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Seed Germination and Cuttings Growth of *Piper Aduncum*

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Abstract. *Sirih hutan* (*Piper aduncum* L) is one of group shrubs tropical species, has potential to be developed as raw material of biomass based electricity. The aim of this research was to know seed germination and cuttings growth of *P. aduncum* plant as the first step in cultivation of this plant. Observation of flowers and fruits were done in secondary forest, while seed germination and growth of shoot cuttings were done in the laboratory. The results showed that *P. aduncum* seeds can be germinated in a relatively short time of 17 to 25 days with a fairly high germination percentage of $90 \pm 8.16\%$ and germination rate of $4.7 \pm 0.34\%$. The growth of seedlings at 2 months old was 4.78 ± 0.42 cm, plant height 3.97 ± 0.27 cm, and relative growth rate $0.33 \pm 0.14\%$. The treatment of synthetic growth regulator had significant effect on shoot growth and root number on the plant stem cuttings. Preparation of seedlings ready to plant in a generative and vegetative for cultivation of these plants in the experimental plot.

1. Introduction

Piper aduncum L (Sirih hutan) is classified as an invasive plant, naturally spreading from Fiji, the Solomon Islands and the United States. It spreads also to Southeast Asia, Melanesia and Papua New Guinea [1, 2]. *Piper aduncum* is usually called sirih hutan (Indonesia), while in Sunda area usually is called Gedepong or Seuseureuhan, which can be found at altitude 1 - 2.000 dpl (above sea level).

In Indonesia, this plant was first introduced in Bogor Botanical Gardens, West Java in 1860. Then spread widely in the island of Java, Sumatra, Seram, Papua and other islands. In Kalimantan, *P. aduncum* was first collected in 1952 [3]. According [4], the spread of invasive *P. aduncum* plants on the island of Borneo, particularly in East Kalimantan occurred through a network of logging roads stretching from the southern part of Kutai Barat Regency to the north in Malinau District. Many grow on the older (old) logging roads in the south and there are no plants in the younger (new) streets in the north. The minimum spread rate is estimated to be five to seven kilometers per year, where a river with a width of 30 m cannot prevent the spread of this plant. On the previous report [5], this plant is found in secondary forest of shifting cultivation fields with a fallow age of 0.5 to 3 years, but not found in the fallow period of 6 to 10 years.

Forest betel plant extracts have the potential to be *Aedes aegypti* mosquito larvae of dengue virus spreader [6], drawing the growth of *Fusarium solani*, causing fusariosis disease in *Piper nigrum* [7].

The above ground biomass from *P. aduncum* reached 48 Mg DM ha⁻¹ at 23 months and three quarters of the biomass consisted of wood. The growth rate of *P. aduncum* averaged 69 kg DM ha⁻¹ per day and increased with higher rainfall [3]. Rapid growth and large biomass production from these

plants could potentially be developed as a source of renewable raw materials for biomass-based power generation, especially for the inland areas of East Kalimantan. On the past report [8], the woody shrub species of *P. aduncum* produce an energy potential of 2.19 Mwh per ton of dry biomass, compatible and can be used as a biomass feedstock for sustainable electricity production. Recently, information on germination and cultivation of this plant has not been widely known and never been cultivated. Based on the above description, our study focused on the generative and vegetative reproduction of *P. aduncum* plant as a preliminary study for the provision of ready-to-plant seedlings in the cultivation of this plant.

2. Materials and Methods

2.1. Study area

Flower and fruit development research were conducted in forest research Faculty of Forestry Mulawarman University, East Kalimantan, Indonesia (Botanical Garden of Mulawarman University of Samarinda), located between the coordinates of 0 ° 25'10 " - 0 ° 25'24" South Latitude and 117 ° 14'00 " - 117 ° 14'14" East Longitude. Seed germination research from fruit and growth of shoot / stem cuttings were done at Physiology Laboratory of Faculty of Mathematic and Natural Sciences, Mulawarman University in Samarinda, East Kalimantan, Indonesia.

