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As

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

Dr. Drs./I Made Sukamerta, M.Pd.

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PROCEEDINGS

ICSD 2015

2nd International Conference on Sustainability Development



PROCEEDING

**2nd International Conference on
Sustainable Development (ICSD)**

“Global Sustainable Development”

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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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TABLE OF CONTENTS

PREFACE.....	iii
TECHNICAL ADVISORY COMMITTEE	iv
ORGANIZING COMMITTEE.....	v
TABLE OF CONTENTS.....	vii
COMMITTEE REPORT	xvi
OPENING SPEECH.....	xviii

KEYNOTE SPEAKERS

CORPORATE SOCIAL RESPONSIBILITY IN SUSTAINABLE OIL AND GAS INDUSTRY: THE PETRONAS INITIATIVE <i>DA Bakar and NA Aziz</i>	<i>1-14</i>
GLOBAL SUSTAINABILITY DEVELOPMENT: REPOSITION OF SOCIAL AND CULTURE OF <i>KOLOK</i> PEOPLE <i>Sundani Nuroso Soewandhi.....</i>	<i>15-16</i>
SLUGGISH BUT STRENGTHENING: INDONESIA'S DECLINING DISECONOMIES OF AGGLOMERATION <i>Jennifer Day.....</i>	<i>17-31</i>

TRACK 1. REGIONAL PLANNING, AGRICULTURE, AND ENVIRONMENT SUSTAINABILITY

DIMENSIONAL ANALYSIS ON THE POLICY IMPLEMENTATION OF ECOTOURISM DEVELOPMENT IN BOGANI NANI WARTABONE NATIONAL PARK, GORONTALO PROVINCE <i>Irwan Bempah, E.K.S. Harini Muntasib, Arzyana Sunka, and Rinekso Soekmadi</i>	<i>33-43</i>
SOIL IMPROVEMENT OF NICKEL POST MINING WITH SAGO WASTE TREATMENT <i>Lies Indriyani , Hasbullah Syaf, and Arsy Aysyah Anas.....</i>	<i>44-52</i>
THE CONSERVATION OF ENDEMIC AND ENDANGERED SPECIES <i>KALAPPIA CELEBICA</i> KOSTERM THROUGH CUTTINGS PROPAGATION AND AMF POTENTIAL ASSESSMENT <i>Asrianti Arif, Faisal Danu Tuheteru, and Husna.....</i>	<i>53-65</i>
QUALITY OF MINING GOLD TAILING CONCRETE:CONSISTENCY AND STRENGTH PROPERTIES <i>Amalia and Murdiyoto.....</i>	<i>66-73</i>
SUSTAINABLE TOURISM DEVELOPMENT IN BALI COASTAL AREAS TO BE CREATIVE DESTINATION <i>I Made Bayu Wisnawa, I Ketut Sutapa, and Luh Komang Chandra Dewi.....</i>	<i>74-88</i>
THE INFLUENCE OF COMPOST ON GROWTH AND PRODUCTION OF LOCAL CASSAVA TYPE <i>Sofyan Samad, Abd Wahab Hasyim, Hamidin Rasulu, and Hasbullah</i>	<i>89-93</i>

