The pyraclostrobin effect on *in vitro* rooting of potato tissue culture

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The potato has been used as important food nowadays. Today, the potato is the fourth largest food crop in the world and grown in almost every country. The annual worldwide production exceeds 300 million tones. Seedling is the most important factor and potentially can be boost by tissue culture techniques. In tissue culture technique plant hormones are signal molecules produced within the plant, which is very important to increase the growth and differentiation. Plant hormones occur in extremely low concentrations or synthetic molecules for special purposes. This research was conducted at Tissue Culture laboratory, Faculty of Agriculture, University of Brawijaya, arranged with Randomized Complete experimental design using four level of treatments on GK Variety potatoes, The treatment was arranged as explained below: Ms + 0, MS + IAA 1 ppm, MS + IBA 1 ppm, MS + Phyraclostrobin. The application of Phyraclostrobin in tissue culture is also potential for potato tissue culture and it will perform better by combine it with hormones.

**Keywords:** *In Vitro, Phyraclostrobin, Potato, Tissue Culture*

**INTRODUCTION**

Potato (*Solanum tuberosum* L.) is a very important horticultural commodity in Indonesia and one of the alternative sources of carbohydrates. Potatoes are grown in upland areas at an altitude of more than 1,000 m asl. Currently the productivity of potatoes is still low, hence, it is necessary to conduct research activities to increase the productivity. The most important factor influence the low productivity is unqualified seeds, while others are low cultivation management and inadequate postharvest handling Husna, (2004). One of the solutions to increase productivity is the use of superior seeds. Seed is one of the important effort to increase production, even until now considered as dominant factor in agricultural production, use of appropriate seed, hence obtained optimal growth.

The low production in Indonesia is due to the fact that there are not yet many seed producers of high quality potato seeds, so the demand for potato seeds can not be met (Rainiyati et al., 2009). Good seed is one of the key factors determining the success of increasing productivity and product quality. The success of the food resilience program and the increase of farmers' incomes nationally depends largely on the ability of providing quality seeds with superior varieties suitable for agro-ecosystem (Husna, 2014). Nowadays, the cost for the seeds can reach 40-50% of total production costs. Therefore, one way that can be used to answer the above challenges and problems is through tissue culture techniques by using the roots organas propagation.

Tissue culture is one of the activities that utilize parts of plants (roots, buds, plant growing
tissues) grow into perfect plants in aseptic conditions. Therefore this technique is one of alternative for potato’s propagation (Molla et al., 2011). Technology for large-scale tissue culture is enhanced for potato production. Currently, almost all seed potato production systems are incorporated in this technology. This technology allows rapid dispersal of plants with cloning, which uses less space and labor than traditional methods Nirmah (2012). For farmers, the technology means improving the quality and quantity of cultivated plants.

Success in technology and application of tissue culture method is closely related to adequate supply of nutrients and in accordance with cell or tissue culture. There are two things that often determine the success of tissue culture, namely the origin of explant and culture media. Propagation in vitro and plant growth is further influenced by growth supplementation in the media (Mengesha et al., 2013). Growth from plant tissue culture can be increased by the addition of hormones. These organic nutrients are a source of vitamins, amino acids and some supplements. The amount of this substance is required for tissue culture that varies with species and genotypes of explants (George et al., 2008).

Köhle et al., 2003 stated that Pyraclostrobin is a strobryulin group, a substance which previously functioned as fungicide, yet has a growth stimulation mechanism, which in plants has been related to reduction in the disease activities as well as increased nitrate uptake and assimilation in small grains. Research has shown that pyraclostrobin was important in stimulating nitric oxide, a key messenger in plants (Conrath et al., 2004).

The objective of this research is to seek how the potential of pyraclostrobin in potato tissue culture technique

MATERIALS AND METHODS

This research was conducted at Tissue culture Laboratory, Faculty of Agriculture University of Brawijaya on May 2017, arranged with randomized complete design with several treatment of plant regulator concentration to enhance the rooting of the potato tissue culture. The treatment was arranged as explained below: Ms + 0, MS + IAA 1 ppm, MS + IBA 1 ppm, MS + Pyraclostrobin.

