
GROWTH DYNAMICS AND YIELD OF *Andrographispaniculata* (BURM. F.) NEES AS INFLUENCE BY PLANT HARVEST AGE

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ABSTRACT

Andrographispaniculata or commonly known as King of bitters is one of the high value herb that globally in demand because of its pharmacological potential as anticancer. Plant harvest age is a critical factor affecting the growth dynamics and yield on herbal plants. Early or late harvesting time subsequently produces a low herbage yield. Therefore, a study was conducted to determine the effects of plant harvest age on growth dynamics and yield of *A. paniculata*. The plant was planted at the University Agriculture Park, Universiti Putra Malaysia, using a randomized complete block design with three replications. The plants were harvested at 14, 16, 18, 20 and 22 weeks after sowing (WAS). Results showed that the maximum increment rate in plant height and number of leaves and branches were at 16 and 22 WAS, respectively. Plant height correlated with the number of leaves ($r = 0.97$) and branches ($r = 0.94$). Specific leaf area decreased by 41% as plant harvest age increased. Plant fresh linearly increased until 20 WAS and tended to plateau thereafter. Plant dry weight increase as plant harvest age increased. Thus, plant should be harvested at 20 WAS or flowering stage in order to obtain desirable growth and yield.

Keywords: plant height, number of leaves, specific leaf area, plant dry weight, correlation.

Introduction

The use of herbal plants as alternative medicines has tremendously increased in the past decade and become a public interest. It is estimated that nearly four billion people around the world rely on herbal products as alternatives to modern medicines for primary source of healthcare (Ekor, 2013). *A. paniculata* is one of the herb plants that has a wide range of medicinal and pharmacological applications. Traditionally, *A. paniculata* extract from the aerial parts are used to get rid of body heat, dispel toxins from the body, prevent common cold and sinusitis (Joselin and Jeeva, 2014), fever, and as well as an antidote against snake and insect poisons (Samy et al.,

2008). Most of the *A. paniculata* products available in the market were highly consumed as stomachic, hepatoprotective, dyspepsia, anthelmintic and bitter tonic (Kataky and Handique, 2010).

Analysis of plant growth characteristic can be conducted in order to determine the optimum growth age/stage that can give a high yield to farmers and producers. According to Anon (2005) a plant growth dynamics can provide a common reference for describing the crop development, so that the farmers or producer can implement agronomic decisions based on a common understanding of which stage the crop has reached. Hunt et al. (2002) stated that plant growth analysis is an explanatory, holistic and integrative method to interpreting plant form and function. It uses simple primary data in the form of weights, areas, volumes and contents of plant components to investigate processes within and involving the whole plant.

Plant height and plant dry weight are important plant growth attributes that represent an increment in size per unit of time. These attributes are commonly used as an index for plant growth to determine the rate of changes in the plant size (Hunt et al., 2003). The other attributes that had been used among researchers in the plant growth analysis are fresh weight, absolute growth rate, relative growth rate (Tessmer et al. 2013), leave area ratio, specific leaf area and leaf root ratio (Hunt et al., 2003; Hunt et al. 2002). According to Tessmer et al. (2013), growth can be defined as an increase in dry mass, volume, length, or area that resulted from the division, expansion, and differentiation of cells. Huijser and Schmid (2011) stated that plant growth and development progresses through another different stages which were vegetative, reproductive, seed set and senescence stage. *A. paniculata* is categorized under an angiosperm plant that undergoes a complete cycle of growth stage and is usually propagated by seeds (Hossain et al., 2014; Chia 2009).

Most of the studies have stated that changes in phenological stage affect the growth characteristics and phytochemical contents of the plants (Ahl and Sabra, 2016; Ben Farhat et al., 2015; Chauhan et al., 2013; Chen et al., 2012; Chouaieb et al., 2012). Intervals of harvesting are vital since delay in harvesting can reduce yield of plants. In some crops, early or late harvesting may result in low yield of leaves due to over maturity, causing poor harvest of herb. Studies on the growth characteristics of *A. paniculata* have not been conducted comprehensively as needed by growers and producers. Thus, information on plant growth dynamics needs to be determined to associate the growth with standard optimum harvesting ages/stages for consistent production of phytochemical contents by the plants. The objective of this study was to determine the effects of plant harvest age on growth dynamics and yield of *A. paniculata*.

MATERIALS AND METHODS

Plant materials

The seeds of *A. paniculata* were scarified using a sand paper (N^o 120, sized 30 cm x 30 cm) and then soaked in hot water (68 ± 2 °C) for three minutes (Talei et al., 2012). The seeds were sown in Petri dishes containing filter papers (No. 2) moistened uniformly with sterile distilled water. The petri dishes were sealed with parafilm and placed in the growth chamber with controlled light source and relative humidity (60-70%). At two leaf stage, the seedlings were transplanted into Jiffy-7® pellets in the nursery unit and maintained until six to eight leaf stages (8 weeks after sowing).

