Optimization of an *in vitro* Regeneration Protocol for Rough Lemon Rootstock (*Citrus jambhiri* L.) *via* Direct Organogenesis

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Abstract

Standardization of a reproducible protocol for *in vitro* rough lemon rootstock mass propagation was conducted at Tigray Biotechnology Center Plc., Plant Tissue Culture Laboratory, Mekelle, Ethiopia in 2015/2016 cropping season. Rough lemon is the frequently used rootstock both in the world and Ethiopia citrus fruit production, particularly in the Tigray region due to its superior performance over other rootstocks. However, seedlings produced through conventional ways are not recommended to be used in orchards due to variability problems caused by its polyembrony nature. To overcome such variations, *in vitro* regeneration of rough lemon rootstocks was performed using nodal segments and shoot tips as explant types. The explants were inoculated on MS medium supplemented with 5% scurose and 250 mg/L streptomycin followed to surface sterilization. The most effective and reproducible auxin (NAA), cytokinin (BA) and gebrillenllic acid (GA3) for *in vitro* shoot and root induction in rough lemon rootstocks were determined. Almost all IBA and BA treatments resulted in almost 100% shoot induction except for at 0.0 and 0.1 mg/L IBA and at 1.5 and 2.0 BA mg/L. Nodal segments induced a higher percentage of explant response with longer shoots in a shorter period of time than shoot tips, which produced more shoots and leaves than nodal segments. The effect different BA and IBA concentrations on various parameters of proliferation were studied. Full strength medium produced more regenerated shoots and leaves per shoot than half-strength MS medium. In addition, longer shoots formed with 0.1 mg/L GA3 than culture medium without this plant growth regulator. Root length decreased with higher concentration of NAA and the longest root (2.5 ± 0.22 cm) was found in the 1.0 mg/L NAA and followed by (1.95 ± 0.22 cm) at 0.5 mg/L of NAA. The rooted plants were successfully established in the greenhouse on the substrate called coco-peat and sand, and their survival rate was found to be 98%. These results suggest that standardization of these factors can help in development of a commercially viable tissue culture system for rough lemon. Moreover, it signifies the need of plant variety based *in vitro* protocol development and optimization across citrus species.

BA concentration (mg/L) (C)					
0.5	99.9a	8.7c	3.17a	16.4a	1.28a
1	99.9a	9.19b	3.13a	14.57b	1.24a
1.5	98.89a	9.57a	2.53b	11.77c	1.05b
2	95.0b	9.57a	2.47b	10.60d	1.03b
Interaction among factors					
A × B	*	*	NS	*	*
A × C	*	NS	NS	NS	*
B × C	*	*	NS	*	*
A × B × C	*	NS	NS	*	*

Shoot tip	3.5a	10.45a	2.62b
MS medium strength (B)			
Full strength	2.75a	10.00a	2.67a
Half strength	1.75b	9.35b	2.75a
GA3 concentration mg/L (C)			
0	1.95b	8.2b	2.65a
0.1	2.95a	12.25a	2.77a
Interaction among factors			
A × B	NS	NS	NS
A × C	*	*	NS
B × C	NS	*	NS
A × B × C	NS	NS	NS

1.5	4.75a	1.3c
2	5.25a	1.27c
Interaction among factors (D)		
A × B	*	NS
A × C	*	*
B × C	*	*
A × B × C	*	*

Table 3: Effect of NAA concentration on root number and length of shoot buds of Citrus jambhiri . [i]simliar letters indicate means which are not significantly different (LSD, P=0.5), comparisons are made in each column within A, B and C, values represent as means. [k]Data were recorded at 4 weeks after culturing explants onto shooting medium. [*]Indicates significant difference. NS non-significant difference.

The rooted plantlets elongated in the rooting media and maximum elongation of 2.62 to 2.79 cm of the regenerated shoots was achieved in half MS strength medium containing 0.5 and 1 mg/L NAA respectively after four weeks of the culture.

After

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