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Molecular Markers and Their Application in Fusarium Wilt Studies in *Musa* spp.

(Penanda Molekul dan Aplikasinya dalam Kajian Layu Fusarium pada *Musa* spp.)

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ABSTRACT

Bananas and plantains (Musa *spp.*) *are an important socio-economic fruit crop grown worldwide. Their production across the regions where they are grown is largely hampered by pests and diseases. Fusarium wilt is a disastrous diseases of bananas caused by the fungal pathogen* Fusarium oxysporum f.sp. cubense (Foc). *Managing it with chemicals, biological control agents and cultural methods is ineffective. Host plant resistance is the most effective and durable approach of managing most pest and disease epidemics in most plant species and could equally be effective in managing Fusarium wilt in bananas. Crossbreeding as one of the ways to introgress disease resistance genes and phenotyping for biotic and abiotic stresses currently used in banana breeding is apparently difficult to apply because of banana's low fertility, gigantic size, and long-life cycle which prolongs its breeding cycle. There is, therefore, a need to apply molecular markers in banana genetic improvement for Fusarium wilt resistance because of their accuracy, speed, robustness and effectiveness of operation. The objective of this article was to review and discuss molecular markers that have been successfully used in studying Fusarium wilt in bananas and some other important crops. Molecular markers discussed in this article include Random Amplified Polymorphic DNA, Sequence Characterized Amplified Region, Simple Sequence Repeat, Inter-Simple Sequence Repeat, and Single Nucleotide Polymorphism. The information discussed in this article informs future decisions to identify suitable marker systems for fine mapping of target regions and accelerated identification of quantitative trait loci for* Foc *resistance in bananas.*

Keywords: Banana; Fusarium oxysporum f.sp. cubense*; molecular markers; quantitative trait loci; resistance*

ABSTRAK

Pisang dan plantain (Musa *spp.*) *adalah tanaman buah-buahan sosio-ekonomi penting yang berkembang di seluruh dunia. Pengeluarannya merentasi sempadan dengan sebahagian besar penanamannya dibantutkan oleh penyakit dan perosak. Layu Fusarium adalah sejenis penyakit pisang yang disebabkan oleh kulat patogen* Fusarium oxysporum f.sp. cubense (Foc). *Kaedah mengawal penyakit ini dengan menggunakan bahan kimia, agen kawalan biologi atau kaedah kultur tidak berkesan. Kerintangan tumbuhan perumah adalah pendekatan yang paling berkesan dan bertahan lama untuk mengawal wabak penyakit dan perosak dalam kebanyakan spesies tumbuhan dan berkesan dalam menguruskan layu Fusarium pada pisang. Pembiakbakaan kacukan merupakan salah satu cara untuk introgres gen rintangan penyakit dan fenotip bagi tegasan biotik dan abiotiknya yang digunakan dalam penanaman pisang namun teknik ini sukar untuk diaplikasikan kerana kesuburan pisang yang rendah, saiz yang besar dan hayat kitaran yang panjang yang turut memanjangkan kitaran pembiakannya. Oleh itu, ada keperluan untuk menggunakan penanda molekul dalam meningkatkan genetik pisang untuk menghalang layu Fusarium kerana ketepatan, kelajuan, keteguhan dan keberkesanan operasinya. Objektif kajian ini adalah untuk meneliti dan membincangkan penanda molekul yang telah berjaya digunakan dalam mengkaji layu Fusarium pisang dan sesetengah tanaman lain yang penting. Penanda molekul yang dibincangkan dalam kertas ini merangkumi DNA Polimorfik Rawak Teramplifikasi, Rantau Jujukan Pencirian Teramplifikasi, Pengulangan Jujukan Mudah, Pengulangan Jujukan Inter-Mudah dan Polimorfisme Nukleotida Tunggal. Maklumat yang dibincangkan dalam kajian ini menunjukkan keputusan pada masa depan dalam mengenal pasti sistem penanda sesuai bagi pemetaan kawasan sasaran lengkap dan pemecutan pengenalpastian lokus khi kuantitatif untuk rintangan* Foc *dalam pisang.*

Kata kunci: Fusarium oxysporum f.sp. cubense*; lokus khi kuantitatif; penanda molekul; pisang*; *rintangan*

INTRODUCTION

Banana and plantains (*Musa* spp. hereafter referred to as banana) are among the most important fruits produced worldwide for food and commercial purposes (Brown et al. 2017; Looney 2016; Tourky et al. 2014). They are the second largest produced fruit after citrus (Tourky et al. 2014).

Banana is cultivated in more than 130 countries worldwide (FAOSTAT 2017). Its current global production is 161 million tonnes, of which close to 10% is deemed for export (FAOSTAT 2017). India is the largest producer of banana with 29.1 Million Metric Tons (MMT), followed by China (13.3 MMT) and the Philippines (8.9 MMT), all

of which contributes about 25% of worlds production (FAOSTAT 2016). Banana trade is valued at about US$ 600 million in the Philippines, US$ 8 million in Malaysia and in Bangladesh at about US$ 0.3 million annually (FAOSTAT 2016; Natsuaki 2011). In many countries of Africa, banana is largely a staple food crop to millions of people and is a source nutrients such as carbohydrates, vitamins and minerals and play a key role in food security (Dotto et al. 2018; Kennedy et al. 2018). By the year 2000, Uganda was the largest producer of bananas, followed by Tanzania and Nigeria among the African countries (Dotto et al. 2018; FAOSTAT 2000). However, according to FAOSTAT (2017), Uganda's banana production reduced substantially to more than a half (Table 1).

