Genetic Variability in West Timor Landrace Maize Populations

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Abstract. Genetic variability information, which is a heritable difference among maize cultivars, is important to a long-term plant breeding program. In the last decade a number of researchers have studied the correlation between phenotypic and genetic variability in maize by using Deoxyribonucleic Acid (DNA) markers. Maize landraces as maize cultivars grown ‘on farm’ by farmers who reproduce their seed every year, and they can be distinguished by specific traits, so maize landraces are composed of widely variable populations, where the variation can be seen between and within populations. Five white maize landrace seed samples were obtained from five farmers in each of three villages, Nunmafo, Amol and Ajaobaki; five yellow landrace maize seed samples were obtained from five farmers in Nunmafo village only; while the five certified improved open pollinated varieties (Lamuru, Piet kuning, Bisma, Harapan, Kalingga) were obtained from the Indonesia Cereal Research Institute (ICRI) Maros. The banding patterns of the samples’ fingerprint were evaluated in the scoring range of 50-700bp fragment weight using fragment size standard ladder (Figure 6.3). Image data was viewed on the computer screen and saved in the SAGA computer program. The gel images were scored manually in Excel 2010 program for presence (score 1) or absence (score 0) of bands, and the binary data used for further analysis. The results of this study for AFLP analysis of West Timor maize landrace populations from three villages and varieties have confirmed that there are extensive genetic variations within West Timor yellow and white maize landrace populations and varieties. There were two clusters of genetic variation among all samples. The first cluster was a general West Timor maize landrace population, with more than 80% similarity among individuals, and the second cluster was a much more diverse grouping with less than 80% similarity. It suggests that even though a high molecular diversity had been found among West Timor maize landrace populations most maize genotypes belong to a West Timor maize landrace cluster. In addition the analysis indicated not only significant difference between maize landraces and maize varieties but also maize landraces from the three villages were genetically significantly different from each other. The molecular marker characterization of West Timor maize landrace populations will be a key step for obtaining an improved understanding of the population so they can be managed carefully for the future. Moreover, due to the genetic diversity captured within local landraces, farmers have the opportunity to perform the phenotypic selections based on plant type and seed yield which are likely to give them significant genetic gain in yield in subsequent seasons. Plant breeders have the additional opportunity of applying molecular marker techniques to assist selection.
1. Introduction
Maize landrace populations are domesticated local plant types that differ from varieties that have been developed by modern plant breeding during a formal breeding programme. Bitocchi [1] have described maize landraces as maize cultivars grown ‘on farm’ by farmers who reproduce their seed every year, and they can be distinguished by specific traits and also maize landraces are composed of widely variable populations, where the variation can be seen between and within populations.

The phenotypic variability of the landrace maize populations is the expression of genetic variability and its response to the environment. There is a significant interaction between genotype and environment in maize traits particularly grain yield [2]. Physiological characteristics and biological explanations can be derived from an analysis of genetic and environment (GE) interaction in maize. Genetic variability information, which is a heritable difference among cultivars, is important to a long-term plant breeding program [3]. In the last decade a number of researchers have studied the correlation between phenotypic and genetic variability in maize by using Deoxyribonucleic Acid (DNA) markers [4]; [2]. The usefulness of the DNA marker-based Amplified Fragment Length Polymorphisms (AFLP) technique, in agricultural, horticultural and environmental research, has been recognised for over a decade [5]. The AFLP technique was developed in 1991 [6] and has since been used to produce linkage maps of a maize mapping populations and for studying the genetic diversity of both maize inbred lines [7].

A distance-based clustering method has been widely used in genetic variability studies to determine the degree of variation among accessions, but more recently a model-based clustering analysis has been applied by many researchers in genetic variation analysis for the same purposes. Distance-based clustering analysis produces a dendrogram of accession clusters within which the number of groups identified is based on an arbitrary level of similarity, decided by the researcher. Hence, Pritchard [8] developed a model-based cluster method to analyse the degree of variation among accessions and evaluate the statistical significance of likely population groupings. This method uses the software package STRUCTURE to derive the most probable number of populations within the total number of individuals tested and then assigns individual accessions to membership of these populations. Accordingly in the last five years, scientists have used both distance-based clustering analysis and the model-based cluster method [9]; [10]. The advantage of the model-based cluster method is that each accession is characterized by its probability of belonging to one or more of the populations (groups) determined by the analysis. Moreover, this program uses a Bayesian clustering which indicates the number of groupings with the highest probability. This method has been widely used in population structure studies in plants, fungi, animals and human genetics [9].

