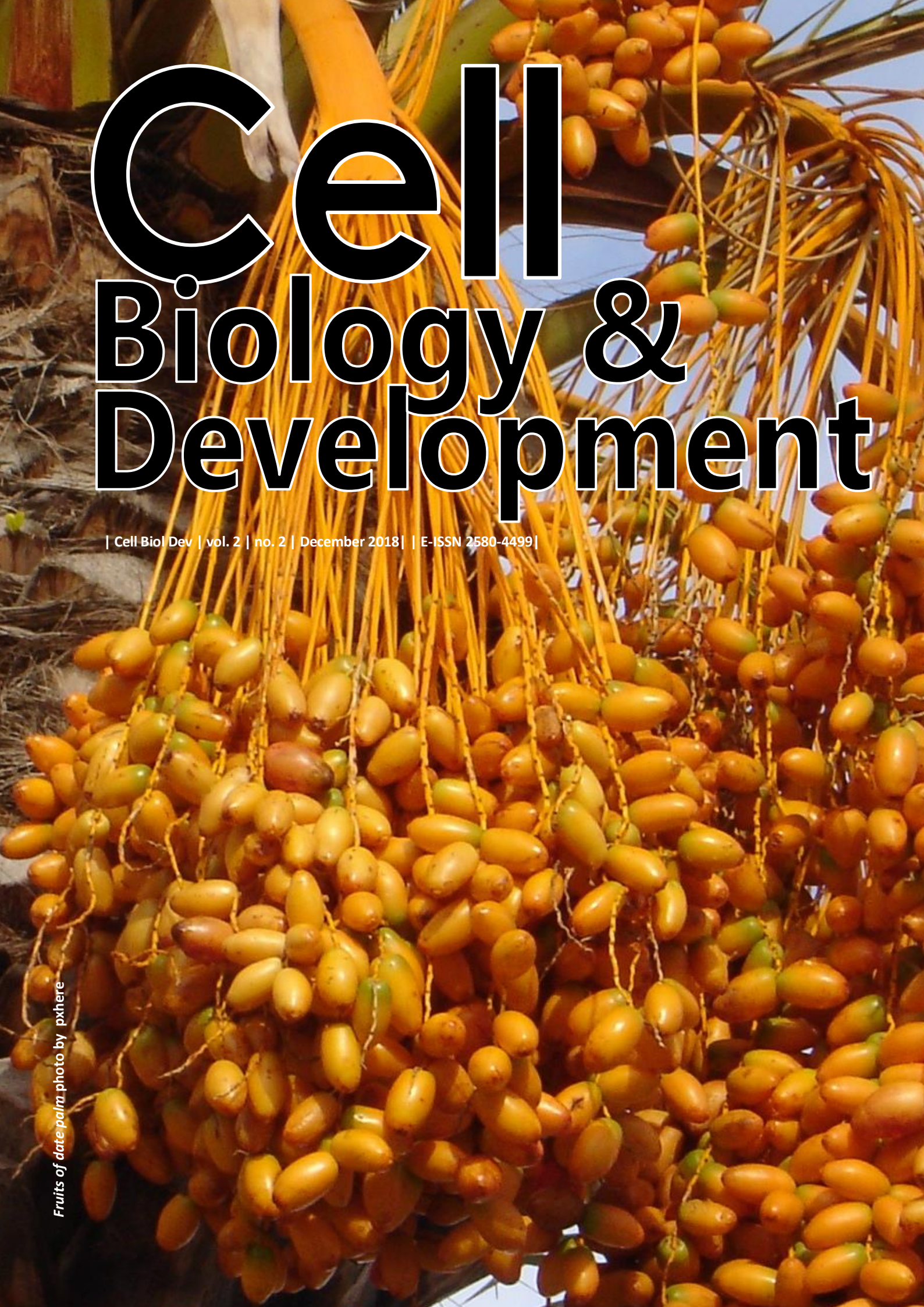

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Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158.

Book:

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Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

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Short Communication: Optimazing culture and differentiation L6 cell, C_2C_{12} cell and primary myoblast cells culture

RONNY LESMANA[1,2,♥], HANNA GOENAWAN[1,2], VITA MURNIATI TARAWAN[1], IWAN SETIAWAN[1], ACE HIDAYAT[2,3], UNANG SUPRATMAN[2,3]

[1]Division of Physiology, Departement of Basic Science, Faculty of Medicine, Universitas Padjadjaran. Jl. Sukarno-Hatta Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia. ♥email: ronny@unpad.ac.id

[2]Central Laboratory, Universitas Padjadjaran. Jl. Sukarno-Hatta Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia

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Manuscript received: 22 July 2018. Revision accepted: 10 October 2018.

Abstract. *Lesmana R, Goenawan H, Tarawan VM, Setiawan I, Hidayat A, Supratman U. 2018. Short Communication: Optimizing culture and differentiation of L6 cell, C_2C_{12} cell, and primary myoblast cells culture. Cell Biol Dev 2: 51-54.* Alteration in skeletal muscle function and structure could result from many chronic diseases, drug treatment, or physical activity. Therefore, understanding the molecular mechanism of skeletal muscle diseases or the adaptation process will be very important and beneficial. In most cases, for example, during aging, the imbalance between anabolic and catabolic processes could initiate a decreased muscle mass and change its fiber structure. Unfortunately, there is no suitable animal model for study, and animal model development is time-consuming at some point. Next, it is good to study and elaborate a model on disease mechanisms, drug targets, and hormonal effects in skeletal muscle, which is not easy and tricky. Finally, the promising methods to study molecular biology properties of muscle are using *in vitro* systems utilizing 2 cell lines derived from mouse (C2C12 myoblast cells) and rat (L6 myoblast. Another establishment technique is using mixed and isolated single fiber primary myocytes culture.

Keywords: Cell line, C2C12, L6 myoblast cell, primary myocytes culture

INTRODUCTION

Alteration in skeletal muscle function and structure often occurs due to different chronic diseases, including cancer, heart failure and diabetes, aging, hormonal imbalance, and genetic myopathies (Lin et al., 2018; Demontis et al., 2013). The inflammatory cytokines are observed due to an imbalance between anabolic and catabolic processes (Demontis et al., 2013). There are many unknown mechanisms of hormonal regulation in controlling the physiological function of skeletal muscle, like thyroid hormone, mechanism of Emery Dreyfuss, Duchenne muscular dystrophy (DMD), exercise regulation, etc. (Bloise et al. 2018).

Limitations in utilizing animal models or performing experiments in vivo using mice, rats, and rabbits lead researchers to use cell lines for study. Experiments using *in vitro* models, like muscle culture systems, may become a very good solution (Liu et al., 2017; Rovetta et al., 2013). Unfortunately, there is limited knowledge to study using mature muscle cell culture derived from myoblast cell culture. and elaborate more on the mechanism of disease, drug target, and hormonal effects in skeletal muscle, and it is not easy and tricky (Rovetta et al. 2013; Koenig and Smith 1985). However, to better understand the disease mechanism in skeletal muscle, the right approach other than in *vivo* experiments using mice and rats is needed. Besides that, the animal ethical issues recommended reducing, refining, and resizing the number of animals used in the experiments as motivation to use the cell line as the best option to work with for study.

Setting an experiment using an in vitro system is one solution whenever there are difficulties in using mice or rats as animal models. Moreover, to set skeletal muscle in vitro experiments, better knowledge about muscle cell line characteristics is needed. Therefore, this study established the *in vitro* study using 2 cell lines derived from mouse: C2C12 myoblast cells and rat: L6 myoblast cells and mixed and isolated single fiber primary myocytes culture.

MATERIALS AND METHODS

Cell culture L6 cells

Rat L6 myoblast derived cell line was a more suitable model for thyroid hormone (TH) study (Koenig and Smith 1985). Therefore, L6 myoblast-derived cells were maintained at 37º C, with 5% CO_2 in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 100 µg/ml penicillin-streptomycin antibiotic. After 3 days of proliferation with DMEM contained with 10% FBS, the medium was changed with DMEM contained with 2% horse serum (HS) for 5-7 days to induce myotubes formation.

Cell culture C_2C_{12} cells

Mouse C_2C_{12} myoblasts were maintained under standard conditions (37 °C, humidified atmosphere, and 5% CO2) in Dulbecco's Modified Eagle's Medium (DMEM) high-glucose supplemented with 10% Fetal Bovine serum (FBS) and 100 µg/ml penicillin-streptomycin antibiotic. Like the Rat L6 differentiation protocol, cells were cultured and incubated for 5 days in differentiating medium (DM) with reduced serum content into 2% FBS. The myotubes were then fixed in absolute methanol for 10 min, stained with 10% Giemsa reactive, and observed using light microscopy.

Primary skeletal muscle culture cell

For four weeks, old Wistar rats were used in the experiment; muscles were excised and cleaned briefly from surrounding connective tissues. Then, muscle tissue was minced with sharp scissors and digested with protease XIV (p5147, Sigma Aldrich, USA). Several centrifugations separated the cells from muscle fiber fragments and tissue debris. After several layers fractioned count by Percoll density, cells were plated at 2.10^4 cells/cm^2 on a 2 cm Petri disc coated with poly-l-lysin and used alpha Modified Eagle Minimum essential. Primary culture myoblast cells were maintained at 37ºC with 5% CO_2 for 48 hours. Finally, to differentiate myoblast cells into myocytes, serum in the medium for differentiation was changed into 2% horse serum and maintained for 5 days (Ono et al. 2015).

RESULTS AND DISCUSSION

Proliferation and differentiation in L6 Cell

L6 cells were seeded with 4.10^4 cells/cm^2 density and followed up its progress using light microscope observation. Most of the cell shape was round at the proliferation state, and there was no long structure. However, after 5 days of differentiation, there are long tubes formation as a sign of myotubes formation of more than 65% in one field inspection. (Figure 1).

Proliferation and differentiation in C_2C_{12} Cell

Next, 5.10^4 cells/cm^2 of C_2C_{12} myoblast cells were seeded in 6 well plates. During proliferation, most of the cell shape was round and no long myocyte structure. However, five days after differentiation began, long structures as characteristic of myocyte cells were observed in >80% cell culture (Figure 2).

Proliferation and differentiation in primary myoblast culture cells

Primary myoblast cells were plated at 2.10^4 cells/cm^2 on the Petri disc. Within 3-4 days, the primary cell showed a growing process. Most of the cell shape is round and cylindrical at the proliferation state. After differentiation for 8-10 days, there was a shift that >90% of myoblast cells were transformed to myotube cell shape with characteristic has long tube structure form (Figure 3).

Discussion

Myopathic changes are observed in 30-80% of patients with hypothyroidism. Patients with more severe or longstanding untreated hypothyroidism are more likely to develop clinically significant muscle disease. Physiological aging also can alter muscle structure and functions (Lin et al., 2018). Four types of hypothyroid myopathy are found: Hoffmann syndrome, Kocher-Debre-Semelaigne syndrome, atrophic form, and myasthenic syndrome (Udayakumar et al. 2005; Vasconcellos et al. 2003). Those are an example of muscle diseases with an unclear mechanism; possibly, the physiological changes appear before or after the structural adaptation of skeletal muscle. Mechanisms of underlying pathological or physiological conditions in skeletal muscle can be studied easier using an *in vitro* system.

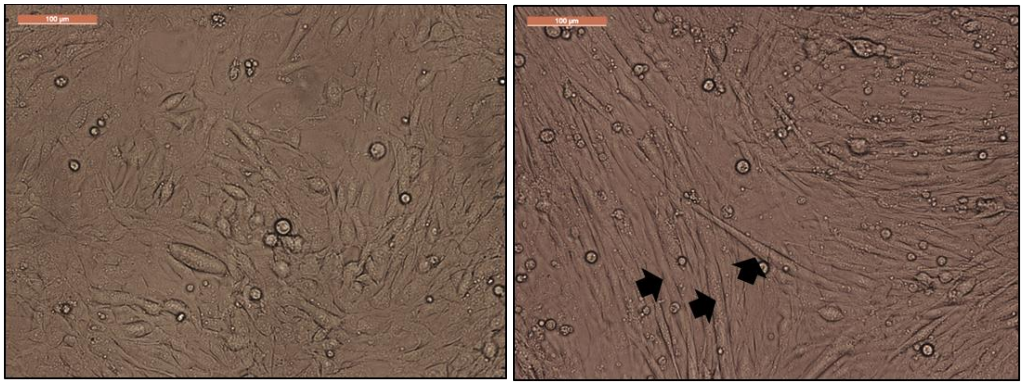

Figure 1. The different appearance between myoblast (Day 1; *left*) and after serum in the medium was adjusted and induced cell to myotubes (Day 5; *right*) in L6 cells

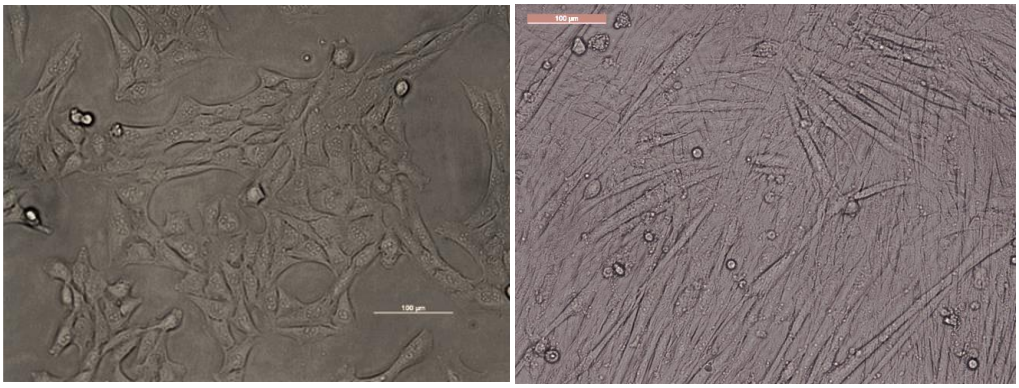

Figure 2. The different appearance between myoblast (Day 1; *left*) and after serum in the medium was adjusted and induced cell to myotubes (Day 5; *right*) in C2C12 cells

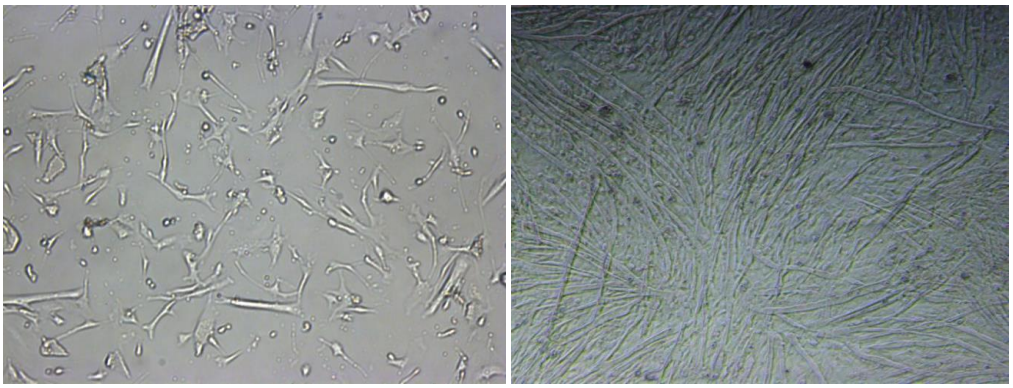

Figure 3. The different appearance between myoblast at day 2 (*left*) and myotubes at day 6 (*right*) in rat primary myoblast culture cell. The Source of muscle is the soleus muscle

Several types of immortalized muscle cell lines can be used for experimenting. The L6 cells are derived from rat myoblast, and the C_2C_{12} cells are derived from mouse myoblast; both cells are the most common cell line utilized for the experiment. Immortalized L6 cells and C_2C_{12} cells are cultured in myoblast form; under a specific condition, the cell will proliferate and differentiate (Rovetta et al., 2013; Koenig and Smith, 1985). An important key to getting long-lasting results is knowing every specific change during this process very well. At the end of the differentiation state, it is common to see a mixed population of myoblast and myotubes. Many researchers used arabinose to eradicate the myoblast from the plate culture to purify myotubes from myoblast. Next, it was possible to characterize these cells, mimic situations like in vivo experiments and set them according to the preferred experiment.

The limitation of the study utilizing L6 and C_2C_{12} cells are immortalized cells, which do not 100% reflect the physiological type of muscle function. However, primary myoblast culture or single fiber culture derived from any muscle can be used to overcome these limitations. Furthermore, primary myoblast culture showed a higher response to hormonal treatment, chemical or manipulation, and its signaling was closer to the *in vitro* experiment results (Iovine et al., 2012; Minami et al., 2011). Finally, optimizing protocol and conditions for in vitro experiments will help us to get the best results in our setting experiment. Therefore, L6 cell, C_2C_{12} cell, and primary myoblast cultures cell could be the answer for studying muscle physiology and pathology in mice and rats.

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Sucrose and coumarin effect on the growth and development of micro-cutting potato plant (*Solanum tuberosum*) variety Granola Kembang

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Abstract. *Manjaswari A, Pitoyo A, Sari SLA. 2018. Sucrose and coumarin effect on the growth and development of micro-cutting potato plant (Solanum tuberosum) variety Granola Kembang. Cell Biol Dev 2: 55-62.* This research aimed to determine the effect of various concentrations of sucrose and coumarin on the micro-cutting potato plant's growth and development. A completely Randomized Design (CRD) with a single concentration combination factor of sucrose and coumarin was treated well. Five levels of sucrose concentrations were 0, 30, 50, 70, and 90 g/L. While coumarin concentrations were 0, 20, 40, 60, 80 mg/L. Observation and data collection was made in the second month after the explant planting. Some parameters observed were morphological changes in explant characteristics, i.e., the percentage of explants response, the emergence of the shoot, shoot height, shoot number, leaf emergence time, number of leaves, the emergence of the root, root number, and root length. The data obtained were analyzed using ANOVA followed by DMRT at level test 5 % to determine if there is a significant difference between the treatments. The result showed that the higher sucrose concentration added into the media could increase the micro-cut growth and development of shoots and leaves; otherwise, it would negatively influence root development. The addition of coumarin in high concentration inhibited all the parameters of growth and development observed. Both combinations of sucrose and coumarin could increase the micro-cut development of shoot at 50 g/L of sucrose and 20 mg/L of coumarin concentration. Those concentrations could produce the best amount and higher shoot for micro-cut multiplications.

Keywords: Coumarin, in vitro, micro-cutting, potato, sucrose

Abbreviations: BAP: Benzyl Amino Purin; DAP: Days After Planting; GA: Gibberellin Acid; MS: Murashige and Skoog

INTRODUCTION

Potato (*Solanum tuberosum* Linn.) is a highland vegetable plant that belongs to the Solanaceae family. Potato is one of the world's main foodstuffs after rice, wheat, and corn (Rubatsky and Yamaguchi 1995) which contains high carbohydrates and relatively small amounts of fat, i.e., 1.0-1.5% (Bambang 1987). Therefore, potatoes as a national superior vegetable commodity are prioritized for development by the Indonesian government because they have prospects in supporting food diversification programs.