Diseases and pests are hypothesized to be the leading cause of the reduced banana production in all banana growing regions of the world (Brown et al. 2017; Ploetz et al. 2015; Tumuhimbise et al. 2018). Diseases caused by fungi, bacteria and viruses reduce the quality of the fruit before and after harvest (Ploetz et al. 2015). Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense* (*Foc*)) is one of the most disastrous fungal diseases of bananas (Ploetz 2015) that causes an estimated yield loss of 60-90% (Kumar et al. 2010). Fusarium wilt is wide spread across most banana growing regions of the world (Mostert et al. 2017). Breeding for host plant resistance is critically a high priority for controlling Fusarium wilt in bananas (Robinson et al. 1998). Progress in the conventional banana breeding however, has been restricted by factors such as low female fertility, polyploidy nature of the crop and long growth cycle resulting into long breeding cycle of 10-17 years (Brown et al. 2017; Nyine et al. 2018) (Figure 1). Phenotyping bananas particularly under controlled, contained and reproducible conditions, is difficult because of the large space required for growth and maintenance of plant populations (Viljoen et al. 2004).

The advent of molecular markers is a major breakthrough to overcome key limitations associated with conventional breeding in many crop species (Lema 2018). Molecular markers that are associated with traits of interest increase our knowledge and resources for the accelerated genetic improvement of crops and have thus been used for marker assisted selection (MAS) (Francia et al. 2005; Khan 2015). Linkage mapping (Figure 2) and genome-wide association studies (GWAS) are two methods used to establish genotype-phenotype relationships, thus establishing a foundation for the development of markers for resistance to pests and diseases (Migicovsky & Myles 2017). Application of molecular markers in genetic improvement of banana for disease resistance could be a suitable strategy to overcome the long and expensive breeding cycle.

Studies have shown that crops such as tomato, bananas cabbage, peas and watermelon contain similar resistance genes within their respective formae speciales of *Fusarium oxysporum*. *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) 14 'Secreted in xylem' (SIX) 1 gene, is a gene that carries 1-3 resistance gene in tomato that has homologs with many formae speciales (f.sp.) of *F. oxysporum* (Catanzariti & Jones 2010). SIX 1 gene homologs formae species includes, *Fusarium oxysporum* f.sp. *cubense* of bananas (*Musa* spp.), *Fusarium oxysporum* f.sp. *pisi* of peas (*Pisum sativum* L.), *Fusarium oxysporum* f.sp. *conglutinans* of cabbages (*Brassica oleracea*) and *Fusarium oxysporum* f.sp. *melonis* of water melon (*Cucumis melo*) (Li et al. 2016: van Dam et al. 2016; Widinugraheni et al. 2018). Consequently, the 1-3 genes were equally suggested to regulate Fusarium wilt in bananas (Widinugraheni et al. 2018). Azam et al. (2017) indicated that *Foc* 4 of bananas is closely related to the *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) of tomatoes by phylogenetic analysis and at the genome level. Therefore, molecular markers applied to study Fusarium wilt in tomatoes cabbages, peas and water melon could provide useful information on markers to be applied in Fusarium wilt studies in bananas.

Molecular markers that have been reported useful in studying the genetic basis of host plant resistance to Fusarium wilt in bananas and other important crop species are discussed in the subsequent sections.

TABLE 1. Banana and plantain production information in most banana-growing countries in Africa between the year 2000 (FAOSTAT 2000) and 2017 (FAOSTAT 2017)

	Area harvested (ha)		Quantity produced (Tonnes)		Yield (hg/ha)	
	Year 2000	Year 2017	Year 2000	Year 2017	Year 2000	Year 2017
Uganda	1,733,000	950,691	10,038,000	3,867,302	104,184	85,351
Tanzania	546,300	757,316	1,263,060	4,058,161	46,247	90,266
Nigeria	386000	489,946	1,969,000	3,164,878	51,010	64,095
Ghana	247900	370,562	1,942,500	4,138,906	107,642	222,963
Rwanda	360470	322,009	2,212,250	1,729,150	61,371	37,197
Cameroon	276,076	419,422	1,790,070	5,781,710	133,896	291,496
Ivory Coast	453,173	434,815	1,931,105	1,996,012	498,225	498,225
Kenya	76408	65,949	1,054,768	774,521	266,883	332,728
Burundi	273050	195,248	1,513,997	1,238,738	55,448	66,666
DR Congo	346,605	1,082,918	1,490,140	5,109,663	82,146	81,060

