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THE STRENGTH OF MS MEDIA AND STERILIZATION TECHNIQUE ON RED DRAGONFRUIT (*HYLOCEREUS POLYRHIZUS)* SEED GERMINATION

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ABSTRACT

This research aimed to findout efficient sterilization method and MS media to germinate dragonfruit seeds *in vitro*, conducting at Biotechnology Laboratory, Agriculture Faculty, Tadulako University, using factorial completely randomized design. The first factor was sterilization technique (S), *i.e*: S1 (The sliced fruit was washed with sterile distilled water three times, then rinsed in Bayclin (household bleach containing 5.25% NaOCl) 5% for 15 minutes, followed by washing in sterile distilled water three times. S2 (The seeds were taken from the fruit meat, then rinsed in 5% Bayclin for 15 minutes, then washed with sterile distilled water three times. S3 (The seeds were taken from the fruit meat, then rinsed in 15% Bayclin for 15 minutes, then washed with sterile distilled water three times. The second factor was the strength of MS media (M), *i.e* full strength MS (M1) and half strength MS for macro and micro nutrients (M2). Each treatment combination consisted of 35 seeds, and was replicated three times. Germination responses were observed as time to germinate, germination percentage and percentage of opened cotyledone seedlings. All datas were subjected to Analysis of Variance and the mean differences among the treatments were analyzed using Honest Significant Difference (HSD) at the level of 1%. The results showed that removing seed pulp, prior to rinsing the seeds in 15% Bayclin for 15 minutes followed by washing in sterile aquadest three times, and cultured in half MS produce the fastest and highest seed germination of 99.05% with 92.38% opened cotyledon seedlings after 2 weeks in culture.

Key Words: Dragonfruit, germination, in vitro, MS strength, sterilization.

INTRODUCTION

Dragonfruit (*Hylocereus* sp.) of Cactaceae family and Hylocereanea subfamily as discribed in Wikipedia enciclopedia (Anonym, 2014a), is a new introduced fruit served as fresh fruit, juice or an ingredient of many processed foods especially deserts.

Like most other Cactaceae, the plant is resistant to drough and tropical heat, by which it can produce good fruits in low rainfall areas such as Palu City and its surroundings (Anonym, 2014b). Eventhough the origin of the plant is from Central and South Americas (Sven Merten, 2003), current biggest producer has been Vietnam and Thailand as published in Axis Research 2011 (Anonym, 2011 and Nguyen Phuong Thao *et al*., 2004). In Indonesia, the plant has been cultivated widely at Pasuruan, Jember, Mojokerto, and Jombang (Kristanto, 2009), but just started to be cultivated in Palu and its surroundings, especially at Sigi Biromaru Subregence. Therefore, it is needed a huge number of good plant source which can be achieve through tissue culture method.

Research on micropropagation of dragonfruit has been severily reported such as the use of plant regulator in initiation and shoot propagation (Rodziah *et al*., 2010; Chaturani and Jayatilleke, 2006; Samudin, 2009a), but efficient protocol in seed sterilization and the use of media to efficient germination is still out of interest.

Table 1. Composition of Full and Half Strength MS Media

Chemical Stock	Full Strength MS (mg/L)	Half Strength MS (mg/L)
NH_4NO_3	1650	825
KNO_3	1900	950
$CaCl_2.2H_2O$	440	220
KI	0.83	0.415
$CoCl_2.6H_2O$	0.025	0.0125
$MgSO_4.7H_2O$	370	185
$MnSO_4.4H_2O$	22.3	11.15
$ZnSO_4.7H_2O$	8.6	4.3
$CuSO_4.5H_2O$	0.025	0.0125
KH_2PO_4	170	85
H_3BO_3	6.2	6.1
$Na_2MoO_4.2H_2O$	0.25	0.125
$FeSO_4.7H_2O$	27.8	13.9
Na_2-EDTA	37.26	18.63
Thiamine-HCl	0.1	0,1
Pyridoxine-HCl	0.5	0,5
Nicotinic Acid	0.5	0,5
Glycine	2.0	2,0
Myo-Inositol	100	100
Sukrosa	30000	30000
Agar	8000	8000

Source : Modified from Taji *et al*. (1997).