There is very little information available on the genetic variability among local landrace maize populations grown in the West Timor region. In response to this need, an investigation into a range of white and yellow-seeded landraces was conducted using the AFLP method to determine the genetic variability and relatedness among these populations using a phylogenetic tree, and to evaluate the genetic differences between white and yellow maize and between landraces grown by different villages.

The reasons for using the AFLP technique in the West Timor local maize study were: the AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNAs of any origin or complexity; the number of fragments detected in a single reaction can be ‘tuned’ by selection of specific primer sets; the AFLP technique is robust and reliable because strict reaction conditions are applied for primer annealing; and the reliability of restriction fragment generation is combined with the power of the PCR technique.

This paper will discuss how the West Timor maize landrace populations and improved maize variety samples were collected and the procedures applied for DNA extraction and analysis. Based on the results obtained, the genetic variability of West Timor maize landrace populations and the relationship among them will be discussed. In particular the differences between sampling sites, yellow and white maize landraces and improved maize varieties will be considered.
2. Materials and methods

2.1. Maize Seed Sample Collection

One hundred populations of West Timor Maize landraces, consisting of 75 populations of white maize and 25 populations of yellow maize, together with 27 populations of improved open pollinated maize varieties including 22 populations of certified maize (Piet kuning, Kalingga, Lamuru, Harapan and Bisma) and 5 populations of uncertified maize (Lamuru) (Table 1) were tested in the genetic variability evaluation.

**Table 1.** List of maize seed samples, kernel colour, source, sampling site and major environment

| Sample group | Sampling code | Number of samples (populations) | Kernel colour | Source | Sampling site (village) | Major environment |
|--------------|---------------|---------------------------------|---------------|--------|------------------------|-------------------|
| 1. Landrace white maize | WJaoabaki (A1 – A5) | 5 | White | Farmer | Ajaobaki | Highland |
| | WJaoabaki (B1 - B5) | 5 | White | Farmer | Ajaobaki | Highland |
| | WJaoabaki (C1 – C5) | 5 | White | Farmer | Ajaobaki | Highland |
| | WJaoabaki (D1 – D5) | 5 | White | Farmer | Ajaobaki | Highland |
| | WJaoabaki (E1 – E5) | 5 | White | Farmer | Ajaobaki | Highland |
| | WAmol (A1 – A5) | 5 | White | Farmer | Amol | Medium-land |
| | WAmol (B1 - B5) | 5 | White | Farmer | Amol | Medium-land |
| | WAmol (C1 – C5) | 5 | White | Farmer | Amol | Medium-land |
| | WAmol (D1 – D5) | 5 | White | Farmer | Amol | Medium-land |
| | WAmol (E1 – E5) | 5 | White | Farmer | Amol | Medium-land |
| | WNunmafo (A1 – A5) | 5 | White | Farmer | Nunmafo | Lowland |
| | WNunmafo (B1 - B5) | 5 | White | Farmer | Nunmafo | Lowland |
| | WNunmafo (C1 – C5) | 5 | White | Farmer | Nunmafo | Lowland |
| | WNunmafo (D1 – D5) | 5 | White | Farmer | Nunmafo | Lowland |
| | WNunmafo (E1 – E5) | 5 | White | Farmer | Nunmafo | Lowland |
| 2. Landrace yellow maize | YNunmafo (A1 – A5) | 5 | Yellow | Farmer | Nunmafo | Lowland |
| | YNunmafo (B1 - B5) | 5 | Yellow | Farmer | Nunmafo | Lowland |
| | YNunmafo (C1 – C5) | 5 | Yellow | Farmer | Nunmafo | Lowland |
| | YNunmafo (D1 – D5) | 5 | Yellow | Farmer | Nunmafo | Lowland |
| | YNunmafo (E1 – E5) | 5 | Yellow | Farmer | Nunmafo | Lowland |
| 3. Certified improved maize | Piet kuning (1 – 5) | 5 | Yellow | BPTP-NTT* | Naibonat | Lowland |
| | KalinggaOri (1 – 5) | 5 | Yellow | Belitsereal** | Maros | Lowland |
| | LamuruOri (1 – 5) | 5 | Yellow | Belitsereal | Maros | Lowland |
| | BismaOri (1 – 2) | 2 | Yellow | Belitsereal | Maros | Lowland |
| | HarapanOri (1 – 5) | 5 | Yellow | Belitsereal | Maros | Lowland |
| 4. Uncertified improved maize | Lamuru F5 (1 – 5) | 5 | Yellow | Farmer | Nunmafo | Lowland |

