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Effect of 2, 4-Dichloro Phenoxy Acetic Acid on Callus Induction in *Plantago ovata* (Isubgol)

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ABSTRACT: This experiment involved the study of effect of 2, 4-D on callus induction in Plantago *ovata* G-2 (Isubgol). Blond phyllium (Isubgol) is a medicinal plant that belongs to family Plantaginaceae. *Plantago ovata* is a winter season herb valued for its mucilaginous husk on its seed for pharmaceutical uses mainly for the constipation and diarrohea. The seeds of *Plantago ovata* were procured from IARI New Delhi. The plants were maintained in the Green houses in Bhopal. *In vitro* sprouts of *Plantago ovata* G-2 was cultured on MS media supplemented with different conc. of 2, 4-D (0.5mg/l to 5mg/l). Callus induction and photoperiod was maintained 16 hour light and 8 hour darkness and temperature was kept 28°C +2°C. Observations were recorded in terms of fresh and dry weight of callus after 40 days of inoculation. The maximum mean fresh weight of callus was obtained from 2.0mg/l of 2, 4-D was 1.73gm+-4.1 in *Plantago ovata* G-2. From the present study it was found that the maximum average fresh weight of callus was seem to be physically friable and yellowish white in color while compact and brownish colored callus were found to be less in amount as compared to healthy callus.

Keywords: *Plantago ovata*; 2, 4 –D (2,4-Dichloro phenoxy Acetic Acid); MS (Murashige and Skoog Medium) and Explant; Callus Induction .

INTRODUCTION: *Plantago ovata* belongs to family Plantaginaceae. The preference of medicines of plant origin, over synthetic medicines is a global concern due to no or less side effects of plant medicines. Over 700 plants are being used in Unani and Ayurveda system of medicine. Blond Psyllium (Isubgol) 2n = 22 *Plantago ovata* Forsk, is one of the important medicinal plant. Its cultivation is mainly centralized in the northern Gujarat and south-west Rajasthan and now in M.P., where the crop is mostly grown without proper soil fertility and weed management. Blond Psyllium (Isubgol) is a winter season medicinal plant valued for its mucilaginous husk on its seed for numerous pharmaceutical uses, principally as a swelling, dietary fibre against chronic dysenteries and habitual constipation (Dwivedi, 2004; Dwivedi et al., 2007 and Farzaei et al., 2013).

The plant is very short stemmed, hairy annual herb. Leaves are simple narrowly linear or filiform. Flowers are small, in cylindrical or ovoid spikes. The fruit is capsule, where the upper half is a blunt conical lid. Seeds are smooth, yellowish brown in colour, ovoid, oblong or boat shaped. The main chemical constituents are linolenic, linoleic, oleic, palmitic, stearic, lignoceric. The embryo yields 14.7% of linoleic acid rich oil. Its husk, locally called bhusi, is valued medicinally for gastric disorders, constipation, diarrhoea, piles and urinary diseases. It is also used in cosmetic and ice cream industry. Psyllium or Isubgol is important cash crop, mainly cultivated for medicinal use and export value. . Being economically an important crop, it was introduced at Indore in Madhya Pradesh in1995 for conducting agronomical studies. India is the whole and sole exporter of *Plantago ovata.*

MATERIALS AND METHODS: The present investigation was carried out at the Saifia Science College, Botany Department, Bhopal (M.P.). These experiments were conducted during 2010-2012. The seeds of *Plantago ovate* G-2 were procured from IARI, New Delhi. Plants were grown and maintained in green house and stem top, leaf tip and young bud were used as source of explants.

Chemicals: The different chemicals were used and collected from different agencies to perform experiments. Vitamins, Chemicals, Amino acids, Sucrose were obtained from Hi media Laboratories Pvt. Ltd., Bombay. Salts of macro and microelements of analytical grade were obtained from Loba chemicals, Hi media, Sigma, Merck etc.

Glass Ware Sterilization: All the Glasswares used in experiments were sterilized by autoclaving. The autoclaved glasswares were then dried in oven at 80-100°C for two hours. Forceps, scalpels, blades and scissors were first autoclaved and then during inoculation sterilized by dipping in absolute alcohol and holding on flame alternately.

Culture media: The basal media by Murashige and Skoog's (1962) was standardized using different concentrations and combinations of growth hormones for *in vitro* callus induction and regeneration from tissues of *Plantago ovata* G-2.

Basal medium adjuvant: The MS medium was supplemented with following adjuvant for initiation of growth, establishment of cultures, maintenance and multiplication of cultures and morphogenesis of the explants
Indol butyric acid (Auxin)
3-Indol acetic acid (Auxin)
Naphthalene acetic acid (Auxin)
2,4-Dichlorophenoxy acetic acid (Auxin)
Kinetin (Cytokinin)
6-Benzyl amino purine (Cytokinin) etc.
To prepare Murashige and Skoog's basal medium, four different stock solutions were prepared

Aseptic manipulation: Aseptic manipulations like explant preparation, surface sterilization, inoculation as well as their sub culturing operations were carried out in the laminar airflow hood.