2.2. Observation of flowers and fruit of *P. aduncum*

Observations were made in the secondary forest of the flowering and fruiting *P. aduncum* plants. Then performed fruit harvesting and morphological observation and fruit anatomy that has been mature physiologically. Seed extraction was done to separate the seeds from the other pieces of fruit [5].

2.3. Fruit and Seed

The harvesting mature fruits were conducted in April - May 2017, the fruit was collected in bulk (mixed from several trees in the same place). The harvesting mature fruits were done with a hooked pole and under a tree laying plastic to accommodate falling fruits. Samples taken as 1 kg of fruit peeled and seeds extracted. [9].

2.4. Seed Extraction

The seeds separate from the other pieces of fruit such as skin, meat, wings, stems then extraction of seeds. In dry extraction, the fruits were done in the open place for 3 - 4 days until the fruit was broken, and the seeds were easy to remove from the fruit. Performed sifting to separate the seeds from the skin of the fruit [9]. While on the wet extraction, the fruit was immersed in a burlap sack for one week (every day watered with moisture) until the outer skin of the fruit becomes soft, then dried until the fruit rupture and seeds were easy to remove from the fruit. Performed sifting to separate the seeds from the skin of the fruit [5].

2.5. Seed germination

The research was done by using completely randomized design with two treatments which were 1) the way of extraction of seeds consist of: wet extraction and dry extraction, 2) germination media. Seeds were sown evenly on germination medium of 20 seeds with 3 replicates for each treatment and observed for 60 days. The parameters observed were germination time of first seed germination (GTFS), germination time of the last germinated seed (GTLS), average germination time (MGT), germination percentage (G), and germination rate (GR).

2.6. The growth of seedlings

The seeds germinated in the laboratory were transferred into a 30 cm high polybag and 15 cm in diameter, filled with a topsoil soil topsoil medium. Tillers were kept in a greenhouse with a shade of

paranet 50% for 1 month. High data, leaf number, wet weight and dry weight and relative growth rate (RGR) were measured at the end of the study.

2.7. Growth of shoot / stem cuttings

Shoot cuttings were done by planting shoots on some planting medium: M0 = top soil, M1 = soil top soil + manure (1: 1), M2 = soil top soil + manure (2: 1), M3 = soil top soil + fertilizer cage (3: 1), and second treatment with synthetic growth regulator; R0 = control, R1 = 50 ppm, R2 = 100 ppm, R3 = 150 ppm and R4 = 200 ppm. Shoots cuttings were maintained in plastic hoods to keep moisture in the greenhouse with a 50% paranet shade for 2 months, then measured for growth and rooting.

2.8. Data analysis

Data obtained from field observations were analyzed descriptively, while germination data and seedbed were analyzed by analysis of variance (Anova) and advanced test with Duncan's Multiple Range Test (DMRT) at 95% levels.

3. RESULTS AND DISCUSSION

3.1. Morphology of flowers and fruit

Observations of the flowers and *P. aduncum* fruit of the forest showed that these plants flower and bear fruit continuously throughout the year. Compound interest grows continuously on the leaf axillary at the end of the branch and the fruit was increasingly ripe often with the increasing of flowers from the end of the branch. The morphology of flowers, fruits and seeds is presented in Figure 1, long ang diameter seed of *P. aduncum* in Table 1.