**Notes:** BPTP is a provincial level of Assessment Institute for Agriculture Technology in Naibonat and Belitsereal (ICRI) is a national level of Indonesian Cereal Research Institute in Maros

Five white maize landrace seed samples were obtained from five farmers in each of three villages, Nunmafo, Amol and Ajaobaki; five yellow landrace maize seed samples were obtained from five farmers in Nunmafo village only; while the five certified improved open pollinated varieties (Lamuru, Piet kuning, Bisma, Harapan, Kalingga) were obtained from the Indonesia Cereal Research Institute (ICRI) Maros. The uncertified Lamuru samples, originating from certified Lamuru harvested and replanted for five seasons were obtained from Nunmafo village.
2.2. DNA Extraction and Amplification

Polymerase Chain Reaction (PCR) with restriction enzyme digestion requires isolation of genomic DNA of suitable purity [11]. Consequently, a successful extraction of genomic DNA that can be digested by restriction enzymes and amplified by PCR is mandatory. Therefore, DNA was extracted from the samples using the protocol based on the detergent cetyl trimethyl ammonium bromide (CTAB). This method was used because it is relatively simple and has been used successfully on a wide range of plant species using either fresh or dehydrated plant material.

For reasons of maintaining the quarantine between Indonesia and Australia, maize seed DNA was extracted in Indonesia. The seeds were germinated individually in pots in the greenhouse of ICRI Maros, and then the ten day old leaves of the seedling were collected for the next steps of DNA extraction. Fresh leaves were cut into small pieces and ground, while adding liquid nitrogen, to the fine powder to prepare for the DNA extraction process using CTAB buffer. Finally, the genomic-DNA was stored at −10°C prior to the AFLP assay.

The concentration of the extracted maize DNA was determined by using the double stranded (ds) DNA method using an “Implen Nano-Photometer” to measure UV absorption [12]; [11]. The AFLP procedure is shown in Figure 2. Two μl of 75ng/μl genomic DNA was digested at 37 °C for two hours with two restriction enzymes, MseI and Pst, after which MseI- and Pst- adapters ligated to the fragments in room temperature overnight [6].

Each 5 μl sample of digested DNA was pre-amplified in a reaction volume of 20 μl containing 30 ng of primers with one selective nucleotide (MseI-C and Pst-A), 1.5 mM MgCl2, 0.4 μl dNTPs, 5X Taq polymerase buffer (10 mM Tris–HCl pH 9, 50 mM KCl, 0.1% Triton®X-100) and 0.5 U Taq DNA polymerase. The pre-amplification ran with a “Biometra T-gradient” Thermal Cycler for 30 cycles consisting of 30 s at 94 °C, 1 min at 56 °C and 1 min at 72 °C.

![Diagram of the AFLP protocol in identifying the fingerprint of West Timor landrace maize](Figure 1)

The pre-amplification products were diluted 1:5 with 1× TE (1 mM Tris–HCl, 0.1 mM EDTA, pH 8) after which 2 μl of diluted DNA was amplified in a 15 μl reaction volume containing 30 ng MseI and Pst primers with six selective nucleotides (MseI-CTC, MseI-CCA, MseI-AGC, MseI-ACG, Pst-AGA labelled with IRD 700 and Pst-AGC labelled with IRD 800), 0.4 μl dNTP’s, 0.9 μl MgCl2 5X Taq polymerase buffer and 0.5 U Taq DNA polymerase (Promega Corporation). The amplification was conducted with a Biometra T-gradient Thermal Cycler. The PCR cycling condition were 12 cycles of
94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, followed by 23 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min. The PCR products were denatured at 95 °C for 5 min after addition of 4 µl of formamide, and then snap-cooled on ice. Samples were stored at 4 °C [6]. The selective amplification products (0.3 µl) were loaded on to a 6.3% polyacrylamide gel containing 7 M urea. 1×TBE buffer was used as running buffer and the electrophoresis was carried out at 1500V constant power for 5 h using a 4300 LICOR DNA analyser.

