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Research Article

In Vitro Regeneration of Acid Lime using Axillary Bud

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Abstract

The present study explores an efficient in vitro regeneration protocol for Citrus aurantiifolia (acid lime) using axillary buds as explants. Acid lime is a commercially significant fruit crop, but its conventional propagation faces challenges due to its slow growth and susceptibility to diseases. In this context, tissue culture techniques offer a promising alternative for rapid and disease-free plant propagation. Axillary buds from mature, healthy acid lime plants were excised and cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators, including cytokinins and auxins, to optimize shoot proliferation and root induction. The highest regeneration rate was observed with MS medium containing 2.0 mg/L BAP (6-benzylaminopurine) and 0.5 mg/L NAA (naphthaleneacetic acid), resulting in efficient shoot multiplication. Rooting was successfully achieved on a medium with 1.0 mg/L IBA (indole-3-butyric acid). The regenerated plants were acclimatized and successfully transferred to the field with a high survival rate. This study demonstrates a reliable and reproducible method for acid lime micropropagation, which can be applied for large-scale commercial production and genetic improvement programs.

Keywords: Regeneration; Acid Lime; Axillary Bud

Introduction

The Citrus species is one of the most important commercial horticulture crops in the world, cultivated in more than 140 countries mainly in the Mediterranean regions of the world (Pandey and Tamta, 2016). Citrus fruits are extensively known for their fine distinctive flavour packed with nutritional and medicinal properties (Jardak *et al*., 2020). The fruits are particularly termed as relished fruits with great amount of vitamin C, vitamin A, amino acids and flavonoids alongside other bioactive compounds with beneficial health-promoting properties (Singh *et al*., 2019). Lemons are the third most important Citrus crop, is widely recognized due to its refreshing flavour and prominent health benefits (Giron´es-Vilaplana *et al*., 2012). Citrus fruits are considered as the number one fruits of the world due to their high nutritional value, great production potential and preparation of large number of fruit products from them. Citrus species are cultivated in most tropical and subtropical regions of the world. They are very attractive due to their distinctive fruits, colors, and attractive smell, unique from other plants. Containing high amounts of vitamin C, they can be consumed raw or extracted for production of highly nutritious beverages. Citrus species can also be used as traditional medicine, whereby the smell of citrus leaves and fruits can overcome headache and nausea (Azim *et al*. 2013). They are widely used to prevent flu and colds and support the immune system (Dhanawade *et al*. 2011). Citrus fruits also used for patients susceptible to health problems such as gastritis, fever and arterial sclerosis. The juice of lemon used in the pharmaceutical industry since it contains a high quantity of citric acid and essential oils (Bansode and Chavan 2012). There are also reports about positive effects of lemon fruits against cancer of gastrointestinal and upper respiratory tracts (Foschi *et al*. 2010) [1-6].

Material and Method

Cultural media

The formulation or the medium on which the explant is cultured is called culture medium. It is composed of various nutrients required for proper culturing. Different types of plants and organs need different compositions of culture media. For this experiment Murashige and Skoog media is decided to use as culture m Constituents of MS media with roles.

Inorganic nutrients

Micronutrients

The essential micronutrients (minor elements) for plant cell and tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo Chlorine (Cl).

- **Iron (Fe):** Involve in photosynthesis, redox reaction. Iron is usually added inalong with a chelator. EDTA is usually used in conjunction with the iron sulphate.
- **Manganese (Mn):** Plays an important role in the Hill reaction of photosynthesis. required in many enzymatic activities. Supplied as Manganese Sulphate
- **Zinc (Zn):** Zn plays an active role in protein synthesis, enzyme cofactor, tryptophan synthesis. Used as Zinc sulphate.
- **Molybdenum (Mo):** Essential for conversation of Nitrate to Ammonium. Supplied as Sodium molybdate.
- **Copper (Cu):** Important role in photosynthesis, respiration, enzyme cofactor for SOD, cytochrome oxidase. Supplied as Cupric Sulphate.

Sterilization of glassware

The glassware's such as culture bottles and the other equipment like forceps, and scalpels wash in running tap water using detergent followed by rinsing with double distilled water and then wrap in paper and subsequently autoclave at 121°C at 15psi pressure for 20 minutes.

