Biosaintifika 9 (3) (2017) 444-450

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Genetic Variability of Local Corn Cultivars from Kisar Island Southwest Maluku Regency using Microsatellite Molecular Marker

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DOI: 10.15294/biosaintifika.v9i3.8512

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History Article

Received 19 January 2017
Approved 21 September 2017
Published 31 December 2017

Keywords

Genetic variability; Micro-Satellite markers; Local corn cultivars

Abstract

This research aim was to reveal the genetic variability of local corn cultivars from Kisar Island using microsatellite molecular marker. The use of microsatellite markers is based on one reasons that the genetic information is polymorphic, and will be better to identify genetic diversity of plants including corn. Corn cultivars used were obtained from farmer in Kisar Island, and three reference varieties as outgroup were obtained from Institute of Cereals in Maros South Sulawesi. Polymerase chain reaction was conducted following the protocols from Fast Start kit (Qiagen-USA). The data obtained then analyzed with MVSP 3.1A Software for dendogram construction. The results showed that the appearance of DNA bands was vary (monomorphic and polymorphic) for local cultivars as well as reference varieties, with one or two bands on each primer. Based on the dendogram, there are three main clusters with similarity index ranged from 20% -100%. It can be concluded that there is very low similarity and distant kinship of local cultivars. The novelty of this research is knowing kinship relationship between local corn in Kisar Island which is not known yet. This results is expected to provide benefits regarding the breeding program, and for instance, it can be an important information regarding the development of local corn as a source in assembly the superior corn cultivars.

How to Cite

Sinay, H., & Karuwal, R. L. (2017). Genetic Variability of Local Corn Cultivars from Kisar Island Southwest Maluku Regency Using Microsatellite Molecular Marker. *Biosaintifika: Journal of Biology & Biology Education*, 9(3), 444-450.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Corn is a plant species that has a very large genetic diversity, widely distributed, and one of perspective species in world plants utilization (Wijayanto, 2007; Saputro *et al.,* 2016). This high genetic diversity was caused by the natural traits of corn as cross-pollinated plants, can lead variation in genetic composition of one varieties with another (Sudjana *et al.,* 1991). According to Iriany *et al.* (2008) nowadays, corn varieties is estimated about 50.000 varieties. This including local varieties, as well as breeding of improved varieties.

In Indonesia, the genetic diversity of corn is the highest in Asia (Pabendon, 2010), this is due to in many area in Indonesia, there are many local corn cultivars with different characteristics. Local cultivar is formed through a process of isolation of genotypes that are changing and adaptation to specific agroclimate (Iriany *et al.,* 2008) such as the diversity of geographical conditions, and islands, as well as bulk fluctuate, causing the diversity of climatic and soil conditions from one region to another (Wijayanto, 2007).

Southwest Maluku Regency, is one area in Maluku which has high diversity of corn germplasm (Pesireron, *et al.,* 2013a). In this area also, corn be the main commodity that already been cultivated since many years ago in any farming activities and in all growing season (Pesireron, *et al.,* 2013a). Formerely, exploration and documentation of corn germplasm has been done by Alfons *et al.* (2003), and found that in Kisar Island Southwest Maluku, there are seven local corn cultivars specific to this island. These cultivars consist of: *Merah Delima Tongkol Cokelat* (MDTC), *Merah Delima Tongkol Putih* (MDTP), *Merah Darah* (MD), *Putih* (PTH), *Pulut* (PLT), *Kuning Genjah* (KG) and *Kuning Dalam* (KD).

A very striking nature of those local cultivars is the diversity of seed color. The presence of local corn cultivars with specific characteristic and high ability in adaptation to specific environmental condition is the main genetic resources that must be conserved to maintain the specific traits that can be used in the construction of superior varieties. According to Juhriah *et al.* (2011) germ plasm is the source of gene that can be used to increase plant diversity. This is usefull in improving some traits of population, and to make corn varieties. Previously, Pabendon *et al.* (2003) stated that germ plasm and the relationship between breeding material is very important to know and understand regarding to the planning program for producing better hybrid. In addition, the high genetic diversity of local corn can also be used to reduce subjection of corn hybrids derived from multinational companies which cause minor attention to the local cultivars or landraces (Pabendon, 2010).

