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Immature Embryo Culture Accelerates Soybean Reproductive Phase: A Potential Biotechnology Approach for Shortening Breeding Cycle

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Abstract

The production of soybean seeds for breeding purposes is usually constrained by a long reproductive phase. One possible way to shorten soybean reproduction cycle is through immature embryo culture. This research studied the effect of pod age and phytohormone composition on the growth of soybean plantlets originated from immature embryos, and to compare potential life cycles of soybean plants generated from embryo culture and from conventional method. Two separate experiments were studied, i.e. pod age experiment (2, 3 and 4 weeks after flowering (WAF) embryos) and experiment of phytohormones indolebutyric acid (IBA) and 6-benzylaminopurine (BAP) combinations. Data were analyzed using the SAS 9.1.3 version statistical software, and treatment means were compared using Duncan's Multiple Range Test. Data indicated that IBA gave better effects on plantlet growth, as compared to no-hormone treatment, but BAP did not provide good effect. The highest stem (28.7 mm), maximum number of root (10.1), and longest root (63.9 mm) per plantlet were obtained from embryos grown on medium supplemented with 1.5 ppm IBA (B1). Four WAF embryos were suitable explants for better soybean plantlet growth, but in most cases, they were not significantly different from 3 WAF embryos. Culture of 3 WAF embryos can potentially shorten soybean's life cycle (for breeding purposes) by 32 days if compared to conventional propagation using mature seeds. This way could potentially help breeders to plant soybean more often in each year.

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Introduction

Soybean (*Glycine max* L. Merrill) is one of the world's most important sources of vegetable oil and protein (Verma *et al*., 2014), and has many uses and high economic values. Indonesian's consumption and demand for soybean is very high, required for the production of various daily popular consumed products, such as for tauco, soy sauce, "tempe" (soy cake), tofu, soy milk, and for animal feed industries. Demand for soybeans in Indonesia currently cannot be fulfilled domestically because the production is still low. Low production of soybean is caused by many factors; one of them is lack of quality seeds available. Breeding programs are required to develop new novel soybean cultivars.

In plant breeding, it is very important to accelerate generations by shortening each plant life cycle, because conventional breeding programs generally require many generations and that is a lengthy process (Roumet and Morin, 1997). Soybean breeding programs generally also require a lengthy period, due to a long reproductive phase. Reproductive phase of soybean plants occupies more than half of its entire life cycle (Roumet and Morin, 1997; Wijayanto *et al*., 2013b). Indonesian-grown soybean cultivars on average take about 2 months for their reproductive phase, from flowering to harvest (Purnawati and Hidajat, 1994), and it is a relatively long period. The long duration of the reproductive phase of soybean plants becomes an issue for breeders to obtain seeds, for subsequent generation cycles. It is therefore necessary to develop a relatively quicker but equally efficient method of soybean propagation technique.

One potential biotechnological technique to shorten the breeding cycle of soybean is young (immature) embryo culture. Embryo culture involves isolating and growing an immature or mature zygotic embryo under sterile conditions on an aseptic nutrient medium with the goal of obtaining a viable plant (Bridge, 1994). This method has been used for diverse purposes in a number of plant species, such as wheat (Farshadfar *et al*., 2014; Hakam *et al*., 2014; Ekom *et al*., 2014), cassava (Yan *et al*., 2014), orchid (Sungkumlong and Deb, 2008), and soybean (Van *et al*., 2008; Santos *et al*., 2006). Embryo culture technique in general has several purposes in plant breeding such as for shortening breeding cycles, overcoming incompatibility, production of haploid plants as well as the source of callus formation (Abbas, 2011; Shen *et al*., 2011). At the age of 2-3 weeks after flowering (anthesis), immature embryo culture can be carried out (Kosmiatin *et al*., 2005) so as to possibly shorten the reproductive cycle, and for soybean crop it can possibly shorten the cycle for about 40 days (Nurdin, 2013).

Age of pods (embryos) influences the success of the embryos to grow on an *in vitro* medium. The older the pod is usually the relatively easier for the embryos to grow. However, the premise of embryo culture research is usually to use embryos as young as possible but still be able to grow and subsequently produce seeds, because in this way it is expected to save as much time as possible to shorten the generative cycle. In addition to the age of the pods, seeds layout (position) has also been reported to affect the growth of soybean plantlets. Generally seed that sits at the base (axial) of the pod will get more assimilates than the seeds in the middle and at the end (terminal) (Wijayanto *et al*., 2013).

