GENETIC VARIABILITY STUDIES ON AVOCADO (*Persea americana* L.) USING INTER-SIMPLE SEQUENCES REPEAT (ISSR) ANALYSIS

KARTIKA RESTU SUSILO

A24062965

DEPARTMENT OF AGRONOMY AND HORTICULTURE

FACULTY OF AGRICULTURE

BOGOR AGRICULTURAL UNIVERSITY

2010
ABSTRACT

KARTIKA RESTU SUSILO. Genetic Variability Studies on Avocado (Persea americana Mill) Using Inter-Simple Sequences Repeat (ISSR) Analysis. Supervised by SOBIR.

Avocado (Persea americana Mill) is an evergreen tree native to Mesoamerica. Open pollination reproduction mode and mutations are the main source of the high genetic diversity in avocado. Differences in climate and soil conditions in Indonesia by region of origin also makes the avocado has a high diversity.

Evaluation of avocado characterization is usually based on morphological descriptions of plants. However, this method requires a long time because the avocado plant is an annual plant. Therefore, other ways to characterize the avocado is needed. Inter-simple sequence repeat (ISSR) markers were used to study genetic diversity relationships among 23 avocado accessions.

Morphological traits were used to assess levels of polymorphism across 23 accessions. Thirty-four morphological traits were scored and were scattered into 21 different loci. The twenty-third accessions grouped into three groups at 0.44 similarity degree.

A total of 37 different amplification fragments were detected ranging from 2 to 7 per primer with an average of 5.2 fragments per primer. A dendrogram was generated using UPGMA (Unweighted Pair Group Method with Arithmetic Averages). This dendrogram classified most of the genotypes analyzed into three major groups.

The UPGMA cluster constructed from combination data of morphological traits and ISSR marker analysis separated the 23 accessions into three major groups at similarity value of 0.51. The most different accession is Lam1. The rest of the accessions in the dendrogram could be divided into two main clusters with accessions of different origin intermixed. The correlation coefficient between the morphological and ISSR matrices was 0.60076. It means that the matrices obtained from the molecular genomic markers and the morphological trait had significant positive correlations.
GENETIC VARIABILITY STUDIES ON AVOCADO (*Persea americana* L.) USING INTER-SIMPLE SEQUENCES REPEAT (ISSR) ANALYSIS

The Thesis submitted to Faculty of Agriculture in partial fulfillment of the requirements for the degree of Bachelor of Agricultural Science

KARTIKA RESTU SUSILO
A24062965

DEPARTMENT OF AGRONOMY AND HORTICULTURE
FACULTY OF AGRICULTURE
BOGOR AGRICULTURAL UNIVERSITY
2010
TITLE : GENETIC VARIABILITY STUDIES ON AVOCADO (*Persea americana* L.) USING INTER-SIMPLE SEQUENCES REPEAT (ISSR) ANALYSIS

NAME : KARTIKA RESTU SUSILO

NIM : A24062965

Supervisor

Dr. Ir. Sobir, MSi
NIP. 19640512 198903 1 002

Head of Department of Agronomy and Horticulture

Dr. Ir. Agus Purwito, MSc. Agr
NIP. 19611101 198703 1 003

Date of Graduate:
BIOGRAPHY

The author was born in Jakarta, on April, 30th 1989 as the oldest of three children of Mr. Djoko Susilo and Mrs. Lali Endang Suciwati. The author graduated from SDN Pedurenan Timur II in 2000 and finished junior high school at SLTPN 1 Bekasi in 2003. The author completed senior high school at SMAN 1 Bekasi in 2006, and in the same year enrolled at Bogor Agriculture University through USMI (Undangan Seleksi Masuk IPB) program and was accepted on Department of Agronomi and Horticulture, Faculty of Agriculture.

During her studied in IPB, the author active in student organizations. The author joined as general treasured of Himagron (Himpunan Mahasiswa Agronomi) in 2008-2009. The author had joined as fund rising staff of FKRD (Forum Keluarga Rohis Departemen) of Faculty of Agriculture since 2008.
PREFACE

All praise and great thankful to Allah SWT who gives the author a chance to finished this thesis by keeping the author in good health and conscience all the way through the completion of this thesis. This thesis with title “Genetic Variability Studies on Avocado (Persea americana L.) Using Inter-Simple Sequences Repeat (ISSR) Analysis” was completed as one of the requirements to achieve the bachelor degree of agricultural science in Agronomy and Horticulture Department of Bogor Agricultural University. This researched was funded by Center of Tropical Fruit Studies, Bogor Agricultural University.

The author is grateful to Dr. Ir. Sobir, MSi. for the advise during the researched and thesis writing process. Thanks also expressed to Dr. Ir. Darda Effendi, MSi. and Dr. Ir. Adiwirman, MS. for willingness as examiners and all of the lectures for guidelines during study at IPB. The author wishes to thanks Molecular Laboratory, Center of Tropical Fruit Studies staff for providing laboratory facilities and to Ms. Sulassih and all of the staffs for technical supporting and helpful suggestions. The special deeply thanks to beloved ‘ibu n bapak’ and all of the family for the supports and prays all this time. The author also thankful to AGH 43’ers, Himagron’ers, Jasmin’ers, and anyone who gave support and help during finishing the author’s study.

Finally, the author wishes this thesis would be useful in the future.

Bogor, November 2010

Author
# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLE</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURE</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Background</td>
<td>1</td>
</tr>
<tr>
<td>Objective</td>
<td>2</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Origin and Botany</td>
<td>3</td>
</tr>
<tr>
<td>Genetic Variability</td>
<td>4</td>
</tr>
<tr>
<td>Morphological Analysis</td>
<td>6</td>
</tr>
<tr>
<td>Molecular Markers</td>
<td>6</td>
</tr>
<tr>
<td>Polymerase Chain Reaction (PCR)</td>
<td>7</td>
</tr>
<tr>
<td>Inter-Simple Sequence Repeat (ISSR)</td>
<td>8</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>Place and Time</td>
<td>9</td>
</tr>
<tr>
<td>Materials and Tools</td>
<td>9</td>
</tr>
<tr>
<td>Methods</td>
<td>10</td>
</tr>
<tr>
<td>RESULT AND DISCUSSION</td>
<td>17</td>
</tr>
<tr>
<td>General Description</td>
<td>17</td>
</tr>
<tr>
<td>Morphological Character Analysis</td>
<td>17</td>
</tr>
<tr>
<td>ISSR Analysis</td>
<td>20</td>
</tr>
<tr>
<td>Integrated Data Analysis</td>
<td>23</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>26</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>27</td>
</tr>
<tr>
<td>APPENDIXES</td>
<td>29</td>
</tr>
</tbody>
</table>
LIST OF TABLE

