

Effect of nodes position on the growth and yield of stem cutting of Sambiloto (*Andrographis paniculata*)

SOLIKIN

Purwodadi Botanic Gardens, Indonesian Institute of Sciences. Jl. Raya Surabaya-Malang Km. 65, Purwodadi, Pasuruan 67163, East Java, Indonesia.
Tel./fax.: +62-341-426046; email: solikin@lipi.go.id, lipisolikin@gmail.com

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Abstract. Solikin. 2018. *Effect of nodes position on the growth and yield of stem cutting of Sambiloto (Andrographis paniculata). Nusantara Bioscience 10: 226-231.* Plant propagation by cuttings can be done to produce relatively uniform quality of plant yield such as sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). The study aimed to determine the effect of nodes position on the growth and yield of stem cutting of sambiloto. The experiment was conducted in Purwodadi Botanic Garden in February-May 2017. The study used randomized block design with the treatment of nodes position i.e. top, middle and base of stem cuttings. Each treatment was replicated three times. The results showed that the top stem cutting produced the highest cutting life, leaf area, leaf dry weight, root and generative organs dry weight. In contrast, the base stem cutting produced the lowest live cutting and plant growth.

Keywords: *Andrographis paniculata*, cutting, growth, propagation

INTRODUCTION

Plant propagation has an important role for conservation, cultivation and development of medicinal plants such as sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). It uses to fulfil the need of raw material of herbal medicine for public and industrial consumption. Plant propagation can be done generatively by seed and vegetatively by grafting, cutting and in vitro culture. Generative propagation by seed changes the plant genetic properties and varying plants quality and quantity. Whereas, vegetative propagation has advantages to produce more uniform plant, genetically, and its products. It is important to improve productivity and quality of medicinal plants.

Sambiloto is commonly propagated by seed. However, vegetative propagation needs to be conducted to produce high quality and uniform simplicia of sambiloto. Plant propagation by cutting is one of the vegetative propagation conducted by many farmers on cultivation of horticultural plants, including medicinal plants, because it is easy to do and not expensive (Sumirat et al. 2013). Cuttings on high-quality plant varieties need to be investigated in order to obtain good propagation methods. It is to make the cuttings able to form the roots and grow into new plants. The success of cuttings to form new roots and shoots is influenced by environmental and genetic factors. Plant genetic affects the survival and rooting of cuttings, including the physiology of the stem and the nodes position of the stem taken as cuttings (Kraiem et al. 2010). Nodes position on the stem also has a significant effect on the development and number of root primordia on stem cuttings (Ma et al. 2015). The distal stem cuttings are the best for planting cassava (*Manihot esculenta*) (Stephen and Chikordi 2015); middle branch on *Jatropha curcas* (Santoso and Parwata 2014), coffee (Rokhani et al. 2014)

and *Ficus carica* (Yulistyani et al. 2014), whereas the top stem cutting is the the best on *Alstonia scholaris* (Mashudi and Adinugroho 2015). The difference of node position of stems cutting on some plant species as propagation materials is interesting to be examined on sambiloto. Propagation study by stem cutting on sambiloto had been done by Muhammad et al. (1996) using top, middle, base stem cuttings (in glass house), however it needs to be re-examined by field experiments with different experimental design, environmental conditions and more growth variables.

This study aimed to determine the effect of nodes position on the growth and yield of stem cuttings of sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees).

MATERIALS AND METHODS

The study was conducted in Purwodadi Botanic Garden at an altitude of about 300 m above sea level from February to May 2017. Climatic condition during the study was in the rainy season. Based on the climatological data in Climatology Station of Purwodadi Botanic Garden during the experiment recorded averages of maximum temperature 29.88°C, minimum temperature 20.48°C, rainfall 473.75 mm per month, relative air humidity 76.5% and 24 rainy days.

The experiment used randomized block design with the treatment of nodes position of stem cuttings, i.e. base, middle and top. Each treatment was replicated 3 times with 14 cuttings each treatment and replication. The cuttings were taken from cultivated plant in the garden of Purwodadi Botanic Garden which was about 16 months old and in the vegetative period (2 times the growth season). The cuttings were taken from the lateral (secondary)

branches that emerges from the main stem before the flower buds formed. The cuttings were cut with sharp cutter scissors into three parts, i.e. top, middle and base. The base and the middle stem cuttings had 3 nodes such as done by Muhammad et al. (1996), containing axillary buds; whereas the top cuttings consist of 5 nodes included shoot tip (to facilitate planting in the field) (Figure 1).

