Effect of various sugar solution concentrations on characteristics of dried candy tomato (Lycopersicum esculentum)

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Abstract. Buntaran W, Astirin PA, Mahajoeno M. 2009. Effect of various sugar solution concentrations on characteristics of dried candy tomato (Lycopersicum esculentum). Nusantara Bioscience 2: 55-61. The aims of the research were to study the effects of sugar syrup concentration on dried candy tomato characteristics and to determine the proper sugar solution concentration that gives the best characteristics of dry candy tomatoes. The research used Randomized Block Design Method with four treatments and six times repetitions. The treatment that be used was immersing the tomato in sugar solution, with concentration of A (40%), B (50%), C (60%), and D (70%) group in 18 hours. The variables measured were water content, ash, vitamin C and organoleptic tests include flavor, color, flavor and texture test. Data were analyzed using ANOVA test (Analysis of Variance) followed by DMRT (Duncan Multiple Range Test). The result showed that sugar solution concentration had different effect on water content, ash content, vitamin C content, texture, and organoleptic test for colour, taste, and flavor of the dry candy tomato. The best characteristics of dry tomato candy was obtained on A (40%) group, with water content of 24.20%, ash content of 0.62%, and vitamin C content of 31.15 mg/100 g. Standard quality of water content for dry fruit candy was maximal 25% (SII No.0718-2003) and maximal allowed ash content for food materials was 1.0% (SII 0272.90). Vitamin C content was not much decreased compared with ripe tomato i.e. 30-40 mg/100. Organoleptic tests result indicated that A (40%) group get the highest score, i.e. 3.98 for taste, 3.89 for flavor, and 3.98 for colour.

Key words: sugar, candy/candied, tomato, Lycopersicum esculentum.

INTRODUCTION

Fruits and vegetables are agricultural products that function as efficient public nutrition support and the source of income for farmers when cultivated intensively. Fruits and vegetables are rich in nutrients, namely vitamins and minerals that are needed by the human body because it can launch a regulator of metabolism as well as substances needed by the human body such as tomatoes, because tomatoes including the group of fruits and vegetables.

Tomato (Lycopersicum esculentum Mill.) Many people love it because it feels good, fresh and slightly acid. In addition, tomatoes contain vitamins and minerals that are useful for the health of the body. Vitamins are contained in tomatoes are vitamin A, vitamin B and vitamin C (Rismunandar 1984). In Indonesia there are many tomatoes in the markets and the price is relatively cheap at the time of harvest. The production centers of tomatoes as vegetables are generally located in cool climates, such as in West Java are in Ciwidey, Pangalengan, Cipanas, and Garut, in there in Wonosobo, Central Java, in Sumatra there are at Berastagi, Bukittinggi and in eastern Indonesia there Lombok and others.

Tomato’s production in Indonesia is still low compared with other countries, that is only 6.3 tons/ha, while Taiwan, Saudi Arabia and India respectively 21 tons/ha, 13.4
tons/ha and 9.5 tonnes/ha (Kartapradja and Djuariah 1992). In 1998-2002, Indonesia’s tomato plantation productivity increased from 7.1 tons/ha to 8.0 tons/ha, with total production increased from 333,729 tons to 396,208 tons or about 0.5% of the world tomato crop (Adiyogo 2004). The low production of tomatoes in Indonesia is probably due to unsuitable varieties planted, technical culture which is not good or eradication of pests/diseases that are less efficient (Wijayani and Widodo 2005).

Fruits and vegetables generally do not survive in a long storage, as well as with tomatoes that are vulnerable to damage, other problems that often arise is the tomato fungal growth on the surface of tomatoes. People need to make tomatoes more durable. Making candied tomato is one of the alternative processing of tomatoes and the preservation methods that are easy, no need to use high technology and can use a simple facility (Apandi 1994). Fresh tomatoes have resistance of 3-4 days, whereas with made candied the endurance is about 3 weeks. This is because the sugar solution can reduce the oxidation process so that it will prevent the relationship between fruit with external oxygen where oxygen is required for necessities of life harmful microbes, other ways sugar can inhibit the growth of plasmolysis of microbial cells with a lower water content is minimized so that the availability of water for the activity of microbial life there.

Candied is one type of snacks that normally use sugar as a sweetener. to obtain a fairly stable level of hardness, it is soaking in a solution of calcium chloride (CaCl2) thus obtained candied tomatoes are not easily damaged, the color is attractive and meets the quality requirements specified (Ekani 1995). Making candy is done by wet and dry way, wet candied are products made from fresh ingredients and soaked in sugar solution whereas the candied dried products made from fresh ingredients, soaked in a solution of sugar or sugar sprinkled thinly over and over again and then dried (SII 0718-83).

The utilization of tomatoes made sweets in addition to be more durable, tomatoes can also add value to confectionery manufacturers themselves, for consumers, of course, eat candied dried tomatoes would be more attractive because it is more practical to live to eat that will ultimately benefit the health of consumers themselves. Today in Indonesia candied tomato processing has not been produced on a large scale, however, with increasingly conscious of health by consuming fruits, particularly tomatoes, the needs of consumers of confectionery continue to rise. In the year 2010 it is estimated 40% of Indonesia’s population will consume processed from fruits including candied, because the Indonesian people are generally fond of foods that are practical and instant (Sutrisno 2007). Candied tomatoes are also required to be exported because there are some overseas countries like sweets, such as Japan, Korea and several Middle Eastern countries so they can bring in foreign exchange earnings and profits for the state amid the world economic situation is not stabilized. According to Director General of Processing and Marketing of Agricultural Products, Department of Agriculture (2007), candied fruit exports in 2006 amounted to 1024.77 tons, of which 50% were candied mango and barking, the rest is the nutmeg, tomatoes and others who predicted every year will increase.

Looking at the prospects of the research about the making of candied dried tomatoes need to be done, this is because consumers’ demand will increase each year of confectionery products, but the manufacturer has not so much, so that the necessary result of research or study more about the candied tomatoes, which in turn results of this study can be utilized by confectionery manufacturers for both small and large scale. Candied dried tomato products that already exist in traditional markets as well as Super Market is generally made without beans, but it is not intact forms of sweets, so a tomato fruit divided by two (partial), whereas the author of candied dried tomatoes carefully made in full one fruit and without seeded tomatoes, whereas in tomato seeds and gel are enveloped contained vitamin C and other substances among licopen Giji and β-carotene are very beneficial to health. That’s what separates candied dried tomato products we make and our perusal.

This study aims to: (i) Determine the influence of sugar solution on the characteristics of candied dried tomatoes. (ii) specify the concentration of sugar solution appropriate to produce candied dried tomatoes with good characteristics.

MATERIALS AND METHODS

Time and place of study
The research was conducted in August-December 2008, at the Quality Testing Laboratory, Center for Development, Empowerment and Education Education Personnel (P4TK) Agriculture, Cianjur, West Java.

Raw tomatoes
The tomatoes used to make candied tomatoes 1 kg so that each test for each treatment takes 6 kg, where the materials were purchased from markets around Cipanas, Cianjur regency. The tomatoes used were local varieties because it is usually a lot on the market and the price is relatively cheap. The sampling technique selected ripe tomatoes where a minimum of 80% red color, uniform size, are still fresh and clean (free from dirt, twigs, soil, dust, etc.), then weighed for each treatment.

Research design
The experimental design using randomized block design (RAK) consists of four treatments and six replications: (i) Group A, soaking in sugar solution concentration of 40%. (ii) Group B, soaking in sugar solution concentration of 50%. (iii) Group C, soaking in sugar solution concentration of 60%. (iv) Group D, soaking in sugar solution concentration of 70%. All the above treatments were given as much as 0.2% CaCl2 as preservatives which can absorb the remaining water.

Procedures
Making candied dried tomatoes. Making candied fruits begins by choosing a ripe and fresh tomato, and then wash
it to clean the dirt that is still attached, then boiled at a temperature of 70-80°C for 5 minutes, followed by stripping the skin. After that the material is soaked in a solution of calcium chloride 0.2% for 1-2 hours, followed by washing to rinse the residual calcium chloride solution is still attached to the outside of tomato fruit. Further material soaked in sugar solution, 40%, 50%, 60%, and 70% for 18 hours, followed by draining to reduce water attached to the candied tomatoes, then dried at 60 °C, to obtain the moisture content of certain (± 25%) as a condition of candied dried tomatoes.

**Determination of water content (SNI 01-2891-1992).** 1-2 g samples were inserted in the cup that has been known weight, then dried in an oven temperature of 105 °C for 3 hours, and cooled in exikator for 15-30 minutes, then the cup and its contents were weighed and dried again for 1 hour, and chill in exikator, weigh again. This process is repeated until a constant weight obtained. Water content was calculated using the formula:

\[
\text{Water content: } \frac{W_0}{W_1-W_2} \times 100\%
\]

Cup + W1 = Weight of sample before being dried
Cup + W2 = Weight of sample after drying
W0 = sample weight

**Determination of ash content (SNI 01-2891-1992).** 1-2 g samples were inserted in the cup that has been known to weigh, and then burnt in the flame until it becomes charcoal, and burned again in Pengabuan furnace at 550 °C to ashes, then cool in exikator for 15-30 minutes and weighed cup and ashes. Ash content was calculated using the formula:

\[
\text{Ash content: } \frac{W_2-W_1}{W_0} \times 100\%
\]

W0 = Weight of sample
W1 = Weight empty cup
W2 = Weight + ash cup

**Determination of vitamin C (SNI 01-2891-1992).** 10-25 g samples were crushed and included in the 250 mL measuring flask, then added distilled into water, then shaken until homogeneous and filtered, then put into a tube of filtrate of 25 mL, included in Erlenmeyer, added 1-2 mL shaken until homogeneous and filtered, then put into a tube measuring flask, then added distilled into water, then samples were crushed and included in the 250 mL fruit for each replication. The Penetrometer is set ignited and the sample on the basis of the tools, just below the needle gauge the level of hardness. Gauge needle attached to the right must be ensured on the surface of the sample, then the start button is turned to start the measurement, automatically within 5 seconds the needle will measure the hardness or texture sample. The texture of the sample can be read on the scale with units of mm (needle prick them), so the texture (hardness and elasticity) material is expressed in (mm/50 g/5 sec) (Dixon and Parekh 1980).

**Organoleptic test (hedonic methods).** Organoleptic test is conducted to determine the level of preference or acceptance of product’s panelists for candied dried tomatoes. This test is performed towards color, flavor and aroma. Panelists consisted of 15 persons, the criteria that is measured the level of preference is as listed in Table 1.

**RESULTS AND DISCUSSION**

The results influence the concentration of sugar solution to some of the characteristics of candied dried tomatoes, where the parameters of observation consists of chemical analysis (water content, ash, vitamin C) and texture (the level of hardness and tenderness materials) and organoleptic test the level of liking for flavor, color and aroma. Statistical analysis showed that each of attempted different treatment effects on water content, ash, vitamin C, and the texture and the organoleptic (color, flavor and aroma) (Table 2).

**Chemical constituent**

**Water content**

Chemical test results of water content followed by Anova statistical test for each treatment with six replicates among treatments showed no effect on the characteristics of candied dried tomatoes (Table 2). The highest water content in dried candied tomatoes at 24.20% was obtained in treatment A (soaking in sugar solution concentration 40%) and lowest 20.82% was obtained on treatment D (immersion in sugar solution concentration 70%). Water content showed significant differences among the treatments, treatment A with 24.20% moisture content was significantly different from treatment B and treatment C 23.25%, 21.36% and 20.82% D treatment.

There is a tendency that the higher concentration of sugar that tested the water content decreases (Table 2), it is because tomatoes are soaked in sugar solution will
experience osmotic pressure is the pressure of sugar molecules on the cell wall (extra cell) fruit until the sugar solution enter into it, as a result of water within the cells of fruit out. The difference of water flow out and flow of incoming sugar will cause the cell structure and texture of the fruit become hard, because of the higher flow of sugar into the osmotic pressure and consequently the stronger the water will more and more that comes out of the material (Apriyantono 2000).

Water content in food ingredients affects the durability of food against the microbial attack. The higher the water content, the more likely the food is easily damaged, where the high water content can be utilized by microorganisms, especially mold to grow and multiply so as to endanger the health of the body due to poisoning (Fellows and Hampton 1992; Astawan 2007;). Drying of food can lead to impaired growth of microorganisms decay (Kolawole et al. 2009).

In addition, water content in food or food ingredient may affect the texture, taste, freshness, durability of materials and consumer acceptance (Winarno 1981). In determining the standard of food that is used, water content is one of the criteria that usually determines the maximum and minimum limits for water content of food or processed food.

Determination of water content needs to be done to determine the condition of food or food ingredient that compared with standard conditions, for example in terms of quality dried candied fruits (SII 0525-2008), the maximum water content of 25% and was the result of research on the manufacture of candied dried tomatoes from all treatment showed meets the standards for being in the range of less than 25% after going through the process of drying for 24 hours at 60°C.

### Ash content

Chemical Test Results ash content, followed by Anova statistical test for each treatment with six repeated experiments showed no effect among the treatments on the characteristics of candied dried tomatoes (Table 2). The highest ash content on dry candied tomatoes at 0.80% was obtained at D treatment (soaking in sugar solution concentration 70%) and the lowest 0.62% obtained in treatment A (soaking in sugar solution concentration 40%). Ash content showed significant differences among treatments, treatment A with 0.62% ash content was significantly different from treatment B and treatment C 0.70% 0.75% and 0.80% D treatment. Ash is combustion of organic substances. Ash content is related to the minerals, including Mg, Na, Ca and phosphorus (Sudarmadji et al. 1996).

The existence of ash content comes from the tomato itself, where according Cahyono (1996), ash content in tomato reached 32.05 mg/100 g. While real differences of various treatments more likely are caused by ash content of sugar which contains 92 mg/100 g ash (Brautlecht 1953), so it can be assumed that the higher concentration of sugar solution used, the higher ash content will be (Table 2) and on the contrary the lower the concentration of sugar solution used the lower the ash content contained in these candied dried tomatoes.

Chemical test results showed that ash content in dry candied tomatoes from all treatments are still relatively safe or meets standards based on the SII 0272.90 permitted where the ash content of food permitted for a maximum of 1.0%. Ash is the remnant of food that are not needed by the body because the ash is a waste, even need to watch out because the high ash content in food or food ingredient can cause damage to the intestine (Riyada 2007).

### Table 1. Parameters/criteria for testing the level of preference (Hedonic Method 2000)

<table>
<thead>
<tr>
<th>Favorite level</th>
<th>Color</th>
<th>Flavor</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1= Not like</td>
<td>Red charred</td>
<td>Less sweet, typical tomato missing</td>
<td>The smell of charred</td>
</tr>
<tr>
<td>2= Somewhat like</td>
<td>Dark red/brown</td>
<td>Sweet, typical tomato missing</td>
<td>The smell of sugar is still strong</td>
</tr>
<tr>
<td>3= Regular</td>
<td>Red fade</td>
<td>Sweet, typical weak tomato</td>
<td>Tomato aroma less</td>
</tr>
<tr>
<td>4= Like</td>
<td>90% red tomato</td>
<td>Sweet, typical of pristine tomato</td>
<td>Moderate tomato aroma, the smell of sugar less</td>
</tr>
<tr>
<td>5 = Very like</td>
<td>Red tomatoes (original)</td>
<td>Sweet, typical of pristine tomato</td>
<td>Strong tomato aroma, the scent of sugar or less</td>
</tr>
</tbody>
</table>

### Table 2. Water content of dried candied tomatoes

<table>
<thead>
<tr>
<th>Treatment of sugar conc. (%)</th>
<th>Water (%)</th>
<th>Ash (%)</th>
<th>Vitamin C (%)</th>
<th>Flavor</th>
<th>Organoleptic</th>
<th>Texture (mm/50 g/5 sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chemical constituent</td>
<td>Organoleptic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: 40</td>
<td>24.20±0.01472</td>
<td>0.62±0.01633</td>
<td>31.15±0.16293</td>
<td>3.98±0.14729</td>
<td>3.98±0.12139</td>
<td>3.89±0.06812</td>
</tr>
<tr>
<td>B: 50</td>
<td>33.25±0.01871</td>
<td>0.70±0.01472</td>
<td>30.17±0.20047</td>
<td>3.55±0.10488</td>
<td>3.85±0.07941</td>
<td>3.79±0.07118</td>
</tr>
<tr>
<td>C: 60</td>
<td>21.36±0.01871</td>
<td>0.75±0.01472</td>
<td>28.86±0.10741</td>
<td>3.18±0.07528</td>
<td>3.75±0.04215</td>
<td>3.62±0.08894</td>
</tr>
<tr>
<td>D: 70</td>
<td>20.82±0.02160</td>
<td>0.80±0.01472</td>
<td>27.62±0.08116</td>
<td>2.93±0.08165</td>
<td>3.73±0.04401</td>
<td>3.38±0.10073</td>
</tr>
</tbody>
</table>

Note: different letters in the same column indicate significant differences (P ≤ 0.05); numbers above are the mean ± SD.
**Vitamin C content**

Chemical test results in vitamin C, followed by Anova statistical test for each treatment with six repeated experiments showed no effect among the treatments on the characteristics of candied dried tomatoes (Table 2).

Vitamin C is classified as soluble in water. Vitamin C can be shaped as L-ascorbic acid and L-dehydro ascorbic acid; both have activity as vitamin C (Winarno 1997). Ascorbic acid is easily oxidized in a reversible become L-dehydro ascorbic acid. Dehydro ascorbic acid is chemically very unstable and can undergo further change to acid L-diketogulononat who do not have a more active vitamin C (Miller 1992).

Levels of vitamin C that was determined using the iodometric iodine (I2) as penitar vitamin C in the example is a strong reductant will be oxidized by I2 in an atmosphere of acid and iodide ion reduces to I2. The indicator used is the kanji with a blue end point and not lost for 10 seconds and then calculated how many mL titration of I2 is used as the basis for calculating vitamin C (Slowinski and Wolsey 2008).

The highest vitamin C content in dried candied tomatoes amounted to 31.15 mg/100 g of material obtained in treatment A (soaking in sugar solution concentration 40%) and the lowest was 27.62 mg/100 g of material obtained in treatment D (immersion in sugar solution concentration 70%). Vitamin C content among the treatments showed significant differences, treatment A with the content of vitamin C 31.15 mg/100 gram significantly different materials with treatment B 30.11 mg/100 g and 28.86 mg/100 g treatment C and treatment materials D 27 , 62 mg/100 g of material. The higher concentration of sugar solution is attempted, the lower its vitamin C content (Table 2). The loss of vitamin C is believed due to a change in the structure of fruit tissue, where the higher the sugar solution is added then lead to more water molecules to move (diffuse) out of the material and water to dissolve the vitamin C, vitamin C and ultimately reduced materials (Hui et al. 2006).

The content of vitamin C and other vitamins in food or food ingredients, including dried tomatoes in the candied are very much needed by the body, because the vitamin serves as regulator and protector of the body from disease and can launch your metabolism.

**Organoleptic**

**Flavor**

The organoleptic test the panelists to think that continued with Anova statistical test for each treatment with six replicates an effect among the treatments on the characteristics of candied dried tomatoes (Table 2). Organoleptic test of the flavor is intended to determine the extent of consumer acceptance of a food product. This taste test conducted by a number of panelists (15 people trained panelists) in which each panelist gives value to the candied dried tomato flavor, the total value of flavor from the panelists will determine the quality or acceptance of products tested.

The highest values organoleptic test results to the taste of candied dried tomatoes for 3.98 obtained in treatment A (soaking in sugar solution concentration 40%) and the lowest 2.93 obtained in treatment D (immersion in sugar solution concentration 70%). Value taste test showed significant differences among treatments, treatment A with a value of 3.98 was significantly different from treatment B and treatment C value of 3.55 and treatment D value of 3.18 with a value of 2.93.

Rasa including the important factor of a food product in addition to color and flavor, these flavors can be derived from properties of the materials used or when the processing is another ingredient that is added, so that the original sense can be reduced or increased depending on the compound supporters, such as the addition of sugar can provide a sweet taste in food products including confectionery tomato itself.

Candied dried tomatoes in treatment A (soaking sugar concentration 40%) are the most preferred product the panelists, this is possible because the beautiful or unique flavor of tomatoes still feels fresh and it's not too sweet, there is a tendency that the higher the concentration of the sugar tested, then beautiful tomato flavor is replaced by the less because of the sweetness of sugar, so that the panelists liked it less and gave a low value. To give a distinctive flavor can be added to synthetic or artificial flavors, although the results do not like the taste of the original.

**Color**

Organoleptic test results to the color of the panelists, followed by Anova statistical test for each treatment with six replications, not all treatments showed no significant effect on the characteristics of candied dried tomatoes (Table 2). Color is one determinant of quality of food products in addition to the nutritional value itself. The visual assessment of color usually comes first, because the color is a view that can attract consumers so that there are many terms of the color of love. In addition, color can be used as an indicator of freshness or maturity (Winarno 1992).

The highest values organoleptic test results of color in dry candied tomatoes at 3.98 obtained in treatment A (soaking in sugar solution concentration 40%) and the lowest 3.73 obtained in treatment D (immersion in sugar solution concentration 70%). Color test value among the treatments does not all show significant differences. Treatment A with a value of 3.98 was significantly different from treatment B value of 3.85 but treatment C and treatment D value of 3.75 with a value of 3.73 was not significantly different.