Research on genetic diversity can be done by utilizing a system of genetic markers for both morphological, and molecular markers (Farooq & Azam, 2002; Bhat *et al.,* 2010). Molecular markers is an effective technique in genetic analysis and has been widely applied in the breeding program especially in plants. According to Farooq & Azam (2002) some genetic marker-based on polymerase chain reaction (PCR) that has been widely used is randomly amplified polymorphic DNA (RAPD), Sequence Characterized amplified regions (Scars), sequence tagged sites (STS), single strand conformational polymorphism (SSCP), single nucleotide polymorphism (SNPs) and microsatellite or short tandem repeats/short sequence repeats (STRs/SSRs). Some of these genetic markers are relatively simple, easy to use, can be automatically tabulated, and sometimes codominant (Farooq & Azam, 2002; Bhat *et al.,* 2010; Fibriana & Hadiyanti, 2016). One of the genetic markers have also been developed extensively is microsatellites markers. The advantages of microsatellite markers is easy to implement, and the degree of information about the number of alleles per locus is very high. Microsatellite markers can be used to detect genetic differences between species in many eukaryotic organisms. The use of microsatellite markers to analyze the genetic diversity of corn have been also reported (Smith *et al.,* 1997; Senior *et al.,* 1998; Gethie *et al.,* 2002; Lie et al ,. 2004; Pabendon *et al.,* 2006; Pabendon *et al.,* 2008; Zhang *et al.,* 2012).

Research on the genetic diversity of corn in Indonesia using microsatellite markers have been reported by Pabendon, *et al.* (2003), and Pabendon (2006), or a combination of morphological markers and microsatellite (Pabendon, *et al.,* 2008, and Fate, *et al.,* 2009). Especially for local corn on Kisar Island Southwest Maluku Regency, there is no research had never done to determine the genetic diversity of these cultivars. The purpose of this study was to determine the genetic diversity of local corn cultivars in Kisar Island SouthWest Maluku Regency based on molecular characterization using microsatellite molecular markers.

The novelty value of this research is knowing kinship relationship between local corn cultivars in Kisar Island of Southwest Maluku Regency which is not known yet. The results of this study are expected to provide benefits to the

breeding of local corn cultivars and the protection and conservation of corn germplasm in South west Maluku Regency.

METHODS

Research Materials

Local corn cultivars derived from farmer in Kisar Island namely: *Merah Delima Tongkol Cokelat*, *Merah Delima Tongkol Putih*, *Pulut*, *Kuning Genjah*, *Kuning Dalam*, *Putih*, and *Merah Darah*. Hybrid variety as reference and used as outgroup obtained from Research Institute of Cereals in Maros-South Sulawesi namely: *Srikandi*, *Lamuru*, and *Anoman*.

DNA Extraction

Leaves of corn used as sample were taken from the second leaf from the tip at 10 days after planting. DNA isolation was done following the protocol of ilustra phytopure kit. As much as 0.1 g of freshleaf was taken, and gently crushed with a mortal and pestle. Furthermore, 500 mL of ilustra phytopure I kit reagent were added, and placed into eppendorf tubes. Then 150 mL reagent of phytopure II were added and shaken gently and incubated at 65ºC for 10 minutes in waterbath. Subsequently the samples were placed in ice for 20 minutes, added with 400 mL of cold chloroform and 20 mL of resin phytopure into the sample carefully and centrifuged at 3000 rpm for 10 minutes to obtain a supernatant.

The supernatant was taken and placed in another tube. After that, cold isopropanol with the same volume as the supernatant was added through the tube, and centrifuged at 10.000 rpm for 10 minutes to obtain a DNA pellet. The supernatant was discarded and the tubes containing pellets dried on tissue then added 100 mL of 70% ethanol and again centrifuged at 10.000 rpm for 5 minutes.

DNA Amplification and Electrophoresis

DNA obtained was used for PCR analysis using 10 types of primer SSR (phi 026, phi 046, phi 047, phi 058, phi 068, phi 069, phi 074, phi 079, phi 116, and phi 120) (Smith *et al.,* 1997; Senior *et al.,* 1998; Qi-Lun *et al.,* 2008), using Fastart PCR kit. DNA that has amplificated from PCR reaction, then ran in gel agarose electrophoresis, and documented with gel doc to see the appearance of DNA banding pattern.

