# Effects of auxins and cytokinins on plant growth

Plant tissue culture can encompasses different types of plant cell, tissues and organs cultures under aseptic control. The importance of tissue culture technology for large scale multiplication and for commercial purposes is well understood. Many commercial growers are using this technique, especially for propagation of ornamental plants. Despite the well-known importance of plant tissue culture, this technique is sometimes restrained by limited knowledge of tissue and organ cultures in some rare plant species. Different roles and effects of plant hormones, also known as growth regulators, was investigated by examining the induction of root and shoot organogenesis in vitro. In most of the plants, the formation of roots can be promoted by auxins while cytokinins promote the shoots formation in vitro. Sometimes, the tissue culture may directly form roots or shoots without go through intervening callus stage. Some commonly used and effective auxins including naphthylene acitic acid (NAA) and some commonly employed cytokinins are kinetin and 6-benzylamino purine (BAP). Of course, there are many plants which can differentiate directly without the use of plant hormones.

Abbreviations: 1AA, indole 3-acetic acid; 1BA, indole-3-butyric acid; NOA, naphthoxy acitic acid; NAA, naphthylene acitic acid; 2, 4-D, dichloro phenoxy acetic acid; BA, benzy adenine; BAP, 6-benzylamino purine; 2ip, isopentyl adenine.

## Introduction

The genus petunia is best used for tissue culturing owing to the success in isolation and rejuvenation of leaf protoplasts in vitro (Frearson 1973). Explants that have active cells to undergo mitosis are mostly suitable for

callus induction. Carrot is also useful in demonstrating the effects of growth regulators on organogenesis.

Plant tissue culture technology is rapidly emerging with the involvement of specific techniques to facilitate in vitro propagation of ornamentals, food crops and industrial crops. The importance of plant tissue culture is to fasten multiplication rate of plant as the natural plants is usually slow growing and time consuming. Besides, tissue culture can be used to reduce labour and space requisites, for micropropagation of exclusive genotype with desired features, to multiply plants with poor seed set and low germinability, and to produce pathogen free stock to enhance vigour and other plant qualities (Singh 2004). Tissue culture technique is also means for cloning such as to make genetically manipulated exclusive clones, somaclones and transgenic plants. The tissue culture is especially useful to proliferate plants which are cross-pollinated, have wide variations of progeny, male sterile lines or virus free through meristem culture (Singh 2004).

On the other hand, there are some limitations of using plant tissue culture due to slow progression in protoplast, cell and organ culture development. Sometimes, it is hard to obtain successfully regenerated whole plant from civilized plant materials.

The tissue culture method includes extract pieces of tissues from a plant and grows on nutrient medium after sterilizing the tissues surface. Typically, a complex nutrient medium contains sucrose, certain amino acids, vitamins, growth regulators, nitrogen salts and other essential elements for plant tissues to survive, grow and thrive (Singh 2004). After weeks, a mass of new

cells called callus will form rapidly around the tissues due to the induction of plant hormones. In several plants such as petunia and carrot, higher levels of auxin relative to cytokinin stimulates root development in vitro while lower levels of auxin relative to cytokinin promotes shoot formation.

Plant hormones are used to intensely improve cell growth, cell differentiation, organogenesis and biosynthesis of certain compounds. A phytohormone is a substance synthesized by plant organs to stimulate certain biochemical, physiological and morphological responses (Baca & Elmerich 2003). Generally, phytohormones can be classified as five classes which are auxins, cytokinins, gibberellins, abscisic acid and ethylene. (see Appendix Table 2)

The objective of this experiment was to commence tissue culture using petunia and carrot and study the effects of different concentrations and proportions of two plant hormones auxin and cytokinin, in nutrient medium used in the practical.

## Materials and Methods

Plant materials

A number of fresh petunia plants and carrots were prepared in laminar flow cabinet. Healthy leaves were removed from the petunia plants using sterilized forceps and scalpel. Carrots were cut horizontally (in a round shape) into 0. 2 cm thick and further cut into 3-4 small pieces if the parameter of the carrots was too large. The removed leaves and carrot slices were briefly drenched in 10% (v/v) sodium hypochlorite (NaOCl) (less than 5 minutes), followed by rinsing in sterile water (rinse three times) to get rid of

surface microbial contaminants before placed into petri dish (2 leaves in a dish; 4 carrot slices in a dish). While for the carrots, the cambium ring was wounded by slightly slice the surface using sterilized scalpel before placing into petri dishes.