The Ministry of Agriculture (2015) stated that the number of imported potatoes, especially processed ones, has increased dramatically from 2012 until now. However, potato production is almost constant, as stated by the Indonesian Central Bureau of Statistics (2014); in 2014, it produced 1,347,815 tons of potatoes, while in 2015, it produced 1,219,269 tons of potatoes. In addition to the increasingly narrow land area, it is difficult for Indonesian potato farmers to obtain high-quality tubers. The local seeds used are generally degenerated and infected with various diseases caused by viruses. Therefore, it is necessary to produce high-quality potato seeding that produces seeds free of viruses and diseases (Mariani 2011).

One attempt to increase the productivity of quality potato seeds is in in-vitro culture techniques. In vitro plant propagation could be made, among others, through somatic embryogenesis, regeneration of adventitious organs, formation of axillary branches, and single-node culture (Pierik 1987). Single node culture is an in vitro culture technique in which one explant contains one node or only one leaf. The results of single node culture are generally referred to as micro cuttings/plantlets. Micro cuttings produced in-vitro can be used in the multiplication process, the formation of micro tubers, and mini tubers (Struik and Lommen 1990). With this in vitro culture technique, it is possible to produce potato seeds that are uniform and the same as the parent (true to type) in large quantities in a short time without depending on the season and free from pests, diseases, and viruses because they are maintained in aseptic conditions (Wattimena 1983).

Problems encountered in potato propagation in vitro are the time it takes to form roots and the formation of strong and healthy plantlets (Monnier 1990; Liz and Levith 1997). The composition of the culture media is very influential on the growth and development of explants. Sucrose is a carbon source commonly used under in vitro culture techniques and serves as a source of energy cells need to grow (Kimball 1994). Faria et al. (2004) stated that various treatments with a sucrose concentration of 60 g/L showed the highest plantlet growth compared to other treatments. Sucrose can also increase the formation of micro tubers of Dewa leaf plants. Sucrose in a concentration of 6% in the

media was able to increase the number of micro tubers significantly.

In addition to sucrose, media components that determine the success of tissue culture are the type and concentration of growth regulators (PGR) used (Ali et al. 2007). So it is because growth regulators have characteristics that can induce or inhibit plant physiological processes. Growth regulators that have inhibitory properties are also called retardants. One example of a retardant substance is coumarin which works by inhibiting the activity of GA, which plays a role in cell elongation and division. According to Sakya et al. (2003), the administration of coumarin can inhibit the emergence of branches in potato plants. Conversely, the treatment without coumarin showed more branches than the coumarin treatment at a concentration of 45 mg/L.

Research on the effect of sucrose and coumarin on potato micropropagation results has been done previously. However, research on the effect of the combination of the two on the yield of potato plant propagation has never been done. Based on the description above, it is necessary to research the effect of giving various concentrations of sucrose and coumarin or their combination on the growth and development of potato micro-cuttings in vitro to produce high-quality and high-quantity seeds. One of the potential potato varieties is the Granola Kembang variety, which has not been widely studied for its in vitro propagation.

MATERIALS AND METHODS

Materials

This research was carried out for three months, from December 2015 to March 2016. This research was conducted at the Biology Laboratory, the Faculty of Mathematics, Sebelas Maret University, Surakarta. The plant materials were derived from plantlets produced by single shoot multiplication of potato tuber (*Solanum tuberosum* Lin.) Granola Kembang variety aged four weeks after planting in MS medium without growth regulators (MS 0) collection from the garden at the Kledung Horticultural Seed Garden, Temanggung. The materials for making the treatment media were Murashige Skoog (MS) instant basal medium with vitamins (Phyto Technology Laboratories), sucrose, standard coumarin HPLC (High-Performance Liquid Chromatography), 99% (Sigma), aquadest, and 96% alcohol.

Procedure

In this study, a single factor Completely Randomized Design (CRD) was used, namely a combination of sucrose and coumarin concentrations. The sucrose concentration consisted of 5 levels, namely 0, 30, 50, 70, and 90 g/L and 80 mg/L and the coumarin concentration consisted of 5 levels, namely 0, 20, 40, 60, and 80 mg/L with 3 times replications.

Tools such as culture bottles, measuring cups, beakers, Petri plates, Erlenmeyer, spatula, tweezers, and dropper pipettes were washed thoroughly, then sterilized using an autoclave at 121°C, under 1.5 atm pressure for 15 minutes.

The treatment media was stocked with 1000 mL for each treatment. Instant MS media (PhytoTechnology Laboratory) in powder form was weighed using an analytical balance of as much as 4.43 g. After that, 4.43 g of instant MS was put into an Erlenmeyer. Sucrose and coumarin were added according to the variation of the concentration used. For example, to make S4K4 treatment, 90 g of sucrose and 80 mg of coumarin are needed. 500 mL of aquadest was added to an Erlenmeyer glass containing instant MS, sucrose, and coumarin, then heated on a hot plate with the help of a magnetic stirrer. The medium was adjusted until the pH reached 5.5 - 5.7. If the pH was too low, 1 N NaOH was added, while if the pH was too high, 1 N HCl was added. Next, 8 g of agar was added to the media solution, then distilled water was added until the volume reached 1,000 mL. After boiling, the media was poured into sterile culture bottles with a volume of approximately 25 mL per bottle. Then the bottle was immediately covered with aluminum foil and glued with plastic wrap. The media in the bottle was then re-sterilized by autoclave at a temperature of 121ºC and steam pressure of 1.5 atm for 2 hours. The sterilized media bottles are cooled at room temperature and stored in the culture room.

The explants were planted aseptically in a Laminar Air Flow Cabinet (LAFC) Using a single nodule cutting technique. Plantlet collection of Kledung Horticultural Seed Garden, Temanggung, Explants of Granola Kembang variety aged four weeks after planting in MS medium without growth regulator (MS 0) were taken and cut into several parts. Each explant contains one node and one leaf blade. The explants were then planted in culture bottles using tweezers. Each culture bottle was planted with one explant. After the explants were planted in the media, the culture bottles were again covered with aluminum foil and glued with plastic wrap.

The explants planted on each treatment medium were incubated at 18-20°C and observed two months after planting. Observations were made on the percentage of explants responding to media, time of shoot emergence, length and number of shoots, time of leaf emergence, number of leaves, time of emergence of roots, length, and number of roots.

Data analysis

The data on changes in morphological characters were analyzed using Analysis Of Variance (ANOVA) and then continued with Duncan's Multiple Range Test (DMRT) at the 95% test level to determine if there was a significant difference between treatments using SPSS 16.0 software.

RESULTS AND DISCUSSION

Percentage of explants responding to media

In this study, combining sucrose and coumarin with different concentrations resulted in different growth responses of single-node explants. Explants showed a direct organogenesis response where explants grew to form shoots and roots without going through callus formation first (Dhaliwal et al., 2003). It is in line with the study where single-node explants were not found to have a callus. Single node explants grow to form shoots, leaves, and roots directly without callus formation first. The growth of single-node explants grown in media with a combination of sucrose and coumarin concentrations was observed up to 56 days after planting and is presented in Table 1.

Treatment without sucrose and coumarin addition in the media resulted in the percentage of explants responding to the media by 80%. The addition of sucrose to a concentration of 50 g/L increased the percentage of explants responding in a medium without coumarin to 100%. The addition of higher sucrose concentration caused a decrease in the percentage of explants responding in the media without coumarin. The percentage of responding to single-node explant media decreased at a concentration of 70 g/L and decreased at a different concentration of 90 g/L, without adding coumarin. According to Marlin (2005), the energy source (sucrose) available in high concentrations causes a decrease in osmotic pressure. In ginger (*Zingiber officinale* Rosc.), the low osmotic pressure in the media causes the explants to be unable to optimally absorb existing nutrients for growth so that their growth will be stunted.

The addition of coumarin 20 mg/L in the media resulted in an explant response with a percentage of 80%. Adding coumarin concentrations of up to 40 mg/L and 80 mg/L in media without sucrose caused a decrease in explants' response to media. The higher concentration of coumarin addition in the media without sucrose would inhibit the growth of single-node explants planted. The addition of coumarin concentrations of 40, 60, and 80 mg without sucrose showed no growth up to 56 days after planting. Hasni et al. (2014) stated that the higher the concentration of coumarin, the higher the percentage of dead plantlets. The coumarin addition at a concentration of 50 mg/L showed an increase in the percentage of dead potato plantlets and increased again at 75 mg/L. It is because the administration of coumarin in the media will inhibit the activity of the gibberellin hormone in the explants. According to Salisbury and Ross (1995), the gibberellin hormone promotes cell division, so if its activity is inhibited, it will inhibit the growth of the explant.

The formation of good micro cuttings can increase the multiplication of micro cuttings and the production of micro tubers in in-vitro conditions, and the production of mini tubers in the screen house. The combination of concentration of 50 g/L sucrose and 20 mg/L coumarin resulted in good explant growth due to the addition of sucrose with neither too high nor low concentration. Sucrose plays a role in cell elongation and expansion. If added in small amounts, it cannot provide an energy source, and if added too much, it will inhibit growth because it will inhibit the absorption of nutrients for plants (Marlin 2005). Furthermore, adding more than 20 mg/L of coumarin showed no growth because coumarin, as a retardant substance characteristic, reduces tissue metabolic activity and can inhibit the process of vegetative growth (Purnomo and Prahadini 1991).

Number of shoots

The first shoot on each explant is an elongation of the bud or node. In this study, the average shoots appeared on the 14th day after planting. Treatment with 50 g/L sucrose and 60 mg/L coumarins showed the earliest shoot emergence 7 days after planting. That also applies to treating 70 g/L sucrose with 20 mg/L coumarins or 40 mg/L coumarins. The last emergence of the shoot in the treatment of 0 mg/L coumarins and 90 g/L sucrose was at 28 days after planting. The number of shoots indicates success in in-vitro propagation techniques. The more shoots formed, the higher the multiplication rate. The number of shoots formed ranged from 1–3 per explant planted in the media (Table 2, Figure 1).

Table 1. The effect of variations in the concentration of sucrose and coumarin on the percentage of success of explants responding to media (%)

Coumarin (mg/L)	Percentage of explants responding to media (%)				
Sucrose (g/L)	0	20	40	60	80
0	80	80	0	0	0
30	100	80	0	0	0
50	100	100	40	60	0
70	80	100	80	0	0
90	60	100	0	0	0

Table 2. The average number of shoots on variations in the concentration of sucrose and coumarin

Coumarin (mg/L)	Number of shoots (shoots/explants)				
Sucrose (g/L)	0	20	40	60	80
0	1,3^{ab}	1,0^{a}	*	*	*
30	1,0^{a}	1,0^{a}	*	*	*
50	2,0^{b}	1,0^{a}	1,0^{a}	1,0^{a}	*
70	1,0^{a}	1,0^{a}	1,0^{a}	*	*
90	1,3^{ab}	2,0^{b}	*	*	*

Note: * shoots do not grow up to 56 DAP

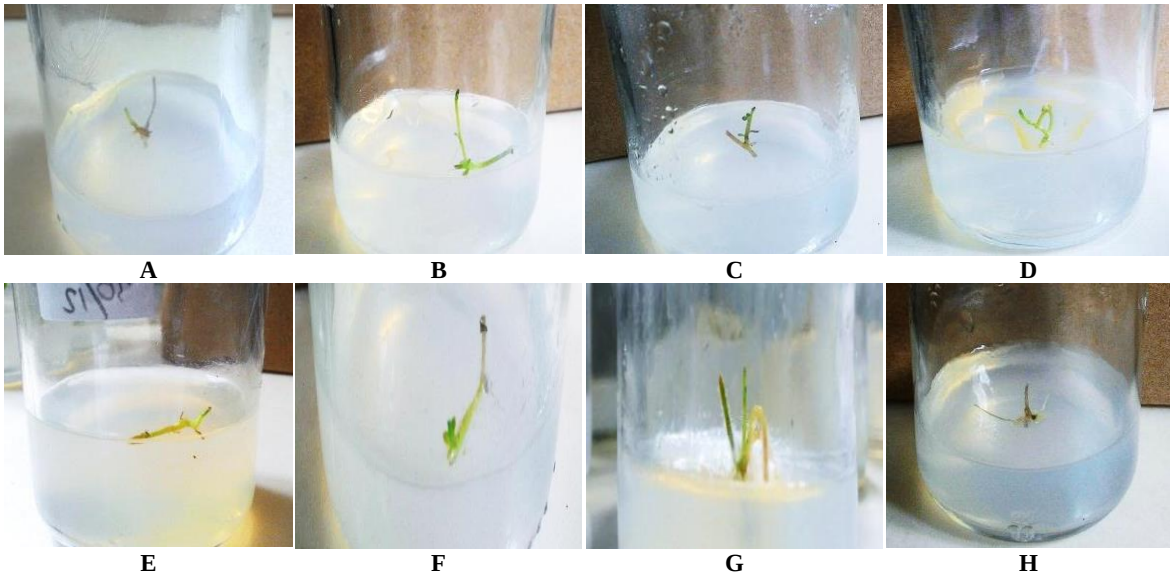

Figure 1. Shoots growing on the 7th day after planting in treatment: A. without sucrose and coumarins; B. sucrose 30g/L without coumarin; C. sucrose 70 g/L without coumarin; D. sucrose 90g/L without coumarin; E. coumarin 20 mg/L without sucrose; F. sucrose 70 g/L and coumarin 20 mg/L; G. sucrose 90 g/L and coumarin 20 mg/L; H. sucrose 90 g/L and coumarin 80 g/L

The treatment without sucrose and coumarin produced an average of 1.3 shoots in each explant planted. Adding a certain amount of sucrose in a medium without coumarin resulted in the number of shoots formed being unstable. The number of shoots formed decreased at a concentration of 30 g/L and increased at 50g/L. At the addition of 50 g/L sucrose in media without coumarin, the shoots formed were at the peak, that an average of 2 shoots per explant planted. The more sucrose added to the media resulted in a higher number of shoots up to a sucrose concentration of 50 g/L. Some of the sucrose in the media lifted into the cells will be converted into energy, and some will be converted into materials needed to stimulate growth (Salisbury and Ross 1995).

The treatment of 20 mg/L coumarins without sucrose produced an average of 1 shoot per explant planted. After adding 20 mg/L, shoots were not able to form. The more coumarins added to the medium without sucrose, the more shoots formed decreased until no shoots were formed at all, namely in the treatment of coumarin concentrations of 40, 60, and 80 mg/L without the addition of sucrose. Paclobutrazol is a retardant substance with an inhibitory function like coumarins, inhibiting the synthesis of gibberellins. Paclobutrazol inhibits the sequence of oxidation reactions from ent-kaurene to ent-kaurenoic acid in the formation of gibberellin acid (GA). When the formation of GA is inhibited, cell division can still occur, but the cell will not elongate, causing the nodes or shoots to become short (Arteca 1996).

The combination of sucrose and coumarin produced several shoots at the concentration of 90 g/L sucrose and 20 mg/L coumarins. With the addition of 20 mg/L coumarins, the more sucrose added was able to increase the number of shoots formed, which reached an average of 2 shoots per explant planted. It is because sucrose is the primary energy source and carbon in in-vitro culture and plays a crucial role in the cell cycle (Tyas et al., 2013). Therefore, more sucrose added could encourage cell division and formation (Dewitte and Murray 2003).

Shoot height

The height of the shoots formed was observed at the end of the observation (56 days after planting). The height of the shoots formed was measured from the node's base to the tip of the shoot. The resulting shoot height reached 8 cm. The average shoot height in the treatment of the combination of sucrose and coumarin concentrations can be seen in Table 3.

Treatment without adding sucrose and coumarin resulted in an average shoot height of 7.13 cm and tended to decrease with the addition of sucrose up to 50 g/L. The addition of 70 g/L sucrose in media without coumarin resulted in the highest shoots reaching 7.5 cm. The addition of sucrose concentration up to 70 g/L increased shoot height and decreased drastically at the addition of 90 g/L sucrose. As a carbon source, sucrose, when absorbed, can affect osmotic pressure, causing cell elongation (Krook et al. 1998).

Table 3. Average shoot height to variations in coumarin and sucrose concentrations

Coumarin (mg/L)	Shoot height (cm)				
Sucrose (g/L)	0	20	40	60	80
0	7,13^{d}	1,03^{a}	*	*	*
30	6,53^{cd}	1,93^{ab}	*	*	*
50	4,23^{bc}	4,43^{bc}	1,93^{ab}	1,76^{ab}	*
70	7,5^{d}	1,7^{ab}	2,73^{ab}	*	*
90	2,46^{ab}	8^{d}	*	*	*

Note: * shoots do not grow up to 56 DAP. Numbers followed by the same letter show no significant difference in DMRT 5%

In the administration of 20 mg/L coumarins without sucrose, there was a decrease in shoot height to an average

of 1.03. The more coumarin added in the medium without sucrose, the lower the shoot height. The formation of shoots is inseparable from dividing active and differentiated meristem tissue and is supported by the presence of organic and inorganic compounds in the media. Administration of coumarins as phenolic compounds can inhibit the work of GAs which function in cell expansion and division (Wattimena 1988).

A good combination of sucrose and coumarin treatments in producing shoot height was the combination of a concentration of 90 g/L sucrose and 20 mg/L coumarins. At 90 g/L sucrose in 20 mg/L coumarin medium, the highest shoot height was 8 cm on average. Krook et al. (1998) stated that sucrose absorbed by cells would be rapidly hydrolyzed to hexose by the cell wall invertase enzyme. Glucose and fructose from the hydrolysis of sucrose enter the cells for metabolism and are then used as a carbon source and energy source for cell division and formation.

Number of leaves

Leaves are essential to plant organs, especially for photosynthesis, so that plants can produce food and experience optimum growth (Arimarsetiowati and Ardiyani 2012). The sucrose and coumarin combination treatment resulted in leaf emergence time and leaf number varying from 7 to 28 days after planting. The first leaf appeared in the treatment of the combination of concentrations of 70 g/L sucrose and 20 mg/L coumarins 7 days after planting. The appearance of the last leaf under observation was in the treatment of the combination of 90 g/L sucrose without coumarin. The number of leaves on the growth of a plant plays a significant role. It is related to vegetative growth, plants' ability to carry out photosynthesis, and various other metabolisms. The combination of sucrose and coumarin concentrations in the media resulted in a different number of leaves for each combination up to 56 days after planting (Table 4).

Table 4. The average number of leaves on variations in coumarin and sucrose concentrations

Coumarin (mg/L) / Sucrose (g/L)	Number of leaves (leaf/explant)				
	0	**20**	**40**	**60**	**80**
0	9,33^{cd}	4,0^{ab}	*	*	*
30	6,67^{bcd}	3,6^{ab}	*	*	*
50	7,33^{bcd}	9,33^{cd}	0,33^{a}	3^{ab}	*
70	19,66^{e}	4,0^{ab}	3^{ab}	*	*
90	5,33^{bc}	10.67^{d}	*	*	*

Note: * no leaves appear until the end of observation (56 DAP). Numbers followed by the same letter show no significant difference in DMRT 5%

Without sucrose and coumarin addition, the number of leaves produced from each explant planted was 9.33. Furthermore, adding sucrose up to 50 g/L in media without coumarin reduced the number of leaves formed. At a concentration of 70 g/L sucrose without coumarin in the media, the highest number of leaves formed was 19.66 on average per explant planted. The more sucrose was added to the media without coumarins, the number of leaves formed increased to a concentration of 70 g/L and decreased when sucrose was given at a concentration of 90 g/L. Carbohydrates are a source of carbon and energy needed when shoot meristem cells divide and enlarge to form new tissue to form leaf primordia (Haryanti et al. 1998).

