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INDUCTION AND REGENERATION OF CALLUS FROM DIFFERENT EXPLANTS IN *CITRUS RETICULATA* (BLANCO.)

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ABSTRACT

The present work was focus on the induction of callus by introduced cotyledon, leaf, stem and shoot explants on MS medium supplemented with BAP, KIN, IAA, IBA and 2, 4-D in different concentration and combination. MS medium alone with BAP 1.0 mg/l and 1.0 mg/l 2, 4-D induce highest percentage of callus, whereas combination of 2, 4-D alone with 0.5mg/l and 1.0mg/l KIN and combination of BAP 1.0mg/l, 1.5 mg/l, 2.0 mg/l, 2.5 mg/l and 3.0mg/l alone with 0.5 mg/l, 1.0mg/l, 1.5mg/l and 2.0 mg/l IAA and IBA were callus reported. Maximum percentage of callus was reported by leaf explant using 1.0 mg/l 2, 4-D combination with 1.5 mg/l BAP. MS medium supplemented with above PGRs either alone or with different combination and concentration all the above explants of C. reticulata were potential capable to regenerated callus.

Key Words: *Citrus reticulata*, Callus induction, Explant, Growth hormones

INTRODUCTION

Citrus is an important horticultural fruit crop, widely cultivated throughout the world for its fine flavor, test, vigor and nutritional quality. Under the cultivation of *Citrus* area Himalayan hill range North India to North China and Burma, Indonesia, Thailand and Caledonia of south range. According to the FAO total *Citrus* producer country in the world are 142 and its most production depends on Northern Hemisphere region about 70% of *Citrus* production in this area. It is considered as a topmost crop fruit of the world due to its high production, nutritional value, fruit products and use in *citrus* industry is known as a major fruit industry.

In India *Citrus* after the Mango and Banana is third largest fruit crop cultivated for food and fed. It is mostly cultivated in 26 States in India Viz. Andhra Pradesh, Maharashtra, Punjab, Gujarat, Madhya Pradesh, Rajasthan, Karnataka, Orissa, Assam and Uttarakhand etc. Most popular cultivated *Citrus* veritiesare mandarin (*C. reticulata*), sweet orange (*C. sinensis*), lemon (*C. aurantifolia*). Nutritionally *Citrus* fruits demandable for their fragrance, containing flavonoids and phytochemicals in rinds and lots of edible juice, it is having high quantities of Citric acid from their characteristic of fine flavors and its biochemical provide health and benefits, being good sources of vitamin C, flavonoids, fiber, and folic acid (Chinelo et al, 2013).

For the propagation practice *Citrus* varieties can be cultivation by both sexual and asexual propagation methods, generally it is cultivated from seed but there are some disadvantages such as seedling produced by plantlets cannot bear fruits before one decade old. Seedling and young plantlets may suffer from disease if unfavorable soil conditions and sometimes plantlets produced by seeds cannot true-true type or similar with the mother plant (Chaudhary M.I. 1994).When the propagation done by grafting, scion and budding etc., this is desire fruiting propagation depend on rootstock selection for disease resistance and hardiness. But these conventional methods of propagation having some barrier, there are more chances of transfer of pathogen plant to plant.

As many commercial *Citrus* crops, subjected to various biotic stresses, virus and viroid's have been recognised as serious problem limiting the vigour, yield and quality. Severe infections have resulted in the exclusion of some cultivars from commercial usage. (Santos et al., 1984) reported that viral diseases are major threats affecting citrus industry. The diseases are graft transmissible through grafting and infected bud sticks. Hence applied bioscience deals with tissue cul-

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ture to rising pathogen free, stress resistance, high yield, improved quality and quantity of plants. The cell and callus cultures are extensively used for development of genetic variants in plant through the selection, screening and somaclonal variation. The *in vitro* techniques tried to induce variation throughout the year and an efficient to age limits of plant or seasonal variation. Currently it has widely accepted for crop improvement such as fruits, vegetable, cereals and medicinal plant. Therefore present research study aims to evaluate standardized protocol of *in vitro* regeneration of callus from different explant and development of variant through caulogenesis.

MATERIAL AND METHODS

Primary source of explant andsurface sterilization:

Fruits of *Citrus reticulata* (Blanco) were collected from different localities of Marathwada region. These fruits were dissected for the seeds and the seeds were washed carefully in running tap water for 7 minutes and followed by distilled water.