A treatment based on test results of the panelists’ favorite level is the most color of the preferred candied dried tomatoes. Red color is thought the still beautiful because of immersion in a solution of sugar which is not too high so as not to damage the tomato flesh tissue in which the pigments or dyes contained therein. There is a tendency of higher concentrations of sugar, then red with dark red and even black due to the caramelization so unpopular with the panelists.
Aroma

The organoleptic test of the scent of the panelists, followed by Anova statistical test for each treatment with six replications, not all treatments showed no significant effect on the characteristics of candied dried tomatoes (Table 2). The highest values organoleptic test results against the candied dried tomato aroma of 3.89 obtained in treatment A (soaking in sugar solution concentration 40%) and the lowest 3.38 obtained in treatment D (immersion in sugar solution concentration 70%). Color test value among the treatments does not all show significant differences, treatment A with a value of 3.89 was not significantly different from treatment B value of 3.79 but treatment C and treatment D value of 3.62 with a value of 3.38 was significantly different.

Based on the appraisal of the panelists on the aroma and after anova was statistically tested treatment A and treatment B value of 3.62 with a value of 3.38 was significantly different from treatment B value of 3.79 but treatment C that produce the most beautiful aroma of fresh tomatoes still smells. Treatment A candied dried tomatoes (soaked in sugar concentration 40%) and treatment B (immersion in 50% sugar concentration), is candied dried tomato aroma most preferably where the panelists, it is believed that soaking in a low-sugar solution is not too damaging aroma tomato so the typical tomato flavor that is still felt in treatment A (soaking in a solution sugar concentration 40%) still the same typical tomato flavor in treatment B (soaking in sugar larutan konseantrasi 50%).

The real difference between perlakuan A to C (soaking in sugar larutan konseantrasi 60%) and treatment C to D (immersion in sugar solution konseantrasi 70%) this is due to the high concentration of sugar into the tomato tissue that happened when water molecules within cells more tomatoes out (diffuse) that allegedly participated soluble tomato aroma. The existence of a solution of sugar in the material, the smell is a volatile compound, so that in conditions of immersion in the solution and drying of high sugar loss smells more and more possibilities.

Treatment A candied dried tomatoes (soaked in sugar concentration 40%) and treatment B (immersion in 50% sugar concentration), is candied dried tomato aroma most preferably where the panelists, it is believed that soaking in a low-sugar solution is not too damaging aroma tomato so the typical tomato flavor that is still felt in treatment A (soaking in a solution sugar concentration 40%) still the same typical tomato flavor in treatment B (soaking in sugar larutan konseantrasi 50%).

The highest value of the texture of the dried candied tomatoes at 4.03 which means the texture is more tender than the material or other candied dried tomatoes obtained in treatment A (soaking in sugar solution concentration 40%), and the lowest is 2.68, which means harder texture compared with the material or other candied dried tomatoes obtained in treatment D (immersion in sugar solution concentration 70%). Value of material texture test showed significant differences among treatments, treatment A with a value of 4.03 was significantly different from treatment B value of 3.81 as well as with treatment C and treatment value of 2.98 D with a value of 2.68 was significantly different.

According Apandi (1994), that the network changes, especially in the cell wall and the progressive dissolution of pectin substances can occur because of the enzyme activity that causes changes in texture in fruits and vegetables. Texture with a lower value means the texture of material harder than the other sample and vice versa texture with a higher value means the texture is more tender than the other samples, this is caused due to a variety of treatments of soaking in sugar solution different.

With the drying of water in the evaporated material, but instead of sugar that is in suspended cells, presumably the higher the concentration of sugar solution is attempted, the more sugar molecules that enter and the more sugar is retained inside the cells of tomato fruit, causing the texture of harder, so does that happen in treatment A (soaking in sugar solution concentration 40%) produces a more tender texture than treatment B, C and D that produce harder texture. The existence of a solution of sugar in the material with the lowest sugar concentration of 40% dissolved solids will cause the harder material (Purnomo 1995).

CONCLUSION

Soaking in a solution of sugar concentration of 40%, 50%, 60% and 70% affects on moisture content, ash content, vitamin C, the results of organoleptic test taste, flavor, color and texture (the level of hardness and tenderness materials). Immersion in 40% sugar solution produced candied dried tomatoes with the best characteristics. Taste sweet enough, the typical tomato flavor is still felt, the aroma is not lost and the color is not broken, where the sense of (3.98), aroma (3.89) and color (3.98).

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The effect of coconut water and naphthalene acetic acid (NAA) application on the in vitro growth of *Paraphalaenopsis serpentilingua* from West Kalimantan

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**Abstract.** Mukarlina, Listiawati A, Mulyani S. 2010. The effect of coconut water and naphthalene acetic acid (NAA) application on the in vitro growth of *Paraphalaenopsis serpentilingua* from West Kalimantan. *Nusantara Bioscience* 2: 62-66. The ‘Ekor tikus’ orchid (*Paraphalaenopsis serpentilingua* J.J. Sm.) is an epidemic orchid in West Kalimantan. Now this orchid is facing a great conservation problem and threatened to be in extinction due to human exploitation. This research was conducted to find out the in vitro growth effect of *P. serpentilingua* by supplementation of NAA and coconut water in culture medium. The experiment was carried out using a Completely Randomized Design with two factors and five replicates. The result showed that supplemented NAA and coconut water on MS medium affected the emergence timing of buds, the average buds number, the average leaf number/buds, percentage of buds, the emergence timing of root, average root number and percentage of root. Medium that supplemented with 1.5 ppm NAA and 10% coconut water showed the fastest emergence timing of apical bud that is 13 days after planting. Medium supplemented with 0.5 ppm of NAA and 7.5% of coconut water showed the highest average number of bud was 11 buds.

**Key words:** *Paraphalaenopsis serpentilingua*, NAA, coconut water.

**INTRODUCTION**

The ‘ekor tikus’ orchid (*Paraphalaenopsis serpentilingua* J.J. Sm) is an endemic orchid in West Kalimantan. This orchid has unique flowers with two branches lips (labellum) like snake’s tongue so that to be called serpentilingua (serpentines is snake, lingua is tongue). This orchid is not only useful for ornament plant, but also for medicine plant. People use this leaf as medicine that neutralize snake’s poison (Chan et al. 1994; Siregar et al. 2005).

Population of these orchid have began decrease and classified as endangered species. One of the reasons is limited factor on the reproduction via seed. Production of seed on the August until December only and the seed do not have food reserve for embryo growth. The seed can be sprouting only if it symbiosis with mycorrhiza. Conservation problem due to human exploitation and forest burned too (Siregar et al. 2005).

Tissue culture technique constitutes an important component of biotechnology and have the potential not only to improve the existing cultivars, but also for the generation of plants in a comparatively short time compared to conventional breeding (Dixon and Gonzales 1994). The successful of tissue culture was influenced by modification of culture medium with add of growth regulator substances and organic compounds. Growth regulator substance Naphthalene Acetic Acid (NAA) from auxin group used to increase *in vitro* root growth. Organic compounds like coconut water was added on the culture medium because it contains amino acid, vitamin, mineral and growth regulator substances like auxin and cytokinin.
that can be exhibit plant growth (George and Sherrington 1984; Hendaryono and Wijayani 1994).

The advantage of application synthetic grow regulator and organic compound like coconut water on the orchid culture medium was much be done (Bey et al. 2006; Untari and Puspaningtyas 2006; Widiastoety and Santi 1994).

This research aims to know effect of application combination concentration NAA and coconut water on the in vitro growth of the orchid _Paraphalaenopsis serpentilingua._

**MATERIALS AND METHODS**

Materials used in the research are explants’ stems that come from plantlets of _Paraphalaenopsis serpentilingua_ (‘ekor tikus’ orchid) (Figure 1) from seed culture in Vacient and Went medium without supplement growth regulator substances, activated charcoal, agar, basal medium Murashige-Skoog (MS), NAA and coconut water.

The media were variously supplemented with NAA alone, coconut water alone or combination NAA (0.5 ppm, 1 ppm, 1.5 ppm) and coconut water (5%, 7.5%, 10%). The pH was adjusted to 5.8 before adding agar.

Cultures were incubated at 25 C at photoperiod of 16h/day with an illumination of 30 µmol m-2 sec-1 provided by 40 W cool white fluorescent light. The cultures were regularly subcultured at four weeks intervals on new medium and twice to do with at least five cultures per treatment (Listiawati et al. 2006) Observation has been done everyday until three months after planting.

Parameter that observed were emergence timing of apical bud and emergence timing of axillary bud (day), number of bud (bud), number of leaf each bud (blade), emergence timing of root (day) and number of root (blade).

The experiment was carried out using a Completely Randomized Design with two factors. First factor is NAA with four level (0 ppm; 0.5 ppm; 1 ppm and 1.5 ppm), and second factor is coconut water with four level (0%; 5%; 7.5% and 10%) and five replicates. The ANOVA test was applied by ANOVA test and continued by using the Duncan Multiple Range Test (DMRT).

![Figure 1. Paraphalaenopsis serpentilingua (‘ekor tikus’ orchid)](image-url)
RESULTS AND DISCUSSION

The emergence timing of bud

The result showed that the apical bud has emergence timing faster than axillary bud. This reason caused that on the tip of stem found the meristem tissue that always to have meristematic characteristic. When the apical meristem was to divide, the axillary meristem to go through dormant so that in the beginning of bud growth especially to go on the apical bud growth. Apical shoot meristem will be synthesis auxin that necessary for apical bud growth. The growth of apical bud will inhibit growth of axillary bud (apical dominance) (Hidayat 1995; Salisbury and Ross 1995).

Treatment without supplemented (control) can be forming apical bud at the 24 day after planting (Table 1). This reason indicated that endogen growth regulator have capable to induction apical bud growth. The plant growth was influenced of internal factors, among of them is endogen growth regulator (Hopkins 1995). Similarly, stem explants of *Paraphalaenopsis serpentilingua* on the treatment with 0 ppm NAA + 0 ppm BAP can be forming apical bud at 37.33 day after planting (Maryam 2008).

Table 1. Effect of supplemented NAA and coconut water on emergence timing of bud

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Emergence timing of bud (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical</td>
</tr>
<tr>
<td>0 ppm NAA + 0% coconut water</td>
<td>24 abc</td>
</tr>
<tr>
<td>0 ppm NAA + 5% coconut water</td>
<td>34 abcd</td>
</tr>
<tr>
<td>0 ppm NAA + 7.5% coconut water</td>
<td>35 bcd</td>
</tr>
<tr>
<td>0 ppm NAA + 10% coconut water</td>
<td>25 abc</td>
</tr>
<tr>
<td>0.5 ppm NAA + 0% coconut water</td>
<td>25 abc</td>
</tr>
<tr>
<td>0.5 ppm NAA + 5% coconut water</td>
<td>46 de</td>
</tr>
<tr>
<td>0.5 ppm NAA + 7.5% coconut water</td>
<td>23 abc</td>
</tr>
<tr>
<td>0.5 ppm NAA + 10% coconut water</td>
<td>28 abcd</td>
</tr>
<tr>
<td>1 ppm NAA + 0% coconut water</td>
<td>53 e</td>
</tr>
<tr>
<td>1 ppm NAA + 5% coconut water</td>
<td>30 abc</td>
</tr>
<tr>
<td>1 ppm NAA + 7.5% coconut water</td>
<td>43 cde</td>
</tr>
<tr>
<td>1 ppm NAA + 10% coconut water</td>
<td>37 bcd</td>
</tr>
<tr>
<td>1.5 ppm NAA + 0% coconut water</td>
<td>40 cde</td>
</tr>
<tr>
<td>1.5 ppm NAA + 5% coconut water</td>
<td>13 a</td>
</tr>
<tr>
<td>1.5 ppm NAA + 7.5% coconut water</td>
<td>26 abc</td>
</tr>
<tr>
<td>1.5 ppm NAA + 10% coconut water</td>
<td>18 ab</td>
</tr>
</tbody>
</table>

Note: Figures in same column in each group followed by the same letter is not significantly different according to DMRT, P<0.05.

Treatment with 1.5 ppm NAA + water coconut 5% is the most efficient concentration for induction apical bud, this case could be showed by fastest of emergence timing of bud that is 13 days after planting (Table 1). Opinion that, there are a proportion on interaction among 1.5 ppm NAA, growth regulators on coconut water and endogen growth regulators, so that they are optimum for induction bud. Optimum interaction among endogen growth regulators and exogenous regulators can be activated enzymes for growth increase (Wattimena 1992).

The fastest emergence timing of axillary bud is in treatment 1.5 ppm NAA + 10% coconut water that is 13 days after planting (Table 1). This reason indicates that interaction among 1.5 ppm NAA, 10% coconut water and endogen growth regulators are efficient to rule the apical dominance. Salisbury and Ross (1995) said that ratio cytokinin higher to auxin will be stimulate growth of axillary bud, but ratio cytokinin lower to auxin will be excite apical dominance.

Number of bud

The combination concentration 0.5 ppm NAA + 7.5% coconut water is the most efficient to give much axillary bud that is 11 buds (Table 2). Opinion that proportion among NAA, growth regulators primary cytokinin on coconut water and endogen growth regulators will be more activated enzymes that needed in bud multiplication. Responds a plant towards growth regulators were depend with species, part of the plant and interaction among growth regulators (Salisbury and Ross 1995; Hopkins 1995).

All treatment conducted without addition of coconut water only to give 0-2 buds (Table 2). This reason can be caused by incapability endogen cytokinin to increase bud multiplication without exogenous cytokinin that come from coconut water. George and Sherrington (1984) said that if the cytokinin on sub optimum condition therefore required exogenous cytokinin to obtain proportion between endogen cytokinin and exogenous cytokinin to bud multiplication.

Application 1.5 ppm NAA in all level concentration of coconut water only give 0-1 axillary bud (Table 2). Axillary bud multiplication only requires efficient concentration of cytokinin without auxin or with low auxin concentration (Wattimena 1992). Endogen auxin and 1.5 ppm NAA interaction can be stimulate synthesis of ethylene. Ethylene on the plant cells can be inhibit plant growth (George and Sherrington 1984; Hopkins 1995). Similarly, application 20 ppm NAA + 150 g/L sweet potato on black orchid (*Coelogyn pandurata* Lindl) culture was give only 1.5 buds (Untari and Puspitaningtyas 2006).

The treatment of 0.5 ppm NAA + 5% coconut water was showed that explants cannot form axillary bud and growth slower than the other treatments. This case can be realized by lasting of emergence timing of apical bud that is 46 days after planting. Eventuality, interaction between endogenous cytokinin and cytokinin on 5% coconut water more effective to form the chlorophyll, whereas all leaves’ plantlets on this treatment greener than leaves’ plantlets on other treatment. Salisbury and Ross (1995) state that once of cytokinin function was increase synthesis of protein that chlorophyll attaches.
### Table 2. Effect of supplemented NAA and coconut water on average number of bud and average number of leaf

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of bud</th>
<th>Average number of leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical</td>
<td>Axillary</td>
</tr>
<tr>
<td>0 ppm NAA + 0% coconut water</td>
<td>1</td>
<td>1.33 a</td>
</tr>
<tr>
<td>0 ppm NAA + 5% coconut water</td>
<td>1</td>
<td>3 abc</td>
</tr>
<tr>
<td>0 ppm NAA + 7.5% coconut water</td>
<td>1</td>
<td>6 bc</td>
</tr>
<tr>
<td>0 ppm NAA + 10% coconut water</td>
<td>1</td>
<td>2 ab</td>
</tr>
<tr>
<td>0.5 ppm NAA + 0% coconut water</td>
<td>1</td>
<td>2 ab</td>
</tr>
<tr>
<td>0.5 ppm NAA + 5% coconut water</td>
<td>1</td>
<td>0 a</td>
</tr>
<tr>
<td>0.5 ppm NAA + 7.5% coconut water</td>
<td>1</td>
<td>11 d</td>
</tr>
<tr>
<td>0.5 ppm NAA + 10% coconut water</td>
<td>1</td>
<td>7.3 cd</td>
</tr>
<tr>
<td>1 ppm NAA + 0% coconut water</td>
<td>1</td>
<td>0 a</td>
</tr>
<tr>
<td>1 ppm NAA + 5% coconut water</td>
<td>1</td>
<td>6 bc</td>
</tr>
<tr>
<td>1 ppm NAA + 7.5% coconut water</td>
<td>1</td>
<td>1.67 ab</td>
</tr>
<tr>
<td>1 ppm NAA + 10% coconut water</td>
<td>1</td>
<td>1.33 a</td>
</tr>
<tr>
<td>1.5 ppm NAA + 0% coconut water</td>
<td>1</td>
<td>1 a</td>
</tr>
<tr>
<td>1.5 ppm NAA + 5% coconut water</td>
<td>1</td>
<td>0.33 a</td>
</tr>
<tr>
<td>1.5 ppm NAA + 7.5% coconut water</td>
<td>1</td>
<td>0.67 a</td>
</tr>
<tr>
<td>1.5 ppm NAA + 10% coconut water</td>
<td>1</td>
<td>1 a</td>
</tr>
</tbody>
</table>

Note: Figure in same column in each group followed by the same letter is not significantly different According to DMRT, P<0.05

### Number of leaf

Variations of average number of leaf are 2.23-5.33 leaves (Table 2). Formating of leaf was related with the emergence timing of bud. The last emergence timing of apical bud is 30 days until 53 days after planting only produce 2.33 until 3.33 leaves, whereas the treatment that gives to emergence timing 13 days until 28 days after planting was produce 3.67 until 5.33 leaves. Proportion among of endogen growth regulators, NAA and growth regulator on coconut water were used to growth apical bud before. Forming of leaves achieved after growth of apical bud. Exogen growth regulators can be reaches growth primordial of leaf (George and Sherrington 1994; Hidayat 1995).

The average number of axillaries bud’s leaves that was achieved in coconut water alone treatments was 2.23 – 2.87 leaves (Table 2). This result showed that cytokinin on coconut water has been able to induction divided of leaf cells. Dixon and Gonzales (1994) state that application of cytokinin without auxin is completely optimum for divide and extend of leaf cells. Otherwise, coconut water was contains some elements that are Ca and vitamins that used to stimulate addition number of leaf (Hendaryono and Wijayani 1995). Application of 15 ppm NAA + 250 mL/L coconut water can be stimulate addition number of leaf of black orchid culture that is 3.3 leaves (Untari and Puspaningtyas 2006).

### Number of root

The result showed that forming root in the plantlets can achieved only on four treatments that are 1 ppm NAA + 7.5% coconut water; 1 ppm NAA + 10% coconut water; 1.5 ppm NAA + 5% coconut water and 1.5 ppm NAA + 7.5% coconut water. Eventuality, the treatments mentioned, have an efficient of proportion among NAA, auxin on coconut water and auxin endogen that stimulate forming of root. Auxin is a fitohormone used to stimulate initiation primordia of root. When, ratio auxin is higher than cytokinin initiation of root can be stimulated (George and Sherrington 1995; Wattimena 1992).

The fastest of emergence timing of root achieved on combination 1.5 ppm NAA + 5% coconut water that is 23 days after planting. Opinion that, ratio NAA and growth regulator on coconut water was efficient to induction growth of root. Otherwise, this treatment has the fastest emergence timing of apical bud that is 13 days after planting. The apical bud will be synthesis auxin, auxin will be translated polar basipetal to induction growth of root (Hopkins 1995; Salisbury and Ross 1995).

### CONCLUSION

Based on the result of analysis, it showed that there was a significant effect of the NAA and coconut water application of emergence timing of bud, number of bud and number of leaf produced by the explants. The treatment of 1.5 ppm NAA and 10 % coconut water has a good effect on the emergence timing of axillary bud that is 13 days after planting. Combination of 0.5 ppm NAA and 7.5% coconut water have a good effect on number of bud multiplication that is 11 buds.

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Evaluation of uniformity, variability, and stability of agronomic traits of doubled haploid rice lines resulting from anther culture

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Abstract. Sasmita P. 2010. Evaluation of uniformity, variability, and stability of agronomic traits of doubled haploid rice lines resulting from anther culture. Nusantara Bioscience 2: 67-72. The formation of doubled haploid lines in anther culture aims to accelerate the acquisition of pure lines. Selection of the desired traits can be done directly to the progeny of anther culture results at early generations. This experiment aims to determine agronomic traits, uniformity, and stability of the doubled haploid lines, and obtain the putative doubled haploid lines as the material for further evaluation to obtain expected lines. The first experiments used completely randomized design which was repeated five times. The treatments were 111 doubled haploid lines of first generation of anther culture results (DH1). The second experiment used split plot design with the main plot treatments were doubled haploid lines resulting from anther culture and the subplot treatment were the second generation of doubled haploid lines (DH2) until the fifth generation (DH5). The results show that each plant within the same line have uniform agronomic traits, while the plants between different lines have different agronomic traits. The results of further evaluation on three out of 111 doubled haploid lines derived from the second to fifth generations show no difference between generations for each trait of the same lines. The results also show that the agronomic traits of the doubled haploid line were stable from generation to generation.

Key words: doubled haploid lines, uniform, stable, promising lines.

INTRODUCTION

Anther culture is one of tissue culture techniques that can be applied to plant breeding programs in order to accelerate the process of obtaining a pure line. The technique is done in vitro technically through two stages, i.e. callus induction stage of pollen contained in the anther, and stage of plant regeneration from the callus. Stages of plant regeneration produces haploid plants, it is obtained through embryogenesis induction from repeated division of monoploid spores of F1 or F2 plants resulting from the crossing among parents those has the desired trait. When the chromosomes are doubled or a spontaneous doubling occurs during culture process, it will obtain homozygous doubled haploid plants. The traits controlled either by dominant genes and recessive genes can be expressed in the early generation of plants.

