Kinetin and Calcium Pantothenate Effects on Shoot Multiplication in *In Vitro* Cultured Cassava Var. Adira 2 and Adira 4

Nurul Khumaida [a,*], Sintho Wahyuning Ardie [a], Candra Catur Nugroho [a], Suwarto [a]

[a] *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
** Corresponding author: Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University,*
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia.
Tel.: +62 251 862 9353; fax: +62 251 862 9353.
nkhumaida@yahoo.com

Abstract

Cassava (*Manihot esculenta* Crantz.) is one of largest carbohydrate sources in the world which also potentially developed as the source of bio-energy and raw materials for severals industries. As the demand for cassava is increasing, it is very important to supply the true-to-type cassava seedling continuously. *In vitro* propagation is one advanced technique that can be applied to meet the increasing demand of cassava seedling. The objective of the research was to study the effect of kinetin and Calcium Pantothenate (CaP) concentration on shoot multiplication of *in vitro* cultured cassava var. Adira 2 and Adira 4. This research was conducted in a completely randomized design with two factors. The first factor was kinetin concentration (0; 1; 1.5; and 3 ppm) and the second factor was concentration of CaP (0; 1; and 2 ppm) used in combination. The results showed that MS medium supplemented with 1 ppm CaP and 1.5 ppm kinetin promoted the growth of cassava explants of Adira 2 variety until 4 Weeks After Treatment (WAT). However, for longer culture period MS medium containing 3 ppm of BAP was better to support the explants growth. MS Medium containing 1 ppm of CaP and 3 ppm of kinetin promoted the growth of Adira 4 variety until 4 WAT. However, for longer culture period MS medium containing 2 ppm of CaP and 3 ppm of kinetin was better to support the explants growth. The highest shoot multiplication rate for 20 weeks old plantlets of Adira 2 and Adira 4 varieties was obtained at MS medium containing 3 ppm of BAP.

Keywords: calcium pantothenate, cassava, kinetin, shoot multiplication

Introduction

Cassava (*Manihot esculenta* Crantz) is the third largest source of carbohydrate for human consumption in the world. It is the principal carbohydrate source for more than 500 million people in the tropical world. Cassava plays a famine prevention role wherever it is cultivated widely. Cassava has adaptability to a range of climatic and edaphic conditions including tolerance to drought, to some pests and diseases relative to other crops, and confers a comparative advantage on cassava under conditions of famine against alternative crops.

More recently, cassava has gained importance as a possible fuel commodity not only in Indonesia but also in the Philippines, China, Thailand, and other countries which have more advanced national bio-fuel programs. In situations where water availability is limited (i.e. not enough for the cultivation of sugar cane), cassava is the preferred feedstock for ethanol production. However, cassava-based industries are facing the main problem of cassava availability in amount and continuity (Suryana, 2009). Therefore, in order to meet the large demand of cassava, farmers need large amount of good quality cassava seedling in a relaitvely short time.

In general, cassava is propagated by stem cutting. Eventhough stem cutting is easy to be done and relatively cheap, it is not always available when needed and it is difficult to assure to have the true to type seedling. *In vitro* technique is one method for mass propagation can be applied for rapid mass propagation of cassava seedlings. Research in cassava shoot multiplication and the induction of embryogenic callus had been done recently (Guohua, 1998; Sudarmonowati *et al*.,

2002; Onuoch and Onwubiku, 2007). Previous results showed that cassava 'Mentega' formed the highest shoot formation when cultured in MS medium containing 2 ppm of BAP (Sudarmonowati *et al*., 2002). In other varieties, Fauzi (2010) found that standard MS medium containing 1.5 ppm of BAP was the most effective medium for shoot multiplication. The objective of this research was to study the effect of Kinetin and Calcium panthothenate (Ca-P) addition in the shoot multiplication of Adira 2 and Adira 4 cassava varieties.

Materials and Methods

The research was conducted from January 2010 to January 2011 at Plant Biotechnology Laboratory, Department of Agronomy and Horticulture, Agriculture Faculty, Bogor Agricultural University. This research was consisted of two separated experiments differed in the cassava variety used as explants (Adira 2 and Adira 4 varieties). The experiment was arranged in Randomized Block Design with two factors and ten replicates. The two factors were kinetin and Calcium panthothenate (Ca-P) concentrations. There were four levels of kinetin concentrations (0; 1; 1.5; and 3 ppm), and three levels of Ca-P concentrations (0; 1; and 2 ppm). A control experiment was made using standard MS medium with an addition of 3 ppm BAP. Each replicate consisted of one bottle culture with one explants.

