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This is to certify that

Dr. Ir. I Ketut Sumantra, MP

As

PRESENTER

of 2nd International Conference on Sustainability Development
Mahasaraswati Denpasar University
Bali, February 28th – March 1st 2015

Dr. Drs. I Made Sukamerta, M.Pd.
Rector of Mahasaraswati Denpasar University

Dr. Ir I Ketut Sumantra, MP
Chairman of the Committee

MAHASARASWATI DENPASAR UNIVERSITY

UNIVERSITI TEKNOLOGI MARA

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PROCEEDINGS

ICSD 2015

2nd International Conference on Sustainability Development

Universitas Mahasaraswati Press

PROCEEDING

2nd International Conference on Sustainable Development (ICSD)

"Global Sustainable Development"

28 February - 1 March 2015
Bali, Indonesia

Edited by:

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Universitas Mahasaraswati Press

PROCEEDING OF
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PREFACE

If we look at the history, modern CSR movement, which has expanded rapidly over the last twenty years, was born as a result of the insistence of civil society organizations and global level networks. The main concern which is voiced is the behavior of corporations; for the sake of profit maximization, it is common to do unfair and unethical practices, and in many cases it can even be categorized as corporate crime. Some of the giant transnational

Yohannesburg meeting in 2002 which was attended by the leaders of the world gave rise to the concept of social responsibility, which is to accompany the two previous concepts namely economic and environmental sustainability. The principle of sustainability is intended to promote growth, especially for the poor in managing the environment and institutional capacity to manage development, as well as the strategy in which the ability to integrate the economic, ecological, social-valued diversity and socio-cultural are of necessary. It is a fact how the local community resistance, in various places and times come to the surface of the companies that are not considered to pay attention to aspects of social, economic and environmental life. Therefore, as its development, researches on sustainability development also experiences their growth.

Research on sustainability development should be disclosed in a broader dialogue as in an international conference. The conference is expected to accommodate the researchers to foster their thoughts on sustainability development in a wider scale. In addition, this conference is expected to generate ideas in all areas of sustainability development.

This international seminar has attracted interest from researchers, experts, and academia. There are 142 manuscripts submitted to the Committee. Having been reviewed, 115 papers will be presented at the seminar, and 10 will be displayed at poster session. The paper included in this proceedings deal with major areas in the field of sustainability development, such as Macroeconomics, Urban and Regional Planning, Sustainable Agriculture and Food Systems, Education, and Community Empowerment.

We would like to take this opportunity to express our sincere appreciation to the members of Technical Advisory Committee who helped review the papers and maintained high standards for the international conference proceedings.

February 2015

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I Wayan Kertiyasa

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3. The honorable Prof Sundani Norono Suwandi from Institute Technology of Bandung (ITB)
4. The honorable Dr. Jennifer Day from Melbourne University, Australia.
5. Honorable distinguished guests, and participants

Om Swastiastu
Assalamu'alaikum Warahmatullahi Wabarakatuh,
Good morning and May the Almighty God give us joy and prosperity.

Excellencies, ladies and gentlemen,

On behalf of the Organizing Committee, it is my pleasure and privilege to welcome all the distinguished speakers, guests, and participants to this 2nd International Conference on Sustainable Development (I C S D).

I also wish to take this opportunity to welcome Prof. Darussalam Abu Bakar our Speaker from MARA University of Technology (UiTM) Malaysia who has an expert on Communication and Broadcasting. Also, Prof.Dr.rer.nat Sundani Nurono Soewandhi, he's a lecturer from Institute Technology of Bandung (ITB), he focuses on crystallographic studies of pharmaceutical solid materials especially on solid interaction and he also created some National Community Programs for Directorate General of Higher Education. And then, Dr. Jennifer Day, lecturer of Urban Planning in University of Melbourne. Her research is in transportation economics, economic development, and urban/regional economics. Currently, she is a lead author in Vice Chancellor's proposal on urbanization to the Australian Agency for International Development (AusAID)

Excellencies, ladies and gentlemen,

Prior to the conference, the Steering Committee has carried out a number of preparation activities, from announcing the call for paper to research centers, universities, and government agencies, up to paper selection. There are 142 manuscripts submitted to the Committee. Having been reviewed, 115 papers will be presented at the seminar, and 15 will be displayed at poster session. The paper included in this proceedings deal with major areas in the field of sustainable

development, such as Macroeconomics, Urban and Regional Planning, Sustainable Agriculture and Food Systems, Education, and Community Empowerment.

The Reviewers are selected for their scientific backgrounds and expertise, which consists of professors and senior researchers from Mahasaraswati Denpasar University and from invited speaker (UiTM and ITB).

