Agrinova: Journal of Agriculture Inovation
Volume 1 (1), 2018, 001-007
Available online at http: journal.ugm.ac.id/agrinova

Article

Effect of Explant Types and Benzyl Amino Purine Concentrations on the In Vitro Regeneration of Several Local Eggplant Cultivars

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Received: 6th May 2018 ; Revised: 3rd June 2018 ; Accepted: 24th June 2018

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DOI:
http:10.22146/agrinova.41750

ABSTRACT

Eggplant as a vegetable is generally fresh consumed. Besides having a high nutritive value, eggplant contains solasonine and solamargine, which act as an antibacterial that can inhibit cancer cells, gastrictic and respiratory disorders. Eggplant also provides a unique system to study in vitro regeneration since it can be induced from different explants by different growth regulators and morphogenetic pathways. This study aims to observe the effect of explant types and BAP concentrations on the in vitro regeneration of three eggplant cultivars, Rimbang, Tanteloh and Limao. Leaf and hypocotyl explants were cultured on MS semi solid medium supplemented with BAP and IBA in combination. BAP tested at 1, 2, and 3 mg.L^{-1} while IBA tested at 1 mg.L^{-1}. Medium containing 1 mg.L^{-1} IBA used for rooting of regenerated shoot. The experiment was arranged in completely randomized design with five replicates. Each replicate consisted of five leaves and hypocotyl explants. Cultures were incubated at 25±10^{o}C, 65% humidity, and 16 hours per day photoperiod. Observation was done for 8 weeks of culture. The result indicated that leaf explants showed shoot initiation within 5 – 9 days of cultured, while hypocotyl explants showed the response within 7 – 12 days of cultured. Shoots formation preceded by swollen on the explants surface followed by emerging light green shoot. Leaf explants cultured on medium supplemented with 3 mg.L^{-1} BAP + 1 mg.L^{-1} IBA showed the maximum number of shoots regeneration while hypocotyl explants showed less of shoots in all medium. Similarly, leaf explants cultured on medium supplemented with 3 mg.L^{-1} BAP + 1 mg.L^{-1} IBA showed the maximum number of roots. Among the cultivars, Rimbang showed the best response than other cultivars.

Keywords: eggplant cultivars; growth regulator; hypocotyl; in vitro regeneration; leaf.

INTRODUCTION

Eggplant fruit contains protein, fat, carbohydrates, calcium, phosphorus, iron, and vitamins A, B and C (Husni et al., 2003), therefore it is generally considered as a fresh consumed vegetable. Besides having a high nutritional value, eggplant also contains an antibacterial secondary metabolic such as solasonine and solamargine (Chakravarthi et al., 2010), which can inhibit cancer cells, gastric and respiratory disorders (Wei et al., 2011). Eggplant also provides a unique system to study in vitro regeneration since plantlet can be induced from different explants by different growth regulators and morphogenetic pathways (Magioli and Mansur, 2005). Genotype is the most important factor affecting organogenesis and explants sources has also been substantiated (Sharma and Rajam, 1995; Kaur et al., 2013). Effect of genotypes is closely related to the factors that affect explants changes, such as growth regulators and culture conditions. Therefore, the composition of medium and growth regulators are varied for each genotype even tissue culture techniques are similar (Arias, 2009).

This study attempts to observe the effect

of explant types and BAP concentrations on the in vitro regeneration of three eggplant cultivars, Rimbang, Tanteloh and Limao.

MATERIALS AND METHODS

Seeds of three eggplant cultivars, Rimbang, Tanteloh and Limao are sterilized with 96% alcohol for a minute and germinated aseptically on MS (Murashige and Skoog, 1962) basal medium with 30 $g.L^{-1}$ sucrose and 4 $mg.L^{-1}$ agar, pH 5.8. Three bottles containing 20 seeds for each cultivar were placed in culture room with 25 ± 1°C, 65% humidity and 16 hours photoperiod per day.

Every combination 1 to 3 $mg.L^{-1}$ BAP and 1 $mg.L^{-1}$ IBA were put in three different beaker glasses containing 1 L MS stock solution and 30 g sucrose then stirred constantly. pH medium is calibrated on 5,8 by adding 1 M NaOH or 0.5 M HCl. Bacto agar was added to solidify a medium and dissolved by heating on hotplate at 250°C. Further, regeneration medium was divided into culture bottles and sterilized by autoclave at 1.1 $kg.cm^{-1}$ (103 kPa) and 121°C for 20 minutes.

