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The suspensor analysis and morphology of somatic embryo of the meristem culture of ginger

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ABSTRACT

The aim of this research was to evaluate the somatic embryo of meristem culture of ginger (Zingiber officinale Rosc.*) through morphological analysis. This research used descriptivee method. Friable embryogenic callus, obtained from meristem culture, was cultured on proliferation medium. Cultured tissue fragments containing several globular embryoids were observed by microscopy to follow development of ginger embryoids. Based at morphology analysis, at first week it grew into somatic embryo. Somatic embryo that rise from embriogenic cells were another contact via a route specific. The somatic embryo formed was consist of an apical region, a basal region and a suspensor region. On the fourth week, however, the morphology of somatic embryo was founded on the shape of globular. It's consist of an apical region and a basal region.*

Key words: *Meristem culture, Morphology, Somatic embryo, Zingiber officinale* Rosc.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is a plant of tropical horticulture that has a value that is very useful as a food seasoning, mix of traditional medicine, cosmetic ingredients, drinks and others. However, the use as a spice or food and beverages remained at the highest portion, ie over 90% of the total volume of ginger that exported [31].

Large white ginger (ginger) more cultivated than the ginger others, because their farm provides several advantages, including the production per hectare is higher, the price of rhizomes per kilogram is more expensive, the market opportunity is wide open both domestically and abroad so that income received much higher [27]. To meet the increasing needs of the community against ginger, it is necessary to do an alternative techniques including tissue culture techniques. According to Bhojwani and Razdan (1996) tissue culture has great potential as a way of vegetative propagation for plants which are reviewed in terms of economic importance. Regeneration of plants using tissue culture techniques can be done through somatic embryogenesis.

Somatic embryogenesis is the process of establishing a complete organ (plantlets) through the formation of embryonic structures from non-sexual cells were induced to form embryos bipolar structure through a series of stages of development as occurs in embryogenesis in vivo but without the fusion of gametes [23][28]. Embryogenesis in plant gamete cells begins with an asymmetric cell division, which produces a smaller apical cell (terminal) and a larger basal cells. The onset of this asymmetric division gives polarity in the embryo. Plant embryo develops from apical cells (terminal), while suspensor evolved from basal cells. The next cell division leading to the globular stage. At this stage three basic network system (dermal, basic, and vascular) is formed based on the typical pattern of cell division. Globular shape of the embryo is then disappear along with the start formation of the cotyledons (leaves embryogenic). Cotyledons are perpendicular can provide torpedo shape embryos, and at this boundary suspensor degenerates and apical meristem of shoots and root apical meristem is determined. This meristem will produce a adult germination structure [20].

The process of somatic embryogenesis different from gamete cell embryogenesis, although almost the same stage of development. Plant propagation via somatic embryogenesis can produce new plants are much more because of somatic embryos derived from single cells so it is easier to monitor the growth process of each individual plant [12]. Meristem cells are regenerated through somatic embryogenesis, are genetically stable and not easily mutated as happened in the callus. Therefore, even though the virus-free plants have been obtained from the callus culture, but somaclonal variation in callus make better be used of meristem culture for regeneration system, especially through somatic embryogenesis [4].

Somatic embryogenesis research on ginger plant has been successfully carried out using leaf explants via callus phase [14], and on the callus culture of stem ginger [24]. Likewise in other plants, for example on anther culture of *Vilis rupestris* cv. Rupestris du Lot. [21], on the zygotic embryo culture and megagametofit *Pinus heldreichii* [30], on the callus culture hypokotil *Carthamus tinctorius* L. [18], on the zygotic embryo culture of *Araucaria angustifolia* [3][29], on the coconut plumula culture [25] on the callus culture of mature zygotic embryos of *Oryza sativa* cv. 5272 [35], and the culture of immature and mature *Abies cilicica* × *Abies cephalonica* hybrid [17].

The units of embryogenic callus potential to become a novice of somatic embryo formation. This aggregate has a rich cells cytoplasm and morphologically identical. Transition from embryogenic unit to form somatic embryos describe the initiation of somatic embryogenesis [9]. How somatic embryo morphology of ginger meristem culture that differentiate it from embryogenic callus? Normal forms of somatic embryos will grow produce mature somatic embryos, at the same time will accelerate the growth and development into normal plantlets. This study aimed to analyze the morphology and somatic embryo suspensor of ginger meristem culture. The use of morphological approach can improve immunokimia method, and can also provide important information about changes in situ during embryogenesis.

