

Efficient protocol for the *in vitro* cloning of *Zizyphus mauritiana* L.

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Conventional way of cloning is applicable to *Zizyphus mauritiana* species with 65% rooting branch cuttings treated with auxins. When rapid and large scale cloning of selected germplasm considered, the method was found unsuitable as it imposed extensive damage to valuable elite stock. Other drawbacks such as time consuming, slow and seasonal influences on rooting, etc. also limited extensive application of this technique, (Bajaj, 1986; Tiwari, 1992; Harry and Thorpe, 1994). Selection and rapid multiplication of 'elite' tree using micropropagation can produce stands which produce higher yields. Further selected varieties/strains which produce uniform, heavy crops of large sized seeds with maximum oil and alkaloid can be cloned to increase yield.

Key words : Mliitipurpse, Mocropropagation, Productivity, *Zizyphus mauritiana*

INTRODUCTION

Zizyphus plants is one of the most valued multipurpose tree of the tropics, Apart from providing fuelwood and small timber *Zizyphus* attributes a wide range of products, In addition, as the *Zizyphus* is not browsed by the cattle's it is a preferred candidate for eco-restoration programmes in areas where high grassing pressure exist. Due to its significant socio-economic importance and its contributions to sustenance of rural economy, Government of Madhya Pradesh has recently declared the state as a *Zizyphus mauritiana* state aimed to promote cultivation, extraction and utilization of *Zizyphus mallritiana* is conventionally propagated via seeds. Seeds are recalcitrant with a very brief longevity. The plant is an out breeding tree results in the production of heterogeneous population, ultimately causes reduction in crop productivity. Cloning therefore, found to be an imperative to achieve significant increase in productivity.

MATERIALS AND METHODS

Fresh shoots of current season's growth, were collected from an identified candidate *Zizyphus* tree. The collected ,shoots were cut into 2-3 cm long pieces having a single node. The processed explants subjected to two hrs running tap water washing prior to sterilization. The explants were surface sterilized using 0.1 % (w/v) mercuric chloride solution containing few drops of Tween-20 for 4 min followed by 5 rinses in sterile double distilled water. Surface disinfested explants were aseptically placed in MS (Murashige and Skoog, 1962) medium containing 3% sucrose and 0.7% agar prepared.

pH of the medium was adjusted to 5.8 using IN KOH/HCl prior to autoclaving at 121°C. Medium supplemented with various levels of Benzyl Adenine (BA) or Kinetin (Kn) with or without auxin (IAA) was tested.

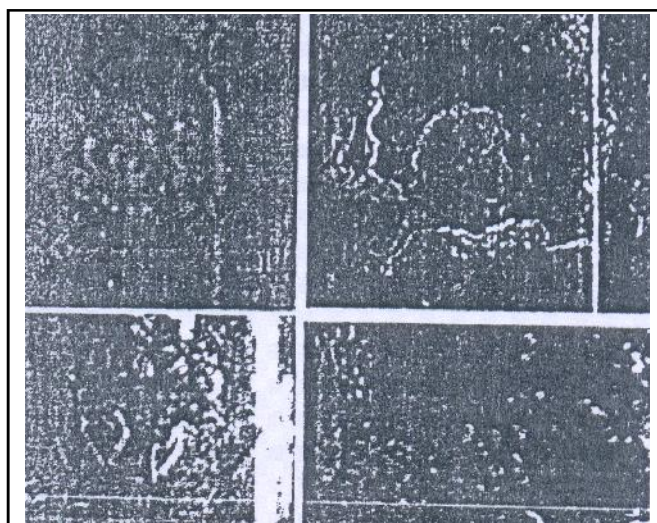
RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Table 1.

Zizyphus mauritiana explants cultured on MS medium containing 3.0 mgrl BA gave maximum (83.3%) axillary bud break. New shoots developed on this medium grew 2-2.5 cm size within 6 weeks. This concentration facilitated development of an average 10.0 new shoots per culture. A transfer of cultures to auxin-cytokinin supplemented MS medium facilitated elongation of shoots. MS medium containing 3.0 mgrl BA + 2.0 mgrl IAA found to be best combination for this purpose. In agreement with several reports (Gill *et al.*, 1996; Roy *et al.*, 1996) on Neem micropropagation MS salt formulation seems to be most suitable and is in contrast to the general assumption that woody perennials respond better to low salt concentration (MacCown and Sellmer, 1987). *In vitro* shoots cut into segments having a single node were subcultured on MS medium containing 3.0 mg^l⁻¹ BA 2.0 mg^l⁻¹ IAA. Addition of high concentration of BA induced multiple shoots (10.0 shoots perculture). Over these shoots need an elongation phase in auxin supplemented medium. Cultures once established were able to multiply and remultiply in every 6 weeks interval without any loss of vigour.

Table 1 : Nodal segments of tissue culture studies on *Zizyphus mauritiana* L.

Media + Growth Regulators (mg ^l ⁻¹)	Explant type and source
Establishment MS + 3.0 BA + 2.0 IAA	Nodal segments from 20 weeks old elite tree
Establishment and Multiplication MS + 1.0 BA + 0.50 IBA	Nodal Segments from 8-10 years old tree
Establishment and Multiplication MS + 1.0 BA + 0.10 NAA + 10% CM + 150 CH	Apical buds and nodal segments from coppice of 28 weeks
Establishment MS - 1.5 BA + 0.05 IAA	Apical buds and nodal segments
Adventitious shoot induction B5+1.0 mg ^l ⁻¹ BA	Cotyledonary nodal explant
Callus induction MS + 2, 4 -D	Nodal
Callus induction MS+0.50 2,4-D + 0.005 BA	Leaf segments from 15-year-old tree

**Plate 1 : Nodal explants on *Zizyphus mauritiana* L.****Conclusion:**

Micropropagation technology of *Zizyphus* has been briefly analyzed. It has the potential to use as major propagation method of *Zizyphus*. An estimation of

efficiency of the protocol revealed above 75% plant recovery. Pilot plant propagation system however, need to be launched for the scaling-up of production to millions. Availability of superior planting material make *Zizyphus* cultivation as an economically viable venture and contribute to sustainability of rural economy.

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