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Effect of Node Positionand Numberof Stem Cuttingon The Growth and Yield of 'katuk' (*Sauropus androgynus* (L.) Merr.)

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Abstract

Katuk (Sauropus androgynus (L.) Merr.) has potential as a vagetable and medicinal plant. This study aimed to determine effect of node position and number of stem cuttings on the growth of katuk. The experiment used Completely Randomized Design with two treatments arranged in split plots. The node position of stem cuttings as the main plot consists of top stem cutting (T, top – 8 nodes under shoot tip), middle stem cutting (M, 8 – 11 nodes under shoot tip) and bottom stem cutting (B, 16-20 nodes under shoot tip). The node number of stem cuttings as a subplot consists of 2 nodes (J1), 3 nodes (J2), 4 nodes (J3), and 5 nodes (J4). Each treatment combination was replicated three times.The results revealed that the top stem cutting resulted in the highest root, total plant dry weight and leaf area, as well stem cutting with 4 nodes produced the highest total plant dry weight and leaf area.

1. INTRODUCTION

Katuk (*Sauropus androgynus* (L.) Merr.) is a vegetable plant which has potential as a medicinal plant. Its leaves can be potentially used for increasing the production of women breast milk after childbirth. Suwanti and Kuswati (2016) reported that the consumption of Katuk's leaves could increase the production breast milk from 53.3 % to 70%. In Malaysia, this plant is traditionally used to cure fever, urinary problems, as well this plant also is consumed as salad, prepared as curry or stir-fried (Bunawan,*et al.*, 2015). It is also known as "multigreen" vegetable due to its perceived superior nutrition and vitamin content incomparison to other vegetables (Singh,*et al.*, 2011). Gireesh *et al.*, (2013) reported that the ethanol and aqueous extracts of leaf or *S. androgynus* contained tannins, saponins, flavonoids, terpenoids, phenolics, steroids, andalkaloids.

As natural wild plant, katuk is commonly propagated by stem cuttings because it is easy to do and cheap. This propagation is influenced by environmental and genetic factors to make the cuttings able to form roots and grow into new plants. Genetic factor affecting survival and rooting of stem cuttings includes the physiology of the stem and the nodes position of the stem taken out as cutting material (Kraiem *et al.*, 2010; Thakur, 2010). Ma *et al*. (2015) reported that nodes position, using as cutting material, significantly affected on development and number of root primordia on stem cuttings of *Ipomoea batatas*. The best stem cuttings for propagation was vary depending upon plants species and position on the stems such as distal stem cuttings was the best for cassava (*Manihot esculenta*) (Stephen and Chikordi, 2015); middle branch on *Jatropha curcas* (Santoso and Parwata, 2014), middle stem on coffee (Rokhani,*et al*., 2014) and *Ficus carica* (Yulistyani, *et al*., 2014), and the top stem cutting on *Alstonia scholaris* (Mashudi and Adinugroho, 2015).

Node number of stem cuttings used as planting materials for propagation need to be considered because it can affect the growth of stem cuttings. Hailemichael, *et al.* (2012) reported that the growth of *Vanilla paniifolia* propagated by stem cuttings with 3-4 nodes was better than those originated from 1 or 2 nodes. In order to obtain high stem cuttings life and plant growth of katuk, it is necessary to study the effect of node position and number of stem cuttings because each plant species may has a different response to propagation by cuttings. Thus, this study aimed to determine the effect of node position and number of stem cuttings on the growth of katuk.

2. MATERIALS AND METHODS

Time and Location

Research was conducted in Purwodadi Botanic Garden from October to December 2017 at an altitude of about 300 m above sea level. Data from Purwodadi Botanic Garden reported that macroclimate in the garden during the study was 18.67 °C (minimum temperature) and 30.97 °C (maximum temperature) in average, rainfall 396 mm per month and relative air humidity 74.45%.

The stem cuttings (6 stem cuttings) were planted as deep as 1 node in upright position into 'katel' media (soil originated from river sedimentation) in each polythene bag (12 cm high. 25 cm diameter). Polythene bags of treatments were arranged in North-South direction while replications were arranged East-West direction. Distances of polythane bags between main plots were 25 cm, 7 cm between replications, and 5 cm between the treatments in subplot. All polythene bags were put into a transparent, curved plastic enclosure with 70 cm high, 100 cm wide and 500 cm long. Shading in experiment location was about 21% with daytime temperatures of 35.2°C, relative air humidity 52.5% inside plastic cove.