I should also inform you that around 200 experts, researchers, and academia from research centers, universities, and government agencies have been invited to the Conference; including our partners from Mara University of Technology (UiTM) Malaysia University of Melbourne, ITB, Udayana University, Ganesha University of Education, Bogor Agriculture Institut (IPB) Bali State Polytechnic, University of Hasanudin, Tadulako University, Halueleo University, Samratulangi University, Bengkulu University, Muhamaddiah University, Malang, Pare-pare, Pelita Harapan University. Ujung Pandang State Polytechnic, Assessment Institute for Agricultural Technology (AIAT) Bali.

To conclude, I would kindly ask the Rector of Mahasaraswati Denpasar University, Bapak Dr. Drs I Made Sukamerta, MPd to give his welcoming remark and to officially open the conference.

I wish you a fruitful discussion on our sessions, and have a joyful stay in Bali. Thank you.

Om Shanti Shanti Shanti Om

Wassalamu'alaikum Warahmatullahi Wabarakatuh.

Chairman of International 2nd International Conference on Sustainability Development
Dr. Ir. I Ketut Sumantra, MP

OPENING SPEECH

Om. Swastyastu

The honourable Prof. Abubakar Darussalam experts in the field of Communication and Broadcasting at University Teknologi MARA (UiTM) at Shah Alam, Malaysia

The Honourable Prof. Sundani Nurono, experts in the field of Community and also as Reviewer of the Higher Education Community Service

The Honourable. Dr. Jennifer Day, Urban Planning in the University of Melbourne an expert in the field of Regional Planning

The Honourable Vice Rectors, Deans, the Quality Assurance and all panelists and other speakers

Distinguished guests Ladies and gentlemen

It's my pleasure to welcome you all to the Opening ceremony of the 2nd International conference on sustainability development (ICSD)", held by Mahasaraswati University. I have also to say a warm welcome that this morning all of us can join this international Seminar with the theme of the Global Sustainable Development. I also would like to welcome the speakers and panelists from within and outside the country. On behalf of Mahasaraswati University, I would like to thank all of you for attending this event and I am very pleased and honored to have the opportunity to join you here at the opening ceremony.

Ladies and Gentlemen

This theme was chosen by the committee based on the result of the insistence of civil society organizations and networks on a global level. The Johannesburg meeting in 2002, which was attended by world leaders led to the concept of social responsibility, as a complement of the two concepts, namely the economic and environmental sustainability. The principle of sustainability is intended to encourage growth, especially for the poor in environmental management and institutional capacity to manage development, to integrate the economic, ecological, and social diversity. Therefore, research on sustainable development should be disclosed in a broader dialogue like in this international conference.

The conference is expected to accommodate the researchers to push their thoughts on sustainable development in a wider scale. In addition, this conference is expected to generate ideas in all fields of sustainable development. I think this theme is very relevant and contextual to the development and dynamics of the era of globalization. These topics are important to be discussed properly and it can be used as a kind of academic forum which would bring benefits to the policy makers in the field of

sustainability of development. Hopefully this seminar can discuss the issues related to the major theme, to improve the current understanding of science in the field of sustainable development.

Ladies and Gentlemen

In this international seminar various experts, researchers, and academicians, from all sectors joined. Therefore I have to thank to all the speakers, presenters, and participants, who have taken the time and leave the daily tasks to participate to the success of this prestigious seminar.

However, we believe that, this seminar will benefit us for an exchange of knowledge and experience as well as many unique issues related to sustainable development, as well as innovative measures to accelerate the competitiveness and sustainability of development. At the same time, we will accommodate a wide range of issues as well closely related to infrastructure development, climate change, rural-urban relations, and sustainable development in general.

On this occasion I would like to express gratitude to the sponsors who have helped financially for this seminar. I also would like to thank the committee who have worked hard for the success of this event and hopefully the seminar can run smoothly and opens up great opportunities for all. I wish you all a very successful and fruitful seminar. Thank you.

Rector of Mahasaraswati Denpasar University
Dr. Drs. I Made Sukamerta, M.Pd

2nd ICSD
INTERNATIONAL CONFERENCE
ON SUSTAINABILITY DEVELOPMENT

Bali, 28 February - 1 March 2015

KEYNOTE SPEAKERS

Prof. Darussalam Abu Bakar
Universiti Teknologi MARA (UiTM), Malaysia

Prof. Sundani Nurono Soewandhi
Bandung Institute ofTechnology (ITB), Indonesia

Dr. Jennifer Day
University of Melbourne, Australia

PHENOTYPIC AND GENOTYPIC OF SALAK (SALACCA ZALACCA VAR. AMBOINENSIS) CV. GULAPASIR ON DIFFERENT GROWING ENVIRONMENTS

I Ketut Sumantra

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Abstract

Salacca (Salak) is one of tropical fruits that was native of Indonesia and preferred by consumers due to the specific fruit flesh taste. The objective of the research was to obtain the phenotypic and genotypic of Gulapasir salacca plant on six growing environmental variability were Saribuana, Pajahan, Bangsing, Telaga, Kecing and Jungutan. Phenotypic characters was observed by morphological characteristics of salacca plant and genotypic character of salacca by an analysis of DNA using *Random Amplified Polymorphic DNA* (RAPD) method. Data were analyzed by using Bartlett's test, cluster analysis using the program Numerical Taxonomy and Multivariate Analysis (NTSYS version 2.1). Salcca plants cv. Gulapasir planted in Tabanan and Karangasem showed a phenotypic and genotypic variation. The coefficient of phenotypic similarity was based on ten quantitative characters ranging from 0.58 to 0.93 while the coefficient of genetic similarity was based on three primer ranges 0.50 - 0.80.