<table>
<thead>
<tr>
<th>No.</th>
<th>Text</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Comparison of Selected Characteristic of Three Horticultural Races of Avocado</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Accessions Used of Avocado</td>
<td>9</td>
</tr>
<tr>
<td>3.</td>
<td>ISSR Primer Used in This Study</td>
<td>16</td>
</tr>
<tr>
<td>4.</td>
<td>Grouping of 23 Avocado Accessions Based on Morphological Characters</td>
<td>19</td>
</tr>
<tr>
<td>5.</td>
<td>Level of Polymorphism Obtained with Seven ISSR Markers in 23 Avocado Accessions</td>
<td>20</td>
</tr>
<tr>
<td>6.</td>
<td>Grouping of 23 Avocado Accessions Based on ISSR Analysis</td>
<td>22</td>
</tr>
<tr>
<td>7.</td>
<td>Eigen Value for ISSR Analysis</td>
<td>23</td>
</tr>
</tbody>
</table>
# LIST OF FIGURE

<table>
<thead>
<tr>
<th>No.</th>
<th>Text</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fruit shape</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Ridges on fruit</td>
<td>11</td>
</tr>
<tr>
<td>3.</td>
<td>Seed shape</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Leaf shape</td>
<td>14</td>
</tr>
<tr>
<td>5.</td>
<td>Leaf base shape</td>
<td>14</td>
</tr>
<tr>
<td>6.</td>
<td>Leaf apex shape</td>
<td>14</td>
</tr>
<tr>
<td>7.</td>
<td>UPGMA Dendogram of 23 Avocado Accessions Based on Morphological Characters</td>
<td>18</td>
</tr>
<tr>
<td>8.</td>
<td>Example of Avocado Fruit</td>
<td>18</td>
</tr>
<tr>
<td>9.</td>
<td>Example of Banding Pattern Profiles Revealed by Primer PKBT 3</td>
<td>21</td>
</tr>
<tr>
<td>10.</td>
<td>UPGMA Dendogram of 23 Avocado Accessions Based on ISSR Analysis</td>
<td>21</td>
</tr>
<tr>
<td>11.</td>
<td>UPGMA Dendogram of 23 Avocado Accessions Based on Morphology and ISSR Analysis</td>
<td>23</td>
</tr>
</tbody>
</table>

## Appendixes

<table>
<thead>
<tr>
<th>No.</th>
<th>Text</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Figure of Avocado Fruit</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td>Figure of Avocado Leaf</td>
<td>32</td>
</tr>
</tbody>
</table>
INTRODUCTION

Background

Avocado (*Persea americana* Mill) is an evergreen tree native to Mesoamerica. Cultivars of Guatemalan, Mexican, and West Indian origin have spread, becoming important crops in many tropical and subtropical regions around the world, such as Florida, California, Australia, South Africa, Spain and Israel. The avocado was introduced to Indonesia in 1750 (Morton, 1987). Avocado germplasm plant in Research and Technology Assessment, Tlekung, Malang was long red avocado, red round, dickson, butler, winslowson, benik, Puebla, fuerte, collinson, waldin, ganter, duke, ryan, leucadia, queen and edranol (DEPTAN, 2010).

Open pollination reproduction mode and mutations proposed as the main source of the genetic diversity in Indonesian avocado. Differences in climate and soil conditions in Indonesia by center of origin also makes the avocado has a high diversity. Diversity can be seen in plant morphology of avocado. Based on Bai *et al.* (2000), in the germplasm collection, characterization of individual accessions and cultivars is very important to elucidate the genetic material of plants that will be used as parental crosses in breeding. Therefore, it is necessary observations and characterizations various types of avocados in Indonesia.

Study for the observation and characterization of avocado could be done based on morphological characters and molecular markers. Evaluation of avocado characterization is usually based on morphological descriptions of plants observed from the beginning of planting until the plants mature and fruit set. However, this method requires time because the avocado plant is an annual plant. In addition, morphological traits are highly influenced by the environment (Shahsavar *et al.*, 2007). Therefore, other ways to characterize the avocado is needed.

Molecular marker is a marker that based on biochemical reactions and molecular structure of genes in a species. Molecular markers more accurately and faster than the characterization of the morphology because it involves genes as objects and not have to wait until the plants mature. One molecular marker that can be used is microsatellite markers using Inter-Simple Sequence Repeat (ISSR).
ISSR amplification is one of molecular marking technique that is useful in genetic fingerprinting to analyze genetic and to identify as well as classify germplasm. According to Zietkiewicz et al. (1994), ISSR markers are molecular typing approaches that have been used to detect variation among plants. This method has been used extensively to identify and determine relationships at the species and cultivar levels. ISSR method widely applicable because it is rapid, simple to perform, does not require prior knowledge of DNA sequence and requires very little starting DNA template. Pradeep Red et al. (2002) said that ISSR technique combines most of the benefits of AFLP and SSR markers with the universality of RAPD.

Avocado characterization that often accomplished was based on morphological description and molecular markers. In Indonesia, studies about avocado identification using morphological trait have been done in Sukabumi (Arham, 2006), Garut (Susanto, 2006), and Bogor (Fazri, 2008). However, genetic variation analysis of avocado using DNA based on molecular markers of Indonesia avocado germplasm have not been done yet.

**Objective**

The objective of this research was to elucidate the genetic diversity among 23 accessions of avocado based on their morphological characters and ISSR analysis.
LITERATURE REVIEW

Origin and Botany

The avocado (Persea americana Mill.) is a evergreen tree species that apparently originated in a broad geographical area stretching from the eastern and central highlands of Mexico through Guatemala to the Pacific coast of Central America (Knights, 2002). Early European travelers during the sixteenth century found avocado in cultivation and distributed throughout Central America and northern South America. This is evidenced by the native names given to avocado in many languages and by archaeological findings (Nakasone and Paul, 2004). The most common English name for this fruit, avocado, is a modification of the Spanish name, aguacate or ahuacate, derived from the Nahuatl word ahuacatl. The common name for the fruit in Dutch is advocaat or avocat, in German Abakate, and abacate in Portuguese.

