In Vitro Propagation Of Buah Merah (*Pandanus Conoideus* Lam) Through Lateral Bud Proliferation

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ABSTRACT

Pandanus conoideus Lam or 'Buah merah' of the Pandanaceae is native to East Indonesia, particularly Papua and North Maluku. Traditionally the fruits are used for health promotion and maintenance as well as for curing several illnesses. Recently, it has been reported that the fruits are potential for cancer medication. As a result, there has been an overexploitation of the plants from their habitats. In order to anticipate their possible disappearance due to overexploitation in the wild, an efficient and effective technology for the mass propagation, conservation and cultivation of these plants should be developed. Generally, buah merah is propagated vegetatively by offshoots and stem cuttings or generatively by seeds. Micropropagation has many advantages over the conventional methods, because the technique allows mass clonal and pathogen-free production of plants at a high rate of multiplication all year round. In this research the effects of 0.1-0.2 mg/l thidiazuron (TDZ), 0.5-1.0 benzyl amino purine (BAP) and 0.25-0.5 mg/l Kinetin (KN) on shoot bud induction and proliferation of *P. conoideus* were investigated using nodal sections or lateral buds of *P. conoideus* on modified Murashige and Skoog medium. Shoots were rooted on MS medium without plant growth regulators (PGR). The results showed that lateral buds of *Pandanus* started to initiate growth after 4-7 days in culture. The best medium for shoot proliferation was MS containing either 0.5 mg/l BAP with 0.1 mg/l TDZ or 1 mg/l BAP with 0.5 mg/l KN, giving a multiplication rate of 16.5 shootlets per shootbud explant after 8 weeks. Rooting of shoots was successfully conducted on MS medium without PGR. Acclimatization of rooted plantlets was achieved on a mixed medium of cocopeat and soil (1:1).

Keywords: Buah merah *(Pandanus conoideus)*, lateral buds, BAP, TDZ, KN

Introduction

Pandanus conoideus Lam or 'Buah merah' of the Pandanaceae is native to East Indonesia namely Papua and North Maluku. The plant can be found from lowland to highland, growing best at 1200-1500 m above sealevel, generally in the open air or slightly in shaded area (Fig.1a). In Papua, there are about 30 different kinds of buah merah which can be divided into 3 groups based on its colour namely red (Fig.1 b), yellow and brown. Traditionally, the fruits are used for health promotion and maintenance as well as for curing several illnesses. The active compounds of the fruits are beta carotene, alpha tocoferrol, oleic acid, linoleic acid and decanoic acid (Yahya & Wiryanto, 2005).

Recently, it has been reported that the fruits of buah merah are potential for cancer medication (Budi & Paimin, 2002; Redaksi Trubus, 2005). As a result, there has been an over exploitation of this potential medicinal plant from its habitats. In order to anticipate their possible disappearance due to overexploitation in the wild, an efficient and effective technology for the mass propagation, conservation and cultivation of these plants should be developed.

Buah merah is generally propagated vegetatively by offshoots and stem cuttings or generatively by seeds (Fig.1 c), but their viability were very low (Budi & Paimin, 2002). Micropropagation has many advantages over the conventional propagation methods, since the technique allows mass clonal and pathogen-free production of plants at a high rate of multiplication all year round (George & Sherrington,1984).

