Morphogenesis Studies on *Amaranthusgangeticus In Vitro*

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Abstract- Research on *Amaranthus gangeticus* were undertaken to investigate the morphogenesis of the plant through tissue culture techniques. The optimal conditions that are required by plant for the purpose of regeneration and also propagation were identified. Various kinds of hormone combinations with different concentrations were used for this purpose. Among the hormone, Benzylaminopurine (BAP) and Napthalene-Acetic-Acid (NAA) were used to initiate the cultures. These plant growth regulators have been found to promote the formation of callus. From the result, explant from leave and stem gave the best response in the formation of callus in certain hormone combinations. Highest percentage of callus (99.7% ± 0.2%) has observed when stem explant was cultured on MS medium supplemented with 2.0 mg/l NAA and 1.0 mg/l BAP. Callus formation was found to be responsive in all medium tested.

Keywords---*Amaranthus gangeticus*, morphogenegis, tissue culture, callus, propagation.

I. Introduction

TISSUE culture is a method to produce complete plantlets from a small plant or explants tissues such as leaves, stems and roots cultured in a sterile or aseptic media for growth. Cell culture techniques and plant tissues were used to study the problems of plant physiology and cells. Its scope has been extended for the purpose of application in the forestry sector, industry, horticulture, and agriculture.

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Factors involved in the selection of tissue explants including resources, physiological age and ontogeny, the season when the explants is taken, explants size and quality of the parent plant [2]. Factors such as genotype, explants orientation and explants inoculation density also affect the success of tissue culture methods [1]. The main purpose of this study is to investigate the response of plants *Amaranthus gangeticus* in *in vitro* systems. In addition, this study involves the use of aseptic seedlings as explants. Many people prefer the use of aseptic seedlings as source of explants. This is because of aseptic seedlings have a lower percentage of contamination from the intact plant. Explants source used include leaves, stems, and roots. Media used in this study is [2]. Among the hormones that are added in the media to study the response of explants in tissue culture system are Benzylaminopurine (BAP) and Napthalene-Acetic-Acid (NAA). These hormones are added in different combinations and concentrations. Plant species that will be examined in this research is a vegetable plant species, *Amaranthus gangeticus*. This plant is native to America was initially known as ornamental plants but then being promoted as a food source of protein, especially for developing countries. *Amaranthus gangeticus* is a leafy vegetable plant species. It has been known that plant leafy vegetables are easier to culture than woody plants. It has shorter propagation period and a simple life cycle compared to woody plants. *Amaranthus gangeticus* has been used as a complementary vegetable dish and has lots of benefits and nutrients. *Amaranthus gangeticus* general aids to improve the work of the kidneys and digestive launch.

II. Materials And Method

In this research, plant cell, tissue and organ culture protocols are being followed. Seeds of *Amaranthus gangeticus* were initially rinsed under running tap water for 1 hour, followed by rinsing three times with distilled water. Then, the seedsare rinsed in 100% sodium hypochlorite solution for 5 minutes added with a mixture of 1 drop of Tween 20. Subsequently, seeds were rinsed in a solution of 70% sodium hypochlorite for 2 to 3 minutes. This was followed by sodium hypochlorite solution at the concentrations of 50%, 30% and finally 10%. The infusion should be for 2-3 minutes for each immersion. The next sterilization process was in the laminar flow chamber. Seeds were rinsed again with distilled water, three times repeatedly. Then, the seeds were rinsed with a solution of 70% alcohol concentration for 1 minute. Final sterilization step was to rinse the explants with distilled water for 4-5 times. Cleaning

measures and sterilization steps should be followed to ensure that resources used are totally sterile and free from the presence of microorganisms. If not, the problem of contamination will occur in culture.

TABLE I
PERCENTAGE OF CALLUS FORMATIONWHEN LEAF, STEM AND ROOT EXPLANTS WERE CULTURED ON MS MEDIUM SUPPLEMENTED WITH VARIOUS COMBINATION OF BAP AND NAA.

CONCENTRATION (mg/l)		EXPLANTS	PERCENTAGE OF CALLUS FORMATION
NAA	BAP		
0.5	2.0	LEAF	14.0±1.5
		STEM	83.0±2.7
		ROOT	70.0±2.7
1.0	1.0	LEAF	56.0±1.5
		STEM	98.2±0.7
		ROOT	47.5±2.1
1.0	2.0	LEAF	32.5±0.8
		STEM	99.7±0.2
		ROOT	86.5±1.3
1.5	0.5	LEAF	26.3±0.5
		STEM	99.7±0.2
		ROOT	53.0±1.0
1.5	1.0	LEAF	22.0±0.8
		STEM	12.1±0.6
		ROOT	12.9±0.5
1.5	1.5	LEAF	10.7±0.6
		STEM	14.6±0.3
		ROOT	12.4±0.5
1.5	2.0	LEAF	61.4±0.8
		STEM	70.1±0.8
		ROOT	65.1±0.4
2.0	1.5	LEAF	52.9±0.7
		STEM	58.8±0.9
		ROOT	55.7±0.4

The sterilized seeds were then culture on MS basal medium. After 4 weeks, aseptic seedlings produced will be used as source of explants (figure 1). Aseptic explant parts such as leaf (figure 2), stem and root will be cultured on MS medium supplemented with different concentration of BAP and NAA (0.5-2.0 mg/l). All cultures were kept in the culture room with 16 hour light and 8 hours dark photoperiod at 25˚C. All responses were observed and recorded.