Field planting was conducted at the University Agriculture Park, Universiti Putra Malaysia, Serdang, Selangor. The experimental plot (12 m wide × 27 m long) consisted of three raised beds which represented three replications. The size of each bed was 1.2 m × 27 m, height of 15-20 cm and with 1.5 m the distance between the raised beds. Ten ton/ha of each rice husk and chicken manure were incorporated to about 15 cm of top soil on each raised bed and left aside for 7 days before field transplanting. The raised bed was covered with Agrosheet® plastic mulching to control weeds. At six to eight leaf stages, the seedlings were transplanted onto the raised planting beds at one seedling/point. There were two planting rows for each bed. On each bed, the distance between the planting rows was 30 cm and between plants within the row was also 30 cm, giving a total of 176 plants per beds. The plots were maintained by carrying standard cultural practices. A sprinkler irrigation system was set up in the field to water the experimental plot of *A. paniculata*. Watering was carried out twice a day, in the morning and late evening, except when it rained. The plants were fertilized using organic based fertilizer (8: 8: 8) at the rate of 250 kg N/ha 3 days after transplanting. The fertilizer was evenly spread around the plants, underneath the plant canopy.

The *A. paniculata* plants were harvested at 14, 16, 18, 20 and 22 WAS (Figure 1). Ten plants of *A. paniculata* were harvested from each bed at about 10 ± 2 cm from the soil surface using a sharp pair of secateurs and then, transferred into plastic containers and transported to the Postharvest Handling Centre at the Vegetable Unit, UPM.

Plant height, number of leaves and number of branches

Plant height refers to the longest distance between the plant base and the tip of the highest shoot buds. The plant height (cm) was measured from the soil level to the highest tip of the shoots by using measuring tape. The plant height was measured immediately after the seedling was transplanted to the beds and subsequently at 2 weeks interval until 22 WAS. Number of leaves

and number of branches were manually counted by hand. For each plant harvest age, ten plants were taken from each replication.

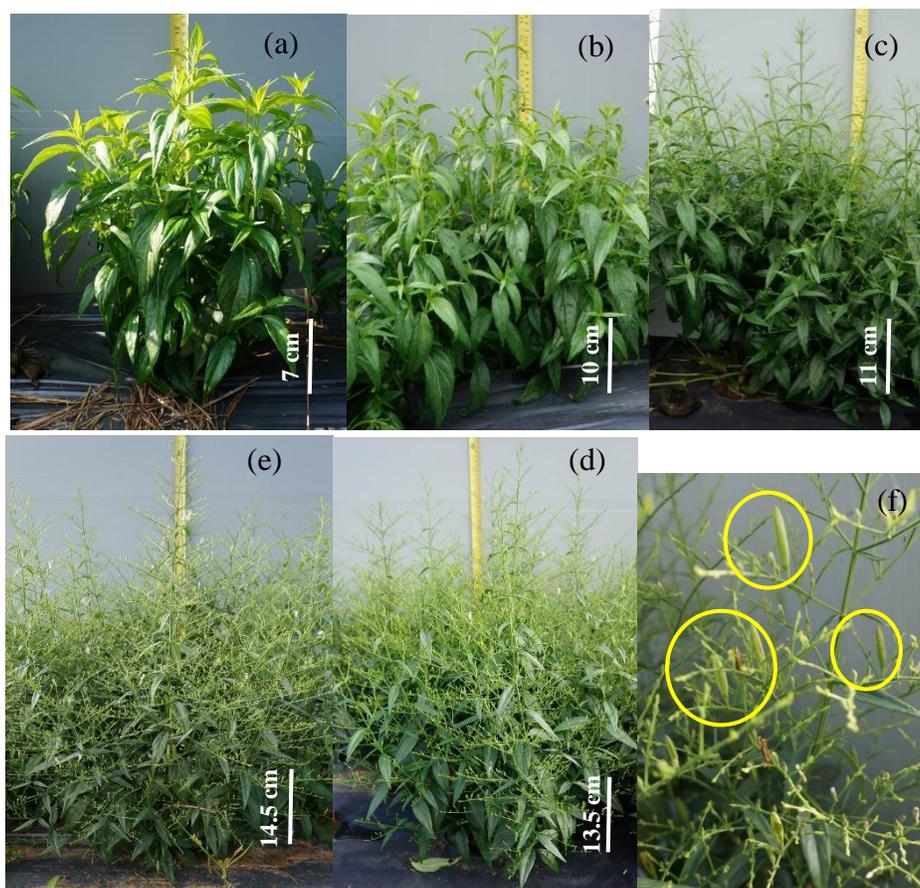


Figure 1. Plant age (weeks after sowing) and phenological stages of *A. paniculata* at (a) early vegetative stage of 14 weeks, (b) mid-vegetative stage of 16 weeks, (c) pre-flowering stage of 18 weeks, (d) flowering stage of 20 weeks, and (e) seed formation stage of 22 weeks. (f) developing of seed pods.

