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# *In vitro* regeneration of plants from mature nodal segments of *Zizyphus mauritiana.* L.

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One third of India's population is dependent on wood fuel for cooking their daily meals, which generates tremendous pressure on the scanty vegetation. Although we have modern technologies and fast developing industrial sector, gas and electricity are neither available nor affordable for this large section of the population. The predominant *Zizyphus* tree species can survive well in high temperature, slight frost and low rainfall. Their roots penetrate deeply in to ground water level and so they do not compete for water with the crop plants (Leaky and Last, 1980). Most of species are scattered widely throughout tropical and subtropical arid regions. Several of these are categorized as "multipurpose trees" and are backbone of rural economy throughout the drier plants of the world. It is because of the dependence on these species that plants have become over exploited. Tremendous pressure exerted by both man and animal, resulted in complete removal of superior germplasm or in some cases plant species have become threatened (Ramawat and Nadwani, 1991). The situation has become compounded by various inherent biological problems.

Key words: Limitations, Prerequisite, Somaclonal variation, Protoplast culture, *Zizyphus*.

## INTRODUCTION

In view of the limitations of conventional breeding techniques, it may not be possible to achieve breeding objectives prioritized for *Zizyphus mauritiana .L.* The biotechnological approaches for fruit crop plants improvement will have to be *in vitro* selection techniques which have been successfully attempted in mango (Litz *et. al.,* 1991) for recovery of anthranose resistant somatic embryos after dual culture of embryogenic suspensions with culture filtrates of *Collectrotrichum gloeosporiodes* obtained from infected leaves and fruits. The use of *in vitro* techniques for collecting and storing rapidly vanishing fruit crop plant *Zizyphus* germplasm deserves top priority. For production of homozygous breeding lines the potential of haploid regeneration for another cultures or from irradiated ovules should be explored. Although iszyme markers have been identified for taxonomical studies in *Zizyphus* HELP markers need be identified to link with morphological as well as horticultural attributes. The improvement of *Zizyphus* through transformation with the help of selectable marker genes will depend upon advances in research on cloned genes having horticultural importance.

## MATERIALS AND METHODS

Experiments with *Zizyphus* nodal, explants using nutrients medium developed in to normal plants when placed in hormone MS medium. In brief, present efforts on selected species led to the limited success in these species. Still a large number of species are not amenable by these methods. Its because of variation between the interspecific species that the results obtained with one material are not replicated for another material.

## RESULTS AND DISCUSSION

Majority of the reports describe development of biotechnology for rapid mass multiplication, and the improvement of trees. Though a considerable progress has been made in tissue culture of tree species, the methods is not widely applicable in its present state for cloning, improvement, somaclonal variation, disease resistance, protoplasts cculture and genetic useful on these lines of work for specific and selected cases for developing clones for fodder, fuel and various types of resistance. In want of basic tissue culture regeneration protocols, work on protoplasts culture (Saxena and Gill, 1987), Somaclonal variation (Rani *et. al.,* 1995), haploids (Gautam *et. al.,* 1993) and genetic transformation (Naina *et. al.,* 1995), are almost lacking. The Zizyphus nodal explants used for initiation of callus were obtained from *in vitro* grown sand were inoculated on MS medium fortified with 1.0 mg/l BAP and 0.5 Kn could initiate white soft callus. Increase

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Table 1 : Effect of BAP + Kinetin on differentiation of Zizyphus mauritiana. L. nodal, derived callus explants.

| Growth regulators | Nodal | |
|---|---|---|
| | % Frequency of growth response | |
| | % Frequency of growth response | Morphogenetic response |
| 0.5 BAP + 0.5 Kn + 0.5 NAA | 50 | Small nodules on response |
| 1.0 BAP + 1.0 Kn + 1.0 NAA | 55 | Small shoot buds |
| 2.0 BAP + 1.0 Kn + 1.0 NAA | 40 | Regeneration + Rooting |
| 3.0 BAP + 1.0 Kn + 1.0 NAA | 42 | Normal callus growth |
| 4.0 BAP + 1.0 Kn + 1.0 NAA | 30 | Small shoot buds |
| 5.0 BAP + 1.0 Kn + 1.0 NAA | 20 | Small shoot bud |
| 6.0 BAP + 1.0 Kn + 1.0 NAA | 15 | Shoot buds + Roots |

