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Short Communication

Evaluation of auxin and cytokinin use for vegetative propagation of *Asystasia gangetica* for forage production

Evaluación del uso de auxinas y citoquininas para la propagación vegetativa de Asystasia gangetica *para la producción de forraje*

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Abstract

The aim of the experiment was to determine the effects of auxin and cytokinin application on vegetative propagation of *Asystasia gangetica* for forage production. Stem cuttings were treated with 9 different hormone levels; control (without hormone), immersion of ends of cuttings in 50, 100, 150, and 200 ppm solutions of auxin (indole 3-acetic acid) and immersion of ends of cuttings in 50, 100, 150, and 200 ppm solutions of cytokinin (benzyl amino purine) for 15 minutes, followed by planting in plastic trays. After 21 days, cuttings were transplanted into soil in polybags in the greenhouse. Forage was harvested 50 days after transplanting to determine yield and quality. The results showed that hormones affected plant height, leaf number, primary branch number, tertiary branch number, yield and nutritional value. It can be concluded that plant hormones can be used for vegetative propagation of *A. gangetica* as forage.

Keywords: Benzyl amino purine, forage, indole 3-acetic acid, plant growth.

Resumen

El objetivo del experimento fue determinar los efectos de la aplicación de auxinas y citoquininas sobre la propagación vegetativa de *Asystasia gangetica* para la producción de forraje. Los esquejes de tallo se trataron con 9 niveles hormonales diferentes; control (sin hormona), inmersión de extremos de esquejes en soluciones de auxina de 50, 100, 150 y 200 ppm (ácido indol 3-acético) e inmersión de extremos de esquejes en soluciones de citoquinina (bencil amino purina) de 50, 100, 150 y 200 ppm durante 15 minutos, seguido de siembra en bandejas de plástico. Después de 21 días, los esquejes se trasplantaron al suelo en bolsas de plástico en el invernadero. El forraje se cosechó 50 días después del trasplante para determinar el rendimiento y la calidad. Los resultados mostraron que las hormonas afectaron la altura de la planta, el número de hojas, el número de ramas primarias, el número de ramas terciarias, el rendimiento y el valor nutricional. Se puede concluir que las hormonas vegetales se pueden utilizar para la propagación vegetativa de *A. gangetica* como forrajera.

Palabras clave: Ácido indol 3-acético, bencil amino purina, crecimiento vegetal, forraje.

Introduction

Asystasia gangetica (L.) T. Anderson is one of the potential herbaceous plants for forage use in integrated farming systems under citrus plantations (Adjorlolo et al. 2014), palm plantations (Ramdani et al. 2017) or other horticultural trees (Junedi 2014) due to its high adaptation to shade (Kumalasari et al. 2019). *A. gangetica* is a good biomass source and nutrient supply for goats, with good levels of crude protein and fiber (Kumalasari et al. 2020a). *A. gangetica* is native to Indonesia and commonly harvested from plants growing wild in plantation areas (Kumalasari et al. 2020b). There is limited research on its cultivation and use of seed is

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limited by the low germination rate (Kumalasari et al. 2018). Therefore, use of vegetative cuttings is one of the alternatives to increase availability of planting material for its cultivation as forage.

Shoot biomass is important for forage plants. Research on hormone use to enhance shoot biomass production (Zaman et al. 2015) found that plant hormone application with biostimulators and fertilizer could increase dry matter yield for perennial ryegrass. Phytohormones have influence on the leaf growth, flower and fruit development (Iqbal et al. 2017), plant senescence (Khan et al. 2014), decomposition and nutrient recycling (Guiboileau et al. 2010). Hormones affect enzyme activity and stimulate cambium activity, providing the resulting vascular tissue formation with a better supply of photosynthetic products, and increase immunity to stress, such as water stress in wheat (Aldesuquy 2000).

Cytokinin and auxin are the two major plant growth hormones that control growth and development in plants (Zhao 2008; Willige et al. 2011). In Indonesia, both hormones are commonly available and applied in the horticultural industry. Cytokinin application stimulates the biosynthesis of ethylene in tissues and nodulation in legumes (Lorteau et al. 2001), while auxin has been shown to contribute to partial restoration of the decrease in the photosynthesis effect in mustard plants (Khan et al. 2002). Auxin also stimulated root development in alfalfa stem cuttings (Ghotbi et al. 2018) and increased chicory root biomass (Nandagopal and Kumari 2004). This study investigated the effects of auxin and cytokinin application for vegetative propagation of stem cuttings, survival, shoot development and forage production and quality of *A. gangetica*.

Material and Methods

The research was conducted in a greenhouse at the Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University (IPB), from September to December 2018 (rainy season). Forage quality was analyzed at the Laboratory of the University Center (PAU) IPB.