Plant fresh and dry weight

The fresh weight (g/ plant) of ten plants was taken immediately after harvest using a weighing balance. After determination of specific leaf area, the plant samples were taken for plant dry weight determination in each replication. The plant samples were cut into 5-7 cm length and packed into labelled paper bags before being oven dried (Memmert ULM 500, Schwabach, Germany) at a constant temperature of 50 ± 2 °C.

Specific leaf area

The specific leaf area is a measurement of the leafiness of the plant on a leaf dry weight basis. Total leaf area per plant was measured in cm^2 by destructive sampling using a leaf area meter (LI-3100, Area meter, Li-cor Inc., USA). Total of five plants were used to determine the specific leaf area. The specific leaf area was computed by using the following formula:

$$\text{Specific leaf area (cm}^2\text{/g)} = \text{Total leaf area/Total leaves dry weight}$$

Experimental design and data analysis

The experimental design used was a randomized complete block design with three replicates. The treatments consisted of five plant age, which are 14, 16, 18, 20 and 22 WAS. The results were statistically analysed using analysis of variance (ANOVA) of SAS version 9.4 (SAS Institute, Cary, NC). Quantitative data on the change in plant height, number of leaves and number of branches were modelled using the logistic growth model (Hunt et al., 2003). Data on plant height was non-linearly regressed against WAS by using the equation $y = A/(1 + be^{-cx})$. The 'A' and $A/(1 + b)$ were the asymptotic level and initial value of each parameter, respectively. The derivative of the above growth function $[dy/dx = (Abce^{-cx})/(1 + be^{-cx})^2]$, where y , x , A , b and c were used in estimating the growth rate (unit/week) of plant height. The variation of number of leaves and number of branches was modelled using different non-linear function of $y = Ae^{bx}$ and the rate of change was calculated using derivative, $dy/dx = Abe^{bx}$.

The relationships between plant age and variables (specific leaf area, plant fresh weight and plant dry weight) were determined using regression analysis. Pearson's correlation coefficients were calculated using the PROC CORR procedure and correlation analysis between dependent variables were performed to measure the strength and the direction of a linear relationship between two variables.

RESULTS AND DISCUSSION

Growth dynamics

Plant height

Changes in plant height of *A. paniculata* were monitored at every two weeks, beginning from the day the seedlings were transferred into the Jiffy-7® plugs. The performance of the plant in terms of height increment is illustrated in Figure 2a, which indicated that the pattern of plant growth well fitted into growth function of $y = A/(1 + be^{-cx})$, where y = plant height, x = WAS, while A , b and c are regression constants (Table 1). The increment in the plant height was slow at the

early stage of the plant from 0 to 10 WAS, and later increased almost linearly up to 20 WAS. After that, the increment in the plant height nearly plateaued, indicating that the growth started to decrease due to the senescence process. The maximum height increment rate was at 16 WAS and the growth rate exponentially decreased over the plant age (Figure 2b).

Table 1. Constants of plant height of *A. paniculata* using a function $y = A/(1 + be^{-cx})$.

Variable	Constant			F value	Approx. Pr> F	Approx. R ²
	<i>A</i>	<i>b</i>	<i>c</i>			
Plant height	88.7689	84.0535	0.2897	3367.41	<.0001	0.999

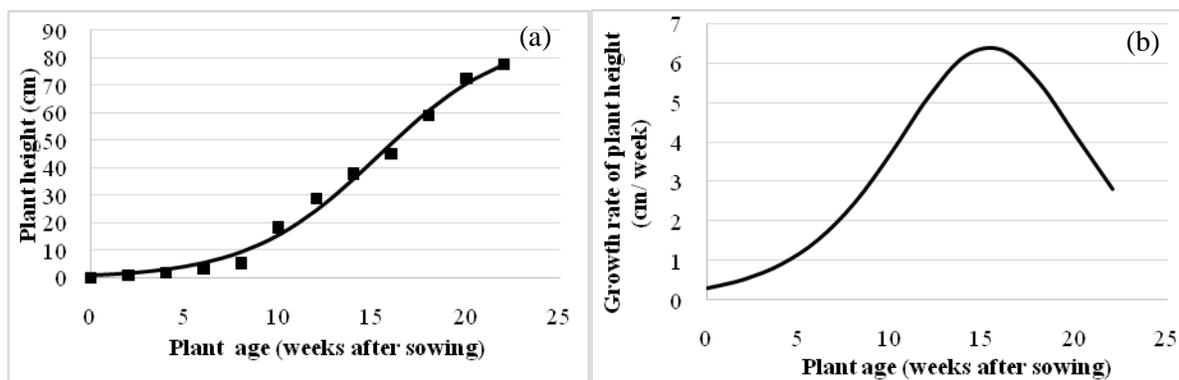


Figure 2. (a) Plant height as a function of plant age, in the form of $y = A/(1 + be^{-cx})$ of *A. paniculata*. The square symbol represent means of four replicates \pm SE, n = 40 and (b) growth rate of plant height as a function of plant age, in the form of $dy/dx = (Abce^{-cx})/1 + be^{-cx})^2$.

