

## DNA markers for early detection of somaclonal variation in *Berangan* banana

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### Abstract

Banana is Malaysia's second most cultivated fruit, with 50% of the cultivation focusing on *Berangan* and Cavendish types. Bananas have been identified as one of the important fruits in the National Agrofood Policy (2011-2020) due to their demand in the domestic and export markets. Thus, FGV is committed to achieving a 50% market share of local banana production through tissue culture propagation techniques. This technique enables the mass production of banana planting material in a short period. However, one downside of this technique is the random occurrence of somaclonal variation, which has been reported for various crops. FGV's clonal propagation aims to produce true-to-type banana planting materials, therefore, somaclonal variants are undesired. Hence, we aimed to establish a marker-based screening method for early detection and elimination of *Berangan* somaclonal variants prior to field planting. *Berangan* leaf samples with and without somaclonal variation were collected from different sites. The samples' DNA was extracted using an in-house cetyltrimethylammonium bromide (CTAB) extraction method and screened using 145 Random Amplified Polymorphic DNA (RAPD) markers. RAPD markers that could distinguish somaclonal variation in the *Berangan* samples were developed into Sequence Characterized Amplified Region (SCAR) markers for routine screening. The SCAR markers were validated for their ability to reproduce the specific bands from the RAPD results. Of the 145 RAPD markers, three markers do not produce any bands, 47 RAPD markers produce monomorphic bands and 95 RAPD markers produce polymorphic bands. Three out of the 95 polymorphic RAPD markers were able to selectively distinguish the somaclonal variation in samples from similar sources. The three RAPD markers were developed into SCAR markers, and they successfully reproduced the specific bands from their respective RAPD results. Hence, the developed SCAR markers will be utilized for the early detection and elimination of *Berangan* somaclonal variants from selected sources.

## 1. Introduction

Banana is Malaysia's second most cultivated fruit, with 50% of the cultivation focusing on *Berangan* and Cavendish types. Over the years, banana consumption and demand in the domestic and export markets have been steadily increasing (Mohamad Roff *et al.*, 2012). In conjunction with that, banana was identified as one of the important fruits under the National Agrofood Policy (2011-2020) for the export market (Tan, 2022). Following up on the National Agrofood Policy (2011-2020), FGV is committed to achieving a 50% market share of local banana production through tissue culture

propagation technique (FGV Holdings Berhad, 2020). This technique enables the mass production of banana planting material with identical genetic composition in a short period. However, one downside of this technique is the random occurrence of somaclonal variation, which has been reported since 1971 for various crops such as sugarcane (Heinz and Mee, 1971), tomato (Van den Bulk *et al.*, 1990), potato (Juned *et al.*, 1991), maize (Williams *et al.*, 1991) and banana (Israeli *et al.*, 1991).

The term somaclonal variation was first used in 1981 when referring to the variabilities observed among the somaclones produced through cell or tissue culture

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techniques (Larkin and Scowcroft, 1981). The variabilities observed could be caused by either genetic or epigenetic modifications that occur during the tissue culture process. The genetic variabilities observed in somaclones enable breeders to rapidly develop new varieties without complex procedures (Krishna *et al.*, 2016). However, somaclonal variants are undesired when the aim is to produce true-to-type planting materials and the somaclonal variants of banana are usually inferior compared to its mother plant (Sahijram *et al.*, 2003). The somaclonal variation reported for bananas can be classified based on the plant stature, foliage, pseudostem pigmentation, inflorescence and fruit. Nevertheless, somaclonal variations involving the inflorescence and fruit such as abnormal bunch orientation, absence of male bud, and a small bunch with short or twisted fingers (Sahijram *et al.*, 2003) are more concerning as the yield would be compromised. Thus, incorporating a quality control step that can distinguish between the normal and somaclonal variant banana during the tissue culture process is crucial and molecular markers could serve this purpose.

Molecular markers refer to the DNA nucleotide sequence differences between individuals, populations, organisms or species. Various types of molecular markers have been developed over the years, each with its advantages and disadvantages. Hence, selecting the molecular markers depends on the research objective, application and practicality of the experimental design (Amiteye, 2021). In some research, incorporating more than one molecular marker in the experimental design provides a better impact on the research. The utilization of Random Amplified Polymorphic DNA (RAPD) markers to distinguish between normal and somaclonal variants of *Berangan* banana has been reported previously by Razani *et al.* (2019, 2020).

RAPD markers amplify DNA fragments using 10-mer random markers designed based on sequences that are generally conserved in multiple species. The amplified DNA fragments would be subjected to gel electrophoresis and the genetic variation among individuals will be differentiated based on the presence or absence of bands. The advantages of RAPD markers are that experimental design is quick and simple, the sample genome sequences are not required for marker design and a small quantity of DNA is required (Babu *et al.*, 2021). Thus, RAPD markers are a good choice for the initial screening stage but it is not suitable for application purpose step as RAPD markers reproducibility is low due to the unspecific amplification and low annealing temperature usage. The reproducibility of the RAPD markers can be improved by developing them into Sequence Characterized

Amplified Region (SCAR) markers.

SCAR markers can be developed by extracting and sequencing the unique bands that differentiate an individual's genetic makeup. Specific markers of 20 to 25 base pairs (bp) with higher annealing temperatures designed for the sequenced unique bands lead to the development of reliable and reproducible SCAR markers (Kiran *et al.*, 2010). Hence, this project aims to establish a reliable and reproducible molecular marker-based screening method for early detection and elimination of *Berangan* somaclonal variants prior to field planting.

## 2. Materials and methods

### 2.1 Genomic DNA extraction

Leaf samples of normal and somaclonal *Berangan* banana variants were collected from two different sites. The somaclonal variations observed for the collected samples are abnormal bunch orientation with small fingers, smaller male buds without fingers and non-flowering after 1 year in the field. The samples' DNA was extracted using an in-house cetyltrimethylammonium bromide (CTAB) extraction method modified from Seng and Zaman (2006) and stored at -20°C, prior to usage. The quality and quantity of extracted DNAs were determined using 1% agarose gel electrophoresis and a spectrophotometer (BMG Labtech, Germany). The quantified DNAs were diluted to 15 ng/μL to be used as a working concentration.

### 2.2 In silico Random Amplified Polymorphic DNA marker selection

There were 1200 RAPD markers listed by Eurofins Genomics and the RAPD markers were selected based on their priming characteristic towards the genomic sequence data of *Musa acuminata* subsp. *Malaccensis* (GenBank accession: GCA\_000313855.2). The essential characteristics are (1) the markers' priming sites match the genome sequence with zero mismatches, (2) the presence of multiple priming sites of the markers in the genome sequence and (3) the distance between the priming sites should be between 200 to 2000 bp.

### 2.3 Random Amplified Polymorphic DNA marker screening

The polymerase chain reaction (PCR) reaction mixtures of 10 μL were prepared with 1× My Taq HS Mix polymerase (Meridian Bioscience, USA), 2 μM RAPD markers (Integrated DNA Technologies, USA) and 15 ng DNA templates. PCR amplifications were performed using a thermal cycler (Eppendorf, Germany) under the following program: (1) initial DNA denaturation at 95°C for 3 mins, (2) 40 cycles of

denaturation at 95°C for 30 s, annealing at 36°C for 30 s and extension at 72°C for 2 mins and (3) final extension at 72°C for 5 mins.

The amplified PCR products were separated through agarose gel electrophoresis using 1.5% agarose gels stained with Red Safe (iNtRON Biotechnology, South Korea) and 1× TAE buffer (Invitrogen, USA). The gels were visualized under UVITEC (Cambridge, England) immediately after the gel electrophoresis was completed. Polymorphic bands that could distinguish the normal and somaclonal variants were identified, excised from the agarose gels, and purified using NucleoSpin® Gel and PCR Clean-up (Macherey–Nagel, Germany) according to the manufacturer's instruction. The polymorphic bands that could distinguish the normal and somaclonal variants were referred to as unique bands.

