Factors affecting organogenesis in plant tissue culture

Organogenesis is the process of forming a specific organ from non-specific mass of meristem or parenchyma cell known as callus. In this experiment, auxin and cytokinin are used to determine the effect of PGR, auxin and cytokinin on the organogenesis in carrots and petunia leaves, in which auxin hormones responsible for growth of roots, phototropism and gravitropism, while, cytokinin helps in inducing the growth of shoots and regulates auxin action. Theoretically, the presence of high cytokinin and low auxin in the media, the shoots will form, meanwhile, in low level of cytokinin and high level of auxin in the media, roots will form. Meanwhile, in the presence of high concentration of both cytokinin and auxin, callus formation will be induced. However, in this experiment, production of the shoots and roots are not compatible with the theory due to improper ratio of auxin and cytokinin and the high toxicity of synthetic cytokinin, kinetin.

Introduction

Plant tissue culture (PTC) is the techniques used to grow plant from any of the plant segment, tissues or cell in a contaminated free environment media such as MS media (Singh & Kumar, 2009). PTC techniques is important plant biotechnology aspect in which it facilitates the production of genetically modified plants and induced rapid multiplication of difficult-to-propagate plant species. Besides that, the ability to produce totipotent plant cell using PTC techniques has significant impact on crop improvement via genetic engineering (Radzan, 2003).

There are two different processes, which involve explant differentiation and growth in PTC, which are organogenesis growth and adventititous roots or shoots growth directly from the explants. Organogenesis is the process of

forming a specific organ from non-specific mass of meristem or parenchyma cell known as callus. Meanwhile, for formation of adventitious roots or shoots means the roots or shoots structure arise from the explants that have been excised (Pernisova et al., 2009). This situation does not usually happen if the plant sample are cultured in a medium with the same ratio of auxin and cytokinin.

The presence of plant growth regulators (PGR) such as auxin, cytokinin, gibberellins, abscisic acid and ethylene has significant impact in the process of plant growth and differentiation. Gibberellins responsible for growth, seed germination and promote fruits growth. As for ethylene helps in controlling of fruit ripening as well as controlling cell division and cell elongation. Meanwhile, abscisic acid act on seed maturation and give the ability to the seeds to response during stress in undergoing dormancy period (Davies, 2010). However, in this experiment only, auxin and cytokinin are involve, in which auxin hormones responsible for growth of roots, phototropism and gravitropism, while, cytokinin helps in inducing the growth of shoots and regulates auxin action (Davies, 2010). In this experiment, NAA will be used as synthetic auxin and kinetin and BAP as synthetic cytokinin.

This experiment was conducted in order to determine the effect of PGR, auxin and cytokinin on the organogenesis in carrots and petunia leaves.

Materials and methods

Plant materials

For petunia leaves samples, the leaves were provided by lab technician in Monash University Sunway Campus. Firstly, 18 petunia leaves were collected

from the petunia leaves petri dish. Then, the petunia leaves were soaked using 10% (v/v) of sodium hypochlorite for not more than 5 minutes. The leaves were then rinsed using sterile water in a laminar flow cabinet. Then, 2 petunia leaves were added in each petri dish with different ratios of NAA: BAP and NAA: Kinetin.

The same methods were implied to the carrot. However, the carrot sample was cut into 27 pieces about 0. 5cm thick on the surface of sterile ceramic tile. After soaking the carrot samples in sodium hypochlorite and rinsed for three times, 3 carrot pieces was transferred into petri dish with different ratios of NAA: BAP and NAA: Kinetin. The result recorded based on table 1 and table 2.

Tissue culture media

MS media supplemented with combination of high cytokinin to low auxin were prepared by the lab technician as follows: NAA: Kinetin ratios (2: 0, 0. 5: 1, 1: 0. 5, and 0: 2) and NAA: BAP ratios (2: 0, 0. 5: 1, 1: 0. 5, and 0: 2). Additional of two control plate with MS media that supplemented with same ratio of auxin and cytokinin were also prepared by the lab technician.