Cuttings were collected from mature *A. gangetica* plants, from the Field Laboratory of IPB 6° 33' 36.72" S Latitude; 106° 43' 32.2248" E Longitude; 179 masl altitude. Selected mature plants growing in natural conditions and flowering and fruiting were used to collect 20cm long shoot cuttings with 2 nodes from the plant crown using pruning scissors. Cuttings were placed in perforated plastic bags immediately after collection for transfer to the greenhouse.

The cut ends of the shoots were immersed for 15 mins in solutions of auxin or cytokinin at 4 levels: 100 cuttings were used for the control (without hormone) and 50 cuttings each were used for immersion in 50, 100, 150, and 200 ppm auxin solutions and 50, 100, 150, and 200 ppm cytokinin solutions. The research used indole 3-acetic acid (IAA) as auxin hormone and benzyl amino purine (BAP) as cytokinin. After immersion for 15 minutes, the stem cuttings were removed and planted in soil in plastic trays (40 cm width × 50 cm length × 15 cm height) arranged in a randomized complete block design. The cuttings were maintained for 21 days in the nursery until the 4-leaf stage, then each plant was transplanted to soil in a 5 kg polybag. The soil in the polybags was fertilized 2 weeks before transplanting with organic fertilizer (cattle manure) at the rate of 250 g manure/polybag (Kumalasari et al. 2020a) and Mutiara inorganic fertilizers (16% N, 16% P_2O_5, 16%K_2O) at the rate of 0.25 g fertilizer/polybag.

The stem cuttings were assessed for the following parameters 30 days after transplanting to the polybags:

- Percentage survival - number of living plants per total number of cuttings planted per treatment;
- Leaf number - number of new leaves formed per cutting;
- Plant height - measured from the base of the plant to the tip of the canopy (cm);
- Number of branches – number of primary, secondary and tertiary branches per cutting.

Plants were harvested 50 days after transplanting by cutting all plants from each treatment approximately 5 cm above soil level followed by direct weighing to determine the fresh weight. Each plant was separated into branches, leaves and young leaves, and each fraction weighed. The fractions were then air-dried outdoors for 2 days and re-weighed to obtain the dry matter yield.

Chemical Analysis

All air-dried plant samples were dried in a forced-air oven at 60 °C for 48 h, and ground to pass through a 1 mm sieve for chemical analyses. The dry matter, crude protein, crude fat, crude fiber and ash contents were determined according to the AOAC procedure (AOAC 2005) The organic and dry matter digestibility were determined by two-stage in-vitro technique (Tilley and Terry 1963).

Statistical Analysis

Data were analyzed statistically with R i386 3.6.1 using Analysis of Variance Test (ANOVA) and the Least Significant Difference Test (LSD).

Results

Survival of cuttings

The cuttings showed variation in stem weight, stem length and number of new leaves with hormone concentration (P<0.001). Hormone type and concentration had significant effects on survival percentage and number of new leaves (P<0.001). The survival percentage of the *A. gangetica* cuttings ranged from 62 to 98% at 30 days after planting. The lowest survival was recorded for cuttings treated with cytokinin while the highest survival was obtained following auxin treatment of 50 ppm (Table 1). The trend was reversed for leaf development with the number of new leaves increased by cytokinin treatment.

Plant growth traits

Hormone treatment significantly affected plant height, total number of leaves, primary branches and tertiary branches (Table 2). Cytokinin application decreased plant height but increased the number of primary branches, while auxin application increased the number of tertiary branches and leaves.

Biomass

Fresh yield of leaf and stem weight were affected by different levels of hormone application (Table 3). Cytokinin application decreased plant biomass while auxin increased biomass.

Table 1. Effect of hormone levels during propagation of stem cuttings of *A. gangetica*.

Treatments	n	Mean stem weight (g/plant)	Mean stem length (cm)	Mean survival percentage (%)	Mean number of new leaves/plant
Control	100	2.36	17.40	77	13.05
A50	50	2.51	17.73	98	6.8
A100	50	2.46	20.43	92	6.4
A150	50	2.48	21.05	92	9.2
A200	50	2.49	20.93	90	9.6
C50	50	2.52	21.22	72	13.4
C100	50	2.49	21.51	64	17.5
C150	50	3.60	21.85	70	14.5
C200	50	3.11	22.68	62	10.6
SEM		0.15	0.86	4.26	1.40
P		<0.001	<0.001	<0.001	<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm.

Table 2. Effect of hormone levels on plant growth traits during propagation of stem cuttings of *A. gangetica*.

