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Review article

Biotechnology of *Zingiber montanum* (Koenig) Link ex A. Dietr.: A review

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ABSTRACT

The present article reviews the biotechnological research in *Zingiber montanum* (Koenig) Link ex A. Dietr. (cassumunar ginger). Studies on biotechnological interventions in this plant were focused mainly on the application of tissue culture techniques. Recently, attentions are being drawn towards the genomic studies which mostly aimed at establishing the genetic relationship of different gene pools. The present review summarizes the various aspects of tissue culture protocol for *in vitro* micropropagation, *in vitro* microrhizome induction, *in vitro* conservation technology and molecular biology studies of this medicinal plant. This review will help in further research of this plant in areas related to tissue culture for propagation and increased production of important secondary metabolites, phylogenetic analysis and genetic engineering. Moreover, it will offer an insight into crop improvement and breeding programs of this important underutilized medicinal plant for improved yield and quality of secondary metabolites.

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Contents

1. Introduction 1
2. Tissue culture studies 2
   2.1. Micropropagation 2
   2.2. *In vitro* rhizome induction and conservation of germplasm 2
3. Molecular biology studies 2
   3.1. Molecular markers in genetic diversity and phylogenetic studies 2
4. Conclusion and future perspectives 3
   Acknowledgement 3
   References 3

1. Introduction

Zingiber montanum (J. Koenig) Link ex A. Dietr. (Family Zingiberaceae), is a perennial aromatic herb with strong camphoraceous odour and spicy, bitter tasting, bright yellow, fleshy, strongly scented rhizomes (Watt, 1972) distributed mainly in India, Indochina and tropical southeastern Asia (Sirirugsa, 1988). It is locally known as "Tekhao Yaikhu" in Manipur and used commonly as folk medicine to combat various ailments and disorders (Burkill, 1966). It is also used as a flavoring agent in many food preparations and as a substitute for true ginger due to its strong aroma (Prakash and Mehrotra, 1996). Phytochemical investigations have revealed that many components are bio-active due to the presence of wide range of active secondary metabolites such as terpenoids, flavonoids, alkaloids, steroids, and benzenoids. Sabinene and terpinen-4-ol have been reported as the main constituents of all the reported oils (Singh et al., 2015). Both *in vitro* and *in vivo* clinical evaluations have shown that rhizomes of this plant possess high antioxidant activity (Jitoe et al., 1992, 1994; Habsah et al., 2000; Chirangini et al., 2004; Vankar et al., 2006; Manochai et al., 2007), anti-inflammatory activity (Wasuwat et al., 1987; Panthong et al., 1990; Ozaki et al., 1991; Masuda and Jitoe, 1994; Masuda et al., 1995; Pongprayoon et al., 1996; Nagano et al., 1997; Oyama et al., 1998; Vimala et al., 1999; Jeenapongsa et al., 2003; Lee et al., 2007), anti-allergic activity (Tewtrakul and Subhadhirasakul, 2007), hypotensive activity (Mokkhasmit et al., 1971), antiarrhythmatic activity (Veerasarn and Komalahiranya, 1971), local analgesic and anesthetic activity (Anantasan and Asayakun, 1971,

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1975), antibacterial and antifungal activity (Kishore et al., 1988; Kishore and Dwivedi, 1992; Dubey et al., 2000; Jantan et al., 2003; Pithayanukul et al., 2007; Tripathi et al., 2008) and antihistaminic action in asthmatic patient (Piromratana et al., 1986). Thus, it has gained importance globally as a potential source of many plant-based drug formulations and its demand is increasing in the pharmaceutical sector.

Biotechnological researches in this plant over the past decades include *in vitro* cell and tissue culture studies, molecular markers in genetic diversity analysis, molecular cloning and gene expression studies. Most common problem faced in the breeding programs of cassumunar ginger is the identification of germplasms as the plant phenotypes are morphologically very similar and thus, limits future use of germplasms for genetic improvement. If the parental genotypes and the genetic similarities of parental accessions were identified and assessed, it would be easier for breeders to select the parents (Kladmook et al., 2010). Thus, the detailed information provided in this review will provide incentive for proper evaluation of the plant which will help in their germplasm conservation and studies on genetic manipulation for economical production of useful medicinal products.

2. Tissue culture studies

Tissue culture techniques provide an indispensable tool for rapid propagation of pathogen-free plants, their sustainable growth and crop improvement. *In vitro* cell and tissue culture studies in cassumunar ginger were mainly focused on micropropagation and *in vitro* conservation through microrhizome induction of the plant.