Data Analysis

Molecular data such as DNA band was scored with the value of one (1) for the band presence and zero (0) for unpresence of DNA band. The data then processed to construct the dendogram using MVSP 3.1a Software.

RESULT AND DISCUSSION

The result showed that performance of the DNA bands varies (monomorphic and polymorphic) both on local corn cultivars (Figure 1-7) as well as reference varieties *Lamuru*, *Anoman*, and *Srikandi* (Figure 8-10).

Total amount of DNA varies between one to two bands on each primer. For *Pulut* cultivar (Figure 3) there were only one DNA bands in 9 primers while in the phi 068 primer, the appearances DNA banding pattern not so obvious. For the *Kuning Genjah* cultivar (Figure 4) there were polymorphic DNA bands with 1-2 bands for all primers. In *Kuning Dalam* cultivar (Figure 5) there

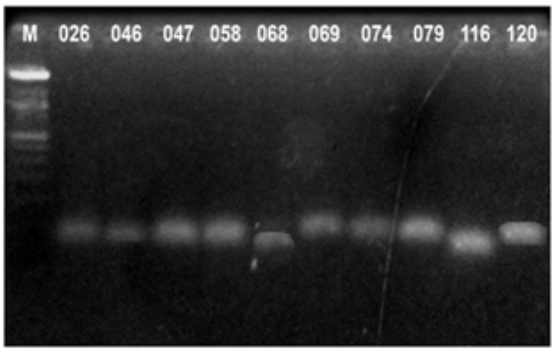

Figure 1. Amplification result of *Merah Delima Tongkol Cokelat* (MDTC) Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

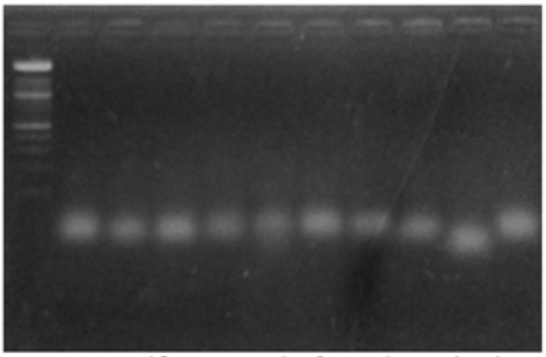

Figure 2. Amplification result of *Merah Darah* Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

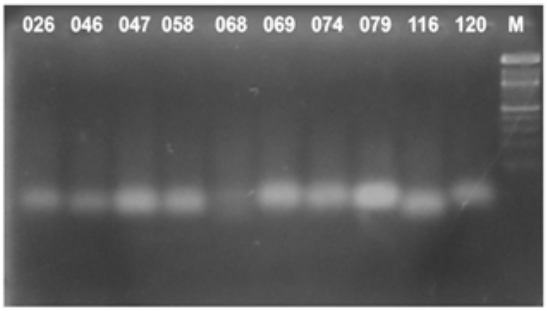

Figure 3. Amplification result of *Pulut Cultivar* with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

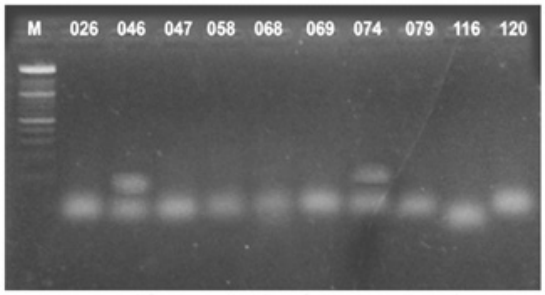

Figure 4. Amplification result of *Kuning Genjah* Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

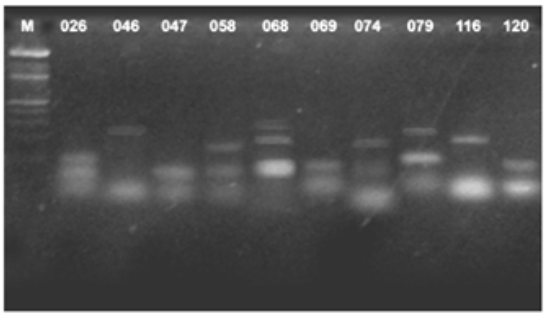

Figure 5. Amplification result of *Kuning Dalam* Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

