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In vitro Propagation and Abrobacterium Mediated Transformation Studies on Chenopodium Album *L*

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Abstract. Tissue explants from different parts of an important Indian medicinal Plant, Chenopodium album *L*, were cultured in vitro and their morphogenetic potential was elucidated in the present investigation. Explants from leaf, stem and node were cultured on MS medium supplemented with different concentration and combination of plant hormones like IAA, NAA, 2, 4-D and BAP. It was observed that stem explants have maximum morphogenetic potential than other explants of chenopodium album. Optimum concentration of individual hormones of the growth of the stem explant was determined. It was found to be 2 mg/l for IAA and NAA, 0.5 mg/l for 2, 4-D and 0.5 mg/l for BAP. Factorial combination of different concentration and combination of plant hormones was tried to ellicit morphogenetic potential of stem explants, compact, green colored and non-friable callus was formed on medium supplemented with IAA of NAA, white friable callus was formed on medium supplemented with 2, 4-D. For maximum callus for-

mation on stem explants a medium supplemented with 2, 4-D 0.5 mg/l and BAP 1 mg/l was found to be ideal callus could be sub cultured indefinatly on this medium for several passages. Multiple shoots were formed on stem explant derived callus on medium supplemented with IAA 1 mg/l and BAP 2 mg/l. Two or three shoots per explant was obtained. The amount of phenol present in the callus extracts on different hormones like 2, 4-D, BAP, NAA, combination of BAP (1 mg/l), 2, 4-D, 0.5 mg/l were found to be nil. But in the care of Agrobacterium tumefaciens transformed callus, the phenol concentration was found to be 123 μg/g.

Keywords: Chenopodium album *L.*, Agrobacterium funefaciens, Phenol, IAA, BAP, 2, 4-D, NAA and coleus amboinicus *L.*

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1 Introduction

Plants have been one of the important sources of medicine. Since the beginning of human civilization. In spite tremendous development in the field of synthetic drugs and antibiotics during the twentieth century, plants still constitute one of the major source of drugs in modern as well as traditional medicine throughout the world. It is significant to note that more than 90 percent of drugs used in traditional system of medicines in a country like India as well as other countries of Asia and Africa come from plants. Even in modern medicine 25 percent of the drugs are plant based [3]. It has been estimated that 80 percent world population depend upon herbal based medicines.

India is sitting on a goldmine of used recorded and well-practiced knowledge of traditional medicine. Traditional medicine is still the ministry of about 75–80% of the world population, mainly of the developing countries, for primary health care. However, the last few years have seen a major increase in their use in the developed world. In Germany and France mainly herbs and herbal extracts are used as prescription drug

and their sales in the countries of Europeans Union were around billion in 1991 and may be over $20 billion now. In USA herbs are currently sold in health food stores with a turnover of about by the turn of century.

The plant improves the appetite, anthelmintic, Laxative, diuretic, aphrodisiac, tonic, useful in biliousness, "Vata" and "Kapha" abdrminal pains, eye diseases, threat troubles, Piles, diseases of the blood, the heart, the spleen (ayurveda). The plant is used as a laxative. It is used in the form of pot herb in piles. The finely powdered leaves are used as a dusting powder about the external genitalia in children.

Soil bacteria, Agrobacterium tumefaciens and A.rhyzogenes which induce crown gall and hairy root disease respectively at wound site on numerous plants. Tumour tissues synthesize novel amino acid and sugar derivatives which are collectively known as opines. The type of opine synthesized in the tumour (for example, nopaline, octopine, agrocinopine, mannopine and agropine) is dependent on the strain of Agrobacterium that initiated tumour formation [2].

The Agrobacterium responsible for tumour formation selectively catabolizes the opine whose synthesis it has induced using it, as a source of carbon and nitrogen. Agrobacterium tumefaciens strains are octopine or nopaline types. Agropine, a sugar derivative is commonly found in hairy root tumours induced by A.rhizogenes. Both tumour and hairy root induction associated with the presence bacterial mega plasmids, they are Ti (Tumour inducing) plasmid in the case of A.tumefaciens and the Ri (Root inducing) plasmid in A.rhizogenes [5]. Caplan *et al.* [1], Ghysen *et al.* [4] and Satchel and Zambryski [6] derived the tumour induction in various plants.