Key words: Phenotypic, genotypic, Gulapasir Salacca, environment

1. Introduction

Bali salacca plants (*Salacca zalacca* var. Amboinensis) is an Indonesian indigenus commodity that has the potential to be developed, both to fulfill the needs of both domestic and export markets. In Indonesia, the salacca has specific advantages compared to other fruits, the fruit can be harvested 2-3 times a year when the management is good. Meanwhile, demand for fruits from other countries is quite high, and can never be fulfilled, because in order to fulfill domestic consumption are still lacking. Per capita consumption of fruits in 2008 was 1.64 kg capita^{-1}year^{-1}, and the salacca needs to reach 420,000 tons per year. This includes the need to export a number of 32.75 tons per year with the aim of Singapore, Hong Kong, Malaysia, and the rest to the needs of the domestic market either as fresh fruit or for processed products (Dimyati *et al.*, 2009). In line with public demand for fruits, fruits predicted demand will increase and potential to be developed as an agribusiness and agro-industries. In addition, the high genetic diversity of salacca allows the plant seed varieties developed to obtain (Ashari, 2002).

Gulapasir salacca are classified as the most superior (Wijana *et al*., 1993), meanwhile demand of the salacca continues to rise, on the other hand the market of Bali salacca continued to decline because of competition from Pondoh salacca. This prompted the

Bali province government to intensively plant Gulapasir, through a program of new plantings on or as a replacement crop Bali salacca (Diperta-Bali, 2009). At the beginning, the developments of the plant Gulapasir salacca were limited in Karangasem regency. Now, it has been extended to the Tabanan, Buleleng, Badung and Bangli regencies (Wijana *et al*., 1993).

Plants *Salacca zalacca* var. zalacca is a decoesis because of the inflorescence are male and female inflorescences on different plants so that the plants male inflorescence salacca will never produce any fruit. During this Salacca zalacca var. amboinensis monoesis classified as plants because it has separate male flowers and female flowers, but there is in one plant (Schuiling & Mogea 1992), so it can be developed by using seeds (Kriswiyanti *et al*., 2008; Darmadi *et al*., 2002), even known to perform sheath bloom before pollination (Guntoro *et al*., 1998; Rahayu *et al.*, 1999). The existence of the embryo in the ovule before blooming flowers and the absence of pollen to germinate on the stigma, then Bali salacca besides reproduction asexually by budding also with no fertilized seeds (agamospermi) (Kriswiyanti *et al*., 2008). It is not known whether this phenotypic property if developed into other areas is still the same as in the area of origin in Sibetan Karangasem.

This research aims to study the variability of phenotypic and genotypic of Gulapasir salacca plants growing on different environments in Bali. The study is expected to provide information and an overview of the phenotypic and genotypic variability Gulapasir salacca plants growing in different conditions in order to get the selection of the parent plant quality seeds.

2. Research Method

This Research began in March 2012 - October 2012 held at six locations were Telaga Sibetan (A-1) 450 m above sea level (asl), Kecing (A-2) 550 m asl, Jungutan (A-3) 670 m asl, Saribuana (T-1) 460 m asl, Pajahan (T-2) 570 m asl and Bangsing (T3) 700 m asl. Tools for phenotypic observation consisted of plastic bags, scissors, ruler, scale, crop sampling tool, and camera. Equipment for RAPD analysis consistsed of freezer, mortar, glass cup, erlenmayer, micro pipette tips eppendorf size and 5 ml, 20 ml, 100 ml and 250 ml, 1.5 ml tube size smaller and 0.5 ml, water bath, microwave, analytical balance, centrifuges, PCR thermolyne amplitron-1, electrophoresis equipment, UV transiluminator, camera. The plant material used for the six locations consisted of 42 plants with an estimated age of the plant between 8 years. RAPD analysis used representative of the population of young leaves from various locations. The material used was a solution of CTAB (cethyl trimethyl ammonium bromide) 10%, 1M Tris-HCl pH 8.0, EDTA (ethylenediamine tetraasetat) 0.5 M pH 8.0, 5 M NaCl, 70% cold ethanol, isopropanol cold, cold absolute ethanol, β-merkaptoetanol 1%, CI solution (chloroform: isoamilalkohol = 24: 1), liquid nitrogen, a solution of 3 M Na-acetate pH 5.2, polyvinyl-polypirollidone (PVPP), TE buffer (tris-HCl and EDTA), ethidium bromide. RAPD-PCR process using the kit consists of a complete brand Bioron buffer (containing MgCl 2), dNTPs, and enzyme taq polymerase. A primer for PCR-RAPD was OPA of the operon. Materials for the electrophoresis loading buffer (2.5% bromfenolblue: sucrose 40%), agarose, EtBr 1% (w / v), TBE buffer, and 1 kb marker.

