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TRAP markers generated with resistant gene analog sequences and their application to genetic diversity analysis of radish germplasm

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ABSTRACT

TRAP is a novel molecular marker technique which has been effectively used in genetic diversity analysis of germplasm and genetic mapping. However, it has not yet been applied to radish. In this study, novel TRAP markers based on expressed sequence tag (EST) and resistance gene analog (RGAs) were developed and applied to the genetic diversity analysis of radish genotypes. With data-mining method, a total of 50 RGAs including 35 unigenes and 15 singletons were identified from the public sequence databases and employed to design the fixed primers of TRAP markers. Fifty-nine RGA-derived fixed primers were combined with five arbitrary primers, and these TRAP primer combinations were tested in two genotypes ('NAU-YH' and 'NAU-DY'). Furthermore, 65 TRAP primer combinations were selected for genetic diversity analysis and 385 polymorphic fragments were produced among 30 radish genotypes. Dendrograms constructed by UPGMA method showed that these genotypes could be clustered into four groups. Interestingly, these groups were in highly accordance with the results of resistance evaluation to Turnip Mosaic Virus (TuMV). A cultivar identification diagram (CID) was made manually to discriminate the 30 radish genotypes using four polymorphic TRAP primer combinations. The results indicated that TRAP is an efficient genetic marker system, which could provide an effective tool for genetic mapping and for marker-assisted selection in radish breeding programs.

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1. Introduction

Radish (*Raphanus sativus* L.) is an important vegetable crop worldwide and the germplasm resources are very abundant, it was reported that more than 2100 radish cultivars have been conserved in China (Kong et al., 2011). Currently, several molecular markers have been used in radish genetic diversity studies, such as amplified fragment length polymorphism (AFLP) (Huh and Ohnishi, 2002), random amplified polymorphic DNA (RAPD) (Madhou et al., 2005), and simple sequence repeat (SSR) (Jiang et al., 2012). Meanwhile, multiple types of makers were also employed to examine genetic diversity in radish germplasm (Liu et al., 2008; Kong et al., 2011; Yamane et al., 2009). But the polymorphism of these marker systems detected is random across the genome (Creste et al., 2010) and may not reflect functional polymorphisms of functionally characterized genes or targeted genes. One such marker system, the target region amplification polymorphism (TRAP) technique, uses known target sequences of the genome as fixed primers in combination with arbitrary primers to generate polymorphism around target sequences (Hu and Vick, 2003).

Various diseases have seriously decreased the yield and quality of radish in resent years. Attempts to control pathogens by chemicals are often ineffective and could cause environmental pollution due to chemical residues. Thus, improving plant resistance to pathogens is an important objective in radish breeding programs. However, selection for resistance to various diseases in radish breeding was difficult to carry out due to it being influenced by the environment which is changeable (Khan et al., 2010). Molecular marker-assisted selection (MAS) can enhance the effectiveness or efficiency of selection in plant breeding and overcome many of the restrictions in phenotypic selection which had been demonstrated in many crops and expected to play an important role in radish disease resistance breeding. Over 70 disease resistance genes (R-genes) at the molecular level have been isolated and characterized from various plant species (Joshi et al., 2011). Most of the R-genes isolated in plants have one or more conserved functional domains, such as nucleotide binding sites (NBS), leucine-rich repeat (LRR), receptor-like transmembrane kinase (RLK), transmembrane

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Table 1
The radish genotypes and their disease resistance phenotypes.

No.	Accession	Root skin	Root shape	Leaf shape	Origin	Disease index	Resistance
1	NAU-YZHKG	Red	Cylindrical	Divided	Chongqing, PRC	45	MR
2	NAU-YZ	Red	Cylindrical	Divided	Chongqing, PRC	45.75	MR
3	NAU-ZSYZH	Red	Cylindrical	Divided	Chongqing, PRC	45.35	MR
4	NAU-TYZQH	Red	Cylindrical	Divided	Nanjing, PRC	23.45	R
5	NAU-LLDHP	Red	Cylindrical	Lathy	Xuzhou, PRC	0.55	HR
6	NAU-SJMSH	Red	Cylindrical	Lathy	Chengdu, PRC	47.7	MR
7	NAU-FFYH	Red	Cylindrical	Lathy	Wuhan, PRC	19.15	R
8	NAU-SH	Red	Cylindrical	Lathy	Wuhan, PRC	15.3	R
9	NAU-NJH	Red	Cylindrical	Divided	Nanjing, PRC	1.05	HR
10	NAU-LLSG	Red	Cylindrical	Lathy	Xuzhou, PRC	6.45	HR
11	NAU-LLHH	Red	Cylindrical	Lathy	Nanjing, PRC	20.75	R
12	NauAU-XZH	Red	Cylindrical	Lathy	Changzhou, PRC	8.45	HR
13	NAU-JNHS	Red	Cylindrical	Lathy	Jinan, PRC	9.7	HR
14	NAU-LLYH	Red	Oblate	Lathy	Nanjing, PRC	1.7	HR
15	NAU-YDH	Red to white	Cylindrical	Lathy	Hangzhou, PRC	30.45	R
16	NAU-XBC	White	Cylindrical	Divided	Nanjing, PRC	45.45	MR
17	NAU-NS40DG	White	Cylindrical	Lathy	Wuhan, PRC	15.4	R
18	NAU-LLDY	White	Cylindrical	Lathy	Guangzhou, PRC	49.65	MR
19	NAU-ZDC	White	Cylindrical	Divided	Hangzhou, PRC	47.3	MR
20	NAU-NPZW	White	Cylindrical	Divided	Guangzhou, PRC	39.6	MR
21	NAU-DCC	White	Cylindrical	Divided	South Korea	28.4	R
22	NAU-XCY	White	Cylindrical	Divided	South Korea	26	R
23	NAU-BXCHH	White	Cylindrical	Divided	Nanjing, PRC	22	R
24	NAU-LU127	White	Cylindrical	Divided	Nanjing, PRC	29.55	R
25	NAU-YBLB	White	Globular	Divided	Yangzhou, PRC	60.2	S
26	NAU-LSZ	White	Oblate	Lathy	Chengdu, PRC	30	R
27	NAU-RG06	White	Globular	Divided	Nantong, PRC	32.25	R
28	NAU-XLM	Green to white	Cylindrical	Lathy	Beijing, PRC	69.05	S
29	NAU-LCQ	Green	Cylindrical	Divided	Jinan, PRC	4.3	HR
30	NAU-SGQT	Green to white	Cylindrical	Divided	Jinan, PRC	16	R

