Genetic characterization of popcorn (Zea mays everta) genotyping based on SSR (simple sequence repeats) markers

Muzdalifah Isnaini, Sri Sunarti, N.N. Andayani and Marcia B. Pabendon
Indonesia Cereals Research Institute, Dr. Ratulangi Street 274 Maros, South Sulawesi, Indonesia
Email: muzjagung@yahoo.co.id

Abstract. Popcorn variety is modified corn that makes good form, crunchy texture, and high yield. Through this research, expected pre-eminent maize national variety can replace role of import maize variety which used in making of popcorn so that the intention of self sufficiency in food and improvement of domestic resource quality can be reached. This research aimed to determine relation of genetic and genetic diversity of popcorn using SSR. The study was conducted at the Molecular Biology Laboratory, Cereals Crop Research Institute. The number of genetic collected popcorn lines consisted of 30 genotypes and tested by 18 markers of SSR. The result show polymorphism value was 0.30 with average value 2 allele/locus and the allele size locus of allele between 106.0-427.0 bp, size measure of minimum allele of umc 1545 while size measure of allele highest of Phi 093. Correlation value of cophenetic (r) equal to 0.82 pertained is good enough (good fit) to looking like matrix of genetic, showing accuracy of subdividing of germplasm into cluster still is good enough. The genetic diversity of popcorn analyzed show genetic viability of popcorn analyzed form 4 cluster and there are 11 tide opportunity of cross. This matter indicated that maize lines of popcorn this have earned to be exploited in making of hybrid maize.

1. Introduction
Popcorn comes from one type of corn (Zea mays everta) where the kernel can pop and expand when heated. Utilization of corn as a favorite food today can be served as a snack which is better known as popcorn [1]. Based on research conducted by [2], the 235 respondents obtained the results that as many as 73 respondents stated that corn can be processed and developed into modern food. The results of this consumer perception assessment illustrate that there are opportunities for processing corn into modern forms of food that can be accepted by the public.

Generally the popcorn sold on the market comes from imported varieties where the gene composition has been modified to produce popcorn with a good shape, crispy texture, and high yield. In the context of food self-sufficiency and improving the quality of domestic resources, this research aims to examine the possibility of national superior varieties of corn to be made into popcorn.

Molecular markers are an excellent tool for breeders and geneticists to analyze plant genomes. This system has revolutionized the field of genetic mapping and can be used to answer questions relating to genetic diversity, classification and phylogeny in germplasm management. One of the molecular markers that has been widely used is SSR (Simple Sequence Repeats) or Microsatellites. This marker has been used in various studies, including studies of genetic diversity or identification of plant varieties [3,4], as conducted by [5], in selecting varieties of popcorn which have high heterozygous.
According to [6], the ease of SSR in the amplification and detection of DNA fragments (Deoxyribose Nucleic Acid), as well as the high polymorphism produced makes this method ideal for use in studies with large sample sizes. In addition the PCR (Polymerase Chain Reaction) technique using the SSRs method only uses DNA in small amounts with a small amplification area, around 100 - 300 bp (base-pair) of the genome.

Identification of lines with the help of molecular markers is also useful in fingerprint analysis, because it can provide information for breeding program planning, especially in the formation of new segregation, new hybrid and synthetic varieties, and in determining the elders used to select new cross-pair. Although information from heterotic groups is not always able to produce the best combination of crosses, this approach can reduce the number of crosses and segregated offspring needed for further evaluation.

The characterization of plant germplasm using molecular markers can provide faster, more effective and accurate results compared to characterization based on morphological characteristics. Molecular characterization can be used together and complement each other with characterization based on morphological features.

Corn genome has 10 pairs of chromosomes which are manifested by heterozygous-heterogeneous genetic composition, which is plastic in adapting to face different environments. Because of this, maize plants have broad adaptations, growing in tropical, subtropical, and temperate regions in the northern and southern hemisphere [7].

This study aims: (1) To determine the genetic relationship and genetic diversity level of popcorn based on SSR (Simple Sequence Repeat) markers, (2) to classify popcorn genotypes based on their kinship, (3) and determine potential pairs in forming recombination for improvement of popcorn corn based on estimated genetic distance values.

