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Original Article

Development of a Mitochondrial SCAR Marker Related to Susceptibility of Banana (*Musa* AAA Cavendish) to *Fusarium oxysporum* f. sp. *Cubense* Race 4

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Abstract

The use of resistant cultivars is an effective method for the control of banana (*Musa* spp.) Fusarium wilt caused by race 4 of *Fusarium oxysporum* f. sp. *cubense* (*Foc*4). However, selection of disease-resistant cultivars requires large-scale field evaluations and is time-consuming. Development of early, reliable, and reproducible selection strategies can speed up this process. Sequence characterized amplified region (SCAR) markers have been widely employed in the resistant breeding of many crops. However, to date, there have been no reports about the presence of plant disease resistance-related SCAR markers in mitochondrial genome yet, which also plays a very important role in plant defenses. In the present study, a sequence-related amplified polymorphism (SRAP) marker, a specific fragment of 829 bp, was identified. This fragment could be amplified from *Foc*4-susceptible but not from the resistant cultivars. It was located in banana mitochondrial genome and mapped near the putative cytochrome c biogenesis ccmB-like mitochondrial protein. This fragment was then successfully converted into a SCAR marker, namely Mito-Foc-S001, which was found to be able to discriminate the resistance from susceptibility to Fusarium wilt disease of bananas with the discriminatory power of the new mark being 96.88%. Thus, this marker can be used in banana (*Musa* AAA Cavendish) breeding for Fusarium wilt disease resistance.

Keywords: banana cultivars; mitochondrial genome; PCR amplifications; SCAR marker; sequencing
Abbreviatios: CTAB: cetyltrimethyl ammonium bromide; EDTA: ethylene diamine tetraacetic acid; PVP: polyvinyl pyrrolidone; RAPD: random amplified polymorphic DNA; SCAR: sequence characterized amplified region; SRAP: sequence-related amplified polymorphism

Introduction

Banana and plantain (*Musa* spp.) are the fourth and the second most important food and fruit crop in the world, respectively (FAO, 2017). Banana and plantain are also the most important fresh fruit in international trade market in terms of production and consumption volume (FAO, 2013). Banana Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most devastating diseases, especially in Asia and Africa. *Foc* has been classified into four physiological races according to the symptoms of different banana cultivars (Pegg *et al*., 1996; Bentley *et al*., 1998). Among them, *Foc* race 4 (*Foc*4) is the most virulent one (Sun *et al*., 2010). This race was first reported in Taiwan in 1969 (Sun *et al*., 1978) and subsequently spread to Australia, Africa and a number of Asian countries (Hwang and Ko, 2004). In China, banana Fusarium wilt caused by *Foc*4 has been successively reported in Guangdong, Guangxi, Fujian, Hainan provinces, including nearly all the main banana-production areas (Li *et al*., 2011). To date, this disease has become the key factor seriously limiting banana production in China (You *et al*., 2008). Since Fusarium wilt is a soil-borne disease, there has been no chemical control approach available and the only way to control it is to plant the cultivars resistant/tolerant to this pathogen (Ploetz, 2006).

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Conventional banana breeding is mainly hampered by the fact that most cultivars are triploid, parthenocarpy and thereafter, sterility. Biotechnological methods can contribute significantly to the genetic improvement of agronomic traits of banana cultivars, including disease resistance (Xu *et al*., 2011; Kovács *et al*., 2013; Umber *et al*., 2016). However, selection of disease-resistant somaclones requires large-scale field evaluations and is time-consuming (Javed *et al*., 2004) whereas sometimes the susceptible plants have a chance to escape detection (Burger *et al*., 2003) due to various other factors. Thus, development of early, reliable, and reproducible selection strategies can speed up the selection procedure and, eventually, improvement of banana production and quality (Javed and Othman, 2005).

