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Full Length Research Paper

Development of specific RAPD markers for identifying albino tea cultivars 'Qiannianxue' and 'Xiaoxueya'

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Albino tea cultivars grow white leaves at low temperature which are valuable materials for processing green tea, but they develop green leaves in summer and autumn seasons. It is difficult to discriminate albino tea cuttings from the normal tea cuttings by leaf colour and plant morphological characteristics. Specific RAPD markers for identifying albino tea cultivars 'Qiannianxue' and 'Xiaoxueya' were developed in the present paper and they can be used in the authentication of the two albino tea cultivars. An amplified fragment (about 1500 bp) from Primer (S 12 (Sangon Biological Engineering Technology and Services Co., Ltd.) was identified in the albino teas and not from the widely cultivated cultivar; Fudingdabai.

Key words: *Camellia sinensis*, albino tea, RAPD maker, cultivar authentication.

INTRODUCTION

White leaves grown on the albino tea cultivars have high level of amino acids which are important tea components responsible for tea quality (Jang et al., 2008; Liang et al., 2003, 2005, 2008) and healthy functions (Yokogoshi et al., 1995; Du et al., 2006, 2008, 2009; Zhang et al., 2007). The cultivars 'Qiannianxue' and 'Xiaoxueya' are two of the major albino tea cultivars grown in Zhejiang Province, China. They grow shoots with albino leaves when the environmental temperature was below 20℃ during early spring season (Du et al., 2008). However, the leaf color becomes green as is the case with the other cultivars commonly grown in the same areas when the environmental temperature is above 20℃, resulting in difficult to identify the authentity of young tea cuttings by leaf color. The malfeasant counterfeited tea cultivar names as albino tea cultivars and the tea farmers have been deceived and suffered from loss. The tea growers are increasingly paying attention to the authentication of albino tea cultivars. Cultivar 'Fudingdabai' is now the widely grown variety in the province. It is important to develop a method to identify the albino tea cultivar from the 'Fudingdabai' in cutting stage.

Various types of DNAbased molecular techniques are utilized to evaluate DNA polymorphism, including random amplified polymorphic DNA (RAPD) (Gupta et al., 2008; Vural and Dageri, 2009). RAPD is a tool widely used to identify genetic variation in ecotypes of crops and in the present paper it was used to develop DNA markers suitable for identifying the albino tea cultivars 'Qiannianxue' and 'Xiaoxueya' from 'Fudingdabai', a widely cultivated tea cultivar in China.

MATERIALS AND METHODS

Materials

First leaf beneath the apical bud on tea shoot of albino tea cultivars 'Qiannianxue' and 'Xiaoxueya' grown in Deshijia Tea Farm (Yuyao City, Zhejiang Province, China) was sampled on 25th April, 2007 and then were frozen in liquid nitrogen and stored at -20ºC until DNA was extracted. To search for DNA markers of the albino tea cultivars which are distinct from common tea cultivar, 'Fudingdabai', the most popular tea cultivar grown in China was used as control in the test.

Chemical reagents used in the present paper were purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China).

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Table 1. RAPD primers that generated polymorphic bands.

Primer No.	Sequence	Primer No.	Sequence
S11	GTAGACCCGT	S21	CAGGCCCTTC
S12	CCTTGACGCA	S22	TGCCGAGCTG
S17	AGGGAACGAG	S24	AATCGGGCTG
S18	CCACAGCAGT	S25	AGGGGTCTTG
S19	ACCCCCGAAG	S27	GAAACGGGTG

Table 2. Distinctive polymorphic bands for Albino cultivars and Fudingdabai

Bands exclusively amplified in Fudingdabai	Bands exclusively amplified in Qiannianxue	Bands generated exclusively in Xiaoxueya	Bands amplified in both albino cultivars
S11-1, S11-2, S11-3, S12-1, S17-1, S17-3, S17-4, S17-8, S18-3, S19-1, S19-2, S21-1, S21-2, S22-1, S22-3, S22-4, S24-1, S27-4	S17-6, S17-9, S24-2, S24-3	S12-3, S22-2, S25-1, S27-1, S17-2, S17-5, S17-7, S27-3, S27-3	S12-2

Genomic DNA extraction

Genome DNA extraction was carried out according to CTAB procedure described by Matasyoh et al. (2008). Concentration of the extracted DNA samples were determined by a GeneQuant Pro RNA/DNA calculator (Biochrom Ltd., Cambridge, England) and diluted to 100 mg ml^{-1} and then stored at -20ºC until used for PCR.

