PROC. INTERNAT. CONF. SCI. ENGIN.
Volume 4, February 2021
Page 100-104

ISSN 1504607797
E-ISSN 1505707533

Factors That Effect of the Optimal Plantlet Growth from Tissue Culture on the Acclimatization Stage

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Abstract. Tissue culture is a method of propagation plants by isolating cell, tissues, protoplasts or plant organs than planting them in a sterile and controlled environment. This method is able to produce superior plants and propagation of plants are difficult to cultivate conventionally in large quantities with a relatively fast time. Acclimatization is an important final stage in the success of tissue culture method. Acclimatization is carried out for adaptation of the plantlet environment from in vitro to in vivo conditions. This is caused in the previous stage the plantlets are heterotrophic, while on acclimatization stage it will be autotrophic. This study aims to provide an information about the factors that affect the success of acclimatization plantlets. This is a review article was conducted using the literature study method by collecting information from journals, books or internet than analyzed in descriptive qualitative to prove scientifically. On this stage, many plantlets fail to grow and even die. for increase growth and reduce plantlets mortality need to be made overcome these problems. This can be overcome by applying acclimatization supporting factors. The result that factors affecting of the success plantlet growth on acclimatization stage including: the condition of the plantlets, light intensity, temperature, relative humidity, types of planting media, nutrition and fertilizing and controlling of pest and microorganisms.

Keywords: Acclimatization; Environment condition; Media; Plantlets; Tissue culture

Running tittle: Acclimatization stage of plantlets

INTRODUCTION

Tissue culture is a method of plant propagation by isolating cells, tissues, protoplasts or plant organs grown on artificial media in a sterile and controlled environment. This method is used to obtain superior plants, pathogen-free and secondary metabolite extraction. In addition, it is dense in producing large quantities of plant seeds in a relatively fast time (Abbas 2011; Nisa and Rodinah, 2005). This method has been used in many plants that have high economic value, rare plants and plants that are difficult to propagate conventionally (Dinarti et al. 2010). Seedlings from tissue culture are called plantlets. Plantlets require an acclimatization process to grow and develop properly.

Acclimatization is the last stage in tissue culture and it is the key to successful plant propagation. Acclimatization is the adaptation of the plantlet environment resulting from tissue culture from heterotrophic conditions (in vitro) to autotrophic conditions (in vivo). Besides, it is able to adapt in environmental conditions, such as high light intensity, temperature, and low humidity (Sukmadijaya et al. 2013; Perez et al. 2016). In the vivo conditions, plants must be able to find their own nutrients in the growing medium and be able to photosynthesize perfectly in the natural environment. This is a critical condition for plantlets because they have high transpiration and low absorption rates. In addition, stomata are not fully functional for photosynthesis. Therefore, many plantlets die at this stage and reducing production results (Kasutjianingati et al. 2020; Zulkarnain 2018).

The issue culture was high mortality rate of plantlets at acclimatization stage. This is indicated by the percentage of living plantlets only reaching 40-60% that survive into adulthood. Marlina and Rusandi (2007) reported that the success of acclimatization *Anthurium* sp. *was* only 10%. The results of research by Nasution et al. (2020) showed that the lowest growth percentage was in the *Dendrobium* sp. reached 46.67%. The success of seedlings was on *Carica papaya* L. only 30% (Malabadi et al. 2011). Seedling plantlets of Black orchids (*Coelogyne pandurata* Lindl.) less than 50% (Adi et al. 2014). Then, the percentage of growth was on mango (*Mangifera* sp. Hybrid) only 20-50% (Husein, 2008). Therefore, the success of acclimatization must be increased to avoid the mortality of plantlets. It is expected to grow well in vivo. Efforts need to be made overcome this problem. This can be overcome by applying acclimatization supporting factors. So that, it is expected that seedlings to be available in field and reduce the failure production. This study aims to determine the factors that affect the success of acclimatization of tissue culture plantlets.

MATERIAL AND METHODS

This study was conducted on September 2020. This was a review article on the factors that influence the successful acclimatization of plantlets from tissue culture results optimally. This study used a literature study method by collecting data in the form of information about acclimatization and plantlets from journals, books and sources from the internet. Then, the data were analysed in descriptive qualitative to prove scientifically.

RESULTS AND DISCUSSION

The result that the factors affecting plantlets growth during the acclimatization stage were plantlet condition, light intensity, relative humidity and temperature, planting media (Zulkarnain 2009).) furthermore, nutritional and pathogen control requirements were also needed (Abbas 2011; Kasutjianingati et al. 2020)).