Tissue culture media

For petunia leaves, a total of 9 petri dishes which is 1 control dish and 8 dishes containing MS media plus phytohormone combinations of auxin (NAA) and cytokinin (kinetin or BAP) were prepared. The 8 dishes stand for NAA: Kinetin – (2. 0: 0. 0, 1. 0: 0. 5, 0. 5: 1. 0 and 0. 0: 2. 0) ratios and NAA: BAP â€" (2. 0: 0. 0, 1. 0: 0. 5, 0. 5: 1. 0 and 0. 0: 2. 0) ratios. The preparation of the culture media for carrots was in the same manner as for the leaves above.

Culture conditions

All the petri dishes were stored in a room temperature (25°C). Other cultural conditions such as light, humidity, oxygen supply were controlled to allow initiation of the tissue cultures to form callus, roots and shoots. Observation was carried out once a week. Whenever there is overgrowth of bacterial, nutrient depletion or contamination occurred in the cultures, the affected leaves and carrots were either transferred to another nutrient medium or discarded.

## Results and Discussion

In few range of plant species, callus can be induced to regenerate plants via organogenesis or embryogenesis. These method of cultures are

advantageous as quicker propagate than shoot culture and genetic variations occur among cell line permit selection of better clones (Singh 2004). Scientists have reported that callus cultures have an important role in morphogenesis and are amenable for storage by growth limitation method or cryopreservation (Singh 2004).

Organogenesis can be identified by initiation of basal callus or shoot bud differentiation. By modifying the ratios of growth regulators such as auxin and cytokinin, or adjust the culture medium composition, organanogenesis can be induced from callus culture.

Roles of auxin and cytokinin

Higher levels of auxin (NAA) promote the root initiation from callus cultures. In contrast to auxins, higher levels of cytokinin support shoot proliferation. The effectiveness of different types of cytokinin such as kinetin and BAP used in this practical, is vary in different plant species. In this practical, BAP is more efficiency in inducing shoot growth in petunia plants compared to kinetin.

According to Reddy and Narasimhulu (1988), the combination of NAA and kinetin in a medium was suitable to induce roots with well-developed laterals; while the medium containing both BAP and NAA were found to be more efficient in regenerating shoots.

This is supported by a research of Jaiwal and Singh (2003), where it was found that BAP alone was the most effective in inducing multiple shoots at high occurrence among other cytokinins. BAP can acts on especially

cotyledonary node explants to produce high amount of shoots per callus (Jaiwal & Singh 2003).

The frequency of root initiation and shoot regenerability of the leaves and carrots were highly variable with various combinations of auxins and cytokinins. This is clearly seen from the observations recorded (in Table 1) on the petunia leaves and carrot slice after four weeks.

## Effect on petunia leaves

Formation of callus

After four weeks, it was observed that callus had formed on the leaf surfaces, especially in the medium containing NAA: kinetin (0. 5: 1. 0) and NAA: BAP (2. 0: 0. 0; 1. 0: 0. 5). The callus produced was milk white, compact and granular. From the result, it showed that the formation of callus is promoted by media containing intermediate auxin/cytokinin ratios. Callus was formed by disorganized cellular propagation as a response to hormones supplied in the medium, either endogenously or exogenously (Banno et al. 2001).

While for the medium of NAA: kinetin (2. 0: 0. 0; 1. 0: 0. 5) and NAA: BAP (0. 5: 1. 0), callus was formed but little. However, there is no callus observed in medium containing NAA: kinetin (0. 0: 2. 0) and NAA: BAP (0. 0: 2. 0). This could be due to some undeveloped (immature) tissues which are morphogenetically plastic in tissue culture (in vitro), hence fewer callus was formed (Walker et al. 1979). Besides, depletion of nutrients for example carbohydrate insufficiency may contribute to less callus formation.