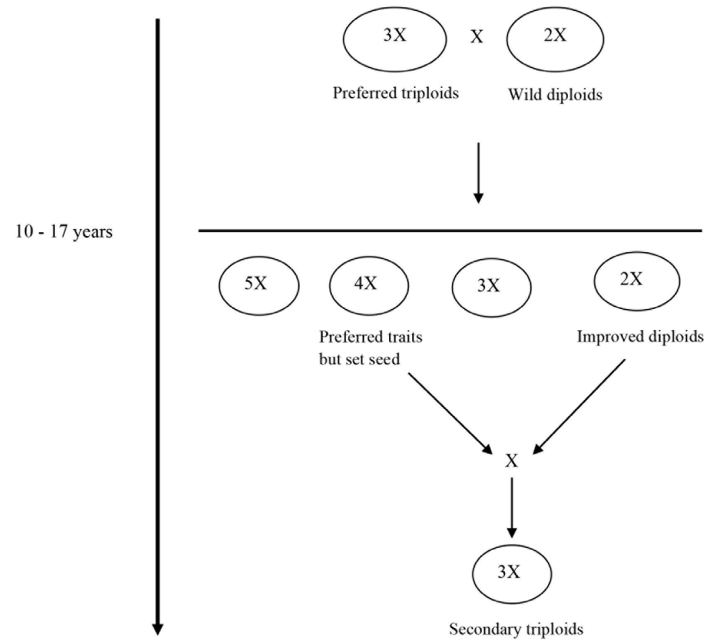

FIGURE 1. The slow and long banana breeding process that involves crossing diploids with triploids to generate tetraploids and the generated tetraploids crossed with improved diploids to generate tertiary triploids with required traits

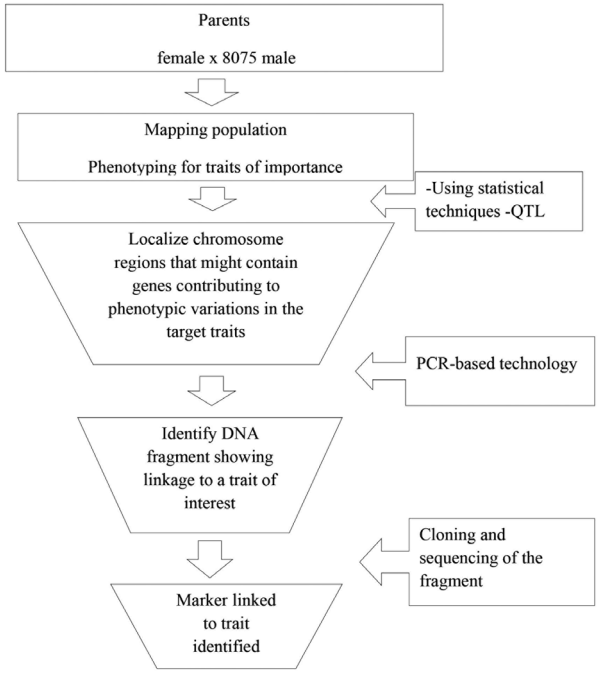

FIGURE 2. Flow chart showing procedures to identify molecular markers associated with traits of importance in plants

RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS

Randomly Amplified Polymorphic DNA (RAPD) is a PCR-based technology based on enzymatic amplification of target or random DNA segments with arbitrary primers. RAPD technique deduces DNA polymorphisms produced by rearrangements or deletions at or between oligonucleotide primer binding sites in the genome using short random oligonucleotide sequences (Adss et al. 2016; Mkada-Driss et al. 2014). RAPDs were among the first molecular markers used to study resistance to Fusarium wilt in bananas. Javed et al. (2004) applied RAPD markers to assess the genetic diversity of wild *Musa acuminata* ssp. *Malaccensis* in Malaysia which showed varying degrees of resistance to *Fusarium oxysporum* f.sp. *cubense* tropical race 4 (*Foc* TR4). In their study, the RAPD primers used provided a large amount of polymorphism (57.69 %) among the seedlings resistant and susceptible to *Foc* Race 4 (TR4). Three primers, primer OPA-03, primer 24 and primer 21 (Table 2), showed banding patterns specific to either resistant or susceptible seedlings. Likewise, Kayat et al. (2004) screened F_1 progenies derived from crossing *malaccensis* (selected male *Foc*-TR4 resistant (R) and female *Foc*-TR4 susceptible (S) to *Foc*-TR4) with *Foc*-TR4. The F_1 progenies were found to be segregating for *Foc*-TR4 and were screened with five RAPD primers. The RAPD primers were able to differentiate F_1 genotypes that were resistant and susceptible to *Foc*-TR4 (Table 2). Prasad et al. (2018) also identified two RAPD primers that could consistently identify bananas resistant to Fusarium wilt race 1. The primers OPN-06 produced bands 1500 bp, 200 bp that were consistent with resistant genotypes whereas the absence of a band at 600bp and presence of a band at 400 bp for primer OPR-07 was consistent with resistant genotypes as well (Table 2).

Besides banana, several studies have identified RAPD markers associated with Fusarium wilt resistance in crops of importance such as chicken pea (Soregaon & Ravikumar 2010; Tullu et al. 1998), watermelon (Wechter et al. 1995; Zheng & Wolff 2000).

TABLE 2. RAPD markers that have been used in studying Fusarium wilt in banana

Primer name	Type of marker	Sequence	Product length (bp)	TM°C	Application in bananas	Author
OPK-03	RAPD	CCAGCTTAGG	485	36	Band consistent with banana genotypes resistant to *Foc* 4 and absent in susceptible ones	(Zambrano et al. 2007)
OPA-03	RAPD	AGTCAGCCAC	400	36	Band consistent with banana genotypes Susceptible to *Foc* 4 and absent in resistant ones	(Javed et al. 2004)
primer 24	RAPD	GTGCGTATGG	300	36	Band consistent with banana genotypes Susceptible to *Foc* 4 and absent in resistant ones	(Javed et al. 2004)
primer 21	RAPD	CGCTGTCCTT	1000	36	Band consistent with banana genotypes resistant to *Foc* 4 and absent in susceptible ones	(Javed et al. 2004)
Primer 25	RAPD	GACAGACAGA	250	36	Band segregating within banana hybrids segregating for *Foc* 4	(Kayat et al. 2004)
Primer 27	RAPD	CTCTCCGCCA	350 and 680	36	Band segregating within banana hybrids segregating for *Foc* 4	(Kayat et al. 2004)
OPN 06	RAPD	GAGACGCACA	1500 and 200		Band consistent with banana genotypes resistant to *Foc* 4 and absent in susceptible ones	Prasad et al. (2018)
OPR 07	RAPD	ACTGGCCTGA	600		Band consistent with banana genotypes Susceptible to *Foc* 4 and absent in resistant ones	Prasad et al. (2018)
OPR 07	RAPD	ACTGGCCTGA	400		Band consistent with banana genotypes resistant to *Foc* 4 and absent in susceptible ones	Prasad et al. (2018)

