Microsatellite-Based Markers Selection to Improve Hybrid Maize Breeding

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ABSTRACT. Information about genetic variability is fundamental in the development of hybrid varieties. Conventional breeding technologies are now supported by molecular tools to enhance the efficiency of hybrid breeding program. Microsatellite markers, for example, is able to detect co-dominant locus which is suitable to use in open pollinated crop such as maize. Results of characterization on a set of elite inbred lines via microsatellite-based markers at ICERI (Indonesian Cereals Research Institute) Molecular Biology, offers essential data for current works in hybrid breeding program. The Information on the level of homozygosity of eight sets of advanced inbred suggests that the level of homozygosity is varied from 2.6-40.0%. This means that a careful selection is required in deciding suitable inbred lines for further phase in hybrid breeding. The genetic diversity of the eight sets of elite inbred showed that there were four sets of inbred which have low degree of polymorphism. This indicated that genetic variability among the inbreds were narrow, where crosses between inbred of the same set should be avoided. Another result was the merging of two sets of elite inbred that own high yielding potential and acid soil tolerant, which provided information on the possibility of heterotic pairs available based on genetic distance values > 0.7. It was also found that, based on several research results, the use of microsatellites-based markers in the improvement of hybrid breeding programs would make the effort to be more focus and efficient.

Key words: maize, microsatellites-based markers, inbred lines, genetic variability.

Introduction

Methodology that has been long used by plant breeders to select hybrid parents from advanced inbred lines is through test cross of selected tester. This is carried out to detect which lines have excellent General Combining Ability (GCA), and then followed by finding out good Specific Combining Ability (SCA). Furthermore, diallel cross on several numbers of excellent GCA inbred lines have been widely used to figure out the heterotic parent among lines (Vassal *et al*. 1993; Ordas 1991; Revilla and Tracy 1997). However, if the amount of elite lines required to be selected are abundant, problem will arise in the time of selecting the best performing lines which is labor intensive due to vast numbers of crossing pairs. On the other hand, there is a possibility that genotypes chosen in the field are still heterozygote dominant which result in only dominant characters are able to be expressed in field trial. If that genotype is selected and segregation occurred, as a result it will create genetic contamination and might reduce homozygosity level of advanced inbred lines which have already been developed.

Genetic marker is able to detect defined gene location in the chromosome which function as a marker for genome analysis. Basically, there are two kind of markers, namely morphological marker and molecular marker. Marker that reveals polymorphism at protein level is known as biochemical marker, while DNA marker reveals polymorphism at DNA level. The use of molecular marker has been intensively studied and developed, especially in maize, commonly for germplasm characterization, pedigree verification, inbred heterotic grouping, heterosis basic knowledge and prediction, tools for selection marker, gene identification and localization (Mladenoviæ Driniæ *et al*. 2004). Informative marker is essential element that needs to be considered in the selection method. However, other factors such as cost, skills required, accuracy level, and multiplication or the marker which will be utilized also needed to be thought-out.

Over various numbers of available molecular markers, microsatellite marker is one that has been widely used in helping hybrid maize development, which CIMMYT has also been utilizing. One of the advantages of micro satellite marker is its ability to detect co-dominant and follows Mendel inheritance pattern (Morgante and Olivieri 1993; Senior and Heun 1993), so that heterozygote locus could easily be separated from the homozygote ones. Homozygote locus will only present with one allele per primer per genotype. If more than one allele present, it indicates that the locus is in heterozygote. This analysis is important to avoid crossing genotypes with high level of heterozygosity whereas in observation through phenotypic character homozygote allele is unable to express due to suppression from dominant allele.

Maize is an open pollinated and genetically is easily contaminated crop. The use of microsatellite marker, also

called Single Sequence Repeat (SSR) in hybrid breeding program turns out to be quite relevant since it can detect co-dominant effect. This means that SSR is able to detect the state of genetic material individually, and determine whether the inbred is homozygote or heterozygote. On the other hand, microsatellite marker is also specific, multi allele in one or two locus, repeatable and representatives for many kind of laboratorial test and can be operated in low facilities laboratory.

