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BIOTECHNOLOGY

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April 2013

Industrial biotechnology for developing countries: The case for genetically modified biofuels in Kenya

Attempts to diversify the energy portfolios of developed countries with green technologies have brought competition between food and fuel for crop production resources to the forefront of public policy debates. Biofuel policies in the European Union (EU) and the United States (US) mandate the long-term use of renewable energy in transportation, independent of production capacity and technical feasibility....

Author(s): Alphanso Williams, Stuart J. Smyth and William A. Kerr

- https://doi.org/10.5897/AJB2012.11918
- Article Number: 7FC4E4422358

Abstract

April 2013

Genomic DNA extraction from sapwood of Pinus roxburghii for polymerase chain reaction studies

A method for extraction of genomic DNA from sapwood tissues of mature tall trees ofPinus roxburghii, where collection of needle tissues is extremely difficult has been standardized. The extracted DNA was comparable to that obtained from the needle tissue in terms of yield and purity. The yield of extracted DNA ranged from 6.98 to 19.668 µg / 100 mg tissue and A260 / A280 ratio ranged from...

Author(s): Anita Rawat, Santan Barthwal and H. S. Ginwal

- https://doi.org/10.5897/AJB12.2733
- Article Number: E152F1C22398

Abstract

April 2013

Characterization of specific random amplified polymorphic (RAPD) DNA fragments related to catechin content for early detection methods in gambier plant (Uncaria gambir (Hunter) Roxb.)

The existence of a target DNA fragments associated with the potential high level ofcatechins in gambier plant was carried out preliminary using RAPD markers. Targetfragment was ligated and transformed into Escherichia coli DH5α strains usingpGem-T Easy vector. The purpose of...

Author(s): Istino Ferita, Hamda Fauza, Irfan Suliansyah, Gustian and Jamsari

- https://doi.org/10.5897/AJB12.2814
- Article Number: B2136D222475

Abstract

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April 2013

Tumor formation in hybrids between Solanum lycopersicum and S. habrochaites

Genetic tumors are neoplastic growths that arise spontaneously in particular genotypes in plants. In the present study, we observed the occurrence of tumors in interspecific hybrids between Solanum lycopersicum L. and Solanum habrochaites S. Knapp and D. M. Spooner. The hybridity of these plants was confirmed based on morphological characteristics, flow cytometry and random amplified...

Author(s): Takahiro Tezuka, Hidenobu Okinaka and Masayuki Oda

- https://doi.org/10.5897/AJB12.1812
- Article Number: 31A2CB022528

Abstract

April 2013

In vitro cell culture of Charybdis congesta for enhanced production of secondary metabolites: Proscillaridin A, Scillaren A and Scilliroside

Callus cultures of Charybdis congesta were initiated in vitro and the effect of growth regulators was tested on callus growth and secondary metabolite production. Among several standard media formulated for use in the present study, MS and B5 were found to be potentially active and facilitated the calculation of callus induction frequency (CIF). The CIF was higher in both MS (70%) and B5...

Author(s): A. Shiva Reddy, P. Sita Devi and S. Ravi Kiran

- https://doi.org/10.5897/AJB2013.12103
- Article Number: 99D9DA522594

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Effects of natural long storage duration on seed germination characteristics of Periploca angustifolia Labill.

This study was carried out to evaluate the effect of long-term natural aging ongermination ability and several biochemical characteristics regarding soluble sugars and polyphenol matter contents and radical scavenging activity
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Author(s): Raoudha Abdellaoui, Aymen Souid, Dhikra Zayoud, and Mohamed Neffati

- https://doi.org/10.5897/AJB10.1862
- Article Number: E5257A222620

Abstract

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April 2013

Variation of gene effects of six agronomic traits with water regimes in durum wheat

This study was carried out to determine the relative importance of additive, dominance and epistatic effects of six agronomic traits evaluated in two crosses under irrigated and rainfed conditions. Separate generations mean analyses revealed that gene effects were dependent upon water regime. Under irrigated regime, only additive and dominance effects were implicated in inheritance of all traits, except plant...

Author(s): F. Bnejdi, M. Saadoun, N. Rassaa and M. El Gazzah

- https://doi.org/10.5897/AJB12.229
- Article Number: D571F9822646

April 2013

Growth, flowering and fruiting in vitro pineapple (Ananas comosus L.) in greenhouse conditions

The experiment was carried out in a greenhouse, located in Tehran city, Iran. The objectives of this study was to evaluate the effects of fertilizer and acidic soil on the foliar and radicular growth of micropropagated plantlets of the pineapple cv. Merr (Ananas comosus L.). We evaluated the growth of that genotype in five different ages of acclimatizing: 1, 2, 3, 4, 5 and 6 months in greenhouse. The...

Author(s): Farah Farahani

- https://doi.org/10.5897/AJB12.1421
- Article Number: E591C4B22675

April 2013

Overexpression of BrSAC1 encoding a phosphoinositide phosphatase isolated from Chinese cabbage (Brassica rapa L) improved tolerance to cold, dehydration, and salt stresses in transgenic tobacco

This study demonstrates the isolation and characterization of cDNA encoding aphosphoinositide phosphatase (PIP) from a stem cell cDNA library of Chinese cabbage (Brassica rapa) seedling. The full length gene (BrSAC1; GenBank accession no., GU434275) contained 1999 base pairs (bp), with an open reading frame of 1785 bp, encoding a polypeptide of 594 amino acids with a predicted molecular weight of 65 kDa,...

Author(s): Kyung Hee Han#, Yu Jin Jung,#, Uuganchimeg Bayarsaikhan, In Hye Lee, Jang Sun Choi, Ill Sup Nou, Yong Gu Cho and Kwon Kyoo Kang, ,

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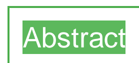

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April 2013

Sensitivity of tomato (Solanum lycopersicum) cultivars from Turkey to bacterial speck (Pseudomonas syringae pv. tomato)

The susceptibility of 93 different tomato cultivars that are commonly grown in greenhouses and field in the western Mediterranean region of Turkey have been assessed for resistance to bacterial speck disease caused by Pseudomonas syringae pv. tomato strains. The disease severity indexes (DSI) varied between zero and four for the tomato cultivars. Seven tomato cultivars showed hypersensitive...