Number of leaves and branches

The number of leaves increased exponentially following the function of $y = Ae^{bx}$ (Table 2) with increasing in WAS (Figure 3a). The number of leaves started to increase at week 8 until the plant reached the reproductive stage at 22 WAS. The maximum of increment rate in number of leaves was at 22 WAS (Figure 3b). A similar exponential trend also can be observed for number of branches. The number of branches increased exponentially following the function of $y = Ae^{bx}$ with increasing in WAS (Figure 4a). The number of branches started to increase at week 10 and continually increased until reproductive stage of 22 WAS. The maximum of increment rate in number of branches was at 22 WAS (Figure 4b). The new leaves and branches of *A. paniculata* was continuously produced even when the plant was at the seed formation stage, indicating that the plant had not reached the senescence stage. The Pearson correlation coefficients revealed that the plant height was strongly correlated with the number of leaves and number of branches (Figure 5a and b). The number of leaves was found to be strongly, and positively correlated with number of branches (Figure 5c).

Table 2. Constants of number of leaves and number of branches of *A. paniculata* using a function $y = Ae^{bx}$.

Variables	Constant			F value	Approx. Pr> F	Approx. R ²
	<i>A</i>	<i>b</i>	<i>c</i>			
Number of leaves	34.6503	0.1672	1551.17	<.0001	34.6503	0.991
Number of branches	0.9118	0.1897	964.56	<.0001	0.9118	0.997

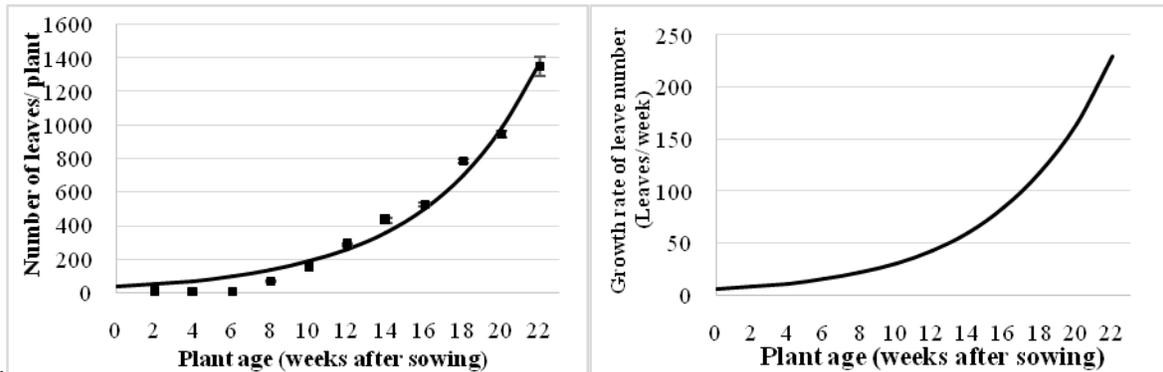


Figure 3. (a) Number of leaves per plant as a function of plant age, in the form of $y = Ae^{bx}$ of *A. paniculata*. The square symbol represent means of four replicates \pm SE, $n = 40$ and (b) increment rate number of leaves as a function of plant age, in the form of $dy/dx = Abe^{bx}$.

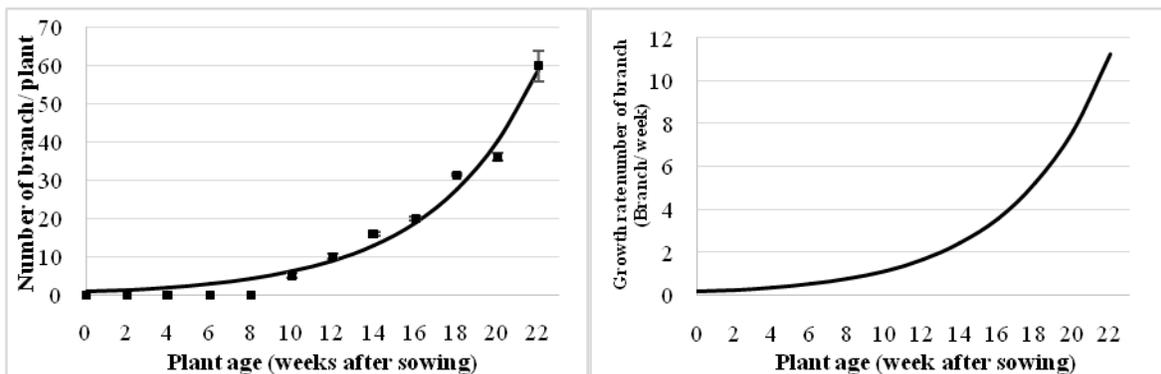


Figure 4. (a) Number of branches as a function of plant age, in the form of $y = Ae^{bx}$ of *A. paniculata*. The square symbol represent means of four replicates \pm SE, $n = 40$ and (b) increment rate of branches number as a function of plant age, in the form of $dy/dx = Abe^{bx}$.