Table1. Diameter and lenght of stem cutting materials of *Sauropus androgynus*(L.) Merr.

Treatments	T		M		B	
	Diameter (mm)	Lenght (cm)	Diameter (mm)	Lenght (cm)	Diameter (mm)	Lenght (cm)
J1	2.42	15.00	3.25	6.67	4.42	17.70
J2	2.75	13.90	3.33	11.5	3.58	23.00
J3	3.33	17.50	3.42	12.8	4.00	27.50
J4	3.77	18.80	3.67	23.00	4.33	29.50

Research Methods

This study used Completely Randomized Design with two treatments arranged in split plots. The stem cuttings of katuk was used as plant material propagation originated from cultivated plants in the garden of Purwodadi Botanic Garden. The node position of stem cuttings as the main plot consisted of top (T, top – 8 nodes under shoot tip), middle (M, 8 – 11 nodes) and bottom stem cutting (B, 16-20 nodes). The node number of stem cuttings as a subplot consisted of 2 nodes (J1), 3 nodes (J2), 4 nodes (J3), and 5 nodes (J4). The combination of the treatments was 12 treatments (Figure 1). Diameters and lenghts of stem cutting on each treatment were showed in Table 1. Each treatment was replicated three times, thus in total there were 36 experimental units.

Maintenance of plants included watering which was done once a week or depended on soil condition in polythene bags. All weeds which were growing around plants and insects attacking on the plants were removed.

Variables were obseved on branchlet number, living of cuttings, leaf area and plant dry weight (root, stem, leaf, total). Plant dry weight, leaf area and stem cutting life were observed at the end of observation. The number of branchlets observed after six days after planting(DAP) until 28 DAP. Measurement of plant dry weight was carried out after the plants was putinto oven at 80°C for 3 x 24 hours until the dry weight was constant. Measurement of leaf area used punch method (Hamoda,*et al*., 2016.Solikin, 2017) with the formula (modified):

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

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 (cm^2)

Data analysis

Data were analyzed by anova using MINITAB 18 program. Mean values of the treatments were tested using the Least Significant Different (LSD) test at α = 5% to know differences between the treatments.

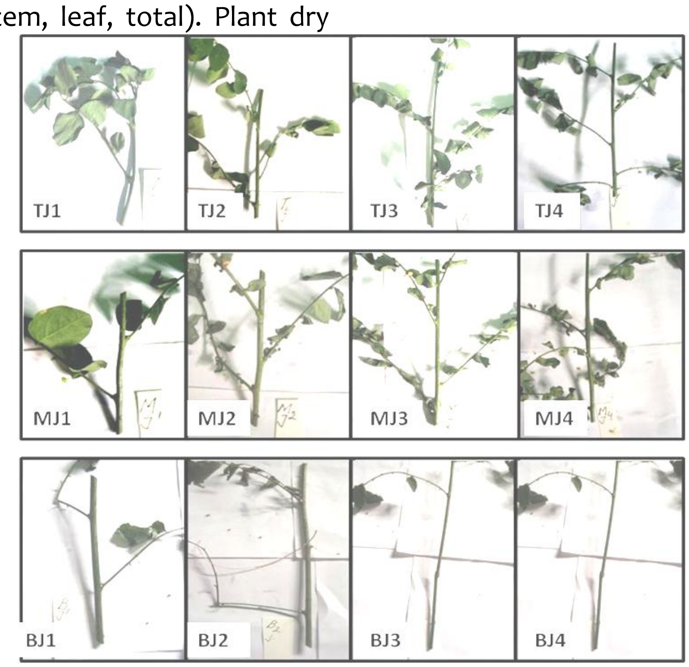

Figure 2. Stem cuttings material of *Sauropus androgynus* (L.) Merr: Top stem cutting and 2 nodes (TJ1), top stem cutting and 3 nodes (TJ2), top stem cutting and 4 nodes (TJ3), top stem

cutting and 5 nodes (TJ4); Middle stem cutting and 2 nodes (MJ1), middle stem cutting and 3 nodes (MJ2), middle stem cutting and 4 nodes (MJ3), middle stem cutting and 5 nodes (MJ4); bottom stem cutting and 2 nodes (BJ1); bottom stem cutting and 3 nodes (BJ2), bottom stem cutting and 4 nodes (BJ3), bottom stem cutting and 4 nodes (BJ4).

