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Review

Biotechnology of *Curcuma*

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Abstract

Curcuma is gaining importance globally as a potential source of drugs to compat a variety of ailments. Biotechnological intervention in the genus is almost two decades old. Though earlier studies of *Curcuma* biotechnology were focused mainly on standardization of tissue culture protocols, recent attention has been towards molecular biology aspects of the genus. This review gives up-to-date information on the various aspects of *Curcuma* biotechnology such as tissue culture protocol standardization, *in vitro* conservation, *in vitro* screening, *in vitro* microrhizome induction and *in vitro* pollination besides molecular biology studies.

Keywords: Biotechnology, *Curcuma*, Isozymes, Genetic fidelity, Molecular biology, Microrhizome, Molecular taxonomy, Phylogeny, Tissue culture

Introduction

Curcuma is gaining importance all over the world as a potential source of therapeutic molecules to combat a variety of ailments as the genus is credited with anti-inflammatory, hypocholestraemic, choleratic, antibiotic, antidiabetic, anticancerous, antihepatotoxic, antiviral, antivenomous and antirheumatic properties besides its use in Alzheimer's disease. Turmeric is also credited with insect repellant properties and also it has use in aroma therapy and perfume industry [1].

Some species of *Curcuma* are valued by the starch industry and floriculturists.

The economically more important *Curcuma* species are given in Table 1.

Biotechnological intervention in *Curcuma* has attracted attention of researchers since the last 2–3 decades. This paper reviews the biotechnological research in *Curcuma*.

In addition to *Curcuma longa* L., the common culinary turmeric, other economically important *Curcuma* species also received the attention of researchers. Biotechnological works in *Curcuma* can be broadly grouped into three categories: tissue culture, molecular markers and genetic transformation.

Tissue Culture

Tissue culture studies in *Curcuma* have been attempted by many workers to standardize protocols for *in vitro* propagation, cell and suspension culture to screen variants with useful traits and for conservation.

Effect of Explants and Media

Among the various explants tried, rhizome buds have been found to be a very useful material for *in vitro* propagation in *Curcuma*. One of the first attempts on micropropagation of turmeric in India was that of Nadgauda *et al*. [2]. These authors could produce plantlets from young vegetative buds (sprouts) obtained from rhizome of two *C. longa* varieties viz. 'Duggirala' and 'Tekurpet' either in Murashige and Skoog (MS) or Smith's medium supplemented with coconut milk, kinetin, benzylaminopurine (BAP) and/or inositol. They also could induce callus from the shoot region of the *in vitro* raised plantlets and multiple shoots from the callus. Subsequently Shetty *et al*. [3] could induce callus and plantlets from sprouting rhizome buds on MS medium supplemented with kinetin 0.2–0.5 mg/l. Parallel to these studies in India,

Table 1 Economically important *Curcuma* spp. [1]

Species	Use
Curcuma longa L. syn *C. domestica* Val.	Spice, medicine, dye, religious, local delicacies, insect repellent, aroma therapy and perfume
C. amada Roxb., *C. mangga* Val. and Zijp.	Spice, medicine, pickles and salads
C. zedoaria Roxb.	Folk medicine, arrow root industry
C. ochrorrhiza Val. and Van Zijp	Malayan traditional medicine
C. pierreana Gagnep.	Vietnamese traditional medicine
C. aromatica Salsb.	Medicine, toiletry articles, insect repellent
C. kwangsiensis S. G. Lec and C. F. Liang syn *C. chuanyujin*, *C. phaeocaulis* Val.	Chinese traditional medicine
C. caesia Roxb.	Spice and medicine
C. comosa Roxb.	Traditional medicine of Thailand
C. angustifolia Roxb., *C. zedoaria* Roxb. *C. caulina* F. Grah., *C. psuedomontana* F. Grah. *C. montana* Roxb., *C. rubescens* Roxb., *C. leucorrhiza*, *C. xanthorrhiza* Roxb., *C. decipiens* Dalz., *C. malabarica* Vel. *et al*., *C. raktakanta* Mangaly and Sabu, *C. haritha* Mangaly and Sabu, *C. aeruginosa* Roxb.	Arrow root industry
C. alismatifolia Gagnep, *C. thorelii*, *C. roscoeana* Wall.	Ornamental (cut flower)

Japanese workers also succeeded to induce callus and plantlets from rhizomes of three *Curcuma* species viz. *C. longa*, *C. zedoaria* and *C. aromatica* in MS medium supplemented with 1-naphthalene acetic acid (NAA) and kinetin or BAP [4].