Polymerase chain reaction (PCR)

The PCR was carried out using S-series of 10-mer RAPD primers from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). Thirty primers; S1 to S30 were tested and those which amplified polymorphic bands (Table 1) were used to evaluate the three cultivars.

The PCR was carried out in 25 µL reaction solution that contains ddH_2O (17.8 µl), 10× buffer (2.5 µl), 25 mmol Mg^{2+} (2.5) µl, 10 mmol dNTP (0.5 µl), 0.5 µ mol l^{-1} RAPD primer (0.33) µl, 100 µg ml^{-1} DNA (1.2 µl) and 5 u $µl^{-1}$ Taq polymerase (0.17 µl).

The thermal amplification was programmed in three phases in a DYAD™ PTC-200 PCR Thermal Cycler (MJ Research, MA, USA), that is, the initial denaturation (at 94ºC for 5 min); the 25 cycles of denaturation (at 94ºC for 30 s), annealing (at 36ºC for 1 min) and extension (at 72ºC for 2 min) and the final extension step was 10 min at 72ºC. The PCR product was checked by electrophoresis on 1.5% (W/V) Agarose gel and photographed using the JD-801 ImageQuant Scanner (Jiangsu JEDA Science-Technology Development Co., Ltd., Nanjing, China).

RESULTS AND DISCUSSION

Among the 30 tested RAPD primers, 10 primers (Table 1) generated polymorphic bands. Compared to control cultivar ‘Fudingdabai’, there were 18 bands which could be generated in cultivar ‘Fudingdabai’ but could not be generated in both albino tea cultivars by the random primers S11, S12, S17, S18, S19, S21, S22, S24 and S27 (Table 2; Figure 1). It suggested that they might be related to some structural genes missed in the albino tea cultivars, compared to the common tea cultivar. These missed genes may be related to the albinism of tea. It offered important information for further studies on the genetic characteristics of albino tea cultivars. Band S12-2 was exclusively generated in the two albino tea cultivars ‘Qiannianxue’ and ‘Xiaoxueya’ by primer S12 (Table 2; Figure 1).

However, polymorphic bands were found between the albino tea cultivars ‘Qiannianxue’ and ‘Xiaoxueya’. Bands S17-6 and S17-9 generated by primer S17 and bands S24-2 and S24-3 by primer S24 were found in ‘Qiannianxue’, but not found in ‘Xiaoxueya’ (Figure 1; Table 2). On the contrary, bands S12-3 generated by primer S12 and band S22-2 by primer S22, band S25-1 by primer S25 and band S27-1 by primer S27 were detected in ‘Xiaoxueya’ but not in ‘Qiannianxue’ (Figure 1; Table 2). Furthermore, bands S17-2, S17-5 and S17-7 generated by primer S17 and bands S27-3 and S27-3 generated by primer S27 were exclusively detected in ‘Xiaoxueya’, which were not amplified in the common tea cultivar ‘Fudingdabai’ and the albino cultivar ‘Qiannianxue’ (Figure 1; Table 2). The polymorphic bands will be useful RAPD markers to discriminate the albino tea cultivars ‘Qiannianxue’ and ‘Xiaoxueya’ from cultivar ‘Fudingdabai’.

This study revealed that there were four groups of bands generated by the ten RAPD primers as shown in Table 1. These were the bands exclusively amplified in Fudingdabai, bands amplified exclusively in Xiaoxueya, bands exclusively amplified in Qianniaxue and bands amplified in both albino cultivars. The bands were generated from genome DNA samples, which were not affected by environmental conditions. Although the albino tea cultivars ‘Qiannianxue’ and ‘Xiaoxueya’ grow green shoots as common tea cultivar at temperature above 20℃, they were identified according to the bands generated