The results of previous studies show that the doubled haploid plants can be obtained directly, together with other plants that have other ploidy on rice anther culture techniques (Chu 1982; Dodds and Robert 1987; Goddard et al. 1996). According to Chen (1983) these plants originated from pollen cells, because only pollen cells that initiate to develop callus and develop into plants regeneration on rice anther culture. The result of genetic analysis shows that 90% of fertile progeny resulted from anther culture were doubled haploid (dihaploid) plants (Chu 1982). Trait of doubled haploid plants of the same line was uniform and remains stable from generation to generation, so selection can be done directly on the early generation plants (Zhang 1989).
The formation of spontaneous doubled haploid plants on rice anther culture is very beneficial, because it does not need to double the haploid plants as material selection. This method has been developed as an alternative in rice breeding to obtain pure lines as selection materials in order to accelerate the development of new superior varieties (Chahal and Gosal 2002).

To obtain genetic variability of doubled haploid plants through anther culture techniques, we use explants (anthers) from plants that have high heterozygosity, F1 or F2 plants (Fehr 1987). Those anthers can be collected from part of rice flower (inside young panicle) at booting stage. Plant genetic variability caused by segregation of genes randomly during meiosis in microspore formation process of used F1 or F2 plants. The traits controlled by dominant genes and recessive genes can be expressed in early generation of doubled haploid plants, so the selection of the desired traits can be done in early generations. According to Zhang (1989) and Chung (1992), selection of main agronomic traits such as yield and grain quality and also tolerance to biotic or abiotic stress which were controlled by minor genes can be done at the generation of DH1 and DH2. Therefore the use of anther culture in breeding programs beside to improve the efficiency of selection, also to reduce the cost, the time and the labor (Chung 1992; Goddard et al. 1996; Niizeki 1997). Application of anther culture in rice breeding program has been reported to create a variety of superior varieties such as in China and Korea (Hu 1985; Li 1992; Chung 1992). Parts of the rice flower are showed in Figure 1.

Sasmita et al. (2002) report that results of anther culture of F1 upland rice obtained genetic material as many as 111 doubled haploid lines. The plants were resulted from regeneration of various callus at regeneration stage in anther culture. The plants originating from one callus or same pollen and expressing uniform phenotype were grouped into one line and they were estimated to be doubled haploid lines (homozygous). These lines could potentially be used as a population of selection material to get a new superior rice line (promising lines). To prove that these lines are pure lines, it is necessary to evaluate the uniformity of agronomic traits of each line and its stability between generations. This experiment aims to obtain information of uniformity and stability of agronomic and morphological traits of doubled haploid lines resulted from anther culture as identifier of pure lines (homozygous).

**MATERIALS AND METHODS**

The experiment was conducted in September 2004 through January 2005 at Greenhouse of Research Institute for Agriculture Biotechnology and Genetic Resources, Bogor, West Java. This study consists of two experiments: first, the evaluation of the uniformity of agronomic traits in the same line and its variability among the lines, and second, the evaluation of the stability of agronomic traits of doubled haploid lines from generation to generation. The genetic material used in the first experiment were 111 genotypes (lines) of first generation of doubled haploid
(DH1) upland rice lines resulted from anther culture, while the material used in the second experiment were three doubled haploid lines of the second generation to fifth generation (DH2, DH5), namely GI-8, IG-19 and IW-56 lines.

The first experiment used a completely randomized design with five replications. The treatments consist of 11 doubled haploid lines (DH1) resulted from anther. One experimental unit was one pot containing two hills of plants for each genotype. The planting and maintenance was done based on upland rice cultivation. Seeds of each genotype were planted in one pot (as a plot) that contains the media of soil and manure. Each pot was planted by two seeds from the same line on the two planting points. Fertilizer was given at a dose of 200 kg Urea, 100 kg SP36, and 100 kg KCI per hectare. Half dose of urea, the whole dose of SP36 and KCI were given as a basic fertilizer mixed with the planting medium, while the remaining half dose of urea was given to the plants at 45 days after seed planting. Weeding was done twice, i.e. 30 and 40 days after seed planting. Pest control was done based on integrated pest management.

Observations was done on 13 agronomic traits at vegetative phase and reproductive phase. At the vegetative phase observation was done on plant height and the number of tiller per hill, while at the reproductive phase was done on flowering time (days), harvesting time (days), plant height at harvest time which was measured from the root neck to the panicle neck (cm), and the total number of tillers and productive tillers at harvest time (tiller/hill). Observations was also done on the yield and yield components, namely, panicle length which was measured from the panicle neck to the tip of the panicle (cm), number of grain/panicle (grains), filled and empty grain number, per panicle, 100 grains weight (g), and the grain production weight per hill (g).

The second experiment used split plot design with four replications. The main plot treatment were doubled haploid lines, namely GI-8, IG-19 and IW-56, while the subplot treatment were generation of those doubled haploid lines, i.e. second (DH2), third (DH3), fourth (DH4) and fifth generation (DH5) of those lines used. Each line grows on plot with 2.4 m x 1.5 m size and plant spacing of 30 cm x 20 cm. The planting and maintenance was done based on upland rice cultivation. Three seeds per hole were planted for each line. One experimental unit consists of five rows of plants. The sample plants were considered to be 10 plants, i.e. plants which were located on the middle row.

Fertilizer was given at a dose of 200 kg Urea, 100 kg SP36, and 100 kg KCI per hectare. Half dose of Urea, the whole dose of SP36 and KCI were given as a basic fertilizer at 15 days after seed planting, while the remaining half dose of urea was given to the plants at 45 days after seed planting. Crop arrangement was done at 14 days after planting by leaving two seeds per planting hole. Weeding was done twice: first, at 14 days after planting, and the second at 45 days after planting. The observation were done on 13 agronomic traits as it was done on the first experiment.

For the first experiment, traits homogeneity of each line was determined based on the Z value or the value of data standardization of each trait of individual plants from all five replications (10 plants). The uniform traits (homogeneous) were the traits that have frequency of observation data below the Z 97.5% curve which was bounded by -1.96 ≤ Z ≤ +1.96 with the data deviation < 20%. Furthermore, the variability among lines were analyzed by analysis of variance. For the second experiment, data was analyzed using analysis of variance, if the generation treatment has no significant effect on a trait, then the trait was considered to be stable treatment has no significant effect on a trait, then the trait was considered to be stable.

**RESULTS AND DISCUSSION**

**Uniformity of the traits**

Uniformity of the traits in the same line as well as the high variability among different lines is an important characteristic of the selection material population. The traits uniformity in the same line is one of pure line identifier ( homozygous), while the population with a high variability among lines is the expected population that providing a great opportunity to get a genotype with the desired trait.

Based on the standardization results onto the 'Z' value from the observation data of each trait for each line, the overall data deviations were < 10%, except for the observation data of plant height and number of tiller on 45 days after planting. The data average deviation from normal distribution of Z values for each agronomic trait of the entire doubled haploid lines tested were presented in Table 1. These data indicate that the average of agronomic trait in the same line following the normal distribution of Z97.5% curve which was limited by the Z value = -1.96 and Z = +1.96, meaning that every trait in the same line shows uniformity.

**Table 1. Data deviations of agronomic traits in the same line and variability among lines.**

<table>
<thead>
<tr>
<th>Agronomic traits</th>
<th>The same lines (Deviation of Z 97.5 (%))</th>
<th>Among lines (F value (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height at 45 days after planting</td>
<td>10.8</td>
<td>97.8**</td>
<td>33.0</td>
</tr>
<tr>
<td>Number of tiller at 45 days after planting</td>
<td>10.6</td>
<td>39.6**</td>
<td>52.7</td>
</tr>
<tr>
<td>Flowering time</td>
<td>6.1</td>
<td>29.7**</td>
<td>13.3</td>
</tr>
<tr>
<td>Harvesting time</td>
<td>6.5</td>
<td>46.3**</td>
<td>11.3</td>
</tr>
<tr>
<td>Plant height at harvest time</td>
<td>7.9</td>
<td>51.0**</td>
<td>22.0</td>
</tr>
<tr>
<td>The number of total tiller</td>
<td>7.2</td>
<td>9.8*</td>
<td>25.4</td>
</tr>
<tr>
<td>The number of productive tiller</td>
<td>8.1</td>
<td>27.6**</td>
<td>40.7</td>
</tr>
<tr>
<td>Panicle length</td>
<td>6.8</td>
<td>23.0*</td>
<td>24.5</td>
</tr>
<tr>
<td>The number of grains per panicle</td>
<td>5.2</td>
<td>46.3**</td>
<td>19.9</td>
</tr>
<tr>
<td>The number of filled grain per panicle</td>
<td>5.9</td>
<td>44.6**</td>
<td>25.1</td>
</tr>
<tr>
<td>Grain sterility</td>
<td>5.5</td>
<td>36.7**</td>
<td>65.3</td>
</tr>
<tr>
<td>The weight of 100 grains</td>
<td>7.2</td>
<td>158.7**</td>
<td>44.1</td>
</tr>
<tr>
<td>Grain weight per hill</td>
<td>8.1</td>
<td>36.8**</td>
<td>57.9</td>
</tr>
</tbody>
</table>

Note: CV = coefficient of genetic variance, * = significant at 5% level, ** = significant at 1% level.
According to Baihaki (2000), the amount of variation in populations of pure lines can be presented as a scale with a normal distribution curve. Theoretically, in a plant population of pure line, there is no genetic variation; variation that occurs is mostly caused by environmental factors. The results of this evaluation show that the first generation rice lines from anther culture are indeed doubled haploid lines or pure line.

Evaluated doubled haploid lines in this study were result of callus induction from pollen and were not derived from anther somatic cells. Results of previous studies have proved that only the initiated (induced) pollen that can develop in anther culture process, whereas the somatic cells were not induced but act as a source of metabolites necessary for the development of callus (Chu 1982; Chen 1983). Plants derived from callus or pollen which were used in this study demonstrate the phenotypic expression which were uniform and can be grouped into one line. The results of this study indicate that lines of the early generations result from anther culture are pure lines (homozygous). These results also supports the previous reports indicating that each individual plant in a population of same doubled haploid lines (from the same callus) have a uniform agronomorphologic traits (Suhartini and Somantri 2000; Goddard 2002).

**Variability of agronomic traits among the lines**

Traits variability of plant populations determines the success of plant breeders in getting a new genotype with expected combination of superior traits. The greater variability of population selection is available, the greater the probability of obtaining a genotype with the expected combination of superior traits. The greater success of plant breeders in getting a new genotype with uniform agromorphologic traits (Suhartini and Somantri 1995) of same doubled haploid lines (from the same callus) have reports indicating that each individual plant in a population of pure lines or pure line.

Results of variance analysis showed that the agronomic traits among the doubled haploid lines were significant different (Table 1). Agronomic traits which have the genetic variability and relatively high coefficients of genetic variance were the plants height at 45 days after planting (33.0%), number of tiller (52.7%), productive tillers (40.7%), sterility (65.3%), weight of 100 grains (44.1%), and grain weight per hill (57.9%). The variance value indicates a great probability to have the expected trait from the evaluated population. These results support the results of research by Dewi (2002) which show that there was great agronomorphologic variability in the population of doubled haploid rice lines (DH1) obtained from anther culture. The agronomic traits appearance of doubled haploid lines obtained from anther culture were presented in Table 1.

Table 2 shows that the agronomic traits of the plant height at harvest time range from short to medium category (72.2 to 119.6 cm), while their productive tiller were in categories of little to many (5.3 to 28.4 tillers). The flowering time of the evaluated lines ranges from 58.4-82.0 days after planting and harvesting time at 96.8 to 131.7 days after planting. Based on the classification by Siregar (1981), harvesting time of the evaluated lines are classified into the category of very early maturing (harvest time < 125 days after planting), medium (115 ≤ harvest time < 125 days after planting), as well as the long maturity (harvest > 125 days after planting).

**Table 2.** Appearances of agronomic traits of doubled haploid lines obtained from anther culture.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Value</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height at 45 days after planting (cm)</td>
<td>34.6</td>
<td>8656.1**&lt;1**</td>
</tr>
<tr>
<td>Number of tiller at 45 days after planting</td>
<td>4.0</td>
<td>1463.5**&lt;2**</td>
</tr>
<tr>
<td>Flowering time (days after planting)</td>
<td>58.4</td>
<td>349.2**&lt;1**</td>
</tr>
<tr>
<td>Harvesting time (days after planting)</td>
<td>96.8</td>
<td>1365.4**&lt;1**</td>
</tr>
<tr>
<td>Plant height at harvest time (cm)</td>
<td>72.2</td>
<td>1574.9**&lt;1**</td>
</tr>
<tr>
<td>The number of total tiller</td>
<td>31.4</td>
<td>404.5**&lt;1**</td>
</tr>
<tr>
<td>The number of productive tiller</td>
<td>5.3</td>
<td>1223.7**&lt;1**</td>
</tr>
<tr>
<td>Panicle length (cm)</td>
<td>18.8</td>
<td>386.9**&lt;1**</td>
</tr>
<tr>
<td>The number of grains per panicle</td>
<td>99.2</td>
<td>1463.5**&lt;2**</td>
</tr>
<tr>
<td>The number of filled grain</td>
<td>78.2</td>
<td>386.9**&lt;1**</td>
</tr>
<tr>
<td>Weight of 100 grains (g)</td>
<td>2.22</td>
<td>1574.9**&lt;1**</td>
</tr>
<tr>
<td>Sterility (%)</td>
<td>8.8</td>
<td>1574.9**&lt;1**</td>
</tr>
<tr>
<td>Grain weight/hill (g)</td>
<td>10.6</td>
<td>1463.5**&lt;2**</td>
</tr>
</tbody>
</table>

**Stability of agronomic traits among generations**

The stability trait of a plant genotype resulted from breeding is a requirement that must be fulfilled before being released as a new variety. Stability analysis basically aims to measure the variation of a genotype in different environments. In this study, the stability is intended to determine variations of a genotype trait on several generations of plants. The experiment was done on the same environment, with the aim that if there are variations, they are only caused by genetic variation. The results of the variability analysis to the effect of genotype, generation, and interaction between genotype and generation to agronomic traits of doubled haploid lines obtained from anther culture are presented in Table 3.

Table 3. Result of variance analysis of the effect of genotype (line), generation, and interaction of genotype and the generation to agronomic traits of doubled haploid lines obtained from anther culture.

<table>
<thead>
<tr>
<th>Agronomic traits</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height at 45 days after planting</td>
<td>8656.1**&lt;1**</td>
</tr>
<tr>
<td>Number of tiller at 45 days after planting</td>
<td>1463.5**&lt;2**</td>
</tr>
<tr>
<td>Flowering time</td>
<td>349.2**&lt;1**</td>
</tr>
<tr>
<td>Harvesting time</td>
<td>1365.4**&lt;1**</td>
</tr>
<tr>
<td>Plant height at harvest time</td>
<td>1574.9**&lt;1**</td>
</tr>
<tr>
<td>The number of total tiller</td>
<td>404.5**&lt;1**</td>
</tr>
<tr>
<td>The number of productive tiller</td>
<td>1223.7**&lt;1**</td>
</tr>
<tr>
<td>Panicle length</td>
<td>386.9**&lt;1**</td>
</tr>
<tr>
<td>The number of grains per panicle</td>
<td>157.8**&lt;1**</td>
</tr>
<tr>
<td>The number of filled grain per panicle</td>
<td>96.0**&lt;1**</td>
</tr>
<tr>
<td>Grain sterility</td>
<td>7.8**&lt;1**</td>
</tr>
<tr>
<td>The weight of 100 grains</td>
<td>2120.0**&lt;1**</td>
</tr>
<tr>
<td>Grain weight per hill</td>
<td>20.8**&lt;1**</td>
</tr>
</tbody>
</table>

Note: **= significantly different at level 1%, ns = not different significantly.
Results of variance analysis show that genotype (line) significantly affect to observed agronomic traits. The evaluated agronomic traits of the three lines are GI-8, IG-19, and IW-56 are presented in Table 4. The results show that the IW-56 line is the shortest line and has the highest number of tiller. At the time of harvest, the plant height was 72.3 cm with the number of productive tiller are 19.2, while the two other lines, namely GI-8 and IG-19, have plant height are 84.2 cm and 86.5 cm, and the number of tiller are 8.6 and 9.9 tillers.

Harvesting time, yield components and yield of the three lines were significantly different in general from each other. The longest harvesting time was shown by the GI-8 (123.7 days) while the two other lines were shorter. The highest yield (grain weight per hill and yield per plot) were achieved by the IW-56 line. It was estimated that the number of tiller per hill which were more gives contribution on the yield of this line. Grain weight per hill and yield per plot of the line were 57.20 g and 2.80 kg (Table 4). The two other lines show yield that were not significantly different from each other and were lower than the IW-56. In general, agronomic traits of the three doubled haploid lines, i.e. GI-8, IG-19 and IW-56, different from one another, but there were no significant differences in agronomic traits among generations (DH2-DH5) for the same line. It means that the agronomic traits are stable from generation to generation. Performance of different generation doubled haploid lines (DH2-DH5) in the same genotype (line) at vegetative stage were presented on Figure 2.

**Table 4.** Agronomic traits of doubled haploid rice genotype resulting from anther culture.

<table>
<thead>
<tr>
<th>Traits</th>
<th>GI-8</th>
<th>IG-19</th>
<th>IW-56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height at 45 days after planting</td>
<td>67.0 a</td>
<td>69.1 a</td>
<td>39.2 b</td>
</tr>
<tr>
<td>Number of tiller at 45 days after planting</td>
<td>9.1 b</td>
<td>9.0 b</td>
<td>16.1 a</td>
</tr>
<tr>
<td>Flowering time</td>
<td>85.9 a</td>
<td>63.4 c</td>
<td>68.9 b</td>
</tr>
<tr>
<td>Harvesting time</td>
<td>123.7 a</td>
<td>103.8 b</td>
<td>107.6 b</td>
</tr>
<tr>
<td>Plant height at harvest (cm)</td>
<td>84.1 b</td>
<td>86.5 a</td>
<td>72.3 c</td>
</tr>
<tr>
<td>The number of total tiller</td>
<td>11.9 c</td>
<td>14.6 b</td>
<td>24.2 a</td>
</tr>
<tr>
<td>The number of productive tiller</td>
<td>8.6 c</td>
<td>9.9 b</td>
<td>19.2 a</td>
</tr>
<tr>
<td>Panicle length (cm)</td>
<td>22.1 c</td>
<td>28.3 a</td>
<td>27.1 b</td>
</tr>
<tr>
<td>The number of grains per panicle</td>
<td>145.2 b</td>
<td>138.4 c</td>
<td>154.9 a</td>
</tr>
<tr>
<td>The number of filled grain per panicle</td>
<td>133.1 b</td>
<td>124.1 c</td>
<td>140.4 a</td>
</tr>
<tr>
<td>Grain sterility (%)</td>
<td>13.8 a</td>
<td>14.5 a</td>
<td>13.5 a</td>
</tr>
<tr>
<td>The weight of 100 grains (g)</td>
<td>3.22 b</td>
<td>4.16 a</td>
<td>2.57 c</td>
</tr>
<tr>
<td>Grain weight per hill (g)</td>
<td>47.27 b</td>
<td>47.31 b</td>
<td>57.20 a</td>
</tr>
<tr>
<td>Yield weight per plot (kg)</td>
<td>1.97 b</td>
<td>2.04 b</td>
<td>2.80 a</td>
</tr>
</tbody>
</table>

Note: number in the same row followed by same letters indicates not different significantly based on 5% of DMRT.
These results are consistent with Zhang’s research result (1989) which indicates that all the traits of doubled haploid rice lines originated from cultured one pollen trough anther culture which were planted from second generation to next generations showed no different significantly. It means that the appearance of all traits in this study are stable from 2nd generation to 5th generation. The previous research also indicate that more than 90% of diploid plants progeny which originated from pollen trough anther culture were homozygous and stable from generation to generation (Chu 1982; Niizeki 1997; Zhang 1989).

Chen (1983) shows that only pollen cells initiating to develop callus and then into plant regenerants in in vitro rice anther culture. In his research, he also indicates that anther wall tissue (tapetum cells) were not induced during the culture but plays an important role as a source of metabolites required for division and further proliferation of pollen. The use of metabolites from the anther wall was indicated by the formation of suspensor cells (multilayer) that connects the anther wall with microspores at the time of developing into globular embryos. This factor also supports the anther culture success rate higher than the culture of pollen and ovule.

Developed lines in breeding plants will eventually be planted by farmers in various different environments, while the phenotype appearance is determined by genetic factors, environmental, and the interaction of genetic and environmental factors. Therefore, the doubled haploid lines resulted from anther culture that have been identified as pure lines as shown in this study, need to be further evaluated through multi-location test to determine their traits stability among environments. The use of stable varieties or lines are useful for the development of a variety in a region, and also in extending the use of seeds as it can be planted in the next several generations.

CONCLUSION

Agronomic traits of doubled haploid rice resulted from anther culture which in the same line (genotype) were uniform, whereas the same agronomic traits among different lines were varied. Resulted doubled haploid lines from anther culture that have been evaluated are really pure lines, so they can directly be used as material selection to obtain lines with the expected superior traits. Agronomic traits of doubled haploid lines were stable from generation to generation, allowing these lines can be used as seed in a long time.

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Effect of seaweed extracts on growth and yield of rice plants

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Abstract. Sunarpi, Jupri A, Kurnianingsih R, Julisaniah NI, Nikmatullah A. 2010. Effect of seaweed extracts on growth and yield of rice plants. Nusantara Bioscience 2: 73-77. Application of liquid seaweed fertilizers on some plant species has been reported to decrease application doses of nitrogen, phosphorus and potassium on some crop plants, as well as stimulating growth and production of many plants. It has been reported that there are at least 59 species of seaweeds found in coastal zone of West Nusa Tenggara Province, 15 of those species were able to stimulate germination, growth and production of some horticultural and legume plants. The aim of this research is to investigate the effect of seaweed extracts obtained from ten species on growth and production of rice plants. To achieve the goal, seaweed (100 g per species) was extracted with 100 mL of water, to obtain the concentration of 100%. Seaweed extract (15%) was sprayed into the rice plants during vegetative and generative stages. Subsequently, the growth and yield parameters of rice plants were measured. The results showed that extracts of Sargassum sp.1, Sargassum sp.2, Sargassum polyctenum, Hydroclathrus sp., Turbinaria ornata, and Turbinaria murayana, were able to induce growth of rice plants. However, only the Hydroclathrus sp. extract could enhance both growth and production of rice plants.