THE POTENCY OF ETTAWAH DESCENDANT GOAT FECES THAT FED IN DIFFERENT LEVEL OF CONCENTRATE AND FORAGE DIETS AS A SOURCE OF SUISTANABLE ORGANIC FERTILIZER <i>Anak Agung Ngurah Badung Sarmuda Dinata, Anastasia Sischa Jati Utami, and I Wayan Sudarma</i>	94-102
THE DISPARITY ANALYSIS OF DEVELOPMENT BETWEEN REGENCY IN THE EX KARESIDENAN MADIUN, PROVINCE OF EAST JAVA <i>Eko Wahjudi and Hendry Cahyono</i>	103-112
THE IMPACT OF TO BALI TOLL ROAD TO THE ECONOMY OF THE BALINESE COMMUNITY <i>I Putu Astawa</i>	113-116
MOSAIC DISEASE: AS A CHALLENGE FOR SOYBEAN PRODUCTION IN SOUTHEAST SULAWESI <i>Muhammad Taufik, Gusnawaty HS, Asmar Hasan, and Muhammad Danial Rahim</i>	117-124
FACILITATION OF BROILER CHICKEN FARMING BASED ON LOCAL RESOURCES FOR THE COMMUNITY OF KABARUAN SUBDISTRICT, TALAUD ISLANDS, NORTH SULAWESI <i>Revolson Alexius Mege, Josephine Louise Pinky Saerang, Jouke Hendrik Manopo, and Alfonds Andrew Maramis</i>	125-133
EFFECT OF CELL-WALL NITROGEN PROPORTION ON PROTEIN UTILIZATION BY RUMINANT LIVESTOCK: A META-ANALYSIS ACROSS DIFFERENT EXPERIMENTS <i>Sari Putri Dewi, Muhammad Ridla, and Anuraga Jayanegara</i>	134-139
THE STRAWBERRY FRUIT CULTIFATION AS AN AGRO TOURISM AT BEDUGUL (CONCERNING TO SUSTAINABLE TOURISM DEVELOPMENT) <i>Solihin and I Ketut Sadia</i>	140-148
ISOLATION AND IDENTIFICATION OF METHANOTROFIC BACTERIA FROM IRRIGATION RICE FIELD IN GOWA, SOUTH SULAWESI, INDONESIA <i>Maimuna Nonci, Baharuddin , Burhanuddin Rasyid, and Pirman</i>	149-155
AN ANALYSIS ON THE PRODUCTIVITY LEVEL OF ARABICA COFFEE IN BALI <i>I Made Kartika and I Made Darsana</i>	156-165
SCREENING OF EXOPOLYSACCHARIDE PRODUCING BACTERIAL FROM POTATO RHIZOSFER ON SEVERAL SOURCES OF CARBON <i>Mu'minah, Baharuddin, Hazarin Subair, and Fahrudin</i>	166-172
THE LIGNOCELULOTIC POTENTIAL OF ROT FUNGAL TO DECOMPOSITION WASTE OF COCOA POD LEATHER <i>Iradhatullah Rahim, Tutik Kuswinanti , Laode Asrul , and Burhanuddin Rasyid</i>	173-179

THE DESIGN OF ECONOMIC, SOCIAL, AND ENVIRONMENTAL PERFORMANCE MEASUREMENT SYSTEM FOR INDUSTRIAL SUSTAINABILITY <i>Ahmad Mubin</i>	180-186
IMPROVEMENT OF PHYSICAL AND CHEMICAL SOIL RAINFED BIOCHAR THROUGH GIVING LAND IN EFFORTS TO INCREASE PRODUCTIVITY <i>I Putu Sujana, I Made Suryana, and I Nyoman Labek Suyas dipura</i>	187-193
THE EFFECTIVENESS OF CAR-FREE DAY AS AN ALTERNATIVE OF PUBLIC SPACES <i>I Gusti Ayu Andani and Cokorda Javandira</i>	194-201
THE ROLE OF AGRICULTURAL SECTOR IN THE ECONOMY IN SOUTH BALI <i>I Ketut Arnawa, Dian Tariningsih, and Luh Kadek Budi Martini</i>	202-210
DAILY ACTIVITIES JAVA DEER (CERVUS TIMORENSIS) IN CAPTIVITY <i>Deden Ismail</i>	211-218
PATHOGENICITY TEST AND THE INHIBITION OF BACCTERIAL ISOLATES OF BACILLUS SP. AGAINST FUSARIUM OXYSPORUM CAUSING WILT DISEASE IN PLANTS SOLANACEAE <i>I Ketut Widnyana</i>	219-226
PHENOTYPIC AND GENOTYPIC OF SALAK (SALACCA ZALACCA VAR. AMBOINENSIS) CV. GULAPASIR ON DIFFERENT GROWING ENVIRONMENTS <i>I Ketut Sumantra</i>	227-237
ANALYSIS OF WATER QUALITY CHANGES IN THE PAKERISAN WATERSHED <i>Deden Ismail, I Gusti Ayu Andani, and Ketut Sumantra</i>	238-248
STUDY ON CATTLE FODDER AVAILABILITY TO SUPPORT THE DEVELOPMENT OF BALINESE CATTLE IN BALI <i>IGN Alit Wiswasta, I Ketut Widnyana, and Bagus Putu Udiyana</i>	249-255
PLANTING TIME ON THE DRY LAND AT SOUTH OF BALI <i>I Made Sukerta and Bagus Putu Udayana</i>	256-265
EROSION CONTROL MODEL AND WATERSHED MANAGEMENT (DAS) IN SOIL CONSERVATION EFFORTS AND REHABILITATION OF CRITICAL LAND IN BALI <i>I Dewa Nyoman Raka, Putu Nirlam Sucika, I Made Nada, and IGN Alit Wiswasta</i>	266-278
TRADITIONAL TECHNOLOGY ON PADDY RICE PLANTING TO YIELD IMPROVEMENT IN MERTASARI FARMER GROUP IN TABANAN REGENCY <i>Bagus Putu Udiyana and Farida Hanum</i>	279-282
INCREASING BALI CATTLE PRODUCTIVITY WITH WASTE MATERIAL TO IMPROVING FOOD SECURITY <i>Anastasia Sischa Jati Utami, Anak Agung Ngurah Badung Sarmuda Dinata, and I Nyoman Suyasa</i>	283-290

