Review of Literature

Lincy A.K “Investigation on direct in vitro shoot regeneration from aerial stem explant of ginger (Zingiber officinale Rosc.) and its field evaluation “, Indian Institute of Spices Research, University of Calicut, 2007
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2. Review of Literature

Ginger (Zingiber officinale Rosc.), a rhizomatous monocotyledon, was first described by Rheede (1962) in ‘Hortus Indicus Malabaricus’ (Nayar and Ravindran, 1995). Ginger belongs to the family Zingiberaceae, in the order Zingiberales. The family Zingiberaceae consists of 47 genera and about 1400 species (Purseglove et al., 1981). The subfamily Zingiberoideae includes the important spice crops ginger (Zingiber officinale Rosc.), turmeric (Curcuma longa L.) and cardamom (Elettaria cardamomum Maton).

2.1. General

2.1.1. Origin

The country of origin is presumed to be in the region of India or China (Purseglove, 1972). According to Bailey (1949), ginger originated in Western Ghats of India. Though a putative wild type Zingiber officinale is collected from Western Ghats of India, its occurrence in wild habitat is yet to be confirmed (Sasikumar et al., 1995).

2.1.2. Botany

Ginger is a herbaceous perennial grown as an annual. The plant is erect, having aerial shoots and underground stem. Aerial shoot (pseudostem) has many leaves borne on very short petioles and with long and narrow sheaths. The leaves are arranged in a distichous manner (Purseglove et al., 1981).
2.1.2.1. Aerial stem
Aerial stem is annual, erect, usually about 50 cm tall, 5 mm in diameter, partly consisting of a pseudostem of overlapping leaf sheaths. The internodal length of aerial stem ranges 3 to 7 cm. (Purseglove et al., 1981).

2.1.2.2. Leaf
The foliage leaf consists of a leaf sheath, a ligule and an elliptical – lanceolate blade. The leaf sheath is about 15 – 18 cm and lamina about 12 – 15 cm long. A distinct mid rib is present in the lamina. Small distichous scale leaves which are brown in color are also present (Purseglove et al., 1981).

2.1.2.3. Roots
Ginger has two types of roots i.e., fibrous and fleshy (Purseglove et al., 1981).

2.1.2.4. Rhizome
The useful part of ginger is the subterranean stem (rhizome) which is modified for the vegetative propagation and storage of food materials. Rhizomes are thick and hard, laterally compressed; often palmately branched. It has nodes with scale leaves and internodes. Each node has an axillary bud except for the first few nodes. The number of nodes in each rhizome varies. The main axis (mother rhizome) and the subsequent branches (primaries) have 6 to 15 nodes. The internodal length of the rhizome branches ranges 0.1 to 1.5 cm. The internodal length is more in secondary and tertiary branches. Each nodes have scale leaves that ensheath and protect the axillary buds (Purseglove, 1972; Ravindran et al., 2005).

2.1.2.5. Inflorescence
Inflorescence arises directly from the rhizome, spiciform 15 – 25 cm long. Single flower is produced in each bract. Flowers are short lived, fragile and each with a bracteole. Fruits are seldom produced (Purseglove, 1972; Ravindran et al., 2005).
2.2. *In vitro* studies

2.2.1. Micropropagation

True-to-type propagation of selected genotypes using *in vitro* culture technique is termed as micropropagation. There are five stages critical for successful micropropagation. They are preparative stage to minimize contamination, initiation of culture, multiplication and elongation of shoots/roots and transfer to greenhouse conditions. The survival, multiplication and field establishment of culture depends upon a variety of factors such as origin of culture, physiological stage of explant, endogenous hormone level and culture environment like nutrient media, photoperiod, CO₂ concentration, temperature etc. Each species is unique in these requirements (Debergh and Read, 1991). Plant regeneration through tissue culture occurs through two methods: organogenesis and embryogenesis.

In ginger, micropropagation studies were carried out by many workers using different explants and media compositions. MS medium (Murashige and Skoog, 1963) was found to be best for micropropagation of ginger. Other basal media used were Gamborg’s B5 (Gamborg *et al*., 1968), Schenk and Hildebrandt’s (Schenk and Hildebrandt, 1972) and Norstog’s (Norstog, 1973) medium. Mainly used cytokinins were BAP, Kinetin and auxins were, 2, 4-D, NAA and IBA. The different explants used for micropropagation are vegetative buds, inflorescence, single flower and roots.

2.2.1.1. Organogenesis

Organogenesis can be obtained either through direct differentiation of shoot buds from explants (direct organogenesis) or through callus formation in explant and subsequent formation of shoots and roots (indirect organogenesis). Organ formation *in vitro* was reported as early as 1939 when White (1939) observed shoot differentiation in callus cultures of Tobacco.
2.2.1.1. Direct organogenesis

Shoots were regenerated directly on explants from numerous species; plants that conventionally propagated adventitiously may be proliferated rapidly in vitro by using not only the conventional organ as a source of explants, but other tissues not normally associated with vegetative reproduction (Meins, 1986).

a) Vegetative bud

Vegetative bud is the most commonly used explant in ginger tissue culture. Hosoki and Sagawa (1977) successfully micropropagated ginger from rhizome buds cultured on MS medium supplemented with 2% sucrose and 1 ppm BA. Pillai and Kumar (1982) used SH medium for micropropagation.

Shoot tip explants of ginger cv. Oshouga were planted in both modified Gamborg's B5 medium and Murashige and Skoog (MS) medium containing various levels of NAA and BA. Explants were cultured at 22°C by rotating slowly (2 rpm) under continuous fluorescent light of 2000 lux. Plantlets were produced in modified B5 and MS medium supplemented with 0.2 to 2.0 ppm of BA in addition to 0.2 ppm of NAA. (Sakamura et al., 1986). Bhagyalakshmi and Singh (1988) used MS medium with 6% sucrose, 20% coconut milk, AA at 100 mg/l, AC at 250 mg/l, BA at 0.5 mg/l, IBA at 0.4 mg/l, and 0.8% agar. A simple and efficient micropropagation method was developed by Inden et al. (1988). The medium used was MS with 5 mg/l BA, 0.5 mg/l NAA and 20 g/l sucrose.