MS medium has been the widely used medium and benzyladenine (BA) or BAP supplementation has been found to have a significant effect on shoot proliferation in *in vitro* clonal propagation of *Curcuma* [5–10]. The beneficial effect of MS medium fortified with BA for callus regeneration and multiple shoot production from fresh rhizome sprouts of *C. aromatica* too was reported [12]. In a study on the effect of BA on clonal propagation of *C. longa* from *in vitro* derived plants, Mogor *et al*. [6] reported non-significant effect of different levels of BA on multiple shoot induction.

Balachandran *et al*. [5] could induce shoots and roots from rhizome explants of *C. longa*, *C. aeruginosa* and *C. caesia* cultured on MS medium fortified with BA or a combination of BA and kinetin. These authors could observe significant species × media composition interaction for multiple shoot production. However, all the *Curcuma* species studied responded well to BA.

Chan and Thong [7] also observed that *C. longa* (*C. domestica*) and *C. zedoaria* produced more number of multiple shoots when cultured on liquid MS medium containing BA and indole 3-butyric acid (IBA). Winnaar and Winnaar [8], Ali *et al*. [9], Tule *et al*. [10] and Rahman *et al*. [11] have also corroborated the above results through their studies on *C. longa*.

The positive effect of BA in combination with the NAA, kinetin, triiodobenzoic acid (TIBA), 2,4-dichlorophenoxy acetic acid (2,4-D), thidiazuron (TDZ) and indole 3-acetic acid (IAA) in either liquid or solid MS medium to induce callus and multiple shoots from rhizome buds, shoot tips and immature inflorescence of different *Curcuma* species such as *C. longa*, *C. amada*, *C. caesia*, *C. aromatica*, *C. zedoaria* are reported in different works [13–30]. Histological studies in *C. zedoaria* revealed the ontogeny of callus from the hypertrophied cortical parenchyma cells of the root explant [31]. Sucrose was found to be the best carbon source in the culture medium for *C. zedoaria* [32]. Longitudinally split rhizomes of the new ornamental bulb, Siam tulip or Summer tulip (*C. alismatifolia*) also responds well to MS medium fortified with BA [33, 34]. However, Yasuda *et al*. [4] reported callus regeneration from rhizomes of *C. longa*, *C. aromatica* and *C. zedoaria* cultured on MS medium containing NAA and kinetin without BA. But for plant regeneration from stem explant of *C. zedoaria* on MS medium the need for NAA or BA is recorded by these workers. A positive effect of NAA in MS medium on rooting of *in vitro* derived turmeric is also reported [10]. The significant effect of TDZ alone in MS (solid or liquid) medium for *in vitro* shoot proliferation and spontaneous rhizogenesis from terminal bud explants of *C. longa* is also recorded [35–37].

A perusal of these works reveals that irrespective of the *Curcuma* species studied, rhizome buds (sprouts) cultured in MS medium containing BA with or without other phytohormones or vitamins favoured callus induction and multiple shoot production. TDZ fortified MS medium was also found good for multiple shoot production in different *Curcuma* species.