Isolation and preparation of explants: *Plantago ovata* G-2 regularly irrigated and maintained in the green house. The plant tissues such as stem tip, leaf tip and young flower buds were selected from healthy plants. The isolated plant tissues were washed with tap water and then double glass distilled water. The explants were cut in proper size in the petriplate and were washed with double glass distilled water. These explants were ready for surface sterilization.

Explants sterilization: The surface sterilization of explants was performed on laminar airflow. The different concentrations of sodium hypochloride (NaOCl) were used for the surface sterilization of explants. Stem tip, leaf tip and young flower buds were suspended in 0.1 to 1.0% NaOCl (sodium hypochloride) (W/V) solution for 1 minute to 10 minutes time duration. The explants inoculated aseptically on MS medium supplemented with various concentrations of auxin.

Culture room incubation: All the inoculated cultures of *Plantago ovata* G-2 were incubated in culture room. The temperature was maintained at 27+2°C. The cultures were kept in light for 16 hours (1000-4000 lux) and 8 hour dark respectively.

Growth of callus: The explants (new sprout bud segments) were cultured on MS media fortified with different concentrations of auxin. Days required for induction of callus were observed. Physical appearances of callus were noted after 40 days. Callus growth was measured. For dry weight, callus tissues were dried for overnight in oven at 65°C for 12 to 18 hours to remove complete water, then, the dry weight was taken. The data was recorded and analyzed statistically for all the observations in terms of fresh weight and dry weight after 40 days.

Embryogenesis in somatic cells and multiple shoot formation: The fresh calli were transferred to different modified MS media fortified with different concentrations of auxin and cytokinin either single or in combination, for the induction of somatic embryos.

Roots induction from isolated shoots: The individual shoots were inoculated on rooting media. The isolated shoots were washed thoroughly with double distilled water and transferred for rooting on full and half strength. MS medium was supplemented with different concentrations of auxin. The cultures were kept under 16 hours light (3000-3200 lux) and 8 hours dark. In individual shoot, root initiation was observed after 15 days of shoot transfer. Each treatment was repeated thrice and the observations for average numbers of roots were recorded after 20 days.

Hardening of *in vitro* raised bamboo plantlets: *In vitro* mature plantlets were about 4 to 6 cm tall, which were ready for the hardening. The plantlets were removed from the medium and roots were washed gently with double distilled water to remove the excess media from the roots. *In vitro* grown root systems appeared to be very delicate; therefore care was taken to retain all the fibre roots intact to the plantlets. These plantlets were transferred into different hardening media constituted such as 1) Soil: Sand, 2) Soil: Sand: Cow dung, 3) Soil: Sand: Cow dung: Vermiculite and 4) Soil: Sand: Cow dung: Biofertilizar (*Azatobactor*).

The temperature, humidity and photoperiod were maintained during hardening the *in vitro* grown plantlets. After 15-25 days, the plantlets were transferred into polythene bags with harden media. Then, the plants were transferred to green house. Throughout the hardening procedure the survival rate of the plant was measured. Observations of successfully grown plantlets were taken after 25 and 30 days and data were statistically analyzed.

RESULTS AND DISCUSSION: *In vitro* sprouts of *Plantago ovata* G-2 were cultured on MS medium supplemented with different concentrations of 2, 4-D for callus induction. The photoperiod was maintained 16 hours light and 8 hours dark and temperature 28°C ± 2°C. The observations were recorded in terms of fresh and dry weight of callus after 40 days of inoculation. The mean observations were recorded. The observations are presented in Table1 and Figure 1. The concentrations of 2, 4-D were used from 0.5 to 5 mg/l. The maximum mean fresh weight of callus obtained from 2.0 mg/l of 2, 4-D was 1.73 gm ± 4.1 in *Plantago ovata* G-2. The callus developed in *Plantago ovate* when MS medium was supplemented with 2.0 mg/l of 2, 4-D.

Table 1: 2, 4-D effect on induction of callus in *Plantago ovata* G-2 after 40 days inoculation.