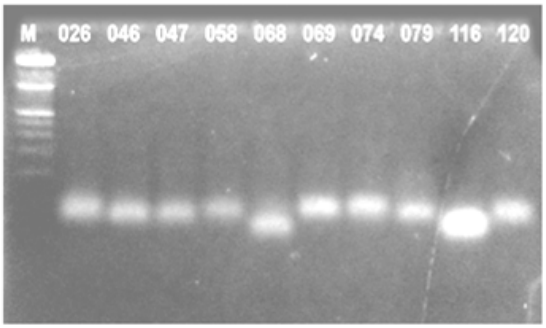

Figure 6. Amplification result of *Putih* Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

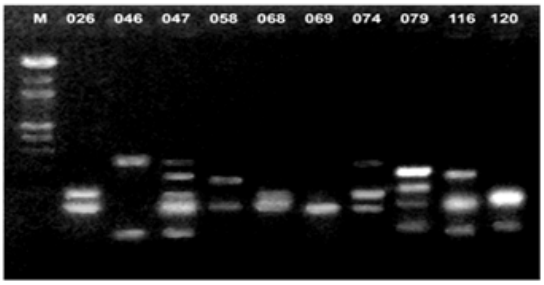

Figure 7. Amplification result of *Merah Delima Tongkol* Putih Cultivar with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

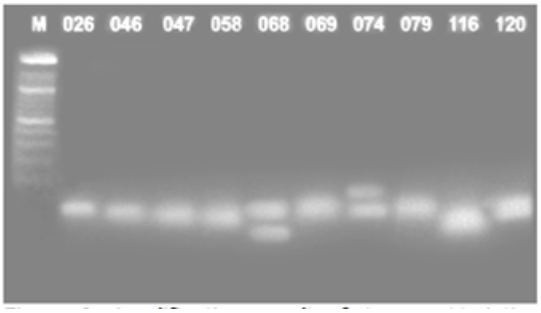

Figure 8. Amplification result of *Lamuru* Varieties with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

were 2-3 polymorphic DNA bands on all 10 primers. For the *Merah Delima Tongkol Cokelat* cultivars. There were 1-5 polymorphic DNA bands in the all 10 primers. For the outgroups or reference varieties (*Lamuru*, *Anoman*, and *Srikandi*) (Figure 8-10) there are 1-2 polymorphic DNA bands for all 10 primers.

DNA fragment on the phi026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers then used for scoring the DNA bands. Results of scoring with a total of 50 bands as the next character were used to make dendogram construction using MVSP 3.1a software (Figure 11).

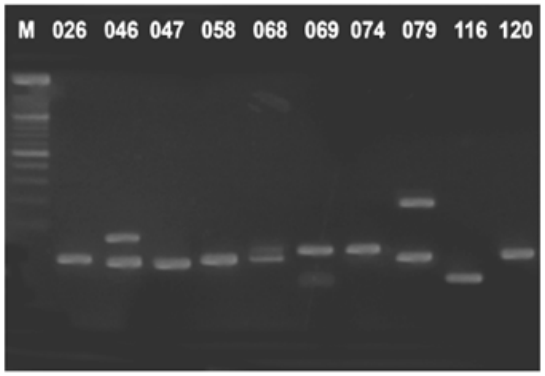

Figure 9. Amplification result of *Anoman* Varieties with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

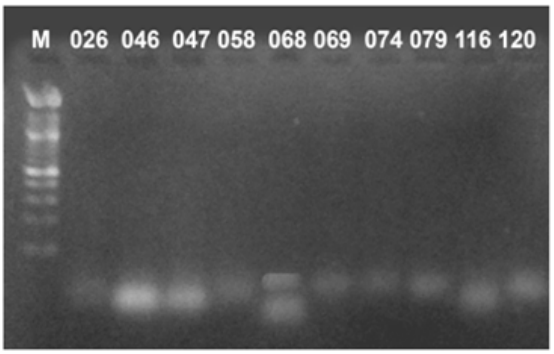

Figure 10. Amplification result of *Srikandi* Varieties with 026, 046, 047, 058, 068, 069, 074, 079, 116, and 120 Primers on the 1.5% of agarose gel electrophoresis. M. 1kb of DNA Marker

