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SRAP MARKERS FOR FLOWER STALK COLOR IN CHINESE KALE

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ABSTRACT

Chinese kale is avegetables native to south China. The flower stalk, the edible part of this plant, has a high nutrition value. Flower stalk color is one of the most important factorsof the quality of Chinese kale. In this study, a bulked segregate analysis (BSA) was used to identify sequence-related amplified polymorphism (SRAP) markers associated with the flower stalk color in Chinese kale*(Brassica alboglabra* Bailey). A segregating F_2population consisting of 196 plants derived from a cross of cv. Zhonghua (green flower stalk) and cv. Hongjiao (mauve flower stalk) was used. A total of 153 pairs of SRAP primers were used to amplify the DNA of the cv. Hongjiao and cv. Zhonghua, and investigate the DNA pools of their mauve and green flower stalk, respectively. Twenty-nine of the primers pairs obtained different bands. The DNA pools of the mauve and green flower stalk were successfully amplified. Only the pair of me2/em14 consistently exhibited different bands in all 29 pairs of primers. The amplification results of the individual plantsin the DNA pools and F_2 generation indicated that me2/em14 was the molecular marker associated with the flower stalk color in Chinese kale.

Key words: *Brassica alboglabra*, SRAP marker, color of flower stalk.

INTRODUCTION

Chinese kale (*Brassica alboglabra* Bailey) is a vegetable native to Guangzhou, China. The flower stalk of Chinesekale, the edible part, is crispy and rich with nutrients, such as Vitamin C, glucosinolates and minerals. The color of flower stalk in Chinese kale isdetermined by the content of chlorophyll, anthocyanin, and other pigments, such as flavonoids and carotenoids, in its epidermis (Liu *et al.*, 2004). Most Chinese kale cultivars have green flower stalks. However, the 'Hongjiao' cultivar, has a mauve flower stalkdue to its high anthocyan in content. Sincethis cultivar has strong resistance to heat and disease, 'Hongjiao' cultivar can be used as a specialtycultivar, also as the parentalmaterial for hybrid production (Chen *et al.*, 2013). Flower stalk color is one of the most important indicative factors of the quality ofChinese kale. The use of molecular marker-assisted selection is an alternative approach that could reduce the time consumption of traditional Chinese kale breeding methods.

Sequence-related amplified polymorphism (SRAP) technology is a simple and efficient marker system that can be used for map construction, gene tagging, genomic and cDNA fingerprinting, and map-based cloning (Li and Quiros 2001; Riaz *et al.* 2001; Ferriol *et al.* 2003; Li *et al.* 2003; Budak *et al.* 2004a, b, c; Lin *et al.* 2004;). SRA Panalyzes coding sequences and in order to identify co-dominant markers (Budak *et al.* 2004b). In this study, a segregating F_2population consistingof 196 plants derivedfrom a cross of cv. Zhonghua, with green flower stalk, and cv. Hongjiao, with mauve flower stalk, was used to detect SRAP markers associated with the flower stalk color gene of Chinese kale.

MATERIALS AND METHODS

Plant material: A segregating F_2population consistingof 196 plants from a cross of cv. Zhonghua (green flower stalk) and cv. Hongjiao (mauve flower stalk) was used to determine the phenotypic distribution and identify SRAP markers associated with the flower stalk color of Chinese kale. This study was conducted in theexperimental vegetable farm at South ChinaAgricultural University in Guangzhou, China, from 2010 to 2011. Two Chinese kale cultivars were crossed, with cv. Zhonghua as the female parent, after manual emasculation to obtain the F_1 hybrid. The resulting F_1 was planted and F_2 generationwas obtained after self-crossing.

DNA extraction: Total genomic DNA samples were extracted from bulked flower stalk tissues using the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al.* 1984). The amount and quality of DNA in each sample were tested by performing electrophoresis on 1% agarose gel stained with ethidium bromide and visualizing the samples under UV illumination.