The genus Persea (Clus.) Miller belongs to the family Lauraceae which has arisen from woody magnolia forebears. In subgenus Persea three species are recognized, P. schiedeana Ness, P. parvifolia Williams, and P. americana Mill. The later species is polymorphic and consist of several separate taxonomy that may be considered botanical varieties or subspecies, which are referred to as ‘horticultural’ races in the popular literature. Within this group are the varieties that make up the commercial avocados, namely P. americana var. americana Mill, the West Indian or lowland avocado; var. guatemalensis Williams, the Guatemalan avocado; and var. drymifolia Blake, the Mexican avocado; all three regarded as geographical ecotypes (Scora, et al., 2002).

The avocado tree is variable in shape, from tall, upright trees to widely spreading forms with multiple branches. Tree can attain heights of 15 – 18 m, with manageable height being controlled by pruning. The dark green leaves are spirally arranged and variable in size from 10 to 13 by 20 to 25 cm long, entire, elliptic or ovate to lanceolate. The juvenile period in avocado can be from 5 to 15 years. Girdling in early autumn 3 years after planting can significantly increase flowering and fruit set (Nakasone and Paul, 2004).
The avocado fruit is a one seeded berry. The single large seed is composed of two cotyledons enclosing an embryo and is surrounded by a thick fleshly mesocarp. The skin varies in thickness to 0.65 cm, depending upon the race (Table 1). The skin colour of the ripe fruit ranges from various shades of green to yellow-green and from reddish to maroon and light to dark purple. The buttery flesh (mesocarp) is greenish yellow to bright yellow to creamish when ripe. Oil content ranges from 7.8 to 40.7% on a fresh weight basis. Size varies from small fruit of some Mexican types, about 227 g or less, to the large Guatemalan types, 1.4 – 2 kg or more (Table 1). In shape, the fruit is usually pyriform to oval and round (Nakasone and Paul, 2004).

Table 1. Comparison of Selected Characteristic of Three Horticultural Races of Avocado

<table>
<thead>
<tr>
<th>Trait</th>
<th>West Indian</th>
<th>Guatemalan</th>
<th>Mexican</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climate</td>
<td>Tropical</td>
<td>Semitropical</td>
<td>Subtropical</td>
</tr>
<tr>
<td>Cold tolerance</td>
<td>Least</td>
<td>Intermediate</td>
<td>Most</td>
</tr>
<tr>
<td>Salt tolerance</td>
<td>Most</td>
<td>Intermediate</td>
<td>Least</td>
</tr>
<tr>
<td>Leaf anise</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Leaf color</td>
<td>Pale yellow</td>
<td>Green with red tinge</td>
<td>Green</td>
</tr>
<tr>
<td>Fruit bloom to maturity</td>
<td>5 months</td>
<td>12 months or more</td>
<td>6 months</td>
</tr>
<tr>
<td>Size</td>
<td>Variable</td>
<td>Variable</td>
<td>Small</td>
</tr>
<tr>
<td>Color of fruit</td>
<td>Green or reddish</td>
<td>Green</td>
<td>Often dark</td>
</tr>
<tr>
<td>Skin thickness</td>
<td>Medium</td>
<td>Thick</td>
<td>Very thin</td>
</tr>
<tr>
<td>Skin surface</td>
<td>Shiny</td>
<td>Rough</td>
<td>Waxy bloom</td>
</tr>
<tr>
<td>Seed size</td>
<td>Variable</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Seed cavity</td>
<td>Variable</td>
<td>Tight</td>
<td>Loose</td>
</tr>
<tr>
<td>Oil content</td>
<td>Low</td>
<td>High</td>
<td>Highest</td>
</tr>
<tr>
<td>Pulp fiber</td>
<td>Less common</td>
<td>Less common</td>
<td>Common</td>
</tr>
<tr>
<td>Pulp flavor</td>
<td>Sweeter, milder</td>
<td>Rich</td>
<td>Anise-like, rich</td>
</tr>
</tbody>
</table>

Genetic Variability

Three horticultural races of avocado are recognized and reflect geographical areas of origin, based on abundance of each race in cultivation. The West Indian avocado is a tree of lowland, hot and humid Central American forests with a short dry season (Knights, 2002). The West Indian race is not native to the West Indies but rather to the lowlands of Central America and possibly to
northern South America. It is tropical in ecological requirements and characterized by producing small fruit with medium thin, leathery skin, low oil content, loose seed and maturing 160 – 240 days after flowering (Table 1). The West Indian race is best adapted to a humid, warm climate and monsoon rains with optimum temperatures around 25 – 28 °C. Higher temperatures depress photosynthesis, thus lowering yields (Nakasone and Paul, 2004).

The Guatemalan race has large fruits, round or nearly so with as much as 3 cm of fruit flesh in a durable rind of 2 – 3 mm thickness with prominent stone cells, a rough or smooth outside texture, of green to sometimes black colour. Variety guatemalensis grows from the warm/humid climates of the tropical rainforests to semi-arid ones, from 100 – 2 300 m altitude on acid soils ranging from low to high fertility (Scora, et al., 2002). The Guatemalan race is adapted to a cool tropical climate but is less tolerant of low temperatures than the Mexican race. Leaves of cultivated types of this race are not anise-scented (Nakasone and Paul, 2004).

The native habitat of Mexican avocados is more elevated (1 400 – 2 500 m) and cooler than West Indian and Guatemalan, with mean annual temperatures from 14.2 to 19.8°C, rainfall from 665 to 1 562 mm and a 6 – 8 month winter–spring dry period (Wolstenholme, 2002). Leaves of the Mexican race are anise-scented and 60% of the essential oil is the monoterpene estragol (Nakasone and Paul, 2004).