The cuttings were planted as deep as 1 node in upright position into a medium consisting of a mixture of soil and compost (2:1). Cuttings were planted in two strips with spacing 7 cm x 10 cm in one treatment, and 15 cm between the treatments in one replication. The size of each plot was 80 cm x 100 cm for each replication (block). Shading on the blocks were recorded each block I (40.39%), block II (9.2%) and block III (21.32%). Plant maintenance included watering (depended on the circumstances) and pests control (animals were captured and discarded).

Observations of the cuttings growth were conducted every week after 2 weeks after planting (WAP) on variables of branchlet number, plant height and leaf number. Early time of flowering was observed and recorded every day after planting at the age of 3 WAP by giving value 0-4 on variable abundance as showed in Table 1.

Observation of plant dry weight for determining plant biomass (root, stem, leaf and generative organ), leaf area and stem cuttings diameter were done at the end of observation. The weighing of plant dry weight was done after the plants put into oven at temperature of about 60°C for 3 days until the dry weight was constant and then weighed with an electric weight counter. Leaf area measurements used a punch method (modified) (Johnson 1967, Hamoda et al. 2016, Solikin 2017); with the formula:



Figure 1. Stem cutting materials of sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). Note: A. Top, B. Middle, C. Base

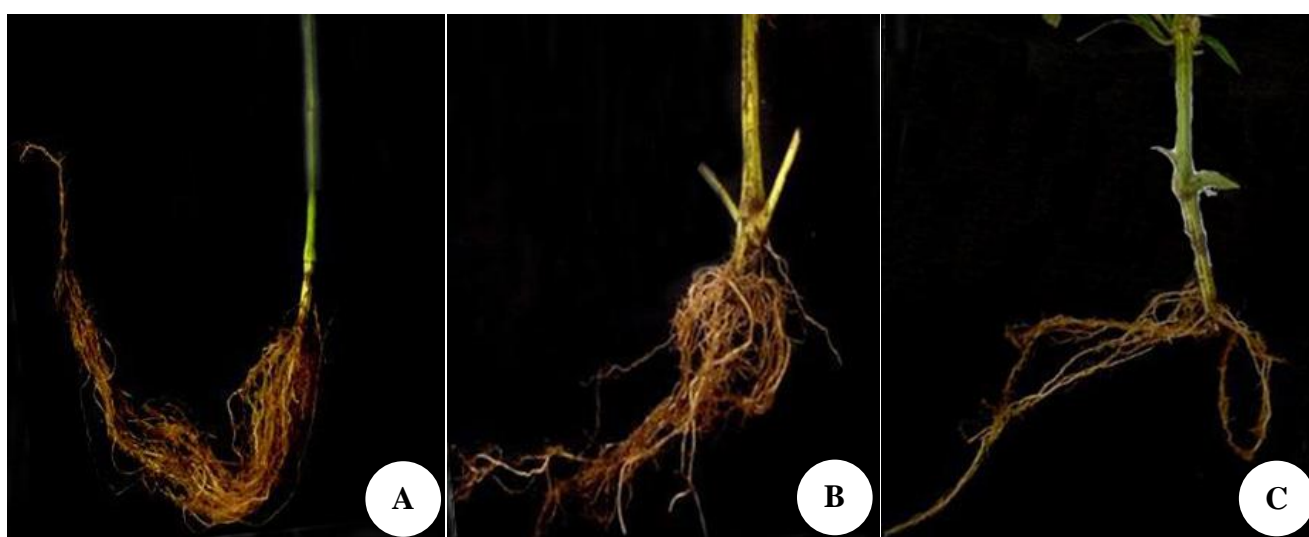


Figure 2. Roots of stem cutting on sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). Note: A. Top, B. Middle, C. Base

$$\text{Leaf area} = \frac{a + b}{a/n} \times c$$

Where:

- a: Dry weight of leaf disks (g)
- b: Dry weight of perforated leaves (g)
- n: The number of leaf disks
- c: The area of each leaf disk (cm²)

The data were analyzed by anova using MINITAB 16 program. If there were any significant difference between the treatments, mean values of the treatments were tested using the Least Significant Different (LSD) test at $\alpha = 5\%$.

RESULTS AND DISCUSSION

Results

There were significant effects on all observed variables, except the total dry plant weight, leaf number and cuttings life (Table 2).

The living of stem cutting

The nodes position of the stem cutting treatments did not give significant affect on the stem cutting life, although they had different stem diameter (Table 3). However, the living of the top stem cutting tended to be higher than the middle and the base stem cutting, i.e., 100% on the top cutting and 78.20% on the base stem cutting.