Treatments	Mean plant height (cm)	Mean leaf number	Number of primary branches/plant	Number of secondary branches/plant	Number of tertiary branches/plant
Control	111.3a	491.1ab	3.8abc	21.3	19.3abcd
A50	109.3a	465.4abc	2.5c	21.2	23.3abc
A100	113.0a	569.4a	2.6bc	21.4	30.8a
A150	120.4a	551.4a	2.9bc	26.4	29.4ab
A200	109.0a	589.3a	2.7bc	23.1	30.4a
C50	83.7b	364.6bc	5.0a	23.8	9.1d
C100	86.0b	375.2bc	5.6a	24.1	12.6cd
C150	78.7b	340.3c	4.5ab	24.0	19.2abcd
C200	81.0b	335.1c	5.4a	23.6	17.3bcd
SEM	0.69	0.45	3.72	0.04	0.22
P	<0.001	<0.001	<0.001		<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Nutritional value

Immersion in increasing levels of cytokinin was associated with an increase of dry matter percentage and ash content but had no significant effect on protein or fiber (Table 4) nor on mineral concentration or digestibility (Table 5).

Table 3. Effect of hormone levels on plant biomass during propagation of stem cuttings of *A. gangetica*.

Treatments	Leaf weight (g/plant)	Stem weight (g/plant)	Total weight (g/plant)
Control	73.8ab	102.6a	176.4ab
A50	88.7a	129.2a	217.9a
A100	100.5a	145.9a	246.4a
A150	91.0a	135.6a	226.6a
A200	97.2a	139.8a	237.0a
C50	46.7bc	55.3b	102.0b
C100	42.4c	55.7b	98.1c
C150	39.5c	50.1b	89.6c
C200	40.9c	50.6b	91.5c
SEM	0.13	0.17	0.14
P	<0.001	<0.001	<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Table 4. Effect of hormone levels on nutrient content of rooted stem cuttings of *A. gangetica*.

Treatments	Dry matter	Ash	Crude fat	Crude protein	Crude fiber
Control	13.11ab	13.11ab	1.78	9.96	25.48
A50	12.02b	15.46a	1.89	10.03	25.87
A100	12.13b	13.65a	1.76	10.74	25.24
A150	12.08b	15.77a	2.24	9.34	27.76
A200	12.11b	14.72a	1.56	9.50	25.29
C50	14.32a	11.96b	2.03	11.75	24.21
C100	14.31a	11.41b	1.83	11.45	24.32
C150	13.85a	12.21b	1.99	11.67	24.19
C200	13.67a	12.85b	3.05	12.53	23.15
SEM	0.04	0.04	0.02	0.09	0.09
P	<0.001	<0.001			

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Table 5. Effect of hormones on forage macro mineral concentration and digestibility of stem cuttings of *A. gangetica*.

Parameters	auxin	cytokinin	SEM
Ca (g/kg)	5.72	4.91	0.09
P (g/kg)	3.34	3.53	0.03
Mg (%)	1.45	1.30	0.26
DMD (%)	59.33	59.09	4.09
OMD (%)	57.15	57.36	4.72

DMD = dry matter digestibility; OMD = organic matter digestibility.

Discussion

This research has shown that plant growth hormone type and concentration are important factors in vegetative propagation of *A. gangetica* by cuttings. Vegetative propagation has the potential to be scaled up as the survival percentage of *A. gangetica* stem cuttings was higher than survival from seed propagation (Kumalasari et al. 2018). Cytokinin hormone treatment had no significant effect on shoot number using stem cuttings of *A. gangetica*, implying that vegetative propagation is also possible without the use of growth hormones as reported for *Jatropha curcas* by Adekola and Akpan (2012).

Auxin treatment increased *A. gangetica* plant height, number of leaves and number of tertiary branches. Rastogi et al. (2013) also reported that *Linum usitatissimum* plant height was maximized after auxin application. Auxin has been reported to increase production of *Onobrychis viciifolia* (Avci et al. 2010) with implications on nutrient and water absorption from the soil (Koç and Acar 2015). These results are consistent with Latef et al. (2021) who reported that auxin application at 150–200 ppm increased *Vicia faba* plant yield in salt conditions. However, Ferguson and Beveridge (2009) reported that auxin application inhibited bud growth on primary branches and affects *Pisum sativum* shoot development. Gaveliene et al. (2005) reported that auxin application stimulated monosaccharide accumulation in plants and affected *Brassica napus* biomass yield.

This research shows that stem cuttings can be used for large scale propagation of *A. gangetica*. Use of growth hormones could stimulate propagation, maintain forage production and also enrich nutrient and mineral content. Application of this vegetative technique would allow rapid multiplication of material in breeding programs and preservation of agriculturally valuable characteristics of selected materials.

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(Note of the editors: All hyperlinks were verified 5 May 2022).

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