Indonesian Journal of Agricultural Science Vol. 16 No. 2, October 2015: 79-86

GENETIC DIVERSITY OF S3 MAIZE GENOTYPES RESISTANT TO DOWNY MILDEW BASED ON SSR MARKERS

Keragaman Genetik Genotipe S3 Jagung Tahan terhadap Penyakit Bulai Berbasis Marka SSR

Amran Muis, Nurnina Nonci and Marcia B. Pabendon

Indonesian Cereal Research Institute
Indonesian Agency for Agricultural Research and Development
Jalan Dr. Ratulangi No. 274, PO Box 173, Maros 90 514, South Sulawesi, Indonesia
Phone +62 411 371529, Fax. +62 411 371961, E-mail: balitsereal@litbang.pertanian.go.id
Corresponding author: amranmuis1@gmail.com

Submitted 17 April 2015; Revised 14 August 2015; Accepted 20 August 2015

ABSTRACT

The compulsory requirement for releasing new high yielding maize varieties is resistance to downy mildew. The study aimed to determine the level of homozygosity, genetic diversity, and genetic distance of 30 S3 genotypes of maize. Number of primers to be used were 30 polymorphic SSR loci which are distributed over the entire maize genomes. The S3 genotypes used were resistant to downy mildew with homozygosity level of >80%, genetic distance between the test and tester strains >0.7, and anthesis silking interval (ASI) between inbred lines and tester lines was maximum 3 days. The results showed that 30 SSR primers used were spread evenly across the maize genomes which were manifested in the representation of SSR loci on each chromosome of a total of 10 chromosomes. The levels of polymorphism ranged from 0.13 to 0.78, an average of 0.51, and the number of alleles ranged from 2 to 8 alleles per SSR locus, an average of 4 alleles per SSR locus. The size of nucleotides in each locus also varied from 70 to 553 bp. Cophenetic correlation value (r) at 0.67 indicated that the Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) was less reliable for differentiating genotypes in five groups. Of the total of 30 genotypes analyzed, 17 genotypes had homozygosity level of >80% so it can be included in the hybrid assembly program.

[***Keywords*:** Maize, downy mildew, resistant genotypes, genetic diversity, simple sequence repeat]

ABSTRAK

Syarat utama untuk merilis varietas unggul baru jagung adalah ketahanan terhadap penyakit bulai. Penelitian ini bertujuan untuk mengetahui tingkat homozigositas, keragaman genetik, dan jarak genetik 30 genotipe S3 jagung. Jumlah primer yang digunakan sebanyak 30 lokus polimorfik SSR yang tersebar pada seluruh genom jagung. Genotipe S3 yang digunakan tahan terhadap penyakit bulai dengan tingkat homozigositas >80%, nilai jarak genetik antara genotipe yang diuji dengan strain penguji > 0,7, dan anthesis silking interval *(ASI) antara galur inbrida dan galur uji maksimum 3 hari. Hasil analisis keragaman genetik berbasis marka SSR genotipe S3 jagung tahan penyakit bulai menunjukkan bahwa 30 primer SSR yang digunakan menyebar secara merata pada seluruh genom jagung, yang ditunjukkan oleh adanya representasi lokus SSR pada setiap kromosom dari total 10 kromosom. Tingkat polimorfisme berkisar antara 0,13-0,78, rata-rata 0,51, dengan jumlah alel 2-8 alel per lokus SSR, rata-rata 4 alel per lokus SSR. Berat molekul basa-basa dalam setiap lokus juga cukup bervariasi dari 70 sampai 553 bp. Nilai korelasi kofenetik (r) pada 0,67 menunjukkan bahwa* Unweighted Pair-Group Method Using Arithmetic Averages *(UPGMA) kurang andal membedakan genotipe dalam lima kelompok. Dari total 30 genotipe yang dianalisis, 17 genotipe memiliki tingkat homozigositas >80% sehingga dapat diikutkan dalam program perakitan jagung hibrida.*

[***Kata kunci*:** Jagung, penyakit bulai, genotipe tahan, keragaman genetik, simple sequence repeat]

INTRODUCTION

Maize is a strategic commodity in Indonesia's economy, especially in animal feed industry (60%). In the eastern parts of Indonesia, such as East Nusa Tenggara and in several other provinces, maize is consumed as a primary food (30%). The demand for maize increases in line with population growth and development of feed and food industries. Nevertheless, the national maize production cannot meet domestic demand, which makes the volume and value of imported maize tend to increase from year to year. Maize is commonly grown by farmers on paddy fields after rice, upland, and hillsides.