Figure 1. a. Plant of *Piper aduncum*, b. Branch and fruits

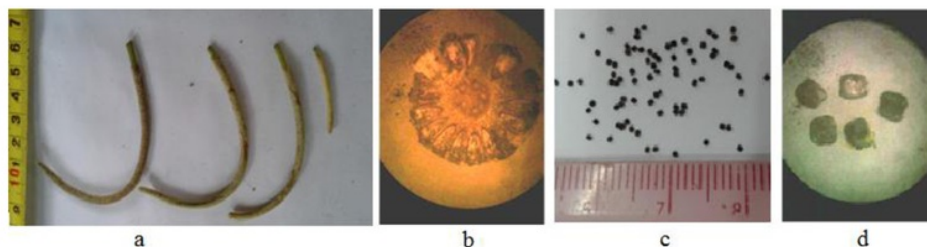


Figure 2. Fruits and seeds of *Piper aduncum* Description: a. flowers, young fruit and ripe fruit physiological, b. cross section of fruit, c. seeds, d. microscope photo seeds (4 x10 magnification)

Table 1. Mean size of fruit and seed of *P. aduncum*

| Organ | Long (cm) | Diameter (cm) |
|--------|------------|---------------|
| Fruits | 11.83±1.07 | 0.35±0.04 |
| Seeds | < 0.1 | <0. 1 |

Fruit was round and long, the measurement of ripe fruit obtained the average length of 11.83 ± 1.07 cm, diameter 0.35 ± 0.04 cm with a small seed size of less than 1 mm.

3.2. Seed germination

The method of seed extract and planting media was influenced and statistically significant (Anova, $P \leq 0.05$) on the seed germination percentage. The percentage of germination and rate of seed germination of wet extraction was greater than dry extraction seeds. The highest percentage of germination on the combination of wet extraction with compost media was 65% while the germination rate (MGT), early germination (GTFS) and germination end (GTLS) seeds from wet extraction were faster than dry extraction seeds (Table 4). The percentage percentage of germination of forest is presented in the table and the figure below.

Table 2. Effect of seed extract method on percentage of seed germination of *Piper aduncum* seed.

| Extraction treatment | G(%) | GTFS (days) | GTLS (days) | GR (%) |
|----------------------|---------|-------------|-------------|----------|
| Wet extraction | 90±8.16 | 17 | 25 | 4.7±0.34 |
| Dry extraction | 0 | 0 | 0 | 0 |

G, mean germination (%); GTFS (days), germination time of the first seed; GTLS (days), germination time of the last seed. GR, germination rate (%)

Table 3. The growth of seedlings of tropical shrubs 2 month

| No | Species | ∑Leaves | Stem height | RGR |
|----|-------------------|-----------|-------------|-----------|
| | <i>P. aduncum</i> | 4.78±0.42 | 3.97±0.27 | 0.33±0.14 |

RGR= relative growth rate

3.3. The growth of stem cuttings

3.3.1. Application of growth regulators

Tropical bush-type plants that have the potential as seeds of energy until now have not been cultivated. In this study carried out vegetative propagation experiments of several types of tropical shrubs, including stem cuttings with the application of plant hormones to stimulate root and shoot growth. This is important in the preparation of ready-to-plant seedlings when these types of plants will be cultivated. The results of stem cuttings measurements with IAA (Indole acetic acid) and NAA (Naphthalene acetic acid) hormone applications are presented in the table below.

Table 4. Effect of plant hormone treatment on *P. aduncum* cuttings growth

| Treatment | ∑ shoots | ∑ leaves | Height | ∑ Roots |
|-----------|----------|----------|--------------------|--------------------|
| K0 | 1.00 | 5.33 | 26.33 ^a | 11.33 ^a |
| K1 | 1.00 | 6.33 | 26.67 ^a | 22.67 ^b |
| K2 | 1.33 | 9.67 | 45.33 ^b | 34.67 ^c |

3.3.2. Application of commercial growth regulators.

In this study used commercially plant growth regulators on the market and commonly used by farmers in vegetative propagation such as grafts or stem cuttings or shoots. The content of these growth regulators were IBA, NAD, MNAA, MNAD and Oysters. The result of analysis of variance using anova showed that media had significant effect on high growth of shoot cuttings ($0.010 < 0.05$), while commercial growth regulator did not significantly affected on the growth of shoot cuttings ($0.319 > 0.05$). The interaction between medium and commercial growth regulator did not significantly affect the growth of plant height ($0.434 < 0.05$). The average growth rate of *P. aduncum* is presented in the Table 5.