GROWTH AND PRODUCTION OF SUPERIOR NEW RICE VARIETIES (<i>INPARI 7</i> AND <i>INPARI 10</i>) ON DIFFERENT PLANTING SYSTEM <i>Putu Suratmini, S. N. Aryawati, I.B. Aribawa and A.A.N.B.Kamandalu</i>	291-297
WASTE UTILIZATION OF AGRICULTURE TO IMPROVE PRODUCTIVITY BALI CATTLE IN SUPPORTING SYSTEM OF SUSTAINABLE AGRICULTURE <i>N. Suyasa and IAP.Parwati</i>	298-305
SUPPORT PROGRAM <i>SIMANTRI</i> (INTEGRATED AGRICULTURE SYSTEM) IN THE PROVISION OF ORGANIC FERTILIZER ON THE DEVELOPMENT OF ORGANIC COFFEE ARABICA DIRECTION (CASE VILLAGE CATUR, BANGLI) <i>Ida Ayu Parwati and N. Suyasa</i>	306-313
EFFECTIVENESS OF DIRECT SEED SOWING SYSTEM AND BALANCED FERTILIZER ON RICE PRODUCTIVITY <i>Ni Putu Pandawani, I Made Diarta, and I Gede Putra Cahyadi</i>	314-323
REVIEW OF SUPPLY CHAIN OF RICE AND BULOG'S FUNGCTIONS IN INDONESIA <i>Kuntoro Boga Andri, Ni Putu Sutami, and I Made Londra</i>	324-333
STUDY ON DAIRY COOPERATIVE DEVELOPMENT IN INDONESIAN <i>Kuntoro Boga Andri and I Made Londra</i>	334-344
PERFORMANCES OF PRODUCTION AND REPRODUCTION OF BALI COWS IN PADANGBULIA VILLAGE, SUKASADA DISTRICT, BULELENG REGENCY <i>I Made Londra, Kuntoro Boga Andri, and Putu Sutami</i>	345-354

TRACK 2. SOCIAL AND COMMUNITY EMPOWERMENT

THE EMPOWERMENT OF SMALL SCALE FOOD INDUSTRY OF DRIED BANANA BY INTRODUCING A SOLAR DRIER OF COPULA MODELS <i>I Wayan Sweca Yasa, Nazaruddin, and Sukmawaty</i>	356-360
ECONOMIC TRANSFORMATION MODEL OF BLIMBING SARI COMMUNITY <i>I Wayan Ruspendi Junaedi</i>	361-372
EMPOWERMENT INFORMAL SECTOR TO DEVELOP FOOD SECURITY THROUGH LOCAL FLOUR - BASED FOOD INDUSTRY <i>Meylia Elizabeth Ranu</i>	373-379
TAROT CARDS AS TOOLS FOR ILLUMANATION AND BETTER UNDERSTANDING OF THE MEANING OF LIFE <i>I Gusti Made Wendri</i>	380-389

