

Potential Development of Purwoceng (*Pimpinella pruatjan* Molk or *Pimpinella alpine* Kds) Plant Scale Industry Using In-Vitro Culture Technique By Means of Rooting Induction

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ABSTRACT

Purwoceng (*Pimpinella pruatjan* Molk or *Pimpinella alpine* KDS) belongs to the Apiaceae family, is an endemic plant in mountainous areas such as the Dieng Plateau, Pangrango Mountain, and mountainous areas in East Java. Parts of the plants, especially roots are efficacious as aphrodisiacs, diuretic drugs and tonics. Based on CITES, this plant belongs to the category of endangered species, so its presence is very rare in nature. This scarcity occurs due to the purwoceng exploitation in nature as traditional herbal medicine without any cultivation or rejuvenation. In situ conservation efforts are almost impossible because the original habitat of this plant has become extinct. Thus, ex situ conservation is more suitable to be applied, by using in-vitro culture techniques. Therefore, a research study is needed on the development of tissue culture techniques in the production of the seeds. Methods of data collection in this study was conducted by literature studies. Therefore, ex-vitro root induction techniques are applied currently, namely rooting induction. The content of the chemical compounds and various properties make purwoceng as a commodity in the raw materials of drugs that have great potential to promote industrial players in the economic field. Based on the results from the literature, the authors can conclude that the potential of purwoceng plants is quite large, but still constrained by the scarcity of seed supply so the application of ex-vitro root induction techniques in in-vitro culture can be a solution in limitation of supply of purwoceng crops for industrial scale.

1. Introduction

Indonesia is known as a country with a very high level of biodiversity. This is because Indonesia is located in the tropics of abundant level of flora. Many types of plants are high-value germplasm sources. Besides functioning as a producer in the food chain, plants have long been known to be used as a source of medicine, one of them is Purwoceng (*Pimpinella pruatjan* Molk or *Pimpinella alpine* KDS). Purwoceng including the Apiaceae family is an annual aromatic herbal plant that grows in highland habitats. All parts of purwoceng plants can be used as traditional medicines, especially roots. Purwoceng is a rare Indonesian native medicinal plant, efficacious as an aphrodisiac (increasing the vitality of male sexuality). For generations

this plant has been used by our ancestors, and has been scientifically proven to increase testosterone in mice (Taufiqurrachman & Wibowo, 2006). In addition, purwoceng is also efficacious as a diuretic drug (smoothing the urine ducts), and tonic (able to increase stamina) (Darwati & Rostika, 2006). Purwoceng belongs to Indonesia's endemic plants that live in mountains such as the Dieng Plateau in Central Java, Pangrango Mountain in West Java, and the mountainous area in East Java. Since ancient ago the people around the Dieng Mountains have used this plant as a mixture of traditional herbs to treat various diseases and health problems. The high consumption of this plant causes the existence of purwoceng is increasingly reduced in its natural habitat. In addition, the scarcity of purwoceng is also

caused by massive genetic erosion, even the population in Pangrango Mountain in West Java and the mountainous areas in East Java are reported to have been destroyed (Darwati & Rostika, 2006). Based on the CITES (Convention on International Trading in Endangered Species of Wild Flora and Fauna) this plant belongs to the category of endangered species. The potential of purwoceng plants is quite large but is still constrained by the scarcity of seed supply and limited land suitable for these crops (Yuhono, 2004). The government has not allowed the herbal medicine industry and drugs to use purwoceng simplicia obtained not from cultivation, because the status of purwoceng plants is still relatively rare (Ditjen POM, 2000). The endangered is mainly due to excessive exploitation without being offset by conservation efforts. Most traditional drug companies (jamu) take or harvest purwoceng plant material directly from their habitat without rejuvenation efforts. Since the main ingredient of the harvested plant is the root, the harvesting action automatically damages the plant as a whole. So this makes the BPOM prohibit the use of purwoceng as a mixture of medicinal ingredients if the plant material is taken from nature. If the conservation and cultivation activities are not carried out, purwoceng plants are likely to become extinct. Purwoceng is still cultivated in a narrow area in the house in Dieng, Central Java (Rahardjo, 2003), Putri Mountain, West Java and Semeru area, East Java (Darwati & Rostika, 2006). So, the solution must be found in increasing the purwoceng plants quality.

2. Materials and Methods

2.1. Materials

The experimental material used was *Pimpinella pruatjan Molk* or *Pimpinella alpine* KDS. Based on literature the tools used include petri dishes, analytic scales, culture bottles, glass beakers, measuring cups, duran bottles, erlenmeyer, hot plates, magnetic stirrers, pH meters, pipettes, aluminum foil, filter paper, autoclaves, Laminar Air Flow (LAF) Cabinet, Bunsen, Refrigerator, Culture Rack, Tweezers, Scalpel, Cling Film, Label Paper, and Hand Sprayer. The ingredients used are *Pimpinella pruatjan Molk* or *Pimpinella alpine* KDS seeds, Murashige media & skoog (MS-1962), casein hydrolyzate, sucrose, agar, Fe-EDTA, sterile distilled water (sdw), distilled water, 20% chlorox, 70% alcohol, HgCl₂ 0.1%, growth regulator GA₃, 6-benzyl aminopurine (BAP), indole-3-butyric acid (IBA) and Mos.