The administration of coumarin with a concentration of 20 mg/L without adding sucrose in the media showed a decrease in the number of leaves formed compared to without coumarin and sucrose. The more coumarins added to the media without sucrose, the lower the number of leaves. However, adding coumarin with a 40, 60, and 80 mg/L concentration did not result in leaf formation in the planted explants. The addition of coumarin in the media made the number of leaves formed less than the treatment without sucrose. The application of coumarin, a phenolic compound, can inhibit plant growth (Prawiranata et al. 1981), as shown in Figure 2.

Figure 2. Leaves growing in treatment: A. without sucrose and coumarins; B. sucrose 30 g/L without coumarin; C. sucrose 50 g/L without coumarin; D. sucrose 70 g/L without coumarin; E. coumarin 20 mg/L without sucrose; F. sucrose 50 g/L and coumarin 40 mg/L; G. sucrose 70 g/L and coumarin 20 mg/L

The best combination of sucrose and coumarin concentrations in producing the number of leaves was the treatment of the combination of sucrose concentration at 70

g/L and without coumarin, in which in this treatment, the highest number of leaves was 19.66 with a significant difference from other treatments when viewed from the results of one way ANOVA statistical test. The number of leaves increased due to increased cell division in leaf primordial. Fatima et al. (2004) stated that in vitro propagation of potato cultivar PARS 70 with MS medium with 8% sucrose resulted in an average of 5.71 leaves formed.

Administration of higher concentrations of coumarin with the same sucrose concentration showed a decrease in the number of leaves formed. As shown in Figure 2, in Figure 2.C, the treatment of 50 g/L sucrose and 0 mg/L coumarins showed more leaves formed than the treatment of 50 g/L sucrose and 40 mg/L coumarins (Figure 2.F). It is because although sucrose was added in the same two media when combined with other substances, such as coumarin, in different concentrations, it showed a difference in the number of leaves formed. The more coumarin added would inhibit the number of leaves formed.

Number of roots

Root formation in plantlets is vital because it can increase growth during the in-vitro propagation process. The combination of the concentration of sucrose and coumarin in the media showed the beginning of the emergence of different roots in each treatment, where the roots that appeared ranged from 7 to 35 days after plants. In this study, the highest number of roots that appeared until the end of the observation was 42.33 roots, namely at the treatment of sucrose concentration of 70 g/L without coumarin. On the other hand, the number of roots was the least in treating the combination of concentrations of 70 g/L sucrose and 40 mg/L coumarins (Table 5).

In treating media without sucrose and coumarin, the average number of roots that grew was 5.66. However, adding sucrose to the media increased the number of roots that appeared. The addition of sucrose resulted in the number of roots increasing until the addition of sucrose at a concentration of 70 g/L was an average of 42.33 roots/explant. Still, there was a decrease in the addition of 90 g/L sucrose. In addition, according to Kazemiani et al. (2012), an increase in sucrose concentration above 30%, namely 40%, increased the percentage of root formation in the MS medium without the addition of growth regulators such as BAP.

With the addition of coumarin concentration of 20 mg/L, the number of roots formed increased to 9.33 roots/explant. However, at higher concentrations, there was a decrease in the number of roots formed. The more coumarin added to the media without sucrose, the lower the number of roots formed. In the treatment of coumarin concentrations of 40, 60, and 80 mg/L without sucrose, no roots were formed until the end of the observation. According to Sakya et al. (2003), coumarin as a growth inhibitor effectively inhibits or suppresses gibberellin activity in plants. Inhibition of gibberellins by coumarins will accelerate the entry of plants into the generative phase so that energy for the process of the branch, node, and root growth will be diverted for tuber formation.

Better treatment of sucrose and coumarin combination for increasing the number of roots formed in explants planted in the media was the addition of sucrose at 70 g/L without the addition of coumarin, with the average number of roots per explant being 42.33. On the other hand, administering paclobutrazol, the same retardant as coumarin, as much as 5.0 mgl/l, actually produces fewer roots than the control. The administration of paclobutrazol at a concentration of 5.0 mg/L reduces the number of roots produced (6.80) when compared to the control (13.6) (Shahid 2007).

Root length

Root length results from the extension of the cells behind the tip meristem; the longer the root, the wider the area of nutrient absorption is expected. This study measured root length from the base where the roots grew (single-node explants) to the tip in mm. The longest roots were formed without sucrose and coumarin treatment, while the shortest leaves were formed in the 50 g/L sucrose and 40 mg/L coumarin treatment (Table 6).

Table 5. The average number of roots to variations in sucrose and coumarin concentrations

Coumarin (mg/L)	Number of roots (root/explant)				
Sucrose (g/L)	0	20	40	60	80
0	5.66^{a}	9.33^{ab}	*	*	*
30	12.33^{abc}	8.33^{ab}	*	*	*
50	25.33^{abc}	3.9^{bc}	4^{a}	28.66^{abc}	*
70	42.33^{c}	24.66^{abc}	3.66^{a}	*	*
90	4.6^{a}	29.33^{abc}	*	*	*

Note: * no roots appear until the end of the observation (56 DAP)

Table 6. The average root length against variations in the concentration of sucrose and coumarin

Coumarin (mg/L)	Root length (mm)				
Sucrose (g/L)	0	20	40	60	80
0	$26,94^{d}$	$9,14^{abc}$	*	*	*
30	$15,38^{bc}$	$7,80^{ab}$	*	*	*
50	$17,56^{c}$	$6,75^{ab}$	$5,02^{a}$	$8,37^{ab}$	*
70	$10,42^{abc}$	$10,44^{abc}$	$9,43^{abc}$	*	*
90	$10,12^{abc}$	$6,34^{ab}$	*	*	*

Note: * no roots appear until the end of the observation (56 DAP)

Figure 3. Roots formed in treatment: A. without coumarin and sucrose; B. sucrose 30 g/L without coumarin; C. sucrose 70 g/L without coumarin; D. sucrose 50 g/L and coumarin 20 mg/L; E. sucrose 70 g/L and coumarin 20 mg/L; F. sucrose 90 g/L and coumarin 20 mg/L.

In the treatment without adding sucrose and coumarin, the average length of the roots formed reached 26.94 mm. In the sucrose addition of 30, the root length decreased to 15.38 mm and increased again in the sucrose addition of 50 to 17.56 mm. After adding 50 g/L sucrose, there was a decrease in the length of the roots formed. The more sucrose added to the medium, the less the length of the roots formed. With the addition of 70 g/L sucrose, the roots formed reached 10.42 mm and decreased again with the addition of 90 g/L sucrose to 10.12 mm. Root length results from the extension of the cells behind the tip meristem (Dewi 2007). The longer the roots, it is expected that the area of nutrient absorption will be wider so that the distribution of nutrients from the media to plants can run smoothly. If the root length is inhibited by increasing the amount of sucrose added, it will affect the subsequent acclimatization after the in-vitro culture process.

The addition of 20 mg/L coumarins without the addition of sucrose in the media caused a decrease in the length of the formed roots to 9.1 mm. The more coumarin added in the medium without sucrose, the shorter the roots. In the treatment of 40, 60, and 80 mg/L coumarins without sucrose, no root formation occurred until the end of the observation. One of the physiological effects of coumarin is to inhibit growth and. root extension (Wattimena, 1988). Prawiranata et al. (1981) suggested that the most common effect of administering phenolics (coumarins) inhibits growth, such as cell division and elongation.

The combination of sucrose and coumarin did not affect the roots because the longest roots occurred in the control medium without sucrose and coumarin. Tyas (2013) said that the highest number of roots was found in media with a concentration of 1/2 MS plus 1% sucrose. The limited concentration of MS media with sucrose affects the number of nutrients pummelo plantlets obtain in culture. The reduction of nutrients in the media caused the explants to grow slowly; leaf formation and shoot length decreased, while root formation and elongation increased as a means of nutrient absorption. Roots formed in the treatment of the combination of concentration of 70 g/L sucrose without the addition of coumarin (Figure 3.C) spread throughout the media. In comparison, in the treatment of the combination of concentration of 70 g/L sucrose and 20 mg/L coumarins (Figure 3.E), the roots formed tended to gather around the mother plant.

In conclusion, adding sucrose could increase the growth and development of micro cuttings on shoots and leaves but has a negative effect on root development. Coumarin in high concentrations in the media would inhibit the growth and development of shoots, leaves, and roots. While the combination would increase the growth and development of micro cuttings shoots. The combination of the concentration of 50 g/L sucrose and 20 mg/L coumarins is the best combination to get the micro cuttings with good average results on each parameter.

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Date palm (*Phoenix dactylifera*) seeds germination

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Abstract. *Mohammed NMI, Said AGE. 2018. Date palm (*Phoenix dactylifera*) seeds germination. Cell Biol Dev 2: 63-68.* This study aimed to examine if pre-germination treatments could effectively promote date palm (*Phoenix dactylifera* L.) seed germination in the Nursery of the Horticultural Sector, Ministry of Agriculture and Irrigation, Khartoum, Sudan. Seeds were soaked in water for 0, 2, 4, 6, 8, 10, and 12 days before being planted in one of the following media: sand:clay (1:1), sand:clay (1:2), sand:clay (1:0), sand:clay (0:1), and sand:clay (2:1). The factorial completely randomized block design was employed in this investigation. The 6-day soaking time resulted in the highest percentage of seeds germinating; as the soaking time increased, the percentage of seeds germinating decreased. The 6-day soaking time also improved seed germination, allowing for a 50 % final germination percentage in the shortest time. Sowing seeds in a 2:1 soil mix decreased germination days, resulting in a substantial increase in germination percentage compared to other soil type mixes evaluated, regardless of soaking duration. In addition, seeds soaked for 6 days and planted in a 2:1 soil mix produced the tallest seedlings.

Keywords: Acceleration, date palm, *Phoenix dactylifera,* seeds germination

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a member of the Arecaceae (Palmae) family, is an important plantation crop in many desert countries in West Asia and North Africa. Almost every portion of the plant is used to produce food or industrial goods. In many developing countries, it plays a significant role in rural communities. Dates are grown in arid climates around the world and sold as a high-value confectionery all over the world (Mahmoudi et al. 2008)

It is one of the world's oldest cultivated plants, dating back over 6,000 years, and is said to be the world's oldest food plant. Many people in arid and semi-arid regions rely on dates for social, environmental, and economic reasons. Due to its hardy plant characteristics and deep root system, it grows well in poor desert soils (Chandra et al. 1992; Sharma and Singh 2013). It was found in the Middle East, North Africa, South Sahel areas, East and South Africa, the South Western United States, Central and South America, and South Western Europe. In 2010, global date production was estimated to be around 7.4 million tons (FAOSTAT 2011). The Arab world accounts for over 70% of total date production. Around 3,000 identified date palm varieties worldwide, while some names are likely synonyms, resulting from a local or national name given to one cultivar that also occurs in another region under a different name (Johnson 2011).

Dates are grown in North Africa and the Middle East and have recently been brought to new production locations in Australia, India/Pakistan, Mexico, South Africa, South America, and the United States. North Africa and the Middle East, Southern Africa, Australia, India/Pakistan, Palestine, Mexico, South America, and the United States are commercial date palm growing countries of the world (Zohary and Hopf 2000; Abd Rabou and Radwan 2017).

Egypt, Saudi Arabia, Iran, the United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya, and Tunisia are the top 10 producing countries. Thousands of date palm cultivars are grown in these nations, including soft, semi-dry, and dry fruits (depending on their water and sugar content at harvest when completely ripe) (Kader and Hussein 2009). The Arab countries own most of the world's date palms and produce most of the total date harvest (FAOSTAT 2009). The importance of date palm culture for its high nutritional, economic, and social qualities is well known, particularly in arid and semi-arid environments where it plays a vital role in influencing microclimate and enhancing the production of other agricultural commodities. As a result, date production, use, and industrialization are all on the rise worldwide (Botes and Zaid 2002).

The date palm is the most important fruit tree in the northern region of Sudan. It has been cultivated there for 3,000 years, and it helps people in northern Sudan make a living (Osman 2001). Although some scattered date palm populations occur in oasis areas in North Kordofan and Darfour, as well as in the eastern section of the country, date palm culture in Sudan is centered along the River Nile banks between latitudes 15.5° and 22° N in River Nile and Northern State (Elshibli and Korpelainen 2008). Sudan produced approximately 119.048 metric tons of date palm fruits in 2010 (FAOSTAT 2011). The date palm industry of Sudan is based on the cultivation of old traditional dry, soft, and semi-soft varieties. Barakawi, Gondaila, Tamoda, and Abdel Rahim are the most important indigenous dry cultivars, while Mishrig Wad Khateeb and Mishrig Wad Laggai are the most important indigenous soft cultivars.

Furthermore, many trees (farmer's varieties) that grow from seeds are collectively called Jaw, signifying that they are seedling varieties (Osman and Boulos 1978).

The following are the three ways for propagating date palms: Seed propagation: seed propagation is undesirable since it results in a differentiated population in which no two palm seedlings are alike, reducing the likelihood of producing high-quality fruit (Pieniążek and Pieniążek 1981; Moustafa et al. 2010). Offshoot propagation is the most common way of propagating date palm trees, whether male or female. Axillary buds on the trunk of a palm tree produce offshoots. First, a professional and qualified laborer must separate date palm offshoots from the mother palm. Then, offshoots are planted in their permanent location in the orchard to grow as new and distinct individuals (Alihouim and Dialami 2010). Finally, tissue culture propagation is a new technique for mass propagation of date palms that uses three different types of tissue culture: shoot tips and buds culture (organogenesis), embryo culture (embryogenesis), and highly differentiated somatic tissues culture that includes leaf, stem, inflorescence, and root sections (Al-Sakran and Muneer 2006).

The main issues with date palm seed propagation are genetic variability and sex ratio, as 50:50 male:female plants are usually generated. It isn't easy to distinguish between male and female trees before flowering. As a result, seed trees are frequently delayed in reaching the flowering stage, and the price of seed propagated tree fruits is frequently low (www.moa.gov.jo/portals). The benefits of date palm seed propagation include obtaining tree stallion, ornamental and windbreaks, resistance to diseases such as eggs cillnesstreese, and specific education such as pollination and hybridization (www.paaf.gov.kw/paaf/ershad/jsp). This study evaluated the effects of water soaking and the planting medium on germination percentages and the days required to achieve 50% final germination on the seeds of the "Barakawi" date palm cultivar.

MATERIALS AND METHODS

Experiments were carried out in the Department of Horticulture, Ministry of Agriculture and Forestry, Al-Mogran, Khartoum, Sudan (Latitude 35°- 15° N; Longitude 33°- 32 °E) utilizing seeds of the "Barakawi" date palm cultivar. Following harvesting, the "Barakawi" fruits were acquired from Khartoum North's Central Market wholesaler. The "Barakawi" cultivar was chosen as a seed source in this research because of its economic value and availability. Seeds were removed, rinsed, and dried under running tap water. Next, the seeds were visually selected for the study based on size homogeneity to reduce potential seed size effects and stored in plastic bags at room temperature until use. There was no pre-treatment applied to the seedlings. Before sowing, all selected seeds were soaked for 0, 2, 4, 6, 8, 10, or 12-days in tap water in a 3-gallon plastic bucket at room temperature; a total of 6 soaking cycles plus unsoaked seeds as a control. Seeds were planted in sand:clay (1:1), (2:1), (1:2) (0:1), and (1:0) mixture (v/v) planting media after the soaking treatments. Every day, the soaking water was changed. Next, 20 seeds were counted out for testing for each treatment. In perforated 27x24x7-cm black plastic bags, 15mm-deep and 3 to 4 cm apart, the sums of 5 soaked seeds were sown on the surface of each medium. Twenty seeds were used in each treatment (5 seeds x four replications). After seeding, the bags were placed in a lath house with natural daylight and day length. Watering was done by hand with tap water every other day, and no fertilizer was used.

A two-way factorial randomized complete block design (RCBD) was used, with each treatment replicated 4 times, with 5 seeds per replication. Twenty seeds were used in each treatment (5 x 4 replications). When the cotyledonary petiole emerged above soil level and was visible to the naked eye, the seed was considered to have germinated. Seeds with visible cotyledonary petiole protrusion through the medium surface were counted daily for germination. The daily germination counts were used to calculate the total germination percentage (G) and mean emergence time (days to 50% of total germination T50). All observations were made with a total of 20 seeds per treatment. The percentages represent the percentage of seeds that germinated. On the Excel computer program, data were subjected to an analysis of variance procedure. At the 5% level, Duncan's multiple range test was used to separate treatment means.

Forty days after seeding, the number of germinated seeds (daily germination counts) was recorded daily and continued for two months after germination. The ratio of germinated seeds to the total number of seeds planted was used to calculate the total germination percentage.

Total germination % (G)

% = ta/a x 100

Where,
%: germination percent.
Ta: total number of germinated seedlings
A: total number of seeds

Days to achieve 50% of the germination percentage.

The number of days required for the germination percentage to reach 50%.

Seedling length

The mean was derived by randomly picking two seedlings from each treatment to measure the length of each seedling. A meter ruler was used to measure the length in centimeters.

RESULTS AND DISCUSSION

Because date palm seeds are genetically heterozygous, there was a lot of diversity in emergence percentages across seeds for each treatment. However, there were clear disparities in response between the treatments for all measured measures. The amount of seed reaction to water

soaking varied depending on soaking time and planting medium, and seed response competence to water soaking decreased as soaking time increased.

Final germination percentage

Table 1 depicts the effect of water soaking duration and sowing media type on the total germination percentage of seeds of the date palm cultivar "Barakawi." With increasing treatment time, the degree of the response to water soaking changed. Over all other pre-water soaking methods examined, the 6-day water pre-soaking led to a considerable increase in total germination percentage (88%). The percentage of germinated seeds increased as the water pre-soaking duration increased from 0 to 6 days. However, When you soak your seeds in water for an extended time, the percentage of seeds germinating decreases. Seeds soaked for 8 days or longer had significantly lower germination percentages than seeds for 6 days. With seeds treated for 12 days, the lowest total germination percentage figure (69%) was recorded. No significant differences existed between the 6-days and 8-days water soaking treatments. After an 8-days soak, the average germination percentage dropped significantly. There were significant variances between the sowing media.

There were no significant variations in total germination percentages between the 1:0 and 2:1 sand:clay mix sowing media, although both sowing media resulted in a considerable increase in total germination percentage compared to other sowing medium types. Seeds are sown in 1:0 sand:clay sowing medium had the highest germination rate (86%), followed by seeds sown in 2:1 sand:clay sowing medium with an 83 percent germination percentage, with no significant difference between the two types of media. Seeds grown in a 0:1 sand:clay medium type had the lowest germination percentage (72%). The germination percentages of the 1:2 and 0:1 sand:clay soil mixes were comparable.

