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In vitro Propagation of desirable plants through cultivation from Leaf explants of *Cocculus orbiculatus*.

Mandaloju Venkateshwarlu
Department of Botany, Kakatiya University, Warangal Urban- 506 009, T.S. INDIA.

ABSTRACT:- Plants usually propagated by seed can now be cultured *In vitro* yield thousands of identical plants a number of agronomically important medicinal plants. Specific characteristics such as disease and herbicide resistance also can be selected for while plants are in culture concentration of cytokinine Induction M. venkateshwarlu (2021). The MS medium supplemented with different concentrations of Cytokinins alone and in combinations with various auxins were used for high frequency shoot regeneration from Leaf explants. *In vitro* shoot and multiple shoot induction was achieved in one of the important medicinal plants of *Cocculus orbiculatus* which has been historically been used to treat a wide assortment of diseases. MS medium supplemented with 1.0 mg/l BAP was found to be optimum to induce shoots (100%) directly from the Leaf explants. Significant increase in the number of shoots per explants was found in MS medium supplemented with 1.0 mg/l BAP and 15 mg/l Adenine Sulphate. Desirable plants are cloned through tissue culture to produce genetically identical plants in a process called *In vitro* clonal propagation. The large variety of medicinal and crop plants in both laboratories and plant nurseries. All the tested combinations have little effect on increasing the number of shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from Leaf explants of *Cocculus orbiculatus* using different concentrations and combinations of cytokinins. Micro shoots developed in the above culture rooting was also induced by BAP, NAA+Kn 0.5mg/l to 5.0 mg/l from callus. Callus tissue has the ability to plant or plant organ in a process called dedifferentiation in plant tissue culture plant growth is usually initiated from leaf explants of Cocculus.

Keywords: Propagation, *Cocculus orbiculatus*, BAP, Kn, *In Vitro* cultivation.

I. INTRODUCTION

MS nutrient medium in the presence of a specific ration of BAP, Kn and L-Glutamic acid, the non dviding callus cells revert to an undifferentiated leaf explants callus tissue. The primary aim of this study has been to gain some knowledge about the genotypic differences for callus initiation and high frequency plant regeneration from long term callus cultures of *Cocculus orbiculatus.* Plant tissue culture methods must be supplied with various concentrations of BAP+Kn (1.0mg/l-5.0mg/l) combination. The culture induces multiple shoots (4-6) to appear from a small shoots. It grows on any kind of soil, but thrives best on well – manured rich loamy soils with abundant water supply. Plant tissue culture organogenesis is a process of differentiation by which plant organs simultaneously adventurous development of callus with plant lets. The existence of genetic variability in the form of wild relatives of domestic crops is the source for continued improvement in yield and resistance to disease or stressful changes in environmental conditions. The seeds are small and edible, and are used in confectionery. Several workers in past have micropropagated some of the important Asclepiadaceae members such as *Ceropegia bulbosa* (Patil, 1998; Britto *et al.,* 2003), Venkateshwarlu (2020) & Thoyajalosa & Rai (2016). *Hemidesmus indicus* (Misra *et al.*, 2003; Patnaik and Kishore, 1996) Venkateshwarlu *et al* (2018) & Venkateshwarlu (2017) and *Holostemma ada-kodien* (Martin, 2002 & 2003). Since very scarce information is available about micropropagation about this important medicinal plant, an attempt was made to develop a reproducible protocol for shoot and multiple shoot induction from leaf explants. Using various concentrations of Benzyl Amino Purine and Adenine Sulphate. In the recent years there has been a major crop plant development. Many of these plants were identified because of their use in traditional *In vitro* culture isolated from leaf explants potential and a limitation of leaf explants M. Venkateshwarlu (2020).

