# STUDIES ON GENETIC VARIABILITY AND IMPROVEMENT OF PEACOCK GINGER (*KAEMPFERIA ROTUNDA* L., ZINGIBERACEAE)

*Thesis submitted*
*in part fulfilment of requirements*
*for the degree of*
*Doctor of Philosophy in Botany*
*of*
*University of Calicut*

**by**
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## CERTIFICATE

Certified that this thesis titled **"Studies on genetic variability and improvement of peacock ginger (*Kaempferia rotunda* L., Zingiberaceae)"** embodies the results of a piece of bona fide research work carried out as part fulfilment of requirements for the degree of Doctor of Philosophy in Botany of University of Calicut by **Mrs. Thushara V.S.** under my guidance and supervision and that no part of the thesis has been submitted for any other degree.

I further certify that such helps or sources of information availed of in this connection have been duly acknowledged.

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# DECLARATION

I, **Thushara V. S.**, hereby declare that this thesis entitled **"Studies on Genetic Variability and Improvement of Peacock Ginger (*Kaempferia rotunda* L., Zingiberaceae)"** being submitted in part fulfilment of requirements for the degree of Doctor of Philosophy in Botany of University of Calicut embodies the results of a bona fide research work done by me under the guidance of **Dr. V.V. Radhakrishnan**, Professor & Head, Department of Botany, University of Calicut, Kerala, India and that no part of it has previously formed the basis for the award of any degree, diploma, associateship, fellowship, title or recognition.

Calicut University

**Thushara V. S.**

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# PREFACE

Plant genetic resources in agri-horticultural crops and their wild relatives are of immense value to mankind as they provide food, fodder, shelter and industrial products. Due to the spread of high yielding varieties and selection pressure, the genetic variability is getting eroded resulting in large scale depletion of variability. This situation thus demands immediate action to conserve such germplasm. Many plants from the family Zingiberaceae are used in various systems of medicine because of their pharmacological and economic importance. *Kaempferia rotunda* L. is such a potential underutilized medicinal plant species of this family, which requires urgent attention in terms of efforts to conserve the genetic diversity and to upgrade its economic potential. The plant is distributed throughout India from Eastern Himalaya to Sri Lanka and the Malay Peninsula to Malay Island. Interest in the exploitation of medicinal plants as pharmaceuticals, cosmetics, flavourings and other natural products has greatly increased in recent years. Threats to genetic diversity and species survival have also increased in the case of medicinal plants as a result of habitat destruction, over-exploitation and land use patterns. Efforts to assess the available genetic diversity and to augment the diversity of such plants are highly essential in order to meet the pharmaceutical needs and to prevent the plants from becoming endangered or extinct. As this species is only marginally cultivated in spite of its medicinal and commercial importance, the present research programme has been designed and executed so as to collect, conserve and evaluate the genetic diversity of the species in the study area and also to identify superior genotypes from the germplasm.

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# Chapter I
# INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Medicinal plants are considered rich source of ingredients, which can be useful in drug development. Before the introduction of chemical medicines, man depended on the therapeutic properties of medicinal plants. The World Health Organization estimates that about 80% of the 5.2 billion people of the world live in the less developed countries and these people rely almost exclusively on traditional medicine for their primary healthcare needs (Davidson-Hunt, 2000; Ahvazi *et al.,* 2012).

The forests of India are the principal repositories of medicinal plants, which are largely collected as raw materials for pharmaceuticals. Most of the medicinal plants are collected from the wild even today. Continued commercial exploitation of these plants has resulted in declining the populations of many species in their natural habitats in India. Drugs of herbal origin have been used in traditional systems of medicines such as Ayurveda and Unani since time immemorial. Medicinal plants used in Ayurveda can provide biologically active molecules for the development of modified derivatives with enhanced activity and/or reduced toxicity (Joy *et al*., 2001).

The Family Zingiberaceae consists of the large number of medicinal plants and they are well-known for their use in ethnomedicine. Gingers are important natural resources, which provide many useful products used as food, spices, condiments, colouring agents, medicines, perfumes, etc. The family Zingiberaceae consists of 53 genera and 1200 species (Kress *et al*., 2002; Jantan *et al*., 2003; Sabu, 2006).

India is one of the richest and diverse regions for gingers having 20 genera and 200 species (Sabu, 2006). The members of Zingiberaceae are annual or perennial tuberous rhizomatous herbs. The rhizome is sympodially branched and composed of prominent distinct segments (Tomlinson, 1956). The important genera having medicinal uses coming under the family Zingiberaceae are *Alpinia, Amomum, Curcuma, Elettaria, Etlingera, Globba, Hedychium*, *Kaempferia* and *Zingiber* (Kumar *et al*., 2013).

*Kaempferia rotunda* L. belonging to the family Zingiberaceae is an important aromatic medicinal herb with tuberous rootstalk and very short stem. In Sanskrit, it is known as *bhumichampaka* and in Malayalam as *chengazhineerkizhangu.* Leaves are simple, few in number, erect, variegated green above and tinged with purple below. The plant produces a sub-globose tuberous rhizome from which many roots bearing small oblong or rounded tubers arise (Warrier *et al*, 1994). The plant has been mentioned in different traditional systems of medicine including Ayurveda and Unani due to immense medicinal properties (Jafri *et al.,* 2001). This species is distributed throughout India in moist localities and is also cultivated (Bantawa and Rai, 2009). The tubers of this plant are widely used as a local application for tumours, swellings and wounds. They are also given in gastric complaints and help to remove blood clots and other purulent matter in the body. The juice causes salivation and vomiting. In Ayurveda, the important formulations using this herb are *Cyavanaprasam, Asokaristam, Baladhatryadi tailam, Kalyanakaghritham, etc*. The popular drug "*Hallakam*" prepared from this is used in the form of powder or as an ointment to reduce swellings and wounds (Joy *et al*., 2001).

This plant requires special attention because it is a source of important formulations in Ayurveda and possess a wide range of beneficial properties such as anti-cancerous (Dhanamani *et al*., 2011; Amri, 2014; Tomar *et al*.,

2014; Kirana *et al*., 2003; Atun and Arianingrum, 2015), anti-microbial (Kumar *et al*., 2015; Dubal *et al*., 2009; Iyenger, 1976; Kabir and Reza, 2014), anti-viral (Aznam *et al*., 2012), anti-oxidant (Sirat *et al*., 2001; Pietta, 2000; Middleton, 1984; Chan *et al*., 2008; Atun and Arianingrum, 2015) and anti-mutagenic (Atun *et al.,* 2013). It has got insecticidal activity against neonate larvae of *Spodoptera littoralis* (Nugroho *et al*., 1996; Tushar *et al*., 2010). In order to meet pharmaceutical needs and also to prevent the plant from becoming endangered or extinct, it is necessary to conserve and improve *K. rotunda* for the benefit of the society.

Plant genetic resources that are available to humans are being eroded rapidly. Reasons for this are many which include introduction of new and uniform cultivars, deforestation, developmental activities such as irrigation and hydroelectric projects, urbanization, changes in agricultural practices, etc. Under these circumstances, there is an urgent need to assemble whatever genetic diversity that is still available, either from farmers' fields or from the wild (Rao and Riley, 1994).

The plant breeder uses various technologies and methodologies to achieve targeted and directional changes in the nature of plants. It is impossible to develop new cultivars without genetic variability. After creating variability, the next task is to discriminate among the variability to identify and select individuals with the desirable genotypes and to multiply them in order to develop new potential cultivars (Acquaah, 2012).

However, the genetic diversity of medicinal plants is being threatened with the over-harvesting of plant materials from their natural habitats, especially from the forests. The effective management of these resources and their habitats is deemed important and necessary to meet the rising demand for its use by an increasing population. Harvesting of medicinal plants by cash-needy collectors has increasingly intensified since these materials have

high commercial value in the markets. Hence, the genetic base of wild medicinal plants in Kerala is being depleted at an alarming rate, leading to loss of diversity of the species and ecological instability (Batugal *et al*., 2004).

Studies on the genetic variability and the genetics of various agronomic characters of *K. rotunda* have been attempted only to a limited extent. Hence, no improved high yielding varieties have been developed so far and no scientific publications on its cultivation practices have been released. Further, it is a common fact that the species density of this valuable medicinal plant is declining currently due to various anthropogenic activities. The release of high yielding varieties of *K. rotunda* will be thus beneficial for the large-scale production, cultivation and conservation of this valuable medicinal plant.

Hence the present study was undertaken with the objective of analyzing the genetic variability of *K. rotunda* by studying the genetic control, phenotypic and genotypic variability, heritability and genetic advance of important morphometric characters and their interrelationship, association and divergence based on accessions collected from different locations of Kerala state of India, so as to facilitate its genetic upgradation and identification of superior genetic material from them for further breeding programmes.

# Chapter II
# REVIEW OF LITERATURE

## 2.1. Introduction

### 2.1.1. The family Zingiberaceae

Zingiberaceae or the ginger family is a large monocotyledonous family of aromatic rhizomatous perennial herbs, comprising of 53 genera and over 1200 species worldwide (Kress *et al*., 2002). It is chiefly present in tropical regions confined to Asia, Africa, Australia and tropical America (Burtt and Smith, 1972; Jain and Prakash, 1995; Borah and Sharma, 2012). Sirirugsa (1999) has reported 18 genera and 120 species from India. According to Sabu (2006) 21 genera and 200 species have been discovered from India and among them 10 genera and around 65 species are present in South India. They mostly grow in damp and shady places. However, some species can fully expose to the sun and grow on high elevation (Sirirugsa, 1999). Zingiberaceae is well known for the presence of different types of secondary metabolites such as flavonoids, phenolic acids, essential oils, oleoresin, etc. (Connell, 1970; Chan *et al*., 2008; Santos *et al*., 2012; Nag *et al*., 2013; Yusuf *et al*., 2013; Taheri *et al*., 2014).

Zingiberaceae species are generally characterized by thickened rhizomes with secretory cells producing essential oil (Mabberley, 1997). Plants of the family Zingiberaceae have been widely used as spices and in traditional oriental medicine and play a major role in the Indian system of medicine, Ayurveda (Amri, 2014; Kumar *et al.,* 2013; Choudhury *et al.,* 2013). The members of Zingiberaceae play an important role in ecological instability particularly in the understorey of tropical and subtropical forests where many species are quite common. The family Zingiberaceae is also an

important natural resource that provides useful products for food, spices and condiments, medicines, dyes and perfumes besides many ornamental species cultivated for their showy flowers. Many species are economically important and also a source for income generation (Singh, 2009). These plants are cultivated for their rhizomes in tropical areas of South and East India (Bhunia and Mondal, 2012).

The members of Zingiberaceae are mostly annual or perennial rhizomatous herbs. The plants propagate by means of fleshy, elongated or tuberous rhizomes. The rhizome is sympodially branched and formed of distinct segments. The rhizomes are differently coloured ranging from deep yellow, pale yellow, greenish blue, pink or combinations of these in different species (Sabu, 2006; Tomlinson, 1956; Kumar *et al*., 2013).

Rhizomes may be small, medium or large in size, which are usually fleshy or hard. The young rhizome and axillary buds are covered and protected by scale leaves. Sessile tubers if present are branched, elongated or condensed and the tips end in an erect leafy shoot or in a flower bearing shoot. Roots may be fleshy or fibrous ending in root tubers. These root tubers are small, rarely long, ovoid or elliptic and watery pearl white inside (Sabu, 2006; Jayasree and Sabu, 2013).

Leafy shoots are generally unbranched and true aerial stem is present in some genera while absent in others. True stem is very short as in *Kaempferia* or is a pseudostem with clasping leaf sheath as in *Curcuma*. The leaves are distichous and they exhibit morphological variation in shape, size, texture and venation pattern (Sabu, 2006; Kumar *et al*., 2013). Leaves are distichously arranged transverse or parallel to the rhizome. The colour of the leaves also shows much variation among different taxa. Leaves are sessile or petiolate and possess sheathing bases. Lamina is elliptic to oblong-lanceolate or circular, variegated sometimes, glabrous or pubescent and it remains

convoluted in the bud, short or long ligule is present in the upper end margin of the sheath. Venation is pinnate to parallel with a prominent mid vein (Pandey, 1997; Sabu, 2006).

Inflorescence is terminal or lateral. Both terminal and lateral inflorescences can be observed in different seasons. Inflorescences are produced on the terminal part of the leafy shoot or from the base of the leafy shoot or on a special leafless branches growing directly from the rhizome. It may be a condensed or elongated spike, raceme or panicle. In some species, white or attractively coloured sterile bracts called comma bracts are seen at the tip of the spikes. Fertile bracts are green or coloured, spirally or distichously arranged, free to the base or adnate with each other laterally. They are deciduous, persistent or may be absent. Bracteoles may be present or sometimes absent (Sabu, 2006).

Flowers are bisexual or unisexual and are seen on the upper part of the inflorescence. They are irregular, zygomorphic, trimerous, dichlamydeous and epigynous. In India, majority of the species flower during monsoon and rarely during summer, while some other species flower throughout the year (Sabu, 2006; Sabu *et al*., 2011). Calyx is tubular, formed with three sepals united to form a calyx tube that is shorter than or equal to the corolla tube. Corolla is formed with three petals and they are fused at the base to form a corolla tube, which may be tubular, or funnel shaped and exhibits wide range of colours that vary from white to yellow, orange, pink or purple. Androecium consists of six stamens arranged in two whorls of three each inserted at the throat of the corolla tube. The plants of this family possess only one functional stamen that is the posterior one of the inner whorl while the remaining two lateral stamens unite to form the labellum. Labellum or lip is the large and most conspicuous attractive part of the flower, which is variously coloured in different combinations of white, yellow or deep yellow,

violet or purple with strips or spots (Sabu, 2006). Staminodes are seen attached to the corolla tube and the fertile anther bears appendages. Anthers are mostly dithecous with longitudinal dehiscence. Ovary is always inferior, usually trilocular with axile placentation, rarely bilocular or unilocular with parietal placentation. Style is long and filiform, usually as long as the stamen and held within the filament below and between anther thecae and appendages above. The stigma is swollen, bilipped and fringed with hairs. Two epigynous glands are present just above the ovary. Fruits are usually dry or fleshy capsules or berries and most of them are dehiscent. Dehiscence of the capsule is loculicidal. Seeds are arillate with starchy perisperm and endosperm (Sabu, 2006; Jayasree and Sabu, 2013; Nair, 1997).

## 2.2. Medicinal gingers

Zingiberacean members form an important group with economic potential and many members of this family provide spices, dyes, perfumes, medicines and some are aesthetic to man. Plants of this family are used for the treatment of many diseases as herbal medicines and they play a major role in the preparation of many ayurvedic drugs (Kumar *et al*., 2013; Sirirugsa, 1999).

The plant *Alpinia galanga* (L.) Wild commonly called greater galangal, known as 'rasna' in Sanskrit has various medicinal properties. This plant has been used traditionally for the treatment of eczema, bronchitis, ulcers and cholera. The seeds of *A. galanga* are used to clean the mouth and for emaciation and to stimulate digestion and as a purgative. It's rhizome is generally used as a spice or source of essential oil and it can be used as anti-microbial, anti-bacterial, anti-inflammatory and flavouring agent. It's flowers and young shoots are used as vegetable or as spice (Al-Snafi, 2014; Verma *et al.,* 2011). Galangal forms the source plant of the ayurvedic drug *rasna* which is a popular remedy for rheumatism, intermittent fevers and respiratory

diseases (Girija and Shree, 2014). Its rhizome yields an essential oil called galangal oil, which possesses marked insecticidal properties (Hussain *et al.,* 2006).

*Alpinia officinarum* Hance. is commonly known as lesser galangal; it's rhizomes are used for their sweet spicy flavour and aromatic scent. It is used as stimulant and digestive in Homeopathy (Grieve, 1931). *A. officinarum* contains high concentrations of the flavonol galangin, which has been shown to slow down the increase and growth of breast tumor cells (Stull, 2016; Masoudi and Movahedi, 2017). It is also used as an aromatic, stomachic, analgesic and antiemetic (Shin *et al*., 2002).

*Alpinia calcarata* Roscoe is a rhizomatous herb which is widely used in the traditional system of medicines in Srilanka. The officinal part is the rhizome, which forms the main ingredient of several preparations like *Rasnadi kasaya, Rasnadi churna, Rasnadi tailam, Asvagandharistam* etc. The drug stimulates digestion, purifies blood and improves voice (Kumar *et al*., 2013; Rahman and Islam; 2015; Sivarajan and Balachandran, 1994).

*Amomum subulatum* Roxb. commonly known as large cardamom is widely cultivated in north-eastern and the central Himalayan regions of India. It has been a well known spice since time immemorial; used as flavouring agent to various dishes indigenous to Nepal, Bhutan, and India. It is also reported as an official drug in Ayurvedic pharmacopoeia due to its curative as well as preventive properties for various ailments. This plant has been recognized for its wide range of physiological and pharmacological properties. It contains 1.95% to 3.32% of essential oil (Gupta, 1986) having a characteristic flavour and possesses medicinal properties like stimulant, stomachic, alexipharmic and astringent properties (Bisht *et al.,* 2011). In the Indian systems of medicine, Ayurveda and Unani, it is used for gastric ulcers

and gastrointestinal disorders, as a liver tonic and appetizer, as diuretic and for the treatment of migraine (Jafri *et al*., 2001).

*Curcuma aeruginosa* Roxb., commonly known as 'pink and blue ginger' is used for the extraction of East Indian arrow root or Travancore starch (Sabu, 2006). It is widely used in Indian System of Medicine as an anti-diarrheal and anti-fungal agent (Jantan *et al.,* 2003; Soorya, 2017). Its rhizome is used externally as astringent for wounds (Srivastava *et al.,* 2006; Nadkarni, 1954).

*Curcuma amada* Roxb. is a unique species having morphological resemblance with ginger but imparts a raw mango flavour. Its rhizome is edible and having characteristic taste of unripe mango (Kumar *et al*., 2013; Saji and Sasikumar, 2004). The rhizomes are aromatic, bitter or sweet sour in taste and show appetizing, carminative, digestive, stomachic, demulcent, vulnerary, febrifuge, aphrodisiac, laxative, diuretic, expectorant, anti-inflammatory and antipyretic activities (Warrier *et al*., 1994). The main use of rhizome is in the manufacturing of pickles and culinary preparations (Policegoudra *et al*., 2011).

*Curcuma angustifolia* Roxb. is commonly called as 'Indian arrow root'. The rhizome of *C. angustifolia* is useful in the treatment of leprosy, jaundice, burning sensations, dyspepsia, fever, loss of taste, bronchitis, asthma, anaemia, leucoderma, stones in the kidney and bladder, urinary discharges, ulcers and diseases of blood. Its rhizome is highly valued as an article of diet. The starch obtained from the dry powdered rhizome is nutritive and medicinal (Murthy *et al.,* 2015; Rajeevkumar *et al.,* 2010).

*Curcuma aromatica* Salisb. is popularly known as wild turmeric or Cochin turmeric. It is a threatened aromatic medicinal plant, well known for its multifaceted properties. The medicinal properties of this plant are being

utilized in many traditional systems of medicines like Ayurveda and Unani. The rhizome extract of the plant is highly effective against many pathogens as well as microorganisms causing food spoilage and food borne diseases. The aboveground appearance of this plant is closely similar to *Curcuma longa* but the rhizomes are less pigmented with a particular camphoraceous smell (Anoop, 2015). It is applied externally for skin diseases, sprain, bruises, snake bite and also to enhance complexion (Sikha *et al*., 2015).

*Curcuma caesia* Roxb. is commonly known as black turmeric. The inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to the presence of essential oil (Pandey and Chowdhary, 2003; Das *et al.*, 2013). It is widely used in Ayurvedic, Unani and Siddha systems of medicine. Traditionally, the rhizomes of *C. caesia* are used in treating asthma, piles, tumours, bronchitis, leucoderma, etc. The paste is applied on bruises and for rheumatic pains in Manipur (Sarangathem and Haokip, 2010; Ranemma and Reddy, 2017).

*Curcuma longa* L. is popularly known as turmeric regarded as 'golden spice' as well as the 'spice of life' (Ravindran, 2007). Since ancient times, turmeric has been used as the principal ingredient of dishes for its colour, flavour and taste. It occupies an important position in social and religious ceremonies in Ayurvedic and folk medicines against various ailments including gastric, hepatic, gynecological and infectious diseases (Gupta *et al*., 2013; Hasan and Mahmud, 2014). The rhizomes are widely employed as a colouring agent and condiment entering largely into the composition of Indian curry powders. A paste of turmeric is applied to facilitate the process of scabbing in the case of small-pox and chicken-pox. An ointment made of turmeric, onions, hemp leaves and warm linseed oil gives great relief when the piles are painful and protruding (Nadkarni, 2005). Turmeric is also used as a safe colouring matter in pharmaceutical industry. It also enters into

formulation of cosmetic soaps particularly effective in skin problems and also is used to remove unwanted hair (Ahmad *et al*., 2010).

Turmeric contains about 5% of diaryl heptanoid colouring materials known as curcuminoids, the chief of which is a yellow pigment curcumin (diferuloylmethane). Extensive research over the past half century has shown that curcumin can modulate multiple cell signaling molecules in biological activity (Gupta *et al*., 2013). It has been shown to cure inflammatory condition, metabolic syndrome, pain, and it helps to manage inflammatory and degenerative eye conditions. In addition, it has been shown to benefit the kidneys (Hewlings and Kalman, 2017). Curcumin has beneficial effects in Alzheimer's diseases and is effective in the treatment of depressive disorders (Mishra and Palanivelu, 2008; Ringman *et al*., 2005: Soleimani *et al*., 2018). Turmeric plays an important role in traditional veterinary medicines. Turmeric powder is widely used as a spice, food preservative, as a household remedy for hepatic disorders, wounds, rheumatism and sinusitis and in religious rituals (Sasikumar, 2005).

*Curcuma mangga* Valeton and Zijp. is called mango turmeric, when the fresh rhizome is cut, it produces a mango like smell (Kaewkroek *et al.,* 2009). It is mainly used for cooking purposes, food decoration as well as a vegetable and traditional medicine (Wong *et al*., 1999). It is also used to treat skin diseases such as red spot, which cause itching and can reduce body heat caused by continuous fever (Raihana *et al*., 2011). For medicinal purposes, it's rhizomes are used in postpartum care, usually to aid in womb healing (Abas *et al*., 2005).

*Curcuma zanthorrhiza* Roxb., commonly called false turmeric, has been used for centuries in traditional systems of medicine to treat several diseases. It is used for hepatitis, liver complaints, diabetes, rheumatism, cancer, hypertension, skin eruptions and heart diseases in folk medicine

(Devaraj *et al.,* 2010). Pharmacologically *C. zanthorrhiza* has anti-microbial, anti-metastatic, anti-cancer and anti-oxidant activities (Athira *et al.,* 2018a; Yasni *et al.,* 1993).

*Curcuma zedoaria* (Christm) Roscoe is also known as white turmeric, zedoaria, krachura or gajutsu (Wilson *et al.,* 2005). It is a well known ethnomedicinal plant that is also used in Ayurveda. Traditionally, it is used for the treatment of menstrual disorders, vomiting, dyspepsia and cancer (Lobo *et al.,* 2009). The rhizome of this plant is used as appetizer and tonic, particularly prescribed to woman after childbirth. A decoction made of long pepper (*Piper longum*), cinnamon (*Cinnamomum verum*), zedoary and honey is given to cure cold (Kumar *et al.,* 2013).

*Elettaria cardamomum* (L) Maton., popularly called cardamom is a perennial herb with thick horizontal rootstock. It is also known as 'Queen of Spices' because of its pleasant aroma and taste and is highly valued from ancient times. Cardamom is often used to flavour tea and coffee. The seeds from the fruit capsules, which are harvested before they are fully ripe, constitute the cardamom of commerce. The essential oil is used as a food flavoring, in perfumery and for flavoring liquors (Radhakrishnan, 2003). It is an essential ingredient in garam masala and is also used as a breath freshener. Powdered cardamom seeds are mixed with ground ginger, cloves and caraway and used regularly for combating digestive ailments. It is used as a powerful pleasant aromatic stimulant, diuretic, stomachic and carminative. Use of cardamom controls nausea and vomiting. It is believed that daily consumption of cardamomum seeds along with a tablespoon of honey improves eye site, ameliorates the nervous system and thus improves health of the person (Korikanthimath *et al.,* 2001).

*Hedychium coronarium* Koenig., known as white ginger lily or butter fly lily is commonly grown as ornamental. It grows in moist tropical forests

and the Himalayas which are the probable centres of origin of this plant (Soares and Barreto, 2008). This plant possesses medicinal as well as economical values. The flower contains essential oil, which is used in high grade perfumes. It pacifies vata, kapha, nasal polyps, fever and worm infection (Verma and Bansal, 2010; Matsumoto *et al.*, 1993). The rhizome of the plant is found to be useful in the treatment of diabetes, headache, cancer, inflammation, apoptosis, invasion and osteoclastogenesis (Bhandary *et al*., 1995; Kunnumakkar *et al.*, 2008).

*Hedychium spicatum* Ham. ex Smith, commonly known as 'spiked ginger lily', is considered as one of the important species for its medicinal and food value (Rawat *et al.,* 2018). It is being used in traditional as well as modern medicine and in cosmetic and perfumery industries. Recently, the crude extract obtained from the rhizome has been used in the preparation of an anticancerous drug, PADMA 28 (Navab *et al.,* 2004; Giri *et al.,* 2010). They enter into the preparation of cosmetic powders used for promoting hair growth and ingredients of herbal vanishing cream. It also acts as antidote for snakebite and is used in veterinary medicine (Sravani and Paarakh, 2011).

*Kaempferia galanga* L. is a stemless herb and a highly priced medicinal plant indigenous to tropical Asia (Kareem, 1997; Shankar *et al.,* 1997). The rhizomes of the plant are widely used in East Asia for a wide range of medicinal applications and for food flavouring in Malaysia (Sadimann, 1992; Wong *et al*., 1992). Traditional health practitioners use these rhizomes for the treatment of psoriasis, tumour and bacterial infections and it is also applied externally for abdominal pain in women and treatment of rheumatism (Hirschhorn, 1983; Nag and Mandal, 2015; Jayasree, 2009). It's rhizome is a constituent of various ayurvedic preparations like *rasnadi kashayam, rasnachandanadi tailam, kachuradi churnam, panchagandhaka*

*churnam* and *chyavana prasam* (Sivarajan and Balachandran, 1994; Thirumulpad, 2004).

*Kaempferia rotunda* L. is a handsome aromatic herb with very fragrant subglobose yellow-white tuberous rhizome used in traditional medicine of Kerala. The rhizomes and root tubers of the plant have a bitter, camphoraceous taste and has been widely used as vegetable and a food flavouring spice in India and south east Asia (Lotulung *et al*., 2008; Sini *et al*., 2014). Benzyl benzoate is a major component of the rhizome of *K. rotunda,* which is used as an insecticidal ethnopharmacotherapeutic agent (Omotuyi *et al*., 2013; Nugroho *et al.,* 1996; Tushar *et al*., 2010). Phytochemical screening of the rhizome oil of *K. rotunda has* reported the presence of four main constituents, which include pentadecane, bornyl acetate, benzyl benzoate and camphor (Woerdenbag *et al*., 2004; Sirat *et al*., 2005).

*Zingiber officinale* Roscoe., commonly known as ginger, is cultivated for thousands of years as a spice and for medicinal purposes. The rhizome of this plant has been used as a medicine in Indian and Arabic herbal traditions since time immemorial (Altman and Marcussen, 2001). It has been used extensively in China for the treatment of headaches, nausea and common cold (Grant and Lutz, 2000) and for the treatment of arthritis, rheumatological conditions and muscular discomfort in herbal medicinal practices in Mediterranean and Western parts (Bordia *et al*., 1997; Shukla and Singh, 2007). In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help in common cold, fever and even painful menstrual periods (Awang, 1992). Experimental reports have shown that the main constituents of ginger are the gingerols, shogaols, zingerone and paradol (Langner *et al.,* 1998). Ginger oil

is used as a flavouring agent in pharmaceuticals as well as perfumery (Joshi, 2000; Nybe *et al.,* 2007).

Ginger plays a major role in curing various diseases in traditional Indian ayurvedic system of medicine. Both fresh and dried rhizomes are used in various formulations (Sivarajan and Balachandran, 1994). Indian traditional medicinal remedies especially for cough and asthma consists of fresh ginger juice with a little of fresh garlic juice mixed with honey (Gupta and Sharma, 2014). Besides these, ginger is very often used to cure many illnesses such as indigestion, tastelessness, loss of appetite, vomiting, allergic reactions, acute and chronic cough, sinusitis, bronchitis, respiratory troubles, pain, backache, painful tooth and swelled gum, etc. (Kumar *et al*., 2011).

*Zingiber montanum* (K.D. Koenig) Link ex Dietr., also known as cassumunar ginger is native plant of India. Cassumunar ginger oil has been pharmacologically studied to prove its potential in pharmaceutical industry. Rhizome extract and oil of cassumunar ginger possess anti-inflammatory, anti-allergic and anti-oxidant activity and are used against asthma and muscle and joint pain (Jeenapongsa *et al.,* 2003; Tewtrakul and Subhadhirasakul, 2007).

*Zingiber zerumbet* (L) Smith is commonly known as shampoo ginger or pinecone ginger. The plant's cones are used as an ornamental, and the milky juice obtained from the pine cones is used as a shampoo (Yob *et al.,* 2011). Some of the traditional uses of its rhizome as botanical medicine include the treatment of inflammation, fever, toothache, constipation, indigestion, diarrhea, worm infestation and severe sprains. It is antispasmodic, antirheumatic and diuretic also (Bhuiyan *et al*., 2009; Zakaria *et al*., 2010; Sulaiman *et al.,* 2010).

## 2.3. The genus *Kaempferia*

Many of the members of Zingiberaceae are well known for their medicinal values and play a major role in Indian systems of Medicine, particularly Ayurveda. Among these, the genus *Kaempferia* has a remarkable position. Linnaeus, in honour of Engelbert Kaempfer, a German physician who was an ardent lover of plants, named this genus of gingers as *Kaempferia.* The genus *Kaempferia* beholds about a dozen species, are shade lovers and the shorties of the ginger world. The flowers are usually inconspicuous, very pretty and pale lavender for most species. *Kaempferia* species are small perennial herbs with short rhizome and tuberous roots (Larsen *et al*., 1999). The genus *Kaempferia* has around 70 species distributed in Asia and Africa. Most are hardy and go dormant in winter. Any amount of sunlight will make the leaves curl protectively into tight rolls (Stephen, 2003). It is a genus of rhizomatous herbs, widely distributed in the tropics and subtropics of northeast India, Asia and Africa (Daimari *et al*., 2016; Rao, 2013). Some species are sources of important medicines and cure human ailments and the rest are grown as ornamentals in gardens. In the genus *Kaempferia*, five species have been recognized from India which include *K. angustifolia*, *K. elegans*, *K. marginata*, *K. parviflora*, *K. galanga* and *K. rotunda* (Aishwarya, 2017). Among these species, *K. galanga* and *K. rotunda* are more valued for their medicinal attributes (Joseph and Kurien, 2008; Saensouk *et al*., 2016). Some workers given the status critically endangered for *K. rotunda* (Devi *et al*., 2015; Ganeshaiah, 2005). The genus *Kaempferia* is completely absent in the wild habitats but two species of *Kaempferia* namely *K. galanga* and *K. rotunda* are available in the homesteads of Kerala (Unnikrishnan, 2004; Borah and Sharma, 2012; Chong *et al.*, 2009; Kumar, 2011). Phytochemical studies have shown that *Kaempferia* plants are rich in cyclohexane oxide derivatives, chalcone derivatives, cinnamates, terpenoids and flavonoids (Prawat *et al.,* 1993).

The probable origin of this genus is Asiatic, perhaps in Myanmar where it is grown as an evergreen in shady forest conditions (Holttum, 1950). From there it appears to have migrated across most of tropical Asia and across Africa. An adaptation to seasonal climates has occurred with the geographical migration. A comparison of literature review and specimens of Asiatic and African species shows a marked difference of flower morphology between the two species of two different geographical areas (Baker, 1893). In the flowers of Asiatic origin, the two lateral petaloid staminodes are so separate from the labellum and the latter is deeply 2-lobed so as to give the appearance of a four petalled flower. Also, in the African species it is seen that two lateral staminodes and the two lobes of the labellum are strongly united and this compound structure consequently gives the appearance of a 3 or 4 lobed petal (Mahanty, 1970). Rats and rabbits seldom attack plants with fleshy rhizomes like *Kaempferia* in which rhizomes are rich in terpenes. *K. rotunda* and *K. galanga* show oil richness where as in *Kaempferia pulchra* no essential oil is detected. Thus this genus includes both aromatic and non aromatic plants. This may be due to the geographic isolation of the species, where species emerged with or without aromaticity (Joseph, 1998).

### 2.4. *Kaempferia rotunda* L.

The genus *Kaempferia* includes about 70 species distributed in Asia and Africa. This genus of gingers has promising ornamental and medicinal potential so that they can be used to develop a variety of commercial products (Abdullah *et al.,* 2015; Sirirugsa, 1999; Kam, 1980; Singh *et al*., 2012).

*Kaempferia rotunda*, commonly called peacock ginger (Cordier and Steenkamp, 2012; Yeoh *et al*., 2015), or Indian crocus (Sikdar *et al*., 2015; Sharma and Pegu, 2011; Borpujari and Dutta, 2015) is known as *bhumicampaka* in Sanskrit, *bhuyicampa* in Hindi (Joy *et al*., 1999; Hussain and Hore, 2007; Barbhuiya *et al*., 2009), *misri dana* in Bangladesh (Sultana *et*

*al*., 2012) nerppicin in Tamil (Warrier *et al*., 2001), *chengazhineerkkizhangu* in Malayalam (Warrier *et al*., 2001; Sini *et al*., 2014; Joy *et al*., 1999; Kumar *et al*., 2011; Sereena *et al*., 2011; Unnikrishnan, 2004), *bui champai* in Nepali (Basnett *et al*., 2015) and *yai-thamna-manbi* in Manipuri (Tushar *et al*., 2010). Linnaeus first used the genus "*Kaempferia*" in 1736 in a catalogue of plants named '*Hortus Cliffortianus*'.

