THE EFFECT OF PGR IN THE REGENERATION OF *Arctium lappa*

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Abstract

Herbs play an important role in traditional treatments of disease since ancient time. People are most likely to consume herbs because they have less toxicity and side effect when compare to chemical drugs. *Arctium lappa*, also called burdock, is a plant that commonly used in Traditional Chinese medicine. It can be used to cure sore throat and remove heavy metals such as mercury in the body. It also has high potential in cosmetic market and to treat cancer and HIV because many active compounds and antioxidant can be extracted from *A. lappa.* Antioxidant prevents the aging of skin and keeps the texture of the skin and the active compound extracted have anti-inflammatory, anti-tumor and anti-HIV properties therefore it is important to mass propagate this medicinal herb. Plant growth regulators (PGR) are commonly used to induce the growth of plants. The aim of this study is to determine the effect of PGR namely kinetin, benzyladenine (BA), indole-3-butyric acid (IAA), naphthalene acetic acid (NAA) in the regeneration of *A. lappa*. The fragments of the root of A. lappa were treated with kinetin, BA, IAA or NAA at different concentrations, 1, 2, 3, 4, 5 mg/L, the number of shoots formed was recorded for a period of three weeks. The results showed that only the first fragment of the root of *A. lappa* showed response to the different concentrations of exogenously applied PGRs. The best concentration of PGR to induce the leaves formation of *A. lappa* was 5 mg/L of kinetin which produced 14 leaves in the sample. BA and NAA were found to produce leaves at low concentrations whereas IBA induced leaf formation at high concentration.

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LIST OF ABBREVIATIONS

A. lappa	*Acrtium lappa*
ANOVA	One-way analysis of variance
BA	Benzyladenine
CKs	Cytokinins
cm	Centimetre
HIV	Human immunodeficiency virus
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
L	Litre
m	Metre
mg	Milligram
mg/ L	Milligram per litre
mm	Millimetre
NAA	Naphthalene acetic acid
PGR	Plant growth regulator
ppm	Parts-per notation
rRNA	Ribosomal ribonucléic acid

CHAPTER 1

INTRODUCTION

Herbs play an important role in traditional treatments of disease since ancient time. Herb also act as a key ingredient in about 25% of modern medicines (Rates, 2001). There are more than 1500 type of herbs can be used as dietary supplement (Wang & Renn, 2002). Almost 80% of the population in Asia and Africa rely on the herbal product to maintain and improve their health. It is estimated that 80% of the people in Indian use herbs as the medicines for treating the disease (Sahoo, Manchikanti & Dey, 2010). The herbal market in the world achieved about 5% to 15% annual growth rate (Citarasu, 2010). The estimate value of the herbal drug market in the worth is 62 billion dollar and it may be growing to 5 trillion dollar in 2050. China produce about 48 billion dollar of herbal product annually and export about 3.6 billion dollar of these herbal product to other country such as Hong Kong, Japan and Singapore a (Citarasu, 2010).

Herbs are widely used as an alternative or complementary treatment of disease. Almost all part of herbs can be used as medicine, for instance, flowers, leaves, seeds and stems, even the roots also has their medicinal potential in treating diseases or can be used as nutritional improvement. People are most likely to consume herbs because they have less toxicity and side effect when compare to chemical drugs. There are many type of antioxidative compounds in the herbs such as carotenoids, vitamin C and flavonoid (Farzaneh & Carvalho, 2015). Antioxidants have the properties of anti-aging and disease prevention (Norhaiza, Maziah & Hakiman, 2009). Antioxidants also can neutralize free radicals by binding to them to avoid or lower the damage of free radicals to the body (Aleksic & Knezevic, 2014). The herbs that show the anti-inflammatory properties are considered as candidate to treat the cancer (Farzaneh & Carvalho, 2015). Serpentine has been extracted from the root of *Rauwolfia serpentina* to reduce blood pressure (Verma & Singh, 2008) and the another plant called *Labisia pumila* can contract the muscle of birth canal and induce the birth of baby (Norhaiza et al., 2009).

Arctium lappa is a plant that commonly used in Traditional Chinese medicine. It origin from China and commonly known as burdock. It has been traditionally used for treating disease or maintaining health in China. Now, they can be found in Asia, Europe and North America (Chan et al., 2010). It can be grown everywhere but it only can produce the best quality root in the well-drained soil (Grieves, 2013).