In Vitro *Screening Studies*

Turmeric is a vegetatively propagated crop and efforts to improve turmeric by conventional breeding is beset with the problems of heterozygosity and lack of sexual cycle. Hence, researchers were seized with the idea of using *in vitro* techniques in turmeric for isolating useful variants using tissue culture techniques. Nadgauda and Mascarenhas [38] tried to develop an *in vitro* screening method to

select high curcumin turmeric lines by estimating the curcumin content from the 'rhizome-like' portions of the *in vitro* grown plants and later on correlating this value with that of the field-grown plants. However, this work failed to lead to any practical results. In fact, there is very high variety $\times$ environment interaction for curcumin content in turmeric and hence this type of *in vitro* screening is unlikely to yield any practical results. Nearly two decades later, Gayatri *et al*. [39] tried to isolate *Pythium graminicolum* tolerant cell lines from *C. longa* variety 'Suguna'. These authors incorporated culture filtrate of *P. graminicolum* in the culture medium and selected tolerant cell lines and grew out them in *in vitro* sick plots. The plants derived from the tolerant cell lines exhibited better disease tolerance as compared to the control. *In vitro* screening of variants on selection media containing culture filtrates of various pathogens, though have academic interest, may not yield any useful practical result as we have observed in case of ginger and black pepper, one of the reasons being selection of non-stable epigenetic cell lines and their subsequent reversion or suppression.

In Vitro *Conservation*

With the advent of commercial exploitation of economically important *Curcuma*, particularly from the wild habitat, the rich biodiversity is gradually getting lost. Attempts have been made by many institutes around the world to collect and conserve these germplasm. Conserving them in the field gene banks as well as in the *in vitro* gene banks is essential to conserve this important genus. Balachandran *et al*. [5] described a short term conservation of *in vitro* raised plants of different *Curcuma* species as an *in vitro* conservation strategy. Subculture intervals for *in vitro* conservation of different *Curcuma* species such as *C. aeruginosa*, *C. caesia* and *C. longa*, are standardized [40]. As compared to other members of the family Zingiberaceae, *Curcuma* species need an earlier subculture interval of 6–8 months under *in vitro* conservation. Ravindran *et al*. and Babu *et al*. [41, 42] reviewed the status of biotechnology of different spices including turmeric on micropropagation, exploitation of somaclonal variation, *in vitro* selection against stresses and *in vitro* conservation.

Production of synthetic seeds in turmeric under *in vitro* condition as a safe means of germplasm exchange and conservation is highlighted [43]. Gayatri *et al*. [44] studied the effect of two types of hydrogels in encapsulating the non-embryogenic vegetative propagule shoot buds of turmeric (*C. longa*) var. 'Suguna'. It was observed that among the two encapsulating agents tried, i.e. sodium alginate and carboxymethyl cellulose, sodium alginate (4%) containing Linsmaeir and Skoog's medium (LSBM) fortified with BAP, dipped in 50 mM calcium chloride solution and incubated for 30 min in orbital shaker was found to be the best matrix and complexing agent for encapsulation and regeneration of *in vitro* buds.

In Vitro *Mutation Induction*

In vitro mutation induction in *C. longa* varieties 'Suvarna' and 'Prabha' using chemical (ethyl methane sulphonate) and physical mutagens resulted in cytotypes with distinct chromosome numbers [37].

In Vitro *Microrhizome Production*

Microrhizomes are very convenient for packing and transportation besides its advantage in germplasm conservation and exchange. Thus, attention of researchers has been also drawn into this aspect of *Curcuma* biotechnology.

Rajan [45] reported the successful *in vitro* microrhizome formation at the base of *C. longa* plantlets raised on MS medium supplemented with BAP, NAA and ancymidol with 10% sucrose and observed that microrhizomes of 8 weeks maturity are ideal for field planting as they exhibited better germination. Inhibitory effect of BA on *in vitro* microrhizome production of *C. longa* too was reported [46]. Nayak [13] observed that an increase in the concentration of sucrose in the medium favoured better microrhizome induction. She observed that a liquid MS medium supplemented with BA with reduced photoperiod favoured microrhizome production at the base of the plantlet after 30 days of culturing, in four varieties of *C. longa* viz. 'Ranga', 'Rasmi', 'Roma' and 'Suroma'. Maturity of the microrhizome was 120 days. Nayak [12] further observed that MS medium fortified with 5 mg/l BA, 60 g/l sucrose and 8 h photoperiod were optimum for *in vitro* microrhizome production in *C. aromatica*.