Table 1. Effect of time of water soaking and type of sowing medium on total emergence percentage (%) of seeds of "Barakawi" date palm cultivar

Medium	Soaking duration (days)							Medium
type	0	2	4	6	8	10	12	mean
Sand: clay (0: 1)	50.20 B	50.20 b	52.20 b	52.00 b	69.25 b	75.00 b	86.25 b	62.18b
Sand: clay (1: 2)	52.00 B	52.50 b	46.70 b	51.20 b	52.00 b	54.50 b	67.25 b	53.75b
Sand: clay (1: 0)	39.00 B	38.20 b	44.20 b	39.00 b	42.00 b	51.25 b	56.50 b	44.32b
Sand: clay (1: 1)	47.70 b	46.20 b	48.00 b	44.00 b	57.50 b	51.00 b	64.00 b	51.21b
Sand: clay (2: 1)	45.50 a	47.50 a	43.00 a	41.20 A	51.25 a	56.25 a	65.00 a	49.96a
Soaking mean	46.90 a	46.95 a	46.80 a	45.50 A	54.40 a	57.60 a	57.80 a	

Note: Means followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan|s Multiple Range Test. Percent data were transformed to the square root of the arcsine of the proportion for analysis. All observations were based on 20 sown date palm seeds per treatment. The percentages refer to the proportion of the seeds that germinated

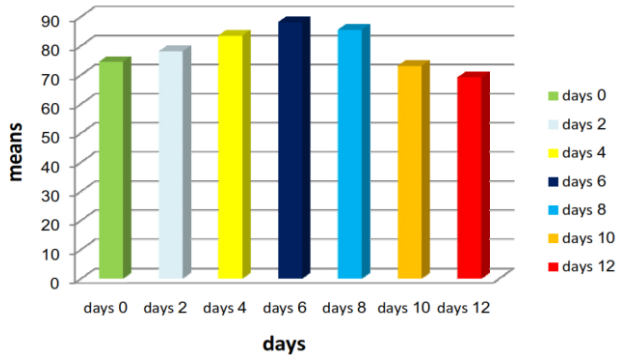

Figure 1. Effect of time of water soaking on total emergence percentage (%) of seeds of "Barakawi" date palm cultivar

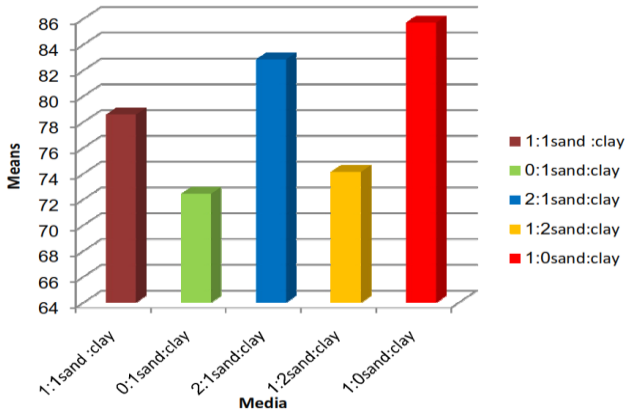

Figure 2. Effect of time of water soaking on total emergence percentage (%) of seeds of "Barakawi" date palm cultivar

Days to 50% germination

The results of the impacts of the period of seed soaking in water before sowing in different soil media on days to achieve 50% (T50) of final germination percentage were shown in Table 2. The 6-days water soaking treatment required the fewest days to accomplish 50% germination of the final germination percentage (45 days). In contrast, the 8-days water soaking treatment required the most days for the growing seeds to reach 50% germination of the final germination percentage (68 days). For seeds submerged for 8 days, a longer soaking duration delayed germination by prolonging T50 values with additional days. Mean days required for germination declined as soaking time in the water was lengthened from 7-days to 12-days. Seeds soaked for more than 6 days of water soaking time required a longer time to germinate than those soaked for 6 days or less water soaking time. Differences among seeds soaked for 6 days and less were no-significant. Irrespective of soaking duration, the1:0 sand: clay soil mix significantly decreased the number of soaking days required by seeds to achieve T50 final germination relative to the other sowing media tested, whereas 44 days were required to achieve T50 of final germination percentage. Other examined soil mixtures resulted in T50 durations. Seeds planted in 0:1 sand:clay required the most days (62 days) to achieve T50 of final germination percentage than seeds planted in other tested soil mixes.

Table 2. Effect of time of water soaking and type of sowing medium on number of days required to achieve 50% final emergence percentage of seeds of "Barakawi" date palm cultivar

Medium type	Soaking duration (days)							Medium mean
	0	2	4	6	8	10	12	
Sand: clay (0: 1)	50.20 b	50.20 b	52.20 b	52.00 b	69.25 b	75.00 b	86.25 B	62.18b
Sand: clay (1: 2)	52.00 b	52.50 b	46.70 b	51.20 b	52.00 b	54.50 b	67.25 B	53.75b
Sand: clay (1: 0)	39.00 b	38.20 b	44.20 b	39.00 b	42.00 b	51.25 b	56.50 B	44.32b
Sand: clay (1: 1)	47.70 b	46.20 b	48.00 b	44.00 b	57.50 b	51.00 b	64.00 B	51.21b
Sand: clay (2: 1)	45.50 a	47.50 a	43.00 a	41.20 a	51.25 a	56.25 a	65.00 A	49.96a
Soaking Mean	46.90 a	46.95 a	46.80 a	45.50 a	54.40 a	57.60 a	57.80 A	

Note: Means followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test

Table 3. Effect duration of water soaking and type of sowing medium on lengths of date palm "Barakawi" seedlings (week's cm) after sowing

Medium type	Soaking duration (in days)							Medium mean
	0	2	4	6	8	10	12	
Sand: clay (0: 1)	21.25 b	21.40 b	22.36 b	22.72 b	22.64 b	22.56 b	22.34 b	22.18b
Sand: clay (1: 2)	22.10 b	22.43 b	22.17 b	22.15 b	22.26 b	21.93 b	22.51 b	22.22b
Sand: clay (1: 0)	22.84 b	23.10 b	21.66 b	22.66 b	22.34 b	22.15 b	20.44 b	22.17b
Sand: clay (1: 1)	22.80 b	22.26 b	22.35 b	22.48 b	23.10 b	22.70 b	21.10 b	22.40b
Sand: clay (2: 1)	24.76 a	25.59 a	26.01 a	26.03 a	24.15 a	24.53 a	25.90 a	25.28a
Soaking Mean	22.75 a	22.95 a	22.91 a	23.20 a	22.90 a	22.77 a	22.46 a	

Note: Means followed by the same letter are not significantly different at P = 0.05, according to Duncan Multiple Range Test

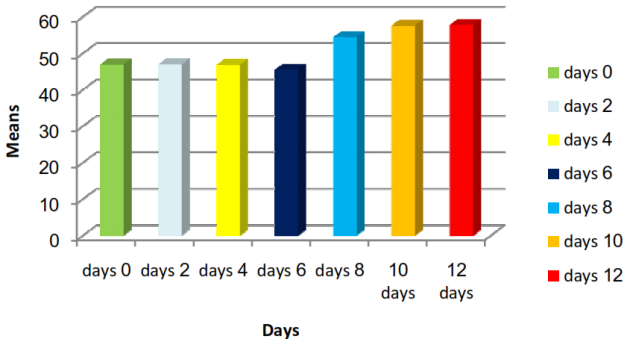

Figure 3. Effect of time of water soaking on number of days required to achieve 50% final emergence percentage of seeds of "Barakawi" date palm cultivar

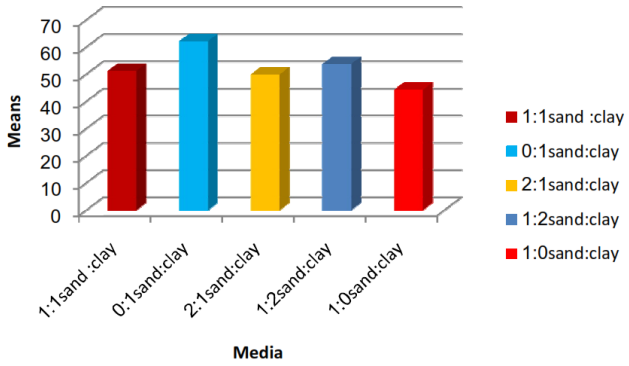

Figure 4. Effect of type of sowing medium on number of days required to achieve 50% final emergence percentage of seeds of "Barakawi" date palm cultivar

Seedling length (cm)

Soaking time treatments had little effect on seedling length. There was no discernible variation in seedling length between the soaking times investigated. With substantial differences from the other media types studied, seeds were sown in 2: 1 sand: clay sowing medium produced the tallest seedlings (25 cm) regardless of soaking time. Table 3 shows no significant difference in seedling length recorded for the other tested media types.

Discussion

The primary morphogenic response evaluated in this study was germination. Date palm seed propagation efforts might benefit from information on seed germination requirements and effective dormancy-breaking procedures. Several attempts have been made to expedite palm seed germination and enhance total germination percentage (Rees 1963; Homquist and Popenoe 1967; Said 1986; Carpenter 1987; Al-Wasel and Warrag 1998). Because soaking is excellent in promoting germination and raising germination percentage, Rees (1963) recommends pre-soaking in water before sowing to enhance palm seed germination. According to our findings, date palm seeds treated for 6 days sprouted substantially faster and with a higher germination rate. Compared to other water-soaking treatments, the day's duration promoted the highest overall germination (88%) and fewer days (45) to 50% of final germination. In addition, at 6 days of water soaking time, the total germination percentage was higher, earlier, and more uniform than most other studied water soaking treatments. Previous studies with date palm seeds (Samarawira and Osuhor 1981; Olumekun and Remison 1985; Abdullah and Maroff 2007) and seeds from other palm species (Loomis 1958; Rees 1963; Nagao and Sakai 1979; Carpenter 1987) have shown that soaking seeds in water before sowing increased total germination percentage and shortened germination time. For resumption of germination, sufficient water and oxygen permeate freely via the operculum and the micropyle opening into the seed. Others have reported (Al-Wasel and Warrag 1998; Carpenter et al. 1993) that mechanical removal of the operculum (embryo cap) of palm seeds alleviated the physical dormancy imposed by the embryo cap, resulting in a significant increase in total germination and a reduction

in germination time, most likely by facilitating water and oxygen diffusion. Replacement of the soaking water once every two days during seed preparation for sowing dilutes and removes germination inhibitors from the seed coat. Water imbibition through the micropyle opening softens the operculum, which is then easily ruptured by the elongating embryo without the risk of embryo damage raised by Al-Wasel and Warrag (1998).

The amount of time a seed takes to absorb enough moisture for germination varies depending on the plant species and the amount of water available. The ideal water soaking length for maximal total germination in date palm seeds ranges from 2 days (Abdullah and Maroff 2007), 3 days (Samarawira and Osuhor 1981), and over 4 days (Olumekun and Remison 1985). Differences in cultivars, developmental stage of source fruits, age of seeds and source, sowing media, and ambient variables at the study site could all contribute to inconsistencies. The most uniform and quick germination happened after 6 days of water soaking. The 6-day soaking period appears to be the bare minimum for "Barakawi" seeds to achieve optimum moisture imbibition, allowing the embryo to remove the operculum mechanically. Increased soaking time resulted in a significant decrease in germination percentages, indicating that "Barakawi" seeds have a narrow water imbibition range for maximum moisture for germination. It's also possible that the effect of water soaking on date palm seed germination is primarily related to the operculum's physical, structural weakening rather than biochemical changes in the embryo or endosperm.

The ability to respond to soaking time decreases as the soaking time increases. The progressive decline in germination percentage and delay in germination speed found when seeds were soaked for longer than 7 days is consistent with a previous observation on seed germination (Maekawa and Carpenter 1991), which shows that increased free water in the planting medium causes a progressive decline in total seed germination percentages and rates. That could be due to the thickening of the micropyle wall and expanding with increased soaking time, resulting in a progressive reduction in the micropyle opening and limiting cotyledonary sheath protrusion via the micropyle opening. Maekawa and Carpenter (1991) hold a similar viewpoint, claiming that when the free water content of the germination substrate increases, seed germination decreases as the micropyle walls thicken and the micropyle opening of the seeds decreases. It's also likely that the prolonged water soaking time had a negative impact on germination by disrupting several physiological processes required for germination. The negative effects of prolonged water soaking on seed germination could, however, be connected to the destruction of the germ pore and/or embryo of some seeds that failed to germinate due to infection with rot-causing soil bacteria before and/or after they began to germinate. Water logging causes, on the other hand, cannot be ruled out as a cause of the harmful effects of prolonged water soaking. The progressive drop in germination percentage could have been caused by suffocation and specific growth inhibitors (s) production during extended water soaking.

The sowing medium is an important factor in the proper germination of seeds. Successful seed germination depends on selecting suitable soil and its proper care. Sands or sand and clay mixtures are commonly used as sowing media for seed germination. According to the current findings, date palm seeds germinate best on a 1:0 sand: clay combination. These findings are similar to those of Banks and Marcus (1999), who recommended utilizing well-drained soil mixes with some moisture-holding capability for palm seed germination. Total germination percentage was higher with a medium of 1: 0 mixtures by volume of sand and clay than the other media evaluated in response to the sowing medium. The 1:0 sand:clay mixture allows rapid and equal water penetration throughout the medium while also regulating moisture reserves and improving aeration. The capacity of date palm seeds to germinate reduces as the clay volume in the sowing media increases compared to sand. The removal of sand from the sowing medium had a substantial negative effect, implying that soil oxygen levels in high clay percentage sowing mediums are restricting or that carbon dioxide or other gas levels are too high for germination. Adding sand to the sowing medium enhanced aeration and decreased water retention, which appears to be due to enhanced drainage and/or aeration compared to the other media evaluated and rapid and uniform water penetration throughout this soil mix medium. This hypothesis is consistent with Hartmann et al. (2002) 's suggestions for using well-drained media for various greenhouse growth goals. Said (1986) arrived at identical findings and had similar results. The findings are consistent with Bani (1988) and Azad et al. (2011), which found that using sand as a rooting medium resulted in a higher total rooting percentage, faster root emergence, longer roots and shoots, and higher survival of rooted cuttings compared to a variety of other horticultural substrates tested.

Soaking treatments had little effect on seedling length. The 6-days soaking treatment, on the other hand, had a non-significantly higher value than the other soaking treatments examined. The lack of effect of soaking treatments on seedling length may be due to competition for assimilates between the newly developed apical meristem of the shoot and the leaf blades (source-sink relationship). With a growing shift in assimilating translocation germination to the meristematic area, growth can be controlled separately from germination right after germination. Soaking triggered metabolic and physiological mechanisms that allowed the embryo to resume active development. The water-soaking treatments have no carry-over effect from seed germination to seedling growth and development.

Seedlings grew the longest in a medium containing a 2:1 ratio of sand and clay by volume compared to the other media examined. Compared to the other media examined, the enhanced seedling duration with this medium could be attributable to rapid and consistent water penetration throughout the medium, high moisture reserves, improved drainage and/or aeration, and high organic matter.

As a result, it is possible to assume that soaking seeds in water helped date palm seeds overcome their intricate dormancy. Soaking date palm seeds for a week as a pre-

sowing treatment seems to be a potential method for speeding up and improving total germination. The technique could be used to design a generalizable seed germination approach for additional plant species. It proved simple, quick, efficient, economical, straightforward, repeatable, and can be practiced year-round. However, more study is needed to maximize total germination percentages and reduce the days required for germination to build a commercially viable system.

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The effect of sucrose concentration on growth and induction of somatic embryogenesis in *Catharanthus roseus* callus

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Abstract. *Herwinaldo DC, Solichatun, Anggarwulan E. 2018. The effect of sucrose concentration on growth and induction of somatic embryogenesis in* Catharanthus roseus *callus. Cell Biol Dev 2: 69-77.* The purpose of this study was to determine how sucrose concentration affected the growth and induction of somatic embryogenesis in Madagascar periwinkle callus cultures (*Catharanthus roseus* (L.) G.Don). This study employed a completely randomized design (CRD) with one treatment factor, namely the addition of sucrose to MS media, and five treatment levels, including 0 g/L, 10 g/L, 20 g/L, 30 g/L, and 40 g/L, each with five replicates. Data was collected in the form of qualitative data, such as the presence or absence of somatic embryos and callus color and texture, as well as quantitative data, such as callus fresh and dry weight. ANOVA was used in quantitative data analysis, followed by the DMRT test at the 5% level. Somatic embryos were not found in all treatments, according to the findings. Variations in sucrose concentration in callus culture significantly affected dry callus weight but not callus fresh weight. The higher the sucrose concentration, the greater the callus's dry weight.

Keywords: Callus, *Catharanthus roseus*, somatic embryogenesis, sucrose

INTRODUCTION

A quarter of all modern medicines are derived from active ingredients isolated and developed from plants. The problem is determining how to maintain the production level of these herbal medicines in the face of limited raw materials for herbal medicines because the majority of raw materials for herbal medicines are derived from the parent plant (Radji 2005).

Plants are totipotent, meaning they can reproduce not only from egg or sperm cells but also from root cells, leaves, stems, and other plant cells. The entire plant body can be regenerated if we use a plant cell. The tissue culture method allows for the indefinite propagation of plant clones. Tissue culture plant regeneration has been shown to produce the same chemicals as the parent plant (Radji 2005). When compared to traditional methods, plant tissue culture has several advantages. These benefits include: (i) bioactive compounds can be formed under controlled conditions and in a relatively short period with tissue culture technology; (ii) culture is free of microbial contamination; (iii) each cell can be propagated to produce certain secondary metabolites; controlled cell growth and metabolic processes can be regulated rationally; (iv) tissue culture is not dependent on environmental conditions such as geography, climate, and seasons; and (v) secondary metabolites can be produced continuously, (vi) does not require a large area (Fowler 1983; Sudirga 2002).

Organogenesis and somatic embryogenesis can be used to multiply culture in tissue culture. Somatic embryogenesis is the process by which somatic cells (haploid or diploid) develop to form new plants without gamete fusion during specific stages of embryonic development. Somatic embryogenesis has received much attention because the number of propagules is limitless and can be obtained in a shorter period. It can help plant breeding programs through genetic engineering (Purnamaningsih 2002).

Secondary metabolites can be produced in vitro using the tissue culture method. Plant cell suspension culture is considered an alternative method for obtaining secondary metabolites on a large scale, particularly alkaloids for pharmaceutical purposes (Radji 2005). Many important compounds are not synthesized in significant amounts in cell culture, according to Croteau et al. (2000), due to the expression of genes encoding alkaloid biosynthesis specific to specific tissues. According to Zhao et al. (2001), the ajmalicine content of compact callus suspension culture is higher than that of crumb callus because compact callus has a higher degree of cellular and tissue differentiation. Somatic embryos, like embryos derived from zygotes, have the potential to produce secondary metabolites because they have high cell differentiation capabilities.