A. lappa is used to cure sore throat and remove heavy metals such as mercury in the body. Its root can be used to treat diabetes, various skin diseases and improve the circulation of the blood under the skin. It contains a lot of antioxidants, thus it can prevent the aging of skin and keep the texture of the skin. The leaves of *A. lappa* can impede the bacteria growth in the oral cavity and the seeds contain compounds that show anti-inflammatory properties which can suppress the growth of tumors (Chan et al., 2010).

Plant growth regulators (PGR) are used to induce the growth of plants. There are several types of PGR, namely cytokinins, auxin, abscisci acid (ABA), ethylene and gibberellins (GAs). In general, cytokinins are used to facilitate the cell division, auxin are used to promote the cell expansion (Hu, 2012), ABA function in detachment and bud dormancy (Skriver & Mundy, 1990), ethylene stimulate the ripening of fruit (*Ethylene in Plant Biology*, 2012) and gibberellins regulate the growth and development of plants such as pollen germination and shoot elongation (Hooley, 1994).

The objective of this study are (a) to determine the effect of cytokinins namely kinetin and 6-benzyladenine (BA) on the regeneration of *A.lappa.* (b) to determine the effect of auxin namely, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on the regeneration of *A. lappa.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Acrtium lappa*

A. lappa is a plant belongs to Compositae family. It can achieve about 1.5-3.0m in height (Gross et al., 1980). It has a long and brown root. It can be used as herb or vegetable for human consumption. It saves a lot of nutrients in the first year to prepare for blooming (Chan et al., 2010). The purple tubular flower of the *A. lappa* is enclosed in a ball shape involucre that surround of multiple prickles. The leaves on the top are small and eye-shaped, the leaves on the bottom are big and heat-shaped (Grieves, 2013). Its seeds have hook which help them to adhere to the cloth or fur for seed dispersal (Gross et al., 1980).

Many active compounds can be isolated from *A. lappa* due to the advance of the analytical technique. These compounds are arctigenin, tanin, trachelogenin, chlorogenic acid, lappaol F, diarctigenin, inulin etc. Arctigenin and tannin show antitumor properties. Arctigenin show the ability to kill nutrient-deprived cancer cells and tanin can inhibit the growth of tumor cells. Trachelogenin and chlorogenic acid exhibits anti-HIV effect which may be a valuable candidate to treat HIV (Chan et al., 2010). Lappaol F and diarctigenin can inhibit the nitric acid (NO) production that induced by lipopolysaccharide (LPS) which may help in inflammatory disease because inflammatory disease involve excess NO production in macrophages (Park et al., 2007).

2.2 PLANT GROWTH REGULATOR

Plant growth regulators (PGRs) produced in one part of plants and transport to another part to action but they also may affect their producing cells. They active at low concentration and control the growth and development process of plants such as stem and root expansion, drop of the fruits and leaves. PCR can be natural or synthetic. The natural PCR are named phytohormones and plant hormones. There are

five classes of phytohormones namely cytokinins, auxins, abscisic acid, ethylene and gibberellines (Basra, 2000).

2.2.1 Cytokinins

Cytokinins (CKs) are one category of phytohormones which play an important role in the lifespan of plants. CK induce the proliferation of the cells with the present of auxins and regulate the growth and development process of plants such as lead senescence and seed germination (Mok & Mok, 2001). In 1913, Gottlied Haberlandt discovered a compound in phloem show the ability to promote cell division in potato parenchyma and named it as cytokinins. Later in 1955, Carlos Miller discovered kinetin and the zeatin was first isolated between 1961 and 1963 (Maheshwari, 2015).

The natural CKs are adenine-derivatives and they are divided to two groups: isoprenoid CKs and aromatics CKs. They are categorized based on the side chain at N^6 position is adenine-derived or aromatic and show some structure differences of the side chain within each group such as the position of hydroxyl group (Sakakibara, 2006). Zeatin and isopentyl are included in isprenoid CKs, benzyladenine and furfuryladenine are the example of aromatics CKs. Kinetin is isolated by structural rearrangement, thus it is not included in the natural CKs (Maheshwari, 2005). CKs are synthesized in the root and transferred to the canopy though xylem to promote the growth of shoot, they are also synthesized in leaves and transferred to roots though phloem to promote the growth of roots (Ferguson & Grafton-Cardwell, 2014).