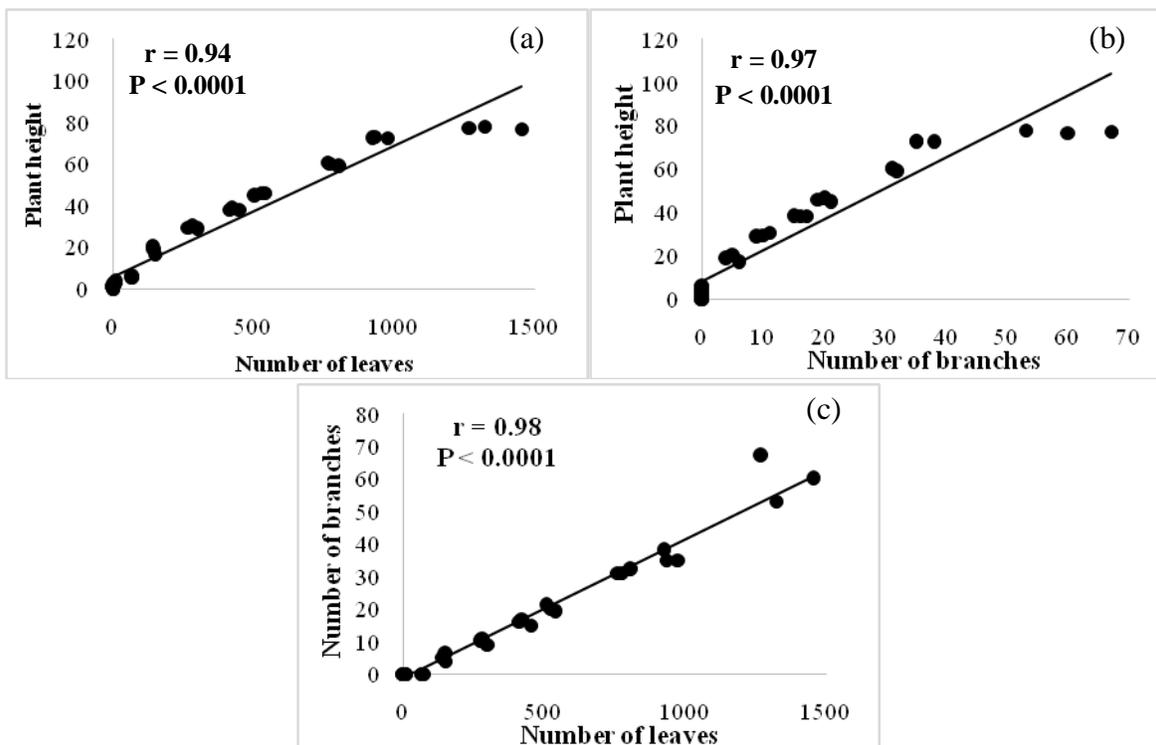


Figure 5. Linear correlations between number of leaves and plant height (a); number of branches and plant height (b); numbers of leaves and number of branches (c) of *A. paniculata* during 22 weeks after sowing. Solid line indicates a significant linear relationship at $P \leq 0.05$. The bullet symbol represent individual replicates, $n = 40$.

Specific leaf area

There was a significant negative quadratic relationship between plant age and specific leaf area (Figure 6). Specific leaf area of *A. paniculata* showed a decreased of 41% when the plants were harvested at 14 to 22 WAS. The specific leaf area decreased as plant age increased, indicating that the plant produced smaller leaves towards seed formation stage compared to the earlier stages of growth. There were strong and negative correlations between specific leaf area and plant height as well as with number of leaves (Figure 7a and b).

