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Propagation of potato seeds *in vitro* in various varieties and planting media

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Abstract

Potato is one of the horticultural commodities that has a very important role as an alternative food ingredient. Efforts to increase potato productivity begin with providing quality seeds. One of the superior seed procurement techniques is the use of stem cuttings to increase the number of seedlings. This study aims to determine the effect of varieties and planting media on the propagation of potato seeds. The study consisted of 3 varieties, namely Granola L, AR 08 Agrihorti, AR 07 Agrihorti and 4 types of planting media, namely rice husk charcoal, rice husk charcoal + compost, rice husk charcoal + cocofeat + compost, rice husk charcoal + rotten rice husk + compost. The combination of treatments was applied to a two-factor factorial study arranged based on a completely randomized design with three replications. The results showed that the Granola L variety gave better results on the length of the apical shoots of potato seedlings, namely 56.33 mm and the number of shoots, namely 3.58 pieces. The AR 07 Agrihorti variety gave better results on the length of lateral shoots, namely 78.11 mm and the number of lateral shoot cuttings, namely 4.69 stems. The planting media of rice husk charcoal + rotten rice husk + compost gave better results on the length of apical shoots, which was 65.93 mm, the length of lateral shoots was 83.41 mm, the number of shoots was 3.22 pieces and the number of lateral shoot cuttings was 4.74 stems. The Granola L variety planted on rice husk charcoal + rotten rice husk + compost gave better results on the number of potato seed segments from in vitro, which was 3.44 pieces.

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Introduction

Potatoes (*Solanum tuberosum* L.) are the fourth main food crop in the world after wheat, rice and corn (Mulyono *et al.*, 2017), one of the horticultural commodities from the vegetable group which has a very important role in Indonesia (Pratika *et al.*, 2020). Potato farmers in Indonesia tend to still use potato seeds from previous harvests as planting material. The weakness of using potato tubers is that they have a low multiplication rate and are susceptible to disease (Mohapatra and Batra, 2017).

Efforts to increase potato plant productivity can be done through improving cultivation techniques, including using superior potato seeds, shoot cuttings and results from tissue culture technology. Tissue culture techniques are an alternative solution for mass plant propagation methods (Efendi *et al.*, 2022), fast and has genetic uniformity that is free from contaminants (Ekawati *et al.*, 2022). However, thus, the main obstacle in increasing potato production is the procurement and distribution of quality potato seeds, not continuous and adequate (Fatchullah, 2016). Suboptimal potato productivity can be caused by the low quality of seeds used, lack of knowledge about technical culture, continuous planting and limited farmer capital (Arifin *et al.*, 2014). One of the superior potato plant seed propagation techniques is the use of stem cuttings starting from G0 seeds obtained from plantlets produced in vitro, by isolating plant parts such as organs, tissues, cell groups, cells and protoplasm aseptically and growing them in a rich growing medium in the form of micro cuttings or micro tubers (Asih *et al.*, 2017), then planted in rice husk charcoal media, if G0 tubers are planted in soil media and harvested when they are 97-100 days old after planting, they will produce G1 tubers (Lestari *et al.*, 2014).

In general, the potato seeds used are in the form of plantlets or G0 tubers and micro tubers are the best alternatives as source seeds. However, due to the lack of information on the potential of micro tubers in the production of source seeds, testing the potential of micro tubers from various potato varieties needs to be done. This is especially necessary for the dissemination of superior national varieties in an effort to accelerate the variety process (Hidayah *et al.*, 2017). Tissue culture technique is one of the techniques for propagating healthy, high-quality, precise, fast and continuous potato seed plantlets (Husen *et al.*, 2019). The obtained plantlets are then mass propagated in situ through rooted stem cuttings in a greenhouse (Kurniawan *et al.*, 2016).

Research result stating that Potato plants of RGH01 and Medians varieties grown from rooted cuttings produced more leaves and branches and greater tuber productivity (Putra *et al.*, 2019).