EMPOWERMENT INTELLECTUAL PROPERTY RIGHTS FOR CREATIVE BUSINES OPPORTUNITY TO INCREASED REVENUE <i>Budi Hermono</i>	390-396
WOMEN AS SOUVENIR VENDORS: AN EFFORT TO THE ACHIEVEMENT OF GENDER EQUALITY THROUGH THE STRENGTHENING OF THE ECONOMIC BASE OF THE FAMILY <i>Ni Made Ary Widiastini</i>	397-407
OPPORTUNITIES AND THREATS: DEVELOPING ADVENTURE TOURISM BASED ON QUALITY OF ENVIRONMENT AND SOCIO-CULTURE IN PANJI VILLAGE <i>Nyoman Dini Andiani and Made Ary Widiastini</i>	408-414
WOMEN'S CONTRIBUTION TO INCREASE INCOME AT COMMUNITY FISHING TEMPE LAKE IN WAJO REGENCY <i>Haerunnisa, Sahriah Rahim, and Andi Siswati</i>	415-432
THE FULFILMENT LEVEL OF TRANSMIGRANT HOUSEHOLDS' BASIC NEEDS IN THE DISTRICT OF LADONGI, REGENCY OF KOLAKA, PROVINCE OF SOUTHEAST SULAWESI <i>Aylee Christine</i>	433-440
THE ROLE FOOD SECURITY FOR PUBLIC CONSUMPTION COMPLIANCE IN MAROS REGENCY, SOUTH SULAWESI PROVINCE <i>Suryawati Salam and Andi Gusti Tantu</i>	441-448
GLOBALIZATION AND REINFORCEMENT OF DAE: A REVIEW OF CULTURAL STUDIES OF DEVELOPMENT OF TOURISM IN BALI <i>I Wayan Winaja</i>	449-455
COMMUNITY DEVELOPMENT THROUGH LOCAL POTENCY SUPPORTS GOVERNMENT PROGRAM OF ONE VILLAGE ONE PRODUCT <i>I Ketututama, I Gede Mudana, and I Made Sukamerta</i>	456-466
TOURISM, GLOBALIZATION, AND GENDER IN BALI <i>I Gede Mudana, I Ketututama, I Made Sukamerta, and Ni Wayan Ardini</i>	467-472
COMMUNITY EMPOWERMENT THROUGH FACILITATION OF SOYBEAN FARMING IN KABARUAN SUBDISTRICT, TALAUD ISLANDS, NORTH SULAWESI <i>Alfons Andrew Maramis, Revolson Alexius Mege, Josephine Louise Pinky Saerang, Jouke Hendrik Manopo</i>	473-481
ECONOMIC EMPOWERMENT FOR EDUCATIONAL FEMALE IN FARMING AREAS : A CASE STUDY OF TABANAN REGENCY <i>Anik Yuesti</i>	482-486
APPLICATION OF SCIENCE TECHNOLOGY AND ARTS FOR OFFERING MAKERS <i>I Ketut Wardana and Anak Agung Yudi Pramaswati</i>	487-496

PERFORMANCE OF CAPTURE FISHERIES IN RESPECT TO PROGRAM OF ECONOMIC EMPOWERMENT OF COASTAL COMMUNITIES IN KARANGASEM REGENCY: PRODUCTION FUNCTION STOCHASTIC FRONTIER APPROACH <i>I Made Tamba</i>	497-502
THE APPLICATION OF SCIENCE AND TECHNOLOGY ON SCHOOL-OUTDOOR EDUCATION IN BUDUK VILLAGE, BADUNG REGENCY <i>I Gusti Agung Putri Wirastuti, I Ketut Wardana, Anak Agung Istri Yudhi Pramawati</i>	503-510
ECOPRENEURE PERSPECTIVE ON GROUP PELITA BALI DENPASAR (APPLIED SCIENCE AND TECHNOLOGY ON PLASTIC WASTE RECYCLING GROUP) <i>Anak Agung Dwi Widyani and Ni Wayan Rustiarini</i>	511-525
REPRESENTATION OF LESBIAN, GAY, AND TRANSGENDER IN <i>PENJARA</i> ANTHOLOGY OF SHORT STORY: A SOCIOPRAGMATIC OBSERVATION <i>Nyoman Deni Wahyudi and Luh Ketut Sri Widhiasih</i>	516-524
EXISTENCE OF THE CONCEPT OF <i>TRI HITA KARANA</i> IN GUARANTEERING RIGHTS OF (LESBIAN, GAY, BISEXUAL, TRANSGENDER (LGBT) PEOPLE <i>I Wayan Gde Wiryawan and I Made Hendra Wijaya</i>	525-532
WOMEN AS SOUVENIR VENDORS: AN EFFORT TO THE ACHIEVEMENT OF GENDER EQUALITY THROUGH THE STRENGTHENING OF THE ECONOMIC BASE OF THE FAMILY <i>Ni Made Ary Widiastini</i>	533-537
THE ROLE OF LOCAL CREDIT INSTITUTIONS IN EMPOWERING COMMUNITY'S ECONOMY:STUDY BASED ON SOCIAL CAPITAL <i>I Gede Cahyadi Putra and I Gusti Ngurah Bagus Gunadi</i>	538-549
WOMEN GROUP EMPOWERMENT THROUGH LIFESKILL TRAINING <i>I Made Suryana and Ida Bagus Widiadnya</i>	550-554
COMMUNITY ENGAGEMENT BASED ON SCHOOL AWARENESS ON SUSTAINABLE DEVELOPMENT <i>Ida Bagus Suryatmaja and I Made Nada</i>	553-556
APPLIED SCIENCE AND TECHNOLOGY ON WARNASARI AND TUKADAYA VILLAGE, JEMBRANA DISTRICT, BALI PROVINCE IN SECOND YEAR <i>I Made Legawa, Tri Djoko Setyono, I Made Sudiana, I Ketut Widnyana, and Ni Wayan Rustiarini</i>	557-561
APPLIED SCIENCE AND TECHNOLOGY FOR THE LOCAL CREDIT INSTITUTION IN BATUAJI KAWAN VILLAGE, TABANAN, BALI <i>I Nyoman Putra Yasa and I Gede Cahyadi Putra</i>	562-571

