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Submission date: 07-Jan-2022 12:23PM (UTC+0700)
Submission ID: 1738396355
File name: culenta-Var-Antiqourum-In-Various-Pesticides-Concentration-1.pdf (315.58K)
Word count: 2194
Character count: 11930

Aseptic Culture Of Apical Bud Of Japanese Taro (C.Esculenta Var. Antiqourum) In Various Pesticides Concentration

Feranita Haring, Yunus Musa, Enny L. Sengin, Rinaldi Syahril, Muhammad Nasrun

Abstract: Series of studies were conducted to obtain Japanese taro propagules. This initial study was conducted at the Plastics House and Laboratory of Seed Production Unit and Plant Micro-propagation, Teaching Industry, Hasanuddin University, Makassar from March to June 2013. Research method using a group randomized design, and the data were analyzed by using analysis of variance and followed by Honestly Significant and Difference test (HSD). The concentration of fungicide and bactericide for aseptic culture of apical buds of Japanese Taro tested ranged from 0-10 g L^{-1}. The results showed that: the use of pesticides (fungicides of Dithane M-45 and Agrept bactericidal) each 10 g L^{-1} with the lowest percentage of contamination (20%), pesticide treatment had no effect on the percentage of time to germinate and browning.

Keywords: aseptic culture, Japanese taro, fungicide, bactericide.

1. INTRODUCTION

Japanese taro/satoimo had become agribusiness commodity and potentially lucrative for export nowadays, beside that, it also can be an alternative agricultural products to overcome the issue of national food security. Bantaeng Regency of South Sulawesi is one of the regency that develop Japanese taro for export to Japan. Seed/tuber limited is a constraint for farmers. These problems can be solved by input of tissue culture techniques in Japanese taro seedling. In Indonesia the development of horticultural nursery industry and perennial crops (forestry) with tissue culture technology is developing rapidly as the potato crop (Wattimena, 1989, in Wattimena, et al.1992) [1], Manau Rattan (Gunawan and Yani, 1986; Gunawan 1990 in Wattimena et al. 1992) [1]. Bananas, Alokasia, and teak (Sulistiani and Ahmad Yani, 2012) [2]. A common obstacle which is faced by researchers in tissue culture is the high contamination.

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All of the sources of contaminants, explants contaminant were hardest to overcome because in this case the sterilization method should be selective, only eliminate unwanted micro-organisms with minimal disruption to the explants. Specifically, the most appropriate method of sterilization which would be obtained from *trial and error* (Gunawan, 1995) [3]. Levels of contamination in aseptic culture is mainly caused by fungi and bacteria, this can be reduced by the maintenance of mother plants in the greenhouse with pest control and intensive crop diseases. Besides, part of the plant used as explants were young tissue, actively growing. Young plant tissue has a higher power regeneration, the cells are still actively dividing, and relatively clean (Zulkarnain, 2009) [4]. A variety of treatments to clean the dirt that is on the surface of the explants with disinfectant. To improve the effectiveness of sterilization, used Tween-20, Tween 80, or a soft liquid detergent as a wetting agent (Yusnita, 2004) [5]. In addition, giving of bactericide and fungicide, can also be done to obtain a better rate for the sterilization of explants to be cultured (Nugroho and Sugito, 2001) [6]. Shoots Sterilization of intact plant by Wetter and Constabel, (1991) [7] starting with 70% ethanol rinsed, and then dipped in a 7% solution of natrium hypochlorite (50% bleach solution) for 5-10 minutes added Tween 20 or Tween 80 (0 , 01%), then rinsed 5-6 times. It is almost the same in research Ying Ko, Ping Kung and Donald (2008) [8], taro shoots sterilization begins with flushing on 70% ethyl alcohol, then soaked in a solution of 7% natrium hypochlorite for 8 minutes and rinsed 4 times with sterile distilled water. Sterilization of bud weevil of Alokacia by Sulistiani and Ahmad Yani (2012) [2] before treatment as above soaked first in a solution of fungicide and systemic bactericide. Based on the explanation above, it has conducted the experiments of the aseptic culture of apical buds of Japanese taro at various various concentrations of the fungicide and bactericide.