*Kaempferia rotunda* is a handsome aromatic herb with very fragrant subglobose yellow-white tuberous rhizome, commonly known as *chengazhineerkizhangu* in the traditional medicine of Kerala. The rhizomes and tubers of the plant have bitter, pungent and camphoraceous taste. It has been widely used as a vegetable and a food flavouring spice in India.

It's rhizomes and tubers are traditionally used for abdominal pain, diarrhoea, cold, obesity and preparation of cosmetics and it is locally applied to tumours, swellings, bruises, wounds and ulcers. It is also considered as stomachic and anti-inflammatory and is given in gastric complaints (Sivarajan and Balachandran, 1994; Kirtikar and Basu, 1987; Prajapati *et al*., 2003; Singh *et al*., 1996; Rout and Thatoi, 2009). In Ayurveda, the important formulations using this plant are *Cyavanaprasam, Asokarishtam, Baladhatryadi tailam, Kalyanakaghritham,* etc. The drug *Hallakam* prepared from this is in popular use in the form of powder or as an ointment application to wounds and to reduce swellings (Sereena *et al*., 2011; Warrier *et al*., 2001). According to IUCN, *K. rotunda* is proposed as vulnerable (Aishwarya, 2017). Despite its widespread use, no toxicological data are available regarding the safety assessment of the rhizome of *K. rotunda.*

### 2.4.1. Habitat and distribution

The plant is widely distributed in the tropics and sub-tropics of Asia and Africa (Sirirugsa, 1999). It is distributed throughout the Indian

subcontinent from eastern Himalayas to Sri Lanka and the Malay Peninsula to Malay Island (Warrier e*t al*., 1994, Anonymous, 1959; Kirtikar and Basu, 1987; Sharan, 2011). It is possibly a native of Indo-China and is cultivated widely in southeast Asia (Babu *et al*., 1997; Pai *et al*., 1970; Pancharoen *et al*., 1996; Larsen, 1980). This plant is grown as undergrowth in mixed forests or in open grassy areas (Aishwarya, 2017). This species is distributed throughout India in moist localities and is also cultivated (Bantawa and Rai, 2009; Joy *et al*., 2001). It is seen naturally growing in the Western Ghat region of Kerala state of India, i.e., in Silent valley and Nelliampathy of Palakkad District, Begur, Kalpetta and Thirunelli of Wayanad District and Mala and Peechi of Thrissur District of Kerala (Warrier *et al*., 2001). The plant grows wild in shaded areas that are wet or humid, predominantly in the forests of South India. It grows in gardens and is well known for their beautiful flowers and foliage. It is also cultivated as an intercrop with other plantation crops (Joy *et al*., 1999). Review of literature revealed that *K. rotunda* first found its name in Van Rheede's '*Hortus Malabaricus'* as "*Malan Kua*". Fischer in Gamble's '*The Flora of Presidency of Madras*' has specified it as 'often cultivated and doubtfully wild'. In South India it is reported as endangered and grows on the Western ghats at higher and low elevations in Karnataka and Kerala (Pushpakaran and Gopalan, 2013; Sabu, 2006; Aishwarya, 2017).

*K. rotunda* is widely distributed in the wet and mixed deciduous forests and monsoon forests of Thailand (Khare, 2007; Saensouk *et al*., 2016; Barbhuiya *et al.,* 2009). Valeton, a botanist reported that this plant is apparently wild in East Jawa, but he considered that it might have escaped from cultivation. In Peninsular Malaysia, it is perhaps common in the north, but can only keep alive in cultivation in the South (Holttum, 1950).

### 2.4.2. Taxonomic hierarchy of *Kaempferia rotunda* L.

According to Angiosperm Phylogeny Group (2009), *Kaempferia rotunda* L. belongs to Kingdom: Plantae, Clade: Angiosperms, Clade: Monocots, Clade: Commelinids, Order: Zingiberales, Family: Zingiberaceae.

### 2.4.3. Agronomy

*K. rotunda* is an easily cultivable species with crop duration of six months, well adapted to shaded situations and rain fed conditions and provides a steady, additional income to farmers. Rich loamy soil having good drainage is ideal for the cultivation of the species. Laterite soil with heavy organic manure application is also well suited for cultivation. Planting is done during the month of May or June in India with the receipt of four or five pre-monsoon showers. Well developed healthy and disease free rhizomes with root tubers can be used as the planting material. Rhizomes harvested can be stored in cool dry place under shade plastered with mud or cow dung. The field is ploughed to a fine tilth and mixed with organic manure. Pits are made at 20 cm spacing in which 5 cm long pieces of rhizomes are planted. Pits are covered with organic manure, rotten straw or leaves. Apply FYM or compost as basal dose by ploughing pits after planting. Apply NPK at the ratio of 50:50:50 at the time of first and second weeding. After planting, mulch the beds with dry or green leaves. Weeding and manuring are done twice. 150-200 g of new rhizomes are obtained from each plant. This is an ideal species for underplanting in coconut plantations (Warrier *et al*., 2001; Joseph and Kurien, 2008). During heavy rainy months, leaf rot disease may occur which can be controlled by drenching 1% Bordeaux mixture. The crop can be harvested after six months of maturity. Drying up of the leaves is the indication of maturity. They are stored in moisture proof sheds. Prolonged storage may cause insect and fungal attack (Joy *et al*., 1999; Prasad and Joseph, 1997).

### 2.4.4. Morphology

*K. rotunda* is a perennial aromatic herb, attains height upto 50-65 cm with tuberous rhizomes that produce numerous tuberous roots. Leaves are few, erect, simple, ligulate, lanceolate, acute, variegated green above and tinged with purple below, upto 45 cm long and 10 cm wide; petiole short channeled; leaf base sheathing. Inflorescences are seen on separate shoots arising from the rhizomes; appear before pseudostems (Borah and Sharma, 2012; Sirirugsa, 1999). Flowers are seen on a short crowded spike pink to pinkish purple in colour, bracteolate, bisexual and trimerous. Calyx is 6 cm long, white in colour, cylindric, splitting down on one side, greenish white in colour. Corolla tube is longer than the calyx, 7 cm long, slender, infundibuliform towards the mouth and petals five in number. Androecium consists of 6 stamens in two whorls of three each. Filaments are short, erect, 5 mm long, sparsely pubescent; thecae 9 mm long, lanceolate or subulate segments. The two laterals of the outer whorl get transformed into petaloid staminodes. Lateral staminodes are 3 cm x 0.5 cm, ovate to elliptic, with acuminate apex, white with violet tinge towards the margin, with a small cleft in the middle. The laterals of the inner whorl are united to form the posterior labellum, which is pinkish white or lilac in colour. Labellum is obcordate, overlapping, deeply divided into 2 suborbicular lobes. The anterior odd one is fertile, which has a long filament and two anther lobes. Ovary 5 mm × 3 mm, inferior, tricarpellary, syncarpous with many ovules on axile placentation; style filiform, terminal, passing through the groove in between the anther lobes; stigma slightly flattened, funnel shaped, margin hairy. Epigynous glands two, filiform, erect, enveloping the lower part of the style (Warrier *et al*., 2001; Sivarajan and Balachandran, 1994; Gamble, 1987; Kirtikar and Basu, 1987; Lalnundunga, 2000; Sharma and Pegu, 2011; Pushpakaran and Gopalan, 2013; Kumar *et al.,* 2004; Kumar *et al*., 2013; Aishwarya, 2017). Leaf shedding occurs during November. New leaf arises during February to

April. Flowering occurs during March to May. The flower blooms on the forest floor and soon disappears before the appearance of the leaves. Fruiting occurs during May to June but is not commonly seen (Saensouk *et al*., 2016; Devi *et al*., 2015; Lalnundunga, 2000; Pushpakaran and Gopalan, 2013; Kumar *et al*., 2013).

### 2.4.5. Cytology

Conventional cytological study among zingiberaceous plants is extremely hindered by the presence of diverse bioactive metabolites, which also affect their genome size estimation using flow cytometry. Sadhu *et al*. (2016) attempted a study on genome size estimation using flow cytometry of zingiberaceous plants. Natural hybridization and subsequent neutralization of these crosses have also been reported in this family (Skornickova and Sabu, 2005; Skornickova *et al*., 2007) indicating a vital role of chromosomal changes in the evolution of Zingiberaceae. They have suggested a novel nuclei isolation buffer, Modified Buffer 01 (MB01) that is capable of isolating good quality nuclei in various species of Zingiberaceae that are rich in phenolic compounds and essential oils. None of the several nuclei isolation buffers used in flow cytometry could be used very successfully for zingiberaceous plants to isolate good quality nuclei from both shoot and root tissues. Genome contents of 21 species belonging to six genera namely *Alpinia, Curcuma, Globba, Hedychium, Kaempferia and Zingiber* were assessed using MB01. The results affirmed that novel nuclei isolation buffer MB01 yielded superior quality of nuclei from plants of different genera of the family from both shoot and root tissues.

The effect of incubation time of colchicines on tetraploid induction of *K. rotunda* was studied by Soonthornkalump *et al*. (2012). Colchicine is a well-known anti-mitotic chemical that is widely used for chromosome doubling to improve several ornamental cultivars (Nuki, 2008). Chromosome

doubling was introduced for a plant breeding programme for improved new tetraploid cultivars (Urwin and Horsnell, 2007). So, tetraploid induction would improve some characters such as larger flower, leaves or stem (Piestsch and Anderson, 2006). Young shoots of *K. rotunda* were used as planting materials for treating with 0.2% colchicine on semi-solid MS medium for 0, 2, 4, 8 and 12 days. The shoots were transferred to a semi-solid MS medium supplemented with 17.8 μM 6-benzyladenine after the treatments. It was observed that the survival rate of the treated plants decreased when colchicine incubation time was increased. The maximum of tetraploid induction percentage (40%) was estimated from the shoots treated with colchicine for four days. There was a report of success in chromosome doubling of edible ginger using 0.2% colchicine but there was none on *K. rotunda* (Adaniya and Shirai, 2001). The results have demonstrated that four days was the optimal incubation time for polyploidy induction. The use of 0.2% colchicine for four days has produced the highest number of tetraploid plants with the highest survival percentage in *K. rotunda*.

Saenprom *et al*. (2018) analysed the somatic chromosome numbers and karyomorphological characters of Thai Zingiberaceae belonging to the genera *Alpinia, Elettariopsis* and *Kaempferia* using root tips. The root tips of *K. rotunda* showed a somatic chromosome number of $2n = 30$. The karyotype is composed of 14 metacentric and 16 submetacentric chromosomes ($2n = 30 =$ 14m + 16sm). The somatic cells of root tips of *K. marginata* showed a chromosome number of $2n = 42$ with a karyotype composed of 28 metacentric and 14 submetacentric chromosomes (2n=42=28m+14sm). This study also indicates that the basic chromosome number of the two species of *Kaempferia* is tetraploid with x = 9, 10, 11. Earlier reports on chromosomes of the species *K. rotunda* also show the diploid number to be 2n = 22, 33, 45, 44, 54, respectively (Eksomtramage and Boontum, 1995; Omanakumari and Mathew, 1991). The reported occurrence of *K. rotunda* with 2n=33 (Chakravorti,

1948) and 2n=54 (Raghavan and Venkatasubban, 1943) shows that this species occur in at least three different cytotypes, all based on the same basic number x=11. The karyotype consisted of 4 pairs of M-type, 10 pairs of m-type, 6 pairs of sm-type and 2 pairs of st chromosomes. Three of the longest chromosomes (1,2,3) and $6^{th}$ and $7^{th}$ chromosomes possessed secondary constrictions on their long arm (Omanakumari and Mathew, 1984). Recently Saenprom *et al.* (2015) conducted a study to explore chromosome numbers of ten *Kaempferia* species in northeast Thailand using root tips with the Feulgen squash technique. Results have shown that *K. rotunda, K. siamensis* and *K. sisaketensis* have the same chromosome numbers of 2n=22.

*K. rotunda* is of Asiatic origin and somatic chromosome number is found to be 33 (Mahanty, 1970). Root tip cells of *K. rotunda* showed 2n=44 ranging in length from 1.33 to 4.32 µm and this agrees with the study reported by Ramachandran (1969) and Omanakumari and Mathew (1984). According to Nopporncharoenkul *et al.* (2017) the diploid and triploid accessions of *K. rotunda* differ in growth and reproductive mode, they are also very similar to each other in morphology. The diploid accession usually produces only one rhizome, whereas the triploid accession has more than three moniliform like rhizomes. The diploid accession can be found as individual, sexually fertile plants, while the triploid accession colonizes by a clump of rhizomes. However, based on the botanical similarity, *K. rotunda* is believed to be an autotriploid.

### 2.4.6. Anatomy

#### 2.4.6.1. Root

Uma and Muthukumar (2014) studied solely on root anatomy findings, highlighting taxonomic and phylogenetic features of some tuberous and rhizomatous gingers. The anatomical characters of roots of Zingiberaceae

resembles that of Cannaceae (Jayakumari and Stephen, 2009), Costaceae (Pazhanichamy *et al*., 2010), Heliconiaceae (Simao and Scatena, 2001) and Musaceae (Sumardi and Wulandari, 2010) included in the order Zingiberales. Roots were sectioned with a freehand or microtome and examined using a variety of staining techniques. The anatomical characters of the roots were thoroughly studied and analysed on 21 qualitative and 16 quantitative characters.

Tuberous roots of *Kaempferia* have a wide, starch-filled cortex with stele diameter similar to non-tuberous roots. Roots are often off-white and arise from the fleshy tubers. The root is circular in cross section. Epidermis is uniseriate, bearing unilocular unicellular hairs. The exodermis is 2 layered with cells containing suberized walls. The cortex has got two regions with intercellular spaces that are radially extended. The cortex is 15 cells in width, parenchymatous, with intercellular spaces triangular in the inner cortical layers. The endodermis is uniseriate with cells having suberized 'U'-shaped thickened walls. The pericycle is uniseriate with thin walled cells. Vascular cylinder is solitary with 8–12 arches, exarch, vascular bundles radially arranged with the xylem distributed in two rows between phloem. Xylem vessels found to be associated with xylem fibres and parenchyma. Vascular bundles are embedded in sclerenchymatous conjunctive tissue. Pith is occupied by parenchymatous tissue in young roots. The metaxylem is seen enlarging towards the pith when the roots transform into tubers. The sclerenchymatous conjunctive tissue reduces as the size of parenchymatous pith increases. Starch grains are present in the inner cortex and pith regions of *Kaempferia* spp., (Warrier *et al*., 2001). Oil globules are present in the inner cortical region.

### 2.4.6.2. Rhizome

Cross section of rhizome is circular in outline with a distinct brown coloured exodermis consisting of 7-10 layers of cells. A correct distinction between the outer and inner zone by endodermis like layer is present and it is termed as endodermoidal layer. Towards the inner side is a large zone of ground tissue composed of thin walled cells with abundance of intercellular spaces. Most of the cells shows the presence of starch grains where as a few contain oil globules. Collateral and closed vascular bundles are seen scattered in the cortex. Each bundle contains 3-4 xylem vessels and small phloem. Each vascular bundle is surrounded by a single layer of parenchymatous cells which are devoid of starch grains. Numerous vascular bundles are seen randomly scattered towards the middle (Warrier *et al*., 2001; Sereena *et al.,* 2011).

### 2.4.6.3. Tuberous root

Transverse section of tuberous root is circular in outline. Outer 4 to 6 layers constitute the brown coloured epidermis. Interior to this is a large zone of parenchymatous cortex containing oil globules and starch grains. Stelar region is similar as that of the normal root (Warrier *et al*., 2001).

### 2.4.6.4. Petiole

Adaxial surface is almost straight with a small V-shaped depression in the centre. Petiole wings are acute towards the margins. Unicellular trichomes are observed in the adaxial epidermis. Cross section of petiole is 'c' shaped and closed in outline. Epidermis is single layered followed by one to two layers of parenchymatous cells. Vascular bundles are arranged along the periphery. These bundles are alternating with conspicuous air cavities. Each air cavity is surrounded by two to three layers of chlorenchymatous cells. Vascular bundles are closed and have two to four xylem vessels and a small

patch of phloem. Schlerenchymatous girdles are seen on both sides of the bundle. The ground tissue is parenchymatous in which small bundles are seen scattered (Warrier *et al*., 2001; Aishwarya, 2017).

#### 2.4.6.5. Lamina

Epidermal cells on the adaxial surface are comparatively larger than those on the abaxial surface. The length to breadth ratio varies with cells in both surfaces. Single layered epidermis is followed by single layered large parenchymatous hypodermis. Mesophyll is undifferentiated and contains many chloroplasts. Vascular bundles are seen as developed in the mesophyll region. The bundle cap is not in contact with either of the epidermis. Towards the mid-rib region, air cavities are prominent in the mesophyll tissue. Vascular bundles are closed and contain 3 to 4 xylem vessels and small phloem. Sclerenchymatous girdles are present on both sides of the bundle. Stomata are of rubiaceous type (paracytic). Stomatal index is calculated as 8.73 and 1.96 of lower epidermis and upper epidermis respectively. A few unicellular trichomes are present on the abaxial epidermis (Warrier *et al*., 2001; Aishwarya, 2017).

#### 2.4.6.6. Floral vasculature

About twenty vascular strands supply the calyx tube and they get interconnected often by oblique or transverse branches. The corolla tube is supplied with about twenty vascular bundles, which further enter into the petals; each are having six to ten bundles. These 12 to 14 bundles are seen usually interconnected due to frequent branching. Similar type of vascular supply is seen in the labellum also. The fertile stamen is supplied with three vascular strands which run parallel to each other along the filament. The middle one passes through the connective tissue in between the anther lobes and get branched at the tip, while the laterals after supplying the anther lobes

continue into the protruded connective and get branched. Ovary is supplied with three vascular bundles. Each one branches into two giving rise to dorsal and ventral branch. Ventral branch supplies the ovules; dorsal branch traverse through the style and enter into stigma without any branching (Warrier *et al*., 2001).

### 2.4.7. Phytochemistry

*K. rotunda* rhizomes contain economically important phytochemicals such as flavonoids, chalcones, stigmasterol, crotepoxide, quercetin, flavonols, β-sitosterol, benzoic acid, protocatechuic acid, syringic acid and some hydrocarbons such as camphor (Hussain and Hore, 2007; Sulianti and Chairul, 2005). A new lectin was isolated from the tuberous rhizome of *K. rotunda* with the molecular weight of 21 ± 1 kDa. In the presence of urea, the lectin did not lose its activity but the activity got abolished completely when treated with EDTA. The lectin showed its activity at the pH ranging from 6.0 to 9.0 and in a temperature range of 30 to 80°C. The cell growth inhibition was due to the induction of apoptosis in the EAC cells which was further confirmed by cell morphological study, caspase-3 inhibitor and apoptosis-related gene expression (Ahmed *et al.,* 2017). Kabir *et al.* (2011) carried out a research to isolate a new letin from tuberous rhizomes of *K. rotunda* with the molecular weight of 29.0±1.0 kDA. Hemagglutination inhibition studies revealed that methyl-α-D-mannopyranoside, D-mannose and Methyl-α-D-glucopyranoside were the most potent inhibitors for KRL and the minimum inhibitory concentration was 0.4, 1.6 and 1.6 mM respectively. Demetallization and remetallization of KRL showed that the lectin activity was dependent on the presence of metal ions. KRL lost its activity crucially beyond the pH value from pH 6 to 8.2 demonstrating the changes in structure of the lectin binding sites or ionization of the group associated with the sugar binding regions (Nelson and Cox, 2001).

On distillation, the rhizome yields oil that contains cineol and probably methyl chavicol (Babu *et al.*, 1997; Behra and Rout, 2003; Ahmed, 2010). Major essential oils of Zingiberaceae species were studied by Joseph (1998). In *K. rotunda* the chemotype belongs to ethyl-p-methoxy cinnamate (27.08%), which is the principal constituent of the essential oil. The other constituents identified were α-pinene (1.46%), β-pinene (4.43%), $\Delta^3$- carene (6.67%), 1,8 cineole (4.13%), camphor (5.18%), ethyl cinnamate (11.64%) and pentadecane (12.44%) (Kumar *et al*., 2013). α-pinene shows high repellant activity against certain insects. Also, it has the property to mimic alarm or alter pheromones of certain termites (Moore, 1974).

Jahan *et al*. (2015) assessed the elemental and fatty acid content of four medicinal plants including *K. rotunda* grown in Bangladesh. Macro (Na, K), micro (Mg, Mn, Fe, Cu, Cr) and heavy metal (Cd, As) elements were estimated quantitatively by flame photometer and atomic absorption spectroscopy technique in the tubers of *K. rotunda*. The ash value of *K. rotunda* was found to be 13.7481%. Elemental analysis revealed that the plant with the highest sodium (17030 ppm) and potassium content (63310 ppm) was *K. rotunda.* The analysis of fatty acid in *K. rotunda* using GC-MS showed that it contains various bioactive constituents including heneicosanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, stearic acid and octadecanoic acid in major concentration. The iron and arsenic content of this plant was found below the detectable levels of 10 µg/g. For all the plants analyzed, *K. rotunda* was found to hold the highest percentage of chromium (28.91 ppm). The content of plant minerals such as magnesium, manganese, copper and cadmium showed 1.95 ppm, 0.603 ppm, 0.2111 ppm and 0.0105 ppm respectively. The presence of these elements can make *K. rotunda* as a potential source of food and drug. These will be helpful in the synthesis of new ayurvedic drugs for controlling various diseases.

Kumar (2014) carried out the chemical characterization of essential oil from the fresh rhizomes of *K. rotunda* by gas chromatography-mass spectrometry (GC/MS method). This study revealed fifty seven components in the rhizomes oil, which accounted to 99.74% of the oil. The major components obtained were endo-borneol (9.30%), dehydro-iso-androsterone acetate (9.12%), naphthalene, decahydro-1, 1, 4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl), [4aS(4aα, 5β,8aα)]- (9.03%) and β-phyllandrene (7.08%). Other constituents were 2-propenoic acid, 3-(3-methoxyphenyl)-ethylester (4.47%), α-pinene (3.39%), ethylcinnamate trans (3.15%), 2,6-octadienoic acid, 3,7-dimethyl-, methyl ester (2.90%) and camphene (2.89%). *K. rotunda* contains flavonoid which shows antiphlogistic and vitamin P activity (Ghani, 2003). Feng (2009) conducted a comparative study on essential oils and phytochemicals of *K. rotunda* cultivated in Malaysia and Indonesia. Hydrodistillation of the fresh rhizomes of *K. rotunda* from Malaysia and Indonesia gave 0.09% and 0.23% oils respectively. The main constituents present in the rhizome oil of Malaysia were bornyl acetate (9.6%), benzyl benzoate (8.4%) and camphor (5.6%), while the rhizome oil from Indonesia was rich in benzyl benzoate (87.7%) and *n*-pentadecane (4.2%). Two new compounds identified from the Malaysian species are 2-(benzoyloxymethyl) phenyl (3-O-acetyl)-β-glucopyranoside and 3-debenzoylrotepoxide A, together with seven known compounds, crotepoxide, 4-benzoyloxymethyl-3,8dioxatricyclo[5.1.0.$0^{2,4}$]octane-5,6-diol 5-acetate, 1,6-desoxypipoxide, curcuminol C, 2' hydroxy-4,4',6'-trimethoxychalcone and naringenin 4',7-dimethyl ether. A new compound isolated from Indonesian species was 3-acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)-cyclohexa-4,6-diene with the seven known compounds namely crotepoxide, 4-benzoyloxymethyl-3,8 dioxatricyclo [5.1.0.$0^{2,4}$] octane-5,6-diol 5-acetate, 1,6-desoxypipoxide, 6-acetylzeylenol, *trans*-docosyl ferulate, benzyl benzoate and benzoic acid. Naringenin 4',7-dimethyl ether, *trans*-docosyl ferulate,

curcumrinol C and benzoic acid were found for the first time from *K. rotunda*. Antibacterial and antioxidant screening assays using disc diffusion method and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method respectively were carried out on the crude extracts and essential oils. The crude extracts and essential oils of *K. rotunda* from Malaysia and Indonesia did not show activities on antibacterial and antioxidant assay.

Phucho and Singh (2017) conducted a study to evaluate the trace elements such as Fe, Ca, Zn, Cu, Mn, Ni, Mg and Cr present in the rhizome of *K. rotunda* collected from Manipur using Graphite furnace-atomic absorption spectrophotometer (GF-AAS) method. This study revealed that the trace element concentrations in *K. rotunda* are Ni, 0.096±0.002ppm, Mg, 0.87±0.005ppm, Cr, 0.07±0.002ppm, Fe, 1.22±0.005ppm, Ca, 0.46±0.01ppm, Zn, 0.14±0.003ppm, Cu, 0.9±0.003ppm and Mn, 0.26±0.002ppm. This result could give the importance about the herbal drugs prepared from the plant materials in herbal remedies and in pharmaceutical companies.

Pancharoen *et al*. (1996) conducted a research to isolate three new cyclohexane diepoxides from rhizomes of *Kaempferia rotunda.* The newly isolated compounds were (–)-(1R,2R,4R,5S,6R,7R) -4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.$0^{2,4}$] octane-5,6-diol6-acetate,(+)-(1R,2R,4R,5S,6R, 7R)-4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.$0^{2,4}$] octane-5,6-diol 5-acetate and (–) - (1R,2R,4R,5S,6R,7R) -4-benzoyloxymethyl -3,8- dioxatricyclo [5.1.0.$0^{2,4}$] octane5,6-diol 6-benzoate together with crotepoxide and(-)-zeylenol. Lallo *et al*. (2014) carried out a study for isolation of secondary metabolites from the methanol extract of *K. rotunda* and obtained twelve compounds including a new compound (–)-3-acetyl-4-benzoyl-1-benzoyloxymethyl-1,6-diepoxycyclohexan- 2,3,4,5- tetrol and eleven known compounds namely, 2-[(benzoyloxy) methyl] cyclohex-5-ene-1,2,3,4- tetrol 1,4-diacetate (Starks *et al.,* 2012), crotepoxide (Kupchan *et al*., 1969), 4-

benzoyloxymethyl-3,8-dioxatricyclo [5.1.0.$0^{2,4}$] octane-5,6-diol 5- acetate (Pancharoen *et al*., 1996), 4–benzoyloxymethyl-3,8–dioxatricyclo [5.1.0.$0^{2,4}$] octane - 5, 6-diol 6- acetate (Pancharoen *et al.,* 1996), (–) –6-acetylzeylenol (Stevenson *et al*., 2007), 2-hydroxymethylcyclohex -5 -en-1,2,3,4-tetrol,1,4-dibenzoate (Pancharoen *et al.,* 1989), (–) - zeylenol (Palframan *et al*., 2012), 2- acetylretopoxide B (Stevenson *et al.,* 2007), benzylbenzoate (Pouchert and Behnke, 1993), benzoic acid (Pouchert and Behnke, 1993) and (–)-1,6-desoxypipoxide (Schulte *et al*., 1982). All of the isolated compounds were tested for their cytotoxic activity against pancreatic and breast cancer cell lines. Cytotoxic activity test revealed that (–)-1,6-desoxypipoxide exhibits moderate inhibitory activities against human pancreatic cell line and human breast cancer cell line without showing inhibitory activity against normal cell line.

Nugroho *et al.* (1996) conducted a research on rhizomes of different species of the Zingiberaceae for insecticidal constituents against larvae of the pest insect *Spodoptera littoralis.* Rhizome extract of *K. rotunda* when incorporated to artificial diets of the insect exhibited significant insecticidal activity in bioassays at a concentration of 2500 ppm. Woerdenbag *et al.* (2004) studied the volatile constituents of rhizomes of two medicinally used Indonesian plants namely, *K. rotunda* and *K. angustifolia* by GC and GC–MS (EI) analysis. They identified a total of 75 compounds. The most abundant constituents reported were benzyl benzoate (69.7%, 20.2%), *n*-pentadecane (22.9%, 53.8%) and camphene (1.0%, 6.2%) in *K. rotunda*. Three known flavanone compounds were isolated from chloroform extract of *K. rotunda* namely, 5- hydroxy -7- methoxyflavanone, 7-hydroxy-5-methoxyflavanone, and 5,7-dihydroxyflavanone by Atun *et al*. (2013).

Sini *et al.* (2014) conducted a toxicity study of the ethanolic extract of rhizome, tuber, rhizome fractions and the essential oil of *K. rotunda* to

authenticate the tribal claims of its use as a safe wound healing and antiulcerogenic drug. The essential oil, the extracts and the rhizome fractions of the plant were found to be non-toxic in the acute dermal toxicity studies when tested topically on the skin of the animal and were safe for use.

Chifdhiyah (2012) carried out a research to determine the effect of *K. rotunda* extract to feed against total amount hemocytes and phagocytic activity in tiger shrimp. The results have revealed that *K. rotunda* extract can be an alternative immunostimulant for tiger shrimp with a dose of 10 g / kg - 15 g / kg of feed. Pillai and Warriyar (1962) examined non-essential oil portion of powdered dried tubers of *K. rotunda*. Powdered tubers soxhleted with petroleum ether gave a white crystalline solid with a yield of 0.4% apart from an essential oil portion.

### 2.4.8. Pharmacognostic activity

#### 2.4.8.1. Anticancer activity

Rhizome of *K. rotunda* shows the presence of wide range of biologically active compounds reported to show anticancer activity (Dhanamani *et al*., 2011; Das *et al*., 2012; Amri, 2014; Tomar *et al*., 2014). Sowmya *et al*. (2014) specified that dried powder of *K. rotunda* rhizome is famous for prevention and used as a traditional medicine for cancer. Kirana *et al*. (2003) conducted a research to screen the ethanol extracts of eleven Zingiberaceae species for anticancer activity. Results have shown that extracts of *K. rotunda* had no effect on the growth of either cell lines at concentrations up to 250 µg/ml. Atun and Arianingrum (2015a) conducted a study to determine anticancer activity of bioactive compounds from *K. rotunda* rhizome extract against human breast cancer. The isolation of bioactive compounds from methanol extract *K. rotunda* was worked out by chromatographic technique. The *in vitro* cytotoxicity test was carried out on

human breast cancer T47D cell lines by MTT assay. These identified bioactive compounds can repair breast tissue and suppress *c-Myc* expression on mice with T47D breast cancer xenograft. Their findings confirmed that *K. rotunda* rhizome is potential to be developed as breast cancer chemotherapeutic agent. Kabir *et al.* (2011) conducted an investigation on antiproliferative activity of new lectin (KRL) isolated from the tuberous rhizome of *K. rotunda.* Antiproliferative activity against Ehrlich ascites carcinoma cells exhibited 51% and 67% inhibition *in vivo* in mice administered 1.25 mg/kg/day and 2.5 mg/kg/day of lectin respectively by injection. The information obtained from this study is insufficient to use KRL as an anticancer agent, but the lectin might be a good subject in cancer research due to the potent antiproliferative activity against EAC cells. Kour (2014) has documented anticancer plant species around the globe. His findings revealed that chloroform soluble extract of *K. rotunda* exhibited anticancer properties.

#### 2.4.8.2. Antioxidant activity

The abundant presence of flavonoids in this plant is interpreted as a consequence of antioxidant mechanisms in the plant (Mohanty *et al*., 2008; Pratiwi *et al*., 2015; Pai *et al*., 1970; Pietta, 2000; Middleton, 1984; Sirat *et al*., 2001). It is generally believed that antioxidants produced by the plant are transported to the rhizomes where they are accumulated. This implies that rhizomes would have higher antioxidant activity than all other plant parts. However, results of this study described that this might not be true as the majority of the species studied had significantly higher phenolic content and antioxidant activity in leaves than in rhizomes (Chan *et al.,* 2008). Similar observations have been made by Herrmann (1988), who reported greater concentrations of flavonoids in leaves of vegetables which are exposed to sunlight.

Chan *et al*. (2008) screened total phenolic content and ascorbic acid equivalent antioxidant capacity of leaves of 26 ginger species belonging to nine genera and three tribes. Species of *Kaempferia* had very low phenolic content and radical-scavenging activity with values ranging from 112 to 146 mg GAE/100 g and 30–77 mg AA/100 g, respectively. Zingiberaceous plants in the genus of *Kaempferia* and *Boesenbergia* including *K. rotunda* were investigated for the inhibitory effect of nitric oxide production using lipopolysaccharide-stimulated RAW264.7 macrophage like cells (Sudsai *et al*., 2013).

Lotulung *et al*. (2008) identified two compounds namely 2'-hydroxy-4,4',6'-trimethoxy-chalcone and (+)-crotepoxide having antioxidant property. The chloroform soluble extract of *K. rotunda* showed significant scavenging effect on the 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals. Among the two compounds isolated, 2'-hydroxy-4,4',6'-trimethoxy-chalcone was found as active constituent while (+)-crotepoxide was inactive.

Atun and Sundari (2016) conducted a study to analyze the total phenolic content and antioxidant activity of the chloroform extract of *K. rotunda*. The total phenolic content was estimated by Follin Ciocalteu method and using gallic acid as standard phenolic. Antioxidant activity test was carried out by β-carotene bleaching method and DPPH method. The result of this research showed that total phenolic content of chloroform extract was 63.1 mg. Of the two types of determination, test for antioxidant activity showed ascorbic acid had higher antioxidant activity compared to chloroform extracts of *K. rotunda*.

Atun and Arianingrum (2015b) conducted a research to synthesize nanoparticle chitosan of chloroform fraction of *K. rotunda* to characterize this product and to study biological activity as an antioxidant. The antioxidant potential of this plant has been evaluated and the presence of flavonoids and

phenolic derivatives found in the plant demonstrated inhibitory action against lipid peroxidation, suggesting that the plant can be useful in diseases like myocardial infarction, atherosclerosis, diabetes mellitus, rheumatoid arthritis, hepatic injury and cancer (Lotulung *et al*., 2008; Prasad *et al.,* 2010).