TRACK 3. ECONOMY AND MANAGEMENT

IMPACT OF CREDIT ON FARMER HOUSEHOLD WELFARE IN INDONESIA <i>Made Wahyu Adhiputra</i>	573-581
ECONOMIC AND SUSTAINABLE DEVELOPMENT <i>Made Antara and Made Sri Sumarniasih</i>	582-594
THE ACCOMODATION THAT DOES NOT SUPPORT SUSTAINABLE TOURISM DEVELOPMENT (CASE STUDY IN UBUD TOURISM AREA) <i>A.A.A. Ngurah Harmini and Nyoman Mastiani Nadra</i>	595-603
RECRUITMENT PROCESS AS AN IMPORTANT STEP FOR SUSTAINABLE ORGANIZATIONAL DEVELOPMENT <i>Aria Andriyadi and Anggraini Sukmawati</i>	604-607
THE INFLUENCE OF ACADEMIC CLIMATE ON UNIVERSITY STUDENTS' BRAWLING THROUGH LOCUS OF CONTROL <i>Sukma Nurilawati Botutihe</i>	608-618
CUSTOMER RELATIONSHIP MANAGEMENT (CRM) AND APPLICATION IN EDUCATIONAL INSTITUTIONS <i>Putu Astri Lestari</i>	619-626
EFFECTIVE LEADERSHIP STYLE IN IMPROVING STAFF MOTIVATION AND SUSTAINABEL SERVICE EXCELIENT IN HIGHER EDUCATION <i>Inten Pertiwi</i>	627-634
TAX COMPLIANCE AND TAX ADMINISTRATION <i>I Nyoman Kusuma Mahaputra</i>	635-639
FINANCIAL AND NON FINANCIAL FACTORS THAT AFFECT THE COMPANY GETS GOING CONCERN AUDIT OPINION <i>Ni Nyoman Ayu Suryandari</i>	640-649
GOING CONCERN OPINION AND AUDITOR CHANGES: THE ROLE OF AUDIT COMMITTEE IN INDONESIA <i>Luh Komang Merawati</i>	650-658
PROFIT INTERPRETATION: TRADITIONAL SELLERS IN DENPASAR (STUDY HERMENEUTIKA INTENSIONALISME) <i>Putu Kepramareni</i>	659-663
BRAND COMMUNITY AS AN EFFECTIVE MEANS OF POSITIVE WORD OF MOUTH <i>Agus Wahyudi Salasa Gama, Ni Wayan Eka Mitariani, and Gede Gama</i>	664-670
SERVICE EXCELLENCE IS A KEY FOR DEVELOPING SUSTAINABLE RESTAURANT BUSINESS <i>I Nyoman Winia and I Ketut Redjasa</i>	671-678
FOOD AND BEVERAGE DEPARTMENT IS AS A HERO IN GETTING PROFIT FOR HOTEL DEVELOPMENT IN ASEAN ECONOMY COMMUNITY (AEC) ERA AND IN FREE TRADING ERA THIS YEAR IN 2015 <i>I Ketut Redjasa</i>	679-684