Plant growth

The nodes position of the stem cutting was significantly affected on plant height and leaf area, whereas the leaf number was not significantly affected by the treatments. Top stem cutting had the highest plant height and leaf area among other stem cutting treatments, i.e., 31.87 cm and 162.4 cm² per plant respectively (Table 4).

Root, stem, leaf, generative organ and total dry weight were produced during plant growth. The highest root dry weight, leaf and generative organs were reached by top stem cuttings, i.e., 0.06 g, 0.49 g, 0.14 g per plant, respectively. The total plant dry weight was not significantly different among the treatments, however there was a trend that the top stem cutting produced the highest plant dry weight, i.e., 1.03 g per plant (Table 5).

Table 1. Category of flowering and fruiting abundance in plants (Arisoesilaningsih et al. 2001)

Value	Abundance (%)	Note
0	0	Characters were not found on observed plants
1	1-25	Characters were found few on observed plants
2	26-50	Characters were found enough more on observed plants
3	51-75	Characters were found enough more on observed plants however not on all branches
4	76-100	Characters were found nearly on all branches of observed plants

Table 2. Recapitulation on the effects of nodes position on the growth and yield of sambiloto (*Andrographis paniculata* (Burm.f) ex Nees)

Variables	P value	Note
Root dry weight	0.005	S
Stem dry weight	0.036	S
Leaf dry weight dry weight	0.003	S
Generative organ dry weight	0.007	S
Total plant dry weight t	0.100	NS
Leaf area	0.001	S
Early time of flowering	0.007	S
Stem cutting diameter	0.001	S
Plant height	0.005	S
Flower number	0.005	S
Leaf number	0.090	NS*
Fruit number	0.014	S
Branchlet number	0.007	S
Cutting life	0.105	NS

Note: S: significant, NS: not significant; *: at the end observation

Table 3. The living stem cutting of sambiloto (*Andrographis paniculata* (Burm.f) ex Nees on the nodes position

Treatment	Stem cutting diameter (mm)	Cutting life (%)
Base	3.80 c	78.20 a
Middle	3.13 b	88.81 a
Top	2.56 a	100.00 a
LSD 5%	0.34	NS

Note: Numbers followed by same alphabets and same column were not different by LSD test at $\alpha = 5\%$, NS: not significant

Table 4. Growth of leaf area, leaf number and plant high of sambiloto (*Andrographis paniculata* (Burm.f) ex Nees on the nodes stem cutting position treatment

Treatment	Leaf area (cm ² per plant)	Plant height (cm)	Leaf number *
Base	68.46 a	20.22 a	12.66 a
Middle	127.40 b	23.85 b	17.35 a
Top	162.40 c	31.87 c	19.46 a
LSD 5%	25.41	0.65	NS

Note: Numbers followed by same alphabets and same column were not different by LSD test at $\alpha = 5\%$. *): 42 DAP, NS: not significant

Table 5. Plant dry weight of sambiloto (*Andrographis paniculata* (Burm.f) ex Nees on the nodes stem cutting position treatment

Treatment	Dry weight (g per plant)				
	Total	Root	Stem	Leaf	Generative organ
Base	0.82 a	0.02 a	0.59 b	0.20 a	0.01 a
Middle	1.05 a	0.03 a	0.54 b	0.39 b	0.08 b
Top	1.03 a	0.06 b	0.34 a	0.49 b	0.14 c
LSD 5%	NS	0.02	0.19	0.12	0.03

Note: Numbers followed by same alphabet and column were not different by LSD test at $\alpha = 5\%$; NS: not significant

Table 6. Growth of flower and fruit of sambiloto (*Andrographis paniculata* (Burm.f) ex Nees on the nodes position of stem cutting treatment

Treatment	Flower number *)	Fruit number*)	Early time of flowering (DAP)
Base	0.54 a	0.14 a	61.85 b
Middle	1.95 b	0.38 a	49.13 b
Top	3.29 c	0.77 b	26.59 a
LSD 5%	0.45	0.26	16.25

Note: Numbers followed by same alphabets and same column were not different by LSD test at $\alpha=5\%$. *): last observation

The early time of flowering, flower number, fruit number and dry weight of generative organs was significantly affected by the nodes position of stem cutting. The stem cuttings derived from the top stem produced the highest flower number, fruit number and generative organ dry weight, i.e., 3.286, 0.766 and 0.138 g per plant, respectively. Early time of flowering on the top stem cutting was the fastest, i.e. 26, whereas the slowest was the base stem cutting, i.e. 61.85 DAP (Table 6).

