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Micropropagation of *Trachyspermum roxburghianum* (DC.) Craib

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Abstract: The family Apiaceae occupies a prominent part in the angiosperms as it represents genus diversity rich in aromatic and medicinal properties. *Trachyspermum*, a genus included, is grown in parts of Asia much valued for its spicy seeds, both medicinally as well as in culinary. The genus has 25 or more species distributed in the temperate regions. *Trachspermum roxburghianum* (DC) Craib plant under study, can be propagated only through seeds and germination to produce healthy seedlings is season dependent. Attempts to produce plantlets irrespective of season achieved through tissue culture and the variants developed from several explants, exhibited *in vitro flowering also.*

Index Terms **- IBA, IAA, *in vitro* flowering, callus**

I. INTRODUCTION

India has one of the richest plant based ethnomedicinal traditions in the world. The western system of medicine is focussing its outlook to Indian systems as there is an alarming awareness of side effects of synthetic drugs along with the evolution of new strains resistant to antibiotics. The increased demand of medicinal plants for commercial utilisation has set an alarm on the conservation status of these exquisites. Habitat loss, genetic erosion, species loss are more distressing factors that has to be dealt with novel modus-operandi. In the present context, conventional breeding methods has to be complemented with modern ones. The inherent advantage of tissue culture over field propagation is the greatest plant production potential from a single plant (De Fossard, 1976). Variation has been a ubiquitious phenomenon associated with tissue culture of single plants (Carlson & Polacco, 1975). *In vitro* cultures therefore aids in formation of novel compounds as well as large scale production of substrates. The family Apiaceae is a large and widely distributed family and was the first to be recognized by taxonomists because of the characteristic umbel inflorescence and cremocarp fruits (Iyengar *et al.,* 1997). Because of their distinctive chemistry, reflected in odour, flavour esculence or toxicity, members of Apiaceae were familiar prehistorically to many people (Constance, 1971). The different properties of the family are due to the chemical principles, which exist in various proportions either in leaves, fruits or roots. Eventhough, the family is a repository of essential oils and oleoresins, only 10% of the known species have been investigated (Kubezcka, 1982). The plant, *Trachyspermum roxburghianum* (DC.) Craib is native to Asia minor and Africa. The plant is mainly grown for its seeds, used as spice; leaves substituted for parsley (Singh *et al.,* 1990). The plant is reported to possess cardiotonic, stimulant, carminative, anthelmintic, digestive and antiseptic properties (Agarwal, 1997). The plant can be propagated only by seeds and no vegetative

propagation is possible. Obtaining the plant in between the year is difficult as the seed germination is poor in the off-season. An alternative method is to perform microprogation that also helps to develop variants.

Plant tissue culture plays a significant role in basic research in the areas of plant pathology, plant physiology, plant metabolites and conservation (Espinosa-Leal *et al.,* 2015; Chandran *et al.,* 2020; Vidyagina *et al.,* 2021; Taalat *et al.,* 2021). Somaclonal variation under quantitative genetic control plays a pivotal role in crop improvement. In context of these ideas, micropropagation of *Trachyspermum roxburghianum* was attempted with leaves, node, internode, inflorescence axis, root and flower explants.

II. MATERIALS & METHODS:

Seeds of *Trachyspermum roxburghianum* (DC.) Craib were collected from the herbal Garden, Kottakkal, Kerala, South India. The seeds were sown and voucher plant specimens were authenticated at Calicut University Herbarium (CALI51311). Nodal segments, internode, peduncle, single flower were used as explants. Small sized explants cleaned and washed thoroughly with labolene, surface sterilized with 0.1% mercuric chloride, rinsed in double distilled water and implanted on nutrient medium. Murashige & Skoog (1962) basal medium with 3% sucrose, 100 mg/l myoinositol and 0.8% agar was used. The induction media included MS medium supplemented with different concentrations and combinations of auxins and cytokinins. The pH of medium was adjusted to 5.7-5.8; media sterilized at 120^0 C for 15 minutes. Each experiment was set up in 10-12 replicates and repeated twice. The cultures were grown in 25 ± 3^0 C with humidity 50-60% under fluorescent day light tubes emitting 2000 lux for 16/8 light/ dark period and were subcultured every 4-6 week.