Rhizome buds excised from Curcuma Spp. and ginger were inoculated on MS medium with different combination of BAP and Kinetin. MS medium with 3.0 mg/l BA was found to be optimum for in vitro clonal multiplication (Balachandran et al., 1990) Choi and Kim (1991) and Choi (1991a) studied the factors affecting in vitro response of shoot tip explant. The nutrient medium used was MS supplemented with 0.5 ppm NAA and 5.0 ppm BAP. Multiple shoots were induced from vegetative bud, when cultured on MS medium supplemented with 2.5 mg/l BA and 0.5 mg/l NAA.
In vitro propagation of ginger using vegetative bud cultured on MS medium containing 2 mg l\(^{-1}\) BA and 0.6 mg l\(^{-1}\) NAA was reported by Huang (1995). Oliver (1996) cultured axillary buds on MS medium supplemented with 8.9 µl BA, 30 g l\(^{-1}\) sucrose and 7 g l\(^{-1}\) agar and studied the rate of shoot multiplication in each sub culturing.

A protocol for shoot development from disc and apex part of sprouting buds was standardized by Samsudeen (1996). MS medium containing 1 µM BAP and 1 µM NAA was used for the study. Nirmal Babu (1997) developed a method for clonal propagation of ginger from vegetative bud cultured on MS medium supplemented with 4 mg l\(^{-1}\) BA and 1 mg l\(^{-1}\) NAA. Disease free plantlets of *Zingiber officinale* were produced by culturing rhizome buds on MS medium supplemented with 3 mg l\(^{-1}\) BA and sub cultured to MS with 2 g Twin 40 (a commercial fertilizer medium containing, N, P\(_2\)O\(_5\) and K\(_2\)O in a ratio of 30:20:10 l\(^{-1}\)) with or with out plant growth hormones (Pandey *et al.*, 1997).

High frequency in vitro multiplication of ginger was reported by Sharma and Singh (1997). The rhizome buds were cultured on MS medium containing 2 mg l\(^{-1}\) Kinetin and 2 mg l\(^{-1}\) NAA and 20 g sucrose. A protocol for micropropagation of ginger using vegetative bud was developed by Palai *et al.* (1997). Vegetative buds were cultured on MS medium with 4 - 6 mg l\(^{-1}\) BA, 1 - 1.65 mg l\(^{-1}\) IAA and 100 mg l\(^{-1}\) Adenine sulphate and 3 % sucrose. The interaction of growth regulators and culture conditions on micropropagation was also investigated. Freitez and Casares (1997) reported organogenesis from in vitro shoot tips cultured on MS medium supplemented with BAP and NAA in different combinations (2, 2.5 & 3 mg l\(^{-1}\) BA and 0.25 mg l\(^{-1}\) NAA).

A micropropagation protocol for ginger using vegetative bud was standardized by Tyagi *et al.* (1998). The vegetative buds were cultured on MS medium containing BAP and Kinetin in different concentrations. Devi *et al.* (1999)
developed a method for micropropagation from bud explants cultured on MS medium supplemented with BAP and Kinetin (both at 0.1 mg/l). Shoot production was increased when the culture was transferred to a medium containing BAP at 4 mg/l. Jasrai et al. (2000) propagated ginger plantlets through tissue culture using apical bud on MS medium augmented with different concentration of BAP.

An efficient tissue culture protocol was developed for ginger with a multiplication rate of 1:6 for every 28 days (Rout et al., 2001). Medium containing high level of BAP (3 mg/l and above) promoted tiny multiple shoots but inhibited root formation. A significant decrease in shoot number per explant was observed at lower (less than 1 mg/l) concentration of BAP. Ginger plantlets were regenerated from meristem tissues cultured on MS medium with 26.6 µM BA, 8.57 µM IAA and 1111.1 µM adenine sulfate and 3% sucrose. Adaniya and Shirai (2001) reported that the shoot tip explants on solid medium containing 2.0 mg/l BA, 0.05 mg/l NAA and 0.2 % (w/v) colchicines for 8 days was most efficient way of inducing tetraploid ginger. The pollen fertility and germinability were also investigated. In the diploid strains, pollen fertility ranged from 0.3 to 6.2% and germination rate from 0 to 0.1% while in the tetraploid strains pollen fertility ranged from 27.4 to 74.2% and the germination rate from 4.8 to 12.9%.

b) *In vitro* aerial stem

*In vitro* propagation of ginger using *in vitro* aerial stem (pseudostem) explants was reported by Ikeda and Tanabe (1989). Leaf aerial stem and decapitated crown sections from *in vitro* plantlets of *Zingiber officinale* were cultured on MS medium containing various concentrations of BAP and NAA. Pseudostem (aerial stem) cultured on solid medium supplemented with 11 µM BA in combination with 0.6 µM NAA produced an average of 5 shoots and 15.3 roots. Decapitated crown sections cultured in liquid medium with 11 µM BA produced an average of 10 shoots and 16.6 roots.
Inflorescence and single flower

There is only one report in ginger regarding the micropropagation using inflorescence. Nirmal Babu et al. (1992a) reported direct regeneration of plantlets from immature inflorescence of ginger. One week old inflorescence cultured on MS medium supplemented with 10 mgl⁻¹ BA and 0.2 mgl⁻¹ 2, 4 – D produced plantlets. Single plantlets were emerged from the ovaries by the seventh week when cultured on MS medium fortified with 10 mgl⁻¹ BA and 0.2 mgl⁻¹ 2, 4-D.

