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Direct regeneration of shoot from axillary bud of *Citrus reticulate*

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Abstract: Young axillary buds of mature plant of *Citrus reticulata* were cultured on MS medium (1992) containing 6-Benzyl amino purine (BAP) 0.25, 0.50,1.00,1.50 & 2.00 mg/l. The best results for multiple shoots were obtained with 1.50 mg/l. The highest degree of Callus was induced on BAP 1.00 mg/l plus NAA 0.25 mg/l. The transfer of multiple shoots on rooting medium containing NAA 0.25 mg/l induced good rooting.

Key words – *Citrus reticulata*, multiplication, growth regulator, explants.

Abbreviations - BAP – Benzyl amino purine, 2, 4-D – 2, 4 Dichlorophynoxyactic acid, MS – Murashige and Skoog (1962), NAA-α-napthalene acetic acid.

Introduction

Citrus reticulata is a member of the family Rutaceae as an important evergreen and aromatic small tree. Its fruits are an important source of vitamin C for human nutrition. They also act as an antiseptic, antirheumatic, antibacterial and antioxidant (Rathore *et al*,2007). It is the number one fruit of the world due to its high nutritional value considerable production of the fruits industry (Chaturvedi *et al*,2001).The Citrus varieties are propagated by both sexual and

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a sexual methods. Generally rootstocks are propagated sexually through seeds, while most of the commercial varieties are propagated by various sexual methods.Commercial cultivars are commonly propagated by air layering or grafting on seedling rootstock and therefore the genetic conservation of commercial rootstock should also be considered(Marin and Duran-vila,1991).The Citrus are generally propagated by traditional methods like budding and grafting. Therefore, there is possibility of virus transmission from the mother plant to the propagated plant. However, *in vitro* micro propagation technology can overcome some constraints to citrus improvement and cultivation and can increase fruit quality and resistance to disease and environmental stresses. *In vitro* micro propagation of *C.aurantifolia* was reported by AL-khayri and AL-Bahrany. Chaturvedi *et al* have comprehensively reviewed the micropropagation of citrus, indicating that result of practical importance is meager.In most cases, the limiting factor has been the difficulty in tissue while maintaining Clonal fidelity. This study was conducted to identify the best type of explants and growth regulator concentration and combination for shoot proliferation callus induction and rooting of regenerated shoots.

Materials and methods:-

Explant Preparation

A high quality fruit, yielding, 10-12year old field plant of *Citrus reticulata* was selected. Few of the branches of the plant were pruned during the month of October to December. Fresh shoot–sprouts were harvested during January to March of the subsequent year and modal shoot segments were used as explants. The explants were disinfected with 0.5% Tween-20 liquid soap solution for 10-15 minutes, then explants were treated with 0.5% $HgCl_2$ solution for 5-6 minutes. After rinsing several times with autoclaved water, the surface sterilized explants were inoculated on MS medium.

Shoot multiplication :

The shoot induction and multiplication was performed on MS medium containing 3% sucrose with different concentrations of BAP (0.25, 0.50, 1.00, 1.50, 2.00 mg /lt) individually.

Callus formation

Callus induction was initiated in 25-150 mm culture tube containing MS medium with 3% sucrose and various concentrations BAP of and NAA. In combination then explants were used for each treatment. Visual observations were taken every three days. The effect of different treatment was quantified on the basis of percentage of shoots showing response for rooting.

Rooting of regenerated shoot

Rooting was performed in culture tubes containing 5 ml of MS medium containing 3% sucrose and solidified with 0.8% agar having different Concs. of NAA individually from 0.25 to 2.00 mg/lt. The 10–15 regenerated shoots were cultured for rooting. Visual observations were taken every three days and the effect on different shoots was quantified on the basis of percentage of shoots showing response for rooting.

Results

Initiation and multiplication of shoots :

Dircet shoot induction was accomplished from young axillary buds on MS medium (1962) in a range of concentration of BAP (Table 1). Out of several concentrations of BAP tested it was found that (1.5 mg/l) was the most effective growth regulator for eliciting shoot formation (Fig. A). About 5-6 number of shoots were derived within 25 -28 days and the regenerated shoots attained a height of 8-10cm within two weeks .Transfer of these multiple shoot on MS medium with same rate of shoot multiplication. The subculture of *in vitro* obtained shoots at an interval of days produced 625 shoots with 100 days .An increase in the concentration of BAP to 2mg /lt. reduced the number of shoots .

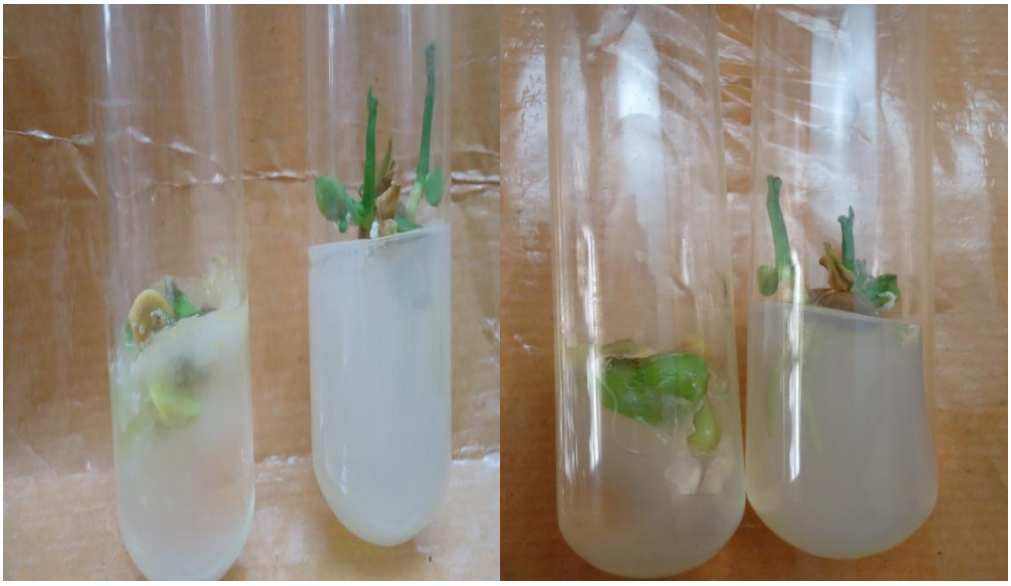

Fig.a Multiple shoots induction on 1.50 mg/l BAP from axillary bud C. *reticulata*

