



Standardization of Callus Induction Protocol From The Petals and Sepals of Garden Rose

Abstract: Roses are propagated *in vitro* because of their widespread acclimation for being an ornamental plant as well as for the compounds derived from them. In the present study, we made an attempt to induce callus from the petals and sepals of garden rose using MS media which was supplemented with different concentrations of hormones 2,4-Dichlorophenoxyacetic acid [(2,4-D)] and kinetin. The maximum induction rate of callus from petals were observed in MS media supplemented with 1.5 mg/l 2,4-D and 0.5 mg/l kinetin. Callus was successfully generated from sepals inoculated in MS media supplemented with 1.5 mg/l of both 2,4-D and kinetin as well as MS media supplemented with 2.5 mg/l 2,4-D and 0.1 mg/l kinetin.

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1. Introduction

Plant tissue culture has widespread applications in clonally propagating leading varieties of plants, producing virus-free plants, conserving germplasm, producing secondary metabolites and to conserve endangered species. Rose is a popular plant which is propagated *in vitro* because of their widespread acclimation for being an ornamental plant as well for the compounds derived from them. Belonging to the Rosaceae

family within the genus *Rosa*, rose is a woody perennial which is often referred to as 'the queen of flowers'. Owing to its beauty, fragrance and flavor, it is commercially used for the production of essence, oils, perfumes and cut flowers. With more than 200 species and 20,000 cultivars, the genus *Rosa* is extensively dispersed in Asia, Europe, North America, and the Middle East ([Hazrat Belal et al 2016](#)). Different parts of the rose plant, such as flowers, leaves, and bark, have the potential to be utilized in a variety of product development sectors, ranging from cosmetics and food to pharmaceuticals. The results of the biochemical analysis unveiled that rose petals are rich in vitamin C and antioxidants. This discovery emphasizes the potential worth of roses as a viable food option ([Vukosavljev et al 2023](#)). The application of roses ranges to the production of numerous food products, which include jams, jellies, cookies, salads, ice-creams, juices, and wines ([Hegde et al 2022](#)). Rose is generally used in the floriculture and cut flower industry throughout the world. India ranks first by contributing 46.54 per cent area under cut rose production in the world ([Bhagat et al 2019](#)). Major production of roses in India is from Karnataka, Tamil Nadu, West Bengal, Madhya Pradesh, Andhra Pradesh, Orissa, Bihar, Haryana, Maharashtra, Chattisgarh, Jammu and Kashmir, Punjab and Gujarat ([R. Govindasamy et al 2023](#)). Presence of noteworthy amounts of anthocyanins, polyphenols, and flavonoids were observed in



rose petal extracts. It was found that the skin anti-inflammatory activity of rose petal extracts is due to its antioxidant properties (Lee et al 2018). This feature makes it exceptionally valuable in the cosmetic industry. Apart from these, roses can also be used for therapeutic purposes. Rose aromatherapy has been proven for relieving pain related to dysmenorrhea and for having a positive impact on anxiety reduction and enhancing the sleep quality of patients. Rose extracts also have anti-diabetic, antimicrobial and seizure reducing properties (Wang, 2023).

In the present study, our aim is to standardize a hormone concentration, namely 2,4-Dichlorophenoxyacetic acid (2,4-D) and kinetin in MS media for callus induction from the petals and sepals of garden rose. By standardizing the media, we will be able to develop a large number of plantlets via tissue culture, thus increasing rose production.

2. Materials And Methods

Media Preparation

Ready to use MS media was prepared and used as per manufacture's instruction. For finding out the optimal media for induction of callus from petals and sepals, the explants were inoculated in MS media supplemented with two growth regulators, 2,4-D and kinetin, either alone or in combination at various concentrations.

The initial set of media concentrations, prepared solely with 2,4-D, were 1mg/l, 1.5 mg/l, 2.0 mg/l, and 2.5 mg/l. A concentration of 1.5 mg/l was achieved in the second set of media, which was prepared exclusively with kinetin. The third set of media concentrations were prepared by utilizing both 2,4-D and kinetin in the following ratios: 1.5 mg/l 2,4-D and 0.5 mg/l kinetin, 1.5 mg/l of both 2,4-D and kinetin, and 2.5 mg/l 2,4-D and 0.1 mg/l kinetin.

The culture media was transferred to tubes and subjected to autoclaving at a temperature of 121°C and a pressure of 15 lbs. for a duration of 15 minutes.

Collection of explants and surface sterilization

The petals and sepals of fully bloomed garden roses were collected. Once the procurement of flowers was done, measures were taken to ensure that the flowers remained sterile. The explants (petals, sepals) were meticulously washed with tween 20 (5%) and then rinsed three times with distilled water. Subsequent sterilization was carried out in aseptic conditions within a laminar airflow chamber. Following this, the explant was exposed to 0.1% mercuric chloride for two minutes and then washed three times with sterile distilled water. The final step involved treating the explant with 70% ethanol for one minute, followed by a thorough rinse with distilled water to eliminate any residual ethanol. Finally, the petals and sepals were transferred to a petri dish containing blotting paper to remove excess moisture.