### 2.4.8.3. Antimutagenic activity

Atun *et al.* (2013) conducted a study to investigate antimutagenic activity of some flavonones from *K. rotunda*. They have isolated three known flavonoids namely, 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone and 5,7-dihydroxyflavanone. The methanol extract and isolated flavanones from *K. rotunda* showed significant antimutagenic effect compared to control group. These results demonstrated that *K. rotunda* has a preventive effect against chromosome fragmentation probably due to its free radical scavenging activity. Chloroform fraction of *K. rotunda* was loaded on chitosan nanoparticles and then was prepared by ionic gelation of chitosan with sodium tripolyphosphate in various compositions. The biological activity of the products as antioxidant was tested using DPPH method. Results of this study revealed that the nanoparticle can be synthesized at the concentration ratio of 10:1 for chitosan/Na-TPP.

### 2.4.8.4. Antihyperglycemic activity

Sultana *et al*. (2012) conducted a research to study antihyperglycemic and antinociceptive activity with methanolic extract of *K. rotunda.* Lowering of blood sugar level by the extract could be achieved through various individual mechanisms or a combination of mechanisms. It is concluded that the rhizome extract might have potentiated pancreatic secretion of insulin, increased glucose uptake from serum, or decreased glucose absorption from gut (Farjou *et al*., 1987; Nyunai *et al*., 2009; Bhowmik *et al*., 2009). The

extract also shows dose-dependent significant antinociceptive activity when administered to mice compared to control animals.

### 2.4.8.5. Antiviral activity

Aznam *et al*. (2012) conducted a research on isolation, identification and antiviral activity of bioactive compounds of *K. rotunda*. Isolation was done by maceration of the dry powder of *K. rotunda* using methanol and fractionation of the methanol extract using the solvents n-hexane, chloroform, and ethyl acetate respectively. The structure was elucidated using NMR spectroscopy. The isolation of bioactive compounds from the hexane fraction showed the presence of one compound pinostrombin or 6-hydroxyl, 8-methoxy-flavanone. Three compounds were obtained from ethyl acetate fraction of *K. rotunda* namely 4'-hydroxy-8-methoxy-flavanone, 6-hydroxy-8-methoxy-flavanone and 4',8-dihydroxy-flavanone. The activity of different solvent extracts of *K. rotunda* against the AI virus H5N1 showed that the hexane extract of *K. rotunda* showed high antiviral activity while methanol extract had antiviral activity that was not significant.

### 2.4.8.6. Antimicrobial activity

Kumar *et al*. (2015) carried out a research to evaluate the antibacterial activity of different solvent extracts, *i.e.*, n-hexane, methanol, ethyl acetate and water of *K. rotunda* against six bacterial strains, *i.e. Staphylococcus aureus* (MTCC 1144), *Haemophilus influenza* (MTCC 3826), *Pseudomonas aeruginosa* (MTCC 2474), *Streptococcus pneumonia* (MTCC 655), *Streptococcus pyogenes* (MTCC 442) and *Lactobacillus acidophilus* (MTCC 447) and one fungal organism *i.e.*, *Candida albicans* (MTCC 227) by the agar well diffusion method. The results of antibacterial screening showed that the ethyl acetate and water extracts had significant antibacterial activity against *Lactobacillus acidophilus, Streptococcus pyogenes* and *Streptococcus*

*pneumonia,* suggesting that the *K. rotunda* rhizomes could be significant to cure respiratory infections, pneumonia and mouth and skin diseases. All the extracts showed low antifungal activity against *Candida albicans.*

Dubal *et al*. (2009) carried out a research on ethanolic extracts of seven different medicinal plants including *K. rotunda* used in folk medicine in Sikkim and 16 different commercially available antimicrobial agents were investigated for their antimicrobial activity against 25 different strains of *Escherichia coli.* Highest antimicrobial activity was detected in the extract of *Eupatorium cannabium*, *Astilbe rivularis* and *Schima wallichi* in comparison to *Artemisia vulgaris*, *Aloe barbadensis* and *Kaempferia rotunda.* Iyenger (1976) in his bibliographic account has indicated *K. rotunda* which is chemically investigated and has also mentioned its antimicrobial activity. Pratiwi *et al*. (2015) carried out a study on antimicrobial effect of Indonesian medicinal plant extracts on planktonic and biofilm growth of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* Cowan I. A few studies have investigated Indonesian medicinal plants for their antibiofilm activities. This study focused particularly on the idea that Indonesian medicinal plants might be a novel source of candidate antibiofilm compounds to be used in treating biofilm associated infections. Biofilm formation of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* Cowan I is inhibited by ethanol extracts of *K. rotunda* at a concentration as low as 0.12 mg/ml. The results obtained in this study indicate that the extracts of *K. rotunda* have potential for antibiofilm agents in the development of new strategies to treat infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms.

Kabir and Reza (2014) carried out a research to study the antibacterial activity of lectin isolated from *K. rotunda* rhizome (KRL) and its induction of apoptosis in Ehrlich ascites carcinoma cells. Lectin showed agglutination activity against *Escherichia coli* and *Staphylococcus aureus*, with partial

inhibition of their growth. MTT colorimetric assay was used to detect the effect of lectin on Ehrlich ascites carcinoma cells *in vitro* and it was found that lectin inhibited 6.2–50.5% cell growth at the range of 7.5–120 µg/ml protein concentration. The inhibition study demonstrated that bacterial agglutination happened by the interaction of lectin with carbohydrates present on bacterial surface (Ghanekar and Perombelon, 1980). Kabir *et al.* (2011) conducted a study to isolate lectin (KRL) from the rhizome extract of *K. rotunda*. The lectin showed toxicity against brine shrimp nauplii with strong agglutination activity against seven pathogenic bacteria. KRL inhibited the growth of six bacteria and did not show any antifungal activity. KRL agglutinated both gram positive and gram negative bacteria. *Bacillus subtilis* was the most sensitive to the lectin as it was agglutinated at 0.3±0.2 µg/ml of protein concentrations. These results have revealed that KRL recognized the surface molecules on both gram positive and gram negative bacteria. Kumar *et al*. (2015) carried out a phytochemical screening of the different solvent extracts of *K. rotunda* rhizomes which revealed the presence of several bioactive phytochemicals including alkaloids, terpenoids, steroids, flavonoids and saponins having significant antibacterial potential against respiratory tract pathogens. Furthermore, the isolation and identification of these bioactive constituents from the extracts would be highly recommended to get effective antimicrobial agents.

Recently, Shaheen *et al*. (2017) conducted a study to isolate endophytic bacteria from different ethnomedicinal plants including *K. rotunda* of Manipur. A total of 104 endophytic actinomycete isolates were obtained. Eighty one putative endophytic actinomycetes were selected for primary screening by cross streak method against bacterial test organisms (*Micrococcus luteus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*). Thirty seven isolates exhibited antibacterial activity and were further screened for other antimicrobial assays against the same bacterial test

organisms as well as indicator rice fungal pathogens namely *Curvularia oryzae, Rhizoctonia solani, Aspergillus niger, Bipolaris oryzae, Fusarium oxysporum* and *Pyricularia oryzae*. Furthermore, they were subjected to various biochemical and physiological characterization tests and were also screened for plant growth promoting traits. Results of this study revealed that *K. rotunda* was a promising source of plant growth promoting (PGP) traits such as production of IAA, siderophore and ammonia.

### 2.4.8.7. Antiallergic activity

Madaka and Tewtrakul (2011) carried out detailed investigations on the anti allergic activities of selected plants in order to acquire scientific support for their uses by Thai traditional doctors. Researches have shown that ethanol and water extract of *K. rotunda* showed much lower antiallergic activity.

### 2.4.8.8. Antiandrogenic activity

Suphrom *et al.* (2017) investigated *in vitro* antiandrogenic activity and observed chemical profiles of hexane, dichloromethane and ethanolic extracts of *K. rotunda* using GC-MS. They found that the hexane extract was the most potent and showed the $IC_{50}$ value in same range to that of positive control. The GC-MS results showed that most of the volatile components including mono and sesquiterpenes were present in the hexane extract. Since, the hexane extract of *K. rotunda* was the most active extract and showed the highest content of terpenoids (mono and sesquiterpenes) than the other two extracts. These compounds detected in different extracts of *K. rotunda* corresponded to that of the previous reports (Kumar, 2014; Sirat *et al.,* 2005; Woerdenbag *et al.,* 2004).

### 2.4.8.9. Anthelmintic activity

Anthelmintic activity of the alcoholic extract of *K. rotunda* has been demonstrated for the first time by Agrawal *et al.* (2011). They studied three concentrations (25, 50 and 100 mg/ml) of methanolic extract which involved in the determination of paralysis and death time of worm. The alcoholic extract showed significant anthelmintic activity at the highest concentration of 100 mg/ml.

### 2.4.9. Nanotechnology

Nanotechnology is the new technology that has begun to grow in the fields of engineering, medicine, electronics, optics and biomedicine (Stern and Mc Neil, 2007). Nanotechnology can facilitate to manipulate drugs to reach targets with a right dose. Some researchers have used it to treat serious diseases such as tumors, cancer, and HIV (Ranganathan et *al.,* 2012). Nanoparticles can be used as drug carriers in the form of colloidal solid with a diameter of 10-1000 nm and are composed of natural or synthetic polymers that can encapsulate drug molecules (Nagpal *et al.,* 2010). Atun and Arianingrum (2017) made an attempt to identify the nanoparticles produced by the chloroform fraction of *K. rotunda* loaded with alginic acid and combination of alginic acid-chitosan and to study its biological activity. Characterization of the products was investigated in particle size, zeta potential and morphology by scanning electron microscopy (SEM). The biological activity of the products as an antioxidant was tested by the DPPH method. The cytotoxic effect of nanoparticles was analyzed using 3-(4,5 dimethyltiazol-2-yl)-2,5-diphenyltetrazoilium bromide assay. The nanoparticle products of chloroform fraction from *K. rotunda* loaded alginic acid and combination of alginicacid-chitosan were successfully obtained by ionic gelation method. The nanoparticle products showed lower activity in

antioxidant and cytotoxic studies against human breast cancer cell lines than the chloroform fraction of *K. rotunda*.

### 2.4.10. Properties and activities

*K. rotunda* is one of the herbs, which is very useful as immunostimulant and antiinflammatory (Jamir *et al*., 2015). The rhizome is colourless with a characteristic camphoraceous smell and is woody in nature with medicinal properties. It flavours musty with a spicy after taste, and gets solublized well in two volume of 80% alcohol (Joseph, 1998). The tubers contain crotepoxide, which is useful for the inhibition of tumours and b-sitosterol (Husain *et al.*, 1992; Kupchan *et al*., 1969; Pai *et al*., 1970; Demuth *et al.,* 1976; Sood *et al*., 2011; Behra and Rout, 2003). Rastogi and Mehrotra (1991) and Asolkar *et al.* (1992) also reported crotepoxide in tubers. Crotepoxide a highly substituted cyclohexane diepoxide linked with anticancer activity, was isolated in the 1960's from *Croton macrostachys* by Kupchan and his coworkers (Kupchan *et al*., 1968; 1969). Since then, crotepoxide has been isolated from *K. rotunda* and *K. angustifolia* (Pancharoen *et al*., 1989; Pai *et al*., 1970; Prasad *et al*., 2010). Crotepoxide showed significant inhibitory activity against Lewis lung carcinoma in mice and Walker intramuscular carcinosarcoma in rats (Kupchan *et al*., 1969; Marco-contelles *et al*., 2004). Tuber contains essential oil, which gives a compound with melting point 149ºC which yielded benzoic acid on hydrolysis (Rastogi and Mehrotra, 1990). Its tubers are used as ointment to heal wounds, mumps, pneumonia, bronchitis, dysentery, obesity, cold, diarrhea and gastric complaints and to reduce swellings (Yusuf *et al*., 2009; Prasad *et al.,* 2010; Sirirugsa, 1991; Ibrahim, 1999; Perry and Metzger, 1980; Hussain and Hore, 2007; Prajapati *et al.,* 2003).

### 2.4.11. Medicinal properties and uses

Leaves of ginger plants have also been used for food flavouring and in traditional system of medicine. *K. rotunda* and *K. galanga* are eaten fresh or cooked as vegetables and used as cosmetic powder, as food flavouring agents and in elephant medicine (Ibrahim, 1999; Pamungkas and Hakim, 2013; Chan, 2009; Johnson, 1999; Mabberley, 1997; Wohlmuth, 2008; Burkill and Birtwistle, 1966; Prakash, 2008). Interestingly, often due to shortages in supply, *K. pandurata* has been used to replace *K. rotunda* as a main component of popular traditional tonics and medicines, especially for women (Kirana *et al.,* 2003). The dried powder of the rhizome of *K. rotunda* is famous for prevention and treatment of cancer and cholesterol (Silalahi *et al*., 2015). According to Rout and Thatoi (2009) the whole plant is reduced to powder and used in the form of ointment and has wonderful efficacy in healing fresh wounds and if taken internally it removes any coagulated blood. Dried powder of rhizome is easily available and sold in Indonesian traditional medicine markets. This powder is made as drink by adding sugar and hot water. Formulation made of ethanol extracts of the rhizomes of *K. rotunda* in combination with the extracts of *Boesenbergia pandurata*, *Allium tuberosum* and *Phyllanthus niruri* possessed platelet activating factor. This extract is used for atopic dermatitis externally for rough skin prevention and it shows preventive effect in various skin diseases like eczema.

Paste of rhizome mixed with red-soil, warmed in fire and applied on affected parts can cure swelling, sprain, fracture and internal blood clot due to accident (Bhattarai, 2017; Roy *et al*., 2004). Decoction of leaves is used for rheumatic pain and for raising body temperature (Sujarwo *et al*., 2015). Benzyl benzoate is a major component of the essential oil of rhizome of *K. rotunda,* which is used as insecticidal ethnopharmacotherapeutic agent (Woerdenbag *et al*., 2004; Omotuyi *et al*., 2013; Nugroho *et al.,* 1996; Tushar

*et al*., 2010). Jantan *et al.* (2008) evaluated the ability of compound isolated from *K. rotunda* to inhibit arachidonic acid (AA) induced platelet aggregation (Cordier and Steenkamp, 2012). Among the compounds tested, 3-deacetylcrotepoxide from *K. rotunda* showed strong inhibition on platelet aggregation induced by arachidonic acid (AA) with $IC_{50}$ values of less than 84 μM.

People of Manipur from time immemorial use *Yu* for medicine, relaxant and offerings. *Yu* is a distilled product of fermented local rice. About 10-20 g fresh rhizome of *K. rotunda* (*Yai-Thamna-Manbi*) is crushed and mixed with 30-40 ml of first class *Yu* and stored for a while. This preparation is prescribed for women on the first day of menstruation for five days at the dose of 30 to 40 ml twice daily before meals. It is also recommended for post-partum women at the same dose for three months, after five days from the delivery date (Singh and Singh, 2006).

The flower contains the toxin benzyl benzoate, which is used to formulate ointments that act to treat scabies (Sowmya *et al.,* 2014). The tuberous rhizome is used for malignancy and also to prevent blood clotting (Ahmed *et al*., 2017; Prakash, 2008). This plant has been traditionally used to treat abdominal pain, sinusitis, high blood pressure, as sputum laxative, diarrheoa and colic disorder (Atun and Arianingrum, 2015; Devi *et al*., 2015; Devi *et al*., 2014). In some districts of Maharashtra the powdered root is widely used to cure mumps and used in the form of poultice to promote suppuration (Mohanty *et al*., 2008). Imam *et al.* (2013) conducted investigations on aqueous and methanolic extracts of *K. rotunda* leaf by using the incision wounds and excision wounds model study in rat. The results have revealed that the aqueous extract and methanolic extract exhibited significant wound healing activity. These findings provide an insight into the usage of *K. rotunda* leaf in traditional treatment of wounds and burns and to

reduce swelling. Its tubers are acrid, thermogenic, aromatic, stomachic, antiinflammatory, abortifacient, sialagogue, emetic and vulnerary (Ahmed, 2010). They are useful in vitiated conditions of vata and kapha, gastropathy, dropsy, inflammations, wounds, ulcers, blood clots, tumours and cancerous swellings (Warrier *et al*., 2001; Devi *et al*., 2015; Joy *et al*., 2001; Ramashankar and Sharma, 2015; Joseph, 1998; Sharma and Pegu, 2011; Gogoi *et al.,* 2013; Sowmya *et al.,* 2014). It promotes digestion and cures ailments related to stomach (Jamir *et al*., 2015; Babu *et al*., 1997). The powder extracted from the dried tubers of *K. rotunda* is made into an ointment and is used for healing wounds (Pushpakaran and Gopalan, 2013; Jagtap, 2015; Shrirame and Gogle, 2014). Panthi and Singh (2013) reported that paste of rhizome is applied in boils of skin to expel the pus and foreign particles.

This plant is considered as an important medicinal plant in ancient Indian system of traditional medicine, namely, Ayurveda. The drug "*hallakam*" prepared from this is in popular use in the form of powder or as an ointment application to wounds and bruises to reduce swellings (Joshi *et al.,* 2011; Jagtap, 2015; Kumar *et al*., 2013). According to Sivarajan and Balachandran (1994) *hallakam* is equated with *K. rotunda* and they reported that in practice *Lagenandra toxicaria* of the family Araceae is also used in Kerala. Controversies do exist in the case of *hallakam* among physicians. Sereena *et al*. (2011) worked out characterization of the reputed ayurvedic drug *hallakam* from its substitute or adulterants. The distinguishing characters evolved from the study help to detect the genuine material and substitute of *Hallakam* and it is concluded that *Lagenandra toxicaria* is a clear case of adulterant. It also improves complexion and cures burning sensation, mental disorders and insomnia (Chopra *et al.,* 1956; Nadkarni, 1954; Sereena *et al.,* 2011; Sivarajan and Balachandran, 1994; Sowmya *et al.,* 2014; Atun, 2014). In Ayurveda, rhizomes are used for curing nausea,

stomach ache and dyspepsia (Sereena *et al*., 2011; Sudershna and Sood, 2008). The decoction is applied with much benefit to wounds with coagulated blood and with any purulent matter (Sivarajan and Balachandran, 1994; Nadkarni, 1998; Joy *et al*., 1999; Singh and Panda, 2005). Root bulb crushed with bark of *Gynocardia odorata* is used for sore throat problems (Lalnundunga, 2000). Juice of its rhizome is used as eye drops for removing cataract and also as a cure for night blindness (Rao, 1981; Ahmed, 2010). Extract from tubers is used for fractures and dislocation of bone (Biswas and Chopra, 1982). Its flower and rhizome can be used as a hepatoprotective agent (Das and Choudhury, 2012). Kumar and Prabhakar (1989) reported that *K. rotunda* formed an ingredient in cardioactive formulations in Ayurveda. Mitra (2014) investigated the role of 40 medicinal plants of North Eastern Himalayas of Indian subcontinent. Results have shown that nine medicinal plants including *K. rotunda* had significant anti gastric ulcer activity under the experimental condition. According to Singh and Khan (1990) 100 g of roots of *K. rotunda* is ground and mixed with honey and animal fat. This preparation is given twice in a day to cure ulcer. Same recipe is given in the dose of 5 g thrice daily for five days to cure typhoid.

### 2.4.12. Formulations using *Kaempferia rotunda*

The tuber is used in about 21 preparations which include *Asokarishta, Anuthaila, Amrithadi taila, Chandanadi taila, Thungadrumadi taila, Thriphaladi taila, Prapundareekadi taila, Manjishtadi taila, Gopanganadi kashaya, Mustharishtadi kashaya, Sathavaryadi kashaya, Kalyanaka ghritham, Chandanadi nethratharpana sarpis, Chargeriyadi ghritha, Mahakalyanaka ghritha, Mahatriphala ghritha, Baladhatryadi taila, Pancha valkadi taila, Vasthyamayanthaka ghritha, Sathavaryadi ghritha, Chyavanaprasa,* etc. (Warrier *et al*., 2001; Joy *et al*., 1999; Joy *et al*., 2001; Jose *et al.,* 2001; Kumar *et al*., 2013; Sivarajan and Balachandran, 1994).

*Asokarishta* contains 4 *tola* (1 *tola*=12 g) rhizome of *K. rotunda* (Sekar and Mariappan, 2008). It is also a constituent of Indonesian traditional medicine (Jamu) which has also been shown to give antinociceptive activity in gastric pain model mice (Sultana *et al.,* 2012; Nahar *et al.,* 2013).

### 2.4.13. Ethnobotany

. The term ethnobotany deals with the study of plants used by primitive and aboriginal people (Anonymous, 1895; Harshberger, 1896). Ethnomedicinal knowledge on plant species has been diminishing continouously because of changing perception of the local people, increasing influence of commercialization and socioeconomic transformation (Gadgil *et al*., 1993; Kunwar and Duwadee, 2003). Family Zingiberaceae involves various medicinal plants and is well known for its use in ethnomedicine. Tushar *et al.* (2010) studied ethnomedical uses of Zingiberaceous plants of northeast India. They documented that rhizomes of *K. rotunda* are used in local medicine by grinding fresh rhizome and making a paste with water. This paste is mixed with other herbs and applied to sprains and covered with bandage to cure sprain ache. Its leaves are used as body lotion (Tushar *et al*., 2010; Sirirugsa, 1999).

Pamungkas and Hakim (2013) conducted an investigation to find out the diversity of species of plants that made up home garden species, to measure the use value of each species using ethnobotany indices. Data were calculated using synthetic indices named Relative Frequency of Citation (RFC) and Cultural Importance (CI) indices. Relative frequency of citation is used to find out probability between number of people who give citation to each species and number of all informants. It describes local importance of each species. Cultural Importance Index (CI) is used to compare the plant knowledge among different cultures and this can also be used to know diversity information within each species if collaborated with diversity

indices (Tardio *et al*., 2008; Signorini *et al*., 2009). RFC and CI indices calculated for *K. rotunda* are 0.3043478 and 0.4347826 respectively.

Tubers of *K. rotunda* are made into paste and applied externally to cure chest pain (Shil *et al*., 2014). Sheikh *et al*. (2015) conducted an extensive survey on anti-diabetic potential of selected ethnomedicinal plants of northeast India. They observed that the boiled extract of rhizome is useful for the treatment of diabetic patients. Sherpa *et al*. (2015) documented medicinal plants of major Sikkim's Himalayan regions. This study revealed that tubers of *K. rotunda* are used to cure swelling, fracture, bruises and insect bites (Chauhan, 2001; Sherpa *et al.,* 2015; Ahmed, 2010). Village people of Tripura use the flower decoction to bath patients with skin infections. Aqueous decoction of rhizome is used in the treatment of jaundice (De, 2016). Rhizomes and roots are applied for proper hair growth and as an ingredient of an indigenous hair lotion of Manipur. Rhizome is locally applied for the treatment of tumour. Rhizomes are taken internally for removing coagulated blood from body and also used in the treatment of abdominal pain and gastric troubles (Phucho and Singh, 2017). Limbu and Rai (2013) explored ethnomedicinal practices among the Limbu community in Eastern Nepal. They use rhizome of *K. rotunda* for treatment of fracture.

Das *et al.* (2012) explored traditional knowledge of ethnomedicinal hepatoprotective plants used by certain ethnic communities of Tripura state. Chakma community of Tripura state uses aqueous decoction of rhizome, half cup per day taken for one week. In Indonesia, rhizome is used as traditional insect repellant (Chan *et al*., 2009). Bantawa and Rai (2009) conducted an ethnobotanical study among the traditional health practitioners *Jhankri*, *Bijuwa* and *Phedangma* in Darjeeling Himalaya. People of Darjeeling Himalaya use paste of rhizome of *K. rotunda* along with the roots of *Laportea terminalis* (Urticaceae) and aerial portion of *Viscum album* (Loranthaceae) to

apply externally on bone fracture and joint dislocation. Affected portion is bandaged for 3-5 weeks depending upon the seriousness of the damage. Sikdar *et al*. (2015) conducted a survey to explore common poisonous plants of Birbhum, Burdwan and Nadia districts of West Bengal. They reported that juice of the tubers of *K. rotunda* cause nausea and vomiting. Tripathi and Goel (2001) reported that Garo tribs of Meghalaya use the rhizome of *K. rotunda* to cure eye diseases (Rao, 2013). This plant is used in the folk medicine system of Bangladesh for the treatment of high blood sugar level commonly observed in patients with diabetes as well as for the treatment of pain (Atun, 2014; Sultana *et al.,* 2012). Ramashankar and Sharma (2015) documented *K. rotunda* as a priority species used for traditional healing practices in northeast India.

Rai and Bhujel (2013) conducted an investigation to document the use of plants in the treatment of livestock by the ethnic people of Darjeeling Himalaya. They documented that freshly collected stem of *Selinum wallichianum* is crushed along with *K. rotunda, Gonostegia hirta* (Blume *ex* Hasskarl) Miquel. and phylloclade of *Viscum liquidambaricolum* Hayata and the prepared paste is plastered on fractured bone in cattle and kept under bandage for 3 to5 days. Treatment is continued for 21 to 27 days till recovery. The whole plant of *Dendrophthoe falcata* is crushed with the rhizome of *Kaempferia rotunda* and root of *Laportea bulbifera* and the prepared paste is plastered externally on fractured bone in cattle and kept under bandage for 5 to 7 days. This treatment is followed for 21 to 27 days by changing the paste as and when required. It will stimulate the articulation of bones.

Sharma and Pegu (2011) explored the plants related to magico religious believes in Dobur Uie (ritual) of Mising (largest tribal community of Assam). The Mising people have themselves categorized *K. rotunda* as very

rare (Medhi and Borthakur, 2011). The Mising of Lakhimpur district believes that growing *K. rotunda* brings peace to the family. Also the tubers of the plant are used in Apong preparation (Gogoi *et al.,* 2013). Apong is a kind of rice beer used as offering drink during rituals of Mising community. According to Rout and Thatoi (2009) bulb paste of *K. rotunda* is locally applied to take out pus from boils and nuts formed in parts of spine.

Basnett *et al.* (2015) documented ethnomedicinal practices using *K. rotunda* for the treatment of sprain and bone fracture by the Nepalese community of eastern Sikkim. Rhizome of *K. rotunda* with entire plant of *Viscum articulatum* of the family Viscaceae and *Bergenia ciliata* of the family Saxifragaceae are made into a paste and the affected area is bandaged with it. Duration of this treatment is recommended as 7 to 8 days or one month. The root of *Urtica dioica* with *K. rotunda* is ground into a paste and affected area is bandaged with it for 10 to 15 days. The juice of the rhizome of *K. rotunda* and the shoot tip of *Azadirachta indica* are applied externally in the affected area and bandaged over it for one week. The root of *Abroma augusta* of Malvaceae, rhizome of *Kaempferia rotunda* and root of *Euphorbia hirta* of Euphorbiaceae are ground into paste mixed with red mung. The affected area is bandaged with the paste till it loosens. This treatment is kept under bandage for 30 days. Entire plant of *Bergenia ciliata*, *Viscum articulatum* and *Euphorbia hirta*, fruit of *Kaempferia rotunda*, root of *Astilbe rivularis* (Saxifragaceae), bark of *Terminalia chebula* and *Terminalia balerica* (Combretaceae) are harvested on Tuesday, Thursday or Saturday, ground individually and the juice is extracted. A type of stone known as "dalsay dhunga" is put into the mixture which is boiled for ten minutes. It is allowed to cool and bandaged on the fractured area for two months. A paste of the rhizome is prepared along with the roots of *Laportea terminalis* (Urticaceae) and aerial portion of *Viscum album* and applied externally and bandaged on bone fracture and joint dislocation for 3 to 5 weeks depending

upon the seriousness of the damage of the bone. *Kaempferia rotunda*, *Euphorbia hirta* and *Urtica dioica* are ground into paste and bandaged the affected portion for one month. Whole plant of *Euphorbia hirta*, rhizome of *Kaempferia rotunda* and bark of *Engelhardtia spicata* are ground properly and the juice is extracted. The mixture is boiled with water and made it into a paste. This paste is used as a bandage on fractured portion for 22 to 25 days. Whole plant of *Bergenia ciliata, Viscum articulatum* and *Euphorbia hirta*, fruit of *Kaempferia rotunda* and root of *Astilbe rivularis* are harvested on Tuesday, Thursday or Saturday, ground individually and the juice of all plants is extracted well. A type of stone known as "dalsay dhunga" is put into the mixture, which is boiled for ten minutes. It is allowed to cool and then bandaged on the fracture for two months.

Das and Choudhari (2012) who conducted a field study in some tribal villages of Tripura reported that flower decoction made of *K. rotunda* is used to bathe patients with skin infection. Aqueous decoction made of rhizome is taken half cup per day for one week to cure jaundice. Sharief *et al.* (2005) recorded medicinal utilities of *K. rotunda* used by Karens tribe of middle Andaman. Paste of rhizome of *Acorus calamus* along with *Piper betle* leaves, rhizome of *Kaempferia rotunda*, *Peperomia pellucida* plant and cloves by adding sugar or salt is applied on the forehead. Sometimes, all these plants are boiled and the vapour is inhaled. This treatment is used to cure headache and cold. Fresh rhizome paste of *K. rotunda* and a pinch of salt diluted with water is taken to treat gastric complaints. This extract is drifted into ears with hen's feather to heal ear pain. A formulation made with leaves of *Millingtonia hortensis*, fresh leaves of *Kaempferia rotunda*, bark of C*itrus medica*, paste of dugong bone (Paani soovar), whale bone and self holding stone, the mixture is diluted with water and this medicine is applied on the head with the help of needle prepared with bat's bone. This treatment is recommended for bodyache and fever. Borpujari and Dutta (2015)

documented plants used as medicine by Singphoos (Tribes of Tinsukia District of Assam) in different ailments and their mode of use. They reported that root tubers of *K. rotunda* are ground after boiling and eaten with rice to cure stomach ailments.

Jamir *et al*. (2012) made an attempt to collect information regarding traditional knowledge on herbal medicines used by the Naga tribes. They documented that rhizome is crushed and ground to a paste and applied over skin burns for relief. Haridas *et al.* (2015) reported that *K. rotunda* was utilized by the tribe Kattunayakans of Nilambur forests of Kerala for relieving stomach pain. Juice of rhizome is administered orally for curing stomach pain. Rout and Thatoi (2009) documented ethnomedicinal uses of plants in Similipal biosphere reserve of Orissa. They found that the paste of *K. rotunda* rhizome along with the root of *Swertia angustifolia* and honey is given orally twice a day to cure ulcer. Biswakarma *et al.* (2015) documented the traditional application of *K. rotunda* in Naxalbari area of West Bengal. They reported that it's rhizome beaten and mixed with turmeric is tied on place where bone is fractured.

### 2.4.14. Economic importance

Utami *et al.* (2014) investigated the effect of enrichment of *K. rotunda* essential oils on cassava starch based edible coating to patin's quality during storage. They determined the ability of *K. rotunda* essential oil to extend shelf life of fish during storage. Fish quality was determined based on microbiological and chemical properties. The results have shown that essential oil enrichment on edible films were able to maintain the patin fillet's quality. At present, consumers' demand has increased for high quality food with an extended shelf life without chemicals. Biopolymer materials generally have good oxygen barrier under dry conditions. Films or coatings generally made of proteins, lipids and polysaccharides are used to extend the

shelf life of seafood and conserve the quality of fish muscle by preventing moisture loss and gaseous exchange (Stuchell and Krochta, 1995; Gennadios *et al*., 1997; Jeon *et al*., 2002; Sathivel, 2005; Artharn *et al*., 2009; Fan *et al*., 2009; Song *et al*., 2010). Vast range of findings has been demonstrated for increasing seafood shelf life using films enriched by natural extracts. The experimental results indicated the effect of *K. rotunda* essential oil on increasing shelf life of seafood products (Woerdenbag *et al*., 2004; Noorihashemabad *et al*., 2015). Therefore, *K. rotunda* essential oils enriched on cassava starch based edible coating could extend fish shelf life and use as alternative fish preservation.

Sukari *et al.* (2010) investigated larvicidal activity of plant extracts from the rhizome of *K. rotunda* against the larvae of dengue fever mosquito *Aedes aegypti*. The crude chloroform and methanol extracts of *K. rotunda* were moderately toxic with $LC_{50}$ values between 60 and 90 µg/ml while petroleum ether extracts of *K. rotunda* were lesser toxic with $LC_{50}$ values more than 100 µg/ml. Yeoh *et al*. (2015) reported that camphene is obtained from *K. rotunda*, which is used to make ointments to treat scabies and itches. It can be used to synthesize camphor and insecticide. A formulation made of *K. rotunda* extracts promoting melanin formation and with tyrosinase inhibitors for skin lightening cosmetics was reported to be safe and effective (Lotulung *et al.,* 2008).

Fitri *et al*. (2017) assessed the diversity and capability of endophytic actinobacteria from five medicinal plants in producing pancreatic lipase inhibitor, which inhibits fat absorption that were traditionally used in treatments for weight loss. Endophytic microbes including actinobacteria have the capability to produce similar bioactive compounds, which have various biological functions including enzyme inhibitor with the host plant (Pujiyanto and Ferniah, 2010). A total of 35 endophytic actinobacteria have

been isolated from the five medicinal plants studied. Out of these five medicinal plants studied, the number of endophytic actinobacteria was dominant in the rhizome of *K. rotunda* (12 isolates). Among these AEKp9 isolate from *K. rotunda* showed an inhibition value of 92.4% and the highest pancreatic lipase inhibitory activity of 65.1% (Iswantini *et al.*, 2010; 2011; Pradono *et al*., 2011). Its rhizomes are used in cosmetics and as a dye and flowers are used in perfumery (Sood *et al.,* 2011; Thatoi and Rout, 2011). It also forms an important constituent of Indonesian traditional medicine (Jamu) for pain relief (Nahar *et al*., 2013). Medhi and Borthakur (2011) reported that rhizome paste of *K. rotunda* yielded black coloured traditional dye that was used to dye cotton fibre.

### 2.4.15. Phylogeny

The most exhaustive work to date on the phylogeny of the family Zingiberaceae is the one by Kress *et al*. (2002). They proposed a new classification based on the evidences from molecular data. They used sequence data from *matK* and ITS for which they carried out maximum parsimony analysis. Kress *et al.* (2002) identified two clades in Zingiberae based on sequence data of *matK* gene. They were *Curcuma* clade and *Kaempferia* clade. *Curcuma* clade was a moderately supported clade while the *Kaempferia* clade, a weakly supported clade which included *Kaempferia*, *Boesenbergia*, *Distichochlamys*, *Scaphochlamys*, *Haniffia*, *Cornukaempferia* and *Zingiber*.