3. Results

The results showed that there were no significant interaction between the node position and number of stem cuttings treatments to all observed variables (Table 2).The treatment of node position of stem cuttingsaffected significantly on all observed variables, except branchlet number and cutting life (Table 2). Whereas the treatment of node number affected significantly on all observed variables, except the branchletnumber and root dry weight (Table 2)

Table 2. Effect of position and number of node on the growth of 'katuk' (*Sauropus androgynus* (L.) Merr.)

Variable	Node position (P)		Node number (J)		(PxJ)	
	P- value	Note	P- value	Note	P- value	Note
Branchlet number	0.164	NS	0.000	S	0.115	NS
Cutting life	1.000	NS	0.541	NS	1.000	NS
Leaf area	0.000	S	0.013	S	0.894	NS
Leaf dry weight	0.000	S	0.013	S	0.894	NS
Stem dry weight (stem +petiole)	0.000	S	0.002	S	0.270	NS
Root dry weight	0.000	S	0.145	NS	0.153	NS
Total plant dry weight	0.000	S	0.007	S	0.473	NS

Note : S=significant and NS= not significant on LSD test at α=5%.

Stem cuttings originating from the top position produced the highest plant growth compared to the cuttings originating from the middle and bottom of stem cuttings. Table 3showed that cuttings originating from the top position produced the highest total dry weight and plant leaf area compared to plants derived from middle and bottom stem cuttings with total dry weight of 5.94 g per plant and leaf area of 1199 cm2 per plant. In contrast, plant growth derived from the bottom stem cuttings was the lowest total plant dry weight and leaf area, i.e. 281 g per plant and 505 cm2 per plantrescpectively.

Stem cuttings with 4 nodes (J3) produced the highest total plant dry weight and leaf area among the other treatments of the node number, although statistically no different from the treatment of 5 nodes cuttings (J4), with the total plant dry weight of 4.92 g per plant and leaf area of 881.48 cm2 per plant (Table 4). In contrast, the 3 nodes cuttings treatment (J2) produced the lowest plant growth with total dry weight and leaf area of 3.54 g per plant and 520.37 cm2 per plant respectively (Table 4).

Table 3. The growth and cutting life of katuk (Sauropus androgynus (L.) Merr.) on node position of stem cutting treatment

Dry weight (g per plant)													
Leaf		Stem		Root		Total		Leaf area		Branchlet number		Cutting life (%)	
3.03	c	1.99	c	0.93	c	5.95	c	1199	c	1.25	a	100	a
2.18	b	1.48	b	0.59	b	4.25	b	862	b	1.36	a	97.20	a
1.28	a	1.11	a	0.43	a	2.81	a	505	a	1.43	a	100	a

Note: Numbers which was followed by same letters in the same coloum were not significant different in LSD test at α=5%.

Table 4. The growth and cutting life of 'katuk' (Sauropus androgynus (L.) Merr.) on node number of stem cutting treatment

Treatments of node number	Dry weight (g per plant)								Branchlet number		Cutting life (%)		Leaf area (cm^2 per plant)	
	Leaf		Stem		Root		Total							
J1	1.13	a	1.33	a	0.66	a	3.95	a	1.02	a	98.10	a	563.96	a
J2	1.04	a	1.27	a	0.51	a	3.54	a	1.09	a	98.10	a	520.37	a
J3	1.76	b	1.78	b	0.70	a	4.92	b	1.57	b	100	a	881.48	b
J4	1.64	b	1.73	b	0.72	a	4.91	b	1.7	b	100	a	818.52	b

Note: Numbers which was followed by same letters in the same coloum were not significant different in LSD test at α=5%

4. DISCUSSION

Living of stem cutting

Living of stem cuttings isaffected by the ability of the cuttings to formroots and to grow as a new plant.The effect of node position and number treatmentswas notsignificanton stem cutting life. Interaction effect of these treatments also was not significant. However,there was a trend that the treatment ofstem cutting with 4 nodes (J3) and 5 nodes (J4) had higher stem cutting life than those of stem cutting with 2 nodes (J1) and 3 nodes (J2). Table 2 showed that the living stem cutting on the treatments of J3and J4 reached 100%, whereas on J1 and J2 were 98.10 %. It may be caused by the treatments of J3 and J4 had longer cuttings than those on J1 and J2 (Table 1) so that they may contain more water,nutrient and nodesthan the treatment of J1 andJ2. The living stem cutting on the middle stem cutting showed lower than the top or bottom stem cuttings (Table 3). This was caused attacking plant pest to the base of some stem cuttings during experiment so the living of cuttings decreased.