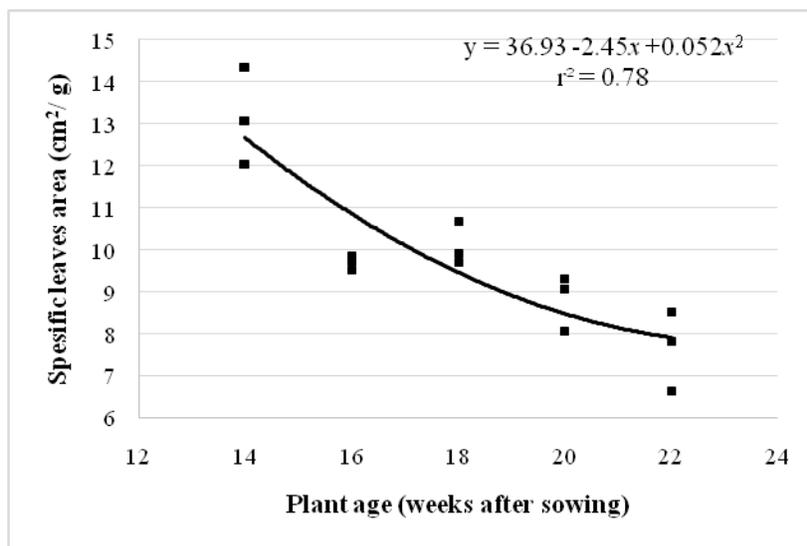


Figure 6. Relationship between plant age and specific leaf area. Solid line indicates a significant quadratic regression trend at $P \leq 0.05$. The square symbol represent individual replicates, $n = 30$.

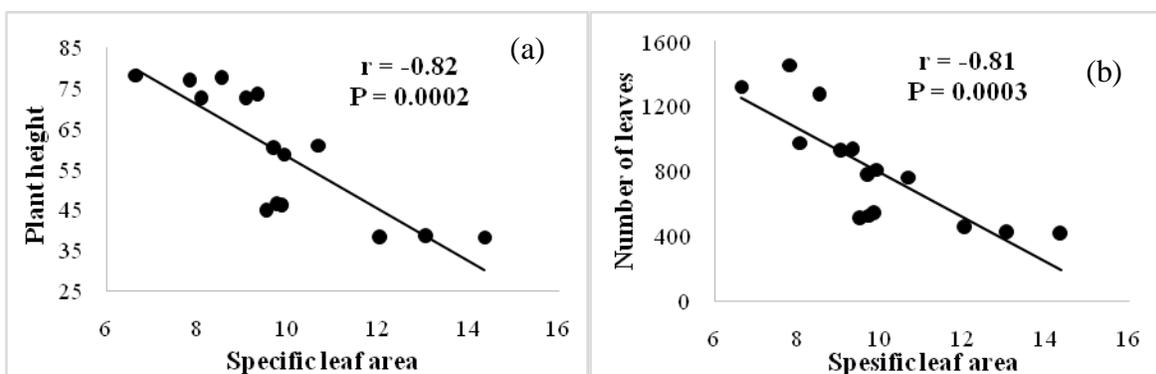


Figure 7. Linear correlations between specific leaf area and plant height (a), and specific leaf area and number of leaves (b) of *A. paniculata* at 22 weeks after sowing. Solid line indicates a significant linear relationship at $P \leq 0.05$. The bullet symbol represent individual replicates, $n = 15$.

According to Hunt et al. (2003), the general pattern of the logarithmic plot of plant growth in the productive environment starts with zero or a negative initial phase of growth, followed by a grand period near to exponential increase. Then, the pattern of plant growth becomes a plateau when the plant reached maturity stage followed by senescence or decay. A study conducted on

growth dynamic of *Celosia cristata* as affected by different growing media also showed a similar pattern of plant height during 42 days of growing duration (Awang et al., 2010). A similar pattern of plant height was achieved by *A. paniculata* when harvested at different harvest age in this current study. As expected, harvest age affected the plant height of *A. paniculata*. Delaying harvesting time indirectly increases the plant height. However, the transport of minerals and water supply upwards becomes more difficult as the height of a plant increases and it also increased risk of breakage or lodging of the stems (Falster and Westoby, 2003).

Kumar and Kumar (2013) reported that the plant height of *A. paniculata* increase by 10% when the plant was harvested from 120 to 170 days after planting. Several studies, such as on *Alpinia zerumbet* (Santos et al., 2012), *Sesamum indicum* L. (Sarkar et al., 2007) and *Hibiscus cannabinus* L. (Webber and Bledsoe, 2002), also reported that the plant height increased as the harvest age increased. An increase in the plant height is the most obvious change in the growth of most plants and plays an important role in order to compete for light. Generally, increased in the plant height is due to the elongation of stem and vascular tissue (Falster and Westoby, 2003; Hunt et al., 2003). Several factors such as planting distance (Kumar and Kumar, 2013; Xiao et al., 2006) and spacing (Islam et al., 2011) also influence the height of plants.

Increased in plant height lead to increase in number of leaves and number of branches. Plant height was strongly and positively correlated with the number of leaves and number of branches (Figure 5a and b). A study done by Kumar and Kumar (2013) reported that the number of branches increased as the harvest age advances. A similar result was obtained by Parashar et al. (2011) who stated that the number of branches and number of leaves were increased by five-fold and ten-fold respectively, when the *A. paniculata* plants were harvested between 30 to 120 days after sowing.

