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Callus induction studies of plant *Piper betle* Linn.

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Abstract : *Piper betle* Linn. is medicinally and economically very important plant. An attempt has been made to study the effect of three Auxins incorporated in Murashige & Skoog medium (MS) on initiation of callus in various explants of *Piper betle* L. . Among three Auxins 2,4-dichlorophenoxy acetic acid (2,4-D) was proved to be best for callus induction in *Piper betle* L.

Keywords : *Piper betle* L. , callus, MS, 2,4-D.

INTRODUCTION

The Piperaceae family comprises 14 genera and 1,950 species, of which the genus *Piper* is the largest, with more than 600 species distributed world wide (Danelutte, Lago, Young & Kato, 2003)[1]. *Piper betle* belongs to the Piperaceae (Samba, Murty and Subrahmanyan, 1987)[2] family. Besides being of high commercial and economical importance, *Piper betle* is medicinally used in different ways. Hence the present studies have been undertaken with a objective to study the effect of different auxins on the initiation of callus in *Piper betle* L.

MATERIALS & METHODS

Explants used for callus initiation were root , leaf and shoot apex of *Piper betle* from field condition. All experiments were performed in Murashige & Skoog medium(MS) (Murashige and Skoog; 1962)[3]. 2,4-dichlorophenoxy acetic acid (2,4-D), Indole-3 acetic acid (IAA) and Napthaleneacetic acid(NAA) were three Auxins used for Callus initiation.

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Preparation of stock solution

Stock solutions of macro and micro elements, organic substances, vitamins and amino acids of MS and Auxins were prepared in the following manner-

(1) **Stock Solution of Macro Elements –** Each Macro Elements of MS was being weighed 10 times and dissolved in double glass distilled water and the solution made 1 litre. The prepared solution was kept in a measuring bottle inside refrigerator and was labelled 100 ml. for one litre.

(2) **Stock Solution of Micro Elements, Iron Sources, Amino Acids and Vitamins –** Stock Solution of each micro elements, iron sources, amino acids and vitamins were being weighed hundred times and dissolved seperately in distilled water and the solution made one litre. Prepared solution were kept in measuring bottle inside refrigerator and labelled 10 ml./litre on each bottle.

(3) **Stock Solution of Auxins –** 250 mg. of each auxin (2, 4-D, NAA and IAA) was measured and dissolved with a few drop of absolute alcohol and the solution was made 250 ml. by adding sterilized distilled water. It was kept inside freezer and was labelled 1000 ppm.

Double glass distilled water was used in all the experiments. The medium was made semi-solid with 0.6% to 0.8% Difco-Bacto Agar. The PH of the medium was adjusted usually to 5.8 using 0.1 N NaOH or 0.1 N Hcl before autoclaving. About 20 ml. of the hot medium was dispensed into each culture test tube (15.0x2.5 cm.) made of corning glass. The test tubes were plugged with non-absorbent cotton wrapped in chese cloth. The culture tubes were autoclaved at ca 1.06 Kg./Sq. Cm. for 15 to 20 minutes. After autoclaving all the culture tubes were put into racks with isodiametric holes and kept in slanting position till they become semisolid. Each experiments, with a minimum of 48 cultures, was repeated thrice. The cultures were usually maintained in diffuse light at 28 ± 2^0C and 60 to 65% relative humidity.

STERILIZATION OF EXPLANTS

All the explants were washed with running tap water for about 10 mins., rinsed in a dilute solutioin (1:100) of cetrimide (ICI trade mark "Cetavlon") for 10 mins. and again thoroughly washed with sterile water. Then explants were kept in 0.25% streptomycin SO_4+0.5% Bavistin (anti-fungal) for 20 mins. and again washed with distilled water. The explants were then surface sterilized with 0.1% $Hgcl_2$ solution for 1 to 2 mins. and again washed with distilled water. Then explants were again surface sterilized with 70% ethyl alcohol for 1 min. and 3 times washed with sterile water.

OBSERVATION

2, 4-D, IAA and NAA were 3 auxins used for callus initiation. The concentration used for each set of experiments for different auxins were from 1 ppm to 10 ppm. Among 3 auxins tested, 2, 4-D proved to be more effective and callus induction was noticed in 70% cultures at 2.5 ppm. On MS + 2, 4-D (5ppm) callus induction was 60% and MS + 2, 4-D (2 ppm) callus was 61%. IAA showed less effect among 3 auxins. On MS + IAA (2.5 ppm) callus induction was noticed in 45% cultures. On MS + IAA (2 ppm) callus induction was 31% and MS + IAA (5 ppm) callus was 30%. NAA showed better effect than IAA. On MS+NAA (2.5 ppm) callus induction was noticed in 66% cultures. On MS + NAA (2 ppm) callus induction was 50% and MS + NAA (5 ppm) callus was 40%. (shown in table:1). The best explants for callus initiation was leaf.