Maize development in Indonesia faced a problem of diseases, especially downy mildew which is a major and important disease on maize. Disease infection on susceptible varieties causes yield loss up to 100% (Sudjono 1988; Wakman 2004).

Downy mildew is caused by fungi *Peronosclerospora* spp. that infect maize plant through spores carried by the wind in the morning. There were 10 species from

three genera that cause downy mildew on maize, namely *P. maydis*, *P. phillipinensis*, *P. sacchari*, *P. sorgi*, *P. spontanea*, *P. miscanthi*, *P. heteropogani*, *Sclerospora macrospora*, *S. philippinensis*, *S. rayssiae* and *S. graminicola* (Shurtleff 1980; Rathore *et al*. 2002; Wakman and Djatmiko 2002, Yen *et al*. 2004). Telle *et al*. (2011) discovered downy mildew pathogen species, namely *P. eriochloae* which has not been reported as a pathogen on maize. In Indonesia, three species of *Peronosclerospora* have been found spreading on different islands, namely *P. maydis* in Lampung, West Kalimantan and East Java, *P. philippinensis* in Sulawesi, and *P. sorghi* in Aceh, North Sumatra and West Java (Mikoshiba *et al*. 1977; Wakman and Hasanuddin 2003; Lukman *et al*. 2013; Muis *et al*. 2013).

The released new high yielding maize varieties must have resistance to downy mildew. The level of resistance of maize varieties to downy mildew varied and many of them were susceptible to downy mildew (Wakman and Kontong 2000). Resistant screening of maize from the germplasm collection show a number of S3 lines which are resistance to downy mildew (Muis *et al*. 2013). Theoretically, for S3 lines, the levels of homozygosity have reached 85%. Thus, these lines can be incorporated into breeding programs of hybrid or open pollinated maize varieties. However, in the implementation of activities, the homozygosity levels sometimes were not as expected because it is influenced by several factors, among which is contamination during selfing. Maize is highly vulnerable to contamination because it is a cross-pollinated plant.

Advances in biotechnology, especially in the field of molecular biology led to the genetic variability of a population that can be observed at the molecular level. DNA analysis has a high efficiency and accuracy, which can assist breeders in identification and prediction of genetic diversity of fungi such as *Peronosclerospora* spp. (Hikmawati 2011).

Ability of affinity plays an important role in crop improvement as it helps breeders to study and compare performance of new line in hybrid combination. The high degree of heterotic value in F1 hybrid formation indicates that the parents are genetically distance than crosses that showed lower heterotic levels (Mungoma and Pollack 1988).Therefore, information on genetic diversity of inbred lines is very important in developing a heterotic level of hybrid combinations.

Use of molecular marker for predicting heterosis on the basis of genetic diversity of parental lines had been demonstrated on rice (Zhang *et al*. 1996), wheat (El-Maghraby *et al*. 2005), soybean (Woody *et al*. 2011), and maize (Kiula *et al*. 2008). SSR-based molecular markers are accurately to obtain information on genetic diversity among maize inbred lines to synthesize a hybrid combination of the most heterozygote (Reif *et al*. 2006; Trindade *at al*. 2010). By using molecular markers, genetic differences between inbred lines of maize can also be directly evaluated. Kanagarasu *et al*. (2013) reported that SSR markers can separate inbreds that clearly analyzed into different groups based on genetic differences which are very important in development of hybrid combination of heterosis. Therefore, whenever inbred lines sourced from local and exotic germplasms included in the development of a hybrid, it is advisable to find out genetic differences to avoid hybrids with narrow genetic distance.

Screening for molecular characterization of genetic pools of potential forms of alleles at a locus, identification of positive impact and look for rare alleles controlling important agronomic traits that greatly facilitate the transfer of genes in new varieties (Xu *et al*. 2009), as well as identification of genetic material tested for heterotic pattern and planning a cross between inbred lines (Hallauer and Miranda 1988) are very important in breeding programs. Therefore, genetic diversity information can be used as genetic resources in developing elite inbred lines and hybrid combinations.

The study aimed to determine the level of homozygosity and genetic variability, and estimate the value of genetic distance between 30 genotypes of maize at S3 generation.

MATERIALS AND METHODS

This research was conducted in the greenhouse and molecular biology laboratory of Indonesian Cereal Research Institute (ICERI) from January to March 2014. A total of 30 genotypes of maize resistant to downy mildew at S3 generation were used in this study, which was the result of screening against downy mildew in the field. Leaf tissue harvested from 10 seedlings at 15 days after planting (DAP) was bulk with equal quantities for DNA extraction. Method for DNA extraction and isolation followed George *et al*. (2004) with modification as described by Khan *et al*. (2004) by applying cetyl trimethyl amonium bromide (CTAB) instead of liquid nitrogen. To determine DNA quantity and quality after isolation, optical density (OD) of samples (3-4 µl per sample) was measured using nanophoto-meter (Implen) at 260/280 wavelength.