Author(s): Ali Turgut and Hüseyin Basim

- https://doi.org/10.5897/AJB12.2740
- Article Number: 9B2CD2822727

Abstract

April 2013

Assessment of the protein quality of twenty nine grain amaranth (Amaranthus spp. L.) accessions using amino acid analysis and one-dimensional electrophoresis

Protein deficiency in diets adversely affects growth and development. Novel source of high quality protein and its utilization is essential in improving the nutritive status of the vulnerable groups. Total protein content and protein fractions of 29 amaranth accessions and a soybean cultivar used as reference were determined. The amino acid composition of ten representative accessions of amaranth was also...

Author(s): Akin-Idowu, Pamela Eloho, Odunola, Oyeronke Adunni, Gbadegesin, Michael Adedapo, Oke, Abiola and Orkpeh, Uterdzua

- https://doi.org/10.5897/AJB12.2971
- Article Number: B1CAC2822764

Abstract

April 2013

Agrobacterium mediated transformation of banana (Musa sp.) cv. Sukali Ndiizi (ABB) with a modified Carica papaya cystatin (CpCYS) gene

Conventional banana breeding for pest and disease resistance is a very difficult and slow process due to the limited sources of resistance, sterility of cultivated banana varieties, high polyploidy levels, long cropping cycle and the lack of rapid screening methods. Molecular breeding using the transgenic approach with candidate genes such as cystatins offers an alternative method to banana improvement....

Author(s): Namuddu, A., Kiggundu, A., Mukasa, S. B., Kurnet, K., Karamura, E. and Tushemereirwe, W.

- https://doi.org/10.5897/AJB12.2478
- Article Number: CB7047322798

Abstract

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April 2013

Genetic transformation of cry1EC gene into cotton (Gossypium hirsutum L.) for resistance against Spodoptera litura

Cotton is the chief fibre crop of global importance. It plays a significant role in the national economy. Cotton crop is vulnerable to a number of insect species, especially to the larvae of lepidopteron pests. 60% insecticides sprayed on cotton are meant to control the damage caused by bollworm complex. Transgenic technology has become a popular option for the development of bollworm resistant...

Author(s): R. Pushpa, T. S. Raveenderan, S. Rajeswari, P. Amalabalu and D. Punitha

- https://doi.org/10.5897/AJB12.1572
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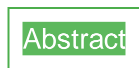
Abstract

April 2013

An improved method of DNA extraction from plants for pathogen detection and genotyping by polymerase chain reaction

Polymerase chain reaction (PCR)-based applications in plant molecular biology and molecular diagnostics for plant pathogens require good quality DNA for reliable and reproducible results. Leaf tissue is often the choice for DNA extraction, but the use of other sources such as tubers, stems, or seeds, is not uncommon. The extraction of DNA from different tissue sources often requires different protocols. In...

Author(s): Kamal Sharma, R. Bhattacharjee, Alieu Sartie and P. Lava Kumar

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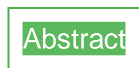
- Article Number: 7FBAC3523213


Abstract

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Haematological changes due to bovine fascioliasis

This study determined the haematological changes due to the infection of fascioliasis in cattle. The haematological indices of blood samples collected from purposely selected Fasciola-infected and non-infected cattle were analyzed using standard methods. Statistical analysis revealed high significant differences between the packed cell volume (PCV), haemoglobin (Hb) and...

Author(s): Egbu, Florence M. I., Ubachukwu, Patience O. and Okoye, Ikem C.

- https://doi.org/10.5897/AJB12.2716
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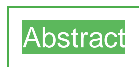
Abstract

April 2013

Sensory diversity of fonio landraces from West Africa

This study aims to establish if there is some sensory variability among fonio landraces. Fonio, the oldest indigenous and very tasty cereal growing in West Africa, is usually consumed as a couscous. Group interviews of consumers were conducted in Bamako, Mali to identify the main quality criteria of a cooked grain. Fonio grain must be swollen, not sticky with a soft consistency,...

Author(s): G. Fliedel, Y. Koreissi, F. Boré Guindo, D. Dramé, I. Brouwer and F. Ribeyre

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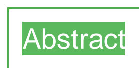
Abstract

April 2013

Lead induced dyslipidemia: The comparative effects of ascorbate and chelation therapy

To investigate the comparative effects of ascorbate and chelating agents on some markers of lipid metabolism in lead exposed rats, 35 male Wistar rats were used. They were grouped randomly into five (n=7); 28 of which were administered 75 mg/kg body weight lead acetate (PbAc) orally for 14 days after which their blood samples were assayed for lead. Three of the groups were further administered 30 mg/kg body...

Author(s): Regina Ngozi Ugbaja, Beno Okechukwu Onunkwor and Demilade Akinbola Omoniyi

- https://doi.org/10.5897/AJB2012.2982
- Article Number: F8828AA22977

Abstract

April 2013

Potential effects of plant growth promoting rhizobacteria (Pseudomonas fluorescens) on cowpea seedling health and damping off disease control

Damping off caused by Sclerotium rolfsii on cowpea results in yield losses with serious socio-economic implication. Induction of defense responses by plant growth promoting rhizobacteria (PGPR) is largely associated with the production of defense enzyme phenyl ammonia lyase (PAL) and oxidative enzymes like peroxidases (PO) and poly phenol oxidase (PPO). In the present study, the effect of plant...

Author(s): S. Nandi, A. Adhikari, S. Dutta, A. Chattopadhaya and R. Nath

- https://doi.org/10.5897/AJB12.2846
- Article Number: 2525E0623012

Abstract

April 2013

Studies on some active components and antimicrobial activities of the fermentation broth of endophytic fungi DZY16 Isolated from Eucommia ulmoides Oliv.

Research into plant-derived endophytic fungi has grown in recent decades. Endophytic fungi still have enormous potential to inspire and influence modern agriculture. In this study, the endophytic fungi DZY16 isolated from Eucommia ulmoides Oliv. was tested for its bioactive components and antimicrobial activities using phenol-sulfuric acid method, high performance liquid...

Author(s): Ding Ting, Sun Wei-Wei, Qi Yong- Xia and Jiang Hai-Yang

- https://doi.org/10.5897/AJB11.2638
- Article Number: C0F7E4123050

Abstract

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April 2013

Isolation and antibacterial activity of anabaena phycocyanin

The isolation and antibacterial activity of anabaena phycocyanin were investigated. The result indicates that three kinds of protein ingredients: PC-A, PC-B and PC-C were obtained using high performance liquid chromatography. The estimated molecular masses of PC-A and PC-B were 14 to 18 kD. PC-B and PC-C had certain antibacterial activity on Bibrio parahemolyticus, Bacillus...