RAPD markers have drawbacks that limit their application in plant studies, such as their dominance nature and low reproducibility. The latter requires highly standardized experimental procedures because of the markers' sensitivity to the reaction conditions (Jarocki et al. 2016). Moreover, RAPD markers generally require well purified and high molecular weight DNA (Athe et al. 2018).

SEQUENCE CHARACTERIZED AMPLIFIED REGION MARKERS

Sequence Characterized Amplified Region (SCARs) are DNA fragments amplified by the PCR using specific 15-30 bp primers, designed from nucleotide sequences established from cloned RAPD and Inter-simple sequence repeat (ISSR) fragments linked to a trait of interest (Nadeem et al. 2018). SCARs are advantageous simply because they are quick and easy to use, have higher reproducibility and are less sensitive to amplification conditions (Pathak 2015; Spooner et al. 2005).

In *Musa* spp. SCAR markers developed from RAPDs have been employed in several Fusarium wilt studies. For instance, Wang et al. (2018) screened banana genotypes (*Musa* spp. AAA Cavendish) that were either resistant or susceptible to Fusarium wilt race 4 with a Sequence Related Amplified Polymorphism (SRAP) marker Me6-em1 which was able to produce bands consistent with susceptible genotypes and absent in resistant genotypes. A SCAR marker SC1-SC2 was later developed from the SRAP marker Me6-em1 that amplified a band at 324 with susceptible genotypes and absent in genotypes resistant to *Foc* 4 (Table 3). Cunha et al. (2015) used a RAPD primer OPP-12 that generated a band of approximately 1700 bp which was consistent in 18 banana cultivars susceptible to Fusarium wilt. From the amplified band a primer denoted SuscPD-F and SuscPD-R (Table 3) was designed and this primer could consistently identify susceptible banana from resistant ones with an accuracy of 93%. Wang et al. (2012) identified RAPD arbitrary primers with two reproducible bands that could identify the two pools of bananas resistant and susceptible to *Foc* race 4. The authors successfully converted two bands into SCAR markers: ScaU1001 and ScaS0901 (Table 3) that were able to distinguish banana genotypes resistant to *Foc* race 4 from the susceptible genotypes. Zambrano et al. (2007) identified a RAPD marker OPK-03 (Table 2) that amplified a band at 485 bp within susceptible banana genotype while absent in the resistant ones. From this band, a SCAR marker that had the capacity distinguish susceptible clones from resistant/tolerant clones was developed.

SCAR markers have also been utilised in studying Fusarium wilt in other crops of importance such as *Fusarium oxysporum* Schlecht. *emend*. f.sp. *pisi* of peas (Okubara et al. 2005), *Fusarium udum* of Pigeon pea (Prasanthi et al. 2009).

TABLE 3. SCAR markers that have been used in studying Fusarium wilt in banana

Primer name	Scar name	Primer sequence		Product length (bp)	TM°C	Application in bananas	Author
		Forward 5'-3'	Reverse 5'-3'				
OPP-12	SuscPD	GAACCAGAGCCAGGGCATAG	TCTATGCGCCTACCCTCCTT	842	55.9	Band consistent with banana genotypes resistant to *Foc* and absent in susceptible ones	(Cunha et al. 2015)
OPU1001	ScaU1001	ACCTCGGCACTCGAAGACACAT	ACCTCGGCACTATTACCCATCAT	1694	55	Amplified in *Foc* 4- resistant banana genotypes ('Williams 8818-1' and Goldfinger)	(Wang et al. 2012)
OPU1001	ScaS0901	TCCTGGTCCCAGTACAAATAC	TCCTGGTCCCTCTGAATTTTC	1429	54	Amplified in *Foc* 4- resistant banana genotypes ('Williams 8818-1' and Goldfinger)	(Wang et al. 2012)
Me6-em1	SC1/SC2	CTCGCCGACACCTTACTTGA	AGGAGAGTCCAGCTCCAGTT	324	60	Band consistent with banana genotypes Susceptible to *Foc* 4 and absent in resistant ones	(Wang et al. 2018)

Like RAPDs, SCAR markers are dominant and detect single locus. They are disadvantageous in that they need sequence data to design the PCR primers, take long to develop and the procedure is expensive, a characteristic that has limited its application in crop studies (Spooner et al. 2005).

SIMPLE SEQUENCE REPEATS MARKERS

Simple sequence repeats (SSRs) are a stretch of repeats of short nucleotide motifs (dinucleotide, trinucleotide, tetranucleotide repeats) (Nadeem et al. 2018). They have advantages over RAPD and SCAR markers such as the multi-allelic nature, good genome wide coverage, high reproducibility and co-dominant nature that enables them to distinguish homozygotes from the heterozygotes (Athe et al. 2018). Owing to the numerous advantages of SSR markers, they have been used for diverse studies in crops species.

