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An efficint protocol for callus regeneration in *Citrus reticulata* through tissue culture

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Abstract: **The *Citrus reticulata* is a industrially important horticultural plant of the family Rutaceae. The present report developed the protocol for callus induction and callus regeneration. The explants were cultured on medium containing M.S. basal medium with various concentrations of NAA. For callus induction. The treatment containing M.S. basal with 1.0 mg/l NAA were best for callus induction and proliferation. The best callus regeneration on M.S. with 1.0 mg/l BAP. The regenerated shoots were successfully rooted on M.S. with 0.5 mg/l NAA**

Keywords: **Citrus reticulata, Callus, multiplication**

Abbreviations: BAP, NAA, MS (Murashige and Skoog 1962)

1. INTRODUCTION

Citrus reticulata is a member of family Rutaceae and has the common name sweet orange. The *Citrus reticulata* has been cultivated in vast Tropical and sub tropical area (Hasan et al, 2016 and Prodhan et al, 2016). Citrus is economically one of the most important fruit crop all over the world. The commercial cultivation of orange in Bangladesh is gaining popularity but recent studies shows that citrus production declining due to several factors like diseases and obiotic stresses Despite the exeesive culitivation, Citrus plantai=tion still has some majar problems such as long juvenitlity, slow growth and imsects, past, diseases, pre and post harvest losses. Genetic improvement of this plant by traditional plant breedion methods tades many years (Kayim and Doe 2016 and Vansmal et al, 2006). Improvement of citrus by conventional breeding methods is hampered various aspects of cutrys biikigt geteriztgicutt, sexual incompability, juvenility etc. (Buttan et al, 1977 and Koltunow 1993).

Such circumstances invitro culture techniques hold potentioal and could offer solution to these problems the in vitro technique has emerged a powerful tool for propagation and improvement of many woody plant species including citrus (Awafef and Badr 2017.). One of the effective methods for citrus propagation is the use of somatic embryogenesis which consequently produces larde number of healthy uniform plants (Gholami et al, 2015) micropropagation of citrus offers rapid propagation of such crops in limited space and time under controlled conditions around the year (Usman 2005). In citrus, the production of embryogenic callus was reported from excised undeveloped ovules (starrantino and Russo 1980.). Juvenile vesicles (Nito and Iwamasa 1990.), anthers (Benelli et al, 2010) from leves, epicotyls, cotyledons (Kiong et al, 2008.).

2. METHOD AND MATERIALS

The young nodal buds were collected from fiald the nodal buds ware surface sterilized with 0.5% HgCl2 solution for 5-6 minitues. Was it with sterile distilled water for three times, inoculate it on simple M. S. medium containing various contencations of BAP, all the culture were Kept under light in growth room at 25 $^{o}_{C}$. The multiple shoots were subcultured on M.S medium containing 2 gm suerose and 0.5, 1.0, 1.5, 2.5 & 3.00 mg/L NAA. All the culture were incubated in growth room at 25± 2$^{o}_{C}$. with photo period of 16 hours & light intencity 2500 lux. The initiating callus culture were again subculture on 1.5 mg/L NAA. Kept it in growth room at. 25± 2$^{o}_{C}$.

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3. RESULTS AND DISCUSSIONS

In the experiments, callus induction was done from nodal bud explant were taken from field plant. Higher efficienty of shoot initiation was observed on M.S medium containing 1.0 mg/L BAP, than other concentrations (table I). the High efficiency callus was produced at M.S containing 1.5 mg/L NAA. Than other concentrations (table II and fig. 1st) the development of maximum callus was observed on 1.5 mg/L NAA. The lower concentration of NAA (0.5 mg/L) is not sufficient to induction of callus. But the NAA concentration 2.0 mg/L is sufficient to induce callus this phenomenon suggest that NAA concertrations and explant type are more important role in callus formation from explants. (Altaf & Khan 2009) reported that hormonal combination for good callus induction for seedling leaf of kinnow mandarin. Al-Taha et al, 2012 who reported successful embryogenic calli in citrus species. Singh et al, 2013 reported that concentration of NAA at 0.5 mg/lt and 0.5 mg/L BAP was most effective in callus initiation in citrus species. Durin study of somatic embryogenesis Kiong et al, 2008 reported that 4.0 mg/L 2,4-D showed highest percent of callus induction.

Table I – Callus induction and Proliferation on M.S. medium with different concentrations of NAA in *C.reticulata*

S.N	Conc. of M.S. + NAA/mg/lt	No of explants innoculated	No of explants survived	Survival rate	No of expalnts induced callus	% of callus induction & proliferation
1	M.S. + 0.5	25	15		05	-
2	M.S. + 1.0	25	25		18	72 ± 2.25
3	M.S. + 1.5	25	20		12	35 ± 2.47
4	M.S. + 2.00	25	18		13	22 ± 1.25
5	M.S. + 2.5	25	15		13	18 ± 1.30
6	M.S. + 3.00	25	15		13	18 ± 1.20

Table II – shoot regeneration on M.S. medium with different concentrations of BAP in *C.reticulata*

S.N	M.S. Media + BAP mg/lt	% of explants with shoot Initiated	No of shoot/Explant (Mean ± SE)
1	0.5	-	-
2	1.00	62	3.21 ± 0.24
3	1.5	46	1.67 ± 0.21
4	2.00	41	1.67 ± 0.21
5	2.5	25	2.30 ± 0.16
6	3.00	10	1.27 ± 0.16

Figure: callus induction, regeneration and rooted shoot of *Citrus reticulata*

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