For each time genotyping a set of inbred based on microsatellite marker, the results consist of several information such as genetic variability which is presented in level of average polymorphism and number of allele detected. Other results are range of heterozygote or homozygote per genotype, dendogram construction to create group of tested genotype based on genetic similarity, and estimation of genetic distance from all possibility crossing pair. Every time the information is obtained, it automatically creates selection of genotypes for used in breeding programs. Therefore in the process of genotyping there are 3-4 selection times. Furthermore, result of genotyping is also able to create finger print data of potential inbred lines to be used for selecting parent of hybrid. This review is aimed to present several results from microsatellite marker which is able to support the hybrid breeding programs. It can also be applied in low facilities laboratories or other satellite laboratories of different commodities.

Detection of Homozygosity/ Heterozygosity Maize Inbred Lines Via Microsatellite-Based Markers (Pre-breeding)

Various numbers of inbred lines created from advanced populations or local germplasm which have advanced generations based on prediction of genetic improvement and is thought to have homosigosity ≥85% will then be submitted to hybrid breeding program. On the other hand the estimation occasionally is incorrect due to interference of several factors, such as pollen contamination or technical error in the field and within the genetic itself because of the occurrence of recombination or cross over. Conventionally these conditions could not be detected. However, with the help of molecular marker like microsatellite which is codominant then the level of homozygosity could be assessed. Thus, it would delete those inbred from the current phase of breeding program for recycled in selfing method to increase the homozygosity level, especially for potential lines.

Table 1. Result for detection on the level of heterozygosity of several sets of inbred based on microsatellite marker.

Set of inbred	Genotype tested	Number of inbred with homozygosity level≤ 85%	Year
Elite inbred high yield	73	30 (40,1%)	2002
Elite inbred high yield	39	5 (12,8%)	2007
Waxy corn inbred	40	1 (2,6%)	2008
Sweet corn inbred	50	19 (38%)	2009
Low N Inbred	10	0 (0%)	2010
Drought tolerance inbred	61	6 (9,8%)	2010
Acidity tolerance inbred	17	2(11,8%)	2010
Early maturity inbred	32	7 (21,9%)	2011
Waxy corn inbred	45	14 (31,1%)	2011
Pro-vitamin A inbred	11	4 (36,4%)	2011
Downy mildew resistant inbred	50	14 (28,0%)	2011

The result for homozygosity of several set of inbred lines at the advanced level (6th generation or above) showed variation in its level (Table 1). If these lines are placed for hybrid program based only on generation of selfing, then crossing pair would not be effective due to high level of heterozygosity. Data presented in Table 1 showed that only 1 set of inbred where all of characterized genotype had level of homozygosity ≥85% which was set of inbred lines tolerant to low N whereas another set of inbreds still had genotype heterozygosity between 2.6-40,1% (85% based on CIMMYT recommendation). With the help of microsatellite marker it helps to detect hetozygote locus so that those inbred are deleted from the current hybrid variety development programs.

Genetic Variability of Inbreds Based-on Microsatellite Marker

Intensive effort has been given to collect and preserve maize germplasm to maintain genetic diversity required for breeding program. Even though the landraces are commercially insignificant, it has certain important characteristic which can be beneficial for breeding program. Breeders pay special attention to various genetic diversity, whether in the population or elite inbred because it can determine the success of a breeding program. Emphasis on the study of genetic diversity based-on molecular marker have been intensively carried out in maize (Messmer *et al*. 1992; Melchinger *et al*. 1991; Ajmon Marsan *et al*. 1998; Dubreuil *et al*. 1996). And it is proven to have an impact toward plant improvement (Hallauer dan Miranda 1988). Through crossing potential parents we can expect a vast range of genetic variability in F2, and high level of heterosis in F1 (Daradjat *et al*. 1991). For the next

Table 2. Information on genetic variability over several sets of inbred from molecular characterization of microsatellite-based markers.

Set of inbred	Number of genotype	Average of polymorfism	Average number of allele	Reseaarch collaborator
Elite inbred high yielding potential (a)	43	0.62	4.8	Pabendon *et al.* 2002
Elite inbred high yielding potential (b)	39	0.61	4.5	Pabendon *et al*. 2007
Waxy corn inbred lines (a)	39	0.56	3.0	Makkulawu *et al*. 2008
Sweet corn inbred lines	31	0.56	3.1	Iriani *et al*. 2009
Low N tolerant inbred lines	10	0.61	3.0	Muzdalifah *et al.* 2009
Drought tolerant inbred lines	57	0.63	4.0	Azrai *et al*. 2010
Early acid soil tolerant inbred	15	0.59	4.0	Srisunarti *et al*. 2010
Ultra early inbred lines	25	0.48	3.2	Makkulawu *et al*. 2011
Waxy corn inbred lines (b)	31	0.44	3.0	Makkulawu *et al*. 2011
Provit A inbred lines	6	0.47	3.0	Yasin *et al*. 2011
Downy mildew resistant inbred lines	36	0.48	3.0	Makkulawu *et al*. 2011

advanced generation, there will be segregation which can reduce the effect of heterosis (Baihaki 1989).