2.2.1.1 Kinetin

Skoog, Miller and associates in University of Wisconsin discovered a compound able to facilitate the cell division in cultured plant cells and stimulate morphogenesis in plant with the presence of auxins in 1955. The compound was named kinetin and also called N^6-furfurylaminopurine (Kung, & Yang, 1998) (Figure 2.2.1.1.1).

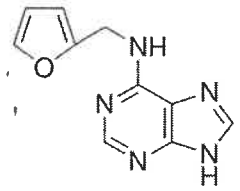

Figure 2.1 The structure of kinetin, a type of cytokinins, which is a plant growth regulator. The molecular formula is $C_{10}H_9N_5O$.

Kinetin shows the ability to regulate gene expression, control cell cycle, inhibit the action of auxin and promote calcium flux, it also act as an anti-aging molecule. Kinetin induces the transcription initiation in RNA polymerase I, which is a gene promoter to transcribe the ribosomal RNA (rRNA) gene to produce protein and form ribosome. Other PGR showed no effect of rRNA transcription therefore kinetin may be a main regulator of transcription process (Gaudino & Pikaard, 1997). Kinetin can activate cyclin-dependent kinase (CDC2) which plays an important role in cell cycle regulation. Kinetin also can inhibit the formation of bud dormancy induced by abscisic acid (Barciszewski, Rattan, Siboska & Clark, 2000).

Maheswari et al (2006) stated that the Murashige and Skoog (MS) medium contained 0.5mg/L kinetin is the best for the callus formation of sorghum because 0.25mg/L kinetin cause abundant root formation and the concentration of kinetin above 0.5mg/L should promote more shoot development than the callus formation.

2.2.1.2 Benzyladenine (BA)

Benzyladenine (BA) also called 6-benzylaminopurine is a synthetic cytokinins which synthesized by Folke. K. Skoog (Kamínek, 2015) (Figure 2.2.1.2.1). The ability of BA to increase the fruits weight and size was demonstrated in 'Empire' apple. BA stimulates the cell division in the fruit cortex to increase the size and it also increase return bloom of the apple tree (Wismer, Proctor & Elfving, 1995). BA can increase the post-harvest life span of broccoli, delay the yellowing by lower the respiration rate to prevent the loss of ascorbic acid and inhibit ethylene production to prevent loss of protein (Siddiqui, Bhattacharjya, Chakraborty, & Dhua, 2011).

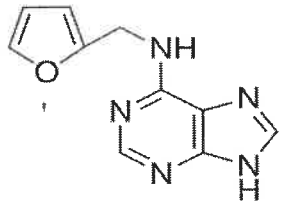

Figure 2.2 The structure of benzyladenine, a type of aromatic CKs. The molecular formula is $C_{12}H_{11}N_5$.

The ability of BA to stimulate the shoots regeneration is demonstrated on *Cicer arietinum L.* (chickpea) (Polisetty et al, 1997) and *Mella azedarach L.* (meliaceae) (Vila, Gonzalez, Rey, & Mroginski, 2005). The concentration of BA to get the highest number of shoots is in the regeneration of chickpea by using seed explants. If the concentration is higher than 0.017 mg/L, it will inhibit the bud growth and the root can only be induced at concentration lower than 0.0028 mg/L (Polisetty et al, 1997). In the regeneration from root explants of meliaceae, 0.1 mg/L of BA achieved the greatest number of shoot buds (Vila, Gonzalez, Rey, & Mroginski, 2005).

2.2.2 Auxin

Auxins were discovered by Charles Darwin and Francis Darwin in 1881 as the first phytohormones. Auxins affect the cell division, cell elongation, cell differentiation and the movement of plants (Teale, Paponov & Palme, 2006). When the plants detect the light in one area, auxin exposed to the light are destructed and accumulated in the opposite dark area, it stimulate the elongation of the cells in dark site and make the plant grow toward the light, this process is called phototropism. Auxins also help in abscission of leaves and fruits. When the amounts of auxins produced by the leaves or fruits reduce, it cause the detachment of leaves and fruits stalk (Kimball, 2006). Auxin can be inhibited by ethylene. If ethylene is inhibited by inhibitors, there is no inhibitory effect on auxins production (Peter, 2010).

2.2.2.1 Inndole-3-butyric Acid

Indole-3-butyric acid (IBA) is a type of auxin discovered by Zimmerman and Wilcoxon in 1935 (Epstein, & Ludwig-Müller, 1993) (Figure 2.2.2.1.1). IBA can be naturally produced in plant but the role of IBA in the development of plants is not understood. When apply exogenously, it can stimulate the formation of adventitious