2. Materials and Methods
The study was conducted in March – June 2014 at the Laboratory of Molecular Biology of the Indonesian Agency for Agricultural Research and Development, Indonesia Cereal Research Institute (ICeRi), Maros. Genetic materials were popcorn inbreeds line collection consisting of 30 genotypes and 18 SSR primers were selected through the Maize GDB corn genome database (Table 1.) [8].

DNA isolation was carried out to obtain DNA with quality and quantity that can be used for the SSR method. In the SSRs method the DNA concentration needed for one reaction is 10 ng/μl, whereas the quality of DNA does not require pure DNA. This DNA isolation process follows the procedure of [9] and [10] adapted to the CTAB method, and modified according to the conditions of the Iceri Molecular Biology Laboratory. The quantity and quality of DNA extracted was measured through horizontal electrophoresis using 0.9% agarose gel.

The amplification process uses the SSR method, used PCR (Polymerase Chain Reaction) machine. The amplification process was 30 cycles, which consisted of several stages in accordance with the CIMMYT protocol [11], namely initial denaturation of 94°C for 2 minutes, Denaturation of 94°C for 30 seconds, Annealing 56°C for 1 minute, elongation of 72°C for 1 minute. The second stage was repeated 29 times and ended with a 72°C elongation cycle and cooling at 4°C. After completion, the results of the amplification are removed from the PCR machine. The results of PCR product amplification were checked through horizontal electrophoresis using 2% agarose gel. If DNA is amplified then DNA separation from the PCR product can be continued on the vertical gel using the Dual Mini-Verticals Complete System MGV-202-33 with polyacrylamide gel, which is capable of producing high resolution.
Table 1. SSR markers and their nucleotide sequences for popcorn plants.

| No. | Primer | Bin  | base arrangement | ∑ Base |
|-----|--------|------|------------------|--------|
| 1   | phi227562 | 1.11 | TGA TAA AGC TCA GCC ACA AGG // ATC TCG GCT AGC GGC AGA | 39 |
| 2   | phi083  | 2.04 | CAA ACA TCA GCC AGA AGA AAG GAC // ATT CAT CGA CGC GTC ACA GTG TAC T | 49 |
| 3   | phi101049| 2.10 | CCG GGA ACT TGT TCA TCG // CCA CGT CCA TGA TCA CAC C | 37 |
| 4   | phi053  | 3.05 | CTG CCT CTC AGA TTA AGA GAT TGA C // AAC CCA AGC TAC TCC GGC AG | 45 |
| 5   | phi102228| 3.06 | ATT CCG AGC CAA TCA ACA // TTC ATC TCC AGG AGC TTT | 39 |
| 6   | phi079  | 4.05 | TGG TGC TCG TTG CCA AAT CTA CGA // GCA GTG GTG GTT TCG ACG AGA CAA | 48 |
| 7   | phi093  | 4.08 | AGT GCC TAC GTC TCG CCT ACA AGA // AGG CCA TGC ATG CTT GCA ACA ATG GAT ACA | 56 |
| 8   | phi331888| 5.04 | TTG CCG AGG TTT GTA GCT G // ACT GAA CCG CAT GCC AAC | 37 |
| 9   | umc1153 | 5.09 | CAG CAT CTA TAG TTT CTC GCT TGC ATT // TGG GTT TTG TTG TTG TTT TTT | 48 |
| 10  | phi423796| 6.01 | CAC TAC TCG ATC TGA AGC ACC A // CGC TCT GTG AAT TCG CTA GCTC | 43 |
| 11  | phi299852| 6.07 | GAT GTG GGT GCT ACG AGC C // AGA TCT CCG AGC TCG GCT A | 38 |
| 12  | umc1545 | 7.00 | ATT CAC TCT TGC ATT GCC TCT ACC // ATG AAC GAG TCC AGT TTC TTG | 48 |
| 13  | phi328175| 7.04 | GGG AAG TGC TCC TTT CAG // CCG TAG GTG ACG GGC GTC | 36 |
| 14  | phi420701| 8.00 | GAT GTT TCA AAA CCA CCC AGA // ATG GCA CGA ATA GCA ACA CG | 41 |
| 15  | phi065  | 9.03 | AGG GAC AAA TAC GTG GAG ACA CAG // CGA TCT GCA CAA AGT GGA GTA GTC | 48 |
| 16  | phi032  | 9.04 | CTC CAG CAA GTG ATG CGT GAC // GAC ACC CGG ATC AAT GAT GGA AC | 44 |
| 17  | phi96342 | 10.02 | GTA ATC CCA CGT CCT ATC AGC C // TCC AAC TTT ACG GAA TCC CTC | 42 |
| 18  | umc1196 | 10.07 | CGT GAT ACT GCT GCA AAG CAG // AGT CGT TCG TGT CTT CCG AAA CT | 47 |