Explant inoculation

Following the transfer, the petals and sepals were precisely cut to the required sizes using a sterile blade. The explant pieces were then introduced into the culture media using forceps that were sterilized by dipping them in absolute ethanol after every use. Subsequently, the inoculated tubes were placed in a culture environment with 24-hour light and humidity conditions.

3. Results And Discussion

Callus induction in petals

Out of the three medias in which the petals were inoculated, callus was induced in the MS media formulation containing 1.5 mg/l 2,4-D and 0.5 mg/l kinetin. The callus was formed

within 7 days and had a greenish and friable appearance. In the rest of the media formulations, the result was negative. Petal derived callus was generated from many plant species including hybrid rose varieties. In our

study, callus was formed within a week, and it was later sub cultured. These calluses could be used for shoot and root regeneration.



Fig 1: Callus growth from petal explants on MS medium supplemented with 1.5 mg/l 2,4-D and 0.5 mg/l kinetin

Callus induction in sepals

Callus from sepals were successfully induced in two media formulations. The first one was 1.5 mg/l 2,4-D and 1.5 mg/l of kinetin in MS media. The callus only began to form 32 days after inoculating the explant and appeared to be pale green and friable.



Fig 2: Callus beginning to form on sepal explants after 32 days on MS media supplemented with 1.5 mg/l 2,4-D and 1.5 mg/l kinetin

The second formulation of MS media in which sepal produced callus was 2.5 mg/l 2,4-D and 0.1 mg/l kinetin. The callus was formed after 14 days and showed a pale yellow and friable appearance. Though sepal samples were least studied in roses, this ratio of 2,4-D and kinetin was found to be better in inducing callus. Callus initiation is usually accompanied by supplementation of suitable callus induction hormones. Growth-supporting medium and sterile conditions are obligatory for its growth and development. Exogenously supplied growth regulators present in the nutrient

medium influence callus formation from the explants.



Fig 3: Callus beginning to form on sepal explant after 7 days on MS media supplemented with 2.5 mg/l 2,4-D and 0.1 mg/l

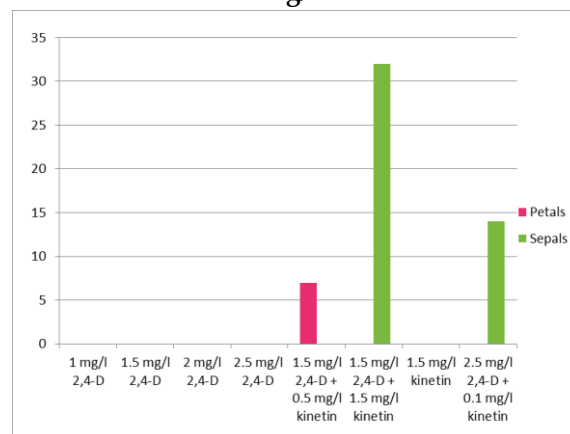


Fig 4: Graph showing days taken to produce callus from the explant inoculated on MS media supplemented with different concentration of growth regulators

Tissue culture plays an important role in developing large number of plants. The initial development of callus is a crucial stage in most



tissue culture experiments. Callus cultures possess significant commercial potential due to their diverse range of applications. It is very much useful in producing secondary metabolites at a larger scale in pharmaceutical industry. In the horticulture industry, ornamental flowers can be genetically modified by transforming the embryogenic calli with *Agrobacterium tumefaciens* where its genes encoding for color, shelf life and resistance against disease could be altered (Efferth, 2019). As roses have a distinguished position in the horticulture field and in various sectors ranging from cosmetics to pharmaceuticals, it is necessary to develop a system where mass propagation of roses is possible in a short time, independent of seasonal variations. This is made possible by callus culture. In regards to the different combinations of plant growth regulators used to investigate the effect of callus initiation, it was observed that a concentration of 1.5 mg/l 2,4-D and 0.5 mg/l kinetin in MS media resulted in a maximum induction rate of callus from the petals of garden rose. In case of sepals, MS media formulations with equal concentration of 2,4-D and kinetin (1.5 mg/l) proved to be effective in inducing callus formation (32 days). A faster rate of callus induction was observed (14 days) when 2.5 mg/l of 2,4-D and 0.1 mg/l of kinetin were incorporated into the MS media. In vitro propagation via callus offers great potential where a complete plant can be generated. In the present study, we were able to standardize a medium for the development of calluses from the sepals and petals of rose flowers, which could pave the way for the horticulture industry to produce a large number of plantlets.

Future Prospects

The standardised protocol will lead way to develop tissue cultured rose plants from the floral parts which benefits the horticulture sector.

Conflict of Interest. There is none among the authors

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