Key words: extract, seaweed, growth, production, rice plants.

INTRODUCTION

Nationwide, the need for nitrogen (N), phosphorus (P) and potassium (K) fertilizers increased from 96,116 tons in 2006 to 739,271 tons in 2007 (Pusri 2008). The increase tends to be caused by the dosage increase of fertilizer used in per unit area. Facts show that farmers use about 300-350 kg of urea per hectare of rice, and about 200-250 kg per hectare to plant vegetables and fruits. This condition certainly not only increases production costs, but also reduces soil fertility, and causes environmental pollution. Ironically, the increase in fertilizer costs, coupled with a variety of economic losses due to excessive fertilization, are not followed with the increase in farmers’ income.

In order to decrease the financial burden of the farmers, government then has raised the fertilizer subsidy from 1.5 trillion in 2006 to around 5 trillion in 2007 (Agency for Agricultural Research and Development 2008). Yet, the provision of subsidy funds did not solve the problem of agricultural production, instead it causes the scarcity/lack of fertilizers in the country due to act of irresponsible speculators who selling the subsidized fertilizer to other countries. Therefore, the efforts to maximize the absorption of nutrients by spraying extracts of natural products that contain stimulants, is a strategic move to suppress the use of excessive doses of inorganic fertilizer.

Results of previous studies reported that some liquid fertilizer products made from raw seaweeds found in some countries, such as Seasol in Australia (Tay et al. 1987), Kelpak in Europe (Beckett and van Staden 1989), SM3, SM6 and Maxicrop in the United States (Hankins and Hockey 1990), Algaenzims in Mexico (Sanchez et al. 2003) and Algifert, Goemar GA14, Seaspray, Cytec and SeaCorp in India (Sivasankari et al. 2006), are proven to increase the absorption of nutrients, which can enhance growth, development and production of various species of agricultural crops.
West Nusa Tenggara (NTB) marine waters are reported to have about 59 species of seaweed (Sunarpi et al. 2005, 2006), 15 species of which can stimulate the germination of watermelon and sesame seeds (Sunarpi et al. 2007), the growth of bean plants (Sunarpi, 2008), the growth and production of tomato plants (Sunarpi et al. 2008; Sunarpi 2008). In addition, Tangaraju (2008) succeeded in lowering the dose of urea fertilizer in rice plants by spraying seaweed extract to the plants. However, there is insufficient information on the effect of seaweed extract which grows in tropical waters on the growth and yield of rice. Given the aforementioned facts, the study aims to determine the effect of several types of seaweed extracts on growth and yield of rice. The results showed that the extract of seaweed Sargassum sp.1, Sargassum sp.2, Sargassum polycistum, Ulva fasciata, Ulva furticculata, Padina sp., Chaetomorpha sp., and Hydroclathrus sp. that influences the growth and yield of rice. This has implications for efforts to reduce the dose of NPK fertilizer use, so it can lower the production costs and reduce environmental pollution on rice plants.

**MATERIALS AND METHODS**

**Design, time and place of study**

The study was designed using completely randomized design (CRD), which consists of treatment of ten kinds of seaweed extract, which is *Turbinaria murayana*, *Turbinaria ornata*, *Sargassum sp.1*, *Sargassum sp.2*, *Sargassum polycistum*, *Ulva fasciata*, *Ulva furticculata*, *Padina sp.*, *Chaetomorpha sp.*, and *Hydroclathrus sp.* The study was conducted in July-November 2009. Samples were taken from several sampling points in the sea of Lombok Island. The seaweed extraction was performed at the Laboratory of Imunobiology, Faculty of Mathematics and Natural Sciences, Mataram University. Rice planting and treatment were done in plastic house at Jatisela Village, Gunung Sari Subdistrict, West Lombok District, West Nusa Tenggara.

**Preparation of seaweed extract**

Seaweed that has been collected on Lombok sea waters, i.e. *Turbinaria murayana*, *Turbinaria ornata*, *Sargassum sp.1*, *Sargassum sp.2*, *Sargassum polycistum*, *Ulva fasciata*, *Ulva furticculata*, *Padina sp.*, *Chaetomorpha sp.*, and *Hydroclathrus sp.*, each was weighed as much as 100 grams, cut into pieces and placed in the blender. After that, 100 mL of distilled water was added (ratio of 1:1 (w/v)), the mixture then blended until smooth, and filtered using filter paper. The slurry was centrifuged for 5 minutes at 4°C at 5000 rpm speed. Following this, the supernatant was transferred to a Falcon tube (designated as an extract with 100% concentration). Finally, 15% extract was prepared by mixing 15 mL of it into 85 mL of water.

**Planting and plant treatment**

The medium used in this study is potting mix composed of soil, sand and manure (1:1:1 (w:w). The three components was then homogeneously mixed, weighed 8 pounds, and put in a plastic pot (size of 5 L). Rice seeds were sowed by spreading them in the nursery pots containing planting medium as described previously. After 21 days, rice seedlings were planted in the medium that had been prepared in the pot, one clump per pot. Inorganic fertilizers, NPK fertilizer each with a dose of 2.4 g urea, 1.2 g KCl gTSP and 0.36 per pot, were applied to the pot 14 days after transplanting. During the course of experimentation, the plant was maintained according to procedure standards for paddy crop, from planting to harvesting the rice crop.

Seaweed extract treatment was done by spraying the whole plant (4 times, 2 times during the vegetative phase and 2 times during the generative phase). Spraying on the vegetative growth phase was done when the rice was 3 weeks and 6 weeks after planting, with spray volume of 20 and 30 mL per pot. During the generative phase the spraying was carried out on the time of flowering and fruit filling, each spray volume was 50 mL per pot.

Growth parameters observed were plant height, number of leaves, number of tillers and weight of stems and roots. Plant height was observed by measuring the plant height from the base of the clump which was exactly above the ground until the end of the highest grove. Number of leaves was observed by counting the number of leaves on each clump, while number of tillers was observed by counting the number of puppies/tillers that grow from each clump. The observation was carried out from 14 days after planting, with intervals of 3 days. Eight of stems and roots were measured after the completion of all other observation. Observations were conducted by calculating the fresh weight of plants.

The crop yield parameters observed were the number of panicles, number of grains (seeds), weight per 100 seeds, and weight of seed per clump. The number of panicles was calculated by counting the number of panicles in each clump of plants per pot. Number of grains (seeds) per panicle was observed by calculating the number of grains (seeds) per panicle in each clump while weight per 100 seeds was observed by measuring the weight per 100 seeds. Weight of seeds per clump was observed measured by measuring the weight of seeds in each clump. All the data obtained are expressed in the form of an average of replications ± SE.

**Data analysis**

Data were analyzed only by calculating the average value of three replications in each test, and presented in graphical form.

**RESULTS AND DISCUSSION**

**Vegetative growth of rice**

The effect of several kinds of seaweed extracts on the growth and production of paddy was observed by measuring some growth and yield parameters of rice...
following the treatments. Growth parameters observed were plant height, leaf number, number of tillers, weight stems and roots.

Most of the seaweed extracts tested did not give positive response to the rice plant height. They mostly decreased the plant height while only the height of rice paddy treated with three extracts, *Sargassum* sp.1, *Ulua furticulata* and *Hydroclathrus* sp., were not suppressed. Interestingly, the height of plants treated with extract of *Sargassum* sp.1 (91.67 cm) was slightly higher than the control plants (91.00 cm) (Figure 1).

Different phenomena is found in the number of leaves. Most of the plants that were given seaweed extract treatments had 5-25 more leaves compared with control plants. The highest average number of leaves was observed in *Sargassum* sp.1- and *S. polycistum*-treated plants, at which 100.33 leaves were found compared to 75.7 leaves in control plants (Figure 2). The big difference in the number of leaves on the plant-treated with extracts and without the extract indicates the effect of seaweed extracts on the number of leaves of rice. This phenomenon may be due to the presence of active compounds, micro-and macronutrients in the extract of seaweeds (macroalgae), which can stimulate plant growth (Abetz 1980; Finnie and Van Staden 1985). Previously, it was suggested that various species of marine algae found in nature or commercially cultivated contain organic compounds which activity resemble the activity of a cytokinin, auxin and gibberellin (Crouch and Staden 1993). These compounds were able to stimulate growth as a result of enhancement of protein synthesis and cell division, and mobilization of nutrients needing for growth (Pascale 1993).

The seaweed extracts increase the number of tillers of rice plants (Figure 3). There were four kinds of seaweed extracts that able to induce the formation of rice plant tillers. The highest number of tillers present in plant-treated with *Hydroclathrus* sp. (26.33 tillers), followed by *Turbinaria ornata* (25 new plants), while the average number of tillers on control plants was only about 18.

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In regard to fresh weight of stems and roots, extracts *Hydroclathrus* sp. was significantly enhanced the growth by increasing fresh weight of rice plant stems and roots (Figure 4). *Hydroclathrus* sp.-treated plants has an average weight of 190.5 g (the weight of control plant is only 130.87 g) and roots fresh weight of 51 g (the weight of control plant is 39.3 g).

Plant weight is affected by the concentration of nutrients, as well as the quantity of photosynthetic products of the plant. Seaweed extract application on plant is suggested to capable of increasing nutrient concentrations in the leaves, through involvement of growth hormone in the process of nutrients absorption and movements in a plant, thus increasing the weight of the plant. It was reported in other systems that seaweed extract known of *Ascophyllum nodosum* contains growth hormones, namely IAA, while brown alga *S. heterophyllum* contains sitokinin (Crouch and Staden 1993). On addition, Smith and Staden (1984) found that cytokinin activity was higher in plants treated with seaweed extract compared to the control-untrated plants.

**Production of rice**

Effect of seaweed extract treatment on rice was also observed in the rice product. In this study, several yield parameters observed were the number of panicles, number of grains (seeds) in each panicle, and weight per 100 seeds. The number of panicles formed depends on the number of tillers present in every plant rice. The number of panicles
that were form will be directly proportional to the number of tillers came out in the rice plant. As explained previously that most types of seaweed extract give a quite positive influence on the growth of rice seedlings. In line with these conditions, the number of panicles on the plant-treated with seaweed extracts were higher than the control plants (without extract). Plants that have the most number of panicles is a plant-treated with *Hydroclathrus* sp. extract with an average number of panicles of 26.33, while the control plants has only 18 units (Figure 5). Seaweed extract-treated plants were capable to produce more grain (seed) per panicle (Figure 6). Control plants were only capable of producing 160.11 grain per panicle, while the plants which were given *Hydroclathrus* sp. seaweed extract produced 171.11 grains per panicle. Although seaweed extracts enhanced the number of seeds per panicle, they did not alter grain weight. In most cases, they promoted similar 100 grain weight per panicle or in other case they even decreased it Figure 7).

Figure 4. The effect of seaweed extract to the branaksan weight of the rice plant: A. Roots, B. Stem.

Figure 5. The effect of seaweed extract to the amount of rice panicle

Figure 6. The effect of seaweed extract on number of grains (seeds) on each panicle rice plants

Figure 7. The effect of seaweed extracts on seed weight of rice plants; A. Weight per 100 seeds, B. Heavy seeds /clump.
The opposite occurs on the weight of grain (seed) in each clump of rice plants. The highest grain weight in each cluster was observed in plant treated with extract Hydroclathrus sp. which is about 66.89 g, while control plants weighed only about 46.80 g (Figure 7). Taken together, the results indicate that application of extracts from some seaweed species could promote growth and production of rice paddy and this imply that it may be possibility to reduce the inorganic fertilizers dosage for rice paddy cultivation.

CONCLUSIONS AND RECOMMENDATIONS

Seaweed extracts that are able to induce vegetative growth of rice plants are the extract of Sargassum sp.1, Sargassum sp.2, Sargassum polycistum, Hydroclathrus sp., Turbinaria ornata, and Turbinaria murayana. However, only extracts Hydroclathrus sp. that can stimulate both growth and yield of rice. Further studies are now underway to examine the effect of combined extracts and solid fractions of seaweed on growth and production of rice plants as well as to test the effect of seaweed solid and liquid fractions on NPK fertilizer use efficiency in rice cultivation.

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The diet of spotted cuscus (Spilocuscus maculatus) in natural and captivity habitat

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Abstract. Saragih EW, Sadsoeitoeboen MJ, Pattiselanno F. 2010. The diet of spotted cuscus (Spilocuscus maculatus) in natural and captivity habitat. Nusantara Bioscience 2: 78-83. The ex-situ conservation of cuscus (Spilocuscus maculatus) under captivating condition is an alternative solution to protect cuscus from extinction. Diets became the main factor in order to support the domestication process. Particular studies on habitat and diet of cuscus have been carried out however there is still limited information on the nutrition aspects of cuscus food. This study aimed to determine the diet type, palatability and nutrient in both natural habitat and captivating condition. The results indicated that there were 19 and 8 plant species identified as cuscus diets in both natural habitat and captivating condition. Cuscus prefers fruits with astringent and sour taste which is contained high crude fiber and low fat.

Key words: cuscus, diets, habitat, nutrient contents, wildlife.

INTRODUCTION

Cuscus (Phalangeridae) is marsupial’s animal which has long tail, round eye and hairy. There are five species of cuscus in Papua: Phalanger gymnotis (ground cuscus, kuskus kelabu), Spilocuscus maculatus (spotted cuscus, kuskus bertotol biasa), Phalanger orientalis (northern common cuscus kuskus timur), Spilocuscus rufoniger (black-spotted cuscus, kuskus totol hitam) and Phalanger vestitus (Stein's cuscus, kuskus rambut sutera) (Petocz 1994). In addition, Menzies (1991) stated Spilocuscus papuensis (Waigeo cuscus, kuskus pulau Waigeo) is endemic species of Waigeo Island, Raja Ampat District, West Papua Province; while Aplin and Helgen (2008) stated Spilocuscus wilsoni (Biak spotted cuscus, kuskus totol pulau Biak) is endemic species to the islands of Biak and Supiori in the Cenderawasih Bay, Papua Province. Moreover, Helen et al. (2004) reported phalagerid genus Spilocuscus are endemic to tropical forest in the Australo-Papuan region.

In Indonesia, cuscus include in species that protected by the government regulation, Ministry of Agriculture Decree No. 247/Kpts/Umi/4/1979, Government Decree No. 7 of 1999. The reduction of habitat cover lead to the decrease of food availability are the most common threats that effect cuscus population and currently, and according to the IUCN criterion cuscus belongs to the group of animal with least concern and not included as extinction species (Bailey and Groombridge 1996). However, Norris (1999) reported that Phalangeridae is still considered to be vulnerable by virtue of restricted distribution.

Most studies on cuscus diet have been undertaken though they were more focused on cuscus habitat and type of foods. Dogomo (2004) fed cuscus with kangkong, banana and star fruit as food, Mansay (2006) reported cuscus consume shoot of Spondias dulcis (kedondong) and Terminalia catappa, fruit of Spondias dulcis, mango, T. catappa, Musa paradisiaca (banana), Carica papaya (papaya), Persea americana (avocado) and flower of avocado. Menzies (1991) reported cuscus consume food...
that contains high crude fiber; Flannery (1990) found cuscus in New Guinea consume Aglia, Alstonia, Ioanea, Ficus spp., Ficus adoaria, Lithocarpus, Elacocarpus, Mishocarpus, Pipturus, Pandanus, Oernathe, Rungia, Poikilospermum amboinase, Rattus exulans, Psysignatus lesueuri, and Pometia sp. Moreover, Dimonmonmau (2000) and Linthin found 32 species of plants which consists of 24 forest plant and eight of agriculture plant are consumed by cuscus in Moor Island. Sawen and Faidiban (2004) found 25 species plants are consumed by cuscus in Yamna Island. Dahruddin and Farida (2005) reported that cuscus food composition in Northern Biak Natural Reserve consists of 76.1% fruit, 13.4% foliage, 9% flowers, and 1.5% shoot. Spilocuscus maculates is one of abundance species of cuscus in Ratewi Island and high life survival in captivity habitat if food availability and cage condition are suitable (Pattiselanno 2007). Fox (2007) reported this species consume fruit and flower, however, base on tooth structure this species also carnivore.

Cuscus was one of several species considered hunting target as animal protein sources by local people in Papua. Therefore, uncontrolled exploitation of cuscus will put this species in endangered situation. Ex-situ captivating is an alternative solution to protect cuscus in one hand, and they can be harvested from captivating breeding on the other hand. To support the captivating condition, information on diets is required to improve ex-situ program beside environmental and habitat condition. Particular information about the nutrition contents on the other hand is still limited. In fact, information on diet content is highly important if the ex-situ captivating is seriously programmed. This study was designed to determine the diets of cuscus included type of food, food palatability and nutrition contents in natural and captivity habitat. This information is important to formulate the diet of cuscus particularly in ex-situ conservation program.

**MATERIALS AND METHODS**

**Study area**

This study was carried out from June to July 2009 in Ratewi (or Ratewo) Island (Figure 1), Nabire Distric, Papua Province. The study site was about 45 minutes by boat from Nabire, and located in 2°50'-3°00’ N and 135°40’-135° E with 357 ha area consisted of primer and secondary forest. The secondary forest was the area utilized for logging activities in the past (Pattiselanno 2007).

**Diet inventory**

General approach is conducted through field observation by collecting and identifying plant species consumed by cuscus in natural habitat. Some transects was set up as purposive in the cuscus habitat in the secondary forest with 5 km long and 0.5 km width and 2 km range between transects. Information was also retrieved by interviewing the cuscus hunter, and observed and identified cuscus feces found in the field. Particular type of food fed to cuscus by local people in captivity was also collected and identified as well. Diets inventory was conducted between June and July 2009 in both natural and captivity.
Food palatability

Food palatability test was done by identifying the most preferable plant species consumed during the study. Information were generated from the most plant species consumed by in the natural habitat, while in the captivity habitat type of food given by the owner and most preferred by the animals were observed and noted. Food palatability was also assessed by categorizing the taste of the plant species which is divided to astringent, sour, sweet and bitter tastes. The part of plants species consumed by cuscus were also observed and categorized into pulp of fruit, epidermis of fruit, shoot and foliage. Combination of food parts was classified under certain category because it was commonly found that cuscus eats more than one category.

Nutrient contents

The nutrition contents of cuscus diets were analyzed in animal husbandry research institute (Balai Penelitian dan Pengembangan Peternakan (Balitnak) Bogor laboratory. The analysis was mainly focused on crude fiber, fat and protein that considered important nutrient content in the diet.

Data analysis

Data were tabulated and nutrient content of cuscus diet were analysis by using SPSS 12.01 in order to figure out the different of nutrient contents of cuscus diets in the both habitats.

RESULTS AND DISCUSSION

Diet inventory

Nineteen plants species were observed consumed by cuscus in the natural habitat, commonly fruits and foliage. Majority of the species (13 species) were belongs to Ficus sp. Complete list of the species consumed are presented in Table 1. Under captivity condition, there are only eight food type were given to cuscus including fruits, human food such fish and papeda (traditional staple food made from sago) and plantation plant.

Number of consumed plant species found in the natural habitat was 19 species, or less than other studies conducted by Linthin (2000) 25 species of forest plant and 8 species agricultural plants considered as cuscus diets in Moor Island closed to Ratewi Island, Dimomonmau (2000) identified 32 species forest plant and agricultural plant in
Moor Island, while Sawen and Faidibani (2004) found 25 species forest plant and agricultural in other study in Yamna Island. In Mandopi, the coast site of Manokwari, 34 plant species from 28 families includes 28 forest plants and 6 crop plants are identified as cuscus diets (Fatem et al. 2008), while Nakoh et al. (2010) found 21 forest plants from 16 families as food plants at Udopi, Manokwari.

Moreover, Dwiyahreni et al. (1999) found bear cuscuses fed on 31 species of plants, including 26 identified trees and lianas. Less species of plant as cuscus food are found in this study maybe due to the accessible area observed because other areas the study site considered as forbidden forest. It might be more plants species are found in forbidden forest. Different results find from different studies indicated that forest condition was the main factor affect cuscus habitat. Forest conversions to other land use purposes such logging concession, crop plantation, mining industries and areas development are among the factors of forest fragmentation. In particular areas where, pristine forests still preserved might have more food plants for animals surrounding the areas where, pristine forests still preserved might have more food plants for animals surrounding the areas.

Thirteen species of forest plant belong to Ficus sp. and six species are Intsia bijunga, Syzygium cf. versteegii (L) Merr & Perry, Calophyllum inophyllum, Merremia peltata, Syzygium sp. and Sonneratia griffithi. Linthin and Dimomonnau (2000) reported that the most common type of cuscus diet in Moor Island of Nabire was fruits and shoot. Similarly, Dahruddin and Farida (2005) found that fruits, foliage, flowers and shoot were identified as cuscus diet composition in Northern Biak Nature Reserve.