In *Musa* spp. for example, SSR markers have been used in several phylogenetic/diversity studies (Amorim et al. 2012; Irish et al. 2014; Kabir et al. 2015). In relation to Fusarium wilt of banana studies, Rebouças et al. (2018) applied SSR markers to study the genetic relationship of banana genotypes showing varying degrees of resistance to Fusarium wilt race 1. However, the groups formed by a dendrogram using SSR did not relate well to the susceptibility or resistance of the genotypes to Fusarium wilt race 1. Still, the SSR markers applied were able to detect genetic variability among the screened genotypes and such markers were suggested to contribute to improvement of bananas for Fusarium wilt resistance (Table 4). In a related study, Pushpakumari et al. (2009) studied the genetic diversity of 14 *Kolikuttu* accessions (AAB) exhibiting varying degrees of resistance to Fusarium wilt using SSR markers. The SSR markers could detect genetic diversity among the screened accessions but were unable to distinguish susceptible genotypes from resistance ones. These markers, however, could be of great importance in studying resistance to Fusarium wilt in bananas. Interestingly, SSR markers have been utilised to study other resistance in bananas. Among such studies, we can mention resistance to salinity (Miri et al. 2014) and resistance to black Sigatoka in *M. acuminata* subsp. *burmannicoides* var. Calcutta 4 (Miller et al. 2010).

Other than bananas, SSR markers have been utilised in studying Fusarium wilt resistance in other crops. For instance, Hawkins et al. (2001) used them to study resistance to *Fusarium oxysporum* f.sp. *niveum* of watermelon. Padaliya et al. (2013) and Patil et al. (2014) used the same type of markers to study resistance to *Fusarium oxysporum* f.sp. *ciceris* of chicken pea, and Lv et al. (2014) in *Fusarium oxysporum* f.sp. conglutinans of cabbages. In all those cases, the SSR markers were found to be associated with Fusarium wilt resistance.

The few disadvantages of SSR markers are that they expensive to generate, laborious to apply in certain species and they require prior sequence knowledge on the region flanking the repeat of interest to design PCR primers (Abdurakhmonov 2016). Having successfully employed SSR markers in studying Fusarium wilt in the above crops, there is a need to apply them in studying Fusarium wilt resistance in bananas.

INTER-SIMPLE SEQUENCE REPEAT MARKERS

Inter-simple sequence repeat (ISSR) are regions of DNA between 100 bp and 3 kb that are found between identical or different microsatellites of opposite orientation. This makes them directly amplifiable by PCR (Abate 2017).

ISSR markers have numerous advantages that make them easy to be applied in several crop studies including high reproducibility due to the longer primer length linkage to coding regions and their likelihood to mark gene-rich regions (Nkongolo et al. 2014). The development of ISSR does not need prior knowledge of the genome to be analysed, hence appropriate for plant genome analysis and are very polymorphic and most importantly highly variable and ubiquitously distributed across the genome (Ng & Tan 2015).

Owing to numerous advantages of ISSR markers, they have been used for several phylogenetic/diversity studies in *Musa* spp. (Borse et al. 2011; Khatri et al. 2011; Lamare & Rao 2015). Li et al. (2012) attempted to use ISSR markers to study banana resistance to Foc race 1. Their work was carried out on 6 banana varieties (Baxi, Yueke No.1, Yueke No.3, Kangku No.1, Fenza No.1, and Nongke No.1) with varying degrees of resistance to *Foc* race 4. The 19 ISSR primers used were able to detect good genetic diversity among the varieties. However, this diversity could not be correlated to *Foc* race 1 resistance.

Other than *Musa* spp., ISSR markers have been used in studying Fusarium wilt resistance in gladiolus (Kumar et al. 2016) and Chickpea Haji-Allahverdipoor et al. (2011). The disadvantages of ISSRs include their dominant nature making it impossible to detect heterozygotes, and the lack of knowledge of allelic bands produced (Gramaje et al. 2014). Owing to the numerous advantages and application in genetic diversity studies, ISSR markers show a potential to be utilised in Fusarium wilt resistance studies in bananas.

SINGLE NUCLEOTIDE POLYMORPHISM MARKERS

Single nucleotide polymorphisms (SNPs) refers to a single base change in a DNA sequence, with a usual alternative of two possible nucleotides (alelle) at a given position (Athe et al. 2018; Jehan & Lakhanpaul 2006). SNPs are the most abundant marker system in eukaryote genomes and are gaining popularity as the next generation of molecular markers for various applications (Arif et al. 2010; Kwon et al. 2019).