In general, Guatemalans have the most useful horticultural genes. They dominate the germplasm of the world’s subtropical avocado cultivars, and the better types are recognized for desirable fruit quality, small seeds, and late fruit maturity. Good quality pure Mexicans are rare, but they have contributed genes for early maturity and cold tolerance, inter alia. West Indian types, or their hybrids with Guatemalans, dominate the world’s tropical and semi-tropical industries. West Indian avocado fruits are renowned for relatively low oil but high sugar content, and a distinctly less ‘nutty’ flavor than found in subtropical cultivars (Scora, et al., 2002).
Morphological Analysis

The analysis of genetic variation or diversity in plant has been assessed by analysis of morphological, physiological, or by horticultural descriptions. These descriptions may not be a reliable measurement of genetic variability caused by environment influence on gene expression and subjected to human judgment (Rajapakse and Ballard, 1997). A further evaluation of this trait requires growing the plants to full maturity prior to identification (Bai et al., 2000).

In avocado, as in other fruit species, morphological characters have traditionally been used to identify the different genotypes (IPGRI 1995). This marker observed vegetative and generative phase of plants like colour, shape, and character on fruit, leaves, branches and other inherited characters. However, the evaluation of these characters is labor intensive and inaccurate due to the influence of environmental factors, subjectivity and the limiting number of discriminating traits (Alcaraz and Hormaza, 2007).

Molecular Markers

Molecular markers have become more common for analyzing germplasm resources in many field crop species. The most familiar application has been the assessment of the amount of the genetic variability present in germplasm collections. Additionally, molecular marker technology is helping in the identification of redundancies and gaps in germplasm collections, screening of new potential accessions, and variety identification (Subudhi et al., 2006).

Recent advantages in molecular techniques have led to the development of assays based on variation in DNA sequences, broadly referred to as DNA (or molecular) markers. DNA markers provide good resolution because, unlike most non-DNA based marker (morphological, biochemical, or physiological), they are (1) unlimited in number, (2) independent of environment, developmental stage, and complex genetic interactions, (3) frequently free of dominant and recessive effect, and (4) easy to score, analyze and interpret (Varshney et al., 2006). Molecular markers have proven to be useful in clarifying genetic relationship among individuals in avocado germplasm (Cuiris-Pérez, et al., 2009).
The use of DNA markers to identify single-gene traits was first applied to avocado by Furnier et al. (1990). They used Restriction Fragment Length Polymorphism (RFLP), to assess genetic relationships within the subgenus *Persea* in which species identification is quite difficult. Other researches to fingerprint different sets of cultivars in avocado were done using minisatellites, VNTRs, or RAPDs.

**Polymerase Chain Reaction (PCR)**

Polymerase chain reaction (PCR) is an effective procedure for generating large quantities of specific DNA sequences in vitro. It is able to detect a single DNA target by producing approximately $10^{12}$ copies of selected sequences in only a few hours. The amplification is normally performed in a small polypropylene tube place in thermal cycler programmed to achieve three selected temperature in turn, known as cycle. Each cycle consist of DNA denaturation, primer annealing, and elongation. DNA denaturation is the converting of duplex DNA to single strands by breaking the hydrogen bands of complementary nucleotide pairs. Then the primers base pair with their complementary sequences in single stand as the annealing process. The last process is elongation, sequential addition of monomer at a time to a polymer (McDowell, 1999).

McDowell (1999) stated that the primers used in PCR are specific oligonucleotide sequences, which hybridized to the DNA molecule. PCR primers are typically 15 – 20 nucleotides long and placed at a distance of 200 – 2 000 bp for primer amplification of the target gene. A suitable *Taq* DNA polymerase buffer is an enzyme from *Thermus aquaticus, Thermus thermophilus*, and other bacterium which is used as thermostable in amplification reaction. A standard amplification as set out below might be 25 – 100 µl in volume, although volume as small as 5 µl has been used successfully. The reaction would contain target DNA, deoxynucleotide triphosphate, a reaction buffer, a thermostable DNA polymerase, and primers.
Inter-Simple Sequence Repeat (ISSR)

Inter-Simple Sequence Repeat (ISSR) is a PCR based method that can rapidly differentiate closely related individuals. This technique involves amplification of DNA segment between two identical microsatellite repeat regions. The ISSR is known as mono microsatellite the form of repetition, in, or trinucleotida which usually consists of 4 – 10 repeating units, extending the DNA strand. Such base composition is characteristic of the nuclear genome and varies between species or populations. On the detection of genetic polymorphisms ISSR without the need first to identify the composition of bases (sequence) of genomic base composition among plants that repeatedly, as long as the composition of these recurring base represents a broad and spread across the genome (Zietkiewicz et al., 1994).

There are several types of ISSR primers are: (1) Un-anchored primers. Amplification with the primer did not occur when the composition of base anchored repeated distance close to the templates (2-3 kb) and its orientation reversed. There are multiple products so that the number of banding pattern produced can be more than one; (2) Anchored primer that is primary with the addition of a few bases are not repeated in the position or '5 or '3 at the beginning or end of the arrangement of bases on the primer used whose function is to ensure the target amplification. Basically, the ISSR to initiate a particular section of DNA strand in the region close between microsatellite repeat using primers anchored in position 5 or 3 'at the beginning / end of the primer with 2 to 4 additional non-recurring bases (Zietkiewicz et al., 1994).

ISSR analysis has been used for cultivar identification in numerous plant species. Recently, several sets of microsatellites have been developed in avocado (Ashworth and Clegg, 2003; Borrone et al., 2007) and used for fingerprinting and diversity studies (Schnell et al., 2003; Chen et al., 2009; Cuiris-Pérez et al., 2009).
MATERIALS AND METHODS

Place and Time

This study was conducted at Pasir Kuda Research Farm, Bogor, in altitude 250 m above sea level. DNA extraction was conducted at Molecular Laboratory, Center of Tropical Fruits Studies (PKBT), Baranang Siang, Bogor. This study was conducted from November 2009 to July 2010.

Materials and Tools

Plant Materials

Avocados are used in this study derived from avocado fruits taken randomly from some traditional markets in Bogor. Region of origin of avocado is known by trader’s narrative. The fruits were differed based on morphological characters. Twenty-three avocado fruits were chosen to identify the morphological characters and molecular analysis.