An observations phenotypic refered to the book's Individual Guide Testing salacca species (Deptan, 2006). DNA analysis by RAPD technique consisted of several activities, namely the isolation, purification and determination of the quantity of DNA, primer selection and amplification reaction.
Performed following the method of DNA isolation procedures were performed by Pamidimarri *et al.* (2009). The salacca young leaves were cut and weighed as much as 0.5 g of the homogenized using a liquid nitrogen. Once smooth add 1 ml of extract buffer containing 2% PVP. The homogenate were added 50 ml β-merkaptoethanol and incubated at 65 °C for 60 minutes. The homogenate was then centrifuged at 8000 rpm at 4 °C for 10 minutes. The supernatant was separated from the pellet and added 1 : 1 PCI (phenol: chloroform: isoamyl alcohol) and then centrifuged at 8000 rpm at 25 °C for 10 minutes. 1.5 ml of the supernatant was put a new tube and added 1: 1 CI (chloroform Isoamyl alcohol) and then centrifuged at 8000 rpm at 25 °C for 10 minutes. The supernatant was put in a new tube and 1.5 ml plus 50 ml 5 M NaCl and 0.6 volumes of isopropanol and incubated for 1 hour at room temperature. The supernatant was added 500 ml 80% ethanol and incubation temperature of -20 °C for 1 hour. Then the obtained DNA was separated by centrifugation at a speed of 8000 rpm at 4 °C for 10 minutes. Discard the supernatant and pellet plus 500 ml of ethanol 70% centrifuged at 8000 rpm at 25 ° C for 10 minutes. Discard the supernatant and dry the pellet at 55 °C to ethanol odor disappeared. Pellets added with 50 ml of TE buffer pH 7.6 and store DNA samples at -20°

DNA samples obtained were electrophoresed on 0.8-1% agarose which was added 1 ml Ethidium bromide. A total of 3 ml of DNA was mixed with 2 ml of loading dye and incorporated into the gel wells and the wells are put on one of the DNA markers. Electrophoresis was performed at 100 volts for 30 minutes or tracking dye on line two of the bottom plate. Visualization results transiluminator electrophoresis using UV and photographed using a Polaroid camera. DNA concentration was determined by comparing the thickness of the DNA samples with DNA Marker.

PCR amplification reaction using 2x Master mix Solution (i-StarTaq) with the following composition: 8 ml ddH2O, 10 ml 2x PCR Master mix Solution (i-StarTaq), 1 ml primer OPA (10 mM) and 1 ml of DNA (25 ng / ml) and were included in the PCR tube (axygene). RAPD then performed with the following program: (1) Predenaturasi with temperature 95 °C for 5 min, (2) amplification reaction lasted for 45 cycles consisting of: denaturation at 95 °C for 1 min, annealing at a temperature of 36 °C for 1 min , extension 72 °C for 2 min, and (3) final extension at 72 °C temperature for 5 minutes. Primary Selection was based Nandariyah (2009) by using primer OPA showed some bands that appear (Table 1).

Table 1 Primary type and arrangement of bases that were used in the amplification reaction

No	Primary name	Base arrangement 5' → 3'
1	OPA 3	AGTCAGCCAC
2	OPA4	AATCGGGCTG
3	OPA 6	GGTCCCTGAC

No	Primary name	Base arrangement 5' → 3'
4	OPA 11	CAATCGCCGT
5	OPA 15	TTCCGAACCC
6	OPA 16	AGCCAGCGAA
7	OPA 17	GAC CGC TTGT
8	OPA 18	AGG TGA CCGT
9	OPA 19	CAA ACG TCGG

From the primary screening were then determined successfully amplifying DNA primers of salacca. Based on the data showed that the primary OPA3, OPA17 and OPA19 could amplify salacca leaf samples from six locations.

Phenotypic data analysis used Barlett test, the ratio between the variance with standard deviation and cluster analysis. The use of the Bartlett test was to determine homogeneity variety of samples taken from two or more populations. Implementation of the analysis carried out with the Minitab version 14. Decision-making based on the P- values were obtained. If the P value > 0.05 means a homogenous phenotypic character, otherwise if P value of <0.05 means that the character of the diverse phenotypic. Analysis of the phenotypic variance of data through comparison with a standard deviation performed on the measured variable phenotypic. Phenotypic variance calculated according to Steel and Torrie (1995) as follows:

$$\sigma^2 f = \frac{\sum X_i^2 - (\sum X_i)^2/n}{(n-1)}$$

Where: σ2f = phenotypic variance
Xi = the value of the average phenotypic to i
n = number of phenotypic tested.