MATERIALS AND METHODS

The main material used is embryogenic callus of a large white ginger meristem culture and growing media. The main tool used is invertid microscope equipped with a camera, a petri dish, and tweezers, and others. In this study, the first thing to do is to provide materials of embryogenic callus and culture medium for proliferation of somatic embryos. Embryogenic callus subcultured into a proliferation medium (MS medium + 3% mannitol). Then stored in an incubation chamber in bright conditions with a 20 watt fluorescent lamp lighting for 16 hours each day at a temperature of 25^{o}C [6].

Observations were made every week in order to obtain somatic embryo morphology. Specimens were observed with a microscope, photographed so that the image obtained somatic embryo morphology. This research uses descriptive method to analyze the morphology of somatic embryo and suspensor on meristem culture ginger.

RESULTS AND DISCUSSION

Formation of somatic embryos already visible after the first week in subculture on proliferation medium. Although at that time the embryo is still average size is very small and elongated. The somatic embryos characterized by yellowish translucent white (transparent) and exposing the outer wall is smooth and shiny as in **Figure 1**. At this time the process of somatic embryo formation seems not stable, because it still shows the diverse embryonic form.

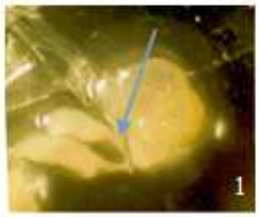

Figure 1. The shape of somatic embryos ginger one week old (magnification of 10 x 1). The arrow shows that somatic embryos associated with other somatic embryos through a channel

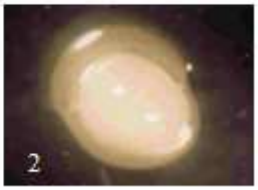

Figure 2. Somatic embryos ginger age of 4 weeks (magnification 10 x 3)

Based on observations, that at the stage of pre embryogenic, every somatic embryos were formed, one of the end portion of the poles are interconnected with somatic embryos to another through a channel as a liaison between somatic embryos, thus forming a set of embryos that will into a globular embryo. Channel that connects the embryo with the other embryo is clearly visible in **Figure 1**. On the surface of the embryo looks a little light brown spots that spread, while the elongated embryo induction. Then in the second week, the embryo grows and develops with big round or oval shape (globular) and there is also a small round. The shape globular embryo is more obvious and

more visible attached to the non-embryogenic callus. Brown spots that spread on the surface of the embryo is also more and more apparent after four weeks of subculture. This can be seen more clearly in **Figure 2**. At week 4, the average of somatic embryos no longer relate among other embryos through a channel that extends, but globular-shaped somatic embryos that had been attached to the callus.

Embryogenic callus requires the right time to differentiate into globular-shaped somatic embryos. In this study, the first week of media proliferation has stimulated the formation of somatic embryos. The quantity of embryos increases, while the shape becomes stable in globular form until the fourth week. This suggests that the potential for proliferation of specific somatic embryo in its growth and is influenced by the composition of the medium. To get the amount and speed of growth and differentiation of somatic embryos on ginger, required proper medium. According to Torres *et al.* (2001) kesinkronan embryonic development as well as the quantity can be increased in the medium MS added mannitol. This media can affect the speed of cell division and stimulate the process of morphogenesis of embryogenic cells [39].

In addition to growing media, plant growth regulators also affect the formation of globular somatic embryo. Growth regulator used at the time of induction of embryogenic callus on meristem culture ginger likely to affect the process of formation of somatic embryos were subcultured in proliferation medium. According to Kim *et al.* (2008) that the initiation of embryogenic suspensor mass frequency also depends on growth regulators. Pescador *et al*. (2008) also found the presence of 2,4-D in the culture medium can interfere with the genetic and physiological proembriogenik culture, so as to affect the morphology of somatic embryos that will be generated. The existence of 2,4-D in the medium can change the PIN protein conformation, is negatively associated with effluk IAA, and alter the normal determination of the axis of the apical-basal somatic embryos. Therefore, the process of proliferation of globular-shaped somatic embryos closely related to PGR given at the time of induction of embryogenic callus. Likewise, when the proliferation of somatic embryo meristem culture, it is not necessary media containing PGR. This is likely due to endogenous PGR has enough available for use in the process of somatic cell division rate and optimize somatic embryo morphogenesis globular shape. As in explants Palm (Phoenix dactylifera) successfully induced into somatic embryo within 2-3 weeks on hormone-free medium [26]. Similarly, the proliferation of somatic embryos on callus culture of meristem ginger. Currently embryo induction, the process of morphogenesis of ginger embryogenic callus developed into embrioid-embrioid which shows the average elongated. One part of the embryo poles interconnected with other embryos via an interface (channel). The channel is attached to one pole of the embryo is thought suspensor (as seen in Figure 1). So morphologically, almost all embryos have the apical region, basal area, and the area suspensor. It is through these channels that is suspected as suspensor, then embrioid-embrioid obtains nutrients to meet their needs, so the potential to differentiate into somatic embryo. According to Kawashima and Goldberg (2009) suspensor is differentiated embryonic area in the terminal that connects the embryo to the surrounding tissues during early development of seed. Suspensor plays an important role in embryonic development, transfer of nutrients and growth factors in the embryo. According to Fransz & Schel (1990) and Laux & Jurgens (1997) suspensor role in somatic embryos suspected to function in taking and transport of nutrients. Transport simplastis of suspensor to the apical cells going through plasmodesmata, which was among the cells [9].

