SUMMARY

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Guggul, the oleo-resin gum complex secreted by Commiphora wightii is useful in perfumery and incense industries; beside its wide range of uses in indigenous 'Ayurvedic' medical systems. However, the current interest in this plant is on account of anticholesterol activities found in guggul. Five Guggulsterols and Guggulsterone Z and E have been isolated from gum-resin of which the latter acts synergistically as anticholesterant.

In view of the fact that a currently used drug (clofibrale) is found to have undesirable side effects, a need has arisen to look for a suitable substitute as anti-cholesterant. Guggul being one of the potential candidates, intensive search of plants rich in active principle is called for. The available literature on this plant reveals that even though substantial work has been carried out on the chemical, pharmaceutical and histochemical aspects, there

are no reports either on multiplying 'super' plants or on efforts to raise the contents of desirable compounds.

The present research work is aimed, therefore, at identifying the elite plants from different localities and propagating them by *in vivo* and *in vitro* methods. The cell cultures too were subjected to experimental manipulations to raise the content of desired compounds in cultured cells.

Clonal propagation of Elite trees :

To screen tree populations, samples of gum exudate of plants growing wild in the ravines of Mahisagar near Vasad, semiarid areas of Kutch and Sindhroat were collected periodically. Using UV-spectrophotometric methods qualitative and quantitative estimates were made. The percentage of ketonic fraction and guggulsterones was calculated. 'Elite' trees were selected on the basis of the percentage of this fraction and used as source of explants for further studies.

Stem cuttings of mature trees had been planted in the University Botanical Garden after various treatments. Over 30% cuttings sprouted after Seradix '3' treatment. However, only thick branches survived after sprouting, requiring plentiful amount of material from elite trees. Such severe pruning is harmful for elite clones. This prompted us to attempt clonal propagation of trees by employing shoot tip culture techniques.

As bud sprouting from mature plants proved very difficult, juvenile plants were used to work out optimal conditions. Highest frequency of shoot formation was registered when Kn and BAP were used together at 4 mg l^{-1}. Glutamine (100 ppm) and thiamine (10 ppm) were found beneficial in controlling the premature leaf drop. Activated charcoal was found to support sprouting and further growth of buds as well. Reduced levels of cytokinins enhanced further growth. Highest frequency of rooting was, on the other hand, obtained when the shoots treated with IAA + IBA (1 mg l^{-1} each) for 24 h were transferred to half strength of MS medium containing activated charcoal. The same protocol was extended to the clonal propagation of elite clones with slight modifications.

Seasons had profound effect on bud growth of Commiphora wightii. Maximum response with minimum contamination was obtained when the explants were collected during April to June. The greater responsiveness of first nodal explants of Commiphora wightii over shoot tip and other nodal explants can be attributed to the absence of apical dominance and presence of axillary buds at a more advanced stage of development.

However, the plantlets derived from mature plants elongated and developed further only after transferring in half strength Wood and Braun's medium after rooting.

Micropropagated plants were established in soil

demonstrating the feasibility of the application of tissue culture approach for raising elite plants of Commiphora wightii. The multiplication rate is not sufficiently high though. However, these results do provide a basis for further research on improvement of clonal propagation of Commiphora wightii.

Secondary Metabolites in the Cell Cultures:

Callus cultures were initiated from different parts, viz. leaf and stem, on a defined medium. For designing standard medium, effects of various nutritional and hormonal parameters on callus growth were studied. The best growth was achieved on MS medium with 2% sucrose containing Kn and 2,4-D both at 0.2 ppm conc. and Inositol 100 ppm. The conc. of Kn and 2,4-D was reduced to one half level to achieve healthy fine suspension.

Since β-C-3 sterols act as precursors of guggulsterones, experiments were designed to raise the content of β-C-3 sterols in suspension culture. When the total β-C-3 sterol content of stem, leaf and calli derived from them was investigated, it was found that the stem contains higher amount of β-C-3 sterols than leaf, as also the callus derived from it. The suspension culture of stem derived callus was employed for further studies. The kinetics of total sterol content indicated that the sterol content drops during lag phase, while increasing slowly during exponential and stationary phases. The parameters studied include different conc. of nitrate, phosphate, macro and micro-elements,

different carbon sources, sucrose, different auxins and cytokinins, as well as feeding with known precursors.

During these studies it was observed that by raising two fold the total nitrate level as present in MS medium, the total sterol content increased to 0.266%; but the increased nitrate level was correlated with reduced cell growth. By raising the phosphate content (1.5 fold) the total sterol content enhanced by 0.213% without any adverse effect on growth. The highest sterol content (0.301%) was achieved with 6% sucrose, the most suitable carbon source. The nature of hormones and their conc. markedly influenced the ability of cells to produce the metabolites. The total sterol content fluctuated at the different conc. of auxins tested. When the various combinations of Kn and 2,4-D were tried, Kn at 0.1 ppm with 2,4-D 0.1 ppm gave a high cell yield; whereas Kn at 0.1 ppm with 0.01 ppm 2,4-D resulted in higher percentage of total sterols. When the levels of macro and micro elements were altered, it was noticed that the macroelements i.e. $CaCl_2$ $MgSO_4$ and $Na_2FeEDTA$ had more pronounced effect than the micro-elements. The total sterol content was markedly increased (0.2%) by feeding Na-mav. at 1.0 and 10 μM conc.

Attempts made to elucidate the effect of two stage culture medium on sterol production, revealed that highest percentage of sterol content was obtained when the cells were grown for 2 weeks in growth medium and then transferred to the production medium for 2 weeks. However,

the sterol yield was maximum when the cells were grown for 3 weeks in growth medium and then for one week in the production medium, because of maximum biomass.

However, the higher β-C-3 sterol pool did not result in higher pool of guggulsterones. An another approach was, therefore, attempted.

There are few indications of enhancement in gumresinosis by ethephon (2-chloroethylphosphonic acid) in some woody plants. This gave us a clue of testing the effect of ethephon in cell cultures. The synthesis of guggulsterones did enhance by incorporation of ethephon or by treating the callus aseptically with ethephon.

Our results also illustrated that the formation of guggulsterones, the major component of resin, could be enhanced by the stress condition caused by ethephon. Possibly the stress caused by ethephon is responsible for enhanced production of guggulsterone.

These findings are encouraging for further studies regarding the manipulation of plant cells, plant enzymes and plant genes for the production of secondary metabolites.

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