Plantlet conditions

Tissue culture will develop into plantlets. The plantlets that are ready to be acclimatized already have complete organs such as stems, leaves and roots. A good plantlet is sized like a plant seed in vivo. Plantlets that grow vigorously with a good root system will be better able to adapt in the extreme temperature and fluctuating external environment. The adaptability of each plant species is different. Furthermore (Valero et al. 2006) said that the criteria for ready to acclimatize plantlets for each plant need to be defined. The establishment of these criteria or standards is important to obtain a high percentage of living plantlets when acclimatizing to the outside environment. According to Sumaryono and Riyadi (2016), the higher size of the plantlets, the higher survival rate of the Kopyor coconut (*Cocos Nucifera*) plantlets. Meanwhile, plantlets that already have 2 up to 3 open and straight leaves were used as one of the standard date plantlets ready to be acclimatized (Abahmane, 2011; Darwesh 2015). In addition, this is supported by (Sumaryono and Riyadi, 2011) said that plantlets that already have 2 up to 3 open and straight leaves were used as one of the standard date plantlets ready to be acclimatized and also for palm oil. Meanwhile, Susinandar (2011) reported that the plantlets that were ready to be acclimatized had 3up to 4 primary roots. In addition, root length also affects the life of the plantlets.

Light intensity

Light intensity is one of the factors supporting the success of acclimatization. In addition, light intensity is a component of climate. Light intensity affects temperature and humidity in the environment, so this needs to be considered for plantlet growth. Freshly removed plantlets from the in vitro environment require gradual adaptation of the light adjusted. Acclimatization requires a light intensity of 30-50% of the natural environment. This is because in vitro plantlets only have a few palisade cells that are smaller in size so they cannot receive light effectively (Abbas 2011; Martins et al. 2015; Zulkarnain 2018). The in vivo environment has a light intensity of 4.000-12.000 lux, so it needs to be lowered by being given 50-60% shade for 3 to 6 weeks then lowering it to 30-50% for environmental adjustments (Royani 2014).

Setting light intensity can use shade in the greenhouse. The level of light intensity varies with each type of plant. Dragon fruit plantlet (*Hylocereus undatus*) growth is effective in giving 2 layers of waring layer (Basri et al. 2013). *Neoregelia concentrica* using 50% light was the best irradiance level (Martins et al. 2015). Light intensity of 45% gives the best results on plant height, number of leaves, number of roots and root length of agarwood plantlets (*Aquilaria beccariana*) (Mulyono, 2014) and optimal light intensity of 900 lux on acclimatization of somatic embryo plantlets Robusta coffee (*Coffea canephora* Pierre ex A. Froehner) (Wahyuningsih 2016) and giving 60% paranet shade on the acclimatization of *Phalaenopsis* hybrid orchid (Fauziah et al. 2020), than 80% shade on plantlets *Nepenthes rafflesiana* Jack. (Sukmadijaya et al. 2013).

Temperature

Temperature is a factor that affects the growth of plantlets at the acclimatization stage. The temperature on the in vivo environment at night can reach 15 ºC and during the day up to 32ºC. This can affect plantlet growth because in previous conditions the temperature in vitro was stable at 23º-25ºC. can cause excessive transpiration in plantlets and water stress (Abbas 2011; Basri et al. 2013; Zulkarnain 2018). This water stress can cause an imbalance between the absorption of light energy and photosynthesis and affect the decrease in cell metabolism, lipid oxidation, protein and nucleic acids (Royani, 2014).

Pinar et al. (2020) reported that a temperature treatment of 26˚C and 80% humidity gave the best seed development results on the Nefir Deniz Banana variety and also at a temperature treatment of 22˚C and 70% humidity gave the results of the development of banana seeds of the Nefir AZ variety. Plantlets of *Phalaenopsis* orchids, at a temperature of 15-25° C with low relative humidity (60 ± 5% RH). Cha-um et al. (2010) explained that in this conditions, total chlorophyll and total carotenoids, net photosynthetic rate, stomatal conductance and low transpiration rate, leading to enhanced growth.

Relative humidity

In vitro plantlets are accustomed to high relative humidity (RH) conditions ranging from 90-100%, so that at the acclimation stage RH is gradually decreased. RH in this condition ranges from 70-80% for optimal plantlet growth. The leaves on plantlets have poorly developed cuticles due to high humidity, causing excessive transpiration on the in vivo environment. Lowering of the RH in vitro results are better wax formation on the cuticular layer, leading to less evaporation. To increase high RH, coverings are carried out using plastic covers, automatic mist system or fog system to reduce evapotranspiration (Fauziah et al. 2020; Pierik 1987; Rachmawati et al. 2020; Royani 2014). In Rubber plantlets (*Hevea brasiliensis* Muell. Arg.) covered with plastic overnight for 6 weeks gave the best results (Sumaryono et al. 2012). Sutthinon et al. (2015) reported that to overcome humidity and water availability using hydroponic methods on the growth of Tiger orchid seeds *Grammatophyllum speciosum* Blume. 90% RH was maintained during early period and slowly reduced to 40% on *Eucalyptus tereticornis* Sm. Plantlets (Aggarwal et al. 2012).

