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In vitro shooting of cotyledon explants of *Zizyphus mauritiana* Lamk

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SUMMARY

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. In contrast, reduction in leaf CO_2 inforporation was noticed in crop plants subjected to soil water logging or anaerobic conditions (Ta and Ho, 2001). 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. 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.

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Key words : *Zizyphus mauritiana, In vitro* Shooting, Cotyledon explants, Regeneration

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. 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. In view of the limitations of conventional breeding techniques, it may not be possible to achieve breeding objectives prioritized for *Zizyphus mauritiana.* L. For production of homozygous breeding lines the potential of haploid regeneration for another culture or from irradiated ovules should be explored.

The use of *in vitro* techniques for collecting and storing rapidly vanishing fruit crop plant *Zizyphus* HELP markers need be identified to link with morphological as well as horticultural attributes. Although isozyme markers have been identified for taxonomical studies in *Zizyphus.* HELP markers need be identified to link with morphological as well as horticultural attributes. Chlorophyll is also used in preparation of medicines, candles, soaps, tooth paste and oil (Kadam and Ahire, 2006). Cooker and Camper (2000) who reported higher percentage of explants displaying callusing in 2.0 mg/L – 1 of both Kn and NAA in *Echinacea purpurea*. The untreated control of shoot cutting shows 38 per cent rooting and 48 per cent survival on their out planting. It indicates easy-to-root nature (Pal. 1988).The propagation of geranium by single node stem cutting Bhattacharya and Rao, (1998) and petioles cultured *in vitro* (Stephaniak and Zenkteler, 1982) have been reported.

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MATERIALS AND METHODS

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

RESULTS AND DISCUSSION

The *Zizyphus* cotyledon 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 o.5 Kn could initiate green callus. Majority of the reports describe development of biotechnology for rapid mass multiplication, and the improvement of trees. Most of the tree species are grown from seeds and are wild population with inter specific variation. Though a considerable progress has been made in tissue culture of tree species, the methods is not widely applicable in its

Table 1: Effect of BAP + NAA + Kn on Cotyledon explants of *Zizyphus mauritiana* L.

Growth regulators	Cotyledon	
	% Frequency of growth response	Morphogenetic response
0.5 BAP + 0.5 NAA + 0.5 Kn	60	Small nodules
1.0 BAP + 1.0 NAA + 1.0 Kn	50	Small shoot buds
1.5 BAP + 1.0 NAA + 1.5 Kn	45	Regeneration + Rooting
2.0 BAP + 1.0 NAA + 2.0 Kn	40	Normal callus
2.5 BAP + 1.0 NAA + 2.5 Kn	30	Small shoot buds
3.0 BAP + 1.0 NAA + 1.0 Kn	35	Small shoot buds
3.5 BAP + 1.0 NAA + 1.0 Kn	20	Small buds + Roots

present state for cloning, improvement, somaclonal variation, disease resistance, protoplasts culture 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.,* 1989), are almost lacking. Increase NAA resulted in the appearance of green globular callus (Table 1, Plate 1, Fig. 1 and 2). The percentage of growth response was comparatively more (50-60%) BAP and Kn were efficient in producting shoots and roots from proximal ends of the cotyledon explants with an increase in the hormonal concentrations. 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.

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. The *Zizyphus* cotyledon explants used for initiation of callus were obtained from *in vitro* grown cotyledon were inoculated on MS medium supplemented with auxins, cytokines and auxin and cytokine 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 soft and hard compact callus. The percentage frequency of growth response was high and was 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.

If one 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). 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 cotyledon explants on a high cytokine 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 behavior of these species, except two examples 70% in *Zizyphus* species per cent

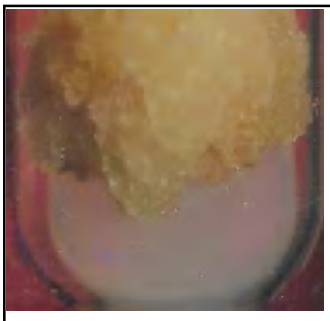

Fig. 1

Fig. 2

Plate 1: Shoot induction from cotyledon explants of *Zizyphus mauritiana* L.

rooting in shoots of nature explants origin remained low. 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.

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.

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