Effect of various concentration of 2, 4-D, IAA and NAA in initiation of callus on the explant of *Piper betle* Linn. on MS.

Result After 4 Weeks			
Concentration of Auxins in ppm	Medium + Phytohormone		
	MS+2, 4-D	MS+IAA	MS+NAA
1	45%	20%	21%
2	61%	31%	50%
2.5	70%	45%	66%
5	60%	30%	40%
7	35%	28%	35%
10	31%	25%	30%
Average of 100 Cultures			

Fig 1: callus initiation in 2,4-D

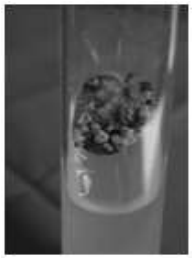


Fig 2: callus initiation in NAA

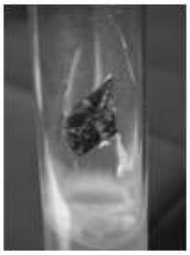
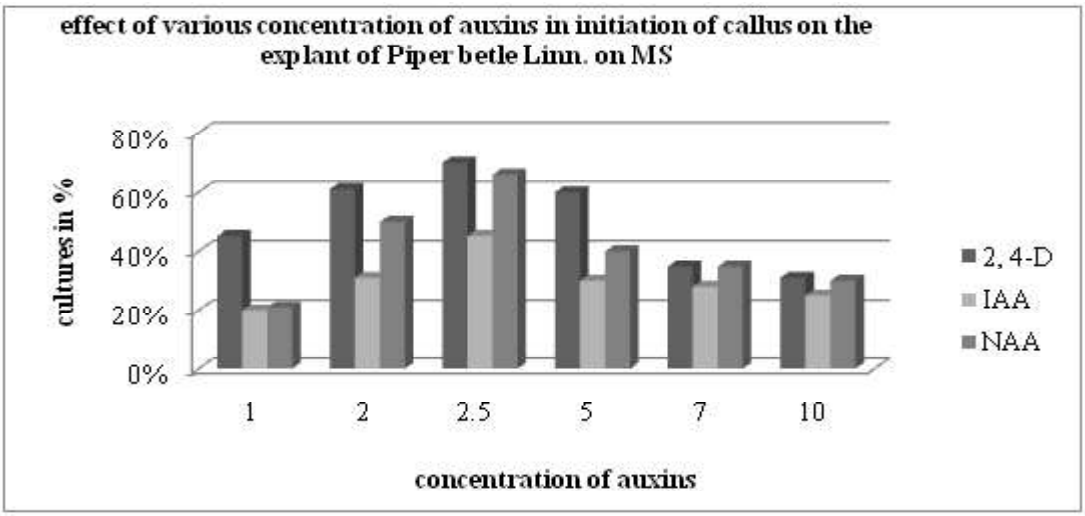


Fig 3: callus initiation in IAA



DISCUSSION

Piper betle belongs to the Piperaceae family(Samba, Murty and Subrahmanyan, 1987)[2]. It is medicinally and economically very important plant. Hence the present investigation has been undertaken with a objective to study the effect of different Auxins on callus initiation of *Piper betle* L. 2, 4-D, IAA and NAA were three auxins incorporated with MS media used for callus initiation. The concentration of each auxin was 1 ppm to 10 ppm. The results obtained in the present investigation on the cultures of *P. betle* L. make it clear that among three auxins 2, 4-D proved to be more effective and callus induction was noticed in 70% cultures at 2.5 ppm. On MS + 2, 4-D (5 ppm) callus induction was 60% and MS + 2, 4-D (2 ppm) callus was 61%. The best explant for callus initiation was leaf. NAA showed better effect than IAA in initiation of callus. On MS + NAA (2.5 ppm) callus induction was noticed in 66% cultures. At 2 ppm 50% and 5 ppm 40% cultures showed callus induction. IAA showed less effect among 3 auxins in callus induction. On MS + IAA (2.5 ppm) callus induction was in 45% cultures, at 2 ppm 31% and 5 ppm 30% results were obtained.

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