Author(s): Meihua Fan, Zhi Liao, Ri xin Wang and Nianjun Xu

- https://doi.org/10.5897/AJB12.2575
- Article Number: 0A235A323074

Abstract

April 2013

Evidence of high production levels of thermostable dextrinizing and saccharogenic amylases by Aspergillus niveus

The aim of this work was to analyze the effect of several nutritional and environmental parameters on amylase production by a novel, isolated from the thermotolerant filamentous fungus Aspergillus niveus. This strain produced high levels of amylolytic activity in Khanna liquid medium supplemented with commercial starch, initial pH 6.5, under static conditions for 72 h. Among the tested carbon sources,...

Author(s): Tony Marcio da Silva, Alexandre Maller, Simone de Carvalho Peixoto-Nogueira, Michele Michelin, João Atílio Jorge and Maria de Lourdes Teixeira de Moraes Polizeli

- https://doi.org/10.5897/AJB12.2830
- Article Number: F8D2EC823125

Abstract

April 2013

Population of Tuta absoluta and natural enemies after releasing on tomato grown greenhouse in Turkey

The study was conducted to evaluate the population of tomato leafminer, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) and efficiency of natural enemies on tomato grown greenhouse in Mediterranean Region of Turkey during 2010 and 2011.Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) andNesidiocoris tenuis (Reuter) (Hemiptera: Miridae) were used for biological...

Author(s): Sevcan Öztemiz

- https://doi.org/10.5897/AJB12.726
- Article Number: B449D8323168

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April 2013

Purification and characterization of angiotensin-1 converting enzyme (ACE)-inhibitory peptide from the jellyfish, Nemopilema nomurai

The Nemopilema nomurai hydrolysate was produced by the reaction of papain, and an angiotensin-I converting enzyme (ACE)-inhibitory peptide was purified by using the molecular cut-offs membrane filter, the gel filtration chromatography with Sephadex LH-20 and the reverse phase chromatographic method using C18and C12 columns. Purification yield of the active peptide was estimated to be 0.2...

Author(s): Chi-Won Lim, Yeon-Kye Kim, So-mi Yeun, Moon-Hee Lee, Ho-Sung Moon, Na-Young Yoon, Ho-Dong Yoon, Hee-Yeon Park and Doo-Seog Lee

- https://doi.org/10.5897/AJB11.2677
- Article Number: 899B28E23188

Abstract

April 2013

Effects of different levels of protein-to-energy ratios on nutrient digestibility and digestive enzyme activity in Leiothrix luteal

Effects of different levels of protein-to-energy ratios (PER) on nutrient utilization, digestive organs index and digestive enzymes activities in Leiothrix luteal were investigated in this research. It was found that different PER feedstuff had no significant effects on the utilization of dry matter (DM), calcium (Ca) and total phosphorus (TP). With the improvement of the...

Author(s): Yuan Shi-bin, Zhou Cai-quan and Qi Sai-fei

- https://doi.org/10.5897/AJB12.1489
- Article Number: CE1892523251

Abstract

April 2013

Research regarding the reproductive capacity and biotechnologies of cows

The decline of fertility in the last decades is a major concern in dairy farms being caused by a series of factors analysed in the present paper. Research took place on a number of 950 dairy cows exploited in a semi-intensive system in the North-East area of Romania. On the whole analysed population, the frequency of reproduction disorders were between 12 (in January) and 27% in July, with a greater intensity...

Author(s): Vasile Maciuc, Cătălin Emilian Nistor and Teodor Bugeac

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Full Length Research Paper

Characterization of specific random amplified polymorphic (RAPD) DNA fragments related to catechin content for early detection methods in gambier plant (*Uncaria gambir* (Hunter) Roxb.)

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The existence of a target DNA fragments associated with the potential high level of catechins in gambier plant was carried out preliminary using RAPD markers. Target fragment was ligated and transformed into *Escherichia coli* DH5α strains using pGem-T Easy vector. The purpose of this study was to obtain information of specific DNA fragments and to generate specific primer combinations associated with potentially high content of catechins. DNA sequence was successfully generated with 206 nucleotides in length. The combination of specific primers, designated as Udtg3 (5'-CAGATCCTTTGCCACACTGA-3') and Udtg4 (5'- CGTACCTGCCAA CATA ACAG - 3') were successfully designed and could be applied as a diagnostic marker in detection of catechin content production of gambier plant.

Key words: Gambier, RAPD, specific primers, MAS, catechin.

INTRODUCTION

Gambier plants (*Uncaria gambier* (Hunter) Roxb.) is one of the export of plantation commodities possesing high economic value and this is based on their commercial role. The gambier resin contains mainly catechins that is necessary in the pharmaceutical industries, cosmetics, paint, and others. Gambier plant in Indonesia is largely dispersed and cultivated in West Sumatra; so-called specific plants of West Sumatera.

Fauza (2009) described four genotypes of gambier mainly found in West Sumatera, namely: Udang, Cubadak, Riau Gadang dan Riau Mancik. Among this genotypes, Udang is the most productive genotype than the other three based on their sap and catechin content yield (Hasan et al., 2000). This was further proved by Ferita et al. (2009) that they showed range productivity of 14-45%; 3-33%; 9-27% and 9-17% for each Udang, Riau Mancik, Ria Gadang and Cubadak respectively.

Developing a superior cultivars containing high level of catechin , is one of gambir breeding program goals. But such breeding goal was hindered by the unavailability of method that can be used for early detection of catechin contents in the seedlings stage. For that reason, it is necessary to identify the genetic characters associated with high catechin level and use them as a marker. The success of the identification and characterization of such marker could speed up the breeding program of this crop.