2.2. Methods

Methods of information collection was conducted by

literature review. Several reference from journals has been gathered and several references from journals have been collected and selected for some relevant information about the topics.

3. Results and Discussion

Purwoceng belongs to Indonesia's endemic plants that live in mountains such as the Dieng Plateau in Central Java, Pangrango Mountain in West Java, and the mountainous area in East Java. Since ancient ago the people around the Dieng Mountains have used this plant as a mixture of traditional herbs to treat various diseases and health problems. The high consumption of this plant causes the existence of purwoceng is increasingly reduced in its natural habitat.

Purwoceng is an Endangered Plant (Rivai *et al.*, 1992), it is also being confirmed in research by Ministry of Environmental and Forestry of India (Rao *et al.*, 2003). In Indonesia this rarity being caused by the exploitation of Purwoceng in nature for herbal medicine sake, without any cultivation or any regeneration worked to regain the number of Purwoceng (Darwati & oostika, 2006).

Meanwhile, Purwoceng is an Endemic mountain plant such as Dieng High Plateau, Central Java, Pangrango Mountain in West Java and Mountain in East Java (Hernani & Yuliani, 1990; Rahardjo *et al.*, 2005). Furthermore, as result of Research, that this time Purwoceng only can be found in Dieng High Plateau, in village cultivation of Purwoceng that is Sekunang Vilage (Rahardjo, 2013; Syahid *et al.*, 2004). Aphrodisiac properties which were empirically believed by people especially who lives in Dieng Plateau of Central Java, has also been scientifically approved. Preliminary research on rats shown that pruatjan containing metabolite was capable to enhance or restore male sexual potencies (Rostiana, 2014).

Of all parts of plants purwoceng having efficacy at the root part. It is become main causes for Endangered Purwoceng in Nature (Darwati & Roostika, 2006). Purwoceng was successfully cultivated outside its natural habitat of Dieng Plateau, but the main problem is scarcity of seeds or seedling materials (Rahardjo, 2003), because when it comes to harvesting, the whole parts of the plant are taken up.

Roostika *et al.* (2009) used In-Vitro Culture with DKW media to Purwoceng and the result is there are shoot development. As the result of in-vitro culture, today shoot can be used for cultivation or in-vitro conservation (Ningsih *et al.*, 2011; Ermayanti *et al.*, 2005, Darwati & Roostika, 2006). The others Mariani (2017) use MS medium supplemented with 0.5 ppm Benzyl Adenine Purine (BAP) and 0.025 mg/l α -naphthaleneacetic acid (NAA) to cultivated Purwoceng.



Figure 1. Growth steps of purwoceng plant, a = whole plant, b = buds flower, c = bloom flower, d = fruits, and, e = the roots of the 6 month old plant. Source: Rahardjo *et al.* (2005).

The purpose of Micropropagation to prevent extinction of Purwoceng, because if Purwoceng extinct it will affect the ecosystem sustainability. Purwoceng is member of Family Apiaceae (Rahardjo *et al.*, 2005) have been used in commercial way as Aphrodisiac, diuretic and tonic (Darwati & Roostika, 2006; Wahyuningrum, 2016). The research of Purwoceng beneficial has been confirmed by Prof. Dr. Susilo Wibowo and the result is Purwoceng can be use as Aphrodisiac (Darwati & Roostika, 2006) because its contain Stigmaterol (Suzery *et al.* 2005). Purwoceng have been proven to increase Testosterone level, and it's been used for several decades (Meiny, 2017). Otherwise for composition research of Purwoceng has result that Purwoceng contain bergapten, isobergapten, sphondin that three matters are furanokumarin group (Sidik *et al.*, 1975) has function to analgetika, sedative, antibacterial, antifungi (Darwati & Roostika, 2006). This effort of purwoceng cultivation is caused by high consumption, let alone efficacious as apriodisiak.

Purwoceng as an aphrodisiac contains a chemical component of steroids, volatiles, furanocumarine, and vitamins, which are found in the canopy and roots (Rahardjo & Darwati 2006). The steroid group consists of sitosterol, stigmaterol (stigmasta-7, 16 dien-3-ol), and (stigmasta-7, 25 dien-3-ol). Steroids are a powerful chemical component in the synthesis of testosterone in humans. These chemical components make purwoceng a traditional medicine to increase male vitality and fertility.

