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Research Article
Simple Sequence Repeats Analysis of New Indonesian Maize Inbred

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Abstract

Background and Objective: The assessment of the genetic diversity of new inbred lines has proven significant to the improvement of maize varieties. The objective of the study was to evaluate the diversity and relatedness of new Indonesian elite maize lines. **Materials and Methods:** Genetic diversity of 48 Indonesian elite maize lines was studied using six SSR markers. Some parameters were estimated including genetic relationship and genetic diversity. Genetic relationship was assessed using Jaccard's similarity coefficient and was used to draw a neighbourhood-joining tree (NJT) by circle cluster using MEGA-pc software. Principal coordinate analysis (PCoA) was used for the estimation of genetic diversity. **Results:** All SSR loci detected in all genotypes were polymorphic. The population in this study is diverse as estimated by its polymorphic index coefficient (PIC). Population of 20 Indonesian maize lines was divided into 3 sub-groups, whereas 28 of mutant population was clustered into 7 sub-groups. **Conclusion:** SSR marker is a meaningful marker for genetic diversity study of Indonesian maize core collection. The forty-eight Indonesian maize genotypes were clustered into three major groups. These findings can be used in breeding methods for the development of populations.

Key words: Indonesian core collection, maize, albizia system, shade tolerant, tropical maize

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Simple sequence repeats (SSRs) is an effective molecular marker to select superior genotypes in early growth with desirable agronomic traits. SSR markers application in crop improvement includes DNA fingerprinting and genetic diversity analysis, assigning inbred lines to heterotic groups, high throughput genotyping and phenotyping, mapping both association and QTL and marker assisted selection for doubled haploid technology[1]. Plant breeders, therefore, applied SSRs in their programs for the reasons of low cost, consistency of product for every marker, a high amenability to multiplex PCR and simplicity and effectiveness[1,2].

Diversity and similarity among inbred are important for breeder in selecting parental lines for developing new superior hybrids by reducing huge number of crosses combination. Some researchers reported the application of SSRs in barley and wheat[2], maize[3,4], cotton[5], groundnut[6], peanut[7], soybean[8], oat[9], carrot[10] and melon[11]. Genetic diversity studies on maize using SSR markers have been reported by many researchers in China[4,12], India[13], Eastern and Southern Africa[14] and Mexico[15]. Masuka *et al.*[14] assessed genetic diversity of parental from CIMMYT ESA for hybrid breeding using SSR markers to avoid the narrowing down of the genetic base. Abakemal *et al.*[16] applied SSR to determine genetic diversity among and within quality protein maize (qpm) and non-qpm line in the hope of conversion of normal maize into quality protein maize. The use of SSR markers to evaluate the genetic diversity of mutant and parental lines was suggested by Ruswandi *et al.*[17] since their ability to cluster those genotypes into different major group.

Maize development program in West Java was initiated from 2001 by crossing CIMMYT lines with local land races and by means gamma ray irradiation. The program produced 20 new inbred lines derived from crossing and 28 new potential mutant lines[17]. Some mutant lines were informed as high yield parental lines for hybrids[18], a shading tolerant line for cultivation under maize/ Albizia agroforestry system[19] and a high nutritious line for industry[20]. This study evaluated the diversity and relatedness of 48 Indonesian elite maize lines using SSR markers.

MATERIALS AND METHODS

The study was carried out from November, 2016 up to June, 2017 at the Plant Breeding and Seed Technology Laboratory, Padjadjaran University, Indonesia. DNA of 48 Indonesian maize genotypes consisting of 20 DR lines and 28 mutant DR were evaluated using SSR markers. The protocol of Asian Maize Biotechnology Network (AMBIONET), Agricultural Biotechnology Center (CIMMYT ABC), Philippines, was used including: DNA extraction, SSR amplification, separation and visualization[21].

Selected primers for important traits, such as: SSR markers for resistance to downy mildew pathogens, tolerance to drought and tolerance to shading was used to amplify their DNA. These SSR markers included Bngl 137, Umc 1149, Phi 112, Phi 113, Phi 36, Bngl 589, Phi 126 and Bngl 125. The polymerase chain reactions (PCR) with Mastercycler ep gradient (eppendorf) were done using KAPA2G Fast Ready Mix (Boston, Massachusetts, USA) which contained 10 mL of reaction mix each of forward and reverse primer and 20 ng of genomic DNA. The profile of DNA amplification were: One cycle of initial denaturation (30 cycles, 93°C, 1 min), annealing (56°C/58°C/60°C, 2 min) and extension (72°C, 2 min). It was, then, followed by 1 cycle of final extension (72°C, 5 min). The SSR products were run on polyacrylamide gel in 1× TBE (70-75 W, 45 min). The gel was stained in Promega silver stain solution.