Seeds of dragonfruit are located in fruit meat, in innert part of the fruit. Thus, they are relatively sterile, do not need to be sterilized hardly, using a lot chemicals. It is a wise procedure to sterilize dragonfruit seeds severely, using a low concentration of soft chemicals, and culture the seeds in a lower minerals concentration, by which the laboratory consumption of chemicals could be saved.

The use of MS (Murasighe and Skoog, 1962) media to germinate seeds of many plants has been common. Chaturani and Jayatilleke (2006) reported that *in vitro* germination percentage (98,5%) of dragonfruit seeds using MS media, was significantly higher than those obtained *in vivo*. Rodziah e*t. al*. (2010) reported the highest germination percentage of red purple dragonfruit seed after three weeks in culture was obtained in MS (87.0%) compared to other basal media, while addition of indolebutyric acid (IBA) alone or in combination with benzylaminopurine (BAP), generally decreased the germination percentage.

Basri (2004) stated that MS media is a high macro and micro nutrients basal media that has been used by nine among ten laboratories in the world. In Biotechnology Laboratory of Agriculture Faculty at Tadulako University, germination of various plant seeds such as kiwi (Kasim, 2012), apple (Yuliana, 2005; Samudin, 2009b) and pear (Ridwan, 2006) has been done using a half strength MS media.

Beside the use of media, an efficient technique in sterilization physically as well as chemically is important to save high chemicals used in laboratories. Commercial products used as sterilizing agents such as Bayclin (a local household bleach containing 5,25% NaOCl), instead of clorox (laboratory standard 100% NaOCl), is more reliable to be used, due to easier to be found in local merkets and significantly cheaper in price. Therefore, it is important to findout an efficient as well as effective procedure in sterilizing and germinating the seeds.

MATERIALS AND METHOD

Explant Source. Healthy fresh mature red dragonfruits obtained from local supermarket, were used as explant source. A three quarter middle part of the fruit was taken, and cleaned with running tap water for 5 minutes. This part would be devided into three parts, to be treated differently in sterilization. The seeds of each sterilization procedure were then cultured in full and half strength MS media. Composition of the full and half strength MS media can be seen in Table 1.

Culture Condition. Full and half strength MS media had been used, with 3% saccarose, 0.1 g/L myoinnositol, 1 ml/L MS vitamins, and 8 g/L agar. The pH of each media was adjusted to 5,7-5,8 using 0,5 N NaOH and HCl, prior to autoclaving at 121^{o}C, 17.5 psi for 15 minutes. All cultures were incubated in continuous light with illumination intensity of 2500 lux at the top of culture vessels using fluorescent lamps.

Design and Experimental Procedures. The research was arranged as factorial

completely randomized design with two factors. First, sterilization technique consisting of three levels i.e:

S1 : The fruit meat including seeds of 3x3x3 cm^3 was washed with sterile distilled water for three times, then rinsed in Bayclin 5% for 15 minutes, followed by washing in sterile distilled water for three times. The sterile fruit meat then was brought to the air flow laminar, and ready to culture.

S2 : The seeds were taken from the fruit meat, then rinsed in 5% Bayclin for 15 minutes, then washed with sterile distilled water three times. The seeds were ready to culture in the air flow laminar.

S3 : The seeds were taken from the fruit meat, then rinsed in 15% Bayclin for 15 minutes, then washed with sterile distilled water three times. The seeds were ready to culture in the air flow laminar.

Second, the strength of MS media consisting of two levels i.e: full (in macro and micro nutrients) MS (M_1) and half strength MS (M_2). Thus, there were 6 treatment combinations. Each combination was replicated three times, therefore there were 18 experimental units. Each unit consisted of 35 seeds. The effects of the treatments were recorded as time to germinate, percentage of germination 1 and 2 weeks after culture, percentage of seedlings with opened cotyledones 1 and 2 weeks after culture and the existance of contamination, including the contamination level and the time to contaminate. All the datas were subjected to Analysis of Variance and the mean differences among the treatments were analyzed using Honest Significant Difference (HSD) at the level of 5% or 1% (Gomez and Gomez, 1995 and Kemas, 2004).