S3 lines to be selected had homozygosity of > 85% and parents cross pairs based on the estimated genetic distance values > 0.6. Thus the cross to be performed will be more efficient and focused. Selection of genotypes with homozygosity > 80% assumed that inbreds are generally homogeneous, so it could be included in a hybrid development program, with a note that homozygosity of the selected inbred was still able to be improved by selfing. Selection of pairs of hybrid was based on genetic distance values of > 0.6, assuming that the two parents are relatively distant resemblance to produce derivatives F1 heterosis.

Number of primers used was 30 polymorphic SSR loci, which are distributed over the entire maize genome. The method used was following the protocol of George *et al*. (2004).

Molecular data were analyzed based on the DNA bands appeared in the electrophoresis plate. The scoring system was the following: score 1 when there were bands and score 0 when there was no band. Incomplete bands, which were not very cleared or blurred, were scored 9 and considered as missing data. The polymorphism information content (PIC) of the primer used was calculated for each of the SSR markers (Smith *et al*. 1997) using the following formula:

$$PIC = 1 - \sum_{1}^{n} f_i^2 \; i = 1, 2, 3, \ldots\ldots n ,$$

where *i* is the frequency of the alleles.

Genetic similarity (GS) of the genotypes was estimated by the Jaccard coefficient (Rohlf 2000) using the following formula:

$$GS = \frac{m}{(n + u)}$$

where:

m = number of DNA bands (alleles) at the same position

n = total number of DNA bands

u = number of DNA that were not in the same position.

Data on the genetic similarity were analyzed using the NTSYS-PC program version 2.1 (Rohlf 2000). Analysis of the matrix of genetic distance was done based on the results of genetic similarity analysis (Lee 1998) using the following formula:

$$S = 1 - GS$$

where:

S = genetic distance

GS = genetic similarity

RESULTS AND DISCUSSION

Hybrid maize is a result of commercial exploitation of heterosis value resulting in a number of commercial hybrids that have been widely available for cultivation. For commercial heterotic, exploitation it is necessary to develop lines through selection, followed by selfing for several generations to obtain pure lines with high level of homozygosity.

The thirty maize genotypes tested have been already at S3 generation, except two genotypes from CIMMYT that have been in the higher generation. In S3 generation all the genotypes tested should have homozygosity level at 87.5%. However, the results indicated that the level of homozygosity varied from 33.33 to 93.33% (Table 1). This can be caused by several factors such as conditions when the genetic material was collected, that the germplasm may be planted together in the field so it is very heterogeneous. The other factor was careless handling of genotypes during crossing so that a number of genotypes were unable to reach homozygosity level of 87.5%. Maize is easily contaminated due to its open pollination nature. CIMMYT recommended that genotype that has homozygosity level of >80% can be included in the hybrid assembly program (personal communication at the Asian Maize Biotechnology Network/AMBIONET project 1989-2005). Table 1 showed that 17 genotypes could be considered for inclusion in the hybrid assembly program, while other genotypes need to be improved by selfing to increase their homozygosity. In addition, growing germplasm in isolated places and no other maize germplasm grown in the area could also increase homozygosity level.

The level of homozygosity and genetic purity is an important key in developing novel hybrid and synthetic maize varieties (Heckenberger *et al*. 2002; Gunjaca *et al*. 2008). In conventional breeding, the hybrid will be starting to develop after the sixth generation to ensure that the inbreeds used as parents had homozygosity of more than 80%. However, by using molecular markers selection can be performed in the third generation to obtain homozygous inbreeds to be used in a hybrid development program. With the assumption that inbreeds with high levels of homozygosity (> 80%) will produce F1 which show uniformity in the field. However, selected inbreeds which were as hybrid parents will continuously be improved through increased homozygosity. Thus, there is a time saving of about 2-3 generations. It is one of the strategy of saving time, lab or and cost.

Table 1. Homozygosity level of thirthy S3 maize genotypes resistant to downy mildew.