Molecular techniques such as random amplified polymorphic DNA (RAPD) can be used to support plant breeding activities, such as identification of specific characters through fingerprinting on gambier. RAPD is one of a DNA fingerprinting technique based on polymerase chain reaction (PCR) technology that can multiply the amount of DNA millions of times from its original amount. The technique has several advantages over hybridization methods, one of which is not necessary to use radioactive materials as used in the process of hybridization, rapid analysis systems, high

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sensitivity, and can identify the organism in small quantities (Babaloka, 2003; Jamsari et al, 2004; Lee et al., 1997; Lopez et al., 2003; Pastrik and Rainey, 1999). Unfortunately, RAPD technique has several drawbacks, for instance this technique was believed to be very sensitive to reaction conditions bringing the consequencies to less consistent results (Yu and Pauls, 1992). Many factors affect the volatility and sensitivity of RAPD markers for example ratio of template DNA and primers, concentration of Mg ions and Taq-polymerase type and even the type of used PCR machine, as well as the low temperature and short RAPD primer that commonly iis used in the procedure (Yang et al., 1996). Based on those arguments, many RAPD based molecular markers were converted into other marker system, for instance sequence tagged sites (STS). STS marker system is a marker system based on DNA sequence information of target region. Primers can be developed from specific region associated with some important agronomical traits.

The purpose of this study was to identifiy DNA specific fragments genetically associated with the potency of catechins production level and further develop specific primer combinations that can be used for early detection system during positive selection of gambier plant containing high level of catechin production.

MATERIALS AND METHODS

Genotype of Udang was used in this study. They were arranged into two groups, which were bulked into high and low catechin content. Each group consisted of five individual plant. The high group was characterized by ≥ 15% of catechin content, while the low group was characterized by < 15% of catechin content.

Molecular experiments such as: DNA isolation, PCR amplification, and cloning of DNA fragments were performed at the Laboratory of Biotechnology and Plant Breeding Faculty of Agriculture, University of Andalas Padang while the sequencing of specific DNA fragments was conducted at the Institute of Biology Molecular Eijkmann Jakarta. Plant materials used in this research was Udang type based on their morphological cahracter. Sampels were collected from gambier collection facility at Faculty of Agriculture-Andalas University.

Plant material and RAPD-based differential fingerprinting

Differential fingerprinting experiment was performed to visualize any polymorphism character between low and high catechin content of gambir collection. DNA isolation based on CTAB protocol as described by Doyle and Doyle (1990) was applied prior to differential screening. RAPD based marker system was applied in pool of low and high catechin content gambir fingerprinting. Each pool contained DNA from five individual plants. Pool was composed based on their catechin content for instance the low pool content of plants having of ≤ 15% of catechin content, and the high pool content plants having of > 15% of catechin content. 98 RAPD-primers were used in the differential screening (Table 1). All PCR reactions were performed using a T-Professional termocycler (Biometra-Germany), using standard PCR condition cycled for 45 times of 5 min, 95°C denaturation, 2 min of 38°C annealing and 5 min of 72°C extension. PCR product was checked by electrophoresis technique on 1.5% agarose. Primers successfully showing polymorphism between low and high pools were used for individual fingerprinting analysis. Similar principle was performed during individual differential screening of DNA composing DNA pool. Only fragments showing stabile performance were choosen for further analysis.

Cloning and sequencing of specific RAPD fragments linked to catechin level

Specific RAPD fragments from individual screening were further used for the cloning step. Fragments were cut from the gel using sterile scalpel and further purified using Wizard-Gel Purification Kit (Promega-USA). Purified fragments were subsequently proceeded for cloning step. Ligation condition and components were applied as recommended by the kit producer. Genetic transformation of recombinant plasmid into *Escherichia coli* strain DH5α were done using heat-shock technique as described by Sambrook and Russell (2001). Transformation suspension were plated on Luria Bertani selective medium containing IPTG and X-Gal to facilitate blue-white selection. Transformant were checked via mini preparation of plasmid DNA as described by Birnboim and Doly, (1979). Successful isolated DNA was further analyzed using PCR technique by applying T7 and SP6 primers. RTG-PCR bead (LG-healt care-UK) was used as PCR cocktail. Amplification reaction was done using the condition as follow: 5 µl of amplification product was controlled by electrophoresis technique on 1% agarose gel. Positive product was subjected directly for sequencing using both primers. Sequencing was done at the Institute of Molecular Biology Eijkmann Jakarta, following polymerase chain termination technique as described by Sanger et al. (1977). Sequence data was edited and subjected for BLAST (http://www.ncbi.nlm.nih. gov / BLAST) analysis for homology search.

Primer design and marker evaluation

Primer design was done by using the Primer3 software accessible on line at http://frodo.wi.mit.edu/primer3.2010. Synthesis of the primer was done at one-base (first-base-Singapore). The designed primers were checked for their optimum temperature using T-Gradient professional PCR machine (Biometra-Germany). Successful primer were tested against their original genome. Only primer pairs capable of producing clear and single fragment were taken for further evaluation. Primer evaluation was aimed for their accuracy among different genetic background of genotypes. Accuracy level was measuerd using the formula:

$$\text{Accuracy (\%)} = \frac{\text{Number of samples with expected product}}{\text{Number of tested samples}} \times 100\%$$

RESULTS

Isolation of DNA

Prior to differential screening of polymorphism between low and high catechin content in the pool step, genomic DNA isolation was performed. DNA generated from fresh youg leaf of each sample showed good condition in quantity as well as in quality (Figure 1).

RAPD-based differential screening

In order to speed up selection step, RAPD-based

Table 1. RAPD primers and sequences used in selection bulk (high and low pool) contents of catechin Udang type.