Plant growth

Plant growth from the stem cuttings related to the leaf area which has an important role to plant photosynthesis for growing of plant cells, tissues and organs.Figure 2 showed that there was relationship between leaf area and plant growth (plant dry weight) of katuk which increasing of leaf area followed by increasing plant dry weight with equation $y = 2.948\ln(x) - 15.10$; $R^2 = 0.875$. It was also showed in Table 3 and Table 4 that the larger the leaf area, the higher the plant dry weight produced by plant such as the dry weight of leaf, stem and root. Enyi (2008) also proved that large leaf area can increase the growth of leaves and stems on D. esculenta. Solikin (2017) reported that increasing leaf areaon Dioscorea sansibarensis Pax from1521 to 9609 cm2 per plant followed by increasing leaf dry weight from 3.80 to 6.63 g per plant and root dry weight from 24.02 gto 21.31 g per plant.

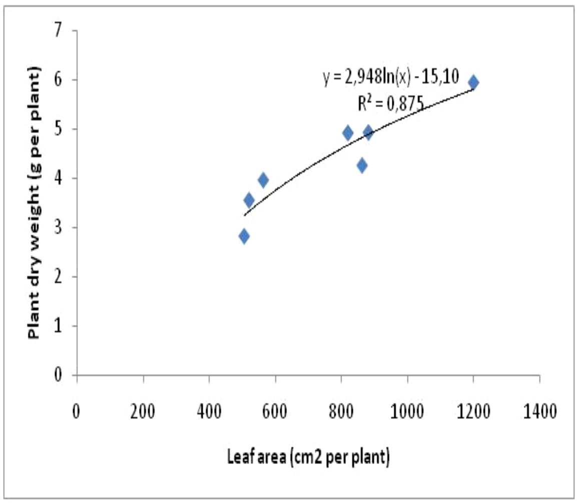

Figure 2. Relationship between node number and branchlet number on node number and position treatments of katuk' (*Sauropus androgynus* (L.) Merr.).

Effecf of node position on the plant growth

Node position did not affect significantly on branchlet number. The branchlet number on the top stem cutting tended to be fewer than those on the bottom and middle stem cutting (Table 4). This may be caused by apical growth on the top cutting more dominant than those on the bottom or midle cutting so the rowing of axillary buds on the top cutting were inhibited by the growing of bud on the shoot tip.The top stem cutting which was used as material propagation containing more younger leaves and they still alive during the observation than those on the middle and bottom stem cutting (Figure 1).

The top stem cutting contains more juvenile tissues, which were actively differentiating compared to the bottom and middle positions (Tchoundjeu and Leakey, 2001) for adventitious roots growing so it produced more roots than the middle and bottom position. These will affect on plant leaf area which has an important role to plant phosynthesis on supplying carbohydrates for the plant growth at the first time and during plants growing. Table 3 showed that the top stem cutting produced the highest leaf, root, stem, total plant dry weight i.e. 3.03 g, 0.93 g, 1.99 g and 5.95 g per plant respectively. In contrast, the bottom stem cutting produced the lowest leaf, root, stem, total plant dry weight, i.e. 1.28 g, 0.43 g, 1.11 g and 2.81 g per plant respectively. Safeer et al. (2013) also proved on Plectranthus vettiveroides that plant leaf area, root weight and shoot weight on top stem cuttings were higher than those on middle and bottom stem cutting. Kassahun and Mekonnen (2012) also reported that the growth of top stem cutting on Stevia rebudiana Bertania was higher than those on middle and bottom stem cuttings.

Thickening of schlerenchymatous cells which the point of origin of adventitious roots on older cells on the bottom stem cuttings may caused decreasing ofroots growth as reported by Darus (1989) on the stem cutting of Acacia mangium. Hartman,et al. (2002) stated that the regeneration of stem tissue declined following with age of stem tissue. It may caused the roots and plant growth on the bottom stem cutting of katuk lower than those on the top stem cuttings as showed in Figure 3. It was also proved by Aini,etal. (2010) on Gonystilus bancanus that the cells activity decreased and mortality increased followed increasing the cuttings age.

Plant growth regulator such as auxin content in the stem cutting is also important to roots and plant growth of stem cuttings. It was produced on meristematic tissue at the leaf buds and the tip of the stem and it distributed to the organs underneath it through the phloem. Thus its content in the top stem cuttings was higher than those in bottom stem cutting. It may caused the roots and plant growth on the top stem cutting of katuk higher than those on the bottom stem cuttings as showed in Table 3. It was agree with studying by Solikin (2018) on stem cutting propagation on Andrographis paniculata (Burm,f.) ex Nees that top node position produced higher plant growth than those on middle and bottom stem cutting.