Table 1. Plant species and type of food consumed by cuscus in natural habitat and captivity in Ratewi island, Napan, Nabire

<table>
<thead>
<tr>
<th>Latin name</th>
<th>Local name</th>
<th>Natural habitat</th>
<th>Captivity habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficus sp.1</td>
<td>Makuku buah merah</td>
<td>Makuku buah kasar</td>
<td>Papaya hutan</td>
</tr>
<tr>
<td>Ficus sp.2</td>
<td>Makuku buah kasar</td>
<td></td>
<td>Pepaya hutan</td>
</tr>
<tr>
<td>Ficus myriocarpa (F. punguen)</td>
<td>Surembo</td>
<td></td>
<td>Kecodondong hutan</td>
</tr>
<tr>
<td>Ficus sp.4</td>
<td>Makuku buah kasar daun halus</td>
<td>Tali wuraram</td>
<td>Belimbing</td>
</tr>
<tr>
<td>Merremia peltata</td>
<td>Beringin daun lebar</td>
<td>Beringin pantai</td>
<td>Pisang</td>
</tr>
<tr>
<td>Ficus sp.5</td>
<td>Beringin pantai</td>
<td>Beringin daun kecil</td>
<td>Kelapa</td>
</tr>
<tr>
<td>Intsia bijunga</td>
<td>Bakau</td>
<td></td>
<td>Ikan</td>
</tr>
<tr>
<td>Ficus paka</td>
<td></td>
<td></td>
<td>Padi (nasi)</td>
</tr>
<tr>
<td>Ficus sp.9</td>
<td></td>
<td></td>
<td>Sagu (papeda)</td>
</tr>
<tr>
<td>Ficus benjamina</td>
<td>Beringin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syzygium cf. versteegii</td>
<td>Jambu hutan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calophyllum inophyllum</td>
<td>Bintanggur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intsia bijuga</td>
<td>Kayu besi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intsia sp.</td>
<td>Kayu besi pantai</td>
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<td></td>
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<tr>
<td>Ficus sp.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Pepaya hutan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spondias dulcis</td>
<td>Kecodondong hutan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avverhoa carambola</td>
<td>Belimbing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musa paradisiaca</td>
<td>Pisang</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocos nucifera</td>
<td>Kelapa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katsuwonas pelamis</td>
<td>Ikan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>Padi (nasi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metroxylon sago</td>
<td>Sagu (papeda)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Under captivity condition, eight type of food recognized as cuscus diets, comprised of agricultural plants, plantation plants and human food. Interviewed to the owners indicate that cuscus diets under captivity condition were mostly depend on the availability of food items found in the surrounding. In this study, papaya and banana were abundant and available so they were common food to cuscus. Human food usually fed to cuscus because the society believed that giving human food to cuscus will lead to ease the domestication process. In domesticated trial experiment, Dogomo (2004) combined fruit and foliage (majority vegetables) as cuscus diets.

Food palatability

It was observed that in the natural habitat, Ficus sp. is the most preferable food consumed and we identified 13 out of 16 species. Pulps of fruits are the most part of the plant species consumed in the natural habitat beside foliage and shoot. Approximately 64.4% of cuscus diets is the combination between pulp of fruit and epidermis of fruit, while 21.1% is shoot. Other combination found in the diets is pulp, epidermis of fruit and shoot (5.3%), and combination of foliage and shoot (5.3%) which is found less favorable compared to others. Astringent and sour are dominant taste of food in natural habitat. Astringent taste is found in Ficus sp., bitter taste in foliage (M. peltata, Intsia sp.) and sour taste in fruit (Syzygium sp.). On the other hand, under captivity condition, pulp of fruits becomes the mayor diet (66.67%) mostly found in banana and papaya. Combination of pulp and epidermis of fruit become the second combination of cuscus diet. Sweet taste (C. papaya and M. paradisiaca) is dominant in cuscus diet, beside sour taste (Spondias dulcis and Avverhoa carambola).

Figure 2. Taste of cuscus diet in the natural (A) and captivity (B) habitat in Ratewi island Napan, Nabire

Among sixteen of plant species consumed by cuscus in the natural habitat thirteen were Ficus sp. Four species of Ficus were also among forest plants consumed in Mandopi, Manokwari (Fatem et al. 2008), while in Udopi, five Ficus species were consumed as well (Nakoh et al. 2010). This information implies that Ficus sp. were most commonly eaten by cuscus and becomes favorable food. Murwanto et al. (2008) reported Ficus benjamina is one of highly palatable food for cuscus. Detail observation has been conducted and it was clear that Ficus sp. were abundantly surrounding the study site. Particular parts of plant species
are most likely to be consumed are pulp and epidermis of fruit. Shoot is another part of plant consumed and found in *Merremia peltata*, *Calophyllum inophyllum* and *Sonneratia griffithi*. According to Fatem et al. (2008), parts of plants being consumed are young leaves or shoots, ripe fruits, husk of fruits and inflorescence. Similarly, Nakoh et al. (2010) reported that fruits were dominantly consumed, followed by leaves. However, Dwiyahreni et al. (1999) found that bear cuscus in North Sulawesi fed mainly on young leaves (54.4%), mature leaves (22.9%), and leave buds (7.8%), whereas in the captivity habitat (U=33; N=24; P=0.162). Other nutrients on the other hand show there is significant different in both habitats for crude fiber content: U=5;00; N=24; P=0.001 and fat content: U=25; N=24; P=0.05 (Figure 3).

Table 2. Nutrient content of cuscus diet in the natural and captivity habitat in Ratewi island Napan, Nabire.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Protein</th>
<th>Crude fiber</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural habitat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ficus</em> sp.1</td>
<td>14.46</td>
<td>32.48</td>
<td>10.19</td>
</tr>
<tr>
<td><em>Ficus</em> sp.2</td>
<td>10.46</td>
<td>41.6</td>
<td>9.4</td>
</tr>
<tr>
<td><em>Ficus myriocarpa</em> (<em>F. punguen</em>)</td>
<td>10.17</td>
<td>37.01</td>
<td>7.04</td>
</tr>
<tr>
<td><em>Ficus</em> sp.4</td>
<td>10.75</td>
<td>36.87</td>
<td>1.77</td>
</tr>
<tr>
<td><em>Merremia peltata</em></td>
<td>11.02</td>
<td>26.52</td>
<td>4.03</td>
</tr>
<tr>
<td><em>Ficus</em> sp.5</td>
<td>22.2</td>
<td>12.43</td>
<td>2.68</td>
</tr>
<tr>
<td><em>Ficus</em> sp.6</td>
<td>12.48</td>
<td>31.16</td>
<td>4.45</td>
</tr>
<tr>
<td><em>Syzygium</em> sp.</td>
<td>5.71</td>
<td>12.74</td>
<td>1.02</td>
</tr>
<tr>
<td><em>Ficus</em> sp.7</td>
<td>7.18</td>
<td>13.32</td>
<td>1.95</td>
</tr>
<tr>
<td><em>Sonneratia griffithi</em></td>
<td>11.2</td>
<td>9.32</td>
<td>2.06</td>
</tr>
<tr>
<td><em>Ficus paka</em></td>
<td>10.6</td>
<td>27.44</td>
<td>6.2</td>
</tr>
<tr>
<td><em>Ficus</em> sp.9</td>
<td>13.81</td>
<td>35.89</td>
<td>12.7</td>
</tr>
<tr>
<td><em>Ficus</em> benjamina</td>
<td>6.55</td>
<td>37.81</td>
<td>4.13</td>
</tr>
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<td><em>Ficus</em> sp.11</td>
<td>14.52</td>
<td>25.9</td>
<td>6.88</td>
</tr>
<tr>
<td><em>Syzygium</em> cf. versteegii</td>
<td>10.94</td>
<td>7.91</td>
<td>1.91</td>
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<tr>
<td><em>Calophyllum inophyllum</em></td>
<td>7.96</td>
<td>34.03</td>
<td>5.51</td>
</tr>
<tr>
<td>Intsia bijuga</td>
<td>19.01</td>
<td>16.16</td>
<td>1.12</td>
</tr>
<tr>
<td>Intsia sp.</td>
<td>28.02</td>
<td>19.29</td>
<td>2.26</td>
</tr>
<tr>
<td>Captivity habitat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Spondias dulcis</em></td>
<td>14.64</td>
<td>13.58</td>
<td>2.46</td>
</tr>
<tr>
<td><em>Cocos nucifera</em></td>
<td>11.01</td>
<td>5.64</td>
<td>45.67</td>
</tr>
<tr>
<td><em>Musa paradisiaca</em></td>
<td>4.46</td>
<td>0.00</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>5.69</td>
<td>4.63</td>
<td>0.74</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td>9.85</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Metroxylon sago</em></td>
<td>8.70</td>
<td>0.00</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The nutrient contents

The protein content of cuscus food in natural habitat was 12.60 ± 5.64g/100 g, while crude fiber and fat were as follows 25.44 ±11.12 g/ 100g and 4.74 ± 3.40 g/100g. Under captivity condition, diet of cuscus consist of 9.06 ± 3.70 g/100g of protein, 2.97 ± 5.34 g/100g of crude fiber and 8.23 ±18.36 g/100g of fat (Table 2). Different plant species have dissimilarity on nutrient content, therefore, fed on variety of plants, and selectivity in both species chosen and items eaten reflects the needs to optimize the mix of nutrients and total bulk of diets (Westoby 1974) and choose species that contain low levels of toxic and digestion-inhibiting chemicals (Milton 1979).

The data shows that the content of protein and crude fiber in the natural habitat were higher than in the captivity. However, non parametric test (U-test) for protein content shows that there is no different between natural and captivity habitat (U=33; N=24; P=0.162). Other nutrients on the other hand show there is significant different in both habitats for crude fiber content: U=5;00; N=24; P=0.001 and fat content: U=25; N=24; P=0.05 (Figure 3).

Figure 3. Boxplot average content of crude fiber and fat in diet of cuscus in Ratewi island, Napan, Nabire, Papua.
captive condition, most of cuscus diets were low in fat, and coconut was the only item contributes to the high fat content, therefore, this might indicated the significant different in fat content between the natural and captivity. Protein and fat content in this study were higher compared to the study of Dahruddin and Farida (2005) in Biak Nature Reserve ((10.98 g/100 g protein and (20.4 g/100g), while crude fiber in this study was relatively similar (25.08 g/100g).

ACKNOWLEDGEMENT

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A habitat selection model for Javan deer (*Rusa timorensis*) in Wanagama I Forest, Yogyakarta

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Abstract. Purnomo DW. 2010. A Habitat selection model for Javan deer (*Rusa timorensis*) in Wanagama I Forest, Yogyakarta. Nusantara Bioscience 2: 84-89. Wanagama I Forest is the natural breeding habitat of Javan deer (*Rusa timorensis* de Blainville, 1822). Habitat changes had affected Timor’s resource selection and caused the deer to move from undisturbed areas to developed areas with agriculture and human settlements. We suspected that this shift was caused by the degradation of natural habitat. The research aimed to identify factors that might influence future habitat selection. Habitat selection was analyzed by comparing proportions of sites actually used to sites that we considered available to use. The results of a logistic regression of site categories showed there are three habitat variables that influence resource selection: sum of tree species (exp$\beta$=1.305), slope (exp$\beta$=1.061), and distance to a water source (exp$\beta$=1.002). The three variables influence the deer existing in a certain site of Wanagama Forest and arrange resource selection probability function (RSPF).

Key words: habitat selection, *Rusa timorensis*, Wanagama I Forest.

INTRODUCTION

Javan deer or also called as Javan rusa, Rusa, Rusa deer, and Timor deer (*Rusa timorensis*) is a Red List Category & Criteria species (Hedges et al. 2008) listed as vulnerable C1 ver 3.1. Population size estimated less than 10,000 mature individuals and an estimated continuing decline at least 10% within 10 years or three generations, whichever is longer, (up to a maximum of 100 years in the future). The declining natural deer population due to the high level of utilization encouraged the Minister of Forestry to issued decree No.301/Kpts-II/1991. This issue is sequel of the Wild Animals Protection Ordinance 1931, No.134 and 226, which stated that Javan deer was one type of animal protected by law in Indonesia. Environmental change affected habitat conditions and it forced the deers to moving out from the forest to more strategic areas, i.e. agricultural and settlement. According to Dewi (2006), some deer populations in the Wanagama Forest I had become pests for agricultural crops around the area.

Selection is the process of selecting wildlife habitat components that are used (Johnson, 1980). Animals choose habitats through a process of spatial hierarchy that can occur on a scale roaming area (*home range*) (Johnson, 1980; Hutto, 1985). Selection of a habitat type is closely related to the resources availability. Manly et al. (2002) explained that the resource selection functions (Resources Selection Function/RSF) is a unified concept to explain the selection of several types of habitat. RSF can be analyzed through two approaches, namely habitat-categorizing and site-categorizing (Alldredge et al. 1998).

Wanagama I Forest consists of various types of vegetation that need to be analyzed in relation to the provision of resource requirements for deer. Shift in the utilization of resource is likely related to changes in resource availability in the forest. Therefore its necessary to identify the factors affecting the habitat selection in the cruising area. This study aims to determine the factors influencing habitat selection by Javan deer in the Wanagama I Forest. The factors then analyzed to establish...
the formulation of habitat selection models based on Resources Selection Probability Function (RSPF) in Forest Wanagama I, Yogyakarta.

**MATERIALS AND METHODS**

This research was conducted in the area of Wanagama I Forest Gunungkidul Regency. The data was taken during the dry season in July until the early November 2008. Utilization of habitat by the deer was estimated by the indirect approach in the form of footprints and droppings the deer (Lavieren, 1982; Strien, 1983). Plots was placed systematically (distance between transect 200 m and distance between plots 100 m). Plots which had population indicators, was called used plot, while plots which had not found population indicators was called unused plots, and both of used and unused was called availability plots (Figure 1). In each plot the habitat variables measured with the sampling protocol technique (Noon, 1981). Habitat variables include all components that affect the welfare of animals (welfare factors), including biotic and abiotic components (Bailey, 1984; Higgins et al. 1994).

Habitat selection determination was approached by site-categorization that explained the selection stated of certain resources by the animals in a site (Alldredge, Thomas, and Mcdonald, 1998). Habitat variables in used plot was compared with the habitat variables in availability plot using logistic regression to find out what factors influence the resources selection (Alldredge, Thomas, and Mcdonald, 1998; Manly et al. 2002).

**Figure 1.** Distribution of used-unused plots of Javan deer habitat research in Wanagama I Forest
Each used plot was marked “1” while availability plots “0”. All of habitat variables plots were selected randomly to get the data sample. In addition, each variable analyzed with Multicolinearity Test to avoid correlation between variables. The data analyzed using SPSS 16.0 for Windows Evaluation Version to determine the resource selection probability functions (RSPF). This function subjected as selection model and simulated using some site in the field.

Wanagama I Forest located in Gunungkidul District, Yogyakarta Province with average annual rainfall 1500-2000 mm. The rainy season usually falls in November-April and the dry season comes in the month of June to September. Based on the distribution of climate types by Schmidt and Ferguson, Wanagama I Forest is included in the D climate types (Anonymous, 1988). Based on measurements of rainfall measuring station near in Wanagama I, i.e. Wonolegi (Playen), in the last 10 years showed that the wettest month is January with the average amount of rainfall on 425.18 mm while the driest month on is August with the average amount of rain 16.55 mm (Anon. 2005).

Wanagama I Forest has diverse vegetation composition and structure. This area is divided into blocks of plants in blocks of vegetation. The species number of forest vegetation in Wanagama I today reaches 190 species (Anon. 2005). Various types of trees planted with homogeneous or heterogeneous pattern (mixed). Based stands of Acacia auriculiformis, Samanea saman, Swietenia macrophylla, Adenanthera sp., Tectona grandis, Pinus merkusii, Delonix regia, Dalbergia latifolia, Acacia auriculiformis, Samanea saman, Swietenia macrophylla, Polytrias amaura, Imperata cylindrica, Melaleuca leucadendron, and Vitex pubescens.

Based on observational data, population movement patterns have 3 major groups, namely: group 1 in the vicinity of Block 5, group 2 in the vicinity of Kemuning and Wonolagi, and Group 3 in the vicinity of Block 14 and 17. Each group moving on a periodic basis to several places in the vicinity. Deer population ages varied, consisting of adults, young, and chicks (Anon. 2005). Meanwhile, according to Supraptomo (2006), the structure of the deer population, especially in the Block 5 was one male versus three females.

The types of Javan deer feed that available especially in Block 5 is very abundant compared to other plots. Vegetation types of Javan deer feed in Wanagama I include: Arachis hypogaea, Manihot utilisima, Euphorbia prostrata, Ipomoea batatas, Leucaena glauca, Swietenia macrophylla, Polytrias amaura, Imperata cylindrica, and Ageratum conyzoides (Purnomo 2003). Furthermore, Purnomo (2003) noted that three kinds of vegetation most preferred feed was peanuts (Arachis hypogaea) (Level of Feeding Preferences/LFP=0.88 on a scale of 1 of 9 kinds of feed), weeds (Imperata cylindrica) (LFP=0.67) and cassava (Manihot utilisima) (LFP=0.33).

There are several water sources in the Wanagama I Forest. However, the main water source for deer in the Wanagama I Forest was Oyo River that not dry all year round. At the time of observation (dry season), the water still available for the deer. Therefore, areas along the River Oyo are the main location of the activity of deer population (Purnomo 2003; Supraptomo 2006).

### RESULTS AND DISCUSSION

#### Analysis of resource selection probability function

The field research resulted 114 plots (total length of transect ± 11.4 km) with 55 categories of used plots and 59 categories of unused plots (Figure 1). Numbers of sample plots are about 100 plots (40 used plots and 60 availability plots). The results of Multicolinearity Test of habitat variables show that the highest correlation between temperature and humidity is 0.494 (49.4%). This value is far below 95%, it means there is no significant multicolinearity. All variables could be subjected for further logistic regression analysis. The logistic regression was used because it did not requiring the normal distribution (Ghozali 2001).

Comparison of used-availability plots is more reasonable than used-unused considering detection of animals present at one time will be different at other times (Keating and Cherry 2004). Logistic regression was performed with backward stepwise method to filter out any variables that come in and produce the best-fit model. Statistics value -2LogL in the table used to screen the independent variables, which included in the model and assessed the data overall fit model (Ghozali 2001).

<table>
<thead>
<tr>
<th>Iteration</th>
<th>-2 Log likelihood</th>
<th>Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant Tree Slope Water</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>120.178</td>
<td>-2.572   0.225 0.05 0.002</td>
</tr>
<tr>
<td>2</td>
<td>119.858</td>
<td>-3.003   0.264 0.07 0.002</td>
</tr>
<tr>
<td>3</td>
<td>119.858</td>
<td>-3.026   0.266 0.06 0.002</td>
</tr>
<tr>
<td>4</td>
<td>119.858</td>
<td>-3.026   0.266 0.06 0.002</td>
</tr>
</tbody>
</table>

Note: a. Method: Backward Stepwise (Likelihood Ratio). b. Constant is included in the model. c. Initial -2 Log Likelihood: 134.602. d. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001. e. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

The value -2LogL in step 8 (Table 1) was 119.858 and X² distribution with df97 (100-3). This value was not significant at α=0.05, which means the model has been fit with the data. In these circumstances, the addition of three independent variables, which is the number of trees, slope, and distance of water would improve the model.

Fit Model could be tested with the Hosmer and Lemeshow Test by testing Ho that the empirical data is state with the model. The result showed the value of Hosmer and Lemeshow is 3.297 and the P value for 0.914 (Table 2). Because the P value is higher than 0.05, it could be concluded that the model is fit and acceptable. Classification Table could be used to test the practicability of the model, i.e. the model ability to estimate true (correct) and false (incorrect) (Table 3). The simulation with availability status (code 0), if there is 60 points in the field, then 49 points will be availability status, so the classification accuracy reach 81.7%. Meanwhile, in the area with used status from 40 points in the field, then 22
points will be used, and contributed to 55% of classification accuracy. Overall, this model could predict the classification accuracy of 71%.

Tabel 2. Hosmer and Lemeshow test of logistic regression analysis

<table>
<thead>
<tr>
<th>Step</th>
<th>Chi-square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.347</td>
<td>8</td>
<td>.311</td>
</tr>
<tr>
<td>2</td>
<td>9.064</td>
<td>8</td>
<td>.336</td>
</tr>
<tr>
<td>3</td>
<td>4.554</td>
<td>8</td>
<td>.806</td>
</tr>
<tr>
<td>4</td>
<td>4.767</td>
<td>8</td>
<td>.804</td>
</tr>
<tr>
<td>5</td>
<td>4.604</td>
<td>8</td>
<td>.803</td>
</tr>
<tr>
<td>6</td>
<td>8.277</td>
<td>8</td>
<td>.942</td>
</tr>
<tr>
<td>7</td>
<td>9.427</td>
<td>8</td>
<td>.972</td>
</tr>
<tr>
<td>8</td>
<td>3.297</td>
<td>8</td>
<td>.914</td>
</tr>
</tbody>
</table>

Tabel 3. Classification table of logistic regression analysis

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Step 8</td>
<td>Presence</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Overall percentage</td>
<td>71.0</td>
<td></td>
</tr>
</tbody>
</table>

Note: 1 used, 0 availability

Independent variables included in the model could be seen in Table 4. There are 3 significant variables, namely the number of tree species, slope, and distance of water sources. The influence value of independent variables affecting the dependent variables of the deer presence could be explained of on the column Exp (ß). Number of tree species variable with a value of Exp (ß) for 1.305 was the highest value, it means if the slope and distance of water sources variables are considered constant then the odds ratio of deer presence will change for 1.305 in every tree species variable with a value of Exp (ß) for 1.305 was the highest value, it means if the slope and distance of water sources variable, if the number of tree species and distance of water sources variables considered constant then the odds ratio of deer presence will change for 1.061 at every change of one unit of the slope variable. Similarly, the distance of water sources variable, if the number of tree species and the slope variables considered constant, then the odds ratio of deer presence will change for 1.002 at every change of one unit of the distance water sources variable.