Ngamriabsakul *et al*. (2004) have done a phylogenetic analysis of the tribe *Zingibereae* using nuclear ribosomal DNA (ITS1, 5.8S and ITS2) and chloroplast DNA (*trn*L (UAA). The tribe *Zingibereae* is monophyletic with two major clades, the *Curcuma* clade and the *Hedychium* clade. Ranges of the sequence at primer sites‘d’ and ‘e’ of *K. rotunda* lacked 32 base pairs. *Kaempferia* species are grouped as a clade with weak support. *Hedychium* is

next separated as sister to the genera of the *Boesenbergia* group which includes *Boesenbergia*, *Caulokaempferia*, *Cornukaempferia*, *Distichochlamys, Kaempferia*, *Scaphochlamys* and *Zingiber*.

The relationships among 11 species of *Boesenbergia*, six species of *Kaempferia*, and two species of *Scaphochlamys* from southern Thailand were analysed using random amplified polymorphic DNA (RAPD) profiles from leaf tissue samples. The dendrogram obtained from cluster analysis, UPGMA and a principal component analysis of the RAPD result validates a higher degree of relationship between *Boesenbergia* and *Scaphochlamys* than between *Boesenbergia* and *Kaempferia.* Data obtained from isozyme electrophoresis of leaf extracts of these plants were also investigated. The dendrogram resulting from cluster and UPGMA analysis revealed a higher degree of relationship between *Boesenbergia* and *Scaphochlamys* than between *Boesenbergia* and *Kaempferia*. These findings were also supported by principal component analysis (Vanijajiva *et al.,* 2003; 2005).

Root anatomy is an important aspect of taxonomy to elucidate plant diversity, phylogeny and evolution (Endress *et al*., 2000). Uma and Muthukumar (2014) have explored root anatomical characters for the first time in the phylogenetic analysis of the family Zingiberaceae. The anatomical characters of roots were extensively studied and analysed laying emphasis on 21 qualitative and 16 quantitative characters. The UPGMA phenogram of the qualitative and quantitative data provided ten principal clusters. The fifth cluster consisted of *Curcuma amada, Zingiber purpureum* and *Zingiber officinale* and *Kaempferia rotunda* was found to have similar morphological characters with a definite distance.

Siriluck *et al*. (2014) conducted a research to find out the genetic relationship of five genera of Zingiberaceae namely *Zingiber*, *Alpinia*, *Bosenbergia*, *Curcuma* and *Kaempferia.* Hierarchical cluster analysis and

inter-simple sequence repeat technique were used to ascertain the genetic relationship among five genera. Banding profiles were subsequently done using ISSR primers. Results obtained from dendrogram analysis indicated that the genera *Alpinia, Bosenbergia, Curcuma*, and *Kaempferia* were clearly observed with the genetic relationship of 81%, 94%, 89%, and 78% respectively. Theanphong *et al.* (2013) investigated phylogenetic relationships of selected *Kaempferia* plants in Thailand based on random amplified polymorphic DNA markers. They screened a total of 40 random primers in which six primers have produced clear and reproducible polymorphic bands. A total of 93 scorable bands ranging from 159 to 2464 base pairs in size were amplified, among which 47 products were found to be polymorphic. According to the UPGMA dendrogram analysis, five *Kaempferia* species could be divided into two clusters. First cluster included two species of *K. marginata* and *K. galanga* and second cluster included three species of *K. parviflora, K. rotunda* and *K. larsenii*. The phylogenetic relationships were associated with the morphological characterization. *K. parviflora, K. rotunda* and *K. larsenii* which clustered into second group have ellictic or oblong leaves, linear leaf blade and pedunculated inflorescence with white or light purple flowers (Picheansoonthon and Koonterm, 2008; Sirirugsa, 1991). The polymorphic banding pattern which is derived from RAPD marker can be developed as RAPD derived sequence characterized amplified regions (SCAR) marker development for rapid detection of *Kaempferia* species.

Sihanat *et al*. (2015) reported phylogenetic relationships of *Kaempferia* species based on AFLP markers. They were screened with thirty primer combinations, of which four primer combinations produced a total of 253 distinct and reproducible bands ranging from 56 to 70 bands with an average of 63.25 bands per primer combination. Based on AFLP bands amplified using four primer combinations, genetic distance was calculated and found

out the similarity index (SI) ranged from 0.02774 to 0.93750. The results have described that the cluster could be divided into two main groups and the phylogenetic relationships were associated with the morphological characters. This is the first report which demonstrates that AFLP fingerprint is a useful technique for the identification of *Kaempferia* species.

### 2.4.16. Taxonomy

The genus *Kaempferia* L. of the family Zingiberaceae forms one of the important medicinal plant groups. Many taxa like *Kaempferia angustifolia* Rosc., *Kaempferia galanga* L., *Kaempferia marginata* Carey, *Kaempferia parviflora* Wall. ex Baker, *Kaempferia rotunda* L., etc are known for their medicinal properties. Taxonomic studies on this plant group are essential to set up the baseline information especially in researches related to drug development (Picheansoonthon and Koonterm, 2009a; 2008b).

Recently, eight taxa were included in a checklist of the vascular plants of Lao PDR (Newman *et al*., 2007) namely *K. angustifolia*, *K. elegans*, *K. fallax*, *K. fissa*, *K. galanga*, *K. harmandiana*, *K. laotica* and *K. rotunda.* Chayan and Chayan (2009) reported another beautiful species *Kaempferia sawanensis* Picheans and Koonterm, sp. nov. found in the mountain in Sawanakhet Province of Southern Laos, near the Vietnamese border. The species found in Lao PDR can be divided into two main groups, the *Kaempferia rotunda* group and *Kaempferia galanga* group. In the *Kaempferia rotunda* group, inflorescence appears before the leaves while in the *Kaempferia galanga* group inflorescence occurs after the leaves. This new species belong to *Kaempferia galanga* group. *K. sawanensis* can be clearly distinguished by its hairiness of most parts, especially on both sides of the leaves. Picheansoonthon and Koonterm (2008a) reported a new species, *Kaempferia champasakensis* from Southern Laos. This new species can be easily distinguished by its pure white flowers with the labellum divided two-

third to the base and the large white ovate-elliptic to suborbicular anther crest with greatly varied apex.

Picheansoonthon and Koonterm (2009a) reported a new species *Kaempferia sisaketensis* from northeastern Thailand. This new species can be easily identified by its two to four elliptic and glabrous leaves. Flowers are pink to violet with deeply bilobed labellum and without the staminodes. Picheansoonthon and Koonterm (2009b) explored and described two new *Kaempferia* species from Southern Laos namely *Kaempferia gigantiphylla* and *Kaempferia attapeuensis*. *K. gigantiphylla* can be easily recognized by its single large prominent veined leaves, hairy on both surfaces and the labellum divided to the middle. *K. attapeuensis* can be easily recognized by its sessile leaves, oblong-elliptic to broadly ovate, horizontal near the ground or upright. Anther crest of *K. attapeuensis* is also varied greatly and it ranged from rectangular shape with the apex varying from bilobed to bifid.

Phokham *et al*. (2013) reported three new species namely *Kaempferia udonensis* Picheans. and Phokham and *Kaempferia picheansoonthonii* Wongsuwan and Phokham from Thailand and *Kaempferia xiengkhouangensis* Picheans. and Phokham from Laos. A new species *Kaempferia lopburiensis* from central Thailand was described by Picheansoonthon (2010). This new species can be easily recognized by its 2–3 large orbicular leaves, red broadly triangular and hairy ligules with rounded or obtuse apices, and broadly obovate or rectangular anther crests with tridentate to crenate apices.

### 2.4.17. Biotechnology

Micropropagation has superiority over conventional method of propagation because of its higher multiplication rate. Field performance of these tissue-cultured plants depends on the selection of the initial material, media composition, growth regulators and environmental factors. Now a

days, some well developed *in vitro* techniques are available to help farmers to meet the demand of the spices and pharmaceutical industry. Babu *et al*. (1997) established a study to develop a protocol for micropropagation of *K. rotunda.* Some researches have shown that vegetative buds and rhizome with axillary buds are best suitable for multiplication and vegetative buds for *in vitro* conservation (Gantait *et al*., 2011). The basal medium used is MS (Murashige and Skoog, 1962) for *K. rotunda*. Rhizome and vegetative bud explants produced multiple shoots with root in about 90 days of culture on MS + 1 $mgl^{-1}$ BA + 0.5 $mgl^{-1}$ NAA. The cultures could be maintained upto one year without sub-culture in 1/2 MS + 10 $mgl^{-1}$ sucrose + 10 $mgl^{-1}$ mannitol in screw capped culture tubes. When the plantlets were transplanted into a mixture of garden soil, sand and vermiculite (1:1:1) while kept in humid chamber for about 20 days and could be established in soil with 90% of success. Tissue cultured plants require three crop seasons to develop normal sized rhizomes. This protocol is ideal for the production and multiplication of disease free plants and somaclones for exploiting somaclonal variation. The slow growth method is recommended for *in vitro* conservation of the species (Babu *et al*., 1993; 1996; 2005; 2012; Ravindran *et al*., 1996; Geetha *et al*., 1995). The use of tissue culture for the biosynthesis of secondary metabolites in plants of pharmaceutical importance holds an alternative to control production of plant constituents. *In vitro* propagation of *K. rotunda* has been attempted by Anand *et al*. (1997).

Soonthornkalump *et al*. (2016) observed morphological and stomatal guard cell characteristics of *K. rotunda* through colchicine induced polyploidy. It demonstrated that colchicine treatment had the potential to produce dwarf line and induce chlorophyll mutation in *K. rotunda.* Tetraploid plants were produced by *in vitro* culturing of young shoots of *K. rotunda* on gelrite modified MS medium supplemented with 0.2 % colchicine for four days in order to improve some characteristics of *K. rotunda*. After the

incubation, all the explants were transferred to culture and subcultured until M1V4 generation for chimera segregation. Ploidy determinations were done by flow cytometry analysis. Stomatal guard cell size, leaf thickness and chloroplast numbers in stomata of polyploid plants were significantly increased as compared to diploid plants. Even though, changes in the anatomical characteristics of stomata were found, the stomatal index of the ploidy levels of *K. rotunda* was not changed. Two solid lines of tetraploid plants, normal green and variegated dwarf, were produced during the experiment. In contrast, chloroplast numbers in stomata and leaf thickness seemed to be a good indicator to distinguish diploidy and polyploidy of *K. rotunda*.

Chirangini *et al*. (2005) regenerated microshoots of rhizomatous buds of *K. galanga* and *K. rotunda* when cultured on MS medium supplemented with plant growth regulators. Multiple shoots were induced on MS medium containing 5.70 µM IAA alone and a combination of 0.57 µM IAA plus 4.65 µM kinetin in the case of *K. galanga*. Muralidharan (1997) worked out micropropagation of *K. rotunda.* The media containing a wide range of the cytokinins, benzylaminopurine (BAP) and kinetin (Kin) were used for regeneration of plantlets. The mineral salts and vitamins used in the basal medium were according to Murashige and Skoog (1962). Sucrose was added at 2 % (w/v) as the carbon source in all the media. Liquid medium was found to be better than solid medium for multiplication and plantlet formation. Natural light was found to be sufficient for illumination of cultures. The plantlets were transferred to soil and more than 80% survival was recorded. The micropropagated plants also showed morphological variations. The most striking feature of micropropagated plants of *K. rotunda* was that the leaves were narrower and did not have the variegation found in normal leaves. In micropropagated plantlets of *K. rotunda* the length to breadth ratio reduced although the length of the leaves had increased. In micropropagated plants of

*K. rotunda* the rhizome production is evident even in plants at the time of transfer to soil after hardening and after the first season's growth, a thick rhizome was produced.

Mustafaanand (2014) developed an efficient protocol for *in vitro* plant regeneration through somatic embryogenesis. Embryogenic callus was induced on MS solid medium supplemented with 2.5 mg/L 2,4-D and 0.5 mg/L BAP. Further advancement of embryonic callus in to embryos occurred on MS medium containing 0.25 mg/L 2, 4-D and 3.0 mg/L BAP. Further plant regeneration was detected on MS medium supplemented with 5.0 mg/L BAP. They successfully encapsulated globoid or torpedo shaped somatic embryos derived from the callus culture. Plantlets developed through embryogenesis successfully got transferred to the field and they showed 50% of establishment in the soil. Geetha *et al.* (1997) developed a protocol for micropropagation of *K. rotunda*. Young sprouting buds of this species could be established in Murashige and Skoog medium supplemented with 0.5 $mgl^{-1}$ kinetin and 1.5 % sucrose solidified with 0.7 % agar. The buds produced multiple shoots and well developed roots in Murashige and Skoog medium supplemented with 0.5 $mgl^{-1}$ α- naphthalene acetic acid and 1.0 $mgl^{-1}$6-benzyl amino purine. A multiplication ratio of 1:6 with an average of five roots per shoot was obtained in the study. It is also evident from the study that the medium supplemented with 2.69 µM NAA plus 2.22 µM benzyladenine was the best for *K. rotunda.*

### 2.4.18. Genetic variability studies in *Kaempferia* species

Induction of genetic variability in *K. galanga* was attempted by Kanakamany (1997). Rhizomes of *K. galanga* cv.Vellanikkara local were treated with eight doses of gamma rays and six concentrations of EMS and until MV3 generations were evaluated. LD50 value of gamma rays was 20 Gy and that of EMS 1.5 per cent were recorded. The highest values for yield

and yield attributing characters were obtained for 7.5 Gy gamma rays and 0.75 per cent EMS. Gamma rays at 15.0 Gy and EMS at one per cent were most effective in inducing variability for rhizome yield and yield attributes. High estimates of broad sense heritability coupled with high genetic advance was observed for number of leaves and rhizome number. This indicates direct selection for improvement of these traits will be effective in plant breeding. Correlation coefficient between yield and yield contributing characters indicated significant positive correlation of yield with number of leaves, tillers, leaf length, plant spread and rhizome number in the untreated control. Mutagenic treatments induced alterations in the association between rhizome yield and components. Path coefficient analysis of important yield characters showed that alterations in plant characteristics for higher yield is possible with 7.5 Gy gamma rays. Changes in plant characters so as to improve the yield is rather difficult with EMS. High frequency of positive variants at lower doses and high frequency of negative variants at higher doses were also observed.

Techaprasan *et al*. (2010) studied genetic variation and species authentication of different *Kaempferia* species grown in Thailand. The selected species were examined by determining chloroplast *psbA-trnH* and partial *petA-psbJ* spacer sequences. After sequence alignments, 1010 and 865 bp in length was obtained for the respective chloroplast DNA sequences. Intraspecific sequence polymorphisms were observed in various populations of *Kaempferia filifolia*, *Kaempferia fallax*, *Kaempferia pulchra*, *Kaempferia elegans*, *Kaempferia rotunda*, *Kaempferia marginata*, *Kaempferia parviflora*, *Kaempferia larsenii*, *Kaempferia roscoeana*, *Kaempferia siamensis* and *Gagnepainia godefroyi.*

Jayasree (2009) analysed the genetic variability, character association and genetic divergence in *Curcuma amada* and *Kaempferia galanga* to

identify the superior genotypes from them. In the case of 50 accessions of *K. galanga*, all the agronomic characters showed continuous distribution showing polygenic genetic control. Among the yield characters, the highest coefficient of variation (CV) was shown by yield per plant followed by number of secondary fingers. In the case of all the agronomic characters analysed, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV). Among the growth characters, the highest PCV and GCV were shown by number of leaves. Among yield characters, the highest PCV was shown by yield per plant followed by number of secondary fingers and the lowest PCV was shown by diameter of mother rhizome. Genotypic coefficient of variation (GCV) also showed the same trend of variation in the case of the yield characters. The highest heritability was shown by yield per plant followed by leaf area and plant height. The highest genetic advance was shown by yield per plant followed by number of secondary fingers and number of leaves. The result of cluster analysis attempted in *K. galanga* showed that 50 accessions were grouped into 8 clusters based on the characters studied.

The above review highlights the genetical and allied studies already carried out in the genus *Kaempferia* in general and the species *K. rotunda* in particular. The review has brought out the fact that efforts to study the genetic diversity and to initiate breeding programmes in *K. rotunda* in Kerala state are scanty. Being a plant species which is very marginally cultivated in spite of its medicinal and commercial importance, the present research programme has been designed and executed so as to collect, conserve and evaluate the genetic diversity of the same in the study area and also to identify superior genotypes from the germplasm.

# Chapter III
# MATERIALS AND METHODS

*Kaempferia rotunda* L. is a valued medicinal plant belonging to the family Zingiberaceae. Scientific researches to study its genetic variability, diversity and crop improvement programmes reported are very scanty. Experimental programmes were designed and carried out from 2016-2019 to evaluate its genetic diversity and variability and to identify elite genotypes from the germplasm collected and maintained for the purpose. The experiments were carried out with an intention to study the genetic control and variation of agro-morphometric characters, genetic variability, character association and genetic divergence with reference to the germplasm collected for the purpose from the different locations of Kerala, India. An effort has also been made to select superior genotypes from the different accessions of *K. rotunda* collected based on their overall performance. Experiments have also been designed to study the growth performance of plants raised from rhizomes of different status such as mother rhizome, primary finger and secondary finger as the planting material.

## 3.1. The experimental field

The experiments were conducted in the experimental plot of the Genetics and Plant Breeding Division of the Department of Botany, University of Calicut, Kerala, India. The experiments were laid out in randomized block design (RBD) with three replications in open field condition. The experimental plot is located at 75°46' E longitude and11°15' N latitude at an elevation of 50 m from MSL. Average temperature of the study area ranges from 17.83°C to 36.83°C with an annual rainfall of 247 cm. The area has got tropical monsoon climate with southwest monsoon rains

from June to August, northeast monsoon rains in October and November and dry spell from December to May with summer showers in March, April and May (Tables 3.1; 3.2; 3.3; 3.4). The agricultural operations in the case of rainfed annual crops start in May and usually come to a close by November-December (Anonymous, 2018).

Table 3.1. Weather data of the experimental plot for 2016

| Months | Rainfall (mm) | Temperature (°C) | | Relative humidity (%) | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| January | 6.7 | 24 | 35 | 45.5 | 76.66 |
| February | 5.9 | 24.83 | 35.17 | 52.16 | 81.16 |
| March | 3.8 | 26.17 | 36.17 | 55.16 | 83.83 |
| April | 6.00 | 26.83 | 37 | 59.33 | 82.33 |
| May | 152.3 | 27.17 | 36.83 | 60.5 | 69.66 |
| June | 787.6 | 25.13 | 32.17 | 73.33 | 86.83 |
| July | 651.6 | 25.17 | 31.83 | 77 | 87.3 |
| August | 224.6 | 24.67 | 32.5 | 71 | 85.33 |
| September | 99.6 | 24.33 | 33.17 | 68.33 | 86.16 |
| October | 91.9 | 23.5 | 34.67 | 66 | 88 |
| November | 40.6 | 24.67 | 35.83 | 62.33 | 85.16 |
| December | 56.4 | 23.5 | 35 | 55.66 | 84 |
| Yearly value | 2127 | 24.99 | 34.61 | 62.19 | 83.03 |

Table 3.2. Weather data of the experimental plot for 2017

| Months | Rainfall (mm) | Temperature (°C) | | Relative humidity (%) | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| January | 32.7 | 23.17 | 35.17 | 47 | 78.16 |
| February | 1.8 | 23 | 35.33 | 56.5 | 81.16 |
| March | 26.3 | 25.5 | 35.67 | 56.66 | 81.5 |
| April | 19.8 | 26.5 | 36.5 | 64 | 85.5 |
| May | 208.1 | 26.67 | 32.17 | 66.83 | 87.16 |
| June | 647.8 | 25 | 27.5 | 88.33 | 92.83 |
| July | 361.7 | 25.17 | 33 | 68.66 | 83.66 |
| August | 459 | 25.83 | 32.5 | 75.33 | 86.66 |
| September | 226.2 | 25.67 | 29.83 | 71.66 | 87.5 |
| October | 213 | 24.67 | 29.5 | 71.83 | 89.16 |
| November | 237.9 | 25 | 30.83 | 64 | 85.83 |
| December | 35.9 | 24.17 | 29.67 | 63 | 83.66 |
| Yearly value | 2470.2 | 25.02 | 32.30 | 62.27 | 78.64 |

Table 3.3. Weather data of the experimental plot for 2018

| Months | Rainfall | Temperature (°C) | | Relative humidity (%) | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| January | 1.90 | 22.16 | 29.83 | 51 | 77.33 |
| February | 3.20 | 22.66 | 31.66 | 49.5 | 77.5 |
| March | 56 | 24.5 | 32.83 | 55.16 | 83.83 |
| April | 41.4 | 25.66 | 32 | 60.66 | 88.33 |
| May | 344.3 | 26 | 31.16 | 63 | 85.16 |
| June | 546.01 | 24.66 | 28 | 74.83 | 87.5 |
| July | 54.46 | 24.16 | 27 | 80.33 | 89 |
| August | 66.72 | 23.66 | 26.83 | 78.66 | 89.83 |
| September | 95.81 | 23.83 | 29 | 63.16 | 89.16 |
| October | 170.79 | 24 | 29.33 | 69.5 | 88.33 |
| November | 104.27 | 23.66 | 30.16 | 59 | 84 |
| December | 81.3 | 23.5 | 30.16 | 57 | 85.5 |
| Yearly value | 1566.16 | 24.03 | 29.83 | 63.48 | 85.45 |

Table 3.4. Weather data of the experimental plot for 2019

| Months | Rainfall | Temperature (°C) | | Relative humidity (%) | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| January | 1.4 | 23.83 | 30.66 | 51.83 | 76.83 |
| February | 1.3 | 25.16 | 32.16 | 54.5 | 80 |
| March | 0.90 | 25.66 | 32.66 | 55.16 | 81.83 |
| April | 33.7 | 27.66 | 33.16 | 60.16 | 82.83 |
| May | 75 | 27.16 | 32.5 | 64 | 82.33 |
| June | 288.8 | 26.5 | 30 | 71.66 | 85.66 |
| July | 593.2 | 25 | 28.16 | 78.83 | 88.83 |
| August | 512.3 | 23.66 | 28 | 80.16 | 91.16 |
| September | 216.04 | 24.83 | 28.83 | 74.66 | 89.83 |
| October | 320.7 | 24.66 | 30 | 68.66 | 89.83 |
| November | 30 | 24.83 | 32.16 | 23.5 | 28.16 |
| December | 35.3 | 17.83 | 24.5 | 25 | 41.83 |
| Yearly value | 2108.64 | 24.73 | 30.23 | 59.01 | 76.59 |

### 3.2. Experimental material

*K. rotunda* accessions collected from different locations of Kerala state of India have been used for the present experiment. *K. rotunda* is an aromatic herb with tuberous rhizomes, which produce numerous tuberous roots. The rhizomes and root tubers of the plant have a bitter, camphoraceous taste and has been widely used as vegetable and spice in India. This plant attains a height up to 50-60 cm. Leaves are few, erect lamina oblong-lanceolate,

purple beneath, variegated green above and tinged with purple below with sheathing leaf base. Inflorescence is appearing before the leaves. Flowers usually appear as crowded spike, which is bisexual, trimerous and bracteolate. Calyx is light violet in colour, transparent and unilaterally split tip with two dorsal ridges. Corolla tube is longer than the calyx, white in colour, narrow, lanceolate with acuminate tip. Androecium consists of six stamens in two whorls. The two laterals of the outer whorl get transformed into petaloid staminodes which are pinkish white in colour. The laterals of the inner whorl are united to form the posterior labellum. The anterior odd one is fertile which has long filament with two anther lobes. Ovary is inferior, tricarpellary, syncarpous with axile placentation. Fruiting is not commonly seen (Warrier *et al*., 2001; Kumar *et al*., 2013).

### 3.3. Experimental programmes

The experimental programmes were carried out from 2016 to 2019, to evaluate the genetic diversity of *K. rotunda* and to identify superior genotypes from the accessions collected from different locations of Kerala state of India. Sixty eight accessions were collected and planted in the experimental plot during the first crop season of 2016 for developing sufficient planting materials and the experimental programmes started in May 2017.

#### 3.3.1. Layout, design, planting and aftercare

The present experiment was laid out in randomized block design with three replications. Sixty eight accessions of *K. rotunda* collected from different locations as mentioned above were used for the experimental programmes. (Table 3.5; Fig. 3.1).

Table 3.5. Accessions collected for the present study

| Accession Number | Place | District | Latitude/ Longitude and elevation |
|---|---|---|---|
| CUR 1 | Kattakada | Thiruvananthapuram | 8°50'N 77°08' E, 90 m MSL |
| CUR 2 | Ponmudi | Thiruvananthapuram | 8°76' N 77°11' E, 938 m MSL |
| CUR 3 | Kannanalloor | Kollam | 8°89' N 76°68' E, 35 m MSL |
| CUR 4 | Aryanadu | Thiruvananthapuram | 8°52' N 77°08' E, 74 m MSL |
| CUR 5 | Karunagappalli | Kollam | 9°05' N 76°53' E, 10 m MSL |
| CUR 6 | Vakkanadu | Kollam | 8°92' N 76°72' E, 22 m MSL |
| CUR 7 | Punnala | Kollam | 9°08' N 76°90' E, 45 m MSL |
| CUR 8 | Eraviperoor | Pathanamthitta | 9°38' N 76°64' E, 131 m MSL |
| CUR 9 | Manthuruthy | Kottayam | 9°52'N 76°64' E,46 m MSL |
| CUR 10 | Kothanalloor | Kottayam | 9°71' N 76°52' E, 24 m MSL |
| CUR 11 | Vadasserikkara | Pathanamthitta | 9°34'N 76°82' E, 33 m MSL |
| CUR 12 | Pezhumpara | Pathanamthitta | 9°34'N 76°85' E,119 m MSL |
| CUR 13 | Kurumpanadom | Kottayam | 9°48' N 76°58' E, 36 m MSL |
| CUR 14 | Chithirapuram | Idukki | 10°00'N 77°06' E, 774 m MSL |
| CUR 15 | Kuttampuzha | Ernakulam | 10°15'N 76° 73' E, 61 m MSL |
| CUR 16 | Cholathadam | Kottayam | 9°61'N 76°85'E, 28 m MSL |
| CUR 17 | Odakkali | Ernakulam | 10°09'N 76°55' E, 75 m MSL |
| CUR 18 | Koovappady | Ernakulam | 10°16 'N 76°48'E, 25 m MSL |
| CUR 19 | Ezhamkulam | Pathanamthitta | 9° 14' N 76°76'E, 54 m MSL |
| CUR 20 | Pottankadu | Idukki | 10°00'N 77°08'E, 920 m MSL |
| CUR 21 | Kuravankuzhy | Pathanamthitta | 9°35'N 76°67'E, 15m MSL |
| CUR 22 | Karumalloor | Ernakulam | 10°13'N 76°28'E, 13m MSL |
| CUR 23 | Upputhodu | Idukki | 9°87'N 77°01'E, 691m MSL |
| CUR 24 | Murickassery | Idukki | 9°91'N 77°00'E, 723m MSL |
| CUR 25 | Erattayar | Idukki | 9°79'N 77°10'E, 765m MSL |
| CUR 26 | Neyyasseri | Idukki | 9°93'N 76°77'E, 60m MSL |
| CUR 27 | Vellathooval | Idukki | 9°97'N 77°02'E, 494m MSL |
| CUR 28 | Thaikkattussery | Thrissur | 10°45'N 76°24'E, 25m MSL |
| CUR 29 | Athani | Thrissur | 10°61'N 76°22'E, 62m MSL |
| CUR 30 | Thathamangalam | Palakkad | 10°68'N 76°70'E, 124m MSL |
| CUR 31 | Mannuthy | Thrissur | 10°3'N 76°26'E, 26m MSL |
| CUR 32 | Kollamkode | Palakkad | 10°61'N 76°69'E, 107m MSL |
| CUR 33 | Nenmara | Palakkad | 10°54'N 76°62'E, 90m MSL |
| CUR 34 | Cherpulassery | Palakkad | 10°87'N 76°31'E, 63m MSL |

| | | | |
|---|---|---|---|
| CUR 35 | Mannarkkad | Palakkad | 10°99'N 76°45'E, 89m MSL |
| CUR 36 | Mazhuvanchery | Thrissur | 10°61' N 76°13'E, 11m MSL |
| CUR 37 | Mullassery | Thrissur | 10°53'N 76°08'E, 7m MSL |
| CUR 38 | Chelakkara | Thrissur | 10°69' N 76°33'E, 38m MSL |
| CUR 39 | Amballoor | Thrissur | 10°43' N 76°26'E, 13m MSL |
| CUR 40 | Kavassery | Palakkad | 10°65'N 76°51'E, 65m MSL |
| CUR 41 | Elavanchery | Palakkad | 10°59'N 76°64'E, 97m MSL |
| CUR 42 | Pattambi | Palakkad | 10°80'N 76°17'E, 28m MSL |
| CUR 43 | Mulloorkara | Thrissur | 10°70'N 76° 25'E, 37m MSL |
| CUR 44 | Peechi | Thrissur | 10°52'N 76°36'E, 79m MSL |
| CUR 45 | Nilambur | Malappuram | 11°30'N 76°33'E, 54m MSL |
| CUR 46 | Kottakkal | Malappuram | 11°00'N 76°00'E, 43m MSL |
| CUR 47 | Kottukkara | Malappuram | 11°1'N 75°98'E, 48m MSL |
| CUR 48 | Alathiyoor | Malappuram | 10°86'N 75°93'E, 17m MSL |
| CUR 49 | Narippatta | Kozhikode | 11°70'N 75°70'E, 11m MSL |
| CUR 50 | Chempanoda | Kozhikode | 11°63'N 75°82'E, 65m MSL |
| CUR 51 | Avitanallur | Kozhikode | 11°49'N 75°80'E, 32m MSL |
| CUR 52 | Vaduvanchal | Wayanad | 11°55'N 76°22'E, 892m MSL |
| CUR 53 | Kallody | Wayanad | 11°76'N 75°96'E, 792m MSL |
| CUR 54 | Kavilumpara | Kozhikode | 11°67'N 75°77'E, 30m MSL |
| CUR 55 | Nenmeni | Wayanad | 11°64'N 76°21'E, 820m MSL |
| CUR 56 | Thrissilery | Wayanad | 11°85'N 76°04'E, 817m MSL |
| CUR 57 | Peechankode | Wayanad | 11°75'N 76°00'E, 758m MSL |
| CUR 58 | Vengappally | Wayanad | 11°61'N 76°04'E,788m MSL |
| CUR 59 | Nadavayal | Wayanad | 11°75'N 76°09'E, 770m MSL |
| CUR 60 | Kottathara | Wayanad | 11°78'N 76°05'E, 726m MSL |
| CUR 61 | Ambalavayal | Wayanad | 11°61'N 76°21'E, 924m MSL |
| CUR 62 | Elerithattu | Kasargod | 12°33'N 75°31'E, 157m MSL |
| CUR 63 | Periyanganam | Kasargod | 12°31'N 75°27'E, 141m MSL |
| CUR 64 | Konnakkad | Kasargod | 12°36'N 75°24'E, 153m MSL |
| CUR 65 | Muzhakkunnu | Kannur | 11°94'N 75°70'E, 9m MSL |
| CUR 66 | Aaralam | Kannur | 11°96'N 75°76'E, 83m MSL |
| CUR 67 | Karivelloor | Kannur | 12°17'N 75°19'E, 13m MSL |
| CUR 68 | Edayannur | Kannur | 11°92'N 75°51'E, 66m MSL |

**Fig. 3.1. Different locations of collection of *Kaempferia rotunda***

#### 3.3.1.1. Planting

Fresh healthy rhizomes of the collected accessions from the germplasm were used for all experimental programmes. 25 to 35 g rhizome fingers with one or two bud fingers were used as the planting material. The rhizomes were planted in open condition, each planted in polythene bags of size 38 cm × 35 cm filled with garden soil, cow dung and sand in 3:1:1 ratio. The first crop was planted during the first week of May 2016 just before the onset of southwest monsoon season. In the case of the experiment carried out to study variability, the same procedure was repeated in 2017 and 2018. An experiment designed and carried out in 2018 to find out whether the status of the planting materials such as mother rhizome, primary fingers and secondary fingers had any influence on the growth and yield of the crop (Figs. 3.2 and 3.3).

#### 3.3.1.2. Weeding and manuring

Weeding was carried out thrice, i.e., on 60, 90 and 120 days after planting depending upon the weed intensity. The plants were irrigated regularly on non-rainy days to maintain the optimum soil moisture. Manuring was also done using NPK (18:18:18) at a dosage of 2 g per plant at monthly intervals starting from the $30^{th}$ day of planting. Recommended package of practices and plant protection measures were followed to raise a healthy crop (KAU, 2011).

#### 3.3.1.3. Recording of data

Data on growth and yield characters were recorded at the end of six months of growth by destructive sampling (Table 3.6). The data were analyzed as described below so as to study the genetic variability, correlation of characters, character association, genetic divergence, overall performance

of the genotypes and performance of plants based on the status of planting materials used.