2. MATERIAL AND METHODS

The experiment was conducted in a plastic house, and in the Laboratory of Seed Production Unit and Micro-propagation of plants, Teaching Industry, Hasanuddin University, Makassar. Implemented in March to June 2013. Tubers Japanese taro obtained from nurseries and garden of PT Global Seafood and farmers garden, Bantaeng Regency, South Sulawesi Province. Tubers planted in a plastic tub, which was filled with rice husk. Before tubers planted, soaked in fungicide and bactericide each 2 g L^{-1}, maintenance was conducted by watering and fertilizing. Fertilization is done by dissolving fertilizer Felo 1.5 g L^{-1} of water, the same water is given once a day watering. After leaf, 2-3 leaves are used as explants . Explants consisted of buds with a little bulb at the base. Explants sterilization stages are as follows: (a) The young plants are taken along the tuber apical and buds and wash in running water while peeling the outer sheath of skin and skin buds with a knife until the white and clean. (b) Enter in the soap solution of Tween 80 (0.01%), soak while occasionally shaken, then rinse with sterile water until the tween foam exhausted. (c) explan were soaked in an antiseptic solution of Povidone Iodine for 15 minutes, then rinsed with sterile distilled water for 3 times. (d) explants were soaked in a solution of fungicide Dithane M-45 according to treatment (0, 2.5, 5.0, 7.5, 10) g L^{-1} for 60 minutes. Then rinsed with sterile distilled water for 3 times. (e) explants were soaked in a solution of bactericidal Agrept (0, 2.5, 5.0, 7.5, 10) g L^{-1} for 60 minutes, then rinse with sterile distilled water 3 times. (f) The next stage in the laminar air flow, explants were soaked in 70% alcohol for 10 minutes, then rinsed with sterile distilled water 1 times. (g) explants were soaked in 50% bleach solution Bayclin for 15 minutes, then rinsed sterile distilled water 4 times. (h) Peel the midrib leaf and damaged tuber parts caused by treatment of Bayclin, then planting on modified medium MS (5 g L^{-1} macro and micro nutrient fertilizers Felo), MS vitamins, 30 g sucrose, 7 g of jelly. Store at incubation room with temperature of 25 ± 2^0C.

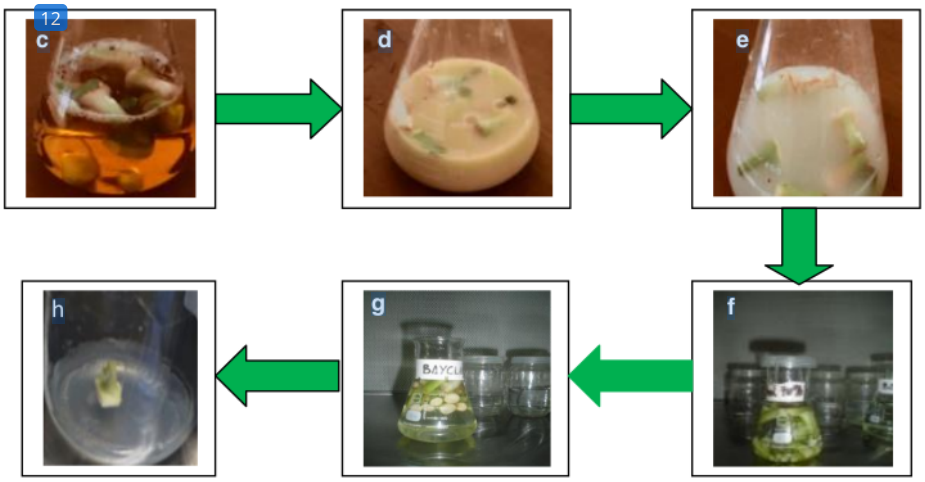

Figure 1. Stages of explants sterilization of apical buds of Japanese taro

3. RESULTS AND DISCUSSION

Observations at aseptic culture of Japanese taro plant consisting of time to germinate of Japanese taro, was not influenced by the concentration of Dithane M-45 and Agrept (Table 1), with the time to germinate between 16 to 17. While the percentage of observations of contamination, the concentration of Dithane M-45 and Agrept each 10 g L^{-1} caused decrease in the percentage of contamination (20%) compared with controls (46.67%), as well as other concentration is the concentration of Dithane M-45 and Agrept respectively 7, 5 g L^{-1}; 5.0 g L^{-1} ; and 2.5 g L^{-1} resulted in contamination percentage respectively 26.67%; 33.33%; and 43.33% (Table 1). The concentration of Dithane M-45 and 2.5 g L Agrept-1 is not different from controls. Whereas, percentage of browning, also was not affected by the concentration of Dithane M-45 and Agrept (Table 1)

Table 1. Sprout Time Japanese taro plants at various concentrations of Dithane M-45 and Agrept