Data analysis was performed based on the results of scoring DNA band patterns that appear on the plate. The results of scoring in binary data, if there is a band, scoring 1 and if there is no band, scoring 0. Band that is blurred or too difficult to be scored are marked as missing data and given score 9. Binary data were analyzed using the NTSYS-PC computer program version 2.1 [12]. For each SSR locus, the average number of alleles per locus and polymorphism level (PIC) were calculated. PIC values [1], [6] were calculated following [13] with the equation:

\[ PIC = 1 - \sum_{i=1}^{n} f_i^2 \]

where \( f_i^2 \) is frequency i allele

Cluster analysis based on genetic similarity matrix was constructed based on UPGMA (Unweighted Pair Group Using Arithmetic Average) using Jaccard coefficient. The distance matrix and dendrogram are formed using the NTSYS-PC (Numerical Taxonomic System) version 2.1 [12] program. The level of genetic similarity is the degree of similarity of characters, in this case the band fragments that are shared from the identified genotypes. The level of genetic similarity (GS) is estimated from allele size data using the Jaccard coefficient [12] with the formula:
cluster analysis is based on 19 SSR loci and is formed based on genetic distance matrix using the UPGMA (Unweighted Pair-Group Method with Arithmetic average) linking method with the NTSYS-pc 2.1 program tool [12]. The value of genetic distance is obtained from the results of genetic similarity analysis [14], with the formula:

\[
S = 1 - GS
\]

s = Genetic distance
S = genetic similarity

The average value of genetic distance in a cluster is greater than the general average, so crossing between clusters can be done.

Bootstrapping analysis using the "winboot" program, to determine the level of confidence of the dendrogram results based on the primary set used in the 30 genotypes identified. The cophenetic correlation coefficient (r) was also calculated followed by the Mantel test [15] to see the goodness of fit from the results of the cluster analysis. Principal Coordinate Analysis (PCA) [16], conducted to determine the relative position of the inbreds analyzed in two-dimensional space.

### 3. Results and Discussion

Profile data of microsatellite markers resulting from inbred popcorn maize characterization A total of 30 popcorn maize genotypes were characterized based on the SSR loci used in this study. Data on allele diversity at each SSR locus is presented in Table 2.

**Table 2. Profiles of 18 SSR markers resulting from the characterization of 30 popcorn lines**

| No. | Primer       | Bin no. | PIC | Number of Allele/locus | Base size (bp) |
|-----|--------------|---------|-----|------------------------|----------------|
| 1   | phi109275    | 1.00    | 0.29| 3                      | 118.0-258.7    |
| 2   | Phi227562    | 1.11    | 0.52| 2                      | 311.0-427.0    |
| 3   | Phi102228    | 3.04    | 0.07| 2                      | 125.3-151.0    |
| 4   | phi053       | 3.05    | 0.56| 3                      | 175.5-228.3    |
| 5   | Phi079       | 4.05    | 0.06| 2                      | 191.8-311.0    |
| 6   | Phi093       | 4.08    | 0.03| 2                      | 398.0-427.0    |
| 7   | phi331888    | 5.04    | 0.19| 2                      | 140.0-151.0    |
| 8   | umc 1153     | 5.09    | 0.33| 2                      | 106.0-112.0    |
| 9   | phi 423796   | 6.01    | 0.33| 3                      | 123.5-175.5    |
| 10  | phi 299852   | 6.08    | 0.52| 3                      | 106.0-200.0    |
| 11  | umc1545      | 7.00    | 0.53| 2                      | 74.0-100.0     |
| 12  | Phi328175    | 7.04    | 0.30| 2                      | 134.5-151.0    |
| 13  | Phi 420701   | 8.00    | 0.16| 3                      | 134.5-175.5    |
| 14  | phi 065      | 9.03    | 0.52| 3                      | 123.5-427.0    |
| 15  | Phi 032      | 9.04    | 0.10| 2                      | 232.7-315.0    |
| 16  | umc 1196     | 10.07   | 0.50| 3                      | 134.5-212.2    |
| 17  | phi083       | 10.2    | 0.10| 2                      | 129.0-232.7    |
| 18  | phi 96342    | 10.2    | 0.38| 2                      | 249.0-331.0    |