SRAP analysis: Bulked segregant analysis: DNA samples obtained from ten plants with green and tenplants with mauve flower stalk were pooled to prepare the flower stalk bulks. A total of 153 primerpair SRAP markers were used to analyze thepolymorphism between the green flower stalk and the mauveflower stalk bulks, as well as the two parental lines. The polymorphicmarkers were utilized in 196 plants of the segregatingpopulation. The PCR products were separated with polyacrylamide gel electrophoresis. After the gel was stained silver, the polymorphicbands were observed, and theprimer combinations capable of producing polymorphicbands were selected.

An SRAP analysis was conducted according to the process proposed by Li and Quiros (2001). As shown in Table 1, nine forward primers and seventeen reverse primers synthesized by the Shanghai Shenggen Science and Technology Company were selected for analysis.

DNA isolation and PCR amplification: Genomic DNA was extracted from fresh flower stalk using theCTAB procedure (Chen *et al.*, 2006). PCR amplification was conducted as follows: initial demoisturized for 5 minutes at 94℃; 85 cycles for: 60 seconds at 94℃, 60seconds at 36℃, 90 seconds at 72℃; followed by35 cycles for: 60 seconds at 94℃, 60 seconds at 50℃, 90 seconds at 72℃; and final extension for 10 minutes.

The test of SRAP marker in F_2 populations: Using the polymorphic primer combinations, the DNA samples of the F_2 individuals was amplified. The polymorphic bandseparation in the F_2 individuals were observed and recorded.

Table 1.List of SRAP primers.

Forward primers	Reverse primers
me1, 5 -TGAGTCCAAACCGGATA	em1, 5 -GACTGCGTACGAATTAAT
me2, 5 -TGAGTCCAAACCGGAGC	em2, 5 -GACTGCGTACGAATTTGC
me3, 5 -TGAGTCCAAACCGGAAT	em3, 5 -GACTGCGTACGAATTGAC
me4, 5 -TGAGTCCAAACCGGACC	em4, 5 -GACTGCGTACGAATTTGA
me5, 5 -TGAGTCCAAACCGGAAG	em5, 5 -GACTGCGTACGAATTAAC
me6, 5 -TGAGTCCAAACCGGTAG	em6, 5 -GACTGCGTACGAATTGCA
me7, 5 -TGAGTCCAAACCGGTTG	em7, 5 -GACTGCGTACGAATTATG
me8, 5 -TGAGTCCAAACCGGTGT	em8, 5 -GACTGCGTACGAATTAGC
me9, 5 -TGAGTCCAAACCGGTCA	em9, 5 -GACTGCGTACGAATTACG
	em10, 5 -GACTGCGTACGAATTTAG
	em11, 5 -GACTGCGTACGAATTTCG
	em12, 5 -GACTGCGTACGAATTGTC
	em13, 5 -GACTGCGTACGAATTGGT
	em14, 5 -GACTGCGTACGAATTCAG
	em15, 5 -GACTGCGTACGAATTCTG
	em16, 5 -GACTGCGTACGAATTCGG
	em17, 5 -GACTGCGTACGAATTCCA

RESULTS AND DISCUSSION

Variationof flower stalk color: The color of flower stalk exhibited variation in the individual of F_2 population. Of the tested 196 F_2 plants, 150 plants had mauve flower stalk, and 46 plants had pure green flower stalk. A distinctsegregation for this trait was, therefore, observed in the F_2population.

Development of SRAP marker associated with the flower stalk color of Chinese kale: The two DNA bulks and the two parental DNAswere screened using 153 SRAP primer combinations. The results indicated that 29 of the amplified primers amplified pairs were polymorphic bands for mauve and green bulks. The primer combinations that detected polymorphism between the two bulks were used to screen the two parents, the two bulks and ten individuals from each of the bulks. The results indicated that primer combination me2/em14 produced one specific band in the mauve plants but not in the green plants, suggesting that these markers, named me2/em14 (600 bp), were associated with a gene that determines flower stalk color (Figs. 1 and 2).

These primer combinationswere used to amplify the 196 F_2 individuals. The specific band was found in 145 of 150 individuals with mauve-colored flower stalks, while the specific band was not found in 44 of 46 individuals with green flower stalk.