Besides the pod (embryo) age, the success of immature embryo culture is also dependent on the use of appropriate growth medium, phytohormones, and plant genotypes (Purnamaningsih and Lestari, 1998; Van *et al*., 2008; Uzun *et al*., 2014a; Pishbin *et al*., 2015). The use of basic medium and appropriate growth hormones was required to obtain optimum results. Differences in the composition of the medium may lead to differences in the growth and development of the explants grown *in vitro* (Kaviani *et al*., 2015).

Auxin and cytokinin are two types of regulatory substances commonly used in culture medium. Auxin and cytokinin often used for *in vitro* culture are 6-benzylaminopurine (BAP) and indolebutyric acid (IBA) (Verma *et al*., 2014).

Auxin plays a role in breaking the embryo dormancy and subsequently for root formation (Wattimena *et al*., 2011). Cytokinins are phytohormones that induce cytokinesis and are essential for diverse developmental and physiological processes in plants, particularly regulate shoot growth (Ko *et al*., 2014).

Uzun *et al.* (2014b) found that regenerated shoots of *Iris sari* and *I. schachtii* were successfully rooted on MS medium with 1 ppm IBA, and additional of BAP produced more shoots than Kinetin. Julkiflee *et al.* (2014) also reported that combinations of 4.44 or 8.88 µM BAP and 8.88 µM NAA produced higher PLBs proliferation rate in *Dendrobium* sonia-28 orchid.

This paper reports the effect of pod (embryo) age and hormone composition in the *in-vitro* culture medium on the growth of plantlets originated from soybean embryo culture, and to compare potential life cycle duration of soybean plants generated from embryo culture and from conventional method.

Materials and methods

Preparation of donor plants

This research was conducted at the Faculty of Agriculture, University of Halu Oleo, Kendari, Indonesia. The materials used were Indonesian soybean cultivar "Argomulyo", B5 medium (Gamborg *et al*., 1968), L6KK medium (Croser *et al*., 2010), growth hormones IBA and BAP, 70 % ethanol, 2.5 % sodium hypochlorite, Tween - 20, and sterile water.

Potting mix medium used to grow soybean plant donor was in the form of a mixture of soil and manure (cow feces) with the ratio of 3:1 (Wijayanto *et al*., 2013a; Wijayanto *et al*., 2013b). Soybean seeds were sown in the potting mix in pots (size 30 cm x 40 cm), with 2 seeds per pot and kept in a glasshouse. For the treatment purposes, seeds were sown in pots with one week interval, for at least 3 consecutive weeks. NPK fertilizer (15:15:15) at a dose of 10 g per pot was applied two (2) weeks after planting, and subsequently every two (2) weeks (Wijayanto *et al*., 2013b).

Culture media and conditions

Two basal media, solid B5 medium (Gamborg *et al*., 1968) and liquid L6KK medium (Croser *et al*., 2010), were used with some adjustments (Wijayanto *et al*., 2013a; Wijayanto *et al*., 2013b). The medium pH was adjusted to 5.5. B5 medium (5 ml) was poured into a 30 mL culture tube. Culture media were sterilized by autoclaving at 121^{0}C and 1.5 atm, for 20 minutes. The culture tube was tilted at 45˚ slope position after sterilization, and allowed to solidify (Croser *et al*., 2010; Wijayanto *et al*., 2013a).

Young pod harvest

Immature pods were carefully harvested at 2, 3, and 4 weeks after flowering (WAF), and then placed in a container containing wet filter papers to maintain humidity during the process of moving from the glasshouse to the laboratory. Two days pre pod desiccation treatment was conducted before the embryos were rescued from the pods (Wijayanto *et al*., 2013a; Wijayanto *et al*., 2013b: Wijayanto *et al*., 2014).

Pod sterilization was conducted according to the routine procedure (Croser *et al*., 2010; Wijayanto *et al*., 2013a; Wijayanto *et al*., 2013b: Wijayanto *et al*., 2014). The immature pods were washed with 70 % ethanol for 5 minutes, then with sodium hypochlorite 2.5 % + 1 drop of Tween-20 for 5 minutes with agitation. The pods were finally rinsed 3 times with sterile water for 5 minutes each, and then were wind dried over sterile filter paper in a laminar air flow cabinet.