Number	Primer name	Sequence	Fragment of high catechin	Fragment of low catechin	Polymorphism
1	OPA-02	TGC CGA CCT G	6	6	0
2	OPA-04	AAT CGG GCT G	0	0	0
3	OPA-05	AGG GGT CTT G	0	0	0
4	OPA-06	GGT CCC TGA C	0	0	0
5	OPA-09	GGG TAACGC C	6	6	0
6	OPA-11	CAA TCG CCG T	4	4	0
7	OPA-13	CAG CAC CCA C	6	6	0
8	OPA-15	TTC CGA ACC C	2	2	0
9	OPA-19	CAA ACG TCG G	5	5	0
10	OPB-01	GTT TCG CTC C	6	6	0
11	OPB-06	TGC TCT GCC C	4	4	0
12	OPB-09	TGG GGG ACT C	2	2	0
13	**OPB-11**	**GTA GAC CCG T**	**4**	**3**	**1**
14	OPB-14	TCC GCT CTG G	1	1	0
15	OPB-15	GGA GGG TGT T	0	0	0
16	OPB-17	AGG GAA CGA G	5	5	0
17	OPC-02	GTG AGG CGT C	8	8	0
18	OPC-08	TGG ACC CGT C	3	3	0
19	OPC-14	TGC GTG CTT G	0	0	0
20	OPC-15	GAC GGA TCA G	1	1	0
21	OPC-16	CAC ACT CCA G	0	0	0
22	OPD-04	TCT GGT GAG G	0	0	0
23	OPD-05	TGA GCG GAC A	1	1	0
24	OPD-06	ACC TGA ACG G	0	0	0
25	OPE-03	CCA GAT GCA C	2	2	0
26	OPE-04	GTG ACA TGC C	0	0	0
27	OPE-05	TCA GGG AGG T	3	3	0
28	OPE-08	TCA CCA CGG T	3	3	0
29	OPE-14	TGC GGC TGA G	6	6	0
30	OPE-18	GGA CTG CAG A	5	5	0
31	OPF-05	CCG AAT TCC C	0	0	0
32	OPF-08	GGG ATA TCG G	5	5	0
33	OPF-12	ACG GTA CCA G	0	0	0
34	OPF-13	GGC TGC AGA A	0	4	4
35	OPF-14	TGC TGC AGG T	0	0	0
36	OPG-04	AGC GTG TCT G	0	0	0
37	OPG-05	CTG AGA CGG A	4	4	0
38	OPG-06	GTG CCT AAC C	1	1	0
39	OPH-03	AGA CGT CCA T	2	2	0
40	OPH-04	GGA AGT CGC C	1	1	0
41	OPH-05	AGT CGT CCC C	2	2	0
42	**OPI-01**	**ACC TGG ACA C**	**2**	**1**	**1**
43	OPI-02	GGA GGA GAG G	0	0	0
44	OPI-03	CAG AAG CCC A	0	0	0
45	OPJ-04	CCG AAC ACG G	0	0	0
46	OPJ-05	CTC CAT GGG G	0	0	0
47	OPJ-06	TCG TTC CGC A	3	3	0
48	OPK-04	CCG CCC AAA C	5	5	0
49	OPK-06	CAC CTT TCC C	5	5	0
50	OPK-07	AGC GAG CAA G	3	3	0
51	OPK-12	TGG CCC TCA C	4	4	0
52	OPK-13	GGT TGT ACC C	5	5	0
53	**OPK-15**	**CTC CTG CCA A**	**6**	**5**	**1**
54	OPL-09	TGC GAC AGT C	0	0	0
55	OPL-10	TGG GAG ATG G	5	5	0
56	OPL-11	ACG ATG AGC C	5	5	0
57	OPL-13	ACC GCC TGC T	5	5	0
58	OPM-04	GGC GGT TGT C	0	0	0
59	OPM-05	GGG AAC GTG T	0	0	0
60	OPM-06	CTG GGC AAC T	3	3	0

Table 1. Contd.

61	OPN-06	GAG ACG CAC A	5	5	0
62	OPN-10	ACA ACT GGG G	0	0	0
63	OPN-11	TCG CCG CAA A	1	2	1
64	OPN-12	CAC AGA CAC C	3	3	0
65	OPN-14	TCG TGC GGG T	3	3	0
66	OPN-15	CAG CGA CTG T	3	3	0
67	**OPN-16**	**AAG CGA CCT G**	**8**	**4**	**4**
68	**OPN-19**	**GTC CGT ACT G**	**7**	**4**	**3**
69	OPO-04	AAG TCC GCT C	5	5	0
70	OPO-05	CCC AGT CAC T	1	1	0
71	OPO-06	CCA CGG GAA G	2	2	0
72	OPP-04	GTG TCT CAG G	0	0	0
73	OPP-06	GTG GGC TGA C	4	4	0
74	OPQ-11	TCT CCG CAA C	0	0	0
75	OPQ-12	AGT AGG GCA C	1	1	0
76	OPR-11	GTA GCC GTC T	3	3	0
77	OPS-03	CAG AGG TCC C	4	4	0
78	OPS-18	CTG GCG AAC T	8	8	0
79	OPU-08	GGC GAA GGT T	4	4	0
80	OPW-01	CTC AGT GTC G	7	7	0
81	OPW-02	CAT CGC CGC A	5	5	0
82	OPW-03	GTC CGG AGT G	6	6	0
83	OPW-11	CTG ATG CGT G	6	6	0
84	OPW-14	GGT CGA TCT C	5	6	1
85	OPW-19	CAA AGC GCT C	4	6	2
86	OPX-01	CTG GGC ACG A	6	6	0
87	OPX-04	CTG GGC ACG A	6	6	0
88	OPX-07	GAG CGA GGC T	7	7	0
89	**OPX-09**	**GGT CTG GTT G**	**4**	**0**	**4**
90	OPX-15	CAG ACA AGC C	7	7	0
91	OPX-19	TGG CAA GGC A	5	5	0
92	OPY-04	GGC TGC AAT G	7	7	0
93	OPY-08	AGG CAG AGC A	6	6	0
94	OPY-09	AGC AGC GCA C	5	8	0
95	OPY-10	TCG CAT CCC T	4	4	0
96	OPY-16	GGG CCA CTG T	8	8	0
97	OPY-20	AGC CGT GGA A	7	7	0
98	OPZ-13	GAC TAA GCC C	0	0	0

fingerprinting differentiation was combined with bulked segregant analysis (Michelmore, et al. 1991). A total of 98 operon RAPD primers (Almaeda, USA) were used in the first step differential screening in the pool level. Results of the screening is shown in Table 1. There were 629 total fragments generated and six fragments on average could be generated from each primer. However, some primer even could not produce any fragment. Six out of 98 primers, OPB-11, OPI-02, OPK-15, OPN-16, OPN-19, and OPX-09 produced polymorphism fragment between low and high catechin content pool (Figure 2A). Figure 2B is an example of polymorphism shown by primer OPK-15. Theses primers were further proceeded to individual differential screening level using individual plant DNA composing the pool. However, only one primer OPK-15 could consistently differentiate between high and low catechin producing plants in both level screening. This was indicated by a single 700 bp fragment (Figure 3). The other five primers were then discharged from further analysis.

