INVESTIGATION ON CALLUS FORMATION IN *CAREYA ARBOREA* ROXB.

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Abstact

Present study was formulated standarize protocol for induction of callus from explant seeds and leaf of *Careya arborea* Roxb. Different types of media (Murashige and Skoog, 1962) were prepared according to various concentrations of growth regulators to standardize a medium protocol for callus induction from *Careya arborea* Roxb. The explants were surface sterilized, cultured and incubated at 24±4°C, under 16 hrs photoperiod, 8 hrs dark. The concentrations of MS medium for development of callus from the explants seeds and leaf of *Careya arborea* Roxb at high frequency level.

Key words: MS medium: Murashige & Skoog Medium, 2,4-D: 2, 4-diphenylphenoxyacetic acid, BAP: 6-benzylaminopurine, IAA: Indole-3- acetic acid, Con.: Concentration, KN: Kinetin, lit: litre, M: mole

Introduction

Careya arborea Roxb. is commonly known as Wild Guava in English and Kumbhi in Hindi.It is a species of Lecythidaceae family (Gamble,2005).*Careya arborea* Roxb. Is a deciduous tree that grows up to 15m-45ft high.It is a medium sized deciduous tree and widely available in India, Sri Lanka, Malay and Peninsula. It flowers during March-April.Seed dispersal take place with the commencement of the rain (Bhat,*et al*.,2004). Its leaves turn red in the cold season. Flowers are yellow or white in colour that become large green berries. Flowers in an erect raceme at the end of branches. It is high economical and pharmacological importance ofsecondary metabolites, industries are deeply interested in utilizing plant tissue culture technology for large scale production of these substances. Plant tissue culture is an important frontier area in plant biotechnology and to support the production of an enormous array of phytochemical compounds in the laboratory conditions (TM Hussain, *et.al.*, 2008) .The use of tissue culture for large scale production of plants, improvement of crops, conservation of valuable germplasm and production of secondary metabolites has been well documented. However, many plants are producing pharmaceutically or pharma-cologically valuable secondary metabolites, which are extremely expensive to obtain by extraction from the plant. The *in vitro* derived phytochemical compound has many selective advantages over the normal and synthetic source of production.The plant has been considered ethonobotanically important due to its use in traditional and health care system for curing severe diseases like skin diseases, bronchitis, tuberculosis, diarrhea, dysentery, ulcer etc (Kirtikar *et al.*, 1999). Stem bark of *Careya arborea* Roxb.is traditionally used in the treatment of tumours, bronchitis, skin disease, epileptic fits, astringents, antidote to snake-venom, abscesses, boil and ulcer (KR Kirtikar,*et.al.*,1975). Fruits are used as decoction to promote digestion. Leaf paste and pulp used as poultice rapidly heals ulcers and root is used for the treatment of tuberculosis and skeletal fractures. Leaf extract is used as an indicator in acid base titration. Qualitative chemical tests revealed the presence of terpenoids, flavonoids(RK Gupt *et al*.,1975), alkaloids, saponins and tannins(Mahato SB,*et al*.,1972) reported from seed of *C. arborea* Roxb.

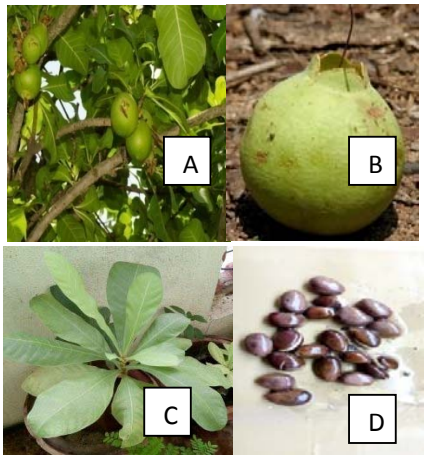

Fig.*Careya arborea* Roxb.

Materials and methods :

*Careya arborea*Roxb. plant was collected from the Melghat region Amravati district, India in the month of March. The plant was identified by Dr. S.P. Rothe , Head of Department Botany, Shri Shivaji college art,Commerce and Science, Akola. Dist Akola, Maharashtra. And grow in the garden of Department of Botany, in this college. *Careya arborea* Roxb.seed were used as source of explants throughout this work. The complete work were perform under aseptic and controlled conditions in plant tissue culture laboratory of Department of Botany, Shri Shivaji Arts, Commerce and Science college, Akola Dist. Akola, Maharashtra .

Seeds and leaf were thoroughly washed under running tap water for 7 minutes to remove the traces of dust. Then the seeds were treatment with concentrated 1N hydrochloric acid 35 minutes toand then rinsing 2-3 time with distilled water. Now, the broken hard coat of the seeds was removed and, the seeds and leaf were then washed and cleaned carefully under distilled water for 30 minutes to remove all the trace of chemicals. Then, the explants of *Careya arborea* Roxb. were transferred to laboratory for further sterilization process.

Place the explants in a beaker and wash the seeds and leaf with sterile distilled water for 2 minute.Now, the seeds and leaf were allowed to soak in 70% ethanol for 60 sec. followed by sterile distilled water twice. Then the seeds and leaf were transfer in 0.1% $HgCl_2$ solution for 8 minutes followed by rinsing 2-4 times with sterile distilled water.After surface sterilization, keep the sterilized seeds and leaf in distilled water in petridish to prevent drying with help of sterile tissue paper. The surface sterilized seeds and leaf were placed on the MS medium with the help of sterile scalpel. This all process take place in front of the laminar air flow chamber and aseptic condition.