Table 2. Accessions Used of Avocado

<table>
<thead>
<tr>
<th>No</th>
<th>Area of Origin</th>
<th>Accessions Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lampung</td>
<td>Lam1</td>
</tr>
<tr>
<td>2</td>
<td>Lampung</td>
<td>Lam2</td>
</tr>
<tr>
<td>3</td>
<td>Lampung</td>
<td>Lam3</td>
</tr>
<tr>
<td>4</td>
<td>Lampung</td>
<td>Lam4</td>
</tr>
<tr>
<td>5</td>
<td>Lampung</td>
<td>Lam5</td>
</tr>
<tr>
<td>6</td>
<td>Lampung</td>
<td>Lam6</td>
</tr>
<tr>
<td>7</td>
<td>Lampung</td>
<td>Lam7</td>
</tr>
<tr>
<td>8</td>
<td>Garut</td>
<td>Gar1</td>
</tr>
<tr>
<td>9</td>
<td>Garut</td>
<td>Gar2</td>
</tr>
<tr>
<td>10</td>
<td>Garut</td>
<td>Gar3</td>
</tr>
<tr>
<td>11</td>
<td>Garut</td>
<td>Gar4</td>
</tr>
<tr>
<td>12</td>
<td>Garut</td>
<td>Gar5</td>
</tr>
<tr>
<td>13</td>
<td>Sukabumi</td>
<td>Suk1</td>
</tr>
<tr>
<td>14</td>
<td>Sukabumi</td>
<td>Suk2</td>
</tr>
<tr>
<td>15</td>
<td>Sukabumi</td>
<td>Suk3</td>
</tr>
<tr>
<td>16</td>
<td>Sukabumi</td>
<td>Suk4</td>
</tr>
<tr>
<td>17</td>
<td>Bogor</td>
<td>Bog1</td>
</tr>
<tr>
<td>18</td>
<td>Bogor</td>
<td>Bog2</td>
</tr>
<tr>
<td>19</td>
<td>Bogor</td>
<td>Bog3</td>
</tr>
<tr>
<td>20</td>
<td>Bandung</td>
<td>Ban1</td>
</tr>
<tr>
<td>21</td>
<td>Bandung</td>
<td>Ban2</td>
</tr>
<tr>
<td>22</td>
<td>Bandung</td>
<td>Ban3</td>
</tr>
<tr>
<td>23</td>
<td>Bandung</td>
<td>Ban4</td>
</tr>
</tbody>
</table>
Chemical Reagents

The chemical reagents used in this research were cetyltrimethylammonium bromide (CTAB), NaCl, mercaptoetanol, Tris HCl, nuclear free water, sand, polyvinilpolypyrrolidin (PVPP), chloroform isoamyl alcohol (CIAA 24:1), isopropanol, cold absolute ethanol, alcohol 70%, loading dye, agarose powder, buffer TAE 1x, ethidium bromide, PCR mix, seven primers ISSR, and DNA ladder 1kb.

Equipments

Equipments used were caliper, ruler, and colour chart. Other equipments used were mortal and pestle, spatula, pincers, Erlenmeyer, micropipette and eppendorf tips, microtube, tube-stakeholders, parafilm papers, waterbath, analytical weighting machine, microwave, centrifugation machine, PCR machine, electrophoresis set, UV transilluminator, digital camera, and other supporting tools.

Methods

Observation of morphological characters conducted on avocado’s fruit and seed characters. Then avocados grown in polybag of 30 cm x 30 cm sized. Medium used were soil and compost with a ratio of 2:1. The isolation of DNA using avocado leaves organ as DNA samples while the plant was 5 month after planting. There were observed on plant height and number of leaves per plant. Average high avocado plant in 7 months after planting was 91.7 cm, while the number of leaves ranged from 25 to 50.

Morphology characterization

Observation of avocado’s characterization is using scoring standard based on Descriptor for Avocado (IPGRI, 1995) that has been modified. The characters that been observe was fruit, seed and leaf. Observation was included both qualitative and quantitative characters of avocado. Thirty-four quantitative and qualitative morphological traits were assessed across the 23 accessions:
1. **Fruit**

a. Fruit weight (g)  
   Average of three fruits

b. Fruit length (cm)  
   Average of three fruits

c. Fruit diameter (cm)  
   Measured at the broadest part. Average of three fruits

d. Fruit shape uniformity
   - 3. Low
   - 5. Intermediate
   - 7. High

e. Fruit shape

   ![Fruit shapes diagram](image)

   1. *oblate*  
   2. *spheroid*  
   3. *high spheroid*

   4. *ellipsoid*  
   5. *narrowly obovate*  
   6. *obovate*

   7. *pyriform*  
   8. *clavate*  
   9. *rhomboidal*

   **Figure 1. Fruit shape**

f. Ridges on fruit

   ![Ridges on fruit diagram](image)

   1. *absent*  
   2. *partial*  
   3. *entire*

   **Figure 2. Ridges on fruit**

g. Fruit skin surface

   - 3. Smooth
   - 5. Intermediate
   - 7. Rough
### 1. Gloss of fruit skin
- Weak
- Medium
- Strong

### 2. Fruit skin colour
- Light green
- Yellow
- Black
- Green
- Purple
- Speckled
- Dark green
- Red
- Other

### 3. Fruit skin thickness
- 1 mm
- 2 mm
- 3 mm

### 4. Colour of flesh next to skin
- Ivory
- Deep yellow
- Other
- Light yellow
- Light green
- Green

### 5. Colour of flesh next to seed
- Ivory
- Deep yellow
- Other
- Light yellow
- Light green
- Green

### 6. Flesh texture
- Watery
- Pastose (doughy)
- Other
- Buttery
- Granular

### 7. Sweetness of flesh
- Low
- Intermediate
- High

### 8. Bitterness of flesh
- Low
- Intermediate
- High

### 9. General taste of flesh
- Very poor
- Fair
- Excellent
- Poor
- Excellent

### 2. Seed
- Seed weight (g)
- Seed length (cm)
- Seed diameter (cm)
d. Seed shape

1. oblate
2. spheroid
3. ellipsoid
4. ovate
5. broadly ovate
6. cordiform
7. base flattened, apex rounded
8. base flattened, apex conical

Figure 3. Seed shape

e. Cotyledon surface
3. Smooth
5. Intermediate
7. Rough

f. Cotyledon colour
1. Ivory
3. Yellow
5. Other
2. Cream
4. Pink

g. Seed coat
1. Seed not free, coat not attached to the flesh
2. Seed not free, coat attached to the flesh
3. Seed free, coat not attached to the flesh
4. Seed free, coat attached to the flesh

