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RESEARCH ARTICLE

IN VITRO REGENERATION STUDIES OF BLACK TURMERIC (*CURCUMA CAESIA ROXB.*)

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ABSTRACT

Curcuma caesia, a member of Zingiberaceae family, is a unique perennial aromatic plants distributed in South and Southeast Asia. The most important components of turmeric are curcuminoids which chemically related to its principal ingredient of curcumin. The detailed studies using curcumin include antioxidative, anti inflammatory, anti carcinogenic, anti viral and anti infectious activities, wound healing and detoxifying properties. Plant tissue culture is an appropriate tool for conserving the rare and endangered *Curcuma spp*. Morphogenetic changes were observed in *Curcuma caesia* explants in the MS media supplemented with different concentrations of PGRs. Kinetin 1 and 2 mg/l and combination of BAP 1mg/l and Kinetin 1mg/l exhibited a better response.

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INTRODUCTION

The members of the Zingiberaceae family are commonly known as gingers. The family comprises of about 50 genera and 1300 species worldwide and is mostly distributed in South and Southeast Asia (Wu and Larsen, 2000). They are unique perennial aromatic plants characterized by their tuberous or non tuberous rhizomes having strong aromatic and medicinal properties (Miquel *et al*., 2002; Ammon, 1991; Charles and Charles, 1992). Their medicinal and culinary uses have been widely discussed and accepted in many traditional recipes. Ginger plants contain many essential oils including terpenes, alcohols, ketones, flavonoid, carotenoids and phytoestrogens (Habsah *et al*, 2000; Mau, 2003). Reports on their antifungal, antioxidant, insecticidal and anti inflammatory activities have also been widely represented (Sirat *et al*., 1996). Naturally, the members of the family Zingiberaceae occur in the Indo-Malaysian sub kingdom in the tropical and subtropical areas. They mostly grow in the damp and humid shady places. Their centre of distribution is in South-east Asia. Among the different genera of Zingiberales, genus *Curcuma* is well known for their commercial and medicinal values. It is considered to be originated in the Indo Malayan Region and it has widespread occurrence in the tropics of Asia to Africa and Australia.

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The genus is composed of about 70-80 species of rhizomatous annual or perennial herbs (Sirirugsa, 1999).Out of these, about 40 species are of Indian origin (Velayudhan *et al*., 1999) The genus *Curcuma* has paramount importance as spice, medicines, dyes, cosmetics, starch and ornamentals. *Curcuma* is gaining importance as a potential source of new drug(s) to combat a variety of ailments. The most important species is *Curcuma longa* is commercially known as turmeric plant. Turmeric is the processed underground rhizome used as spice, herbal medicines, dyeing agents and cosmetics since vedic age. The most important components of turmeric are curcuminoids which refer to a group of phenolic compounds, which chemically related to its principal ingredient of curcumin. The detailed studies using curcumin include antioxidative, anti inflammatory, anti carcinogenic, anti viral and anti infectious activities (Sirat *et al*., 1996). Recently, the wound healing and detoxifying properties of curcumin have also received considerable attention. (Joe and Lokesh, 2004) Another important species is *C.Caesia* also known as black turmeric. It has a distinctive bluish black rhizome and it is native to north east and central India. The rhizomes have a high economical importance owing to its wonderful medicinal properties .It is used in the treatment of leprosy, piles, bronchitis, asthma, fever, wounds, pimples, allergies, migraine, impotency, fertility, menstrual disorders, tootache, vomiting etc. But presently, black turmeric is on the verge of extinction due to biopiracy. And there are also other useful curcuma species having medicinal properties and other uses like in floriculture,

cosmetic, pharmaceutical and as a source of starchy food. Tissue culture or *in vitro* culture is the culture of plants, seeds, embryos, organs, explants tissues, cells and protoplasts on nutrient media under sterile condition. It has shown magnificient development since 1975 resulting in the production and regeneration of viable individual of many plant species .In addition, since 1980 plant tissue culture has evolved into major and indispensable element in many fundamental science and applied biotechnology. The technique has been successfully used for the commercial production of pathogen free plants an to conserve germplasm of rare and endangered species (Fay, 1992; Mikulik, 1999). *In vitro* culture techniques provide alternative means of crop improvement and a tool of crop improvement. Advance biotechnological methods of culturing plant cells and tissues provide new means of conserving and rapidly propagating valuable, rare and endangered medicinal plants. Thus, plant tissue culture technology will be an appropriate tool for conserving the rare and endangered *Curcuma sp*. in the tropical countries since they are sterile seedless species. Conventionally, rhizomes are used as planting material which encounter several disadvantages such as low multiplication rate by rhizome separation method, requirement of large amount of rhizome for propagation in the next season and senescence and degeneration due to intrinsic pathogens an disease incidence in field and storage. There has been various reports on in vitro regeneration of *Curcuma sp*. (Nadgauda *et al*., 1978).

