DNA-fingerprinting of downy mildew resistance (DMR) and quality protein maize (QPM) lines using eleven simple sequence repeats (SSR)

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Maize cultivars possessing resistance to downy mildew pathogen and high quality protein is the ultimate goal of maize breeding program in Indonesia. A collaborative research between Lab. of Plant Breeding, Padjadjaran University (UNPAD) and Indonesia Corn Research Institute (ICERI) has been established to develop maize having these important traits. A set of experiment was conducted at the Field Experimental Station, Faculty of Agriculture, Padjadjaran University in Jatinangor and Lab. of Molecular Biology, Biotechnology Research Institute for Food Crop. Bogor to finger-print downy mildew resistance and quality protein of maize lines to be used as parental lines in the breeding program. Five of downy mildew resistant (DMR) inbred lines and six of quality protein maize (QPM) inbred lines were used in the study. Molecular characterization using eleven simple sequences repeats (SSR) was done to profile these inbred lines. There were three groups of parental lines based on Jackard coefficient similarity at 0.3.

Keywords DNA fingerprinting . Downy mildew resistance . Quality protein maize . Simple sequence repeats

Maize cultivars possessing resistance to downy mildew pathogen and high quality protein as indicated by high lysine and tryptophan is the ultimate goal in any maize-breeding program in Indonesia. It is because Indonesia produces low quality of maize since percentage of lysine in the grain less than FAO standard of 4%. In addition, downy mildew is the main problem in maize production area of Indonesia in Lampung, South Sulawesi, East Java, and Central Java.

Breeding for QPM and resistance against *P. maydis* requires a great effort as QPM is ruled by opaque2 gene which is a recessive gene (AMBIONET, 2002), thus resistance to downy mildew is controlled by additive with dominant and epistatic effects (Capio, 1990; Ruswandi, *et al*. 2002[b]). In addition, severe epiphytotic condition is necessary in selection that makes excellent genes, such as yield lost (Kaneko and Aday, 1980). Therefore, selection for these two important traits spends much more time to complete.

Application of molecular marker is the most effective solution to breach those constrains since molecular marker could aid selection for resistance to downy mildew pathogen in the absence of the

artificial inoculation. This technology could eliminate confounding effects of environmental variation associated with conventional breeding. Thus, molecular marker, particularly simple sequence repeats (SSR) analysis can be completed during the early seedling stage and can select both recessive opaque2 and resistance to *P. maydis* gene (Ruswandi, *et al*., 2002[b]; Khairallah, 2000).

SSR is widely used as marker aided selection in crop improvement, including in maize. The importance features of SSR had been clearly described by Senior *et al*. (1996). SSR is PCR-generated marker so that polymorphism can be detected in short time and early stage of crop. SSR is easy to amplify and to detect that make possible for automation. SSR is very robust which make application of SSR as marker aided selection is cost effective and efficient. SSR marker is inherited as co-dominant that makes ease identification of pure hybrid. Furthermore, SSR and QTL map for resistance to downy mildew pathogen (Ruswandi et al., 2002[a]) and SSR marker for recessive opaque2 gene (Khairallah, 2000) is available as a requirement to apply marker aided selection (MAS).

The objectives of the experiment were to identify polymorphism of DMR and QPM inbred lines and to fingerprint downy mildew resistance (DMR) and quality protein maize lines using eleven SSR markers.

Materials and methods

Eleven maize inbred lines including six QPM lines, namely CML 161, CML 162, CML 163, CML 164, and CML 172, and 5 DMR lines, i.e. MR 10, AMATLCOHS, Pi 345, Nei 9008, and Ki 3, were isolated their DNA for genotyping using the SSR marker system.

Maize DNA samples were isolated following the technique described by Hoisington *et al*., (1997) with slight modifications (Ruswandi, *et al*., 2001). Concentrations of DNA samples were estimated after gel electrophoresis by comparing DNA quantity with known DNA standard markers.

A total of 11 primers were used to amplify QPM and DMR parental lines. The primers used were 20 mer oligo-nucleotides from commercially available primers such as: phi374118, phi109275, phi328175, phi053, phi087, phi034, phi109188, phi063, phi072, umc1304, and phi423796.

The polymerase chain reactions were performed using optimum amplification conditions as described by Ruswandi *et al*., (2001). Amplification reactions was performed in 10μL total volume containing 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.16 mM dNTPs, 0.4 mM each forward and reverse primer, 1U Taq polymerase and 20 ng of genomic DNA. Amplification was carried out in an MJ Research PTC- 100 Programmable Thermocycler with the following profile: one cycle of 1 min 93^o C initial denaturation, 30 cycles of 1 min 93^o C denaturation, 2 min 56^o C/58^o C/60^o C annealing and 2 min 72^o C extension, followed by 1 cycle of 5 min 72^o C for final extension. Amplicon were resolved on

poly-acrylamide gel in 1xTBE running at 70-75 W for 45 min to one hour. The gel was silver stained as described by the manufacturer (Promega, 1998).

