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Aseptic Culture of Apical Bud of Japanese Taro (C.Esculenta var. Antiqourum) in Various Pesticides Concentration

by Rinaldi Syahril

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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 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) explants 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°C.

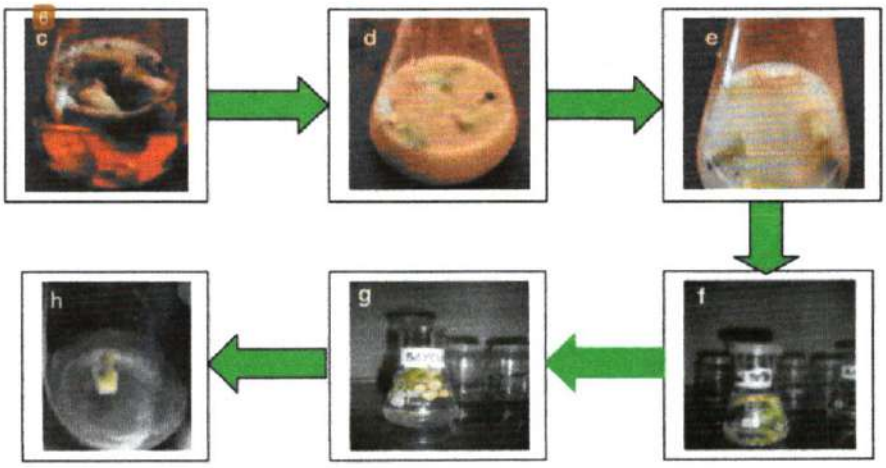

Figure 1. Stages of explants sterilization of apical buds of Japanese taro

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.

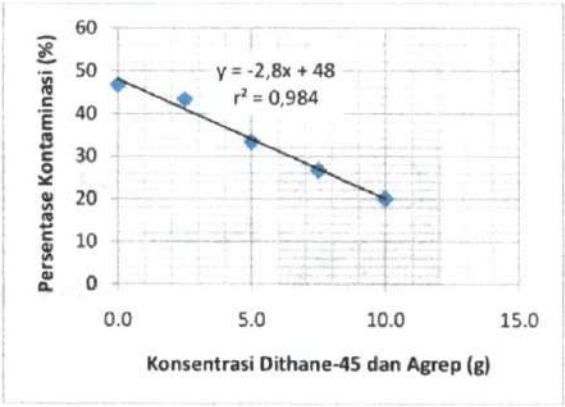

Figure 2 Percentage of contamination at various concentrations of the fungicide Dithane M-45 and bactericidal Agrept

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)

1999; Baba *et al*., 2011) and in some grass-legume mixtures; it is relatively resistant to heavy grazing compared to other legumes (Olanite *et al*., 2004). In introducing legumes into grassland stand, good establishment is essential. To ensure such a good crop, high levels emergence and seedling growth are important parameter to allow a good competition against weeds and a rapid growth against competing grasses.

Rapidity and emergence percentage have been shown to be influenced by sowing depth. Sowing depth at an optimum depth is an important aspect to obtain higher emergence and good quality of seedlings. Sowing at shallow depth generally stimulates more seed germination and seed emergence than seeds sown on the soil surface, because the former provides a moist environment around them and prevents seeds and seedlings from drying out, as well as preventing damaged by insects.

Deeper sowing usually delays and reduces emergence and vigor of seedlings, frequently leads to poor establishment, despite more deeply sown seeds having a greater chance of accessing soil moisture (Buakum *et al*., 2013). Excessive sowing depth prevents seed from emerging above the soil surface and thus prevents their survival. Therefore, determining optimum planting depth for individual species is critical for establishing productive stands in the field.

Emergence and seedling growth in the field are also related to light level. Light is generally known as one of the ecological factor exerting the greatest effect on germination, seedling emergence and development (Baiyeri, 2006). The ability to grow rapidly when shaded is an important adaptation mechanism when species deprived of their full complement of sunlight (Bjorkman *et al*., 2000). Both in pure and mixture with grasses, competition for light from weeds and grasses will inhibit the establishment of legume in pastures. It has been reported that legume inter-seeding into grassland is most successful when competition from grasses is reduced through herbicide application or by grazing swards to low height before seeding legumes (Teutsch and Fike, 2003).