Microtuber formation was influenced by the media used, culture environment (temperature and lighting duration), sucrose concentration and plant growth regulators (Emaraa *et al.*, 2017; Shukla and Joshi, 2018).

Planting media plays an important role in the growth and yield of potato plants. Generally, soil media mixed with rice husk charcoal and manure is used to facilitate drainage, improve the physical and biological structure of the soil and increase the soil's water absorption capacity (Lestari *et al.*, 2014). The planting medium for cuttings will affect root growth which will ultimately affect tuber growth. The media commonly used for planting potato cuttings is a mixture of soil and sterilized manure (Hamdani and Dianawati, 2020), or by providing organic materials in the form of compost, rice husk charcoal and cocopeat. This media can be used as a mixture with sand, soil, manure because it has the property of easily absorbing water and nutrients, does not clump, good porosity for potato seed propagation media that require homogeneous, sterile and isolated areas, so that the propagation process and the resulting seeds meet certification standards and are in accordance with technical requirements (Hamdani *et al.*, 2020), and is expected to provide optimal results for the growth of in vitro potato plantlet cuttings (Kurniawan *et al.*, 2016). The use of a mixture of organic materials

such as rice husk charcoal, rotten rice husks, compost and cocopeat as a component of the composition of the planting media is expected to create support for the growth of rooted cuttings of potato seedlings from in vitro. This study aims to determine the effect of varieties and planting media on the propagation of potato seedlings from in vitro and is useful for farmers to obtain planting materials for rooted cuttings of potato plants from mass propagation.

Materials and methods

Place and time

The research was conducted at the Green House of the Horticultural Seed Garden Installation of the Food Crops, Horticulture and Plantation Service of South Sulawesi Province, Indonesia, from January to May 2023.

Materials and tools

The materials used are Granola L potato plantlets, AR 07 Agrihorti, AR 08 Agrihorti, compost, rice husk charcoal, cocopeat, rotten rice husks, 70% alcohol, tissue. The tools used are cutting trays, scissors, cutting baskets and potrays, hand sprayers, scissors, label paper, plastic covers, digital cameras, nameplates and stationery.

Research design

Three varieties of in vitro potato seeds, namely Granola L, AR 08 Agrihorti and AR 07 Agrihorti were planted in four types of planting media, namely rice husk charcoal, rice husk charcoal + compost, rice husk charcoal + cocopeat + compost, and rice husk charcoal + rotten rice husk + compost, so that 12 treatment combinations were obtained. The treatment combinations were applied to a two-factor factorial study arranged based on a completely randomized design with three replications, so that there were 36 research plot units in total (each unit contained 9 plants). Observed variablesare the length of apical shoots, the length of lateral shoots, the number of internodes, the number of shoots, and the number of lateral shoot cuttings produced. The observation data were analyzed for variance using the F test. If the results of the analysis of variance were significant or very significant, it was continued with the Honestly Significant Difference test at the α level of 5%.

Results and discussion

Potatoes are a major food crop that plays an important role for society and has economic value to support the diversification of highly nutritious foods (Amarullah *et al.*, 2019), as an alternative material after rice, wheat and corn (Hidayah *et al.*, 2017). For this reason, efforts are needed to provide quality, location-specific potato seeds at low cost. Provision of potato seeds can be done through tissue culture techniques, because this technique has the advantage of being able to isolate the apical part to obtain plantlets with virus-free cultures (Furnawanthi *et al.*, 2017). The stages of plantlet production using in vitro culture techniques are the isolation of explants from virus-free meristem tissue, after the meristem grows into a plantlet, the next step is to plant the potato cuttings on Murashige and Skoog (MS) media which has complete and effective nutrients for the germination process or shoot formation (Hamdani and Dianawati, 2020). The advantage of producing in vitro potato seeds is that the planting material used is efficient, the environmental conditions are aseptic and controlled so that the resulting plantlets are free from pathogens (Husen *et al.*, 2018), high propagation rate, relatively small production area with large and uniform seedling yields (Chaidir *et al.*, 2021), fast, and the resulting seeds have the same characteristics as the parent (Putri *et al.*, 2021).