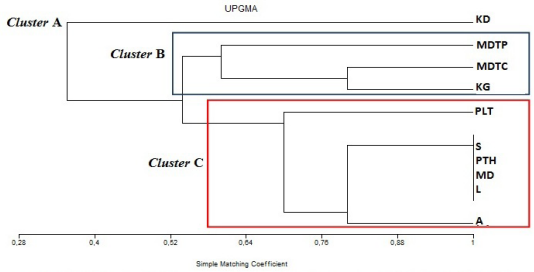

Figure 11. Dendogram of local corn cultivars and reference varieties based on UPGMA algorithme with *Simple Matching Coefficient Method*

Based on the molecular characterization, the local corn cultivars and reference varieties were devide into three main clusters (cluster A, B, and C). Cluster A consists of only one cultivar that was *Kuning Dalam* (KD) cultivar. Cluster B consists of three local cultivars *Merah Delima Tongkol Putih* (MDTP), *Merah Delima Tongkol Cokelat* (MDTC), and *Kuning Genjah* (KG), while the cluster C consists of local cultivars *Pulut* (PLT), and *Merah Darah* (MD), and also reference varieties *Srikandi* (S), *Lamuru* (L), and *Anoman* (A).

According to Stuessy (1990), kinship is defined as a pattern of relationships or total similarity between groups of plants based on their nature or specific character of each group of plants. The distance of the kinship is affected by the similarities and differences between the characteristics of the local corn cultivars indicated by the similarity index. The value of the similarity between corn cultivars tested, ranged between 20% -100%. The high index shows that the similarity between corn plants tend to high, otherwise when the similarity index is small, the similarity is tend to small. The fenetic approach, if the similarity index reaches a 85%, the similarity between the operational taxonomical unit (OTU) is considered as a species, 65% for genus, and 45% for family.

Merah Delima Tongkol Putih, *Merah Delima Tongkol Cokelat*, and *Kuning Genjah* cultivars were clustered into same cluster (Cluster B) which means that these cultivars have high similarity index (70-80%). The value of the similarity between *Merah Delima Tongkol Cokelat*, and *Kuning Genjah* cultivars as much as 80%, *Merah Delima Tongkol Putih* and *Merah Delima Tongkol Cokelat* as much as 70%, while between *Merah Delima Tongkol Putih* and *Kuning Genjah* only about 50%. This result showed that in cluster B even though these cultivars are in same cluster, but *Merah Delima Tongkol Putih* cultivars were separated from *Merah Delima Tongkol Cokelat*, and *Kuning Genjah* cultivars.

Srikandi, *Lamuru*, and *Anoman* varieties as a reference (outgroup) looks to be on the same cluster (cluster C). However, in this cluster it involves the local cultivar *Merah Darah* (MD), and *Putih*. The similarity value of C cluster reaches 100%, indicating that reference varieties and local cultivars *Merah Darah* and *Putih* had a very high similarity. In cluster C also showed that the *Pulut* cultivar and *Anoman* varieties were separate from the *Srikandi*, *Lamuru* and local cultivars *Putih*, and *Merah Darah*.

The similarity value among *Anoman, Srikandi*, *Putih*, *Merah Darah*, and *Lamuru* as much as 80%, while the similarity value between Anoman varieties with *Pulut* cultivars as much as 70%. This result indicated that the *Anoman* varieties was closely related to *Srikandi* varieties, and *Lamuru*, also the local cultivars *Putih*, and *Merah Darah*, or in other words *Srikandi* varieties, and *Lamuru*, as well as the local cultivar *Putih*, and *Merah Darah* is more similar to *Anoman* varieties than the *Pulut* cultivar.