Two- to four-week-old regenerated plants were subcultured to MS medium with different auxin concentrations for rooting and standardized. The rooted plants were removed from the tube and potted in the sterilized mixture of soil and sand (1:1). They were initially irrigated with ½ MS solution for one week. Established plants were transplanted to the field in pots and watered regularly. These plants were then utilised for further studies.

III. RESULTS:

The MS medium was used with varied hormonal combinations for initiating multiple shoot cultures. Among different explants used, positive response was shown by nodes, flower and inflorescence axis. The nodal segments were cultured on MS medium with combinations of BA and IAA, BA and 2,4- D, Kin with NAA, 2,4-D alone. Multiple shoot initiation was noted in 1 mg/l BA and 7 mg/l IAA. Frequency of shoots and percentage of initiation was lower in medium containing 1 mg/l BA and 8 mg/l IAA. Several other combinations were also used, but positive response was observed only with higher concentration of IAA and lower concentration of BA. The culture establishment was in the form of clustered shoots from the proliferated callus as well as shoot elongation from axillary buds of nodal explants. The use of KIN (1 mg/l) and NAA (6 mg/l) showed only meagre growth. 2,4-D (2 mg/l) showed profuse callus in nodal explants. These calli on transfer to MS medium fortified with BA (1 mg/l) and IAA (7 mg/l) showed multiple shoot development. Using NAA (2 mg/l) fortified in MS medium, callus proliferation was observed, but comparatively lesser than 2,4-D (2 mg/l). 2,4- D (1 mg/l) with BA (2 mg/l) had no effect on nodal explant. The callus obtained was nonfriable in nature. *In vitro* flowering was observed in plants regenerated from nodal explants in mS medium with BA (1 mg/l) and IAA (7 mg/l), both in plants regenerated from callus as well as axillary buds.

Flowers were used as explants and callus growth started within two weeks from the basal portion of the flower and later green patches appeared all over the flower. Flower explant in BA (2 mg/l) with IAA (8 mg/l) showed callusing but shoot development was less. KIN (1 mg/l) and NAA (6 mg/l) resulted in little response in *in vitro* development. BA (1mg/l) and IAA (7 mg/l) showed callusing and shoot development in 60% of cultures.

The inflorescence axis showed profuse callusing in 70% cultures with BA (1mg/l) and IAA (7 mg/l) followed by multiple shoot initiation and *in vitro* flowering. callusing was low in BA (2 mg/l) and IAA (8 mg/l).

The use of leaf, internode and root in MS medium fortified with BA (1 mg/l) and IAA (7mg/l) showed negative response in the case of development *in vitro.* There was inverse relationship between callus age and morphogenic differentiation. Maximum response was elicited from 2-week oold calli, while regeneration was poor from 6-7 week old calli.

The clumps of multiple shoots were separated and subcultured for multiplication. These were further inoculated for rooting on different concentrations of auxins, IAA and IBA. Profuse rooting was observed in MS medium containing IBA (3mg/l). 70% of cultures showed rooting profusely. Rooting was also observed in MS with IBA (5 mg/l) as well as IAA (3 mg/l), but lesser than IBA (3 mg/l). the cultures were mainly inoculated in MS with IBA (3 mg/l) for rooting, which were later transferred to sterile soil: sand mixture. About 76% survival was obtained in pots. The figures showing the results are shown below. The regenerants and parent were further subjected to detailed molecular and cytological analysis.

Fig. 1-4 : Nodal explant – callus formation; 5-9: Multiple shoot formation & inflorescence development; 10-15: Callus development in different combinations, root and shoot initiation

Fig. 16-19: Nodal explant – callus formation, axillary shoot formation; 20-24: callus formation, *in vitro flowering;* 25- 30: Axillary shoots development and *in vitro* flowering from axillary shoots

