Conference Paper

Simple Sequence Repeats (SSR) Marker Screening Related to Orange Fleshed Sweet Potato F1 Genotype Resistance against Scab (*Sphaceloma batatas* Saw.)

Nurfitriani Rista[1], Fitri Widiantini[1], Anna Aina Roosda[2], Endah Yulia[1], and Agung Karuniawan[2]

[1]Department of Plant Pests and Diseases Faculty of Agriculture Universitas Padjadjaran, Jln. Raya Bandung – Sumedang Km 21 Jatinangor 45363, Indonesia
[2]Department of Plant Breeding Faculty of Agriculture Universitas Padjadjaran, Jln. Raya Bandung – Sumedang Km 21 Jatinangor 45363, Indonesia

Abstract

Orange fleshed sweet potato contains high beta carotene as vitamin A precursor. However, its production is limited by the presence of scab disease caused by *Sphaceloma batatas* Saw. The disease is able to cause yield loss up to 60%. Best controlling method is using resistant varieties. However, the development of resistant varieties are involving long procedures which is time consuming. The long procedure of resistance varieties selection can be shorted cut using molecular markers such as SSR (Simple Sequence Repeats). Specific SSR markers for sweet potato resistance against scab has not been found. This study aimed to screen SSR molecular markers which were related to resistance to scab. The study used 5 resistant genotypes, 5 susceptible genotypes, and 6 SSR primers. PCR analysis showed that those SSR primers were polymorphic. Furthermore, the biplot analysis result demonstrated that several markers allele were related to plant resistance against scab. This finding indicated that these particular SSR markers can be used in sweet potato breeding program as marker assisted selection to develop resistant variety against scab disease.

Keywords: *Sphaceloma batatas*; orange fleshed sweet potato; SSR markers.

Corresponding Author:
Agung Karuniawan
akaruni1@unpad.ac.id

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1. Introduction

Indonesia is the fifth-largest sweet potato producer in the world after China, Nigeria, United Republic of Tanzania, and Uganda [1] with a total area harvested in 2013 was 161,850 ha and productivity of 147.47 quintals/ha [2]. In Indonesia, 89% of the sweet potato production is used as food with the level of consumption of 7.9 kg/capita/year, while 11% of production is used for industrial raw materials [3]. Considering the high demand of sweet potatoes for food and processing materials, factors affecting sweet

potato production need to be considered. The plant disease is one of the limiting factors that are able to reduce plant production up to 14% worldwide [4].

Scab (*Sphaceloma batatas* Saw.) is one of the important diseases in sweet potato. In Indonesia, scab causes the decline of local sweet potato yield from 20 ton/ha to 7 – 10 ton/ha. Scab causes sunken and scabby lessions on petioles and leaf veins. Severe infected plants show brittle and distinct erect of terminal that can be distinguished from far [5]. Jackson & McKenzie [6] reported that in Papua New Guinea, the sweet potato yield decreased by 60% due to scab. Further Jackson & McKenzie [6] also reported that in Fiji and Tonga, some varieties of sweet potatoes were very susceptible to scab, causing the plants failed to produce tubers. Accordingly, the use of resistant varieties is necessary to suppress the yield loss caused by scab. However, developing resistant varieties takes a long period of time. Using molecular markers assisted selection (MAS) such as SSR (Simple Sequence Repeats) can short cut this process. SSR molecular markers have high levels of heterozygosity and high polymorphism [7, 8]. Some SSR markers have been effectively used in the process of selecting paddy cultivars resistant to blast disease [9]. However, specific SSR markers for sweet potato resistance against scab has not been developed.

Therefore, development of orange-fleshed sweet potato resistant to scab disease has been started at the Plant Breeding Laboratory Faculty of Agriculture, Universitas Padjadjaran. As much as 144 F1 genotypes of orange-fleshed sweet potatoes were generated from polycross between 29 local landraces [10]. Widiantini et. al. [11] reported among these 144 F1 genotypes, 5 F1 genotypes were highly resistant and 5 F1 genotypes were susceptible to scab during two growing seasons. Therefore, these 10 F1 genotypes can be used to obtain specific SSR marker related to scab disease. Six SSR markers were used in this study to find their correlation with the resistant and susceptible F1 genotype of sweet potato plants.

