High Frequencies Repetitive Shoots organogenesis of Potato (*Solanum Tuberosum* L.) Under Partial Immersion Bioreactor Culture

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Abstract. Potato plays important role in nutritional security, poverty alleviation, and income generation in Indonesia. Potato production restricted and inconsistent by lower quality of seedlings application. Solid medium technique for long time has been using for producing pathogen free plants. This procedure has typically costly as labor intensive and longer durability of production. Liquid culture medium allows the close contact to explants which stimulates and facilitates the nutrients and hormones uptake leading to higher rate multiplication and shoot and root growth of higher quality seedling mother plants. The aim of study is to introduce partial immerse bioreactor system (PIB) for high frequencies of granola Kembang (GK) organogenesis and survival rate during weaning stage in nursery. A 20 shoots of GK were transferred to PIB containing MS medium aerated with 0.5, 1.0 and 1.5 vvm air atmosphere during 30 days of culture. High rate repetitive shoots organogenesis were observed from PIB culture, significantly different to semisolid culture. Averagely 500, 616 and 602 new shoots were harvested from 0.5, 1.0 and 1.5 vvm treatment respectively, which are 30, 37 and 36 folds compared to semisolid culture. Approximately 95-98% of regenerated shoots were survived in acclimatization chamber for both PIB and semisolid culture.

Keywords: Potato Production, Seedling Quality, Bioreactor System, Organogenesis, Plant Nursery

1 Introduction

The potato (*Solanum tuberosum* L.) has been growing worldwide as the major food crop in many countries [1]. Potato tubers are containing a several minerals [2] and dietary protein, that help to increase the circulation of cholesterol level, therefore reduce blood cholesterol

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[3]. Potato production either quality and quantity has becoming important to be increased as human population increased.

In Indonesia, most business activity of potato seed production are by recycling planting materials from previous tuber harvest. Typical traditional seed preparation such as tuber, has several risk on diseases and insect distribution and increased pesticides application that reduced environment for sustainable use and food qualities as well. Those factors have commonly found in developing countries which reducing potato production at most [4]. All those issues due to limited number production of diseases free (healthy) plantlets derived meristem, expensive process and distribution. Application of proper technology of seed production would ensure stable production of healthy planting materials for distribution and reducing contamination.

Two technology, that is micropropagation using solid culture for producing clone derived isolated meristem and tuber/minituber multiplication [5] play as an integral part of potato seed production in Indonesia. However, this techniques is need to be improved, difficult to automatic and scaling up quantities of plantlets in short period of time. A simple micropropagation techonology that facilitate rapid, efficient and large-scale healthy plantlets should be developed for supplying planting materials to meet requirement of large plantation areas and distantly areas. Bioreactor based on liquid medium has been identified as appropriate technology for producing large number of potato shoots in Indonesia. The objective of study is to introduce partial immerse bioreactor system (PIB) for high frequencies of GK organogenesis and survival rate during weaning stage in nursery. High quality seeds would increase productivity, reduce pathogen distribution and agrochemical application therefore promoted sustainability in food production and good agricultural practices.

2 Materials and Methods

Sequential experiments were conducted in Filant laboratory Tissue Culture Laboratory. The meristem were initiated in 2021 and shoots proliferation in semisolid medium were routinely conducted in 2021 and 2022 as described below.

2.1 Culture Establishment

The genotipe material was Granola Kembang, typically grown in East Java and some areas in Java, Indonesia. Shoot apical were collected from mother plant and meristem were isolated and cut transverse thin cell layer for producing multiple shoot from small size explants.

Shoots were carefully cleaned and flushed using tap water, and placed into a laminar air-flow cabinet (LAFC). Explants were sequential dipped in 70% ethanol for 5 minutes followed by 10% sodium hypochloride for another 5 minutes and washed 3 times with autoclaved water for 1 minute. Explants were then placed on full strength Murashige and Skoog (MS) (Duchefa M220), 30 g/L sucrose, and 13% (w/v) agar, pH 5.7±0.1, to induce new shoots. Media were autoclaved at 121°C for 15 min. New shoots were regularly selected and placed on new medium after 2 weeks culture. Habituated shoots grown in the medium then were selected for further shoots production activities. After 5 subcultures biweekly in semisolid, an individual 10 shoots apical were transferred to liquid double layer cultures containing 50 mL medium for another 4 times biweekly subculture as standard pre-culture prior to multiply in bioreactor system.