Total: 38
Average: 0.30

74.0-427.0
The popcorn genotype analyzed had a polymorphism level ranging between 0.03 found in phi 093 primers and 0.56 in phi 053 primers with an average value of 0.30. This shows a lower level of polymorphism compared to research conducted by [17] in the maize inbred collection where the average PIC value obtained was 0.53. The results were lower in this study because the average number of alleles per locus was lower. With a high polymorphism value 0.72, the high number of alleles was obtained because the average number of detected alleles was quite high [18]. Several studies conducted such as [19], the average PIC value obtained was 0.45. [20] found an average PIC value 0.54, [21] reported a PIC value 0.50 and [22] with a PIC value 0.51.

| No. | Popcorn genotypes | % Heterozigosity | %Homozigosity |
|-----|------------------|------------------|--------------|
| 1   | ACMMRM1          | 11,11            | 88,89        |
| 2   | ACMMRK13         | 5,56             | 94,44        |
| 3   | ACMMRK12         | 16,67            | 83,33        |
| 4   | ACMMRK11         | 11,11            | 88,89        |
| 5   | ACMRK7           | 16,67            | 83,33        |
| 6   | ACMRK16          | 16,67            | 83,33        |
| 7   | ACMRK10          | 16,67            | 83,33        |
| 8   | ACMRK6           | 11,11            | 88,89        |
| 9   | ACMRK10          | 11,11            | 88,89        |
| 10  | ACMRM3           | 11,11            | 88,89        |
| 11  | ACMRK1           | 5,56             | 94,44        |
| 12  | AKM11            | 0,00             | 100,00       |
| 13  | AKM8             | 11,11            | 88,89        |
| 14  | AKM7             | 11,11            | 88,89        |
| 15  | AKM4             | 16,67            | 83,33        |
| 16  | AKM6             | 16,67            | 83,33        |
| 17  | AKM9             | 16,67            | 83,33        |
| 18  | AKM17            | 11,11            | 88,89        |
| 19  | AKM19            | 16,67            | 83,33        |
| 20  | AKM20            | 16,67            | 83,33        |
| 21  | BKBBRK2          | 11,11            | 88,89        |
| 22  | BKBBRK7          | 16,67            | 83,33        |
| 23  | BKBBRK10         | 5,56             | 94,44        |
| 24  | AKRK2            | 5,56             | 94,44        |
| 25  | AKRK3            | 5,56             | 94,44        |
| 26  | AKRK4            | 5,56             | 94,44        |
| 27  | AKRK5            | 11,11            | 88,89        |
| 28  | AKRK6            | 11,11            | 88,89        |
| 29  | MMRMAK10         | 0,00             | 100,00       |
| 30  | PCMS8            | 16,67            | 83,33        |

A high PIC value indicates that the variation between the analyzed genotypes is quite large. If the genetic viability obtained is higher and it will be more flexible in the selection of the desired
characters to exploit heterocyst. There are 38 alleles detected from 18 primers with an average value of 2 alleles / loci and ranging from 2-3 alleles, this value is relatively low compared to research conducted by [23] with an average of 5.4 alleles in the population corn using 61 SSR loci; [21] reported 3.85 alleles per locus using 27 SSR loci. [17] reported a range of 2-4 alleles with a total of 23 alleles in an inbred genetic diversity study of 27. The allele size is between 106.0-427.0 bp, the lowest allele size is in the primary umc 1545 while the highest allele size is in the phi 093 primer. The percentage of homozygosity of the popcorn corn lines is presented in Table 3. The homozygosity percentage of popcorn PCMS8 line was 83.33, and there were even 100% homozygotes for the 2 primers used, namely AKMKRM11 and MMRMAK10. This shows that these popcorn corn lines can already be utilized in hybrid assembly.