There are two major advantages of using biotransformation system, (i) More than one reaction can be accomplished using cell cultures that express a series of enzyme activities. (ii) In some instance even non-producing cell cultures can be used to synthesize the desired product using appropriate precursors.

2 Materials and methods

2.1 Extraction of explants

Chenopodium Album *L* belongs to the family chenopodiaceae which is evenly distributed in the drier parts of the India which is a major Indian medicinal plant used in Ayurveda, siddha and Unani systems of medicine. Leaf explant and shoot opices were used for the present study. The explants were excised with the help of sterile forceps and blade. The nodes were cut into 0.5–1 cm sized segments and care was taken that each explant included the midrip portion. Apical shoot buds measuring, 10–15 mm in length with 2–3 leaf primordial attached were also used.

2.2 Surface sterilization of the Explant

Surface sterilization was done by using mercuric chloride and alcohol. The explants were treated with 0.1 percent mercuric chloride for 1 to 2 minutes and washed twice with sterile distilled water. Then the materials were rinsed in 70 percent alcohol for 2 to 3 minutes. Then the explants were thoroughly washed twice with sterile distilled water.

2.3 Inoculation

Before starting inoculation, culture tubes containing media, instruments like sprit lamp, sterilized forceps, scissors, petridishes and sterized distilled water were transferred to UV chamber and there were exposed to UV light, for 30 minutes. After that the surface sterilized explants were inoculated. The leaf explants was implanted on the medium with abaxial surface in contact with nutrient medium. The culture room was maintained at a temperature of 25 ± 2 °C. The cultures were kept under the light intensity of 2,000

lux at the level of culture tubes, using white fluorescent lamps. Photo-period of 12 h per day was maintained. The relative humidity of the room was maintained at 70 percent.

Explants and calli were subcultured every 5–6 weeks. The tubes containing culture materials were externally sterilized with 70 percent alcohol. The materials were transferred to fresh medium with the help of sterile forceps in the inoculation chamber. After subculture they were transferred to the culture room. Fresh and dry weight of ht explants was measured before inoculation and after 5 weeks of culture. Regular observation at an interval of two days was made for the formation of callus, change of colour and initiation of the root or shoot.

3 Confirmation test for agrobacterium mediated transformation

Basically, two separate experiments were carried out of confirm the transformation of Agrobacterium tumefacians having Ti plasmid in to chenopodium album.

3.1 *in vitro*

In the first experiment, callus were excised from transformed callus of chenopodium album in the medium containing shoot inducing medium (IAA 0.5 mg/l + BAP 1.0 mg/l) containing 200 mg/ml kanamycin 100 mg/ml cerfotaxine. Transformed tissues cultured after the heat or antibiotics treatments for removing the bacteria selectively have an required new properties that are stably inherited and by which the tissue can be distinguished from normal callus. Which is inoculated in the medium without growth regulators, auxins and cytokinin.

3.2 *in vivo*

In the second experiment, the two different type of callus were collected form 2, 4-D and Ti induced 2, 4-D callus. 2, 4-D extract was treated as control and Ti induced callus extract treated as sample the two extracts were centrifuged individually where as the inoculam. Using a syringe needle or razor blade, made shallow wounds in the surface of the plant stem, inoculate the sample wound sites with the callus supernatant solution of two different sample cover the inoculation site with a piece of sterilized wrapper. The plants maintained at normal gardening condition and observe tumour formation over period of one to three weeks.

3.3 Estimation of phenols (Arnow's method)

Arnow's reagent specifically reacts with ortho dihydric phenols by producing a pink coloured complex; the intensity of which is measured in a colorimeter.

3.4 Arnow's reagent

Dissolve 10 g of sodium nitrate ($NaNO_3$) and log of sodium molybdate ($NaMo_2$) in 100 ml of distilled water. Store the reagent in a brown bottle and reagent is stable for a year.

Pipette 1 ml of the alcohol extract into a test tube, add 1 ml of 0.5 N of Arnow's reagent, 10 ml of distilled water and 2 ml of 1 N NaoH. Soon after the addition of the alkali; pink colour appears. Maintain a reagent blank without the extract. If the colour intensity is high, dilute to 25 ml and read the absorbance of the solution at 515 nm. Calculate the OD phonols present in the sample from a standard curve prepared with catechol.

Figure 1: Habit of chencopodium album L.