2.2.1.1.2. Indirect organogenesis

Indirect organogenesis through callus induction and regeneration in ginger was reported by a number of workers. Different explants were used for callus induction. The significant results are summarized below:

a) Vegetative bud / shoot meristem

In ginger, callus induction and regeneration were first reported by Illahi and Jabeen (1987). Shoot bud derived callus on sub culturing onto MS medium supplemented with various concentrations of 2, 4 – D and BA developed plantlets. Malamug et al. (1991) induced callus from shoot tip explants of ginger. The medium used was MS with 2, 4-D at 0.5 mgl⁻¹ and NAA at 1 mgl⁻¹. These calli produced plantlets when cultured on medium (MS major elements, organic addenda, 2% sucrose and 0.8% agar) with 5 mgl⁻¹ BA and 1 mgl⁻¹ NAA. Samsudeen (1996) regenerated plantlets from shoot bud derived callus cultured on MS supplemented with 0.1µM 2, 4-D with 50 µM BAP or Kinetin.

Plant regeneration from two callus types in ginger was reported by Ishida and Adachi (1997). These workers studied the varietal differences on callus induction and regeneration. Callus developed by culturing shoot meristem domes of three cultivars of ginger on MS medium containing 2 mgl⁻¹ 2, 4-D. ‘Taiwan’ variety produced compact callus, ‘Oshouga’ produced sticky callus and ‘Indo’ produced both compact type and sticky type of calli. Compact callus of ‘Taiwan’ and ‘Indo’ varieties
produced only roots in MS with BA. Sticky callus of Indo and ‘Oshouga’ varieties produced adventitious shoots on MS medium containing 0.1 mg l\(^{-1}\) NAA and 1.0 mg l\(^{-1}\) BA. These shoots readily formed plantlets on MS medium containing 0.5 mg l\(^{-1}\) NAA and 1.0 mg l\(^{-1}\) BA. Rout and Das (1997) reported \textit{in vitro} organogenesis in ginger. Callus derived from shoot primordia showed best organogenesis on MS medium supplemented with BA at 5 mg l\(^{-1}\), IAA at 1 mg l\(^{-1}\), adenine sulphate 100 mg l\(^{-1}\) and 3% sucrose.

b) Aerial stem

Clonal multiplication of ginger using \textit{in vitro} aerial stem (pseudostem) cuttings was standardized by Choi (1991b). Explants consisted of the base of aerial stem or portion from the top, middle or bottom with one leaf blade attached. All formed callus \textit{in vitro} and from which several plantlets differentiated, although response was best from the base or middle portion explants. Callusing was best on medium containing 0.5 ppm NAA, while shoot and root formation were best on medium containing 0.1-1.0 ppm NAA and 1.0 ppm BA.

c) Leaf explant

\textit{In vitro} plantlet regeneration from leaf derived callus in ginger was reported by Nirmal Babu \textit{et al.} (1992b). Callus induction and proliferation were obtained from leaf tissues cultured on MS medium supplemented with various levels of NAA and 2,4-D. The induced callus produced plantlets when transferred to MS medium containing 0.9 \(\mu\)M 2, 4-D and 44.4 \(\mu\)M BA.

d) Anther

Callus induction from anther tissues of tetraploid ginger was reported by Samsudeen \textit{et al.} (1997). Anthers from diploid and tetraploid plants gave callus on MS medium with 2 mg l\(^{-1}\) 2, 4-D. Seven days cold treatment for anther explant and incubated in light were the other conditions required for callus formation. Samsudeen \textit{et al.} (2000) regenerated ginger plantlets from anther derived callus cultures. The media used was MS medium supplemented with 5-10 mg l\(^{-1}\) BA and 0.2 mg l\(^{-1}\) 2, 4-D.
Investigation of floral structure and plant regeneration through anther culture in ginger was reported by Kim et al. (2000). In this study, callus formation on anther explant was compact and embryogenic when cultured on N6 medium supplemented with 2 mg l\(^{-1}\) NAA. Plant regeneration occurred on MS medium with BA at 1-2 mg l\(^{-1}\).

2.2.1.2. Somatic embryogenesis

Plant regeneration via somatic embryogenesis in ginger was standardized by Kacker et al. (1993). Embryogenic calli were induced by culturing leaf segments taken from \textit{in vitro} shoot cultures on MS medium supplemented with Dicamba 2.7 μM and plantlets were regenerated when the embryogenic cultures were transferred onto MS medium containing 8.9 μM BA. Nirmal Babu et al. (1996) regenerated plantlets from somatic embryos of ginger. Ovary explants from 1-2 week old ginger flowers developed profuse callus on MS medium supplemented either with 2,4-D 1 mg l\(^{-1}\) or 2,4-D 0.5 mg l\(^{-1}\) + BA 1 mg l\(^{-1}\). The callus produced white globular embryoid like structures when cultured on MS medium supplemented with 10 mg l\(^{-1}\) BA + 0.2 mg l\(^{-1}\) 2,4-D. The embryoids developed into plantlets with better rooting when 1 mg l\(^{-1}\) NAA added to the medium. Somatic embryogenesis and its regeneration in ginger were also reported by Tyagi et al. (1998). Somatic embryos were induced from leaf derived calli and were regenerated when cultured onto MS with 2.7 μM dicamba and 8.9 μM BAP.

Somatic embryogenic cultures of four ginger cultivars were established by Hua et al., (2005). Somatic embryogenic calli were induced from ginger shoot tips on MS solid medium supplemented with 1.0 mg l\(^{-1}\) 2,4-D and 0.2 mg l\(^{-1}\) Kinetin which contain only half concentration of NH\(_4\)NO\(_3\). Rapid growing and well dispersed suspension cultures were established by sub culturing this callus in the same liquid MS medium. The embryogenic callus was transferred onto solid media (MS + 0.2 mg l\(^{-1}\) 2,4-D + 5.0 mg l\(^{-1}\) BA + 3% sucrose + 0.7% agar). Somatic embryos produced shoots and roots on MS solid medium with 3.0 mg l\(^{-1}\) BA and 0.1 mg l\(^{-1}\) NAA. The relationship between the dry weight of suspension cultures and pH changes in
medium is also discussed. A protocol for plant regeneration via somatic embryogenesis of ginger was reported by Suma and Keshavachandran (2005). Callus, derived from young vegetative buds cultured on MS medium supplemented with BA and 2,4-D. Among the plant growth regulators tested, a combination of 2,4-D 1 mg l⁻¹ and BA 0.5 mg l⁻¹ was most effective in inducing and maintaining embryogenic cultures. The somatic embryos germinated on half strength MS medium with 3% (w/v) sucrose and BA 3 mg l⁻¹.