Catharanthus roseus (L.) G.Don (Madagascar periwinkle) is a synonym for *Lochnera Rosea* Reichenb. Ex Endl. and the basionym *Vinca rosea* L., both of which are members of the Apocynaceae family. Madagascar periwinkle is used not only for traditional medicine but also by modern medical experts due to its substance content (Suryowinoto 1997). This plant is significant because it contains two antitumor alkaloid compounds, vinblastine, and vincristine, found in the leaves, and ajmalicine, found in the roots (El-Sayed and Verpoorte 2004). The *C. roseus* produces very complex indole alkaloids. Important

compounds like vinblastine and vincristine only produce a very small proportion (about 0.00025 percent of dry weight), making extraction and purification difficult and expensive (Hopkins 1999).

Environmental stress (Sukarman et al. 2000), as well as organogenic tissue culture methods (without going through embryogenesis) through acidification and addition of tryptophan (Pitoyo 2002), the addition of elicitor (Fitriyani et al. 1999), root transformation (Ciau-Uitz et al. 1994), and suspension culture with airlift bioreactor, have all been used to increase the content of secondary metabolites, particularly ajmalicine, in *C. roseus* (Esyanti and Muspiah 2006). The somatic embryogenesis method is rarely used in *C. roseus*, including efforts to increase its secondary metabolites (Junaid et al. 2007). According to Junaid et al. (2006), the formed somatic embryos have the potential to be used as raw materials for genetically modifying embryonic precursor cells to increase alkaloid content.

The effect of growth regulators on somatic embryogenesis in *C. roseus* callus and suspension cultures has been studied (Junaid et al. 2007), but the effect of sucrose variations on media has not been studied yet. The optimal sucrose concentration for somatic embryogenesis in other species has been determined, such as peanut (*Arachis hypogea* L.) by Srilestari (2005), which is 40 g/l, and neem (*Azadirachta indica* A. Juss) by Shrikhande et al. (1993). Sucrose is important in the process of somatic embryogenesis because it serves two functions: it provides energy and regulates osmotic concentration. The purpose of this research is to find out how sucrose concentration affects the initiation of somatic embryos.

The objectives of this study were as follows: (i) To investigate the effect of variations in sucrose concentration in MS media on the growth of callus culture of *C. roseus*. (ii) To investigate the effect of sucrose concentration variations in MS media on the induction of somatic embryogenesis in *C. roseus* callus culture.

MATERIALS AND METHODS

Research time and place

This study was carried out for six months in the Biology Sub-Lab, Central Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.

Materials

Explant source plant material

The plant parts used as a source of explants were several pieces of hypocotyl derived from the seeds of the tapak dara *C. roseus* var. roseus (purple flower) that germinates. The seeds obtained were from the *C. roseus* plant grown in a greenhouse. The chemicals used include Murashige-Skoog (MS) media base.

Experimental design

The experimental design carried out in this study was a completely randomized design (CRD) with one treatment factor, namely the addition of sucrose with five concentration levels 0 g/L, 10 g/L, 20 g/L, 30 g/L, and 40 g/L) with five replicates.

Tool sterilization

The culture bottles and tools were washed with detergent, rinsed with water, and dried. After drying, aluminum foil was wrapped around the culture bottles and tube-shaped tools, while the paper was wrapped around the other tools (Petri dishes, scalpels, tweezers, spatulas, and pipettes). All tools and culture bottles were sterilized in an autoclave for one hour at 121°C and 1.5 atm.

Stock solution preparation

The MS media stock chemicals were weighed and then dissolved in 50 mL of distilled water in a beaker while stirring with a magnetic stirrer. Following the dissolution of the material, the volume is set to 100 mL, and the solution is placed in a stock bottle and labeled. After dissolving the Na_2EDTA solution, only the crushed Fe_2SO_4 was added to make a FeEDTA solution. Once the chemical has been dissolved, the volume is set to 100 mL and placed in a stock bottle before being labeled. All bottles containing stock solution are wrapped in aluminum foil and refrigerated.

Media preparation

Callus initiation media

The refrigerator is raided for stock solutions. A one-liter beaker is placed on a hot plate, and one-third of it is filled with distilled water. Afterward, each stock solution was added by the MS medium composition listed in Appendix 1. In a beaker, sucrose was added and stirred until completely dissolved. The distilled water is added until the ¾ beaker's capacity is reached. A pH meter is used to determine the acidity (pH) of the solution. If the pH of the solution does not reach the desired range of 5.6-5.8, it is adjusted by adding HCl if it is too high or KOH if it is too low. After the pH was optimized, up to 1 mg of a growth regulator (ZPT) in the form of 2,4-D (2,4-Diclorophenoxyacetic acid) was added to the solution. The media solution was stirred until all ingredients were dissolved, then agar and distilled water were added until a total volume of one liter was reached. The media is heated to boiling before being transferred to hot culture bottles. The culture bottles containing the media were then wrapped in aluminum foil and sterilized in an autoclave for 15 minutes at 121°C and 1.5 atm pressure (Junaid et al. 2007).

Treatment media

The preparation of the treatment media was almost the same as the callus initiation medium, but the PGR 2,4-D was replaced with NAA 1 mg/L and BA 1.5 mg/L (Junaid et al. 2007). Sucrose was added to the treatment medium according to the predetermined concentration.

Induction of callus formation

Explant sterilization

Surface sterilization of *C. roseus* seeds was accomplished by first washing the seeds with running

water, then immersing them in a 0.5 percent mercury chloride solution for 2 minutes, followed by another wash with distilled water.

In vitro seed germination

The sterile seeds were placed in a culture bottle with sterile tissue moistened with sterile distilled water. The bottle was then tightly wrapped in aluminum foil and kept in the dark place. In a laminar airflow, the germinated hypocotyl was removed from the culture bottle and cut into several pieces (Junaid et al. 2007).

Initiation of callus from hypocotyl

The hypocotyl was placed aseptically in a laminar airflow cabinet in a culture bottle containing MS medium containing 1 mg/L of 2,4-D. The culture bottles were then tightly sealed with aluminum foil and placed on a culture rack to incubate. Culture racks are sprayed with 70% alcohol at least once every three days to prevent contamination.

Callus planting on treatment media

Callus was transferred to the treatment medium aseptically in a laminar airflow cabinet using sterile tweezers from the callus initiation media. After inserting the callus, the culture bottles were tightly sealed with aluminum foil and incubated on the culture rack for one week. The callus formed after one week of planting is harvested.

Qualitative and quantitative tests on callus

Callus qualitative testing was performed, among other things, by observing callus morphology to determine whether embryogenic or non-embryogenic callus were formed. The callus' shape, color, and texture were all observed. During the incubation period, it was also observed on what day the embryogenic callus appeared. Weighing the overall weight of the callus, both wet and dry weights were used for quantitative testing.

The initial wet callus and the final callus fresh weight were weighed to determine the wet callus weight. The fresh weight of embryogenic callus was calculated by weighing the callus, culture bottles, media, and aluminum foil. The increase in callus fresh callus was calculated as the difference between the initial and final callus fresh weight. The callus dry weight was calculated by measuring the callus weight after it had been dried in a 50°C oven. Weight is measured every 24 hours until a constant weight is obtained.

Data analysis

The data obtained are both qualitative and quantitative. Qualitative data in the form of morphological data, such as callus color and texture, as well as the presence or absence of somatic embryos. Wet and dry callus weights were measured quantitatively. The qualitative data were analyzed descriptively, while the quantitative data were statistically analyzed using the ANOVA test and then the DMRT test at the 5% level.

RESULTS AND DISCUSSION

Callus growth on initiation media

The hypocotyl *C. roseus* explant was grown under aseptic conditions. Murashige-Skoog (MS) media was used because it contains a high mineral salt content and is widely used in in vitro culture (Lestari and Purwaningsih 1996). MS media was used as the initiation medium, along with an auxin hormone in the form of 2,4-dichlorophenoxyacetic acid (2,4-D).

After a week of being planted on initiation media, the callus on explants began to form. Because the cut end of the hypocotyl is in direct contact with the media and contains a transport bundle, callus forms. According to Gunawan (1998), cell autolysis causes callus formation on injured explants, and compounds produced by these damaged cells stimulate cell division in the next layer.

Because callus is a meristematic wound covering tissue, it will form from the wound of the explant or the edge of the explant slice, according to Suryowinoto (1996). This is also possible because there is one type of plant response to tissue or cell injury. According to Leon et al. (2001), injury to plant tissue or cells activates plant self-defense mechanisms both locally and systemically (in uninjured tissue) by changing the direction of metabolic pathways and inducing the expression of specific genes, but only in damaged tissues. Which will form an irregular cell structure, dedifferentiate, release stored compounds, and lose a significant amount of water. A callus will form from this irregular cell structure. The callus is an undifferentiated mass of cells.

Because callus histologically originates from the division of many parenchyma cells around the transport bundle except the xylem, it can appear around the carrier bundle. The callus that forms on the hypocotyl incision is caused by faster explants peripheral cells dividing faster due to increased oxygen availability, CO_2 release, and the presence of light (Gunawan 1988).

The use of 2,4-D hormone aids in the formation of callus on explants. According to Agustina (2003), 2,4-D can increase osmotic pressure, protein synthesis, cell wall permeability to water, and soften cell walls, followed by a decrease in cell wall pressure that allows water to enter the cell, resulting in a decrease in cell wall pressure. Over time, cell volume increases, and the callus expands. Protein synthesis stimulated by 2,4-D will also promote growth. Because it grows and absorbs water from its surroundings, the callus expands in size over time.

The formed callus was then subcultured several times in the same media composition to obtain a sufficient number of the callus to be subcultured into the treatment medium. Callus that has grown on a medium must be moved regularly within a certain time frame. A long culture period in fixed media depletes nutrients and water. Running out of water can happen not only because of growth but also because the media occasionally evaporates water. In addition to running out of nutrients, the cells in the callus secrete compounds from metabolism that inhibit callus growth, so the resulting callus must be subcultured to maintain life and continuous propagation (Gunawan 1988).

Because new media keeps the callus in an exponential phase, subculture also serves to multiply it.

Growth and development of callus on treatment media

Morphology (color and texture) of callus on treatment media

The initiated callus was then transferred to the treatment medium, which was MS media supplemented with NAA and BA hormones. The color and texture of the callus were observed after a week. Table 1 displays callus color and texture data, while Figure 1 displays callus morphology photos.

Some calluses were white when they were first initiated, some that turned yellowish, and some that had browning to dark brown. Light yellow callus develops when the callus begins in an area exposed to light, causing the callus to become pigmented (yellow) (Sudirga 2002). Callus that is still white and yellow or has not browned is thought to have not experienced senescence or aging. This is most likely due to the BA content of the treatment media, which slows the aging process. Wattimena (1998) describes BA as a cytokinin that slows the process of cell senescence by inhibiting the overhaul of chlorophyll grains and proteins in cells.

Table 1. Color and texture of *C. roseus* callus on treatment media

Treatment media	Callus morphology	
	Color	Texture
A1	Dark brown	Compact
A2	Brownish-yellow	Compact
A3	Brown	Compact
A4	Yellow	Compact
A5	Yellow	Compact
B1	Dark brown	Compact
B2	Clear white	Compact
B3	Yellow	Compact
B4	Clear yellow	Compact
B5	Brown	Compact
C1	Dark brown	Crumb
C2	Clear yellow	Crumb
C3	Clear yellow	Compact
C4	Clear yellow	Compact
C5	Brownish-yellow	Compact
D1	Cloudy yellow	Crumb
D2	Yellowish white, slightly brown	Crumb
D3	Yellow	Crumb
D4	Yellow	Crumb
D5	Brown	Crumb
E1	Yellowish white	Crumb
E2	Yellowish white	Crumb
E3	Yellow	Crumb
E4	Yellowish white	Crumb
E5	Yellowish white	Crumb

Notes: A1, A2, A3, A4, A5: callus on media with the addition of sucrose of 0 g/L repetitions of 1, 2, 3, 4, and 5; B1, B2, B3, B4, B5: callus on media with the addition of sucrose of 10 g/L repetitions of 1, 2, 3, 4, and 5; C1, C2, C3, C4, C5: callus on media with the addition of sucrose of 20 g/L repetitions of 1, 2, 3, 4, and 5; D1, D2, D3, D4, D5: callus on media with the addition of sucrose of 30 g/L repetitions of 1, 2, 3, 4, and 5; E1, E2, E3, E4, E5: callus on media with the addition of sucrose of 40 g/L repetitions of 1, 2, 3, 4, and 5

The brown color of the callus may be due to natural aging symptoms and the production of phenolic compounds in response to tissue injury caused by cutting. Some phenolic compounds are auxin protectors and antioxidants that act as inhibitors of the peroxidase enzyme-catalyzed IAA oxidation process. Generally, phenolics are extremely labile and easily oxidized to phytotoxic compounds (Debergh and Zimmerman 1991). This can be avoided by introducing activated charcoal into the media (Dodds and Roberts 1995).

Figure 1 shows that brownish callus was still obtained in the sucrose treatments of 0 g/L to 30 g/L, while callus grown at a sucrose concentration of 40 g/L was still fresh. The difference in callus color is caused by the age of the hypocotyl used. Callus that has browned is most likely the result of hypocotyl fragments near the root (older age), whereas callus that is still young is the result of hypocotyl fragments near the shoot's tip (younger age).

Observation revealed that some callus textures were compact while others were crumbly. A compact callus has a tight, dense, and difficult to separate cell structure, larger vacuoles, and a thicker polysaccharide wall. This large vacuole allows the callus to store water in the cell, increasing its water content. According to Zhao et al. (2001), compact callus is formed by NAA, which does not induce the synthesis of cellulase and pectinase enzymes, which have lytic activity on the middle lamella and provide a compact structure so that the bonds between cells are not tenuous.

Callus crumbs have a loose arrangement of easily separated cells that are meristematic and actively dividing (Street 1993). Steves and Sussex (1994) mentioned that cells with crumb textures are irregular and relatively small in size, with a large nucleus and thick cytoplasm. The presence of endogenous auxin hormone, produced internally by explants that have grown to form the callus, also stimulates the formation of callus with a crumb texture.

A somatic embryo in the callus

For a week, the callus was examined every day to determine the presence or absence of somatic embryos. There was no embryogenic callus in any of the treatments. Somatic embryos can be formed directly in the mesophyll of endogenous explants, according to Trisnawati et al.'s (1999) research on Allium sativum L, but they can be degraded back into a callus. Only somatic embryos (exogenous) formed on the callus surface could be observed.

The type of explant, the developmental stage, and the interaction between the explant and the growth medium are the parameters that determine the success of somatic embryogenesis (Debergh and Zimmerman 1991). Different types of explants require different times in the auxin-containing induction medium of somatic embryos. This is because the role of exogenous auxin in the induction of somatic embryos depends on the type of explants used in the study. Explants derived from petioles, hypocotyls, and cells isolated from suspension cultures in carrot (*Daucus carota* L.) required 1, 2, and 7 days of auxin administration

in the initiation medium before becoming competent to undergo incubation embryogenesis after being transferred to auxin-free media (Zimmerman 1993). This study found no somatic embryos, possibly because the callus had been in the initiation medium for too long. Initially, the goal of initiating callus in a medium containing 2,4-D for a long enough period was to obtain enough callus to subculture in the treatment medium. Still, this step was ineffective in growing somatic embryos. Junaid et al. (2007) discovered that the effective time for *C. roseus* hypocotyl callus culture in the initiation medium was approximately one month before being transferred to the treatment medium.

The explants used may differ depending on the type of explant source plant and the explant's developmental stage. Using meristematic explants generally results in a higher success rate in somatic embryo formation. Explants in the form of the axis of young and adult zygotic embryos, cotyledons, buds, epicotyl, and hypocotyl can be used (Purnamaningsih 2002). There are also differences in the hypocotyl cells' meristematic abilities. Cells near the shoot tip were younger and more meristematic than older cells near the root.

Explants will also interact well with the growth medium and produce somatic embryos if the media contains enough substances required to induce somatic embryogenesis. Interactions include nutrients in the media, particularly nitrogen sources, which are the main factor that stimulates morphogenesis in vitro culture and will also indirectly affect culture pH, carbon source (sugar), and growth regulators (Purnamaningsih 2002).

Junaid et al. (2007) found that the same treatment medium was very suitable and effective in producing embryogenic callus from hypocotyl *C. roseus* explants. When the media was applied to the same explants in this study, it produced only non-embryogenic callus. This is possible because the hypocotyl used is too old, resulting in differences in meristematic abilities compared to younger tissue and variations in the level of endogenous growth regulators in the hypocotyl used. Young tissue in the hypocotyl can certainly divide faster than adult tissue. The length of the hypocotyl indicates the age of the plant.

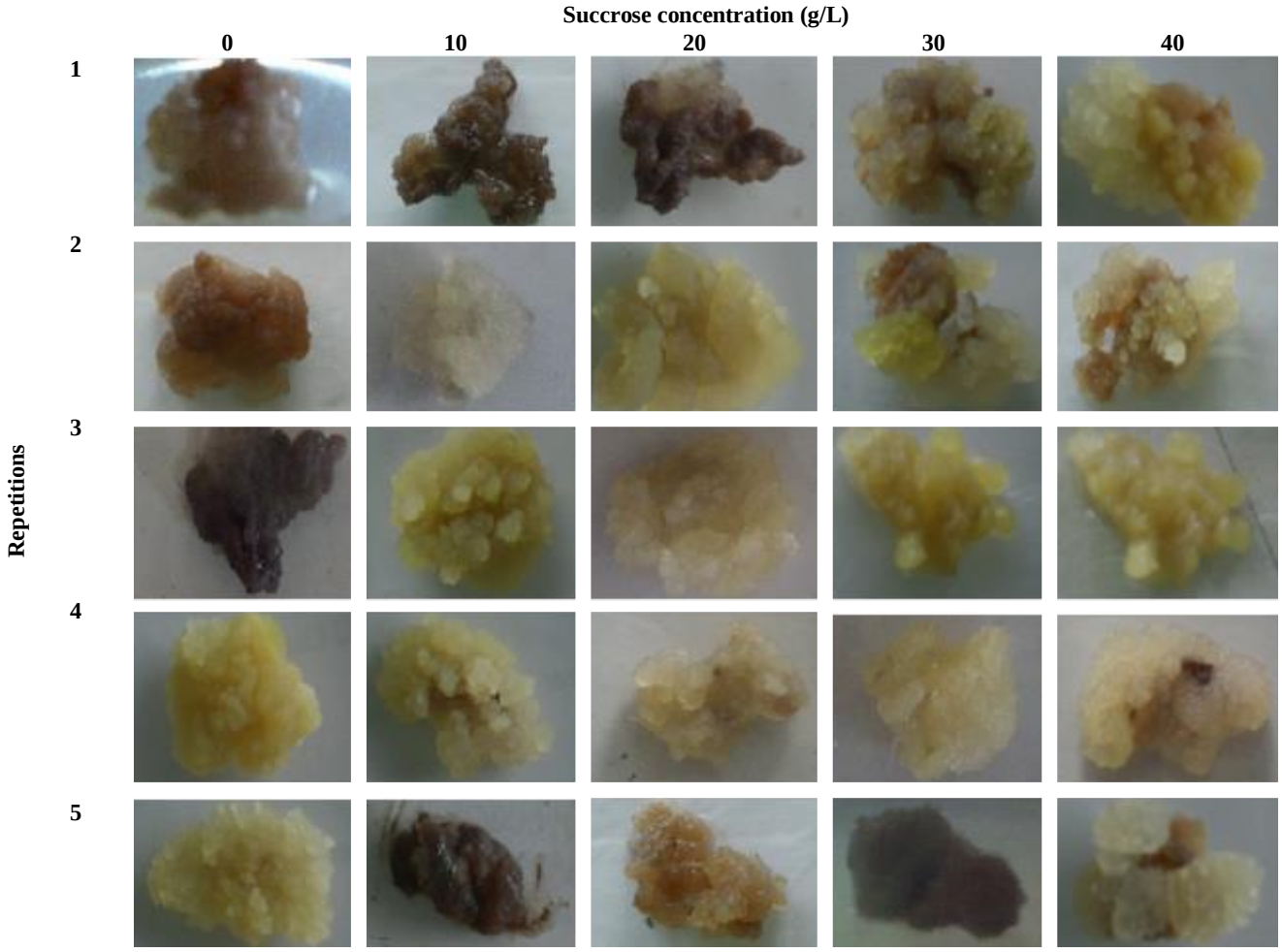

Figure 1. Displays callus morphology

When compared to other methods such as suspension culture, in vitro culture, particularly somatic embryogenesis, has the potential to produce secondary metabolites more effectively. Culture methods commonly used to produce secondary metabolites in large quantities have encountered several challenges, one of which is that important medicinal compounds such as vinblastine, catharanthine, ajmalicine, and serpentine are produced at extremely low levels in *C. roseus* cell culture. Many factors contribute to this, but the lack of cellular and tissue differentiation in cell suspension cultures is thought to be the most important (Moreno et al. 1996 in; Zhao et al. 2001). Biosynthetic products are influenced by molecular, cellular, and organ differentiation (Heble 1996). Embryogenesis is one type of organized development, according to Torres (1989). This is demonstrated by the ability of embryos formed in vitro to develop into complete plantlets through stages similar to normal embryogenesis. Because of its high level of cell differentiation, somatic embryogenesis has the potential to produce secondary metabolites, particularly drug compounds, making it more effective than other culture methods.