Furthermore, the standard deviation of the phenotypic variance was calculated as Darajat, (1987) and Mansyah (2002):

$$Sd_{\sigma 2f} = \frac{\sqrt{\sigma 2f}}{(n-1)}$$

Criteria for assessment of the broad or narrow calculated according Mansyah (2002) as follows:
If $\sigma^2 f > 2\ Sd_{\sigma 2f}$ means that the wide phenotypic variability
If $\sigma^2 f < 2\ Sd_{\sigma 2f}$ means that narrow phenotypic variability
Decision-making was based on the two tests conducted with the criteria listed in Table 2.

Cluster analysis using the program NTSYSpc (Numerical Taxonomy and Multivariate Analysis) version 2.1 (Rohlf, 2000). NTSYSpc was a program used to acquire and demonstrate the structure of multivariate data, such as used on data from a sample derived from two or more different populations. Cluster analysis method selected SAHN (Sequential, Agglomerative, Hierarchical and Nested Clustering). The phenotypic similarity were used Dice coefficient with UPGMA method

(Unweight Pair Group Method Arithmetic) qualitative similarity function (SIMQUAL).

Table 2. Criteria of phenotypic variability based on Bartlett test and comparison of variance and standard deviation (Mansyah, 2002).

Bartlett test	Ratio of variance and standard deviation	Fenotipic Variability
Varied	Wide	Wide
Varied	Narrow	Nerrow
Highly significant varied	Wide	Wide
Highly significant varied	Narrow	Wide
Not varied	Wide	Nerrow
Not varied	Nerrow	Nerrow

For cluster analysis, each character is divided into sub-character. The determination of sub-characters was refered to Individual Testing Handbook (PPI) species of Salak (MOA, 2006). To determine the correlation between the characters phenotypic used a correlation analysis through comparative analysis using the NTSYS program function MXCOMP

The data obtained from the genotypic results RAPD gel was a DNA banding pattern of a certain size. The difference between the plants was shown on the banding pattern. If there was no difference between plant DNA banding pattern means there was no genetic variation and mean the opposite occurs when genetic variation. Assessment cluster analysis performed on the tape consistently clear and sharp. Band that appears along given a score of 1, and if not given a score of 0. Cluster analysis using UPGMA method with SIMQUAL function. To determine the correlation between praimer done through the program NTSYS correlation analysis using comparative analysis MXCOMP function. Genotypic similarity matrix was calculated based on the Dice coefficient with the formula:

$$S = \frac{2\,n\,ab}{na + nb}$$

Where : S = similarity coefficient
a and b = two compared individuals
n ab = number of DNA bands in the same position both individual a or b
na = number of individual DNA bands on a
nb = number of DNA bands in the individual b.

3. Results And Discussion

3.1. Phenotypic Observation based on Bartlett Test and Standard Deviation

The observation of 17 characters phenotypic of Gulapasir salacca of Karangasem and Tabanan regencies consisting of ten characters of quantitative and seven qualitative characteristics presented in Table 3. Results in Table 3 showed the comparison of

the value of the variance and standard deviation of the wide variation found in the ten quantitative characters were tested. Quantitative analysis of ten characters with Bartlett test showed a significant variance in the character of the length of flower without the sheath, number of fruit bunches^{-1} and thick flesh. Based on the merger of two test variance with Bartlett and standard deviation values showed three quantitative characters shows phenotypic variability criteria with a wide range of flower without covering sheath length, number of fruit bunches^{-1} and thick of fruit flesh. The third character indicates a high value on the salacca grown in Telaga Sibetan followed Kecing and Jungutan village.

Variations location not caused differences in qualitative characteristics which included color of leaf, midrib color, the color of thorns, flowers and fruit. However, the quantitative characters showed different values. The amount of fruit, the length of flowers and thick of flesh fruit was bigger and display found in Telage, Kecing and Jungutan, while the length of leaf found in Saribuana, Pajahan and Bangsing. Salacca planting location with altitude above 650 m asl, there was a decrease in the length of sheath, number of flowers bunches^{-1}, fruit number and fruit flesh thickness.