Molecular biomarker technologies have become the powerful tools in crop improvement through their applications in germplasm characterization and fingerprinting, genetic analysis, linkage mapping, and molecular breeding. Sequence characterized amplified regions (SCARs) were originally derived from eight random amplified polymorphic DNA (RAPD) markers linked to disease resistance genes in lettuce (Paran and Michelmore, 1993). SCARs are PCR-based markers that represent single, genetically defined loci identified by PCR amplification of genomic DNA with pairs of specific oligonucleotide primers. They may contain high-copy, dispersed genomic sequences within the amplified region (Paran and Michelmore, 1993). Today, SCAR markers have been widely employed in the resistant breeding of many crops, such as wheat (*Triticum aestivum* L.) (Mandoulakani *et al*., 2015), tomato (*Solanum lycopersicum* L.) (Panthee and Ibrahem, 2012), sweetpotato (*Ipomoea batatas*) (Nakayama *et al*., 2012), sorghum [*Sorghum bicolor* (L.) Moench] (Singh *et al*., 2006), cotton (*Gossypium hirsutum* L.) (Wang *et al*., 2009), barley (*Hordeum vulgare*) (Genger *et al*., 2003) as well as banana (Wang *et al*., 2012; Cunha *et al*., 2015; Das *et al*., 2016; Silva *et al*., 2016).

However, all the reported SCAR markers linked to plant resistance are located in nuclear chromosomes. Mitochondria are one of the most plant cell organelles and have been suggested to play a very important role in plant defenses (Gleason *et al*., 2011; Laluk *et al.,* 2011; Ambrosio *et al*., 2013; Qamar *et al*., 2015; Fuchs *et al*., 2016). Furthermore, mitochondrial DNA (mtDNA) is also widely used as molecular markers in the fields of systematic evolution, cytoplasmic male sterility and so on (Ji *et al*., 2014; Gao *et al*., 2015; Shu *et al*., 2016). However, to date, there have been no reports about the presence of plant disease resistance or susceptibility-related SCAR markers in mitochondrial genome yet. Whether SCAR markers related to plant disease susceptibility or resistance are present in mitochondrial genome remains to be determined.

In the present study, we developed a novel SCAR marker, which was located in mitochondrial genome and associated with *Foc*4 susceptibility of *Musa* AAA Cavendish, through sequence-related amplified polymorphism (SRAP)-based screening. This marker could discriminate the resistance from susceptibility to Fusarium wilt disease of bananas with the discriminatory power of the new mark being 96.88%. The availability of this novel SCAR marker will provide an essential tool to diagnose *Foc* resistant germplasm and facilitate molecular marker-assisted selection of novel banana cultivars resistant to this pathogen.

Materials and Methods

Plant material

A total of thirty-two banana (*Musa* AAA Cavendish) cultivars with different degrees of resistance or susceptibility to *Foc*4 were used in the present study and their characteristics were presented in Table 1. The test of their resistance or susceptibility was carried out in the inoculated field for identification of resistant banana germplasm after a 3-year period (from 2012-2015) of observation and investigation.

DNA extraction

DNA was extracted by a modified cetyltrimethyl ammonium bromide (CTAB) method described by Javed *et al*. (2004) with modifications. In brief, fresh leaves (0.2 g) were ground to powder in liquid nitrogen with cross-linked polyvinyl pyrrolidone (PVP), suspended in 800 μL of preheated extraction buffer [4% CTAB, 1.4 M NaCl, 20 mM ethylene diamine tetraacetic acid (EDTA), 100 mM Tris-HCl [pH 8.0] and 1% 2-mercaptoethanol at 65 °C for 30 min, followed by centrifugation at 12000 rpm for 10 min. The supernatant was saved and extracted twice with equal volumes of chloroform/isoamyl alcohol (24:1). The supernatant was combined and centrifuged at 12000 rpm for 6 min. DNA was pelleted with equal volumes of pre-cooled isopropanol (-20 °C). After being rinsed with 75% pre-cooled ethanol, DNA sample was dried under vacuum and then dissolved in 50 μL of TE buffer [10 mM Tris-Hcl (pH 8.0), 1 mM EDTA, pH 8.0]. The DNA concentration was determined with an ultra low volume spectrometer (BioDrop μLite, Biochrom/UK). The concentration was adjusted to 50 ng.μL^{-1} with ddH_2O before use.

Cloning and sequencing of SRAP products

For screening the molecular markers involved in *Foc*4 resistance of banana, three susceptible ('Baxijiao', 'NK4-1', and 'L3-3') and four resistant cultivars ('Kangku 1', 'Nantiahuang', 'Kangku 5', 'Nongke 1') were used as the plant materials (Table 1). One hundred pairs of primers with each primer containing the original 10 bases were synthesized and used for SRAP, which are available from Thermo Fisher (China) Co., LTD. Guangzhou Branch (Table 2).