2.3. Data Scoring and Analysis
The banding patterns of the samples’ fingerprint were evaluated in the scoring range of 50-700bp fragment weight using fragment size standard ladder. Image data was viewed on the computer screen and saved in the SAGA computer program. The gel images were scored manually in Excel 2010 program for presence (score 1) or absence (score 0) of bands, and the binary data used for further analysis.

The binary data set was analysed using the DICE Coefficient in the qualitative data program of NTSys-pc version 2.21c software package to produce a similarity matrix. Cluster analysis of the matrix value was analysed by employing the unweighted pair-group method with UPGMA (arithmetic mean) provided in the SAHN program of NTSys-pc and a dendrogram was produced using “Three plot” [13]; [14]. The tested cultivars would be classified as a similar genetic group when the value of estimated coefficient is higher than 0.6 [15].

2.4. Model-based clustering analyses
A STRUCTURE version 2.2, computer analysis program, was used to infer the genetic structure and the number of clusters or populations (K) of all samples. The analysis was run on the whole AFLP dataset, consisting of 6 independent runs performed for each value of population (K value), with the K values varying from one to six. Each run was set to a burn-in period of 10 000 iterations followed by 100 000 Monte Carlo Markov Chain (MCMC) iterations [8]. This process was run to deduce the cluster based on the similarity of the pattern of the dataset and is marked as delta K (ΔK) [16].

The computer software package “Clumpp” version 1.1.1. [17] was used to permute one output from the 10 independent cluster outputs produced by STRUCTURE. Graphs were constructed in Microsoft Excel 2010.

2.5. Analysis of molecular variance (AMOVA)
Molecular variances were analysed using the Arlequin computer program version 3.5 [18]. This programme was used to analyse population genetic data. The software package Transformer 3 was used to convert the format of the AFLP data from the Microsoft Excel file format to the Arlequin file format before running the analysis. One thousand permutations were used in the analysis [19]. Genotypic data were grouped into clusters in order to test the genetic variation within the white maize landraces from the three villages, the yellow maize landraces, the certified improved varieties the uncertified improved variety, and between yellow and white maize landraces.

3. Results and Discussion
The investigation of 100 West Timor maize landrace populations and 27 improved maize varieties through AFLP analysis showed that there were genetic differences among and within groups. The structure and genetic relationships between and among these populations will be discussed based on the cluster analysis.

3.1. DNA Quantity
The quantity of DNA isolated from the samples is the most important factor for successful and reliable AFLP analysis [20]. The quantity of the DNA extract of the West Timor maize landraces and varieties varied, ranging between 45ng/µl and 600ng/µl of the total extract volume of 0.5µl (Table 2). About 25% of the samples had a DNA concentration below 200ng/µl, about 46% of the samples contained DNA between 200ng/µl and 400ng/µl and 29% of the samples yielded more than 400ng/µl DNA in the extract.
volume of 0.5µl. This result confirmed that all of the DNA extractions were suitable for the AFLP procedures because the DNA concentration of all samples was more than 20ng/µl [20].

**Table 2.** The proportion of the genomic DNA quantity of all samples based on the DNA concentration in the extract volume of 0.5µl

| Concentration (ng/µl) | Number of samples | Percentage (%) |
|-----------------------|-------------------|---------------|
| 20 - 40               | 0                 | 0             |
| 40 - 100              | 5                 | 3.9           |
| 100 - 199             | 27                | 21.3          |
| 200 - 299.9           | 37                | 29.2          |
| 300 - 399.9           | 21                | 16.5          |
| 400 – 600             | 37                | 29.1          |
| Total                 | 127               |               |

3.2. Fingerprint and Number of Bands

Eight primer combinations amplified 63,881 fragments in the 127 genotypes of West Timor landrace maize populations and improved maize varieties. The presence and absence of bands of 40 genotypes can be distinguished from their fingerprints (Figure 2).

**Figure 2.** Fingerprint of 40 West Timor maize landrace samples under 255 bp in size assessed using Mse CTC and Pst AGA primers

The fingerprint bands were clearly seen and comparable among the samples. The banding patterns of each genotype were evaluated in the scoring range of 50-700 bps (Figure 2). The absence of a band in the sample’s lane indicated that the DNA in the fragment was missing at the distance between about 50 to 53 bp and between 72 to 83 bp. For instance, WAmolD4, WAmol E3, and YLamuru ori5 had no band at about 50bp while WAmol C5, WAmol D5, WAmol E4, WAmol E5, YKalinggaori 3, YKalinggaori 4 and YLamuruori 2 had two bands and YKalinggaori 5 and YLamuruori 4 had one band
at the same fragment weight. From the fingerprints, the genetic variability of all samples can be analyzed.