For the surface sterilization70% ethanol and 0.3 % mercuric chloride (HgCl2) were used. The healthy collected Seeds were surface sterilized with70% ethanol for 1 minute followed by0.3% $Hgcl_2$ (three minutes) after that three subsequent rinses with sterilized glass distilled water, all these above process carried out in a laminar airflow. Sterilized seeds were dissected and removing the seed coat without any damages and aseptically inoculated in test tube as well as culture vessels.

Medium and storage condition:

In the present research work MS medium (Murashige and Skoog, 1962) was used for all the experimental work such, germination of seeds, initiation of callus and regeneration of shoots from callus by using different explants of *C. reticulata*. MS medium fortified with 3% sucrose and gelled with 0.3%, Clerigel along with various concentrations of PGRs like BAP, KIN, IAA, IBA and 2, 4-D with different concentration and combination. After the adding of growth regulators the pH was adjusted 5.8. Then the media was steam sterilized under 15 psi pressures and 121° C temperature in an autoclave. After the autoclave these media was transfer to laminar air flow for solidified and inoculation of explant. For the incubation period culture vessels were sifted to incubation room at a constant 25 ± ^{0}C temperature for 30 Days and, a growth chamber with 16 hours of photoperiod of cool white fluorescent tubes light and 60 % relative humidity.

RESULTS

Sterilized seeds of *C. reticulata* were dissected and remove the seed coat without any damages and aseptically inoculated on MS medium along with various concentrations of PGRs for germination of seeds. After the 25 days these seeds were germinated and produce more than two seedlings it is considered as *C. reticulata* seeds have a polyembryonic. The present work was concentrated on induction of callus from cotyledon, leaf, stem and shoot explants these segments excised from *in vitro* grown seedling or plantlets. All these explants of *C. reticulata* regenerated callus when it was inoculated on MS medium Supplemented with optimum concentration of 2, 4-D either alone or in combination of BAP and KIN.

Effect of 2, 4-D on Initiation of callus

Excised leaf, cotyledon, shoots and stem segments were introduced on MS medium supplemented with 2, 4-D at range 0.5-2.0 mg/l along with BAP 1.0 mg/l and Kin 0.5 mg/l for the induction the callus. When these explants were inoculated on MS medium along with BAP 0.5-2.0 mg/l combination with 0.5-2.0 mg/l IBA or IAA, able to induce callus but MS medium alone with KIN at various ranges of concentration and combination produce very low percentage of callus was reported (table 1).

Leaf excised explant was inoculated on MS medium supplemented with 2, 4-D 1.2 mg/l either alone or combination with BAP 1.0 mg/l derived highest percentage of induction of callus, these calli observe brownish color and fibril in nature were also recorded. Leaf callus was transfer on MS medium supplemented with different optimized concentration and combination of BAP, KIN, IBA and IAA they do not shown regeneration of shoot activity. The cotyledon and shoot explant were inoculated on MS medium supplemented with 2, 4-D, BAP, KIN, IAA and IBA at different concentration develops callus, 2, 4-D 1.0 mg/l alone with 1.0mg/l BAP produce maximum percent of callus was recorded. These calli were derived from cotyledon and shoot tip explant, it must be regenerated by shoots also reported in the presence study; these callus was raised from cotyledon explant these are observed in greenish color and compact in nature.

Effect of BAP on Initiation of callus

The leaf excised explant was inoculated on MS basal medium addition with 1.5 mg/l BAP in combination 1.0 mg/l IAA, or 1.5 mg/l IBA produce highest percentage of callus. But it was inoculated on MS contain KIN 1.0 mg/l 1.5, mg/l and 2.0 mg/l either alone combination of 1.0 mg/l 1.5 mg/l and 2.0 mg/l produce average percentage of callus was also observes.