Table 5. Influence of planting medium and commercial growth regulator on the average height of *P. aduncum* at 2 months.

| Treatment | M0 | M1 | M2 | M3 | Mean |
|-----------|-------------------------|--------------------------|-------------------------|------------------------|-------------|
| R0 | 16.40±14.29 | 20.73±1.16 | 16.33±14.44 | 22.50±3.28 | 18.99±9.23 |
| R1 | 26.83±1.75 | 24.83±6.64 | 22.00±1.90 | 0.00±0.00 | 18.42±11.65 |
| R2 | 22.76±3.57 | 8.83±15.29 | 7.50±12.99 | 7.00±12.12 | 11.52±12.19 |
| R3 | 21.96±19.05 | 17.40±15.51 | 13.86±12.03 | 8.00±13.86 | 15.31±14.12 |
| R4 | 26.30±9.89 | 8.80±15.24 | 6.33±10.97 | 5.16±8.95 | 11.65±13.28 |
| Mean | 22.85±10.5 ^b | 16.12±12.3 ^{ab} | 13.21±11.3 ^a | 8.53±11.0 ^a | |

Further test results with DMRT showed that giving planting medium decreased the growth of plant height cuttings. The highest average at M0 was 22.85 ± 10.50 and the lowest on M3 namely 8.53 ± 11.0 . M1 is not significantly different from M2 but is different from M0 and M3. The result of analysis of variance showed that media and commercial growth regulator have significant effect on the growth of leaf buds leaf cuttings ($0.043 < 0.05$), ($0.045 < 0.05$). The interaction between medium and commercial growth regulator did not significantly influence on the growth of shoot leaves leaf number ($0.075 < 0.05$). The average number of piper plant leaves is presented in the Table 6.

Table 6. Influence of planting medium and commercial growth regulator to the average number of leaves of plant of *P. aduncum* age 2 months.

| Treatment | M0 | M1 | M2 | M3 | Mean |
|-----------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|
| R0 | 4.00±3.46 | 5.67±0.58 | 3.33±3.05 | 5.67±0.58 | 4.67±2.27 ^b |
| R1 | 2.33±0.58 | 4.67±2.52 | 6.00±1.00 | 0.00±0.00 | 3.25±2.67 ^{ab} |
| R2 | 3.67±0.58 | 3.00±5.19 | 1.33±2.31 | 0.67±1.15 | 2.17±2.79 ^a |
| R3 | 5.33±0.58 | 3.00±3.60 | 1.67±1.53 | 1.67±2.89 | 2.91±2.61 ^{ab} |
| R4 | 5.00±2.00 | 1.33±2.31 | 0.67±1.15 | 1.00±1.73 | 2.00±2.41 ^a |
| Mean | 4.07±1.91 ^b | 3.53±3.14 ^{ab} | 2.60±2.58 ^{ab} | 1.80±2.48 ^a | |

Further test results with DMRT showed that the planting medium decreased the growth of the number of leaves of plant cuttings. The highest average at M0 is 4.07 ± 1.91 and the lowest on M3 is 1.80 ± 2.48 . M0 was not significantly different from M1 and M2 but significantly different from M3. Administration of commercial growth regulator also decreased the growth of the number of leaves of plant cuttings. The highest average at M0 was 4.67 ± 2.27 and the lowest at R4 was 2.00 ± 2.41 . R0 was not significantly different from R1 and R3 but significantly different from R2 and R4.

The result of analysis of variance showed that media and commercial growth regulator have significant effect on growth of root root cuttings ($0.00 < 0.05$), ($0.036 < 0.05$). The interaction between media and commercial growth regulator did not significantly affect the growth of leaf cuttings ($0.213 > 0.05$). The average height of pipe plant is presented in the Table 7.