Planting media

The media is one of the factors that influence plantlet growth. Media as a place for the establishment of plants and function for the development of plant roots. According to Hartman and Kaster (2002) an ideal growing medium has the characteristics of containing essential nutrients, loose structure, good aeration and drainage, sufficient moisture, free of pests and toxins. The plantlet roots resulting from tissue culture were still weak and had a few root hairs, so it was not effective when transferred to in vivo. Each type of plantlet has a different media compatibility. Types of media that can be used include cocopeat, rice husk charcoal, wood charcoal, Sphagnum moss, cadaka root, zeolite and coconut husk (Adi et al. 2014; Andriani 2017; Kurniasih et al. 2017; Saiful 2017; Yosepa et al. 2013).

Kasutjianingati, et al (2020) reported that the zeolite rock media results in *Vanda sanderina* orchid plantlets growth. The best planting medium for *Anthurium* sp. used rice husk charcoal, fern and cocopeat (Julhendri et al. 2013; Rachmawati et al. 2020). *Gastrochilus matsuran* using sphagnum moss 80% survived (Kang et al. 2020). Pencil orchid (*Papillionanthe hookeriana* Rehb.f) on fern root media and wood shavings (Febriani et al. 2019). *N. rafflesiana* Jack. was effective on cocopeat media (Sukmadijaya et al. 2013) than Composition of sand media, husk charcoal and *Hydrilla verticillata* in "banana taro" plantlets (*Musa paradisiaca* var. sapientum L.) (Rodinah 2015).

Availability of nutrients

Nutrition is an important component in plant growth. Rooting plantlets that have just been transferred to in vivo are still weak and cannot absorb nutrients optimally. The addition of nutrients to the media aims to enrich the media and provide nutrients that are readily absorbed by the roots. This is because the plantlets are still in adaptation to the environment (Kasutjianingati et al. 2020). Fertilization through design is widely applied at the acclimatization stage. This is because is absorbed more quickly by leaves and is able to absorb 90% of nutrients compared to 10% through roots (Maera et al. 2014). The addition of different nutrients or fertilizers according to the type of plantlet. The nutrients provided can be in the form of N, P, K, leaf fertilizer and manure fertilizer (Dwiyani 2019; Julhendri et al. 2019; Rodinah, et al 2015).

Latif et al (2020) reported that giving vitamin B1 and Atonic can increase plant height, number of leaves and shoots with frequency of watering every 3 days on Pencil orchid (*Papillionanthe hookeriana* Rehb.f). Phloroglycinol and IBA administration was effective in rooting *Carica papaya* L. var. Maradol Raja (Perez et al., 2016). The used of Gandasil D and Hyponex foliar fertilizers with a 10-day watering frequency gave the best yields of plant height, number of leaves, tillers and fresh weight for *Dendrobium sp.* (Dwiani, 2012). Growmore fertilizer 2 g L^{-1} was given a better effect on the growth of *Phalaenopsis* hybrid orchid plantlets (Maera et al. 2014).

Free of detrimental pests and pathogens

During the acclimatization stage, plantlets must be free from pests and pathogens that can interfere with plantlet growth. Plantlets are easily infected with detrimental fungi and pathogenic if the remaining in vitro media is not sterile. In addition, the planting medium needs to be sterilized first by heating, sterilizing or soaking in a solution of bactericides and fungicides before used (Sukmadijaya et al. 2013). However, plantlets need to be in symbiosis with useful fungi and bacteria like Mycorrhizae, Rhizobium on Leguminosae and Rhizobacteria for roots and avoid drought stress (Abbas 2011; Aggarwal et al. 2012; Zulkarnaen 2018).

Plantlet sterilization is carried out to remove and prevent detrimental bacteria or fungi on the roots. Sterilization of plantlets can use a bactericide and fungicide solution 2 g L^{-1} for 15 minutes on *Thyponium flagelliform* L. Blume plantlets (Royani, 2014) and soaking plantlets for 3 minutes on *Gerbera jamesonii* (Irsyadi 2018, unpublished data). Sumaryono et al. (2012) reported that the use of the fungicide "Dithane" 2 g L^{-1} for 1 minute was used on Rubber plantlets (*Hevea brasiliensis* Muell. Arg) and 2 g L^{-1} for 10 minutes on soybean (*Glycine max*) plantlets (Wijayanto and Boer 2013).

CONCLUSIONS

The factors that affecting of the optimalization at the acclimatization stage were different in each plants type. There are appropriate temperature, relative humidity and light intensity, the best type of media, addiction of nutrients or fertilizer and free of detrimental pests and pathogens.

ACKNOWLEDGEMENTS

The author would like to thank to the Library Universitas Muhammadiyah Yogyakarta for providing the needs in this study. I also thanks to friends, especially N. A. Rudin and A. Salma who have helped and shared information.

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