Table 3. Phenotypic characters (quantitative) based on Bartlett test and ratio of the variance (σ) with standard deviation (Sd)

Characters	Locations						Bartlett Test		Ratio σ dan Sd				
	A1	A2	A3	T1	T2	T3	X^2 hit.	P-value	$\sigma^2 f$	$Sd\sigma_{2f}$	$2Sd\sigma_{2f}$	C	CC
Number of leaflets	75.57	76.43	75	76.29	75.57	75	4.17 ns	0.525	3.231	0.044	0.11	L	S
Length of leaf (cm)	57.86	57.89	57.81	59.14	59.57	59.43	2.85 ns	0.724	6.827	0.063	0.127	L	S
The Width of leaf (cm)	3.74	3.86	3.81	3.73	3.7	3.63	0.30 ns	0.908	0.161	0.011	0.021	L	S
The length of sheath (cm)	27.57	27.59	27.21	26.27	26.27	25.76	0.69 ns	0.632	8.198	0.068	0.136	L	S
The length of flower without sheath (cm)	12.81	12.76	12.74	12.64	12.69	12.54	11.84*	0.037	0.086	0.012	0.014	L	L
Number of flowers bunches $^{-1}$	1.86	1.71	1.71	1.57	1.57	1.43	0.21 ns	0.958	0.333	0.014	0.028	L	S
The number of fruit bunches^{-1}	21.29	20.57	18.57	18.29	19.29	16.86	21.21 **	0.001	5.93	0.059	0.121	L	L
The number of seed fruit^{-1}	1.57	1.57	1.14	1.57	1.57	1.14	0.64 ns	0.671	0.251	0.012	0.024	L	S
Thick flesh fruit (cm)	0.69	0.64	0.57	0.61	0.61	0.4	15.60 **	0.008	0.013	0.003	0.005	L	L
Ratio L/D	0.63	0.59	0.81	0.73	0.7	0.61	5.40 ns	0.369	0.01	0.002	0.004	L	S

Remarks: A= Karangasem; A1=Telaga; A2=Kecing; A3 =Jungutan; T= Tabanan: T1= Saribuana; T2=Pajahan; T3 = Bangsing; Ckl= Brown; hj = green; htm = black; mm = pink;ns = not significant * = significant; ** = High significant; C= Criteria; CC= Combined criteria ; L= Wide;S= Narrow

3.2. Phenotypic Variation based on Cluster Analysis

Cluster analysis of the phenotypic character in six different locations, the range of values obtained coefficient 0:58 - 0.93. At the level of similarity of 58.62% was

obtained two different groups, namely the group consisting of salacca from Telaga-Sibetan (A1) Kecing (A2), Jungutan (A3), Sarinbuana (T1) and Pajahan (T2) with a distinctive thick fruit size, fruit number and length of flower. Into two groups at the similarity level of 62.06% was made up of salacca from Bangsing (T3), salacca of Saribuana (T1), salacca of Kecing (A2), Salacca of Jungutan (A3) and Pajahan (T2). In this group of prominent characteristic lies thick on the size of the fruit, flower length, number of fruits. Salacca from Bangsing (T3) the size of the characters were thinner and shorter. At the rate of 72.41% the similarity consisted of salacca from Saribuana (T1), Kecing (A2), Jungutan (A3) and Pajahan (T2). Prominent features in this group were the size of a thick fruit, flower length and number of medium-sized fruit (Figure 1).

From the dendrogram was known that grouping based on similarity phenotypics in addition to accession also based on location. Plants of the same height were likely to join the same group, as shown by the salacca coming from the Kecing (A2) and the salacca of Jungutan (A3), as well as the salacca of Saribuana (T1) and the salacca of Pajahan (T2). In line with this research, Mansyah (2002) reported the mangosteen plant phenotypics from the same location was in group, this could be caused by environmental influences at each location.

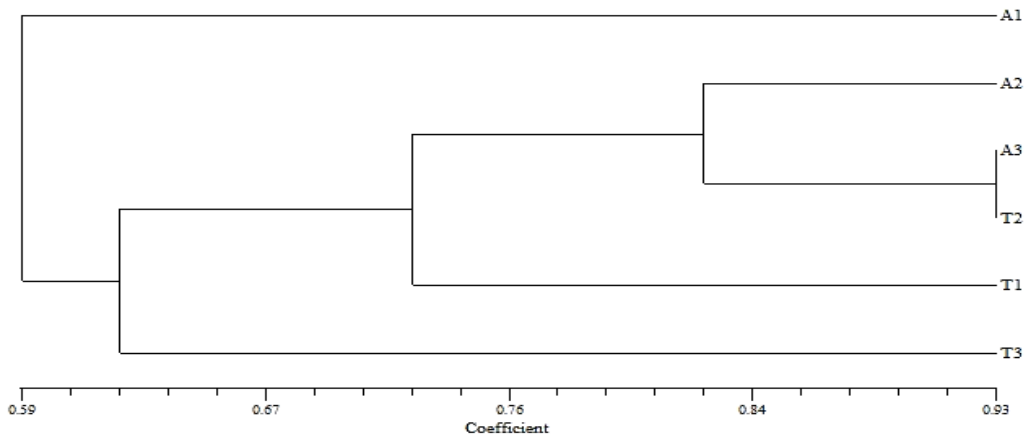

Figure 1. Phenotypic Dendrogram of Gulapasir salacca from six different locations (A= Karangasem: A1=Telaga; A2=Kecing; A3 =Jungutan; T= Tabanan : T1= Saribuana; T2=Pajahan; T3 = Bangsing)