Plant material used was one node of axillary shoot from *in vitro* culture of Adira 2 and Adira 4 cassava varieties provided by Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRD). MS medium was used as basic medium of all treatments. Surface sterilization was conducted by dipping the axillary shoots in Agrept (2 g L^{-1}) and Dithane (2 g L^{-1}) solution for 2 h, and consecutively into Chloramfenicol (2 g L^{-1}) solution overnight. Sterilization by dipping in 10% NaClO solution for 5 min, then into 5% NaClO for 2 min was conducted in the laminar air flow cabinet. Sterilized shoots were planted in the precondition medium with 4 -5 explants in each bottle for 4 weeks. Explants used for experiment were micro cutting with single node of cassava (approximately 1-3 mm in length) cultured in the precondition medium. Those explants were transferred into MS medium containing kinetin or Ca-P according the treatments.

Planted explants were cultured in the dark for one week to induce shoot initiation. After that, cultures were transferred into 24 h light condition with 21^{o}C for 9 weeks. Observations were conducted on *in vitro* culture and acclimatized plants. On the *in vitro* culture, the first shoot formed and the time of callus formation were observed daily. Percentage of callus formation (%), number and percentage explants with shoot (%), number of shoot per explants, callus diameter (mm), callus color, structure of callus, length of shoot (mm), shoot morphology, number of leaves, number of explants with root, number of roots, and number of nodes were observed weekly until 10 Weeks After Planting (WAP). Data was test for normality then tested with F-test at α = 5%. Data with significant difference was tested with Duncan' Multiple Range Test.

Results and Discussion

The use of Ca-P in several concentrations only affected the callus diameter of Adira 2 variety, while kinetin addition significantly affected number of shoot and callus diameter of Adira 2 and Adira 4 varieties. Interaction between Ca-P and kinetin concentration only affected the callus diameter of Adira 4 variety. The contamination rate in this research was relatively low for Adira 2 variety (2.12%) and Adira4 variety (1.77%), respectively. Most of the contamination in the culture was caused by bacteria. Fungi contaminated the culture media in a lower level than bacteria.

As shown in Table 1, percentage of explants with shoots of Adira2 variety were higher except for three combination showing lower percentage (less than 50%) that were 1.5 ppm of

kinetin, 1 ppm of CaP, and 2 ppm CaP. All treatment combination did not significantly affect the shoot formation. In the other hand, medium containing 3 ppm of BAP resulted in 100% callus formation. Some treatments that failed to induce callus formation were in medium without kinetin or low kinetin concentration (less than 1.5 ppm). For Adira 4 variety, the highest percentage of explants with shoots was resulted in medium containing 0.5 ppm of kinetin. In general, percentage of explants with shoot was relatively high (more than 50 %) for all treatments, except for medium containing 3 ppm of kinetin (44.44 %). The highest number of callus formed was achieved in medium containing high level of cytokinin (3 ppm BAP and 3 ppm kinetin). In contrary, the root formation was inhibited with the presence of high level of cytokinin. Root formation was induced in medium containing low concentration of cytokinin.

Table 1. Percentage of shoot forming explants, callused explants, and rooted explants of Cassava Adira2 and Adira 4 variety at 10 WAP

Treatment		Adira 2			Adira 4		
		Shoot forming explants	Callused explants	Rooted explants	Shoot forming explants	Callused explants	Rooted explants
Ca-P (ppm)	Kinetin (ppm)	……%.....			……%.....		
0	0	75.00	0.00	75.00	66.67	11.11	44.44
	1	50.00	0.00	50.00	57.14	0.00	42.86
	1.5	33.33	0.00	0.00	80.00	0.00	40.00
	3	100.00	33.33	0.00	44.44	44.44	33.33
1	0	25.00	0.00	75.00	50.00	16.67	50.00
	1	50.00	25.00	25.00	77.78	22.22	44.44
	1.5	100.00	33.33	33.33	66.67	22.22	22.22
	3	75.00	25.00	25.00	77.78	66.67	10.00
2	0	33.33	0.00	33.33	70.00	40.00	50.00
	1	75.00	0.00	25.00	71.43	0.00	57.14
	1.5	100.00	50.00	100.00	66.67	11.11	33.33
	3	100.00	33.33	33.33	60.00	60.00	20.00
3 ppm BAP		100.00	100.00	0.00	75.00	50.00	0.00

At the 1 WAP, shoot of cassava was initiated at the nodes. Number of shoot formed in every treatment was compared to number of shoot formed in the control treatment (medium containing 3 ppm BAP, B3) and tested by Dunnet test as shown in Table 2.