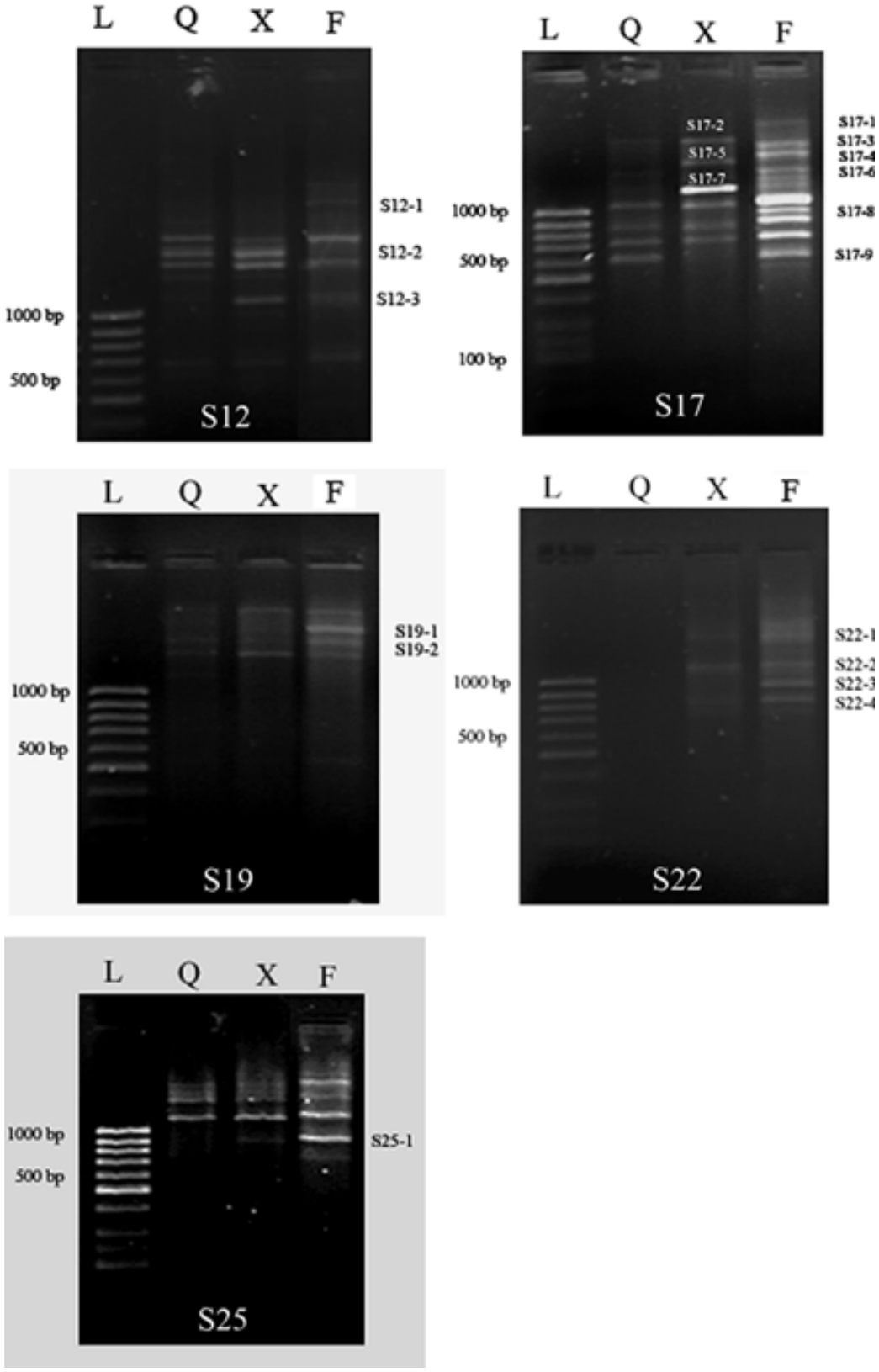

Figure 1. RAPD markers generated by random primers S12, S17, S19, S22 and S25. L = DNA Ladder; Q = Cultivar 'Qianianxue'; X = Cultivar 'Xiaoxueya'; F = Cultivar 'Fudingdabai'. The bands were numbered by primer number-the polymorphic band number, that is, S11-1 means the largest polymorphic band generated by primer S11.

by the ten primers in Table 1. However, only one widely cultivated cultivar ‘Fudingdabai’ was used as control test in the present study. The result of the present study is suitable for identifying the two albino tea cultivars from ‘Fudingdabai’, instead of all green tea cultivars. Further study should be carried out using more control tea cultivars.

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