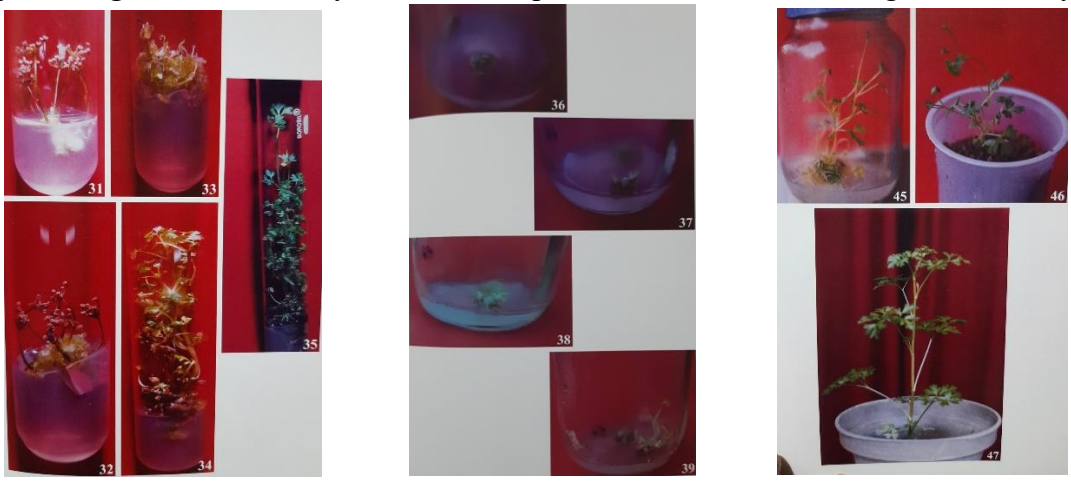

Fig. 31- 35: Peduncle- Callus formation, shoot development, i*n vitro* flowering; 36-39: Single flower explant- callus formation, shoot development; 45- 47: Root development, Hardening, I*n vitro* developed somaclone.

IV. DISCUSSION

Plant tissue culture have become a powerful tool for studying and understanding the basic and applied aspects in plant biotechnology. A new micropropagation protocol was developed to obtain the variant *in vitro* plant which showed variations in the cytological and molecular analysis, although no morphological variations were observed. Emergence of shoots directly from the cultured explants and from their callus will be useful in the propagation of true-to-type plants and also in the induction of variations, respectively. BA was more effective in shoot proliferation than KIN in the present study which was similar in similar works (Sen & Sharma, 1991).Chawla & Benzel, 1987 observed that shoot forming ability can be improved with auxin and Benzyl amino purine which was also in conformation with present study. Hu & Wang (1983) proved superiority of 2,4-D over other auxins for induction of callus and strongly antagonize any organized development. This is in agreement with present results as profuse callusing only was observed in 2,4-D. According to Von & Woodward (1988), Hakman & Fowke (1987) presence of auxin together with cytokinin is indispensable for induction and formation of organogenic callus , which is in conformity with the results of present study. Axillary shoot initiation observed which is supported by the fact that BA is the most effective synthetic cytokinin for stimulating axillary bud proliferation for different plant systems (Gangopadyay *et al.,* 1998). Sharief & Jagadishchandra,1999 hypothesised that the balance of growth regulators as well as their concentration is critical in determining the direction of morphogenesis, which is in conformity with the present study. Immature inflorescence has been recognised as an important source of totipotent cultures in many dicots and monocots (Eapen & George, 1997). The inflorescence explants and isolated flower buds of turmeric differentiated to plants under *in vitro* conditions (Salvi *et al.,* 2000) which was in conformity with results observed in the present study. Since floral buds are modified vegetative buds, it is quite likely that immature floral buds have capacity to revert back to vegetative buds under appropriate *in vitro* conditions (George & Sherrington, 1984). *In vitro* flowering was observed from nodal explants, peduncle via callogenesis resulting in plantlets as well as directly from axillary bud developed in MS medium with IAA (7 mg/l) and BA (1 mg/l). The observations that different kinds of cytokinins have different effect on *in vitro* flowering has also been made by Wagner *et al.* (1989). Flower bud initiation frequently observed in the present study, from the vegetative tissue cultured *in vitro* is consistent with many of the earlier reports (Reddy & Naraimhulu, 1988). The tissue culture process could cause the apex to undergo a morphogenetic change, which results in the formation of pseudo-inflorescence as an evocation of phenotypic plasticity rather than as a clear-cut transition from juvenile to mature stage, characterized by flowering (Giellis, 1999). IBA generally employed in rhizogenesis (Pijut Pula *et al.,* 1994) was effective in the present study in inducing rhizogenesis.

Any step towards understanding the basis of tissue culture induced genetic variations should be helpful in developing more stable and manipulable somatic cell systems.

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