They are a new marker technology with several advantages such as high information content and depicting an extremely high level of polymorphisms (Jehan & Lakhanpaul 2006). SNPs are highly amenable to automation, thus eventually can become cost-effective. Since most of them are non-gel based, they are less

TABLE 4. SSR markers that have been used in studying Fusarium wilt in banana

Marker type	Primer name	Forward 5'-3'	Reverse 5'-3'	Primer name	Forward 5'-3'	Reverse 5'-3'	Application in bananas	Author
SSR	MaCEN3	GGAGGAGGAA GAGGAGAAGG	TGAACTGACA CCCTGAGCAC	MaCEN1	AGATGATGAC CCCCACCTC	TTCTCCTTATC CCGTGGTTG	Study the genetic relationship of banana genotypes showing varying degrees of resistance to Fusarium wilt race 1	Rebouças et al. (2018)
	MaCEN4	TGTCAGATAGG TCGGAGTTG	AGTGCTCTTG TTAGGTTTCC	MaC-CEN18	GCTTCGTAC CGCTCTCAC	GCGTTCATCC ATTTTCATC		
	MaCEN5	ATCTCGCTCA CCTCGTCTTC	TCATAGACAG CCCAGCAGAA	MaC-CEN19	TTCCTGCCT TTGCCTGTA	GGTTTACCCA TTGCTCTGAC		
	MaCEN6	TTCTGCTGGG CTGTCTATGA	AAGGGCAGTT CACAACACAA	MaC-CEN23	ATAGAAGGAA ACGGGAAATC	AAAGGAGTTTG TGTAGGAAGC		
	MaCEN10	ATCTGTGGGC TTATGGTCGT	GCAGGTTTGG GAGAAGACAT	MaC-CEN34	GAGAATGGCA AATGTCAAGT	GGTCCCAGTG TGTTATTGTC		
	MaO-EC02	GGGGAAGGT GGTGTAGGA	GGCAAATGGA AGAGAGGAG	MaO-EC09	GGACTTGTATTT TGTGCTTCTTC	ATCATCTCCA GCCATCTCC		
	MaO-EC05	TGGAGTCGCT TTTTGCTTTT	GTGGTGATTT CCGAGTGGTT	MaO-ED01	TGTTCCACAG GTTTCTCCA	CGCATCTATGA CAACAGCAA		
	MaO-EC11	GCACAACCTTA CTCCCATCAC	CACTACAACTCA CCCTTCCAATC	MaC-CEN17	AGAAACAAAC AGATACCCGA	TTCCCTTATGT AGTAGCACCA		
	MaO-FD02	GGCATCACA CACGCAAAA	ATTACATTCCC AGCCCACAC	MaC-CEN39	TGGTGCTGAA TTGAATCTGA	CGCCACGAAT ACATCTATCT		
	MaO-FG09	TTCTTTCCTGA CCACCTTTTTC	CCAAGTATCAC ACCAACACCA	MaC-CEN42	AATCTTGGTT GGCTTCCTGA	CAAATAAACC TGGGGCATTC		
	MaO-FH03	CTATGGGCGTG AGTGCATTGAA TCCCAAGTTTG GTCAAG	TCTCTTTCCCT CTCTTGCCAT	MaC-CEN44	GAAGGCAGG GAACACGAA	TGAGAAGAGC GAGAGAAGCA		
	MaO-2B10	ACGAGGAGCA GGAAAGTAGC	TTCTCCTTATC CCGTGGTTG	MaC-CEN46	TGTAAGGAGC CTCTGTGTGC	GAGATGGGAT TGGTGTTCGT		
	MaO-EC12	AACCCAACAC CAAAGGAAGA	TGTGGGCGGA GAAATAAATC	MaC-CEN52	TCACTCGGCA GTTCACAAAG	GACTTCATCT TCGGCAATGG		
	MaO-ED09	TGTCAGATAG GTCGGAGTTG	CGCATCTATGA CAACAGCAA	MaC-CEN56	CGAGGAGCAG GAAAGTAGC	TGTGGGCGGA GAAATAAATC		
	MaO-EH12	AACCCAACAC CAAAGGAAGA	ATGGAAGCATG TGGAGGAAC	MaOCEN03	ACCACGAGGAGC AGGAAAGTAGC	TTCGGGATAG GAGGAGGAG		
	MaC-CEN13	TTGTTCTCCTT GTGCTCTTTGA	TCTCTTTCCCT CTCTTGCCAT					

time consuming compared to the rest of the markers (Labuschagne et al. 2015). Being binary or co-dominant, they efficiently discriminate between homozygous and heterozygous genotypes (Arif et al. 2010; Athe et al. 2018). SNPs have been used in important studies in *Musa* spp. For example Sardos et al. (2016) carried out a genome-wide association study on 105 gene bank accessions from the global banana collection at the International Transit Centre (ITC) for parthenocarpy and identified SNP markers associated with the seedless trait. Mahendhiran et al. (2014) also identified 7 SNPs in the AGPase LSU gene in *Musa* that caused changes in the amino acids in the different accessions of diploid and triploid bananas. Their study suggested that SNP marker could be designed from this result to aid in the screening of AGPase LSU gene in banana genomes. Mmeka et al. (2013) identified 7 SNPs from two genes PSY 11 and 35 *LYB* 7 that can be used within diploid and triploid landraces for mapping quantitative trait loci for genes brought about by climatic variation within plantain landraces over time. The SNPs identified could as well show the phylogenetic relationship within those plantain landraces. D'Hont et al. (2012) sequenced the banana genome and established 523-megabase genome of a *Musa acuminata* doubled-haploid genotype. From the sequence data, 18 conserved non-coding sequences (CNSs) were identified in the syntenic position in eudicotyledons from which SNPs can be developed to study bananas. The identification of CNSs in *Musa* sp. opened doors to study CNSs even beyond monocotyledons. However, to the best of our knowledge, there is no report on the use of SNPs to study Fusarium wilt in *Musa* spp.

SNP markers have been utilised in studying Fusarium wilt resistance in crops other than *Musa* spp. such as pigeon pea (Singh et al. 2016), tomatoes (Gonzalez-Cendales et al. 2016), and watermelon (Lambel et al. 2014; Ren et al. 2015).