#### 2.4 Sequence Characterized Amplified Region marker development

The purified PCR products were quantified and subjected to agarose gel electrophoresis to ensure that the unique bands were correctly obtained. The purified PCR products were ligated into the PGEM-T Easy Vector System (Promega, USA) and transformed into chemically competent *E. coli* DH5α cells (Yeastern Biotech, Taiwan). The positive clones were confirmed through colony PCR and agarose gel electrophoresis. The colony PCR amplification was performed using a thermal cycler (Eppendorf, Germany) with initial DNA denaturation at 95°C for 3 mins, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min. The final extension was performed for 5 mins at 72°C.

The positive clones were grown in Lennox broth (LB) medium (Conda, Spain) with 100 µg/µl ampicillin (MedChemExpress, USA) and extracted using NucleoSpin® Plasmid EasyPure (Macherey–Nagel, Germany). The extracted plasmids were sent for nucleotide sequencing to First Base Laboratories, Malaysia. The complete nucleotide sequences of the forward and reverse strands were aligned and assembled using BioEdit software. The assembled sequences were subjected to homology search in the nucleotide BLAST algorithm of NCBI (<https://blast.ncbi.nlm.nih.gov>) and suitable SCAR markers were designed for the assembled sequence of the RAPD unique bands.

#### 2.5 Sequence Characterized Amplified Region marker validation

The DNA templates used in RAPD analysis were used with the newly developed SCAR markers for PCR amplification. The PCR reaction mixtures were prepared with 1× My Taq HS Mix polymerase (Meridian

Bioscience, USA), 2 µM RAPD markers (Integrated DNA Technologies, USA) and 15 ng DNA templates in total volume of 10 µL. PCR amplifications were performed using an Eppendorf Mastercycler Pro S (Hamburg, Germany) with initial denaturation at 95°C for 3 mins, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C for 30 seconds and extension at 72°C for 2 mins. The final extension was performed at 72°C for 5 mins.

The amplified PCR products were separated through agarose gel electrophoresis in 1× TAE buffer using 1.0% agarose gels stained with Red Safe (iNtRON Biotechnology, South Korea). The gels were visualized under UVITEC (Cambridge, England) immediately after the gel electrophoresis was completed. The gel image of the SCAR marker and the RAPD marker were compared to check the SCAR markers' ability to specifically reproduce the unique bands of the RAPD markers.

### 3. Results

#### 3.1 Random Amplified Polymorphic DNA marker screening

From a total of 1200 RAPD markers, 145 were selected based on the *in silico* analysis. The selected RAPD markers were subjected to PCR and agarose gel electrophoresis to test their capability in detecting *Berangan* somaclonal variants. Out of the 145 RAPD markers, three markers did not produce any bands while 47 markers generated only monomorphic bands among the tested samples. Meanwhile, 95 markers yielded polymorphic bands with a percentage ranging from 5 to 92% of polymorphism (Table 1). Although 95 markers were able to produce polymorphic bands, only three markers (OPA-15, OPAG-14 and OPE-09) were able to produce polymorphic bands that could selectively distinguish the normal and the somaclonal variant *Berangan* from similar sites (Figure 1(a)).

OPA-15 RAPD marker produced 15 bands with amplicon sizes ranging from 400 to 3000 bp while OPAG-14 RAPD markers produced 12 bands with amplicon sizes ranging from 400 to 2000 bp. Both OPA-15 and OPAG-14 RAPD markers have two polymorphic bands contributing to 13.33% and 16.67% of the markers' polymorphism, respectively. However, only one of the polymorphic bands of each marker are unique band that able to differentiate the normal and somaclonal variants. The unique band was present only in the normal samples from site 1 for OPA-15 markers. Meanwhile, for the OPAG-14 marker, the unique band was present in some somaclonal variant samples from site 2. On the other hand, for the OPE-09 RAPD marker, eight bands with 500 to 3000 bp were observed. Of the eight bands,

Table 1. Details of RAPD markers with various parameters reveal the discriminatory power of each marker.

No	Markers	Markers sequence	No. of Bands	No. of MB	No. of PB	% of polymorphism	Amplicon band size	Ability to distinguish normal vs somaclonal variant
1	<b>OPA-15</b>	<b>TTCCGAACCC</b>	<b>15</b>	<b>13</b>	<b>2</b>	<b>13.33</b>	<b>400-3000</b>	<b>Yes</b>
2	OPA-19	CAAACGTCGG	10	9	1	10.00	400-2800	No
3	OPAA-09	AGATGGGCAG	6	5	1	16.67	400-1800	No
4	OPAA-20	TTGCCTTCGG	11	8	3	27.27	400-1800	No
5	OPAB-06	GTGGCTTGGA	10	8	2	20.00	300-1500	No
6	OPAB-15	CCTCCTTCTC	5	5	0	0.00	500 -1500	No
7	OPAC-02	GTCGTCGTCT	11	11	0	0.00	400-2500	No
8	OPAD-20	TCTTCGGAGG	10	10	0	0.00	300-2500	No
9	OPAE-05	CCTGTCAGTG	4	4	0	0.00	600-2500	No
10	OPAE-11	AAGACCGGGA	6	6	0	0.00	700-1800	No
11	OPAF-02	CAGCCGAGAA	10	9	1	10.00	500-2000	No
12	OPAF-03	GAAGGAGGCA	18	17	1	5.56	300-3000	No
13	OPAF-09	CCCCTCAGAA	2	2	0	0.00	700-1000	No
14	OPAF-18	GTGTCCCTCT	15	15	0	0.00	400-2000	No
15	OPAG-06	GGTGGCCAAG	14	13	1	7.14	250-1800	No
16	OPAG-09	CCGAGGGGTT	17	17	0	0.00	300-3000	No
17	<b>OPAG-14</b>	<b>CTCTCGGCGA</b>	<b>12</b>	<b>10</b>	<b>2</b>	<b>16.67</b>	<b>400-2000</b>	<b>Yes</b>
18	OPAG-16	CCTGCGACAG	13	11	2	15.38	200-2000	No
19	OPAH-10	GGGATGACCA	14	13	1	7.14	200-1800	No
20	OPAI-08	AAGCCCCCA	13	12	1	7.69	300-2000	No
21	OPAJ-08	GTGCTCCCTC	15	15	0	0.00	300-2000	No
22	OPAJ-18	GGCTAGGTGG	8	7	1	12.50	300-1500	No
23	OPAJ-19	ACAGTGGCCT	11	11	0	0.00	300-1800	No
24	OPAK-02	CCATCGGAGG	10	10	0	0.00	200-2500	No
25	OPAK-05	GATGGCAGTC	9	9	0	0.00	250-1500	No
26	OPAK-09	AGGTGGCGT	9	9	0	0.00	400-1000	No
27	OPAK-15	ACCTGCCGTT	7	3	4	57.14	500-1800	No
28	OPAK-19	TCGCAGCGAG	15	14	1	6.67	200-2500	No
29	OPAL-01	TGTGACGAGG	15	13	2	13.33	200-2500	No
30	OPAL-06	AAGCGTCCTC	9	6	3	33.33	500-3000	No
31	OPAM-01	TCACGTACGG	6	3	3	50.00	500-1800	No
32	OPAM-03	CTTCCCTGTG	8	7	1	12.50	400-3000	No
33	OPAN-05	GGGTGCAGTT	11	7	4	36.36	100-500	No
34	OPAO-01	AAGACGACGG	15	15	0	0.00	300-2000	No
35	OPAO-07	GATGCGACGG	12	11	1	8.33	300-2000	No
36	OPAO-13	CCCACAGGTG	11	11	0	0.00	200-2500	No
37	OPAO-15	GAAGGCTCCC	7	7	0	0.00	400-2500	No
38	OPAP-11	CTGGCTTCTG	3	3	0	0.00	500-1000	No
39	OPAP-15	GGGTTGGAAG	9	8	1	11.11	300-2500	No
40	OPAR-04	CCAGGAGAAG	7	6	1	14.29	500-3000	No
41	OPAR-07	TCCTTCGGTG	4	3	1	25.00	600-1500	No
42	OPAR-13	GGGTGGGCTT	12	9	3	25.00	300-2500	No
43	OPAR-17	CCACCACGAC	12	10	2	16.67	200-1800	No
44	OPAS-09	TGGAGTCCCC	10	7	3	30.00	300-2500	No
45	OPAT-02	CAGGTCTAGG	10	4	6	60.00	300-2500	No
46	OPAT-03	GACTGGGAGG	18	14	4	22.22	200-2000	No
47	OPAT-11	CCAGATCTCC	9	9	0	0.00	200-2000	No