2.2.2. Effect of cultural conditions and growth hormones on in vitro culture

In vitro responses of explant to various growth hormones may vary according to the type of explant, plant genotype, cultural conditions and kind of medium. In vitro growth and morphogenesis are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth regulators produced endogenously. Auxin and cytokinins are most important for regulating growth and morphogenesis in plant tissue culture (George, 1993a). A balance between an auxin and a cytokinin determine the nature of organogenesis. A high ratio of cytokinin to auxin induced shoot differentiation whereas the reverse favoured root formation; intermediate ratios induced callus formation (Skoog and Miller, 1957).

A study on factors affecting in vitro response of shoot tip explants of ginger was conducted by Choi (1991a). The optimal concentration of sucrose in the medium was found to be 3%, and rooting of shoots was enhanced by the addition of 2 g l⁻¹ activated charcoal. Rout and Das (1997) studied the effect of media constituents on in vitro responses of callus. Organogenesis was best on media supplemented with benzyl adenine (5 mg l⁻¹), IAA (1 mg l⁻¹), adenine sulfate (100 mg l⁻¹) and 3% sucrose. D-Glucose influenced rooting; fructose, maltose and mannitol had no effect on rooting. Shoot bud regeneration was best at pH 5.7 or 5.8 and under 24 h illuminations. The rate of shoot bud regeneration was positively correlated with the concentration of plant growth regulators. Liquid media were less effective than solid media for root
development. Shoots were rooted on half strength MS supplemented with IBA or IAA (1 mg/l) and 2% sucrose.

The effects of media variables and light conditions on micropropagation were studied by Palai et al. (1997). These authors observed that shoot bud multiplication decreased as BA concentration increased from 6 to 8 mg/l, while IAA had an intermediate effect; NAA, IBA and 2,4-D were ineffective with regard to induction of multiple shoots. Although a high rate of shoot multiplication was noted under both 14 and 24 h of illuminations, shoot elongation was poor under the 14 h photoperiod.

The in vitro growth and development of ginger exposed to different levels of NAA (0, 0.25, 0.5, 0.75, 1.0 mg/l) or BAP (0, 0.5, 1.0, 1.5, 2 mg/l) and NAA: BAP (0.5:1.0 mg/l) in solid and liquid media was studied by Arimura et al. (2000). NAA increased shoot length in both solid and liquid media. NAA at 0.5 mg/l promoted the highest number of roots and the longest roots. BAP influenced the number of shoots with maximum response at 1 mg/l. In liquid media, the absence of BAP induced higher number of shoots. Shoot length was influenced by NAA and BAP. Root number and length were enhanced in liquid media regardless plant growth regulator treatment.

2.2.3. Studies on cost effective media for micropropagation

A simple and cost effective medium for propagation of ginger (Zingiber officinale Rosc.) was standardized by Sharma and Singh (1995). Explants from in vitro cultured shoots of Z. officinale cv. Himachal Local were cultured on MS medium with analytical grade sucrose, ordinary sugar and raw sugar, or on potato extract medium with sucrose, dextrose or sugar. The effects of supplementing the medium with 2 mg/l BA, omitting agar and replacing distilled water with tap water in the preparation of the medium were also investigated. The highest number of shoots/explant was produced on MS medium + ordinary sugar and no growth regulators. Use of tap water and omission of agar also gave good results. These techniques can used
to reduce the cost of micropropagation. Economics of mass in vitro propagation of vanilla and ginger was studied by Rao et al. (1998). The cost of production of tissue cultured plantlets of ginger and vanilla was determined, based on 14168 plantlets of the former and 3560 of the latter, produced in the tissue culture laboratory during 1994–95. Production costs were Rs. 2.77/plantlet for ginger and Rs. 5.64/plantlet for vanilla. Ginger had a higher multiplication rate and required shorter subculture intervals than vanilla.

2.2.4. Screening of micropropagated ginger plants for disease resistance.

Selection of Pythium tolerant ginger by subjecting cell cultures to Pythium culture filtrate and subsequently regenerating plants from the surviving cells was attempted by Kulkarni et al. (1984). Regenerated plants were again screened for tolerance to Pythium which were enabled to isolate three lines tolerant to Pythium.

Virus diseases of ginger were controlled in tissue culture by heating to 50°C for 5 minutes. Cultures were grown in MS medium with 2 mg/l BA + 0.2 mg/l IAA, then in the same medium with half the concentrations of BA and IAA, followed by the final transfer to a B5 medium with 0.2 mg/l IAA. The cultivation of virus free stock to give high yields of 5 t/667 m² is discussed (Gao et al., 1999).

2.3. Histological studies

2.3.1. Tissue cultured plant materials

Histological origin of in vitro regenerated organs of ginger (Zingiber officinale Rosc.) was described by Freitez et al. (1998). Samples were taken during the initiation and multiplication phases of Z. officinale cultures. During the initial phase, progress was observed in the development of shoot tips in terms of the number and size of cells and foliar and axillary bud development. Likewise at 21 days the endogenous differentiation of a root primordium or meristemoid was observed,
starting from the cells of the procambium with their respective vascular connections. At 28 days, there was a great proliferation of vascular bundles oriented towards the adventitious axillary buds, the latter originating from *de novo* tissue. In the multiplication phase a great profusion of buds and adventitious roots without any type of polarity was observed, similar to a rosette; finally achieving a high *in vitro* multiplication rate. Histological analysis confirmed that the development of buds as well as of roots occurred through organogenesis without passing through callus tissue, through the process of induction and differentiation of the tissues. In spite of their adventitious nature, the plants obtained were morphologically and genetically uniform.

Histological examination of embryogenic calli was done by Kacker *et al.* (1993). The study revealed the presence of nodular structures containing richly cytoplasmic cells delimited by a single layered epidermis. The first sign of embryogenesis was marked by the appearance of white globular structures that were attached to the surface of nodular callus by a distinct stalk. Ontogeny of shoot and roots from ginger callus was described by Nirmal Babu (1997).