Unlike the marker systems discussed earlier, SNPs made it possible to create saturated, if not, supersaturated genetic maps, thereby enabling genome-wide tracking, fine mapping of target regions, the rapid association of markers with a trait, and accelerated cloning of gene/QTL of interest (Mammadov et al. 2012). Therefore, SNP marker system can be utilised in studying *Foc* in *Musa* spp., linkage map construction and identification of QTLs for *Foc* resistance.

MOLECULAR ASPECTS OF HOST PLANT RESISTANCE AGAINST FUSARIUM WILT

Sequencing of 18 RNA-Seq libraries from plant resistance inducers (PRIs) by benzothiadiazole (BTH) pathogen resistance responses in bananas showed 14 genes signalling perception and transduction, transcription factors, disease resistant proteins, plant hormones and cell wall organization-related genes from BTH treated Cavendish banana. The ethylene and auxin biosynthesis and response genes that were found to be up-regulated in leaves and roots of BTH treated Cavendish bananas. The genes upregulated were identified to be responsible for tolerance for Fusarium wilt race 4 in bananas (Cheng et al. 2018) (Table 5). Dale et al. (2017) showed in their study that two Cavendish lines transformed with genes RGA2 and Ced9 isolated from Fusarium wilt TR4 resistant diploid bananas and nematode derived, respectively, could not be infected by *Foc* TR4. Alignment of the RGA2 transgene protein sequence from TR4-resistant *M. acuminata* ssp. *Malaccensis* indicated that the expression of RGA2 is strongly associated with resistance to *Foc* TR4 (Table 5). Azam et al. (2017) identified four genes (I, I-1, I-2, I-3) that show resistance against *Fusarium oxysporum* f.sp. *lycopercisi* (Fol) in tomatoes to study resistance against Fusarium race 4 in bananas. The protein sequences and docking studies among both resistant and pathogenic proteins showed that the I, I-1, I-2 and I-3 can be used as resistant genes against *Foc* race 4 in bananas. Magambo et al. (2016) were able to identify a gene Mced9 through cloning and transformation to be responsible for tolerance to *Foc* race 1 in cv.'Sukali Ndiizi. Through greenhouse screening of cv.'Sukali Ndiizi plants transformed with a synthetic, plant-codon optimise Mced9 gene, transgenic lines showed significantly lower internal and external disease symptoms compared to susceptible control.

Bai et al. (2013) performed transcriptome sequencing of bananas 'Yueyoukang 1' highly resistant to *Foc* 4 and Brazilian' (AAA, Cavendish) a cultivar susceptible to *Foc* 4 after challenging them with *Foc* 4. Results indicated that Yueyoukang 1' had much faster defense response against *Foc* 4 infection than 'Brazilian'. 'Yueyoukang 1' expressed defense genes associated with CEBiP, BAK1, NB-LRR proteins, PR proteins, transcription factor and cell wall lignification were expressed stronger than 'Brazilian', indicating that these genes play important roles in banana against *Foc* 4 infection (Table 5). Mohandas et al. (2013) transformed a banana cultivar Rasthali (which known to be susceptible to *Foc* 1) with an onion-derived gene encoding antimicrobial protein (Ace-AMP1). Screening results showed that the transgenic banana had less disease symptoms of *Foc* 4 as compared to the non-transformed control (Table 5). Mahdavi et al. (2012) genetically engineered bananas susceptible to *Foc* race 4 with rice thaumatin-like protein gene (TLP) (gene ID: 4352852 Os12g0628600). The transgenic banana Pisang Nangka (AAB) with TLP gene showed enhanced resistance to *Foc* race 4 as compared to the control after screening. This TLP gene (Table 5) was recommended to enhance resistance to Fusarium wilt in bananas. Ghag et al. (2012) used Petunia floral defensins, PhDef1 and PhDef2 (antimicrobial protein), to transform a banana cv. Rasthali that is known to be susceptible to *Foc* 1. Evaluation of the transformed bananas lines showed that over expression of these genes in the transformed bananas led to significant resistance against *Foc* 1 compared to non-transformed ones (Table 5). Dequan et al. (2009) identified resistance gene analogues (RGAs) associated with Fusarium wilt race 4 in bananas named GF1-GF20 through sequencing of the cloned transformants. The amino acids deduced from RGAS were associated with resistance genes (R genes) Fom-2, I2C-1, I2C-2, and I2 which indicated that the conservation of the

TABLE 5. Genes and gene-specific primers associated with tolerance to Fusarium wilt in banana