Replicated DNAs were scored based on the presence (1) or absence (0) of amplified products. Estimation of major allele, allele number, gene diversity, polymorphic index coefficient (PIC) used the program PowerMarker[22]. Genetic distances (GD) between pairs of lines were calculated as GD = 1-GS, where GS was Jaccard similarity coefficients. GS and GD were assessed using NTSYS-pc software[23]. The GD were estimated to construct a neighbourhood joining tree (NJT) by circle cluster using MEGA-pc software version 6[24]. Principal coordinate analysis (PCoA) was also estimated for assessing of genetic diversity.

RESULTS AND DISCUSSION

Major allele, allele number, gene diversity and PIC of line, mutant and line-mutant population assessed by SSR markers are presented in Table 1. All SSR loci detected were polymorphic as shown by its allele/locus and its major allele frequency. The average of allele/locus was 8 for line, 14 for mutant and 16.5 for line-mutant. The mean of major allele frequency was 0.34 for line, 0.28 for mutant and 0.21 for line- mutant. Allele number in this study was higher than that estimated by Makumbi *et al.*[25], Krishna *et al.*[26], Babu *et al.*[27] and Pandey *et al.*[28,29], but lower than values previously assessed by Rocandio-Rodriguez *et al.*[15]. Abakemal *et al.*[16] explained that the variation in average of allele number from extensive studies could have originated from the source of germplasm, sample size and repeat length of the SSRs used. Choukan and Warburton[30] justified that dinucleotide SSR exhibits high

Table 1: Allele frequency and number, gene diversity and PIC of line, mutant and line- mutant population

Population	Marker	Major allele frequency	Sample size	Allele number	Gene diversity	PIC
Line	112	0.45	20	6.00	0.71	0.66
Line	113	0.50	20	4.00	0.64	0.57
Line	36	0.20	20	10.00	0.87	0.86
Line	589	0.50	20	6.00	0.70	0.67
Line	131	0.15	20	14.00	0.92	0.91
Line	126	0.25	20	8.00	0.83	0.80
Mean of line		0.34	20	8.00	0.77	0.74
Mutant	112	0.29	28	9.00	0.84	0.82
Mutant	113	0.71	28	4.00	0.46	0.43
Mutant	36	0.18	28	14.00	0.90	0.89
Mutant	589	0.25	28	8.00	0.85	0.83
Mutant	131	0.11	28	20.00	0.94	0.94
Mutant	126	0.14	28	13.00	0.91	0.90
Mean of mutant		0.28	28	11.33	0.81	0.80
Line-Mutant	112	0.19	48	13.00	0.89	0.88
Line-Mutant	113	0.42	48	7.00	0.75	0.72
Line-Mutant	36	0.17	48	20.00	0.92	0.92
Line-Mutant	589	0.21	48	13.00	0.88	0.87
Line-Mutant	131	0.13	48	27.00	0.95	0.94
Line-Mutant	126	0.17	48	19.00	0.92	0.92
Mean of line-mutant		0.21	48	16.50	0.88	0.87

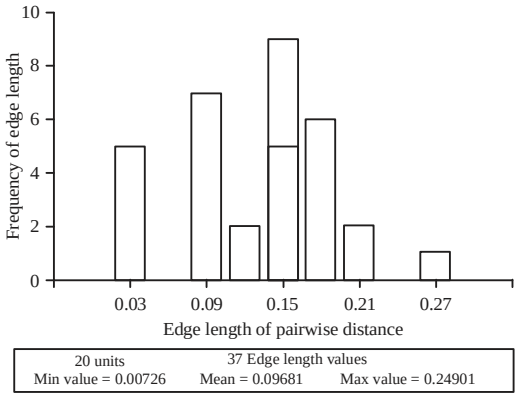

Fig. 1: Histogram of genetic distance from 20 Indonesian maize lines based on shared-allele distance
Coefficient of co-phenetic correlation between genetic distance and allele frequency based on shared allele distance of lines at 0.93

