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***Research Note* :**

ROLE OF PROLINE AS COADJUTANT ON DIRECT REGENERATION OF CITRUS ROOTSTOCK ROUGH LEMON (*Citrus jambhiri* Lush.)

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ABSTRACT: At present, about 61 per cent area of fruit crops in the Punjab state is occupied by Kinnow mandarin which is mainly propagated on rough lemon rootstock. Polyembryony, sterility, poor viability of hybrid seeds, unknown mode of inheritance and long juvenility present major problems in citrus improvement through conventional breeding programme. Tissue culture and biotechnological methods provide fast improvement to a particular crop and their success rests upon the reproducible and efficient regeneration protocols. The experiment carried out on effect of proline on tissue culture aspects of rough lemon *(Citrus jambhiri)* revealed that proline might have reduced the effect of NAA and can replace ABA in direct regeneration of citrus rootstock rough lemon.

Keywords: *Rough lemon, root stock, regeneration, biotechnology, protocol.*

In India, citrus is the third largest fruit industry after mango and banana, covering approximately 0.91 m ha area with an annual production of 7.9 MT (Anon, 1). In Punjab, citrus ranks first with an area of 49,244 ha and annual production of 10,15,628 MT (Anon, 2). At present, about 61 per cent area of fruit crops in the Punjab state is occupied by Kinnow mandarin which is mainly propagated on rough lemon rootstock. It is a fast growing rootstock, induces large fruit size, higher yield besides, being also tolerant to drought, exocortis and tristeza viruses. Rootstock is a major contributor to tree performance and longevity, as it determines tolerance to various biotic and abiotic stresses. Although, this rootstock has well adapted under Punjab conditions, but its susceptibility to *Phytophthora* fungus has become a major cause of citrus decline (Castle and Baldwin, 3). Polyembryony, sterility, poor viability of hybrid seeds, unknown mode of inheritance and long juvenility present major problems in citrus improvement through conventional breeding programme.

Tissue culture and biotechnological methods provide fast improvement to a particular crop and their success rests upon the reproducible and efficient regeneration protocols. Tissue culture and micro propagation protocols have been described for a number of citrus species and explant sources (Duran-Vila *et al.,* 4). However, very little work has been carried out on effect of proline on tissue culture aspects of rough lemon and therefore, this study was planned accordingly.

Fresh fruits of citrus rootstock rough lemon (*Citrus jambhiri* Lush.) were first washed with Teepol solution and then used for extracting seeds, which were made free of testa (outer covering) and surface sterilized under aseptic conditions with 0.1 per cent mercuric chloride ($HgCl_2$) for 10 minutes. The seeds were thoroughly washed with sterile distilled water thrice before inoculation to circumvent the deleterious effects of mercuric chloride. The seedlings of rough lemon were raised *in vitro* on MS (basal) medium by aseptic culturing of surface sterilized seeds.Three week old epicotyl segments excised from *in vitro* raised seedlings were used as explants. MS (Murashige and Skoog, 1962) media fortified with single concentration of BAP and NAA and different concentrations of proline were used for the study. The culture vessels were incubated at 25±2°C temperature in continuous fluorescent white light (2000 lux) with 16h/8h light /dark periods. The response to per cent callusing and adventitious bud formation was recorded.

Effect of proline on rough lemon epicotyls

Media composition C_1 [MS + NAA (10 mgl^{-1}) + BAP (1.0 mgl^{-1})] was considered as control as it gives the good quality callus and proline was added to improve callus quality. Proline was added at 280, 420, 560 and 700 mgl^{-1} to the C_1 and it was observed that with the increase in dose of proline there is a decrease in callus induction and increase in adventitious bud formation (Table 1). Proline dose of 280 mgl^{-1} induced 5.50 per cent callus in 20.50 days, whereas, no other

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Table 1: Effect of proline on callus induction in *Citrus jambhiri* using epicotyl segments as explant.

Treatment	Media composition	Sucrose (%) (w/v)	Proline (mgl^{-1})	Induction (%)	Days to initiation	Results
C_1 (Control)	MS + NAA ($10mgl^{-1}$) + BAP (1 mgl^{-1})	3	-	79.43	10.50	Creamish callus formed
CP_1		3	280	5.50	20.50	Creamish callus formed
CP_2		3	420	8.00	23.40	Adventitious buds formed
CP_3		3	560	35.00	26.50	Adventitious buds formed
CP_3		3	700	41.00	29.00	Adventitious buds formed

Plate 1 : Adventitious bud formation on epicotyl segments of *Citrus jambiri* Lush when cultured on MS + NAA (10 mgl^{-1}) + BAP (1.0 mgl^{-1}) + Proline (560 mgl^{-1}).

supplementation did the same. The callus growth was more on the upper surface of explant and it was friable and whitish in colour. It was interesting to note that addition of 560 ppm proline to C_1 induced adventitious buds formation in 35 per cent explants near the cut ends within 26.50 days of culturing [Plate 1]. Whereas, the increase in addition of proline to 700 ppm induced adventitious buds formation in 41.0 per cent explants within 29.00 days of culturing.The buds were of slow growth and required frequent subculturing.

Sub-cultured calli of *C. sinensis* cv. Valencia Late had the best embryogenic callus response from proline (150 mgl^{-1}), when the source of ammonium was reduced to 75 per cent and MS salts diluted to half (Rodriguz and Villalobos, 7). Similarly, Prerez and Ochoa (6) reported adventitious bud formation in Mexican lime (96%) and mandarin cv. Monica (88%), when internodal segments were cultured on MS with vitamins from B_5 medium, sucrose (5%), BAP (33.3 μMl^{-1}) and NAA (5.4 μMl^{-1}). The highest adventitious bud regeneration frequency (85.2%) and bud formation efficiency (3.7 per responsive inter-nodal stem segment) in Newhall Navel orange was obtained (Huang *et al.* 5) in the media supplemented with BAP (1.0 mgl^{-1}) + NAA (0.5 mgl^{-1}) + ABA (0.2 mgl^{-1}). Hence, in this study it became clear that proline might have reduced the effect of NAA and can replace ABA in direct regeneration of citrus rootstock rough lemon.

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