Differences in varieties and planting media affect the initial growth of rooted cuttings (plantlets) *in vitro* potato bits. The Granola L variety produced the highest average value againstthe length of the apical shoots of potato seedlings was 56.33 mm and the number of shoots was 3.58 pieces. Meanwhile, the AR 07 Agrihorti variety produced the highest average value for the length of lateral shoots, namely 78.11 mm and the number of lateral shoot cuttings, namely 4.69 stems. Furthermore, vvariety 08 Agrihorti produced the lowest average value for apical shoot length, namely 45.45 mm, number of shoots, namely

2.61 pieces and number of lateral shoot cuttings, namely 3.67 stems, while the Granola L Variety produce an averagelowest value againstthe length of the lateral shoots is 63.83 mm.

Plant varieties are a group of plants of a type or species characterized by plant shape, plant growth, leaves, flowers, fruits, seeds, and expression of genotype characteristics or combinations of genotypes that can distinguish from the same type or species by at least one determining trait and when propagated do not change. As with the results of this study which show the advantages of each potato plant variety studied. The Granola L variety produces the highest average value for the length of the apical shoots of potato seedlings, namely 56.33 mm and the number of shoots is 3.58 pieces and the AR 07 Agrihorti variety produces the highest average value for the length of lateral shoots, namely 78.11 mm and the number of lateral shoot cuttings is 4.69 stems (Table 1). This shows that the Granola L potato plant variety has the potential to produce very good apical shoot length and number of shoots to grow with optimal quality and quantity. While the AR 07 Agrihorti variety produces a large number of lateral shoot lengths and lateral shoot cuttings, which are very suitable for meeting the needs of many cuttings in a short time. The longer the apical shoots and lateral shoots produced, the greater the number of leaves and the continuity of the photosynthesis process will increase.

Table 1. Effect of variety and planting media on apical shoot length, length of lateral shoots, number of shoots, and number of lateral shoot cuttings 15 days after planting

Treatment	Long apical bud (mm)	Long lateral shoots (mm)	Number of shoots (fruit)	Number of lateral shoot cuttings (stems)
Varieties:				
Granola L	56.33^{a}	63.83^{b}	3.58^{a}	4.64^{b}
AR 08 Agriculture	45.45^{b}	64.83^{ab}	2.61^{b}	3.67^{b}
AR 07 Agriculture	51.81^{b}	78.11^{a}	2.67^{b}	4.69^{a}
planting media:				
Rice husk charcoal	59.48^{a}	55.96^{b}	2.55^{a}	3.92^{a}
Rice husk charcoal + compost	61.11^{a}	64.96^{ab}	3.00^{a}	4.44^{a}
Rice husk charcoal + cocopeat + compost	62.56^{a}	71.37^{ab}	3.04^{a}	4.22^{a}
Rice husk charcoal + rotten rice husk + compost	65.93^{a}	83.41^{a}	3.22^{a}	4.74^{a}

The numbers in one column for each treatment factor followed by the same letter are declared not significantly different according to the 5% BNJ test.

The presence of shoots and leaves formed on plant cuttings is very important for the root system. Shoots produce auxin growth regulators that play a role in root formation while leaves produce photosynthate (carbohydrates) that are translocated to the root system, shoot buds and other growing plant organs. The more cuttings, shoots and leaves that are formed, the higher the protein, nitrogen and carbohydrate content which functions to stimulate the formation of shoots and plant roots. Food reserves in the form of carbohydrates and nitrogen stored in shoot segments/stems can increase the size and number of shoots formed due to natural growth hormones that function as plant growth and development regulators (Pattanagul *et al.*, 2001).

