Journal of Tropical Crop Science Vol. 4 No. 3, October 2017
www.j-tropical-crops.com

Optimation of *In vitro* Lateral Shoots Multiplication of Papaya (*Carica papaya* L.) "Callina" with BAP and NAA

Darda Efendi*[A, B] and Mirza R. Putra[B]

[A] Center for Tropical Horticulture Studies, Bogor Agricultural University, Kampus IPB Baranangsiang, Bogor 16144.
[B] Department of Agronomy and Horticulture, Bogor Agricultural University, Jl. Meranti, Kampus IPB, Dramaga.

*Corresponding author; email: darda@apps.ipb.ac.id

Abstract

Papaya is a popular fruit and is grown commercially in many subtropical and tropical countries. Papayas are generally grown from seeds; therefore the offsprings are not true-to-type and could come in three sexes, female, male, and hermaphrodite. Clonal propagation is required to obtain to grow true-to-type hermaphrodite papayas. In this research, we developed an *in vitro* protocol for shoot multiplication from lateral shoots from *in vitro* germinated papaya seedlings. The *in vitro* propagated plant materials could potentially be used as a source of papaya micro-cuttings, or as scion for papaya grafting. The experiment was set up as a factorial experiment with NAA at 0, 0.1 and 0.5 mg.L^{-1}, and BAP at 0, 0.1, 0.5, and 1.0 mg.L^{-1} in a completely randomized block design. BAP interacted with NAA in affecting the shoot production per explant. The optimum BAP and NAA concentration to produce lateral shoots was 0.54 mg. L^{-1} and 0.1 mg.L^{-1}, respectively. Media without NAA reduced the number of lateral shoots and number of leaf per explant at any BAP concentration.

Keywords: hermaphrodite, seeds, true to type, clonal propagation, micro cuttings.

Introduction

Papaya (*Carica papaya* L.) is a fruit commonly eaten fresh; immature papayas can also be consumed as vegetables. Papayas are commercially grown in many subtropical and tropical countries, including Indonesia. Papaya production in Indonesia has increased from 695,214 ton in 2010 to 899,358 ton on 2012 and 830,491 in 2014 (BPS 2014). Raw papaya pulp contains 88% water, 11% carbohydrates, have low calories and rich in vitamin A and C. The carotenoid content of papaya was 175 *µ*g per 100 g edible portion (Ncube et al., 2007).

Papaya is a polygamous species grow in three sexes: male, female, hermaphrodite. The male produces only pollen and never set fruit. The female trees produce small, inedible fruits unless pollinated. The hermaphrodite can self-pollinate since its flowers contain both male stamens and female ovaries. Almost all commercial papaya orchards contain only hermaphrodite trees as their fruit quality is superior to fruits from female trees.

Sexual propagation is the preferred method for commercial papaya propagation because papaya is a cross-pollinated crop, and sexual propagation can result in high degree of variation among populations in terms of yield, fruit size, fruit shape, and taste (Keng and Teo, 1994). Vegetative propagation can be used to overcome the problems of sexual propagation of papaya and produce genetic uniformity or true to type progeny. Vegetative propagation can preserve the identity of an elite clone or cultivar. Therefore an efficient vegetative propagation method is required develop true-to-type progenies. Development of an efficient *in vitro* protocol for regeneration system would be very useful for mass propagation of clonal papaya. Propagation by tissue culture has benefits over conventional propagation methods including can produce many propagules in a relatively short time and can improve plant quality (Tahardi et al., 2000).

Research on *in vitro* papaya micropropagation have been reported including Drew (1988, 1992), Drew et al. (1991, 1993), Lai et al. (1998), Yu et al. (2000), Fitch et al. (2003), Dhekney (2004), and Saksena and Sharma (2014). Saksena and Sharma (2014) reported a standard protocol for the commercial micropropagation of four papaya varieties, CO_2, Madhu, Honey Dew and Taiwan, and reported that 0.1 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP in a liquid media could be used for *in vitro* propagation of papaya. Mumo et al. (2013) reported an optimum IBA concentration for root induction of 2.5 mg.L^{-1}. NAA is a synthetic auxin and has been reported to be more effective than

natural auxin indole-3-acetic acid (IAA) in inducing cotton cell elongation *in vitro* (Singh, et al., 2009).