Accession	Origin	Kernel color	Pedigree	Homozygosity (%)
2/45L.Kandora	Tana Toraja, South Sulawesi	White	LK-35-21-33	76.67
3/19L.Kandora	Tana Toraja, South Sulawesi	White	LK-36-19-33	76.67
7/40L.Gorontalo	Gorontalo	Yellow	LG-34-32-40	33.33
9/30L.Tongo	Palu, Central Sulawesi	Yellow	LT-42-47-30	80.00
15/1L.Bebo	Tana Toraja, South Sulawesi	Yellow	Lbe-27-41-39	83.33
23/12L.Majene	West Sulawesi	Yellow	LM-5-11-35	83.33
27/11Jg.Entok	West Java	Yellow	JE-1-11-39	76.67
59/8P.Umurgenjah	-	White	PUG-32-8-8	83.33
70/34Sulut(28A)	North Sulawesi	Yellow	SU-7-2-3	76.67
83/50Pensaijan(1)	Timor Tengah Utara, East Nusa Tenggara	Yellow	Pen-32-16-50	80.00
90/23Penliatanah(155A)	Kupang, East Nusa Tenggara	White	PT-25-6-23	93.33
110/23L.K.Kalsel(99A)	South Kalimantan	Yellow	LKK-9-47-47	76.67
112/3L.K.O.Kalsel	Tanah Laut, South Kalimantan	Brown Yellow	JKK96A-29-3-3	80.00
120/30L.Mamuju	West Sulawesi	Yellow	Lmam-5-37--20	66.67
128/26Jole(48B)	Donggala, Central Sulawesi	Yellow	Jole48B-7-24-13	66.67
135/29Jole(48B)	Donggala, Central Sulawesi	Yellow	Jole48A-33-29-40	83.33
137/5L.Bodoketek(45C)	Lamongan, East Java	Yellow	LBK-7-4-23	83.33
141/41BL.Sulut(37A)	North Sulawesi	Yellow	SU37A-21-46-21	96.67
143/41BataraMulen(63)	-	Red	Bmul-3-26-41	50.00
152/44Penone(76A)	Timor Tengah Utara, East Nusa Tenggara	Yellow	Penone-8-27-44	76.67
156/49BarallePute(71)	Polman, West Sulawesi	Yellow	Bar Put-28-8-28	80.00
161/13Mal01-2-4-1-1	CIMMYT	White	Mal01-2-4-1-1-6	86.67
162/6Mal01-2-4-1-3	CIMMYT	White	Mal01-2-4-1-3-6	83.33
183/30L.Jg.Putih(83A)	Kendari, Southeast Sulawesi	White	LP-29-8-39	80.00
184/38L.Jg.Putih(83A)	Kendari, Southeast Sulawesi	White	LP-32-5-16	90.00
188/39Jg.K.Kalsel	Tanah Laut, South Kalimantan	White	JKK96A-36-34-18	86.67
Pulu24(S3)	Bantaeng, South Sulawesi	White	Pul20-40	50.00
54/30P.S.Bone	Bone, South Sulawesi	Yellow	PSB-2-3-50	80.00
BataraKoasa57	Jeneponto, South Sulawesi	White	Bko-27-26-30	73.33
40/31Pulu(S2)	Bantaeng, South Sulawesi	White	Pul-43-12-31	73.33

The result of analysis showed that the levels of polymorphism (PIC) ranged from 0.13 to 0.78, average at 0.51. The number of alleles obtained were 125 alleles, ranged from 2 to 8 alleles per SSR locus with an average of 4 alleles per SSR locus. The size of nucleotides in each locus also quite varied from 70 to 553 bp. These data indicate that genetic variability of 30 maize genotypes analyzed was high enough to have the opportunity for recombination in forming F1 hybrids (Table 2). Pabendon *et al*. (2009) performed molecular characterization of 39 inbred elite maize formed in ICERI using 30 SSR primers and obtained 133 alleles in the range of 2-8 alleles per locus or an average of 4.5 alleles per locus. Average polymorphism level was 0.60 with an estimated genetic distance of 0.20-0.85. PIC demonstrates the informativeness of SSR loci and their potential to detect differences among inbred lines based on their genetic relationship. According to Botstain *et al*. (1980), PIC values exceeding 0.50 indicate highly informative loci; values ranging from 0.50 to 0.25 indicate moderately informative loci, and those below 0.25 indicate uninformative loci.

Dendrogram constructed using 30 SSR markers based on UPGMA showed that the genetic similarity coefficient ranged from 0.35 to 0.70 (Fig. 1), indicating that there was no genotype genetically very similar (0.7) but also as contrast no different (0.35). Based on the analysis of bootstrapping, formed cluster of 30 genotypes had low confidence level of grouping at 0.1-80.2% (complete data not shown in the dendrogram). This is because a number of genotypes showed heterozygosity level of >20% so that allele variation was quite high. For such conditions, these genotypes should not be included in the development of hybrid program. It would be more efficient to determine heterotic group if the genotypes have homozygosity level of >80%.