Table 2 indicates that every treatments affected number of node when they were compared to the control medium B3, except for C0K3−B3 (9 WAP), C0K3−B3 and C1K3−B3 (10 WAP). In contrast, for Adira 4 variety none of the treatment affected number of node when compared to the control medium. Based on the regression analysis, kinetin addition could be increased above 3 ppm. For Adira 2 variety, the regression equation for shoot formation and kinetin concentration was linear ($y = 0.401x – 0.095$, $R^2 = 0.866$). This equation means that every 1 ppm increase of kinetin concentration would increase shoot formation 0.401 per explants. For Adira4 variety, the regression equation for shoot formation and kinetin concentration was also linear ($y = 0.278x + 0.224$, $R^2 = 0.613$). This equation means that every 1 ppm increase of kinetin concentration would increase shoot formation 0.278 per explants.

Table 2. Number of cassava shoot of Adira2 and Adira4 variety in the in vitro culture at 9 and 10 WAP

Treatment combination	Adira 2		Adira 4	
	Age (WAP)		Age (WAP)	
	9	10	9	10
C0K0-B3	−3.75*	−3.75*	−0.71 ns	−0.71 ns
C0K1-B3	−4.00*	−4.00*	−0.88 ns	−0.80 ns
C0K1.5-B3	−3.00*	−4.17*	−0.58 ns	−0.58 ns
C0K3-B3	−2.17 ns	−2.17 ns	−0.93 ns	−0.93 ns
C1K0-B3	−4.25*	−4.25*	−0.75 ns	−0.88 ns
C1K1-B3	−4.00*	−4.00*	−0.60 ns	−0.60 ns
C1K1.5-B3	−4.17*	−3.17*	−0.71 ns	−0.71 ns
C1K3-B3	−3.00*	−2.75 ns	0.53 ns	0.29 ns
C2K0-B3	−4.17*	−4.17*	−0.48 ns	−0.58 ns
C2K1-B3	−3.75*	−3.75*	−0.60 ns	−0.66 ns
C2K1.5-B3	−3.50*	−3.50*	−0.71 ns	−0.71 ns
C2K3-B3	−2.50*	−3.50*	1.33 ns	1.23 ns
F test	*	*	*	*

Note : Variety was not compared to each other; C0, C1, C2 = 0, 1, 2 ppm CaP, respectively; K0, K1, K1.5, K3 = 0, 1, 1.5, 3 ppm kinetin, respectively; B3 = MS + 3 ppm BAP; ns = not significant at P = 0.05; * = significantly different (P<0.05)

Table 3. Number of leaf of cassava Adira2 and Adira4 variety at 3, 7, and 10 WAP

Treatment		Adira 2			Adira 4		
		Age (WAP)			Age (WAP)		
CaP (ppm)	Kinetin (ppm)	3	7	10	3	7	10
0	0	1.25	2.00 b	2.75	0.80 jkl	0.30	0.33
	1	1.00	0.33 b	0.00	0.70 jkl	0.33	0.43
	1.5	0.50	0.25 b	0.00	0.60 jkl	0.14	0.20
	3	1.25	1.33 b	1.33	0.40 jkl	0.22	0.11
1	0	0.25	0.25 b	0.25	1.10 j	1.00	0.67
	1	0.50	0.50 b	1.25	1.00 jk	0.67	0.33
	1.5	2.00	2.00 b	1.00	1.20 j	0.33	0.44
	3	0.50	0.50 b	0.25	1.20 j	1.10	0.56
2	0	0.00	0.00 b	0.00	1.10 j	0.30	0.40
	1	1.00	1.25 b	1.25	0.90 jk	0.33	0.57
	1.5	1.00	1.67 b	2.50	0.60 jkl	0.20	0.22
	3	0.75	1.33 b	1.67	0.00 l	0.70	2.00
3 ppm BAP		1.00	5.50 b	7.50	0.11 kl	0.50	1.00
F test		ns	*	ns	*	ns	ns

Note: Variety was not compared to each other; Numbers followed by the same letter in the same columns are not significantly different based on DMRT at level α = 5%.

Leaf formation of cassava explants started at the 2 WAP. The leaf formation rate was slower than the leaf senescence rate at the 4 WAP and resulted in the decreased of leaf number in every observation point. The leaf senescence might be caused by the production of ethylene gas, nutrient deficiency, and toxicity. Magdalita *et al*. (1997) found that the accumulation of ethylene fasten the senescence of leaves in *in vitro* culture. Nutrient deficiency might caused by longer

culture, thus periodically subculture might be needed at 4 WAP. Leaf senescence might be also caused by endogen auxin-cytokinin imbalance in the plants tissues (Lizawati *et al*., 2009). As shown in Table 3, the highest number of leaf for Adira 2 variety at 10 WAP resulted in medium containing 3 ppm of BAP. In contrast, medium containing 1 ppm of kinetin, 1.5 ppm of kinetin, and 2 ppm of CaP resulted in lowest leaf number (0). The highest number of leaf for Adira 2 variety at 10 WAP resulted in medium containing 2 ppm of CaP in combination with 3 ppm of kinetin, while medium containing 1.5 ppm of kinetin resulted in the lowest number of leaf formed.