II. MATERIALS AND METHODS

The resulting plants differ from the *in vitro* production of leaf explants. Although the processes of somaclonal variation new potentially desirable traits are obtained it they can be stably maintained progeny crop plants. The leaf segments after removing the leaves were cut into 2cm pieces, each containing a single node region and washed under running tap water for 15 min, followed by brief washing with sterile distilled water. Node explants (1.25 cm) were surface sterilized in 70% (v/v) ethanol for 60 sec followed by 0.1% (w/v) mercuric chloride for 6 min. explants were thoroughly washed in sterile distilled water and blot dried on sterile Whatmann 1 mm filter paper. For shoot induction, leaf explants were again trimmed into 1.0 cm and transferred to MS medium supplemented with 0.1 – 1.0 mg/l BAP.The isolated in vitro raised explants segments

observations were recorded on yield and various yield traits on normal loading plants selected randomly. MS medium supplemented with different concentrations of Benzyl Amino Purine (BAP) (0.1-1.2mg/l were used for shoot induction. For multiple shoot induction MS medium supplemented with 1.0 mg/l BAP and 5-20 mg/l Adenine Sulphate were used. The pH of all media was adjusted to 5.75 before adding 0.8% agar and autoclaved at 151b and 121°C for18 min. All the media were kept at 26±2°C for 3 days before use. Cultures were incubated at 26±2° under a 16/8 h photoperiod for 26-28 days at a relative humidity of 65%. Node explants (1.0 cm long) were used as explants for multiple shoot induction on MS medium fortified with 1.0 mg/l Benzyl Amino Purine and 5-20 mg/l Adenine Sulphate. After two weeks of culturing at 26± 2° under a 16/8 photoperiod shoots were sub cultured onto fresh medium for proliferation. A number of combinations factors influence the presence of somatic embryos during plant tissue culture. Biotechnological Applications *in vitro* production of axillary bud explants. Venakateshawrlu M (2021).

III. RESULTS AND DISCUSSION

The resulting plant differs from the original parent plant this multiplication high production cultivation occurs randomly so that the regenerated mature plants. The results scored on the above mentioned aspects (shoot and multiple shoot induction) are summarized in the following order. In order to assess the effect of different concentrations of Benzyl Amino Purine (1.0-2.0 mg/l) on shoot induction from *Cucurbit maxima* nodal explants were surface sterilized and inoculated onto MS media supplemented with various concentrations of Benzyl Amino Purine. Shoot induction was monitored after 24-28 days of inoculation by counting the number of shoots induced from each explant. Shoot induction was observed in all the concentrations of Benzyl Amino Purine tested with variation in per cent response of shoot induction. The highest per cent of shoot induction was observed in MS with 1.0 mg/l Benzyl Amino Purine followed by 80.4 and 80.2 in the medium containing 0.8 and 0.7 mg/l Benzyl Amino Purine respectively. The number of shoots produced from nodal explants on medium with 1.0 mg/l BAP was 3.8 with an average height of 2.5 cm. We found an increase in the per cent response of shoot induction and number of shoots with an increase in the concentration of Benzyl Amino Purine from 1.0 mg/l to 2.0. The percentage of explants exhibiting shoot induction was found to be between 40-80 is most of the concentrations of Benzyl Amino Purine tested except MS medium supplemented with 1.0 mg/l Benzyl Amino Purine. After 26-28 days of culture, nodal explants derived shoot cultures were subcultured to MS medium fortified with same concentration of hormone for shoot elongation. Significant elongation has been achieved in medium with 3.0 and 4.0 mg/l Benzyl Amino Purine. There was no significant variation in shoot length between the different concentrations of Benzyl Amino Purine except in the case of medium with 2.0 mg/l producing average shoot length of 1.5-2.0 cm. The effect of Benzyl Amino Purine in inducing shoot induction was already reported in some of the important medicinal plants of Asclepiadaceae family members such as *Ceropegia bulbosa* (Patil, 1998; Britto *et al.*, 2003), *Gymneme elegans* (Komalavalli and Rao, 2000) and in *Holostemma ada-kodien* (Martin, 2002). The promotive effect of Benzyl Amino Purine on shoot induction and multiplication was well understood in various plants like *Phytolocca decanta* (Demeke and Huges, 1990), *Saussuriea lappa*, *Clerodendran colebrookianum* (Mao *et al.*, 1995), *Trichopus zeylanicus* (Krishnan *et al.*, 1995) and in *Woodfordia fruticosa* (Krishnan and Seeni, 1994). To analyse the shoot induction ability of leaf explants from *in vitro* multiplied plants, nodal explants were used as an ideal source of explants for reculturing. Additional 3-6 shoots per node explants on MS medium fortified with 1.0 mg/l indicate the effectiveness of explants on multiple shoot induction without surface sterilization. A similar effect of the hormone in enhancing shoot induction has been reported in one of the Asclepiadaceae family members, *Ceropegia candelabrum* (Beena *et al.*, 2003). As expected, contamination rate has been drastically reduced in recultured leaf explants. (Table 1, Plate 1).

