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In Vitro Propagation of Medicinal Herbs in Korea

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Abstract

Mass production of forest medicinal plants is related to quality control of raw medicinal materials. Plant tissue culture is an important technology to produce high-quality plant materials. Numerous factors are reported to influence the success of *in vitro* regeneration of medicinal plants. Embryogenesis is known to be the most effective techniques and it has developed in some medicinal plant species. Various *in vitro* cultural condition for direct and/or indirect somatic embryogenesis systems have developed in *Epimedium koreaum*, *Bupleurum falcatum*, *Paeonia lactiflora*, *Chrysanthemum zawadskii*, *Houttuynia cordata* etc. In this study, we provide the present statue and information of *in vitro* propagation techniques that is able to apply as an efficient system for rootstock propagation system of forest medicinal plants.

Key Words: medicinal herb, micropropagation, *in vitro* propagation

Introduction

Among native plants in forest, 826 species of forest medicinal plants are known as available for medicinal usage (NIFoS 2017). The number of potentially usable species is expected to increase due to the development of natural substances and new functional food and drugs. Among the items supported by forestry and mountain villages development promotion act (Article 8 - supported for developing and promoting different sources on income concerning forest products), 18 medicinal herbs such as peony and 17 medicinal trees such as elm. In the result of medicinal herbs income analysis, initial investment cost such as purchasing root or seedlings were showed to the high at around 30% (Kang et al. 2015). In forest parts, *in vitro* propagation has been carried out at the National Institute of Forest Science (NIFoS) since 1980. Mainly poplar, rigitaeda pine, fruit tree and rare plants have been developed (Moon et al. 2010). However, the mass propagation of forest medicinal herbs has been partially carried out by universities and research institute, but the practical application of technology such as rootstock production system using tissue culture method is insufficient. In this study, we provide the present status and information of plant tissue culture technology that can enable the effective propagation and improvement of medicinal herbs.

Current Status and Suggestions

As the interest on forest medicinal plants resources has significantly increased, mass production technologies of economically important medicinal herbs have been developed and those technologies were provided to forest farmers for income increase. Raw materials of forest medicinal plants have good quality for highly functional component, bioequivalence evaluation division, and standardization processing. Also, plantlet in mass production of forest medicinal plants is related to quality control of raw medicinal

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material. In order to increase the utilization of forest medicinal plant resources, it is necessary to develop the effective seedling and/or root stock production technologies based on *in vitro* culture. So, it will be adapted to product plant stock of new medicinal plant resources from Korea native herbs. The functional component content and quality of forest medicinal plants depend on growing condition such as climate, temperature, soil condition and seedling and/or rootstock quality. In forest medicinal plants studies, a series of process for selection of economically important plants and mass production must be performed.

The cultivation area of forest medicinal herbs in Korea is smaller than other crops, and the production technology of seeds and seedstocks is not established. Medicinal herbs are proliferated as higher vegetative propagation than seedling propagation, making it difficult to the supply, nursery, rootstock demend (Jang et al. 2013). In breeding process, seedling propagation is required to the trait fixation duration from wild type relatively longer than vegetative propagation. It should be noted that vegetative propagation would be advantaged to propagate same clone, but degradation of propagation efficiency and applicability are limited.

For all these reasons, tissue culture technology, one of the vegetative propagation methods, is commercially available and can be used for forest medicinal herbs propagation as regeneration method for disease-free materials production (Tripathi and Tripathi 2003; Debnath et al. 2006). In particular, tissue culture can be used as an alternative method for low yield due to low productivity and germination rate and to maintain pure line. And it utilizes a part of plant tissue, so it is possible to mass-product clone stocks that are genetically identical to the mother plant in a short period of time. The mass proliferation of clone stock using tissue culture technology can lead to mass production of plantlets and useful materials using bioreactors, thus to establish the establish industrial base.

The totipotency of plant is able to induce *in vitro* embryogenesis and/or organogenesis from plant explant allowing the same clone to propagate as the mother plant (Skoog and Miller 1957). Several studies have been reported on the successful propagation system of plants using seeds and/or plant explants including axillary buds, leaf, root etc (Table 1). The most effective method for plant mass production is known as somatic embryogenesis. Plant from somatic embryo is similar to ordinary seeds except that there is no endosperm and seed coat.

It is possible to form stem and root like the plant, can be apply to the mass propagation as effective technics. Among the supported items by the Forest Service in Korea, researches on the mass propagation of forest medicinal plants using *in vitro* culture techniques were investigated. Among them, seed germination, bud culture, callus development and somatic embryo induction were investigated. Numerous factors are reported to influence the success of *in vitro* propagation of medicinal plants. NIFoS scientists have researched the control of *Gastrodia eleta* disease which is achieved through *in vitro* propagation of healthy plants. The reported *in vitro* culture techniques of several major forest medicinal plants were summarized.

Angelica gigas

Ultrasonically treated seeds were germinated in the most effective on medium with 0.1 mg l^{-1} GA_3 when heated to methanol. Callus induction was carried out from stem, root, and hypocotyl explants. Especially, somatic embryos were observed from stem and hypocotyl on 1.0 mg l^{-1} 2,4-D and 0.1 mg l^{-1} NAA supplemented medium respectively (Lee et al. 2012).