According to Bennett and Leyser (2006), most of the axillary bud will form as lateral branches which lead to the formation of the primary shoots, including the production of leaves, flowers, axillary meristems and branches. A study on the growth of *Cannabis sativa* L. showed that longer cultivation period of the plants resulted in increased plant height and number of axillary buds (Yoshimatsu et al., 2004), indirectly increasing the number of leaves and number of branches. This is in agreement with Yan et al. (2012) who stated that herbaceous plants have more axillary buds per unit plant body size that are available for reproductive units or lateral spread.

Leaf area per unit dry mass or specific leaf area is one of the important traits associated with the growth and survival of the plant (Li et al., 2005). Scheepens et al. (2010) mentioned that specific leaf area also can be an indicator of an eco-physiological characteristics such as relative growth

rate, stress tolerance and leaf longevity. It also reflects the photosynthetic capacities of plant species and characterizes plant adaptation to the environment (Karavin, 2013). The author also reported that there was no significant relationship between plant age of *Quercuscerris* L. var. *cerris* and specific leaf area. Conversely with the results reported by Liu et al. (2008) and Nouvellon et al. (2010), the specific leaf area decreased with the plant age. In the present study, the specific leaf area decreased when the plants were harvested from 14 to 22 WAS.

Advances in plant age will increase the leaf density and leaf thickness, which closely associated with specific leaf area (Poorter et al., 2009). Increase in leaf thickness is due to the increase in the numbers of spongy or palisade mesophyll layers as the plant age advanced. The increases in the leaf density and leaf thickness lead to the increase of leaf dry mass, resulting in a decrease in specific leaf area (Karavin, 2013). Specific leaf area was strongly and negatively correlated with plant height (Figure 7a). Similar relationships have been obtained by England and Attiwill(2005) who found that the specific leaf area was negatively correlated with plant height. The authors stated that increase in plant height results in increase in leaf water stress due to gravity and/or increased path length resistance which indirectly decreased in the specific leaf area.

As discussed earlier, an increment in the plant height resulted in reducing leaf size. The leaf size becomes smaller due to the reduction of turgor pressure and cell volume, which affected the cell formation and cell expansion in the leaves (Woodruff et al., 2004). This leads to the increasing number of leaves since plant continuously produces the small leaves as the plant height increase. According to Digel et al. (2016) the transition from vegetative to reproductive stage was associated with the ontogenetic changes in leaf size and morphology. Reduction in leaf at early flowering stage because of the fate of existing leaf primordia is changed with the transition to flowering initiated by Squamosa Promoter-Binding Protein. Even though there were reduction in the leaf sizes, the leaf mass still increases as the plant harvest age increased, resulting in decreasing specific leaf area (Karavin, 2013). This is in agreement with the results of the current study, whereby the number of leaves was strongly and negatively correlated with the specific leaf area, indicating that increase in number of leaves resulted in a decrease in specific leaf area (Figure 7b).

Yield

Plant fresh and dry weights

Plant fresh weight showed a significant, positive and a cubic relationship with plant age, with 98% ($r^2 = 0.98$) of the variability being due to plant harvest age (Figure 8). The increment of plant fresh weight was slow at the early stage, from 14 to 16 WAS and later increased almost

linearly up to 20 WAS. At 22 WAS or seed formation stage, the increment in the plant fresh weight nearly plateaued, indicating the growth started to decrease due to the senescence process.

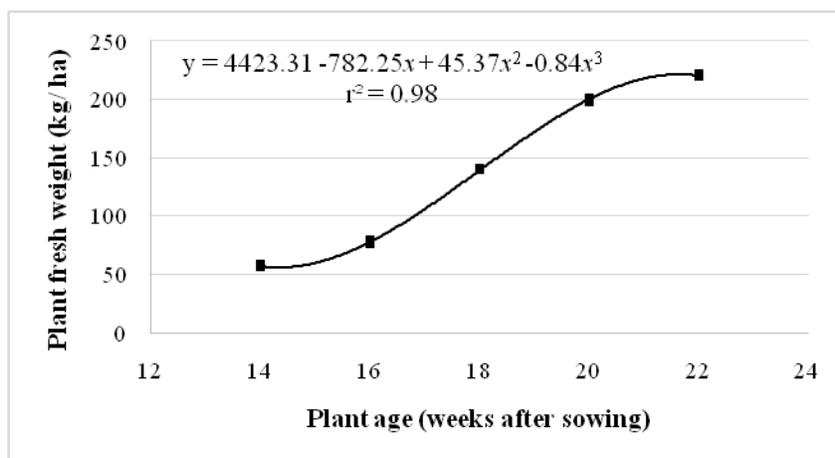


Figure 8. Relationship between plant age (weeks after sowing) and plant fresh weight. Solid line indicates a significant cubic regression trend at $P \leq 0.05$. The square symbol represent individual replicates, $n = 30$.