### 2.3.2. Aerial stem

No report is available on the anatomical studies of aerial stem in ginger. Tomlinson (1969) described the internal structure of aerial stem of Zingiberaceous plants in general. In the internodal region cortex and central cylinder are always distinct, usually separated from each other by a narrow fibrous cylinder; cortex fairly wide, including one or more rings of vascular bundles; central cylinder including many scattered vascular bundles. In the node region main leaf traces entering stem gradually and not forming a vascular plexus. Axillary bud present in each node.

### 2.3.3. Leaf

Ginger leaves are isobilateral. The upper epidermal cells of leaf are polygonal. In the lower epidermis the cells are polygonal and irregular. Oil cells are present in
the epidermis. Unicellular hairs are present in the lower epidermis of the foliar leaves. Ginger leaves are amphistomatic. A distinct substomatal chamber is present. Tomlinson (1969) gave a brief note on the petiolar anatomy of ginger. The short petiole shows a swollen pulvinus like appearance. Two bundles arcs, air canals and assimilating tissues are absent in the pulvinus. Collenchymatous thickening of the cells of bundle sheath is present in the pulvinus.

Comparative foliar anatomy of ginger plants under three different growing conditions was reported by Freitez and Casares (2004). In vitro, acclimatization and field experiments were conducted to describe the foliar anatomy of ginger that may influence their establishment. Samples were prepared from transverse sections of the foliar lamina. There were anatomical difference in stomata location, cuticle thickness and vascular sheath location and shape.

2.3.4. Rhizome

The developmental pattern, growth and branching behaviour of rhizomes of ginger were studied. Axillary buds are present at each node. Secondary and tertiary branches originate from the axillary bud from the adaxial sides of the leaf or scale leaf (Remashree et al., 1998). The T.S of rhizome has a distinct layer of epidermis consisting of a single raw of rectangular cells. Within this is the cork composed of irregularly arranged, tangentially elongated, slightly brown colored cells and an inner zone of 6 to 12 regular rows of thin walled rectangular to slightly tangential elongated cells arranged in radial rows. Inner to the cork is the cortex, composed of thin – walled large hexagonal to polygonal parenchymatous cells. The cortical region is heavily loaded with starch grains and oil cells. Many scattered, collateral, closed vascular bundles are present, within which are mostly concentrated in the inner cortical zone. The inner limit of the cortex is marked by a single – layered endodermis composed of thin – walled rectangular cells. Inner to this is the stele consists of parenchymatous cells. It also contains starch grains, oil cells and vascular bundles (Ravindran et al., 2005).
In ginger, rhizome enlargement is due to the activity of three meristematic zones (primary thickening meristem, actively dividing parenchyma and secondary thickening meristem). The scattered vascular bundles are developed from the primary thickening meristem (PTM) or procambial cells. Unlike in many monocots, in ginger there is a special meristematic layer along with the endodermoidal layer and this layer consists of cambium like cells. The presence of cambium like cells is an important feature in rhizome development. From this layer, inverted and irregularly distributed groups of xylem and phloem are formed along the intermediate layer. The cells outer and inner to the cambial layer become filled with starch grains (Remashree et al., 1997; Ravindran et al., 2005). Minute glands containing essential oil and resin are scattered throughout the rhizomes, but numerous in the epidermal tissue (Guenther, 1952; Mangalakumari et al., 1984).

2.3.5 Embryology of angiosperms

Embryogeny: The zygote divides transversely, resulting into a small apical cell toward the interior of the embryosac and a large basal cell towards the micropyle. Then these cells followed 4 celled and 8 celled stages. It is at the octant stage of the proembryo that the destinies of various cells become determined. Wardlaw (1955) remarked that in monocotyledonous embryo the shoot apex occupies a lateral position in the somewhat cylindrical embryo and the cotyledon is terminal. A mature embryo of *Triticum* has a single cotyledon called scutellum. In median longitudinal section of the mature embryo it appears laterally attached to the embryonal axis. The portion of the embryonal axis below the level of scutellum is the radicle, which bears an apical meristem and a root cap at the lower end. The radicle and its cap are enclosed in coleorhiza, which is the undifferentiated lower part of the proembryo. The portion of the embryonal axis above the level of scutellum is the epicotyl. It comprises a shoot apex with some leaf primordial, enclosed in a hollow foliar structure, the coleoptile (Batygina, 1969).
2.4. Genetic fidelity analysis in ginger

Genetic fidelity of micropropagated plants are always a concern for horticulturists. RAPD (Random Amplified Polymorphic DNA) markers were used to evaluate the genetic stability of micropropagated plants of *Zingiber officinale* Rosc. (Rout et al., 1998). These authors reported that RAPD profiles did not indicate any polymorphism among the micropropagated plants. Suja (2002) and Nirmal Babu et al. (2003) used RAPD profiles amplified by 11 Operon primers as an index for estimating genetic fidelity of selected ‘variants’ among micropropagated and callus regenerated ginger plants. They observed differences in RAPD profiles of some micropropagated plants. It indicated that micropropagation even without any callus phase induced variations.

2.5. Hardening

The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the *ex vitro* conditions to prevent high mortality after transfer to the environment of the field (Hazarika, 2003).

For acclimatizing the micropropagated ginger plantlets, a number of hardening media have been used. Plant acclimatization was completed with laboratory hardening under 85 µE m\(^{-1}\) s\(^{-1}\) light for 7 days and then plants were transplanted into substrates in humidified chambers in the shade (Freitez and Casares, 1997). Hardened plants could establish in the field with a survival rate of 80-98% (Roy and Wamanan, 1990; Samsudeen, 1996; Nirmal Babu, 1997; Sharma and Singh, 1997; Palai et al., 1997). Jasrai et al. (2000) could established tissue cultured ginger plantlets in media containing garden soil, sand and compost in the ratio of 40:20:40 and got 98% survival.
2.5.1. Biological hardening

Four antagonistic bacterial isolates, *Bacillus subtilis*, *Bacillus* sp., *Pseudomonas corrugata* 1 and *P. corrugata* 2, isolated from the rhizosphere of tea plants growing in different geographical locations in India, were tested as microbial inoculants for hardening of tissue-cultured tea plants raised in the laboratory prior to the transfer to open land. Bacterial inoculations resulted in enhanced survival (up to 100, 96, and 88%), as against 50, 52, and 36% survival observed in the corresponding control plants, in rainy, winter and summer seasons, respectively. Rhizoplane and rhizosphere soil analyses showed that the major biotic factor responsible for mortality following the transfer of tissue culture raised plants to soil was fungal attack (*Fusarium oxysporum*). Bacterial inoculations also resulted in plant growth promotion of tissue culture as well as seed raised plants of tea (Pandey et al., 2000).