Shoot multiplication rate could be increased by periodically subculture (Hartmann and Kester, 1983). In this research, the subculture was done twice. The first subculture was done at 10 WAP, while the second subculture was done 4 weeks after the first subculture. The multiplication rate for Adira 4 variety was observed only at the first subculture. As shown in Table 4, the highest multiplication rate was achieved in the medium containing 3 ppm of BAP for both varieties.

Table 4. Shoot multiplication rate of in vitro grown cassava Adira 2 and Adira 4 varieties

Variety	Treatment		Replication	∑ Initial explants	∑ Initial shoots	∑ Shoot first sub-culture	∑ Shoot second sub-culture	Total number of Shoot
	Kinetin (ppm)	CaP (ppm)						
Adira 2	0	0	1	1	1	5	2a	8
	3	0	1	1	1	3	3b	7
	3	0	3	1	5	3	–	8
	Means ± Standard deviation							7.50 ± 0.71
	1	1	1	1	1	5	3bc	9
	1.5	1	1	1	1	2	–	3
	3	2	1	1	1	1	2	4
	3 ppm BAP		1	1	7	17	34	58
	3 ppm BAP		2	1	2	2	–	4
	Means ± Standard deviation							31.00 ± 38.18
Adira 4	1	0	4	1	1	2	–	3
	3	1	7	1	3	4	–	7
	3	1	8	1	2	1	–	3
	Means ± Standard deviation							5.00 ± 2.83
	0	2	1	1	2	1	–	3
	3	2	3	1	6	8	–	14
	3	2	6	1	6	11	–	17
	3	2	7	1	5	1	–	6
	3	2	9	1	8	1	–	9
	Means ± Standard deviation							11.50 ± 4.93
	3 ppm BAP		10	1	6	27	–	33

Note : Variety was not compared to each other; a = sub-cultured to MS0 + 3 ppm kinetin; b = sub-cultured to MS0 + 2 ppm CaP + 3 ppm BAP; c = sub-cultured to MS0 + 2 ppm CaP + 3 ppm kinetin

Conclusions

The use of several kinetin concentrations (0; 1; 1.5; dan 3 ppm) gave a positive linear response to number of shoots for both varieties (Adira 2 and Adira 4). In contrast, concentration of kinetin had negative linear response to number of roots. Media composition with 1 ppm of CaP in combination with 3 ppm of kinetin promoted the growth of cassava explants of Adira 4 variety until 4

WAT, but for longer culture period MS medium containing 2 ppm of CaP and 3 ppm of kinetin was better to support the explants growth. The highest shoot multiplication rate was achieved in the medium containing 3 ppm of BAP for both varieties.

References

Fauzi, AR. 2010. Induksi Multiplikasi Tunas Ubi Kayu (*Mannihot esculenta* Crantz.) var. Adira 2 secara In Vitro. Skripsi. Fakultas Pertanian. Institut Pertanian Bogor. Bogor. 67 hal.

Hartmann, H.T. DE. Kester. 1983. Plant propagation, principles, and practice, p. 223-280. *In* E. Cliffs (*ed*.). Prentice-Hall inc. New Jersey.

Guohua, M. 1998. Effects of cytokinins and auxins on cassava shoot organogenesis and somatic embryogenesis from somatic embryo explants. Plant Cell Tissue Organ Cult. 54:1-7.

Lizawati, T. Novita, R. Purnamaningsih. 2009. Induksi dan multiplikasi tunas jarak pagar (Jatropha curcas L.) secara *in vitro*. J. Agron. Indonesia 37: 78-85

Magdalita, PM., DG. Ian, AD. Roderick, WA. Stephen. 1997. Effect of ethylene and culture environment on development of papaya nodal culture. Plant Cell Tissue Organ Cult. 49:93-100.

Onuoch, CI., NIC Onwubiku. 2007. Micropropagation of cassava (*Mannihot esculenta* Crantz) using different concentrations of benzylaminopurine (BAP). J. Engineering Appl. Sci. 2:1229-1231.

Sudarmonowati, E., R. Hartati, T. Taryana. 2002. Produksi Tunas, Regenerasi dan Evaluasi Hasil Ubi Kayu (*Mannihot esculenta*) Indonesia Asal Kultur Jaringan di Lapang. Pusat Penelitian Bioteknologi. LIPI: Cibinong.

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