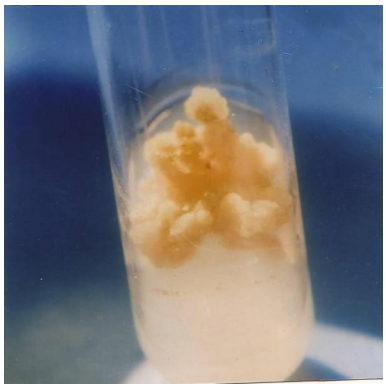

Figure 2: Light brown colour friable callus formed on the medium containing 2, 4-D 0.5 mg/l.

Figure 3: Shoots were developed from the nodal explant on the medium containing IAA 0.5 mg/l + BAP 0.5 mg/l.

Figure 4: Shoots were developed from the nodal explant on the medium containing IAA 1 mg/l + BAP 2 mg/l.

Figure 5: Shoots were developed from the nodal explant on the medium containing IAA 2 mg/l + BAP 0.5 mg/l.

4 Results and discussion

Different explants like leaf, stem and node of chenopodium album were cultured on different concentration 0.5, 1.0, 2.0, 5.0 mg/l of hormones IAA, 2, 4-D and BAP. It was observed that only stem explants showed growth response like enlargement and initiation of callus. Out of the different concentrations of the IAA (0.5–5.0 mg/l) used the maximal growth in terms of fresh and dry weight was observed at 2.0 mg/l. The concentration of IAA above 2.0 mg/l was found to be inhibitory effect (Table 1).

2, 4-D, BAP and NAA in the concentrations of 0.5–5.0 mg/l was used and it was found that concentration of 0.5 mg/l yielded the maximal fresh and dry weight from the stem explants. Transformed callus induced by the Gram-negative bacterial Agrobacterium tumefaciens and found on the MS medium without growth hormones. Auxins and cytokinins callus proliferation was started after 5 days from the date of inoculation. The callus was friable and dark brown coloured was occurred on the medium with out any supplement.

Crown gall was initiated after 7 days from the date of inoculation. Galls prolif-

Table 1: Effect of different auxins on growth of stem explants of chenopodium album.

No	Conc.	Weight at harvest (mg)					
	(mg/l)	IAA		NAA		2, 4-D	
		Fresh wt	Dry wt	Fresh wt	Dry wt	Fresh wt	Dry wt
1	0.5	24.7±20	5.2± 0.46	12.5 ± 1.5	3.5 ± 0.42	115 ±228	16.0 ± 2.0
2	1.0	35.2±3.2	6.1 ±0.50	16.2 ± 2.2	4.6 ±0.45	85±22.8	13.5 ± 1.9
3	2.0	74.5 ± 11.3	11.0 ± 1.3	26.8 ± 10.1	8.1 ± 0.74	67 ± 12.8	9.1 ±1.5
4	5.0	31.8 ± 4.4	4.8 ± 0.51	12.4 ± 3.4	3.4 ± 0.41	39 ± 3.7	5.8 ± 1.3
Medium: MS Basal.				Age of Culture: 4 weeks			
Initial fresh weight: 12 ±1.20 mg				Initial dry weight : 2.1 ± 0.10 mg			

eration was observed at the site of wound. The galls were green in colour and well proliferated. After three weeks the gall color was changed to light brown color and size was reduced. No observations was recorded in the wound site, which the site of 2, 4-D induced callus suspension inoculated. Phenol estimation was done, in the callus extracts on various hormones like. 2, 4-D, BAP, NAA, BAP (1mg/l) + 2, 4-D (0.5 mg/l) + IAA (0.5 mg/l) + BAP (1 mg/l) + 2, 4-D (0.5 mg/l) and tumefaciens transformed callus. The following are the results obtained.

The amount of phenol present in the callus extracts on different hormones like 2, 4-D, BAP, NAA combination of BAP (1 mg/l), 2, 4-D 0.5 mg/l and the combination of IAA 0.5 mg/l, BAP 1 mg/l, 2, 4-D 0.5 mg/l) were found to be nil. But in the case of tumefaciens transformed callus the phenol concentration was found to be 123 µg/g. Infection of plant induces changes in phenolic substances (Mahadevan., 1996). Varieties differ in their response with regard to phenolic changes, initially an increases in the susceptible ad resistant varieties but with symptom development, phenols decrease

in the susceptible cultivate while in the resistant varieties, phenols accumulate. It has been suggested that the major difference between resistant and susceptible varieties is in the velocity of accumulation of phenols; 'faster' is typical of resistant varieties.

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