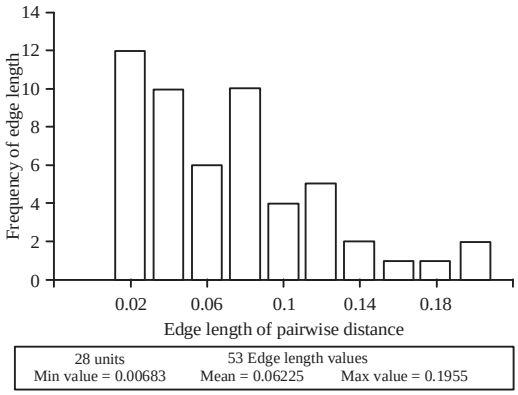

Fig. 2: Histogram of genetic distance from 28 Indonesian maize mutant based on shared-allele distance
Coefficient of co-phenetic correlation between genetic distance and allele frequency based on shared allele distance of mutant at 0.90

numbers of alleles than other types of SSR loci. The high numbers of lines to evaluate increase chance of finding rare alleles as compared to small samples. They also stated that gene diversity corresponds to the projected heterozygousity for diploid data.

The line, mutant and mutant population in this study are diverse as estimated by its gene diversity and polymorphic index coefficient (PIC). The average of gene diversity was 0.77 for line, 0.81 for mutant and 0.88 for line- mutant. The mean of PIC was 0.74 for line, 0.80 for mutant and 0.87 for line-mutant. PIC indicated the inequitable power of any locus by considering the frequency and the number of alleles per locus in the population designating gene diversity of population[21]. PIC value of maize population of this study was higher than that estimated by Pandey *et al.*[29] and Abakemal *et al.*[16]; but lower than values previously assessed by Rocandio-Rodriguez *et al.*[15]. Choukan and Warburton[30] and Xia *et al.*[31] reported that high PIC values were as a result of wide diversity and number of genotypes used and SSR repeat type. Based on the above results, it is concluded that of this study mutant population is more diverse than their original line population. Therefore, mutation breeding can be used to improve genetic base population in Indonesia.

Genetic distance of line population, mutant population and line-mutant population are shown in Fig. 1-3, respectively. Genetic distance of line population based on their combined

allele matrix differ from 0.01 up to 0.25 with an average of 0.10 (Fig. 1). This shows that there are high similarities within lines in the population. The allele frequency and genetic distance is closely related. The grouping of the lines shows that it is genetically fit, as is indicated by the cophenetic correlation coefficient of 0.93. Genetic distance of mutant population differed between 0.00-0.19 with an average of 0.06 (Fig. 2). Coefficient of cophenetic correlation of the mutant, however, is 0.90 showing similarities among the mutants. Genetic distance of line- mutant population varied between 0.00-0.25 with an average of 0.07 (Fig. 3). The coefficient of cophenetic correlation between genetic distance and allele frequency based on their shared allele matrix of 0.93 showed similarities among the mutants.

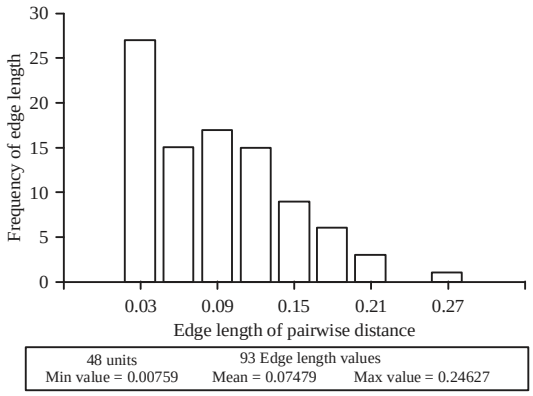

Fig. 3: Histogram of genetic distance from 48 Indonesian maize genotypes based on shared-allele distance

Coefficient of co-phenetic correlation between genetic distance and allele frequency based on shared allele distance of lines and mutant at 0.93

The population of 20 Indonesian maize lines was divided into 3 sub-groups (Fig. 4). They were ten lines derived from the combination of downy mildew resistant (DMR) and QPM lines (DR #1-10), five lines originated from the selection of DMR lines (DR # 11-15) and five lines formed by phenotypic selection of QPM lines (DR #16-20). This grouping is similar to the NJ analysis in which each group was clustered following DMR or QPM traits. The mutant population was clustered into 7 sub-groups (Fig. 5). The position of the mutant in the sub-group was randomly distributed. Mutation, therefore,