Seed germination and emergence is species specific. Seeds of some species germinate and emerge equally in light and dark (Norsworthy and Oliveira, 2005), some require light to stimulate seedling growth (Li *et al*., 2012; Kalmbacher, 2013), and others are favored by low light intensity (Gardener *et al*., 2001; Jellicorse *et al*., 2009).

There is limited documentation on the effect of depth of sowing and shade on emergence, growth and biomass production of centro. Such information is useful to provide agricultural extension delivery to farmers for increased forage productivity. The present study was designed to determine the effects of sowing depths of seeds and shade on emergence and seedling growth of centro.

2. Materials and Methods

2.1. Experimental Site and Materials

A pot experiment was conducted in June 2013 in grassland experimental field of Faculty of Animal Science, Hasanuddin University Makassar, Indonesia (5°14' S, 119°42' E) with 7 m elevation above sea

levels. The mean annual rainfall was 1,465 mm per year which falls mainly from December until March which accounts for more than 60% of the total annual rainfall.

Seeds of *Centrosema pubescens* were obtained from plant growing naturally in the campus of Hasanuddin University South Sulawesi, Indonesia, one month before experiment started. After air drying, seeds were stored under temperature of 4° C. The floatability method was used to assess seed viability. The seeds were placed in a 1 litre container that was later filled with water; floating seeds were assumed to be unviable, while sunken seeds to be viable. The floating seeds were discarded from the experiment.

2.2. *Experimental Procedure*

This study was arranged in split plot completely randomized design, with two light levels (shade 50% and full sunlight) as the main plot and sowing depth (2, 4, 6 and 8 cm) as subplot with three replications, making 24 pots in total. The pots (14 cm in height, bottom diameter of 12 cm and top diameter of 16 cm) were first filled with soil to a certain depth at which 40 seeds each were placed. The pots then filled with additional soil to obtain the desired planting depth. The soil was clay loam texture of ultisol soil that had been passed through a 5 mm sieve. Artificial shade – 50% of full sunlight was provided by black plastic screen fixed in polyvinyl frames with dimension of 215 x 80 x 38 cm and placed under field conditions.

The pots were watered with tap water to field capacity. Once a day, pots were checked for seedling emergence. Seedlings were thinned to five plants per pot after full cotyledon extension stage was attained. Cumulative emergence over the entire duration of experiment was measured.

Experiment was terminated at 16 days after sowing when cotyledon began to yellow. At harvest, all seedlings were measured for percentage of emergence, rapidity of emergence, seedling height, seedling diameter, number of leaves, shoot and root biomasses, seedling biomass and shoot-root ratio. Emergence of seedling was noted as the first appearance of any portion of seedling above soil surface. Stem height of seedling was measured from soil surface to the tip of the uppermost stem, using a ruler. The diameter of stem seedlings was measured with micrometer screw gauge at 1 cm above soil surface.

At harvest, soil was washed from below ground hypocotyl and roots in running water. Roots from below ground hypocotyl were removed and mixed with basal roots. Each shoot and roots of seedlings were oven dried at 65°C for 72 hours to determine their biomass dry weight. Shoot seedling biomass was measured as dry weight of seedlings minus roots.

2.3. *Data Analysis*

SPSS version 16 statistical software were used to analyze the effects of light levels, sowing depth sand their interaction on emergence percentage, rapidity of emergence, seedling height, seedling diameter, number of leaves, shoot and root biomasses, seedling dry weight and ratio of shoot to roots. To meet assumption of ANOVA, data for percentage of emergence were arc-sin-transformed prior to analysis. Least significant difference (LSD) was used to compare treatment means.

