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Adventitious Shoot Proliferation from Aseptically Germinated Seedlings of *Cuminum cyminum*

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ABSTRACT

In the present investigation a protocol has been standardized for enhancement of seed germination and regeneration from the various explants (epicotyl, hypocotyl and root) obtained from aseptically germinated seedlings. Seeds were treated with various physico-chemical factors like chilling, acid scarification, crushing and dry storage. The seeds were also given hormonal (Gibberellins and Benzyl Amino Purine) treatment. Explants from the aseptically germinated seedlings were inoculated on Murashige and Skoog's (MS) medium supplemented with IBA, IAA and Kinetin (0.5-5 mgl^{-1}). The seeds responded well after the chilling treatment (0-1°C) and germinated after one week on MS Basal medium. Best adventitious shoot proliferation was observed in epicotyl explants inoculated on MS medium supplemented with IBA, IAA, and Kinetin within two weeks. The protocol will provide a continuous supply of disease free cumin shoots through out the year, which can be used as a natural source of cuimaldehyde.

KEY WORDS: *Cuminum cyminum,* germination, Gibberellin, scarification, cuminaldehyde.

INTRODUCTION

Cuminum cyminum L. (Umbelliferae) is widely used in traditional system of medicine as an anti-oxidant (1), digestive, anti-spasmodic, anti-genotoxic, anti-hyperglycemic (2,8), galactogogue (3), anti-convulscent, diuretic and stomachic. It mainly consists of cuminaldehyde (7), cuminol, myricene, cymene, pinene and terpenes. This important medicinal plant is an allogamous crop and available variability in the crop is limited. It is highly susceptible to disease like wilt blight and powdery mildew from seedlings to maturity. The plant is also associated with seed dormancy problem. The embryogenesis (4) in plant tissue culture technique(5) help to rectify above problems.

MATERIALS AND METHODS

Materials

Cuminum cyminum Seeds were obtained from the local market of New Delhi and authenticated by Department of Botany, Faculty of science, Jamia Hamdard, New Delhi.

Seeds were checked for viability by taking a 100 ml flask half full of water and dipped seeds into it. The seeds, which were settled on the bottom, they were viable seeds and selected for the study.

METHODS

The seeds of cumin were treated with various physico-chemical factors and hormonal treatment on Murashige and Skoog (MS) basal medium The basal MS medium (Murashige and Skoog's, 1962) Table 5 with 3% sucrose and 0.8% agar was adjusted to pH 5.7 with 0.1 N HCL or 0.1 N NaOH solutions, using digital pH meter (Macro-scientific Work, New Delhi) prior to autoclaving. The basal MS medium was supplemented with the auxins (IAA, IBA, NAA and 2,4-D) and cytokinins (BAP or Kinetin) either alone or in combinations accordingly.

Preparation of Culture Medium

DDW and analytical grade chemicals were used to make stock solutions and medium. Stock solutions of the Macronutrients (10X), Micronutrients (20X), Vitamins (50X) and Iron Source (25X) were prepared separately accordingly Table 1, by dissolving all the constituents in a conical flask on a magnetic stirrer. Iron source was stored in amber colored bottle to prevent oxidization by light. Meso-inositol was prepared fresh at the time of medium preparation. IAA, IBA, NAA, 2, 4-D and BAP were dissolved in 95% ethanol and kinetin was dissolved in 1.0 N NaOH. Stock solutions of the growth regulators were made by dissolving 10 mg of each hormone in 10 ml of the respective solvents to get 1 mg/ml of the solution. All the stocks were stored at 4°c. The medium was prepared by adding stock solutions one by one, sucrose and growth regulator were supplemented as and when required. The ph was adjusted and agar was melted and added to the medium before pouring into culture

vessels (Glass flasks/tubes Borosil). The culture vessels were plugged with cotton plugs. The medium was sterilized in an autoclave at 121 °C under 15 lb/Square inch pressure for the 20 min. prolong autoclaving was avoided for it may lead to decomposition of the medium components. After sterilization tubes / flaks were kept for solidification of medium at 25 °c ± 2 °c in an aseptic room. The tubes were kept at an angle for slant formation during solidification