Dendogram showed that the grouping based on morphological phenotypic (Figure 1), Gulapasir salacca grown in Karangasem and Tabanan in different locations showing the variation in phenotypic with phenotypic similarity level 58.62% -93.10%. The occurrence of phenotypic variability in a population of one type of living thing in nature could be caused by hybridization, mutation and gene flow naturally from the kind of living creature that was the same or different. From the cluster analysis were known, besides accession grouping based on similar phenotypic was also based on location. Plants from the adjacent heights tend to merge into the same group, as shown by the salacca coming from Kecing and Jungutan, as well as from from Pajahan and Saribuana. The results of this study were consistent with findings Mansyah *et al.* (2003) on mangosteen and Rai *et al.* (2008) on wani plants.

3.3. Genotypic of Gulapasir salacca Based on RAPD Analysis

The results of the analysis showed that of the nine RAPD primers used three DNA primer capable to amplify. The third primary was OPA3, OPA17 and OPA 19. Primary OPA3 produce the highest number of 15 bands with number 11 or the percentage of polymorphic 73.33% polymorphic and monomorphic band consisted of 4 monomorphic or monomorphic percentage (26.66%). OPA17 the number 8 pieces all were polymorphic and OPA19 amounted 5 of bands, were polymorphic (Table 4). The mean level of polymorphism of the three primary was 85.71%. According to McGregor *et al*. (2000), polymorphism was a picture difference amplification of DNA fragments obtained were observed and scored as the presence or absence of sequence differences indicating the presence of variations. Banding pattern resulted from the three primary amplification were presented in Figure 2.

Based on the interpretation of amplification with OPA-03, number of bands that appear numbered 8-10 tape with salacca samples of Kecing (A2) and Saribuana (T1) resulted in the highest band, respectively 10 Interpretation of the diversity of the DNA banding pattern indicated a diversity of band patterns on Gulapasir salacca planted in different areas, namely the size of the 4000 bp band only owned salacca of Telaga (A1), and the size of the thick bands of Karangasem salacca were thicker than the Tabanan salacca. This was most likely due to the competition primer template DNA that caused one of the fragments amplified in large quantities and other fragments amplified in small amounts, so that only a few were detected as a band after amplification (Williams *et al*., 1990).

Table 4. Level of polymorphism of three primers used based on the pattern of DNA bands of Gulapasir salacca of six different locations.

Primer	nucleotide sequences 5'……3'	Total number of bands	The number of polymorphic	The number of monomorphic
OPA 3	AGT CAG CCAC	15	11 (73.33%)	4 (26.66%)
OPA 17	GAC CGC TTGT	8	8 (100%)	0
OPA 19	CAA ACG TCGG	5	5 (100%)	0
Total		28	24 (85.71%)	4 (14.28%)

Based on the dendrogram in Figure 3 was known, the degree of similarity accessions ranged from 50.74% - 80.00%. Grouping at the level of 50.74% similarity accession was devided into two groups. The first group was derived from the Karangasem salacca, which consisted of 3 accession salacca of Telaga-Sibetan (A1), Kecing (A2), Jungutan (A3), and the second group consists of the Tabanan were Saribuana salacca (T1), Pajahan (T2) and Bangsing (T3). At a higher level of similarity that was 56.66%, the first group was divided into two groups: A1 and A2, A3, while the second group at 76.66% similarity level was divided into two T3, and T2, T1.