*Piriformospora indica* is a novel plant growth promoting root endophyte. Regenerated plantlets of tobacco subjected to two different biological hardening techniques showed 88-94% survival when inoculated with *P. indica* as compared to 62% survival of un inoculated controls under similar conditions. The tendency of the plantlet to overcome the stress in terms of maximum revival capacity was in the case of *P. indica* as compared to the control. The fungus has the potential to render protection to the micropropagated plantlets and help them escape the 'transient transplant shock' (Sahay and Varma, 1999).

2.6. Field evaluation

2.6.1. Morphological and yield characterization in micropropagated plants.

Field evaluation of micropropagated and conventionally propagated ginger in subtropical Queensland was reported by Smith and Hamil (1996). In the first generation \textit{ex vitro}, micropropagated ginger plants had significantly reduced rhizome yields with smaller knobs and more roots as compared to the control plants. Micropropagated plants had a greater shoot: rhizome ratio. Shoots from micropropagated plants were also significantly smaller, with a greater number of shoots per plant. Rhizomes collected from micropropagated plants were smaller than that of control plants. By the second generation \textit{ex vitro}, there was no significant difference in any of the parameters measured between the treatments.

The morphological and yield characteristics of \textit{in vitro} derived plants; micropropagated plants derived from vegetative buds (MP), callus regenerated plants (C) along with the conventionally propagated ones (CP) were studied by Samsudeen (1996). The characteristics observed are number of tillers – 1.40 to 10.00 (MP), 1.20 to 8.30 (C), 2.80 to 9.20 (CP); number of leaves - 11.40 to 57.60 (MP), 8.30 to 64.90 (C), 27.60 to 69.10 (CP); height of the plant- 8.40 to 36.90 cm (MP), 7.60 to 35.80 cm (C), 15.50 to 36.70 cm (CP) and rhizome weight – 10.10 to 57.80 g (MP), 8.40 to 34.80 g (C), 15.10 to 41.50 g (CP). Sharma and Singh (1997) reported the field performance of \textit{in vitro} derived plants. The micropropagated plants were morphologically identical to the mother plants and were free of ginger yellows disease. They performed well under field condition and well developed rhizomes obtained from tissue cultured plants did not rot during storage for up to 6 months.

Morphological characterization of 4 year old micropropagated as well as callus regenerated plants was reported by Nirmal Babu (1997) in comparison with conventionally propagated plants. Direct regenerated plants and callus regenerated plants as separate groups when compared with conventionally propagated plants...
revealed a good amount of variation with regard to plant height, number of nodes per finger and leaves per plant, girth of rhizome, number of nodes per finger, internodal distance and yield per plant. Micropropagated and callus regenerated plants have higher mean values with regard to plant height, number of tillers, number of nodes per finger and yield per plant compared to controls. With regard to the width of rhizome, internodal distance all three groups are at par.

Morphological features and yield of tissue culture raised ginger plants were studied by Rao et al. (2000). The features observed were; number of leaves/tiller (10.00 – 13.00), length of sixth leaf from top (22.00 – 23.00 cm), breadth of sixth leaf from top (2.20 - 2.30 cm), plant height (45.00 - 55.00 cm), number of tillers/plant (14.00 – 19.00) and fresh weight of rhizome/plant (55.00 – 66.00gm). The vegetative growth was normal and the yield was more or less comparable with that of conventionally propagated ginger plants. It took four months and fifteen days extra to harvest the rhizome in case of tissue cultured plants.

Field evaluation of ginger plants (Zingiber officinale Rosc.) obtained in vitro and from sections of rhizome was reported by Freitez et al. (2003). The horticultural performance of ginger (Z. officinale Rosc.) plants cultured in vitro and propagated from rhizome sections was evaluated under full sunlight and partial shade in Tarabana, Lara State, Venezuela. The results showed significant differences for the type of propagation and the condition of light, except for the variables, number of leaves and root mass, respectively. The number of shoots, and fresh and dry mass of shoots was higher in in vitro propagated plants, which were shorter, than those propagated by rhizome. Rhizome mass was greater in plants propagated conventionally, but root mass was smaller than in those propagated in vitro. The in vitro plants produced numerous small rhizomes, with a high number of fleshy roots and tuberous structures at the tips. All the evaluated variables were superior in partial shade, independently of the type of propagation, with the exception of roots mass in those plants produced from rhizome sections.
2.6.2. Morphological and yield characterization in conventionally propagated ginger

Field evaluation of ginger plants was reported by a number of workers. Rattan (1989) indicated that number of leaves per plant had maximum direct contribution to yield per plant, followed by rhizome breadth.

Multiple regression analysis by using morphological characters indicated that the final yield could be predicted fairly accurate by taking into consideration plant height, number of leaves and breadth of last fully opened leaf at the 90th and 120th days after planting (Rathnambal et al., 1980). Morphological variability in ginger was reported by a number of workers (Table 1).