Callus fresh weight

The fresh weight of the plant can be used to determine the growth of cell volume and mass (Salisbury and Ross 1995). Cell elongation will occur, followed by cell enlargement and an increase in fresh weight. The increase in the fresh weight of the callus indicates that the cell growth process has occurred.

The fresh weight of the explant culture was calculated by weighing the callus, along with the bottle and aluminum foil cap, before and after subculture into the treatment medium and then calculating the difference to obtain the value of the callus's fresh weight gain. Fresh weight can be used to assess the growth of explant culture results, but it cannot be used as a standard because fresh weight is affected by cell water content, plant metabolism, and plant moisture conditions (Sitompul and Guritno 1995). Table 2 and Figure 2 display the average data for callus fresh weight gain.

Because the fresh weight of the callus in the treatment media decreased over time, all values from the average increase in fresh weight were negative. The ANOVA test results showed that the results were not significantly different. This means that the treatment of varying sucrose concentrations did not affect the increase in fresh weight of *C. roseus* callus.

Because callus fresh weight measurement is still highly dependent on callus water content, the decrease in fresh callus weight is most likely due to a decrease in callus water content. The difference in fresh weight loss is thought to be due to the tissue's ability to store water and different nutrients, specifically the ability to carry out diffusion, osmosis, and cell turgor pressure regulation (Sriyanti 2000). The accumulation of secondary metabolites that are toxic to cells, as well as the occurrence of lysis, contribute to the decrease in fresh weight (cell death).

Table 2. The average fresh weight gain of *C. roseus* callus (g) after incubation for a week on the treatment media.

Sucrose concentration (g/L)	**0**	**10**	**20**	**30**	**40**
Average fresh weight gain of callus (g)	- 0.285[a]	- 0.439[a]	- 0.615[a]	- 0.295[a]	- 0.670[a]

Note: numbers followed by the same superscript letter in the same line show no significant difference in the 5% DMRT test

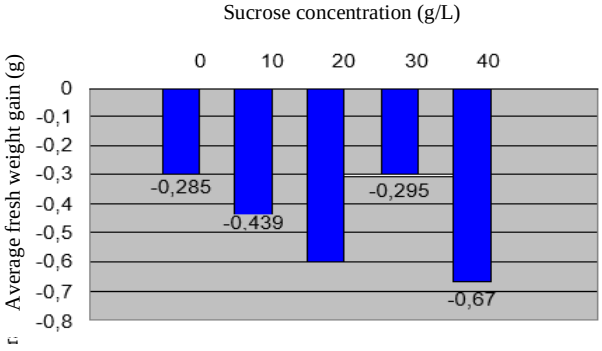

Figure 2. Average fresh weight gain of *C. roseus* callus

The decrease in fresh callus weight may also be due to the callus having endogenous auxin hormone, causing the hormone content in the media to become too high. Auxin's role is to change the osmotic pressure in the cell, which affects the biochemical processes in the cell (Wattimena et al. 1992). The high concentration of auxin reduces osmotic pressure, which results in the release of water from the cell (Palupi et al. 2004). The difference in fresh weight loss between treatments was most likely due to differences in callus endogenous auxin levels. Endogenous auxins contribute to increased cell permeability to water. Because the callus was formed from hypocotyls of varying ages, the endogenous auxin level in each callus varied. As a result, the level of cell permeability to water in each callus varies (Abidin 1990).

Dry weight

Plant products are typically expressed in terms of total dry matter rather than fresh weight. The plant's total dry weight manifests the processes and events during plant growth (Sitompul and Guritno 1995). Unlike fresh weight, dry weight provides an acceptable estimate of a culture's metabolic activity (Dodds and Roberts 1995).

According to Gardner et al. (1991), fresh weight measurements are less representative of growth parameters because the numbers fluctuate depending on the humidity conditions of the plant. Because the dry weight of the callus was measured from a constant weight, the number of measurements on the dry weight of the callus did not vary. Dry weight was obtained by baking the callus harvested at the end of the treatment until the weight was constant. According to Sitompul and Guritno (1995), drying the material aims to stop metabolic activity in the material, as

opposed to a fresh weight measurement, which is still influenced by metabolic activities such as transpiration, causing difficulties in measuring and gaining constant weight. The amount of biomass produced highly depends on the rate at which these cells divide and multiply, which can be influenced by the medium's composition (Wattimena et al. 1992).

The ANOVA statistical test on dry callus weight revealed that sucrose administration at various concentrations significantly affected callus dry weight in each treatment. Table 3 shows that the highest average callus weight was obtained with media containing 40 g/L sucrose, which was 0.037 g, significantly different from all treatments. Because there was no decrease in dry callus weight in this study, the results were not optimal.

The media used in plant tissue culture is critical to its success. Plant tissue culture media not only supply macro and micronutrients but also carbohydrates in the form of sugar. This sugar is a carbon source that plants use to replace the carbon they get from the atmosphere in the form of CO_2, which is a component of photosynthesis. Sucrose is an important carbon source used as a building block for cells, according to George and Sherrington (1984). With enough sucrose, cell division, cell enlargement, and further cell differentiation can occur normally. Sucrose provides energy and carbon for callus growth as well as cell constituents. The abundant availability of sucrose enables the occurrence of sufficient energy and essential materials for biomass growth and formation.

Sucrose serves several functions, including being a carbon and energy source, regulating osmotic pressure, a key factor in membrane stabilization, acting as a stress protector, and a signaling molecule (Tomaz et al. 2001; Lipavska and Konradova 2004). Meanwhile, Iraqi and Tremblay (2001) attribute the importance of sucrose in the media to the following factors: (i) sucrose is hydrolyzed by the enzymes invertase and sucrose synthase into hexose, namely glucose and fructose, which can be directly utilized by plants; (ii) the result of sucrose hydrolysis increases the osmotic concentration of the medium; and (iii) sucrose acts as a signal for storage protein synthesis.

The increase in dry callus weight was accompanied by an increase in sucrose concentration, as shown in Figure 3. The increase in sucrose concentration is thought to cause the formation of metabolites that promote callus division and growth. Sucrose in the media stimulates callus cell division (Suskendriyati et al. 2004).

Table 3. *Catharanthus roseus* callus dry weight (g) after incubation for a week on treatment media

Sucrose concentration (g/L)	0	10	20	30	40
Callus dry weight (g)	0.015a	0.017a	0.024ab	0.033bc	0.037c

Notes: numbers followed by the same superscript letters in the same line show no significant difference in the 5% DMRT test

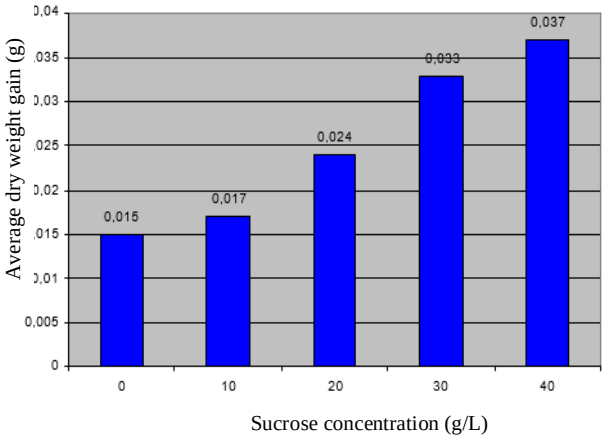

Figure 3. The average dry weight of *Catharanthus roseus* callus (g)

According to Sitompul and Guritno (1995), some sucrose transported into cells is metabolized to produce energy and carbon. Others are converted into essential materials such as cell wall materials, proteins, and other growth materials. Sucrose will be hydrolyzed into glucose and fructose in the plant body. Both are essential for cell growth. Cells will use phosphorylated glucose and fructose for further metabolism, according to Strum (1999). Glucose can enter glycolysis and the Krebs cycle to produce ATP and NADH, which are used to fuel growth. Fructose also plays an important antioxidant role in membrane stabilization (van den Endel and Vallumu 2009). In addition to producing energy, Sucrose metabolism also provides a carbon skeleton, among other things, which can be used to produce other essential products in plants, such as secondary metabolites (Salisbury and Ross 1995).

Media containing a high sucrose concentration will be more concentrated than media containing no sucrose. Because a medium with a high concentration contains many molecules, the diffusion movement is directed toward a location with a scarcity of molecules or a low concentration. This condition causes cells in explant tissue grown on media containing the highest amount of sucrose, namely 40 g/L, to receive nutrients more quickly. Furthermore, sucrose is hydrolyzed into glucose and fructose when sterilized at the proper temperature. Glucose provides energy for cells to grow and develop to form new cells. This results in the highest dry weight of 0.037 g in the treatment with the highest sucrose concentration. The lowest callus dry weight was obtained on media without sucrose, which was 0.015 g. The callus did not receive enough material to form biomass in this medium because the number of carbohydrates (sucrose) that served as a source of energy and carbon was very low, if not non-existent.

The fresh and callus dry weight measurements yielded different results in the study. The fresh weight measurement revealed a decrease in callus growth, whereas adding sucrose to the dry weight measurement increased as the sucrose concentration increased. The callus's fresh weight is still heavily influenced by its water content. The ability of a callus to absorb and store water is influenced by

its texture. According to Abidin (1990), cells in the outer layer that are in contact with the media absorb water more easily than cells in the inner layer. Because of the uneven callus texture, not all callus cells, particularly those on the inside, were able to touch the media. As a result, the callus' ability to absorb and store water differs. Callus cells with larger vacuoles store more water than callus cells with small vacuoles. In this study, a decrease in fresh weight does not imply a decrease in growth because dry weight is the more appropriate parameter to indicate an increase or decrease in growth. Only a decrease in water content, not a decrease in growth, affected the decrease in fresh weight. The callus's higher dry weight and the increasing sucrose concentration indicated that the callus was still growing. This is thought to cause the pattern difference between callus's wet and dry weight.

Based on the research, it could be concluded that: (i) Sucrose administration at various concentrations (0; 10; 20; 30; 40 g/L) had a significant effect on dry callus weight but did not affect fresh weight. The *C. roseus* callus (ii) Sucrose administration at various concentrations (0; 10; 20; 30; 40 g/L) had no effect on the formation of *C. roseus* callus embryos.

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Morphology and cytology of five soybean varieties (*Glycine max*) treated with phosphate fertilizer

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Abstract. *Darsono DC, Nandariyah, Sugijono. 2018. Morphology and cytology of five soybean varieties (*Glycine max*) treated with phosphate fertilizer. Cell Biol Dev 2: 78-87.* The demand for soybeans (*Glycine max* (L.) Merrill) tends to increase. The first step to increasing soybean productivity is identifying the plant's character. This study aims to study plants' morphological and cytological characteristics (chromosome number) and determine the effect of phosphate fertilizer application on changes in plant morphological and cytological characteristics (chromosome number) in five soybean varieties. The research was carried out from April 2009 to March 2010. Observations of plant morphology were carried out at the Jumantono Dry Land Research and Development Center (*Pusat Penelitian dan Pengembangan Lahan Kering Jumantono*), Karanganyar, Central Java, Indonesia (07°37' latitude and 110°56' east longitude). The number of chromosomes was analyzed in the Laboratory of Plant Breeding, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, and the Laboratory of Animal Anatomy, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Plant morphology research was arranged factorially using a Completely Randomized Block Design (RAKL). The treatment factors were varieties (Argomulyo, Anjasmoro, Kaba, Sibayak, and Wilis) and doses of P fertilizer (0, 18, and 36 kg P_2O_5/ha (equivalent to 0, 50, and 100 kg SP-36/ha). Therefore, there were 15 treatment combinations, and each combination was repeated 3 times. The observation of the number of chromosomes used the squash method. Quantitative data were analyzed by Analysis of Variance (ANOVA), if significantly different, continued with Duncan's multiple distance test (DMRT) level of 5%. The qualitative analysis was analyzed and presented descriptively. The results showed that each of the five soybean varieties' morphological characteristics differed. Applying phosphate (P) fertilizer from a dose of 0, 18, or 36 kg P_2O_5/ha could affect plant morphology in the form of an increase in plant height, length of the main root, number of branch roots, length of stem internode, length of petiole, leaf area, and weight of 100 seeds in each soybean variety. The number of chromosomes in five soybean varieties is the same, namely 2n = 40. The application of phosphate (P) fertilizer did not affect the number of chromosomes in the five soybean varieties.

Keywords: Cytology, *Glycine max*, morphology, phosphate fertilizer, soybean

INTRODUCTION

Indonesia is a country that has large and fertile land as natural resources. Therefore, climatic conditions, temperature, and humidity that are suitable for the growing needs of staple food crops cause almost all food crops to grow relatively well. One food crop that is very important for the Indonesian population is soybean (*Glycine max* (L.) Merrill).

Soybeans have been known for a long time as one of the plant sources of vegetable protein. Soybean seeds can be processed into food and beverage ingredients, such as tempeh, soy sauce, tauco, bean sprouts, milk, and soy juice drinks. As a food ingredient, soybeans are very nutritious for growth and maintain the condition of body cells. Soybeans contain many elements and important food substances such as protein, fat, carbohydrates, etc. The nutritional value of 100 g of soybean seeds is 330 calories, 35% protein, 18% fat, 35% carbohydrates, and 8% water (Budiastuti et al. 1997).

At this time, the demand for soybeans tends to increase in line with the increase in population, per capita income, and public awareness of the healthy menu. However, the pace of demand still cannot be matched by the increase in soybean production rate, so Indonesia must import it. For example, the demand for soybeans in 2004 was 2.02 million tons. However, domestic production only reached 0.71 million tons, so to meet the needs, the government must import 1.31 million tons since only about 35% of the total needs can be fulfilled by domestic production itself (Swastika et al. 2008). Therefore, it is necessary to increase soybean production to meet soybean needs and reduce import dependency.

It is necessary to pay attention to several aspects of cultivation, especially proper fertilization, to increase the productivity of soybean plants and use superior varieties (Amir et al. 2015). Fertilizers have an important role in plant life, especially plant physiology. Physiological processes that take place optimally can encourage plants to respond to growth in appearance and high yields. One of the important macronutrients for soybean plants is phosphorus (P). Phosphorus plays an important role in growth and production because it can provide the energy needed for plant metabolic activities.

Each soybean variety has a different appearance, and it is it's characteristic. Therefore, as an initial step to increase soybean productivity, it is necessary to identify plant characteristics, especially the character of the variety to be cultivated. Identification of these characters can be made

genetically or morphologically. The genetic identification is useful to support the development of soybean plants, especially concerning plant breeding activities, both direct and indirect applications. The use of genetic information in plant breeding indirectly increases the knowledge of the genetic composition of a plant species and can be used directly to improve plant characteristics.

The morphological identification of soybean varieties is intended to identify the characteristics of soybean plants from their external appearance. Descriptions based on morphological characters can generally be used to determine the relationship between soybean varieties that can be seen directly because these morphological characteristics arise through the interaction between genetic traits and the environment in which the plant grows. The combination of genetic and morphological identification is expected to be useful for obtaining complete information about the nature and characteristics of soybean plants. These are useful in soybean production efforts to obtain optimal results.

The aims of this study were: (i) to study plant morphology and cytology characteristics (chromosome number) in five soybean varieties. (ii) to determine the effect of phosphate fertilizer application on changes in plant morphological and cytological characteristics (chromosome count) in five soybean varieties.

MATERIALS AND METHODS

Place and time of research

Research on plant morphology was carried out at the Dry Land Research and Development Center (*Pusat Penelitian dan Pengembangan Lahan Kering*), Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, located in Jumantono, Karanganyar, Central Java, Indonesia, with latosol soil types at positions 07°37 latitude and 110°56 east longitude and an altitude of 180 m above sea level. In addition, cytological research (chromosome count) was carried out at the Laboratory of Plant Breeding, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, and the Laboratory of Animal Anatomy, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The research was conducted from April 2009 to March 2010.

Research material

The materials in this study included seeds (Argomulyo, Anjasmoro, Kaba, Sibayak, and Wilis varieties), fertilizer (Urea, SP-36, KCl), 1 N HCl solution, 2% aceto-orcein solution, Carnoy solution 2 (6 ethanol : 3 chloroform : 1 glacial acetic acid 45%), alcohol 96% and aquades.

Research tools

The tools in this research included a hoe, measuring tape, seed drill, nameplate, camera, tweezers, flacon, glass preparations, cover glass, light microscope, and photo-microscope.

Research design

The design used in the soybean plant morphology study was a Completely Randomized Block Design (CRBD), which consisted of two factors. The factor I: soybean variety (V), consists of 5 levels: (i) V1: Argomulyo variety, (ii), V2: Anjasmoro soybean, (iii) V3: Kaba variety, (iv) V4: Sibayak variety, (v) V5: Wilis variety. The factor II: dose of phosphate fertilizer (D), consists of 3 levels: (i) D0: 0 kg P_2O_5/ha or equivalent to 0 kg SP-36/ha, (ii) D1: 18 kg P_2O_5/ha or equivalent to 50 kg SP-36/ha, (iii) D2: 36 kg P_2O_5/ha or equivalent to 100 kg SP-36/ha. Based on the treatment of these two factors, 15 treatment combinations were obtained. Each treatment combination was repeated 3 times (as blocks) so that there were 45 treatment plots. Sampling was done by random sampling in each treatment, with 3 samples for each treatment plot.