Observations were performed at ages 14 to 28 weeks after planting. The variables are: (a) length of plant, (b) length of root, (c) number of root, (d), number of shoot, (e) number of leaves. After the analysis of Variance, the Duncan test performed to see the different between each treatment.

RESULTS AND DISCUSSION

RESULT

On Figure 1, treatment influenced root length and length of plant at 28 DAP. The treatment of MS + IBA 1 ppm resulted in the lowest root length recorded 2.020 cm, contrary on plant length indicates the highest plant length recorded 9.325 cm. Media MS + Pyraclostrobin produces the best root length recorded 9.142 cm

![Figure1. Root and Plant Length in 28 DAP](image_url)
Figure 2. Number of Roots in 14 and 28 DAP

Figure 3. Number of Shoots in 14 and 28 DAP
Figure 4. Number of leaves in 14 and 28 DAP

On root number, 14 DAP and 28 DAP there is a significant effect on treatment (Figure 2). The application of MS + IBA 1 ppm showed the highest increase of root number recorded 106.91% from 14 DAP to 28 DAP. The result of the observation of shoot number is presented in Figure 3. The application of MS + IBA 1 ppm showed that number of shoot has not occurred until the age of 14 DAP. In general, the use of Ms + 0 media and the use of Ms + IAA 1 ppm resulted in high shoot rates compared to 2 other treatments on GK varieties. Figure 4 shows the MS medium and various plant hormones did not result in differences in the number of leaves in GK varieties potato plants. Treatment of Ms + 0 and Ms + IAA 1 ppm showed higher leaf number compared with Ms + IBA 1 ppm and Ms + Pyraclostrobin treatment.

DISCUSSION

The successful of tissue culture method is dependent on the composition of the media used. Plant tissue culture media provides not only macro and micro nutrients, but also carbohydrate sources, which are generally in the form of sucrose or sugar, to replace the carbon normally obtained from the atmosphere through photosynthesis. The better results will be obtained if the media are added vitamins, amino acids, and growth regulators (Zulfiqar et al., 2009).

The growth of shoot generally requires growth regulators. Stages and growth type determine the type and concentration of growth regulators required. Auxin commonly used in shoot culture is IAA, NAA, IBA, picloram. Commonly used cytokines are BAP, 2-ip or Kinetin. In shoot cultures it is very common to use relatively higher concentrations of cytokinin than auxin (Karjadi, et al., 2008). Strong and healthy shoot growth required 3 kinds of growth regulator are Kinetin or BAP as source of cytokinin, NAA, IAA or Picloram as source of auxin and GA3 in concentration ranged from 0.01 - 5 mg /l or third growth regulator substances in balance condition (Karjadi, 2006).

The result of root length observation showed that Ms + 0 and Ms + IAA 1 ppm treatment were significantly different with Ms + IBA and Ms + Pyraclostrobin treatment media. While the parameters of plant length showed the same thing, where the treatment media of Ms + 0 and Ms + IAA 1 ppm was significantly different with the treatment of Ms + IBA and Ms + Pyraclostrobin media. Comparison of root length observation and plant length on Ms + 0, Ms + IAA 1 ppm and Ms + Pyraclostrobin media had more influence on root formation than plant length. However, different on the treatment medium Ms + IBA 1 ppm effect on the length of the plant. According to Gustafron et al., (2006) The process of root elongation begins with stimulation by endogenous auxin.
The presence of auxin has been proven to stimulate organogenesis and lead to the formation of roots. Auxins are able to increase growth by encouraging the formation of a number of cells in plants, but they do not divide, so many of them are polyploids with some nuclei (Farzana's, 2007). This also has been proven by Septiana, et al., (2014) that low concentrations of IAA (0.1 ppm - 0.2 ppm) inhibit root cell growth on pineapple. IAA is one type of auxin. Auksin serves to spur the process of root formation and root growth better. Mengesha et al., (2013), auxin is a substance that stimulates the extension so as to increase the formation of roots and shoots of plants. In in vitro cultures, the role of auxin is to stimulate the growth of callus, roots, cleavage and elongation of cells and organs. Martin et al., (2004) Auxin in plant tissue can work actively even in the dark, but auxin synthesis takes place in a bright state. Root length increase is caused by the process of cell division in the root root meristem, followed by the process of elongation and cell enlargement.