Physico-chemical treatment

Physico-chemical treatment includes chilling treatment, acid scarification, crushing and dry storage. In chilling treatment seeds were kept in refrigerator at temperature 0-1°C for 3 and 6 days then inoculated on Murashige and Skoog (MS) basal medium. In acid scarification the seeds were treated with sulphuric acid in various concentrations (100%, 50%, 25%) for 5 minutes and then inoculated. In dry storage the seeds were stored at 40°C and 50°c for 2 days. The seeds were slightly crushed and inoculated to reduce the time of germination.

Hormonal Treatment

Seeds were given hormonal treatment by dipping in hormonal solution (Gibberellin 1 and 2 mgl^{-1} and BAP 1 and 2 mgl^{-1}) for 2, 4, 6 hours. The seeds were surface sterilized with mercuric chloride solution (0.2%) for 5 min., followed by washings with sterile double distilled water for six times under aseptic conditions. Finally the seeds were dipped in 70% alcohol for 45 sec. and were inoculated on Murashige and Skoog (MS) medium. The 3% sucrose (w/v) added in the medium and pH was adjusted to 5.5-5.8, solidified with 0.8% (w/v) agar and autoclaved at 121°C for 15 min. All the cultures were maintained at 25° ± 2°C under a light intensity of 3,000 lux provided by cool white fluorescent lamps. After 12 days explants from the aseptically germinated seedlings were inoculated on Murashige and Skoog (MS) medium supplemented with Indole acetic acid, Indole butyric acid and kinetin in concentration ranging from 0.5-5 mgl^{-1}.either alone or in combinations.

RESULTS AND DISCUSSION

Germination of Seeds

Viable seeds were surface sterilized with mercuric chloride solution (0.2%) for 5 min., followed by washing with sterile DDW for six times under aseptic conditions. Finally the seeds were dipped in 70% alcohol for 45 sec. and then rinsed with sterilized DDW. The seeds responded well to various physico-chemical factors (Table 2). The best results were obtained after chilling treatment, followed by crushing of seeds. These particular treatments germinated the seeds after 7^{th} day with 75% germination rate (Fig.1) and 5^{th} day with 50% germination rate (Fig.2) respectively in contrast to germination, which normally takes 2-3 weeks.

Table 1: Composition of basal Murashige and Skoog's medium

S.No.	Constituents	Quantity mgl^{-1}
MACRONUTIENTS		
1.	Poassium Nitrate (KNO_3)	1,900.00
2.	Ammonium nitrate (NH_4NO_3)	1,650.00
3.	Calcium Chlorode ($CaCl_2.2H_2O$)	440.00
4.	Magnesium Sulphate ($MgSO_4.7H_2O$)	370.00
5.	Potashium dihydrogen phosphate(KH_2PO_4)	170.00
MICRONUTRIENTS		
1.	Boric Acid (H_3BO_3)	6.200
2.	Manganese Sulphate ($MnSO_4.4H_2O$)	22.300
3.	Potassium Iodide (KI)	0.830
4.	Zinc Sulphate ($ZnSO_4.7H_2O$)	8.600
5.	Sodium molybadate ($Na_2MoO_4.2H_2O$)	0.250
6.	Copper Sulphate ($CuSO_4.5H_2O$)	0.025
7.	Cobalt Chloride ($COCl_2.6H_2O$)	0.025
VITAMINS		
1.	Nicotinic Acid	0.500
2.	Thiamine Hydrochloride	0.200
3.	Pyridoxine Hydrochloride	0.500
IRON SOURCE		
1.	Ferrous Sulphate ($FeSO_4.7H_2O$)	27.800
2.	Disodium E.D.T.A. ($Na_2EDTA.2H_2O$)	37.300