BUDGET AND PROFITABILITY ANALYSIS AS A MANAGEMENT TOOLS TO MAXIMIZE PROFIT AT THE CHEDI CLUB TANAH GAJAH UBUD HOTEL <i>I Ketut Sugiarta</i>	685-690
THE EFFECT OF NATIONAL SOCIAL SECURITY ON THE EMPLOYEES PERFORMANCE OF THE CHEDI CLUB HOTEL TANAH GAJAH UBUD BALI <i>I Gusti Ayu Hayatti Yowani</i>	691-695

TRACK 4. HEALTH AND EDUCATION

THE EFFECT OF JIGSAW II – STAD AND SELF-CONFIDENCE ON STUDENTS’ SPEAKING SKILL <i>Dewa Ayu Ari Wiryadi Joni</i>	697-707
THE ADJUSTMENT MADE IN THE TRANSLATION OF ENGLISH MONOTRANSITIVE CLAUSE INTO INDONESIAN: A SOCIOSEMIOTIC APPROACH TO TRANSLATION <i>A.A. Istri Yudhi Pramawati</i>	708-719
SLOW DEEP BREATHING REDUCES HIGH BLOOD PRESSURE IN HYPERTENSIVE PATIENTS <i>Dame Elysabeth, Sedia Simbolon, and Belet Lydia</i>	720-725
HEALTH EDUCATION OF PRE- HOSPITAL ASSESSMENT FAST (FACE, ARM, SPEECH, TIME) IMPROVES CADRES KNOWLEDGE ON EARLY DETECTION OF STROKE <i>Dame Elysabeth, Sedia Simbolon, and Belet Lydia</i>	726-731
THE EFFECT OF I-SEARCH AND SELF-EFFICACY ON STUDENTS IN EFL ACADEMIC WRITING <i>Anak Agung Putri Maharani</i>	732-742
DIDACTICISM IN VERBAL ART: A CASE WITH THE POEMS “PROMISE”, “MENGHADAPI MAUT”, AND “LUH” <i>I Wayan Resen</i>	743-756
POWER OF MEDITATION AS AN ENERGY GENERATOR THE HUMAN SPIRITUALITY (PERSPECTIVE HINDUISM THEOLOGY) <i>Pande Wayan Renawati</i>	757-765
THE DEVELOPMENT OF EARLY CHILDHOOD EDUCATION IN SEMARANG CITY <i>Siti Hasanah and Wildana Latif Mahmudi</i>	766-775
THE HEALTHY AND HYGIENIC BEHAVIOR: ANALYTICAL FROM SOCIAL CONSTRUCTION PERSPECTIVE <i>Muria Herlina</i>	776-790
PRINCIPALS’ ROLE IN INCREASING TEACHER JOB SATISFACTION <i>Grace Jenny Soputan</i>	791-797

PRODUCTION OF PHOSPHATE (P) FROM FUNGAL ISOLATES COLLECTED FROM RHIZOSPHERE OF AROMATIC RICE TANATORAJA <i>Abri, Tutik Kuswinanti, Enny Lisan Sengin, and Rinaldi Sjahir</i>	798-802
LOCAL CULTURE BASED MODEL AND CONCEPT IN GENETICS LEARNING AS THE EFFORT TO ENHANCE UNDERSTANDING AND CULTURE PRESERVATION <i>Gusti Ayu Dewi Setiawati</i>	803-813
THE EFFECT OF MODIFIED COLLABORATIVE STRATEGIC READING AND VOCABULARY MASTERY ON THE READING COMPETENCY OF THE SECOND SEMESTER STUDENTS OF ENGLISH EDUCATION STUDY PROGRAM OF MAHASARASWATI DENPASAR UNIVERSITY <i>Paramita Dharmayanti, P. A.</i>	814-825
ROLE PLAY IN SPEAKING CLASS <i>Luh Ketut Sri Widhiasih and Nyoman Deni Wahyudi</i>	826-832
ENGLISH LEARNING ACTIVITIES FROM SCRAP PAPERS; PUTTING ECO-PEDAGOGY INTO PRACTICE <i>Ni Luh Putu Dian Sawitri</i>	833-836
FUNCTIONS AND MEANINGS OF <i>GENJEK KADONG ISENG</i> IN KEEPING SOCIAL LIFE SUSTAINABILITY <i>Ida Bagus Nyoman Mantra</i>	837-843
THE IMPROVEMENT OF ENGLISH SPEAKING SKILL THROUGH <i>TRI PREMANA</i> BASED LEARNING AT THE ENGLISH STUDY PROGRAM OF FKIP UNMAS DENPASAR <i>I. A. Md Sri Widiastuti and I. B. N Mantra</i>	844-849
ENGAGING STUDENTS THROUGH DEMOCRATIC APPROACH <i>I Gde Putu Agus Pramerta</i>	850-861
SOCIAL CAPITAL AND SOCIAL NETWORKING ANALYSIS OF LEARNERS ON FIRST GRADE, SECOND GRADE AND HIGHER EDUCATION IN BALI <i>Cornelius Sri Murdoyuwono and Ni Gst. Ag. Gde Eka Martiningsih</i>	862-872
REVIEW OF POTENCY ANTIOXIDANT FROM TEMPEH TO PREVENT ATHEROSCLEROSIS <i>I G A Ari Agung</i>	873-878