Table 3 showed that the bottom stem cutting produced the least root dry weight, i.e. 0.43 g per plant and the other handthe top stem cutting produced the highest root dry weight (0.93 g per plant).The top stem cutting mayhad more meristimatic tissue and auxin than those on the bottom stem cuttings so it had potential to grow faster than the middle and bottom stem cuttings which had older cells.It was also proved on stevia (Stevia rebaudiana) (Yusmaini, 2009) andPlechtranthus amboinicus (Apriani and Suhartanto, 2015).

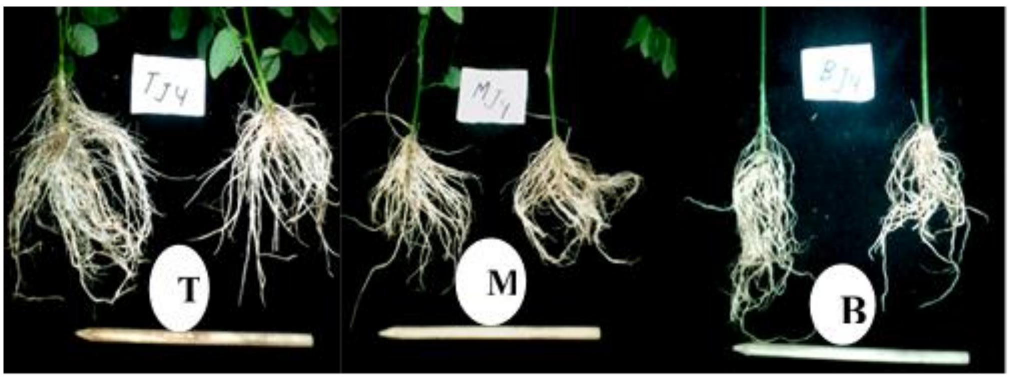

Figure 3. Roots growth of four nodes stem cuttings on top (T), middle (M) and bottom (B) stem position on katuk (*Sauropusandrogynus* (L.) Merr

Effect of node number of stem cutting on the plant growth

The treatment of node number of stem cuttings affected significantly on plant growth and branchlet number (Table 2). Tulaini (2014) also reported that the node number of stem cuttings influenced significantly on the growth of katuk. The treatment of stem cuttings with 4 nodes (J3) produced the highest plant growth amongst the other treatments with leaf dry, stem and total plant dry weight of 1.76, 1.78 and 4.92 g per plant, respectively, although it was not differ significantly from J4. Rahmania and Kurniawati (2014) also reported that the growth of Orthosiphon aristatus Bl. Miq on 4 and 5 nodes of stem cuttings were higher than those on 2 and 3 nodes stem cuttings.This wascaused by the highest leaf area produced by this treatment (881.48 cm2 per plant). On the other hand, treatment of J2 having the lowest leaf area (520.37cm2 per plant) produced the lowest plant growth with total plant dry weight of 3.54 g per plant (Table 4).

The treatment of J3 and J4 produced higher branchlet number than those on J1 and J2 i.e. 1.51 and 1.70 branchlet per plant respectively (Table 2). Rahmania and Kurniawati (2014) proved on Orthosiphon aristatus Bl. Miq that 4-5 nodes of stem cuttings produced branchlet more than those on 1-3 nodes of stem cuttings. These may be caused by the treatment of J3 and J4 longer and had more axillary buds on the stem cutting than those on J1 or J2 (Table 1).Figure 4 showed that increasing of node number on the stem cutting from 2 nodes (J1) to 5 nodes (J4) followed by increasing of branchlet number with equation $Y=0.252x +0.715$; $R2= 0.914$. The growth of lateral branchlet was also efforted by the growing of roots and leaf area at these treatments such as showed in Table 2 that the root dry weight and leaf area of J3 was the highest between other treatments i.e. 0.70 g per plant and 881.48 cm2 per plant, respectively. On the other hand, the treatment of J2 had root dry weight and leaf area i.e. 0.51 g per plant and 529.37 cm2 per plant, respectively. Naidu and Jones (2009) reported on Eucalyptus that longer stem cuttings (10-13 cm) produced higher root dry weight, stem dry weight and leaf number and than the shorter cuttings of 5-8 cm long.