3. Leaf
a. Leaf blade length (cm)
Average of five leaves
b. Colour of mature leaves
1. Light green
2. Green
3. Dark green
c. Groove on petiole
0. Absent
1. Present
d. Leaf texture
1. Soft
2. Semihard
3. Hard
4. Very hard
e. Leaf shape

1. ovate
2. narrowly obovate
3. obovate
4. oval
5. roundish
6. cordiform
7. lanceolate
8. oblong
9. oblong-lanceolate

Figure 4. Leaf shape

f. Leaf base shape

1. acute
2. obtuse
3. truncate

Figure 5. Leaf base shape

g. Leaf apex shape

1. very acute
2. acute
3. intermediate
4. obtuse
5. very obtuse

Figure 6. Leaf apex shape

h. Anise smell

Leaf must be crushed

3. Weak
5. Intermediate
7. Strong
DNA extraction

The total genomic DNA was extracted by using CTAB (cetyltrimethylammonium bromide) method with some modifications. Leaf tissues (100 mg) were ground in 1000 µl of CTAB extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% [p/v] b-mercaptoethanol, 2% [p/v] CTAB) and heated at 60°C for 30 min. DNA was extracted with one volume of a chloroform:isoamyl alcohol mix (24:1) and precipitated in presence of isopropanol (40% [v/v] final concentration). The DNA pellet was washed with 70% ethanol, dried, and dissolved in 100 µl of nuclear free water.

DNA quality was determined by electrophoresis using 0.8% (w/v) agarose gel of Promega. Gel was prepared using TAE buffer 1x by warm it up at microwave. Gel was put on electrophoresis tank with same buffer. Then 4 µl of each 23 DNA extracted sample was pipette into wells of gel with 1 µl of loading dye 6x. Electrophoresis ran at 90 volt for about 30 minutes. After electrophoresis running, the product should be readily visible in an ethidium bromide-stained gel under UV light.

ISSR analysis

Polymerase chain reactions (PCRs) were carried out in 25 µl final volume of reaction mixture containing 2 µl genomic DNA suspension, 2 µl of primer, 12 µl Taq polymerase (green master Promega), and 9 µl nuclear free water. Amplification was carried out in a thermocycler. The aim of primer selection was
to determine which primer could amplify target DNA. Twelve primers from PKBT were initially screened for well amplified and polymorphic bands among accessions. Primer that could visualize DNA bands means that primer was able to amplify all of 23 accessions. Seven ISSR primers were finally selected for the full screen of all accessions.

Table 3. ISSR Primer Used in This Study

<table>
<thead>
<tr>
<th>No.</th>
<th>Primer</th>
<th>Annealling</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PKBT 2</td>
<td>53 °C</td>
<td>AC AC AC AC AC AC AC AC TT</td>
</tr>
<tr>
<td>2</td>
<td>PKBT 3</td>
<td>53 °C</td>
<td>AG AG AG AG AG AG AG AG T</td>
</tr>
<tr>
<td>3</td>
<td>PKBT 4</td>
<td>53 °C</td>
<td>AG AG AG AG AG AG AG AG AA</td>
</tr>
<tr>
<td>4</td>
<td>PKBT 5</td>
<td>53 °C</td>
<td>AG AG AG AG AG AG AG AG TA</td>
</tr>
<tr>
<td>5</td>
<td>PKBT 6</td>
<td>53 °C</td>
<td>AG AG AG AG AG AG AG AG TT</td>
</tr>
<tr>
<td>6</td>
<td>PKBT 7</td>
<td>53 °C</td>
<td>GA GA GA GA GA GA GA GA A</td>
</tr>
<tr>
<td>7</td>
<td>PKBT 8</td>
<td>54 °C</td>
<td>GA GA GA GA GA GA GA GA GA C</td>
</tr>
</tbody>
</table>

The PCR program was single initial denaturation for 4 min at 94°C, followed by 35 cycles of denaturation (30 second at 94°C), annealing (30 second at 53 to 54°C according to the individual primers), and extension (60 second at 72°C). The program ended with final extension at 72°C for 5 minutes. Amplified products were separated by electrophoresis on 1.0% agarose gels (Promega corporation), stained in ethidium-bromide (0.1%). The 100 bp DNA ladder was used as a standard molecular weight. Photographs were taken using digital camera.

Data Analysis

Only consistent, bright, reproducible (i.e., band absence was randomly verified) ISSR bands were scored as present (1) or absent (0), where each character state was treated independently. Genetic similarity and cluster analyses were performed by subjecting character state data to empirical examination using Numerical Taxonomy and Multivariate Analysis System software version 2.02Rev (NTSYS-PC 2.02Rev). Grouping was carried out using Un-weighted Pair Group Method and Arithmetic Average (UPGMA) cluster analysis. Goodness of fit for cluster analysis was revealed by cophenic correlation.
RESULT AND DISCUSSION

General Description

Avocados grown in a screen house that was covered paranet density of 65% at Pasir Kuda Research Farm, Bogor, in altitude 250 m above sea level. Temperatures on during the study ranged from 25.3 – 27.1 °C with the highest temperature 27.1 °C in April and the lowest 25.3 °C in January. Humidity ranged between 77 – 86% with the highest humidity occurred in March and June, the lowest humidity occurred in April. Wet month with rainfall more than 200 mm lasted for 8 months, while the dry months occurred only in April. The highest rainfall occurred in February amounted to 689 mm and the lowest occurred in April amounted to 149 mm. However, the use of parnet 65% can able to reduce rainfall in the screen house.

Maintenance includes watering, weed control, and the control of pests and diseases. Watering was done twice a day. Weed control was done manually. Avocado pests included caterpillars (Cricula trisenestrata Helf), and Aphis gossypii glov. Control of pests and diseases was done by spraying pesticides.

Morphological Character Analysis

The total number of vegetative and generative characteristics were observed from 23 accessions was thirty-four. There is a high uniformity (100%) in several traits, namely leaf base shape, crotch angle of leaf petiole, and groove on petiole. Therefore, the number of characters that were observed was 31 characters. Thirty-one characters were scattered into 121 different loci.