One of the leading papaya cultivars in the Indonesian market is "Callina" (IPB9). Papaya "Callina" has small fruits of about 23 cm length and 9.5 cm in diameter, has orange pulp with sugar content of 10.1-11.2°brix (Sujiprihati et al., 2010). Papaya "Callina" has been propagated mainly by seed and can be harvested at eight to nine month after planting (Sujiprihati et al., 2010). A clonal propagation method is being developed to produce true-to-type "Callina". The objective of this study was to examine the effect of NAA and BAP on *in vitro* lateral shoots multiplication of papaya "Callina" to develop an efficient *in vitro* regeneration system.

Materials and Methods

The research was conducted at the Tissue Culture laboratory of the Center for Tropical Horticulture Studies, Bogor Agricultural University from December 2015 to June 2016. Seeds of papaya "Callina" (IPB 9) were grown on MS media supplemented with Naphthalene Acetic Acid (NAA), 6-Benzyl Amino Purine (BAP), sugar, and agar. The culture vessels were put under a 16h photoperiod with 1000 ± 100 lux of cool white fluorescent light, and temperature of 22 ± 2°C.

The experiment was set up in a completely randomized block design with two factors, BAP at 0, 0.1, 0.5, and 1.0 mg.L^{-1}, and NAA at 0, 0.1, and 0.5 mg.L^{-1}, totalling12 treatments. Each treatment was repeated ten times to give a total of 120 experimental units. Each experimental unit consists of four explants in one bottle, so there are a total 480 explants in 120 culture bottles.

Scoring was conducted weekly on the number of shoots, number of leaves and shoot height produced per explant for eight weeks. Collected data were subjected to analysis of variance (ANOVA) and correlation test using the General Linear Model procedure. Means were separated using the DMRT at 5% level of significance in SAS version 9.

Results and Discussion

Recapitulation of F-test

NAA and BAP interaction had significant effects on the number of shoots starting the first week after culture, and very significant effects from week two to week six (Table 2). These results are consistent with Weaver's report (1972) that application of NAA and BAP was effective and could increase *in vitro* multiplication. Silva et al. (2007) reported that lateral shoots would make good explants for further shoot

Table 1. Recapitulation of the variance of papaya number of shoots per explant, number of leaves per explant, and plantlet height

Parameters	Weeks	F-test			CV
		NAA	BAP	NAA:BAP	
Number of shoots	1	0.2842 ns	0.413 ns	0.0116*	13.77
	2	0.0001**	0.0001**	0.0008**	19.52
	3	0.0001**	0.0001**	0.0009**	16.73
	4	0.0001**	0.0001**	0.0085**	20.88
	5	0.0001**	0.0001**	0.0047**	20.56
	6	0.0001**	0.0001**	0.0007**	20.74
	7	0.0001**	0.0001**	0.0743 ns	20.74
	8	0.0001**	0.0001**	0.1713 ns	20.95
Number of leaves	1	0.0426*	0.6321 ns	0.2739 ns	23.44
	2	0.0503 ns	0.0097**	0.0002**	25.10
	3	0.0062**	0.0046**	0.0022**	26.00
	4	0.0023**	0.0005**	0.0001**	24.05
	5	0.0878**	0.0001**	0.0001**	26.81
	6	0.0024**	0.0001**	0.0001**	24.83
	7	0.0209*	0.0001**	0.0001**	28.35
	8	0.051 ns	0.0001**	0.0001**	28.98

Note: **= very significant at α 1%; *= significant at α 5 %; ns= non-significant according to DMRT.

multiplication. Saksena et al. (2014) and Mumo et al. (2013) also demonstrated that application of NAA and BAP could induce lateral shoot multiplication of papaya. NAA and BAP interaction significantly increased the height of plantlet at week one, two, four and six after treatment. The development of the *in vitro* papaya explants from germinated seeds to five-week-old plantlet can be seen on Figure 3.