The research results in table 1 show the planting mediaRice husk charcoal + rotten rice husk + compost produced the highest average value for the length of apical shoots, namely 65.93 mm, the length of lateral shoots was 83.41 mm, the number of shoots was 3.22 pieces and the number of lateral shoot cuttings was 4.74 stems. Furthermore, the rice husk charcoal planting medium produced the lowest average value for the length of apical shoots, namely 59.48 mm, the length of lateral shoots was 55.96 mm, the number of shoots was 2.55 pieces and the number of lateral shoot cuttings was 3.92 stems.

Planting media is one of the important factors for the success of in vitro potato cuttings to form a good root

system and shoot growth. Planting cuttings in a greenhouse using planting media with a crumbly, loose structure and containing lots of organic materials can provide optimal results if other environmental factors such as the availability of water, nutrients, carbon dioxide, sunlight are suitable for the growth of potato plantlet cuttings. Currently, the use of rice husk charcoal, rotten rice husk charcoal, cocopeat and compost as agricultural waste that is cheap, easy to obtain and light is starting to be in great demand by the public.

The results of this study indicate that the planting media of rice husk charcoal + rotten rice husk + compost produces the highest average value for the length of apical shoots, namely 65.93 mm, the length of lateral shoots is 83.41 mm, the number of shoots is 3.22 pieces and the number of lateral shoot cuttings is 4.74 stems. This is suspected that the planting media of rice husk charcoal + rotten rice husk + compost has a good planting media structure for root growth and development, mineral nutrient content that is optimal enough for root formation, apical shoots, number of shoots and number of lateral shoot cuttings. The content of organic matter in the growing media can increase the cation exchange capacity of the soil, improve soil structure, increase the availability of nutrients and increase the soil's ability to bind water (Rafindo *et al*., 2022).

A rice husk charcoal has light and coarse characteristics so that it has good porosity and low water absorption, contains quite high Si, which is 16.98%, even so Si is not an essential nutrient for plants. The presence of Si is known to improve the physical properties of the planting medium, so that it affects the solubility of P in the soil. If the Si element in the soil is less than 5%, then the plant stands are not strong and easily collapse. The nutrient content of rice husk charcoal is SiO_2 52%, C 31%, N 0.32%, P 0.15%, K 0.31%, Ca 0.69%, Fe 180 ppm, Mn 80.4 ppm and Zn 14.10 ppm (Kurniawan *et al.*, 2016). Furthermore, compost is the result of fermentation of organic waste such as straw, husks, leaves, grass, and garbage that can be used to improve the physical, chemical and biological properties of the soil. Compost functions as a soil conditioner, which plays a role in improving soil structure, especially dry soil. Also as a soil ameliorator that functions in improving the cation exchange capacity of the soil. Good compost to be used as a planting medium is one that has undergone perfect weathering, blackish in color because it is formed from stable organic material (Nurmalasari *et al.*, 2021), odorless, has a low water content, and has room temperature. Compost contains nitrogen nutrients that function to accelerate the vegetative growth of plants, also contains phosphorus and potassium nutrients that play a role in the process of photosynthesis, make water use more efficient, form stronger branches, accelerate rooting so that plants are stronger and increase plant resistance to disease. Compost also contains micronutrients Fe, Cu, Zn, Cl, B, Mn and Mo which can help the plant growth process (Imas *et al.*, 2017). The use of rice husk charcoal, rotten rice husks, cocopeat and compost as components of the planting medium can create support for the growth and development of potato plants.

Table 2. Effect of interaction between varieties and planting media on the number of segments of *in vitro* potato seedlings 15 days after cuttings

Growing media	Number of sections		
	Granola L	AR 08 Agriculture	AR 07 Agriculture
Rice husk charcoal	3.00^{ax}	2.00^{by}	2.11^{cy}
Rice husk charcoal + compost	3.11^{ax}	2.56^{ay}	2.67^{bx}
Rice husk charcoal + cocopeat + compost	3.33^{ax}	2.89^{ax}	3.11^{ax}
Rice husk charcoal + rotten rice husk + compost	3.44^{ax}	3.00^{ax}	3.33^{ax}

Note: Numbers followed by the same letters are not significantly different according to the 5% BNJ test.