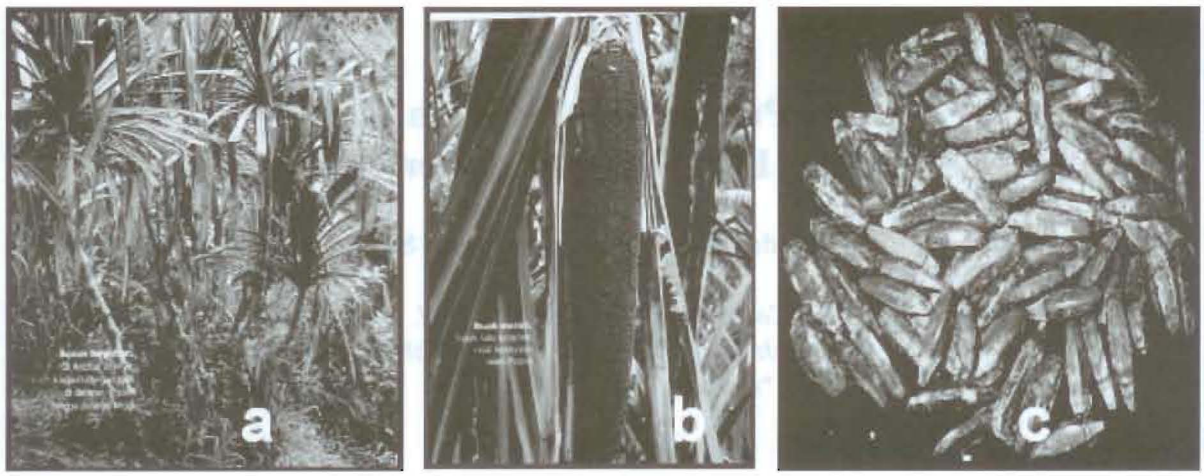

Figure 1. *In vitro* propagation of buah merah (*Pandanus conoideus*). (a). Mature plants in their wild habitat in Papua, (b). The fruit of buah merah, (c). Infertile seeds

However, the application of this technique has not yet been reported on this plant.

Cytokinin is a group of plant growth regulator (PGR) which has an important role in cell division, breaking dormancy, apical dominance, stimulates growth of dormant buds and influence shoot growth (Mok *et al.*, 2000). In plant tissue culture, cytokinin such as thidiazuron (TDZ), benzylaminopurin (BAP), and kinetin (KN) are generally very effective in supporting the formation and proliferation of *in vitro* shoots (George & Sherrington, 1984).

In this research, nodal sections or lateral buds of *P. conoideus* were used as explants with the aim of proliferating high quality shoots and regenerating plantlets. The effects of thidiazuron (TDZ), benzyl amino purine (BAP) and Kinetin (KN) on shoot bud induction and proliferation were investigated.

Materials and Methods

Preparation and inoculation of explants

Young plants of *P. conoideus* about 1-month old collected from Papua, Indonesia were grown in the glasshouse of Zhejiang University, PR China. Shoots of about 15 cm in height were collected from the glasshouse. The shoots were cut into ± 5 cm length and were then washed in running tap water, immersed in 70 % ethanol for 30 seconds, then rinsed several times with distilled water.

Under a laminar air flow cabinet, the shoots were further sterilized in 0.1 % $HgCl_2$ for 12 min and then rinsed three times with sterile distilled water. The shoots were transferred to a sterile Petri-dish containing a filter paper, after their leaves were removed, the shoots were cut into internode segments with lateral buds of about 1 cm in length for inoculation on the prepared medium.

All cultures were incubated in a growth room at 25°C and under a 16-h photoperiod with light intensity of 30 µmol/m2/sec provided by cool white inflorescent tubes. Subculture to the same medium was done every 4 weeks.

Preparation of medium

A basal medium of Murashige and Skoog (MS) (1962) containing 3% sucrose and 2.5 g/l phytagel was used in the experiment. The pH of the medium was adjusted to 5.8 by the addition of 1 N KOH or 0.1 N HCl before autoclaving at 121° C for 15 min at 1 atm.

Treatments

Shoot proliferation was induced by the addition of BAP (0, 0.5 and 1.0 mg/l) in combination with TDZ (0, 0.1, and 0.2 mg/l) and BAP (0, 0.5, and 1.0 mg/l) in combination with KN (0, 0.25, and 0.5 mg/l) into the medium. Totally there were 18 treatments with 2 replicates.

Rooting and Acclimatization

Rooting of shoots was induced in MS medium without the addition of PGR. A hundred rooted plantlets were removed from culture, rinsed in tap water to remove the agar medium and planted in plastic pots containing soil and coco peat medium (1:1). The potted plants were watered to saturation and covered with

transparent plastic bags for about 2 weeks and placed in the greenhouse.