Table 4. Variables in the equation of logistic regression analysis

<table>
<thead>
<tr>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(ß) for EXP(ß)</th>
<th>95.0% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step Tree</td>
<td>0.266 0.12</td>
<td>4.876 1</td>
<td>0.027</td>
<td>1.305</td>
<td>1.03</td>
<td>1.652</td>
</tr>
<tr>
<td>8(a) Slope</td>
<td>0.06 0.027</td>
<td>4.712 1</td>
<td>0.03</td>
<td>1.061</td>
<td>1.006</td>
<td>1.12</td>
</tr>
<tr>
<td>Water</td>
<td>0.002 0.001</td>
<td>11.116 1</td>
<td>0.001</td>
<td>1.002</td>
<td>1.001</td>
<td>1.003</td>
</tr>
<tr>
<td>Constant</td>
<td>-3.026 0.854</td>
<td>12.556 1</td>
<td>0.049</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The resulting model is as follows:

\[ \pi(x) = \frac{\exp(-3.026+0.266x_j+0.06x_k+0.002x_a)}{1 + \exp(-3.026+0.266x_j+0.06x_k+0.002x_a)} \]

\[ \pi(x) = \text{probability of the deer presence} \]

\[ x_j = \text{number of tree species} \]

\[ x_k = \text{slope} \]

\[ x_a = \text{distance of water sources} \]

The number of tree species could be used to describe biodiversity at a site. The diversity of tree species would increase the height variation, especially the quantity of feed resources and the availability of cover required by animals (Bailey, 1984; Higgins et al. 1994). The diversity of vegetation could serve the environment and create a balance of wildlife communities and ecosystems within (Bolen and Robinson, 1995). Javan deer would looking for places that with a higher variety of tree species to meet the needs of their group. The composition of the tree was also associated with the provision of grass and understory plants that affect the grazing behavior of animals (Mligo and Lyaru 2008).

According to the field observation, locations of deer’s activity center (Block 5 and 17) had a variety of feed vegetation such as Arachis hypogaea, Manihot utilisima, Euphorbia prostrata, Ipomoea batatas, and Ageratum conyzoides. In fact, according to report, understory plants abundance in Block 5 were dominated by Ageratum conyzoides (Important Value / IV = 0.20 on a scale of 1 of the 8 species of understory plants), Eupatorium odoratum (IV = 0.15, and Imperata cylindrica (IV = 0.15) (Anon. 2005). Meanwhile, the three dominant shurbs, that potentially as a cover for the deer in Block 5, were Flacourtia indica (IV = 0.44 of the 3 types of shrubs dominant), Glyricidae maculate (IV = 0.31), and Santalum album (IV = 0.25) (Anon. 2005).

Slope had a positive effect in the model which means that Javan deer tend to like the steep places. A steep slope is usually far from the human interference activity, and relatively high vegetation density. Several locations with flat or slightly sloping in the Wanagama I Forest had intercropping systems of agricultural land, and tumpang sari, for example in the Block 14, Block 16, and Block 18. Farmers will have intensive activity especially in the mornings at 06.00 until 09.00 am and the afternoon between 15:00 until 18:00 pm. In the steep locations, usually the vegetation was found in the form of forest or dense bush. Therefore, the steep areas were strategic place for animals to protect themselves from predators and the disturbance of human activity. In the steep slope category areas (25-45%), Javan deer would tend to used locations with greater opportunity rather than simply used it as resource availability. Phenotype deer's body had a strong leg that could move very swiftly (Dradjat 2002; Semiadi 2002). Therefore, it could be said that the slope is not a limiting factor for deer movement (Semiadi 2002).
Distance of water sources has a positive correlation in the model, although coefficient value Exp (ß) lowers than the number of trees and slopes. A permanent water source in the Wanagama I Forest is the Oyo River, while the surrounding area is an agricultural area with the high intensity of human activity (Dewi 2006). Therefore, the distance of water sources positively correlated with human activity. The deer will move away from human activity because it is sensitive and had a sense of smell. In the more distant locations than 1,200 meters from the River Oyo, deer are most likely going to attend.

Deer had characteristic that could withstand drought (Dradjat 2002). To manage their water needs, the deer will drink in the river or water source during rainy season, while in the dry season the deer will take the leaf buds, which contain lots of water (Djuwantoko 2003). Therefore, deer in the Wanagama I Forest chose a place with far from the human activity, although its far from water sources.

Simulation model

One benefit of the resource selection model is that this model could predict the chances of the animal’s presence in one place. Resource selection model of Javan deer in the Wanagama I Forests was used to predict the deer presence in some points in the field. RSPF in Wanagama I Forest formed based on the model can be seen in Figures 2, 3 and 4.

CONCLUSION

There are three habitat variables that determine resources selection, i.e. the number of tree species, slope, and distance of water sources. These variables showed strong influences of the deer presences and formed a RSPF in the Wanagama I Forest. Furthermore, the vegetation management of Wanagama I Forest had to consider with habitat especially concerned with the minimum number of available tree species in the forest. Problem solving of deer disturbance in agricultural could be improved through efforts to improve the quality of habitat in the forest.

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Review: Colchicine, current advances and future prospects

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Abstract. Ade R, Rai MK. 2010. Colchicine, current advances and future prospects. Nusantara Bioscience 2: 90-96. Colchicine is a toxic natural compound and secondary metabolite commonly produced by plants like Colchicum autumnale and Gloriosa superba. It is originally used to treat rheumatic complaints, especially gout, and still finds its uses for these purposes today despite dosing issues concerning its toxicity. It is also prescribed for its cathartic and emetic effects. Initially oral colchicine has not been approved as a drug by U.S. Food and Drug Administration (FDA). But now FDA approved colchicine as a drug for some disorders. Colchicine's present medicinal use is in the treatment of gout and familial Mediterranean fever. It is also being investigated for its use as an anticancer drug. In neurons, axoplasmic transport is disrupted by colchicine. Due to all the pharmacological application of colchicine, there is urgent need to enhance the properties and increase the production of colchicine with the help of in vitro technologies. The present review is mainly focused on the chemistry of colchicine, its medicinal uses and toxicity.

Key words: colchicine, photoisomerization, colchicinamide, toxicity, polyploidy

INTRODUCTION

Colchicine is a traditional drug for gout (Wendelbo and Stuart 1985), and has been in use for treating acute gout dates back to 1810. It is obtained from corms of Gloriosa superba and also from Colchicum autumnale (Family Liliaceae). Since the approval of colchicine as drug for gout in 2009 by Food and Drug Administration (FDA, USA) there has been revival of interest in colchicine research and applications (Schlesinger 2010). Colchicine is an extremely poisonous alkaloid, originally extracted from Colchicum autumnale (autumn crocus, meadow saffron) medicinal plants. It is used to treat rheumatic complaints.

Colchicine was first isolated in 1820 by the two French chemists Pelletier and Caventon and extract of Colchicum plant was first described as a treatment for gout in De Materia Medica of Padanius Dioscorides. It was later identified as a tri-cyclic alkaloid and its pain relieving and anti-inflammatory effects for gout were linked to its binding with the protein tubulin. The molecular formula of colchicine is C22H25NO6 and its chemical name is N-[ (7S)-5, 6, 7, 9-tetrahydro-1, 2, 3, 10-tetramethoxy-9- oxobenzo[a]heptalen-7-yl) acetamide].

The term ‘colchicine’ is originated from area known as “Colchis” near black sea. C. autumnale grows wild in Europe and Africa while Gloriosa is distributed in Africa and Asia including foothills of Himalayas, Burma, Indonesia, Malaya, etc. Thomson was the first who proposed the early idea of action of colchicine in gout treatment. Gout and uric acid metabolism is same way proposed the early idea of action of colchicine in gout treatment. Gout and uric acid metabolism is same way deposition of micro-crystals of uric acid in joints and may be due to defective regulatory mechanism for endogenous purine synthesis but conflicting results for the action of colchicine on synthesis and extraction of urates have been recorded. The colchicine interrupts the cycle of new deposition, which appears to be essential for the maintenance of acute gout. The frequent side effect has been recorded, but colchicine remains the ideal drug for acute gout. Modification of the side chain of rings does not eliminate anti-gout activity as long as the configuration of anti-gout activity as long as the configuration of C-ring confirms to that of colchicine. It suppresses cell
division by inhibiting the development of spindles, from a pool of subunit during a distinct phase of cell cycle and then depolymerized during other phases.

Colchicine can solve an important problem of fuchsia breeding. The maximum fuchsia species are diploid or tetraploid. The crossing between diploid and tetraploid results often in a triploid, which is mostly sterile because the process of meiosis requires the pairing of similar chromosomes and also due to lack of mechanism so as to allow the alignment of three similar chromosomes. Triploid plants are not able to produce prolific reproductive cells therefore they remain sterile and unusable like parents. The advantage of polyploidy plants that all plant parts are bigger (flowers, leaves) a lot of big double fuchsias are polyploidy. A special problem of colchicine which induced ploidy, particularly in vegetatively propagated crops, is the chimerism caused by the simultaneous occurrence of tissue of different ploidy levels in one plant or plant part.

CHEMISTRY OF COLCHICINE

Colchicine is also known as methyl ether of colchicine. It is a major alkaloid of Gloriosa superba and Colchicum autumnale. N-formyl-N-de-acetyl colchicine and 3-demethyl colchicine designated as substances A, B, C respectively have occurred in liliaceae family (Figure 1). The study of isolation started in 1820 and present method of Ziesel-methoxyl determination has its foundation in the determination of these functional groups in colchicine possessing only one asymmetric carbon atom at position C7. The alkaloid morphine and strychnine are now known.

Colchicine (C22H25NO4) is not an alkaloid, because the nitrogen atom is not basic, which is part of acetamide function, four oxygen atoms are present as 4-methoxy group, and remaining oxygen is unreactive towards reagent that affect acylation and affords no carbonyl derivative. Acid hydrolysis of colchicine in varying degrees of rigidity provides method used for the selective breaking cleavage of the functional groups. Dilute acid affords colchicine, an acidic substance that can be methylated to colchicine and iso-colchicine by diazo-methane. The assigned colchicine 9-methyl phenanthrene structure and the structural formula for ring. Proof of cycloheptane structure for ring-B was obtained by synthesis of dl-colcholin methyl ether, N-acetyl colcholin methyl anhydride, the degradation products of colchicine. Dewar (1945) reported troponol structure for ring-C and it was responsible for coinining the term troponol for cycloheptatrienolon,. It was proved that ring -C was 7 member by the synthesis of octa-hydro demethoxy des oxides acetamid colchicine, a degradation product of colchicine in which ring C-remain intact, so the correct structure of colchicine is assigned as methyl ether of colchicine. Colchicine is optically active by virtue of the single asymmetric carbon atom at position C-7. The absolute configuration at this center was established by oxidation of colchicine to N-acetyl-L-glutamic acid.

The synthesis of colchicine has attracted widespread attention as a synthetic object (Seganish et al. 2005). The starting material was 7-8-9 trimethoxy-benzo-suberone and end product was plus minus trimethylcolchicine acid which had been converted earlier to colchicine by resolution N-acetylatyng and O-methylamine, while in case of Alexander et al. (1994) the starting material was purpurogallin trimethyl ether and end product is similar to Van tamelan synthesis. In Nakamura synthesis, starting material is pyrogallolmethyl ether herring A and C formed first and then constructed to form end product.

![Chemical structure of colchicine](image)

**Figure 1.** Chemical structure of colchicine.

Colchicinamide

The well-designed synthesis of Woodward is conflicting and complete departure from the other approaches since it begins with the construction of a supplementary ring carbon atom 6, 7, 7a, 8, 12a and nitrogen atom of the future colchicine molecule. The N-atom masked in the stable isothiazole system until it is in the final step. Simple isothiazole was previously unknown. In this synthesis starting material substituted thiazole and end product al-colchicine.

Photoisomerization

When colchicine is irradiated by light, photoisomerization occurs and structure of α-β-γ-lumicolchicine so formed has been elucidated. Now the process and methodology are currently at the cross road between the effectiveness of synthetic and natural compound in the improvement of human ailments. In comparison with allopathic or chemotherapy or antibiotic therapy, there are tremendous difficulties, allopathy has taken strong roots in most urban areas, the rural population of India has much faith in the usefulness and healing powers of age-old system of Ayurveda that is original system of medicines. Concentrated research in identifying and characterizing newer medicinal and aromatic plants can place us in a position of growth of National economy. Also we can help by fortifying the very grass root of Ayurveda by scientific interpretation to the pharmacodynamics of the many medicinal plant bases used in traditional treatments of the past. During the last three and half decades, various workers engaged in the field of Medicinal and Aromatic plants in India, have increased manifold and so the output of research data on the subject. There is similar stepping up in research and development work in the growing and processing of medicinal and aromatic plants in many other developing countries like Asia, Africa and Latin America.
germinated well at 24°C after being transferred to solid regeneration medium and an initial period of low temperature (2°C) for 10 days, and could directly and rapidly regenerate vigorous plants. A high doubling efficiency of 83-91% was obtained from 500-mg/L colchicine treatments for 15 hour with low frequency of polyplid and chimeric plants. The experiment has shown that treatment duration of 30 hour revealed less positive effects on embryogenesis and doubling efficiency, especially at higher colchicine concentration (1000 mg/L). Poor embryogenesis and embryo germination were observed from ordinary microspore culture without change of induction medium and colchicine treatment, and several sub-cultures were required for induction of secondary embryogenesis and plant regeneration (Bourgau et al. 2001; Hadacek et al. 2002).

PLANT SOURCE OF COLCHICINE

Gigantic important flora has been a major source of secondary metabolites, which is now a main source of pharmaceuticals, food additives, fragrances and pesticides (Figure 2).

Colchicum spp.

Al-fayyad et al. (2002) studied determination of colchicine from Colchicum autumnale, and several others species, for example, in corms of Colchicum hierosolymitanum and Colchicum tunicatum colchicine was reported in an appreciable amount. The effect of different NPK (Nitrogen, Phosphorous and Potassium) fertilizer levels on colchicine content of the two colchicum species at different growth stages were evaluated by High Performance Liquid Chromatography. Results indicates that increasing NPK fertilizer levels significantly improve colchicine content in different plant parts and stages. The highest colchicine content observed in corms was at maturity stage 0.766 mg/g and 0.688 mg/g dry weight with C. hierosolimitanum and C. tunicatum respectively.

Gloriosa superba

Gloriosa superba is one of the important species in the world particularly, Asia and Africa produces two important alkaloids colchicine and gloriosin which is present in seeds and tubers (0.7% to 0.9%) and other is lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine have been isolated from the plant (Chulabhorn et al. 1998). It is used in almost all diseases, like cancer, gout, scrofula and act as antipyretic, antihelmintic, purgative and antiabortive. It is also source of gloriosin and colchicocides, which are very costly, being highly demanded by pharma industries. (Finnie and van Staden 1989; 1991). Due to excessive use of the plant for diverse medicinal purposes the species is on the verge of extinction and included in Red data book (Sivakumar et al. 2003a; 2003b; 2004; 2006).

Gloriosa superba also known as Malhar glory lily is a perennial tuberous climbing herb, widely scattered in the tropical and sub-tropical parts of India. It is called as

(Sudipto and Sastry 2000). This fact is powerfully reflected in the reports of many United Nation agencies, which has been advocating greater attention to those crops as a means of socio-economic uplift. However, in fact revitalization of interest in natural plant products as these are biologically more well-matched with human system and relatively less toxic than the synthesis. Thus, the growing of medicinal and aromatic plants has got a great boost during the last two decades. Evidently, need was felt for scientific literature on the growing and processing of these plants. Under such a situation retrieval of the information becomes a very painstaking process for the research and development.

Nguyen et al. (2005) studied the common pharmacophor for a diverse set of colchicine site inhibitor using a structure-based approach. In which the modulation of structure and function of tubulin and microtubule is most important route to anticancer therapeutics therefore small molecule bind to tubulin and cause mitotic arrest are of enormous interest. A large number of synthetic and natural compounds with dissimilar structures have been shown to bind at the colchicine site, one of the major binding sites on tubulin, and inhibit tubulin assembly. Using the recently determined X-ray structure of the tubulin colchicinoid complex as the template, and also employed docking studies to determine the binding modes of a set of structurally diverse colchicine site inhibitors. These binding models were subsequently used to construct a comprehensive, structure-based pharmacopoeia.

Raimond et al. (2004) reproted the tubulin regulation from a complex with colchicine and stathmin-like domain. The microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. Their dynamic instability is controlled by numerous compounds and proteins including colchicine and stathmin family proteins. The way in which microtubule instability is regulated at the molecular level has remained controlled, mainly due to lack of appropriate structural data. The structure at 3.5 Å resolution of tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3 is the interaction of RB3-SLD with two tubulin heterodimers in a curved complex capped by the SLD amino-terminal domain, which prevents the incorporation of the complex tubulin into microtubules. A comparison with the structure of tubulin in protofilaments shows change in the subunits of tubulin as it switches from its straight conformation to a curved one. These changes correlate with the loss of lateral contacts and provides a validation for the rapid microtubule depolymerization characteristic of dynamic instability. Moreover, the tubulin-colchicine complex sheds light on the mechanism of colchicine activity. Colchicine binds at a location where it prevents curved tubulin from adopting a straight structure, which inhibits assembly.

Zhou et al. (2000, 2002) reported increasing embryogenesis and doubling efficiency by immediate colchicine treatment of isolated microspores in spring Brassica napus in which immediate colchicine treatment of isolated microspores with the concentrations 50 and 500 mg/L for 15 hour stimulated embryogenesis and produced large amounts of healthy-looking embryos. These normal embryos
‘Mauve beauty’, ‘Purple prince’, ‘Modest’, ‘Orange gem’, ‘Salman glow’ and ‘Orange glow’ (Bose and Yadav 1989). It is adapted to different soil textures and climatic variations. The leaf juice is used to kill-lice in hair, the tuber contains the bitter principles, superbine and gloriosin, which in large doses are fatal; however, in small doses they are used as tonic, antiabortive, and purgatives (Bellett and Gaignanl 1985; Somani et al. 1989, Finnie and van Staden 1991; Samrajeeva 1993). The white flour prepared from the tubers is bitter and used as a stimulant.

Ghosh et al. (2005; 2006) reported colchicine production by using aluminium chloride as an elicitor. Root cultures of *Gloriosa superba* were treated with 5 mM methyl jasmonate and 125 M AlCl3 which enhanced the intracellular colchicine content of the roots by 50-folds and 63-folds respectively. 10 mM of CaCl2 and 1 mM CdCl2 enhanced biomass significantly (7 to 8.6-fold respectively), while the maximum release of colchicine into the medium was obtained with 10 mM CdCl2. Casein hydrolysate, yeast extract and silver nitrate had no significant effect on growth and colchicine accumulation in root cultures.

Muzaffar and Brossi (1991) investigated the chemical structures of colchicine and related analogs, including allo-compounds with a six-membered ring. It was reported that colchicine cardiotoxicity by ingestion of *Gloriosa superba*, in which the clinical features of colchicine toxicity in a patient following ingestion of *G. superba* tubers were studied. Gastroenteritis, acute renal failure, cardiotoxicity and haematological abnormalities were the main toxic manifestations. There was no hypotension and no neurological manifestations. Electrocardiographic changes were noteworthy and have not been reported previously.

Sivakumar et al. (2004) reported colchicine production in *Gloriosa superba* calli by feeding precursors, phenylalanine and tyrosine. The lack of biosynthetic precursors and signal inducing enzyme activity are responsible for the lower production of colchicine in *vitro*. B5 medium nutrient grown calli have a low content of colchicine indicating that an optimal precursor level is required to increase PAL and TAL activity for colchicine accumulation. These results suggest that precursors are an important regulatory factor in colchicine accumulation in *vitro*.

**Other plants**

Jha et al. (2005) reported production of forskolin, withanolides, colchicine and tylophorine from plant source by using biotechnological approaches in which three alkaloids such as forskolin from *Coleus forskohlii* Briq. with anoloid from *Withania somifera* (L.) Dunal and colchicine from *Gloriosa superba* are discussed (Mukherjee et al. 2000; Furmanowa et al. 2001). The *Coleus forskohlii* Briq., a member of the family Lamiaeae is a common and ancient medicinal plant of India, and is used traditionally in Ayurvedic medicine (Bhattacharyya and Bhattacharya 2001; Engprasert et al. 2004). A large-scale screening of medicinal plants by the Central Drug Research Institute, Lucknow, India, in 1974 revealed the presence of a hypotensive and spasmylytic component of *C. forskohlii* that was named coleonol. Further investigation (Saksena et al. 1985) determined micropropagation and *in vitro* culture for production of forskolin. Forskolin synthesis in transformed cultures transformed cell and organ cultures have proved valuable transformed cell suspension culture withanolides from *W. somifera*. Cell and tissue culture of *G. superba* for production of colchicine. There are few reports on micropropagation of *Gloriosa* sp. Since the active principle is mainly concentrated in the tubers, multiplication of tubers in *vitro* is essential. *In vitro* tubers have several advantages. They are harder, easier to handle, can be transported dry, there is no dormancy period thereby year-round cultivation is possible. These *in vitro* generated plantlets could serve as a source of cultures for studying the relationship between secondary metabolite accumulation and tissue different. The productivity of the culture systems (transformed/ untransformed) needs to be improved significantly and to be shown to be competitive with field plants for production of target secondary metabolites on an industrial scale (Brodelius et al. 1994). The lack of understanding of the molecular mechanism of regulation of secondary metabolism is the main bottleneck in attempts for further study.

**POISONING OF COLCHICINE**

Colchicine is often used to treat gout and acute rheumatoid arthritis and is known to relieve pain effectively (Neuwinger 1994). The mode of action of colchicine in gout is unknown, however, it is believed to decrease lactic acid production by the leukocytes and consequently, decrease urate crystal deposition and the subsequent reduction in phagocytosis with the inflammatory response. It also alters neuromuscular functions, intensifies gastrointestinal activity by neurogenic stimulation, increases sensitivity to central depressants, and depresses the respiration.

