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Short Communication

A molecular marker linked to the male gender of *Actinidia arguta* Siebold & Zucci

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Abstract

To distinguish the female plants in early stage, BSA and RAMP techniques were employed to screen and characterize molecular markers linked to hardy kiwi gender. One RAMP marker GA(CT)$_4$/S129$_{450}$, was found to be tightly linked to the male gender. Then the RAMP maker was cloned, sequenced, and conversed into SCAR marker. And early stage selection was carried out using the SCAR marker on 142 F_1 progeny from crossing between Yongshan 11 and Yongshan 5. It is the first report on distinguishing the gender of hardy kiwi in early stage.

Key words: *Actinidia arguta*, gender identification, RAMP, SCAR

Attempts to commercialize hardy kiwi (Siebold & Zucc.) [*Actinidia arguta* Plamch. ex Miq.] also known as tera vine are underway on a small scale in South America, New Zealand, Europe, Canada, the USA and China (Huang 2003). Rational selection of male and female individual in the cultivation helps improve the economic benefit. However, seed to blooming stage or to productive stage often takes 3-5 years, long childhood, dioecism, and hard in distinguishing male and female during seedling period, bring huge difficulties in improvement and breeding of hardy kiwi. Hence, early stage selection of gender is very important for hardy kiwi production and genetic breeding.

Molecular marker technologies have become important in the plant heredity and breeding, which could be used to assist selection in the early stage of plant growth (Dunemann et al. 2012). Making good use of the technology could improve the selection efficiency and shorten the breeding period. In recent years a lot of important genes quality traits controlling of plants were marked (Liu et al. 2009). Random amplified microstatellite polymorphism (RAMP) technology using arbitrary decamer oligonucleotide and micosatellite primer pairs could preferentially amplify genomic sequences containing low numbers of microsatellite motifs. Unlike microsatellite needing the information from the genome in advance, or RAPD acting as a dominant marker, RAMP are advantageous with respect to cost, easy to perform, speedy, accurate and acting as a co-dominant marker (Cheng et al. 2001; Liu et al. 2009). Bulked segregant analysis (BSA) is also one of the most useful methods in finding linkage of marker with gene of interest. Further, development of more specific and reproducible markers like RAMP based Sequence Characterized Amplified Region (SCAR) could increase application of the molecular techniques (Dnyaneshwar et al. 2006). In this paper, the results of combination with RAMP and BSA to identify linked molecular maker, and a SCAR marker based on RAMP of male gender of hardy kiwi are presented.

As parent hardy kiwi plants, Yongshan 5 was taken as female while Yongshan 11 as male accession. Twenty female and 20 male accessions from different origins (Table 1) and a total of 142 F_1 plants of

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Table 1. A list of accessions used in the experiment

No. of female accessions	No. of male accessions	Origin
1-5	11-15	Yongshan
6-10	16-20	Qiaojia
21-25	31-35	Huizhe
26-30	36-40	Ludian

Yongshan 5 x Yongshan 11 were used in the experiment.

Hardy kiwi DNA used for PCR detection was isolated according to Liu et al (2009).

The Bulk Segregant Analysis (BSA) was used to screen markers co-segregated with female or male gender as described by Liu et al. (2009). Equal amounts of DNA from 10 female (1-10) and 10 male (11-20) accessions were mixed to form a female pool and a male pool, respectively. Amplifications were carried out as described by Liu et al. (2009).

Cloning and sequencing the RAMP amplicon were performed as described as Dnyaneshwar et al (2006). Based on the sequenced RAMP amplicon a pair of SCAR oligonucleotide primer (L1 forward: CCATGCAGGTCTCATCTAAC; and R2 reverse: CATTGGCCTTGACTCTCTCT), which could amplify approximately 450 bp of the genomic *A. arguta* DNA was designed.

The SCAR primers pair was used for PCR amplifications of genomic DNA from F_1 plants of Yongshan 5 and Yongshan 11. Thermal cycling conditions for amplification using SCAR primers were optimized as: 94°C for 5min; 45 cycles at 94°C for 30s, 56°C for 30s. and 72°C for 2min, and a final extension at 72°C for 8min.

Two hundred primer pairs were used to screen the female and male pools. Only 9 (4.5%) out of 200 primer pairs produced polymorphism. Then, these RAMP primer pairs were further used to test the other 20 (21-40) individuals (Table 1), whose gender had already been known. Results showed that only $GA(CT)_4$/S129 could reproducibly produced polymorphism $GA(CT)_4/S129_{450}$ (about 450bp) in plant Yongshan 11 and other male plants but no corresponding band was obtained in Yongshan 5 and other female plants (Fig. 1).