3.1. The level and pattern of genetic diversity
The results of cluster analysis or grouping based on UPGMA on genetic similarity matrix were obtained by dendrogram through NTSYS analysis as shown in Figure 1. The cophenetic correlation value (r) was 0.82, which shows the accuracy of genetic grouping into the cluster is still quite good [17] suggested that the value of cophenetic correlation coefficients illustrates the accuracy of genotypic grouping, which can be generated based on the estimation of genetic similarity between lines characterized by the number of primers used. [11] suggested that the more polymorphism primers used, the greater the value of r, where the value of r> 0.9 (very good fit), 0.8 <r <0.9 (good fit), 0.7 < r <0.8 (poor fit), and r <0.7 (very poor fit).

Dendrogram results showed that almost all genotypes can be distinguished between each other. Numbers above the line indicate the level of confidence of the grouping based on bootstrapping analysis using the Winboot program. The higher level of grouping trust means the stronger genetic similarity of the lines in the group. So if the crossing is done between individuals in the same group (with a high level of grouping trust) which means that these individuals are genetically strong, then the chances of inbreeding will be higher. Therefore crossing in the same group must be avoided. According to [24], non-clustered lines based on phenotype, environmental adaptation, seed type or color, age of harvest, or heterotic response, but pedigree-related lines are usually in the same cluster. The level of kinship (genetic similarity) in the lines of popcorn tested ranged from 0.54 - 1.00. When crossing lines are drawn on a kinship scale between 0.54 and 0.66 four groups or clusters are formed.

![Figure 1. Dendrogram of 30 lines of popcorn based on genetic similarity using 18 primers SSR markers](image-url)
Information on the kinship relationships of the hybrid composition lines was obtained by conducting cluster analysis of 30 lines using 18 SSR markers, in which 4 clusters were obtained. Group I is the largest group consisting of ACMMRM1, ACMMRK12, ACMMRK11, ACMMRK6, ACMMRK13, ACMMRK10, ACMMRK16, ACMMRK10, ACMKRK7, ACMKRK11, ACMKRK18, ACMRK9, AKMRM, ACMKRK10, ACMKRK7, ACMKRK11, ACMKRK18, ACMRK16, ACMKRK16, ACMKRK10, ACMKRK7, ACMKRK11, ACMKRK9, AKMKRM, ACMRRK6, ACK. Group II is composed of popcorn lines, namely ACMKRK1, BKBBRK10, AKRRK2, AKRRK3, AKRRK4 and AKRRK5. While group III consisted only of one line of popcorn, namely ACMRRK16 and group IV consisted of 2 lines namely MMRMAK10 and PCMS8.

The level of grouping confidence of the three groups is still low, around 8.4 for group I, 28.1 for group II and 34.7 for group III. This indicates that there are still a number of genotypes that are unstable or have the potential to shift to other clusters if a primer is added. To increase the level of confidence of the grouping it is necessary to add the number of primers used for analysis.

### 3.2. Matrix Genetic Analysis

Analysis of genetic distance between inbred lines reflects pedigree kinship to ensure the adequacy of the evaluation using molecular markers data [25]. There are 11 chances of crossing popcorn with genetic distance values ≥ 0.55 that can increase variability or have the opportunity to produce heterocyst, namely PCMS8 x ACMMRM1, PCMS8 x ACMMRK13, MMRMAK10 x ACMMRK13, PCMS8 x ACMKRK7, MMRMAK10 x ACMKRK7, PCMS8 x ACMKRK13, MMRMAK10 x ACMKRK13, PCMS8 x ACMKRK7, MMRMAK10 x ACMKRK10, MMRMAK10 x ACMKRK10, MMRMAK10 x ACMKRK8, BKBBRK2 x ACMRRM3, and BKBBRK2 x ACMRRK1. Opportunities for crossing all pairs occur between clusters and mostly were K4 and K1 (Table 4).