Each accession was planted in three replications and each plot consisted of 18 plants. Data were recorded from 9 plants each and the mean was calculated. Observations on growth and yield characters were recorded appropriately as shown in Table 3.6. Leaf area was calculated using the formula:

Leaf area = leaf length × leaf breadth × conversion factor

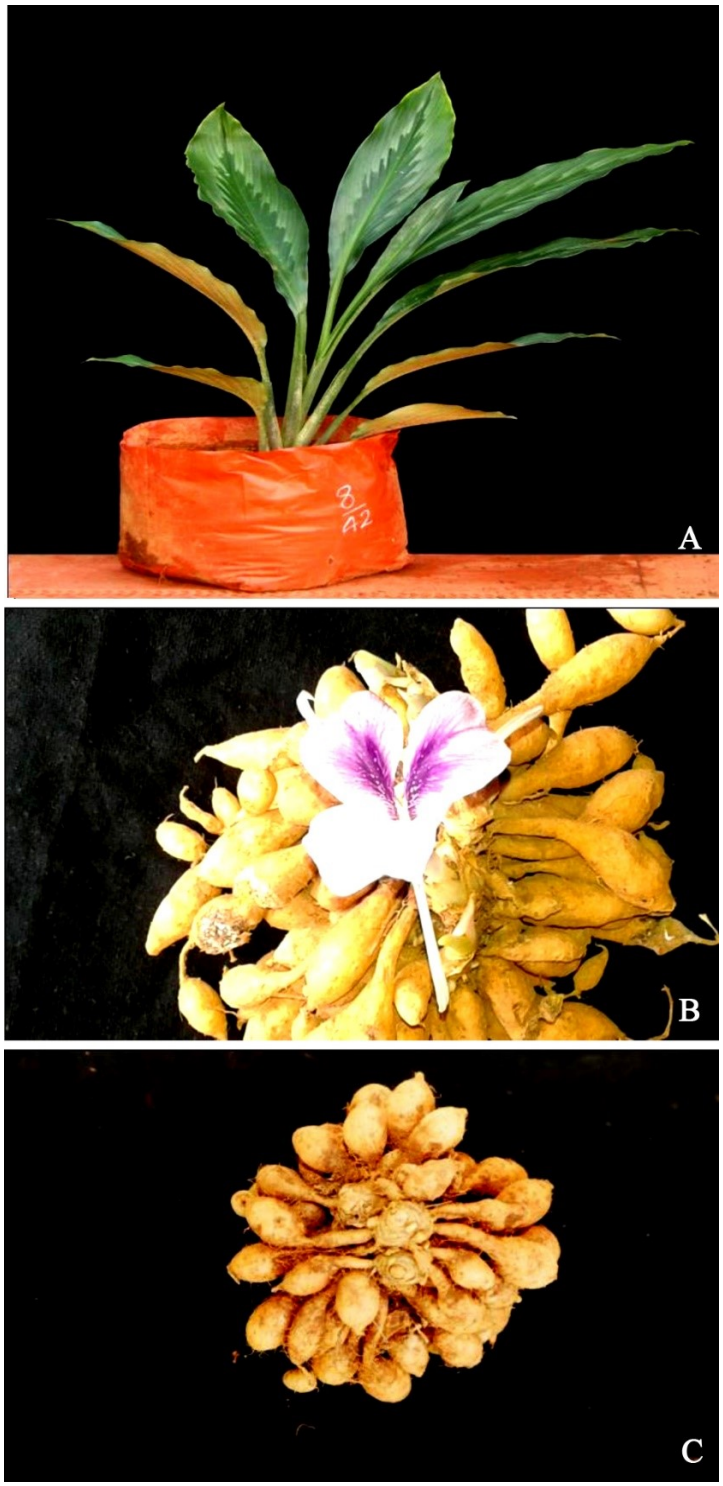
The conversion factor for leaf area was calculated as shown in Table 3.7. To find out the conversion factor, leaves of five plants were selected and measured randomly and appropriate calculations were made.

Table 3.6. Characters of *Kaempfeia rotunda* observed in the study

| Sl.No. | Character |
|---|---|
| **Growth characters** | |
| 1 | Plant height (cm) |
| 2 | Number of tillers |
| 3 | Number of leaves per tiller |
| 4 | Leaf length (cm) |
| 5 | Leaf breadth (cm) |
| 6 | Leaf area ($cm^2$) |
| **Yield characters** | |
| 7 | Number of primary fingers |
| 8 | Number of secondary fingers |
| 9 | Length of primary finger (cm) |
| 10 | Diameter of primary finger (cm) |
| 11 | Length of secondary finger (cm) |
| 12 | Diameter of secondary finger (cm) |
| 13 | Length of mother rhizome (cm) |
| 14 | Diameter of mother rhizome (cm) |
| 15 | Yield per plant (g) |

**Fig. 3.2. *Kaempferia rotunda* in the experimental layout**

**Fig. 3.3. *Kaempferia rotunda* L. – single plant, inflorescence and rhizome**




**(A) Single plant; (B) Inflorescence; (C) Rhizome**

Table 3.7. Calculation of conversion factor for leaf area

| Species | Conversion factor for leaf area | | | | | |
|---|---|---|---|---|---|---|
| *Kaempferia rotunda* | R1 | R2 | R3 | R4 | R5 | Mean |
| | 0.59 | 0.60 | 0.61 | 0.63 | 0.61 | 0.61 |

Different statistical methods adopted in the present experimental programmes for different purposes are explained below.

### 3.3.2. Genetic variability

The efficiency of selection depends on the extent of genetic variability observed in the case of any species. Genetic improvement is normally achieved by selecting the genotypes with desirable traits from the available population. Moreover, the unthreatened occurrence of the species shows high genetic variability. Genetic variability of *K. rotunda* has been studied using the following tools of analysis.

#### 3.3.2.1. Frequency distribution of growth and yield characters

Frequency distribution analysis of the germplasm of *K. rotunda* collected has been carried out to analyze the nature of frequency distribution of characters in the germplasm and also to study the nature of genetic control of the characters. Data on 612 plants belonging to all the accessions collected and grown in 2016 were used for the experiment.

#### 3.3.2.2 Phenotypic and genotypic variability

Phenotypic and genotypic variability of the population was assessed presently based on the morphometric characters of the plants. Data collected from nine plants each in the case of each replication were averaged for the three replications in the case of each accession.

### 3.3.2.2.1. Variability of agronomic characters

Variability of morphometric characters in the case *K. rotunda* under study has been analysed presently by the analysis of mean and standard deviation of the characters. Analysis of variance (ANOVA) was carried out to test the significance of variation between the genotypes studied. F value was calculated for the study and its significance was tested with reference to the standard F table (Fischer and Yates, 1963). CD was calculated using the following formula:

$$CD = t_{0.05} \times \sqrt{\frac{2VE}{r}}$$

Where $t_{0.05}$ is for error degree of freedom; VE denotes the error mean square and r denotes the number of replications.

Genotypic and phenotypic variances of the different characters were estimated using the following formulae (Singh and Choudhary, 1985).

$$\text{Genotypic variance } (\sigma 2g) = \frac{\text{MSS for Treatment} - \text{MSS for error}}{\text{Number of replications}}$$

Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$

Where, $\sigma^2 e$ is the error variance.

Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953). The coefficients of variation are very useful since different traits are measured in different units.

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma g}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma p}{\bar{X}} \times 100$$

Where σg is the genotypic standard deviation; σp is the phenotypic standard deviation; $\bar{X}$ is the grand mean of a character.

### 3.3.2.2.2. Heritability of agronomic characters

The extent of variation due to genetic differences among the genotypes can be used to assess the relative contribution of the genotype and environment in the form of heritability. Heritability and correlation are important parameters for quantitative traits because they can be used to predict the response to selection in plant breeding. The parameter heritability (broad sense) involves all types of gene action and thus forms a broad estimate of heritability (Chahal and Gosal, 2002). According to Jain (1982) heritability is the fraction of total variance that is heritable and is estimated as the percentage of genotypic variance over phenotypic variance.

$$\text{Heritabili ty (Broad sense) } H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

### 3.3.2.2.3. Genetic advance

Genetic advance is the genetic improvement of the progeny possible through selection over the original population (Chahal and Gosal, 2002). Genetic advance is estimated as per Singh and Choudhary (1985) using the following formula:

$$GA = \frac{KH^2\sigma p}{\bar{X}}$$

Where $H^2$ is the heritability (broad sense); σp is the phenotypic standard deviation; K denotes the selection differential which is 2.06 at 5% intensity of selection in large samples (Allard, 1960).

### 3.3.3. Correlation of characters

Heritable characters of organisms show different degrees of interrelationships due to common sharing of alleles. This leads to the phenomenon of systematic interrelationship between the variables termed correlation. Correlation is due to common sharing of genes as in the case of characters, which are heritable. Correlation coefficients of fifteen morphometric characters of 68 accessions of *K. rotunda* have been worked out presently as per Rangaswamy (1995).

### 3.3.4. Character association

Study of association of characters is worked out for grouping of variables and data reduction so as to find out the lead characters that can be employed in selection processes in plant breeding. Factor analysis by means of principal component analysis has been done for the purpose using the statistical software STATISTICA, based on fifteen morphometric characters of *K. rotunda*.

### 3.3.5. Genetic divergence

A study on genetic divergence is helpful in grouping of different accessions into different clusters. Study of genetic divergence among the sixty eight genotypes of *K. rotunda* was carried out by principal component analysis using the statistical software STATISTICA following UPGMA (Unweighted Pair Group Method with Arithmetic Mean) of Sneath and Sokal (1973).

### 3.3.6. Performance analysis of the accessions collected

Comparative performance of the accessions of *K. rotunda* has been analyzed presently based on fifteen growth and yield characters with the help

of performance index calculated as per Amaravenmathy and Srinivasan (2003). The performance index is calculated using the following formula:

$$\text{Performance index} = \frac{\text{Accession mean of the character}}{\text{Grand mean of the character}}$$

### 3.3.7. Study of performance based on the status of planting materials used

An experiment was designed and carried out during the first crop season of 2018 to evaluate the performance of the crop of *K. rotunda* in relation to the status of the seed materials used. The planting material used for the experiment consisted of seed rhizomes of three different statuses such as the mother rhizome, primary fingers and secondary fingers collected from the experimental seed stock maintained in University of Calicut. A crop of 68 plants for each type of planting material was raised from fresh, healthy and disease free rhizomes of about 3 cm – 5 cm length and 25 g - 30 g weight and the same agronomic practices as mentioned in the case of the previous experiments were adopted for the present study. The plants were harvested simultaneously after six months of maturity.

The growth and yield characters were recorded after six months of growth. The yield characters were recorded by destructive sampling. The data were pooled and subjected to statistical analysis for comparative performance of the plants developed from planting materials of different status.

### 3.3.8 Pharmacognostic standardization of *Kaempferia rotunda*

#### 3.3.8.1. Microscopic studies

Fine sections of the rhizome were taken using automatic MT3 microtome. The sections were stained with diluted aqueous saffranin, washed

thoroughly and mounted in 40% glycerin and observed under the microscope. Trinocular Leica DM 3000 microscope attached with Leica DFC 295 digital camera connected to the computer and Leica application suite software was used for the study. Images obtained were examined thoroughly and compared the anatomical characteristics.

In order to estimate the presence of various cell inclusions like starch grains and oil globules the following methods were adopted.

- **Starch grains**- To examine the presence of starch, the sections were stained with iodine solution. Starch grains got turned blue in colour.
- **Oil globules**- To examine the presence of fixed oil, the sections were stained with sudan red. If present, the oil droplet got coloured orange pink.

### 3.3.8.2. Powder analysis

For examining the cell structure in powder form, the rhizomes were powdered, sieved and stained with appropriate stain, mounted in glycerin and observed under microscope. Transferred the images of powder characters to the computer using the computer controlled microscopic system and camera.

#### 3.3.8.2.1. SEM analysis of rhizome powder of *Kaempferia rotunda*

The rhizome powder was subjected to SEM viewing for the detailed surface ornamentation patterns. For SEM analysis, the specimens were (rhizome powder of *K. rotunda*) placed on aluminium stubs using double sided adhesive tape and sputter coated with gold using a Hummer VII gold coating apparatus. They were observed and photographed under JEOL Model JSM – 6390LV SEM and Gemini SEM 300 under different magnifications.

### 3.3.8.3. Phytochemical analysis of *Kaempferia rotunda* using GC-MS

#### 3.3.8.3.1. Preparation of plant material

The rhizomes collected were cleaned and washed with distilled water and dried at room temperature. The dried rhizomes were ground to fine powder using a mortar and pestle and the powder was preserved in air sealed plastic covers.

#### 3.3.8.3.2. Preparation of samples

Powdered rhizomes of *K. rotunda* were extracted with methanol by soxhlet extraction method. The extract was filtered. The crude extracts were concentrated by rotary evaporator at 40°C and the concentrated extracts were used for GC-MS analysis.

#### 3.3.8.3.3. GC-MS analysis

Model QP2010S was employed for GC-MS analysis. A cross linked factor four capillary column Rxi-5Sil MS with 30 m × 0.25 mm ID and 0.25 µm film thickness was utilized. Carrier gas used was helium at a flow rate of 1 ml/min. Injection volume was 1 µl. The split ratio was 20.0. The temperature programme for the chromatographic analysis was set at 80°C for 4 min and then heated up at a rate of 5°C min to 280°C. Run time was 50 min. Quantification was done using percentage peak area calculations and identification of individual compounds was done with the help of NIST 11 and WILEY 8 search. The relative concentration of each compound in the methanolic extract was found out based on the peak area integrated by the analysis programme.

#### 3.3.8.3.4. Identification of compounds

Interpretation of mass spectrum of GC-MS was carried using the database of NIST 11 and WILEY 8 search. The relative percentage amount of each compound was calculated by comparing its average peak area to the total area.

# Chapter IV
# RESULTS AND DISCUSSION

The family Zingiberaceae is widely distributed throughout the tropical part of the world with more than 50 genera and 1200 species (Kress *et al*., 2002). *Kaempferia rotunda* L. is a valued medicinal plant since ancient times for its medicinal uses. The present study was designed to analyze the genetic variability, character association, genetic divergence and performance of the genotypes of the species available in Kerala state of India, based on collections made from different locations of Kerala. The experiments were carried out in the experimental plot of the Genetics and Plant Breeding Division of the Department of Botany, University of Calicut, Kerala, India from 2016 to 2019. The observed data are analyzed and discussed below under appropriate titles.

## 4.1. Genetic variability

### 4.1.1. Genetic control of growth and yield characters

Analysis of the frequency distribution of agronomic characters of crop plants will help to discover the mechanism of genetic control involved in them and also to analyze the distribution pattern of dominant and recessive contributing alleles or factors in the gene pools of these characters. Hence, a study in relation to the genetic control of six growth characters namely plant height, number of tillers, number of leaves per tiller, leaf length, leaf breadth and leaf area and nine yield characters namely yield per plant, number of primary fingers, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome of a population consisting of 612 plants of *K. rotunda* derived from different

locations of Kerala state of India has been conducted presently based on frequency distribution analysis.

All the characters showed continuous frequency distribution as evidenced by their frequency curves presented in Tables 4.1 to 4.15 and Figs. 4.1 to 4.15. Continuous frequency distributions with all possible intermediates indicate the polygenic control of these characters.

Quantitative characters with polygenic control show normal frequency distribution when the allelic combinations are distributed in the gene pool of the population as per the principles of probability and when the dominant and recessive alleles are in equal frequencies. The bell shaped normal distribution curve shows skewness when there is variation in the frequency of the dominant or recessive alleles in the distribution (Chahal and Gosal, 2002). Among the vegetative growth characters of *K. rotunda* studied presently, plant height, leaf length and leaf breadth showed continuous distribution with accumulation of higher number of dominant alleles thus shifting the skewness of the distribution towards the distal end of distribution curve. The gene pool of these characters shows an accumulation of higher number of dominant alleles even when maintaining good genetic base ranging from comparatively lower to higher values. However, in the case of plant height, leaf length and leaf breadth the frequencies of the classes with higher values of the characters are comparatively low. Selecting the promising genotypes with maximum accumulation of dominant alleles is necessary for the development of superior varieties and for maintaining good genetic base. The characters such as number of tillers and number of leaves per tiller showed skewness of the distribution towards the proximal side thus indicating the accumulation of higher number of recessive contributing factors. This indicates that the frequency of plants with higher accumulation of dominant contributing factors of these characters is very low and hence, more scientific selection

studies are to be carried out to develop promising varieties with higher accumulation of dominant alleles of these characters.

Among the yield characters studied, yield per plant, length of primary finger, number of secondary fingers and length of secondary finger showed skewness of the distribution towards the proximal end thus indicating the presence of higher number of recessive alleles. Diameter of mother rhizome and diameter of secondary finger showed skewness of the distribution towards the distal side of the distribution curve indicating the accumulation of dominant alleles for these characters. It also confirms the essentiality of selection for better phenotypes and genotypes with higher accumulation of dominant alleles so as to develop superior varieties. This is a desirable phenomenon in view of both the characters directly contributing towards the rhizome yield of plants. Higher frequency of dominant contributing factors is the prerequisite for the development of superior varieties.

Among all fifteen growth and yield characters studied, leaf area, number of primary fingers, diameter of primary finger and length of mother rhizome showed a balanced distribution of genotypes almost equally towards the proximal and distal sides of the frequency distributions indicating the existence of equal frequency of the dominant and recessive alleles contributing to these characters. This can be considered as an ideal situation in the case of non-selected natural population. Dominant allele combinations with better expression of characters are to be selected while practicing selection.

Table 4.1. Frequency distribution of plant height

| Plant height (cm) | Number of plants |
|---|---|
| 15-25 | 1 |
| 25-35 | 8 |
| 35-45 | 28 |
| 45-55 | 86 |
| 55-65 | 123 |
| 65-75 | 167 |
| 75-85 | 127 |
| 85-95 | 50 |
| 95-105 | 4 |

Fig. 4.1. Frequency curve of plant height

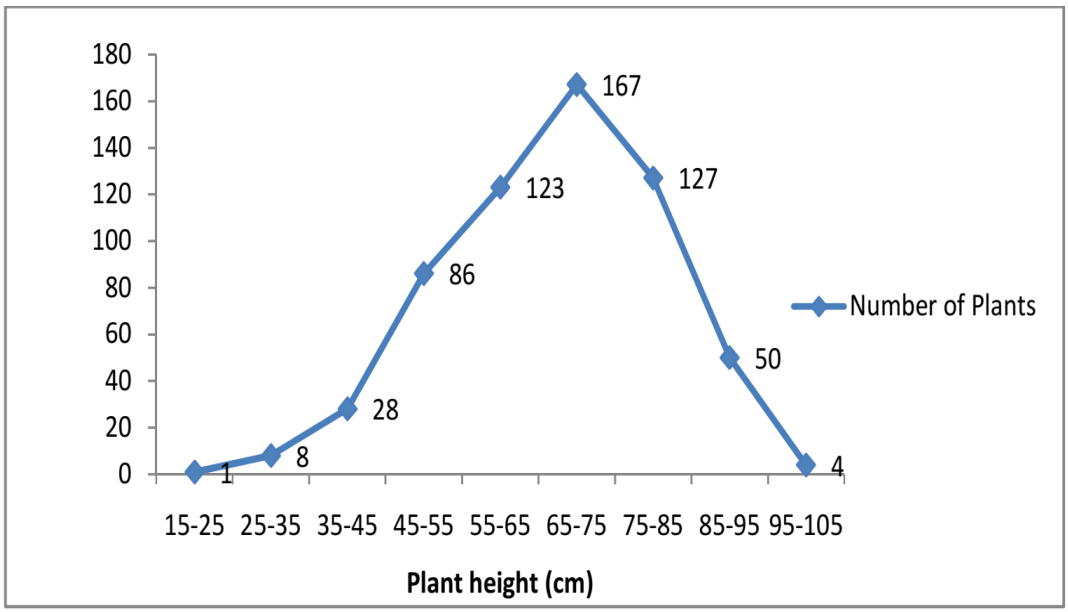


Table 4.2. Frequency distribution of number of tillers

| Number of tillers | Number of plants |
|---|---|
| 0-1 | 68 |
| 1-2 | 140 |
| 2-3 | 210 |
| 3-4 | 150 |
| 4-5 | 19 |
| 5-6 | 4 |
| 6-7 | 3 |

Fig. 4.2. Frequency curve of number of tillers

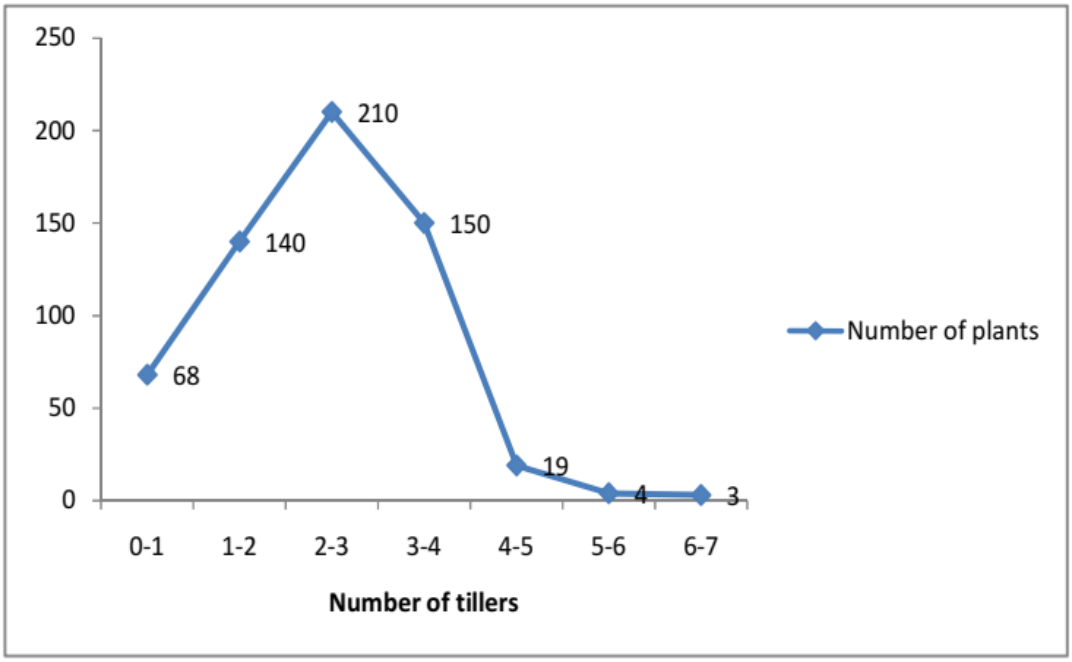


Table 4.3. Frequency distribution of number of leaves per tiller

| Number of leaves per tiller | Number of plants |
|---|---|
| 3–5 | 160 |
| 5–7 | 263 |
| 7–9 | 119 |
| 9–11 | 34 |
| 11–13 | 12 |
| 13–15 | 4 |
| 15–17 | 2 |

Fig. 4.3. Frequency curve of number of leaves per tiller

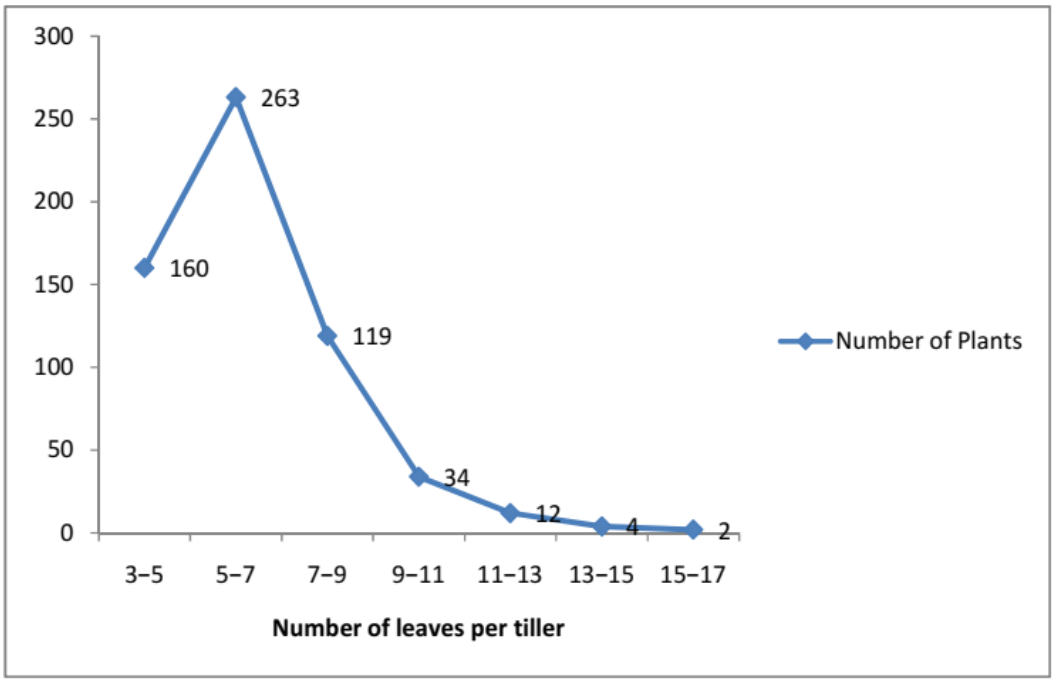


Table 4.4. Frequency distribution of leaf length

| Leaf length (cm) | Number of plants |
|---|---|
| 10–20 | 7 |
| 20–30 | 101 |
| 30–40 | 283 |
| 40–50 | 195 |
| 50–60 | 8 |

Fig. 4.4. Frequency curve of leaf length

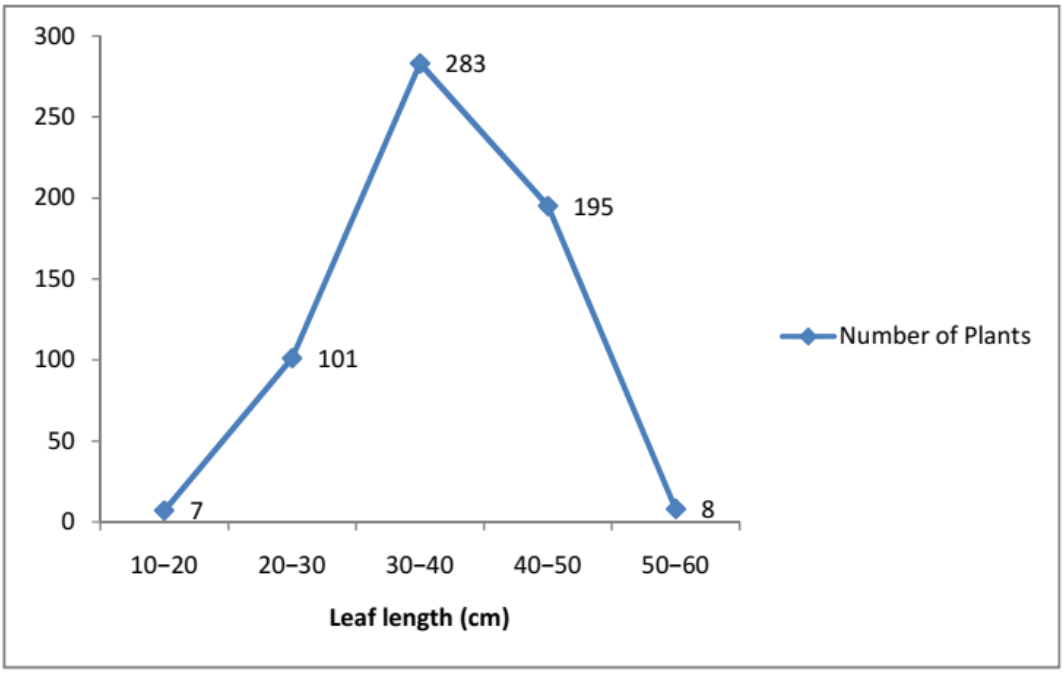


Table 4.5. Frequency distribution of leaf breadth

| Leaf breadth (cm) | Number of plants |
|---|---|
| 0–2.5 | 1 |
| 2.5–5 | 13 |
| 5–7.5 | 200 |
| 7.5–10 | 335 |
| 10–12.5 | 45 |

Fig. 4.5. Frequency curve of leaf breadth

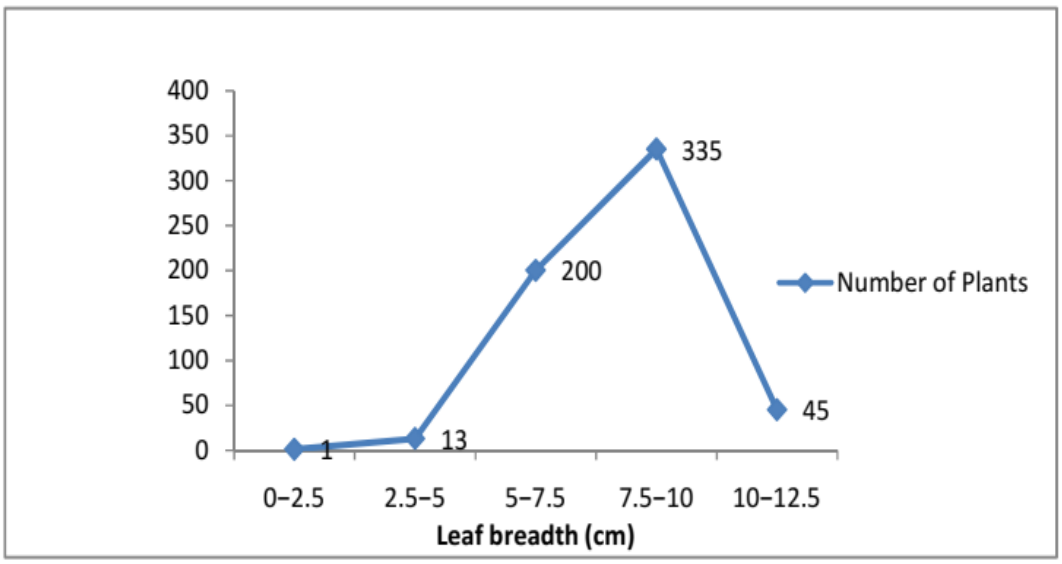


Table 4.6. Frequency distribution of leaf area

| Leaf area ($cm^2$) | Number of plants |
|---|---|
| 0-50 | 4 |
| 50-100 | 42 |
| 100-150 | 132 |
| 150-200 | 190 |
| 200-250 | 156 |
| 250-300 | 60 |
| 300-350 | 10 |

Fig. 4.6. Frequency curve of leaf area

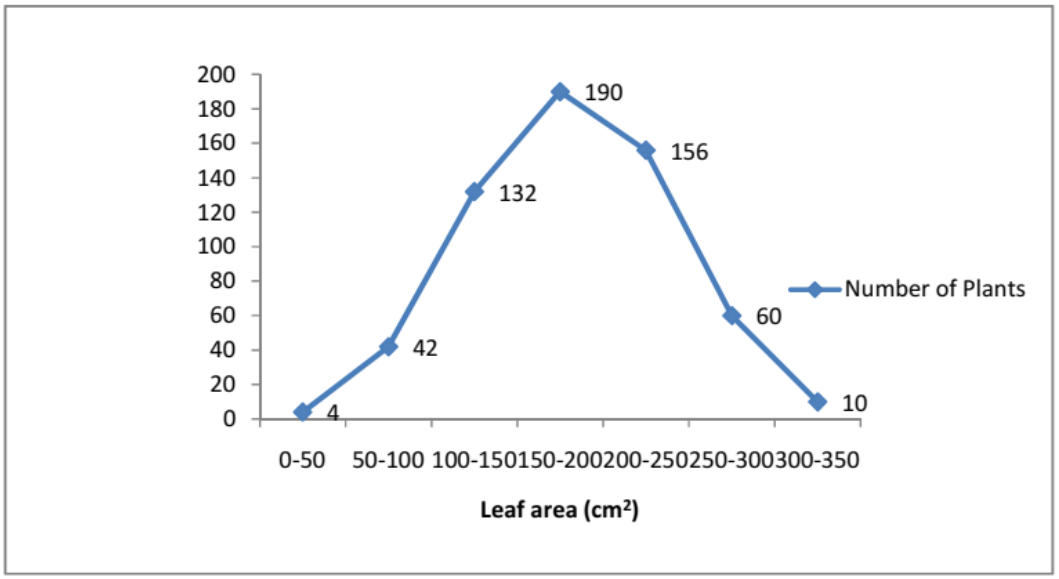


Table 4.7. Frequency distribution of yield per plant

| Yield per plant (g) | Number of plants |
|---|---|
| 0-100 | 196 |
| 100-200 | 245 |
| 200-300 | 90 |
| 300-400 | 36 |
| 400-500 | 19 |
| 500-600 | 4 |
| 600-700 | 4 |

Fig.4.7. Frequency curve of yield per plant

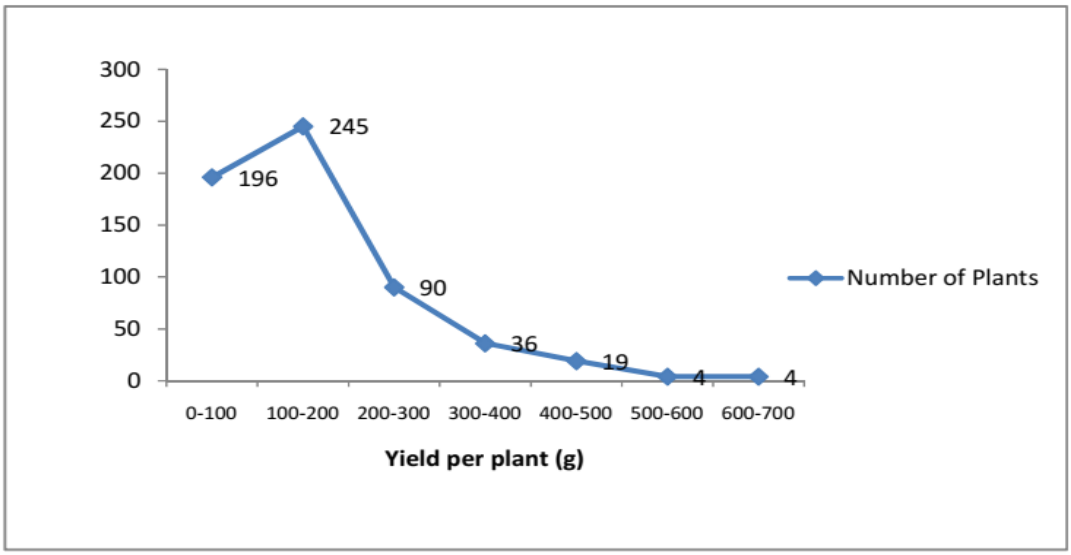


Table 4.8. Frequency distribution of number of primary fingers

| Number of primary fingers | Number of plants |
|---|---|
| 1–4 | 44 |
| 4–7 | 146 |
| 7–10 | 292 |
| 10–13 | 89 |
| 13–16 | 23 |