2. Materials and Methods

2.1. DNA extraction

Five resistant F1 genotypes, 5 susceptible F1 genotypes, and 6 SSR primers (Macrogen, Inc) used in the study are presented at Table 1 and Table 2. Total genomic DNA was extracted from the leaves using the modified CTAB extraction protocol by Doyle & Doyle [12]. Quality of DNA was checked on 1.5% agarose gel electrophoresis stained with 1x gel red a constant voltage of 65 V for 70 min. Quality of DNA visualized using gel documentation system. The quantity of DNA was observed using Spectrophotometer at a wavelength of 260 nm and 280 nm.

Table 1: Ten genotypes orange fleshed sweet potato list.

No.	Genotype code	Genotype name	Resistant/susceptible
1	96	F1 (9) LAD	Resistant
2	98	F1 (25) HAR	Resistant
3	99	F1 (2) MPAND	Resistant
4	105	F1 (2) F1 (12) T1	Resistant
5	119	F1 (1) Bras2	Resistant
6	23	F1 (5) CTRA	Susceptible
7	95	F1 (7) KUMEROT	Susceptible
8	111	F1 (3) Kumer	Susceptible
9	227	F1 (1) CCRA	Susceptible
10	230	F1 (17) KUMEROT	Susceptible

Table 2: SSR primer list.

Primer code	Primer	*Sequence* (5′ *to* 3′)		TA (°C)	Reference
		Forward	Reverse		
A	Xbarc75	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTCTA	52	Zhou *et al.*
B	Xbarc147	GCGCCATTTATTCATGTTCCTCAT	CCGCTTCACATGCAATCCGTTGAT	52	Zhou *et al.*
C	Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	52	Zhou *et al.*
D	Xgwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	52	Zhou *et al.*
E	Xgwm389	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	52	Zhou *et al.*
F	XBARC87	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC	52	Zhou *et al.*

2.2. PCR reaction

The PCR reaction mix includes the following: PCR reactions were performed in a total volume of 10 μl. Concentrations of the components were 7 μl *Kappa Ready Mix* 2G (KAPA Biosystem), 1 μl *template* DNA, 1 μl *forward* primer (10 μM), dan 1 μl *reverse* primer (10 μM). PCR reactions were carried out in a Eppendorf Mastercycler Gradient thermal cycler. PCR profile started with 3 min at 94°C, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s, extension at 72°C for 30 s. A final extension at 72°C for 5 min was included. PCR products were separated on 1.5% agarose gel and electrophoresed at 65 V for 70 min. Agarose gel stained with 1x gel red (Biotium) to detect the amplicons in 0.5x TBE buffer. Gels visualized under UV light using G-box.

2.3. Data analysis

All genotypes were observed for the presence or absence of SSR band. The data were entered into a matrix with discrete variables, 1 as the presence of a SSR band and 0 was for the absence of the SSR band. This matrix was then used for further analysis using Biplot analysis [13]. Biplot analysis performed using XLStat version 2009 and Biplot version 2007.

3. Results and Discussion

Molecular markers used for MAS should permit efficient screening of large populations and show a high degree of reproducibility to guarantee optimal cost-effectiveness [14]. SSR is one of the molecular markers. SSR has advantages than other markers. SSR markers combine reliability and genomic abundance with high levels of polymorphism [14]. SSR markers do not require sophisticated DNA extraction methods [15].

Classical breeding program of sweet potato constrained by its hexaploid genomic. Therefore, the use of molecular markers can speed up the development of improved cultivar program. Six SSR markers were evaluated for the presence of alleles in the sweet potato F1 genotypes. The visualization results of each primer can be seen in Fig 1. All primers optimized for each PCR program and the annealing temperature showed polymorphic. Polymorphic bands are primers that produce more than one alleles in the PCR product. Polymorphic is if there are two or more alleles at the same locus. Polymorphic genes is when one allele has a frequency of less than 0.99 [16].