The genetic distance matrix of 30 popcorn lines using 18 SSR markers, genetic distance ranged from 0.05 to 0.60. If the average genetic distance in the cluster is smaller than the general average, crossing within the cluster must be avoided. Conversely, if the average genetic distance in a cluster is greater than the general average, crossing between inbreeds in the same cluster can be done [26].

| Crossing                     | Genetic distance value | Cluster   |
|------------------------------|------------------------|-----------|
| PCMS8 x ACMMRM1              | 0.58                   | K4 x K1   |
| PCMS8 x ACMMRK13             | 0.55                   | K4 x K1   |
| MMRMAK10 x ACMMRK13          | 0.57                   | K4 x K1   |
| PCMS8 x ACMKRK7              | 0.58                   | K4 x K1   |
| MMRMAK10 x ACMKRK7           | 0.58                   | K4 x K1   |
| ACMRRM3 x ACMKRK7            | 0.55                   | K3 x K1   |
| PCMS8 x ACMKRK10             | 0.60                   | K4 x K1   |
| MMRMAK10 x ACMKRK10          | 0.58                   | K4 x K1   |
| MMRMAK10 x ACMKRK8           | 0.56                   | K4 x K1   |
| BKBBRK2 x ACMMRM3            | 0.58                   | K1 x K3   |
| BKBBRK2 x ACMRRK1            | 0.55                   | K1 x K2   |

*Note: K = cluster*

Crosses between cluster IV and cluster I about 8 crosses, cluster III and cluster I totally 2 crosses and cluster I and cluster II got 1 cross. One of the benefits of molecular markers is that they can form clusters based on genetic similarities and opportunities for heterocyst recombination, the selection process can be carried out faster and relatively cheaply so that the number of crosses can be
streamlined. However, the achievement of high heterocyst is not only based on genetic distance but also involves other factors such as the genetic potential of the inbred itself so that field verification is also needed [27].

4. Conclusion

1. The analyzed popcorn lines has an average polymorphism level around 0.30 with an average value of 2 alleles /locus and allele sizes between 106.0-427.0 bp. The cophenetic correlation value (r) of 0.82 is quite good fit on the genetic similarity matrix.

2. The genetic diversity of the popcorn lines analyzed showed moderate genetic variability.

3. The analyzed popcorn lines form 4 clusters and there are 11 pairs of crossing opportunities.

Reference

[1] Smith, J.S.C., E.E.L. Chin, H. Shu, O.S. Smith, S.J. Wall, M.L. Senior, S.E. Mitchell, S. Kresovich, J. Ziegle. 1997. An Evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RLPFS and pedigree. *Theor. Appl. Genet.* 95:163-173.

[2] Juniawati.2003. Implementasi Algoritma Genetika Untuk Mencari Volume Terbesar Bangun Kotak Tanpa Tutup Dari Suatu Bidang Datar Segi Empat. *Jurnal Ilmiah LPPM Universitas Surabaya*.

[3] Blair, M.W., O. Panaud, and S.R. McCouch. 1999. Intersimple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and finger-printing in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 98:780-792.

[4] Bredemeijer, M. and J. Cooke. 2002. Construction and testing of a microsatellite data base containing more than 500 tomato varieties. *Theoretical and Applied Genetics* 105 (6-7): 1019-1026.

[5] I.B.O. Eloi, C.A. Mangolin, C.A. Scapim, C.S. Goncalves and M.F.P.S. Machado, 2012. Selection of high heterozygosity popcorn varieties in Brasil based on SSR marker. *Genet. Mol. Res.* 11 (3):1851-1860.

[6] Senior, M.L., E.C.L. Chin, M. Lee, J.S.C. Smith, and C.W. Stuber. 1996. Simple sequence repeat markers developed from maize sequence found in the genebank database; *Map contraction. Crop Sci.* 36: 1676-1683.