Explants	Concentration 2,4-D mg/l	Average fresh weight of callus gm±SE	Average dry weight of callus gm±SE	Physical nature of callus* (Based on 25 observation)
In vitro, sprout of *Plantago ovata* G-2	0.0	1.34± 2.9	0.5 ± 1.1	F, YW
	0.5	1.52 ± 2.7	0.1 ± 0.9	F, C
	1.0	1.59 ± 3.9	1.3 ± 1.1	F, C, YW
	1.5	1.5 ± 1.7	1.1 ± 0.9	F, C, YW
	2.0	1.73 ± 4.1	1.3 ± 2.0	F, C, YW
	2.5	1.53 ± 0.9	1.0 ± 1.3	F, C, YW
	3.0	1.43 ± 1.7	0.9 ±1.0	F
	3.5	1.26 ± 1.4	0.8 ± 0.4	F, C
	4.0	1.23 ± 11.3	0.7 ± 0.3	F, YG
	4.5	1.69 ± 1.	1.0 ± 1.1	F
	5.0	1.6 ± 1.9	0.8 ± 0.6	F, C, YG
Observations based on mean of 30 explants ± SE				

*Abbreviations: * = visually observed; F = Friable; C = Compact; YW = Yellowish White; YG = Yellowish green*

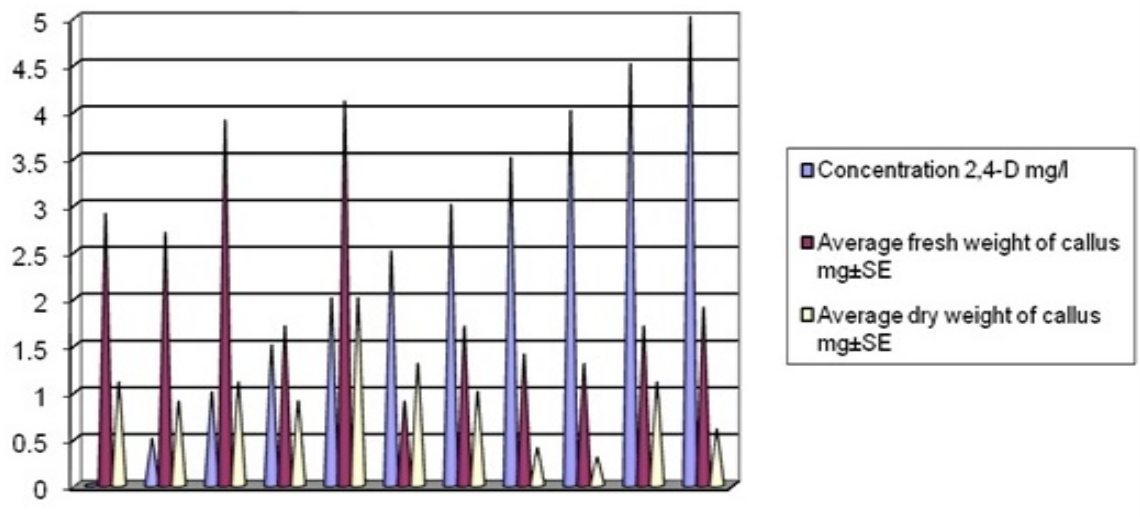

Figure 1: 2, 4-D effect on induction of callus in *Plantago ovata*, G-2 after 40 days inoculation.

The main aim of this study was to develop the embryogenic callus. Therefore, emphasis has been given to initiate the callus and weight of callus was taken as fresh and dry weight of callus.

In vitro sprout obtained from young flower buds were used as explants for the induction of callus. The physical appearance of the callus was also observed and is shown in the table. The physical nature of the callus gives indication for future development of the callus. If callus was friable and yellow white, it seems to be more healthy and durable in nature. It

remained in culture for longer period and continued its growth till the complete media exhaust. However, compact nature and brownish colour showed to be less responsive for further growth and finally the cell death occurred.

It has been observed from table -1, that the maximum average fresh weight of callus seems to be physically friable and yellowish white in colour. However, the compact brownish colored callus was found to be less in amount as compared to the healthy callus.

The known MS Murashige and skoogs Medium was used throughout the experiment. In the present investigation, when *In vitro* sprouts were cultured on MS medium supplemented with 2,4-D 2.0mg/l, they gave a small amount of callus weighing 1.73+-4.1gm of *plantago ovata* G-2 after 40 days of culture respectively (Table 1). Callus obtained was soft, white, friable, green and compact in nature. Other combination were also tested, but could not found satisfactory results.

CONCLUSION: In this research paper, 2,4-D effect was studied for the induction of callus in *In vitro* technology. The known MS Murashige and Skoog medium was used in this experiment. The growth of callus was measured in terms of their average fresh and dry weight after 40 days of inoculation of explants. Young flower buds were used as explants for the induction of callus. After 40 days of inoculation physical appearance of callus was observed for the growth of callus as it was shown in Table 1 and Figure 1. Future development of callus depends upon the physical nature of callus. The different concentrations of 2,4-D were used from 0.5 to 5mg/l. The maximum mean fresh weight of callus was obtained from 2.0mg/l of 2-4 D was 1.73gm ±4.1 in *plantago ovata* G-2. In the present investigation, when *in vitro* sprout were cultured on MS medium supplemented with 2,4-D 2.0 mg/l they gave a small amount of callus weighing 1.73 ± 4.1 gm of *Plantago ovata* G-2 after 40 days of culture respectively (Table 1).

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