Fig.4.8. Frequency curve of number of primary fingers

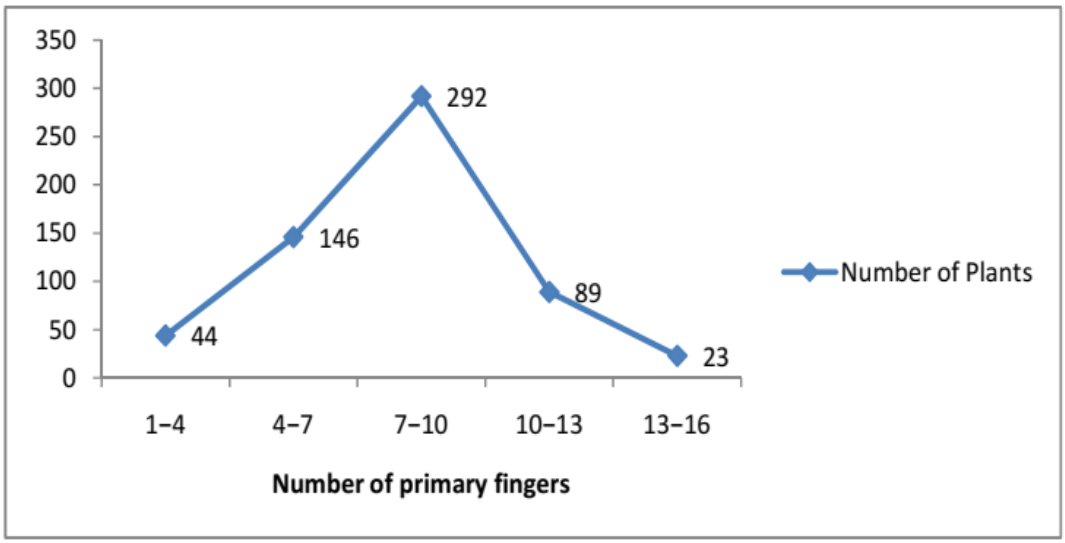


Table 4.9. Frequency distribution of length of primary finger

| Length of primary finger (cm) | Number of plants |
|---|---|
| 0–2 | 19 |
| 2-4 | 306 |
| 4-6 | 247 |
| 6-8 | 21 |
| 8-10 | 1 |

Fig.4.9. Frequency curve of length of primary finger

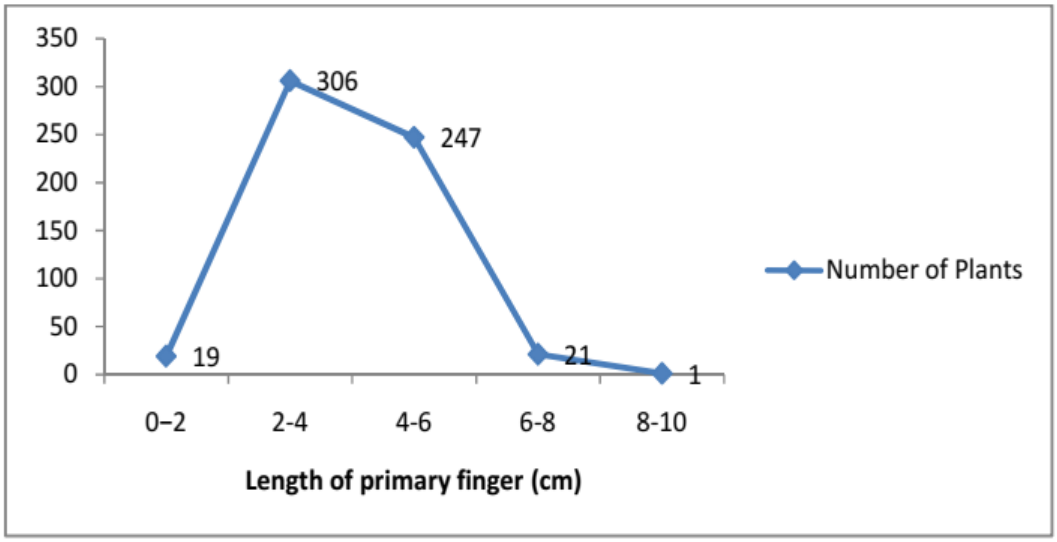


Table 4.10. Frequency distribution of diameter of primary finger

| Diameter of primary finger (cm) | Number of plants |
|---|---|
| 0.5-1 | 16 |
| 1-1.5 | 75 |
| 1.5-2 | 144 |
| 2-2.5 | 197 |
| 2.5-3 | 118 |
| 3-3.5 | 40 |
| 3.5-4 | 4 |

Fig.4.10. Frequency curve of diameter of primary finger

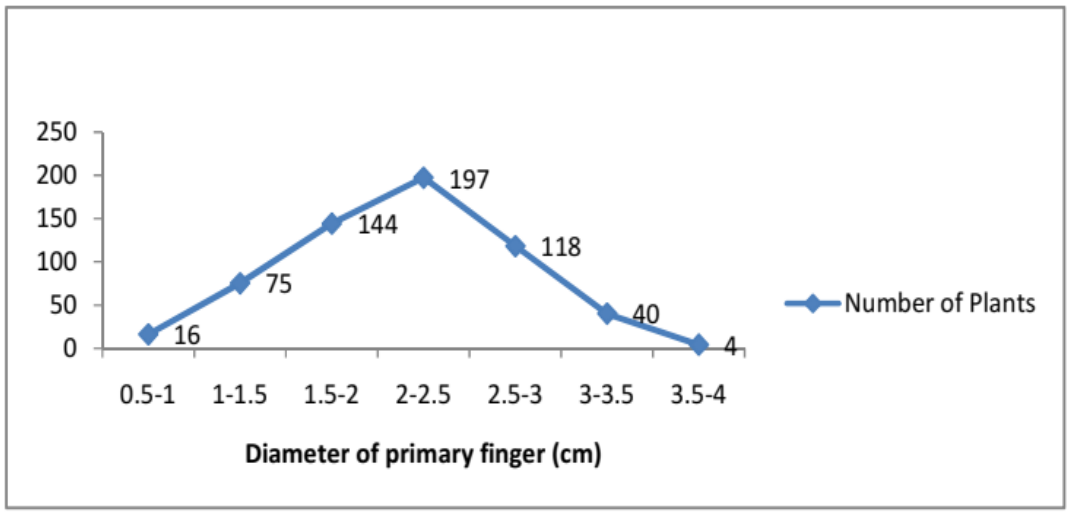


Table 4.11. Frequency distribution of number of secondary fingers

| Number of secondary fingers | Number of plants |
|---|---|
| 1-8 | 174 |
| 8-16 | 257 |
| 16-24 | 130 |
| 24-32 | 26 |
| 32-40 | 7 |

Fig.4.11. Frequency curve of number of secondary fingers

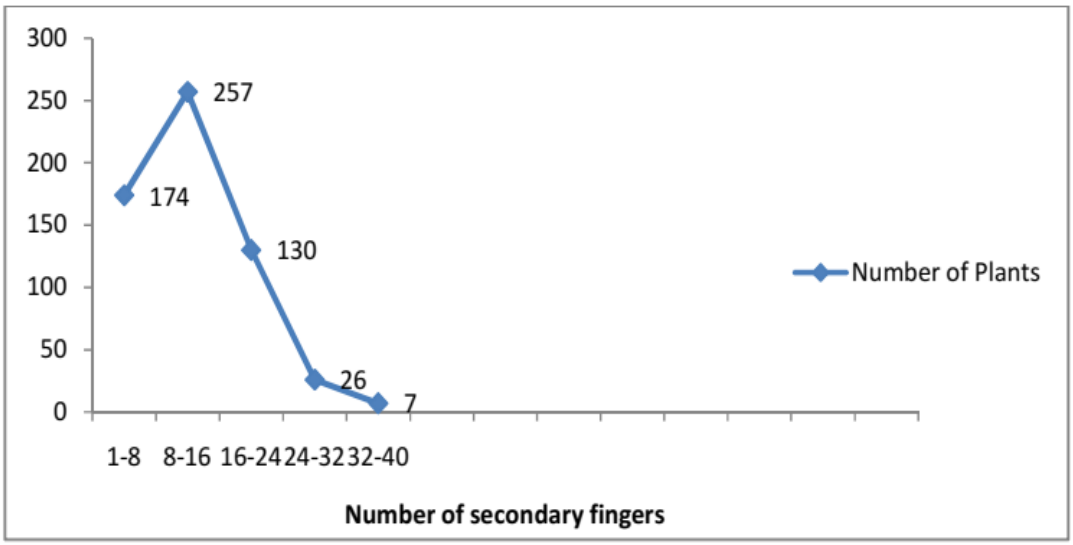


Table 4.12. Frequency distribution of length of secondary finger

| Length of secondary finger (cm) | Number of plants |
|---|---|
| 0–2 | 133 |
| 2–4 | 408 |
| 4–6 | 48 |
| 6–8 | 4 |
| 8–10 | 1 |

Fig.4.12. Frequency curve of length of secondary finger

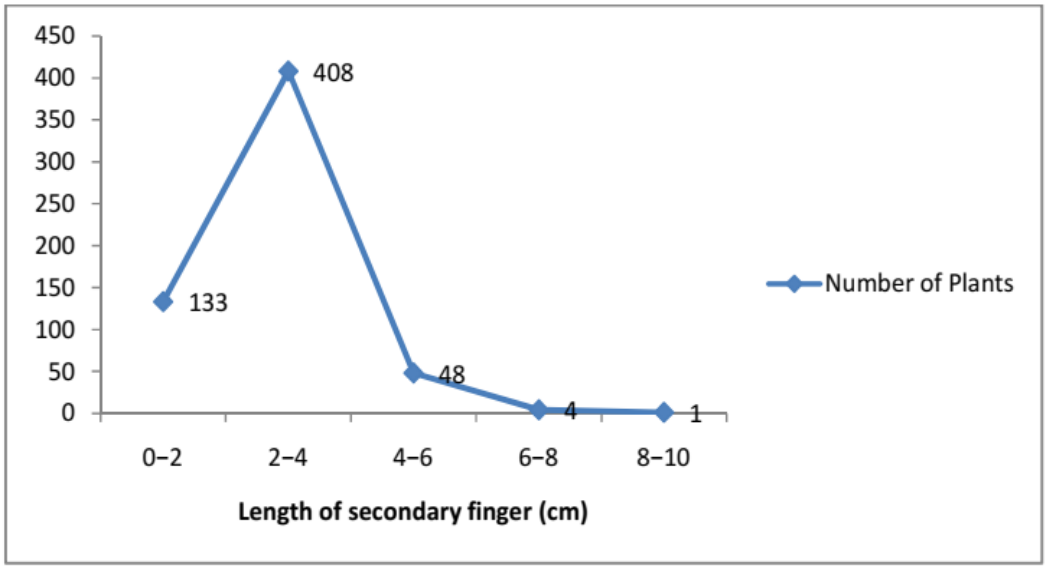


Table 4.13. Frequency distribution of diameter of secondary finger

| Diameter of secondary finger (cm) | Number of plants |
|---|---|
| 0–0.5 | 32 |
| 0.5–1 | 107 |
| 1–1.5 | 245 |
| 1.5–2 | 143 |
| 2–2.5 | 55 |
| 2.5–3 | 10 |
| 3–3.5 | 2 |

Fig.4.13. Frequency curve of diameter of secondary finger

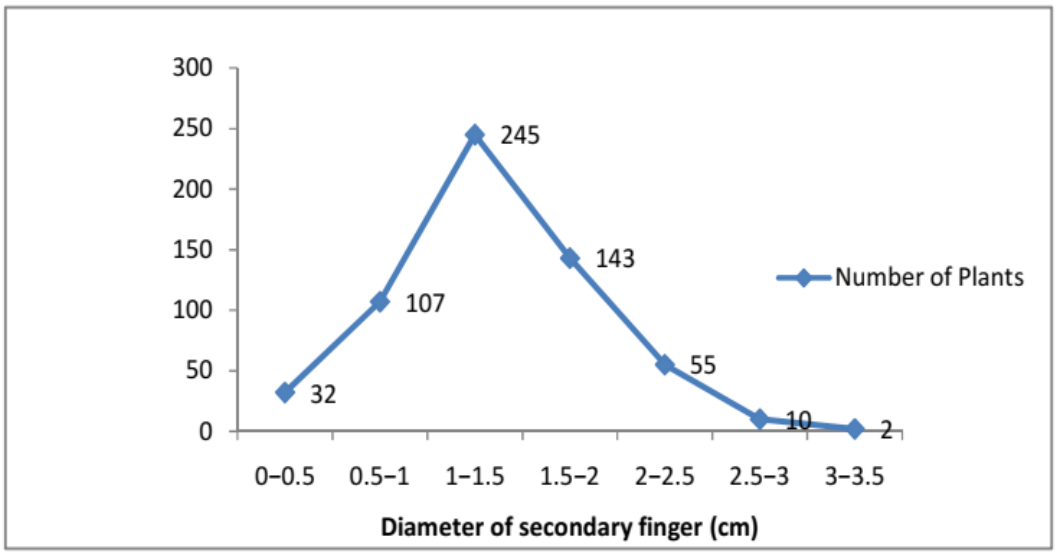


Table 4.14. Frequency distribution of length of mother rhizome

| Length of mother rhizome (cm) | Number of plants |
|---|---|
| 0–2 | 1 |
| 2–4 | 115 |
| 4–6 | 390 |
| 6–8 | 82 |
| 8–10 | 6 |

Fig.4.14. Frequency curve of length of mother rhizome

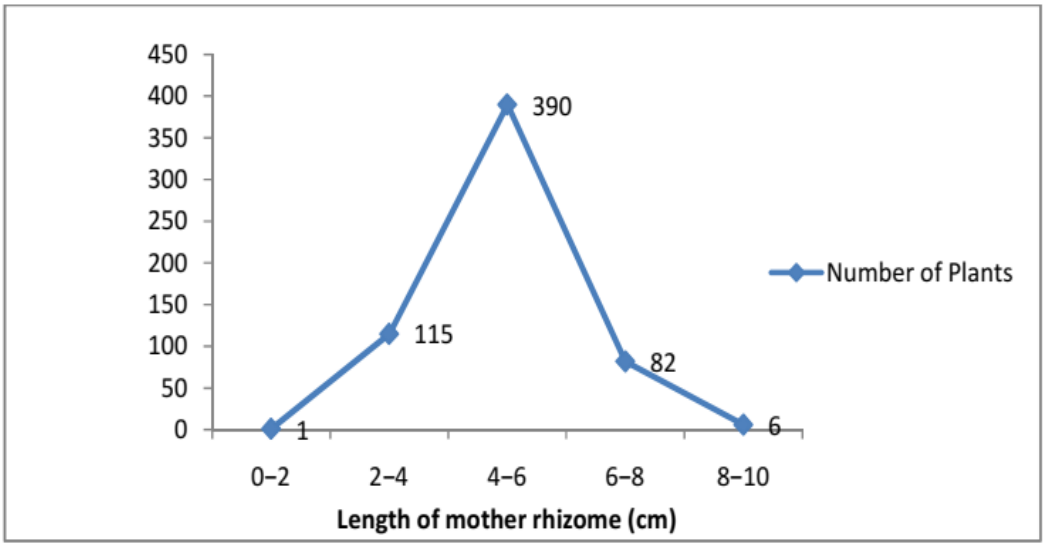
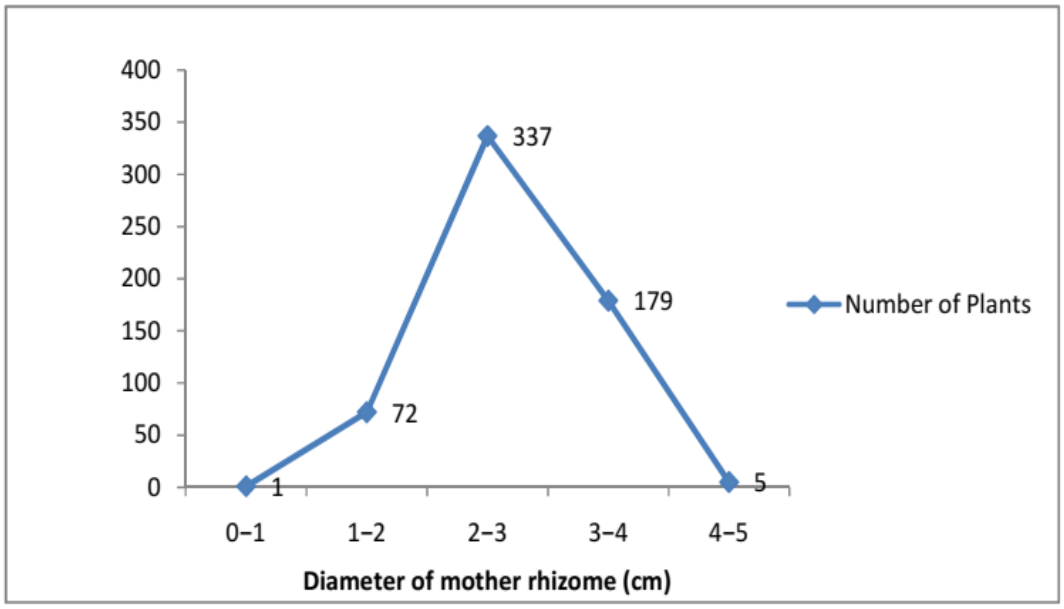


Table 4.15. Frequency distribution of diameter of mother rhizome

| Diameter of mother rhizome (cm) | Number of plants |
|---|---|
| 0–1 | 1 |
| 1–2 | 72 |
| 2–3 | 337 |
| 3–4 | 179 |
| 4–5 | 5 |

Fig.4.15. Frequency curve of diameter of mother rhizome



Even though, high yielding plants are available in the germplasm their frequency is very low. Selection for yield and yield component characters is very important in the genetic stock of *K. rotunda* occurring in Kerala. The above analysis shows that the genetic base of *K. rotunda* in the study area is comparatively broad and there exists no threat of narrowing of genetic diversity which threatens the existence of *K. rotunda* in natural growing areas and homesteads where they are conventionally cultivated. However, due to crop rotation, industrial agriculture and shift to monocropping, underutilized crops including *K. rotunda* face acute threat in their natural and cultivated habitats. Hence, steps should be taken to conserve the species in its natural cultivated habitats so that the diversity of the species is maintained possibly and the unexplored potential of this species is available to the future generations to study and develop plant based drugs and nutraceuticals that improve the quality of human life significantly.

From the study of Athira (2019) in *Curcuma zanthorrhiza*, characters such as number of tillers, leaf length, leaf area, yield per plant, number of primary fingers, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger and length of mother rhizome showed skewness of the distribution towards the distal end of the distribution curve indicating the contribution of dominant alleles for this character. It also confirms the essentiality of selection for better phenotypes and genotypes with higher number of dominant contributing alleles so as to develop superior varieties.

Frequency distribution analysis has been studied by different workers in different crops like coffee (Raghu *et al*., 2003; Nikhila *et al*., 2002; Dharmaraj and Sreenivasan, 1992 and Sreenivasan and Ram, 1993), rice (Paramasivan and Sreerangaswamy, 1988), vanilla (Umamaheswari and Mohanan, 2004), *Cassia tora* (Chandramohanan and Mohanan, 2005),

*Withania somnifera* (Khandalkar *et al*., 1993; Misra *et al*., 1998), *Curcuma amada* and *Kaempferia galanga* (Jayasree, 2009), *Curcuma aeruginosa* (Soorya *et al*., 2016) and West Indian arrowroot (Shintu *et al*., 2016a). Such works have been useful in studying the genetic control of agronomic characters and the distribution of alleles in the respective gene pools.

### 4.1.2. Phenotypic and genotypic variability

Phenotypic variability is the total variability that is observable. It involves both genotypic as well as environmental variation and hence it changes under different environmental conditions. Such variations are studied in terms of phenotypic variance. Genotypic variability is the inherent variability which remains unaltered by environmental conditions. This type of variability is more useful to a plant breeder while practicing selection or hybridization. The present study is an effort to analyze the germplasm of *K. rotunda* collected and conserved for the purpose as mentioned above so as to study the extent of heritable and non heritable components of variability in the germplasm.

Phenotypic and genotypic variability of agronomic characters in *K. rotunda* have been studied presently based on six growth and nine yield characters using 68 accessions of the species (Tables 4.16 and 4.17). All the fifteen growth and yield characters showed statistically significant variations between the accessions. All the characters showed statistically significant variation at 1% significance level except number of tillers.

Plant height showed a mean value of 66.98 cm with the range varying from 16.8 to 100.5 cm and a coefficient of variation of 14.74%. Number of tillers per plant ranged from 1 to 7 with a mean value of 2.89 and coefficient of variation of 15.17%. Number of leaves per tiller ranged from 1 to 19 with the mean value of 6.45 and coefficient of variation of 15.66%. Leaf length

varied from 10.2 cm to 59.9 cm with a mean value of 36.57 cm and coefficient of variation of 12.69%. Leaf breadth showed a mean value of 7.94 cm with the range varying from 1.9 cm to 12.9 cm and coefficient of variation of 10.33%. Leaf area ranged from 17.10 $cm^2$ to 342.96 $cm^2$ with the mean value of 180.58 $cm^2$ and coefficient of variation of 18.77%. Yield per plant varied from 20 g to 700 g with a mean value of 163.24 g and coefficient of variation of 44.71%. Number of primary fingers showed a mean value of 8.47 with a range from 1 to 16 and coefficient of variation of 18.42%. Length of primary finger ranged from 1cm to 8 cm with a mean value of 3.79 cm and coefficient of variation of 15.83%. Diameter of primary finger ranged from 0.50 cm to 3.66 cm with a mean value of 2.13 cm and coefficient of variation of 16.90%. Number of secondary fingers ranged from 1 to 38 with the mean value of 11.63 and coefficient of variation of 31.73%. The mean value of length of secondary finger was 2.71 cm with a range varying from 0.40 cm to 7.4 cm and coefficient of variation of 20.66%. Diameter of secondary finger varied from 0.22 cm to 3.24 cm with a mean value of 1.35 cm and coefficient of variation of 19.26%. Length of mother rhizome ranged from 1.5 cm to 10 cm with the mean value of 4.86 cm and coefficient of variation of 12.55%. Diameter of mother rhizome varied from 0.79 cm to 4.29 cm with a mean value of 2.70 cm and coefficient of variation of 12.96%.

Among the six growth characters the highest coefficient of variation was shown by leaf area (18.77%) followed by number of leaves per tiller (15.66%) and the lowest coefficient of variation was shown by leaf breadth (10.33%). Plant height showed a coefficient of variation of 14.74%, number of tillers showed a coefficient of variation of 15.17% and leaf length showed a coefficient of variation of 12.69%. Among the nine yield characters the highest coefficient of variation was shown by yield per plant (44.71%). The coefficient of variation of number of primary fingers was 18.42%, length of primary finger 15.83%, diameter of primary finger 16.90%, number of

secondary fingers 31.73%, length of secondary finger 20.66%, diameter of secondary finger 19.26%, length of mother rhizome 12.55% and diameter of mother rhizome 12.96%. Among the growth characters leaf area has been found to be the most variable character followed by number of leaves per tiller.

Table 4. 16. Genetic variability of the growth characters in *Kaempferia rotunda* studied.

| Accession Number | Plant height ** | Number of tillers * | Number of leaves per tiller** | Leaf length ** | Leaf breadth ** | Leaf area ** |
|---|---|---|---|---|---|---|
| CUR 1 | 60.56 ±1.46 | 3.10±0.05 | 5.77±0.12 | 31.93±0.77 | 7.33±0.17 | 145.01±6.22 |
| CUR 2 | 62.84±0.89 | 3.44±0.09 | 5.49±0.04 | 33.35±0.47 | 7.53±0.06 | 154.18±3.40 |
| CUR 3 | 64.08±0.40 | 3.33±0.08 | 7.35±0.21 | 34.00±0.25 | 7.28±0.10 | 154.28±2.78 |
| CUR 4 | 57.42±0.92 | 2.49±0.02 | 6.97±0.07 | 30.10±0.56 | 6.51±0.09 | 127.09±2.96 |
| CUR 5 | 62.83±0.51 | 2.22±0.13 | 6.62±0.18 | 36.00±0.42 | 6.98±0.08 | 155.31±3.38 |
| CUR 6 | 51.20±0.55 | 2.44±0.09 | 6.12±0.17 | 30.14±0.17 | 6.10±0.03 | 116.86±0.97 |
| CUR 7 | 69.06±1.04 | 3.33±0.08 | 6.18±0.03 | 36.84±0.37 | 8.45±0.05 | 193.70±1.99 |
| CUR 8 | 45.83±0.35 | 2.66±0.08 | 5.64±0.03 | 27.12±0.03 | 8.54±0.07 | 143.67±1.35 |
| CUR 9 | 43.96±0.50 | 2.77±0.02 | 6.58±0.07 | 28.36±0.51 | 7.67±0.15 | 138.91±4.94 |
| CUR 10 | 44.14±0.37 | 3.22±0.02 | 5.16±0.04 | 27.13±0.10 | 8.97±0.01 | 150.62±0.88 |
| CUR 11 | 48.14±0.47 | 3.33±0.08 | 4.76±0.07 | 29.40±0.22 | 8.87±0.10 | 161.87±2.71 |
| CUR 12 | 48.55±0.57 | 2.99±0.07 | 5.7±0.11 | 28.33±0.15 | 8.34±0.11 | 145.06±1.96 |
| CUR 13 | 58.64±0.94 | 3.05±0.08 | 5.47±0.03 | 34.03±0.39 | 8.53±0.10 | 179.95±3.62 |
| CUR 14 | 66.55±0.87 | 2.99±0.08 | 5.9±0.04 | 36.82±0.56 | 7.93±0.09 | 179.96±3.77 |
| CUR 15 | 66.88±0.63 | 3.33±0.08 | 5.96±0.04 | 37.98±0.33 | 8.91±0.03 | 206.3±2.17 |
| CUR 16 | 70.09±0.14 | 3.44±0.14 | 5.21±0.05 | 39.31±0.22 | 8.42±0.10 | 204.61±3.16 |
| CUR 17 | 64.05±0.50 | 3.33±0.04 | 4.67±0.02 | 38.28±0.16 | 9.15±0.13 | 214.84±3.59 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CUR 18 | 61.63±0.58 | 2.88±0.08 | 5.88±0.28 | 35.64±0.45 | 8.46±0.03 | 186.48±2.66 |
| CUR 19 | 72.09±1 | 2.66±0.08 | 5.93±0.11 | 41.17±0.47 | 8.77±0.04 | 220.67±1.73 |
| CUR 20 | 66.85±0.37 | 2.88±0.12 | 4.51±0.04 | 39.86±0.20 | 9.25±0.04 | 229.36±1.53 |
| CUR 21 | 71.28±0.19 | 3.10±0.08 | 5.44±0.03 | 39.28±0.08 | 9.96±0.05 | 239.55±0.80 |
| CUR 22 | 71.99±1.40 | 2.44±0.08 | 7.1±0.20 | 37.79±0.54 | 7.94±0.06 | 184.55±2.11 |
| CUR 23 | 74.60±1.46 | 2.44±0.12 | 5.75±0.14 | 39.83±0.72 | 8.02±0.03 | 196.94±3.81 |
| CUR 24 | 64.85±0.77 | 2.66±0.11 | 6.68±0.16 | 34.73±0.26 | 7.48±0.10 | 168.78±1.65 |
| CUR 25 | 75.08±0.22 | 3.44±0.06 | 5.65±0.06 | 38.43±0.22 | 8.29±0.08 | 196.16±2.31 |
| CUR 26 | 70.28±1.01 | 3.33±0.04 | 5.16±0.07 | 38.45±0.47 | 8.99±0.11 | 213.96±4.59 |
| CUR 27 | 72.45±0.81 | 3.10±0.13 | 6.25±0.28 | 41.02±0.33 | 8.03±0.08 | 202.94±1.14 |
| CUR 28 | 71.78±0.88 | 3.44±0.10 | 6.01±0.05 | 40.52±0.43 | 8.90±0.04 | 221.05±1.30 |
| CUR 29 | 69.93±0.71 | 2.88±0.02 | 5.05±0.10 | 39.05±0.47 | 9.17±0.09 | 222.85±4.10 |
| CUR 30 | 82.25±0.71 | 2.88±0.02 | 7.21±0.15 | 48.37±0.44 | 8.89±0.06 | 251.64±2.83 |
| CUR 31 | 64.43±1.44 | 3.55±0.06 | 4.84±0.05 | 37.40±0.78 | 7.88±0.20 | 199.46±6.79 |
| CUR 32 | 69.06±0.55 | 3.66±0.04 | 5.71±0.08 | 41.78±0.32 | 8.32±0.01 | 213.87±1.70 |
| CUR 33 | 49.78±0.72 | 3.22±0.02 | 5.46±0.16 | 28.56±0.55 | 7.73±0.15 | 137.30±5.04 |
| CUR 34 | 76.13±0.25 | 4.33±0.15 | 7.32±0.15 | 38.96±0.15 | 8.16±0.09 | 195.04±2.17 |
| CUR 35 | 83.08±0.81 | 3.38±0.04 | 6.95±0.04 | 41.64±0.38 | 8.22±0.08 | 210.80±3.48 |
| CUR 36 | 66.01±1.22 | 2.44±0.06 | 7.21±0.31 | 36.30±0.55 | 7.32±0.06 | 164.28±3.43 |
| CUR 37 | 68.55±0.87 | 2.66±0.04 | 7.56±0.44 | 36.63±0.27 | 6.82±0.01 | 153.99±1.46 |

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|---|---|---|---|---|---|---|
| CUR 38 | 62.09±0.99 | 2.66±0.04 | 5.40±0.01 | 36.17±0.49 | 7.81±0.04 | 173.85±3.28 |
| CUR 39 | 60.89±0.25 | 2.33±0.04 | 6.66±0.15 | 34.79±0.12 | 7.11±0.10 | 152.85±2.68 |
| CUR 40 | 61.02±0.33 | 2.77±0.05 | 6.51±0.18 | 35.4±0.16 | 7.51±0.04 | 164.40±1.91 |
| CUR 41 | 75.86±0.99 | 1.88±0.09 | 7.62±0.20 | 37.66±0.14 | 6.66±0.02 | 154.41±0.52 |
| CUR 42 | 61.93±1.41 | 2.11±0.02 | 7.97±0.17 | 36.22±0.77 | 8.17±0.14 | 187.64±5.86 |
| CUR 43 | 68.64±0.71 | 2.33±0.12 | 6.97±0.18 | 35.04±0.26 | 6.86±0.05 | 148.74±1.29 |
| CUR 44 | 75.61±0.73 | 2.88±0.06 | 8.16±0.11 | 37.24±0.47 | 7.70±0.15 | 181.46±5.56 |
| CUR 45 | 80.37±0.40 | 2.88±0.02 | 7.99±0.17 | 40.66±0.26 | 8.82±0.05 | 220.44±2.88 |
| CUR 46 | 78.14±0.60 | 2.55±0.05 | 6.82±0.01 | 40.21±0.24 | 7.79±0.02 | 188.53±0.76 |
| CUR 47 | 86.72±1.03 | 2.66±0.04 | 8.49±0.21 | 45.67±0.26 | 8.84±0.06 | 246.31±1.05 |
| CUR 48 | 73.99±0.70 | 3±0.12 | 5.90±0.20 | 35.77±0.79 | 6.98±0.22 | 157.66±7.78 |
| CUR 49 | 67.40±0.29 | 2.66±0.04 | 7.27±0.08 | 37.54±0.22 | 8.04±0.02 | 186.29±1.64 |
| CUR 50 | 66.15±0.56 | 2.55±0.06 | 6.82±0.12 | 34.43±0.25 | 7.16±0.08 | 153.64±2.21 |
| CUR 51 | 64.05±0.34 | 2.55±0.05 | 6.01±0.06 | 35.10±0.16 | 7.22±0.09 | 158.28±1.74 |
| CUR 52 | 65.51±0.78 | 3.49±0.02 | 6.04±0.10 | 32.82±0.37 | 6.98±0.06 | 141.17±2.77 |
| CUR 53 | 52.14±0.97 | 2.66±0.12 | 6.10±0.03 | 27.79±0.42 | 6.38±0.06 | 109.38±1.81 |
| CUR 54 | 57.80±0.76 | 2.88±0.17 | 6.84±0.16 | 30.19±0.38 | 6.56±0.08 | 123.4±2.46 |
| CUR 55 | 74.33±2.50 | 2.66±0.07 | 7.82±0.18 | 41.90±0.50 | 7.56±0.06 | 200.58±3.41 |
| CUR 56 | 66.13±0.62 | 3.10±0.06 | 5.58±0.11 | 35.52±0.08 | 7.32±0.08 | 161.74±1.84 |
| CUR 57 | 73.76±1.07 | 2.77±0.06 | 6.18±0.06 | 37.28±0.44 | 7.61±0.11 | 175.60±4.60 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CUR 58 | 67.14±0.35 | 2.10±0.05 | 6.54±0.19 | 34.48±0.11 | 7.2±0.02 | 157.80±0.62 |
| CUR 59 | 66.68±0.69 | 2.88±0.05 | 6.95±0.04 | 36.06±0.45 | 7.32±0.12 | 163.46±4.38 |
| CUR 60 | 76.44±0.85 | 2.99±0.08 | 7.68±0.17 | 40.84±0.14 | 8.13±0.12 | 202.90±2.86 |
| CUR 61 | 85.39±0.76 | 3.11±0.02 | 9.17±0.09 | 43.45±0.52 | 9.01±0.19 | 238.03±4.88 |
| CUR 62 | 71.02±1.22 | 3±0.12 | 6.34±0.08 | 36.34±0.52 | 7.29±0.07 | 165.73±3.29 |
| CUR 63 | 80.40±0.47 | 1.94±0.05 | 8.01±0.18 | 45.28±0.10 | 9.08±0.17 | 252.05±5.44 |
| CUR 64 | 70.86±1.31 | 3.44±0.06 | 7.06±0.04 | 38.85±0.50 | 8.64±0.11 | 207.78±4.33 |
| CUR 65 | 52.11±0.26 | 3±0 | 6.11±0.04 | 28.94±0.26 | 7.60±0.17 | 138.28±4.28 |
| CUR 66 | 83.79±0.73 | 2.83±0.05 | 8.15±0.18 | 44.04±0.22 | 7.95±0.12 | 191.75±6.47 |
| CUR 67 | 67.64±0.58 | 2.55±0.05 | 7.57±0.12 | 36.3±0.36 | 7.99±0.08 | 179.74±3.38 |
| CUR 68 | 73.75±0.15 | 2.66±0.07 | 6.62±0.07 | 40.12±0.17 | 8.54±0.06 | 213.43±1.84 |
| Mean | 66.98 | 2.89 | 6.45 | 36.57 | 7.94 | 180.58 |
| Range | 16.8-100.5 | 1-7 | 1-19 | 10.2-59.9 | 1.9-12.9 | 17.10-342.96 |
| CV | 14.74 | 15.17 | 15.66 | 12.69 | 10.33 | 18.77 |
| SD | 9.87 | 0.44 | 1.01 | 4.64 | 0.82 | 33.90 |
| CD at 5% | 11.15 | 1.02 | 1.86 | 5.25 | 1.27 | 44.69 |

**Significant at 1% level, *Significant at 5% level.

Among the yield characters, yield per plant is the most variable character followed by number of secondary fingers and the least variable character is the length of mother rhizome. Such a significant variability with respect to growth and yield characters indicates the strong genetic base of the species. Statistically significant levels of variability in the case of all the characters studied points out the possibility of selection of promising genotypes based on the above characters in further improvement programmes.