Information on pedigree of maize is useful in planning of crossing for maize hybrid from potential inbred lines by creating groups of heterotic pattern. This is possible through the application of pedigree/heterosis data from morphological character or via molecular marker which can detect DNA variation level (Smith and Smith 1992). It is more reliable in set apart variation among germplasm or in genotypic identification (Caetano Anolles 1996). However for interpreting data then molecular data can not solely be used for application in the field because genotypic data itself could not be expressed. Therefore phenotypic information is needed to find whether or not molecular marker data obtained can provide more effective and accurate information as a tool for hybrid maize program (Smith dan Smith 1992). Level of polymorphism over numbers of primer or marker or locus which are used is to describe character variation each genotype has for every locus or character being analyze.

A result of characterization data of a set of inbred, is able to identify four sets of inbred with low level of polymorphism. They are early maturity inbred, waxy corn (b) inbred, pro-vitamin A inbred, and downy mildew tolerance inbred (Table 2). This result shows that genetic variation among those four sets of inbred are low or closely related. There are possibilities that all of them are originated from the same population. Therefore we have to carefully determine crossing pairs between sets of inbred to acquire high heterosis.

Grouping and Heterotic Pattern Based on SSR Marker

Grouping and heterotic pattern of inbred lines have significant implication, where in the long term it can determine the success of breeding programs. The establishment of group and heterotic pattern functions as a helping tool in the selection of abundant germplasm. Furthermore, it can provide direction of selection, crossing and testing for parental combination as candidate for hybrid. Without sufficient information of heterotic pattern of the parents, selection for crossing pairs might cause inaccurate genetic material to gain an optimum result (Lee 1998).

Classification of elite inbred lines and established inbred into heterotic group is one of the key factors in managing of hybrid program (Kantety *et al*. 1995). Therefore, if heterosis can be predicted before crossing then the number of crossing coul be decreased. Analysis of gene effect for SSR marker was very informative on study conducted by Mohammadi *et al.* (2002). It showed the importance of over dominance gene in maize to express heterosis towards yield and yield component. Paterson *et al.* (1991) reported that genetic marker can improve the ability to study gene effect individually and enable to assign which phenomenon influence more in present of heterosis,whether it is dominant, over dominant or combination of both effects.

Determination of cluster is made through pedigree data, the value of coefficient similarity or dissimilarity and information from breeder involve in producing the lines. Moreover it is also based on the chance of heterosis arise when there is a crossing between cluster. Consequently, dendogram that is constructed will benefit in selecting potential parent. Figure 1 shows a result of dendogram with 43 high yielding inbred lines calculated based on UPGMA (*Unweighted Pair Group Arithmetic Analysis*). An analysis of bootstrapping which resulted in confidence level grouping by WinBoot program is able to construct three heterotic groups. For two heterotic group of cluster A and B each consist of six inbreds, while one group of cluster C only consist of two inbreds. The confidence level of grouping for each cluster is 63% (A), 99.3% (B), and

100% (C). The highest strapping of inbred is clustered in C, while inbreds that are outside from those three clusters the grouping is still weak or unstable and the chance to move to different cluster is higher if other primer is added. If the cluster is constructed by PCoA (*Pricipal Coordinate Analysis*) in two dimensions it can clearly reveal relative position of each cluster. The selection of crossing pair between heterotic groups, in which it is supported by the value of genetic distance estimation, is more accurate (Figure 2).