There was a significant positive quadratic relationship between plant age and plant dry weight (Figure 9). Plant dry weight 4.5 times higher when the plants were harvested from 14 to 22 WAS, indicating the plant dry weight increased as plant age increased. Plant age did influence the plant fresh and dry weights. The plant dry weight increased as the plant age increased, although the plant fresh weight reached a diminishing phase at 20 WAS, where the growth of the plant becomes slower towards 22 WAS or seed formation stage. According to Bhan et al. (2006) the dry herbage yield of *A. paniculata* was twelve-fold increase when the plants were harvested from vegetative to 50% of fruiting phase. Similar results also have been reported by Parashar et al. (2011) who reported that plant fresh and dry weights of *A. paniculata* were minimum at 30 days after sowing and maximum of 120 days after sowing. In contrast, Kumar and Kumar (2013) reported that the dry herbage yields of *A. paniculata* was highest when the plants were harvested at 135 days after planting as compared with the plants harvested at 120, 150 and 170 days after planting.

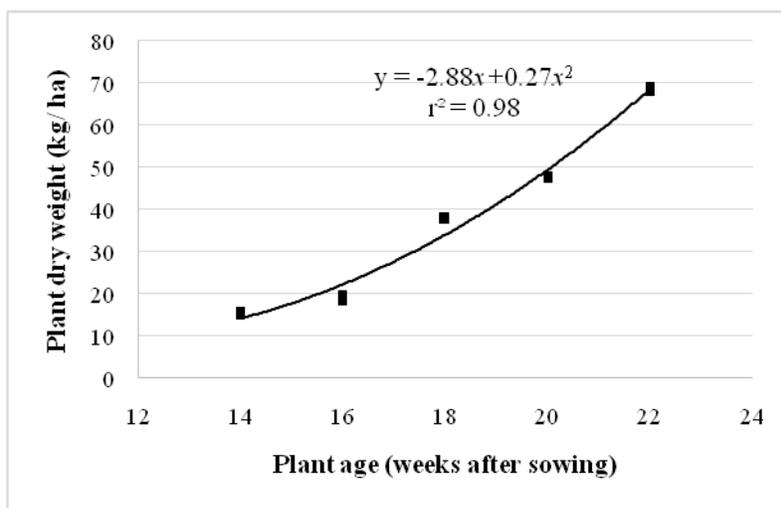


Figure 9. Relationship between plant age (weeks after sowing) and plant dry weight. Solid line indicates a significant quadratic regression trend at $P \leq 0.05$. The square symbols represent individual replicates, $n = 30$.

Goss and Gray(2013) stated that the increase of plant fresh weight was influenced by the water uptake by the plant roots, which was finally being stored in the vacuole. Wink(1993) reported vacuoles contained water and dissolved materials that occupied 80 to 90% of the mature plant cells. This showed that at an advanced plant age, the plant tends to have larger vacuole. The plant fresh weight was strongly correlated with plant dry weight, indicating that increase in plant fresh weight definitely increased the plant dry weight (Table 3). The increasing plant fresh weight and plant dry weight in this current study were due to the increase in the number of leaves and number of stems as the plant age increased. This can be proven by the results of the analysis of Pearson correlation coefficients which showed that the plant fresh and dry weights were strongly correlated with the number of leaves and number of branches (Table 3).

Table 3. Correlation coefficients (r) between plant fresh weight (FW), plant dry weight (DW), number of leaves (NL) and number of stems (NOS) of *A. paniculata* harvested at 14, 16, 18, 20 and 22 weeks after sowing.

	FW	DW	NL	NOS
FW	-	0.97**	0.95**	0.90**
DW		-	0.99**	0.97**

^{ns}, ** = Non-significant at $P > 0.05$ and highly significant at $P \leq 0.01$, respectively, ($n = 15$).

CONCLUSION

A. paniculata plants showed a remarkable growth performance by producing maximum plant height, number of leaves and branches with higher plant fresh and dry weights when harvested at seed formation stage, at 22 WAS. Meanwhile, the maximum height increment rate was at 16 WAS. The maximum increment rate in number of leaves and branches were also at 22 WAS. The correlation coefficients showed that the plant height was strongly correlated with the number of leaves and number of branches. Conversely, specific leaf area decreased as plant harvest age increased, indicating that the plant produced smaller leaves towards senescence stage. The increment in the plant fresh weight nearly plateaued when plant harvested at 22 WAS. Meanwhile, the plant dry weight increase as the plant harvest age increased. In conclusions, *A. paniculata* plant should be harvested at 20 WAS or flowering stage in order to obtain desirable growth and yield. Furthermore, plant entering the senescence stage when harvested at 22 WAS or at seed formation stage.

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