MB: monomorphic band, PB: polymorphic band.

Table 1 (Cont.). Details of RAPD markers with various parameters reveal the discriminatory power of each marker.

No	Markers	Markers sequence	No. of Bands	No. of MB	No. of PB	% of polymorphism	Amplicon band size	Ability to distinguish normal vs somaclonal variant
48	OPAT-13	CTGGTGGAAAG	12	10	2	16.67	300-3000	No
49	OPAU-08	CACCGATCCA	14	13	1	7.14	200-2000	No
50	OPAU-09	ACGGCCAATC	16	12	4	25.00	250-1500	No
51	OPAU-17	TTGGCATCCC	11	10	1	9.09	300-2000	No
52	OPAU-18	CACCACTAGG	12	12	0	0.00	500-3000	No
53	OPAV-04	TCTGCCATCC	14	14	0	0.00	350-2000	No
54	OPAV-08	TGAGAAGCGG	10	7	3	30.00	200-1200	No
55	OPAW-02	TCGCAGGTTC	9	6	3	33.33	300-2000	No
56	OPAW-12	GAGCAAGGCA	11	11	0	0.00	200-1500	No
57	OPAW-17	TGCTGCTGCC	7	6	1	14.29	400-2500	No
58	OPAX-05	AGTGCACACC	12	1	11	91.67	400-2000	No
59	OPAY-17	GGTGATTCGG	0	0	0	0.00	-	No
60	OPAY-19	AACTTGCCCC	17	16	1	5.88	300-2500	No
61	OPAZ-07	CACGAGTCTC	12	7	5	41.67	200-2000	No
62	OPB-18	CCACAGCAGT	10	8	2	20.00	200-2000	No
63	OPBA-14	TCGGGAGTGG	16	16	0	0.00	200-1800	No
64	OPBA-20	GAGCGCTACC	11	9	2	18.18	100-1500	No
65	OPBB-06	CTGAAGCTGG	13	10	3	23.08	300-2000	No
66	OPBB-10	ACTTGCCTGG	6	6	0	0.00	600-3000	No
67	OPBB-16	TCGGCACCGT	11	10	1	9.09	300-1000	No
68	OPBC-02	ACAGTAGCGG	10	10	0	0.00	300-1200	No
69	OPBC-12	CCTCCACCAG	19	18	1	5.26	300-3000	No
70	OPBC-18	GTGAAGGAGG	11	10	1	9.09	300-1500	No
71	OPBG-06	GTGGATCGTC	10	10	0	0.00	250-1200	No
72	OPBG-10	GGGATAAGGG	7	7	0	0.00	300-1500	No
73	OPBG-14	GACCAGCCCA	7	7	0	0.00	300-1000	No
74	OPBG-20	TGGTACCTGG	7	5	2	28.57	650-2500	No
75	OPBH-03	GGAGCAGCAA	9	8	1	11.11	250-1800	No
76	OPBH-08	ACGGAGGCAG	10	10	0	0.00	300-1500	No
77	OPC-03	GGGGGTCTTT	11	10	1	9.09	500-2500	No
78	OPD-01	ACCGCGAAGG	6	6	0	0.00	400-1300	No
79	OPD-09	CTCTGGAGAC	14	12	2	14.29	300-1500	No
80	OPD-14	CTCCCCAAG	5	4	1	20.00	400-1200	No
81	<b>OPE-09</b>	<b>CTTCACCCGA</b>	<b>8</b>	<b>7</b>	<b>1</b>	<b>12.50</b>	<b>500-3000</b>	<b>Yes</b>
82	OPE-17	CTACTGCCGT	10	10	0	0.00	300-2500	No
83	OPF-06	GGGAATTCGG	10	9	1	10.00	400-2000	No
84	OPF-13	GGCTGCAGAA	16	16	0	0.00	300-2000	No
85	OPF-14	TGCTGCAGGT	15	15	0	0.00	250-2000	No
86	OPF-18	TTCCCGGGTT	12	11	1	8.33	200-1000	No
87	OPF-20	GGTCTAGAGG	13	11	2	15.38	200-1500	No
88	OPG-03	GAGCCCTCCA	13	13	0	0.00	200-1500	No
89	OPG-07	GAACCTGCGG	12	10	2	16.67	200-2000	No
90	OPG-16	AGCGTCTCC	12	12	0	0.00	200-1000	No
91	OPG-18	GGCTCATGTG	9	8	1	11.11	300-1500	No
92	OPG-19	GTCAGGGCAA	6	6	0	0.00	300-1200	No
93	OPG-20	TCTCCCTCAG	5	4	1	20.00	300-2500	No
94	OPH-01	GGTCGGAGAA	11	10	1	9.09	300-1200	No

MB: monomorphic band, PB: polymorphic band.

Table 1 (Cont.). Details of RAPD markers with various parameters reveal the discriminatory power of each marker.