Primer/Gene name	Forward 5'-3'	Reverse 5'-3'	Primer name	Forward 5'-3'	Reverse 5'-3'	Application in banana	Author
PAE	GGCTCTCCTTT CTGGATGTTC	TCAGCAAGGC ACTTGACTTTT	PR-3	GGCTCTGTGG TTCTGGATGA	CCAACCCTCC ATTGATGATG	Resistant to *Foc* race 4	(Van Den Berg et al. 2007)
PR-1	TCCGGCCTTA TTTCACATTC	GCCATCTTCA TCATCTGCAA	catalase 2	AAGCATCTTGT CGTCGGAGTA	CGCAACATCGA CAACTTCTTC		
CL9130.Contig2_ BXA-1	TCAGTTTCCTCC AGTCATACCCC	CGGAAGACCTT GGATTTGTAACT	Unigene4625_ BXA-1	ACTTATGAAGGC GAGCACAACC	ACTTGAAGAGG TGGCACAATC		
Unigene15900_ BXA-1	CACGGAGGAG GGTGAGAAG	TGCCACACA CAGCATCGC	CL629.Contig1_ BXA-1	ATCATTTCCAAG TCTTCCTCCAC	CGACCTCATTCT CTCGTTTGTATC	Resistant to *Foc* race 4	(Bai et al. 2013)
Unigene13692_ BXA-1	CTCCACCACG CCAAGAACG	TTGATTCGGG TGATTGATGG	CL2138. Contig7_BXA-1	TCCTGGGAATC CACCAAAGAC	TTTACTTGCTGC TCCAAACTCTC		
Unigene14075_ BXA-1	CGAATCGTGGGA ACATTTGGGTA	CCGAGTTGGCA GAGTCGTAGG	Unigene4215_ BXA-1	AATAAAGCCCG AACCAATCCC	AGTCAATGGAT GCGACGACAG		
Ma5G15040	CACCGTCCGC TCCATTAACT	AGGATGGTGA GGTGCACAAG	Ma9G16540	GGGACGAAGC TGGAGATAGC	TAGGCATCAG GATTGGCGAC		
Ma4G22520	TTCGCGGAGA CCATTGACTT	TACTCGGCTT CTCGGATTGG	Ma6G19890	TGCCATCAAC TGACATCCCT	TGTTAGAGCTA GCCCCAGGAT	Tolerance to *Foc* race 4	(Cheng et al. 2018)
Ma10G04450	GGCAAATGGG TGTCGGAGA	GCTAACCGAG CATCAACGTC	ACTIN	AATTGGGGCC GAAAGGTTCA	GGTGGGGCAA CCACCTTTAT		
Ma11G08430	TCTGTCATGC ATGGGTGCTG	ACACGCGCAA TAACAGTTCC					
PhDef1	CCTGCAGGATGGCT CGCTCCATCTGTTTC	GGTACCCTACACCATC ATATCTGCCTCAAGC	*PhDef2*	CCTGCAGGATGGCTC GCTCCATCTGTTTC	GGTACCTTACT CCATCATATCT TCTTCGACCA	Resistant to *Foc* race 1	(Ghag et al. 2012)
Kinase	GTNYTNGAY GAYGTNTGG	TAGTTGTRAYD ATDAYYYTRC	P-loop/GLPL	GGNGGNRTIG GIAARACIAC	GAGGGCNAR NGGNAAICC	Resistant to *Foc* race 4	Dequan et al. (2009)
RGA2gene	ATGGCTGGTGTCA CATCACAGGCAG	TCAGGTGGTGCTA CAGCGACATGG				Resistant to *Foc* race 4	Dale et al. (2017)
Ace-AMP1	CAGCATCGACC TTCATACTGTTG	GTTAATCCTGC CGCATTGAAT				Resistant to *Foc* race 1	(Mohandas et al. 2013)
TLP	CCATCCATGGGT GGCGTCTCCGGC	CCATGTGCACTTA TGGGCAGAAGA				Resistant to *Foc* race 4	(Mahdavi et al. 2012)

disease resistance genes evolution was identified (Table 5). Van Den Berg et al. (2007) sequenced 13 non redundant genes that were homologous with Fusarium wilt race 4 defense related genes and cell wall strengthening from the tolerant GCTCV-218. From their study, four genes PAE, PR-1, PR-3, catalase 2 were identified to be associated with tolerance to *Foc* 4 in GCTCV-218 and Williams (Table 5). The genes identified for tolerance or resistance to Fusarium wilt could be used as molecular markers for identifying to *Foc* bananas.

Conclusion

The understanding of plant defense mechanisms against Fusarium wilt in bananas and genes underlaying defense mechanisms is critical in developing resistant bananas. RAPD molecular markers have been widely applied in plant studies because they are quick and easy to use, they require low quantities of DNA to perform and are cheap. SCAR markers mostly derived from RAPD have utilised in several plant studies because they are easy and quick to apply also, highly reproducible and because of their high specificity. On the other hand, SSR markers have been widely used in several plant studies due to their codominance that enables them to distinguish heterozygotes from homozygotes, high reproducibility and are amenable to automation. Owing to advantages of RAPD, SCAR and SSR markers, they have been applied to locate QTL for Fusarium wilt resistance and subsequently applied for MAS in crops such as chick pea/pigeon pea, tomatoes, cotton, cabbages and watermelon. RAPD and SCAR markers were used for MAS against Fusarium wilt in bananas but other modern marker technologies such as SSR and SNPs have not been fully utilised for QTL location and subsequent application for MAS against Fusarium wilt in bananas.

Application of modern molecular markers technologies for marker assisted selection in bananas will greatly improve conventional breeding of banana. However, genetic mapping of the *Musa* genome lags behind other crop plants of comparable market value. This could be attributed to the complex nature of banana crop that has made it had to develop suitable mapping populations segregating for desirable traits. Linkage mapping, QTL analysis and subsequent application of MAS have successfully aided breeding for resistance to Fusarium wilt in many crops' species other than bananas. New genotyping technologies now allow generation of much larger sets of genotypic data, based on SNPs. SNPs are abundant in many genomes and are highly amenable to high-throughput detection platforms; two factors that have made them popular for QTL analysis. Therefore, it is important to develop Fusarium wilt segregating populations in bananas, apply new marker techniques such as SNPs and identify QTL for Fusarium wilt resistance to enable MAS.

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