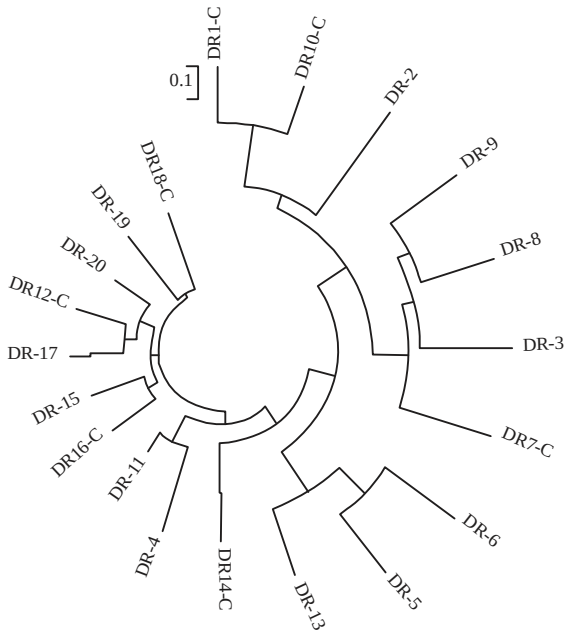

Fig. 4: Neighbour-joining tree based on shared-allele distance matrix on 20 lines population

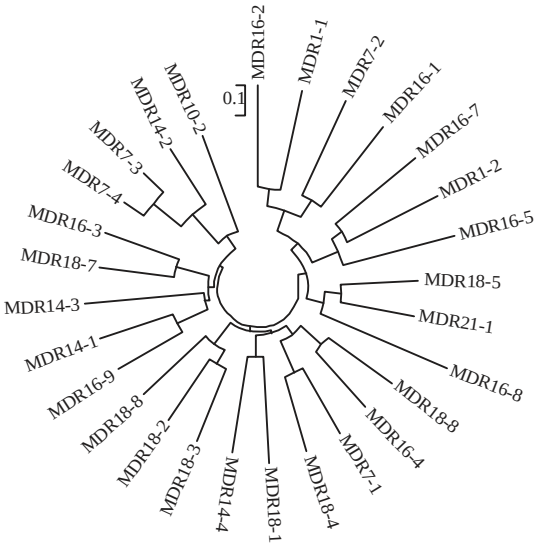

Fig. 5: Neighbour-joining tree based on shared-allele distance matrix on 28 mutant population

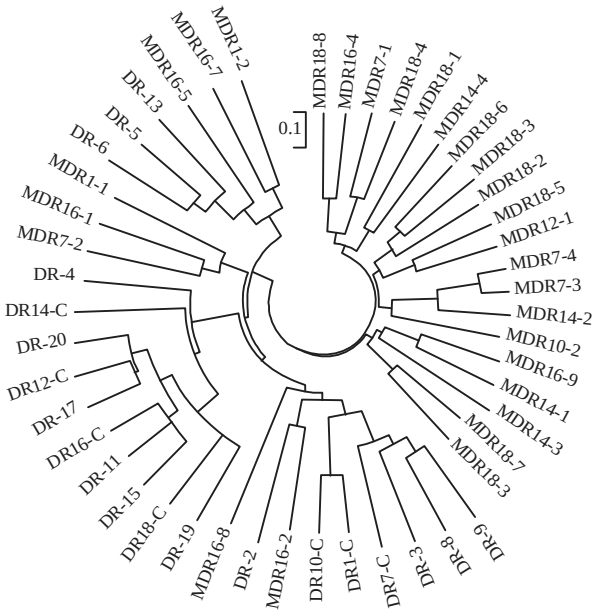

Fig. 6: Neighbour-joining tree based on shared-allele distance matrix on 48 lines, both non-mutant and mutants Indonesian maize

