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RESEARCH ARTICLE

MULTIPLE SHOOT INDUCTION FROM SHOOT TIP EXPLANTS OF ZIZYPHUS MAURITIANA. L

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Abstract

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). One third of India’s population is dependent on wood fuel for cooking their daily meals, which generates tremendous pressure on the scanty vegetation. The predominant Zizyphus mauritiana 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). Large scale variation for yield and quality of fruits have been reported by several workers (Mitra and Bose, 1990). In contrast, reduction in leaf CO₂ incorporation was noticed in crop plants subjected to soil water logging or an anaerobic conditions (Ta and Ho, 2001).

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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 I mango (Litz et al., 1991) for recovery of anthranose resistant somatic embryos after dual culture of embryogenic suspensions with culture filtrates of *Collectotrichum gloeosporioides* obtained from infected leaves and fruits. The improvement of Zizyphus mauritiana through transformation with the help of selectable marker genes will depend upon advances in research on cloned genes having horticultural importance. The plant tissue culture methods also provide base for the improvement of crop to induce somaclonal variations, In Vitro mutations, genetic transformation of medicinally important genes and development of somatic hybrids plant regeneration protocol is required. Embryo genic callus induction and plant let proliferation of *Solanum nigrum* Venkateshwarlu M (2017). Shoot regeneration better than other cytokinins (Hussain et al 2007). Phyto chemical analysis of *Solanum Surattense* young leaves evaluated for the presence of bioactive compounds using various polarity solvents petroleum (Venkateshwarlu et al 2018). The use of in vitro techniques for collecting and storing rapidly vanishing fruit crop plant HELP markers need be identified to link with morphological as well as horticultural attributes. Chlorophyll are also used in preparation of Medicines, Candles, Soaps, tooth pasts 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 1.0 mg/1-2.0 mg/1 NAA in *Echinacea purpurea*. The propagation of geranium by single node stem cutting Bhattacharya and Rao, (1998) and petioles cultured in vitro

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(Stephaniak and Zenkteler, 1982) have been reported. The failure of auxins to promote rooting has also been reported in some plants (Nanda, 1970). The untreated control of shoot cutting shows 50 per cent rooting and 60 per cent survival on their out planting. It indicates easy-to-root nature. (Pal. 1988).

Materials and Methods:-

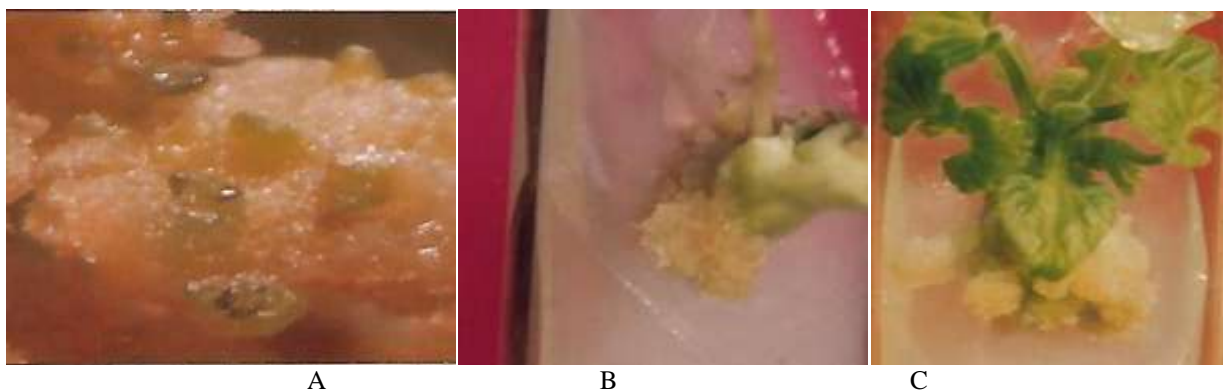
Experiments with *Zizyphus* shoot tip using nutrients medium developed in to normal plants when placed in hormone MS medium. Its because of variation between the interspecific species that the results obtained with one material are not replicated for another material. In brief, present efforts on selected species led to the limited success in these species. Still a large number of species are not amenable by these methods. In the present investigation we present the result of our efforts to develop a protocol for plant regeneration through stem node explants in *Zizyphus mauritiana*. L A medicinally important plant. Shoottip explants of different sizes were cultured with the induction medium consisting of MS Salts and Vitamins 6% Sucrose supplemented with BAP, 1.0mg/l to 4.0mg/l NAA (1.0mg/l to 3.5mg/l) and 2,4-D (1.5mg/l to 3.5mg/l). PH 5.7-5.8 The percentage of explants responding was evaluated after 4-6 weeks of cultures. The cultures were transferred to fresh medium after an internal of 4-6 weeks.

Results and Discussion:-

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 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. The *Zizyphus mauritiana*. L shoot tip 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 callus. 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. Increase NAA resulted in the appearance of green globular callus (Table 1, Plate-I, Fig. A,B,C). 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 (50-60%) BAP and Kn were efficient in producing shoots and roots from proximal ends of the shoot tip explants with an increase in the hormonal concentrations (Table 1, Plate 1 A,B,C).

Table 1:- Effect of BAP + NAA + Kn on Differentiation shoot tip explants of *Zizyphus mauritiana*. L.

Growth regulators	Shoot tip explants	
	% 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	48	Regeneration + Rooting
2.0 BAP + 1.0 NAA + 2.0 Kn	45	Normal callus
2.5 BAP + 1.0 NAA + 2.5 Kn	40	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	25	Small buds + Roots



A

B

C

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. The *Zizyphus mauritiana* L. shoot tip used for initiation of callus were obtained from in vitro grown shoot tip 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 compact 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.

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. 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 mauritiana* 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 mauritiana* per cent 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 extreme desert (arid region) grown in natural habitat, except *Zizyphus mauritiana*. High rate of success using *Zizyphus* explant may be attributed to the absence of extrinsic factor causing permanent changes in the growth.

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