Callus Induction and Caulogenesis of Spinach (Spinacia Oleracea) Plant by Different Plant Growth Regulators

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Abstract - In the last forty years the flow of biological discovery has swelled from a trickle into a torrent, driven by a number of new methodologies developed in Plant Tissue Culture technique. Most of the current interest in medicinal plants has stemmed from the fact that in the recent past, a number of promising drugs have been developed from these plants based on the traditional knowledge and the new insights gained by modern methods of research. Plant Tissue Culture is a collection of experimental method of growing large number of isolated cells, organs or tissues under sterilized and in controlled condition. It utilizes the property of totipotency of cells and plants. Spinach (Spinacia oleracea) belongs to the natural family Amaranthaceae. In this research work, our objective is to study the callus induction and Caulogenesis of spinach (Spinacia oleracea) plant by plant tissue culture method and also find out the affect of different type of plant growth regulators like 2,4-D, BAP, NAA under sterile laboratory condition.

Keyword: - Plant growth regulators, explants, caulogenersis, tissue culture, spinach.

Abbreviations: MS, Murashige and Skoog; NAA, –Naphthalene acetic acid; BAP, 6-Benzyl-amino purine; 2,4-D, 2,4-Dichlorophenoxyacetic acid.

I. INTRODUCTION

Spinach (*Spinacia oleracea*) is a leafy vegetable of the Chenopodiaceae family[1]. Spinach isdioecious and genetically is a diploid with 2n = 12chromosomes2. It requires longdays, moderately deep and highly fertile soil for its growth[3]. The leaves have expectorant properties and their juice is used catarrh and bronchitis. The preparation, also, is applied to the skin ringworm and their coetaneous disease. This plant is high nutrational value. It is rich source of vit-A, vit-C, vit-K, vit E, iron, magnesium, zinc, calcium, manganese, protein is an important vegetable crop of which dioecy in nature has madecultivar improvement difficult using traditional breeding methods; therefore, production of high amount of disease free spinach is critical. Use of Plant Tissue and Cell Culture can be an invaluable aid in the constant search for new drugs from as yet unexplored or underexplored plants. In spinach tissue culture, although callogenesis was described by[4-5] reported on the first adventitious bud formation only in 1973. Kumar and co worker studiedthe effects of NAA and BAP on spinach shooting and they presented that MS media containing 2 mgl-1 NAAwas the best media for shooting[6]. In addition, Knoll and colleague demonstrated that optimum shoot regeneration was from explants of apical and middle root regions on medium with 20 mM NAA and 5.0 mM GA3[7].

II. MATERIAL AND METHODS

Explants preparation:- Tender leaves and inflorescences from healthy plants of spinacia oleracea. were used in the present study. The explants were collected, washed thoroughly under running tap water for 15 min. Explants segments were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, followed by washings with sterile double distilled water inside the laminar airflow chamber to remove traces of $HgCl_2$.

Preparation of Plant Tissue Culture Media:- Gelling agent (agar-agar) was added as per requirement and the medium was steamed to melt the gelling agent. Heat labile constituents like antibiotics were filter-sterilized by passing through a Millipore membrane (0.22 µm pore size) ("Millipore Corporation", USA) and added aseptically to the autoclaved medium just before gelling. All the plant growth regulators used during the course of the present work were added before autoclaving the medium. Determined direct shoot formation from leaf explants of in vitro-grown seedlings using MS (Murashige and Skoog) medium containing IAA and BA[8]. Explants culturing: - Explants were cultured individually on MS containing different concentrations (0.25 – 2.0 mg/l) of 2, 4-D. The pH was adjusted to 5.6 ± 0.1. Culture maintenance: - All the cultures were maintained in a growth room under darkness and the temperature was maintained at 25 ± 2°C, with 50 - 80% relative humidity.

III. RESULT AND DISCUSSION

Callus formation was observed after 4 days of incubation. Plasmolysis in the explants material was observed. From

the 6th day onwards the callusing is initiated. After 14 days, callus has grown to a noticeable size (Figure-1). Different concentration of 2, 4-D were using and the size of the callus is best under 2 mg/lt concentration of 2,4-D. The size of the callus was 0.56 cm in diameter (Table-1).