Table 1: *In vitro* Propagation of desirable plants through cultivation from leaf explants of *Cocculus orbiculatus*

S.No.	BAP+KN+L-Glutamic acid concentration	Shoot length (cm)	No of shoots produced
	(mg/l)	(Mean ± SE)	leaf explants/Callus Response
			(Mean ± SE)
1	BAP+Kn 1.0mg/l	1.20±0.42	1.12±0.44+Callus
2	BAP+Kn 2.0mg/l	2.50±0.07	1.20±0.42+Callus
3	BAP+Kn 3.0mg/l	1.2±0.44	1.22±0.46+Callus+Shoot
4	BAP+Kn 4.0mg/l	1.70±0.09	24±0.42+Shoot (2-4)
5	BAP+Kn 5.0mg/l	1.40±0.06	1.50±0.40+Callus

6	BAP+L-Gltamic acid 1.0mg/l	1.82±0.05	1.6±0.47 Small buds
7	BAP+L-Gltamic acid 2.0mg/l	1.8±0.05	2.1±0.36 Callus+Shoots(2-4)
8	BAP+L-Gltamic acid 3.0mg/l	2.04±0.06	2.1±0.38 Small shoot
9	BAP+L-Gltamic acid 4.0mg/l	2.08±0.08	1.4±0.40 Shoot (3-6)
10	BAP+L-Gltamic acid 5.0mg/l	2.9±0.08	3.6±2.6 shoots

Callus may be serially sub cultured and grown for extended periods but its composition and structure may change with tissue as certain cells are favored growth by MS Medium. In the present study, Adenine Sulphate when used in combination with Benzyl Amino Purine induced multiple shoots. Among the combinations tested, Benzyl Amino Purine (1.0) with 5.0 mg/l Adenine Sulphate produced maximum number of shoots with intermittent callus at the basal cut end of the various concentrations Sulphate with Benzyl Amino Purine and naphthalene acetic acid (Misra *et al.,* 2003). Explant length viability culture conditions such as growth hormone concentrations and selective explants segments the degree of success of finding plant variants *in vitro* culture. Additional example of beneficial variation include stress resistance such as drought tolerance improved quality number of different factors influence the presence of variations during plant tissue culture.

Plate 1: *In vitro* Propagation of desirable plants through cultivation from leaf explants of *Cocculus orbiculatus.*

IV. CONCLUSION

The selection of leaf explants dividing cells are callus cultured on MS media with nutrient and growth regulators combination to support the callus with small buds. Explants from both mature and immature organs can be induced to from callus and then plant regeneration. The great potential of *In vitro* methods of leaf explants from *Cocculus orbiculatus* large scale plant production multiplication can be tapped by cutting down the cost of production per plant by applying low cost tissue culture methods. Plants are examined to establish whether the expressed in the desired tissue plant cells and tissue to be transformed include *in vitro* culture leaf explants

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Corresponding Author: Mandaloju Venkateshwarlu
Department of Botany, Kakatiya University, Warangal Urban- 506 009, T.S. INDIA.