COMMITTEE REPORT

1. The honorable Rector of Mahasaraswati Denpasar University.
2. The honorable invited speaker, Prof. Darussalam Abu Bakar from MARA University of Technology (UiTM) Malaysia
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 Nuroso 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 Nuroho, 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 I C S D
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 of Technology (ITB), Indonesia

Dr. Jennifer Day
University of Melbourne, Australia

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

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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). Salacca 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⁻¹year⁻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 (Dimiyati *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 & Mogeia 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 Sibatana 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 Sibatana (A-1) 450 m above sea level (asl), Kucing (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 consisted of freezer, mortar, glass cup, erlenmeyer, 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 -merkaptobetanol 1%, CI solution (chloroform: isoamilalkohol = 24: 1), liquid nitrogen, a solution of 3 M Na-acetate pH 5.2, polyvinyl-pirolidone (PVPP), TE buffer (tris-HCl and EDTA), ethidium bromide. RAPD-PCR process using the kit consists of a complete brand Bioron buffer (containing MgCl₂), 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 referred 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:

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

Where: σ^2f = phenotypic variance
 X_i = 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 $\sigma^2f > 2 Sd_{\sigma^2f}$ means that the wide phenotypic variability

If $\sigma^2f < 2 Sd_{\sigma^2f}$ 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 referred 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:

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

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	σ^2f	Sd σ_{2f}	2Sd σ_{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), Saribuana (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.

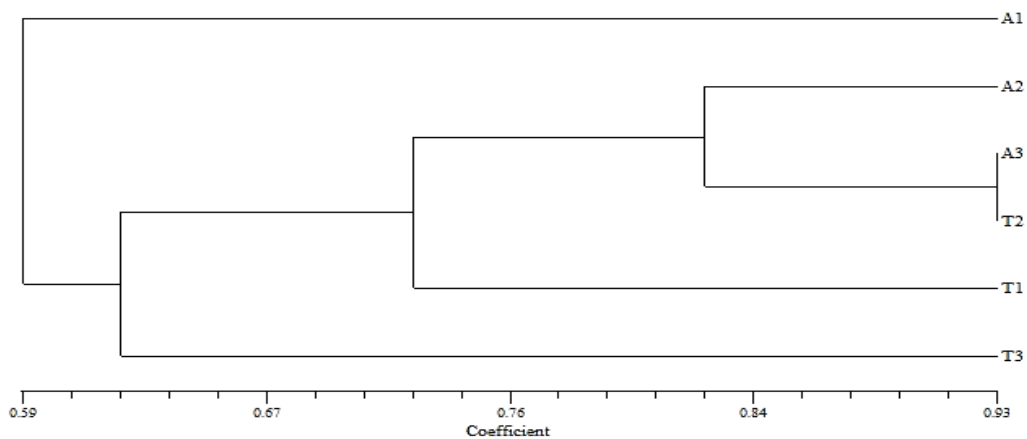


Figure 1. Phenotypic Dendrogram of Gulapasis salacca from six different locations (A= Karangasem: A1=Telaga; A2=Kecing; A3 =Jungutan; T= Tabanan : T1= Saribuana; T2=Pajahan; T3 = Bangsing)

Dendrogram showed that the grouping based on morphological phenotypic (Figure 1), Gulapasis 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 Kencing (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 divided into two groups. The first group was derived from the Karangasem salacca, which consisted of 3 accession salacca of Telaga-Sibetan (A1), Kencing (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.