Primers which produce more than one allele on PCR products increase the probabilities of getting a primer that is associated with the character of the resistance to scab. This is because genetic markers must be able to distinguish individuals in the population studied [17]. In Fig 1, all primers optimized for each PCR program and the annealing temperature showed polymorphic bands, produced more than one alleles in the PCR product. The emergence of allele showed a score 1 and no emergence of allele showed a score 0. The primers used in this study had a minimum number of allele 1 and maximum number of allele 4. Six primers showed polymorphic bands and used for the Biplot analysis.

Based on Fig 2, genotypes number 98 and 99 are genotypes with resistant category which is in the same sector and flanked by two imaginary lines. The genotype can be characterized by primer A (allele 1) and primer B (allele 2). Genotypes 96, 105, and 119 were resistant genotype which located at the same sector with primer A (allele 2 and 3), primer C (allele 2 and 3), primer D (allele 2), primer E (allele 3 and 4) and primer F (allele 1). These primers can be used as the identifier primer related to scab resistance.

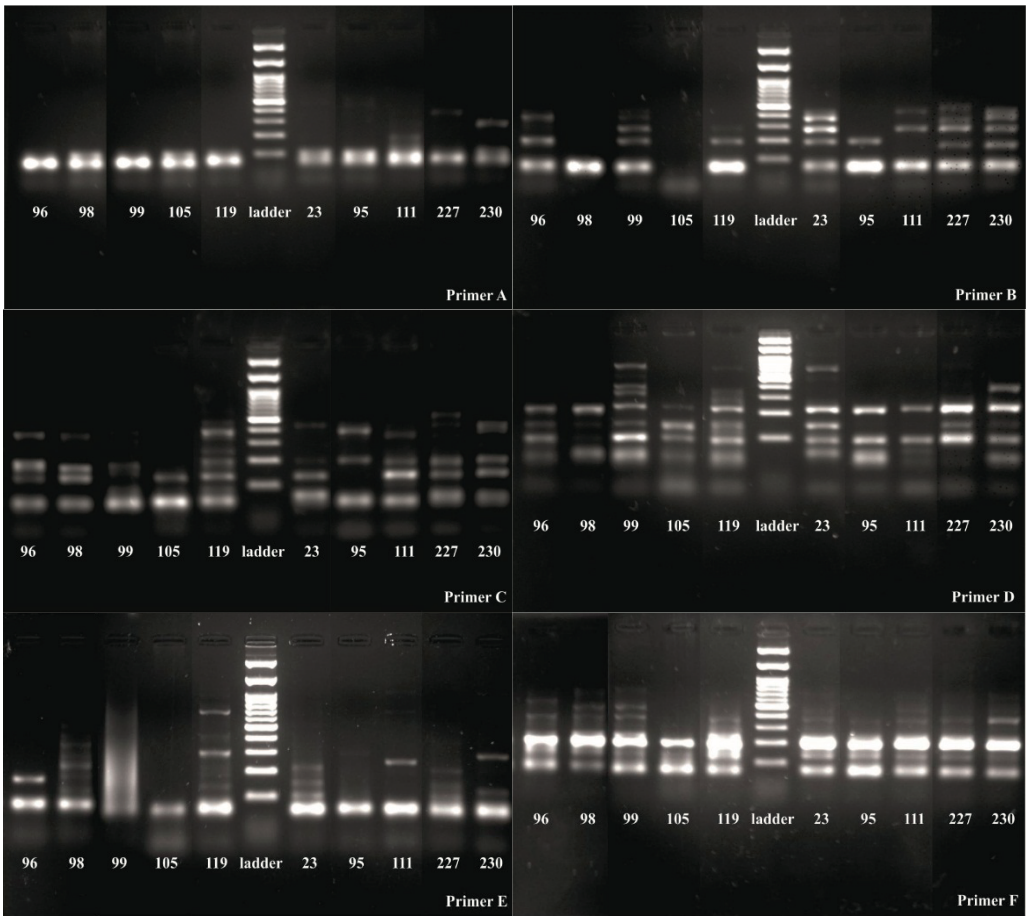

Figure 1: PCR products on six SSR primers.