RESULTS AND DISCUSSIONS

Results.

The results of this experiment show that the strength of MS media and sterilization method significantly affected all parameters observed, while interaction between the treatments was highly significant on the percentage of opened cotyledone seedlings one and two weeks after culture, and significantly affected the time to germinate as well as the percentage of seed to germinate one and two weeks after culture.

The means of the time to germinate (days in culture) by the treatments can be seen in Table 2.

Table 2. Time to Germinate (Days in Culture)

Treatment	Media		HSD 5%
S	M1	M2	0.7412
S1	4.0000^{b}_{x}	4.0000^{b}_{x}	
S2	4.0000^{b}_{y}	2.6667^{a}_{x}	
S3	3.0000^{a}_{y}	2.0000^{a}_{x}	
HSD 5%	0.5951		

* Remarks : Means Within a Column and a Row Followed by the Same Letter do not Differ Significantly using HSD (P = 0.05).

Tabel 3. Percentage of Seed Germination 1 Week in Culture

Treatment	Media		HSD 5%
S	M1	M2	0.1809
S1	0.0476^{a}_{x}	0.2381^{a}_{y}	
S2	0.0667^{a}_{x}	0.5143^{b}_{y}	
S3	0.5429^{b}_{x}	0.6762^{c}_{y}	
BNJ 5%	0.1453		

* Remarks : Means Within a Column and a Row Followed by The Same Letter do not Differ Significantly using HSD (P = 0.05).

Tabel 4. Percentage of Seed Germination 2 Weeks in Culture

Perlakuan	Media		BNJ 1%
S	M1	M2	0.2785
S1	0.1809^{a}_{x}	0.6000^{a}_{y}	
S2	0.4476^{a}_{x}	0.9524^{b}_{y}	
S3	0.9486^{b}_{x}	0.9905^{b}_{x}	
BNJ 5%	0.2236		

* Remarks : Means Within a Column and a Row Followed by the Same Letter do not Differ Significantly using HSD (P = 0.05).

Table 5. The Percentage of Seedlings with Opened Cotyledones 1 Wic

Perlakuan	M		BNJ 1%
S	M1	M2	0.1705
S1	0.0000^{a}_{x}	0.00^{a}_{x}	
S2	0.0000^{a}_{x}	0.1333^{a}_{y}	
S3	0.0000^{a}_{x}	0.5333^{b}_{y}	
BNJ 1%	0.0962		

* Remarks : Means Within a Column and a Row Followed by the Same Letter do not Differ Significantly using HSD (P = 0.05).

Tabel 6. The Percentage of Seedlings with Opened Cotyledones 2 Wic

Perlakuan	M		BNJ 1%
S	M1	M2	0.2334
S1	0.1143^{a}_{x}	0.3429^{a}_{y}	
S2	0.1238^{a}_{x}	0.8286^{b}_{y}	
S3	0.8190^{b}_{x}	0.9238^{b}_{x}	
BNJ 5%	0.1952		

* Remarks : Means Within a Column and a Row Followed by the Same Letter do not Differ Significantly using HSD (P = 0.05).

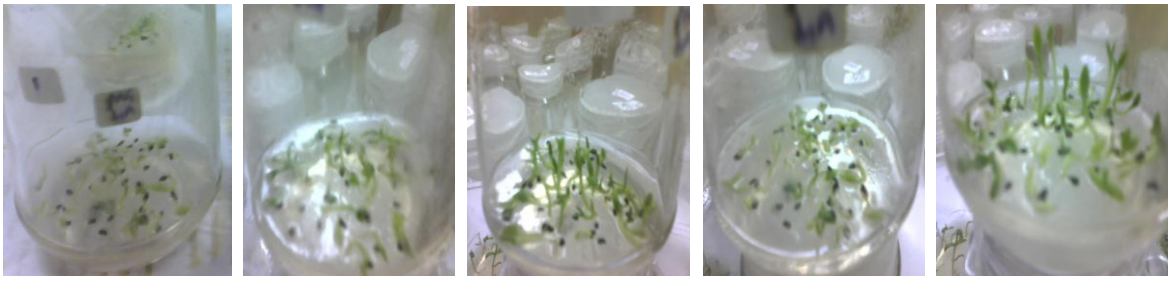
The results show that seeds separated from the fruit meat prior to rinsing in 15% Bayclin for 15 minutes, then washing in sterile distilled water three times (S3M2), and then cultured in half MS, were the fastest to germinate (2.00 days in culture) compared to all other treatments. The seeds of S2M2 treatment started to germinate in 2.67 days in culture (dic), of S3M1 3.00 dic and of the rest treatments 4.00 dic.