Similarity analysis (Figure 7) showed the 23 accessions observed have similarity ranged from 0.40 – 0.74. The cophenet correlation value was 64% (r = 0.6465) that means the dendogram has a very poor fit with the similarity value, where the 64% of similarity matrix were described by the dendogram.
At 0.44 similarity degree, the twenty-third accessions grouping into three groups i.e.: group I only included Lam1; group II included Lam2, Lam7, Suk1, Suk2, and Gar1; and group III. Group III, the largest group, could be divided into three sub-groups separated from each other with a similarity value of 0.46. The first sub-group included Lam3, Suk4, Suk3, and Bog3. The second sub-group included Lam4, Lam5, Gar5, Bog1, Bog2, Ban1, Ban2, Ban3, and Ban4. The other sub-group included the rest of the accessions.

Figure 7. UPGMA Dendogram of 23 Avocado Accessions Based on Morphological Characters

Figure 8. Example of Avocado Fruit

Morphology data analysis of accessions supported close similarities between accessions Gar1 – Suk2 and accessions Bog1 – Ban2, both of them have
genetic similarity = 0.74. The phenomenon shows that accessions from different origin could have some similarity in morphology.

Compared to other groups, the group I has more far genetic distance. This phenomenon could be proved based on the morphological characters that differentiate among the accessions. Lam1 had a clavate of fruit shape, weak gloss of fruit skin, and rough skin surface (Figure 8). It also had green colour of flesh next to skin, broadly ovate of seed shape, and oblong-lanceolate of leaf shape. Lam1 had strong anise smell character, which did not find in other accessions (Table 4).

Group II has some similarities morphological characters, such as 2 mm of fruit skin thickness, light green colour of flesh next to skin, light yellow colour of flesh next to seed, watery flesh texture character, intermediate sweetness of flesh, low bitterness of flesh, and seed not free, but coat not attached to the flesh.

Table 4. Grouping of 23 Avocado Accessions Based on Morphological Characters

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Accessions</th>
<th>Common Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Lam1</td>
<td>weak gloss of fruit skin, rough skin surface, green colour of flesh next to skin, broadly ovate of seed shape, oblong-lanceolate of leaf shape</td>
</tr>
<tr>
<td>II</td>
<td>Lam2, Lam7, Gar1, Suk1, Suk2</td>
<td>light green colour of flesh next to skin, light yellow colour of flesh next to seed, watery flesh texture, intermediate sweetness of flesh, seed not free, coat not attached to the flesh</td>
</tr>
<tr>
<td>III – sub group 1</td>
<td>Lam3, Suk4, Suk3, Bog3</td>
<td>intermediate of fruit skin surface, green colour of flesh next to skin, low bitterness of flesh, seed not free, coat not attached to the flesh</td>
</tr>
<tr>
<td>III – sub group 2</td>
<td>Lam4, Lam5, Ban1, Ban2, Ban3, Ban4, Gar5, Bog1, Bog2</td>
<td>weak gloss of fruit skin, green colour of flesh next to skin, fair general taste of flesh, entire leaf margin</td>
</tr>
<tr>
<td>III – sub group 3</td>
<td>Lam6, Gar2, Gar3, Gar4</td>
<td>green colour of flesh next to skin, yellow colour of flesh next to seed, buttery flesh texture, low bitterness of flesh, good general taste of flesh, undulate leaf margin</td>
</tr>
</tbody>
</table>
Group III, the largest group, could be divided into three sub-groups separated from each other with a similarity value of 0.46. The first sub-group included Lam3, Suk4, Suk3, and Bog3 with a similarity coefficient 0.46. All members of this subgroup had intermediate of fruit skin surface and undulate leaf margin (Table 4). The second sub-group included all of accessions origin from Bandung, two accessions from Lampung (Lam4 and Lam5), and one accession from both Garut (Gar5) and Bogor (Bog1). This sub-group had watery flesh texture character. The third sub-group included three accessions from Garut and one accessions from Lampung. They showed some morphological similarities, for example undulate leaf margin and the general taste of flesh was good.

ISSR Analysis

ISSR amplification of 23 DNA samples extracted from avocado accessions resulted in multiple banding profiles by 7 primers. Six band number and 100% percentage polymorphism were obtained by PKBT 3, PKBT 4, PKBT5, and PKBT 8. The number of fragments per primer ranged from 2 (PKBT 7) to 7 (PKBT 2) with an average of 5.2. It generated a total of 37 fragment bands and 94.5% showed as polymorphic fragments (Table 5). A polymorphic primer was one which presented at least one different band among the 23 accessions; that polymorphic bands were those that was missing in at least one of the accessions analyzed (Cuiris-Pérez, et al., 2009).

Table 5. Level of Polymorphism Obtained with Seven ISSR Markers in 23 Avocado Accessions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Total Band</th>
<th>Total Polymorphic Band</th>
<th>% Polymorphic Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKBT 2</td>
<td>7</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>PKBT 3</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>PKBT 4</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>PKBT 5</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>PKBT 6</td>
<td>6</td>
<td>5</td>
<td>83%</td>
</tr>
<tr>
<td>PKBT 7</td>
<td>2</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>PKBT 8</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>35</td>
<td>94.5%</td>
</tr>
</tbody>
</table>
Representative banding patterns observed with primer PKBT 3 are shown in Figure 9. The amplified fragment sizes ranged from 250 to 1000 bp. These results indicate the usefulness of ISSRs as a tool in distinguishing accessions with diverse origins. However, the loci detected in this study were not associated with any morphological trait.

![Figure 9. Example of Banding Pattern Profiles Revealed by Primer PKBT 3](image)

A dendrogram based on UPGMA analysis of the ISSR data was shown in Figure 10. According to this analysis, the genetic similarity between the accessions ranged from 0.54 to 0.94. The 23 accessions grouped as three major groups with a threshold genetic distance of 0.64. The cophenetic correlation value...
was 84% \((r = 0.84022)\) which means the dendogram has a good fit with similarity value.