Preparation of stock solutions

Separate stock solutions of macronutrients, micronutrients, iron, glycine and various vitamins prepare by dissolving each chemical separately in small quantity of double distilled water and making up the require volume with double distilled water. The stock solutions of growth regulators prepare by dissolving them in small quantity of appropriate solvents, heating gently and then making up the volume with double distilled water.

1.	Macronutrients	50ml
2.	Micronutrients	1ml
3.	Potassium iodide	1ml
4.	Vitamin	1ml
5.	Glycine	1ml
6.	Meso-Inositol	2ml
7.	Agar-Agar	2.5gm
8.	Iron	5ml
9.	Sucrose	30gm

Table a

Figure a

Hormones for acid lime

Preparation of MS medium

Murashige and Skoog medium is a plant growth medium used in the laboratories for cultivation of plant cell culture include different organic (vitamins) and inorganic salt, major salts (macronutrients), minor salts (micronutrients) nutrient and iron stock (Murashige and Skoog (1962). 30g of sucrose in approximately 200 ml in double distilled water. After dissolution of sucrose the stock solutions were added in following order and mixed well.

- Stock solution A (macronutrients) - 50 ml
- Stock solution B (micronutrients) - 1 ml
- Stock solution C (Iron) - 5 ml
- Stock solution D (vitamin) - 1 ml
- Stock solution F (glycine) - 1 ml

Then growth hormones add. The total volume makes up to 1000 ml by addition of distilled water. The pH of liquid medium adjusts at 5.6 to 5.8 with the help of 1N NaOH or 1N HCl. Heat the solution, at the time of heating add cleri gel. After dissolving cleri gel autoclave the media. The culture medium poure into culture bottles (25-30 ml in each bottle approximately)

Surface sterilization of explants

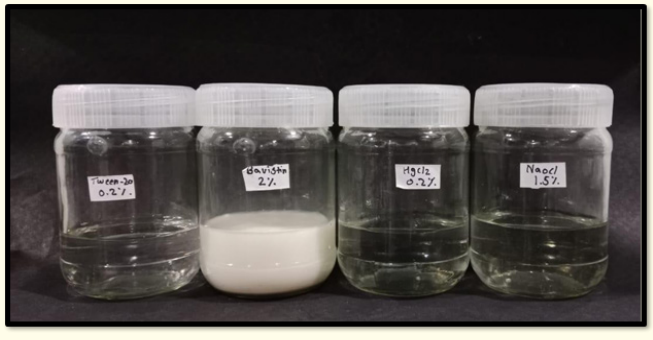

Figure b

The explants cut into pieces of approximately 1-2 cm. Wash with distilled water for 5 minutes. This is follow by treatment with 0.2% Tween20 for 10 minutes. Washe again with distilled water. Explants were then treat with 1% Bavistin for 15 min. After that, it wash with distilled water. Then treat with 0.1% HgCl2 for 10 minutes. Wash again with distilled water. Then treat with 1.9% NaOCl for 5 minutes.

Inoculation of explants

All inoculations and aseptic manipulation carry out in a laminar air flow cabinet. Laminar air flow (LAF) switches ON and power switch press to turn ON power in system, then blower switch on. Once the blower is turn ON, press the UV light switch to start the UV for a minimum 15 minutes before starting the work. The working surface of the laminar air flow cabinet clean by swabbing with 70% ethyl alcohol. The instruments like scalpel, forceps sterilize by an alcoholic dip and place in glass beed sterilizer inside the laminar airflow cabinet. After surface sterilization, explants transferred to sterile tissue papers with the help of sterile forceps under strict aseptic condition in laminar airflow cabinet. The explants cut into very small pieces with sterile scalpel. The bottles containing initiation medium prepare as given in, the inoculations performe by placing explants on the surface of the medium with the help of sterilized