Phenotypic coefficient of variation (PCV) was found to be higher than genotypic coefficient of variation (GCV) for all the characters studied. This shows the presence of environmental influence to some degree in the phenotypic expression of characters. High PCV and GCV describes that the genotypes show evidence of much variation among themselves with respect to morphological characters. Low values of PCV and GCV specify that the genotypes do not show much variation among themselves with respect to such morphological characters (Mishra *et al*., 2015 and Baye *et al*., 2005). All the characters studied showed higher PCV when compared to GCV, indicating the additive nature, polygenic control and differential degrees of environmental influence on these characters (Table 4.18). The PCV of the observed characters varied between 13.10% for leaf breadth and 51.55% for yield per plant, while the GCV ranged between 8.28% for number of tillers and 40.88% for yield per plant. Among the yield characters, the highest PCV (51.55%) and GCV (40.88%) were observed for yield per plant. Plant height showed a PCV of 17.01% and GCV of 13.45% and number of tillers showed a PCV of 23.45% and GCV of 8.28%. Number of leaves per tiller showed a PCV of 21.55% and GCV of 11.63%. Leaf length showed a PCV of 14.66% and GCV of 11.59% and leaf breadth showed a PCV of 13.10% and GCV of 8.56%. Leaf area showed a PCV of 22.62% and GCV of 16.51%. In the case of the yield characters, both the values of PCV and GCV were comparatively higher. Length of mother rhizome showed the lowest values of GCV

(10.91%) and PCV (15.43%) compared to the other yield characters. The differences between PCV and the corresponding GCV were higher in the case of number of tillers and number of secondary fingers indicating comparatively higher influence of environment on the phenotypic expression of these characters compared to the remaining characters. Characters with lesser difference between PCV and GCV show the limited role of environment on these characters. If the values of heritability and genetic advance of these characters coincide with the above mentioned observations, it favours direct selection based on phenotypic performance of these traits for further improvement of the species.

Similar studies on genetic variability of species based on statistical significance of the variations between accessions and analysis of phenotypic and genotypic coefficient of variation have been worked out by earlier researchers and the data generated from these studies have been used for further improvement programmes. Radhakrishnan *et al.* (2006a) conducted such studies in cardamom, Baye *et al.* (2005) in potato, Alkkudsi *et al.* (2013) in cotton, Aditya *et al.* (2011) in soybean, Sethi *et al*. (2016) in cashew, Chandramohan *et al.* (2016) in rice, Roychowdhary and Tah (2011) in Dianthus, Doss *et al.* (2012) in mulberry, Reddy *et al.* (2013) in linseed, Meena *et al.* (2014) in coriander, Shintu *et al.* (2016b) in West Indian arrowroot, Kumar *et al*. (2014) and Maruti and Rani (2015) in maize, Jayasree *et al.* (2006) and Jayasree *et al.* (2014a) in mango ginger, Kahanom *et al.* (2008) in tomato, Mishra *et al.* (2015) in strawberry and Anju (2015), Singh *et al*. (2003), Lynrah *et al.* (1998) and Mishra *et al.* (2015) in *Curcuma longa*. Hence the present work will enable the breeders and farmers to understand the mode and extent of genetic variability associated with *K. rotunda* in the study area.

Table 4. 17. Genetic variability of the yield characters of *Kaempferia rotunda* studied.

| Accession Number | Yield per plant (g)** | Number of primary fingers** | Length of primary finger (cm)** | Diameter of primary finger (cm)** | Number of secondary fingers** | Length of secondary finger (cm)** | Diameter of secondary finger (cm)** | Length of mother rhizome (cm)** | Diameter of mother rhizome (cm)** |
|---|---|---|---|---|---|---|---|---|---|
| CUR 1 | 123.88±5.39 | 10.22±0.45 | 4.45±0.11 | 2.16±0.03 | 17.33±0.64 | 3.76±0.15 | 1.46±0.04 | 5.54±0.17 | 2.9±0.07 |
| CUR 2 | 94.16±3.01 | 9.88±0.15 | 4.4±0.02 | 2.44±0.03 | 12.88±0.50 | 3.02±0.04 | 1.7±0.02 | 6.37±0.13 | 3.07±0.05 |
| CUR 3 | 135.55±2.84 | 10.44±0.09 | 3.99±0.04 | 2.41±0.04 | 16.88±0.46 | 2.95±0.02 | 1.6±0.02 | 4.93±0.08 | 2.76±0.03 |
| CUR 4 | 155.27±0.87 | 5.66±0.19 | 3.13±0.04 | 1.48±0.02 | 6.00±0.24 | 2.28±0.02 | 1.00±0.03 | 3.30±0.08 | 1.99±0.06 |
| CUR 5 | 86.11±2.63 | 6.32±0.14 | 3.72±0.04 | 1.89±0.02 | 9.66±0.36 | 2.56±0.06 | 1.35±0.02 | 3.84±0.06 | 2.52±0.02 |
| CUR 6 | 84.72±3.25 | 10.44±0.05 | 3.00±0.07 | 1.64±0.02 | 9.22±0.44 | 2.28±0.11 | 1.06±0.04 | 4.64±0.05 | 2.50±0.04 |
| CUR 7 | 139.88±5.70 | 9.21±0.15 | 4.66±0.08 | 2.81±0.03 | 15.21±0.64 | 3.68±0.12 | 1.64±0.04 | 5.75±0.10 | 3.26±0.06 |
| CUR 8 | 116.66±4.63 | 8.77±0.16 | 4.23±0.0 | 2.17±0.04 | 18.55±0.38 | 2.29±0.05 | 1.33±0.03 | 5.08±0.05 | 2.65±0.02 |
| CUR 9 | 125±0.15 | 10.22±0.10 | 3.64±0.09 | 1.98±0.03 | 14.77±2.87 | 2.28±0.02 | 1.16±0.03 | 4.72±0.11 | 2.57±0.07 |
| CUR 10 | 116.66±2.29 | 8.66±0.04 | 3.99±0.05 | 2.22±0 | 11.55±0.05 | 2.64±0.10 | 1.41±0.03 | 5.18±0.01 | 2.9±0.01 |
| CUR 11 | 128.33±4.35 | 8.77±0.13 | 3.56 ±0.03 | 1.98±0.02 | 10.99±0.15 | 2.76±0.04 | 1.29±0.01 | 5.01±0.11 | 2.72±0.02 |
| CUR 12 | 97.77±3.39 | 8.99±0.11 | 3.47±0.04 | 2.04±0.03 | 17.88±0.28 | 2.67±0.03 | 1.15±0.01 | 4.74±0.02 | 2.61±0.01 |
| CUR 13 | 148.61±7.98 | 8.27±0.03 | 3.65±0.07 | 2.56±0.04 | 12.00±0.42 | 2.35±0.04 | 1.43±0.03 | 4.49±0.10 | 2.79±0.01 |
| CUR 14 | 117.21±2.53 | 7.66±0.18 | 4.47±0.13 | 2.76±0.04 | 13.55±0.86 | 3.23±0.04 | 1.72±0.04 | 5.65±0.06 | 3.24±0.02 |
| CUR 15 | 165.55±5.79 | 10.21±0.09 | 4.35±0.07 | 2.42±0.02 | 15.88±0.59 | 2.97±0.01 | 1.58±0.01 | 5.40±0 | 3.03±0.03 |
| CUR 16 | 129.44±5.27 | 9.88±0.13 | 4.13±0.09 | 2.32±0.02 | 13.10±0.44 | 2.85±0.02 | 1.49±0.04 | 5.72±0.02 | 3.02±0.05 |
| CUR 17 | 105.55±0.71 | 8.05±0.13 | 4.11±0.12 | 2.75±0.03 | 13.22±0.50 | 2.69±0.08 | 1.66±0.02 | 5.13±0.05 | 3.04±0.03 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CUR 18 | 144.99±5.00 | 8.88±0.10 | 2.24±0.03 | 2.24±0.04 | 16.33±0.19 | 3.08±0.05 | 1.38±0.03 | 4.83±0.06 | 2.83±0.05 |
| CUR 19 | 122.22±0.91 | 7.33±0.15 | 3.92±0.11 | 2.27±0.03 | 8.22±0.30 | 2.67±0.10 | 1.15±0.04 | 5.40±0.08 | 2.8±0 |
| CUR 20 | 114.16±3.70 | 8.72±0.09 | 3.99±0.06 | 2.63±0.07 | 11.44±0.91 | 3.14±0.06 | 1.65±0.04 | 5.40±0.04 | 3.16±0.02 |
| CUR 21 | 157.77±5.27 | 11.33±0.04 | 4.74±0.05 | 3.03±0.08 | 16.44±0.53 | 3.29±0.10 | 1.64±0.07 | 5.61±0.07 | 3.32±0.02 |
| CUR 22 | 115.55±3.04 | 8.22±0.02 | 4.10±0.13 | 2.06±0.01 | 11.10±0.39 | 2.77±0.10 | 1.47±0.01 | 5.12±0.04 | 2.82±0.03 |
| CUR 23 | 99.99±2.67 | 6.77±0.10 | 3.33±0.09 | 1.80±0.02 | 7.10±0.47 | 2.20±0.08 | 0.96±0.02 | 4.3±0.02 | 2.63±0.03 |
| CUR 24 | 76.11±4.21 | 6.99±0.18 | 4.05±0.10 | 2.05±0.02 | 7.55±0.33 | 3.15±0.02 | 1.32±0.02 | 4.75±0.03 | 2.72±0.04 |
| CUR 25 | 146.10±2.39 | 8.88±0.05 | 5.25±0.10 | 2.38±0.03 | 13.55±0.36 | 3.49±0.06 | 1.49±0.03 | 5.65±0.06 | 2.9±0 |
| CUR 26 | 103.88±0.42 | 9.88±0.05 | 4.56±0.10 | 2.67±0.01 | 13.77±0.08 | 3.18±0.04 | 1.83±0.04 | 5.57±0.05 | 3.37±0.02 |
| CUR 27 | 93.88±1.35 | 6.55±0.05 | 3.79±0.03 | 2.04±0.02 | 8.33±0.12 | 2.51±0.06 | 1.33±0.02 | 5.14±0.08 | 2.58±0.05 |
| CUR 28 | 147.21±3.14 | 7.55±0.17 | 4.12±0.03 | 2.47±0.03 | 14.11±0.47 | 3.64±0.06 | 1.87±0.05 | 4.87±0.05 | 3.07±0.01 |
| CUR 29 | 89.44±5.05 | 4.10±0.13 | 2.71±0.03 | 1.55±0.05 | 4.22±0.2 | 2.16±0.02 | 1.04±0.04 | 4.76±0.07 | 2.41±0.04 |
| CUR 30 | 121.66±1.58 | 7.33±0.11 | 4.39±0.12 | 2.21±0.03 | 9.22±0.12 | 3.17±0.07 | 1.37±0 | 5.35±0.05 | 3.05±0.01 |
| CUR 31 | 176.10±1.91 | 5.33±0.07 | 3.83±0.04 | 2.14±0.02 | 6.32±0.14 | 2.56±0.03 | 1.26±0.03 | 5.35±0.05 | 2.69±0.04 |
| CUR 32 | 131.66±0.53 | 9.11±0.10 | 4.59±0.03 | 2.8±0.06 | 15.55±0.67 | 3.25±0.08 | 1.88±0.05 | 5.82±0.09 | 3.06±0.02 |
| CUR 33 | 111.66±3.45 | 7.88±0.02 | 3.63±0.06 | 1.99±0.03 | 10.44±0.40 | 2.37±0.04 | 1.2±0.03 | 4.35±0.02 | 2.21±0.03 |
| CUR 34 | 164.44±3.73 | 9.66±0.04 | 4.1±0.06 | 2.67±0.06 | 18.88±0.49 | 3.86±0.05 | 1.78±0.02 | 5.33±0.07 | 3.12±0.04 |
| CUR 35 | 104.99±3.68 | 9.88±0.20 | 3.85±0.05 | 2.37±0.04 | 12.88±0.33 | 3.54±0.06 | 1.77±0.03 | 5.32±0.14 | 2.98±0.09 |
| CUR 36 | 134.44±0.96 | 7.22±0.08 | 3.84±0.08 | 2.08±0.01 | 10.44±0.62 | 2.62±0.06 | 1.47±0 | 4.36±0.03 | 2.68±0.03 |
| CUR 37 | 190.55±14 | 3.88±0.10 | 3.44±0.08 | 2.00±0.04 | 4.88±0.19 | 1.56±0.05 | 1.10±0.05 | 4.38±0.02 | 2.37±0.02 |
| CUR 38 | 117.77±1.46 | 9.55±0.13 | 3.94±0.02 | 2.11±0.03 | 13.44±0.16 | 2.53±0.04 | 1.48±0.04 | 5.66±0.05 | 3.16±0.01 |
| CUR 39 | 131.66±6.62 | 7.88±0.10 | 3.45±0.06 | 2.13±0.02 | 13.99±0.07 | 2.50±0 | 1.31±0.02 | 4.78±0.05 | 2.84±0.01 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CUR 40 | 133.11±7.01 | 8±0 | 3.38±0.03 | 2.04±0 | 8.22±0.2 | 2.42±0.06 | 1.19±0.04 | 4.4±0.02 | 3.04±0.02 |
| CUR 41 | 132.77±8.02 | 9.10±0.25 | 3.78±0.04 | 1.89±0.03 | 13.10±0.26 | 2.47±0.03 | 1.27±0.02 | 5.09±0.06 | 2.55±0.02 |
| CUR 42 | 165.27±6.56 | 9.77±0.31 | 4.13±0.05 | 2.19±0.04 | 9.61±0.45 | 2.48±0.09 | 1.28±0.05 | 5.03±0.09 | 2.68±0.05 |
| CUR 43 | 127.77±5.32 | 7.22±0.19 | 2.61±0.02 | 1.38±0.03 | 4.88±0.08 | 2.15±0.02 | 0.85±0 | 4.35±0.06 | 2.18±0.03 |
| CUR 44 | 278.32±12.14 | 8.21±0.18 | 3.92±0.08 | 1.97±0.03 | 11.66±0.18 | 2.52±0.05 | 1.35±0.04 | 4.21±0.10 | 2.60±0.04 |
| CUR 45 | 349.99±2.13 | 8.88±0.20 | 4.09±0.04 | 1.92±0.02 | 13.10±0.33 | 2.57±0.02 | 1.23±0.02 | 4.50±0.03 | 2.4±0.01 |
| CUR 46 | 198.88±3.04 | 7.10±0.28 | 3.50±0.07 | 2.00±0.03 | 9.10±0.13 | 2.57±0.06 | 1.14±0.02 | 4.04±0.04 | 2.06±0.01 |
| CUR 47 | 233.88±4.48 | 11.44±0.21 | 3.85±0.04 | 2.11±0.02 | 15.77±0.48 | 2.64±0.06 | 1.23±0.01 | 5.11±0.04 | 2.93±0.04 |
| CUR 48 | 197.77±9.17 | 8.55±0.15 | 3.98±0.02 | 2.13±0.03 | 11.55±0.60 | 2.85±0.01 | 1.30±0.03 | 4.26±0.03 | 2.56±0.04 |
| CUR 49 | 249.44±7.60 | 8.11±0.15 | 3.98±0.03 | 2.05±0.02 | 11.55±0.26 | 2.59±0.07 | 1.37±0.06 | 4.65±0.08 | 2.76±0.05 |
| CUR 50 | 187.77±7.33 | 9.66±0.26 | 3.60±0.05 | 2.26±0.02 | 14.44±0.34 | 3.05±0.05 | 1.56±0.01 | 5.56±0.08 | 2.90±0.04 |
| CUR 51 | 155.55±2.02 | 7.77±0.11 | 3.81±0.08 | 2.14±0.04 | 6.99±0.04 | 2.69±0.03 | 1.35±0.03 | 4.80±0.03 | 2.75±0.01 |
| CUR 52 | 111.38±4.26 | 9.61±0.06 | 3.96±0.07 | 2.04±0.03 | 9.66±0.14 | 2.45±0.07 | 1.2±0.02 | 4.69±0.06 | 2.65±0.05 |
| CUR 53 | 81.10±2.53 | 6.55±0.33 | 2.54±0.01 | 1.29±0.01 | 6.44±0.16 | 1.62±0.02 | 0.8±0.01 | 3.97±0.03 | 2.05±0.01 |
| CUR 54 | 98.33±4.32 | 8.33±0.08 | 2.33±0.09 | 1.42±0.05 | 8.44±0.66 | 1.32±0.08 | 0.87±0.06 | 3.64±0.02 | 1.94±0.03 |
| CUR 55 | 258.33±12.37 | 9.33±0.21 | 3.94±0.13 | 2.15±0.04 | 12.55±0.24 | 2.48±0.05 | 1.42±0.05 | 5.05±0.07 | 2.74±0.01 |
| CUR 56 | 190.83±8.05 | 9.88±0.18 | 3.09±0.03 | 2.02±0.02 | 9.77±0.42 | 2.62±0.05 | 1.29±0.03 | 4.92±0.05 | 2.56±0.03 |
| CUR 57 | 168.33±3.16 | 8.77±0.12 | 3.25±0.04 | 1.84±0.02 | 5.88±0.12 | 2.18±0.01 | 1.17±0.02 | 4.6±0.05 | 2.23±0.01 |
| CUR 58 | 158.88±2.53 | 6.88±0.10 | 2.46±0.02 | 1.46±0.03 | 6.33±0.15 | 1.57±0.04 | 0.94±0.02 | 3.38±0.03 | 1.92±0 |
| CUR 59 | 226.66±5.73 | 10.11±0.05 | 3.14±0.03 | 1.85±0.02 | 11.99±0.45 | 2.24±0.07 | 1.21±0.03 | 4.30±0.02 | 2.47±0.04 |
| CUR 60 | 312.77±8.24 | 10.22±0.24 | 3.20±0.01 | 1.65±0.02 | 11.99±0.40 | 1.94±0 | 1.03±0 | 4.14±0.03 | 2.25±0.02 |
| CUR 61 | 363.33±10.16 | 8.66±0.23 | 4.67±0.08 | 2.32±0.05 | 14.55±0.16 | 4.15±0.02 | 1.81±0.03 | 4.82±0.08 | 2.54±0.06 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CUR 62 | 273.88±19.38 | 6.22±0.04 | 3.62±0.02 | 2.08±0.04 | 11.16±0.60 | 2.7±0.03 | 1.30±0 | 4.57±0.05 | 2.55±0.05 |
| CUR 63 | 252.77±12.72 | 10.88±0.23 | 3.96±0.08 | 1.90±0.04 | 10.38±0.34 | 2.22±0.03 | 1.07±0.02 | 5.17±0.07 | 2.82±0.05 |
| CUR 64 | 401.10±7.03 | 9.99±0.35 | 4.61±0.05 | 2.76±0.03 | 20.77±1.17 | 3.77±0.12 | 1.85±0.03 | 5.30±0.08 | 3.11±0.03 |
| CUR 65 | 120.07±3.98 | 8±0.12 | 3.70±0.08 | 1.80±0.03 | 9.55±0.44 | 2.37±0.05 | 1.18±0.02 | 3.76±0.02 | 2.10±0.03 |
| CUR 66 | 243.88±9.73 | 7.55±0.26 | 3.28±0.08 | 2.00±0.01 | 10.05±0.49 | 2.73±0.10 | 1.3±0.05 | 4.71±0.07 | 2.50±0.02 |
| CUR 67 | 321.66±2.25 | 8.77±0.35 | 4.19±0.03 | 2.13±0.03 | 14.44±0.35 | 3.24±0.11 | 1.34±0 | 5.00±0.02 | 2.51±0.02 |
| CUR 68 | 239.99±9.32 | 7.66±0.04 | 4.09±0.02 | 2.1±0.01 | 10.11±0.34 | 2.70±0.03 | 1.22±0.05 | 4.95±0.03 | 2.54±0.02 |
| Mean | 163.24 | 8.47 | 3.79 | 2.13 | 11.63 | 2.71 | 1.35 | 4.86 | 2.70 |
| Range | 20-700 | 1-16 | 1-8 | 0.50-3.66 | 1-38 | 0.40-7.4 | 0.22-3.24 | 1.5-10 | 0.79-4.29 |
| CV | 44.71 | 18.42 | 15.83 | 16.90 | 31.73 | 20.66 | 19.26 | 12.55 | 12.96 |
| SD | 72.99 | 1.56 | 0.60 | 0.36 | 3.69 | 0.56 | 0.26 | 0.61 | 0.35 |
| CD at 5% | 82.05 | 2.23 | 0.90 | 0.43 | 5.78 | 0.80 | 0.43 | 0.86 | 0.47 |

**Significant at 1% level

Table 4.18. Genotypic variance, phenotypic variance, GCV, PCV, heritability and genetic advance in the case of the growth and yield characters of *Kaempferia rotunda.*

| Sl.No. | Characters | Genotypic variance | Phenotypic variance | Genotypic coefficient of variation | Phenotypic coefficient of variation | Heritability (Broad sense) (%) | Genetic Advance (%) |
|---|---|---|---|---|---|---|---|
| 1 | Plant height (cm)** | 81.18 | 129.75 | 13.45 | 17.01 | 62.57 | 21.87 |
| 2 | Number of tillers * | 0.06 | 0.46 | 8.28 | 23.45 | 13.04 | 6.30 |
| 3 | Number of leaves per tiller** | 0.57 | 1.94 | 11.63 | 21.55 | 29.38 | 13.04 |
| 4 | Leaf length (cm)** | 17.95 | 28.71 | 11.59 | 14.66 | 62.52 | 18.87 |
| 5 | Leaf breadth (cm) ** | 0.46 | 1.09 | 8.56 | 13.10 | 42.20 | 11.39 |
| 6 | Leaf area ($cm^2$)** | 889.5 | 1668.9 | 16.51 | 22.62 | 53.30 | 24.92 |
| 7 | Yield per plant (g)** | 4452.73 | 7081.21 | 40.88 | 51.55 | 62.88 | 66.48 |
| 8 | Number of primary fingers** | 1.78 | 3.74 | 15.70 | 22.79 | 47.59 | 22.33 |
| 9 | Length of primary finger (cm)** | 0.26 | 0.57 | 13.46 | 19.79 | 45.61 | 18.59 |
| 10 | Diameter of primary finger (cm)** | 0.11 | 0.18 | 15.49 | 19.72 | 61.11 | 24.82 |
| 11 | Number of secondary fingers** | 9.25 | 22.3 | 26.14 | 40.58 | 41.48 | 34.69 |
| 12 | Length of secondary finger (cm)** | 0.23 | 0.49 | 17.71 | 25.83 | 46.94 | 24.98 |
| 13 | Diameter of secondary finger (cm)** | 0.04 | 0.11 | 14.81 | 24.44 | 36.36 | 18.31 |
| 14 | Length of mother rhizome (cm)** | 0.28 | 0.57 | 10.91 | 15.43 | 49.12 | 15.62 |
| 15 | Diameter of mother rhizome (cm)** | 0.09 | 0.18 | 11.11 | 15.56 | 50 | 16.02 |

**Significant at 1% level, *Significant at 5% level.

### 4.1.3. Heritability (broad sense) of agronomic characters

Heritability is the amount of observable variation in a population that is attributable to individual genetic differences. Heritability in broad sense is the ratio of genetic variability to the phenotypic variability that is heritable. It aids in measuring the extent of variation due to non-genetic factors. Heritability in narrow sense is the part of observed variance, which is caused by additive genetic variance. Heritability reveals the heritable portion of variability present in different characters. High heritability does not always mean better response to selection since it is also inclusive of non-additive genetic factors (Singh, 1983). However, the study of heritability together with genetic advance would be more reliable and useful in formulating selection procedure (Johnson *et al*., 1955; Archana, 2013).

Fifteen growth and yield characters of *K .rotunda* have been studied presently for broad sense heritability. Growth characters showed heritability varying from 13.04% in the case of number of tillers to 62.57% in the case of plant height. Number of leaves per tiller exhibited 29.38% of heritability, leaf length exhibited 62.52% of heritability, leaf breadth showed a heritability of 42.20% and leaf area showed a heritability of 53.30%. Heritability of yield characters ranged from 36.36% in the case of diameter of secondary finger to 62.88% in the case of yield per plant. Number of primary fingers showed heritability of 47.59%, length of primary finger showed heritability of 45.61%, diameter of primary finger showed heritability of 61.11%, number of secondary fingers showed heritability of 41.48%, length of secondary finger showed heritability of 46.94%, length of mother rhizome showed a heritability of 49.12% and diameter of mother rhizome showed heritability of 50% (Table 4.18). The above analysis indicates the occurrence of comparatively higher heritability in the case of yield characters as compared to growth characters. The characters such as plant height, yield per plant,

diameter of primary finger, leaf length and leaf area showed heritability above 50% and these characters would respond to selection in a better way and the improvement of these parameters could be achieved through direct selection. These characters can be considered for improvement with simple selection in the development of better *K. rotunda* varieties. According to categorization of Babu *et al*. (2012) heritability is low below 30%, medium between 30% and 60% and high above 60% and accordingly the characters studied in the present investigation showed a low heritability for number of tillers and number of leaves per tiller. All the other characters such as leaf breadth, leaf area, number of primary fingers, length of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome showed medium heritability.

Similar works on heritability and the influence of environment on phenotypic variations have been carried out by earlier workers in different crops like wheat (Ali *et al*., 2008), coriander (Tripathi *et al*., 2000), almond (Shah *et al*., 2010), mulberry (Doss *et al*., 2012), ginger (Yadav, 1999; Ravishankar *et al*., 2013), sorghum (Bello *et al*., 2007), linseed (Singh *et al*., 2015), dianthus (Roychowdhary and Tah, 2011), cluster bean (Rai *et al*., 2012), bottle guard (Damor *et al*., 2016), cashew (Sethi *et al.,* 2015), teak (Hrideek *et al*., 2018) and coriander (Nagappa *et al.,* 2018). Characters showing high heritability along with higher genetic potential were useful in selection in breeding programmes.

### 4.1.4. Genetic Advance

Genetic advance is another selection parameter that shows the magnitude of the expected genetic gain from one cycle of selection (Hamdi *et al.,* 2003; Abasianyanga and Balu, 2017). Genetic advance indicates the quantum of improvement of a character that is possible through selection

(Allard, 1960). According to Panse and Sukhatme (1957), if a character is governed by non-additive gene action it may give high heritability but low genetic advance, where as if it is governed by additive gene action heritability and genetic advance would be high. Higher estimates of heritability along with high genetic advance will provide good scope for further improvement programmes (Addisu and Shumet, 2015).

Genetic advance in *K .rotunda* ranged from 6.30% for number of tillers to 66.48% for yield per plant. Yield and yield contributing characters showed higher genetic advance as compared to growth characters. Among the growth characters the highest genetic advance was shown by leaf area (24.92%) followed by plant height (21.87%), leaf length (18.87%), number of leaves per tiller (13.04%) and leaf breadth (11.39%) and the lowest genetic advance was observed in number of tillers (6.30%). Among the yield characters highest genetic advance was shown by yield per plant (66.48%) followed by number of secondary fingers (34.69%), length of secondary finger (24.98%), diameter of primary finger (24.82%), number of primary fingers (22.33%), length of primary finger (18.59%), diameter of secondary finger (18.31%) and diameter of mother rhizome (16.02%) and the lowest genetic advance was shown by length of mother rhizome (15.62%). High values of genetic advance are indicative of additive gene action whereas low values are indicative of non-additive gene action (Singh and Narayanan, 2006). The characters showing high genetic advance would provide good scope in crop improvement programmes.

Similar works on genetic advance have been conducted by earlier workers in different crops like banana (Kavitha *et al*., 2008), glory lily (Rajagopal and Kandhasamy, 2009), elephant foot yam (Anil *et al*., 2011), *Curcuma longa* (Singh *et al*., 2008), *Curcuma aromatica* (Neethu *et al*., 2017), *Curcuma zanthorrhiza* (Athira *et al*., 2018b), mulberry (Doss *et al.,*

2006), cucumber (Yadav *et al*., 2012), ashwagandha (Gami *et al*., 2015), small cardamom (Hrideek *et al*., 2015), barley (Hailu *et al*., 2016), soybean (Neelima *et al*., 2018), finger millet (Wolie *et al*., 2013) and linseed (Singh *et al*., 2019). Studies on genetic advance of growth and yield characters of *K. rotunda* are new to science and it will provide better knowledge on genetic gain feasible through selection.

### 4.2. Correlation of agronomic characters

Most of the agronomic characters of crop plants are polygenic in nature. These characters show varying levels of interrelationships due to common sharing of genes between the characters. The relation existing between two or more variables under consideration is termed correlation. Correlation analysis is a fundamental tool that helps to discover the degree as well as direction of relationship prevailing between the variables. Yield is a character that results from the association and expression of different yield related components. Hence, knowledge on the degree of this interrelatonsip through correlation studies can identify characters that could be used as indirect selection criteria for yield, thus improving the efficiency of the selection process. Study of correlation provides the information that how strongly characters are genetically associated with one another. The influence of each character on yield could be known through correlation analysis with a view to find out the extent and nature of relationships prevailing among yield and yield contributing traits. If the observed correlation is due to multiple effects of the same gene, selection for one character will improve another. Hence, correlation among traits influences the effectiveness of selection. Thus, the estimates of correlation among yield contributing characters pave the basis for selection of superior genotypes from the diverse breeding populations.

Correlation analysis of fifteen agronomic characters of *K. rotunda* has been carried out presently using the data obtained from sixty eight accessions with an intention to identify the interrelationship existing between different characters. In the present study length of primary finger, diameter of primary finger, length of secondary finger, length of mother rhizome and diameter of mother rhizome exhibited significant correlation with the maximum number of characters followed by leaf length, leaf breadth, diameter of secondary finger, leaf area and number of secondary fingers (Tables 4.19 and 4.20).

Plant height showed significant positive correlation with number of leaves per tiller, leaf length, leaf breadth, leaf area, yield per plant, length of primary finger and length of secondary finger. Number of tillers displayed significant positive correlation with number of leaves per tiller, leaf breadth, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Leaf length showed significant positive correlation with plant height, number of leaves per tiller, leaf breadth, leaf area, yield per plant, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Leaf breadth showed significant positive correlation with plant height, number of tillers, leaf length, leaf area, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Leaf area showed significant positive correlation with plant height, leaf length, leaf breadth, yield per plant, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Number of primary fingers showed significant positive correlation with number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger,

diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Number of secondary fingers showed significant positive correlation with number of tillers, leaf breadth, yield per plant, number of primary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Length of primary finger showed significant positive correlation with plant height, number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Diameter of primary finger showed significant positive correlation with number of tillers, number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Length of secondary finger showed significant positive correlation with plant height, number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, diameter of primary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Diameter of secondary finger showed significant positive correlation with number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, length of mother rhizome and diameter of mother rhizome. Length of mother rhizome showed significant positive correlation with number of tillers, number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger and diameter of mother rhizome. Diameter of mother rhizome showed significant positive correlation with number of tillers,

number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger and length of mother rhizome. Yield per plant showed significant positive correlation with plant height, number of leaves per tiller, leaf length, leaf area and number of secondary fingers. It indicates that these characters have some inherent interrelationship with yield per plant, suggesting the possibility of better yield through the improvement of these correlated characters.

Study of correlation of different agronomic characters of *K. rotunda* is new to science and hence the present study would provide better understanding of relationship existing between various quantitative characters in this valuable medicinal plant. However, similar studies on correlation of different agronomic characters have been conducted by earlier workers in different crops. Studies of Ilker (2011) in sweet corn revealed that ear weight could be used as a selection criterion due to its highly positive direct effect on fresh grain yield and indirect effects on all other characters. Row number per ear and ear length could be considered for selection criteria in sweet corn breeding.

Das *et al*. (2010) observed that fruits per plant, flowering branches, bunches per plant, collar diameter and secondary branches per plant had high positive correlation with seed yield per plant in *Jatropha curcas*. Selection for the characters such as flowering bunches per plant and fruits per plant would be highly effective in bringing out improvement in yield in *J. curcas*. followed by 100 grain weight and plant height. These traits contributed maximum to higher grain yield compared to other characters. Thus, selection for these characters helps in selection of superior cross combinations for improvement of yield.