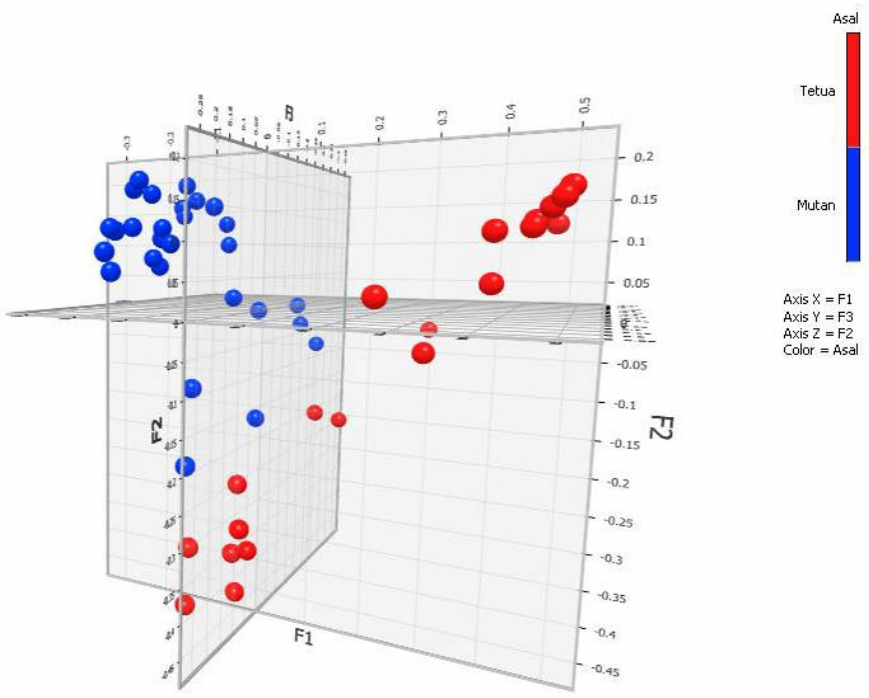

Fig. 7: Population grouping of inbred lines (ancestors) and mutant based on principal coordinate analysis (PCoA)

randomly and unpredictably changes the gene and the position of the locus. From the whole analysis of line-mutant population, there were three grouping based on their positions, i.e: line, mutant and line-mutant (Fig. 6). This shows that there is genetic differentiation based on SSR markers. The different groupings in the line and mutant populations indicated new genetic constitution in the mutant. On the contrary, the mutant- line grouping showed that mutation does not extremely change the whole genetic constitution. This means that mutation randomly changes genetic constitution of the line and express new traits.

The principal coordinate analysis (PCoA) was completed to check the diversity structure of new Indonesian maize population (Fig. 7). The result showed that the population of

line and mutant can clearly be differentiated into genetic groupings based on their breeding methods. There are 3 genetic groups of population, namely lines population, mutant population and mixed mutant-lines population. This means that line and mutant population genetically differed. But, within the line and within mutant populations close relations with many similarities were shown. The PCoA have been widely used to confirm the structure and to acquire knowledge about genetic diversity of many crops, such as oat[9], rice[32], maize[33], lentils[34], chickpea[35], durum wheat[36] and garcinia[37]. This information, in turn, can be used for utilization of the genetic resources in genomic and breeding programs. Boczkowska and Tarczyk[9] had confirmed the diversity of polish oat landraces into three different landraces group using PCoA. Based on similar analysis using PCoA, Choudhury *et al.*[32] clustered North-Eastern region of India rice into three groups based on their population structure. Using the same analysis of PCoA, Semagn *et al.*[33] studied genetic diversity among open-pollinated maize varieties in Eastern and Southern Africa and grouped them into 2-4 clusters depending on maturity groups, breeding programs, mega-environments and specific agronomic traits. Khazaei *et al.*[34] categorized lentil accessions into three major groups, namely: South Asia, Mediterranian and Northern temperate, which reflected world's agro-ecological zones based on PCoA. This study revealed that the clusters reflected the origins, pedigrees and breeding histories of the germplasm. Based on the results of our study, it is suggested that maize breeding programs, particularly hybrid programs have to deliberately use substantial genetic diversity within Indonesian maize inbred, as clustered mutant and non-mutant lines, in order to avoid narrow genetic diversity.

CONCLUSION

SSR markers were valuable for revealing genotypic diversity with PIC as indicated by the range from 0.57-0.94. The forty-eight Indonesian maize genotypes were clustered into three major groups are related with the breeding method to apply in the development of the populations. Thus, SSR markers are a meaningful marker for genetic diversity study of Indonesian maize breeding materials.

SIGNIFICANCE STATEMENTS

This study discovers the diversity of Indonesian maize mini core collection that can be beneficial for maize breeding program in Indonesia. This study will help researchers to uncover the critical areas of plant genetic resources of tropical maize that many researchers were not able to explore. Thus a new theory on utilization of new resources of gene into maize breeding may be arrived at.

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