In a study on the factors affecting *in vitro* microrhizome production in turmeric (*C. longa*), Shirgurkar *et al*. [47] observed that dark cultures on half strength MS medium supplemented with 80 g/l sucrose and lower concentrations of BA along with kinetin induced better microrhizome formation. BA at higher concentrations completely inhibited the microrhizome induction. Further, it was also observed that the size of the multiple shoots used for culturing also affected the formation of microrhizomes in turmeric.

Sunitibala *et al*. [18] also observed better microrhizome formation in MS medium with 6–8% sucrose plus NAA and kinetin in *C. longa*.

In Vitro *Pollination*

Though turmeric sets seed naturally and true turmeric seedlings-derived varieties are released, *in vitro* pollination is also attempted in this crop [48, 49]. Seed set and

Table 2 Summary of tissue culture research in *Curcuma*

Sl. no.	Species	Explant	Media	Result	Reference
1	*C. longa*	Sprouting rhizomes	MS + Kn (0.2–0.5 mg/l)	Induced callus and plantlets	[3]
2	*C. longa*, *C. zedoaria* and *C. aromatica*	Rhizome	MS + NAA (0.1–1.0 ppm) + Kn (0.1 ppm) + BAP (0.3 ppm)	Induced callus and plantlets	[4]
3	*C. longa*, *C. caesia and C. aeruginosa*	Rhizome	MS + BA (0.5 mg/l) or BA (1.0 mg/l) + Kn (1.0 mg/l)	Simultaneous production of shoots and roots. BA was found very significant in shoot production. The different species responded differently to the media except *C. caesia*	[5]
4	*C. longa and C. zedoaria*	Rhizomes/*in vitro* derived plants/rhizome sprouts	MS + BA (1.0–2.0 mg/l) and IBA (2.0 mg/l)/ MS + BA (2.0 mg/l)/MS + NAA (0.1–1.0 mg/ l) + IBA (0.1–1.0 mg/l) + IAA (0.1–1.0 mg/ l)/MS + BA (0.1–1.0 mg/l) + Kn (0.25 mg/l)	Produced multiple shoots/ rooting. MS with NAA was found good for rooting	[6–11]
5	*C. aromatica*	Freshly sprouted shoots/ plantlets	MS + BA (1.0, 3.0, 5.0 and 7.0 mg/l)/MS + BA (1.0, 3.0 and 5.0 mg/l) + Kn (0.5 mg/l)/ MS + higher concentration of sucrose (60 g/ l) + BA (5.0 mg/l) + reduced photoperiod (8 h)	Multiple shoot development, microrhizome production, induced microrhizomes	[12]
6	*C. longa*	Immature inflorescence/ sprouted buds/leaf base/ vegetative buds	MS + BAP (5.0 mg/l) and 2,4-D (0.1–0.2 mg/l)/ MS + BAP (1.0–2.0 mg/l) + NAA (0.1 mg/l) + TDZ (1.0–2.0 mg/l) + IAA (0.1 mg/l)/MS + BA (5.0–10.0 mg/l) + TIBA (0.1 mg/l) or 2,4-D (0.1 mg/l)/MS + Kn (0.1–1.0 mg/l)	Shoot regeneration, multiple shoot production	[14–17]
7	*C. longa*	Rhizome buds	MS + NAA (1.0 mg/l) and Kn (1.0 mg/l), MS + NAA (0.1 mg/l) and BAP (2.0 mg/l), MS + 2,4-D (2.5–3.0 mg/l)	Rapid shoot multiplication, callus induction	[18]
8	*C. longa*	Rhizome buds	MS + BA (4.0 mg/l) + IBA or IAA (0.25–1.0 mg/l)	An increase or decrease in the concentration of BA or NAA reduced frequency of shoot multiplication	[19]
9	*C. longa*	Shoot with rhizome bits	MS + IBA (0.8–2.0 mg/l) + BAP (1.0 mg/l) + GA (0.1 mg/l) + NAA (0.1 mg/l)	Active uniform shoots, highest survival, maximum number of shoots. Explants of 2.5–3 mm size produced better shoots. Roots were produced in induction medium containing NAA	[20]
10	*C. longa*	Bud explants	MS + Kn (1.0 mg/l) + BA (1.0 mg/l)	Multiple shoot induction	[21]
11	*C. amada*	Rhizome	MS + BA (4.4 μM + NAA (1.0 μM/MS + 2,4-D (9.0 μM)/MS + BAP (8.88 μM) + NAA (2.7 μM)	Multiple shoot production, Friable callus	[22]