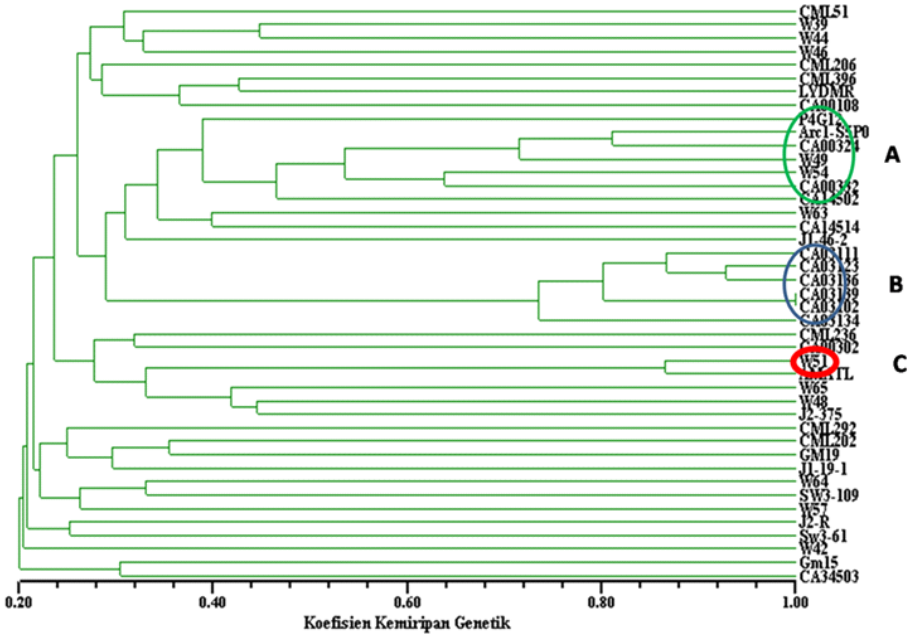

Figure 1. Dendogram construction of 43 elite inbred lines based on UPGMA with 30 SSR markers (Source: Pabendon *et al*. 2003).

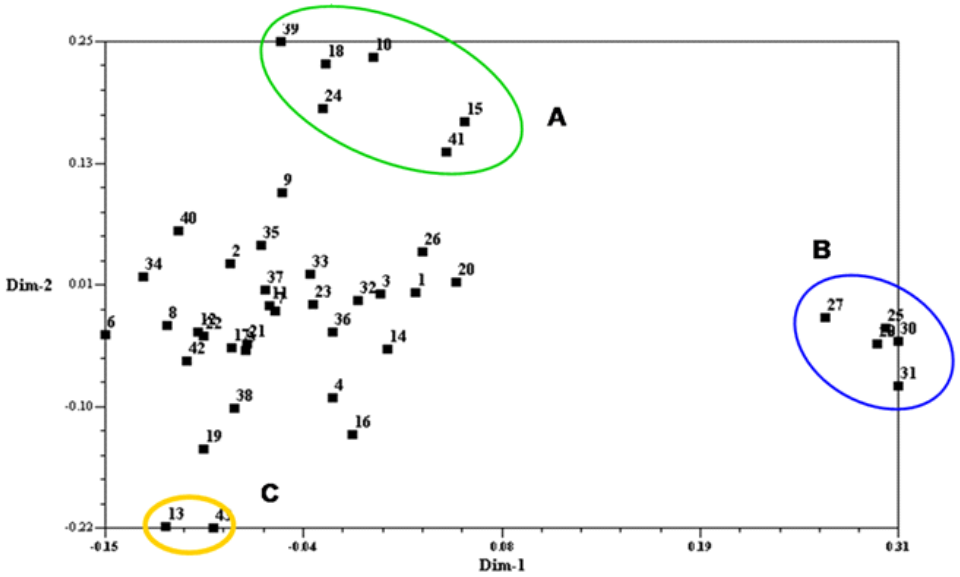

Figure 2. Relative position of 43 inbred lines from characterization via SSR marker which constructed using PCoA (*Principal Coordinate Analysis*).

Result of molecular characterization of inbred lines in China and Indonesia through SSR-based markers at different laboratory (George *et al*. 2004) showed that lines from southern China which have high variation are constructed in six clusters from total seven clusters made. From all of the clusters, there are lines that representative to be used as a tester. Lines originated from Indonesia are in cluster five out of six clusters made, whereas two main clusters each have representative tester. CIMMYT's lines developed for Asian region shows narrow genetic background which forms two separate clusters out of seven clusters and it also forms three clusters out of six clusters each in China and Indonesia.

Genetic Distance Estimation Versus Specific Combining Ability (SCA)

Bohn *et al*. (1999) stated that selection of parental hybrid, especially in maize, for the development of base population is crucial. This is because the success of the program is determined mostly by the selection for the next phase of breeding, which also affect the optimum allocation of resources in hybrid breeding program. An alternative strategy that need to be examined and considered is the analysis technique which is based on the assumption that SCA expressed in a hybrid is closely related with the genetic distance between parental lines (Lee *et al*. 1989). Therefore, if breeder could predict the crossing prospect of developed lines, before being tested in the field it could eventually increase the efficiency of the breeding program, by focusing only on the effort to prospective pairs of parents.