Number of Shoots per Explant

Explants grown on media containing 0.1 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP produced seven shoots at two weeks after treatment (WAT), and the shoot number increased to 10.6 at six WAT. The control explants only produced 1.5 shoots at two WAT and 1.9 shoots at six WAT (Table 2). The increased number of shoots with NAA 0.1 mg.L^{-1} and BAP 0.5 mg.L^{-1} was consistent with the report by Saksena and Sharma (2014) and Mumo (2013). Mumo (2013) further stated that increasing BAP concentration to 2 mg.L^{-1} and NAA to 1 mg.L^{-1} or higher reduced the number of shoots per explant (Mumo, 2013). BAP were also reported as an effective cytokinin to increase proliferation and shoot growth of other tropical fruits pineapple (*Ananas comosus* L.) (Al-Saif et al., 2011), and banana (Paulos et al., 2013).

Regression of NAA 0.1 mg.L^{-1} and BAP 0.5 mg.L^{-1} (Figure 1) demonstrated a very high coefficient of determinant of R^2 = 0.9871. High coefficient of the determinant of close to 1 indicated that the regression model is good and can be used for calculating optimum BAP concentration, which was 0.54 mg.L^{-1} for BAP and NAA 0.1 mg.L^{-1} to produce 10.5 shoots per explant. The regression of NAA 0 mg.L^{-1} or 0.5 mg.L^{-1} with BAP had very low R^2 of 0.0802 and 0.3673, respectively.

Table 2. Effect of NAA and BAP on number of shoots per explant

Treatments	Weeks after treatment					
NAA : BAP	1	2	3	4	5	6
0 : 0	1.00 a	1.50 cd	1.60 cd	1.60 de	1.90 cde	1.90 cde
0 : 0.1	0.90 ab	3.90 b	4.40 b	5.90 b	6.70 b	6.70 b
0 : 0.5	0.90 ab	1.80 cd	2.30 cd	3.20 cd	4.10 c	4.10 c
0 : 1.0	0.90 ab	1.60 cd	2.40 cd	3.90 c	4.20 c	4.20 c
0.1 : 0	0.21 c	0.61 d	1.00 d	1.00 e	1.00 e	1.00 e
0.1 : 0.1	0.80 ab	2.00 cd	2.40 cd	2.70 cde	2.70 cde	3.20 cde
0.1 : 0.5	**1.20 a**	**7.00 a**	**8.00 a**	**8.20 a**	**10.60 a**	**10.60 a**
0.1 : 1.0	0.90 ab	2.30 c	3.00 c	3.30 cd	3.50 cd	3.50 cd
0.5 : 0	0.90 ab	1.00 cd	1.10 d	1.30 de	1.30 de	1.30 de
0.5 : 0.1	0.90 ab	1.00 cd	1.30 d	1.60 de	2.40 cde	2.40 cde
0.5 : 0.5	0.40 bc	1.00 cd	1.20 d	1.20 de	1.20 de	1.20 de
0.5 : 1.0	0.90 ab	1.20 cd	1.30 d	1.90 cde	2.30 cde	2.50 cde

Note: values with the same letters on the same column were significantly different according to Duncan's Multiple Range Test (DMRT) α 5%.

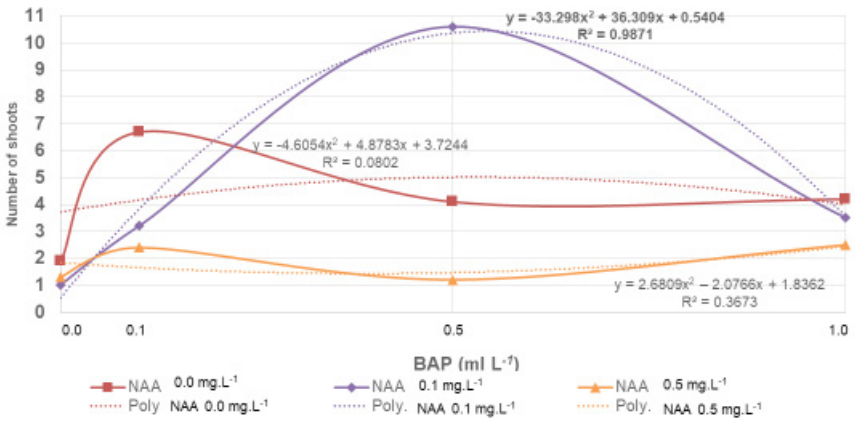

Figure 1. Regression of NAA and BAP concentrations on the number of the shoots per explant