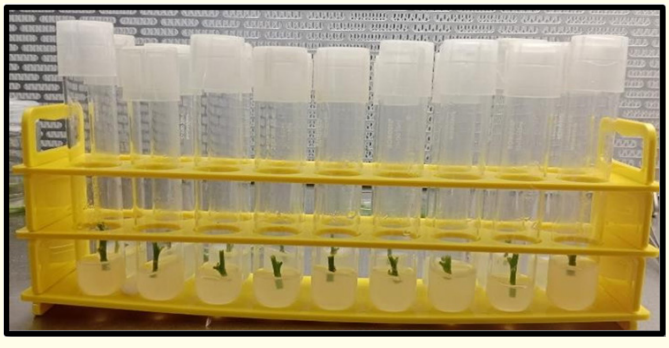

Figure c

long forceps and replacing the cap of the bottle. During inoculation the explants properly position on the medium and gently presses with forceps to secure their firm contact with the medium. After vertically inoculating explants into culture bottles, mouth of the bottles was tightly cap end and properly seal with kiln film to avoid entry of external air. Labeled the tray with marker. The bottles transfer to growth room. Record the data after three weeks of initiation in the terms of average shoot length (cm), average number of shoots per explants and days require for sprouting.

Result

Treatment	Medium + Hormones	No. of Test Tubes Inoculated	No. of Test Tubes Showing Results of Initiation	Response %
T1	Only medium	18	4	22%
T2	0.5 BAP mg/l + 0.2NAA mg/l	18	8	44%
T3	1.0 BAP mg/l + 0.4 NAA mg/l	18	12	66%
T4	1.5 BAP mg/l + 0.6 NAA mg/l	18	17	94%

Table b

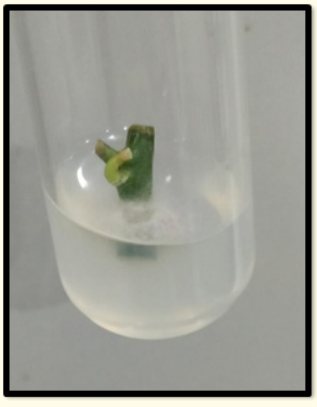

Figure d: T1- Only medium.

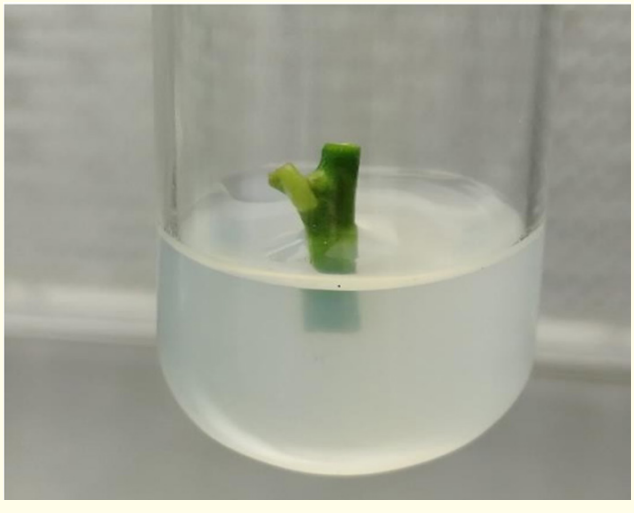

Figure e: T2- BAP 0.5mg/l + NAA 0.2mg/l.

In vitro shoot multiplication of *Citrus aurantiifolia*

Treatment	Medium + Hormones	No of bottle inoculated	Average number of leaves per shoot
T1	0.5 BAP mg/l + 0.2NAAmg/l	12	0
T2	1.0 BAP mg/l + 0.4 NAAmg/l	12	2
T3	1.5 BAPmg/l + 0.6 NAAmg/l	12	2.8

Table c

Figure f

Conclusion

The present study was carried out at Krishi Vigyan Kendra (KVK) Baramati. After two weeks of initiation response was evaluated by measuring parameters such as number of shoots, length of shoots and days required for sprouting. After three weeks of initiation, the highest growth was obtained when the MS medium was supplemented with different concentration of growth hormones. In the effect of hormones, increasing the level of BAP and NAA from 0.5mg/l + 0.2 mg/l to 1.5 mg/l + 0.6 mg/l has increased shoot numbers and shoot length.

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