Sequence characteristic of RAPD specific fragment linked to catechin production

Specific RAPD fragmentt putatively linked to the cateching production was subjected to the subsequent analysis. Cloning procedures of specific fragments into pGemT-Easy vector (Promega-USA) was performed mainly as described by manufacturers recommendation. Transformation suspension was plated onto LB selective medium containing IPTG, X-Gal and supplemented with ampicilin. Selection of transformants were facilitated by expression of Lac-Z gene allowing blue-white selection procedure.

In further step, recombinant colonies were subjected in

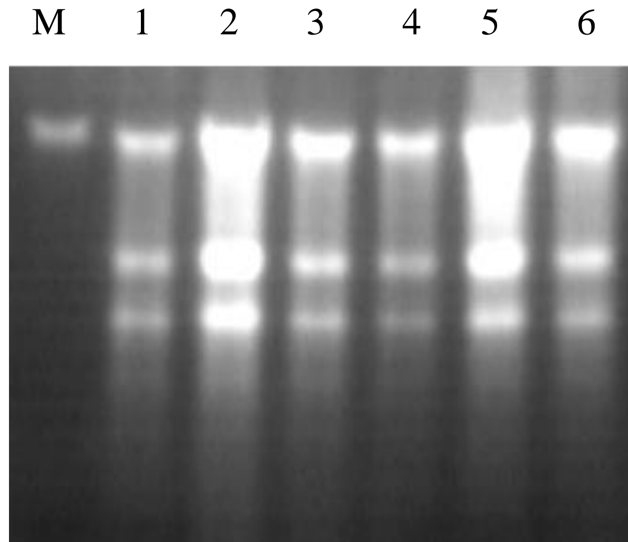
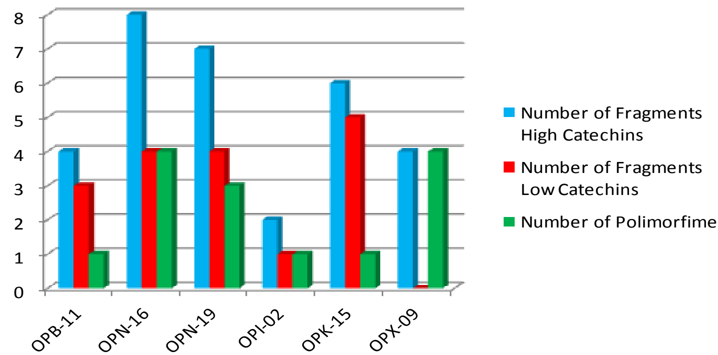


Figure 1. Electrophoresis performance of DNA from 6 *Uncaria gambir* plants. M = λ DNA (50 ng/µl), U1-U6 are samples.



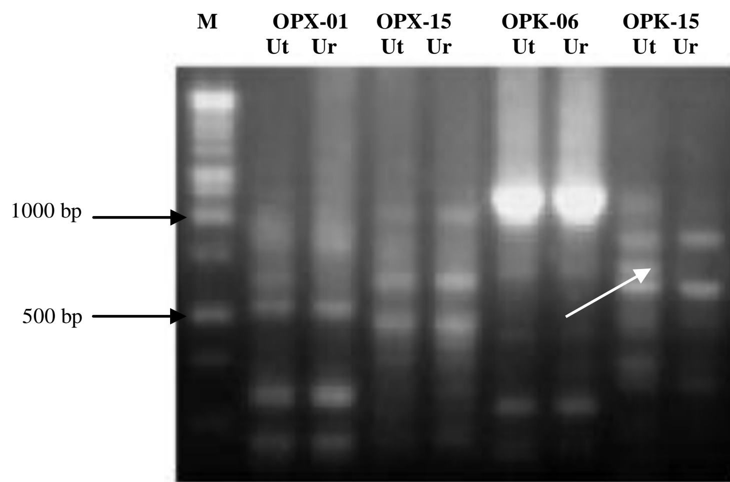


Figure 2. A. Diagram appearance of fragments amplified by RAPD primers showing polymorphism between DNA pool with high catechin and low catechin DNA gambier. **B.** The results of PCR amplification with primer OPX-01, OPX-15, OPK-06 and OPK-15 on a sample of Udang high catechin (Ut) and Udang low catechin (Ur). M = 1kb ladder. The arrow is showing polymorphism.

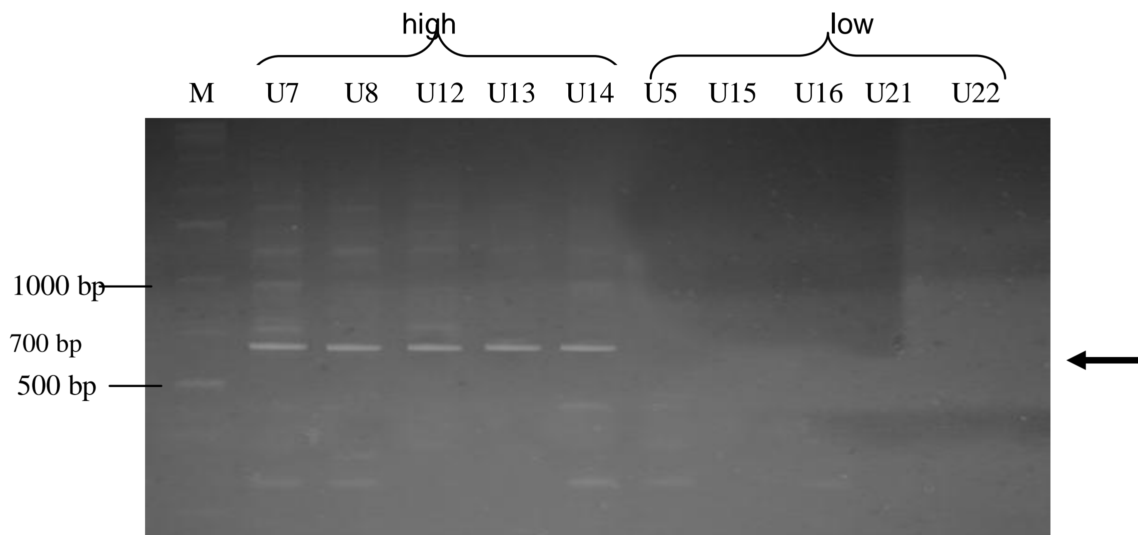


Figure 3. The results of PCR amplification with OPK-15 primer, with sample: U7, U8, U12, U13, U14, (Udang high catechins), and U5, U15, U16, U21, U22 (Udang low catechin). M = 1 kb ladder (Fermentas, USA). The arrows show the position of the fragment polymorphisms.