Polymorphic index content (PIC) was calculated following the formulae suggested by Smith *et al.*, (1997).

PIC = 1 - $\sum f_i^2$ I = 1,

where f_i^2 is the frequency of the i^{th} allele

Results and discussion

DNA fingerprinting of 11 QPM and DMR lines were presented in Table 1. Numbers of alleles' QPM and DMR lines were ranging from 2 up to 5 alleles, with the average alleles of 3.6. Size of PCR products we ranged from 121 up to 232 base pairs (bp).

Table 1. Fingerprinting of 11 QPM-DMR lines based on 11 SSR analyses

No.	Primer pairs	Bin No.	Repeat type	Size-ranged	PIC value (%)	Alleles #
1	phi374118	3.02	ACC	217-232	80	5
2	phi109275	1.00	AGCT	121-140	74	4
3	phi328175	7.04	AGG	121-138	74	4
4	phi053	3.05	ATAC	168-193	70	4
5	phi087	5.06	ACC	151-174	63	3
6	phi034	7.02	CCT	123-144	62	4
7	phi109188	5.00	AAAG	163-177	61	5
8	phi063	10.02	TATC	f1,g1,g2,g3	57	4
9	phi072	4.00	AAAC	g1,h1,h2	51	3
10	umc1304	8.02	$(TCGA)_4$	132-139	46	2
11	phi423796	6.01	AGCC	127-135	26	2
	Average				60	3.6

PIC values ranged from 26% up to 80%, with the average PIC of 60%. This means that the marker to be used were highly discriminative. Smith *et al.* (1997) explained that PIC give an estimate of the discriminatory power of a locus by considering the number of alleles that are expressed and also the relative frequencies of those alleles. SSR loci phi 374118 was the most polymorphic marker as showed by its polymorphic index coefficient (PIC) at 80% (Table 1). This locus also had the highest number of alleles at 5. On the other hand, SSR loci phi 423796 was the

least polymorphic marker as showed by its PIC at 26 %. In addition, this locus had the lowest number of alleles at 2.

It is also noted that not all marker having high number of alleles could result on high PIC. For example, phi109188 which having 5 alleles and PIC at 61% had less discriminatory locus than phi087 that revealed 3 alleles at 63%. Smith *et al.* (1997) illustrated that a marker locus that reveals five alleles, but where one allele is found in very high frequency (e.g. freq. = 0.9), has overall less discriminatory capability than a locus that also has five alleles, but in which those alleles are found in more equal frequencies.

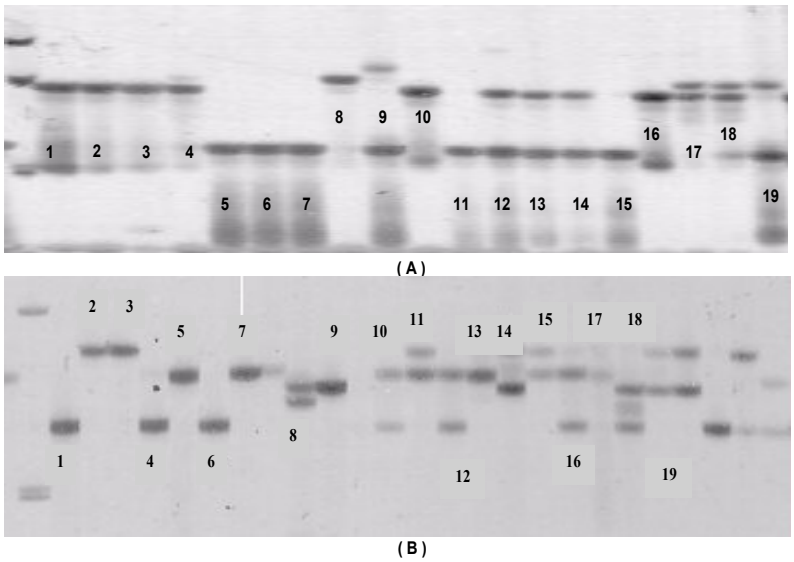

Figure 1. Profile of DNA amplified using (A) SSR Phi 034, (B) SSR Phi 374188

(1) CML161, (2) CML162, (3) CML163, (4) CML164, (5)CML 165, (6) CML172, (7) Nei9008, (8) Ki 3, (9) P 345, (10) MR 10, (11) AMATLCOHS 9-1, (12) Ki 3 x CML172, (13) Ki-3 x CML 165, (14) MR 10 x CML172, (15) Nei9008 x CML172, (16) Nei9008 x CML164, (17) P 345 x CML164 , (18) P 345 x CML163, (19) Nei9008 x CML163

