



Establishment and maintaining cultures from stem node explants of *Corallocarpus epigaens* (Rottler & Willd)

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Abstract

The predominant *Corallocarpus epigaens* 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). The situation has become compounded by various inherent biological problems. 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 Major critical prerequisite for all transformations procedures is the ability in establishing and maintaining a culture with highly responsive plant tissues. Frequently sub cultured suspension cultures and cells taken from the early log phase are almost suitable Vasil and Vasil (1979). Mohan *et al* (1998). Plant regeneration culture. Tropical hardwood tree species that has several applications in the lumber industry and is used in agro forestry systems (Galeano *et al* 2013 & 2015) 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, 1999).

Keywords: establishment, stem node, *Corallocarpus epigaens*, BAP, NAA and Kn

Introduction

In view of the limitations of conventional breeding techniques, it may not be possible to achieve breeding objectives prioritized for *Corallocarpus epigaens*. Although isozyme markers have been identified for taxonomical studies in *Corallocarpus epigaens* Help markers need be identified to link with morphological as well as horticultural attributes. The biotechnological approaches for fruit crop plants improvement will have to be *in vitro* selection techniques which have been successfully attempted in mango (Litz *e. 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 improvement of *Corallocarpus epigaens* through transformation with the help of selectable marker genes will depend upon advances in research on cloned genes having horticultural importance. The use of *in vitro* techniques for collecting and storing rapidly vanishing fruit crop plant *Corallocarpus epigaens* deserves top priority.

Material and Methods

MS Medium supplemented with 1.0-5.0mg/l BAP kinetin individually and in combination with (2.0-4.0mg/l) NAA (1.0mg/l, 4.0mg/l) was used for shoot regeneration. Attentive of inoculation both stem node explants were terminated with the help of a scalped under septic conditions. 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 inter specific species that the results obtained with one material are not replicated for another material. Experiments with *Corallocarpus epigaens* stem node, explants using nutrients medium developed in to normal plants when placed in hormone MS medium. The cultures were sterilized with 0.1 mercuric chloride solution for three min and repeatedly washed with strelized distilled water. The percentage of growth response was comparatively more (40-60%) BAP and Kn were efficient in producing shoots and roots from proximal ends of the stem node explants with an increase in the hormonal concentrations. In want of basic tissue culture regeneration protocols, work on protoplasts culture (Saxena and Gill, 1987) ^[15], Somaclonal variation (Rani *et. al.*, 1995) ^[14], haploids (Gautam *et. al.*, 1993) and genetic transformation (Naina *et. al.*, 1995) ^[11]. 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.

Results and Discussion

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. 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) ^[7]. Explants obtained from mature tree are recalcitrant to regenerate and inherent problems like contamination and browning are associated with these explants. While increased nitrate nitrogen was effective in increasing the number of adventitious shoots in *Z. mauritiana* (Mathur *et. al.*, 1995) ^[10] medium manipulations were not helpful in achieving high frequency multiplication from nature explants. Ugendhar et al (2011) has been also reported that high amount of cytokinin and lower amount of auxins is the best combination for somatic embryogogenesis. Rooting of shoots obtained from nature explants on a high cytokinin medium was uncertain with low frequency in *Corallocarpus epigaens* 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 75% in *Corallocarpus epigaens* species percent rooting in shoots of nature explants origin remained low.

The *Corallocarpus epigaens* stem node explants used for initiation of callus were obtained from *in vitro* grown sand were inoculated on MS medium fortified with 2.0 mg/l BAP and 1.0 Kn could initiate white soft callus. Increase in the concentration from 3.0 to 4.0 mg/l BAP, Kn and NAA resulted in the appearance of green globular callus. Most of the 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 *Corallocarpus epigaens* in which such selection and graft led to the multiplication of superior materials and development of the established varieties (Mann and Saxena, 1981) ^[15]. The addition of 1.0 BAP mg/l + 1.0 Kn mg/l + 0.5 NAA mg/l to MS medium resulted in white 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. The *Corallocarpus epigaens* stem node 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 propagation of teak via cuttings has been reported. (Santos *et al* 2014) ^[3] resistance to weathering strong and special content which produces premium timber (Feroz et al) 2013. PVD Venkateshwarlu *et al* (2018) ^[12] (Table-1, Plate-1)

Table 1: Establishment maintaining cultures from stem node explants of *Carollacarpus epigaenus* (Rottler & Willd)

Growth regulators (mg/l)	Stem node explants	
	% Frequency of growth response	Callus response of growth
1.0 BAP + 0.5 Kn + 0.5 NAA	40	Callus
2.0 BAP + 2.0 Kn + 1.0 NAA	35	shoot buds(2-4)
3.0 BAP + 3.0 Kn + 1.5 NAA	30	Plantlets
4.0 BAP + 4.0 Kn + 2.0 NAA	25	Normal callus
5.0 BAP + 5.0 Kn + 2.5 NAA	20	Green callus Small shoot buds (4-6)
6.0 BAP + 6.0 Kn + 3.0 NAA	15	shoot bud+Roots