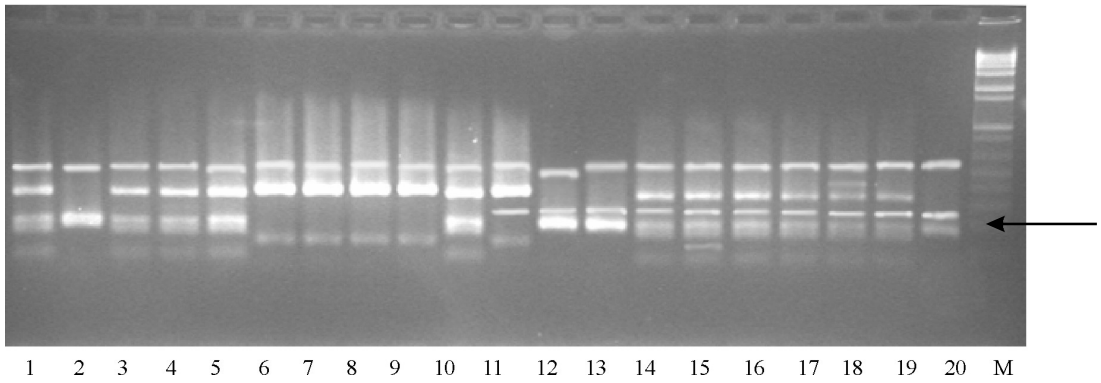

Fig. 1. Amplification products of $GA(CT)_4$/S129 in 10 female and 10 male hardy kiwi accessions. 1-10 = female accessions; 11-20 = male accessions; the arrow in the figure indicated the polymorphic band $GA(CT)_4/S129_{450}$

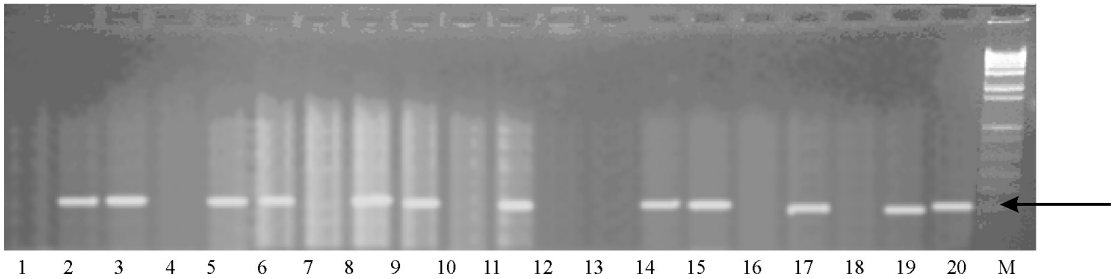

Fig. 2. Portion of amplification products of SCAR in hardy kiwi F_1 plants. 1-20 = different accessions of F_1 generation

Based on the sequence of the RAMP amplicon, a pair of SCAR primers were designed, and the SCAR primer pairs were used to further select the progeny seedlings of Yongshan 5 and Yongshan 11 involved crossing. Portion of amplification products of SCAR in hardy kiwi F_1 plants are depicted in Fig. 2. The amplified products of SCAR showed that there were 65 plants without the marker in 142 F_1 hybrid plants.

In this study, twenty female and 20 male accessions were characterized with the help of molecular maker of genders. Combined with BSA and RAMP, we found a marker $GA(CT)_4/S129_{450}$, linked to the male gender against hardy kiwi, and a RAMP based SCAR was obtained. The SCAR identified in this study could be used in molecular assisted breeding of female and male hardy kiwi plants which could avoid planting a lot of low economic male plants to cut down on workload and also could identify the female plants in the early stages of breeding, increasing selection veracity and efficiency.

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References

Cheng H. Y., Yang W. C. and Hsiao J. Y. 2001. Genetic diversity and relationship among peach cultivars based on Random Amplified Microsatellite Polymorphism (RAMP). Bot. Bull. Acad. Sin., **42**: 201-206.

Dnyaneshwar W., Preeti C., Kalpana J. and Bhushan P. 2006. Development and Application of RAPD-SCAR Marker for Identification of Phyllanthus emblica LINN. Biol. Pharm. Bul., **29**: 2313-2316.

Dunemann F., Gläss R., Bartsch S., Eldin M. A. S., Peil A. and Bus V. G. M. 2012. Molecular cloning and analysis of apple HcrVf resistance gene paralogs in a collection of related Malus species. Tree Genet. Genomes, **8**: 1095-1109.

Huang H. W. 2003. Adavances in Actinidia Research (II). Beijing: Science Press, 351-356 (in Chinese).

Liu X. Z., Yang Y. M., He C. S., Li H. L. and Zhang H. Y. 2009. A RAMP marker linked to the tobacco black shank resistant gene. Afr. J. Biotechnol., **8**: 2060-2063.