Table 1. Morphological variability in ginger

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Character</th>
<th>Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plant height (cm)</td>
<td>35.41 – 49.67</td>
<td>Muraleedharan and Sakunthala, 1975.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49.90 – 64.00</td>
<td>Saikia and Shadeque, 1992.</td>
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<td></td>
<td></td>
<td>23.13 – 88.60</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
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<td></td>
<td></td>
<td>4.00 – 6.80</td>
<td>Saikia and Shadeque, 1992.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.75 – 37.50</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
</tr>
<tr>
<td>3</td>
<td>Leaf number/plant</td>
<td>56.66 – 146.75</td>
<td>Muraleedharan and Sakunthala, 1975.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.00 – 58.00</td>
<td>Saikia and Shadeque, 1992.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.00 – 52.00</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
</tr>
<tr>
<td>4</td>
<td>Rhizome yield/plant</td>
<td>116.00 – 286.96</td>
<td>Muraleedharan and Sakunthala, 1975.</td>
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<td></td>
<td></td>
<td>25.00 – 57.00</td>
<td>Saikia and Shadeque, 1992.</td>
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<tr>
<td></td>
<td></td>
<td>55.00 – 770.00</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
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</tbody>
</table>

References

2.6.3. Effect of planting season in growth and yield of ginger

Ginger needs a warm climate and cannot withstand cold and hence its planting should be adjusted to avoid the winter cold. In India ginger is planted with commencement of South west Monsoon. It generally takes place in May to June (Khan, 1959). Planting time is crucial in ensuring high yield. Too early planting while soil temperature is low will affect germination. If planted too late, the growing period will be shorter and the rhizome yield will be affected. Studies have shown that delay in planting results in a lower yield of ginger. As vegetatively propagated plants, the whole growing period is utilized for vegetative growth only. Growth period takes more than 135 – 150 days from sprouting. Harvesting of ginger is generally done between January and February (Govindarajan, 1982).

2.6.4. Effect of shading in growth and yield of ginger

Ginger belongs to the medium light plant and endures high temperature. If shading or other measures to lower the temperature and hold humidity are not implemented, ginger seedlings will be weak and short, resulting in yield declines. Shading can make ginger leaves sustain a higher photosynthetic efficiency and high dry matter accumulation, thereby increasing the unit area yield (Zhenxian et al., 2000). 50 – 60 shading is suitable for better growth of ginger (Shaottui and Zhenxian, 1998, Xizhen et al., 2001).

2.7. Correlation and path analysis

Mohanty and Sarma (1979) studied the genetic variability and correlation for yield and other variables in ginger germplasm and reported significant positive correlations between rhizome yield and various yield attributes such as plant height, leaf and tiller number, length and width of leaves.

One hundred collections of ginger evaluated for plant height, leaf number, tiller number, leaf length and width, days to maturity, dry recovery as well as rhizome yield/plant revealed good variability for tiller number and rhizome yield/plant.
Moderate variation was observed for plant height, leaf number, tiller number as well as length and width of leaves. These characters had positive significant association with rhizome yield. Plant height, followed by leaf length, had maximum direct effects on rhizome yield (Sasikumar et al., 1992).

Das et al. (1999) reported very high positive direct effect of stomatal number, leaf area, leaf number and plant height on rhizome yield.

Singh (2001) evaluated sixteen clones of ginger for certain metric traits in a field experiment in Himachal Pradesh, India, during the Kharif season of 1996-97. Correlation studies revealed that rhizome yield per plant was positively and significantly correlated with plant height, number of leaves per plant, number of tillers per plant, leaf length and leaf width. The maximum indirect effects on yield were observed for number of leaves through plant height, followed by leaf length through plant height.

The number of tillers, tiller height, leaflet number, leaflet length, leaflet breadth, rhizome length and width, thickness of basal pseudostem, thickness of primary, secondary and tertiary rhizomes and fresh weight of rhizomes recorded from 40 ginger genotypes were studied by correlation and path analysis (Abraham and Latha, 2003). There was a high positive association between yield and leaflet number followed by tiller height, rhizome length, leaf length and thickness of the secondary rhizome. Path analysis revealed that characters such as leaflet number, rhizome length, thickness of the secondary rhizome, rhizome width and leaflet length have high positive direct effect on yield.

2.8. Quality evaluation

Ginger rhizome contains volatile oil, fixed oil, pungent compounds, starch and other saccharides, proteins, crude fiber, waxes, coloring matter and trace minerals. The presence of vitamins and amino acids also has been reported. The relative
percentage of these components varies with cultivar, soil and climatic differences. Starch is the most abundant of the constituents, comprising of 40 – 60 % of the weight of the dry rhizome (Lawrence, 1984).

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The powdered rhizome contains 3 – 6% fatty oil, 9% protein, 60 – 70% carbohydrates, 3 – 8% crude fibre, about 8% ash, 9-12% water and 2-3% oil (Kalpagam and Nirmala, 2003).

By steam distillation or extraction with supercritical carbonic acid dioxide, ginger rhizome gives an essential oil with a high content of mono and sesqui terpene derivatives (α – zingiberene). By extraction with solvents, an oleoresin containing the pungent principles of ginger is obtained.

2.8.1. Ginger oil

Dry ginger contains 1.25 to 3.00 % essential oil (volatile oil), which imparts the characteristic aroma to the spice. The oil is devoid of the pungent taste of ginger. Salzer (1977) and Sankarikutty et al. (1980) suggested the following determinants of quality for ginger oil.

a) Citral and citronillyl acetate are important co determinants of odor.

b) Zingiberene and beta – sesquiphellandrene are the main components of the freshly prepared oil. These components are converted to ar – curcumine with storage.

c) The ratio of zingiberene + beta sesquiphellandrene to ar – curcumine is indicative of the age of the oil.
Oil composition of fresh and dried ginger rhizome of Nigeria was investigated by means of a combination of column chromatography, high resolution GC and GC–MS. The essential oil contained mainly mono and sesquiterpenoids of which Geraniol, Neral, 1, 8 - Cineol, Zingiberene, β - Sesquiphellandrene were the major components (Ekundayo et al., 1988).

2.8.2. Ginger oleoresin

The oleoresin, obtained by extraction of the spice with volatile solvents, contains the aroma as well as the taste principles of the ginger in highly concentrated form. Oleoresin represents the wholesome flavor of the spice – a cumulative of the sensation of smell and taste. It consists of the volatile essential oil and nonvolatile resinous fraction comprising taste components, fixatives, antioxidants, pigments and fixed oils naturally present in the spice. The oleoresin is therefore, designated as “true essence” of the spice.