Further test results with DMRT showed that the planting medium decreased the growth of the number of plant root cuttings. The highest average at M0 was 21.07 ± 10.30 and the lowest at M2 was 3.40 ± 4.67 . M0 was significantly different from M1, M2 and M3. Application of commercial growth

regulator also decreased the growth of the number of leaves of plant cuttings. The highest average at R0 was 13.33 ± 10.08 and the lowest at R2 is 5.42 ± 7.84 . R0 was not significantly different from R1 but significantly different from R2, R3 and R4.

Table 7. Influence of planting medium and commercial growth regulator to the average number of Roots of *P. aduncum* age 2 months.

| Treatment | M0 | M1 | M2 | M3 | Mean |
|-----------|--------------------------|------------------------|------------------------|------------------------|--------------------------|
| R0 | 19.00±16.46 | 8.33±6.50 | 8.00±7.55 | 18.00±5.29 | 13.33±10.1 ^b |
| R1 | 28.67±5.13 | 5.33±7.50 | 5.67±5.51 | 0.00±0.00 | 9.92±12.33 ^{ab} |
| R2 | 16.67±5.67 | 3.67±6.35 | 1.00±1.73 | 0.33±0.58 | 5.42±7.84 ^a |
| R3 | 16.67±14.47 | 4.67±8.08 | 2.00±2.64 | 2.00±3.46 | 6.33±9.67 ^a |
| R4 | 24.33±6.03 | 1.00±1.73 | 0.33±0.58 | 0.33±0.58 | 6.50±11.09 ^a |
| Mean | 21.07±10.30 ^b | 4.60±5.97 ^a | 3.40±4.67 ^a | 4.13±7.60 ^a | |

4. Discussion

Field observations showed that *P. aduncum* flowering and fruitful continuously. The flowers grow on the leaf's armpit and the physiological ripe fruit had a length of 11.83 ± 1.07 cm, diameter 0.35 ± 0.44 cm. Small seeds with dimensions less than 1 mm. Reported [10] that *P. aduncum* flowers consist of compound interest, jar form, one or two pairs, protective leaves 0.5-1.25 mm. Fruit in the form of buni fruit, was short stalk, long grain 12-14 cm.

In this study, the wet extraction treatment yielded high seed germination which was $90 \pm 8.16\%$, while the extraction of dry beans had no seeds that germinated. On the report [11] stated that the percentage of germination of *P. aduncum* reached 49-68% under different thermal regimes in the presence of light, without prior permanence hydrated in darkness for a month (no) and after permanence (yes). *P. aduncum* seeds are orthodox that can be stored for a long time [12]. According to author name [13] the growth of *P. aduncum* seedlings is very slow.

In this study, the treatment of planting media and plant growth regulators were remarkable on the growth of cuttings of *P. aduncum* crops. The best planting medium was topsoil soil, the application of manure decreases the growth of leaf number, plant height and number of roots. Likewise, in commercial application of commercial growth agents, the best growth in control (without the application of growth regulators). This is in contrast to those reported by author name [14] that the growth regulator treatment enhanced the growth of cuttings of Agarwood crops (*Aquilaria malacensis* Lamk.). On the report [13] reported that for multiplication of plants in large quantities, cuttings are a more efficient method, namely by the exposure of the hormones IAA and NAA with the media from the sand.

5. Conclusion

P. aduncum seeds can germinate in a relatively short time of 17 to 25 days with a fairly high germination percentage of $90 \pm 8.16\%$ and germination rate of $4.7 \pm 0.34\%$. The growth of seedlings at 2 months old is 4.78 ± 0.42 cm, plant height 3.97 ± 0.27 cm, and relative growth rate $0.33 \pm 0.14\%$. The treatment of synthetic growth regulator has significant effect on shoot growth and root number on plant stem cuttings. Preparation of seedlings is ready to plant in a generative and vegetative for cultivation of these plants in the experimental plot.

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