2.2 Partial Immersion Bioreactor (PIB) Cultures

 https://doi.org/10.1051/bioconf/20236901026

Shoots from double layer culture were used in PIB experiment. This experiments were conducted to obtain the best condition for shoots to grow in PIB condition. About 20 explants were inoculated into 3 liter Air-lift Bioreactor equipped with plastic net to separate 250 mL liquid phase; as described above without agar and explants. The PIB were continuously supplied by filterized air at 0.5, 1.0, and 1.5 vvm (air volume/culture volume/minutes), regulated by a flowmeter. Those PIB cultures were kept in a culture room having temperature at 23±2°C and a 16 hours radiation at 35 μmol/m^2/s by cool white fluorescent lamps.

2.3 Experimental Setup, Data Collection, and Statistical Analysis

The PIB and semisolid cultures (as control) were conducted using a completely randomized design, repeated 3 times. Explants were harvested at 30 days of culture and shoots number, shoot length, total shoot length and number harvested (@3 cm), survival rate, root number, new leaves and stem diameter were measured. Data collection were analyzed by analysis of variance. A comparison among treatment were carried out using least significant differences (LSD). significant difference were considered at probabilities less to or equal to 0.01.

3 Results and Discussions

A growth comparison of GK shoots after 30 days incubation in solid and PIB culture at different aeration rate revealed that repetitive shoots multiplication and shoots development were more efficient in PIB culture (Table 1). Potato shoots growth in semisolid and PIB culture were identified significant differences ($p \leq 0.01$). The PIB system from all treatments, presented massive growth of shoots compared to semisolid culture. Shoot number per mother explants, shoot length, total shoot length were increased similarly as the aeration rate increased. Maximum responses of

Fig.1. High repetitive shoots organogenesis of Granola Kembang supplied with air atmosphere at 1.0 vvm in PIB (left) and semisolid culture (right)

Repetitive shoots production were observed maximum when explants supplied with 1.0 vvm air atmosphere on 250 mL liquid medium, producing 23 shoots/explants. In the case of shoot length and total shoots length, maximum response were found at aeration rate of 1.5

vvm, that produced 23 new shoots per explant, but non significant with aeration rate of 1.0 vvm (Table 1). In fact, PIB system produced more shoots during incubation compared to semisolid cultures. About 2-4 explants produced 18 to 26 new shoots within 30 days of cultures while semisolid culture produced 1-3 new shoots per explant.

Table 1. The Effect of Aeration Rate on Granola Kembang Shoots Production on Partial Immersion Bioreactor and Solid Culture After 30 Days of Incubation.

Type of culture	Shoots number	Shoot length (cm)	Total Shoot length (cm)	Harvested shoot (@3 cm)
PIB 0.5 vvm	19.67±0.25 b	15.27±0.19 b	300.47±5.39 b	500.78±8.98 b
PIB 1.0 vvm	23.53±0.19 c	15.73±1.18 b	369.87±26.15 c	616.44±43.58 c
PIB 1.5 vvm	22.67±0.90 c	16.00±0.19 b	361.47±6.72 c	602.44±11.20 c
Semi Solid	1.73±0.09 a	5.87±0.09 a	9.87±0.66 a	16.44±1.10 a

Note: Data represent the mean values ± standard deviations. Different letters indicated significant differences at $P<0.01$

Aeration rate regulation during micropropagation for faster shoots multiplied, biomass development and growth has been reported in several crop species, such as in citrus embryogenesis [6] and cell culture [7], banana [8], and ginseng [9]. Continuous air supplied would increased oxygen and/or CO_2 dissolved in liquid media. The more air flowing into vessel, the more oxydative metabolites is potentially working on plant growth in PIB system, leading to actively plantlet growth and multiplication; and more internode numbers and auxillary shoots can be harvested.

In the PIB system, the roots and lower part of stem explants were partially immersed into the liquid medium. This lower part of explants were continuously immersed in medium. Therefore nutritions can be supplied continuously supplied and induced the shoots grew rapidly (Fig.1). Typical immersion culture in PIB system managed by a net that plants placed on and separated it from liquid phase, therefore avoid complete submersion. The physiological disorders: such as hyperhydricity, then avoided as limitation contact between liquid and whole plants.