![Figure 3](image.png)

**Figure 3.** Fragment pattern over 40–90bp range of West Timor maize landraces and improved maize varieties assessed using Mse CTC and Pst AGA primers. Each lane represents one genotype or sample. The absences of fragments are marked by ellipses.

The total band number of the eight marker combinations in the AFLP analysis of West Timor maize landrace populations and improved maize varieties was large enough to differentiate genotypes among all samples (Table 3). The total number of bands was 503 of which 485 (96%) were polymorphic. Combination markers Mse1-CCA and Pst1-AGC amplified the highest band number (89 bands) followed by combination Mse1-CTC and Pst1-AGC (82 bands). However, combination of Mse1-CCA and Pst1-AGA or of Mse1-AGC and Pst1-AGC amplified 100% polymorphic bands. The number of DNA bands of all samples varied within populations in villages, among populations from the three villages and between maize landrace populations and improved maize varieties.

### 3.3. Distance and Model Based Clustering Analysis

The genetic variation of all samples can be analysed through both distance based clustering analysis, and model based clustering analysis. The distance based clustering analysis results are presented in dendrograms (Figure 6 and 9). The dendrogram of Figure 6.6 indicates that there is a very significant genetic variation among the West Timor maize landraces and varieties analysed, showing similarities of between 55% (0.55 in similarity coefficient) and 97% (0.97 in similarity coefficient). The lower the similarity of the coefficient among maize the greater the genetic differences among them. Zhu [21] have proposed that similarity coefficients of less than 0.8 among populations may indicate geographical isolation/poor gene flow.
Table 3. Total bands and polymorphic bands in the AFLP analysis of 100 West Timor maize landrace population and 27 improved maize varieties

| Primer combination       | Number of bands | Percentage (%) |
|--------------------------|-----------------|----------------|
|                          | Total bands     | Polymorphic bands |
| Mse1-CTC and Pst1-AGA (800) | 50              | 47             | 94          |
| Mse1-CTC and Pst1-AGC (700) | 82              | 77             | 94          |
| Mse1-CCA and Pst1-AGA (800) | 54              | 54             | 100         |
| Mse1-CCA and Pst1-AGC (700) | 89              | 86             | 97          |
| Mse1-AGC and Pst1-AGA (800) | 39              | 37             | 95          |
| Mse1-AGC and Pst1-AGC (700) | 51              | 51             | 100         |
| Mse1-ACG and Pst1-AGA (800) | 68              | 65             | 96          |
| Mse1-ACG and Pst1-AGC (700) | 70              | 68             | 97          |
| Total bands              | 503             | 485            | 96          |

Figure 4. UPGMA clustering dendrogram of similarities among 127 entries of landraces and improved maize growing in West Timor

The dendrogram also shows that the 127 entries were divided into two groups (group A and B). Group A, that had a coefficient of similarity above 0.8, consists of white maize landraces from Ajaobaki (25 entries), Nunmafo (25 entries) and Amol (5 entries), yellow maize landraces from Nunmafo (25 entries),
and all yellow improved maize varieties (27 entries), while group B, with a coefficient of similarity less than 0.8, is made up of 20 white maize landrace entries from Amol.

The two improved maize varieties, Y Lamuru-ori 1 and Y Lamuru-ori 2, were the most genetically similar (97%) whereas the most genetically dissimilar lines were two white maize landrace populations from Amol village (65%), WAmol A4 and WAmol C2. White and yellow maize landrace population from Ajaobaki and Nunmafo village were between 85% and 95% similar. In the model based clustering analysis of the AFLP dataset, the log-likelihood values divided the 100 West Timor maize landrace populations and 27 maize varieties into two clusters, when the highest values of probability cluster or number of populations (ΔK) [16] was examined (Figure 5). This result is in agreement with the distance based clustering analysis. The 127 entries were distributed into cluster A which consisted of 108 entries and cluster B which has 19 entries (Figure 8).