12	*C. amada*	Rhizome	B5 medium + NAA (0.5 mg/l) + BA (4.0 mg/l)	Simultaneous production of roots and shoots	[23]
13	*C. caesia and C. zedoaria*	Rhizome	MS + BAP (4.0 mg/l) + NAA (1.5 mg/l)	Best media for multiple shoot production in *C. caesia*	[25]
			MS + BAP (1.0 mg/l) + NAA (0.5 mg/l)	Best media for multiple shoot production in *C. zedoaria*	[25]
14	*C. aromatica* and *C. amada*	Bud explant	MS + 2,4-D (2.0 mg/l)/MS + BAP (2.0 mg/l) + NAA (1.0 mg/l)	Callus induction and multiple shoot regeneration from rhizome buds of *C. aromatica* and *C. amada*	[26]
15	*C. longa*, *C. aromatica*, *C. amada and C. zedoaria*	Nodal explants/rhizome	MS + NAA (4 mg/l) + Kn (5 mg/l)	Multiple shoots were produced in all the *Curcuma* sp.	[28]
16	*C. zedoaria*	Shoot apex of rhizome and *in vitro* plants	MS + BAP (2.0 mg/l)	Multiple shoot production	[30]
17	*C. zedoaria*	Root explants	MS + NAA (1.0 mg/l)	Callus induction	[30]
18	*C. zedoaria*	Root explants	MS + NAA (13.4 µM) + BAP (2.2 µM)	Callus induction	[31]
19	*C. zedoaria*	Root explants	MS + NAA (13.4 µM) + BAP (2.2 µM)	Callus induction. Sucrose is the best carbon source	[32]
20	*C. alismatifolia*	Rhizome	MS + BA (13.32 µM)	Multiple shoot production	[33, 34]
		Inflorescence	MS + TDZ (0.5 mg/l) + IMA (4.0 mg/l) + BAP (0.1 mg/l) + IAA (0.1 mg/l)	Shoot and root regeneration	
21	*C. longa*	Bud explants	MS + TDZ (18.17–72.64 µM)	Multiple shoot production	[35–37]
22	*C. longa*	Vegetative buds derived calli	LSBM + 2,4-D (3.0 mg/l) (selection media with culture filtrate of *P. graminicolum*)	Tolerant calli line obtained	[39]
23	*C. longa*	Embryogenic vegetative buds	LSBM + BAP (4.0 mg/l) + sodium alginate (4%) + calcium chloride (50 µM)	Better medium for synseed production	[44]
24	*C. longa*	Plantlets	MS + BA (3.0 mg/l), NAA (0.1 mg/l) and Ancymidol (0.5 mg/l) + 10% sucrose	Good for microrhizome induction *in vitro*	[45]
25	*C. longa*	Plantlets	MS + BA (12.0 µM) + NAA (0.3 µM) + 9% sucrose	Suitable for *in vitro* microrhizome induction when incubated in the dark	[46]
26	*C. longa*	Plantlets	MS + 80 g/l sucrose + BA (0.88–2.2 µM) + Kn (0.92 µM) + 5% coconut water	Lower concentration of BA along with high concentration of sucrose is found good for *in vitro* microrhizome production	[47]
27	*C. longa*	Inflorescence	MS (half strength) + NAA (0.5 mg/l) + BAP (1.0 mg/l) + Kn (1.0 mg/l)	*In vitro* seed set and development	[49]

development could be observed in the *in vitro* cultured and inter-pollinated spikes of the short duration turmeric varieties, whereas pollination between short and long duration varieties or between long duration varieties failed to set seed. Half-strength MS medium fortified with NAA, BAP and kinetin was used for *in vitro* pollination studies.