3. Results and Discussion

3.1. Seedling Emergence

A two way ANOVA showed that emergence percentage was significantly affected by sowing depth, but not by shade and interaction between sowing depth and shade (Table 1). Seedling emergence occurred at all sowing depth, however seedling emergence was the highest at 2 cm sowing depth and then decreased with increasing sowing depth (Table 2). Increasing sowing depth from 2 cm to 8 cm reduced seedling emergence by 34.33%. Poor emergence at deep sowing is consistent with that of Agbo (2012) and Aikins *et al.* (2006) that deep sowing can significantly affect crop emergence and yield and this may be attributed to relatively more favorable oxygen, light, temperature and soil moisture conservation capacity at shallower depth.

It seems a greater fraction of seed reserves was exhausted by the time of seedling emergence because seedling that emerged from deeper sowing depth showed a smaller size.

The highest seedling emergence at 2 cm of sowing depth indicates that seedlings of centro had the highest potential for successful establishment at that depth. Emergence is an important event that affects the success of crop. Rapid, uniform and complete emergence of vigorous seedling, leads to higher yield potential by shortening the time from sowing to complete ground cover, allows the establishment of optimum canopy structure to minimize plant competition, maximize yield and provide plants with time and spatial advantages to compete with weeds (Soltani *et al.*, 2001).

The days to emerge were significantly longer with increasing sowing depth (Table 2), which is also in line with Ren *et al.* (2002) and Juan *et al.* (2011). For all sowing depth, the fastest seedling emergence occurred from 2 cm sowing depth and delayed with increasing sowing depth. Seeds sown at 8 cm depth took significantly longer time to emerge than those at 6, 4 and 2 cm sowing depth.

Delaying of emergence at deeper sowing might be due to seedlings requiring more energy resources to penetrate the thicker soil layer after germination from greater depth and thus required more time before

Table 1. Influence of sowing depths, shade levels and their interaction on emergence, morphological characteristics and seedling biomass of *Centrosema pubescens*.

Parameter	Sowing depth	Shade	Sowing depth x Shade
Emergence percentage	**	NS	NS
Days to emergence	**	NS	NS
Seedling height (cm)	**	*	NS
Stem diameter (cm)	NS	*	NS
Number of leaves (/plant)	NS	NS	NS
Shoot biomass (g/plant)	*	*	NS
Roots biomass (g/plant)	*	NS	NS
Seedling biomass	*	*	NS
Shoot-root ratio	NS	*	NS

*P <0.05 ** P <0.01 NS indicates not significant

finally emerging from the soil surface and probably more susceptible to establishment failure (Burmeier *et al*., 2010). This implies that "shallow emerger" like centro has a competitive advantage over seedlings originating from greater depths because they can start assimilating before the others have even reached soil surface, which may increase their establishment chances.

There was no significant difference between unshaded and shaded plants on emergence percentage (Table 1). However, data from Table 2 shows that emergence of shaded plants was higher than that of unshaded plants, which consistent with previous works (Baiyeri, 2006; Gardener *et al*., 2001). Becker *et al*. (1988) noted that seeds under full sunlight might have experienced both heat and moisture stress in the upper layer of the soil. However, the days to emergence in this study was not influenced by shade levels.

3.2. *Morphological Traits*

The morphological traits, except number of leaves were significantly influenced by sowing depth and/or shade (Table 1). Seedling height was the greatest at 2 cm of sowing depth and decreased with increasing sowing depth (Table 2). Similar effect of sowing depth on seedling height and seedling diameter has been reported by Seeiso and Materechera (2011) and Wu *et al*. (2011). Lower seedling height at deeper seeding might be attributed to longer seedling emergence as influenced by difficulty of seedlings to push their shoots through the thick soil layer.

There was a significant effect of shade on seedling height and seedling diameter (Table 1) in which seedling height was greater under shade than under full light (Table 2). This is consistent with other studies which show that in a light-limited environment, photosynthetic allocation patterns favor shoot elongation and hence, increase light harvesting capabilities (Wang *et al*., 1994). However, the greater seedling height under shade in this study occurred at the expense of seedling diameter that was significantly lower under shade. This study corroborates the findings reported by Akhter *et al*. (2009) and Mayoli *et al*. (2009). Apical dominance tends to increase when plants are subjected to shade, due to a decrease in the production of photosynthetic and the high levels of auxin at the stem apex bud (Woodward and Bartel, 2005).