Table 2. Effect of Physico-chemical Factors on Germination of Cuminum cyminum L

S.No.	Physico-chemical factor	Concentration	Contact Period	Observations A-germination period(Days) B-germination rate
1	Acid Scarification	100%	5 min.	A - --- B - ---
2	Acid Scarification	50%	5 min.	A - ---- B - ---
3	Acid Scarification	25%	5 min.	A - ---- B - ---
4	Dry Storage	40°C	2 days	A-12th B- 25%
5	Dry Storage	50°C	2 days	A-12th B- 25%
6	Crushing	Slight	-----	A- 5th B- 50%
7	Chilling	0-1°C	3 days	A- 9th B- 50%
8	Chilling	0-1°C	6 days	A- 7th B- 75%

Table 3.Effect of Hormonal Treatment on Germination of Cuminum cyminum L.

S.No.	Hormone treatment	Concentration (mgl^{-1})	Contact period	Observation A– germination period B-germination rate
1	BAP	1	5 min.	A-22nd B-25%
2	GA3	1	2 hr.	A-14th B-50%
3	GA3	1	4 hr.	A-14th B-50%
4	GA3	1	6 hr.	A-14th B-50%
5	GA3	2	2 hr.	A-12th B-75%
6	GA3	2	4 hr.	A-12th B-75%
7	GA3	2	6 hr.	A-12th B-75%

Table 4. Effect of IAA and Kn on Epicotyl Segments of Cuminum cyminum L.

Hormonal Combination (mgl^{-1})	1st week			2nd week			3rd week			4th week		
	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds
Control	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _
MS+IAA$_{(0.5)}$ +Kn$_{(2.0)}$	_ _ _	_ _ _	_ _ _	1	9	_ _ _	1	9	_ _ _	1	9	_ _ _
MS+IAA$_{(0.5)}$ +Kn$_{(3.0)}$	_ _ _	_ _ _	_ _ _	1	9	1	1	10	1	1	10	1
MS+IAA$_{(0.5)}$ +Kn$_{(5.0)}$	_ _ _	_ _ _	_ _ _	3	12	2	3	12	2	3	12	2
MS+IAA$_{(1.0)}$ +Kn$_{(2.0)}$	_ _ _	_ _ _	_ _ _	2	11	_ _ _	2	11	_ _ _	2	11	_ _ _
MS+IAA$_{(1.0)}$ +Kn$_{(3.0)}$	_ _ _	_ _ _	_ _ _	2	14	1	2	15	1	2	15	1
MS+IAA$_{(1.0)}$ +Kn$_{(5.0)}$	_ _ _	_ _ _	_ _ _	4	18	3	4	22	3	4	22	3

Table 5. Effect of IBA and Kn on Epicotyl Segments of Cuminum cyminum L.

Hormonal Combination (mgl^{-1})	1st week			2nd week			3rd week			4th week		
	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds	No.of Shoots	No.of Leaves	No.of buds
Control	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _
MS+IBA$_{(0.5)}$	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _
MS+IBA$_{(0.5)}$ +Kn$_{(0.5)}$	_ _ _	_ _ _	_ _ _	1	7	_ _ _	1	7	_ _ _	1	7	_ _ _
MS+IBA$_{(0.5)}$ +Kn$_{(1.0)}$	_ _ _	_ _ _	_ _ _	1	9	_ _ _	1	9	_ _ _	1	9	_ _ _
MS+IBA$_{(0.5)}$ +Kn$_{(2.0)}$	_ _ _	_ _ _	_ _ _	2	14	1	2	16	1	2	16	1
MS+IBA$_{(0.5)}$ +Kn$_{(5.0)}$	_ _ _	_ _ _	_ _ _	3	17	2	3	21	2	3	21	2