Observations

Observation was done after 4 and 8 weeks on the number of shoots produced by each treatment. The data were statistically analyzed using least significant difference (LSD) test.

Results and Discussion

Nodal sections of lateral buds of *Pandanus* grown on MS basal media with several combination of TDZ and BAP or BAP and KN (Table 1) started to grow after 4-7 days in culture (Fig 2 a). The buds began to swell, then developed into new shoot buds. However, in a medium without plant growth regulators new shoot buds appeared after 4 weeks. Similar results were also shown in shoot cultures of Indian pandan (*Pandanus amaryllifolius)*, which could be continuously multiplied using the same combination of plant growth regulators, namely BAP and KN (Thimmaraju *et al*, 2005).

In this experiment, all media produced shoots but the highest number of shoots was obtained on treatment containing 2 combination of plant growth regulators BAP and TDZ or BAP and KN. The addition of 2 cytokinins produced highest number of adventive shoots compared with if only TDZ or BAP added. When KN was added into the medium without any other plant growth regulator, only 1-2 shoots were formed after 4 weeks (Table 1).

TDZ and BAP are the most effective cytokinins in inducing shoot proliferation *in vitro* in many plant species compared with other cytokinins (George & Sherrrington, 1984) .In this investigation, the highest number of *in vitro* shoots of buah merah (7.5 shoots after 4 weeks and 16.5 shoots after 8 weeks) was attained on MS medium containing 0,5 mg/l BAP and 0,1 mg/l TDZ (Fig. 2 b). The addition of BAP in combination with KN also showed the highest proliferation of adventive shoots (7 shoots after 4 weeks and 16 shoots after 8 weeks). It appears that the combination of these two PGR has a synergic effect in proliferating adventive shoots. The same phenomenon could also be seen on *Arachis stenosperma* and *A.villosa* where the addition of TDZ at low concentration (1.0 µM) in combination with BAP and IAA could increase the regeneration percentage of *in vitro* shoots of *Arachis* spp. (Laxmi & Giri, 2003).

In low concentration TDZ induces higher *in vitro* shoots proliferation than other cytokinins in many plant species, such as on Lentil (Khawar *et al,* 2004), whereas BAP have been reported to be effective in stimulating shoot proliferation in several plants like *Sida cordifolia* (Sivanesan & Jeong, 2007) and sungkai (*Peronema canescens*) (Imelda *et al.*, 1999). In buah merah, BAP alone at 1 mg/l or 0.5 mg/l could stimulate adventive shoots even though it was less than when it was added in combination with other cytokinins. KN is a less effective cytokinin compared with BAP or TDZ in inducing shoot formation (Table 1). The addition of KN as a single plant growth regulator showed a lack in its ability in proliferating *in vitro* shoots. KN at 0.25 or 0.5 mg/l can only produce 1-2 shoots within 4 weeks, but interaction between BAP and KN significantly influence shoot bud induction and proliferation of *P. conoideus*. Similar observations have been reported in other plants such as *Chlorophytum borivilianum* (Purohit *et al*, 1994) and *Hibiscus cannabinus* (Herath *et al*, 2004).

However, prolonged culture containing 0.022 mM BAP resulted in vitrified plantlets. A higher percentage of normal plantlets of *P. amaryllifolius* was recorded with decreasing concentrations of BAP for four consecutive sub-cultural passages (0.022 mM, 0.011 mM, 0.005 mM, 0.002 mM) before transferring to rooting medium (Gangopadhyay, 2004).

Rooting of *in vitro* shoots is generally done on a medium containing root-promoting plant growth regulator such as IBA or NAA. Gangopadhyay (2004) used solid and liquid MS media supplemented with 0.01 mM IBA and 0.002 mM KN for root induction in *Pandanus amaryllifolius*. However, in certain plant species like buah merah, roots can be directly formed without the addition of auxin (Fig.2 c, d). The same condition has also been found in *in vitro* shoots of banana (Imelda, 1991), and *Amorphophallus muelleri* (Imelda *et al*, 2006). *In vitro* shoots of *P. amaryllifolius* can also be rooted in a half-strength MS solid medium without PGR (Thimmaraju *et al,* 2005).