Table 2. Profile of 30 SSR loci used in analysis of genetic diversity of 30 S3 maize genotypes resistant to downy mildew.

SSR primer's code	Bin number	PIC value	Number of alleles	Allele size(pb)
phi109275	1.00	0.69	6	118.00 - 195.10
phi227562	1.12	0.27	**2**	340.00 - 427.00
phi96100	2.00	0.68	4	273.80 - **553.00**
phi109642	2.00	0.60	3	126.80 - 200.00
phi083	2.04	0.60	3	121.67 - 163.25
phi101049	2.09	0.65	4	232.66 - 427.00
phi374118	3.02	0.63	4	218.37 - 302.14
phi102228	3.04	0.14	5	121.67 - 212.25
phi053	3.05	0.69	4	165.70 - 280.00
phi072	4.00	**0.13**	3	140.00 - 186.00
phi093	4.08	0.30	3	269.66 - 407.66
phi109188	5.00	0.37	5	147.33 - 232.67
phi331888	5.04	0.34	4	129.00 - 183.67
umc1153	5.09	0.56	3	104.50 - 134.50
phi423796	6.01	0.50	5	121.67 - 181.62
phi452693	6.06	**0.78**	4	121.67 - 216.33
phi299852	6.08	0.48	6	112.00 - 216.33
umc1545	7.00	0.70	3	**70.00** - 91.00
phi034	7.02	0.67	4	125.00 - 200.00
phi328175	7.04	0.57	6	91.00 - 193.87
phi420701	8.00	0.36	**2**	280.00 - 398.00
umc1304	8.02	0.65	4	131.75 - 193.87
phi233376	8.03	0.46	6	140.25 - 242.00
umc1279	9.00	0.39	**8**	85.60 - 183.66
phi065	9.03	0.59	4	162.00 - 311.00
phi032	9.04	0.40	2	242.00 - 269.67
phi448880	9.05	0.53	3	153.80 - 216.33
phi96342	10.02	0.32	3	273.50 - 427.00
umc1061	10.06	0.71	7	91.00 - 193.00
umc1196	10.07	0.54	5	121.67 - 208.16
Total			125	
Average		0.51	4	70 - 553

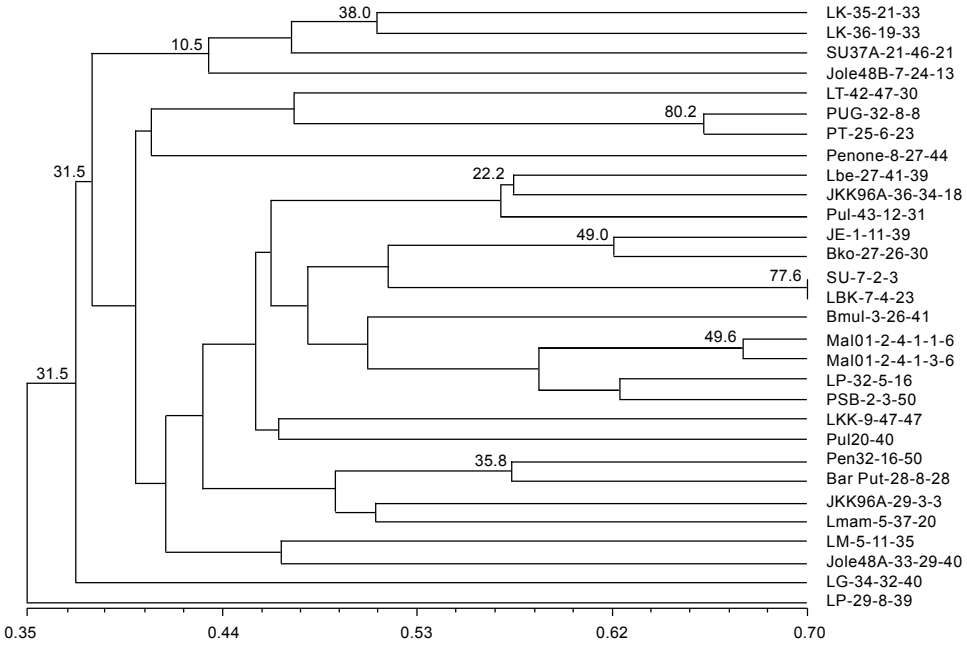

Fig. 1. Dendrogram of 30 S3 maize genotypes resistant to downy mildew using 30 SSR primers constructed by UPGMA.