No	Markers	Markers sequence	No. of Bands	No. of MB	No. of PB	% of polymorphism	Amplicon band size	Ability to distinguish normal vs somaclonal variant
95	OPH-04	GGAAGTCGCC	11	10	1	9.09	200-1200	No
96	OPH-09	TGTAGCTGGG	10	9	1	10.00	300-3000	No
97	OPH-17	CACTCTCCTC	16	12	4	25.00	200-2500	No
98	OPI-02	GGAGGAGAGG	20	18	2	10.00	200-2500	No
99	OPI-04	CCGCCTAGTC	9	9	0	0.00	600-2000	No
100	OPI-16	TCTCCGCCCT	11	6	5	45.45	200-1500	No
101	OPI-17	GGTGGTGATG	15	15	0	0.00	300-2000	No
102	OPL-03	CCAGCAGCTT	11	10	1	9.09	300-1500	No
103	OPL-06	GAGGGAAGAG	10	8	2	20.00	500-2000	No
104	OPL-08	AGCAGGTGGA	11	9	2	18.18	200-1500	No
105	OPL-12	GGGCGGTACT	15	15	0	0.00	300-3000	No
106	OPL-15	AAGAGAGGGG	20	18	2	10.00	200-2000	No
107	OPL-16	AGGTTGCAGG	6	6	0	0.00	200-1000	No
108	OPL-17	AGCCTGAGCC	10	8	2	20.00	300-2000	No
109	OPL-18	ACCACCCACC	10	5	5	50.00	200-1500	No
110	OPM-01	GTTGGTGGCT	13	9	4	30.77	300-1500	No
111	OPM-11	GTCCACTGTG	0	0	0	0.00	-	No
112	OPM-13	GGTGGTCAAG	11	11	0	0.00	300-3000	No
113	OPM-14	AGGGTCG TTC	12	8	4	33.33	200-2000	No
114	OPM-18	CACCATCCGT	8	5	3	37.50	200-1000	No
115	OPN-01	CTCACGTTGG	10	7	3	30.00	400-2000	No
116	OPN-02	ACCAGGGGCA	12	10	2	16.67	200-1800	No
117	OPO-06	CCACGGGAAG	14	14	0	0.00	200-2000	No
118	OPO-11	GACAGGAGGT	15	12	3	20.00	300-3000	No
119	OPQ-19	CCCCCTATCA	13	10	3	23.08	300-2500	No
120	OPQ-20	TCGCCCAGTC	11	7	4	36.36	300-2000	No
121	OPR-05	GACCTAGTGG	4	4	0	0.00	400-1000	No
122	OPR-19	CCTCCTCATC	16	14	2	12.50	150-2000	No
123	OPR-20	ACGGCAAGGA	12	11	1	8.33	300-2000	No
124	OPS-05	TTTGGGGCCT	12	9	3	25.00	450-2500	No
125	OPS-20	TCTGGACGGA	10	10	0	0.00	400-2000	No
126	OPT-03	TCCACTCCTG	3	3	0	0.00	300-1800	No
127	OPT-06	CAAGGGCAGA	11	9	2	18.18	200-200	No
128	OPU-04	ACCTTCGGAC	5	3	2	40.00	200-2000	No
129	OPU-05	TTGGCGGCCT	10	9	1	10.00	400-1200	No
130	OPU-09	CCACATCGGT	8	5	3	37.50	200-2000	No
131	OPV-10	GGACCTGCTG	9	9	0	0.00	300-1200	No
132	OPV-20	CAGCATGGTC	14	13	1	7.14	300-2500	No
133	OPW-12	TGGGCAGAAG	14	14	0	0.00	400-1800	No
134	OPW-15	ACACCGGAAC	13	12	1	7.69	300-3000	No
135	OPW-20	TGTGGCAGCA	13	12	1	7.69	300-2000	No
136	OPX-02	TTCCGCCACC	15	12	3	20.00	400-3000	No
137	OPX-18	GACTAGGTGG	7	5	2	28.57	500-1300	No
138	OPY-01	GTGGCATCTC	17	15	2	11.76	250-2500	No
139	OPY-02	CATCGCCGCA	13	11	2	15.38	300-3000	No
140	OPY-10	CAAACGTGGG	11	9	2	18.18	200-3000	No
141	OPY-20	AGCCGTGGAA	7	6	1	14.29	300-1800	No

MB: monomorphic band, PB: polymorphic band.

Table 1 (Cont.). Details of RAPD markers with various parameters reveal the discriminatory power of each marker.

No	Markers	Markers sequence	No. of Bands	No. of MB	No. of PB	% of polymorphism	Amplicon band size	Ability to distinguish normal vs somaclonal variant
142	OPZ-04	AGGCTGTGCT	23	18	5	21.74	200-2500	No
143	OPZ-13	GACTAAGCCC	0	0	0	0.00	-	No
144	OPZ-14	TCGGAGG TTC	13	13	0	0.00	300-2000	No
145	OPZ-16	TCCCCATCAC	20	15	5	25.00	300-3000	No

MB: monomorphic band, PB: polymorphic band.

one of them is a unique polymorphic band that was observed in some somaclonal variant samples from site 1. The three unique bands from each RAPD marker were extracted and their respective SCAR markers were developed.

3.2 Sequence Characterized Amplified Region marker development

The extracted three unique bands were successfully cloned and sequenced. The sequence and other details of the unique bands are tabulated in Table 2. The size of the unique bands are 1840 bp, 874 bp and 1426 bp for OPA-15, OPAG-14 and OPE-09, respectively. BLAST search for the unique band sequences in the NCBI platform suggests no significant similarity for OPA-15 and OPAG-14. Conversely, five fragments of OPE-09 unique band sequence shared 98 to 99% identity to the *Musa acuminata* subsp. *Malaccensis* strain Doubled-haploid Pahang (DH-Pahang) mitochondrion sequence (Sequence ID: HG996478.1) with an E value ranging from 0.0 to 2e-17 (Table 3). The SCAR markers were designed and synthesized for the unique band sequence since there were no similarities found for OPA-15 and OPAG-14 while OPE-09 sequence homology is by fragments.

Table 3. OPE-09 unique band sequence show homology with the *Musa acuminata* subsp. *Malaccensis* strain Doubled-haploid Pahang (DH-Pahang) mitochondrion sequence (Sequence ID: HG996478.1).

No	Nucleotide	Base pair	Identity (%)*	E value
1	3 -575	573	99	0.0
2	593-843	251	99	1e-123
3	698-873	176	99	3e-80
4	1272-1381	110	98	1e-43
5	1166-1225	60	98	2e-17

\*The sequence similarity was identified based on a BLASTn (megaBLAST) search against the nr/nt database (NCBI).

3.3 Sequence Characterized Amplified Region marker validation

The designed SCAR markers were then used to amplify the DNA samples used for RAPD screening to test their specificity towards the unique bands. The SCAR markers were able to successfully reproduce the specific unique bands from their respective RAPD results (Figure 1). Hence, the developed SCAR marker for OPA-15 was able to distinguish normal samples from site 1. Meanwhile, the OPE-09 and OPAG-14 SCAR markers were able to distinguish some somaclonal variants from site 1 and site 2, respectively. Thus, the developed SCAR markers can be utilized for the early detection and elimination of *Berangan* somaclonal