Acclimatization is an adaptation process for *in vitro* plantlets from the *in vitro* condition, in which both temperature or humidity was under control, to the *ex vitro* green house condition that

are more fluctuative. This process is an important step for acclimatization of all regenerated plants produced by tissue culture since *in vitro* plants are very sensitive to temperature and humidity fluctuation in the greenhouse and easily attacked by diseases. Acclimatization of some tree plants was still difficult due to their physiologal, morphological and anatomical abnormalities induced during the *in vitro* culture (Ziv, 1994). In this research, the acclimatization of buah merah plantlet on soil and cocopeat medium was resulting in 90 % of 100 planlets survived (Fig 2 e).

Table 1. The effect of plant growth regulators on shoot bud initiation of buah merah

Treatment No	PGR (mg/l)			Number of shoots	
	BAP	TDZ	KN	4 weeks	8 weeks
1	0	0	-	1,0 ± 0,29 *a*	2,0 ± 0,33 *a*
2	0	0.1	-	4,0 ± 0,26 *b*	5,0 ± 0,00 *c*
3	0	0.2	-	5,0 ± 0,29 *bc*	8,0 ± 0,29 *de*
4	0.5	0	-	4,0 ± 0,17 *b*	7,0 ± 0,50 *d*
5	0.5	0.1	-	7,5 ± 0,29 *f*	16,5 ± 1,00 *i*
6	0.5	0.2	-	6,0 ± 0,50 *cde*	16,0 ± 0,58 *i*
7	1.0	0	-	5,0 ± 0,50 *bc*	12,5 ± 1,00 *f*
8	1.0	0.1	-	6,5 ± 1,00 *def*	14,0 ± 0,58 *gh*
9	1.0	0.2	-	6,0 ±1,00 *cde*	15,5 ± 0,58 *hi*
10	0	-	0	1,0 ± 0,29 *a*	2,0 ± 0,33 *a*
11	0	-	0.25	1,0 ± 0,50 *a*	3,0 ± 0,29 *b*
12	0	-	0.5	2,0 ± 0,50 *a*	4,0 ± 0,29 *bc*
13	0.5	-	0	4,0 ± 0,50 *b*	7,0 ± 0,29 *d*
14	0.5	-	0.25	5,0 ± 0,50 *bc*	8,0 ± 0,29 *de*
15	0.5	-	0.5	5,5 ± 1,00 *cd*	9,5 ± 0,29 *e*
16	1.0	-	0	5,0 ± 1,00 *bc*	12,5 ± 0,58 *fg*
17	1.0	-	0.25	6,0 ± 1,00 *cde*	14,0 ± 0,58 *gh*
18	1.0	-	0.5	7,0 ± 1,00 *ef*	16,5 ± 0,87 *i*

Note : Means within column followed by the same letter are not significantly different according to LSD test at 5% level.

Figure 2. *In vitro* propagation of buah merah (*Pandanus conoideus*) (a). Shoots appear from lateral buds, (b). Shoots proliferation in MS medium+0.5 mg/l BAP+0.1 mg/l TDZ (c). Plantlets in rooting medium (MS without PGR), (d). Rooted plantlets, before acclimatization (e). Young plants from tissue culture in pots containing soil and cocopeat medium

Conclusion

Buah merah (*P. conoideus*) can be propagated through lateral bud proliferation. The best medium for shoot proliferation was MS + 0.5 mg/lBAP + 0.1 mg/l TDZ or MS + 1 mg/l BAP +0.5mg/l KN giving a multiplication rate of 16.5 shootlets/explant after 8 weeks. Rooting of shoots was successfully conducted on MS medium without PGR. Acclimatization of rooted plantlets gave a 90 % survival rate.

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