Percentage of seed germination 1 and 2 weeks in culture (wic) can be seen in Table 3 and 4.

Half MS gave higher germination percentage at all sterilization methods 1 and 2 wic. In the first week, there were 68% seeds germinated in S3, which is significantly higher than those obtained from the other sterilization methods. Similar pattern happened in the second week, where 99.05% seeds germinated from S3, eventhough there were only a little differences compared to S2 in the same strength MS and S3 in the other full MS (95.24%).

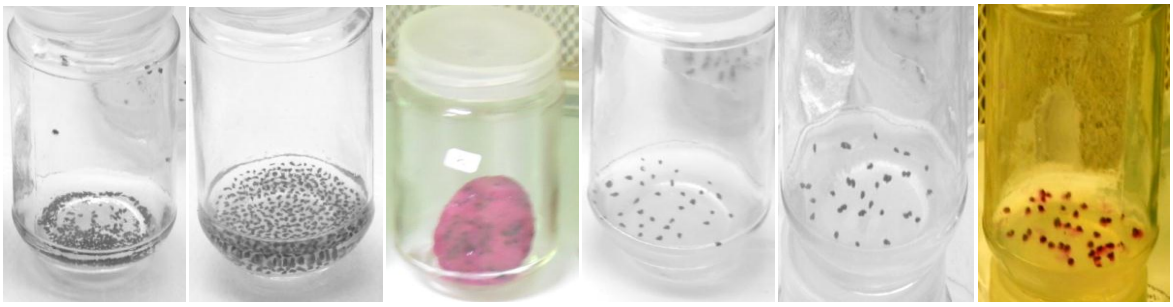
The percentage of seedlings with opened cotyledones 1 and 2 wic can be seen in Table 5 and 6.

In the first week, 53% of the seedlings cultured in half MS and sterilized as S3 had opened cotyledones, which is significantly the highest compared to other treatments. While in the full MS none of seedlings had the opened cotyledones. In the second week, most of the seedlings cultured in half MS and sterilized as S3 had opened cotyledones (92.38%), followed by those obtained from S2M2 (82.86%) and S3M1 (81.90%).



A B C D E

Figure 1A: Seedlings of S1. 1B: Seedlings of S2. 1C: Seedlings of S3. 1D: Seedlings of Full MS. 1E. Seedlings of Half MS.



A B C D E F

Figure 2.
Results of Different Sterilization Methods.
2A &2D. S3 Seeds, 2B &2E. S2 Seeds and 2C &2F. S1 Seeds

DISCUSSIONS

The results show that half MS media, was significantly better to germinate dragonfruit seeds than full MS. Similarly, removing seeds pulp, followed by rinsing in 15% Bayclin (household bleach with 5.25% NaOCl) for 15 minutes and washing in sterile aquadest three times (S3), also gave a better result (faster and higher percentage of seed germination). Combination of both treatments gave 99.05% germinating seeds and 92.38% opened cotyledon seedlings after 2 weeks in culture (Figure 1A-1E).