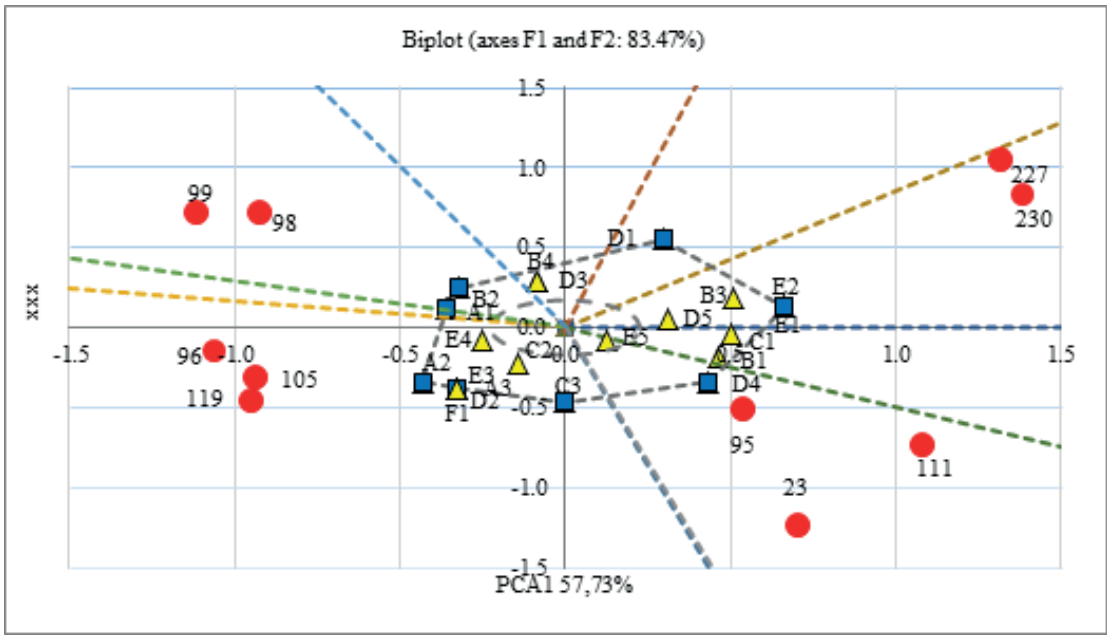

Figure 2: Biplot genotype resistant related to SSR primer.

Genotype 227 and 230 were susceptible genotype which can be characterized by primer B (allele 3), D (allele 5), and E (allele 1 and 2). Primer D (allele 4) and E (allele 5) can characterize genotypes 23, 95, and 111 which were susceptible genotypes. Therefore, these primers suggested can be used for characteristic identification of scab susceptibility. Primer B allele 1 (B1), B allele 4 (B4), C allele 1 (C1), D allele 1 (D1), and D allele 3 (D3) did not characterize any genotype.

All primers can characterize plant resistance to certain alleles. Primer A - F is the identifier primer of wheat plant resistance against scab [18]. Sweet potato resistance against scab can be characterized by the primers, presumably because it has similar genes to wheat plant resistance against scab.

Biplot chart is a visualization of disease resistance grouping characters that is represented by the genotype and molecular markers as an identifier. Biplot correlations are at the cumulative percentage of 83.47% with two PC-forming groupings. Selection of molecular markers associated with a particular phenotypic character has effectiveness up to 80% by using discriminant analysis Biplot [19]. O'Rourke et al. [20] stated that the analysis Biplot can effectively describe the pattern of relationships, interactions, and the identifier of a character and genotype if it has a cumulative percentage of 10% for one component and 70%-80% for the combined components. Thus, Biplot in this study effective to describe the resistance correlation pattern models genotype and molecular markers SSR.

4. Conclusion

Primer A (allele 1, 2 and 3), primer B (allele 2), primer C (allele 2 and 3), primer D (allele 2), primer E (allele 3 and 4) and primer F (allele 1) can be used as marker associated with resistance to scab.

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