Research that studies purwoceng phytochemicals is quite a lot. Purwoceng root contains bergapten, isobergapten, and sphondin which are all included in the furanokumarin group. In the purwoceng root contains

coumarin compounds, saponins, sterols, alkaloids, and several kinds of sugar compounds (Darwati & Rostika 2006). Research conducted by Suzery *et al.* (2004) showed the presence of stigmaterol compounds in purwoceng roots based on spectroscopic data with UV-Vis, FTIR, and GC-MS. Hernani & Rostiana (2004) also reported the presence of chemical compounds identified qualitatively, namely bergapten, marmesine, 4-hydroxy kumarin, umbeliferon, and psoralen. Vitamin E is found in plant canopy but is not found in plant roots. Bergapten, sitosterol, and vitamin E have the highest levels when the plant enters the generative phase, the plant begins to flower. Bergapten serves as an increase in the vitality of the human body.

To preserve this endemic variety of plants, in Indonesia *Pimpinella pruatjan* Molk or *Pimpinella alpine* KDS cultivation has been started in vitro since 1990. In vitro culture efforts have now succeeded in getting callus for both cultivation and in-vitro conservation (Ningsih *et al.*, 2011; Ermayanti *et al.*, 2005, Darwati & Roostika, 2006). The success of this in-vitro cultivation technique must also be supported by root induction techniques.

In general, the media used in in vitro culture use Murashige & Skoog (MS) media (Lestari *et al.*, 1991; Lestari & Purnamaningsih, 1994; Wang, JW *et al.*, 2002; Rostika *et al.*, 2005; Darwati & Rostika *et al.*, 2006; Han M *et al.*, 2010; Lestari EG, 2011; Root induction in purwoceng plants has been successfully carried out in vitro (Syahid S. F *et al.*, 2005). However, in-vitro culture requires techniques and procedures that are quite complicated, which requires a lot of media and requires a long time. Therefore, currently many ex-vitro root induction techniques are applied, namely rooting induction of

compound shoots on acclimatization medium under aseptic conditions so as to summarize the time and procedure. This ex-vitro culture method has been successfully applied to various types of plants for example on the plants of palm *Elais guineensis* Jacq, *Ceropegia bulbosa* Roxb, *Mentha piperita* L (pepper), Blueberry, *Vitis vinifera* L, *Fraxinus pennsylvanica*, *Liquidambar styraciflua* L, *Aloe vera* L, *Nicotiana tabacum* L, *Rhododendron* sp. etc. (Isutsa et al., 1994).

The ex-vitro technique has several advantages, namely a shorter time, the media used is more economical and the plantlets are easily adapted. Based on that facts, ex-vitro root induction techniques need to be tried on purwoceng plants. Root induction starts from shoots that can be obtained through in-vitro culture or seed germination. According to Ningsih et al. (2011) shoots obtained from in-vitro culture are difficult to differentiate because their ability has decreased. Besides that, the shoots obtained from in-vitro culture that have undergone a conservation process need to be regenerated first. Therefore, in this study using shoots obtained from seed germination to get pre-shoots in a short time and in optimal conditions.

For the formation of plant organs such as rooting formation requires growth regulating substances. Plant growth regulators are already owned by plants (endogenous hormones) themselves. But in increasing the productivity of crop cultivation, it is often necessary to add exogenous hormones. The interaction between exogenous growth regulators and endogenous substances can trigger tissue growth and development (Lestari E.G 2011).

Exogenous hormones for shoot formation are generally used cytokines, BAP, whereas for root formation auxin is used, which are indole-3-acetic acid (IAA), α -naphthalenacetic acid (α -NAA), and indole-3-butyric acid (IBA). According to Zulkarnain (2009) administration of auxin or cytokines is a very important action in regulating cell division, elongation and differentiation, as well as the formation of plant organs in tissue culture systems. Both are needed depending on the ratio / ratio of cytokines to auxin or vice versa. The existence of one particular growth regulator can increase the activity of other growth regulators. The type and concentration of plant growth regulators that are right for each plant is not the same because it depends on the genotype and the condition of plant tissue physiology (Lestari, E.G, 2011). Thus, it is necessary to examine the effect of type and concentration of auxin on the physiological response of the roots of purwoceng plants.

Like in-vitro root induction, ex-vitro induction also

goes through the bud formation stage. The addition of IBA can induce changes in the activity of the enzyme peroxidase and IAA oxidase and its effectors (phenolic) which cause endogenous hormone balance for root formation. Then the results of further orthogonal testing that the use of NAA auxin at a concentration of 0.8 mg / l can induce in vitro purwoceng roots (Syahid S. F et al., 2005). NAA hormone is suspected to have an influence on physiological response in purwoceng plants ex-vitro. Moreover, to be able to determine the type of hormone, the optimal concentration of hormones and physiological influences must be carried out by further research.

4. Conclusions

The potential of purwoceng plants is quite large, but still constrained by the scarcity of seed supply and suitable land for these plants so that the application of ex-vitro root induction techniques in in-vitro culture can be a solution in limitation of supply of purwoceng crops for industrial scale.

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