Embryo rescue and culture

Isolation of young immature seeds from the pods and isolation of immature embryos from the seeds were performed aseptically in a laminar air flow cabinet, using a sterile scalpel. Seeds were clamped with tweezers, and then cleaved by separating the cotyledons (Wijayanto *et al*., 2013). Furthermore, embryos were rescued by using the tip of a surgical knife. The embryos were cultured on the solidified B5 medium in a 30 ml polycarbonate tube. Filter-sterilized growth hormones (depending on the treatments) were added into the sterilized L6KK liquid medium.

L6KK liquid medium containing growth hormones was added (1 mL per tube) to the solid B5 medium after completion of embryo planting (Wijayanto *et al*., 2013b). Unless otherwise stated, all culture bottles were incubated in a culture room at 24 °C under cool white fluorescent light (35 µmol photons m^{-2} s^{-1}) with 16-h (day)/8-h (night) photoperiods. Cultures were maintained in the culture room and embryo growth was observed every day, for signs of germination, plantlet growth and development.

Acclimatization

Plantlets with well-developed root system were ready for acclimatization (approximately at 30 days after planting, WAP). The plantlets were taken out of the tubes, and the roots were washed with water to remove the trace of agar (Slamet, 2011). The plantlets were grown in pots filled with sterilized soil-rice husk (2:1) medium. All plantlets were kept covered with the polycarbonate tubes to maintain humidity. Humidity was gradually decreased by opening the cover, gradually over approximately a 2-weeks-period.

Experimental design and data analysis

Two sets of experiment were studied, i.e. the nature of pod (embryo) ages and the combinations of growth hormones IBA and BAP. Both experiments were set up separately in a completely randomized design (CRD) of various treatment levels, considering previous experiment (Wijayanto *et al*., 2014).

The experiment of pod ages consisted of 3 (three) treatment levels, namely 2 WAF (U1), 3 WAF (U2) and 4 WAF (U3). The experiment data were presented descriptively. While the experiment of growth hormone combinations consisted of seven treatment levels, namely: without growth hormone (B0), 1.5 ppm IBA (B1), 1.5 ppm + 0.25 ppm BAP (B2), 1.5 ppm IBA + 0.5 ppm BAP (B3), 1.5 ppm IBA + 1 ppm BAP (B4), 1.5 ppm IBA + 2 ppm BAP (B5) and 1.5 ppm IBA + 4 ppm BAP (B6).

The observed variables were the percentage of germinated embryos, plantlet height, leaf number, root number and root length. Experiment data were analyzed using the SAS 9.1.3 version statistical software. Treatment means were further compared using Duncan's multiple range test (DMRT) at 5% probability level. The germination data were presented descriptively.

Results and Discussion

Immature embryo germination

The results showed differences in the percentage of embryo germination (Table 1). All cultured 3 and 4 weeks after flowering (WAF) embryos were germinated (100 %), whereas only 57% embryos aged 2 WAF were germinated. The differences in the embryo germination percentages (Table 1) may be caused by the differences in initial size and physiological nature of the cultured embryos (Clarke *et al*., 2006), which may also imply its food reserve content.

Table 1. Effect of pod age on the percentage of embryo germination.

Pod age	Embryo size	Germinated
	(mm)	embryos (%)
U1(2 WAF)	1.0	57
U2 (3 WAF)	3.5	100
U3 (4 WAF)	4.1	100

Previously, Kosmiatin and Mariska (2005) has reported that the smaller the size of the embryos is also the more fragile and easier the embryos to be broken. They further stated that germination will increase with increasing age of the embryos due probably to more food reserve content. This is consistent with the results of the research, in which more mature embryos (3-4 WAF) produced more germinated embryos than those embryos at the young cotyledon stage (2 WAF). Similar results were also reported by Kapila and Sethi (1993) who found that embryo size was positively correlated with success in embryo rescue and recovery of plantlets.

Table 2. Effect of pod age on the growth of soybean plantlets.