(TM) and cytoplasmic protein kinase (PK) (Sanseverino et al., 2009). Similar sequences could be mined according to the sequence information of the conserved domains from other plant species and known as resistance gene analogs (RGAs). With the development of genomics programs, more RGAs have been isolated via data mining, making the RGAs serve as molecular markers or combine with other molecular markers for plant genetic mapping, gene tagging and cultivar fingerprinting possible (Xiao et al., 2006). For instance, an STS marker based on the resistance gene-analog polymorphism was developed in wheat to tag *Yr26* against wheat stripe rust (Wen et al., 2008). In grapevine, four candidate RGA-SSCP markers with significant associations to resistance to downy mildew and anthracnose were identified (Tantasawat et al., 2012). Identifying more RGAs and developing molecular markers for resistance analysis would enhance the efficiency of crop breeding for disease resistances significantly.

As a fairly new marker technique, TRAP fixed primers are derived from expressed sequence tag (EST) sequences of the database or gene sequences, and arbitrary primers are designed with either an AT- or GC-rich core to anneal with an intron or exon, respectively. TRAP approach has been successfully used in many crops, including lettuce, sugarcane, *Porphyra*, *Pelargonium*, *Spinacia oleracea*, sunflower and wheat (Alwala et al., 2006; Qiao et al., 2007; Palumbo et al., 2007; Hu et al., 2007; Yue et al., 2009). Many reports have shown that TRAP is a very effective tool in germplasm evaluation and genetic linkage mapping of interested traits (Suman et al., 2012; Chen et al., 2012; Xie et al., 2012). The cultivar identification diagram (CID) is a new approach to identify plant cultivars efficiently by using polymorphic markers (Korir et al., 2012). This new approach developed allowed DNA markers to be more efficiently utilized in distinguishing plant cultivars.

To demonstrate the applicability of TRAP in radish, we designed fix primers with EST or RGAs sequences to generate TRAP markers and applied them to genetic diversity analysis of 30 radish genotypes. Since these polymorphic TRAP markers were generated with RGAs, we examined the relationship among these marker genotypes with the TuMV resistance phenotypes among the 30 radish genotypes. A cultivar identification diagram (CID) was made manually to discriminate these genotypes based on the polymorphic TRAP markers.

2. Materials and methods

2.1. Plant materials

Two radish genotypes, 'NAU-YH' (resistant to TuMV) and 'NAU-DY' (susceptible to TuMV) were used to test the polymorphism of the primers which were utilized in TRAP markers. A set of 30 radish advanced inbred lines which were evaluated for the resistance to TuMV during the seedling stage were selected for genetic diversity analysis by TRAP (Table 1).

Mechanical inoculation of TuMV pathogens was carried out at the third-leaf stage of radish according to the report of Ma et al. (2010). The resistance of genotypes was evaluated according to the disease index (DI) and the resistance levels based on the disease index (DI) were divided into five classes of high resistance (HR) with $0.00 < DI \leq 11.11$, resistance (*R*) with $11.11 < DI \leq 33.3$, medium resistance (MR) with $33.3 < DI \leq 55.5$, susceptibility (*S*) with $55.5 < DI \leq 77.8$ and high susceptibility (HS) with $77.8 < DI$ (Fig. 1).

2.2. Genomic DNA extraction

Total DNA was isolated from leaves using a modified CTAB-chloroform-isoamyl alcohol procedure (Liu et al., 2008), and the DNA was diluted to a final concentration of 20 ng/μl with 1× TE buffer and stored at 4 or −20 °C for further use.

2.3. Data mining of RGAs

The selection of RGAs was referenced to resistance genes which have been isolated in plant species conferring resistance against

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