Number of Leaves per Explant

Media with 0.1 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP resulted in the highest number of leaves per explant (Figure 1) which was six-fold of the control (Table 1), possibly due to NAA and BAP at these concentrations were optimum for multiplication, Therefore addition of 0.1 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP can be used for further *in vitro* lateral shoot multiplication. Application of NAA and BAP will possibly improve sub-optimal levels of endogenous auxin and cytokinin to reach a more optimal balance for multiplication, as reported by Minocha (1987).

Figure 2 demonstrated that the regression of NAA 0.1 mg.L^{-1} with BAP of 0, 0.1, 0.5, and 1 mg.L^{-1} at eight WAT has a coefficient of determinant of close to 1 (R^2 = 0,9988), indicated that regression model is good and can be used to calculate optimum concentration of BAP in combination with NAA 0.1 mg.L^{-1}. The BAP concentration to produce the highest number of leaf per explant, i.e. 43.5 at week eight, was 0.53 mg.L^{-1}. Similar to the number of shoots, regression of NAA at 0 mg.L^{-1} or 0.5 mg.L^{-1} with BAP had a very low coefficient of the determinant, i.e., 0.3906 and 0.6293, respectively.

Table 3. Effect of NAA and BAP on number of leaves per explant

Treatments	Weeks after treatment						
	2	3	4	5	6	7	8
NAA : BAP							
0 : 0	2.00 bc	2.30 bcd	4.00 bcd	5.00 cde	6.40 cde	6.70 cd	7.10 de
0 : 0.1	2.70 b	4.90 b	8.20 b	12.5 b	16.5 b	21.2 b	24.4 b
0 : 0.5	1.90 bc	4.00 bc	6.00 bc	9.80 bcd	12.6 bc	16.1 bc	20.9 bc
0 : 1	1.30 bc	3.40 bcd	7.60 b	9.80 bcd	11.8 bcd	16.8 bc	21.7 bc
0.1 : 0	0.80 c	0.90 d	1.30 d	1.60 e	1.80 e	2.50 d	2.60 e
0.1 : 0.1	1.40 bc	2.90 bcd	5.10 bcd	8.50 bcde	11.8 bcd	13.3 bcd	15.3 bcde
0.1 : 0.5	**5.20 a**	**7.40 a**	**14.6 a**	**24.4 a**	**34.7 a**	**39.8 a**	**43.6 a**
0.1 : 1	1.70 bc	3.20 bcd	5.30 bcd	6.40 bcde	8.70 bcde	11.4 bcd	11.4 bcde
0.5 : 0	1.30 bc	1.80 cd	3.10 cd	5.20 cde	6.00 cde	6.20 cd	7.10 de
0.5 : 0.1	1.70 bc	2.00 cd	4.50 bcd	7.30 bcde	9.90 bcd	11.8 bcd	13.6 bcde
0.5 : 0.5	1.10 bc	1.30 cd	2.10 cd	3.90 de	4.30 de	6.30 cd	9.60 cde
0.5 : 1	1.30 bc	2.60 bcd	4.60 bcd	11.1 bc	11.1 bcd	15.4 bc	18.0 bcd

Note: values with the same letters on the same column are not significant according to Duncan's Multiple Range Test (DMRT) α 5%.

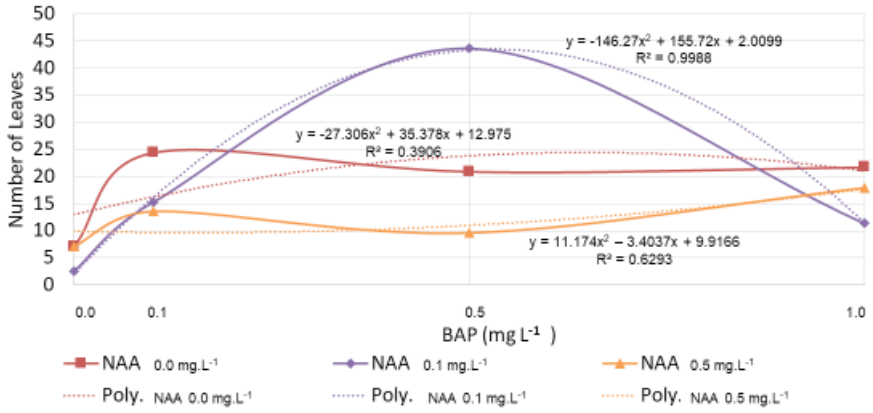

Figure 2. Regression of NAA and BAP concentration on the number of the shoot at six weeks after planting