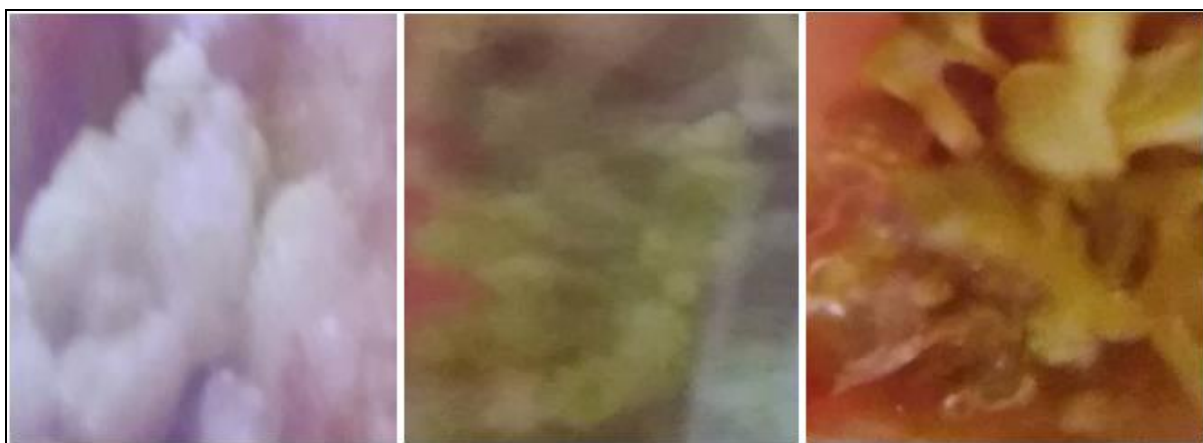


Plate 1: Establishment maintaining cultures from stem node explants of *Carollacarpus epigaenus* (Rottler & Willd)

Conclusion

High rate of success using *Corallocarpus epigaens* explants may be attributed to the absence of extrinsic factor causing permanent changes in the growth. 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. Therefore, there is urgent need to refine the available technology for rapid multiplication of selected germplasm and develop methods for improvement of tree species.

References

1. Vasil V, Vasil IK. Isolation and culture of Cereal protoplasts Part-2 embryogenesis and plantlet formation from protoplasts of pennisetum americanum Theor. Appl. Genet,1979:56:97-99.
2. Ugnder T, Venkateshwarlu M, Shekar GPV, Srilatha T, Reddy JK. High frequency somatic embryogenesis and plantlet regeneration from shoottip explants of soybean. SG-Rese. Rep,2011:01:146-150.
3. Santos AFA Almedida BC, Gava FH, Favare HG, Filho JB, Costa RB, Brondani GE. Clones production of Tectona grandis, Advances in forestry science,2014:1:75-82.
4. Galeano E Vasconcelos TS, Vidal M, Mejia Guerra ML, amd Carrer H. Large scale transcriptional profiling of lignified tissues in Tectona grandis BMC Plant Biology,201515:221.
5. Foroz SM, Almand R, Das P, Mamun AA. Community ecology and Spatial distribution of trees in a tropical wet evergreen forest in Kaptaqi national park in Chittagong Hill tracts, Bangladesh. Journal of forest Research,2013:25:311-318.
6. Pandey D, Brown C. A global overview in Unasyuva No.201, Teak. Int J Forestry. Forest Ind. (FAO),2000:51:2000/2.
7. Gupta PK, Pullaman G, Timmis R, Kreitinger M, Carlson WC, Grob J, Welty E. Forestry in the 21st Century. The biotechnology of somatic embryogenesis. Biotechnology,1993:11:454-459.
8. Leaky RRB, last FT. Biology and potential of prosopis species in arid environments, with particular reference to Prosopis cineraria. J. Arid, Environ,1980:3:9-24.
9. Litz RE, Mathews VH, Hendrix RC, Turgalevitch C. Mango somatic cell genetics. Acta. Hortic,1991:291:133-140.
10. Mathur N, Ramawat KG, Nandwani D. Rapid in vitro multiplication of Jujube through mature stem explant. Plant cell Tiss. Org. Cult,1995:43:75-77.
11. Naina NS, gupta PK, Mascarenhas AF. Genetic transformation and regeneration of transgenic neem (Azadirachta indica) plants using Agrobacterium tumefaciens. Curr. Sci, 1989, 184-187.
12. Venkateshwarlu PVD, Dinesh Kanna M, Nagaraju N, Ugnder T. Protoplast Isolation of Soybean *Glycine max* (L) Merrill from leaf explants The Phar Inno Journal,2018:7(10):661-665.
13. Ramawat KG, Nandwani D. Propagation of prosopis species problem, perseverance and perspectives. Annals arid zone,1991:30:247-258.
14. Rani V, Parida A, Raina SN. Random amplified poly morphic DNA (RADP) markers for genetic analysis in micropropagated plants of Populus deltoides. Marsh. Plant. Cell.Rep,1995:14:459-562.
15. Saxena PK, Gill R. Plant regeneration from mesophyll protoplasts of the tree legume Pithecellobium dulce Benth. Plant. Sci, 1987, 257-258.
16. Warhade MI, Badere RS. Seasonal variation in the shoot regeneration potential of the nodal explants of *Roja Setigera* journal of Indian Botanical Sci,2017:96:199-208.
17. Mohan ML, Krishnamurthy KV. Plant regeneration in Pigeon pea (*Cajanus cajan* (L) Millsp) by organogenesis plat cell Rep,1998:17:705-710.