2.1. Micropropagation

Cassumunar ginger propagates vegetatively through rhizomes but the rate of propagation is very slow giving only 4–6 plants per rhizome per year (Chirangini and Sharma, 2005). So, more planting material is needed to exploit its medicinal properties. Propagation through axillary bud multiplication is an easy and safe method and also assures uniformity and consistent production of true-to-type plants within a short span of time (George, 1993; Salvi et al., 2002). *In vitro* regenerated plants also have more advantages than the conventionally propagated plants in terms of productivity and disease resistance (Bhattacharya et al., 2014). In case of cassumunar ginger, the first attempt on micropropagation was that of Poonsapaya and Kraisintu (1993)· They reported propagation of cassumunar ginger through multiple shoot induction (average of 13 shoots within eight weeks) on LS (Linsmaier and Skoog) medium supplemented with 4 mg/l BAP (6- benzylaminopurine). Rooting was observed when shoots were cultured to the same medium or to a medium with low concentration of NAA (α- naphthalene acetic acid). Further, Chirangini and Sharma (2005) reported the induction of microshoots (8 microshoots per explant) effectively from rhizomatous buds inoculated on MS media supplemented with NAA (0.54 μM or 2.69 μM) and BAP (4.44 μM). However, explants inoculated on MS media supplemented with kinetin (2.32 μM) induced single shoots in higher percentage (80%). Subsequently, Hamirah et al., 2010 also reported micropropagation of cassumunar ginger using sectioned buds inoculated on liquid Gamborg B5 media supplemented with 0.5 mg/l TDZ (Thidiazuron), 3 mg/l BAP and 1 mg/l 2ip- R (2-isopentyl adenine riboside) singly. Multiple shoot induction was reported in all the media supplemented with three different plant growth regulators but the highest shoot multiplication was observed on MS media supplemented with 0.5 mg/l TDZ, producing an average of 8.1 shoots per explant.

In all the reported studies on micropropagation, the survival rates of the explants were low due to contamination of cultures and different strategies were employed by different workers to tackle the problem. Poonsapaya and Kraisintu (1993) used antibiotics such as amoxicillin, neomycin, chloramphenicol, and cloxacillin were used as sterilizing agents which increased the survival rate of the cultured shoot tips up to more than ten percent while Chirangini and Sharma (2005) used mercuric chloride (0.2%) with few drops of 1N HCl as sterilizing agent which proved effective. On the other hand, Hamirah et al. (2010) surface sterilized the explants using tetracycline (15 ml/l) and Plant Preservative Mixture (2 ml/l) to effectively control the contamination of cultures.

2.2. *In vitro rhizome induction and conservation of germplasm*

Since microrhizomes produced *in vitro* also have the potential to be used by commercial growers as disease-free planting material irrespective of seasonal fluctuations and can be sown like seeds (Bhat et al., 1994; Sharma and Singh, 1995), *in vitro* propagation of cassumunar ginger is directed at rhizome induction for efficient acclimatization, easy transportation and to minimize injury during transport.

Microrhizome induction of cassumunar ginger was first reported by Chirangini and Sharma (2005). In their study, *in vitro* derived shoots were used as explants and innoculated on MS media devoid of any plant growth regulators with varying concentrations of sucrose (3–9) for the induction of microrhizomes. Within eight weeks of incubation, microrhizomes were observed and the best responses were shown by media supplemented with 7 and 9% sucrose (upto 6 microrhizomes per tube). However, micorhizomes with highest average fresh weight of 0.81 g was observed in MS media supplemented with 5% sucrose.

With the advent of commercial exploitation of this economically valuable medicinal plant, conserving their germplasm in the field gene banks as well as in the *in vitro* gene banks has become essential. *In vitro* conservation protocol of cassumunar ginger through *in vitro* rhizome induction was standardized by Tyagi et al. (2006). They reported the formation of *in vitro* rhizome from rhizome buds inoculated on MS media supplemented with 9% sucrose, BAP (1 mg/l) and NAA (0.1 mg/l). Light treatments had a significant effect on the survival of cultures (50%) up to14 months and use of maleic hydrazide (2 mg/l and 4 mg/l) increased the survival of the 12 and 14 month old cultures (up to 50–60%).

3. Molecular biology studies

Molecular biology studies in cassumunar ginger over the past decades were focused on the use of molecular markers for identification of this species, genetic diversity analysis among the species and phylogenetic studies.

3.1. Molecular markers in genetic diversity and phylogenetic studies

Bua-in and Paisooksantivatana (2010) have reported the use of RAPD (Random amplified polymorphic DNA) marker to study the genetic diversity of clonally propagated cassumunar ginger and its relation with other *Zingiber* species. In their study, genetic variability among the genotypes collected from different locations of Thailand was detected by using twenty-nine random primers. High molecular variance (87%) was observed within *Zingiber montanum* accessions and the results showed that genetic diversity was exhibited in *Zingiber* both at interspecific and intraspecific level. Kladmook et al. (2010) have also assessed the genetic diversity of cassumunar ginger by using 12 AFLP (Amplified fragment length polymorphism) primers and found that high molecular variance (84%) was detected within samples from the same region. Species – specific AFLP marker were also used for identification of *Zingiber*

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