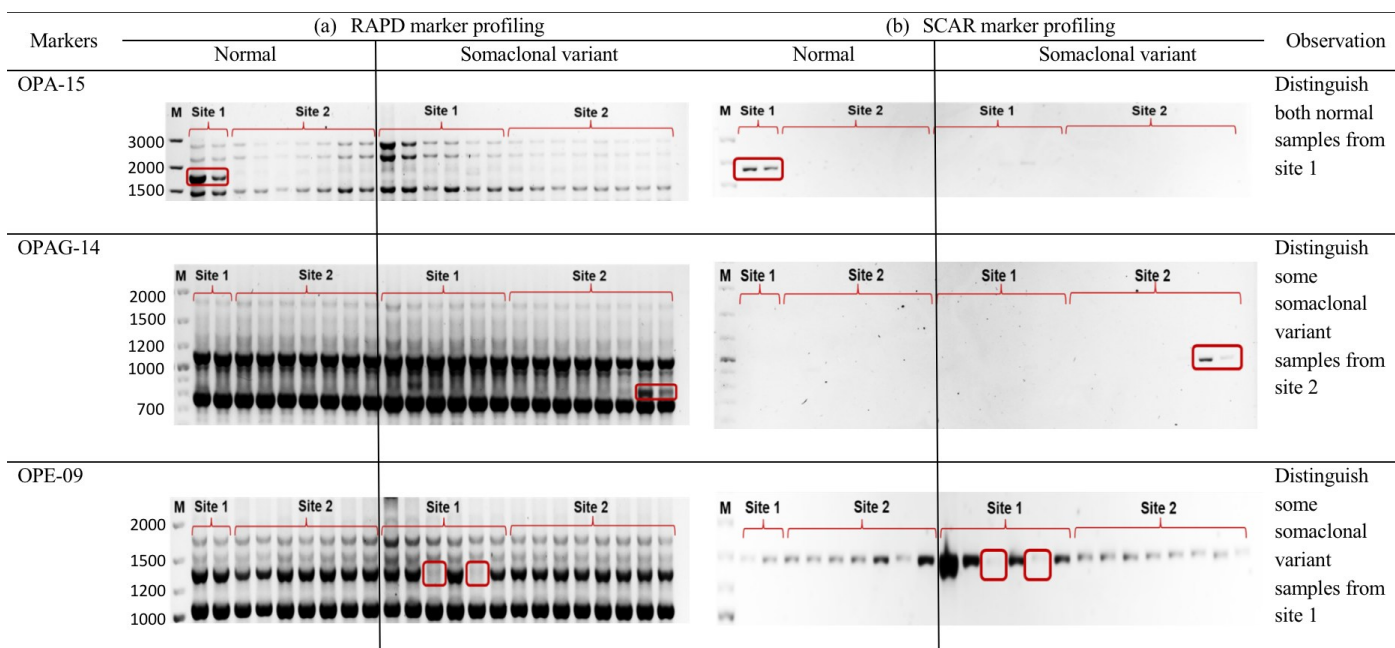


Figure 1. (a) RAPD and (b) SCAR marker profiling of OPA-15, OPAG-14 and OPE-09 markers. Lane “M” indicates the GeneRuler™ 100 bp Plus DNA Ladder with the fragment size (bp) 3000, 2000, 1500, 1200, 1000, 900, 800 and 700.

Table 2. RAPD marker unique band sequence, size, blast result and its SCAR markers sequences.

Markers	Sequence	Amplicon size	Blast result	SCAR markers sequence
OPA-15	<p>1 TTCGAAACCC CCAACACCTA AGAAGTTAA AGAATTACT CAGAGAGCTG CTAGATCCGG</p> <p>61 ACATGGTATC AGTGTTTATG GTGGTTATTA CATGCCGGAT TTGCTAGTA TCTGTGGAG</p> <p>121 CAGGATGCTC TTTGTGGTGG GTGGTTTGG TAAAGTTGAA TGAAGAGTCT GCTTTATCAT</p> <p>181 GTGTCAAAT C666CCCGCA CTCGAGTGTG TCGTCTGCAT TCTTGTATG ATCAAGCTCG</p> <p>241 CAAACCCGGG GGGTTTAAAGG ACTGCTTGGG TACTGTCTGT TGTATGTTA AGGTAAGGTT</p> <p>301 GTTGTCTGT ATCTTTTCCA GAGGCATGCT CTGTGTAAAG ACTATCATTA TCCTTAGATG</p> <p>361 AAGAAGGAGA AGAGGATATC GTAAATGCC TAGAGATGCC TCTAACGAT TGGCTATTCG</p> <p>421 TTGCTTGAAG CTGGGTTCTT AGGGTTTCCA TGGTATTTCC GGGAGATAAC GCATATTCG</p> <p>481 AGAGTAGCTG ATCAACCACT TGTAAATCTA GCTGGTCAAG CTTTGTGCA GCGAGTACTT</p> <p>541 TAGCAGCTTG TCGANTATTA TGTTTGGTAT CTGGGATAGT AAGAGGTTGC TCTGAAATCA</p> <p>601 GTAAAGACTT TTAATAGTAT TGTTTAAACCA GTACAGACGA TTGACAGGTTG GGGTTTGTAT</p> <p>661 TGGTAAATGA CCGGTAAGTG TCTTTAAMGT CCGTAGAGCG CCAAGTCTCG ATGATGACTT</p> <p>721 GTATCTCTTC TGGCATATCG TGAATGGTGT GCGGCTTGTG AATATCTTGA CTTTCTGACTT</p> <p>781 TATGATCGAG ACTCGGGAAA TAACTTTTGT AATATGACAC CTTTAAATCG AGCTATATCT</p> <p>841 AACCTTATATG AGGGGTCGGA AGTCACTTCT ACTACAGACG GTTGTGCTCC TGCCTAGAGA</p> <p>901 TCTACAAGCA GCTCGAAMAA GTTGGTGTGT TTGAATGTCT TTG666666CTC TTGCTCGCGA</p> <p>961 GGTTTTGTGG CTTTGAAGTA ATGTCATATA CCACTGTGCA GAAAATGTC AGCAAAACAT</p> <p>1021 TGGAAATGAT CTTATTCGAG CAGTTCTGAT GTATCTCCCT TTAATTTGCT AAATGTATCA</p> <p>1081 AGAGCAGTGA TTAICTCTCG AATAGATGCG TGAGATAGGG TTTTGA666 GCGGCTCAT</p> <p>1141 TATCATGATA CCGGTAAAT AGEBAATGC CTTGTGGGCT TGGTACTCTA G6AGGTG6AC</p> <p>1201 ATCTTAAATA AATGTCCATA TATCGAAMAT GTAAT1666G ACCCTTIGAT C1GCTGGTGC</p> <p>1261 TCTACTAAT TAGCAGTGA CTTTTGGCTA TATTAGTTAG AATACTGCT GCGCACGTT</p> <p>1321 CAGGCGAAMAA CCGGCGTGA TCAAACTCT CTTTATGGTA TGTGCTATA ATATCTGTAG</p> <p>1381 CAATAGCATT CCGATCGGAG TCTATTTCTG CCGCAATTC TGTATG66CT AAATTTGTGA</p> <p>1441 TAATGTCTAG ATGCTGTCC TGTGTCATTT CTGTGG66G GTTTTTAG6C GAATATGTA</p> <p>1501 AATGGTGTAT AGCGTATTTA CTTTCCAACT CAAGTGCAG CACAACGA TATGCGAAGT</p> <p>1561 TGGCAAMAA ATGATCGTGT TCGATTAAAT ACTTATAGTA GTCAGTATC GTTGAAGCT</p> <p>1621 GAGCATTGGA GTCTTGTGAT GCGTTGATG CAATCTTGG TTTAAATCC TCTAATTT</p> <p>1681 TGTATCTAG GTTGTGTCAG CTTGGGATAG TAGTACTCTC CATCAGTGTG AATAAAAAAT</p> <p>1741 AGCTCATCTA CCGAGTAGAG GTTTTTATCC TATCATCAAT GATCTGTGCT AGT1666TCT</p> <p>1801 G6AA</p>	1840	No significant similarity was found	Forward: CCCAACCACTAAAGAAAGTTAAAGAA Reverse: CCGAAACCCAACTAGACAAGAT
OPAG-14	<p>1 CTCTGGCGA ATAGTAGAT CTAGTGGATT GTCTACTCTA GAAGCTAATA TAGCGCGCGT</p> <p>61 AGTTGCTAAT ATTTACTCCG AAATAGCGCG TATACTTCT TTAGTTTAC TATCCGTGGA</p> <p>121 GAAGCGTCC GAGTGTACT ATACTATAGT ATACTATGCG AATTAAGTC TATTCATAG</p> <p>181 TGTAAITAT AGT666TCT AG6AACCC66 TCGCTTCTA GCACTCCTT TACTTACTAA</p> <p>241 GTATCTGTT AGTAGAGCT ACTAAACCT ACTACTAAGC CTTAAATAG CCGTATATCT</p> <p>301 AGTTATTTAC TTTCCGCG TAAAGGAGC CTAITGCGGG CTTCTTACAT ATAAGAAAG</p> <p>361 CCGGGAAAC TACTGTAAA GGTGCTACT SCCAATATC ATGACTTATC CATGGCTCCC</p> <p>421 ATGCTTTTCC ACAAGATGC CCGGATTTTG TGAAGG66G CCGTGT66G CTGCGTTAAC</p> <p>481 CTAAAGCAA GCATCTGAC GTATCTG6TT AGCGAAGCCG CGAGATGCTC GCGCTACAGT</p> <p>541 CTGGCTT6G TTTGCAAGA CTGCGCTGTC CTTCACTTCT CCGCTGCTC TTCTCTCCC</p> <p>601 TCTTCCAGG CAGAAT666 ATTTTACAG C66CTCGAG TGTACTGAGC TAGACAATTA</p> <p>661 TCGCTCATCA TATCATGATA AG6ACAACT CCG666CAAC TGTGCTCT CCGAGCAGC</p> <p>721 GCATCATCG TGTG66CTT CCGTAGC6GA ACAACTG6CG GTATAGCTAA CCCAAACCT</p> <p>781 GCACTACTAT ACTCAAGCA CCGTGTCTCG CCGTATTTTA GATCAAGACT CAAGAGTGGG</p> <p>841 CCGCG6ACTG ATCAAITTAC CCGTGTG6CG AGAG</p>	874	No significant similarity was found	Forward: CCGCCGTAGTTGCTAATATTCTA Reverse: CTCGGCGAAGCGGGTAAAT
OPE-09	<p>1 CTTACCCGA CCAATAGAT ATTGG6ATG TAACTA6CT G6AACTCTCT TAGGCACAA6</p> <p>61 TCGCTCTCC TCGAAMCAT TAATAGATC TCTCTT6CT C6T6T6T6 CTG6T6T6 CTGCTTGGT</p> <p>121 AGCAAGCA6 TAACTACTG ACCCTAAGC G6AACTCA6 TTAGCACA6 ATCTG66CTT</p> <p>181 ACCTCTCTT CTAGTCTCT ATGATTTTCT TCTTTGCTCT TGG6T66CTT TAGAGT6TCT</p> <p>241 GAGGCTTAC AAGTTTCTAT ACTCTGTTTG ACTTATCGAA AATAACTCT TATGTGTTAG</p> <p>301 GTCG6AAMA TCCAATCATA AAGTACTG6C TTTGCG6AA TACCCTAGCT CCGCTCATAA</p> <p>361 CAAGTATAG TAAATCTCTG A6TAACT6G TANTCTGAGA GTGCTTTCGT G6AGTAN6CT</p> <p>421 GCA6AGCCA GATAATAGT GTAGTTTCA AGATCTT6G CAG6AGCAGT TCGAGT66AA</p> <p>481 GAGAGTATC CAGTTTCAAG TCGACTG6C TCTTAA6CG A6G6A6A6CT CCG6AG666A</p> <p>541 ACCCTAAGA TTTTAG6CA AATTTTACTA TACG6CCA GACACGATA G6CAAGCA6C</p> <p>601 TAGTAAAGC GTTGG666G A6G666CTC TAGTGGAGT C6GATAGCT TTAACAGTGA</p> <p>661 GAGAGG6GA GATCAAGCA GATCAATCA TGAATCATCA GAAGAGCAG GCGCTTTCGG</p> <p>721 AGTGTCAAT CTAGAGGTT CACTAGTAG T66AAAGCTT ATCCCTCTCT TAGT6AGTGT</p> <p>781 GA6CTTCTT ATAGT6TGA TCAAG6CTTA GTAGCATCAT AGT6GTTCT ACGA6CTTAA</p> <p>841 CTGCTTCTT CTTCAACTA CTATAG66C CTATGATCTG ATCGCCCTA TCCATACTTC</p> <p>901 GCTCTTACT CTTAAGAT G6AATGTTA TATAAGATG AAACGATAT TCTTATGAG</p> <p>961 A6G6ATATC TAGTCAAGT AATGAGTCTG CCGGACCTC GCATTGA66G GATATGAAC</p> <p>1021 AAGG6ATGA ACAAGGATG A6AAGACTAG G6ATTC6ACA GTATTTGTTA CAGAGTATC</p> <p>1081 G6AGCCTAG AATCAAGTA TTAGCC6CC AACCAGAA TTAAGATGTT GACCC6CCA</p> <p>1141 G6AGCTAGC AATTA6CTAC TAGAGCCAA GCAACGAATA CATTATAGA AGAGACTGAA</p> <p>1201 T6GTT66CA G6ACCACTA GTAGT66TAA AGTTTCACTA GTAGTAGTAG TCA6AGAA</p> <p>1261 GTTAAAGAA C6AGAG6GAT A6G666GATG GATCGAGCTA GTTGTGTTACT TGCATGGTT</p> <p>1321 CCGGTTGTTA GTTGTGTTCT TCTTGTG6AG ACTAGT666T GTCTGTGTT6C TAGTGTGTT</p> <p>1381 AAGCTAGGT TTTACTG66T TCTTCTTTTA GTTAGTCTGG GTGAAG</p>	1426	5 parts of the sequence match with the <i>Musa acuminata</i> subsp. <i>Malaccensis</i> strain Doubled-haploid Pahang (DH-Pahang) mitochondrion sequence (Sequence ID: HG996478.1) (Table 3)	Forward: CTCTCTACCCCGACCATACATA Reverse: GTCACCTTCAACCCGAACTAAC