The results of the study in Table 2 show that the interaction between varieties and planting media significantly affected the number of potato seedling segments from *in vitro*. The interaction between the

Granola L variety and the planting media of rice husk charcoal + rotten rice husk + compost produced the highest average number of potato seedling segments from in vitro, which was 3.44 pieces and was not significantly different from the interaction between the Granola L variety and the planting media of rice husk charcoal, the AR 07 Agrihorti variety with the planting media of rice husk charcoal, the Granola L variety with the planting media of rice husk charcoal + cocopeat + compost, the AR 07 Agrihorti variety with the planting media of rice husk charcoal + rotten rice husk + compost, the Granola L variety with the planting media of rice husk charcoal + rotten rice husk + compost, the AR 08 Agrihorti with the planting media of rice husk charcoal + rotten rice husk + compost and the AR 08 Agrihorti with the planting media of rice husk charcoal + cocopeat + compost. The interaction between the AR 07 Agrihorti variety and the rice husk charcoal + compost planting media produced the lowest average number of *in vitro* potato seed segments, namely 2.11 pieces, and was significantly different from other treatments.

It is suspected that potato plantlets grown on a planting medium of rice husk charcoal + compost have a root system that easily absorbs water, also functions as a nutrient binder (when there is excess nutrient) which will be used slowly according to plant needs. The availability of nutrients can initiate cell division and elongation and good root system (Zahara *et al.*, 2017), formation of shoots, roots, leaf and optimal photosynthesis process (Ramesh and Ramassamy, 2014). Other research results state that the number of potato microtubers increases according to the nitrogen content of the growth medium (Iranbakhsh *et al.*, 2011), the mineral nutrients from the planting medium that are absorbed will increase the quality of the tubers produced (Altindal and Karadogan, 2010), and the size and weight of the tubers will increase (Iranbakhsh *et al.*, 2011).

Soil media, rice husk charcoal media, cocopeat media, humus media, and manure media have the same ability to increase the percentage of plant growth, plant height, number of leaves, shoot length and stem diameter of Granola potato varieties. *In vitro* culture techniques on Granola potato plants can produce explant regeneration, undergo morphogenesis and grow into plantlets (Kurniawan *et al.*, 2016).

The acclimatization period is a critical period for the survival of potato plants from tissue culture. Acclimatization is a stage in tissue culture which is the process of adaptation of living plantlets in aseptic and heterotrophic conditions and then transferred to non-aseptic conditions and must live in autotrophic conditions. Tissue culture plants almost never photosynthesize, the cuticle layer does not develop, the vascular tissue between the roots and shoots does not develop and the stomata do not function properly. These conditions cause plants to be less able to survive after acclimatization because they are not yet able to photosynthesize optimally and adapt to the ex vitro environment. During the acclimatization period, plantlets from autotrophic propagation are better able to survive, because since in the culture bottle the plants have started photosynthesizing and respiration so that they are easier to adapt to the ex vitro environment (Rai *et al.*, 2015).

Conclusion

The results of the study showed that Granola L. variety gave the best results against the length of the apical shoots of potato seedlings was 56.33 mm and the number of shoots was 3.58 pieces. The AR 07 Agrihorti variety gave the best results in terms of the length of lateral shoots, namely 78.11 mm and the number of lateral shoot cuttings, namely 4.69 stems. Planting media rice husk charcoal + rotten rice husk + compost gave the best results for the length of apical shoots, namely 65.93 mm, the length of lateral shoots, namely 83.41 mm, the number of shoots, namely 3.22 pieces and the number of lateral shoot cuttings, namely 4.74 stems. The interaction between the Granola L variety and the rice husk charcoal + compost planting media produced an average the highest number of potato seed segments from *in vitro* results was 3.44, which was not significantly

different between varieties AR 07 Agrihorti and AR 08 Agrihorti varieties.

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