![Population of All Samples](image.png)

**Figure 5.** Structure analysis for determining the number of population groups; most likely number of cluster (K) for all samples according to values of ΔK calculated for each K

Both distance and model based clustering analysis show that all samples could be divided into two groups. Based on this the 100 West Timor maize landrace populations and 27 maize varieties were grouped in cluster A consisting of 108 entries composed of a mix of landraces and varieties. Nineteen other entries were grouped in cluster B containing only maize landraces from Amol village.

In the A group, most samples could be assigned to a common West Timor maize landrace population as they shared more than 80% common ancestry (Figure 8), while the minority which shared less than 80% similarity were considered as introgression from other areas or countries that originated from a different source, like East Timor. The results confirm that there was a weak relationship between maize landrace populations from Amol and landrace populations from Ajaobaki and Nunmafo villages and maize varieties. However, the big genetic variation in group B (white Amol maize landraces) probably also has advantage for future breeding programs to produce a new variety.
Figure 6. DNA structure of 100 West Timor maize landrace and 27 improved maize varieties. Each individual is represented by a horizontal bar while colours are representative of the inferred populations.

As the Amol village samples were very different from all other samples, they were removed and the model-based cluster analysis was re-run to examine the effect of these “outlier” samples on the analysis. These results are presented in Figure 9. The results indicate that there were three groups (Figure 9) based on the relationship between samples, provided the threshold was raised from 0.8 to 0.85 similarity index. Group A was mainly comprised of white maize landrace populations from Nunmafo and Ajaobaki village, group B consisted of all yellow lines plus some white lines and group C was primarily made up of improved maize varieties. The similarity index between individual genotypes within the three clusters was quite variable. Clusters A and B each ranged from 85% to 94%, while cluster C varied between 87% and 97%. However, some entries did not sort as expected. For instance, Piet kuning and Lamuru F5 variety did not go to the varieties group (group C), these lines have closer similarities to the white maize landrace populations (group A). Similar situations occurred with some white maize landrace population in the predominantly yellow group (Group B) and some yellow maize landrace populations in the varieties group (group C).
Figure 7. UPGMA clustering analysis dendrogram of 102 entries (75 entries maize landraces and 27 varieties after taking out from the database the 25 maize landraces from Amol village)

The close genetic similarities of the Piet kuning variety to the maize landrace populations showed that this variety has a similar ancestry to the maize landrace populations. This is possible because Piet kuning variety is a local variety which was derived from West Timor maize landrace populations using intra population selection method and released in 2005 [22]; [23]. All Lamuru F5 accessions, which have been grown by farmers for five years, also showed strong similarity to landrace maize from Nunmafo and Ajaobaki. Accordingly, cross pollination between the Lamuru variety and maize landrace populations is likely to have occurred in this environment. Hence, Lamuru F5 would have mixed genetic composition between the original Lamuru and local maize landraces as can be seen at Figure 11.

In the model based clustering analysis of the AFLP data set (after subtracting 25 landrace maize entries from Amol), log-likelihood values divided the 75 West Timor maize landrace populations and 27 maize varieties into three clusters, as the highest values of ΔK was examined (Figure 10). Then, the three independent cluster output was permutated by the clumpp program and the result was used to produce a population structure graph (Figure 8). The graph shows that all samples were grouped into three clusters.
Figure 8. Structure analysis for determining the most likely number of population clusters (K) for samples without Amol Village samples according to values of ΔK calculated for each K.

Figure 9. DNA structure of 75 West Timor maize landrace and 27 improved maize varieties (after taking out 25 samples of Amol village entries from the dataset). Each individual is represented by a horizontal bar and shading represents inferred population.
The population structure graph, Figure 9, reveals that some samples such as YBisma-ori 1, WAjaobaki A1 and WAjaobaki D5 were assigned into a single group, whereas others were of mixed origin, i.e. representative of more than one group, e.g. YNunmafo E1 and YPiet kuning.

The population structure analysis result again confirmed the distance cluster analysis that members of each cluster included both grain colours, different sampling sites, landraces and varieties. Cluster A constituted white maize landrace populations from Nunmafo and Ajaobaki village and also Piet kuning and Lamuru F5. Likewise, cluster B consisted of white Ajaobaki maize landrace populations and yellow Nunmafo maize landrace populations, while cluster C was made up of improved maize varieties and yellow Nunmafo maize landrace populations. These results indicate that West Timor maize landrace populations have considerable genetic variation within and between locations.