PCR amplifications were performed according to the manufacturers' instructions and run in a Bio-Rad PCR (Bio-Rad T100, Bio-Rad, Hercules, CA, USA) with a total reaction mixture of 25 μL, containing 2.5 μL of 10×buffer (with Mg^{2+}), 2μL of 0.25 mM dNTPs, reverse and inverse primers at a final concentration of 0.4 μM, 1 μL of DNA template (50 ng.μL^{-1}) and 1.5 unit of TaqDNA polymerase (Tiangen, Beijing, China). Reactions were programmed to an initial denaturation for 5 min at 94 °C, followed by 5 cycles as follows: 1 min at 94 °C denaturation, 1 min at 35 °C annealing and 1 min at 72 °C elongation and then by 35 cycles consisting of 1 min at 94 °C denaturing, 1 min at 50 °C annealing and 1 min at 72 °C elongation, and final elongation at 72 °C for 8 min.

Table 1. The 34 banana（*Musa* AAA Cavendish）cultivars and their reactions to *Foc*4 in the field and predicted by the SCAR marker

Code	Cultivar	Morbidity to *Foc*4 natural in the field (%)	Resistance evaluation to *Foc*4 in the field	Predicted by the SCAR marker
1	'Baxijiao'	100.0	HS	S
2	'NK4-1'	100.0	HS	S
3	'L1N2'	80.0	S	S
4	'L3-3'	100.0	HS	S
5	'WS2'	97.1	HS	S
6	'Nantianqing'	41.7	MR	R
7	'Dongjiao 1'	36.0	MR	R
8	'Kangku 1'	25.1	R	R
9	'Somalia'	100.0	HS	S
10	'Tianbao Ai'	100.0	HS	S
11	'Williams'	100.0	HS	S
12	'Longzhou Zhongba'	100.0	HS	S
13	'Cuba Xiangya'	100.0	HS	S
14	'G6-2'	26.3	R	R
15	'Yueke 1'	30.5	MR	R
16	'Nongke 1'	42.9	MR	R
17	'Kangku 5'	5.0	HR	R
18	'Zhangxuan 8-1'	90.0	S	S
19	'Guangdong 2'	100.0	HS	S
20	'Dongguan Gaoba'	100.0	HS	S
21	'Qiwei'	100.0	HS	S
22	'Honghe Ai'	100.0	HS	S
23	'Pubei Ai'	100.0	HS	S
24	'Moxigexiangjiao'	90.0	S	S
25	'Dongguan Zhongba'	90.0	S	S
26	'Hekou Gaoba'	100.0	HS	S
27	'Helanxiangjiao'	100.0	HS	S
28	'Beida Ai'	90.0	S	S
29	'Nalong Zhongba'	100.0	HS	S
30	'Nantianhuang'	50.0	MR	R
31	'BXM51'	34.0	MR	R
32	'Ke 2'	55.0	MS	R

HR: highly resistant (morbidity≤10%); R: resistant (10%＜morbidity≤30%);
MR: moderately resistant (30%＜morbidity≤50%);
MS: moderately susceptible (50%＜morbidity≤70%);
S: susceptible (70%＜morbidity≤90%);
HS: highly susceptible (morbidity＞90.0%);
*Foc*4: *Fusarium oxysporum* f. sp. *cubense* race 4;
SCAR: sequence characterized amplified region.

Table 2. Primers for PCR and sequences of SRAP primer sets

Forward primer	Sequence (5′-3′)	Reverse primer	Sequence (5′-3′)
me1	5′-TGAGTCCAAACCGGATA-3′	em1	5′-GACTGCGTACGAATTCAA-3′
me2	5′-TGAGTCCAAACCGGAGC-3′	em2	5′-GACTGCGTACGAATTCTG-3′
me3	5′-TGAGTCCAAACCGGACC-3′	em3	5′-GACTGCGTACGAATTGAC-3′
me4	5′-TGAGTCCAAACCGGACA-3′	em4	5′-GACTGCGTACGAATTTGA-3′
me5	5′-TGAGTCCAAACCGGTGC-3′	em5	5′-GACTGCGTACGAATTAAC-3′
me6	5′-TGAGTCCAAACCGGAGA-3′	em6	5′-GACTGCGTACGAATTGCA-3′
me7	5′-TGAGTCCAAACCGGACG-3′	em7	5′-GACTGCGTACGAATTGAG-3′
me8	5′-TGAGTCCAAACCGGAAA-3′	em8	5′-GACTGCGTACGAATTGCC-3′
me9	5′-TGAGTCCAAACCGGAAC-3′	em9	5′-GACTGCGTACGAATTTCA-3′
me10	5′-TGAGTCCAAACCGGAAT-3′	em10	5′-GACTGCGTACGAATTCAT-3′

SRAP: sequence-related amplified polymorphism.