Table 3. Matrix of genetic distance of nine S3 yellow maize genotypes resistant to downy mildew and having a homozygosity level of > 80%.

Genotype	LT-42-47-30 (CS)	Lbe-27-41-39 (SS)	LM-5-11-35 (WS)	Pen-32-16-50 (ENT)	JKK96A-29-3-3 (SK)	Jole48A-33-29-40 (CS)	LBK-7-4-23 (WJ)	SU37A-21-46-21 (NS)	JKK96A-36-34-18 (SK)	Total
LT-42-47-30 (CS)	0.00									
Lbe-27-41-39 (SS)	0.56	0.00								
LM-5-11-35 (WS)	0.69	0.60	0.00							
Pen-32-16-50 (ENT)	0.51	0.53	0.56	0.00						
JKK96A-29-3-3 (SK)	0.57	0.56	0.51	0.49	0.00					
Jole48A-33-29-40 (CS)	0.61	0.70	0.53	0.68	0.58	0.00				
LBK-7-4-23 (WJ)	0.57	0.58	0.63	0.53	0.62	0.54	0.00			
SU37A-21-46-21 (NS)	0.57	0.60	0.65	0.60	0.61	0.58	0.60	0.00		
JKK96A-36-34-18 (SK)	0.59	0.43	0.66	0.56	0.47	0.63	0.63	0.55	0.00	
The number of crosses that provide positive heterotic opportunities (genetic distance > 0.6)	2	3	3	2	2	1	2	0	0	15

CS = Central Sulawesi; SS = South Sulawesi; WS = West Sulawesi; ENT = East Nusa Tenggara; SK = South Kalimantan; WJ = West Java; NS = North Sulawesi.

For formation of F1 hybrid resistant to downy mildew, genotypes used had homozygosity level of >80%. For forming F1 hybrids with yellow kernel, there were nine genotypes that can be used in formation of F1, while for white kernel F1 hybrids, there were five genotypes (Table 3). For yellow kernel maize, the number of crosses that provide positive heterotic opportunities with genetic distance values of >0.6% was 15 pairs crossing, with genetic distance values ranged from 0.60 to 0.70. However, the success of crossing is determined by the value of anthesis silking interval (ASI) between the two parents. If the ASI is not more than 3 days, then the chance of success of the cross is quite high.

Estimated genetic distances of the genotypes in general were not too high even the genotypes came from different islands. It shows that Indonesian local maize germplasms have similar genetic background that is not too different; it means that transfer of genetic materials between islands is quite high. To increase genetic variability of donor lines which are resistant to downy mildew from local germplasm, it can be recombined with introduced lines that have high yield potential.

Table 4 shows that 15 pairs of F1 hybrid maize with yellow kernel were resistant to downy mildew that provide an opportunity to obtain heterosis. Conventionally, the method of lines (OPV) is most commonly used for heterotic pattern formation (Azevedo *et al*. 2003). Microsatellite markers allow to assist cross method, both in reducing the number of crosses, as well as in forming a new heterotic pattern due to the addition of a potential elite line (Reif *et al*. 2003). Thus, field-testing can be planned more efficiently and economically.

Table 4. F1 maize lines resistant to downy mildew that have a chance of generating positive heterosis based on estimates of genetic distance values >0.60.

F1 lines	Genetic distance values
LM-5-11-35 x LT-42-47-30	0.69
Jole48A-33-29-40 x LT-42-47-30	0.61
LM-5-11-35 x Lbe-27-41-39	0.60
Jole48A-33-29-40 x Lbe-27-41-39	0.70
SU37A-21-46-21 x Lbe-27-41-39	0.60
LBK-7-4-23 x LM-5-11-35	0.63
SU37A-21-46-21 x LM-5-11-35	0.65
JKK96A-36-34-18 x LM-5-11-35	0.66
Jole48A-33-29-40 x Pen-32-16-50	0.68
SU37A-21-46-21 x Pen-32-16-50	0.60
LBK-7-4-23 x JKK96A-29-3-3	0.62
SU37A-21-46-21 x JKK96A-29-3-3	0.61
JKK96A-36-34-18 x Jole48A-33-29-40	0.63
SU37A-21-46-21 x LBK-7-4-23	0.60
JKK96A-36-34-18 x LBK-7-4-23	0.63

CONCLUSION

Genetic variability of 30 genotypes of maize resistant to downy mildew was high enough indicated by a fairly high degree of polymorphism from 0.13 to 0.78,

with the average of 0.51. Amongst a total of 30 genotypes analyzed, 17 genotypes had homozygosity of >80% so it can be included in the hybrid maize assembly program. Thirty genotypes produced a number of clusters with a confidence level of grouping is low or unstable. Fifteen F1 hybrids with yellow kernel and four hybrid with white kernel were able to produce heterotic combination. This study recommends to establish heterotic groups by adding a primer or increasing percentage of homozygosity of all genotypes to be >80%. ASI information must be obtained for each promising genotype so that it's easier to predict heterotic potential in hybrid maize assembly program.