Sterilization with the S3 procedure, resulted in seeds free from attaching pulp (Figure 2A). These seeds were easy to take, move into the media and were also easy to be arranged on the media with the help of a pincer, when culturing in the Laminar (Figure 2D). In contrast with that results, using only 5% Bayclin in the same procedure (S2), resulted in a very stikky gel structure attaching to the seeds. These seeds tended to stik together, were difficult to be seperated thus they were hardly to take, move into the media and were also difficult to be arranged on the media with the help of a pincer (Figure 2B and E). While the seed picked up one by one from the fruit meat (S1), did not result a stikky like-gel structure, relatively easy to handle in laminar than the seeds of S2. These seeds however, were surrounded severily by red fruit meat, and to pick up the seeds from the fruit meat, the operator must provide a sterile large Petridish for the work (Figure2 C and F).

Regarding the process of germination, Hopkins (1997) explained that plant seed germination is initiated by imbibition of water to the seed coat tissue, which is driven primarily by physical surface-acting or matric forces, due to hydraulic properties of water. Therefore, removing barrier on water imbibition, such as removing pulp from the seed coats in this research, results in favourable condition for seed germination.

Some plant physiologists such as Hopkins (1997) and McDonald (2015)

explained that environmental conditions whichare necessary for germination are essentially water, oxygen and adequate temperatures during imbibition and even along the subsequent stagesof growth and development after imbibition in germination process. Nutrient requirement is not stated as essential during germination, therefore, it is reliable if half MS gave a better result to germinate the seeds. Some researches have reported a better results on seed germination using half MS on various species, such as Shadang *et al*. (2006) and Ali *et al*. (2011) for orchid, Kainde and Wagania (2010) for Indonesian mahoni (*Swietenia mahagoni*), and Mishra *et al*. (2013) for a multipurpose tree *Pterocarpus marsupium* Roxb, and in same Laboratory, Kasim (2012) have succeed to germinate kiwi seed using half MS. According to McDonald (2015) and Gunawan (1988), since MS medium has a relative high macro and micronutrient content, it is often used in half or eventhough quarter strength.

In relation with sterilizing agents, the use of Bayclin (household bleach containing 5.25% NaOCl), in concentration of 5% has been effectively to sterilize such explant in this research, as zero contaminated culture was found. However, this such low concentration could not remove attached pulps from the seeds, resulted in barrier to water imbibition by the seed coats, which is in turn slowing the germination process. In the same Laboratory, seeds of various species such as apple (Samudin, 2009b), pear (Ridwan, 2006), dragonfruit (Samudin, 2009a) and kiwi (Kasim, 2012) have been succeed to be sterilized using 15% Bayclin for 15 minutes exposure.

The effectiveness of sodium hypochlorite as disinfectant, has been widely published. A Canadian agency of provincial health services authority, the BC Centre for Disease Control (2003), in a guide to selection and use of disinfectants classify hypochlorites including sodium hypochlorite into intermediate level disinfectant. It is stated that hypochlorites are the most widely used of chlorine disinfectants, and sodium hypochlorite is an available one in liquid form, commonlyas aqueous solutions of 4 to 6% sodium hypochlorite, which are readily available as "household bleach". They have a broad spectrum of antimicrobial activity, are unaffected by water hardness, are inexpensive and fast acting, and have a low incidence of serious toxicity (Gamage, 2003). Therefore, the use of sodium hypochlorite as disinfectant in tissue culture practice, whether as laboratory standard clorox or as a household bleach has been widely reported.

The use of local household bleach containing 3.5% sodium hypochlorite has been stated as the most simple, effective and economical explant surface sterilant for *c*owpea, rice and sorghumseeds by Oyebanji *et al*. (2009), producing highest reduction in bacterial and fungal contamination (0%) for 20-45 minutes exposure.

In this research, the 15% Bayclin (household bleach containing 5.25% NaOCl) is equal to 0.788% NaOCl, a much lower concentration of NaOCl compared to other researchers mentioned above. Thus, the sterilization method used in this experiment is more economically reliable to produce clean dragonfruit seeds explant.

CONCLUSSION

Based on the results and discussions, can be concluded that removing seed pulp, prior to rinsing the seeds in 15% Bayclin (household bleach with 5.25% NaOCl) for 15 minutes followed by washing in sterile aquadest three times, and cultured in a half MS produce fast and high seed germination of 99% with 92% opened cotyledon seeds after 2 weeks in culture.

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