Pod (Embryo) Age	Plantlet height (mm)		Leaf number	Root Number		Root length (mm)
	10 DAP	20 DAP	20 DAP	10 DAP	20 DAP	20 DAP
U1 (2 WAF)	26.3^{c}	38.3^{b}	1.8	4.9^{b}	15.8^{b}	42.7^{c}
U2 (3 WAF)	36.9^{b}	43.0^{ab}	1.9	11.1^{a}	20.3^{a}	75.0^{b}
U3 (4 WAF)	44.4^{a}	47.7^{a}	1.9	16.1^{a}	22.9^{a}	94.0^{a}

Notes: The values followed by the same letters in the same column are not significant by DMRT α = 0.05.

The combination of male gametes and female gametes could produce a zygote (pro-embryo), which then develops into an embryo. The development of seed (embryo) is through the stages of zygotic, globular, heart, and cotyledons (Tilton and Russel, 1984; Santos *et al.*, 2006). Embryos at the end of heart stage can be grown *in vitro*. Obstacles that often be faced include the composition of the medium, and the very small size and mushy embryo (0.5 mm), so can easily be broken (Tilton and Russel, 1984).

Table 3. Effect of different compositions of hormones on plantlet growth (at 20 DAP) in *Glycine max*. L. Merr.[x]

Hormone treatments	Plantlet height (mm)	Leaf number	Root number	Root length (mm) [y]
No hormone	20.2^{d}	1.7	7.0^{b}	61.6^{a}
1.5 ppm IBA	28.7^{a}	2.0	10.1^{a}	63.9^{a}
1.5 ppm IBA + 0.25 ppm BAP	23.8^{c}	2.0	2.5^{c}	26.8^{b}
1.5 ppm IBA + 0.5 ppm BAP	27.9^{ab}	2.1	1.2^{c}	20.7^{bc}
1.5 ppm IBA + 1.0 ppm BAP	23.7^{c}	2.0	1.0^{c}	19.0^{c}
1.5 ppm IBA + 2.0 ppm BAP	25.6^{bc}	2.0	1.0^{c}	20.5^{bc}
1.5 ppm IBA + 4.0 ppm BAP	23.2^{c}	1.8	1.0^{c}	17.2^{c}

Notes: The values followed by the same letters in the same column are not significant by DMRT α = 0.05

[x]) Source: Nuryeni (2014)

[y]) 30 days after planting.

In this research, germination of *in vitro* cultured 2 to 4 WAF embryos is certainly supported by the culture medium; the exact composition of the culture medium will provide good growth for the embryo, and serves as a substitute for the endosperm which will supply nutrients to the embryo, especially for young embryos which require a complete composition and specific medium. This result is in line with report by Bridgen (1994) and Muhammad *et al.* (2015) that continued development of young embryos requires complex media supplemented with combinations of vitamins, amino acids, growth hormones, and, in some cases, natural extracts. Previously, Christianingsih (2008) stated that the role of gibberellins in cell elongation is to induce the formation of hydrolase and proteolase enzymes to release amino acid tryptophan as an auxin precursor, thereby increasing auxin levels. An increase in auxin level and inhibition of abscisic acid (ABA) will break the embryo dormancy, so the embryo can germinate and grow (Wattimena *et al.*, 2011; Bouiamrine *et al.*, 2013). Research results showed that cultured 3 and 4 WAF embryos began to germinate on the first day of culture, while 2 WAF embryos took at least 3 days to begin germinating. Besides the amount of food reserve content, the differences were probably also due to the difference in the composition of the medium, in which the medium was still not optimum to support very young embryos to germinate (Kosmiatin and Mariska, 2005). In the heterotrophic phase, young embryos depend on the endosperm and the surrounding maternal tissues, and require a more complex medium and higher osmotic pressure than older embryos (Bridgen, 1994).

Results of this research were also similar to the report by Tilton and Russel (1984) that reported that embryos will better develop at old cotyledon stage, while embryos cultured at very early cotyledon stage can result in stunted growth on the medium.

Table 4. Effect of embryo age on soybean generation cycle.