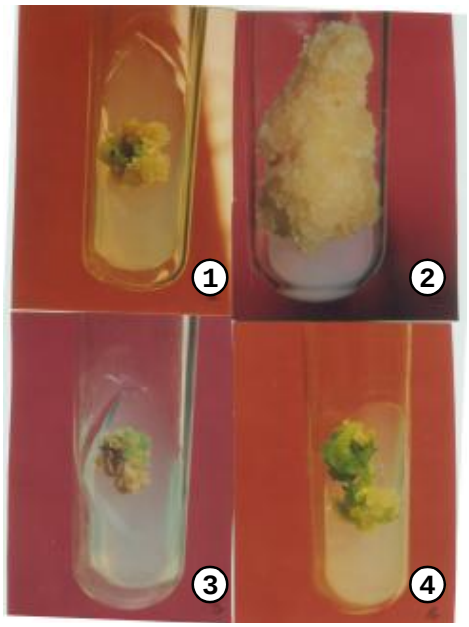


Plate 1 (Fig. 1, 2, 3 & 4) : Regeneration of plants mature nodal segments of *Zizyphus mauritiana.* L

NAA resulted in the appearance of green globular callus (Table 1, Plate-I, Fig. 1 & 2) . Most of the tree species are grown from seeds and are wild population with interspecific variation. So far no detailed selection procedures have been adopted to select the superior material leaving aside the cloning and propagation of such species except a few like *Zizyphus mauritiana* in which such selection and graft led to the multiplication of superior materials and development of the established varieties. The percentage of growth response was comparatively more (40-60%) BAP and Kn were efficient in producting shoots and roots from proximal ends of the nodal explants with an increase in the hormonal concentrations (Table 1, Plate 1,).

The *Zizyphus* nodal explants used for initiation of callus were obtained from *in vitro* grown seedling and were inoculated on MS medium supplemented with auxins, cytokinins and auxin and cytokinin combinations. The effect had evoked different morphogenetic responses. The addition of 1.0 BAP mg/l + 1.0 Kn mg/l + 0.5 NAA mg/l to MS medium resulted in while soft and hard copact callus. The percentage frequency of growth response was high and is 50% at 1.5 BAP mg/l + 1.0 Kn mg/l + 0.5 NAA mg/l.

Development of regenerative system involves use of plant material obtained from selected trees. These plants growing in arid and semi arid conditions are difficult material to handle and manipulate in the culture as they are recalcitrant to growth. By using *in vitro* techniques, a desired tree selected on the basis of its past performance can be cloned at rapid rate, which by conventional method may take years. If we compare the conventional methods of propagation with those of nonconventional ones using cell culture techniques, the advantages are apparent, like short growth cycle, small space requirement, high multiplication rate easy detection of mutants, stable genetic characters possibility of producing haploids and improvement of plants (Table 1, Plate 1, Fig. 3 & 4). It is only after the development of suitable reproducible technology that the improvement programmes can be taken up through tools of genetic engineering (Gupta *et al., 1993)*. Explants obtained from mature tree are recalcitrant to regenerate and inherent problems like contamination and browning are associated with these explants. Use of antioxidants and absorbents (PVP, Cysteine, ascorbic acid and dithiothreitol) was effective to control the browning in *C pendulus* (Bhardwaj and Ramawat, 1993). While increased nitrate nitrogen was effective in increasing the number of adventitious shoots in *Z. mauritiana* (Mathur *et al.*, 1995) medium

manipulations were not helpful in achieving high frequency multiplication from mature explants.

Rooting of shoots obtained from nodal explants on a high cytokinin medium was uncertain with low frequency in *Zizyphus* species varied responses in terms of number of roots, with or without callus and time required were obtained by different groups on rooting behaviour of these species, except two examples 70% in *Zizyphus* species per cent rooting in shoots of nature explants origin remained low. It may be concluded that rooting may be problematic in certain tree species when nature explants are used, otherwise there is little evidence of any difficulty in rooting from *in vitro* shoots.

It is imperative that success is high with plants of semiarid regions maintained under irrigation than those plants of extrement desert (arid region) grown in natural habitat, except *Zizyphus* species. High rate of success using *Zizyphus* explant may be attributed to the absence of extrinsic factor causing permanent changes in the growth. Efforts are still required to develop highly regenerative systems similar to those developed in *Zizyphus* in other species work on protoplasts culture and genetic engineering for the improvement has yet to beginning a major way. Therefore, there is urgent need to refine the available technology for rapid multiplication of selected germplasm and develop methods for improvement of tree species using cell culture and somatic cell genetics.

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