Cotyledon and shoot tip explant produce highest percentage of callus, when it was introduced on MS basal medium containing BAP 2.0 mg/l combination with 1.5 mg/l IAA and 1.0, 1.5 mg/l IBA. The callus produced by cotyledon

explants, it was compact and yellowish and greenish in color observe, it is able to induce direct somatic embryos then later on shoot. These explants were introduced on MS medium supplemented 1.0 mg/l 2, 4-D in combination with different concentration of BAP and KIN produce maximum percentage of callus, but it cannot regenerate the somatic embryos and shoots. Nodal and stem explants were inoculated on MS medium containing 0.5 mg/l, 1.0 mg/l, 1.5 mg/l, and 2.0 mg/l 2, 4-D in combination with BAP induce highest percentage of callus. After the 21 day these calli were subculture on MS medium supplemented with different concentration and combination of BAP, IBA and IAA, result relevant to cotyledon explants shoot proliferation observed. Total biomass of callus was calculated such as fresh weight, dry weight and moisture content, gram per culture as shown (table 2).

DISCUSSION

The stem or nodal explants were developed highest percentage of callus on MS medium supplemented with 1.5 mg/l 2, 4-D alone with 1.0 mg/l BAP was recorded. That callus was raised from stem explant it is observed in yellowish color. The optimum concentrations of 2, 4-D at 1.0-1.5 mg/l were produced maximum percent of callus. Recently Isnaini and Riyanto (2014), similar results were reported that *Citrus* species have capacity regenerate callus from embryo, leaf, stem, nodal, shoot tip and cotyledons explants, out of that these calli derived from nodal segment, it was able to develop shoots by using optimum concentration and combination of BAP + IBA + NAA and 2, 4-D. But in present study shoot proliferation was achieved through cotyledon explant by using different concentration and combination of BAP + IBA + NAA as shown table 1. All the results on induction of calli were significantly associated with used optimized concentration from 2, 4-D. The callus induced by stem explant it was whitish in color observed in present study. These results summarized by, Chayanika et al, (2011) cotyledon explant of *C. reticulata* inoculated on MS basal medium contains 5 mg/l BAP + different concentration of NAA produce 80 % of shoots. Ibrahim M. (2012) also reported that cotyledon explant cultured on MS basal medium containing 4.0 mg/l BAP + 0.1 mg/l NAA induce callus, these callus produce shoots indirectly, when subculture on MS media containing 1-2 mg/l BAP + 0.1 mg/l NAA the after one month.

CONCLUSION

Present work was concluded that all the vegetative part of *C. reticulata* induce maximum percent of callus when the use of 2, 4-D above 1.0 mg/l either alone or in combination BAP. Callus derived from cotyledon and nodal region, those are able to regenerate shoot proliferation, when it was subculture on MS medium alone with BAP + IBA or IAA with optimized concentration.

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Table 1: Effect of 2, 4-D and BAP on induction of callus using different explant.

Source of explants	Conc. 2, 4-D + BAP(mg/l)		After days	% of callus	Nature of callus	No. of Shoot proliferation
Leaf	0.5	1.0	21 ± 1	55	Fibril	-
	1.0	1.0	21 ± 1	80	brownish	-
	1.5	1.0	21 ± 1	70	brownish	-
	2.0	1.0	21 ± 1	65	brownish	-
Nodal	0.5	1.0	21 ± 1	60	whitish	-
	1.0	1.0	21 ± 1	75	whitish	4
	1.5	1.0	21 ± 1	70	whitish	2
	2.0	1.0	21 ± 1	55	whitish	2
Cotyledon	0.5	1.0	21 ± 1	65	compact	-
	1.0	1.0	21 ± 1	80	greenish	8
	1.5	1.0	21 ± 1	70	embryonic	8
	2.0	1.0	21 ± 1	60	embryonic	5
Shoot tip	0.5	1.0	21 ± 1	50	yellowish	-
	1.0	1.0	21 ± 1	70	yellowish	-
	1.5	1.0	21 ± 1	60	yellowish	-
	2.0	1.0	21 ± 1	55	yellowish	-

Table 2: Biomass contents of in vitro grown callus after 30 days (gram/cultures)

Explants	Medium +PGRs		Fresh weight	Dry weight	Moisture contents
Leaf	1 mg/l BAP +	2,4-D, 0.2-2.0mg/l	6.12±0.581	1.54±0.294	4.58±0.112
Nodal	1 mg/l BAP +	2,4-D, 0.2-2.0mg/l	6.18±0.239	1.34±0.177	4.84±0.102
Cotyledon	1 mg/l BAP +	2,4-D, 0.2-2.0mg/l	6.36±0.435	1.46±0.252	4.9±0.192
Shoot tip	1 mg/l BAP +	2,4-D, 0.2-2.0mg/l	6.3±0.641	1.32±0.345	4.98±0.198