After amplification, 4 uL of 6 × loading buffer was added to the PCR amplified products. 8-10 μL of PCR products were fractionated by gel electrophoresis on 1.8% (w/v) agarose gels in 0.5 × Tris-borate-EDTA (TBE) buffer (pH 8.0). Gel was run at 120 V for 1 h, stained with ethidium bromide, visualized on a UV transilluminator and photographed with gel imaging system (UVI FireReader, UK).

The amplified products of the linked SRAP were excised from agarose gels, and the DNA was purified by Tiangel Midi purification kit (Tiangen) according to the manufacturers' instructions followed by sending to Thermo Fisher (China) Co., LTD. Guangzhou Branch for sequencing. The purified products was amplified with BigDye® Terminator v3.1 Cycle Sequencing Kit [Thermo Fisher Scientific, Applied Biosystems, Foster City, CA, USA], and the PCR products were purified using the Mag-Bind® RxnPure Plus (OmegaBiotek, Norcross, GA, USA). Finally, the products were sequenced using a 3730xl DNA analyzer [Thermo Fisher Scientific (Applied Biosystems)].

SCAR design and analysis

The sequence obtained from SRAP was analyzed by MAGE6.0 Software, followed by blasting in NCBI and *http://banana-genome-hub.southgreen.fr/blast*. Four pairs of primers for SCAR were designed with NCBI/primer-BLAST based on the sequence. They were also synthesized by Thermo Fisher (China) Co., LTD. Guangzhou Branch (Table 3). To screen and develop the SCAR marker, DNA samples isolated from three resistant cultivars ('Nantianqing', 'Dongjiao 1', and 'Kangku 1') and five susceptible ones ('Baxijiao', 'NK4-1', 'L1N2', 'L3-3', and 'WS2') were used as the templates, respectively. The optimized PCR reaction mixture was 20 μL in total volume, containing 2 μL of 10×buffer (with Mg^{2+}), 2 μL of dNTPs (2.5 mM), 1 μL of reverse and inverse primers (10 μM), 1 μL of DNA (50 ng.μL^{-1}) and 1.0 unit of TaqDNA polymerase. Reactions were programmed to an initial denaturation at 94 °C for 5 min, and then to 35 cycles as follows: 30 s at 94 °C denaturation, 30 s at 60 °C annealing and 30 s at 72 °C elongation. The final elongation step was prolonged to 7 min at 72 °C.

PCR products were fractionated by gel electrophoresis on 1.2% (w/v) agarose gels in 0.5 × TBE [pH 8.0]) buffer. Gel was run at 110 V for 25 min, stained with ethidium bromide, visualized on a UV transilluminator and photographed with gel imaging system.

Validation of the SCAR marker

The linked SCAR marker obtained in the present study was validated with another 24 banana cultivars with different degrees of resistance to *Foc*4 (cultivars No. 9-32 in Table 1), including the two cultivars originally used as the templates of SRAP. PCR reaction system was referred to the method described in 1.4.

Results

DNA quality

The quality of the extracted DNA was checked with BioDrop μLite (Biochrom/UK). The concentration of DNA samples was between 80 and 150 ng.μL^{-1}, the OD_{260}/OD_{280} ratios of all the samples were in the range of 1.8 to 2.0, indicating that the quality is good enough to be used for further experiment.

Screening of SRAP molecular markers involved in disease resistance

As shown in Fig. 1, a special band of 839 bp was amplified from DNA templates derived from three susceptible cultivars ('Baxijiao', 'NK4-1', and 'L3-3') by PCR with primer me6-em1 out of 100 pairs of SRAP primers tested. However, it was absent in all the resistant cultivars.

Development of a SCAR marker involved in banana resistance to Foc4

The PCR amplified products were collected from cultivars 'Baxijiao', 'NK4-1', and 'L3-3'. The qualities and concentrations were good enough. These products were sequenced and compared with that of MAGE6.0. The result showed that their sequences were exactly the same.