The dendrogram of Figure 9 and DNA structure analysis (Figure 11) also reveal that yellow Lamuru F5, the uncertified Lamuru samples, originating from certified Lamuru harvested and replanted for five seasons, fall in group A together with yellow Piet kuning and white landraces from Nunmafo and Ajaobaki. It suggests that the yellow Lamuru F5 has genetically diverged from the certified original Lamuru (group C), due to cross-pollination with neighboring landraces in the village where it was grown. Mislabeling may also have occurred, although in this case the Lamuru F5 would be expected to cluster with yellow-seeded landraces from Nunmafo.

3.4 Analysis of Molecular Variance (AMOVA)

Classical genetic-variance-based methods (AMOVA) and differentiation statistics revealed that the genetic diversity of West Timor maize landrace population was structured at the three spatial scales studied: between white and yellow maize populations, among sampling locations, and between landrace populations and varieties.

AMOVA (Table 4) revealed significant difference between white and yellow maize, among sampling sites, between maize landrace population and improved maize varieties, and within each of the groups. White maize populations were significantly different from yellow maize populations, contributing 7% (P < 0.01) of the total genetic variation while the variation within the group was higher, contributing 93% (P < 0.01).

Table 4. Analysis of molecular variances (AMOVA) of West Timor maize landrace population and some varieties

| Source of variation                              | Degree of freedom | Sum of squares | Variance components | Percentage of variation (%) |
|--------------------------------------------------|-------------------|---------------|---------------------|-----------------------------|
| Among groups (White and Yellow)                  | 1                 | 164.5         | 2.2                 | 7.0**                       |
| Within group                                     | 125               | 3669.8        | 29.4                | 93.0**                      |
| Among sampling locations (Ajaobaki, Amol and Nunmafo villages) | 2                 | 514.8         | 9.5                 | 26.6**                      |
| Within group                                     | 72                | 1876.5        | 26.1                | 73.4**                      |
| Among groups (Yellow landraces and Yellow varieties) | 1                 | 120.0         | 4.9                 | 19.6**                      |
| Within group                                     | 40                | 809.1         | 20.2                | 80.4**                      |

Note: ** Significant at P < 0.01

A significant difference was also observed among the three sampling sites, contributing 26.6% (P < 0.01) of the total genetic variation, which was less than the variation within the group, which contributed
73.4% (P < 0.01). The analysis also gave the same result with the comparison between yellow maize landrace population and yellow improved maize varieties, where the variation between the groups was 19.6% (P < 0.01) and within group was 80.4% (P < 0.01).

The big differences observed between maize landrace populations from Amol village and the two other white maize landrace populations from Ajaobaki and Nunmafo village may be due to the accessibility of the village. Amol village is situated in remote area of West Timor and has a poor transportation system, including bad roads and irregular public transportation, from and to the capital city of East Nusa Tenggara Province (Kupang). In comparison, Ajaobaki and Nunmafo village are situated in the area of West Timor closer to Kupang. Moreover, Amol village is very close to the border between Indonesia and East Timor (Figure 6.1) where maize seed exchange across borders could easily occur between farmers from West Timor and East Timor this seed may introduce different maize genetic types. [24] has indicated that maize came to several sites of Timor Island, some were brought by the Dutch and others by the Portuguese. In other words, the differences observed among maize populations may be due to isolation by distance of different founder populations [25]). However this needs further investigation.

4. Conclusions
The results of this study for AFLP analysis of West Timor maize landrace populations from three villages and varieties have confirmed that there are extensive genetic variations within West Timor yellow and white maize landrace populations and varieties. There were two clusters of genetic variation among all samples. The first cluster was a general West Timor maize landrace population, with more than 80% similarity among individuals, and the second cluster was much more diverse grouping with less than 80% similarity. It suggests that even though a high molecular diversity had been found among West Timor maize landrace populations most maize genotypes belong to a West Timor maize landrace cluster. In addition the analysis indicated not only significant difference between maize landraces and maize varieties but also maize landraces from the three villages were genetically significantly different from each other.

The molecular marker characterization of West Timor maize landrace populations will be a key step for obtaining an improved understanding of the population so they can be managed carefully for the future. Moreover, due to the genetic diversity captured within local landraces, farmers have the opportunity to perform the phenotypic selections based on plant type and seed yield which are likely to give them significant genetic gain in yield in subsequent seasons. Plant breeders have the additional opportunity of applying molecular marker techniques to assist selection.

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