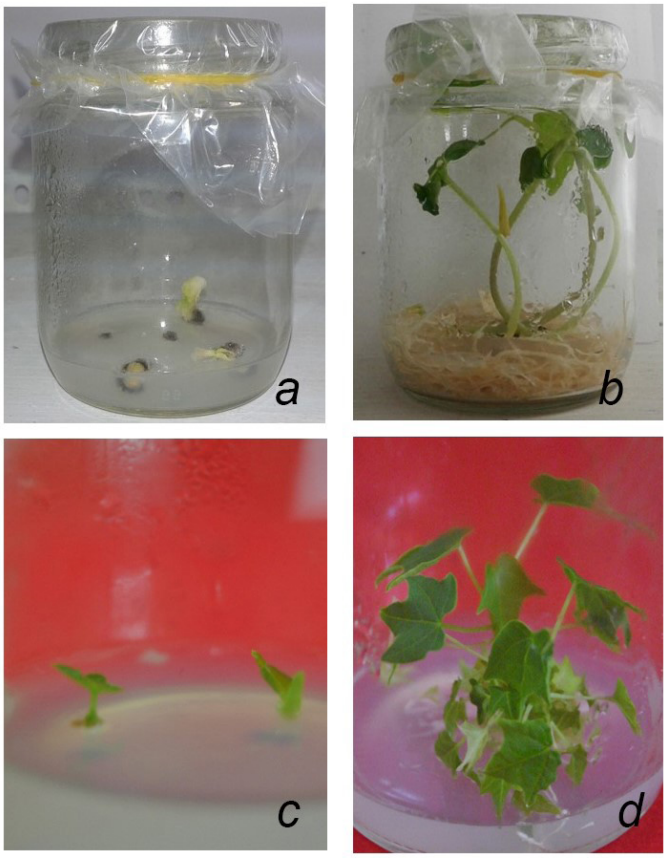

Figure 3. Development of papaya culture *in vitro*: a) seed germination and b) one-month-old *in vitro* seedlings of papaya "Callina" as a source of shoot tip explants on MS0 media; c) two-week-old shoot tip explants and d) five-week-old papaya plantlets on MS media supplemented with 0.1 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP.

This study has provided an advancement for propagation of papaya "Calina". The results of this study could be used as a basic protocol for further development of papaya multiplication *in vitro*.

Conclusion

NAA interacted with BAP to affect the number of shoots produced and the number of leaves of *in vitro* multiplication of "Callina" (IPB 9) papaya. The optimum BAP concentration to efficiently produce the highest multiplication rate was 0.53 to 0.54 mg.L^{-1} and NAA 0.1 mg.L^{-1} which resulted in 10.6 shoots and 43.5 leaves after eight weeks of culture.

Acknowledgment

The authors thanked Directorate of Research and Community Services, Directorate General of Research Strengthening and Development, Ministry of Research, Technology and Higher Education of The Republic of Indonesia for funding this research through the contract number 079/SP2H/LT/DRPM/II/2016 dated 17 February 2016. The authors also thanked the stakeholders of Center for Tropical Horticulture Studies, Bogor Agricultural University, for their continuous support for this research.

References

Al-Saif, A.M., Sharif Hossain, A.B.M., and Taha, R. M. (2011). Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro*. *African Journal of Biotechnology* **10**, 5291-5295.

Badan Pusat Statistik [BPS]. (2014). Basis Data Statistik Pertanian http://aplikasi.pertanian.go.id/bdsp/newkom.asp. [June 12, 2015]

Dhekney, S.A. (2004). "Molecular Investigations, Cryopreservation and Genetic Transformation of Papaya". Thesis. University of Florida, Florida, USA.