Plant materials used as explants were leaf and hypocotyl of 30 days-old seedlings. Five slices of leaves and hypocotyl each sized (2x10) mm and 10 mm were cultured on different induction media with 5 replications. All cultures are incubated on culture room with 25 ± 1°C, 65% humidity and 16 hours photoperiod. The culture was monitored every week for 8 weeks to determine days of shoot initiation, and induced shoot number. Data were analyzed using a completely randomized factorial design with 95% confident level. Duncan Multiple Range Test (DMRT) procedure was used to compare the different among the means. ANOVA and mean comparison are performed using the software SAS System for Window 9.0.

RESULT AND DISCUSSION

Plant regeneration from tissue culture is a vital component of plant biotechnology (Rao, 1992). A proficient and reproducible in vitro regeneration system is considered a vital part of successful genetic transformation (Batti et al., 2014). Eggplant is highly responsive to various tissue culture techniques (Sidhu et al., 2014). Plantlet regeneration is possible via organogenesis and embryogenesis (Jagatheswari & Ramesh, 2014). The combination and concentration of plant growth regulators should be determined through complex and empirical process (Tanaka et al., 2012).

Various protocols on in vitro regeneration have been carried out using various auxin and cytokinin (Magioli et al., 1998; Mukherjee et al., 1991). Cytokinin such as BAP (benzyl amino purine) (Sharma and Rajam, 1995), kinetin (Gleddie et al., 1983), zeatin (Mukherjee et al., 1991), and thiadizuaron (Magioli et al., 1998) either alone or in combination with NAA (Napthalene acetic acid) was reported quite effective to induce shoot development. There was an increase in number of adventitious shoots formed with increasing BAP (Benzyl Amino Purine) (Pal and Singh, 2012), however higher BAP had negative effect on organogenesis leading to shoot vitrification (Zayova et al., 2012).

Genotypes play an important role in organogenesis of the shoot directly from the explants and the regeneration potential also varied with the tissue system used (Sidhu et al., 2014). Different explants had different response to regeneration (Gisbert et al., 2006; Kanna and Jayabalan, 2010). In vitro regeneration of different explants types such as cotyledon, hypocotyls, leaf and root through organogenesis has been reported by Kamat and Rao (1978), Fassuliotis et al. (1981), Allichio et al. (1982), and Franklin et al. (2004). It was generally accepted that the regeneration efficiency is influenced by genotypes and explants types (Mallaya & Ravishankar, 2013). Genotypes and explants were the most important factor affecting the plant cell development pattern and its further regeneration (Huda et al., 2007; Mir et al., 2008).

In this study, we reported in vitro regeneration from hypocotyl and leaf explants of three eggplant cultivars (Rimbang, Tanteloh and Limao) which cultured on MS solid medium containing a combination of 1 $mg.L^{-1}$ IBA and

Table 1. The period of shoot initiation on leaf and hypocotyl explants.

Explants	BAP ($mg.L^{-1}$)	Days of culture			
		Rimbang	Tanteloh	Limao	**Average**
Leaf	1	6	9	9	**8**
	2	5	7	8	**7**
	3	5	7	5	**6**
	Average	**5**	**8**	**7**	**7**
Hypocotyl	1	7	12	10	**10**
	2	7	9	8	**8**
	3	7	9	7	**8**
	Average	**7**	**10**	**8**	**8**

Table 2. Number of shoots of different cultivars and explants induced on different BAP concentrations.

Explants	BAP ($mg.L^{-1}$)	Cultivar			
		Rimbang	Tanteloh	Limao	**Average**
Leaf	1	4.2 a	1.4 b	1.0 b	2.2
	2	1.2 b	1.4 b	1.8 b	1.5
	3	6.0 a	1.8 b	1.8 b	3.2
	Average	3.8	1.5	1.5	2.3
Hypocotyl	1	1.0 b	1.0 b	1.0 b	1.0
	2	1.2 b	1.0 b	1.0 b	1.1
	3	1.0 b	1.0 b	1.0 b	1.0
	Average	1.1	1.0	1.0	1.0

Remarks: ** = Means followed different letter were significantly different (P<0.05) by Duncan's multiple range test.