The tissue culture work in *Curcuma* is summarized in Table 2.

Molecular and Biochemical Markers

Use of molecular markers in *Curcuma* is confined to a few isozyme based characterizations of germplasm accessions of *C. longa* as well as species diversity analysis phylogenetic analysis and genetic fidelity studies of micropropagated plants. DNA marker studies for identification of *Curcuma* species have been reported only recently.

Isozyme Markers

Shamina *et al*. [50] studied the variability in 15 *C. longa* accessions collected from different geographical locations in India with respect to six isozymes viz. acid phosphates, superoxide dismutase, esterase, polyphenol oxidase, peroxidase and catalase. Polyphenol oxidase and peroxidase were more consistent and reproducible though acid phosphatase produced maximum number of bands. A high degree of polymorphism (63.8–96%) was observed in the population studied for these isozymes. Accessions collected from the same geographical area showed highest similarly (above 90%), indicating genetic similarity of the accessions which were collected based on vernacular names. However, two seedlings (true turmeric seedlings) included in the study stood out distinctly from the rest in the dendrogram which were clonal lines, indicating the potential to create new alleles through sexual recombination.

Apavatjrut *et al*. [51] used isozyme markers to resolve the taxonomic confusion in the genus *Curcuma*. These authors could identify eight isozymes that could discriminate the *Curcuma* species such as *C. zedoaria*, *C. xanthorrzhiza*, *C. rubescens*, *C. elata*, *C. aeruginosa*, along with two unidentified *Curcuma* species. The phenetic analysis based on the isozyme data showed the species, *C. rubescens*, is distinct from the other species, which was in agreement with the morphological observation. The isozyme pattern of the species cautioned about solely relying on the morphological/growth/reproductive characters for species delimitation in *Curcuma*.

Paisooksantivatana *et al*. [52] used allozyme polymorphism at seven *loci* to study the level of genetic diversity in the natural and domesticated population of *C. alismatifolia* of Thailand and Japan and observed that the natural populations are more diverse than the cultivated ones. And among the natural population, the ones in the high-altitude areas of Thailand were found to carry more polymorphic loci, indicating their pristine status.

Genetic Fidelity Studies

Random Amplified Polymorphic DNA (RAPD) study has been routinely used to ascertain the genetic fidelity of tissue culture-generated turmeric plants. Salvi *et al*. [14, 15] reported both lack of genetic fidelity and high degree of genetic fidelity of the micropropagated *C. longa* plantlets. Further, Salvi *et al*. [16] observed that plants regenerated directly from shoot tips of turmeric are more uniform, whereas callus-regenerated plants showed polymorphism in DNA banding pattern (RAPD). Praveen [37] too corroborated this observation in his studies on *C. longa*. Syamkumar *et al*. [53] developed a viable genomic DNA isolation protocol from fresh rhizome of *C. longa* as means to develop a Polymerase Chain Reaction (PCR)-based varietal discrimination technique for turmeric varieties, from rhizome DNA. Remya *et al*. [54] perfected a new protocol for isolation of genomic DNA from turmeric powder.

Apart from DNA-based genetic fidelity studies, chemical fidelity study is also attempted in *Curcuma*. GC pattern of essential oil of tissue culture-derived plants of *C. zedoaria* exhibited same pattern as that of the mother plants [4].