Giving Ms + IBA 1 ppm indicates the increment of the number of roots in comparison with other media treatments. Septiana et al., 2014 states the growth of an explant in shoot form will be influenced by the type of additional hormone given or by the concentration of the additional hormone. IAA in low concentrations more quickly forms the roots than IAA at higher concentrations. This occurs because the IAA if given at the right concentration can spur cell division, cell lengthening and play a role in combustion, IAA is a type of auxin that is often used in the combustion medium. According to Lestari, (2011) added that in the process of organ formation such as shoot or root is an interaction between exogenous growth regulator substances that are added to the media with endogenous growth regulator produced by plant tissue. This can be caused by the influence of endogenous auxin formed in leaves that are then transported to the base so that the root is formed. Harjadi (2009) states that auxins are generally synthesized in actively growing tissues, such as shoot meristems, leaf primordia, expanding young leaves, seeds and fruit.

In general, the use of Ms + 0 media and the use of Ms + IAA 1 ppm resulted in high shoot rates compared to 2 other treatments on GK varieties (Figure 5). While the treatment of Ms + 0 and Ms + IAA 1 ppm showed higher leaf number compared with treatment of Ms + IBA 1 ppm and Ms + Pyraclostrobin There are two things that very often determine the success of tissue culture, namely eksplan and culture media used. Propagation in vitro and plant growth was further influenced by growth supplementation on Mengesha et al., (2013) media. Auxin-like growth regulators such as NAA, IAA, IBA, and 2,4-D function in increasing osmotic pressure, cell permeability, reducing pressure on cell walls.
increasing plasticity and developing cell walls, and increasing protein synthesis. In addition, auxins play a role in stimulating the elongation and enlargement cells. Widianto et al. (2014) showed that teak plant can only grow on the media which added 2 ppm BAP + 0.05 ppm NAA. Further Basri (2008) showed that the use of MS medium with 0.25 ppm IBA + 1.5 ppm NAA suitable for multiplication of chrysanthemum varieties Yellow, Fuji, and Elen. van Lingen and Tawn Talk while White Fuji varieties more responses with media usage added 1.50 ppm BAP + 0.50 ppm NAA (Firoozabady & Moy 2004).

The application of Phyraclostrobin on tissue culture practices is potential to develop due to it increase the plant vigor mainly on shoot and root length, uniformity of plant and leaves. The mode of action in tissue culture practices improvements are not yet clear, however it is strongly related with activity of peroksidase in which triggered by the application of pyraclostrobin. This enzyme is responsible for development of monomer chains, lignification, wound cicatrization, phenol oxidation, pathogen defense, cell elongation regulation and other (KAO, 2003) Application of Phyraclostrobin in tissue culture could be use with combination with another substance like IBA, to add the Agcelence effect on plant.

Research on potato micropropagation was also carried out by Karjadi (2007) to produce the best combination of kinetin, IAA, and GA3 plant hormones in increasing the growth of Granola cultivar potato plantlets. The medium used is the basic medium MS + 30g sugar; 0, 1 mg GA3; 100ml coconut water and 6 g order per liter. The result was no interaction from the combination of the three hormones. According to Poudel et al., (2005), the types and concentrations of effective growth regulators for a particular plant species and cultivars will respond differently to other plant species and cultivars. This is confirmed from the results of research conducted by Mervat et al.,(2009), stated that the addition of plant hormones with accuracy is very important in organogenesis and it is related to the interaction of plant hormones used with endogenous substances contained in plant tissue. Harjadi, (2009) stated that changes in the concentration of growth regulating substances are not always related to the actual response. The growth regulator does not work alone in generating a response, because of the interaction of some compounds. The growth regulator substances are chemicals with on or off signals that cause a spate of events in the cell to eventually produce physiological responses.

CONCLUSION
The application of Phyraclostrobin on tissue culture practices is potential to develop due to it could increase the plant vigor particularly on shoot and root length also uniformity of plant and leaves.

CONFLICT OF INTEREST
The authors declared that present study as performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

KPW, KWT and EB designed and performed the experiments and also wrote the manuscript. PMP, AS and AHZ collection and data analysis. All authors read and approved the final version.

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