Group I consisted of 15 accessions, which could be separated as three subgroups at 0.72 genetic distances. Sub-group I included Lam1, Lam2, and Lam5. Sub-group II included Lam4, Suk1, and all of Garut accessions. Sub-group III included Lam6, Lam7, Suk2, Suk3, and Suk4. In this subgroup highest genetic similarity was for accessions Suk3 and Suk4. Group II included only Lam3 which was separated from other groups with similarity coefficient of 0.62. This accession had the greatest genetic dissimilarity. Group III consisted of all accessions come from Bandung and Bogor.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Accessions</th>
<th>Common characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – sub group 1</td>
<td>Lam1, Lam2, Lam4, Lam5, Gar1, Gar2, Gar3, Gar4, Gar5, Suk1</td>
<td>green colour of flesh next to skin, intermediate sweetness of flesh, very acute to acute of leaf apex shape</td>
</tr>
<tr>
<td>I – sub group 2</td>
<td>Lam6, Lam7, Suk2, Suk3, Suk4</td>
<td>intermediate sweetness of flesh, low bitterness of flesh, seed not free, coat not attached to the flesh</td>
</tr>
<tr>
<td>II</td>
<td>Lam3</td>
<td>strong gloss of fruit skin, black fruit skin colour</td>
</tr>
<tr>
<td>III</td>
<td>Bog1, Bog2, Bog3, Ban1, Ban2, Ban3, Ban4</td>
<td>acute of leaf apex shape, entire leaf margin</td>
</tr>
</tbody>
</table>

Based on field observations, it is known that group I – sub group 1 and group I – sub group 2 had the same morphological characters on taste of flesh sweetness and seed coat. They had differences in seed weight, seed length and seed diameter character. Seed of sub group 2 was more heavy and bigger than seed of sub group 1.

Group II consisted of only one accession, namely Lam3. Lam3 has a spheroid shape character, a strong level of shiny skin, ripe black fruit, seed shape spheroid, and the cotyledon color pink. All accessions were from Bogor and Bandung included in Group III. This group has a common character among other acute shape of leaf apex and entire leaf margins (Table 6).
The Eigen value cumulative percentage result in ISSR analysis was give a number ≥ 70% at the fourth component as showed in Table 7. These mean the principal component analysis (PCA) plot for ISSR can be used to analyze.

Table 7. Eigen Value for ISSR Analysis

<table>
<thead>
<tr>
<th>i</th>
<th>Eigen Value</th>
<th>Percent</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.56936244</td>
<td>50.5195</td>
<td>50.5195</td>
</tr>
<tr>
<td>2</td>
<td>0.67744180</td>
<td>13.3200</td>
<td>63.8395</td>
</tr>
<tr>
<td>3</td>
<td>0.27553305</td>
<td>5.4176</td>
<td>69.2571</td>
</tr>
<tr>
<td>4</td>
<td>0.24526308</td>
<td>4.8224</td>
<td>74.0796</td>
</tr>
</tbody>
</table>

Integrated Data Analysis

Molecular markers are more reliable than morphological traits for assessing genetic diversity, but morphology still has the advantage of providing a direct, in-the-field tool for gauging plant performance. Thus, it would be useful if a more detailed analysis of the correlation between molecular markers and morphological traits were able to identify key morphological characteristics of particular value for genetic diversity evaluation. Scora, et al. (2002) said that neither the morphological nor the molecular markers are superior and that the two tools should complement each other.

Figure 11. UPGMA Dendogram of 23 Avocado Accessions Based on Morphology and ISSR Analysis
The genetic distances among the 23 accessions were calculated according to the two assessment methods, morphological traits and the ISSR molecular markers. Matrices were constructed from these two respective genetic distance scores and their correlations assessed according to the Mantel test. The correlation coefficient between the morphological and ISSR matrices was 0.60076. The significant positive correlations between the matrices obtained from the molecular genomic markers and the morphological trait matrices also indicate that morphological characters can provide a useful measure of genetic diversity amongst accessions.

Combination of morphology and ISSR data analyzed give a new analysis output, which not much different from the single data output. The UPGMA dendogram constructed from combination data of morphological traits and ISSR marker analysis separated the 23 accessions into three major groups at similarity value of 0.51. The most different accession is Lam1, which group separately from the rest of the accessions. This accession had dark green colour, rough skin surface, seed free but coat not attached to the flesh, and broadly ovate of seed shape. The rest of the accessions in the dendrogram could be roughly divided into two main clusters with accessions of different origin intermixed. In the upper group of the dendrogram could be found all of Garut and Lampung accessions. All of Sukabumi accessions, except accession Suk3 also included in these group. The second group contains all of Bandung and Bogor accessions. Accessions Ban1 and Ban2 had the closest genetic similarity.

A clear grouping based on avocado race cannot be determined by using the combination of morphological traits and ISSR markers analysis. The seven primers and thirty-four morphological traits were able to reveal the variation among the twenty-third accessions, but were not representative to assume the genetic distance of similarity. There was no patterns show population grouping. It was because seven primers and thirty-four morphological traits not enough to ‘read’ the whole genome. Therefore, other research use more primers should be done to get more accurate results in similarity degree value.

The polymorphism detected in this work with the morphological traits and ISSR markers is an indication of genetic variation existing among 23 accessions
of avocado. The similarity ranges 0.47 to 0.74 is board, its show a high variation among accessions. The fact that avocado is an out crossing species have all favored a certain degree of genetic variation within this species (Cuiris-Pere e et al., 2009). In addition, none of the accessions were found to be duplicates, therefore there is no need to discard any of the individuals; these should be kept and used for breeding purposes.
CONCLUSION

The morphological analysis showed genetic variation among the 23 accessions observed. It could be seen from the broad ranged of similarity value (0.40 – 0.74). The twenty-third accessions grouping into three groups at 0.44 similarity degree. The number of morphological characters that were observed was 34 characters.

A total of 37 fragment bands from 7 ISSR primers were revealed and 94.5% showed as polymorphic fragments. ISSR markers revealed a relatively high level of genetic distance. Genetic similarities among all accessions ranged from 0.54 to 0.94. For the ISSR dendrogram, cophenetic correlation was estimated at 0.84, corresponding to a good level of fit. The UPGMA grouped the accessions into three main groups.

Morphological characters and ISSR molecular analysis were valuable for the determination of genetic diversity and relationships amongst 23 accessions of avocado. Morphological traits and molecular markers were representative of different characters showed by matrix correlation value (r = 0.60076). According to this analysis, the genetic distances among the 23 accessions ranged from 0.47 to 0.74, as three major groups with a threshold genetic distance of 0.51.
REFERENCES


DEPTAN.2010. Dokumen Deptan. [http://dokumen.deptan.go.id] [27 October2010]


APPENDIXES
Appendix 1. Figure of Avocado Fruit
Bogor Agricultural University

Hak Cipta Dilindungi Undang-Undang
Appendix 2. Figure of Avocado Leaf