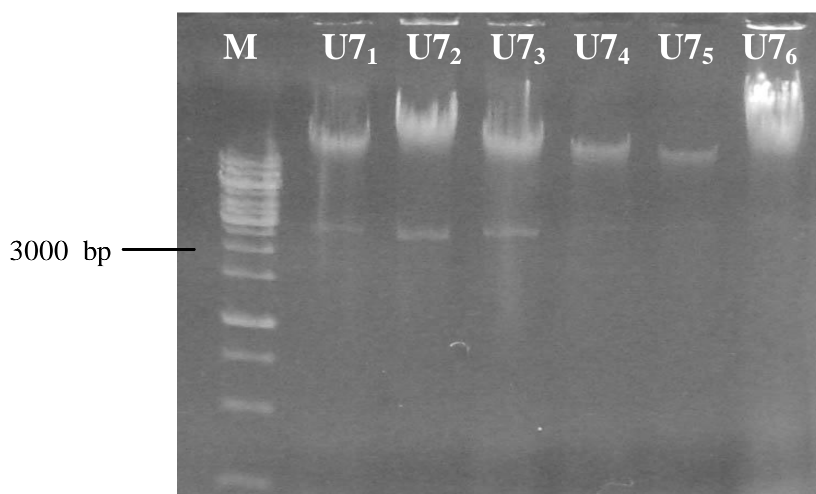


Figure 4. The results of plasmid DNA isolation. M = 1 kb ladder marker (Fermentas, USA); $U7_1$ - $U7_6$, sample of Udang 7.

plasmid DNA isolation. Six white colonies were used as sample for DNA isolation, and all of the samples seemed contained insert (Figure 4). Proving insert availability, PCR based analysis was performed. This was done by using T7 and SP6 primer combination (Figure 5).

Sequencing of specific RAPD fragments linked to catechin content

In order to unravel nucleotide composition of specific RAPD fragments, five samples U7, U8, U12, U13, and U14 were subjected for sequencing. After trimming and editing umbiguous nucleotide of five sequence data, 349 bp in average of nucleotide sequence was produced. They ranged from 314 to 445 bp. Plasmid sequence was eliminated by means of Vecscreen software available at NCBI website (http://www.ncbi.nlm.nih.gov/ VecScreen/). After performing this step, we could determine the inserted sequence ranging from 206 to 294 bp in length.

Marker development linked to catechin level content

Designing of specific primer was performed using Primer3 software available at http://frodo.wi.mit.edu/primer3/. Based on availability of sequence data, five primer pairs were designed.

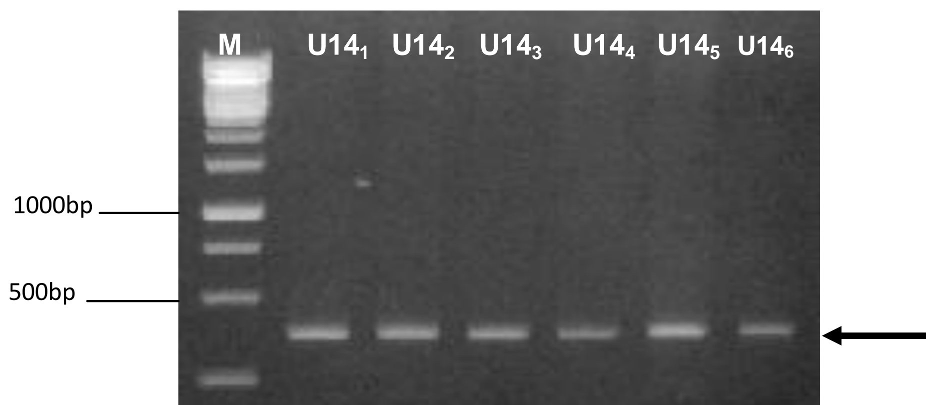


Figure 5. The results of PCR amplification with T7 and SP6 primer. M = 1 kb ladder marker-Fermentas, USA); $U14_1$ -$U14_6$, sample of Udang 14. Arrows are fragments of the insert.

Table 2. Primer sequences designed with the Primer3 program.

Name	Sequence	Size prediction
Udtg3	CAGATCCTTTGCCACACTGA	178 bp
Udtg4	CCTGCCAACATAACAGCGTA	

Synthesis of the primer sequence was ordered via first base (Firstbase-Singapore). However, after testing all the designed primer, only one primer pair which was named Udtg3 and Udtg4 (Table 2). This primer pair was generated from U14. After testing these primer pair via PCR-based technique, we could observe a single fragment of about 178 bp in lenght from all five samples identified as high-content catechin producing plant group (Figure 6).

In order to investigate the accuracy of the primer, we further tested the primer in more wide genetic background. For this purpose, we used 24 plants after previously identifying their catechin level production. There were ten samples positive fragments as expected product, seven samples from high contents of catechins, three samples from low contents of catechins, and 14 samples of negative fragments.

$$\text{Accuracy (\%)} = \frac{\text{Number of samples with expected product}}{\text{Number of tested samples}} \times 100\%$$

$$\text{Accuracy (\%)} = \frac{[7 \text{ (high catechin)} + 3 \text{ (low catechin)}]}{24} \times 100\%$$

Accuracy (%) = 41.7%

DISCUSSION

Gambier extract contains several important components which are useful for health and pharmaceutical process. In general, the extract of gambier contain catechin (1 to 33%), Tannates catecu acid (20 to 55%), pirocatecol (20 to 30%), gambier flaoresenci (1 to 2%), red catecu (3 to 5%), quercetin (2 to 4%), fixed oil (1 to 2%), wax (1 to 2%), and small amounts of alcohol (Nazir, 2000). Among those, catechin is one of the most important substances, since it is more commonly used for many industrial activity. Based on this reason, more study on catechin and its production aspect will be more interesting in the future. However, study on the molecular aspect as we performed in this study is scarce. That is why this our preliminary results will be very valuable for Gambier role for industrial side.