Dhekney, S.A. Kandel, R., Bergey D.R., Sitther V., Soorianathasundaram, K., and Litz R.E. (2016). Advances in papaya biotechnology. *Biocatalysis and Agricultural Biotechnology* **5**, 133–142,

Drew, R.A, McComb J.A., and Considine,J.A. (1993). Rhizogenesis and root growth of *Carica papaya* L. *in vitro* in relation to auxin sensitive phase and use of riboflavin. *Plant Cell Tissue and Organ Culture* **33**, 1-7.

Drew, R.A. (1992). Improved techniques for *in vitro* propagation and germplasm storage of papaya. *HortScience* **27**, 1122 -1124.

Drew, R.A. (1988). Rapid clonal propagation of papaya *in vitro* from mature field grown trees. *HortScience* **23**, 609-611

Drew, R.A. and Miller, R.M. (1984). Nutritional and cultural factors affecting rooting of papaya (*Carica papaya* L.) *in vitro*. *HortScience* **64**, 767-773.

Drew, R.A. and Smith, N.G. (1986). Growth of apical and lateral buds of pawpaw (*Carica papaya* L.) as affected by nutritional and hormonal factors. *HortScience* **61**,535-543.

Fitch, M.M., Terry, L., Saito, N., Yamamoto, G. Dela Cruz, A., Yeh, Aileen, W., White, S., Maeda, S.H., Ferreira, S., and Moore, P. (2003). Photoautotrophic rooting and growth of papayas *in vitro* Abs. 572. Annual Meeting of American Society of Plant Biologists Plant Biology p. 131, July, 25–30, Honolulu, Hawaii.

Keng C.L. and Teo K.H.C. (1994). Culture of papaya explant in solid-liquid media sequence as a rapid method for producing multiple shoots. *Journal of Tropical Agricultural Science* **17**, 103-106.

Lai, C.C., Yu, T.A., Yeh, S.D., and Yang, J.S. (1998). Enhancement of *in vitro* growth of papaya multishoots by aeration. *Plant, Cell, Tissue and Organ Culture* **53,** 221-225.

Minocha, S.C. (1987). Plant growth regulators and morphogenesis in cell and tissue culture in forest trees *In* Cell and Tissue Culture in Forestry Vol. I. (J.M. Bonga and D.J. Durzan, eds) p.50 – 66. Martinus Nijhoff Publishers.

Mumo, N.N., Rimberia, F.K., Mamati, G.E., and Kihurani, A.W. (2013). *In vitro* regeneration of selected Kenyan papaya (*Carica papaya* L.) lines through shoot tip culture. *African Journal of Biotechnology* **12**, 6826-6832.

Ncube, T.N., Greiner, T., Malaba, L.C., and Gebre-Medhin, M. (2001). Supplementing lactating women with pureed papaya and grated carrots improved vitamin A status in a placebo-controlled trial. *The Journal of Nutrition* **131**, 1497-1502.

Paulos, M., Joshi, V.R., and Pawar, S.V. (2013). Effect of BAP and NAA on *in vitro* shoot establishment and proliferation of banana (*Musa paradisiaca*) Cv.Grand Naine.

International Journal of Science and Research **4**, 310-323.

Saksena, P. and Sharma, N.C. (2014). *In vitro* propagation in four dioecious varieties of papaya (*Carica papaya*). *World Journal of Applied Science Research* **4**, 24-31.

Singh, B., Cheek, H.D., and Haigler, C.H. (2009). A synthetic auxin (NAA) suppresses secondary wall cellulose synthesis and enhances elongation in cultured cotton fiber. *Plant Cell Report* **28**,1023-1032. doi: 10.1007/s00299-009-0714-2.

Sujiprihati, S., Gunawan, E., Darma, K., Kurniawan, A., Hidayat, and Gunarso (2010). “Deskripsi Papaya Varietas “Callina”. Center for Tropical Horticulture Studies. Bogor Agricultural University, Indonesia.

Weaver, J.R. (1972). Plant Growth Substances in Agriculture. University of California, Davis WH. Freeman and Co. San Fransisco.