According to Capron *et al*. (2009) and Arnold *et al*. (2002) embryogenic somatic cell initiation process is almost equal to that of the zygotic embryogenesis. At the beginning of zygotic embryogenesis, the apical-basal axis and the radial pattern of the tissue surrounding the embryonic cells only in the size of about hundreds of cells. Initial axial pattern coordinate system for the initiation of embryonic shoots and roots. The embryonic cell divides into two cells are very different to the internal composition and subsequent cleavage patterns. The apical cell contains dense cytoplasm and is the site of protein synthesis is very active, while the basal cell and its descendants are very vacuolization. Early cleavage stages of apical cell derivatives are extremely reproduce. In contrast, basal cells divide horizontally produce filaments called suspensor structures. Most mature embryos derived from the apical cell. However, the apex of the root is derived from basal cells, such as the top suspensor cells (hypofisis) be incorporated in the formation of the embryonic root meristem. The same was found in Arabidopsis zygote [20]. Arnold & Woodward (1988) also found on the embryonic callus culture of zygotic embryos, the proliferation of somatic embryos formed from the regional of embryonic and suspensor area. Embryonic area composed of a small maristematis cells and suspensor area composed of cells vakuolated almost lengthwise. Then according to Bronsema *et al*. (1997) that the early development of globular embryos that occurs after induction in younger *Zea mays* embryo tissue, somatic embryos were formed consisting of the apical region and suspensor region. The cells in the apical region of small, rich cytoplasm, and active mitosis. These cells contain a lot of starch and microtubule bundle. While suspensor cells larger and more vacuolization. High metabolic activity in both these cell types, is seen with many organella, a layer of vesicles, and body multivesikuler [9]. Jones and Ross (1989) argues suspensor cells derived from embryonic basal cells. According to Stasolla *et al*. (2002) also embryogenic tissues were incubated for 4-6 weeks to contain the mass of somatic embryos, and morphologically similar to zygotic embryos. In embryogenic tissue, a newly formed young embryo each with embryo cells that dense cytoplasm and suspensor cells that

vacuolated is continuously generated by the proliferation of embryo mass. Embryos at the globular stage will grow to the elongation phase, which is a sign of a change of isodiametrik to the growth of bilateral symmetry, and the beginning stages of heart [38]. At the globular embryo contained cuticle layer but not on suspensor [32].

Then according to Hazubska-Przybył *et al*. (2008) the embryogenic tissue is different in the proportion of various types of pro-embryogenic mass, and presence / absence of early somatic embryos which showed that somatic embryos differ in embryogenic potential. This shows that in this study, in addition to media and PGR factors that may affect the potential of embryogenic tissue at the initiation stage of mass pro-embryogenic callus culture of meristem ginger, may also be associated with the connecting channel is considered suspensor in the embryo. In the proliferation of somatic embryos, potential differences of embryogenic tissue can be seen clearly in Figure 1. In the figure can be seen that there are some embryos that have different shapes and sizes. There are several embryos are still small and elongated shape, and there are embryos that are large and oval shaped. This shows that in the early stages of embryogenesis, embryogenic tissue has a different potential. There are allegations that the connective tissue between an embryo which is considered as the suspensor is in addition to the potential to affect the formation of the embryo, also has the potential to affect the growth and development of normal somatic embryos. This is likely due to the suspensor role as a transport network (haustoria) that absorb nutrients from the growing medium. Then at the stage of embryonic development, the storage material that accumulates will be used in certain metabolic processes during embryonic development [10]. In this study, while induction of embryogenic cells on the progembryogenic mass of the ginger meristem culture, the cells undergo cell division to form the apical region, basal area, and suspensor area, up towards development of the formation of globular embryo. At meristem culture of ginger, allegedly suspensor plays a role in the early formation of somatic embryos. But after 3-4 weeks possibility suspensor cells are degraded so that the basal part of a somatic embryo is attached to the callus. Therefore, at week 4 the process of morphogenesis showed that the shape of somatic embryos on average globular form attached to the non-embryogenic callus. Then the spots that are part of the embryo's surface, the more obvious dark brown. These traits are thought to be a sign that the somatic embryo is becoming more mature than in the first week after the culture. If made schematic of Figure 1, associated with Figure 2, it will be obvious that the age of 4 weeks somatic embryos of ginger, globular shape without suspensor section (Figure 3). According to Filonova *et al.* (2000) that the formation of the embryo cells and suspensor cells occurs at the beginning of embryogenesis. Jayasankar *et al.* (2003); Yeung & Menkel (1993) also found somatic embryos derived from embryogenic callus initiated from proembriogenik mass which has basic structure suspensor, but after evolving towards mature somatic embryos, suspensor structures be little or no visible. Based on the analysis of developments that occurred between embryo with suspensor, although suspensor looks play an important role in embryogenesis, but on the maturation stage of embryos, suspensor can not survive.

