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Short Communication

Effects of 2, 4-Dichlorophenoxyacetic Acid on the *in vitro* growth of explants (*Clerodendron* spp.)

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Callus cultures and suspension cultures began with pieces of tissues and cells from the leaf but culture conditions were manipulated to create an unorganized state. The initiation of callus was induced from dicotyledonous tissue explants (*Clerodendron* spp.) first by preparing MS basal medium and then by adding varying concentrations of 2, 4-Dichlorophenoxyacetic Acid or 2, 4-D. Growth was observed after each week for a period of 3 weeks and observations were documented. After all the observations were noted down; subsequent analysis and interpretation of the results followed. This led to a valid scientific conclusion for the whole study process. Though there was disturbance due to heavy bacterial contamination in majority of the petriplates, but the effect of plant-status and environmental factors on callus initiation cannot be fully ignored. Growth response studied was more vigorous in low concentrations of 2, 4-D as compared to higher levels.

Keywords: Callus, 2, 4-D, inoculation, *Clerodendron*, plant tissue culture

2, 4-Dichlorophenoxyacetic acid or 2, 4-D is an important plant growth regulator (PGR) which has been extensively utilized in culturing of plant tissues *in vitro* (Wain and Wightman, 1954; Dixon and Gonzales, 1994; Chang et al., 2000). It is a synthetic auxin (Pavlica et al., 1991) and is known to cause abnormalities in the plant cell chromatin and chromosome (Pavlica et al., 1991; Hayashi et al., 1998) and mutations in mammalian cells cultured *in vitro* (Suter et al., 1980). The applications of 2, 4-D on shallot root tip cells have been reported to cause mutagenic and cytotoxic effects (Pavlica et al., 1991) and on fibroblast cells (V79 Chinese hamster) have

been examined and compared (Suter et al., 1980). It is reported that 2, 4-D may induce changes in mitotic activity, in the structure of chromosome and chromatin and during the cell cycle (Pavlica et al., 1991). When used at a higher concentration of 10 µg/ml or more, 2, 4-D may act as a potent mutagen and cytotoxic agent which affects the V79 CHO cells in culture (Suter et al., 1980). The results in both mammalian and plant systems were in agreement showing mutagenic activity of 2, 4-D in the concentration range higher than usually used in establishing plant tissue culture (greater than 5 µg/ml).

Species of the genus *Clerodendron* (or *Clerodendrum*) has been subjected to *in vitro* tissue culture studies resulting in multiple root generation (Goyal et al., 2010). Also, insecticidal properties against mosquitoes have been identified in the leaf extract of *Clerodendrum* (Goyal et al., 2010). It is now well known that for the rapid propagation and conservation of plants, plant tissue culture is an efficient contrivance (Prakash et al., 1999). With this motivation the current work was designed where I desired to subject the explants to varying concentrations of scarcely used synthetic auxin 2, 4-D rather than more commonly used auxins like Naphthalene Acetic Acid (NAA) or Indole Acetic Acid (IAA). This is supported by the fact that 2, 4-D has predominantly been used for callusing purposes only, and not shoot and root induction.

Materials and Methods

Materials used

(a) Glassware: petriplates (medium size), conical flasks (250 ml), glass-bottles (500ml), beaker (400 ml, empty). (b) Chemicals (for standard MS medium): macro nutrients I, macro nutrients II, micro nutrients, Fe-EDTA, Myo-Inositol, sucrose, vitamins (Pyridoxine HCl, Thiamine HCl, Nicotinic Acid), Glycine, agar (0.8%). (c) Others: explant (leaves from *Clerodendron* spp.), micropipettes (adjustable channels), pH meter, parafilm, autoclave.

Medium

Standard MS medium (Murashige and Skoog, 1962) was used for the study along with agar (0.8%) for the preparation of plates. The pH of the medium was maintained at 5.8.

Methodology

Pre-prepared MS Stock Solutions were used.

(a) Preparation of Medium (100 ml): appropriate volumes of ingredients were

mixed together. Then, 3 gm of sucrose were added along with a little distilled water along with appropriate volumes of plant growth regulators (hormones). The volume was made to 90 ml. After adjusting the pH to 5.8, the final volume was made to 100 ml. Finally, the gelling agent (agar) was added and the solution was melted.

(b) Sterilization: All the materials were autoclaved at 121°C at 15 psi pressure for 20 minutes for moist sterilization.

(c) Pouring of medium: After autoclaving, the medium was poured in petriplates (3 petriplates each for 3 concentrations: 0.1 mg/l, 0.5 mg/l and 1 mg/l.)

(d) Inoculation: the explants (leaves) were cut in small pieces, surface-sterilized and finally inoculated. Parafilm papers were wrapped around each petriplate to make them air-tight and avoid contamination from air-borne microbes.

Results and Discussion

Callusing is the method of growing callus from the starting material or an explant. The idea of callusing is to develop the whole plant from a small part. It is an important concept in plant tissue culture technique which provides to critically analyse the organism (plant) from the tissue level. Callus cultures are influenced by various factors. One such factor in context to the current work is the growth hormone, 2, 4-D.

In the current study, various concentrations of 2, 4-D were used. Careful observations over a period of up to three weeks (see Table 1) depict that low concentration (0.1 mg/ml) of the growth hormone facilitates successful callus induction. Though there is a mild response to the higher levels of the growth hormone, but this is not significant and may require further investigation.

Table 1: Effects of 2, 4-D on the *in vitro* growth of explants

Concentration of 2, 4-D	Petriplate	Period (week)	Observation
0.1 mg/l	1	First	No growth
		Second	Swelling
		Third	Callus(friable)
	2	First	No growth
		Second	Contamination
		Third	Contamination
	3	First	Contamination
		Second	Contamination
		Third	Contamination
0.5 mg/l	1	First	Contamination
		Second	Contamination
		Third	Contamination
	2	First	No growth
		Second	Contamination
		Third	Contamination
	3	First	No growth
		Second	Swelling
		Third	Contamination
1 mg/l	1	First	Swelling after 2-3 days
		Second	Contamination
		Third	Contamination
	2	First	Contamination
		Second	Contamination
		Third	Contamination
	3	First	Swelling after 4-5 days
		Second	contamination
		Third	contamination

An important factor to consider may be the status of the plant. In this case, the explant (*Clerodendron* spp.) is not much competent to take up or assist in callusing. The anatomy and morphology of the plant needs to be further examined in order to validate and rationalize this line of thought.

As there is sufficient manual intervention, microbial contamination cannot be overlooked. Though adequate measures were adopted to minimize the occurrence of undesirable growth, still majority of the plates were contaminated. It calls for further stringent needs for a full proof sterile environment. Even the slightest exposure will

lead to contamination of the entire plate. Hence environmental factor is also important for proper callusing.

Conclusions

2, 4-D can be effectively used to study the response of culture explants in controlled environments. Stringent control of environmental factors is needed in order to minimize microbial contamination. Also, the effect of plant-status and environmental factors on callus initiation cannot be fully ignored. In the current work, low concentrations of 2, 4-D stimulated better growth response as compared to the higher levels. The work may provide a base to design future experiments trying to optimize a media for successful plant tissue culture in presence of 2, 4-D over a wide range of explants.

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