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Research Article

THE INVESTIGATION OF MICROPROPAGATION ON *KIGELIA PINNATA*.D.C.

M. Dhanasekaran[1]*, GC. Abraham[2] and S. Mohan[2]

[1]PG and Research Department of Botany, The Madura College, Madurai-11, Tamilnadu, India.
[2]PG and Research Department of Botany, The American College, Madurai-2, Tamilnadu, India.

ABSTRACT

Kigelia pinnata D.C. the midsized ornamental tree of the family Bignoniaceae has been studied in tissue culture. The preliminary observation on tissue culture using embryos and cotyledons show that there is a scope for pursuing cloning and callus mediated organogenesis in this species. *Kigelia pinnata D.C.* is propagated primarily by seed. Vegetative propagation using cutting is possible but success rate is very low. Although *kigelia pinnata D.C*.is reported to be produced from hardwood cutting under experimental conditions, frequency of plant recovery in both seeds and cuttings are small in magnitude. So that invitro propagation methods are introduced in order to produce more number of seedlings. This paper revealed that the micropropagation and what are the methods followed to get plantlets of *kigelia pinnata D.C*. through plant tissue culture.

Keywords: Organogenesis, cloning, vegetative propagation, callus, hardwood cutting.

INTRODUCTION

The success of plant tissue culture techniques has made possible large scale cloning of plant species. Micropropagation can also be an effective way to eliminate viruses and other pathogens and produce plantlets. *Kigelia pinnata D.C.* is propagated mainly by seed. It is not frost resistant, but young plants will survive if protected for 3 years. Seed viability is maintained for more than 5 years in air tight storage at an ambient temperature with 11-15% humidity. Germination can be triggered by soaking seeds in hot (or) boiling water for one minute. To avoid browning of the medium, frequent transfer of explants to a fresh nutrient medium has been earlier reported[1]. Regulation of auxin and cytokinin balance has been recognized as a key factor in the control of cell division and organogenesis in most dicot plants[2]. Over exploitation without commensurate replenishment of natural stands has posed a severe threat to the very existence of this precious timber tree[3]. Natural regeneration has major constraints such as prolonged dormancy and low germinability of seeds[4]. Micropropagtaion of tree species offers a rapid means of producing clonal planting stock for afforestaton biomass production and conservation of elite and rare germplasm[5, 6]. Invitro techniques are being increasingly applied for clonal propagation of selected tree species to supplement conventional methods which have their limitations especially when a large number of genetically uniform propagules are required[7,8]. On the other hand, the characteristic induction of rooting by IAA was reported to be totally absent when the medium was added with BAP[9]. Chandra and Pal[10] have observed callusing along with shoot differentiation from the cotyledonary explants of *Vigna radiata*. In *Mucuna pruriens,* Chattapodhayay et al[11] have also developed a micropropagation protocol for the induction of multiple shoot regeneration from juvenile explants like epicotyls and hypocotyls. The high

level of shoot regeneration obtained from leaf explants compares well with the other cultivars reported by various workers [12].

MATERIAL AND METHODS

Culturing

Adequate amount of shoots and fruits of the plant *kigelia pinnata D.C*. were collected. The seeds are undergo various treatment such as acid, scarify, warmwater, vernalization, GA_3 and pre-seed treatment (invitro culture). MS medium was prepared. The pH of the medium was adjusted to 5.8 before adding agar. The medium was poured in culture vessels and sterilized at 1.08kg/cm^2 for 15 minutes in an autoclave. All the aseptic manipulates were carried out under laminar air flow chamber. The embryos are considered as explants, put in a basal medium. The callus are raised. The invitro raised shoot of the plant put in a medium containing basal medium and 1µm of BAP after 6 weeks of incubation. This invtro raised shoot transferred to BAP medium with l µm. After multiple shoot induction, this explant transferred to the combination medium (1µm of NAA and 1µm of BAP). After every inoculation the culture vessels are kept in the culture room at 26±2^{o}c under 16 hours photoperiod with cool white fluorescent lamps (40mmole/m^2/s) having 70-80% humidity.

Hardening

After proper invitro development, the plantlets were taken out of the flasks in such a way that no damage was caused to their root system. The roots were washed gently under running tap water to remove adhering medium and kept under running tap water for few minutes, so that they do not wilt after transfer to pots. The survival and establishment of plantlets were studied after transplanting the plantlets into pots containing a mixture of sterilized sand and soil (1:1). When the plantlets showed signs of establishment in pots with appearance of new leaves, they are kept under natural room temperature.

Table 1: Impact of seed treatment on germination and seedling establishment

Treatments	Germination characteristics			
	Imbibition (in Frequency%)	Percent Germination (Based on cracking of seed coat)	Radicle growth (in Frequency)	Shoot emergence (in Frequency)
Pre seed treatment (pot culture)	0	0	0	0
Acid (HCl) treated	0	0	0	
Scarified	0.6	0.6	0.1	0
Presoaked in Warm water	0.2	0	0	0
Vernalized	0	0	0	0
Presoaked in GA_3	0	0	0	0
Pre seed treatment (pot culture)	05	0.2	0.2	0
Pre seed treatment (In vitro culture)	0.9	0.7	0.7	0.4

Table 2: In vitro responses of K*igelia pinnata* (in frequency percentage)

Medium composition	Greening of expalnts (in eye ball units)	Callus Development	Shoot Growth	Rhizogenesis	Scope For de novo organogensis
MS Basal medium (BM)	+	0	0	0.1	0
BM+GA_3 (2)	++	0	0.2	0.3	0
BM+NAA(1)	+	0.3	0.1	0.3	0
BM+2,4-D (0.5)	-	0.7	0	0	0
BM+ BAP(5)	++	0.2	0.2	0.6	0
BM+TDA(10)	++	0.1	0.2	0.4	0
BM+NAA(1)+BAP(10)	++	0.4	0.5	0.6	0
BM+NAA(5)+BAP(1)	+	0.3	0.2	0.2	0.2
BM+ TDA(5)+GA_3(1)	++	0	0.6	0.1	0
BM+BAP(20)+AS(50)	++	0	0.5	0	0
BM+BAP(20)+NAA(5)+ GA_3(3)	+	0	0.2	0	0.3

RESULTS

The repeated attempts are made to isolate seed and achieve germination in this species through conventional seed treatment is failed (table.1). The pre incubation resultant treated seeds in sucrose free minimal medium allowed germination of embryos but at very low frequencies. The embryo served as a good primary explants and over all invitro responses shown in (table.2)

CONCLUSION

The difficulties faced in getting the plant germinated in pot cultures and in vitro trials speak of the reasons sparse distribution. We found that the plant produces fairly good number of fruits and seeds but is constrained seriously due to the indehiscent nature of its fruits. The excessively hard and impermeable quality of its seed coat never allows the embryos to germinate successfully under normal Field conditions. But by the selected treatments there is a certainly scope to pursue rapid clonal propagation in this species.

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