Table 3. Number of root from shoot originated from different cultivars, explants and BAP concentration induced on MS containing 1 $mg.L^{-1}$ IBA

Explants	BAP ($mg.L^{-1}$)	Cultivar			
		Rimbang	Tanteloh	Limao	**Average**
Leaf	1	3.0 cdefg	2.8 defg	3.4 bcdef	3.1
	2	6.00 ab	3.8 bcde	5.0 bc	4.9
	3	5.2 bc	5.0 bcd	9.2 a	6.5
	Average	4.7	3.9	5.9	4.8
Hypocotyl	1	1.8 fg	1.60g	3.2 cdefg	2.2
	2	2.4 efg	3.6 bcdef	3.6 bcdef	3.2
	3	3.6 bcdef	3.8 bcdef	4.0 bcde	3.8
	Average	2.6	3.0	3.6	3.1

Remarks: ** = Means followed different letter were significantly different (P<0.05) by Duncan's multiple range test.

1,2 and 3 $mg.L^{-1}$ BAP. Explants were taken from 30-day-old seedlings which are germinated aseptically in vitro. Such old seedlings were recommended by Zayova et al. (2012). It was shown that shoots are observed after 5-12 days of culture. The shoots formation preceded by swollen on the explants surface followed by emerging light green shoot. The small young leaves were formed after the shoot 1 cm height. Similar characteristic was

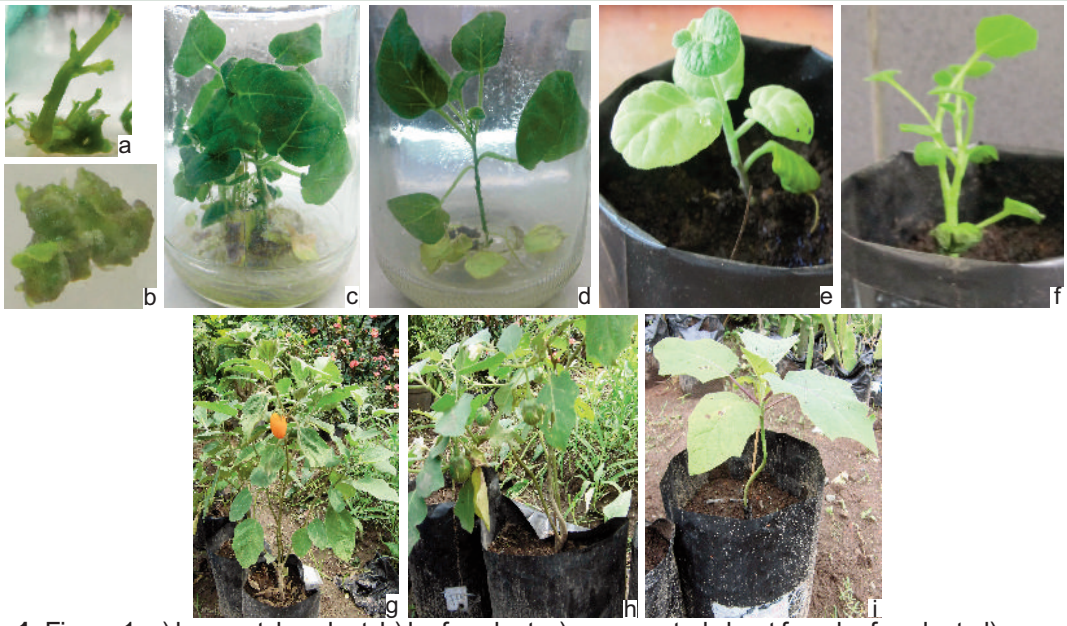

Figure 1. Figure 1. a) hypocotyl explant. b) leaf explant. c) regenerated shoot from leaf explant. d) regenerated shoot from hypocotyl explant. e) hardening of plantlet obtained from hypocotyl explant. f) hardening of plantlet obtained from leaf explant. g) cultivar Tanteloh. h) cultivar Limao. i) cultivar Rimbang (g, h, i = all cultivar after hardening in greenhouse).

reported by Sarker et al. (2006).

The bud formation was influenced by explants types. Shoots were formed within 5-9 days of culture on the surface of the leaf explants which were cultured on all medium, while shoots from hypocotyl explants were observed within 7-12 days of culture (Table 1).

Leaf explants provided a faster response than the hypocotyl explants in all BAP concentrations. All explants were able to form shoots. Hartman et al. (1990) stated that early actively growing tissue is the best explant for in vitro culture. Pierik (1997) suggested the use young and soft tissues as an explant because it is generally easier to proliferate than old woody tissues. Young tissues usually have high regenerative capacities and frequently used as in vitro material.