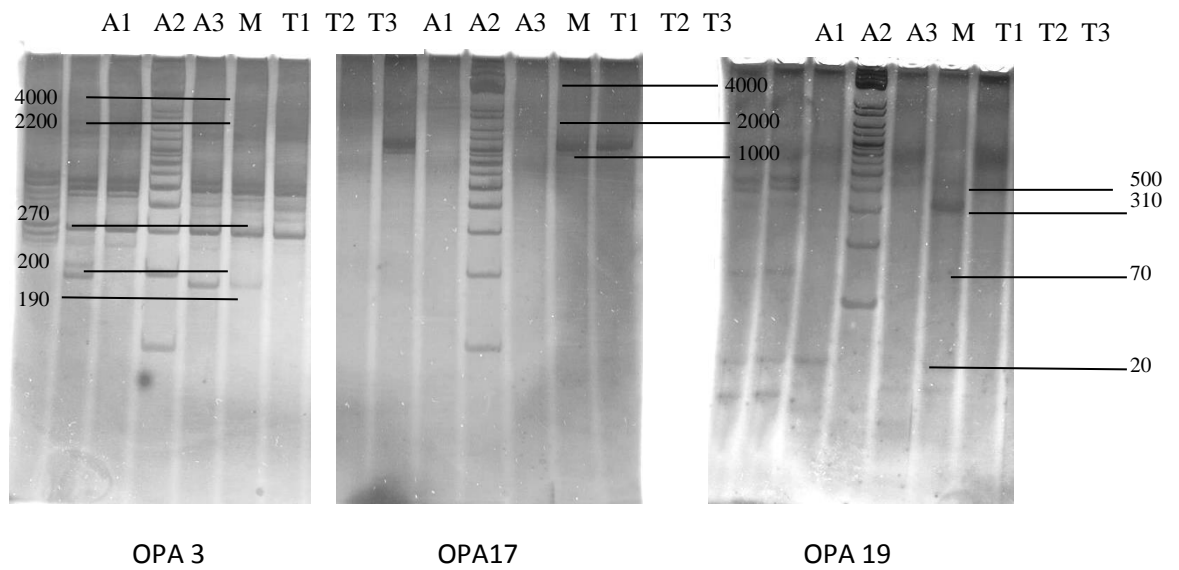


Figure 2. DNA banding pattern of salacca from various locations Based on 3 random primer: OPA3, OPA17, OPA Description: M = Marker 1 kb, A = Karangasem: A1 (Telaga), A2 (Kecing), A3 (Jungutan), T = Tabanan : T1 (Saribuana), T2 (Pajahan), T3 (Bangsing).

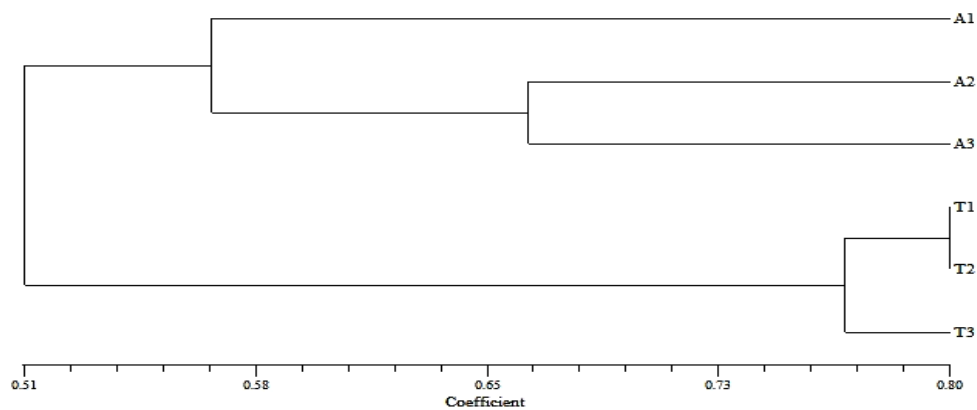


Figure 3. Dendrogram DNA banding pattern of Gulapisir salacca from six different locations (A = Karangasem; A1 = Telaga; A2 = kecing; A3 = Jungutan, T = Tabanan: T1 = Saribuana; T2 = Pajahan; T3 = Bangsing)

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 Gulapisir 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 Gulapisir 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.

Conclusion 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 Phenotype of Gulapasir salacca was done vegetatively by grafting system or by tissue culture techniques.

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