In contrast, semisolid culture showed lower growth activity probably due to lack of oxygen. Agar culture has limitation compared to liquid cultures by which shoots are generally produced. Liquid culture is superior to agar culture due to a better contact between media and explants and more surface of explants being involved in medium absorption. In term of practices, the activities of transferred a dozens of explants in the liquid cultivation can be made at once, while in semisolid required multiple subcultures. The most different of PIB over solid culture is on vessel size. The PIB system uses larger vessel, therefore mechanical stress that caused abnormalities might be avoided. Furthermore, ethylene would be produced during mechanical stress reducing shoots growth of potato plants [10]. Fortunately, continuous supplies of gas in different aeration rate into PIB vessel eliminated gas contamination like ethylene and supported gas exchange. Gas filter in PIB plays important role in aseptic status and filter leaking should be avoided which lead to further contamination. However, culture management would ensure shoots production under fully control.

The results suggested that the revetitive organogenesis of plantlets in vitro can be controlled by aeration rate of PIB culture, volume of vessel and room ecosystem, as explained previously [11]. Intensively shoots multiplication and development in PIB culture increased 11 to 13 folds over semisolid culture at duration stated. Ideal condition in a PIB has increased high rate multiplication of organogenic propagules (of healthy mother plants) and reducing cost of micropropagation to produce transplants for mass production.

A major constraint on mass production of plantlet through micropropagation is lower survival rate during adaptation into external natural conditions. In vitro shoots during

https://doi.org/10.1051/bioconf/20236901026

transferred to natural conditions are potentially affected negatively by abiotic stress; higher heat level, irradiation, humidity and biotic stress; pathogen and insect. Therefore, additional step namely acclimatization is required for successful plantlets or shoots establishment and survival in natural condition. Optimal conditions during in vitro culture is required to increase vigor of plantlet and to preserve their survival rate during acclimatization.

It was then verified that, after 30 days of culturing in both PIB and semisolid culture, plantlets of GK had a survival rate of ranged 95.3 % to 98.0 % without significant differences (Fig. **2**). Its seem that developed shoots derived PIB and semisolid have equal capacity to induce new root, leaves and stem growth during acclimatization stage. High rate survival of plants is regulating by suitability between in vitro (either PIB and semisolid) condition and ex vitro environment regulated, and a guarantee of success after field planting, as previously reported [12]. It was indicated that hyperhydricity that normally found in liquid culture were not presented negatively effect in shoots multiplied and developed under PIB system.

Table 2. The Effect of Aeration Rate on Granola Kembang Shoots Survival Rate, Root Number per Explant, New Leaves Developments and Stem Diameter After 30 Days of Incubation.

Type of culture	Survival rate	Root number	New leaves	Stem Diameter (cm)
PIB 0.5 vvm	95.3%±0.01 a	2.80±0.16 a	2.80±0.16 a	1.28±0.02 a
PIB 1.0 vvm	96.0%±0.03 a	2.93±0.19 a	3.33±0.19 a	1.29±0.05 a
PIB 1.5 vvm	96.0%±0.02 a	3.00±0.33 a	3.00±0.43 a	1.24±0.03 a
Semi Solid	98.0%±0.02 a	2.67±0.34 a	3.00±0.16 a	1.31±0.07 a

Note: Data represent the mean values ± standard deviations. Different letters indicated significant differences at $P<0.01$

Mass potato micropropagation to create high population of healthy plantlet is commonly started from healthy shoots initiated from meristem culture. Over the years, potato shoots derived nodal segments are commonly practices for producing healthy planting materials and has been successfully producing disease-free potato seed in Indonesia and worldwide. The pathogen in potato planting materials is a major concern of potato seed quality as international standard. High quality planting material production therefore is essential for improving domestic potato productivity and to ensure minimum impact on agricultural practices due to non healthy seed application. Improved procedure that is shorter and non laborious steps for producing large number of new shoots is required not only to supplying quality seed but also safe agricultural environment.