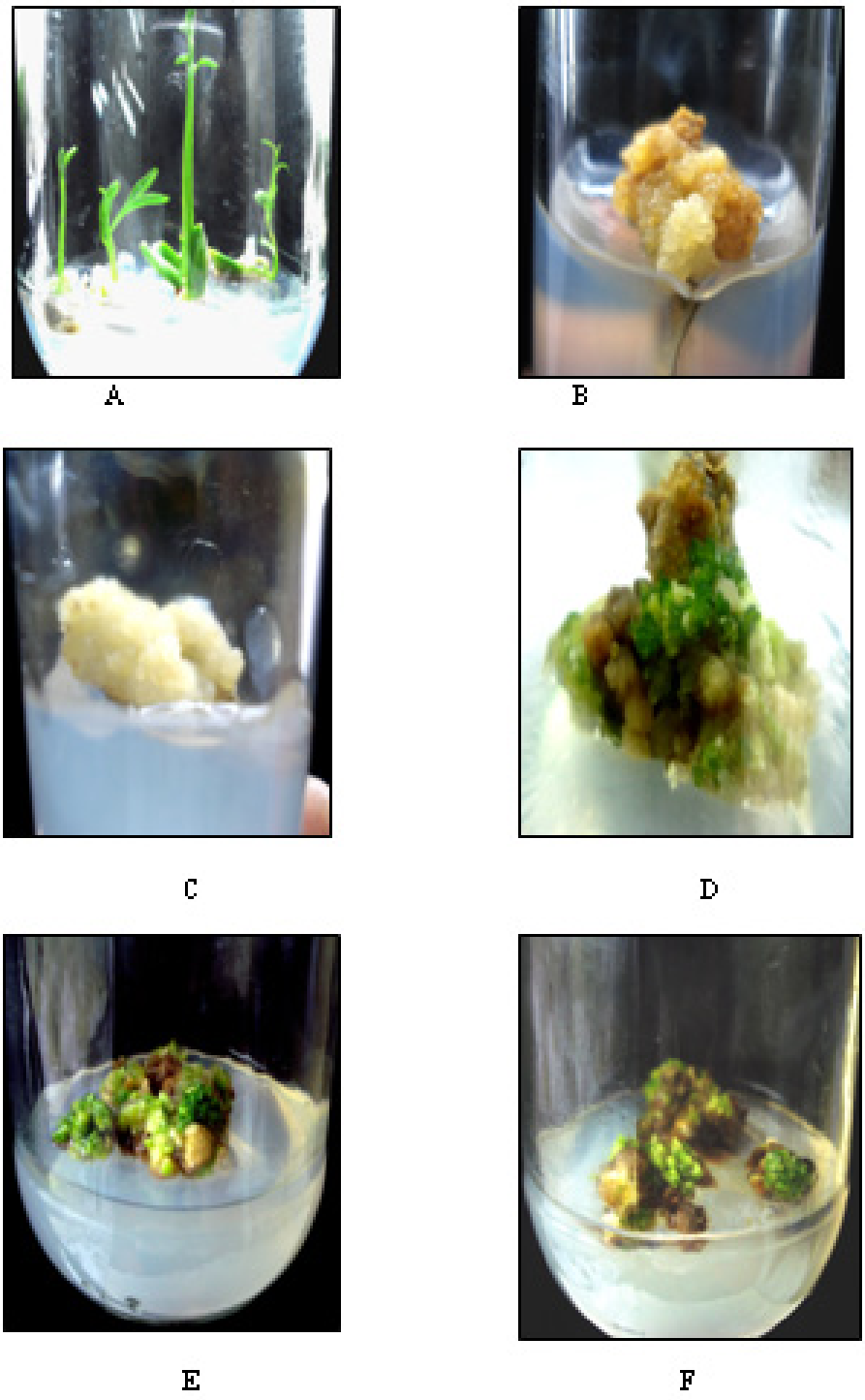

Photo plate: A. *Invitro* Seed germination, B. Callus from leaf, C. White callus from stem, D. Callus from nodal region, E. embryonic callus from cotyledon, F. Shoot proliferation.