[7] Vasal S.K., and S. Taba. 1988. Conservation and utilization of maize genetic resources. In: R.S. Paroda, R.K. Parora, and K.P.S. Chandel (Eds.). Plant Genetic Resources-Indian Perspective. Proceeding of the National Symposium on Plant Genetic Resources NBPGR, *New Delhi*. p. 91-107.

[8] Maize Genetics and Genomics Database (2010). *http://www.maizegdb.org*.

[9] George, M.L.C., E. Regalado, W. Li, M. Cao, M. Dahan, M.B. Pabendon, Warbuton, X. Xianchun, and D. Hoisington. 2004. Molecular Characterization of Asian maize inbred lines by multiple laboratories. *Theor Appl Genet* 109:80-91

[10] Khan, I.A., F.S. Awang, A. Ahmad, and A.A. Khan. 2004. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Molecular Biology Reporter* 22:89a-89e.

[11] CIMMYT. 2004. Protokol untuk Karakterisasi Jagung secara Genetipik menggunakan Marka SSR serta Analisis Data. *Metro Manila*, Philippines.

[12] Rohlf, F.J. 2000. NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.1. *Applied Biostatistic Inc*.

[13] Nei, M. 1987. Estimation of average heterozygosity and genetic distance from a small number of individuals: *Genetic* 89:583-590.

[14] Lee, M. 1998. DNA markers for detecting genetic relationship among germplasm revealed for establishing heterotic groups. Presented at the Maize Training Course, *CIMMYT, Texcoco, Mexico, August 25*, 1998.
[15] Mantel. 1967. The detection of disease clustering and generalized regression approach. *Cancers Res.* 27: 209-220.

[16] Dillon, W.R. and M. Goldstein. 1984. Multivariate Analysis Methods and Applications. *John Willey and Sons*. p. 581.

[17] Pabendon, M.B, M. Dahlan, Sutrisno, dan M.L.C. George, 2006. Karakterisasi Kemiripan Genetik Koleksi Inbrida Jagung Berdasarkan Marka Mikrosatelit. *Jurnal Agro Biogen* 2 (2):45-51.

[18] Peijic, I., P. Ajmon-Marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino, and M. Motto. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theor. Appl. Genet.* 97:1248-1255.

[19] 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 using microsatellite markers. *Africant Journal of Biotechnology* Vol. 12 (39), pp.5723-5728.

[20] Choukan R, Hossainzadeh A, Ghannadha MR, Warburton ML, Talei AR, Mohammad SA., 2006. Use of SSR data to determine relationships and potential heterotic groupings within medium to late maturing Iranian maize inbred lines. *Field Crops Res.* 95:212-222.

[21] Legesse BW, Myburg AA, Pixley KV, Botha AM., 2006. Genetic diversity of African maize inbred lines revealed by SSR markers. *Hereditas*, 144:10-17

[22] Agular CG, Schuster I, Amaral Junior AT, Scapim CA, Viera ESN. 2008. Heterotic groups in tropical maize germplasm by testcross and simple sequence repeat markers. *Genet. Mol. Res.* 7(4):1233-1244.

[23] Wu, Y.S., Zhing Y.L., Sun R., and Wu S.Y. 2004. Genetic diversity of waxy corn and popcorn landraces in Yunnan by SSR markers. *Acta Agron. Sin* 30: 36-42.

[24] Warburton, M.L., J.M. Ribaut, J. Franco, J. Crossa, P. Dubreuil& F.J. Betran. 2005. Genetic characterization of 218 elite CIMMYT maize inbred lines using RFLP markers. *Euphytica* 142:97-106.

[25] Vas Patto, M.C., Satovic, Z., Pego,S., dan Fevereiro, P., 2004. Assessing the genetic diversity of Portuguese maize germplam using microsatellite markers. *Euphytica*, 137 (1), 63-72.

[26] Pabendon, M.B., Mejaya M.J., Koswara J., danAswidinnoor H. 2007. Analisis Keragaman Genetik Inbrida Jagung Berdasarkan Marka SSR dan Korelasinya dengan Data Fenotipik F1 Hasil Silang Uji. *Jurnal Penelitian PertanianTanaman Pangan* Vol. 26 No. 2.

[27] 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*, 14: 301-308.