At least microgram of DNA could be produced from each sample. CTAB method in this case seemed to be suitable for genomic DNA isolation of *Uncaria gambir*. This result shows the suitability of CTAB method in *Uncaria gambir* species DNA isolation. This is surprising, since Uncaria gambir contain polyphenolic compounds in high amount. Normally, DNA isolation from tissue containing polyphenol compunds in high amount is tedious and needs more effort to dissolve the protein (Smalla et al., 1993). However, only CTAB-based protocol could produce genomic DNA in high quantity and quality. One of the reason in such case, could be lying on the type of tissue used for DNA isolation. Fresh young leaf tissue had soft and releasable cells, making lysis procedure more effective. Combined with high incubation temperature (65°C), lysis activity could be effectively improved (Bruce et al., 1992). Average percentage of white colony was 88.1% and blue colony was 0.7%.

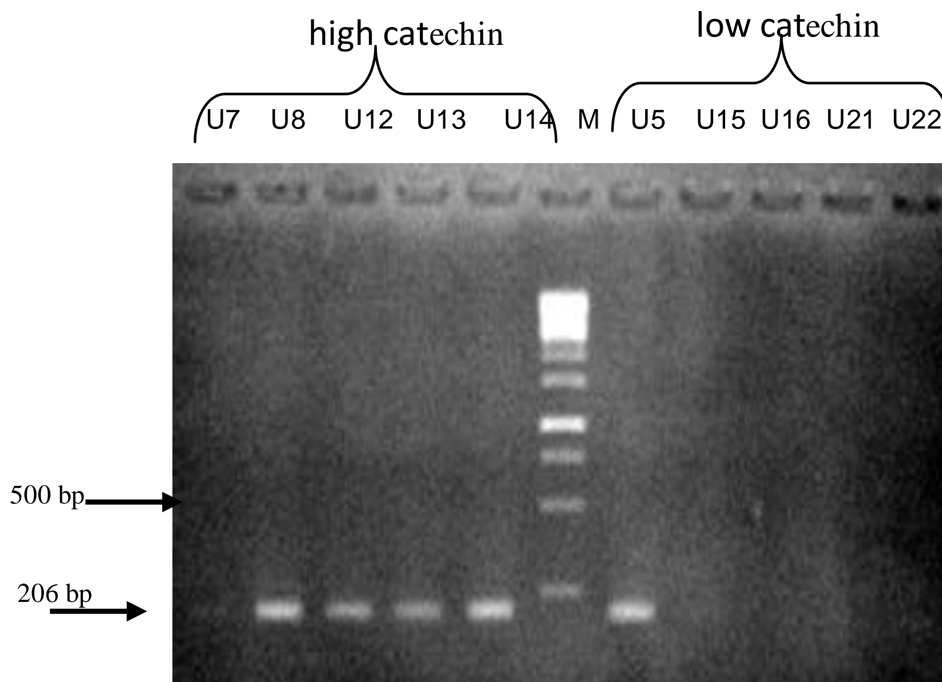


Figure 6. The result of PCR product in testing of primer pair Udtg3 and Udtg4. U7, U8, U12, U13, U14 are samples from high catechin content, while U5, U15, U16, U21, U22 belong to low level catechin content group. M = 1 kb marker (Fermentas-USA).

Transformation eficiency which reached 88.1% observed in this experiment is relatively high compared to the results published by other authors elsewhere (Jamsari, 2008).

The presence of white colonies indicated that the Lac-Z gene expression which change the X-Gal compounds became blue and was no longer able to perform its function, since specific RAPD fragment has interrupted the integrity of Lac-Z gene. Lac-Z gene encodesa β-galactosidase enzyme that catalyzes the breakdown of lactose (Xgal) into glucose and galactose.

After PCR step by T7 and SP6 primer, it could be concluded that 59.6% of the colonies contained positive insert, and only 40.4% did not contain insert. This result shows, that transformation eficiency in this experiment was higher than what was obtained by Jamsari (2008) for similar work with *Colletotrichum capsici* and *Colletotrichum gleosporides*. They got tranformation eficiency of 45 and 47.6% for both Colletotrichum species respectively.

All six samples used for PCR analysis produced approximately a 400 bp single fragment. However, in fact not all six samples were truly white, some of the colonies showed light blue colour, indicating that *Lac-Z* gene expression could still take place. This is actually commonly observed, when the inserted fragment is relatively short. Counting of the 400 bp of PCR product generated from T7 and SP6 primer, showed that the RAPD specific fragment that was successfully inserted to the pGem T Easy vector could be only about 250 bp. This is calculated by reducing PCR product with plasmid fragment flanked by T7 and SP6 primer.

However, comparison between cloned RAPD fragments with PCR product generated from T7 and SP6 primer pair showed inconsistencies. The cloned RAPD fragments was about 700 bp, meanwhile PCR product obtained was only about 400 bp. This results indicate that RAPD fragments cloned in this experiments were not single product. By comparison, short fragment in this case (about 250 bp) is more easier than the longer fragment (about 700 bp). The inconsistencies results is regarded as one of the drawback of the RAPD marker system as described by Yu and Pauls (1992). Furthermore, its sensitivity was affected by many factors for instance ratio of template DNA and primers, concentration of Mg^{2+} ions and type of Taq-polymerase used and also the type of PCR maschine used in the experiment (Meunier and Grimont, 1993; Rajput, et al., 2006; Jamsari, 2008). For this reason, RAPD marker system was preferentially converted to other marker system such as STS, CAPS or SCARs (Jamsari, 2004).

BLAST analysis of the five sequence data exhibited no sigificant hit, indicating no sequence of *Uncaria sp.* was available currently. Sequence allignment of those five nucleotide sequence showed differences in the sequence. Substitution, and insertion-deletion (indel) events could be observed among them. Indels events were observed in 16 position, while substitution was

observed involving 1 to 3 bases (data not shown).

The length of PCR product was in accordance with our expectation. However, applying the primer pair againts five low-content catechin producing plant group still produced an unexpected result since one among them (U15) which belonged to thelow level group produced similar fragment (Figure 6).

The percentage of Udtg3/Udtg4 primer accuracy was still low at 41.7% in detected gambier plant for high contents of catechins potential. The low accuracy of the primer, which is determined by high proportion of recombinants indicated that genetically primer loci of Udtg3/Udtg4 is located far enough from the gene of interest. Besides, low accuracy of primer was developed in this study, probably due that catechins content is quantitatively controlled by many genes. In this manner, every single gene contributes only small part in the catechin production. It could be possible that among the genes involved in the catechin production, some of them is/are major gene(s) located anywhere in the chromosome. However, until now, genomic study of *Uncaria* species is very rare.

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