Table1: Effect of MS medium containing different conc. of BAP on shoot growth from axillary buds of *citrus reticulata.*

S. N.	BAP conc. (mg/l)	Shoot induction (%)	No of shoots/ Explant (Mean ± SE)	Shoot length(cm) (Mean ± SE)
1	0.0	0.0	0.0	0.0
2	0.25	20	0.20±0.02	1.20±0.10
3	0.50	40	1.40±0.12	3.20±0.31
4	1.00	80	3.40±0.24	9.58±0.31
5	1.50	20	1.20±0.20	1.12±0.12
6	2.00	0.0	0.0	0.0

Callus formation

When explants were inoculated on MS medium supplemented with BAP in combination NAA. The callus formed on the basal part of the explants containing the meristimatic tissue. The callus was soft in texture and friable in structure. The callus did not form on medium lacking growth regulators. The callus formation was greatest on the medium containing BAP 1.00 mg/l plus NAA 0.50 mg/l. The addition of BAP or NAA alone did not produce callus. The high conc. of BAP with NAA also inhibited the callus formation. The sub cultured callus grew satisfactorily on revised MS medium supplemented With BAP+NAA same concentrations. (Table II). Proliferated callus was structurally indistinguishable from the initially induced from the shoot tip explants.

Fig.b Callus induction on 1 mg/l BAP + 0.50 mg/l NAA in *C. reticulata*

Table2: Effect of MS medium containing different concentrations of BAP and NAA on callus formation from axillary buds of *C. reticulata*

S.N.	BAP Conc . (mg/l)	Conc of NAA (mg/l)	Morphological response

1	0.0	2.00	Nil
2	0.25	1.50	Nil
3	0.5	1.00	Slight callus
4	1.00	0.50	Proliferated callus
5	1.50	1.00	Nil
6	2.00	0.0	Nil

Root induction :

The root induction was observed within three weeks after transfer of isolated multiple shoots on rooting medium. (Fig.c).The results indicate that the NAA concentration supplied to MS medium signification influenced the root formation. The best rooting treatment was 1.0 mg/l NAA. Since it gave the highest percentage of root induction (Table III). The percentage of rooting ranged from 20– 80%.

Fig.c Various stages of *C. reticulata* from multiple to rooting.

Table3: Effect of MS medium containing different concentrations of NAA on root formation and root length from isolated plantlets of *C. reticulata*

S. N.	Conc. Of NAA (mg/lt)	Root Formation (%)	Number of roots/Explant (Mean± SE)	Root length(cm) (Mean±SE)
1	0.0	0.0	0.0	0.0
2	0.25	80.0	2.21± 0.02	1.68± 0.20
3	0.50	40.0	1.12 ± 0.20	6.35 ± 0.24
4	1.00	20.0	0.60 ± 0.02	2.80 ± 0.18
5	1.50	0.0	0.0	0.0
6	2.00	0.0.	0.0	0.0

Discussion:

The present study was designed to identify the ideal condition for micro propagation of *C. reticulata* (orange) because not much work has been done on the tissue culture and micro propagation of this plant. Citrus seeds have a very short life because they are injured by drying during storage and thus loose their viability.

We tried various concentrations and combination of hormones for shoot multiplication ,callus induction and root formation. The MS medium supplemented BAP 1.5 mg/lt for shoot multiplication from axillary bud explants. This result was a similarity with the results of Costa *et al* (2002). And correlated results with shawkat Ali and Bushra mirza (2006). BAP was required for shoot bud activation and multiple shoot formation from nodal explants. A Superiority of BAP as cytokinin over kinetin has been recorded in many woody plants. BAP is more stable, less expensive cytokinin than the others (Rathore *et*

al,2007). Cytokinin induce bud break by activation of meristems and cause shoot proliferation (Murashige , 1962).

Axillary bud explants is best in our investigation. The outgrowth of axillary bud is well correlated with cytokinin level in the bud. It has been suggested that cytokinin independently regulates the growth of axillary bud (Shimizu and mori, 2001)

We tried various concentrations and combinatios of BAP and NAA for callus induction. The MS medium supplemented with 1.00mg/l BAP addition with NAA 0.50mg/l was the best medium for callus from axillary bud explants, DAS *et al* (2000) have reported callus development in sweet orange (*Citrus sinesis*) on MS medium supplemented with 1mg/l NAA

Low rooting efficiency has been previously reported as a major problem for *in vitro* production of Citrus plants (Duran-vila *et al*, 1989). Pena *et al* (1995) used MS medium supplemented with 2mg/lt. NAA for rooting of sweet orange and got only 3.2% rooting after 3 months of the transfer of shoots to Rooting medium. In our results on MS medium with 0.25mg/l NAA showed good rooting in *C. reticulata*. At higher concentration of NAA upto 2.00 mg/l decreased the percentage of rooting.

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