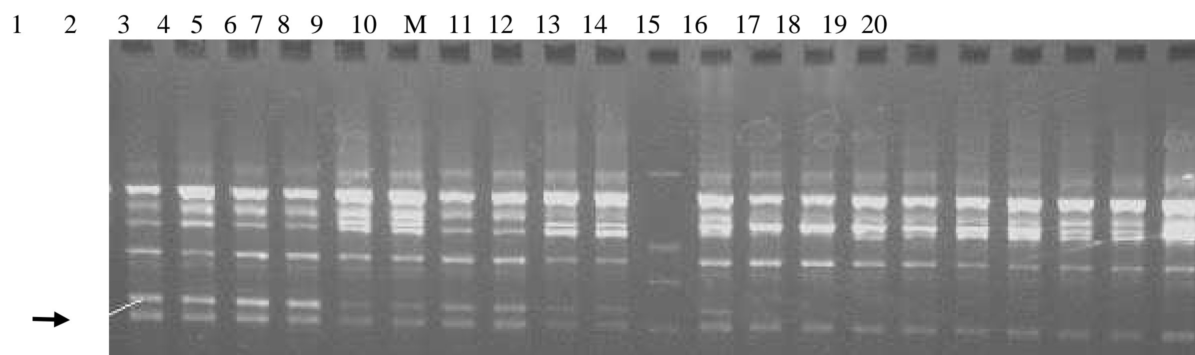

Fig. 1: me2/em14 primerscreening of the individuals in the two pools

M: DL2000 DNA marker, 1~10: 10 individuals fromthe mauve pool; 11~20: 10individuals fromthe green pool; thearrow indicates the different band

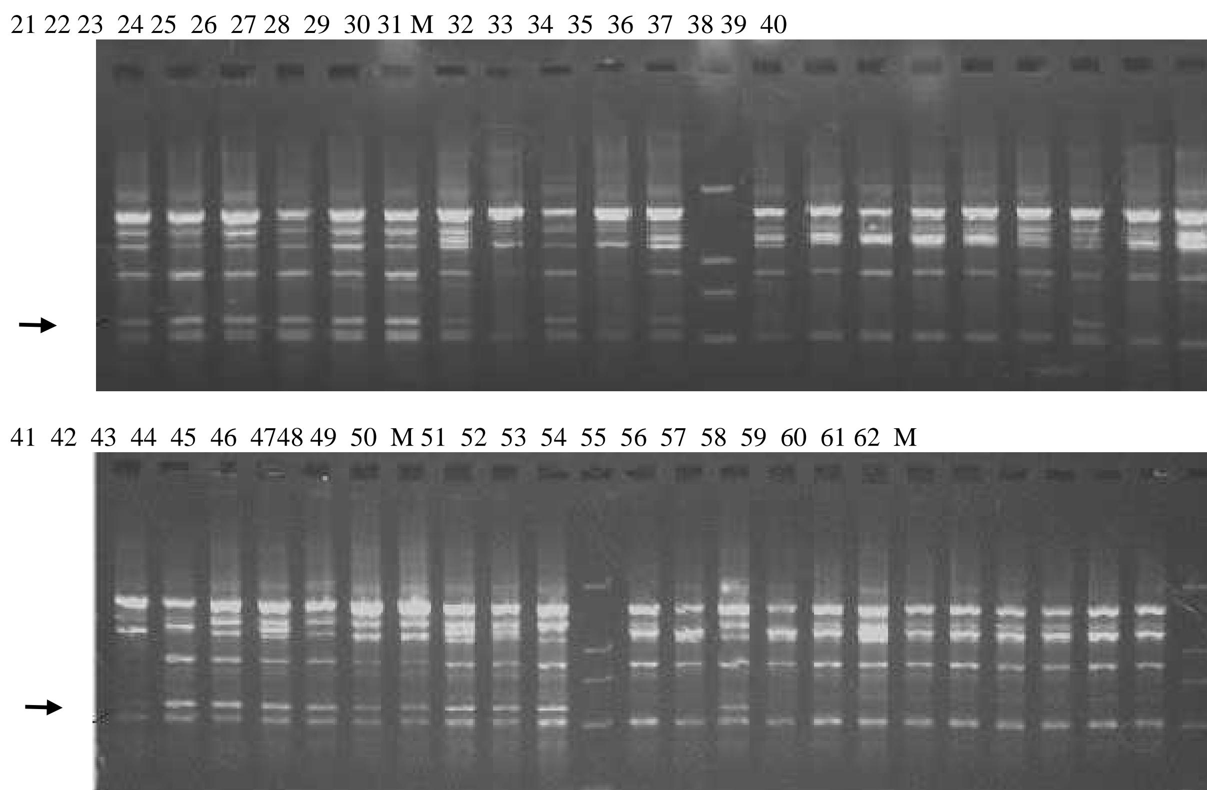

Fig. 2: PCR amplification of 20 mauve and 20 green individuals with primer combination me2/em14.