However reports on micropropagation of wild species of *C.caesia* are rare. Thus the present study was undertaken to standardardize a protocol for the *in vitro* regeneration of black turmeric which is highly threatened and overexploited for commercial values.

MATERIALS AND METHODS

Mature rhizomes were collected from healthy *C. caesia* plants maintained in the Botanical garden of Modern College, Imphal, Manipur, India. The rhizomes were cleaned thoroughly by repeated washing with running tap water and maintained in the laboratory for sprouting. After three weeks the sprouted rhizomes were washed along with few drops of labolene and then treated with Bavistin (Carbendazim 50%w/w) for 10-15 minutes and rinsed with running tap water for 3 times. This was followed by surface sterilization under aseptic conditions with 70% (v/v) ethanol for 5 minutes. After this the sprouts were treated with 0.1% (w/v) mercuric chloride for 5 minutes. Then the sprouts were again rinsed with sterile distilled water 3 times and the sprouts were ready to use as the explants for the experiment.

Culture of Explants

The sprouts were wounded in all the directions with a fine sterile blade to remove the scales and excised it into small pieces.

Approximately the excised sprouts measuring 0.5 × 0.5 cm^2 were used for explants for the present study. The young buds were excised from the sprouting rhizomes and used as explants. The explants were inoculated on MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of Plant Growth Regulators (PGRs). In this experiment we used cytokinins,1-3mg/l of 6-benzylaminopurine(BAP) and Kinetin(Kn) alone and in combinations of BAP and Kn. All the media were supplemented with 30g/l sucrose,8g/l agar and 100mg/l myoinositol.

The pH of all the media were adjusted to 5.8 with 0.1M NaOH prior to addition of the agar and approximately 25ml of the medium was dispensed into culture tubes (25×150mm, Borosil, Mumbai, India). The medium was autoclaved at 121^oC for 20 minutes under 15lbs psi pressure. The chemicals used for the experiment were manufactured by M/S Himedia Company, Mumbai, India. Each culture tube received one explants. Five replicates were taken for each treatment. The cultures were maintained in a growth chamber at 25±1^oC under 16/8h (light/dark) photoperiod with light intensity of 1000lux.Observations were recorded every week for each of the cultures for any changes.

RESULTS AND DISCUSSION

The data on the effect of MS basal media supplemented with different concentration of Plant Growth Regulators (PGRs) on *C. cae*sia young bud explants are given in the Table 2. During the culture periods in the controls (MS medium without PGRs), neither shoot nor root induction was observed throughout the observation. MS media supplemented with growth regulators responded the varied degree of shoot and root formation by the explants based on the hormone and the concentration used (Fig. 2). The explants initiated shooting within 2-8 weeks upon culturing on different MS media supplemented with different concentration of growth regulators (Table 1). Among the ten different treatment of growth regulators, kinetin 1mg/l and 2mg/l and combination of BAP 1mg/l and kinetin 1mg/l shows a better response than any other treatment (Fig 2C). However, MS media supplemented with 3mg/l of kinetin and BAP do not show any promising results.

Table 1. Order of weeks taken by the C. caesia explants to show the 1st morphogenetic change, to form shoots, leaves and roots when treated with MS media supplemented with different concentration of PGR

S.No.	MS + PGRs (mg/l)		0rder (weeks) of 1st morphogenetic change	0rder (weeks) of shoot formation	0rder (weeks) of leaf formation	0rder (weeks) of root formation
	BAP	Kn				
1	0		-	-	-	-
2	1	0	-	-	-	-
3	2	0	-	-	-	-
4	3	0	-	-	-	-
5	0	1	4	-	-	-
6	0	2	2	5	6	6
7	0	3	-	-	-	-
8	1	1	4	5	6	8
9	1	2	-	-	-	-
10	2	2	4	-	-	-

Denotes absence of data

Table 2. Morphogenetic changes showed by the *C. caesia* explants in the MS media supplemented with different concentration of PGRs