Ingestion of colchicine typically leads to profuse vomiting and diarrhea, which can be bloody, followed by hypovolemic shock and multisystem organ failure within 24-72 hours. Coma, convulsions, and sudden death might also occur. Subsequent complications include bone marrow suppression with resultant leukopenia, thrombocytopenia and possibly sepsis.

**Laboratory diagnosis**

There are two methods of detection of colchicine, (i) Biological- in which colchicine is detected in urine, serum, or plasma as determined by a commercial laboratory, and (ii) Environmental - colchicine in environmental samples can be determined as per rules of Food and Drug Administration.

**Case classification**

**Suspected:** A case in which a potentially exposed person is being evaluated by health-care workers or public health officials for poisoning by a particular chemical agent, but no specific credible threat exists.
Figure 1. Several main plant source of colchicines. A. *Colchicum autumnale*, B. *Gloriosa superba*, C. *Coleus forskohlii*. (photos from several sources)

Probable: A clinically compatible case in which a high index of suspicion (credible threat or patient history regarding location and time) exists for colchicine exposure or an epidemiologic link exists between this case and a laboratory-confirmed case.

Confirmed: A clinically compatible case in which laboratory tests have confirmed exposure.

Colchicine is FDA-approved drug in USA recently for the treatment of gout and also for familial Mediterranean fever, amyloidosis, and scleroderma (Kallinich et al. 2007). Side effects include gastro-intestinal upset and neutropenia. Starting the drug early during an attack of gout can exacerbate the symptoms. High doses can also damage bone marrow and lead to anemia. It's not used in the treatment of cancer, as the dose required would lead to intolerable side effects.

Toxicity

Poisoning resembles intoxication with arsenic: symptoms start 2 to 5 hours after the toxic dose has been ingested and include burning in the mouth and throat, fever, vomiting, abdominal pain and kidney failure. Death from respiratory failure can follow (Goldbart et al. 2000). There is no remedy. It was later identified as a tricyclic alkaloid and its pain relieving and anti-inflammatory effects for gout were linked to it binding with the protein tubulin. It inhibits the cytoskeleton by binding to tubulin, one of the main constituents of microtubules. Apart from inhibiting mitosis, a process heavily dependent on cytoskeletal changes, it also inhibits neutrophil motility and activity, leading to a net anti-inflammatory effect.

**COLCHICINE IN CELL DEVELOPMENT**

Pharmacology

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a "mitotic poison" or spindle poison. Since one of the defining characteristics of cancer cells is a significantly increased rate of mitosis, this means that cancer cells are significantly more vulnerable to colchicine poisoning than normal cells. However, the therapeutic value of colchicine against cancer is limited by its toxicity against normal cells.

Apart from inhibiting mitosis, a process heavily dependent on cytoskeletal changes, colchicine also inhibits neutrophil motility and activity, leading to a net anti-inflammatory effect. Colchicine also inhibits urate crystal
deposition, which is enhanced by a low pH in the tissues, probably by inhibiting oxidation of glucose and subsequent lactic acid production in leukocytes. The inhibition of uric acid crystals is a vital aspect on the mechanism of gout treatment. It is also used as an anti-inflammatory agent for long-term treatment of Behcet's disease. The Australian biotechnology company “Giaconda” has developed a combination therapy to treat constipation-predominant irritable bowel syndrome which combines colchicine with the anti-inflammatory drug olsalazine.

The British drug development company “Angiogene” is developing a prodrug of colchicine, ZD6126 (also known as ANG453) as a treatment for cancer. Colchicine has a relatively low therapeutic index. Colchicine is “used widely” off-label by naturopaths for a number of treatments, including the treatmet. Side-effects include gastro-intestinal upset and neutropenia. High doses can also damage bone marrow and lead to anaemia. Note that all of these side effects can result from hyper-inhibition of mitosis.

**Induction of polyploidy**

Since chromosome segregation is driven by microtubules, colchicine is also used for inducing polyploidy in plant cells during cellular division by inhibiting chromosome segregation during meiosis. Half the resulting gametes therefore contain no chromosomes, while the other half contain double the usual number of chromosomes (i.e., diploid instead of haploid as gametes usually are) and lead to embryos with double the usual number of chromosomes (i.e. tetraploid instead of diploid). While this would be fatal in animal cells, in plant cells it is not only usually well tolerated, but in fact frequently results in plants which are larger, faster growing, and in general more desirable than the normally diploid parents for this reason, this type of genetic manipulation is frequently used in commercial plant breeding. In addition, when such a tetraploid plant is crossed with a diploid plant, the triploid offspring will be sterile, which may be commercially useful in itself by requiring growers to buy seed from the supplier, but also can often be induced to grow a “seedless” fruit if pollinated (usually the triploid will also not produce pollen, therefore a diploid parent is needed to provide the pollen). This is the method used to create seedless watermelons, for instance. On the other hand, colchicine's ability to induce polyploidy can be exploited to render infertile hybrids fertile, as is done when breeding triticale from wheat and rye. Wheat is typically tetraploid and rye diploid, with the triploid hybrid infertile. Treatment with colchicine of triploid triticale gives fertile hexaploid triticale.

When used to induce polyploidy in plants, colchicine is usually applied to the plant as a cream. It has to be applied to a growth point of the plant, such as an apical tip, shoot or sucker. Seeds can be presoaked in a colchicine solution before planting. As colchicine is so dangerous, it is worth noting that doubling of chromosome numbers can occur spontaneously in nature, and not infrequently. The best place to look is in regenerating tissue. One way to induce it is to chop-off the tops of plants and carefully examine the lateral shoots and suckers to see if any look different.

**COLCHICINE IN MEDICINES**

**Colchicine poisoning and potential acute health effects**

It is extremely hazardous in case of skin contact (corrosive, irritant, sensitiser, permeator), of eye contact (irritant), of ingestion, of inhalation. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering, ingestion, of inhalation. The substance is toxic to blood, kidneys, lungs, the nervous system, the reproductive system, liver, mucous membranes. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage. Repeated exposure to an highly toxic material may produce general deterioration of health by an accumulation in one or many human organs. Repeated or prolonged inhalation of dust may lead to chronic respiratory irritation. The substance is toxic to blood, kidneys, lungs, the nervous system, the reproductive system, liver, mucous membranes.

**Action and clinical pharmacology**

Although its exact mode of action in the relief of gout is not completely understood, colchicine is known to decrease the inflammatory response to urate crystal deposition by inhibiting migration of leukocytes, to interfere with urate deposition by decreasing lactic acid production by leukocytes, to interfere with kinin formation and to diminish phagocytosis and the subsequent anti-inflammatory response. The anti-inflammatory effect of colchicine is relatively selective for acute gouty arthritis. However, other types of arthritis occasionally respond. It is neither an analgesic nor a uricosuric and will not prevent progression to chronic gouty arthritis. It does have a prophylactic, suppressive effect that helps to reduce the incidence of acute attacks and to relieve the residual pain and mild discomfort that patients with gout occasionally experience.

**CONCLUSION**

Colchicine has been approved as the drug for gout by Food and Drug Administration, USA in 2009. Thereafter, the interest of the scientist have revived. Since colchicine has wide array of properties and applications from ancient periods to modern era of medicine, it is necessary to understand its pharmacology. It is a pressing need to enhance the properties and percentage of colchicine by
application of in vitro technologies. In addition to that, besides chemical synthesis, in vitro biological synthesis by using precursors would be a novel method for the production of colchicine.

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REFERENCES


Review: Biodiversity conservation strategy in a native perspective; case study of shifting cultivation at the Dayaks of Kalimantan

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ABSTRACT

Abstract. Setyawan AD. 2010. Biodiversity conservation strategy: in a native perspective: case study of shifting cultivation at the Dayak of Kalimantan. Nusantara Bioscience 2: 97-108. Native tribes generally are original conservationists; they build genuine conservation strategy of natural resources and environment for sustainable living. Dayak is a native tribe of Kalimantan that has been living for thousands of years; they use shifting cultivation to manage the communal forest lands due to Kalimantan’s poor soil of minerals and nutrients, where the presence of phosphorus becomes a limiting factor for crops cultivation. In tropical forests, phosphorus mostly stored in the trees, so to remove it, the forest burning is carried out. Nutrients released into the soil can be used for upland rice (gogo) cultivation, until depleted; after that, cultivators need to open a forest, while the old land was abandoned (fallow) until it becomes forest again (for 20-25 years). The consecutive land clearing causes the formation of mosaics land with different succession ages and diverse biodiversity. This process is often combined with agroforestry systems (multicultural forest gardens), where the will-be-abandoned fields are planted with a variety of useful trees that can be integrated in forest ecosystems, especially rubber and fruits. These systems of shifting cultivation are often blamed as the main factor of forest degradation and fires, but in the last 300 years, this system has little impact on forest degradation. But, this is relatively low in productivity and subsistent, so it is not suitable for the modern agriculture which demands high productivity and measurable, mass and continuous yield, as well as related to the market. The increased population and industrial development of forestry, plantation, mining, etc. make the communal forest become narrower, so the fallow periods are shortened (5-15 years) and the lands are degraded into grasslands. In the future, shifting cultivation remains one of the Dayaks option to meet the needs of rice, but agroforestry should be developed because of its higher economic value.

Key word: shifting cultivation, agroforestry, Dayak, Kalimantan, conservation, biodiversity.

INTRODUCTION

Borneo (or Pulau Kalimantan in Indonesian) is the third largest island in the world and has a very high biodiversity compared to many other areas. On this island lived about 15,000 species of flowering plants with 3,000 species of trees (267 species of dipterocarp), 221 species of terrestrial mammals and 420 bird species (MacKinnon et al. 1996). In addition, there are still many new species waiting to be found and named. In 1994-2004 in Borneo, it is discovered 361 new species (Rautner et al. 2005), even in-depth exploration for 18 months in 2005-2006 found 361 new species (Rautner et al. 2005), even in-depth exploration for 18 months in 2005-2006 found 361 new species.
species (WWF 2007). This area is home to large mammals which is very rare, such as Borneo orangutan (*Pongo pygmaeus pygmaeus*), Asian elephant (*Elephas maximus*), Sumatran rhinoceros (*Dicerorhinus sumatrensis*), Borneo clouded leopard (*Neofelis nebulosa diardi*), Borneo banteng (*Bos javanicus lowi*) and sun bear (*Helarctos malayanus*), etc.

The high biodiversity in Borneo is due to diverse ecosystem on it, where there are seven different ecoregions. Most of the island is covered by lowland rain forest; the other lowland areas are peat swamp forest, heath (*kerangas*), and freshwater swamp forest in the southwest, and also mangrove forest. In addition, there are also mountain rain forest highlands, above 1,000 m asl., which is located in the center and northeast of the island with its mountain peak of Mount Kinabalu, Sabah. In the region there are alpine meadows and bushes that keep many endemic species, including orchids (Setyawan 2002).

There are several native tribes in Kalimantan based on ethno-linguistic (Figure 1); one of them is the Dayak tribe. This tribe mostly lives in the hinterland and is still dependent on forest livelihoods. Actually, the original tribe spread across the island of Borneo, from coastal to mountainous areas in central and northeast. But the tribe who lived on the coastal area generally has acculturated with the Malays and Muslims, such as Banjar and Kutai people, so they are often identified as a clump of the Malays. Moreover they do not practice the Dayak culture. Dayak tribes who embraced Islam generally no longer identify themselves as Dayaks except in West Kalimantan.

Dayak tribe has lived in Borneo since thousands of years and practice management systems of natural resources and its ecosystem sustainably. They practice shifting cultivation (slash-and-burn or swidden agriculture) to produce upland rice/dry land rice (*gogo paddies*) and form mosaics of agroforestry lands with different age for biodiversity managing. The practice is chosen because the Kalimantan soil is generally poor of mineral nutrients, due to the absence of volcanoes, so the main source of minerals is plants that accumulate these mineral nutrients. By burning trees and shrubs, it is expected that minerals will return to the soil and then, they can be absorbed by would-be-planted food crops. In 4-5 times of rice harvest (1-2 years), the minerals in the soil usually start to thin out, so the cultivators have to move and open new forest for fields. The old fields are abandoned in order to become forest again as nutrient accumulators, and they will be cut down and burned again to provide nutrients for crops. This system requires sufficient land area, with relatively limited results (subsistence), so it is considered ineffective and inefficient by the government and entrepreneurs who need land.

This paper aims to express the conservation of biological diversity, associated with shifting cultivation practices conducted by the Dayak tribe in Kalimantan.

**DEFORESTATION OF KALIMANTAN FOREST**

Species richness and diversity of the ecosystems of Borneo are threatened by high rates of deforestation and habitat conversion throughout the island. Extensive tropical forests of Borneo have the most rapid rate of extinction in the world (Sunderlin and Resosudarmo 1996) due to logging practices, forest plantations and oil palm plantations, mining, forest fires, dam building, creation of wetland peat and others (Notohadiprawiro 1998; Rautner et al. 2005). This encourages the high rate of biological extinction in Kalimantan. Therefore, sustainable forest management and conservation initiatives become more important in tropical forest which deforestation rates continue to be worried about (Joshi et al. 2004). In addition, the inclusion of various economic activities in the above often leads to conflict with Dayak tribe which its community land (*alayat*) sometimes is taken without proper indemnity or compensation (Jawan 1996; Bujang 2005; Rousykin 2005; SAM 2007). In the past, such disputes can be settled down by customary law, but nowadays, with the capital power, plantation and forestry companies do not admit customary law, so the case of destruction and violence are often happened (Gonner 1999; King 1999).

Timber industry, such as plywood, timber, furniture, paper pulp rapidly evolving in the 1980-1990s led to a large number of natural forest in Borneo to be cut down (Velasquez and Shimizu 2001; Butler 2003; Engel and Palmer 2008). Furthermore, palm oil plantations and forest industry plants quickly loot the last remnants of primary forest (Majid-Cooke, 2002; Henson and Chang, 2003; Fitzherbert et al. 2008; Koh and Wilcove 2008; Marti 2008). Palm plantation is one of the greatest threats to the forests of Borneo (Wakker 2006). In 2003, in Sabah and Sarawak, its area reached 1.6 million hectares, while in the Indonesian part of Borneo about 1 million hectares (Rautner et al. 2005). Another threat is the mining, because Kalimantan has the largest coal deposits in the world, and the rich variety of other minerals such as gold, lead, diamond and precious stones (Maunati 1998; Fatah et al. 2007). Large dam construction in Bakung, Sarawak is worried about having an impact on local ecosystems (Rousseau 1995; Williams et al. 1995). The failed project of clearing peat lands for food crops in Central Kalimantan is proven to alter the natural landscape, causing drought and fires (Vayda 1999; Boehm and Siegert 2001). All economic activities above are real threat to the preservation of natural resources and ecosystems of Borneo.

Forest fires from land clearing activities are other threats to wildlife in Kalimantan. Burning forests is a traditional method of the Dayak tribe to open agricultural land and it has been done in a sustainable manner for thousands of years, where the burned area is limited and the burning frequency is 20-25 years (Ave and King 1986). The procedure for land clearing has also been regulated and supervised by customary law leaders. But the growing number of people either by birth or migration entrants, such as transmigrants, company workers, government officials, and others, causing land area for each resident narrowed, so that the frequency of burning land becomes shorter (5-15 years). This is compounded by the entry of various forestry companies, plantation, mining and others which took over a large number of customary land previously used for shifting cultivation.
A number of plantation and forestry companies are also suspected doing land burning, because this is the most effective, fast and inexpensive method to clear land, though it causes the release of carbon compounds into the atmosphere that affect global warming. The land burned by the company is certainly far more extensive than the one burned by individuals shifting cultivators. Regulation on prohibition of open burning of land for plantations has been made, but every dry season the burning is repeated. Long dry season due to El Niño in 1997-1998 led to burning to clear land turned into the biggest fire in the whole of Kalimantan (Vayda 1999; Siscawati 2000; Fuller et al. 2004; Buttler 2005), of which 6.5 million hectares of land burned (Rautner et al. 2005) and thousands of orang-utans died.

The rate of deforestation in Indonesia is very high. In 1950-2000, 40% of Indonesia's forests have been cleared, equivalent to a loss of 2 million hectares of forest each year (Engel and Palmer 2008). In Kalimantan, in the mid-1980s forest cover was about 75%, but in 2005 left only 50%. In 1985-2005, Kalimantan lost an average of 850,000 hectares of forest every year. In 2000-2002 deforestation throughout
the island of Kalimantan rose to 1.3 million ha per year, of which 1.2 million hectares per year occurred in Indonesian Borneo and 100,000 hectares per year in North Borneo (Sabah and Sarawak). If this continues, the forest cover will decrease to less than one-third by 2020 (Rautner et al. 2005).

THE DAYAKS

Origin of the Dayaks

Dayak tribe is descendants of Austronesian migrant who gradually sailed from Taiwan to the archipelago since the 4000-6000 years ago and reached Borneo 4,500 years ago (Blust 1984/1985 1999; Gray and Jordan 2000; Diamond and Bellwood 2003). They replaced or assimilated with the Austro-Melanesians who have inhabited the Borneo from 35,000-45,000 years ago (King 1993; Rautner et al. 2005). In genealogy, the assimilation of ethnic Dayak and other Borneo population causes the formation of several sub-ethnic, namely Mongolid Dayak, Malayoid Dayak, Austro-Melanesoid Dayak and mixed Dayak (Lumbat 1992).

Dayak, which means upstream or inland, is the collective name for various indigenous groups on Kalimantan Island. Dayak tribe has a loose grouping, where there are many sub-tribes, each of which has a dialect of the language, customs, laws and culture of its own territory, but their general appearance showed the same characteristics and is easily identified (Grimes 2000). This tribe is to share physical features, architecture, language, oral traditions, customs, social structure, weapons, agricultural technology and similar views of life (Davis 1993). They have a genuine belief of Kaharingan (animism), although many are now an official religion follower (Kana 2004; Winzeler 2008). Dayak tribes practicing shifting cultivation generally live along the river in the outback of Borneo, sometimes they live communally in a traditional long house (a kind apartment in modern society), and apply customary law (Harrison 1984; Deschamps and Hartman 2006). They are traditionally highly dependent on hunting wild animals to satisfy protein needs. The main wild animals hunted are wild boar (Sus barbatus) (Deschamps and Hartman 2006).

Dayak tribe is divided into six major clusters, namely: Kenyah-Kayan-Bahau generally living in the eastern part of Kalimantan, Odanum in the southern part of Kalimantan, Iban (Sea Dayak) in the northwestern inland to the coastal area of Borneo, Klemant (land Dayak) in the northwestern outback of Borneo, Murut in northern Borneo, and Punan (Penan) in the center to the east of Borneo (Lontaan 1975). Dayak tribes generally live in the outback of the island, especially on the banks of the river by making use of about 200 rivers that flow into the inland as transportation routes, but many who live in the hills (Jessup and Vayda 1988). There is also a tribe that lived in the hinterland to the coast, for example Dayak Iban in West Kalimantan and Sarawak. As descendants of sailors, initially Dayaks are suspected living along the coast, but the arrival of the Malay-Sriwijaya of Sumatra and Malaya as well as the arrival of the Javanese at the Majapahit era and at the Islamic sultanate of Demak-Pajang-Mataram cause them to move into hinterland part of Borneo.

Dayak language is an Austronesian language family (Grimes 2000). The language is divided into about 450 different ethnolinguistic groups, with speakers of about 3-4 million, with a density of about 14 people per square kilometer (Cleary and Eaton 1996) spreading across four provinces of Indonesia, West Kalimantan, Central, South, East and; two Malaysian states, Sabah and Sarawak as well as in the Sultanate of Brunei Darussalam (Davis 1993). In the Indonesian part of Borneo, there are more than 140 languages are still used, whereas there are 50 languages in Sabah and in Sarawak for more than 30 languages (Rautner et al. 2005). But no language is spoken more than 100,000 people; even some of the language is only spoken by about 500 people, making it very vulnerable to extinction. One of the Dayak languages, namely Odanum-Hamlet-Manyaan of Barito river valley located in the northern part of South Kalimantan and the eastern part of Central Kalimantan has a compatibility with the Malagasy language used in Madagascar, so it is suspected that from this region is the origins of the population of Madagascar (Dahl 1951, 1977; Dewar and Wright 1993; Bellwood et al. 1995).

Kalimantan has a population of about 15 million people, with the main composition of the Malays, Dayaks, and Chinese. Dayak tribe has a number of about 3-4 million, of which the largest group is the Iban Dayak consisting of 710,000 people living in the northwest of the island. In addition, there are also ethnic of Javanese, Madurese and Bugis in significant quantities. Most of the inhabitants of Borneo live in coastal cities, while in rural areas; they generally live along the river (MacKinnon et. al.1996). In the hinterland, there is also Dayak Punan, which some members still live subsistence lifestyles and practice life of nomadic hunter-gatherers (Arnold 1958; King 1964; Whittier 1964; Langub 1975).

Dayaks as the indigenous tribe

Generally, original inhabitants (tribe) are genuine conservationists; they build a strategy for biodiversity and environment conservation to sustain the needs of sustainable living. Since thousands of years ago, the Dayak people of Borneo use technology and traditional knowledge, namely shifting cultivation to manage natural resources and biodiversity in the forest. They build and use certain steps as a strategy for the conservation of natural resources and environment. At first, they learn the limitations of natural resources, where the excessive and unwise use of it will reduce its availability and sustainability. Traditional knowledge is the unique local knowledge owned by a particular culture or society. This knowledge is the accumulation of human knowledge and understanding of the universe, including the spiritual relationship with the Almighty, the relationship with nature, and relationship with humans, and it is reflected in language, organization, values and law system, to be the ethics that govern the behavior of a society. Dayak tribe always believes that there is a limitation of natural resources, thus requiring conservation, except for certain
types of resource availability which exceeds demand (Uluk et al. 2001). Review of the literature shows that people who intentionally build conservation strategy usually has limited natural resources and easy to decline. The strengthening conservation strategies in the traditional culture is very important to help surviving in the limited natural resources, especially when natural resources run out.

