Extended Abstract Journal of Biotechnology and phytochemistry 2020

Vol 2:2

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

Molla Gereme Taye*, Brhanu Debesay, Yikunoamilak Tesfahun and Assefa Brhanu

Department of Quality Assurance, Research and Development, Tigray Biotechnology Center, Ethiopia

***Corresponding Author:** Molla Gereme Taye, Department of Quality Assurance, Research and Development, Tigray Biotechnology Center, Plc., Mekelle, Ethiopia, Tel: +251960132552, Email: mogereme1982@gmail.com

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.

Citrus (Citrus spp.) is grown throughout tropical and sub-tropical regions of the world and it is grown in nearly 49 countries around the world. Citrus fruits are very important fruits, ranking first with respect to fruit production in the world. In Ethiopia it is one of the most economically important fruit crops grown by smallholders and commercial farmers. The total area coverage and the annual production of citrus were estimated 5,947 ha and 77,087 tons, respectively. Besides, Ethiopia is the second most populated nation in Africa where agricultural sector is the leading national income supporting more than 80% of the population Abraham. However, Ethiopian agricultural system is predominantly characterized by high level of subsistence production and low improvement of traditional farming practices resulting in declining of agricultural productivity. In fact, persistent dependency on rainfall and frequent occurrence of drought and other natural calamities add up for low agricultural production, and the same is true for citrus fruit production.

Added to the above heated issue, citrus species are infected with systemic diseases caused by fungi, viruses, bacteria, mycoplasma etc. Attack of pathogens does not always lead to the death of the plant but very often the infection caused by pathogens considerably reduces the yield and quality of the plant. While pathogens are nearly always transferred in plants through vegetative propagation, viral diseases occur in virtually all seed propagated as well as vegetative propagated crop species. Elimination of pathogens is highly desirable to optimize the yields and also to facilitate the movement of materials across the international boundaries. Hence, application of tissue culture biotechnology in the field of agriculture seems very crucial so as to increase agricultural productions including citrus for the purposes of feeding the population with no need of international aids.Although tissue culture of citrus species is well studied, several publications report strong genotype dependence, moreover citrus tissue culture is mostly confined to more common species like *C. reticulata, C. aurantifolia, C. aurantium* etc. There have been few reports on micro-propagation of *C. grandis*. However, adopting a repeatable and reproducible *in vitro* regeneration protocol for rough lemon rootstock remains difficulty. Sometimes, plants obtained from secondary organogenesis from disorganized tissues such as callus may not be true to type due to somaclonal variation. Rapid cloning of elite genotype through *in vitro* adventitious shoot propagation is extensively employed for many fruit species. Attempts to propagate pummelo from shoot tip explants of *in vitro* grown seedling have been made.Regardless of the previous attempts made, most of the existing citrus collections are propagated conventionally and conserved in field gene banks in different citrus growing countries. Such collections are vulnerable to biotic and abiotic hazards. Ageing seeds of Citrus species are recalcitrant and lose viability within a short time. Therefore, bearing in mind the problems associated with conventional propagation and the need to develop *in vitro* regeneration protocols for specific cultivars, the aim of the present study was to develop an efficient protocol for *in vitro* clonal mass propagation and conservation of germplasm of

this elite citrus cultivar by inducing multiple shoots on shoot tip and nodal segment explants. Specifically, special attention was given for the determining of the concentration of BA, NAA, IBA and GA3 for *in vitro* shoot and root induction of rough lemon rootstock.