Discussion

Stem cutting life

The living of stem cuttings is determined by the ability to form new roots and shoots. Auxin as one of plant growth regulator has an important role to the root growth of stem cuttings. The stem cutting life of the treatments (top, middle, base) was not significantly different, however there was a tendency that the stem cutting life and growing into new plant increased from base to top stem cutting. This may be caused by higher auxin content in this cutting as the nodes position nearer to the tip of stem. On the contrast, the auxin content tends to decrease from the top to base stem. Thus, although the diameter of base cutting was greater than those of the top cutting, there was not significant different to the cutting life, even tended to decrease (Table 3). Aini et al. (2010) reported that the survival of the top stem cuttings on *Gonystilus bancanus* was the highest, followed by middle and base stem cuttings. Gardner et al. (2013) stated that auxin was produced on meristematic tissue at the leaf buds and the tip of the stem, then distributed to the organs underneath it through the phloem so that the auxin content of the top stem cuttings was larger than the base cutting.

The cutting life on the top stem cutting was the highest, i.e. 100%, whereas the lowest was the base stem cutting, i.e. 78.2%. This was also reported by Muhammad et al. (1996) that the top stem cutting of sambiloto had the highest living ability. The cutting life on this experiment was higher (100%) than those reported by Muhammad et al. (1996) i.e 90%. The high cutting life can be caused by higher auxin content and faster stem tissue regeneration on the top stem cutting compared to the base stem cutting so that the ability to form roots and shoots larger. Kassahun and Mekonnen (2012) proved on stevia (*Stevia rebaudiana*) that top cutting of this plant had higher ability to live and rooted compared to the middle cutting.

The regeneration of stem tissue also plays an important

role to stem cuttings to form roots and shoots. Sclerenchyma cell is thickening in older tissue (base cuttings), inhibited root initiation in *Acacia mangium* (Darus, 1989), causing a slower cell activity, and a higher mortality on *Gonystilus bancanus* (Aini et al. 2010). Hartman et al (2002) stated that the regeneration of stem tissue declined following with age of stem tissue. It was shown in Table 3 and Table 6 that the lowest life and the least root growth were on the base stem cutting. High level content of auxin and stem tissues regeneration on the top stem cuttings will elevate roots and shoots growth faster and increases the cutting life. This was showed by the root growing denser and heavier (Figure 2) with the highest dry weight (0.061 g per plant) (Table 7). It was contrast to the base stem cuttings, which was the lowest root dry weight (0.017 g per plant) (Table 7). Apriani and Suhartanto (2015) also proved that the top stem cutting of *Plechranthus amboinicus* had the ability to form roots and live higher than the middle and base stem cuttings. Santoso and Parwata (2014) also reported on *Jatropha gossypifolia* that the stem cuttings originating from older stems (main branches) had the ability to grow more slowly.

Growth of cutting

Vegetative. The plant growth from cutting is related to the leaf area which has an important role to plant photosynthesis for the growth of plant cells, tissues and organs. The plant growth originating from top cuttings was larger than middle and base stem cuttings. This was showed by the larger leaf area, higher plant (Table 3) and heavier root dry weight (Table 7). Aini et al (2010) also reported that the dry weight of root, stem and leaf on the top cuttings of *Gonystilus bancanus* was higher than those of middle and base stem cuttings. Safeer et al. (2013) also proved on *Plechranthus vetiveroides* that plant height, leaf number, leaf area, root weight and shoot on top stem cuttings were higher than those of middle and base stem cutting.

The shoot growth on the top stem cutting was also the fastest and the highest among other cutting treatments. As shown at Figure 3 that the branchlet number of the cuttings at the age of 14, 21 and 42 DAP was the highest. On the other hand, the shoot growth on the middle and the base stem cuttings were slower than those on the top stem cuttings at the same time. This may be caused by higher growth of branchlets on the top stem cutting as the higher auxin content and axillary bud number when planted (Figure 1). Node number of the stem cutting may relate to the growth of branchlets originating from axillary leaves. The top stem cuttings used in the experiment had more nodes than the middle and base stem cutting so that the growing possibility of branchlets or axillary buds more than the cuttings originating from middle or base stem.

The branchlet number produced on the top stem cuttings continued to increase starting from 14 DAP until the age of 42 DAP to reach about 6 branchlets, whereas the branchlet growth of the base stem cutting was the lowest among all the treatments (2 branchlets) (Figure 4). Apriani and Suhartono (2015) also proved that the branchlet number at the top stem cuttings of *Plechranthus amboinicus* Spreng was the largest (2.9), whereas the base and the middle cuttings were 1,2 and 2.2 branchlets, respectively.