Dayak are the indigenous tribes of Borneo. According to the World Bank, indigenous people have the following characteristics: (i) live in ancestral territory, (ii) are an entity separate from other groups, (iii) use the native language, (iv) has a traditional political and social institutions; and (v) subsistence (Colchester 1999). Biodiversity conservation strategy of indigenous tribe is part of knowledge and traditional technologies of the tribe. Local wisdom is often more appropriate to apply to the local environment than the western system of knowledge and technology that is "scientific" (Slikkerveer 1999). Traditional knowledge and technologies are developed and are accumulated over generations within the scope of certain cultures and regions, including: health, agriculture, plants, forestry, irrigation, and others. Traditional knowledge has the potential to support the development of rural areas, such as traditional herbal medicine, livestock medicine, intercropping agriculture, garden of talun, disease management, wild food plants, architecture and others (Richards 1989; Warren et al. 1989; 1994).

In the world of agriculture, a holistic point of view of traditional knowledge has been developed for food production and natural resource management, including: concept, perception, belief, cosmology; attitude, experience, skills, technology, artifacts, seeds, plants, crop type, and also institutions, procedures, and processes used (Slikkerveer 1994). Recent research in traditional systems of knowledge and technology in various fields produces inter-disciplinary approaches, including anthropology, ecology, sociology, science, and etnosains, which include ethnobotany, etnosejarah, and etnoekologi. Dayak tribe owns all of the terms of indigenous.

Native tribes and the destruction of Indonesian forests

Indonesia has 10% of tropical rain forests of the world, ranking third after Brazil and Zaire. Most studies of deforestation in Indonesia stated that about one million ha of about 100 million ha of remaining forest lost each year (World Bank 1990; FAO 1990). Some authors assume that shifting cultivation is a major cause of deforestation (FAO 1990; World Bank 1990; Barbier et al. 1993). Though admitting a significant influence of shifting cultivation on deforestation, other authors give a greater emphasis on government policy and development projects in forestry and plantation sector (Dick 1991; WALHI 1992; Ascher 1993; Dauvergne 1993; Porter 1994; Thiele 1994; World Bank 1994; Angelsen 1995; Dove 1996; Ross 1996). The latter group of researchers assumes that the effects of shifting cultivation have been exaggerated. Traditional shifting cultivation is not a threat for forests, even necessary for the conservation and management of Indonesian forest remnants in the future (Colfer 1993; Hasanuddin 1996). These debates often occur because of unclear concepts and terminology used. So, it needs to be made clear the parties that affect the forest as well as key terms and concepts used, such as forests, deforestation, degradation, and causes of damage (Sunderlin 1997).

Dayak and other Indonesian tribes have endured for decades to gain recognition of civil rights, and rights to manage forests and water residence. At first the state has no special protection systems against indigenous people, but now there is a significant progress so that the strategies and tactics used are imitated by many indigenous tribes in other countries (Alcorn and Toledo 1998; Alcorn 2000). Dayak tribes face two typical problems of worldwide tropical forests, namely the struggle to adapt to new technology and to withstand the onslaught of entrants, employers and governments who claim their natural resources (ILO 1996). The Dayak tribes are the remnants of natural ecosystems dwellers (ecosystem people), namely the people who adapt to and dependent on local ecosystems to meet the intent of his life (Dasmann 1991). Collective identity, cultural traditions, and practices of management developed are capable of maintaining the ecosystems productivity resilience (Berkes 1999), although there is pressure of changes both on a local scale from members of the society itself, as well as on a national scale from the government (Alcorn 1991). Unlike most people associated with the global economy (biosphere people), the native tribes are dependent on local ecosystems and are affected directly by changes in the ecosystem (Dasmann 1991). The failure of indigenous tribes in adaptation of pressure to change often causes them to be marginalized and brings out violence.

Dayak tribes and their habitats

Humans began to build settlements and adapt to ecological and political changes in the forests of Borneo since 35,000-45,000 years ago (King 1993; Rautner et al. 2005). The indigenous people who live in the interior of Borneo are collectively known as Dayaks. Dayak indigenous territory is rich in natural resources, and it becomes a habitat for large numbers of fish, birds, plants, including many endemic species and 300 species of Dipterocarpaceae which has high economic value, and also a large number of mammals such as orangutans, Borneo banteng, Sumatran rhinoceros, wild buffalo, sun bears, and Asian elephants (POTTER 1993; Cleary and Eaton 1996). For centuries, the Dayak tribes develop various forms of agriculture, fishing, hunting, and forest products harvesting, which are the move-turn in accordance with environmental changes. These changes follow the general pattern that constantly changes the forest environment (Padoch and Peluso 1996). Dayak natural resource management has adapted to suit a variety of natural and anthropogenic events, such as drought, famine, fire, flood, war, and fluctuations in the population, making it possible to live and survive.

Indigenous Dayak tribes as in other Southeast Asian develop agricultural systems (agroecosystem) which are adapted from the tropical forest ecosystem. It is governed by customary law, i.e. regulations made and enforced according to the consensus of indigenous peoples. Dayak
indigenous institutions play an important role in managing ecosystems (Folke 1997). Dayak vision of prosperity suggests that rivers, soils, and forests are very important for ethnic identity. Same vision is shown in the mosaic patterned of shifting cultivation system in the forests they live. In shifting cultivation, mosaics patterns are formed consisting of a collection of natural forest, artificial forests, vacant land, and the fields in accordance with ecological conditions and local topography, namely hills, wetlands, or river valley. The only land without forest is wetland. Landscape lands have different shapes, but forest cover is substantially always there. Research in 1996-1999 shows that of the 21 communities that lands are mapped, with an area between 900-126000 ha, where the community has made an agreement to preserve the forests from logging or mining, forest vegetation covers around 50-99% of the communal land, where approximately 29% of primary forests (Sunderlin 1997). Kalimantan is designed as a logging concession area, but around 63% is still forested plains, and about 35% is the remaining forests of Indonesia (Potter 1993).

Kalimantan forests are mostly located in areas claimed by the Dayak tribe (communal lands). Most of these lands are the forested hills that can not be penetrated. In some places, this communal forest is isolated in fragments surrounded by very large monocultures lands belonging to oil palm plantation companies (Alcorn 2000). In the past, millions hectares of land are covered by a mosaic of shifting cultivations which form the landscape with high resilience. But now, many Dayak tribes follow the entrants and turn the land into oil palm plantations, so the mosaic pattern of shifting cultivations which are rich in biodiversity are difficult to be applied again (Potter 1993). National centralization of land use decisions led to the establishment of plantations, agriculture, and degraded land which are poorer than the ecosystem biodiversity in shifting cultivation (Alcorn 2000).

**Shifting cultivation and agroforestry systems**

Shifting cultivation is a traditional way of farming that is very old. Shifting cultivation is mainly found in highland forests. In a system of shifting cultivation, the main crops cultivated are gogo rice. First, the selected land is cleared by burning, and the ash is used to enrich the soil. It is followed by brief periods of rice cultivation (about 4-5 times of harvest). After the soil fertility is depleted, the farmer leaves the land in order to let secondary forest grow or converts it into agroforestry by planting rubber trees, fruit trees and other crops. After an interval of 20-25 years, soil fertility will return, so a new cycle of shifting cultivation can start (Lim 2001). Given the importance of gogo rice and rubber in this system, it is very important for the government to provide both superior strain of this species, so that local communities can be more effective in managing forest resources sustainably (Arifin 1998).

Agroforestry is done by changing the primary forest into artificial forests planted with various species of beneficial plants. This system has a high density of species with a relatively diverse and complex structure. This system combines productivity, biodiversity and economic value (Belcher et al. 2005). Dayak tribe has long practiced agroforestry systems. When the fertility of the land in shifting cultivation started to decrease, they plant various useful crops, so when the land is completely abandoned, the planted trees is already quite high and can compete with shrubs and grasses that grow later. In West Kalimantan, agroforestry is known as tembawang which based on the rubber tree (Ansari 1996; Sardjono 1990, 2003), in East Kalimantan agroforestry is known as lembo which is based on fruit trees (Sundawati 1993, 2003) and simpukung which is based on fruit trees, rattan, bamboo, wood and other useful plants (Mulyoutami et al. 2008). With the formation of canopy, agroforestry systems can be used to suppress the growth of grasslands (Hairiah et al. 2000; Purnomosidhi and Rahayu 2002).

The traditional systems above require very little or no agrochemical inputs at all, and the only sustainable way of cultivation of rice to poor areas of mineral nutrients such as Borneo (Dauvergne 1993). According to Lawrence and Schlesinger (2001), land infertility in Kalimantan is caused by low phosphorus content. The trees which are deep-rooted and grow on fallow land can raise levels of organic phosphorus significantly, so they can improve soil fertility. This research is supported by Sanchez and Buol (1976) and Richter and Babbar (1991) which state that phosphorus is the limiting factor in agricultural production in the tropics. The productivity of gogo paddies in shifting cultivation system is far below the wetland paddies. Most government officials blame this system, consider it inefficient, unable to raise living standard (subsistence), cause damage, and become a source of forest fires and a major cause of deforestation. So, they all become the reasons to forbid shifting cultivation and settle the cultivators to some settled villages (Dauvergne 1993; Faithful 1999). It is prevalent in indigenous tribes throughout Southeast Asia (Padoch et al. 2007).

Adverse effects of deforestation has been widely recognized, that is a major cause of land degradation, biodiversity loss and threatening of species extinction, as well as contributing to global warming (Gillis 1988; Dick 1991). WCED (1987) shows that deforestation and environmental destruction are positively correlated with poverty and shifting cultivation, especially in developing countries. Those who are poor and hungry often damage the environment to survive; they will cut down the forest and cultivate marginal lands repeatedly, resulting in land degradation. Arifin (1993, 1998) considers the charge is not fair because it blames the victim and ignores the role of shifting cultivation in conserving the environment. Even if poor people do environmental vandalism, mostly because it is the only choice left to live.

**Dayaks shifting cultivation system**

Native tribes in the tropics generally practice the shifting cultivation system, by forming mosaics of land to ensure the availability of resources in the future (Figure 2). Dayak tribes historically have practiced shifting cultivation system by planting gogo paddies, followed by long fallow periods, intensive agroforestry and natural resource extraction. Shifting cultivation is a complex system in
which forest land is cleared in rotation for a certain frequency. This system is also marked by the burning of land to restore minerals to the soil from forest plants, thus increasing fertility and then it can be planted with gogo paddies and other food crops such as maize and cassava (Crevello 2004).

Dayak tribes use shifting cultivation system that forms the mosaic with high resilience, and has a richer biodiversity due to low population density. This system can survive because it has a broad market for non-timber products, a diverse and extensive ecosystems and is exploited only by one community, and also strong traditional institutions that are resistant to the colonial administration (Alcorn 1990; Alcorn and Toledo 1998; Messerschmidt 1993; Warner 1991). Description of Dayak traditional knowledge in natural resource management has been widely publicized (Cleary and Eaton 1992; Cofer 1993; 1997; Dove 1985; King 1993; Padoch and Peters 1993).

Dayak tribe use disturbance to form a space for food crops and use forest succession process as a resource of production (Alcorn 1989). Gogo paddies occupy a principal position in a shifting cultivation system, so highly respected, and is surrounded by various rituals and they can cause the formation of work activities that bind communities. Dayak tribe has a dependency on a variety of natural resources such as fishing, hunting, forest production, and agriculture, but their social ties play a role in maintaining the integrity of the entire system against various disasters such as droughts, fires, and floods. The use of natural markers and augury to determine the location of shifting cultivation land cause random locations are chosen because of the lottery and experience (Dove 1996).

Figure 2. Shifting cultivation system shows the land use mosaic of two adjacent communities (2500 ha) surrounded by oil palm plantations (white area) in West Kalimantan. Adapted by Alcorn (2000) from maps provided by PPSDAK Pancur Kasih.
Dayak tribe generally establishes a permanent settlement at a place and (formerly) lives together in a longhouse (van Beukering et al. 2008). There is also a community that moves from one place to another to follow the shifting cultivation field (Joshi et al. 2004). Dayak indigenous territory usually consists of settlements, rivers and ponds, dry farm field, undisturbed primary forests as a source of regeneration and animals hunting, bush and secondary forest which is the remnants of shifting cultivation, various agroforestry, such as mixed fruit orchards, rubber and rattan, and woody plants. Gogo paddies remain the center of land use change and management of shifting cultivation systems (Joshi et al. 2004). The position of the fields is sometimes far from the settlement so they make huma (newly cleared dry field) for temporary shelter and keep main fields from wild boar attacks. *Huma* is left as the soil fertility started to deplete. When left behind, this land often has been planted with a variety of useful plants that will form agroforestry.

Customary law regulates the establishment and harvesting of forest landscapes, where the conservation, biodiversity and sustainability is very important. Dayak tribe has traditional knowledge to maintain soil fertility, they also know the species of wild plants, economically useful plants, plants which are ecologically useful, plants as indicators of soil fertility, plants having medicinal value etc. (Joshi et al. 2004).

In Loksado, South Kalimantan, every Dayak family has a duty to process the fields of 2 hectares per year for rice farming. Families who do not comply with these provisions are prohibited following the ceremony. This customary law is still an obligation to be carried out, although some families have saved quite a lot, so much that his family can not spend it for 15 years, even if they stopped planting rice. Increased population causes the land needed to fulfill customary obligations also increased, while most of the shifting cultivation land should be left fallow temporary to avoid land degradation. It is necessary to open new land in primary forest. In Loksado, every year an area of 10-40 hectares of primary forest was opened to meet the obligations of this law (Boer 2006).

Hardwood plant regeneration in former shifting cultivation land may be failed if fallow periods are shortened and the frequency of land clearing is increased. In Loksado, narrowing of the land due to population growth and other modern pressures cause fallow periods to be shortened from 20 years to 5-15 years, so the soil fertility can not be regained and erosion happened. Natural regeneration of timber plants as a signal of the return of fertility has failed to form; otherwise the reed dominates because of its resistance to fire. secondary forest is difficult to grow naturally on this land and thus require the help of tree planting to assist the process of succession (Boer 2006). The similar thing is happened in East Kalimantan where soil characteristics are also prone to erosion, so that the opened forests must be reclaimed (Stadtmueller 1990). Primary forest damage due to shifting cultivation is much smaller than the extraction of timber and oil palm plantations (Lawrence 1998).

The practice of shifting cultivation is the most dominant type of land use on a large number of ethnic Dayak. The combination of rubber cultivation, maize, cassava and rice, and harvesting non-timber forest products including wild animals often become the dominant form of land use (Dove 1985; Colfer et al. 1996). In peatlands, the Dayak combines the shifting cultivation system with the burning and is combined with rubber agroforestry using mineral from the riverbed as a planting medium (van Beukering et al. 2008).

There are also several ethnic groups that combine shifting cultivation and agroforestry with extensive agriculture such as oil palm and rubber plantations, as well as hunting, collecting forest products and domestication (Dove 1986; Colfer et al. 1996; Sellato 1996, 2002). In some regions, rattan harvest from the wildwood has to be replaced with the domestication of rattan intercropped with a variety of useful trees (Dove 1985; Colfer et al. 1996). Dayak tribe did not develop animal husbandry despite extensive grasslands in the early stages of succession of shifting cultivation. Livestock do not play a major role in land use patterns in Borneo. Cattle and buffalo as private property is limited and does not have a major impact on the type of land use (Dove 1986; Colfer et al. 1996; Sellato 1996, 2002). Changes in land use patterns are possible, it should be supported as long as it gives benefit to the community and protects the environment (Kartawinata et al. 1992).

Dayak tribes believe that the crop of shifting cultivation depends on the close agreement between the farmers and the world of spirits that control the harvest. Forests and forest products is very important, so a different set of forest is managed with different intensities according to the purpose (Padach and Peters 1993). Dayak tribes exploit much subsistence of forest products; they use about 200 species of medicinal plants from forests (Caniago 1999). Rituals associated with excess or shortage of fruit crops show the importance of the principle of the exchange and give each other (Dove and Kammen 1997). Because most indigenous fruit crops are seasonal, the scarcity of crops led to public awareness of the importance of relationships with nature and with others (Alcorn 2000).

Dayak tribe has set the balance between economic dependence on forest products with the production of gogo paddies. For Dayak tribes, shifting cultivation is an action to be taken (Dove and Kammen 1997). Most of the Dayak rituals associated with rice cultivation. In the management of shifting cultivation, rice has a spirit that must be treated carefully and appreciated highly (Djuweng 1998). This belief supports the shifting cultivation system resilience. In the 1930s, when rubber prices is uncertain, an indigenous elder’s dream about people who are forced to eat the rubber due to the absence of rice spreads rapidly throughout Borneo, and warns the residents to maintain the system of shifting cultivation and integrate rubber plantations in this system (Dove 1999).

Logging concessions have taken over the Dayak indigenous forests and cause ecological damage. Only about 4% of owner of HPH (forest concession) that comply with the regulations set by the government in forest.
exploitation (Potter 1993). In 1998, the coalition of the Institute of Indigenous Peoples' Council sued this matter and asked the government to withdraw the status of state forests to renew the boundary between state forests with indigenous forests, and take back all the rules and policies related to exploitation and violation of community rights (Coup 1998). But the Forestry Law No. 41/1999, which was made to respond to this, did not make much change on the situation; it was more like a lip service for indigenous peoples. This shows that the pressure to the government and Parliament should be more powerful, reformation needs to be done in order that the change is bigger, and local leadership must be more powerful to organize the weakened community ties (Anonymous 1998).

People often do not realize the boundaries of their customary forest. Just after the logging by industrialist or the conversion of forest land into oil palm plantations, they claimed that the forest is theirs. On the other hand, the community also helps clearing the forest and supplies the illegal timber to the lumber mills, inter-island shipping, and even exporting to neighboring countries, Malaysia. Unconsciousness territorial boundaries will also cause loss of ecological protection responsibilities, such as forest clearance leads to loss of useful species which are usually abundant. Upstream society does not care about the impact faced by the downstream communities due to logging activities and forest clearing (Alcorn 2000). The spread of smoke in the dry season and floods during the rainy season are the real result of deforestation.

Preservation of biological resources in shifting cultivation systems

In East Kalimantan, after one year of gogo paddies cultivation, Benuaq Dayak tribes often plant their land with a variety of useful trees, such as fruit trees, rattan, and bamboo, so the land is developed into agroforestry (simpukang). These artificial forests become an important resource for gathering fruits, medicines, timber, fuel wood, rattan, and wild animals. Various simpukang serve the function of ecological, economic, religious and cultural. In addition, they also leave certain areas as protected reserves forest (bengkar). With this combination, Benuaq Dayak tribe has built a system of natural resource management that are relatively sustainable. The logging activity and oil palm plantations are the biggest threat to the system (Joshi et al. 2004).

System of shifting cultivation and agroforestry and the collection of forest products are relatively sustainable compared to plantations, farms, and forest harvesting. Development of settlement and cultivation activities undertaken during the last 300 years do not cause permanent deforestation and do not cause the extinction of species (Gonner 2001). A total of 35 species of local fruit are harvested from agroforestry forests in West Kalimantan and sold to Pontianak, with a market value in every year more than Rp. 500 billion (Armand 1996). Other native plants produce trade goods such as rattan, resins, and vegetable oils (Peters 1996). Some introduced species become the economic value of forests. Rubber plantations introduced in the early 1900s has caused Indonesia to be one of the world's largest rubber producing countries (Dove 1996). These rubber-producing forests have extremely high diversity species (Penot 1999).

In West Kalimantan, agroforestry has a high level of biodiversity, and does not differ significantly from primary forest. On transect with the length of 1,500 m in primary forests there can be found 102 species of birds, whereas in the artificial forest 101 species are found. The pressure of hunting activity on both locations is different because of differences in land cover, but the species in both habitats are relatively similar, where the value of Sørensen's similarity index was 68%. Hunting activity provides a high pressure on several species of animals such as wild boar (Sus barbatus), deer (Tragulus spp., Muntiacus spp., Cervus unicolor), honey/tree bear (Helarctos malayanus), hornbills (Bucerotidae), partridge (Phasianidae), parrots (Gracula religiosa), and freshwater turtles (Testudines). All prey species are still survived, due to a reserve forest that is difficult to reach and the annual flood that allow the breeding of waters species (Gonner 2001).

At this time, socio-cultural changes threaten the old practices in conserving forests. The receipt of the official religion and the abandonment of the original trust cause a number of traditional rituals no longer performed, whereas these rituals are part of Dayak holistic perspective in viewing the human and nature. In addition, traditional knowledge about the value use of plants, such as medicine and toxic substances, is declining, where only the older generation and shamans who still understand it. The absence of traditional knowledge that is replaced by more relevant knowledge to survive in today's world, causes ignorance of the benefits of these plants so that conservation efforts no longer exists (Gonner 2001).

CONCLUSION

Traditional knowledge is very useful to preserve the indigenous environment because of the increasingly limited of natural resources, and the increasing of population. Shifting cultivation by Dayak tribe in Borneo is traditionally not only to fulfill their daily lives but also to maintain the balance of ecosystems and biodiversity. The measured disturbance which is done in shifting cultivation system causes the growth of new seeds. On the other hand, forest concessions and large plantations where there has been land clearing and monocultures farming significantly interfere the preservation of ecosystems and reduce biodiversity.

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