M: DL2000 DNA marker, 21~31and 41-50: 20 individuals fromthe mauve pool; 32~40 and 51-62: 20individuals fromthe green pool; thearrow indicates the different band

SRAP, a simple and co-dominant molecular marker that is highly sensitive to structural DNA differentiation, is used in agriculture to identify DNA markers associated with the important genes (Lin *et al.* 2004). In this study, two local Chinese kale cultivars were used:cv. Zhonghua, which has a green flower stalk, and cv. Hongjiao, which has amauve flower stalk. The F_2 population of these two cultivars exhibited a variety of flower stalk color. The SRAP molecular marker of the two parents and the two DNA pools revealed a large number of bands and a high polymorphism rate. In this study, the SRAP marker associated withflower stalk color was identified by using a larger F_2 population, which consisted of 196 individualsderived from a cross of cv. Hongjiaoandcv. Zhonghua and different SRAP primer combinations.

Conclusion: The marker associated with mauve-colored flower stalks in Chinese kale was determined by analyzing 196 plants derived from F_2 segregating population resulting from a cross between the cv. Hongjiao and cv. Zhonghua Chinese kale variants. This marker could be used for the molecular marker-assisted selection of Chinese kale.

REFERENCES

Budak, H., R. C. Shearman, R. E. Gaussoin, and I. Dweikat (2004a). Application of sequence-related amplified polymorphism markers for characterization of turf grass species. Hort Science, 39: 955-958.

Budak, H., R. C. Shearman, I. Parmaksiz, and I. Dweikat (2004b).Comparative analysis of seeded and vegetative biotype buffalo grasses based on phylogenetic relationshipusing ISSRs, SSRs, RAPDs, and SRAPs. Theoreticaland Applied Genetics, 109, 280-288.

Budak, H., R. C. Shearman, I. Parmaksiz, R. E. Gaussion, T. P. Riordan, and I. Dweikat (2004c).Molecular characterization of buffalo grass germplasm using sequence-related amplified polymorphism markers. Theoretical and Applied Genetics, 108, 328-334.

Chen, H. Z., S. L. Hopper, X. L. Li, L.G. Ljungdahl and C. E. Cerniglia (2006).Isolation of extremely AT-rich genomic DNA and analysis of genes encoding carbohydrate degrading enzymes from Orpinomycessp strain PC-2.Curr.Microbiol., 53: 396-400.

Chen R., S. Song, X. Li, H. Liu, and D. Huang (2013). Phosphorus Deficiency Restricts Plant Growth butInduces Pigment Formation in the Flower Stalk of Chinese Kale. Horticulture, Environment, and Biotechnology, 54(3): 243-248.

Ferriol, M., B. Pico, and F. Nuez (2003).Genetic diversity of a germplasm collection of *Cucurbitapepo* using SRAP and AFLP markers. Theoretical and Applied Genetics, 107:271-282

Li, G., M. Gao, B. Yang, and C. F. Quiros (2003).Gene for gene alignment between the Brassica and Arabidopsis genomes by direct transcriptome mapping.Theor.appl.Genet.,107:168-180

Li, G. and C. F. Quiros (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet, 103:455-461

Lin Z., X. Zhang, and Y. Nie (2004). Evaluation of application of a new molecular marker SRAP on analysis of F_2 segregation population and genetic diversity in cotton. Acta Genetica Sinica, 31: 622-626. (in Chinese)

Liu, H., Q. Huang, and R. Chen (2004).Changes of pigments concentration of flower stalkin Chinese Kale under different light intensities. Acta Hort., 477-482.

Riaz, A., G. Li, Z. Quresh, M. S. Swati, and C. F. Quiros (2001). Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breeding, 120: 411-415.