Plant morphology

Land preparation

Land preparation aims to make the physical condition of the land lose and reduce weed populations. Land preparation is done by hoeing the land to be used for soybean cultivation, and then plots are made with a size of 1 m x 1.5 m.

Planting

Selected soybean seeds (5 varieties) were planted in planting holes with a spacing of 20 cm x 25 cm. Each planting hole is filled with 3-4 soybean seeds and then covered with soil.

Fertilization

Fertilizer for soybeans are: Urea 100 kg/ha, SP-36 according to treatment, namely 0; 50; and 100 kg/ha and KCl 100 kg/ha. The application of urea fertilizer was carried out in two stages at the beginning of the planting period with half the total dose. At the same time, the rest was given when the plants were 4 WAP. SP-36 and KCl fertilizers were applied at the beginning of planting.

Sprinkling

Water plays a very important role in the growth of soybean plants, from the beginning of growth to the period of pod filling. Watering is carried out until it reaches field capacity and starts 5-7 days after the seedlings grow. Subsequent irrigation is carried out when the soil looks dry.

Plant replacement and thinning

Plant replacement is done on plants that die or do not grow at age of one week after planting. Thinning was done at 3 WAP, leaving two plants for each hole.

Weeding

Plant weeding is carried out simultaneously with additional fertilization and according to the condition of the weed population around the plant.

Yield

Harvesting occurs when the plants are ready to harvest (depending on the variety), with 80% of the pod population evenly brownish yellow, the stems are dry, and some leaves have dried and fallen off. Harvest implementation at 80 DAP.

Observation

Observations were made visually covering the vegetative (roots, stems, leaves) and generative (flowers, fruit/pods, seeds) parts based on their morphological appearance. Observations on the roots, stems, leaves, and pods were conducted two weeks before harvest. Seed observation was carried out at harvest. At the same time, the observation for flowers was performed when soybean plants began to flower.

Cytology (chromosome number)

Material preparation

The material was taken from the meristematic root tip ± 5 mm. The root tip is used as a preparation material because it is the most meristem organ related to its function as a nutrient-seeking tool that always divides to move in search of nutrients (Setyawan and Sutikno 2000). Root cutting was carried out at 08.00-08.30.

Pre-treatment

Pre-treatment was carried out for the separation and decomposition of chromosome density, purification of the cytoplasm, and softening of the tissue (Gunarso 1988). Pre-treatment was carried out by immersing the material in distilled water for ± 24 hours at a temperature of 5-8°C.

Fixation

Fixation was done to eliminate the tissue without causing changes to the cell components (Gunarso 1988). Fixation was carried out using Carnoy 2 solution (6 ethanol: 3 chloroform: 1 glacial acetic acid 45%) and stored in the refrigerator for ± 24 hours, then washed gradually every 10 minutes in a row with 70% alcohol, 50% alcohol, and 30% alcohol and aquadest (distilled water).

Hydrolysis

According to Setyawan and Sutikno (2002), hydrolysis was carried out to obtain cells that were spread out in chromosomal observations by dissolving the middle lamella of meristematic cells with no strong attachment. Hydrolysis was performed by immersing soybean roots in 1 N HCl solution for 10 minutes at room temperature (±25^{o}C).

Washing

The hydrolyzed root tips were then washed with distilled water 3 times. The washing was intended to remove the influence of the previous treatment.

Coloring

Chromosome staining was carried out by immersing the material in a 2% aceto-orcein solution for ± 24 hours at a temperature of 5-10°C. Aceto-orcein is suitable for root tips because of its fast penetration and long-lasting storage (Setyawan and Sutikno, 2000).

Squashing

± 0.5 mm of the tip of the meristematic root was taken and placed on a glass slide. The material was dripped with 45% acetic acid, covered with a cover glass, and then squeezed with the thumb. This preparation was used for the observation of the number of chromosomes.

Observation

Chromosomes were observed using a lighted microscope. Observations were made at the prometaphase stage, which showed good distribution. Chromosomes at the prometaphase stage have a much longer size and a much clearer appearance than in other stages (Parjanto et al. 2003). The results were then photographed with a photo-microscope.

Observation variable

Plant characteristics (morphology)

Plant height (cm), roots (main root length (cm), number of lateral roots), stem (stem color, stem shape, number of internodes, internode length (cm), number of branches, hair color), leaves (compound leaf shape and arrangement, compound leaf stalk length (cm), leaf area (cm^2), leaf tip shape, leaf base shape, leaf attachment method, top leaf color, leaf structure system, hair color), flower (when flowers appear (DAP), color flowers, flower location, number of flowers/plants, pods, including pod color, pod length (cm), number of seeds/pod, number of pods/plant, hair color), seeds (seed coat color, seed shape, the weight of 100 seeds (grams).

Number of chromosomes (cytology)

Chromosomes that appeared on observation with a microscope were photographed, and the number of chromosomes could be counted from the printouts.

Data analysis

Observational data was divided into two types, namely qualitative data and quantitative data. First, qualitative data were analyzed and presented descriptively to identify the morphological characteristics of soybean plants and the number of chromosomes. Analysis of Variance (ANOVA) analyzed quantitative data, and if there was a significant difference, it was continued with Duncan's multiple distance test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

This study was divided into two stages: morphological observations of soybean plants in the field and cytological observations (number of chromosomes) using material from soybeans germinated in the laboratory.

Plant morphology

The morphological characteristics of five soybean varieties can be seen in Table 1.

Argomulyo varieties

Argomulyo variety is a determinate type of soybean. This variety has an upright habitus with slightly woody stems with a plant height of 31.69-36.57 cm. The stem is cylindrical and green. The entire surface of the stem is covered with brown hairs. The stem has 2-4 branches. The

number of internodes on the main stem is 8-11, with an average internode length of 2.87-3.24 cm.

The root system of the Argomulyo variety is in the form of a taproot. The taproot (*radix primaria*) is a central root that continues to grow into a main root and branches into smaller roots and is often referred to as a branch root (*radix lateralis*) (Rukmana and Yuniarsih 1996). The main root has a length of 14.93-20.27 cm. The main root forms branches with a total of 18-26 branch roots.

The leaves on the Argomulyo variety (Figure 1) are compounds that are trifoliate (three leaflets), although sometimes there are leaves with four or more leaflets. The leaves are green with a compound petiole 9.83-15.85 cm long. The shape of the tip of the leaf (apex) is pointed (*acutus*). The leaf tip is called pointed if the two edges of the leaf tip on the right and left of the leaf venation gradually move upwards and their meeting at the top of the leaf tip forms an acute angle (<90°). The shape of the leaf base is rounded (*rotundatus*). Leaf venation is pinnate. The leaves were alternately attached to the right and left of the stem. There are brown hairs on the surface of the leaves. The Argomulyo variety has a leaf area of 188.7-335.9 cm^2.

The flower of the Argomulyo variety is shaped like a butterfly. Flowers are called butterflies because they have a crown consisting of 5 free crowns, but 2 of the crowns are usually united in the form of a lifeboat or boat. These two attached crowns are usually narrow and located at the bottom, called the keel. The one opposite the keel is called the flag, and the wing is between the two halves (Tjitrosoepomo 2007). The flowers are purple. Flowers grow in the axils of the leaves and the tips of the stems or branches. The number of flowers in one plant reaches 50-80 flowers. The Argomulyo variety is a short-lived type of soybean. This soybean begins to flower when the plant is 28 DAP and can be harvested from 75 DAP.

Soybeans of the Argomulyo variety are in the form of pods with one or more internodes due to false partitions. The leaves of the legume are covered with brown trichomes (feathers). Young pods are green in color, while ripe/dark green pods are dark brown. The color of the pod is influenced by the carotene and xanthophyll pigments, the color of the feathers, and the absence of anthocyanin pigments. The pod may contain 1-5 seeds, but most pods contain 2-3 seeds (Hidajat 1985). The pods have a length of 4.03-4.57 cm. The length of this pod is influenced by the number and size of seeds in the pod. Although the number of flowers per plant is quite large, about 20-80% experience loss (Caldwell 1973), so only a few can form pods. In this Argomulyo variety, the number of pods in one plant is 25-43.

The shape of seeds differs depending on the variety, which can be round, slightly flat, or ovoid (Rukmana and Yuniarsih 1996), but most of the seeds are ovoid/oval (Hidajat 1985). The seeds of this Argomulyo variety are oval, slightly flattened, and yellow. The seeds of the Argomulyo variety are large, with a weight of 100, reaching 15.5-17.28 g/100 seeds.

Anjasmoro varieties

Anjasmoro variety is a determinate type of soybean. This variety has an upright habitus with slightly woody stems with a plant height of 43-50.3 cm. The stem is cylindrical and green. The entire surface of the stem is covered with white hairs. Branched stems are of 2-4 branches. The number of internodes on the main stem is 10-13, with an average internode length of 3.39-4.06 cm.

The root system of the Anjasmoro variety is a taproot. The main root has a length of 20.43-26.5 cm. The main root forms branches with a total of 19-27 branch roots.

The leaves on the Anjasmoro variety are compounds that are trifoliate (three leaflets), although sometimes there are leaves with four or more leaflets. Green leaves with compound petiole 11.03-16.77 cm long. The shape of the leaf tip (*apex*) is blunt (*obtusus*). The leaf tip is called blunt if the leaf edge, which was originally still a bit far from the main leaf venation, quickly goes to a meeting point to form an obtuse angle (<90°). The shape of the leaf base is rounded (*rotundatus*). The leaf venation is pinnate. The way of attachment of leaves is alternately attached to the right and left of the stem. On the surface of soybean leaves, there are white hairs. The leaves of the Anjasmoro variety are among the widest when compared to other varieties, with a leaf area of 239.5-562.8 cm^2. Wide leaves allow sunlight absorption so that the photosynthesis process will be more effective.

The flower of the Anjasmoro variety is shaped like a butterfly and is purple. Flowers grow in the axils of the leaves and the ends of the stems or branches. The number of flowers in one plant reaches 64-91 flowers. The Anjasmoro variety begins to flower when the plant is 36 DAP and can be harvested from 82 DAP.

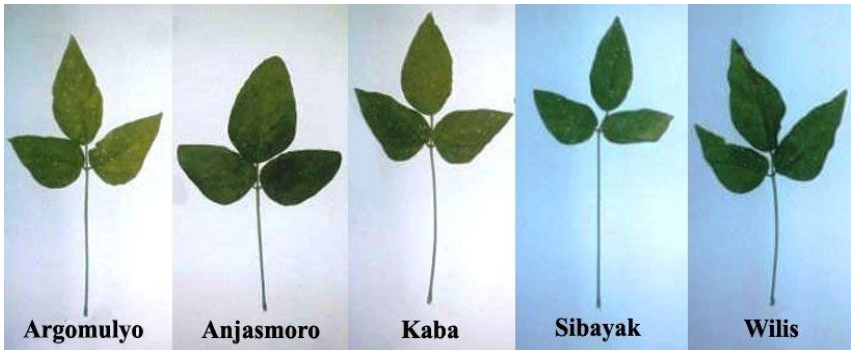


Figure 1. Soybean leaves

Table 1. Morphological characteristics of five soybean varieties

Morphological characteristics	Average value				
	Argomulyo	**Anjasmoro**	**Kaba**	**Sibayak**	**Wilis**
Plant height (cm)	32.6	44.53	45.73	56.67	44.59
Root					
Root length (cm)	16.36	20.69	19.91	21.54	19.46
Number of root branches	19.78	21.87	21.37	22.11	20.11
Stem					
Stem color	Green	Green	Green	Green	Green
Stem shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Number of internodes	9.78	11.22	11.44	12.34	11.67
Internode length (cm)	2.94	3.51	3.73	4.45	3.73
Number of branches	2.11	3.22	2.89	4.45	2.66
Hair Color	Brown	White	Brown	Brown	Brown
Leaf					
Compound leaf shape and arrangement	Trifoliatus	Trifoliatus	Trifoliatus	Trifoliatus	Trifoliatus
Compound petiole length (cm)	11.14	13.38	15.04	15.60	14.89
Leaf area (cm^2)	293.3	342.31	179.58	231.11	242.68
Leaf tip shape	Pointed	Blunt	Pointed	Pointed	Pointed
Leaf base shape	Rounded	Rounded	Rounded	Rounded	Rounded
Leaf attachment	Right-left	Right-left	Right-left	Right-left	Right-left
Top sheet color	Green	Green	Green	Green	Green
Leaf venation	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate
Hair Color	Brown	White	Brown	Brown	Brown
Flower					
Flower emergence time (DAP)	31.67	37	33.33	41	36
Flower color	Violet	Violet	Violet	Violet	Violet
Flower location	At the end of the stem and in the axils of the leaves	At the end of the stem and in the axils of the leaves	At the end of the stem and in the axils of the leaves	At the end of the stem and in the axils of the leaves	At the end of the stem and in the axils of the leaves
Number of flowers/plant	62.67	73.33	67.67	71	65.67
Pod					
Color of the ripe pod	Dark brown	Light brown	Brown	Light brown	Dark brown
Pod length (cm)	4.32	3.21	3.44	3.39	3.53
Number of seed/pod	2-3	2-3	2-3	2-3	2-3
Number of seed/plant	31.44	35.67	37	45.44	35.89
Hair Color	Dark brown	White	Brown	Brown	Dark brown
7. Seed					
Seed coat color	Yellow	Yellow	Yellow	Yellow	Yellow
Seed shape	Slightly flat oval	Slightly flat oval	Slightly oval	Oval	Slightly flat oval
100 seeds weight (gram)	14.76	14.89	10.89	11.43	11.45

Soybeans of the Anjasmoro variety are pod-shaped with young green pods and light brown when ripe. As on the surface of the stems and leaves, the surface of the pods is covered with white trichomes (feathers). Most pods contain 2-3 seeds. The pods are 3.05-3.57 cm long. The number of pods in one plant can reach 32-50 pieces.

The seeds of this Anjasmoro variety are oval, slightly flattened, and yellow. The seeds of the Anjasmoro variety are also large, with a weight of 100 seeds reaching 14.24-17.34 g/100 seeds.

Kaba varieties

The Kaba variety is a determinate type of soybean. This variety has an upright habitus with slightly woody stems with a plant height of 41.43-56.23 cm. The stem is cylindrical and green. The entire surface of the stem is covered with brown hairs. Branched stems with 2-3 branches. The number of segments on the main stem is 10-13, with an average length of 3.46-4.68 cm.

The root system of the Kaba variety is in the form of a taproot. The main root has a length of 15.97-24.8 cm. The main roots form 20-26 branches.

The leaves on the Kaba variety are compound-shaped and trifoliate (three leaflets), although sometimes there are leaves with four or more leaflets. The leaves are green with a compound petiole length of 13.8-16.57 cm. The shape of the tip of the leaf (*apex*) is pointed (*acutus*). The shape of the leaf base (base) is rounded (*rotundatus*). The leaf venation is pinnate. The way of attachment of leaves is alternately attached to the right and left of the stem. On the surface of soybean leaves, there are brown hairs. The leaves of the Kaba variety have a leaf area of 145.54-471.1 cm^2.

The Kaba variety's flower is shaped like a butterfly and purple. Flowers grow in the axils of the leaves and the ends of the stems or branches. The number of flowers in one plant reaches 59-89 flowers. The Kaba variety began to flower when the plant was 31 DAP and could be harvested from 81 DAP.

Soybeans of the Kaba variety are pod-shaped with young green pods and brown when ripe. The surface of the pod is covered with brown trichomes (hair). Most pods contain 2-3 seeds. The pods are 3.32-3.83 cm long. The number of pods in one plant is 27-63 pieces.

The seeds of this Kaba variety are slightly oval in shape and yellow in color. Soybean seeds of the Kaba variety were categorized as a medium, and the weight of 100 seeds was 10.74-12.81 g/100 seeds.

Sibayak varieties

Soybean of the Sibayak variety is a determinate type of soybean. This variety has an upright and large habitus (compared to the other 4 varieties). Slightly woody stems with a plant height of 53.7-66.1 cm. The stem is cylindrical and green. The entire surface of the stem is covered with brown hairs. Branched stems with 3-5 branches. The number of internodes on the main stem is 11-14, with an average internode length of 4.09-4.57 cm.

The root system of the Sibayak variety is a taproot. The main root has a length of 20.8-26.37 cm. The main root forms 19-26 branch roots.

The leaves on the Sibayak variety are compound in the form of trifoliatus (three leaflets), although sometimes there are leaves with four or more leaflets. The leaves are green with a compound petiole length of 13.63-17.87 cm. The shape of the tip of the leaf (*apex*) is pointed (*acutus*). The shape of the leaf base (base) is rounded (*rotundatus*). The leaf venation is Pinnate. The attachment of leaves is criss-cross, attached to the right and left of the stem. There are brown hairs on the surface of soybean leaves. The leaves of the Sibayak variety are wide, with a leaf area of 174.29-605.14 cm^2.

The flower of the Sibayak variety is shaped like a butterfly and is purple. Flowers grow in the axils of the leaves and the ends of the stems or branches. The number of flowers in one plant reaches 60-104 flowers. Sibayak soybean varieties began to flower when the plant was 37 DAP and could be harvested around 90 DAP.

The soybeans of the Sibayak variety are pod-shaped. Young pods are green, and ripe pods are brown. The surface of the pod is covered with brown trichomes (hairs). Most pods contain 2-3 seeds. The pods are 3.32-3.92 cm long. The number of pods in one plant can reach 38-58 pieces.

The seeds of the Sibayak variety are oval and yellow. Soybean seeds of the Sibayak variety are medium with a weight of 10.15-12.92 g/100 seeds.

Willis varieties

Soybean of the Wilis variety is a determinate type of soybean. This variety has an upright habitus with slightly woody stems with a plant height of 40.13-50.43 cm. The stem is cylindrical and green. The entire surface of the stem is covered with brown hairs. The stems have 2-4 branches. The number of internodes on the main stem is 10-14, with an average internode length of 3.29-4.24 cm.

The root system of the Wilis soybean variety is a taproot. The main root has a length of 20-27.63 cm. The main root forms branches with a total of 19-32 branch roots.

The leaves on the Wilis variety are compound and trifoliate, although sometimes there are leaves with four or more leaflets. The leaves are green with a compound petiole length of 13.33-18.55 cm. The shape of the tip of the leaf (*apex*) is pointed (*acutus*). The shape of the leaf base (base) is rounded (*rotundatus*). The leaf venation is pinnate. The attachment of leaves is criss-cross, attached to the right and left of the stem. There are brown hairs on the surface of soybean leaves. The leaves of the Wilis variety have a leaf area of 215.28-314.06 cm^2.

The soy flower of the Wilis variety is like a butterfly and is purple. Flowers grow in the axils of the leaves and the ends of the stems or branches. The number of flowers in one plant reaches 59-95 flowers. Soybeans of the Wilis variety began to flower when the plants were 33 DAP and could be harvested at around 85 DAP.