The pungency of ginger is contributed by gingerols (Nambudiri et al., 1975). The oleoresin of ginger contains gingerols as well as degenerated products. Other pungent components in ginger oleoresin are shogoal, paradol, zingereone, gingediol, gignerdiold and gingediacetae. On long term storage gingerol become converted to shogoal.

2.8.3. Biochemical variability in micropropagated plants

Rhizomes formed in vitro from shoot cultures of ginger were analyzed for flavor and pungency compounds. Essential oil from in vitro developed rhizome contained same constituents as in the original rhizomes, but quantitative difference was observed. Oil composition also depended on the composition of the basal medium. Oil extracted from the rhizome grown in the modified B5 medium, the acyclic oxygenated monoterpenes predominated, while the oil from the rhizome grown in modified MS medium consisted mainly of sesquiterpenes. (Sakamura et al., 1986; Charlwood et al., 1988; Sakamura and Suga, 1989).
Quality analysis of rhizomes obtained from micropropagated plants was done by Bhagyalakshmi and Singh (1988). They reported micropropagated plants were at par with conventionally propagated ones except that they need longer (additional 2 months) crop duration for the same effect. In another study, Bhagyalakshmi et al. (1994) studied the periodic fluctuations in the yield and rhizome composition of various compounds in micropropagated ginger plants. Conventionally propagated ginger is usually harvested after 8 months and micropropagated plants harvested at the same time were comparable qualitatively and quantitatively to the control rhizomes in the composition of starch, ash, acetone extract and volatile extract but only qualitatively similar in total oleoresin content, flavor profile, TLC aromagram and GC analysis, with less of each component than control rhizomes.

Biochemical parameters of ginger somaclones were studied by Samsudeen (1996). The somaclones had significant exploitable variations with regard to biochemical characters like, content of oleoresin, dry recovery and fiber content. In the case of micropropagated plants, oleoresin content of 3.3 - 9.3 %, fibre 3.7 - 6.7% and dry recovery 12.1 - 36.4% were recorded. In callus regenerated plants an oleoresin content of 3.4 - 9.4%, fibre 1.3 - 5.3% and dry recovery of 14.5 - 37.5 % were recorded as compared to control plants (oleoresin content –3.9 - 5.0%; fibre – 4.3 - 5.5%; dry recovery – 19.6 - 26.4%). Nirmal Babu (1997) corroborates this result. Variations were observed among tissue cultured and callus regenerated plants in their oleoresin content, fiber content and dry recovery. In the case of micropropagated plants (oleoresin 2.4 -7.6%; fibre 3.8 – 6.7%; dry recovery 21.2 – 31.8%) and in the callus regenerated plants (oleoresin 1.6 – 4.5%; fibre – 3.6 – 6.0%; dry recovery – 21.9 – 29.4%) whereas in the control plants (oleoresin 5.20%; fibre 5.90%; dry recovery 28%). Qualitative studies on in vitro derived ginger plants were carried out by Rao et al. (2000). The oleoresin (11.34%) and oil contents (3.60%) in the exotic material from Jamaica were found to be superior to the local ginger varieties viz. Kurupampady (oleoresin – 7.44% and oil content – 2.13%) and Cochin ginger (oleoresin – 7.43% and oil content 2.38%).
2.8.4. Biochemical evaluation in conventionally propagated ginger

Biochemical properties of ginger were studied by various workers. The details are given in the Table 2.

Table 2. Quality/biochemical parameters of ginger rhizome.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Character</th>
<th>Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dry recovery</td>
<td>17.70 – 25.09%</td>
<td>Muraleedharan and Sakunthala, 1975.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.00 – 28.00%</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
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<td>2</td>
<td>Volatile oil</td>
<td>1.25 – 2.81%</td>
<td>Natarajan et al., 1970</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00 – 2.70%</td>
<td>Natarajan et al., 1972</td>
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<td></td>
<td></td>
<td>1.13 – 2.56%</td>
<td>Saikia and Shadeque, 1992</td>
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<td></td>
<td></td>
<td>1.80 %</td>
<td>Peter and Kandiannan, 1999</td>
</tr>
<tr>
<td>3</td>
<td>Oleoresin</td>
<td>3.72 – 7.10%</td>
<td>Muraleedharan and Sakunthala, 1975.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.90 – 10.50%</td>
<td>Nybe et al., 1980.</td>
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<td></td>
<td></td>
<td>3.00 – 10.80%</td>
<td>Sreekumar et al., 1980.</td>
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<tr>
<td></td>
<td></td>
<td>3.20 – 9.50%</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
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<td>4</td>
<td>Crude fiber</td>
<td>1.40 – 9.50%</td>
<td>Natarajan et al., 1970</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.80 – 9.80%</td>
<td>Natarajan et al., 1972</td>
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<tr>
<td></td>
<td></td>
<td>3.40 – 6.40%</td>
<td>Nybe et al., 1980.</td>
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<tr>
<td></td>
<td></td>
<td>3.50 – 6.00%</td>
<td>Sreekumar et al., 1980.</td>
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<td></td>
<td></td>
<td>4.56 – 8.05%</td>
<td>Saikia and Shadeque, 1992</td>
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<td></td>
<td></td>
<td>2.10 – 7.00</td>
<td>Sasikumar et al., 1992; Ravindran et al., 1994.</td>
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<td></td>
<td></td>
<td>7.17%</td>
<td>Peter and Kandiannan, 1999</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrate</td>
<td>60.00 – 70.00%</td>
<td>Kalpagam and Nirmala, 2003.</td>
</tr>
<tr>
<td>6</td>
<td>Starch</td>
<td>41.54 – 55.06%</td>
<td>Natarajan et al., 1970</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.40 – 59.00%</td>
<td>Natarajan et al., 1972</td>
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<tr>
<td></td>
<td></td>
<td>39.80 – 53.00%</td>
<td>Saikia and Shadeque, 1992</td>
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<td></td>
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<td>53.00 %</td>
<td>Peter and Kandiannan, 1999</td>
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