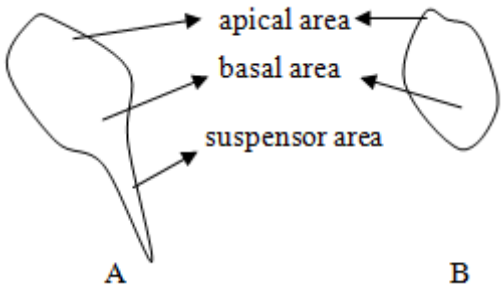

Figure 3. A. Schematic of the somatic embryo of ginger 1 week old, and B. Somatic embryo age of 4 weeks

There are allegations that the existence of suspensor at the beginning of embryogenesis is to assist the growth and development of proembriogenic mass to the globular embryo stage, because suspensor cells play a role as a means of transport of nutrients from the medium into the embryogenic cells. Therefore, it can be said that the existence of suspensor structure on the proembryogenic mass into globular stage to modulate progression from the proembryogenic mass stage to the globular embryo stage, so as to speed up the regeneration of plants leading to the maturation stage. The second allegation, because the basal part which differentiate into root apical meristem was competent to absorb nutrients from the medium, then the suspensor structures slowly degenerate in accordance with the somatic embryo development towards maturation. Thus, structural modifications related to the transport of nutrients that are not needed when somatic embryos resulting in a nutrient-rich environment. So the state of nutrition can affect the development of suspensor. Medium condition of the Embryogenic culture can stimulate and control mechanisms of cell differentiation during somatic embryogenesis [2]. According to Franz & Schuler, (1990) the supply of nutrients to the transmission unit could have been influenced by the basal cells, which functions as an area taking nutrients from the cells of the callus. Therefore, it can be presumed that the callus cells have the ability to absorb the nutrients contained in the medium, to be used by the somatic embryos in morphogenesis. Yeung and

Meinke (1993) also found suspensor functioning in early embryogenesis and then degenerates during the latter stages of development and no on the embryos mature. Classically, suspensor is the first passive role in embryonic development by holding the embryo in a fixed position in the seed. Based on the structure of the biochemistry and physiology, suspensor active role in the early development of a way to spur continuous growth of the embryo proper, then normally the embryo tapping the potential development of suspensor [20]. According to Filonova *et al*. (2000) programmed cell death responsible for the degradation of the mass proliferation of embryogenic when grown into somatic embryos. Programmed cell death in cells of proembryogenic masses and embryo formation interrelated processes, both stimulated the removal or reduction of most of auxin and cytokinin. Programmed cell death is to eliminate cells of the embryo-suspensor during the early embryogenic. During the phase of programmed cell death and cell embryo-suspensor showed progressive autolysis, resulting in the formation of a large central vacuole. Almost all the process of formation of somatic embryos of proembryogenic mass, occurred on the media that does not contain or reduction of auxin and cytokinin. This condition is thought to stimulate normal somatic embryo formation and accelerate the growth and development of somatic embryos. This condition is also the possibility of activating programmed cell death so that part of the embryo that no longer function will degenerate, such that at the beginning of the formation of somatic embryos is needed role suspensor to transport nutrients into the structure of somatic embryos, but in line with the growth and development of somatic embryos, then suspensor will be degraded, while the somatic embryos grown and germinated by having the roots and shoots. Root replaces the function suspensor absorbing nutrients from the culture medium. Likewise, this is likely to occur in the process of formation of somatic embryos of proembryogenic mass on the ginger meristem culture, until the embryo has become mature and germinate and grow into plantlets strong. While the suspensor region of the embryo begins degraded during proembryogenic mass changes to form globular somatic embryo. There is a possibility that the state of the media that does not contain PGR can activate programmed cell death so that when the growth and development of proembryogenic mass heading to form mature somatic embryos, while the suspensor area began degraded. It can be seen a change in morphology in Figure 1 to form globular embryo in Figure 2. In Figure 1 is shown the relationship between the suspensor region with the young embryo. Then in Figure 2, in line with the growth and development of proembryogenic mass seen that somatic embryos have no its part suspensor again. Larsson *et al.* (2008) also found the polar auxin transport model is important for normal embryo development. Model, that where auxin is transported out of suspensor cells to embryonal masses during on the embryogenic early. The transportation is very important for the development decisions of embryonic cells and suspensor, and affect both the amount of programmed cell death and pattern of the embryo.