There was no significant effect of sowing and shade on number of leaves. However, number of leaves was higher under full light than under shade conditions. Wadud *et al*. (2002) and Cookson and Granier (2006) observed similar trend of number of leaves under shade. This may be due to lower production of photosynthetic under low light levels.

3.3. *Seedling Biomass*

There was a significant effect of sowing depth and shade on shoot biomass, root biomass and seedling biomass (Table 1). Shoot, root and seedling biomasses were the highest at 2 cm of sowing depth and grown in full light conditions and decreased with increasing of sowing depth and lowering light intensity (Table 2) and this was similar to that reported by Paul *et al*. (2012) and Wu *et al*. (2011). Decreased seedling biomass when sowing depth increased may be due to

Table 2. Emergence, morphological traits and seedling growth of *Centrosema pubescens* as influenced by sowing depths and shade

Treatments	Percentage of emergence	Days to emergence	Seedling height (cm)	Seedling diameter (cm)	Number of leaves per plant	Shoot biomass (g/plant)	Root biomass (g/plant)	Seedling biomass (g/plant)	Shoot-root dry weight ratio
Sowing depth									
2 cm	57.465^{d}	2.671^{a}	4.330^{c}	0.773^{a}	4.800^{a}	0.143^{c}	0.023^{b}	0.166^{d}	6.217^{a}
4 cm	52.025^{c}	3.002^{ab}	3.750^{c}	0.903^{a}	4.930^{a}	0.124^{bc}	0.019^{a}	0.143^{c}	6.526^{a}
6 cm	42.080^{b}	5.173^{b}	3.000^{b}	0.893^{a}	4.670^{a}	0.116^{b}	0.017^{a}	0.127^{b}	6.834^{a}
8 cm	37.920^{a}	6.005^{b}	2.200^{a}	0.863^{a}	4.930^{a}	0.099^{a}	0.015^{a}	0.114^{a}	6.566^{a}
Shade									
0%	44.58^{a}	4.167^{a}	2.010^{a}	1.037^{b}	0.154^{a}	0.126^{b}	0.025^{b}	0.154^{b}	4.500^{a}
50%	52.28^{a}	3.250^{a}	4.480^{b}	0.806^{a}	0.125^{a}	0.113^{a}	0.012^{a}	0.125^{a}	9.416^{b}

Means of each parameter followed by different letters in each column are significantly different at $P < 0.05$ level.

slower time to emergence and hence delaying time to start photosynthesis.

Both shoot biomass and root biomass were depressed by shade, leading to a decreased of seedling biomass. However, root biomass was more depressed under shade. Shade reduced shoot biomass by 11.50% while roots biomass by 64%, which implies that under shade conditions plants allocated more of their dry matter to shoots than to roots growth and development.

Shoot biomass and root biomass were both influenced by sowing depth and shade. The allometric relationship between shoot growth and root growth as expressed by shoot-root dry weight ratio in different seeding depth and light levels are shown in Table 2. The results were not significantly different between sowing depths but shoot-root ratios in shaded plants were significantly higher than that of unshaded plants.

The relatively high shoot and root ratio in shade-grown plants may be an environmentally induced adaptation which permits higher rates photosynthesis under low light intensity. The high shoot-root ratios indicated that seedlings under shade conditions located more of their dry matter to shoot than to root and this may decrease resistance of plant to draught conditions. because the higher shoot-root ratio reduced draught tolerance in crop plants (Eshanullah *et al*., 1999).

4. Conclusion

It can be concluded that sowing seed at 2 cm significantly improved in the percentage of emergence, time to seedling emergence, seedling diameter and seedling biomass weight. The shade produced improvement in seedling height, shoot biomass and shoot-root ratio. Considering the soil and weather conditions of the experiment, centro seeds should be sown at a depth of 2 cm to obtain the best growth and dry matter yield.

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4. CONCLUSION

Based on the results of the experiment the concentration of Dithane M-45 and Agrept to produce aseptic propagules 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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