Murashige and Skoog media was used as the culture media for Medium which will be preparing in sterile distilled water. The MS media 2.47mg/l and 8gm of agar with 30gm sucrose will dissolve in 1000 ml of sterile double distilled water and boil the mixture to dissolve the agar, and different types of media were prepared according to different concentrations of growth regulators (2,4-D, NAA, Kn & BAP. All the media were adjusted the pH value between 5.7-5.8 by using of 1N HCl or 1N NaOH before autoclaving. Then the media were dispensed into test tube and culture vessels and autoclaved the culture vessel and test tube containing media at 15 lbs/m^2 for 15 mins at 121^{o}C temperature. Keep the vessel at room tempetature for solidifying the media .then prepare slants.

Surface sterilized explants seeds and leaf were inoculated into test tube and culture vessels containing MS media supplemented with different concentrations and combinations of plant growth regulators for callus induction . All the culture vessels were placed under white fluorescent tube light and exposed to 16 hrs of photoperiod, 8 hrs of dark period. Callus induction rate on each media formulation was observed and the data were recorded after every week.

Result And Discussion

Due to this study highly standardized protocol for the callus investigation of *Careya arborea* Roxb was established. different concentrations like NAA &2,4-D,Kn &2,4-D were used to standardize a medium protocol for the induction of callus from the seeds and leaf explant of *Careya arborea* Roxb. Surface sterilized explants were cultured on MS medium supplemented with different concentrations of auxins such as 2,4-D and NAA in combination with 6-BAP and Kn. After two month , callus initiation from the seeds anf leaf explant was observed in different types of media.High amount of callus induction was observed on the modified MS medium supplemented with 2.0 mg/L of 2,4-D combination with 0.5 mg/L BAP and 1.5 mg/L NAA. On callus formation from the seeds of

Careya arborea Roxb. And also the results indicated that the callus growth from the seeds was significant effected by the type and concentration of growth regulator. Most frequently 2,4-D is used to initiate the callus growth. It was found the modified MS medium supplemented with 2.0 mg/L of 2,4-D combination with 0.5 mg/L BAP, 1.5 mg/L Kn was good callus growth. More positive result also found in leaf explant using 1.0mg/LKn and2.0 mg/L 2,4-D.

Table-1: Callus obtained from Seeds explant .

Amount of Callus:No Callus (-); Low (+); Moderate (++); High (+++).

Sr.No.	Growth Regulators mg/L. NAA + 2,4 -D		Explant used	Time required to obtain callus	Amount of Callus
1.	0.6	0.5	Seeds	8 Weaks	+
2.	1.5	1.5		8Weaks	++
3.	1.5	1.0		8Weaks	++

Table-2: Callus obtained from Seeds explant.

Sr. No	Growth Regulators mg/BAP+NAA +2,4-D			Explant used	Time required to obtain callus	Amount of Callus
1	0.2	1.0	0.5	Seeds	8 Weaks	++
2	0.4	1.5	1.0		8Weaks	++
3	0.5	1.5	2.0		8Weaks	+++

Table-3: Callus obtained from Seeds explant .

Sr.No.	Growth Regulators mg/LBAP+Kn+ 2,4-D			Explant used	Time required to obtain callus	Amount of Callus
1.	0.3	0.4	1.0	Seeds	8 Weaks	++
2.	0.5	1.5	2.0		8Weaks	+++
3.	0.5	0.3	0.2		8Weaks	+++

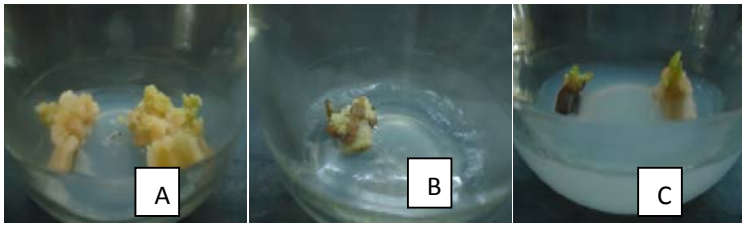

Fig.Callus induction from the seed

Table-4: Callus obtained from Leaf explants.

Sr.No.	Growth Regulators mg/LKn+ 2,4-D		Explant used	Time required to obtain callus	Amount of Callus
1.	1.5	1.0	Leaf	8 Weaks	++
2.	1.0	2.0		8Weaks	+++
3.	0.3	0.2		8Weaks	++

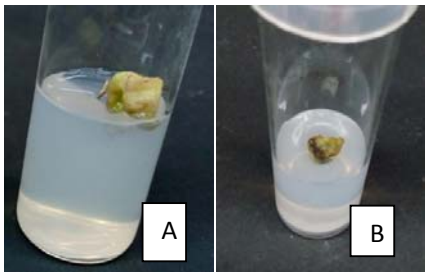

Fig.Callus induction from theLeaf explant.

Conclusion

The present work shows that the callus induction shows positive and promising results .The culture medium with different concentration and growth regulators.,And can for further research and also other tree species.

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