Soybeans of the Wilis variety are pod-shaped. Young pods are green, and ripe pods are brown. The surface of the pod is covered with brown trichomes (hair). Most pods contain 2-3 seeds. The pods are 3.31-3.86 cm long. The number of pods in one plant can reach 33-62 pieces.

The seeds of this Wilis variety are oval, slightly flattened, and yellow. Soybean seeds of the Wilis variety are medium with a weight of 100 seeds of 11.1-12.67 g.

The advantages of each variety can be identified based on the morphological characteristics of the Argomulyo, Anjasmoro, Kaba, Sibayak, and Wilis varieties. The Anjasmoro variety is a variety that tends to be superior to other varieties. This variety has more prominent characteristics, especially in the color of the hair (white), the shape of the tip of the leaf (blunt), the widest leaf area, the longest root length, the highest number of branch roots, and the largest seed size (14.89 g/100 seeds). This variety is a medium-aged soybean (can be harvested from 82 DAP) and can produce many pods.

The Sibayak variety is the variety with the highest plant habitus. This variety has many branches, producing the highest number of pods and medium-sized seeds (11.43 g/100 seeds). In addition, this variety has the longest lifespan because it can only be harvested from the age of 90 DAP.

Argomulyo variety is the variety with the shortest habitus and plant life. This variety can be harvested from the age of 75 DAP. This variety can produce large seeds (14.76 g/100 seeds) even though the number of produced pods is the least compared to other varieties.

Farmers commonly cultivate the Wilis variety. This variety can be harvested from the age of 85 DAP. This variety could produce many pods and medium-sized seeds (11.45 g/100 seeds).

The Kaba variety has a high plant habitus and a few branches. This variety is also commonly cultivated by farmers today. This variety can be harvested from the age of 81 DAP. This variety could produce many pods (second most after Sibayak). The seed size of the Kaba variety is medium (10.89 g/100 seeds) but smallest compared to other varieties.

Effect of phosphate fertilizer (PH) on plant morphology

The results showed that applying phosphate (P) fertilizer from a dose of 0.18 to 36 kg P_2O_5/ha can increase plant growth (the higher the dose, the higher the plant growth)(Figure 2). Applying P fertilizer to the soil will increase the P element in the soil so that the element can be sufficiently available for plants. Element P is the second essential macronutrient after N for plant growth. Elemental P is important because it is directly involved in almost all plant life processes. Still, this element is slightly available in the soil, especially in dry land undergoing advanced weathering (Sanyal et al. 1993), so it needs P element intake from outside.

Plant growth can be defined as an irreversible process of increasing plant size and volume. This growth can occur due to the activity of cell division, enlargement, and elongation. Cells require energy for these activities. Elemental P is needed in adenosine diphosphate (ADP) phosphorylation to adenosine triphosphate (ATP). ATP is an energy compound needed in plant metabolic processes. The presence of sufficient energy causes physiological processes in plants to take place optimally so that it can encourage plants to provide optimal growth responses as well. However, each variety has a different response to the dose of P fertilizer, depending on the genetic characteristics of each variety. Mursito (2003) suggests that different genotypes will show different appearances after interacting with certain environments.

Photosynthesis occurs in the leaf organs. Therefore, the plant's broad leaves will receive a lot of sunlight. Therefore, it can support the process of photosynthesis to run well. Photosynthesis results are distributed to the stems, leaves, and roots (Gardner et al. 1991) to support plant growth (vegetative phase). Based on the analysis results, it was found that increasing the dose of P fertilizer increased leaf area (Figures 3 and 4). Leaf area is also influenced by the genetic characteristics of each variety, especially leaf shape. An increase will follow the increase in leaf area in the length of the petiole because the petiole is the part of the leaf that supports the strands and is responsible for placing the leaf blades in such a position so that they can get as much sunlight as possible.

The stem, as the area of photosynthesis, utilizes the photosynthate for the lengthening and widening of the stem (Figure 5). Therefore, the increase in the length and width of the stem indirectly increases the number of stem segments, which in turn affects the length of the stem segments and the number of branches in the plant. The analysis showed that increasing the dose of P fertilizer could increase the length of the stem segments but did not affect the increase in the number of stem segments and branches in soybean plants. It is presumably because genetic factors of each variety influence the number of stem and branch segments in soybean plants.

The root is the part of the plant that functions to absorb water and nutrients from the soil to be transported through the stem to the leaves as photosynthetic material (Figures 6 and 7). Therefore, deep and broad roots will absorb more water and nutrients from the soil. The analysis showed that increasing the dose of P fertilizer increased the length of the main roots and the number of branch roots. Sutiyoso (2003) cit. Restiati (2006) stated that one of the functions of P is to bind solar energy and convert it into chemical energy, for example, in photosynthesis. Therefore, the provision of P elements can stimulate root growth so that large and strong roots will be formed.

The appearance of flowers marks the shift from the vegetative phase to the generative phase. One of the nutrients playing a very important role in flowering and fruiting in plants is the P element. P element can stimulate flowering in plants. After the plant enters the generative phase, the photosynthate results are more widely used to form generative organs (flowering, pod formation, and seed filling). The analysis showed that increasing the dose of P fertilizer had no significant effect on the flowering time and the number of flowers produced (Figure 8). However, applying P fertilizer to soybean plants could increase the number of flowers and accelerate the emergence of flowers in each soybean variety (the number of flowers was higher, and flowers appeared faster in soybeans fertilized with P).

Fruit formation is an important event in crop production. These processes are controlled by the environment, especially photoperiod and temperature, as well as by genetic or internal factors, particularly growth regulators, photosynthetic yield, and nutrient supply (Gardner et al. 1991). Irdiawan and Rahmi (2002) stated that the pod-filling phase requires full sunlight and good water content for some time, but too much water in the soil can interfere with the pod-filling process. The analysis showed that increasing the dose of P fertilizer had no significant effect on the number of pods produced by each soybean variety. Nevertheless, applying P fertilizer to soybean plants could still increase the pods in each soybean variety (Figure 9).

Inside the pod, there are generally 2-3 soybean seeds. Seeds are the goal of soybean cultivation. The shape and size of soybean seeds vary according to the genetic characteristics of the variety, ranging from small (about 7-9 g/100 seeds) to medium (10-13 g/100 seeds) to large (> 13 g/100 seeds) (Figure 10). The parameter of 100 seeds weight is generally used to determine the size and quality of seeds. The analysis showed that increasing the dose of P fertilizer could increase the weight of 100 seeds in each soybean variety. In addition, Wicks et al. (2004) stated that better plant growth accompanied by increased photosynthesis would increase the photosynthate supply to the seeds.

Cytology (chromosome number)

The results showed that each soybean variety had the same number of chromosomes, 2n = 40 (Figures 11-15). According to Bione et al. (2000), the genus Glycine, including several soybean cultivars, has a diploid number of chromosomes (2n = 2x = 40). The figures show that the size of the chromosomes is small, and there are quite a lot of them, so they often overlap in the observations. Species with many chromosomes have smaller chromosome sizes than species with fewer chromosomes (Suryo, 2003 cit. Sarasmiyarti 2008).

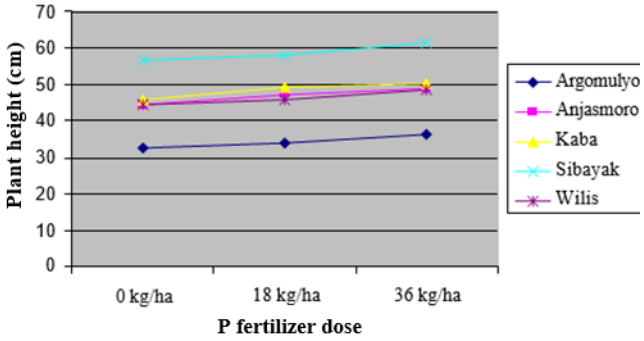


Figure 2. Effect of P fertilization on soybean plant height

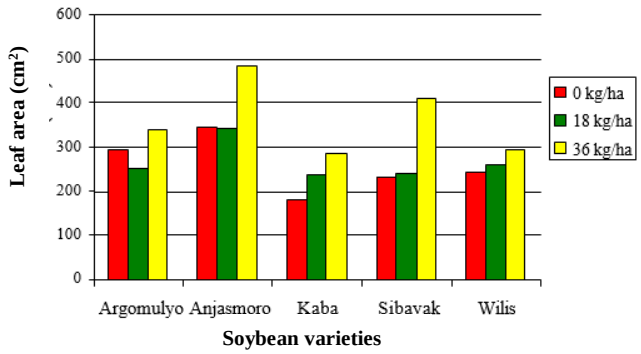


Figure 3. Effect of P fertilization on soybean leaf area

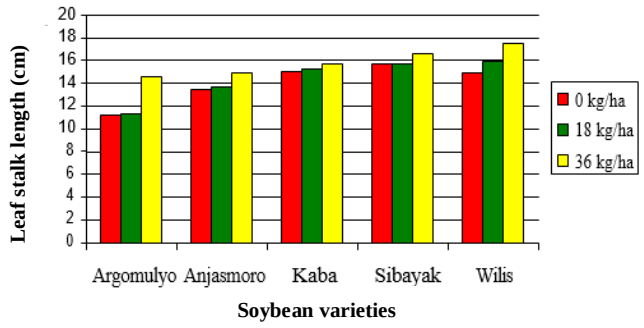


Figure 4. Effect of P fertilization on soybean leaf stalk length

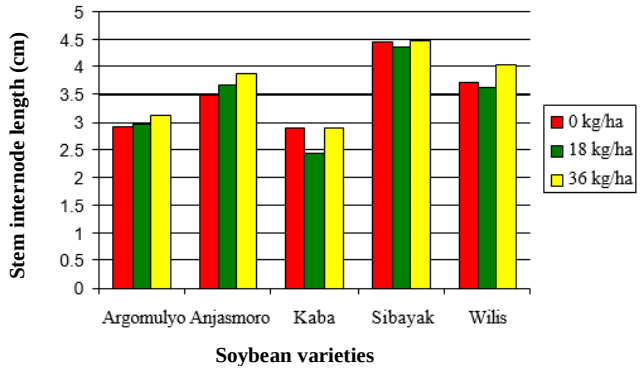


Figure 5. Effect of P fertilization on soybean stem internode length

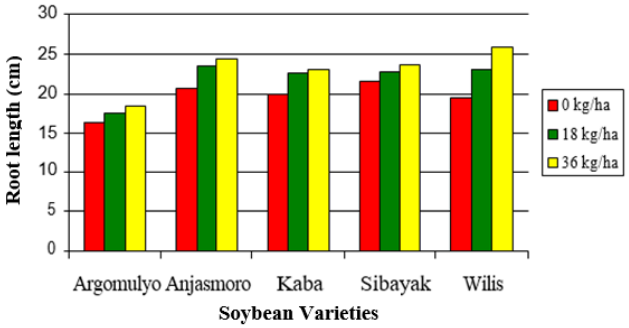


Figure 6. Effect of P fertilization on soybean root length

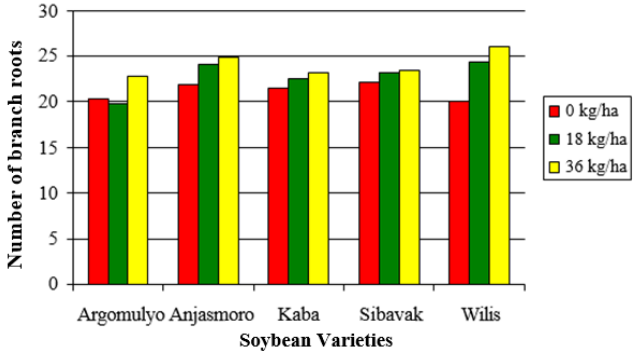


Figure 7. Effect of P fertilization on the number of soybean branch roots

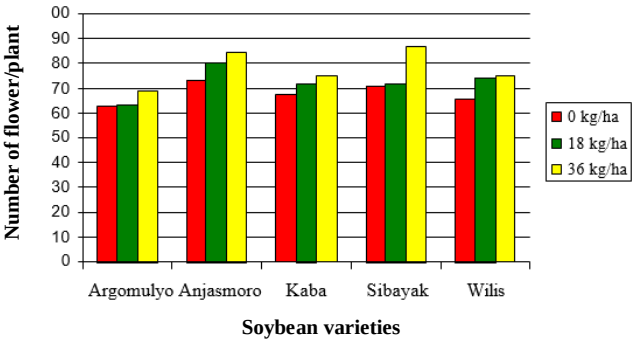


Figure 8. Effect of P fertilization on the emergence of soybean flowers

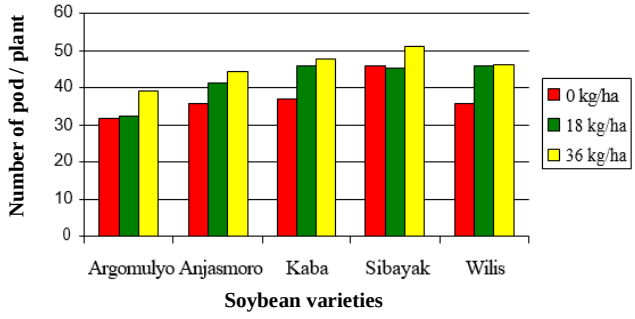


Figure 9. Effect of P fertilization on the number of soybean pod

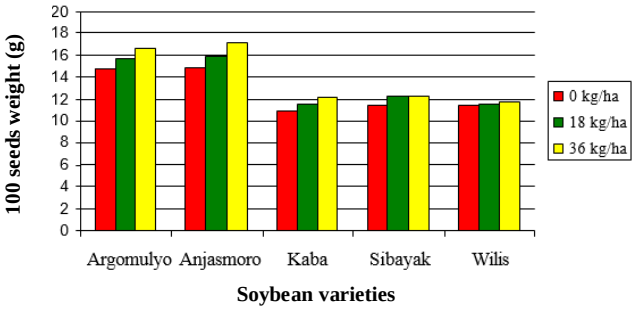
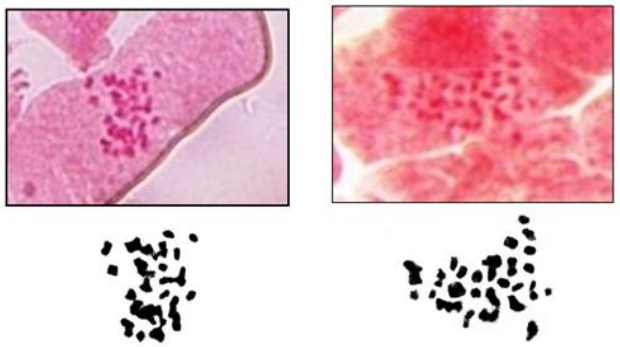
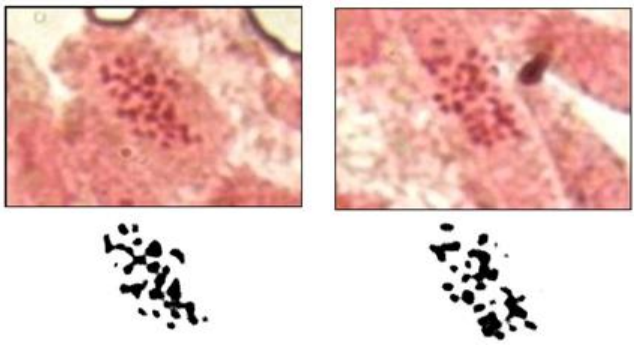


Figure 10. Effect of P fertilization on the weight of 100 soybean seeds



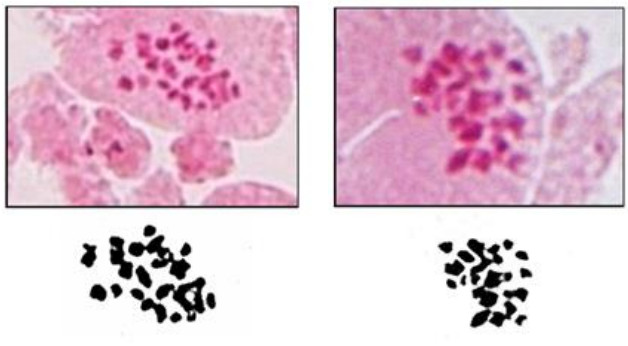
A **B**

Figure 11. Chromosomes of Argomulyo soybean variety. A. Without P fertilizer application, B. With P fertilizer application



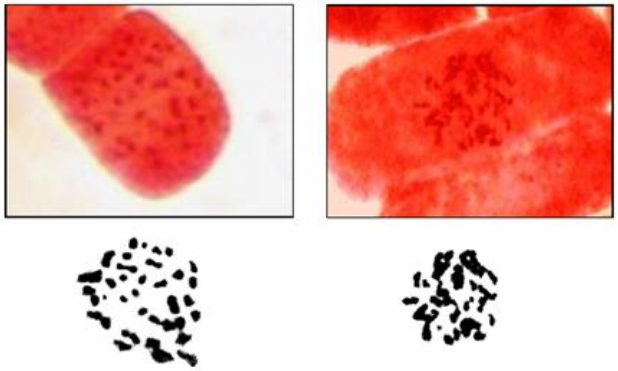
A **B**

Figure 12. Chromosomes of Anjasmoro soybean variety. A. Without P fertilizer application, B. With P fertilizer application



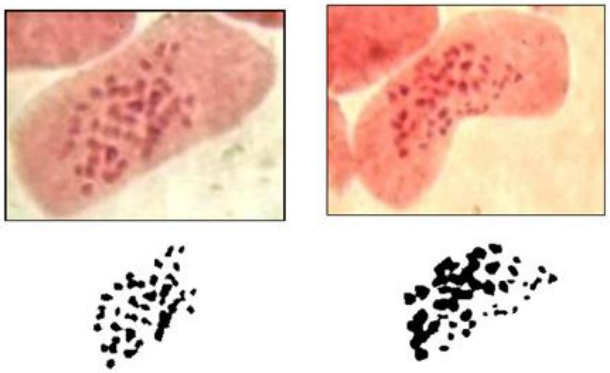
A **B**

Figure 13. Chromosomes of Kaba soybean variety. A. Without P fertilizer application, B. With P fertilizer application



A **B**

Figure 14. Chromosomes of the Sibayak soybean variety



A **B**

Figure 15. Chromosomes of Wilis soybean variety. A. Without P fertilizer application, B. With P fertilizer application

The study also showed no difference in the number of chromosomes between the five soybean varieties in the treatment without P fertilizer application and with P fertilizer application (the application of P fertilizer did not affect the chromosome number of each treatment). It shows that environmental factors do not affect the genetic characteristics of an individual, according to Johansen (1911) cit. Heddy (1990), the coinage of the term genotype was to designate basic traits that have not been affected by environmental factors, and the term phenotype was to designate visible traits.

Based on the research, it can be seen: (i) the morphological characteristics of the five soybean varieties are different in each variety; (ii) application of phosphate (P) fertilizer from a dose of 0, 18, or 36 kg P_2O_5/ha can affect plant morphology such as an increase in plant height, root length, number of branch roots, stem internode length, petiole length, leaf area, and weight at each of 100 seeds in each soybean variety; (iii) the number of chromosomes in the five soybean varieties is the same, namely 2n = 40; (iv) the application of phosphate (P) fertilizer did not affect the number of chromosomes in the five soybean varieties.

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