Culture condition

The transferred petunia leaves and carrot pieces in the 18 petri dishes were incubated for 28days in 25±2°C temperature, and photoperiod of 16hours in light & 8hours in dark as provided in plant culturing room in Monash University Sunway Campus. In addition, for every 4 days, the tissue cultured was checked to whether there are presents of contamination and to transfer the samples into new MS plates.

Results and discussion

Effect on carrot slices

Based on the result obtained in table 1, it shows that for carrot samples, the formation of callus can be seen all of petri dish with various ratios of either BAP: NAA or Kinetin: NAA. Theoretically, the formation of callus is due to the high concentration of both the cytokinin and auxin in a growth media (Duncan et al., 1985). Since all the plates are containing callus, it can be deduced that the growth of callus can be formed by excision of the plant cell. Meanwhile, for formation of roots, only carrot samples from MS media supplemented with BAP: NAA with ratio of 0. 0: 2. 0 which also shows formation of roots. This is because, according to the theory, in the presence of high cytokinin and low auxin in the media, the shoots will form, meanwhile, in low level of cytokinin and high level of auxin in the media, roots will form. However, in the presence of high concentration of both cytokinin and auxin, callus formation will be induced (Chawla, 2002). However, it can be seen that there is no production of shoots in other plates except in MS media containing 0. 0BAP: 2. 0NAA. This may occur due to unsuitable ratios of cytokinin and auxin in MS media that may not favorable for carrot species to induce formation of shoots.

Effect on petunia leaves

By referring to table 2, it shows that petunia leaves sample that shows formation of callus, roots and shoots is the MS media supplemented with BAP: NAA with ratio of 0. 5: 1. 0 and the control plate. This occur due to suitable ratio of cytokinin and auxin suplemeted in the MS media. Furthermore, only MS media supplemented with BAP: NAA with ratio of 0. 0:

2. 0 shows the growth of shoots excluding the MS media supplemented with BAP: NAA with ratio of 0. 5: 1. 0 and the control plate. Theoretically, the productions of shoots are triggered by high concentration of cytokinin and low concentration of auxin. However, in the ratio of 0. 0BAP: 2. 0NAA, it is predicted that formation of roots will be induced, instead, shoots are forming. This occur due to presence of zeatin in petunia leaves. Zeatin is a natural cytokinin presence in the most green leaves that undergoing senescence (Singh et al., 1992). The presence of cytokinin in the media may mask the reaction of auxin hormone on the explants presence in the media (Nakagawa et al., 2006). Therefore, the production shoots are triggered instead of roots. Based on the result in table 2, the production of roots and shoots are low due to due to the excessive amount of alcohol used during the sterilization process. Besides that, shoots and roots only produce in MS media supplemented with BAP: NAA and not in MS plate with Kinetin: NAA. This may occur due to the toxicity of the synthetic kinetin, that are not suitable for the growth of either shoots or roots on petunia leaves.

There were also presence of contamination in the cultured plate of carrot sample, which involve MS media supplemented with BAP: NAA with ratio of 2. 0: 0. 0 and MS media supplemented with Kinetin: NAA with ratio of 2. 0: 0. 0. Meanwhile, for petunia leaves sample, the cultured plate that have been contaminated by fungal infection are MS media supplemented with BAP: NAA with ratio of 0. 0: 2. 0 and ratio of 2. 0: 0. 0 as well as MS media supplemented with Kinetin: NAA with ratio of 0. 0: 2. 0 and ratio of 0. 5: 1. 0. This may occur due to experimental error, in which contamination may occur due to improper techniques while doing PTC such as lack of using aseptic

techniques to make sure sterile condition during transferring the samples into the MS plate.

Conclusion

In conclusion, suitable medium supplemented with correct ratio of cytokinin and auxin are the most important key in order to get successful induction of organogenesis in vitro. Besides that, sterile environment is also one of the important factors in determining the successfulness of PTC and in order to obtain expected