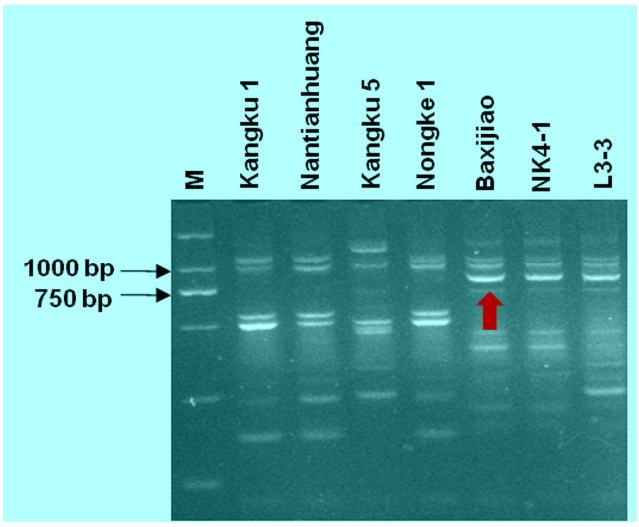

Fig. 1. The PCR products amplified with SRAP primer set (me6-em1) in 7 banana (*Musa* AAA Cavendish) cultivars either resistant or susceptible to *Foc*4. M: 2000 bp marker; lanes 1-4: resistant cultivars; lanes 5-76: susceptible cultivars; *Foc*4: *Fusarium oxysporum* f. sp. *cubense* race 4; SRAP: sequence-related amplified polymorphism. Red arrow indicates the specific band of 829 bp

Table 3. Primers for PCR and sequences of SCAR primer sets

Primer set	Sequence of primer(5′-3′)	Tm	Product length (bp)
SC1/SC2	CTCGCCGACACCTTACTTGA/AGGAGAGTCCAGCTCCAGTT	60	324
SC3/SC4	CTTATTGCTTCCTCGCCGAC/CAGTAGGGGTCTCGTTGCTT	59	293
SC5/SC6	CTCGCCGACACCTTACTTGAT/GGGGTCTCGTTGCTTGTCTC	60-61	277
SC7/SC8	TCGCCGACACCTTACTTGAT/ACAGTAGGGGTCTCGTTGCT	59	282

SCAR: sequence characterized amplified region

No assignment to a particular chromosome or resemblance with known resistance or susceptibility genes was revealed when blasted this fragment in NCBI. However, when being compared with Banana Genome Hub database (*http://banana-genome-hub.southgreen.fr/blast*), it showed a 99.64% similarity to a fragment located in mito4 of the *M. acuminata* spp. *malaccensis* accession Pahang ($2n$ = 22) genome (start from the third nucleotide). This fragment is located between nucleotides 145, 680 and 146, 506 and mapped near the putative cytochrome c biogenesis ccmB-like mitochondrial protein.

To develop SCAR markers, we selected four pairs of primers presented in Table 3 to amplify the PCR products from 8 banana cultivars with different degrees of resistance to *Foc*4.

Fig. 2 showed that a single and clear band of 324 bp was amplified with primers SC1 and SC2 and appeared only in five susceptible cultivars ('Baxijiao', 'NK4-1', 'L1N2', 'L3-3', and 'WS2') but not in the three resistant ones ('Nantianqing', 'Dongjiao 1', and 'Kangku 1'). This fragment is located in the position from 216 to 539 bp of the original SRAP band (located between the mitochondrial genome nucleotides 145, 894 and 146, 217). The quality of the band was good enough to serve as a SCAR marker and thus, was considered a suitable candidate for the development of the SCAR marker, and finally selected. On the other hand, some other special bands were also obtained with the other three pairs of primers, which also could distinguish the resistant cultivars from the susceptible ones; however, their qualities were not good enough to be used as a SCAR marker (data not shown).