ACKNOWLEDGEMENT

We are grateful to the Ministry of Research and Technology Republic of Indonesia for funding this study through Research Incentive SIN of 2014 Program.

REFERENCES

Botstain, D., R.L. White, M. Skolnick and R.W. Dawid. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

De Azevedo Duarte, I., J.M. Ferreira and C.N. Nuss. 2003. Screening potential of three maize topcross testers. Pesquisa Agropecuária Brasileira 38: 365-372.

El-Maghraby, M.A., M.E. Moussa, N.S. Hana and H.A. Agrama. 2005. Combining ability under drought stress relative to SSR diversity in common wheat. Euphytica 141: 301-308. DOI: 10.1007/s10681-005-8066-7.

George, M.L.C., E. Regalado, M. Warburton, S. Vasal and D. Hoisington. 2004. Genetic diversity of maize inbred lines in relation to downy mildew. Euphytica 135: 145-155.

Gunjaca, J., I. Buhinicek, M. Jukic, H. Sarcevic, A. Vragolovic, Z. Kozic, A. Jambrovic and I. Pejic. 2008. Discriminating maize inbred lines using molecular and DUS data. Euphytica 161: 165-172.

Hallauer, A. and J. Miranda. 1988. Quantitative Genetics in Maize Breeding. 2nd edition. Iowa State University Press, Ames, USA. 468 pp.

Heckenberger, M., M. Bohn, J.S. Ziegle, L.K. Joe, J.D. Hauser, M. Hutton and A.E. Melchinger. 2002. Variation of DNA fingerprints among accessions within maize inbred lines and implication for identification of essentially derived varieties. I. Genetic and technical sources of variation in SSR data. Mol. Breed. 10: 181-191.

Hikmawati. 2011. Karakterisasi morfologi dan studi keragaman genetik isolat-isolat penyebab bulai *Peronosclerospora* spp. pada tanaman jagung berbasis *Simple Sequence Repeat* (SSR). Program Pascasarjana Universitas Hasanuddin, Makassar. 100 hlm.

Kanagarasu, S., G. Nallathambi, K.N. Ganesan, S. Kannan, V. G. Shobhana and N. Senthil. 2013. Determination of genetic polymorphism among indigenous and exotic maize inbreds using microsatellite markers. Afr. J. Biotechnol. 12(39): 5723-5728.

Khan, I.A., F.S. Awan, A. Ahmad and A.A. Khan. 2004. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Mol. Biol. Rep. 22: 89a-89e.

Kiula, B.A., N.G. Lyimo and A.M. Botha. 2008. Association between AFLP-based genetic distance and hybrid performance in tropical maize. Plant Breeding 127(2): 140-144.

Lee, M. 1998. DNA markers for detecting genetic relationship among germplasm revealed for establishing heterotic groups. Presented in the Maize Training Course, CIMMYT, Texcoco, Mexico, 25 August 1998.

Lukman, R., A. Afifuddin and T. Lubberstedt. 2013. Unraveling the genetic diversity of maize downy mildew in Indonesia. J. Plant Pathol. Microbiol. 4: 162. DOI: 10.4172/2157-7471.1000162.

Mikoshiba, F., M. Sudjadi and A. Soediarto. 1977. Dispersion of conidia of *Sclerospora maydis* in outbreaks of maize downy mildew disease in Indonesia. Trop. Agric. Center, Japan. pp. 186-189.

Muis, A., M.B. Pabendon, N. Nonci, dan W.P.S. Waskito. 2013. Keragaman genetic *Peronosclerospora maydis* penyebab penyakit bulai pada jagung berdasarkan analisis marka SSR. Jurnal Penelitian Pertanian Tanaman Pangan 32(3): 139-147.

Muis, A., M.B. Pabendon, N. Nonci, dan W. Purbowasito. 2013. Skrining galur-galur S2 jagung terhadap penyakit bulai dan pembentukan galur-galur S3 tahan penyakit bulai. Prosiding Seminar Insentif Riset SINas (INSINAS 2013) pp. 212-222.

Mungoma, C. and L.M. Pollack.1988. Heterotic patterns among ten corn belt and exotic maize populations. Crop Sci. 28: 500-504.