variants from selected sources.

#### 4. Discussion

A total of 1200 RAPD markers were narrowed down to 145 RAPD markers through *in silico* analysis by predicting the priming ability of the RAPD markers towards the *Musa acuminata* subsp. *Malaccensis* (GenBank accession: GCA\_000313855.2 genome. Premkrishnan and Arunachalam (2012) performed similar work on the *Arabidopsis thaliana* genome while Li *et al.* (2006) performed on the oil palm genome. Although the *in silico* analysis can predict the possible binding site of the RAPD markers to the genome of interest, the *in vitro* result might deviate from the prediction. This could be due to incomplete genome assembly and possible mismatch of the markers to the annealing site due to lower annealing temperature. The occurrence of markers binding to the sequences despite the mismatch due to lower annealing temperature, causes amplification of more DNA fragments compared to the prediction of *in silico* analysis. Hence, the number of amplicons observed during agarose gel electrophoresis is often more than the *in silico* prediction (Premkrishnan and Arunachalam, 2012). Similarly, in this project, the number of bands observed for most of the screened RAPD markers is higher than the number of bands predicted through *in silico* analysis. Thus, each of the bands observed in agarose gel was summarized as valid bands based on its polymorphism in Table 1 despite its deviation from *in silico* prediction. Nevertheless, the *in silico* prediction is used as a guideline to filter for the potentially meaningful RAPD markers in amplifying the banana genome.

However, three RAPD markers, OPAY-17, OPM-11 and OPZ-13 do not produce any amplicon despite the *in silico* prediction of one amplicon for each RAPD marker. This could be because the *in silico* RAPD selection was performed using the *Musa acuminata* subsp. *Malaccensis* (GenBank accession: GCA\_000313855.2) or known as double haploid Pahang (DH Pahang) instead of the *Berangan* genome itself. The priming site for the three markers are present in the reference genome but most likely the nucleotide sequences are different in the *Berangan* genome at the suggested priming site and the mismatch was not tolerated, thus, the amplification of the expected single band was absent. There were 95 polymorphic RAPD markers that revealed genotypic differences between the samples. However, only three RAPD markers reveal the genotypic differences between the normal and somaclonal variants. The unique bands from each RAPD marker were cloned and sequenced for homology search but no significant similarity were found for OPA-15 and

OPAG-14 markers, while fragments of OPE-09 match with *Musa acuminata* subsp. *Malaccensis* strain Doubled-haploid Pahang (DH-Pahang) mitochondrion sequence. This suggests that there are possible gaps between the assembled banana genome.