Pod (embryo) age	Life cycle (days)
2 WAF embryo	78
3 WAF embryo	85
4 WAF embryo	92
Mature embryo (seed)	117

Effect of pod age on plantlet growth

As predicted, the highest plantlets were resulted from older embryos (4 WAF), although the plantlet height (observed at 20 days after planting, DAP) was not significantly different from that of 3 WAF embryos. Similarly, 3 WAF embryos produced root number that was not different with that of 4 WAF embryos (Table 2). This result suggested that 3-weeks-old pods (embryos) were comparable with 4-weeks-old pods (embryos) as source of material (explants) used for immature embryo culture of soybean. In the case of root length, 2, 3, and 4 weeks-old-embryos had significant effect on rooting capacity, and the longest root (94 mm) was obtained from 4 WAF embryos. Different with the other observed growth variables, leaf number was not significantly affected by the pod age. In general, all pod age treatments produced similar number of leaves on plantlets (Table 2). The effect of seed (embryo) age has similarly been reported by Yan *et al*. (2014). They reported that seeds from fruits harvested at an earlier age are more suitable for embryo rescue purposes. The ideal stage for embryo rescue in cassava was from 32 to 36 days after pollination (DAP).

Overall, cultured 4 WAF embryos produced the fastest plantlet growth, but they were not significantly different with that of 3 WAF embryos. This indicates that 3 and 4 WAF embryos are appropriate embryo stages and presumably qualified for a cultured life, as it has been thoroughly studied by Santos *et al*. (2006). On the other hand, 2 WAF embryos were the least efficient explants, although they were still able to produce plantlets (Table 2). In line with Kosmiatin and Mariska (2005), it is presumably that 4 WAF embryos are categorized as the mature embryo stage, while 3 WAF and 2 WAF embryos are still in old and young cotyledon stages, respectively.

More matured embryos (3 and 4 WAF) have higher reserved food (protein) than younger embryos (2 WAF). At the age of 2 WAF, embryo growth can be improved by modifying growth medium and/or growth regulators. Slower growth of 2 WAF embryos was allegedly because of the not-optimum concentration of plant growth regulator in the medium. IBA application in a culture medium can stimulate root growth (Tilton and Russel, 1983). In line with the growth of roots, the synthesis of endogenous cytokines in plantlets is also increased, which in turn will trigger protein synthesis (Short and Torrey, 1972). Based on the data of plantlet height and root length (Table 2 and Fig. 1), it was shown that the highest plantlet was obtained from 3 and 4 WAF embryos. Photosyntate was used to increase the number of cells, resulting plantlets to grow taller and bigger (Pertamawati, 2010).

Effect of BAP and IBA on plantlet growth

The use of phytohormones IBA and BAP significantly affected most observed growth variables (plantlet height, root number and root length). Auxin and cytokinin are two types of regulatory substances commonly used in culture medium. Auxin is intended mainly for root formation, while cytokinin is intended for bud formation. (Uzun *et al*., 2014a).

Height, root number and root length of plantlets at 20 days after planting (DAP) were varied with different BAP and IBA concentrations (Table 3.). Differences in height, root number and root length of plantlets from immature embryos cultured with different combinations of BAP and IBA were significant. The highest stem (28.7 mm), the maximum number of root (10.1), and the longest root (63.9 mm) per plantlet were obtained from embryos grown on culture medium supplemented with 1.5 ppm IBA (Table 3). The addition of tested BAP concentrations had negative effects on plantlet height, and even more on root length and root number, in which root length and root number of plantlets grown at BAP-containing medium were significantly lower than those at medium without hormone (Table 3).

Research data have shown that the use of 1.5 ppm IBA produced the highest plantlets when compared with other treatments, especially with treatment without hormones (Table 3). This implies that the use of 1.5 ppm IBA in the medium is accordance with the needs of plantlet growth. According to Triatminingsih *et al.* (2001) and Verma *et al.* (2014), auxin such as IBA, roles in stimulating cell elongation, cell division, xylem and phloem tissue differentiation, adventitious root formation, and apical dominant. Result of this research is in agreement with the report of Uzun *et al.* (2014b) that regenerated shoots of *Iris sari* and *I. schachtii* were successfully rooted on MS medium with 1 ppm IBA. However, on the other hand, Ulfah (2009) found that the use of IBA and BAP on turmeric (*Curcuma domestica* Val.) culture simultaneously gave good results on the growth of shoots, in this research addition of hormone BAP was unfortunately not beneficial to soybean plantlet growth. All treatments with added BAP in the medium produced lower growth values than the treatment of 1.5 ppm IBA + without hormone BAP. This may indicate that either hormone BAP was unsuitable for embryo and plantlet growths, or the concentration of BAP in the medium was still not optimum, as reported by Kaviani *et al.* (2015). The effect of cytokinin for shoot organogenesis in soybean depends upon the cytokinin concentration and explant interaction (Verma *et al.*, 2014).