Pabendon, M.B., M.J. Mejaya, J. Koswara and H. Aswidinnoor. 2009. SSR-based genetic diversities among maize inbred lines and their relationships with F1 phenotypic data of Mr4 and Mr14 testcrosses. Indones. J. Agric. 2(1): 41-48.

Rathore, R.S., A. Trivedi and K. Mathur. 2002. Rajasthan downy mildew: The problem and management perspectives. Proceedings of the Eight Asian Regional Maize Workshop: New Technologies for New Millenium. Bangkok, Thailand, 5-8 August 2002. pp. 366-379.

Reif, J.C., A.E. Melchinger, X.C. Xia, M.L. Warburton, D.A. Hoisington, S.K. Vasal, D. Beck, M. Bohn and M. Frisch. 2003. Use of SSRs for establishing heterotic groups in subtropical maize. Theor. Appl. Genet. 107: 947-957.

Reif, J.C., M.L. Warburton, X.C. Xia, D.A. Hoisington, J. Crossa, S. Taba, J. Muminovic, M. Bohn, M. Frisch and A.E. Melchinger. 2006. Groping of accessions of Mexican races of maize revisited with SSR markers. Theor. Appl. Genet. 113: 177-185.

Rohlf, F.J. 2000. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System Version 2.1. Applied Biostatistics, Inc.

Shurtleff, M.C. 1980. Compendium of Corn Diseases. Second Edition. The American Phytopathological Society. p. 105.

Smith, J.S.C., E.C.L. Chin, H. Shu, O.S. Smith, S.J. Wall and M.L. Senior 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.) - Comparisons with data from RFLPs and pedigree. Theor. Appl. Genet. 95: 163-173.

Sudjono, M.S. 1988. Penyakit jagung dan pengendaliannya. *Dalam* Subandi, M. Syam dan A. Widjono (Ed.). Jagung. Pusat Penelitian dan Pengembangan Tanaman Pangan. Bogor. hlm. 205-217.

Telle S., R. Shivas, M. Ryley, and M. Thines. 2011. Molecular phylogenetic analysis of *Peronosclerospora* (Oomycetes) reveals cryptic species and genetically distinct species parasitic to maize. Eur. J. Plant Pathol. 130: 521-528.

Trindade, A.P.R., R.J. Barth Pinto, A.T. Amaral Júnior, C.A. Mangolin, M.F.P Silva Machado and C.A. Scapim. 2010. Genetic diversity of breeding popcorn lines determined by SSR markers. Electronic J. Biotechnol. 13(1): 1-9.

Wakman, W. dan M.S. Kontong. 2000. Pengendalian penyakit bulai pada tanaman jagung dengan varietas tahan dan aplikasi fungisida metalaksil. Penelitian Pertanian Tanaman Pangan 19(2): 38-42.

Wakman, W. dan H.A. Djatmiko. 2002. Sepuluh spesies cendawan penyebab penyakit bulai pada tanaman jagung. Makalah disajikan pada Seminar PFI di Universitas Negeri Jenderal Soedirman, Purwokerto, 7 September 2002.

Wakman, W. dan Hasanuddin. 2003. Penyakit bulai (*Peronosclerospora sorghi*) pada jagung di dataran tinggi Karo Sumatera Utara. Makalah disajikan pada Seminar Nasional PFI di Bandung.

Wakman, W. 2004. Penyakit bulai pada tanaman jagung di Indonesia : masalah, penelitian dan cara mengatasinya. Prosiding Seminar Tahunan PFI Komda Sulsel.

Woody, J.L., A.J. Severin, Y.T. Bolon, B. Joseph and A.D. Diers. 2011. Gene expression pattern is correlated with genomic and genetic structure in soybean. Genome 54: 10-18. DOI: 10.1139/G10-090.

Xu, Y., D.J. Skinner, H. Wu, N. Palacios-Rojas, J.L. Araus, J. Yan, S. Gao, M. Warburton and J. Crouch. 2009. Advances in maize genomics and their value for enhancing genetic gains from breeding. Int. J. Plant Genomics 2009: 1-30.

Yen, T.T.O., B.M. Prasanna, T.A.S. Setty and R.S. Rathore. 2004. Genetic variability for resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) in the tropical/sub-tropical Asian maize germplasm. Euphytica 138: 23-31.

Zhang, Q.F., Z.Q. Zhou, G.P. Yang, C.G. Xu and K.D. Liu. 1996. Molecular marker heterozygosity and hybrid performance in indica and japonica rice. Theor. Appl. Genet. 93: 1218-1224. DOI: 10.1007/BF00223453.