The RAPD markers are developed into SCAR markers as it is more locus specific, thus, enhancing the markers' reproducibility and reliability. The developed SCAR markers distinguish the somaclonal variants selectively for samples from similar sources but not specific towards a particular type of somaclonal variation of the same source. The samples of the different locations were generated from different mother plants at different times, which could have contributed to the functionality of the markers towards particular locations. Since, the source of the somaclonal variation could be pre-deposited in the mother plant or induced during the tissue culture process (James *et al.*, 2007). Although standard procedures are adhered to during the tissue culture propagation, samples cultured and grown at different times are prone to have variables and it could have contributed to different genetic modifications despite similar phenotypic observation of somaclonal variants. Similarly, in an experiment conducted using Amplified Fragment Length Polymorphism (AFLP), clonal materials that were generated at the same time showed DNA polymorphism but it was not reflected phenotypically and it was considered a latent variation (James *et al.*, 2007). Therefore, the phenotypically observed somaclonal variation could be derived from the mother plant or induced during propagation but the genetic variations are not necessarily expressed as phenotypes.

The somaclonal variation contributed by the mother plant or tissue culture propagation can either be a genetic or epigenetic modification (James *et al.*, 2007). Genetic modification can occur at the chromosomal level or nucleotide sequence level. The chromosomal modification often affects the chromosome number or structure of the chromosome due to deletion, duplication, insertion and translocation. Meanwhile, the nucleotide modification is contributed by deletion, insertion, duplication or substitution of the nucleotides (Duta-Cornescu *et al.*, 2023). Contrarily, the epigenetic modification does not alter the genome and its sequence instead it refers to the heritable chromatin modulation due to the DNA methylation and histone modification. The chromatin modulation alters the DNA accessibility for transcription and regulates gene expression (Azizi *et al.*, 2020). As the somaclonal variation could be contributed by both genetic or epigenetic modification, hence it is best to study both modifications using similar markers as conducted by Linacero *et al.* (2011). By

doing so, it is possible to detect whether the observed phenotype of the somaclonal variants is contributed by genetic modification, epigenetic modification or both. This is because, at the same locus, genetic modification occurs in some plants while epigenetic modification occurs in other plants derived from similar mother plants (Linacero *et al.*, 2011). Hence, there are possibilities of epigenetic modification for other samples at the locus in which the OPE-09 and OPAG-14 detect genetic modification.

To date, the somaclonal variation in bananas has been detected or screened through morphological markers and molecular markers (Deepthi, 2018). Morphological markers are the visual screening of qualitative and quantitative traits that have been recorded at different growth stages such as acclimatization, nursery and open field (Abdellatif *et al.*, 2012; Smith *et al.*, 2000). Besides, microscopic morphological markers also were utilized by Moradi *et al.* (2017) through the histological study of the shoots for the plantlets to study the somaclonal variation in bananas. Meanwhile, molecular markers are classified as biochemical markers and DNA markers. In biochemical markers study, the chemical processes and substances are investigated which focuses on the proteins and secondary metabolites profile. In a study conducted by Smith *et al.* (2000), the boron and calcium profile were studied as the somaclonal variation observed is similar to symptoms caused by boron and calcium deficiency. However, the boron and calcium levels between the somaclonal variant and normal are not significantly different. Contrastingly, in a gas chromatography-mass spectrometry (GC-MS) metabolites study between normal-healthy, cucumber mosaic virus (CMV)-infected and dwarf somaclonal variants of 'Williams' cultivar, 82 metabolites show significant differences among their sample groups (Cevallos-Cevallos *et al.*, 2018).

Various DNA-based molecular markers such as RAPD markers (Abdellatif *et al.*, 2012), representational difference analysis (RDA) (Oh *et al.*, 2007), sequence-related amplified polymorphism (SRAP) (Ky *et al.*, 2021), and cytogenetics approach (Moradi *et al.*, 2017) has been developed and studied in banana over the years. The RAPD markers were utilized to study the genetic differences between normal and 23 types of plant stature and foliage somaclonal variants observed in the 'Grand Naine' cultivar. The RAPD primers show a high percentage of polymorphism and the data were used for cluster analysis and principal coordinate analysis (PCORDA). The result from the analysis suggests the genetic difference between the normal and somaclonal variants which could be used as a guideline to eliminate undesired somaclonal variants (Abdellatif *et al.*, 2012).

In contrast to RAPD analysis, the RDA technique effectively isolates unique sequences of complex and highly related genomes through restriction enzyme digestion, ligation to adaptors, PCR amplification, repetitive differential hybridization and amplification (Michiels *et al.*, 1998). The application of the RDA technique to study the dwarf somaclonal variant of various banana cultivars can identify a labile portion of the genome affecting the height of the plant (Oh *et al.*, 2007). The dwarf somaclonal variation was also studied by (Prathibha *et al.*, 2021) in the 'Grand Naine' cultivar using SRAP markers which target coding sequence and resulted in the identification of eight SRAP markers combination exhibiting the DNA polymorphism between dwarf and normal 'Grand Naine' cultivar. Meanwhile, in the cytogenetics approach, the chromosomal difference between the mother plant and ramets was studied and the variability observed in the chromosome number and morphology probably caused the somaclonal variation (Moradi *et al.*, 2017). However, none of the studies conducted explored bunch or fruit-related somaclonal variations that affect the yield, therefore, our current work is crucial in addressing this gap.

#### 4. Conclusion

In conclusion, the RAPD markers profiling selectively distinguishes the *Berangan* somaclonal variants depending on the source of the samples. The successfully developed SCAR markers are feasible for future screening as only the specific unique bands are amplified. Although the developed OPAG-14 and OPE-09 markers were unable to identify all types of somaclonal variants, it provided a preliminary screening result to detect any possible occurrence of somaclonal variants. Meanwhile, the OPA-15 marker was able to identify possible normal plantlets. Hence, the developed SCAR markers can be incorporated together with the morphology inspection utilized for the early detection and elimination of *Berangan* somaclonal variants from selected sources while retaining possible normal plantlets for field planting to prevent resource wastage.

#### Conflict of interest

The authors declare no conflict of interest.