Callus (green and dark brown in color)

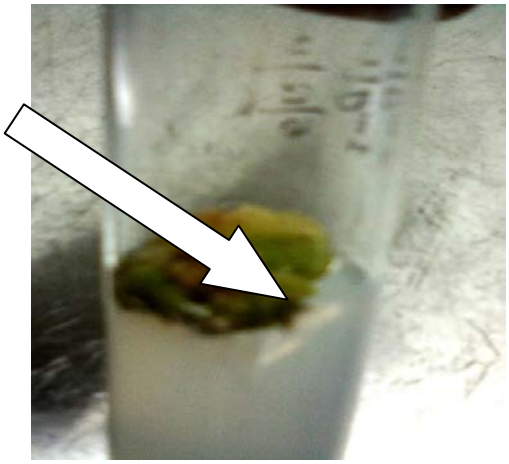

Fig 1. Callus induction from spinach plant (*Spinacia oleracea*)

Table 1. Callus growth under different concentration of 2, 4-D.

Concentration of 2,4-D (mg/lt)	Size of Callus (diameter in cm)	Callus positivity
1	Nil	-
1.25	0.22	+
1.5	0.28	++
2	0.56	+++
2.5	0.33	++
3	0.29	++

Callus obtained from explants was used for shoot culture on MS medium supplemented with different concentrations of NAA and BAP for caulogenesis (Table-2). Calluses were cultured individually on MS containing different concentrations of BAP and NAA. All the cultures were maintained in a growth room under 16hr photoperiod: 8 hr darkness and the temperature were maintained at 25 ± 2°C, with 50 - 80% relative humidity.

Table 2. Different concentration of NAA and BAP for caulogenesis from induced callus.

BAP (mg/lt)	NAA (mg/lt)
1	0
1	0.1
1.5	0.5
2	1

IV.CONCLUSION

In this part of the research work, we have tried to redifferentiate a dedifferentiated tissue. My goal was to regenerate the whole of the plant. The first step towards the same was supposed to be Caulogenesis, followed by rhizogenesis. The former requires a high concentration of cytokinin, while the latter demands a high concentration of auxin. In this part of the research work we just concentrated on shooting of the induced callus. For the same we have used BAP as the primary Plant growth regulator (Cytokinine), supported by some nominal amount of NAA as the Plant growth regulator (Auxin). Detailed concentrations of the same are mentioned above.

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REFERENCE

[1] Al-Khayri JM, "Genetic transformation in *Spinacia oleracea* L.(spinach)", In: Bajaj YPS (ed.) Biotechnology in Agriculture and Forestry. Springer, Berlin Heidelberg, New York, pp. 279–288, 1995.

[2] Khattak JZK, Torp AM, Andersen SB, "A genetic linkage map of *Spinacia oleracea* and localization of a sex determination locus", Euphytica, Vol. 148, pp. 311–318, 2006.

[3] Akhtar N, Ibrar M, Aman N, "The effects of different soaking times and concentration of GA3on seed germination and f growth of *Spinacia oleracea* L", Pak. J. Pl. Sci, Vol. 14 (1), pp. 9-13, 2008.

[4] Hildebrandt AC, Wilmar JC, John H, Riker AJ, "Growth of edible chlorophyllous plant tissues in vitro", Am. J. Bot, Vol. 50, pp. 248-254, 1963.

[5] Neskovic M, Radojevic L, "The growth of and morphogenesis in tissue cultures of *Spinacia oleracea* L", Bulletin de l'Institut de Beog VIII, Vol. 1(4), pp. 35–37, 1973.

[6] Kumar S, Kumar M, Kumar Mukherjee K, "Direct organogenesis in Indian spinach", Plant Cell Tiss. Org. Cult, Vol. 67, pp. 191–194, 2001.

[7] Knoll KA, Short KC, Curtis IS, Power JB, Davey MR, "Shoot regeneration from cultured root explants of spinach (*Spinacia oleracea* L.)", a system for Agrobacterium transformation. Plant Cell Rep, Vol. 17, pp. 96–101, 1997.

[8] Murashige, T. and F. Skoog, 1962, "A revised medium for rapid growth and bioassats with tobacco tissue culture", Physiol. Plant, Vol. 15, pp. 473-497, 1997.