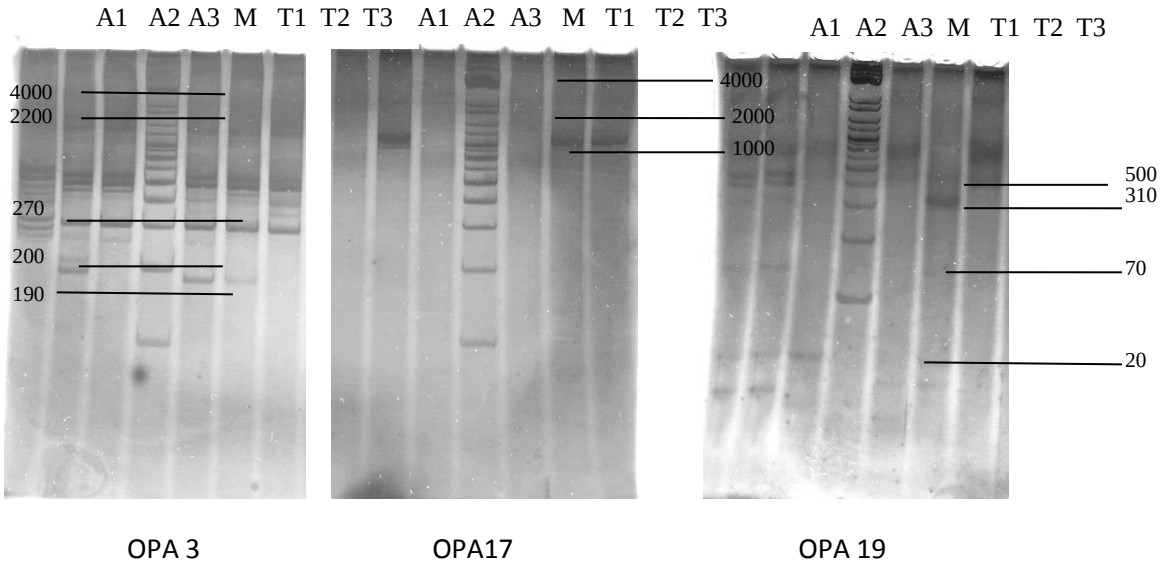

Figure 2. DNA banding pattern of salacca from various locations Based on 3 random primer: OPA3, OPA17, OPA Description: M = Marker 1 kb, A = Karangasem: A1 (Telaga), A2 (Kecing), A3 (Jungutan), T = Tanbanan : T1 (Saribuana), T2 (Pajahan), T3 (Bangsing).

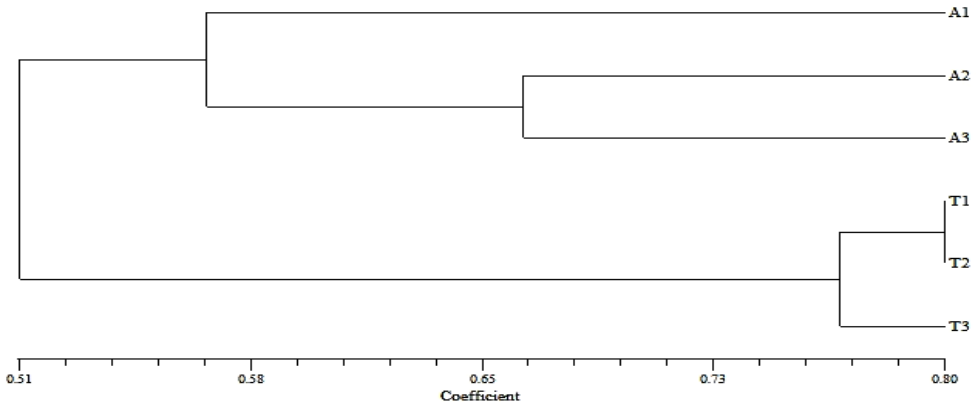

Figure 3. Dendrogram DNA banding pattern of Gulapasir salacca from six different locations (A = Karangasem; A1 = Telaga; A2 = kecing; A3 = Jungutan, T = Tabanan: T1 = Saribuana; T2 = Pajahan; T3 = Bangsing)

Figure 3 showed the salacca coming from adjacent locations tend to merge into one group which was like the salacca of Kecing (A2) and Jungutan (A3), Saribuana (T1) and Pajahan (T2). Figure dendrogram and correlation matrix showed the grouping of Gulapasir salacca from Tabanan having the same degree of genetic similarity. This might be due to the seed that was planted in Tabanan was not derived from Telaga (A1), Kecing (A2) and Jungutan (A3) or salacca may be planted in Tabanan came from local farmers who came from same environments and elders, so as to have a close resemblance level. Instead, Karangasem salacca (A1, A2 and A3) had further similarity level with Gulapasir salacca of Tabanan derived from Telaga (A1). This

might be due to these plants originated from different elders. In accordance with the statement Cahyarini *et al.* (2004), said to be much similarity when less than 0.6 or 60%. From these groupings that gave meaning Gulapasir salacca grown in Telaga-Sibetan showed different characters with other locations. It could be explained that the salacca samples taken from the Telaga-Sibetan Karangasem were the parent plants which had been certified by the Department of Agriculture Karangasem regency for use as a source of seed, so that the superior properties were more dominant than the Gulapasir salacca from another location, either the number of bunches fruit, length of flowers, and thick fles fruit.

Conclussion and Suggestion

The results of this study concluded that the Gulapasir salacca planted in Tabanan and Karangasem showed phenotypic and genotypic variation. Phenotypic similarity coefficient based on ten quantitative characters ranged from 0.58 - 0.93 and the coefficient of genetic similarity based on three primary ranges from 0,50 - 0.80 which was divided into two main groups, namely groups of Karangasem and Tabanan Gulapasir salacca. For the program of expansion of Gulapasir salacca planting in new development areas, the selection of mother plants for seed candidates were advised to take the seeds from the plants that were already adapted to the local environment. To reduce variation in plant propagation Phonotype of Gulapasir salacca was done vegetatively by grafting system or by tissue culture techniques.

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