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#### References

- Abdellatif, K.F., Hegazy, A.E., Aboshama, H.M., Emara, H.A. and El-Shahed, A.A. (2012). Morphological and molecular characterization of somaclonal variations in tissue culture-derived banana plants. *Journal of Genetic Engineering and Biotechnology*, 10(1), 47-53. <https://doi.org/10.1016/j.jgeb.2012.05.002>
- Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon*, 7(10), e08093. <https://doi.org/10.1016/j.heliyon.2021.e08093>
- Azizi, P., Hanafi, M. M., Sahebi, M., Harikrishna, J.A., Taheri, S., Yassoralipour, A. and Nasehi, A. (2020). Epigenetic changes and their relationship to somaclonal variation: a need to monitor the micropropagation of plantation crops. *Functional Plant Biology*, 47(6), 508-523. <https://doi.org/10.1071/FP19077>
- Babu, K.N., Sheeja, T.E., Minoo, D., Rajesh, M.K., Samsudeen, K., Suraby, E.J. and Kumar, I.P.V. (2021). Random amplified polymorphic DNA (RAPD) and derived techniques. In Besse, P. (Ed.) *Methods in Molecular Biology*. Vol. 2222, p. 219-247. New York, USA: Humana New York. [https://doi.org/10.1007/978-1-0716-0997-2\\_13](https://doi.org/10.1007/978-1-0716-0997-2_13)
- Cevallos-Cevallos, J.M., Jines, C., Maridueña-Zavala, M.G., Molina-Miranda, M.J., Ochoa, D.E. and Flores-Cedeno, J.A. (2018). GC-MS metabolite profiling for specific detection of dwarf somaclonal variation in banana plants. *Applications in Plant Sciences*, 6(11), e01194. <https://doi.org/10.1002/aps.3.1194>
- Deepthi, V.P. (2018). Somaclonal variation in micro propagated bananas. *Advances in Plants and Agriculture Research*, 8(6), 624-627.
- Duta-Cornescu, G., Constantin, N., Pojoga, D.M., Nicuta, D. and Simon-Gruita, A. (2023). Somaclonal variation-advantage or disadvantage in micropropagation of the medicinal plants. *International Journal of Molecular Sciences*, 24(1), 838. <https://doi.org/10.3390/ijms24010838>
- Heinz, D.J. and Mee, G.W.P. (1971). Morphologic, cytogenetic, and enzymatic variation in *saccharum* species hybrid clones derived from callus tissue. *American Journal of Botany*, 58(3), 257-262. <https://doi.org/10.1002/j.1537-2197.1971.tb09971.x>
- FGV Holdings Berhad. (2020). FGV produces one million banana clone varieties to meet market's demand. Retrieved on August 23, 2023 from FGV Holdings Website: [https://www.fgvholdings.com/press\\_release/fgv-produces-one-million-banana-clone-varieties-to-meet-markets-demand/#:~:text=FGV%20is%20targeting%20to%20produce,consumption%20demand%20for%20the%20fruit.](https://www.fgvholdings.com/press_release/fgv-produces-one-million-banana-clone-varieties-to-meet-markets-demand/#:~:text=FGV%20is%20targeting%20to%20produce,consumption%20demand%20for%20the%20fruit.)
- Israeli, Y., Reuveni, O. and Lahav, E. (1991). Qualitative aspects of somaclonal variations in banana propagated by *in vitro* techniques. *Scientia Horticulturae*, 48(1), 71-88. [https://doi.org/10.1016/0304-4238\(91\)90154-Q](https://doi.org/10.1016/0304-4238(91)90154-Q)
- James, A.C., Peraza-Echeverria, S., Peraza-Echeverria, L. and Herrera-Valencia, V. (2007). Variation in micropropagated plants. *Acta Horticulturae*, 748, 55-63. <https://doi.org/10.17660/ActaHortic.2007.748.4>
- Juned, S.A., Jackson, M.T. and Ford-Lloyd, B.V. (1991). Genetic variation in potato cv. record: evidence from *in vitro* 'regeneration ability'. *Annals of Botany*, 67(3), 199-203. <https://doi.org/10.1093/oxfordjournals.aob.a088123>
- Kiran, U., Khan, S., Mirza, K.J., Ram, M. and Abdin, M.Z. (2010). SCAR markers: a potential tool for authentication of herbal drugs. *Fitoterapia*, 81(8), 969-976. <https://doi.org/10.1016/j.fitote.2010.08.002>
- Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M. and Sadh, R.K. (2016). Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech*, 6, 54. <https://doi.org/10.1007/s13205-016-0389-7>
- Larkin, P.J. and Scowcroft, W.R. (1981). Somaclonal variation - a novel source of variability from cell cultures for plant improvement. *Theoretical Applied of Genetics*, 60(4), 197-214. <https://doi.org/10.1007/BF02342540>
- Li, J.J., Pei, G.L., Pang, H.X., Bilderbeck, A., Chen, S. S. and Tao, S.H. (2006). A new method for RAPD primers selection based on primer bias in nucleotide sequence data. *Journal of Biotechnology*, 126(4), 415-423. <https://doi.org/10.1016/j.jbiotec.2006.05.003>
- Linacero, R., Rueda, J., Esquivel, E., Bellido, A., Domingo, A. and Vázquez, A.M. (2011). Genetic and epigenetic relationship in rye, *Secale cereale* L., somaclonal variation within somatic embryo-derived plants. *In vitro Cellular and Developmental Biology. Plant*, 47(5), 618-628. <https://doi.org/10.1007/s11627-011-9407-y>
- Michiels, L., Van Leuven, F., van den Oord, J.J., De Wolf-Peeters, C. and Delabie, J. (1998). Representational difference analysis using min quantities of DNA. *Nucleic Acids Research*, 26(15), 3608-3610. <https://doi.org/10.1093/nar/26.15.3608>
- Mohamad Roff, M.S., Tengku Abdul Malik, T.M. and Sharif, H. (2012). Challenges to banana production in Malaysia: a threat to food security. *The Planter*, 88(1030), 13-21. <https://doi.org/10.56333/>

- tp.2012.001
- Moradi, Z., Farahani, F., Sheidai, M. and Nejad Satari, T. (2017). Somaclonal variation in banana (*Musa acuminata colla* cv. Valery) regenerated plantlets from somatic embryogenesis: histological and cytogenetic approaches. *Caryologia*, 70(1), 1-6. <https://doi.org/10.1080/00087114.2016.1198665>
- Oh, T.J., Cullis, M.A., Kunert, K., Engelborghs, I., Swennen, R. and Cullis, C.A. (2007). Genomic changes associated with somaclonal variation in banana (*Musa* spp.). *Physiologia Plantarum*, 129(4), 766-774. <https://doi.org/10.1111/j.1399-3054.2007.00858.x>
- Prathibha, K.Y., Shubha, S., Ravishankar, K.V., Manjula, A.C. and Keshamma, E. (2021). Identification of molecular markers to detect somaclonal variations in banana cv. Grand Naine (AAA) using sequence related amplified polymorphism (SRAP) molecular marker technique. *Internationa Journal of Creative Research Thoughts*, 9(8), b754-b759. <https://doi.org/10.17148/IARJSET.2022.9427>
- Premkrishnan, B.V. and Arunachalam, V. (2012). *In silico* RAPD priming sites in expressed sequences and iSCAR markers for oil palm. *Comparative and Functional Genomics*, 2012(1), 913709. <https://doi.org/10.1155/2012/913709>
- Razani, M., Kayat, F., Mohamed Redwan, R. and Susanto, D. (2019). Effect of somaclonal variation in *Musa acuminata* cv. Berangan through micropropagation using RAPD. *Biotechnology (Faisalabad)*, 18(1), 9-14. <https://doi.org/10.3923/biotech.2019.9.14>
- Razani, M., Kayat, F., Mohamed Redwan, R. and Susanto, D. (2020). RAPD analysis of *Musa acuminata* cv. Berangan plantlets in nursery stage from long-term subculture. *Pertanika Journal of Tropical Agricultural Science*, 43(2), 183-192.
- Sahijram, L., Soneji, J.R. and Bollamma, K.T. (2003). Analyzing somaclonal variation in micropropagated bananas (*Musa* spp.). *In vitro Cellular and Developmental Biology - Plant*, 39(6), 551-556. <https://doi.org/10.1079/IVP2003467>
- Seng, T.-Y. and Zaman, F.Q. (2006). DNA extraction from mature oil palm leaves. *Journal of Oil Palm Research*, 18, 1-6.
- Smith, M.K., Hamill, S.D., Doogan, V.J. and Daniells, J. (2000). Characterisation and early detection of an offtype from micropropagated Lady Finger bananas. *Australian Journal of Experimental Agriculture*, 39 (8), 1017 - 1023. <https://doi.org/10.1071/EA99098>
- Tan, B.C. (2022). Can banana be a success story for Malaysia? *Journal of Agribusiness Marketing*, 9(1), 13-22. <https://doi.org/10.56527/jabm.9.1.2>
- van den Bulk, R.W., Löffler, H.J.M., Lindhout, W.H. and Koornneef, M. (1990). Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis. *Theoretical and Applied Genetics*, 80(6), 817-825. <https://doi.org/10.1007/BF00224199>
- Williams, M.E., Hepburn, A.G. and Widholm, J.M. (1991). Somaclonal variation in a maize inbred line is not associated with changes in the number or location of Ac-homologous sequences. *Theoretical and Applied Genetics*, 81(2), 272-276. <https://doi.org/10.1007/BF00215733>