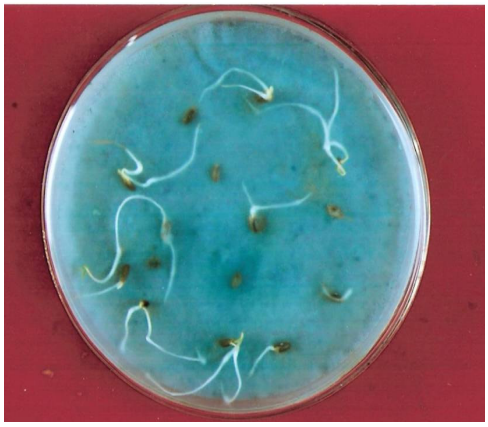

Fig:1 Germinated seedlings of Cuminum cyminum. Linn

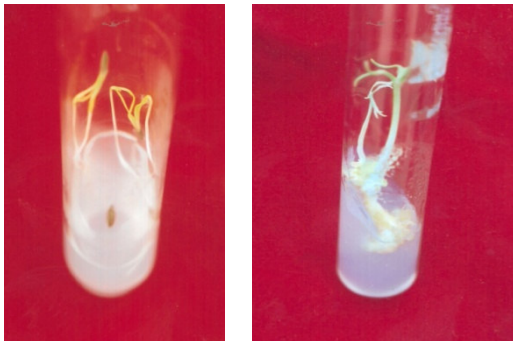

Fig: 2 and 3 Germinated seedlings of Cuminum cyminum Linn. On MS Medium

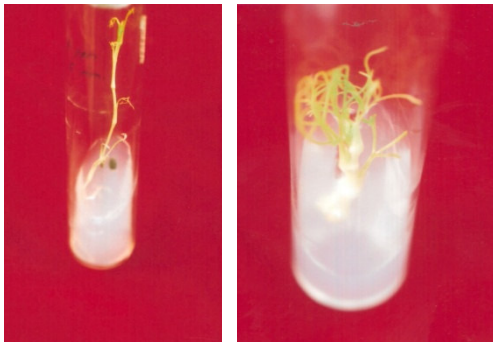

Fig:4 MS Medium + 1.0 mgl^{-1}IAA + 5.0 mgl^{-1} Kinetin ; Fig:5 MS Medium +0.5 mgl^{-1}IBA + 0.5 mgl^{-1}Kinetin

The seeds also responded to various hormonal treatments (Table 3). Gibberrelic acid when given in low concentration it responds slight but when concentration of gibberrelic acid increased up to 2 mgl^{-1} it gives better result. The seeds were germinated on 12th day with 75% germination rate (Fig.3).

The above results reveled that Acid Scarification has no significant value for seed germination because it damage the seeds of cumin. The hormonal treatment also gives some good response but chilling treatment is the best method for enhancement of seed germination.

Regeneration

Epicotyl segments obtained from aseptically germinated seedlings inoculated on MS medium supplemented with various combination and concentrations of IAA (0.5-1.0 mgl^{-1}) and Kinetin (1.0-3.0 mgl^{-1}) [Table 4] or IBA (0.5 mgl^{-1}) and Kinetin (1.0-5.0 mgl^{-1}) (Fig.5), [Table 5]. The best result was observed in combination of IAA (1.0 mgl^{-1}) + Kinetin (5.0 mgl^{-1}) (Fig.4). Epicotyl segment in this hormonal combination give four shoots with 18 leaves after 2nd week. The number of leaves increases with time up to 22 after 4th week.

Buds were also induced on MS medium supplemented with above hormonal combination after 14 days.The frequency of multiple shoots and shoot length was higher on medium containing Kinetin (5.0 mgl^{-1}), which subsequently decreased with further increase or decrease in concentration of Kinetin. IBA has no prominent effect on shoot proliferation in case of cumin callus but IAA shows excellent response.

CONCLUSIONS

Exhausted literature survey of germination of cumin reveled that there are lot of problems in germination of cumin seed. In this paper a protocol has been established to break the dormancy and enhance the germination of cumin seeds. Chilling of seed break dormancy up to 75% and the epicotyl segments obtained from aseptically germinated seedlings can be used as a source of producing a continuous supply of multiple shoots *in vivo* as well as *in vitro* condition.

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