There was dependency among cultivars, IBA concentrations and explants types on the number of induced shoot. This result was similar to the report of Muir et al. (2011) and Pal et al. (2012) where genotype and explant interaction show highly significant effect on organogenesis. Cultivar Rimbang showed significant maximum number of shoot on MS medium supplemented with 3 $mg.L^{-1}$ BAP + 1 $mg.L^{-1}$ IBA, though such shoot numbers was not significantly different from MS media supplemented with 1 $mg.L^{-1}$ BAP + 1 $mg.L^{-1}$ IBA. Cultivar Limao and Tanteloh produced a limited shoot number on all induction media (Tabel 2).

Explant type influences the shoot in vitro development in eggplant. Leaf explants changed and formed the shoots more efficiently than explants from other organs (Husni et al., 2003; Pal & Singh, 2012). For herbaceous plants, leaf explant is preferred due to high number of competent protoplasts which have a high regeneration potential. Leaf can be considered as good explants to be used as a material for crop improvement through somatic hybridization, transformation and in vitro selection. Leaf and cotyledon explants are more responsive than derived from hypocotyl explants in tissue culture of eggplant (Alicchio et al., 1982). In addition, the incision along the sides of leaf provides an opportunity to produce more shoots than explants hypocotyls that have only a small incision at both ends (Figure 1A, 1B, 1C, 1D).

This result was not in line with earlier study conducted by Sharma and Rajam (1995), where leaf explants produced the least number of shoots than hypocotyl and cotyledon explants. This effect may be due

to the different genotypes and combination of growth regulators. In this research, the combination of IBA and BAP was exploited, whereas Jagadheeswari & Ramesh (2014) put explant on media containing NAA and BAP, and on the other hand Sharma and Rajam (1995) used IAA and BAP combination.

To induce rooting of regenerated shoot, medium containing 1 mg.L^{-1} IBA were used for both leaf and hypocotyl explants. Sharmin et al. (2008) reported that the optimal growth of root obtained on the medium containing 1 mg.L^{-1} IBA, whereas Batti et al. (2014) suggested using hormone free MS media or 0.5 mg.L^{-1} IAA supplemented MS media. For number of roots, in vitro induced shoot of Limao in 3 mg.L^{-1} BAP containing media produced the highest root number, although the root numbers were similar to Rimbang shoot produced on 2 mg.L^{-1} BAP. The minimum number of roots was shown on Rimbang, Tanteloh and Limao shoots induced on MS media containing 1 mg.L^{-1} (Tabel 3). Shoot induced from leaf explant responded better than hypocotyl explant. Maximum number of root was obtained from shoot induced from leaf explant cultured on MS medium containing 3 mg.L^{-1} BAP + 1 mg .L^{-1} IBA.

Shoots with good root system were transplanted to polyethylene bags containing sterile soil and compost with 1:1 ratio covered with transparent plastic wrap to maintain the humidity and put in light for acclimatization (Figure 1E and 1F). After one week, the plantlets were moved to screening house and transparent plastic wrap was opened for 2 hours, 4 hours, 8 hours, and 12 hours every day on a regular basis. When the plant was able to adapt to the outside conditions, the plastic wrap was removed. After 2 weeks the plant was transferred to the field (Figure 1G, 1H and 1I).

CONCLUSIONS

From this investigation, it can be concluded that when leaf and hypocotyl cultured on MS media containing BAP and IBA and incubated in light, shoot initiation occured within 5 – 12 days of cultured. Shoots formation preceded by swollen on the explants surface followed by emerging light green shoot. There was significant interaction among genotypes, explant types and BAP concentrations on the shoot induction capacity. The ability of shoot induced in vitro to produce root depended on the genotype and explant origins, and also the induction media. Leaf explants cultured on medium supplemented with 3 mg.L^{-1} BAP + 1 mg.L^{-1} IBA showed the maximum number of shoots regeneration. Similarly, shoot induced from leaf explants cultured on medium supplemented with 3 mg.L^{-1} BAP + 1 mg.L^{-1} IBA produced maximum root numbers, though both of the ability was determined by cultivars. Among the cultivars, Rimbang showed the best response to shoot induction, whereas Limao for root production. It was also able to acclimatize some plantlets to the field and the plantlet can produce some fruits. These findings suggested that direct organogenesis protocol has been developed and could be explored for somaclonal variation induction, tetraploid and transgenic eggplant production.

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