Validation of the SCAR marker

To validate the SCAR marker obtained, we used 24 banana cultivars with different degrees of resistance, including the two cultivars originally used as the templates of SRAP. The results from the PCR amplification with primers SC1 and SC2 indicated a high degree of specificity of this SCAR marker (namely Mito-Foc-S001), because a 324 bp band appeared in all the 17 susceptible cultivars tested ('Somalixiangjiao', 'Tianbao Ai', 'Williams', 'Longzhou Zhongba', 'Cuba Xiangya', 'Zhangxuan 8-1', 'Guangdong 2', 'Dongguan Gaoba', 'Qiwei', 'Honghe Ai', 'Pubei Ai', 'Moxigexiangjiao', 'Dongguan Zhongba', 'Hekou Gaoba', 'Helanxiangjiao', 'Beida Ai', and 'Nalong Zhongba') but was absent in the 6 resistant ones ('G6-2', 'Yueke 1', 'Nongke 1', 'Kangku 5', 'Nantianhuang', and 'BXM51') and 1 susceptible one ('Ke 2') (Fig. 3).

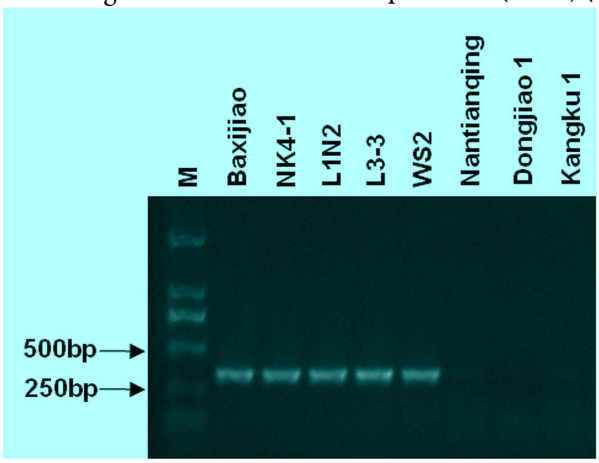

Fig. 2. The PCR products amplified with DNA templates derived from eight banana (*Musa* spp. AAA Cavendish) cultivars with SCAR primer set (SC1-SC2) either resistant or susceptible to *Foc*4. M: 2000 bp marker; lanes 1-5: susceptible cultivars; lanes 6-8: resistant cultivars; *Foc*4: *Fusarium oxysporum* f. sp. *cubense* race 4; SCAR: sequence characterized amplified region

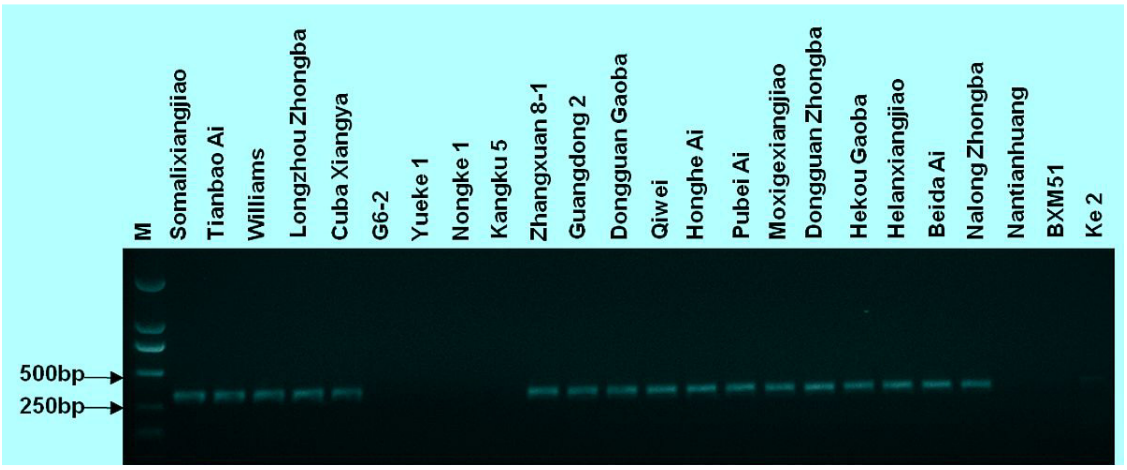

Fig. 3. Analysis of the SCAR marker in 24 banana (*Musa* spp. AAA Cavendish) cultivars either resistant or susceptible to *Foc*4. M: 2000 bp marker; lanes 1-5 and 10-21: susceptible cultivars; lanes 6-9 and 22-24: resistant cultivars; *Foc*4: *Fusarium oxysporum* f. sp. *cubense* race 4; SCAR: sequence characterized amplified region

The PCR amplification results of all 32 test banana cultivars, was in agreement with the resistance evaluation to *Foc*4 in the field for 31 out of 32, which indicates a discriminatory power of 96.88% (Figs. 2 and 3). This SCAR marker is reliable although there is a minimal error.