Direct adventitious organs formation

In the control medium, a direct adventitious root formation was observed on the leaf petiole. This is known as indirect organogenesis where adventitious root is arising directly from plant tissues without an intervening callus phase. Since there is no addition of any phytohormones in the control medium, the leaf tissues may use ammonium, the basic nutrient in the MS medium as sole nitrogen source to support the formation of adventitious root.

Besides, there is also adventitious roots growing on the explants in the medium containing NAA: kinetin (0. 5: 1. 0) and NAA: BAP (2. 0: 0. 0; 1. 0: 0. 5). These roots were originated from the callus on leaf tissues with the aid of auxin (NAA) hormones (direct organogenesis). On the contrary, no roots being observed in the medium of NAA: kinetin (2. 0: 0. 0, 1. 0: 0. 5 and 0. 0: 2. 0) and NAA: BAP (0. 5: 1. 0 and 0. 0: 2. 0). It has been seen that in these mediums, only a very little amount of callus or no callus was formed. A minute amount of callus is unable contribute to the root initiation.

In addition, intensely green shoots was seen to be formed in the medium containing NAA: BAP (1. 0: 0. 5; 0. 5: 1. 0, 0. 0: 2. 0) only. The shoots were developed from subepidermis or epidermis layer as these layer have mitotically active cells for shoot proliferation. According to Singh (2004), adventitious embryos can develop directly into seedlings but in vitro shoots proliferating on a medium containing cytokinin virtually always lack roots. Only when the plantlets are transferred to a medium lacking cytokinin, rooting can occur spontaneously.

## Effect on carrot slices

Formation of callus

From the practical result, there was more callus proliferated from the cut surface of carrot as a result of wounding at cambium rings. A small percentage of callus had formed in the control dish and all the medium containing NAA: kinetin. Similar callus formation was observed in the NAA: BAP phytohormone combinations in nutrient medium, but the callus formed was relatively large compared to the NAA: kinetin phytohormone combinations.

The nature of morphogenesis in callus depends on the parent plants tissues as well as the composition of medium used in vitro (Walker et al. 1978). Concentration of hormones in the tissue culture medium is a crucial factor to determine the morphogenesis. The organ formation is identified by quantitative interaction instead of absolute concentrations of the growth regulators participating in callus growth and development.

Direct adventitious root formation

Adventitious roots were only found at medium containing NAA: kinetin (0. 5: 1. 0). As the initial callus size was very small, fewer roots produced. Carrot tissues were highly prone to microbial contamination. Unlike petunia leaves, carrots lack of wax to protect the tissues from microorganisms.

There was no shoots formed in all culture medium. This may be due to insufficient endogenous cytokinin produced by the explants to promote shoot growth and elongation (Lee et al. 1997). Subepidermal cell proliferation

occurred on the adaxial (upper) surface by forming a diffuse cambium following by phellogenic cambium.

Besides, the root and shoot organogenesis can be influenced by season or climate, donor states of the plant, stage of development and physiological status of the parent plant. In terms of nutrient source, some tissue cultures require both nitrate and ammonia ions while others prefer casein hydrolysate, yeast extract or certain amino acids (Singh 2004). Some improvement can be made by using different basal media such as, Murashige and Skoog (1962, Ms), White (1963, WM), Nitsch (1951, N), Gamborg et al. (1968, B5), then select the best that give ideal response. Otherwise, adjust chemical ingredients of the media for better cell growth.

## Conclusion

There are few pathways for tissue culture to form callus and develop roots and shoots such as embryogenesis and organogenesis. The formation of callus is enhanced by a balanced ratio of auxins and cytokinins. Once callus becomes competent in vitro, continual supply of nutrient will determine the formation and growth of root. As mentioned earlier, higher ratio of auxins to cytokinins induces root growth, while lower ratio of auxins to cytokinins initiates the formation of shoot. Petunia leaves are suitable for tissue culturing as can be seen by the number of roots and shoots formation compared to carrots. It is harder to generate roots from carrot callus as carrots are more susceptible to contamination since there is no wax layer to protect them from microorganism.