III. RESULTS AND DISCUSSION

In this research, the optimum combination of hormone for callus formation was 2.0 mg/l BAP + 1.0 mg/l NAA (figure 3). This combination of hormone produces of 99.7±0.2 percent of callus formation when stem explant was used as source of explant. Besides that, the percentage of callus formation on root explant was also high, 86.5±1.3 percent of callus formation. Callus formation was 61.4±0.8 percent when leaf explant was cultured on MS medium supplemented with 2.0 mg/l BAP + 1.5 mg/l NAA. The least responsive treatment was observed when leaf, stem and root explants were cultured on MS medium supplemented with 1.5 mg/l BAP + 1.5 mg/l NAA (Table 1).

Fig. 1 Aseptic seedlings of *Amaranthus gangeticus*

Fig. 2 *In vitro* culture of leaf explant

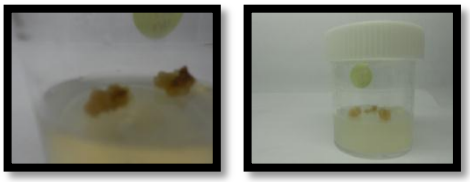

Fig. 3 Callus formation when stem explant was cultured on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA.

Various combinations of hormones were used in this study to analyze and identify the effects of hormones on morphogenesis of *Amaranthus gangeticus*. Explants used in this study were leaves, stems and roots.It was found that stem, root and leave explants responded to hormone treatment and callus formation was induced. BAP and NAA helped in the formation of callus. The optimum hormone observed to induce callus was 2.0 mg/l BAP + 1.0 mg/l NAA when stem explants were used as source of explant. NAA also helped in the formation of callus, but it plays a role in producing roots. However, there are also explants which were unable to give any response to hormone treatment. These explantsdid not produce callus but continue to form roots when NAA hormone was used. This usually occurs for stem explants.The callus produced mostly yellow, it was found that the callus produced through a combination of hormones NAA and BAP are mostly yellow in color. Callus formation occurs only around the explants, especially leaf, root and stem explants. It was found that the explants that are cut too large will prevent callus formation because it will be curled and could not touch the surface of medium containing a mixture of combination hormone. Only explants that touch the surface of the media could form callus.

Light is essential for plant run photomorphogenesis. Cultured plants do not run photosynthesis actively as a carbon source is already derived from sucrose supplied through the

medium. According Murashige (1977) [3], he assumes that the light period of 16 hours of light and 8 hours dark is best for most plants. For example, multiple shoots were regenerated from plant cell cultures of *Allium cepa* cultured in the light period of 16 hours of light and 8 hours dark.In addition to light, the use of disposable culture is also very important. Ventilation for cultured plant depends on culture containers used. Therefore, the media poured into the culture containers should be sufficient rate only. If the media is poured in too much, then the space in the former culture began to decline. This will affect the growth of the culture. Larger culture containers will be able to supply more an air to the culture.

The resource, the size and age of explant used also plays a role in morphogenesis *in vitro*. Explant sources commonly used are the stems, leaves, roots and tips of shoots. It is known that young explants responded better than mature explants [5]. Explants that are too small will lose the ability to carry out regeneration. The explants are too large will cause it rolls and does not touch the surface of the media. When this happens, the explants not be able to interact with the media and resulted in explants cannot give any response and change. Therefore, it is better to use the medium of explants only.

Media plays an important role in morphogenesis *in vitro*. Appropriate media need to be used in order to determine desired results. Among the most common types of media used in the study of tissue culture media was MS [2], B5 medium [6], N6 medium [7], media MT [4], and media LS. Each type of media has its role and function in morphogenesis. Media to be used also depends on the type of tissue culture to be performed and the type of plant species to be used. Tisserat (1985) has reported that MS medium (Murashige and Skoog, 1962) is suitable for most types of plants. Media MS has been widely used around the world. In this study, MS medium was suitable to use to study the morphology of *Amaranthus gangeticus*.

IV. Conclusion

Growth and productivity of plants depend on types of plants growth regulators used with suitable method and concentration. The environmental factors also plays important role in the growth optimization of plants. In the present research, it was found that the application of plant growth regulators at lower concentration helps in the organogenesis and callus induction of *Amaranthus gangeticus in vitro*.

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