Molecular Taxonomy

Because of high morphological variations present in the genus *Curcuma* molecular markers are very useful. In order to clear the confusion about the true botanical origin of *Curcuma* species used in Chinese and Japanese medicines, molecular markers based on 18 S RNA gene and trnk gene sequences were developed. Though initially developed to discriminate the live specimens of *C. longa*, *C. phaeocaulis*, *C. wenyujin*, *C. aromatica* besides the Japanese and Chinese populations of *C. zedoaria* this technique was later extended to identify the genuine *Curcuma* spp. in the marketed drugs. These authors used Amplification Refractory Mutation System (ARMS) analysis of 18 S RNA and trnk genes on plants of the different species and could obtain species-specific fragments as well as between the Chinese and Japanese population of *C. zedoaria* [55, 56].

Though not directly connected to the molecular taxonomy of *Curcuma*, Sasikumar *et al*. [57] developed a PCR-based method for detection of adulteration of marketed *C. longa* (turmeric) powder with that of a wild species, *C. zedoaria*. These authors postulated the scope for developing species-specific Sequence Characterized Amplified Region (SCAR) markers for identifying the *Curcuma* species.

DNA markers-based genetic diversity analysis of *Curcuma* species is now initiated in India. Syamkumar and Sasikumar [58] developed molecular genetic fingerprints of 16 *Curcuma* species of India using Inter Simple Sequence Repeats (ISSR) and RAPD markers to elucidate the genetic diversity among the species. As reported by Apavatjrut *et al*. [51] in their isozyme-based characterization of *Curcuma* species, the dendrograms constructed based on the DNA makers in this study also did not fully endorse the morphology based discrimination of the *Curcuma* species. This study highlighted the limitations of the conventional taxonomic tools in resolving the confusion prevailing in the genus and suggested the need for molecular markers in conjunction with morpho-taxonomic and cytologic studies while revising the genus.

Phylogenetic Studies

A phylogenetic analysis of the tribe Zingiberaceae, performed using unclear ribosomal DNA (1TS1, 5.8 S and ITS2) and chloroplast DNA (*trn* L (UAA) $5'$ exon to *trn* F (GAA)) confirmed the monophyletic status of the tribe with two clades, the *Curcuma* clade and the *Hedychium* clade [59]. Kress *et al*. [60] attempted phylogenetic study of the Zingiberaceae including few *Curcuma* species based on molecular tools. The study suggested the need for molecular data to clearly discern the members of Zingiberaceae, agreeing with the earlier observations of Apavatjrut *et al*. [51] and the recent work of Syamkumar and Sasikumar [58].

Genetic Transformation

Many biotechnological tools such as somaclonal variation mutation breeding and induction of polyploids have been attempted and reported in *Curcuma*. But till recently, no reports are available on the genetic transformation in *Curcuma*. Recently, Shirgurkar *et al*. [61] have reported an efficient protocol for genetic transformation and shoot regeneration of turmeric via particle bombardment. They bombarded the callus cultures initiated from shoots with gold particles coated with plasmid pAHC25 containing the *bar* and *gus* A genes each driven by the maize ubiquitin promoter. They selected the transformants on medium containing glufosinate and established the transgenics. This first report would open vistas for further studies to clone useful genes as well as to transform turmeric for useful traits.

Conclusion

Biotechnology research in *Curcuma* initiated in the late 1970s has touched upon different aspects like tissue culture protocols, *in vitro* conservation, microrhizome induction and *in vitro* pollination besides molecular biology studies (isozymes, phylogeny and DNA markers). It is evident from the literature that MS medium supplemented with BA along with other phytohormones is the best tissue culture media for *Curcuma*. A significant effect of the hormone TDZ is also reported in a few cases. But a critical evaluation of the literature reveals that except for the molecular biology works, other aspects of *Curcuma* biotechnology need more focused attention of the workers. Though tissue culture protocol has relevance in genetic modification of *Curcuma*, its successful application as a large-scale plantation practice is doubtful, unless a cost-effective direct regeneration protocol is developed. Molecular biology research such as DNA marker studies will be worth pursuing from the point of bio-prospecting of the genus as there exists lot of confusion about the taxonomy of the genus. Another relevant area of interest would be identification and characterization of the key enzymes involved in the biosynthesis of medicinally important molecules of *Curcuma*.

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