S.No.	MS+PGRs(mg/l)		Morphogenetic changes after a no. of weeks			
	BAP	Kn	2nd week	3rd week	4th week	5th week
1	0	0	No change	No change	No change	No change
2	1	0	No change	No change	No change	No change
3	2	0	No change	No change	No change	No change
4	3	0	No change	No change	No change	No change
5	0	1	Turns yellowish & bulging	Turns cream colour & show outgrowth	Turn pale & outgrowth proliferates	Pale & proliferates
6	0	2	Turns green	Shooting initiated	Rooting initiated	Shoot & rooting initiated
7	0	3	No change	No change	No change	No change
8	1	1	No change	Turns green	Shooting initiated	Shoot & rooting initiated
9	1	2	No change	No change	No change	No change
10	2	2	Turns green	green	green	green

Table 3. Effects of MS medium supplemented with BAP and Kinetin on C. caesia on shoot and root development after 8 weeks of culture

S.No.	BAP (mg/l)	Kn (mg//)	Mean shoot length (cm)	Mean no. of roots
1	1	1	1	5.0
2	0	2	2.2	2.0

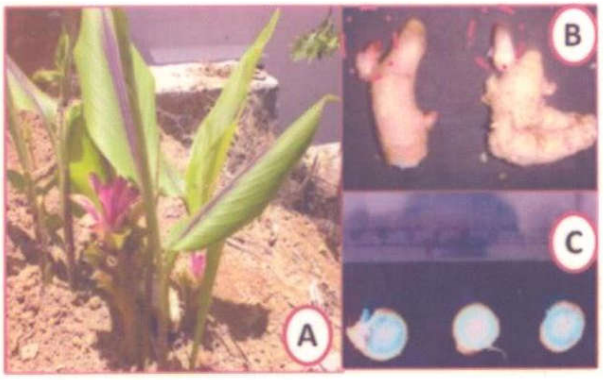

Figure 1:A - A flowering *C. caesia* plant; B - sprouted rhizomes after three weeks of sprouting (inset - sprouts); C - Characteristic Bluish black rhizome of *C.caesia*

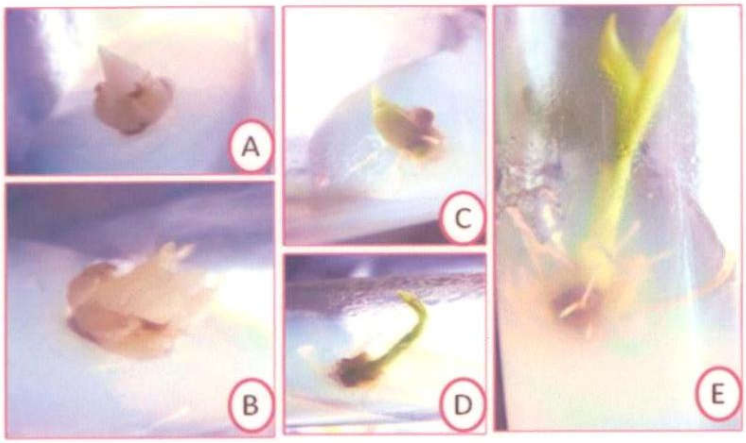

Figure 2: A - Fourth week old culture turning cream yellowish and showing outgrowth on MS media supplemented with Kn (1 mg/l); **B** - Sixth week old culture turning pale and the outgrowth proliferates on MS media supplemented with Kn (1mg/l); **C** - shoot and root initiation on the MS media supplemented with BAP(1mg/l) + Kn (1mg/l) in the sixth week old culture ; **D** – shooting in the fourth week old culture on MS media supplemented with Kn (2mg/l); **E** – Rooting and shooting in the eight week old culture on the MS media supplanted with Kn (2mg/l).

Induction of root and shoot was recorded with MS medium fortified with kinetin 2mg/l and combination of kinetin 1mg/l and BAP 1mg/l as indicated in Table 3 (Fig. C-E). The shooting initiations were observed only at the cut edges of the explants. In the present study, the ability of shoot and root induction along with the healthy looking leaf and root induction was found superior in MS media supplemented with Kinetin compared to BAP. This is in disagreement with the reports in other cucurma spp. (Tyagi *et al*., 2004). Thus, the present study showed that tissue culture presents an efficient system of in vitro propagation for rapid multiplication, production of disease free plants, non-seasonal production of plants, germplasm conservation and facilitating their easy exchange.

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