Results of this research are consistent with the results of Sofia (2007) and Rejthar *et al.* (2014) which stated that the high concentration of BAP decreased plant height, especially at a concentration of 4 ppm. Rejthar *et al.* (2014) further reported that shoot multiplication and growth was suppressed by supplementation of BA and kinetin, regardless of concentration used.

Use of cytokinin and auxin in the medium stimulates the proliferation of shoots due to the influence of the synergism between the two hormones, if the concentrations are appropriate (Gaba, 2005; Pishbin *et al.*, 2015). According to Arnold *et al.* (2002), the balance of auxin and cytokinin concentrations, in general, affects the growth of shoots and roots. Similarly, Julkiflee *et al.* (2014) and Pishbin *et al.* (2015) indicated that appropriate combinations between phytohormones in culture medium for certain plant species or tissues are essential to obtain better *in vitro* plantlet growth. Cytokinins induce cytokinesis and are essential for diverse developmental and physiological processes in plants. Cytokinins are mainly synthesized in root vasculature and transported to the shoot, where they regulate shoot growth (Ko *et al.*, 2014).

Research data also showed that the highest number of roots was obtained in treatment 1.5 ppm IBA (Table 3). This is in line the statement of Zulkarnain (2009) that auxin can assist in cell division and cell elongation and root formation. In addition, the addition of low auxin concentrations can increase the formation of adventitious roots, whereas high concentrations can stimulate callus growth and morphogenesis. Furthermore, according to Mattjik (2005), the addition of sufficient auxin in the culture medium will stimulate root formation. Similar to plantlet height, the lower number of roots was obtained in all other treatments with added BAP in the medium. The low number of roots was allegedly due to the concentrations of IBA and BAP were probably still not quite right. The addition of higher BAP concentrations in culture medium in general gave lower number of roots.

This was consistent with research report by Sofia (2007) that the higher the concentrations of BAP in the medium, the more it will decrease the number of formed roots.

This is due to the administration of high concentration of exogenous cytokinin coupled with the presence of endogenous cytokinin would inhibit root formation and root growth.