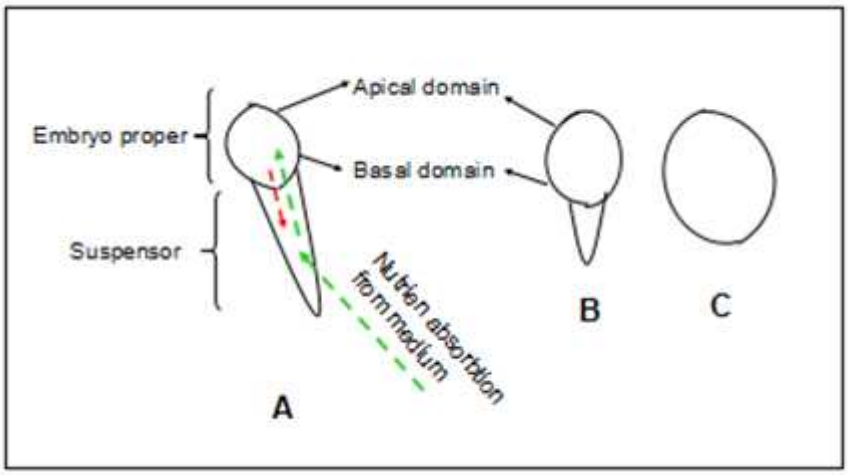

Figure 4. The process of formation of globular somatic embryo at the proliferation stage of proembryogenic mass. A: Embryos with its suspensor, suspensor absorbs nutrients from the medium without PGR, is transferred to the embryo is marked with a dotted line is green. The condition medium without PGR is able to stimulate mass proembriogenik proliferate, differentiate, and morphogenesis. In line with the process of differentiation, the embryo produces hormones result in increased concentrations of growth regulators so regardless to suspensor (indicated by the dotted line in red) results in degraded suspensor. B. Embryos with its suspensor started degraded. C. Globular somatic embryo without suspensor

According to Umehara and Canada (2005) embryonic development is regulated by suspensor that connects the embryo with the donor network. Various factors stimulant and inhibition is associated with the interaction between the embryo and its suspensor. There are two ways of communication between the embryo and suspensor during early stages of embryogenesis. Suspensor provide nutrients and growth regulators in the embryo. Instead, the embryos are releasing negative regulator for tapping the potential of embryonic of the cells suspensor. Degradation of suspensor development will provide a place for the development and maturation of embryos. Zhang and Somerville (1997) also found apical cells and their derivatives are usually suppress embryogenic potential of basal cells and derivatives during early embryonic development. Thus it can be stated that the process of formation of

globular-shaped somatic embryos at the proliferation stage of proembryogenic mass of embryogenic callus culture on the ginger meristem, can be seen in Figure 4.

CONCLUSION

Somatic embryos are grown and proliferate of the embryogenic cells induced from embryogenic callus on meristem culture ginger. At the beginning of the formation of somatic embryos, these embryos have apical region, basal area, and the area suspensor clear. The embryos differentiate to form globular, attached to the non-embryogenic callus. This callus plays an important role in absorbing nutrients from the growing medium, then transported to the growth of somatic embryos. In the fourth week, normal somatic embryo morphology on meristem culture ginger is a globular shape which consists of the apical and basal parts. Every cell in proembryogenic mass of the ginger meristem culture has a different potential in differentiation and morphogenesis. It can be affected by various factors including; medium composition and growth regulators, either at the time of induction callus or induction proembryogenic mass.

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