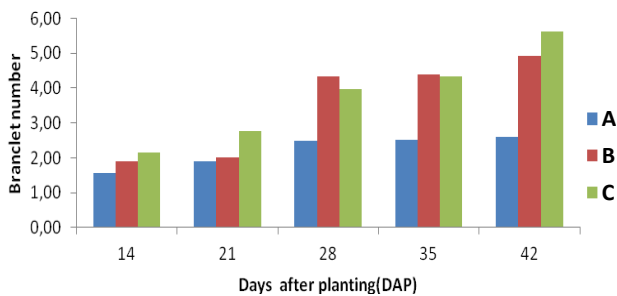


Figure 3. Growth of branchlet number on sambiloto (*Andrographis paniculata* (Burm.f) ex Nees. Note: A. Top, B. Middle, C. Base

The highest leaf number was reached by middle stem cutting, although it was not different statistically from top cutting. This was caused by early leaf number of the stem cutting as plant material when planted (Figure 1). The middle stem cuttings had more tertiary branches, however the size was smaller than the top cutting so that the leaf area and dry weight was lower than the top cutting.

Generative. Generative phase of sambiloto is an important time to determine plant harvest. The beginning of this phase related to the highest content of andrographolids as the main active secondary metabolite compound (Solikin, 2006). The stem cutting treatment had significant effect to the early period of flowering and growth of generative organs. The early time of flowering (the time when flower buds appeared) on the top stem cutting was faster than those derived from the middle and base stem cuttings (Table 5), which was about 26.59 DAP, whereas on the base stem cutting was the slowest i.e. 61.85 DAP. Table 5 showed that the early time of flowering was accelerated as the position of nodes nearer toward the tip of the stem. This was caused by the genetic factor of sambiloto.

The flowering type of sambiloto is determinate and flowering started from the tip or top internodes of the stem (terminal), followed by axillary branches on internodes beneath it. This causes the early time of flowering on the top stem cutting of sambiloto faster than those on the middle and the base stem cuttings. The early time of flowering on the middle and the base cutting was slower than those on the top stem cuttings because they need longer time to grow axillary buds or branchlets before flowering (generative phase) than those on the top stem cutting. The faster formation and growth of branchlets supported by greater photosynthesis in the top stem cuttings (the largest leaf area), the higher flower number, generative and fruit number i.e. 3.286, 0.766 and 0.138 g.per plant, respectively.

Biomass. Plant biomass, determined by total plant dry weight, was not significantly different between the top, middle and base cutting treatment. This was caused by the dry weight and diameter of the base and the middle stem cutting planted were greater than the top stem cuttings, so that the total dry weight was not significantly different (Table 2). The diameter of the base and middle stem cutting used as cutting material was greater than the top

stem cutting, i.e. 3.79 and 3.12 mm respectively (Table 3). This condition produced higher dry stem weight whereas the top stem cutting had the diameter of 2.55 mm so that the stem dry weight was lower. However, the root, leaf, and generative dry weight on top stem cutting was greater than the middle and the base stem cutting. This showed that the production of biomass during the plant growth on the top stem cutting was the highest among to other stem cuttings. Santoso and Parwata (2014) also reported that biomass production from the younger stem cuttings (softwood) was higher than biomass production derived from the older stem (middle and base) cuttings.

The stem diameter did not guarantee the successful plant growth of stem cuttings of sambiloto. This was proven by the life percentage of cuttings on the base stem cutting, which had the largest stem diameter, showed the lowest cutting life, as well as plant growth (leaf, branchlet number and biomass weight). Apriani and Suhartanto (2015) showed that on *Plectranthus amboinicus* Spreng, the living cuttings on the base and the middle stem cutting were the lowest compared to the top stem cutting. This can be caused by difference auxin content and regeneration ability of stem cuttings tissue. The tissues of the base and middle stem were older than the top stem, so they are harder, woody and form lignin. They can reduce the ability of tissue regeneration to form the roots. Hartmann et al. (2002) stated that the potential reduction of root formation in woody and hard woody stem tissue was caused by decreasing content of phenol compounds. Kochhar et al. (2005) stated that the older and woody stem cuttings, the more changes and adaptation of chemicals alteration for lignification. This caused the formation of roots, buds and leaves becomes slower.

In conclusion, the top stem cutting propagation of sambiloto produced the highest plant survival and growth with stem cutting life, leaf area, leaf dry weight and total dry weight of 100%, 162.40 cm² per plant, 0.491 g per plant, 1.033 g per plant respectively.

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