogenet. Genome Res.* **2008**, *120*, 331–338. [CrossRef] [PubMed]
38. Chen, Z.J. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annu. Rev. Plant Biol.* **2007**, *58*, 377–406. [CrossRef] [PubMed]