Pabendon *et al.* (2002) perform characterization via molecular-based markers build upon genetic similarity of a numbers of inbred lines at ICERI. The cluster analysis showed that MSJ1 and MSJ2 were at different group. Estimation of genetic distance of J1 vs J2 was high enough, for example between J2-R-144 vs J1-46 was 0.80, while J2-R-144 vs J1-19-1 was 0.72. This means that information of SCA is consistent with the value of genetic distance through molecular marker. Therefore, clustering lines in heterotic group before field testing will enable breeder to reduce the cost and time of testing because GCA test is not required. Another reason is that it can also avoid crossing lines within the same heterotic group.

Pabendon *et al*. (2009) reported that low genetic distance between parental hybrids (<0.7) produced lower seed weight, while high genetic distance (>0.7) produced higher. The correlation value were medium which indicated that values of genetic distance from medium to high could not clearly predict the seed weight, GCA, and heterosis. Environment effect was thought to be the reason that caused low correlation value. In 2010, Pabendon *et al*. observed two sets of data from using two testers Mr-4 and Mr-14 to study correlation between genetic distance based on microsatellite marker with seed weight of testcross. Result of analysis generally showed trend that low genetic distance caused low seed weight while the opposite was high.

Data in Table 3 showed characterization result of several inbreds which provide the range of genetic distance based on genetic distance matrix. The percentage of chance from potential heterotic pairs within each set of inbred varied because the genetic variability condition of each set was different. However the information is useful because crossing pair are more focused on potential heterotic pairs.

According to Birchler *et al*. (2003) the challenge in developing molecular model for heterosis is to make a correct correlation between phenotypic character with many causative molecular effect occurr in hybrid. Information about correlation between genetic distance

Table 3. Information on the estimation of genetic distance of several inbreds characterized via microsatellite-based markers.

Set of inbred	Number of genotype	Range of genetic distance	Possible crossing pair	Number of possible heterotic pairs (genetic distance ≥0,7)	Percentage of possible heterotic pair (%)
High yield elite inbred (a)	43	0,00-0,89	903	822	91,0
High yield elite inbred (b)	34	0,21-0,88	496	358	72,2
Waxy corn inbred (a)	19	0,30-0,84	171	76	44,4
Sweet corn inbred	31	0,18-0,85	1128	295	26,2
Low N inbred	10	0,37-0,84	45	34	75,6
Drought tolerance inbred	57	0,47-0,87	1485	1133	76,3
Acidity tolerance inbred	15	0,29-0,80	105	98	93,3
Early maturity inbred	25	0,11-0,94	300	247	82,3
Waxy corn inbreds (b)	31	0,00-0,83	528	103	19,5
Pro-vitamin A inbred	7	0,41-0,76	21	6	28,5
Downy mildew resistant inbred	36	0,12-0,85	703	195	27,7

and grain yield is also vary. Parentoni *et al*. (2001) used RAPD marker on corn and resulted in phylogeny match and followed pedigree data, even though the correlation between genetic distance and positive SCA remains weak.

Several data showed that highest yield was not always obtained from crossing pair of high genetic distance. Barbosa *et al*. (2003), analyze clusters to form heterotic grouping of inbred and find significant correlation between genetic distance and grain yield. On the other hand Lanza *et al.* (1997) did not find significant correlation between genetic distance and grain yield. Dias *et al*. (2004) reported that contrast genetic difference and heterosis was not always linier, and Sant *et al*. (1999) added that non linier relationship occurred between genetic distance and grain yield due to environmental effect. Therefore to obtain accurate correlation, field observation data should be placed in more than one locations or seasons due to the effect of environment.

Conclusions

Detection of homozygosity level through molecular marker on elite inbred lines should be assessed to avoid pairing inbred with low homozygosity ≤85%.

Information on genetic variability is important to select potential elite inbreds which will be used in the development of hybrid maize.

Hybrid parental selection based on genetic distance or heterotic group is proven to be effective in estimating the value of heterosis.

Hybrid breeding program is aimed to fully utilize local germplasm in the development of new varieties or improvement of existing varieties. Conventionally it is difficult to screen potential genes especially for biotic/abiotic stresses within local germplasm/population. Molecular biology laboratory is equipped with genomic-based facilities which can be optimally used to explore those potential genes. However, there is a need to increase the human capacity for better implementation.

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