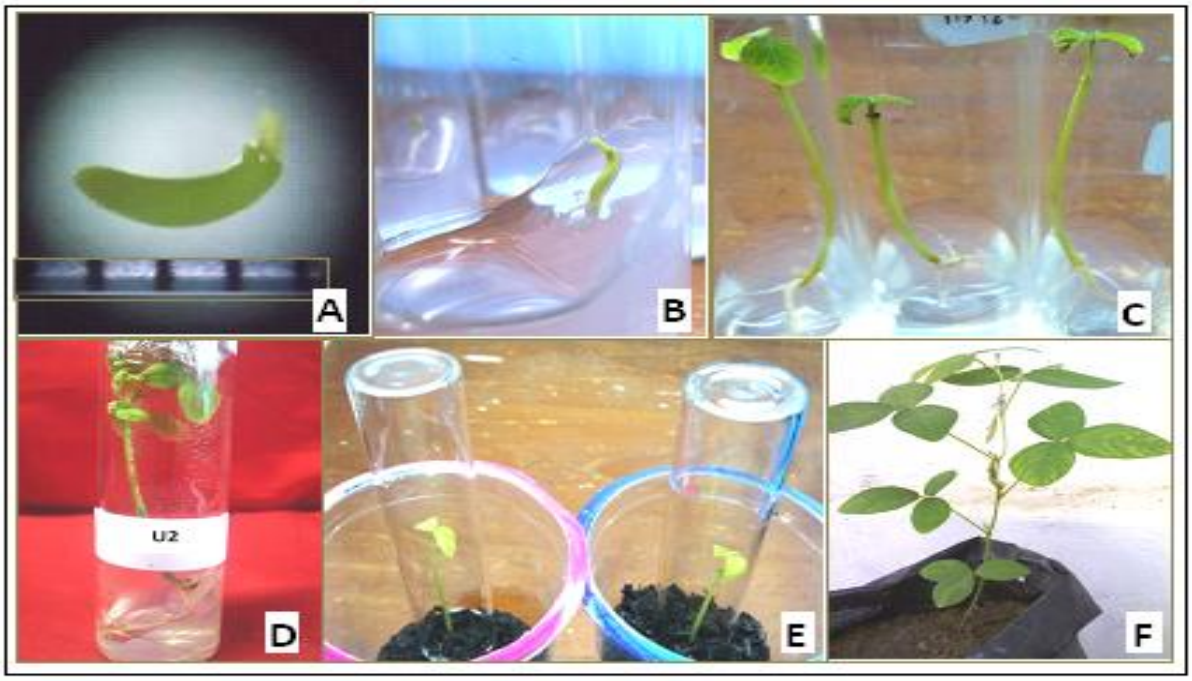

Fig. 1. Growth of soybean *immature embryos*: A) *immature embryo* (approx 3-4 mm) rescued from a 21 days-old soybean pod (Wijayanto *et al*., 2013), B) *immature embryo* grown on B5 culture medium (day 3), C) Soybean plantlets at 14 days after culture, and D) Soybean plantlet at 4 weeks after planting (Wijayanto *et al*., 2013; Wijayanto *et al*., 2015), E) Plantlet acclimatization on soil + burned rice husk medium, and F) *immature embryo*-derived soybean plant, producing few pods.

Similarly, root length was highest in treatment of 1.5 ppm IBA, although it was not significantly different than without hormone. Other treatments with added BAP produced much lower root length (Table 3).

This further indicates that the use of higher BAP concentrations causes stunted root growth. This is consistent with statement of Ilyas (2005), that the provision of BAP with a concentration of 10 ppm gave better leaf formation but slower growth of root length. Furthermore Fereol *et al*. (2002), working with garlic, stated that the combination of cytokinin and auxin did not always stimulating organogenesis in plants.
Overall, research results have indicated that the addition of IBA gave better effects on plantlet growth, as compared to no hormone treatment, but on the other hand the addition of BAP did not provide good effect on plantlet growth.

Comparison of soybean generation cycle from different embryo (seed) stages

Table 4 summaries the time period required to complete a generation cycle of soybean when propagated with different ages of cultured embryos. The data indicate that immature embryo culture can significantly reduce life cycle of soybean when compared with conventional propagation method using mature seed. This result is similar with the findings of Sharma and Gill (1983) that isolated embryos can, in some instances, reduce the generation time in wheat hybrids by 40 days.

The 2 weeks after flowering (WAF) embryos could potentially produce plantlets, but they were much less efficient than 3 and 4 WAF embryos (Table 1 and 2). As previously shown (Table 2), the growth of soybean plantlets from 3 WAF embryos was comparable to and just as efficient as 4 WAF embryos.

Data in Table 4 shows that immature embryo culture of 3 WAF embryos can potentially shorten soybean's life cycle (for breeding purposes) by 32 days from conventional method of propagation using mature seeds (Table 2 and Fig. 1). This way could help soybean breeders to plant soybean more often in each year.

Embryo culture can shorten the breeding cycle by overcoming dormancy in seeds. Dormancy may be caused by endogenous inhibitors, light requirements, low temperatures, dry storage requirements, and embryo immaturity (Bridgen, 1994).

Seed dormancy factors may be localized in the seed coat, the endosperm, or both. By removing the embryos from the influences of these factors, the embryos germinate and grow quickly and the breeding cycle is shortened (Sharma and Gill, 1983; Shen *et al*., 2011).

Conclusion

Indolebutyric acid (IBA) in general provides better effects on soybean embryo germination and plantlet growth, while the addition of 6-benzylaminopurine (BAP) does not provide good effect on plantlet growth. Research data show that immature embryo culture of 3 WAF embryos has potential to shorten soybean's life cycle by 32 days if compared to conventional propagation using mature seeds. This research has shown that immature embryo culture can be used to accelerate reproductive cycle of soybean. Therefore, this method could potentially help soybean breeders to plant soybean more often in each year.

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