Samples of amplified DNA using SSR marker were shown in Figure 1. It was fond that not all bands revealed by SSR markers followed Mendelian inheritance as co-dominant. For example DNA sample of AMATLCOHS 9-1, an inbred developed by CIMMYT, amplified using SSR marker phi 374188 (Figure 1, samples B-11) showed as an F_1 rather than an inbred. Smith *et al.* (1997) described some factors that could explain that phenomena, i.e.: (i) that residual heterozygosity remaining within an inbred at the time it was originally used to make the parental cross for subsequent progeny development by successive self-pollination and selection, (ii) the progeny line could have been contaminated by out-sourced pollen due to either poor pollen control during its development or by physical mixing of seed from another genotype, (iii) a parental stock could have

changed genetically by mutation after the time it was used to make the parental cross from which the progeny line was derived.

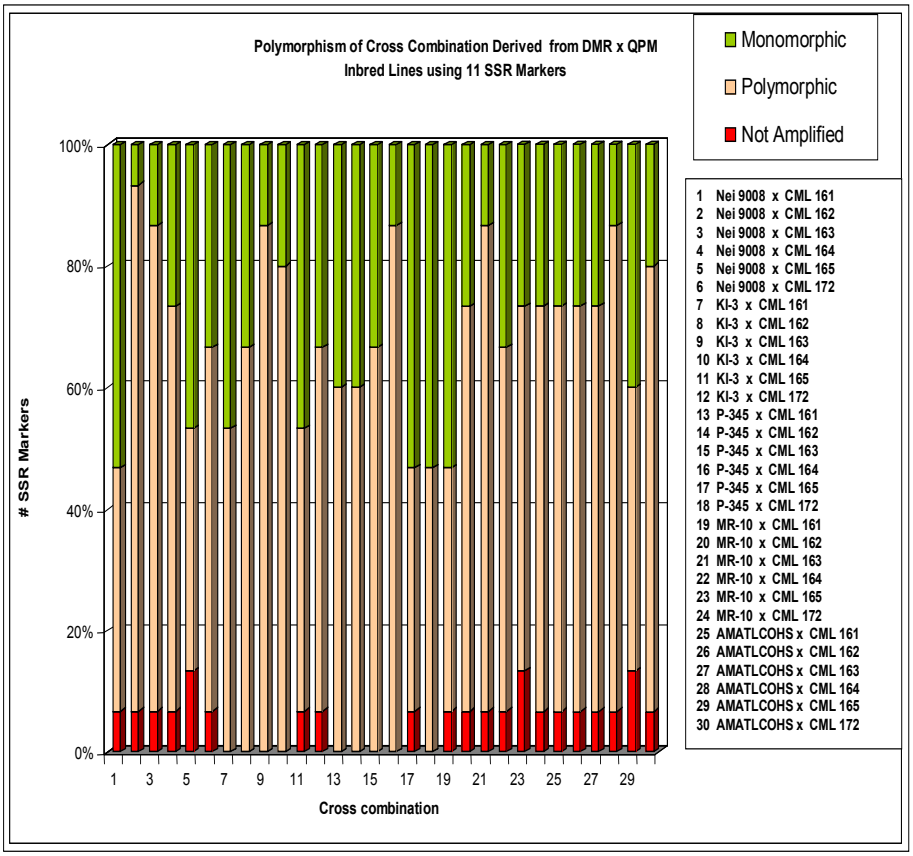

Figure 2. Polymorphism of cross combinations derived from DMR x QPM inbred lines

Polymorphism of cross combinations derived from DMR x QPM inbred lines was presented in Figure 2. Cross combinations having highest polymorphism at 86.7% were: Nei 9008 x CML 162, Ki 3 x CML 163, and P 345 x CML 164. Cross combinations having lowest polymorphism at 40 % were: Nei 9008 x CML 161, Nei 9008 x CML 165, P 345 x CML 165, and MR 10 x CML 161.

DNA profiling is very important stage in molecular marker aided selection for the next reasons: (i) to molecularly map genome, (ii) to protect collection from any conflict, (iii) to identify any changes in DNA level, (iv) to study genetic relationship of the germplasm, (v) to monitor the purity of inbred lines, (vi) to test hybrid derived from crossing between two inbred lines.

Conclusion

As conclusions: (i) all SSR markers that used in fingerprinting were polymorphic; (ii) SSR marker was useful in profiling DNA of parental lines to be used in developing QPM and DMR.

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