Paeonia lactiflora

Embryogeneic callus were induced in flower tissue in 2,4-D supplemented MS medium, after then somatic embryos were induced from non-supplemented PGRs for 80 days as the 38% (Chung et al. 1995). In supplemented with activated charcoal, the embryo development rated increase to 60% in same study. In other study, somatic embryos were mass induced from *in vitro* germinated plant in BA, NAA and GA_3 supplemented medium (Jana et al. 2013).

Cnidium officinale

In order to improve *in vitro* regeneration and root formation derived from shoot tip, optimal propagation condition were studied (Lee et al. 1994). The roots was induced as 80% within 30 days after culture on medium with 1.0 mg l^{-1} IBA and 0.05 mg l^{-1} supplemented. It was transplanted out of the field and 67% survived in 75 shading condition (Kim et al. 1997). Leaf, petiole, and flower explants were used as materials, but embryogeneic calli

Table 1. *In vitro* propagation via various organ and tissue culture of medicinal plants

Species	Explant	Result	Reference
Epimedium koreaum	Root, Leaf	Callus/plantlet regeneration	Han et al. 2000
	Leaf, Stem	Shoot production	Yu et al. 2002
Atractylodes macrocephala×*A. japonica*	Axillary bud, Root	Plantlet regeneration	Koo et al. 2011
Artemisia annua	Seed	Plantlet regeneration	Choi et al. 2007
Bulpleurum latissimum	*In-vitro* plantlet	Somatic embryo	Cho and Soh 1995
	Anther-derived callus	Somatic embryo	Shon et al. 2004
	Seed	Plantlet regeneration	Kim et al. 2007
Paeonia lactiflora	Anther-derived callus	Plantlet regeneration	Chung et al. 1995
	Cotyledonary	Plantlet regeneration	Jana et al. 2013
Gastrodia eleta	Seed	Symbiotic germination	Park et al. 2012, 2013
	Seed tuber	Vegetation stem	Kim et al. 2013
Chrysanthemum zawadskii	Leaf	Plantlet regeneration	Park et al. 2004
Houttuynia cordata	Stem	Plantlet regeneration	Choo et al. 1996
Angelica gigas	Seed	Germination, Plantlet regeneration	Lee et al. 2012
Cnidium officinale	Shoot tip	Plantlet regeneration	Lee et al. 1994
	Inflorescence	Somatic embryo	Cho et al. 2000
Aralia cordata var. *continentalis*	Cotyledon	Somatic embryo	Lee and Soh 1994
	Inflorescence	Plantlet regeneration	Lee and Soh 2000
Adenophora triphylla	Stem	Plantlet regeneration	Chen et al. 2001

were induced only in flower tissue and developed into somatic embryos (Cho et al. 2000). In somatic embryo formation stage, studies have also been conducted to improve the efficiency by controlling media conditions, carbon source types and concentrations, and nitrogen source (Chae et al. 1994).

Gastrodia elata

The aim of *in vitro* production was to develop a method for disease-free tuber production and to overcome the degradation caused by physiological disorder and soil contamination. The water agar (WA) and McCown woody plant medium (WPM) was used to induce protocom and 1.0 mg l^{-1} thidiazurun (TDZ) supplemented WA medium was the most favorable for protocom proliferation (Park et al. 2013).

Epimedium koreaum

In primary culture stage, the callus and plantlet were induced from type and concentration of plant growth regulators (PGRs) after sterilizing the apical and axillary explants of rhizome (Han et al. 2000). Callus and plantlet regeneration were induced by primary sterilization in 0.1% (v/v) $AgNO_3$ solution and secondary immersion sterilization in 0.3% (v/v) NaOCl solution. In callus subculture, 1/2 MS medium with 0.02-0.2 mg l^{-1} naphthaleneacetic acid (NAA) and 1.0 mg l^{-1} benzylaminopurine (BA) was used for subculturing callus. However, plant regeneration from callus was not easy (Yu et al. 2002).

Bupleurm falcatum

The morphology of cotyledons of somatic embryos derived from the leaf tissues in the medium containing 2,4-D was studied. Somatic embryos from embryogenic calli were grown in medium without PGRs. Cotyledons were varied due to the effect of 2,4-D and common type of cotyledons were 65% (Cho et al. 1995).

Dendranthema zawadskii var. *latilobum*

In vitro regeneration research was carried out to establish the propagation system and breed varieties. Axillary buds were regenerated in MS medium containing NAA and BA than other PGRs treatment. RAPD analysis was performed on regenerated plants. As a result, mutations are observed in passed fourth generation so that *in vitro* culture times will need to be adjusted (Kim et al. 1998). In the study of *in vi-*

tro cutting, the medium supplement of BA and NAA was effective on rooting (Park et al. 2004).

Conclusion

As the interest on forest medicinal plants resources has significantly increased, mass production technologies of economically important medicinal herbs have been developed and those technologies were provided to forest farmers for income increase. Raw materials of forest medicinal plants have good quality for highly functional component, bioequivalence evaluation division, and standardization processing. In order to increase the utilization of forest medicinal plant resources, it is necessary to develop the effective seedling and/or root stock production technologies based on *in vitro* culture. So, it will be adapted to product plant stock of new medicinal plant resources from Korea native herbs. The functional component content and quality of forest medicinal plants depend on growing condition, such as climate, temperature, soil condition and seedling and/or rootstock quality. In forest medicinal plants studies, a series of process for selection of economically important plants and mass production must be performed.

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