Cuttings with 2-4 nodesoriginated from the top, middle and bottom of the stem cutting began to sprout at the age of 6 DAP and reach the highest at age of 14 DAP. The number of branchlets that formed not all grow and develop into branches. It was caused by the apical dominance of shoot growth so that only the top buds (1-3 buds) could grow and develop well. Figure 4 showed that high branchlet number were reached at 14 DAP, then decreased and constant at about 19 DAP. This showed that the competition of nutrients, light, water and photosynthate in plants was low before 14 DAP, so that the buds that were present at the leaf's axilscould grow and developed. However, after this phase, competition between the branchlets on nutrients, light and photosynthatecontinued to increase so that only 1-3 upper branchlets could grow and develop well, as showedin Figure 4 that the average number of branchlet on each cuttings that can develop and grow well ranged from 1-3 branchletsat 28 DAP.

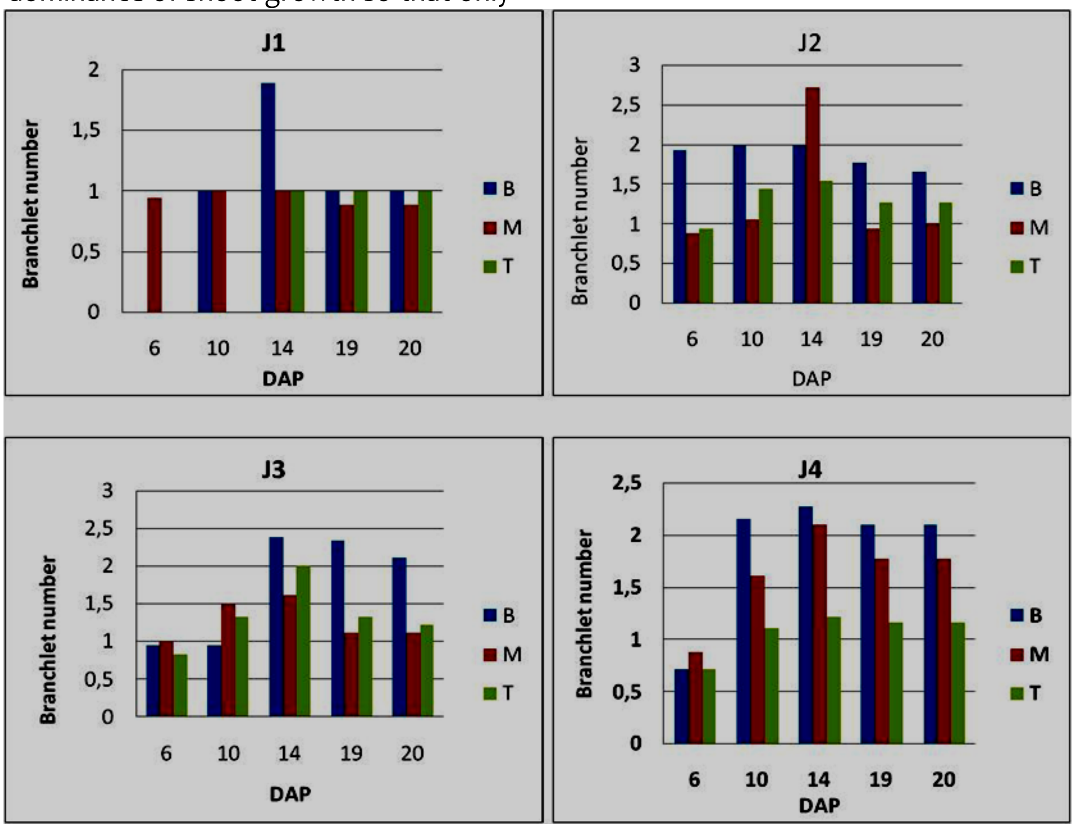

Figure 4. Branchlet number on node position and number of stem cutting treatments on katuk (*Sauropus androgynus* (L.) Merr.) : J1= 2 nodes, J2=3 nodes, J3= 4 nodes, J4= 5 nodes, B=bottom, M=middle, T=top

5. Conclusion

The top stem cutting was the the best material for stem cutting which produced the highest root, total plant dry weight and leaf area i.e 0.93 g, 5.94 g and 1199 cm2 per plant, respectively. Stem cutting with 4 nodes produced the highest total plant dry weight and leaf area , i.e. 4.92 g and 881.48 cm2 per plant.

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