Based on the dendogram, it can be seen

that *Kuning Dalam* (KD) cultivar were separate from other cultivars. This mean was that *Kuning Dalam* cultivar had a low level of similarity index with other cultivars. The similarity index of *Kuning Dalam* cultivar with other cultivars range between 20% -50%. The similarity index below 65% indicated that there are a very long or very different genetic variation. Overall, based on the fenetic analysis with SSR markers it can be known that most cultivars have similarities below 85%. This suggests that the similarity between local corn cultivars with one another is not too high. These results are quite provide an overview and information on the phylogenetic relationship between local corn cultivars in Kisar Island South west Maluku. It is alleged that local cultivars have close genetic relationship because it is at the same location. But apparently based on the results of the analysis with the molecular characterization of microsatellite markers, it is known that the local corn cultivars has kinship distant and very quite different. These results indicate that although these cultivars were grown in the same environment, but they are not similar in their genetic material. This is consistent with the statement of Jose *et al.,* (2005) that the genotype in the same area do not always shows similarity in their genetic arrange. This is allegedly caused by the genotype has a different arrangement of genetic material, resulting in the appearance of different phenotypes, even if they were grown in the same environment.

It is known that the molecular characterization of an investigation to complement and reinforce the allegations obtained from the morphological characterization. Thus, the results of morphological characterization show much variety and at least a great similarity between local corn cultivars, strengthened by the results of molecular data were indicate local corn cultivars in Kisar Island SouthwestMaluku were genetically different, resulting in the appearance of phenotypically different, despite being on a the same neighborhood.

Kinship distant of local corn cultivars in Kisar Island means that there is a high genetic variation and this is a very important information regarding to the breeding program. Corn cultivars can be used as a source of genes for corn germplasm conservation in the future. According to Sukartini (2007), accessions ties distant resemblance is good for plant breeding activities. Instead accessions has very close resemblance relationship is not good for plant breeding activities for at least the possibility of genetic variation within the species.

Nevertheless, all of cultivars with distant kinship, the *Kuning Dalam* cultivar seems to be more difference than the other local cultivars. Morphologically, these cultivars have very little in common with other local cultivars. For example, in the female flower characters. Other six local cultivars shows purple color when appears, except for *Kuning Dalam*, it has pink color of female flower when appear. The differences of *Kuning Dalam* cultivars with other six local cultivars also shown in fruit characters. All of six cultivars have a large category of cob diameter with small category, while the *Kuning Dalam* cultivar has a very large cob diameter with the diameter and medium category. These results then confirmed to the result of molecular characterization that placed this Cultivar in to one cluster alone with a very low similarity value, which means that this cultivar has a far kinship relationship with other local cultivars.

The results of Sinay & Karuwal (2013) studies based on the trials of drought resistance at the early vegetative phase of six local corn cultivars from Kisar Island Southwest Maluku Regency under greenhouse condition, also found that the *Kuning Dalam* cultivar had higher proline and total soluble sugars content than the other local cultivars, as well as reference varieties *Srikandi*, *Lamuru* and *Anoman*.

Another result also reported by Sinay *et al.,* (2015) about the proline content and yield of local corn cultivars on natural conditions in Kisar Island, which indicates that the *Kuning Dalam* cultivar has the highest proline content (in line with the results of greenhouse experiments, according to Sinay & Karuwal, 2014), as well as high yields such as the number of seeds per cob, the number of rows of seeds, the cob weight, the cob length, and the cob diameter.

Based on these observation result in the field or in their natural habitat in Kisar Island, shows that *Kuning Dalam* cultivar is the most widely grown and consumed by the people. Also the result of an experiment under greenhouse condition, and under natural condition, give an information that *Kuning Dalam* has a better or higher response than other local cultivars. Therefore this cultivar can be recommended as a source of germplasm that is urgently needed in its preservation and development as a superior corn cultivar in the future, although other aspects must continue to be made regarding its potential development as a candidate for superior corn cultivars, mainly through multilocation testing.

The study concluded that local corn cultivars of Kisar Island Southwest Maluku Regency

have low similarity and distant kinship. The novelty value of this research is knowing kinship relationship between local corn cultivars in Kisar Island of Southwest Maluku Regency which is not known yet. The results of this study are expected to provide benefits regarding the breeding program of local corn cultivars and for instance, this result can be an important information regarding the development of local corn cultivars as a source in assembly the superior corn cultivars.

CONCLUSION

Based on these findings, it was concluded that the results of the molecular characterization using microsatellite markers grouping local corn cultivars from Kisar Island into three main groups with similarity index ranged from 20%-100%. Molecular characterization results indicate that low similarity or distant kinship between local corn cultivars in Kisar Island Southwest Maluku Regency.

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