Type of treatment	Germinate time (hst)	Percentage of contamination (%)	Percentage of browning (%)
crontrol (0)	tn	46.67 a	40.00 tn
2,5	23.37	43.33 a	33.33
5,0	17.67	33.33 b	36.67
7,5	22.93	26.67 c	30.00
10,0	20.83	20.00 d	33.33

Description: - numbers followed by the same letter in the same column are not significantly different at α HSD test α 0.05 - Tn = not significant at variance

From the results obtained concentrations of Dithane M-45 and Agrept no effect on the time to germinate. This is presumably because the media is not added growth regulators. According to Wetherell (1982) [9] the interaction and balance between auxin and cytokinin in the medium and produced by plants endogenously determining the direction of the development of a culture. Further added by Wattimena et al. (1992) [1] in addition to genotype, explant tissue physiology, and the growth environment, growth and morphogenesis is also influenced by the media, including the composition of the media and plant growth regulators. The percentage of contamination can be reduced with the addition of Dithane M-45 fungicide and bactericide Agrept, presumably because both are systemic pesticides. According Sulistiani and Ahmad Yani (2012) [] before soaking with the bleach solution, explants are soaked in a row in a fungicide and bactericide solution to increase the success of the sterilization process. A high percentage of browning is thought to occur because the taro release phenolic compounds that accumulate around scar tissue as a result of the cuts and no antioxidant or absorbent material phenolic compounds into the media. According Thaib (1977) [10], at the end of the culture of a palm trunk, giving the composition of the balance of growth regulators can minimize browning. Further added by Zaid (1985) [11], auxin can inhibit polyphenol syntesis so can reduce the browning of explants, while cytokines can stimulate explant browning. Sulistiani and Ahmad Yani (2012) [9], in plants such as bananas and Japanese taro contain high phenolic compounds, browning can be overcome with the addition of an antioxidant (ascorbic acid, citric acid, L-cysteine hydrocloride) or absorbing material that absorbed phenolic compounds such as activated charcoal or polyvinylpyrolidone into the planting media. It can also planting te explants earlier on MS media without growth regulators, then add liquid MS media with growth regulators.

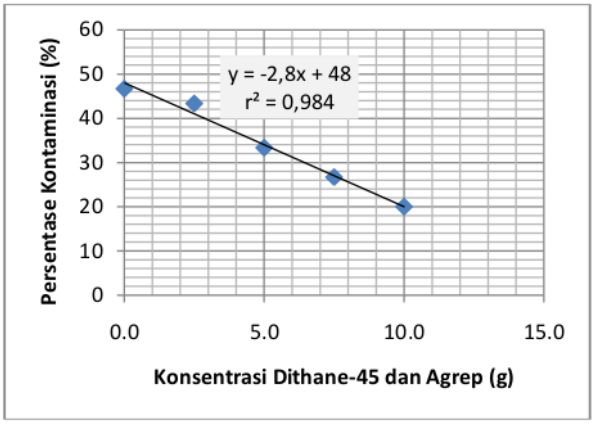

Figure 2 Percentage of contamination at various concentrations of the fungicide Dithane M-45 and bactericidal Agrept

Figure 2 shows the percentage of the response to the contamination of concentration Dithane M-4 and Agrept give the linear effect, with the equation $y = -2,8 x + 48$ (r^2 =0,984) that can be interpreted any additional units of g L^{-1} concentrations of Dithane M-45 and Agrept will lower the percentage of contamination. Can be seen in the picture above that the addition of 2,5 to 10,0 g reduced the percentage of contamination.

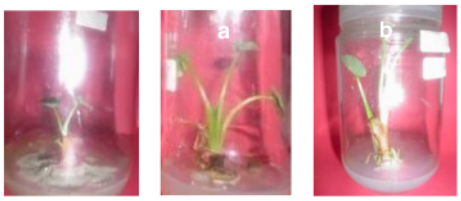

Figure 3. Plantlets were contaminated (a) sap (b) and not contaminated (c)

4. CONCLUSION

Based on the results of the experiment the concentration of Dithane M-45 and Agrept produce aseptic prppagules of taro plant obtained, that the concentrations of Dithane M-45 and Agrept each 10 g L^{-1} can reduce the contamination percentage to 20%. Time to germinate and the percentage of browning is not affected by the experimental treatment performed. To obtain the propagules number of Japanese taro are aseptically in large quantities, in addition to reduce contamination, browning problems also need to find a solution.

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