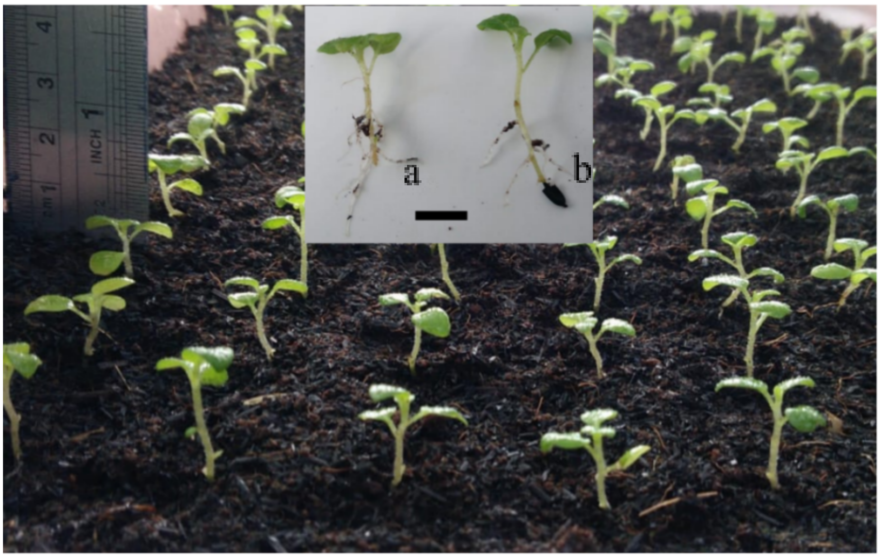

Fig. 2. High rate survival of Granola Kembang shoots derived PIB (a) and semisolid (b) after 30 days of acclimatization. (bar = 1 cm)

In our system, a shoots culture establishment and production were sequentially conducted of meristem isolation and establishment (2 months), induction of axillar and/or adventitious shoots and habituating in solid medium (2-3 months), followed by liquid static (2 months, biweekly subculture and repetitive production in bioreactor system (1 month). Production stage of potato shoots, is the ultimate step that ensuring high productivity and costless. The used of advanced user friendly technology “PIB culture system” for small lab operation is then proposed for fulfilling high demand planting material during planting season. Several types of bioreactor vessels and system have been used for shoots propagation of potato in the past, such as Life Reactor© culture [13], TIS (either in EBB and flood), continuous immersion (either with net and without net) [14], 1.5 L glass bottle type bubble bioreactor (GBTBB) and 10 L bottle type bubble bioreactor (BTBB) [15], Semi-Continuous Bioreactor [16], nutrient spray bioreactor (NSB) [17], TIS bioreactors (SETIST) [18] and [19]. However, some techniques, uses expensive machinery and compressed air to supply air to the vessel.

An optimum protocol of GK mass propagation shoots was recently was recently available using PIB system. Recently studied confirmed that modified system of air-lift bioreactor with net has potentially used for scaling up shoots production of potato is rapid, low cost, hyperhydricity free, vigorous, higher survival rate on fitted acclimatization chamber and therefore solved the most limitation on low quality planting material. However, pilot project to evaluate technology in relevance quantities and planting areas should be conducted.

4 Conclusion

High rate repetitive organogenesis of GK shoots were observed from PIB culture, significantly different to semisolid culture. Averagely 500, 616 and 602 new shoots were harvested from 0.5, 1.0 and 1.5 vvm treatment respectively, which are 30, 37 and 36 folds compared to semisolid culture. Approximately 95-98% of regenerated shoots were survived in acclimatization chamber from both PIB and semisolid culture. A PIB culture system supplied by 1.0 vvm aeration rate has potentially used on potato shoots production; interm of high productivity, faster and low cost operational.

The authors express gratitude to Doni Edo and Sri Rahayu, laboratory assistants, for excellent lab works and nurseries activities.

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Credit Authorship Contribution Statement

Dita Agisimanto conceptualized, supervised and conducted the experiments, observation, analyzed the data, and wrote and review the manuscript and corresponding author, **Nur Hayati Hadiba** shared research ideas, supervised and conducted the experiments, observation, data collection and interpretation, wrote the original manuscript, materials and facilities support, **Nirmala Friyanti Devy** shared research ideas, supervised and conducted the experiments, observation and data interpretation, and wrote the original manuscript, **Hardiyanto** shared research idea, problem statement and premises, conducted the experiments, observation and data interpretation, and wrote the original manuscript, **Farida Yulianti** shared research idea, conducted the experiments, observation, data collector, documentation and analysis, and wrote the original manuscript. All the author involved along planning, conducting experiments and submitting and editing the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.