Discussion

As mentioned above, banana Fusarium wilt is one of the most devastating diseases found in a large majority of the banana-producing regions in the world except the Mediterranean, Melanesia, Somalia, and some islands in the South Pacific (Ploetz, 2006). To date, no methods for efficient control of this disease are available. The best way to control this disease is to plant the resistant cultivars. Identification of molecular markers linked to disease resistance is an excellent alternative to enable banana breeders to make marker-assisted selection for disease resistance without challenging the host with the pathogen. Javed and Othman (2005) studied the resistance of *Musa acuminata* to *Fusarium oxysporum* using the technique of RAPD. The SCAR markers associated with the resistance to this disease was first reported by Wang *et al*. (2012). They detected the resistance of clones from 'Williams' (*Musa* spp. AAA) using two SCAR markers, namely ScaU1001 and ScaS0901, which were amplified in 'Gold finger' (*Musa* spp. AAAB) and one clone from 'Williams'. These SCAR markers could not be detected in 5 susceptible cultivars tested. Recently, Cunha *et al*. (2015) generated a SCAR marker from RAPD markers which could identify susceptibility to *Fusarium oxysporum* f. sp. *cubense* for some banana cultivars belonging deferent genotypes in South Brazil. They validated this SCAR marker on 28 banana cultivars resistant or susceptible to infection by *Foc*. Their result showed that this new marker could discriminate two contrasting groups from each other with the discriminatory power of 93%.

In the present study, we identified a novel and specific SCAR marker from 100 pairs of SRAP primers associated with susceptibility to *Foc*4. We tested the specificity of this marker against a total of 32 banana cultivars exhibiting different degrees of resistance or susceptibility to *Foc*4. Nine resistant cultivars including the most resistant germplasm in China and a few from other countries and 23 susceptible cultivars including the main cultivars grown in China and some other countries as well as a number of the local cultivars in China were purposely selected, respectively. The new SCAR marker, namely Mito-Foc-S001, could distinguish the resistant group from the susceptible one with the discriminatory power of 96.88%. The sequence of PCR product from primers SC1 and SC2 had high homology with a fragment in mito4 of banana, indicating that this SRAP-based SCAR marker is a gene located in the mitochondrial genome instead of in the nuclear chromosomes. Though no a particular chromosome or resemblance with known resistance or susceptibility genes have been assigned with the fragment identified in this study, further developments may enable us to reveal similarities of this sequence to the known genes.

Mitochondria are semi-autonomous organelles that possess their own genomes and are able to synthesize a small number of proteins essential for the functions of mitochondrial respiratory chain. They are the main fuel stations of eukaryotic cells because they are a major source of ATP. In addition, they are vital for cellular homeostasis, participating in diverse metabolic pathways, calcium signalling and reactive oxygen species buffering or production. These functions link mitochondrial activities to the environment and with other cellular compartments such as chloroplast (Welchen *et al*., 2014). Many earlier reports revealed that mitochondria contributed substantially to responses to plant stress and defense responses (Gleason *et al*., 2011; Laluk *et al*., 2011; Ambrosio *et al*., 2013; Qamar *et al*., 2015; Fuchs *et al*., 2016) and/or play a fundamental role in innate immunity signalling (Vellosillo *et al*., 2013). Defects in mitochondrial functions could increase susceptibility of plants to pathogens (Pu *et al*., 2016). Thus, it was suggested that the SCAR marker obtained in the present study also likely plays a very important role in banana susceptibility to *Foc*4. However, no assignment to a particular chromosome or resemblance with known resistance of susceptibility genes was revealed. The information about this novel marker is quite limited. Further work should be focused on acquiring these pieces of information and investigating the specific functions of mitochondrial genes in banana defenses.

Conclusions

Our results demonstrate that the SCAR marker Mito-Foc-S001, could discriminate the resistance from susceptibility to Fusarium wilt disease of bananas with the discriminatory power of the new marker being 96.88%. The application of the marker is simple and efficient, and has a great advantage for the screening of large number of banana individuals, since only DNA extracted and a simple PCR are required. Thus, this marker can be used in banana (*Musa* AAA Cavendish) breeding for Fusarium wilt disease resistance.

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