Table 4.19. Correlation of agronomic characters in the case of *Kaempferia rotunda* studied.

| | Plant height | Number of tillers | Number of leaves per tiller | Leaf length | Leaf breadth | Leaf area | Yield per plant | Number of primary fingers | Number of secondary fingers | Length of primary finger | Diameter of primary finger | Length of secondary finger | Diameter of secondary finger | Length of mother rhizome | Diameter of mother rhizome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 1 | | | | | | | | | | | | | | |
| Number of tillers | 0.00464 | 1 | | | | | | | | | | | | | |
| Number of leaves per tiller | 0.51365* | -0.36938* | 1 | | | | | | | | | | | | |
| Leaf length | 0.91717* | 0.02233 | 0.38415* | 1 | | | | | | | | | | | |
| Leaf breadth | 0.25712* | 0.34738* | -0.18543 | 0.45825* | 1 | | | | | | | | | | |
| Leaf area | 0.71829* | 0.19175 | 0.13748 | 0.87899* | 0.80860* | 1 | | | | | | | | | |
| Yield per plant | 0.48740* | -0.02019 | 0.60828* | 0.40732* | 0.17537 | 0.35396* | 1 | | | | | | | | |
| Number of primary fingers | 0.04241 | 0.20433 | 0.09997 | 0.04850 | 0.23231 | 0.14606 | 0.16955 | 1 | | | | | | | |
| Number of secondary fingers | -0.00240 | 0.41869* | 0.03939 | 0.03319 | 0.38342* | 0.19721 | 0.23690* | 0.67455* | 1 | | | | | | |
| Length of primary finger | 0.24930* | 0.39181* | -0.01871 | 0.31533* | 0.48774* | 0.45768* | 0.16703 | 0.33917* | 0.53382* | 1 | | | | | |
| Diameter of primary finger | 0.17000 | 0.56116* | -0.24105* | 0.30369* | 0.58463* | 0.49161* | 0.04570 | 0.37505* | 0.65371* | 0.75996* | 1 | | | | |
| Length of secondary finger | 0.30623* | 0.52590* | -0.02286 | 0.33130* | 0.45526* | 0.44354* | 0.16673 | 0.34373* | 0.63394* | 0.71634* | 0.75498* | 1 | | | |
| Diameter of secondary finger | 0.21972 | 0.57378* | -0.12251 | 0.30393* | 0.46849* | 0.42960* | 0.08447 | 0.36168* | 0.66333* | 0.73240* | 0.88920* | 0.83889* | 1 | | |
| Length of mother rhizome | 0.16570 | 0.42442* | -0.27472* | 0.30792* | 0.49920* | 0.45186* | -0.08343 | 0.43872* | 0.51280* | 0.67640* | 0.73739* | 0.64086* | 0.68741* | 1 | |
| Diameter of mother rhizome | 0.15310 | 0.42236* | -0.26887* | 0.32934* | 0.54542* | 0.49197* | -0.10519 | 0.42649* | 0.57637* | 0.67689* | 0.85184* | 0.68815* | 0.79148* | 0.83485* | 1 |

*Significant at 5% level

Table 4.20. Details of the characters correlated.

| Characters | Number of characters showing significant positive correlation | Characters correlated |
|---|---|---|
| Plant height | 7 | Number of leaves per tiller, leaf length, leaf breadth, leaf area, yield per plant, length of primary finger, length of secondary finger |
| Number of tillers | 9 | Number of leaves per tiller, leaf breadth, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Number of leaves per tiller | 7 | Plant height, number of tillers, leaf length, yield per plant, diameter of primary finger, length of mother rhizome, diameter of mother rhizome |
| Leaf length | 11 | Plant height, number of leaves per tiller, leaf breadth, leaf area, yield per plant, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Leaf breadth | 11 | Plant height, number of tillers, leaf length, leaf area, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Leaf area | 10 | Plant height, leaf length, leaf breadth, yield per plant, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |

| | | |
|---|---|---|
| Number of primary fingers | 7 | Number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Number of secondary fingers | 10 | Number of tillers, leaf breadth, yield per plant, number of primary fingers, length of primary finger, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Length of primary finger | 12 | Plant height, number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, diameter of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Diameter of primary finger | 12 | Number of tillers, number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Length of secondary finger | 12 | Plant height, number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, diameter of primary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Diameter of secondary finger | 11 | Number of tillers, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, diameter of primary finger, length of secondary finger, length of mother rhizome, diameter of mother rhizome |
| Length of mother rhizome | 12 | Number of tillers, number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of |

| | | |
|---|---|---|
| | | secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger, diameter of mother rhizome |
| Diameter of mother rhizome | 12 | Number of tillers, number of leaves per tiller, leaf length, leaf breadth, leaf area, number of primary fingers, number of secondary fingers, length of primary finger, length of secondary finger, diameter of secondary finger, length of mother rhizome |
| Yield per plant | 5 | Plant height, number of leaves per tiller, leaf length, leaf area, number of secondary fingers |

Studies of Gazal *et al*. (2018) in maize showed that grain yield per plot was positively correlated with 100-seed weight, ears per plot, chlorophyll content, plant height, ear height and number of kernels per row indicating the importance of these characters in selection for yield.

According to Rajyalakshmi *et al*. (2013) rhizome yield was significantly and positively correlated with plant height, number of tillers per plant and number of leaves per plant in turmeric. Correlation studies were also conducted by Banerjee and Kole (2004) in fenugreek, by Rao *et al*. (2008) in mango ginger, by Paul and Bari (2013) in elephant foot yam, by Majumder *et al.* (2012) in Mango, by Akinwale *et al*. (2011) and Sarawgi *et al.* (1997) in rice, by Kassahun *et al*. (2013) in coriander, by Khan *et al*. (2009) in pointed gourd, by Okuyama *et al.* (2004) in wheat, by Tena *et al.* (2016) in sugar cane, by Shintu (2017) in arrow root, by Hrideek *et al*. (2015) in vetiver, by Selvarasu and Kandhasamy (2013) in glory lily and by Walle *et al.* (2018) in cow pea.

### 4.3. Character association

Polygenic characters show different levels of association with each other due to the influence of the same sets of alleles on different characters.

Grouping of characters based on this relationship is an efficient tool to group them and also to identify lead variables in each group. This type of an approach is very useful in reducing the bulk of variables that are being handled in breeding programmes. Character association in *K. rotunda* has been analyzed presently using factor analysis. It is accomplished through the identification of lead characters from each group under study (Nikhila *et al*., 2008). Factor analysis is an efficient tool to determine character association, to group different variables into different factors and for data reduction by identifying lead characters. Grouping is carried out based on the extent of relative contribution of variance by a variable to each factor based on factor loading calculated for the study. Characters with higher factor loading could be considered as lead characters and based on this, selection of superior genotypes could be easily practiced in such a way that other characters associated with the lead characters get automatically selected (Hrideek *et al*., 2008).

Character association in *K. rotunda* has been found out presently with the help of factor analysis using fifteen variables. Factor analysis resulted in grouping of the fifteen characters into three factors based on factor loading (Tables 4.21- 4.23). The first factor was associated with three growth and seven yield characters such as number of tillers, leaf breadth, leaf area, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome with factor loadings of -0.561726, -0.701499, -0.688183, -0.815597, -0.908291, -0.697642, -0.848075, -0.886737, -0.815030 and -0.866097 respectively. Since these characters belong to the same factor group, it is presumed that they share common alleles to a considerable extent in their expression and among them diameter of primary finger and diameter of secondary finger with the maximum factor loading are considered as the lead characters. The improvement of these lead characters will lead to

the simultaneous improvement of the other characters associated with them. Plant height, number of leaves per tiller, leaf length and yield per plant are grouped under the second factor with respective factor loadings of -0.829121, -0.747636, -0.769417 and -0.662790 respectively. The third factor group was found to be associated with number of primary fingers with a factor loading of -0.587466. In the case of the first factor, the variables that showed positive factor loading were number of tillers, leaf breadth, leaf area, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome.

From the analysis it could be observed that diameter of primary finger, diameter of secondary finger and diameter of mother rhizome can be regarded as the lead characters among the fifteen characters under study in *K. rotunda* and while practicing selection and further crop improvement of this valuable medicinal plant, these characters shall be given preference of selection.

The percentage of variance contributed by the characters of the first factor is 45.87%, that contributed by the characters of the second factor is 19.99%, that contributed by the characters of the third factor is 10.26%. These three factors cumulatively contribute 76.12% of the total variance of the present study population based on the characters studied (Table 4.22).

Table 4.21. Factor analysis in the case of *Kaempferia rotunda* – Factor loadings

| Characters | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| Plant height | -0.391931 | **-0.829121** | 0.121279 |
| Number of tillers | **-0.561726** | 0.359516 | 0.063133 |
| Number of leaves per tiller | 0.078180 | **-0.747636** | -0.527882 |
| Leaf length | -0.513012 | **-0.769417** | 0.284222 |
| Leaf breadth | **-0.701499** | -0.123744 | 0.326282 |
| Leaf area | **-0.688183** | -0.560532 | 0.368340 |
| Number of primary fingers | -0.488531 | 0.122187 | **-0.587466** |
| Number of secondary fingers | **-0.697642** | 0.225920 | -0.553630 |
| Length of primary finger | **-0.815597** | 0.051847 | -0.068903 |
| Diameter of primary finger | **-0.908291** | 0.220105 | 0.038651 |
| Length of secondary finger | **-0.848075** | 0.065925 | -0.119067 |
| Diameter of secondary finger | **-0.886737** | 0.178536 | -0.070793 |
| Length of mother rhizome | **-0.815030** | 0.227604 | 0.117072 |
| Diameter of mother rhizome | **-0.866097** | 0.238975 | 0.119813 |
| Yield per plant | -0.202747 | **-0.662790** | -0.461463 |

Table 4.22. Factor analysis in the case of *Kaempferia rotunda*- Eigen values and percentage of total variance

| Factors | Eigen value | Percentage of total variance | Cumulative Eigen value | Cumulative percentage of variance |
|---|---|---|---|---|
| 1 | 6.880730 | 45.87154 | 6.88073 | 45.87154 |
| 2 | 2.998695 | 19.99130 | 9.87942 | 65.86283 |
| 3 | 1.538332 | 10.25555 | 11.41776 | 76.11838 |

Table 4.23. Factor analysis in the case of *Kaempferia rotunda*- characters showing association as per factor analysis

| Factor | Characters |
|---|---|
| 1 | Number of tillers, leaf breadth, leaf area, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome, diameter of mother rhizome |
| 2 | Plant height, number of leaves per tiller, leaf length, yield per plant |
| 3 | Number of primary fingers |

Factor analysis has been utilized for data reduction and grouping of variables by earlier investigators like Tadesse and Bekele (2001) in grass pea, Hrideek *et al*. (2006) in chilli, Radhakrishnan *et al*. (2004) and Hrideek *et al*. (2008) in small cardamom, Nikhila *et al*. (2008) in robusta coffee, Filipovic *et al.* (2014) in maize, Yol *et al*. (2010) in sesame, Khan *et al*. (2000) in linseed, Shintu (2017) in West Indian arrowroot, Denton and Nwangburuka (2011) in *Solanum anguivi* and Umamaheswari and Mohanan (2011) in *Vanilla planifolia*. Study of association of characters in different crops is an important approach in determining the relationship between quantitative morphometric characters of agronomically important plant species since such studies could provide genetic foundation for further breeding and improvement in such species.

## 4.4. Genetic divergence

The success of any breeding programme depends on the availability of genetic diversity in the base population. The analysis of genetic diversity and relationships among germplasm accessions facilitates the selection of parents with diverse genetic constitution (Murphy *et al*., 1986; Souza and Sorrels, 1991). The characterization on morphological variability is an important tool to identify accessions with desirable traits. The characterization and grouping of germplasm helps the breeders to avoid duplication in sampling populations. The main aim of using cluster analysis in plant breeding trials is to group the

accessions into several homogeneous groups such that those accessions within a group have a similar response pattern across the locations. Classifying genotypes based on their agronomic characters with multivariate techniques could reduce the time and expenditure for crop improvement (Shrestha, 2016). In this context, sixty eight accessions of *K. rotunda* have been subjected to genetic divergence analysis through cluster analysis using the software STATISTICA, following UPGMA (Unweighted Pair Group Method with Arithmetic mean) method to identify the closeness and distance that pertain between the accessions on the basis of fifteen agronomic characters.

Cluster analysis grouped the entire accessions into three clusters at a linkage distance of 0.998 (Fig. 4.16). The first cluster is occupied by sixty six accessions, showing maximum accommodation of genotypes which are related. The second cluster is occupied by one accession namely CUR 13 collected from Kottayam district and third cluster occupied by CUR 58 collected from Wayanad district. The first cluster at a linkage distance of 0.996 bifurcated again into two sub clusters, the first constituting of 55 genotypes and the second consisting of 11 genotypes. These sub-clusters got again divided repeatedly in to different groups with maximum genetic closeness.

The genotypes CUR2 and CUR 16; CUR14 and CUR 25; CUR 17 and 51; CUR 46 and CUR 50; CUR 5 and CUR 52; CUR 48 and CUR 62; CUR 8 and CUR 68; CUR 24 and CUR 49; CUR 38 and CUR 47; CUR 12 and CUR 19; CUR 18 and CUR 45; and CUR 22 and CUR 36 were found to be more genetically related with regard to the characters subjected to the study. All these twelve groups come under the first cluster and they bifurcate at a linkage distance of 0.865. Cluster I has got genotypes from all the thirteen districts. Hence, each cluster is a mixture of genotypes collected from different geographical areas and it indicates that geographical separation is not a major criterion for genetic closeness and distance between the accessions studied. Genotypes belonging to same clusters show higher levels

of similarity and it is generally presumed that they exhibit genetic proximity and those belonging to different clusters are genetically distant from each other thus showing higher levels of divergence in genetic makeup. Since there is scope to think that accessions showing genetic closeness might have evolved from similar parental lines, there is lesser scope for the selection of genetically divergent parents for crosses from the same cluster. Distantly related genotypes can be considered as genetically diverse (Table 4.24). However, selection within groups for promising genotypes for use in selection programmes will lead to the development of promising and improved planting material. Being a clonally propagated crop, diverse accessions of *K. rotunda* could be selected for further improvement programmes based on further assessment of performance so that exploitation of genetic variability and production of high yielding varieties is possible.

**Fig.4.16. Dendrogram showing the diversity of the sixty eight accessions of *Kaempferia rotunda* studied**

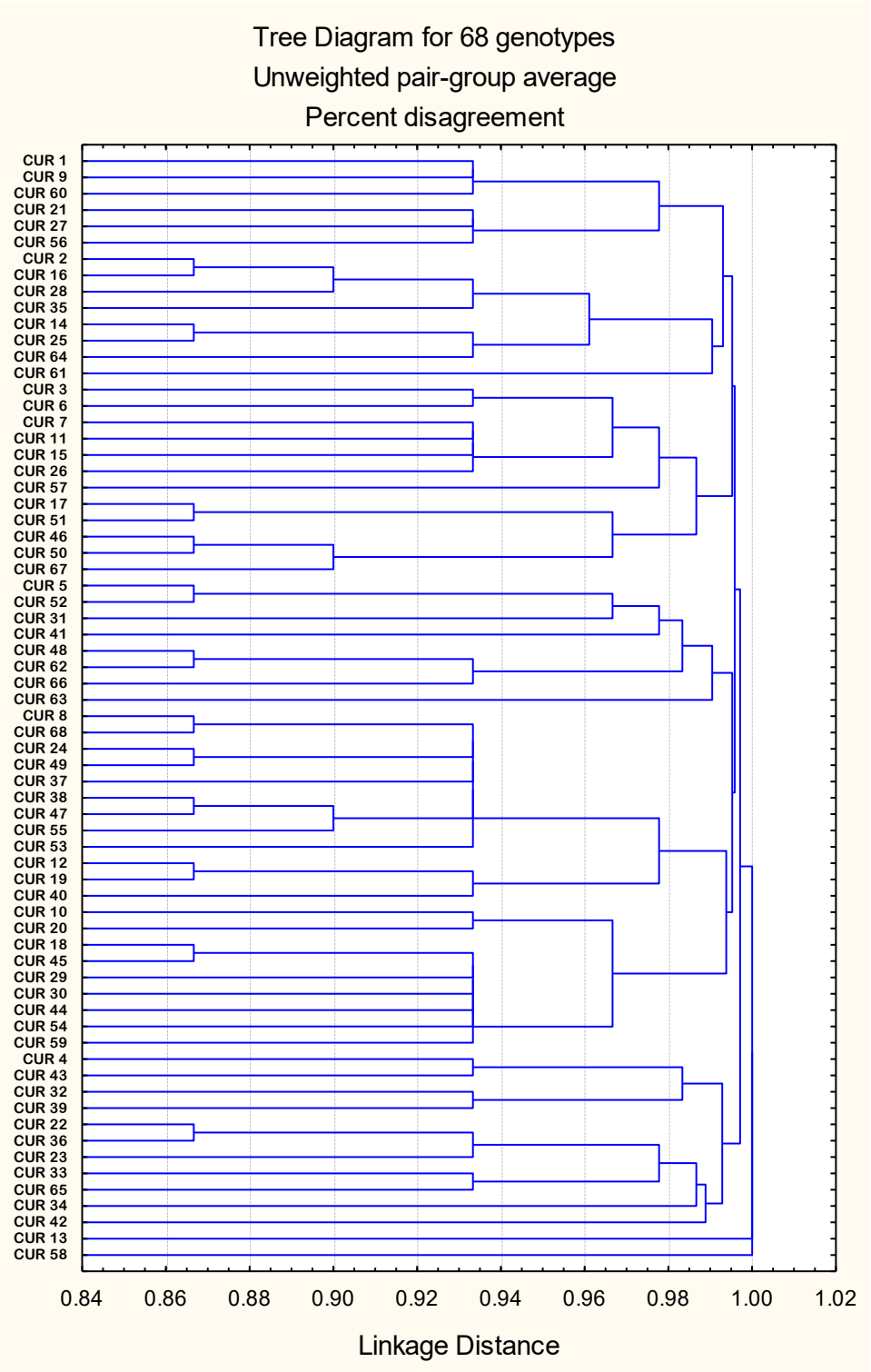


Table 4.24. Clustering of the genotypes studied in *Kaempferia rotunda*

| Cluster number | Sub cluster number | Accessions |
|---|---|---|
| I | 1A | CUR 1, CUR 9, CUR 60, CUR 21, CUR 27, CUR 56, CUR 2, CUR 16, CUR 28, CUR 35, CUR 14, CUR 25, CUR 64, CUR 61, CUR 3, CUR 6, CUR 7, CUR 11, CUR 15, CUR 26, CUR 57, CUR 17, CUR 51, CUR 46, CUR 50, CUR 67, CUR 5, CUR 52, CUR 31, CUR 41, CUR 48, CUR 62, CUR 66, CUR 63, CUR 8, CUR 68, CUR 24, CUR 49, CUR 37, CUR 38, CUR 47, CUR 55, CUR 53, CUR 12, CUR19, CUR 40, CUR 10, CUR 20, CUR 18, CUR 45, CUR 29, CUR 30, CUR 44, CUR 54, CUR 59 |
| | 1B | CUR 4, CUR 43, CUR 32, CUR 39, CUR 22, CUR 36, CUR 23, CUR 33, CUR 65, CUR 34, CUR 42 |
| II | | CUR 13 |
| III | | CUR 58 |

Similar works on cluster analysis have been carried out by earlier workers like Radhakrishnan *et al.* (2006b) in cardamom, Koij and Saba (2015) in white bean, Murphy *et al.* (1986), Khodadadi *et al.* (2011) in wheat, Souza and Sorrels (1991) in oats, De *et al.* (1988) in rice, Subramanian and Subbaraman (2010), Kahraman *et al.* (2014) in common bean, Shrestha (2016) in maize, Gupta *et al.* (2017) in grapes, Broich and Palmer (1980) in soybean, Janaki *et al*. (2016) in chilli and Punitha *et al*. (2010) in sunflower. Such studies have proved their importance in finding out the genetic distance and genetic closeness of different genotypes of various crops.

### 4.5. Performance analysis of the *Kaempferia rotunda* accessions collected

Performance analysis of sixty eight accessions of *K. rotunda* has been carried out presently on the basis of the performance indices of major morphometric characters. The cumulative performance index calculated as

described earlier can be used to find out the most promising genotypes with the desirable characters for further improvement of the species. Of the sixty eight accessions of *K. rotunda*, accession number CUR 64 ranked first with a cumulative performance index of 19.53. The accessions CUR 61 and CUR 34 have been placed at the second and third rank with cumulative performance index of 18.99 and 17.97 respectively. The accessions CUR 21, CUR 47, CUR 32, CUR 7, CUR 45, CUR 28 and CUR 15 ranked from 4 to 10 with a cumulative performance index of 17.78, 17.37, 17.17, 17.00, 16.80, 16.73 and 16.71 in that order (Table 4.25 and 4.26; Figs. 4.17 to 4.26). These superior accessions possess significantly maximum values of agronomic traits compared to the other accessions and these superior accessions can be subjected to further crop improvement programmes so that promising genotypes with better agronomic characters can be made available to the farmers.

Similar experiments on performance analysis of different crops for the selection of superior genotypes from the existing germplasm have been carried out by earlier workers like Chaudhary *et al*. (2006) and Salimath *et al*. (2014) in turmeric, Anand *et al*. (2006) in pepper, Ahmad *et al*. (2007) in tomato, Elavarasan *et al*. (2013) in cabbage, Jayasree *et al*. (2014b) in mango ginger and Chandramohan *et al*. (2016) in rice.

Table 4.25. Performance analysis of the accessions of *Kaempferia rotunda* studied -Mean value of the characters

| Accession Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 1 | 60.56 | 3.10 | 5.77 | 31.93 | 7.33 | 145.01 | 123.88 | 10.22 | 4.45 | 2.16 | 17.33 | 3.76 | 1.46 | 5.54 | 2.90 |
| CUR 2 | 62.84 | 3.44 | 5.49 | 33.35 | 7.53 | 154.18 | 94.16 | 9.88 | 4.4 | 2.44 | 12.88 | 3.02 | 1.70 | 6.37 | 3.07 |
| CUR 3 | 64.08 | 3.33 | 7.35 | 34.00 | 7.28 | 154.28 | 135.55 | 10.44 | 3.99 | 2.41 | 16.88 | 2.95 | 1.60 | 4.93 | 2.76 |
| CUR 4 | 57.42 | 2.49 | 6.97 | 30.10 | 6.51 | 127.09 | 155.27 | 5.66 | 3.13 | 1.48 | 6.00 | 2.28 | 1.00 | 3.30 | 1.99 |
| CUR 5 | 62.83 | 2.22 | 6.62 | 36.00 | 6.98 | 155.31 | 86.11 | 6.32 | 3.72 | 1.89 | 9.66 | 2.56 | 1.35 | 3.84 | 2.52 |
| CUR 6 | 51.20 | 2.44 | 6.12 | 30.14 | 6.10 | 116.86 | 84.72 | 10.44 | 3.00 | 1.64 | 9.22 | 2.28 | 1.06 | 4.64 | 2.50 |
| CUR 7 | 69.06 | 3.33 | 6.18 | 36.84 | 8.45 | 193.70 | 139.88 | 9.21 | 4.66 | 2.81 | 15.21 | 3.68 | 1.64 | 5.75 | 3.26 |
| CUR 8 | 45.83 | 2.66 | 5.64 | 27.12 | 8.54 | 143.67 | 116.66 | 8.77 | 4.23 | 2.17 | 18.55 | 2.29 | 1.33 | 5.08 | 2.65 |
| CUR 9 | 43.96 | 2.77 | 6.58 | 28.36 | 7.67 | 138.91 | 125.00 | 10.22 | 3.64 | 1.98 | 14.77 | 2.28 | 1.16 | 4.72 | 2.57 |
| CUR 10 | 44.14 | 3.22 | 5.16 | 27.13 | 8.97 | 150.62 | 116.66 | 8.66 | 3.99 | 2.22 | 11.55 | 2.64 | 1.41 | 5.18 | 2.90 |
| CUR 11 | 48.14 | 3.33 | 4.76 | 29.40 | 8.87 | 161.87 | 128.33 | 8.77 | 3.56 | 1.98 | 10.99 | 2.76 | 1.29 | 5.01 | 2.72 |
| CUR 12 | 48.55 | 2.99 | 5.70 | 28.33 | 8.34 | 145.06 | 97.77 | 8.99 | 3.47 | 2.04 | 17.88 | 2.67 | 1.15 | 4.74 | 2.61 |
| CUR 13 | 58.64 | 3.05 | 5.47 | 34.03 | 8.53 | 179.95 | 148.61 | 8.27 | 3.65 | 2.56 | 12.00 | 2.35 | 1.43 | 4.49 | 2.79 |
| CUR 14 | 66.55 | 2.99 | 5.90 | 36.82 | 7.93 | 179.96 | 117.21 | 7.66 | 4.47 | 2.76 | 13.55 | 3.23 | 1.72 | 5.65 | 3.24 |
| CUR 15 | 66.88 | 3.33 | 5.96 | 37.98 | 8.91 | 206.30 | 165.55 | 10.21 | 4.35 | 2.42 | 15.88 | 2.97 | 1.58 | 5.40 | 3.03 |
| CUR 16 | 70.09 | 3.44 | 5.21 | 39.31 | 8.42 | 204.61 | 129.44 | 9.88 | 4.13 | 2.32 | 13.10 | 2.85 | 1.49 | 5.72 | 3.02 |
| CUR 17 | 64.05 | 3.33 | 4.67 | 38.28 | 9.15 | 214.84 | 105.55 | 8.05 | 4.11 | 2.75 | 13.22 | 2.69 | 1.66 | 5.13 | 3.04 |
| CUR 18 | 61.63 | 2.88 | 5.88 | 35.64 | 8.46 | 186.48 | 144.99 | 8.88 | 2.24 | 2.24 | 16.33 | 3.08 | 1.38 | 4.83 | 2.83 |
| CUR 19 | 72.09 | 2.66 | 5.93 | 41.17 | 8.77 | 220.67 | 122.22 | 7.33 | 3.92 | 2.27 | 8.22 | 2.67 | 1.15 | 5.40 | 2.80 |
| CUR 20 | 66.85 | 2.88 | 4.51 | 39.86 | 9.25 | 229.36 | 114.16 | 8.72 | 3.99 | 2.63 | 11.44 | 3.14 | 1.65 | 5.40 | 3.16 |
| CUR 21 | 71.28 | 3.10 | 5.44 | 39.28 | 9.96 | 239.55 | 157.77 | 11.33 | 4.74 | 3.03 | 16.44 | 3.29 | 1.64 | 5.61 | 3.32 |
| CUR 22 | 71.99 | 2.44 | 7.10 | 37.79 | 7.94 | 184.55 | 115.55 | 8.22 | 4.10 | 2.06 | 11.10 | 2.77 | 1.47 | 5.12 | 2.82 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 23 | 74.60 | 2.44 | 5.75 | 39.83 | 8.02 | 196.94 | 99.99 | 6.77 | 3.33 | 1.80 | 7.10 | 2.20 | 0.96 | 4.30 | 2.63 |
| CUR 24 | 64.85 | 2.66 | 6.68 | 34.73 | 7.48 | 168.78 | 76.11 | 6.99 | 4.05 | 2.05 | 7.55 | 3.15 | 1.32 | 4.75 | 2.72 |
| CUR 25 | 75.08 | 3.44 | 5.65 | 38.43 | 8.29 | 196.16 | 146.10 | 8.88 | 5.25 | 2.38 | 13.55 | 3.49 | 1.49 | 5.65 | 2.9 |
| CUR 26 | 70.28 | 3.33 | 5.16 | 38.45 | 8.99 | 213.96 | 103.88 | 9.88 | 4.56 | 2.67 | 13.77 | 3.18 | 1.83 | 5.57 | 3.37 |
| CUR 27 | 72.45 | 3.10 | 6.25 | 41.02 | 8.03 | 202.94 | 93.88 | 6.55 | 3.79 | 2.04 | 8.33 | 2.51 | 1.33 | 5.14 | 2.58 |
| CUR 28 | 71.78 | 3.44 | 6.01 | 40.52 | 8.90 | 221.05 | 147.21 | 7.55 | 4.12 | 2.47 | 14.11 | 3.64 | 1.87 | 4.87 | 3.07 |
| CUR 29 | 69.93 | 2.88 | 5.05 | 39.05 | 9.17 | 222.85 | 89.44 | 4.10 | 2.71 | 1.55 | 4.22 | 2.16 | 1.04 | 4.76 | 2.41 |
| CUR 30 | 82.25 | 2.88 | 7.21 | 48.37 | 8.89 | 251.64 | 121.66 | 7.33 | 4.39 | 2.21 | 9.22 | 3.17 | 1.37 | 5.35 | 3.05 |
| CUR 31 | 64.43 | 3.55 | 4.84 | 37.40 | 7.88 | 199.46 | 176.10 | 5.33 | 3.83 | 2.14 | 6.32 | 2.56 | 1.26 | 5.35 | 2.69 |
| CUR 32 | 69.06 | 3.66 | 5.71 | 41.78 | 8.32 | 213.87 | 131.66 | 9.11 | 4.59 | 2.8 | 15.55 | 3.25 | 1.88 | 5.82 | 3.06 |
| CUR 33 | 49.78 | 3.22 | 5.46 | 28.56 | 7.73 | 137.30 | 111.66 | 7.88 | 3.63 | 1.99 | 10.44 | 2.37 | 1.20 | 4.35 | 2.21 |
| CUR 34 | 76.13 | 4.33 | 7.32 | 38.96 | 8.16 | 195.04 | 164.44 | 9.66 | 4.1 | 2.67 | 18.88 | 3.86 | 1.78 | 5.33 | 3.12 |
| CUR 35 | 83.08 | 3.38 | 6.95 | 41.64 | 8.22 | 210.80 | 104.99 | 9.88 | 3.85 | 2.37 | 12.88 | 3.54 | 1.77 | 5.32 | 2.98 |
| CUR 36 | 66.01 | 2.44 | 7.21 | 36.30 | 7.32 | 164.28 | 134.44 | 7.22 | 3.84 | 2.08 | 10.44 | 2.62 | 1.47 | 4.36 | 2.68 |
| CUR 37 | 68.55 | 2.66 | 7.56 | 36.63 | 6.82 | 153.99 | 190.55 | 3.88 | 3.44 | 2.00 | 4.88 | 1.56 | 1.10 | 4.38 | 2.37 |
| CUR 38 | 62.09 | 2.66 | 5.40 | 36.17 | 7.81 | 173.85 | 117.77 | 9.55 | 3.94 | 2.11 | 13.44 | 2.53 | 1.48 | 5.66 | 3.16 |
| CUR 39 | 60.89 | 2.33 | 6.66 | 34.79 | 7.11 | 152.85 | 131.66 | 7.88 | 3.45 | 2.13 | 13.99 | 2.50 | 1.31 | 4.78 | 2.84 |
| CUR 40 | 61.02 | 2.77 | 6.51 | 35.4 | 7.51 | 164.40 | 133.11 | 8.00 | 3.38 | 2.04 | 8.22 | 2.42 | 1.19 | 4.4 | 3.04 |
| CUR 41 | 75.86 | 1.88 | 7.62 | 37.66 | 6.66 | 154.41 | 132.77 | 9.10 | 3.78 | 1.89 | 13.10 | 2.47 | 1.27 | 5.09 | 2.55 |
| CUR 42 | 61.93 | 2.11 | 7.97 | 36.22 | 8.17 | 187.64 | 165.27 | 9.77 | 4.13 | 2.19 | 9.61 | 2.48 | 1.28 | 5.03 | 2.68 |
| CUR 43 | 68.64 | 2.33 | 6.97 | 35.04 | 6.86 | 148.74 | 127.77 | 7.22 | 2.61 | 1.38 | 4.88 | 2.15 | 0.85 | 4.35 | 2.18 |
| CUR 44 | 75.61 | 2.88 | 8.16 | 37.24 | 7.70 | 181.46 | 278.32 | 8.21 | 3.92 | 1.97 | 11.66 | 2.52 | 1.35 | 4.21 | 2.60 |
| CUR 45 | 80.37 | 2.88 | 7.99 | 40.66 | 8.82 | 220.44 | 349.99 | 8.88 | 4.09 | 1.92 | 13.10 | 2.57 | 1.23 | 4.50 | 2.4 |
| CUR 46 | 78.14 | 2.55 | 6.82 | 40.21 | 7.79 | 188.53 | 198.88 | 7.10 | 3.50 | 2.00 | 9.10 | 2.57 | 1.14 | 4.04 | 2.06 |
| CUR 47 | 86.72 | 2.66 | 8.49 | 45.67 | 8.84 | 246.31 | 233.88 | 11.44 | 3.85 | 2.11 | 15.77 | 2.64 | 1.23 | 5.11 | 2.93 |
| CUR 48 | 73.99 | 3.00 | 5.90 | 35.77 | 6.98 | 157.66 | 197.77 | 8.55 | 3.98 | 2.13 | 11.55 | 2.85 | 1.30 | 4.26 | 2.56 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 49 | 67.40 | 2.66 | 7.27 | 37.54 | 8.04 | 186.29 | 249.44 | 8.11 | 3.98 | 2.05 | 11.55 | 2.59 | 1.37 | 4.65 | 2.76 |
| CUR 50 | 66.15 | 2.55 | 6.82 | 34.43 | 7.16 | 153.64 | 187.77 | 9.66 | 3.60 | 2.26 | 14.44 | 3.05 | 1.56 | 5.56 | 2.90 |
| CUR 51 | 64.05 | 2.55 | 6.01 | 35.10 | 7.22 | 158.28 | 155.55 | 7.77 | 3.81 | 2.14 | 6.99 | 2.69 | 1.35 | 4.80 | 2.75 |
| CUR 52 | 65.51 | 3.49 | 6.04 | 32.82 | 6.98 | 141.17 | 111.38 | 9.61 | 3.96 | 2.04 | 9.66 | 2.45 | 1.2 | 4.69 | 2.65 |
| CUR 53 | 52.14 | 2.66 | 6.10 | 27.79 | 6.38 | 109.38 | 81.10 | 6.55 | 2.54 | 1.29 | 6.44 | 1.62 | 0.8 | 3.97 | 2.05 |
| CUR 54 | 57.80 | 2.88 | 6.84 | 30.19 | 6.56 | 123.4 | 98.33 | 8.33 | 2.33 | 1.42 | 8.44 | 1.32 | 0.87 | 3.64 | 1.94 |
| CUR 55 | 74.33 | 2.66 | 7.82 | 41.90 | 7.56 | 200.58 | 258.33 | 9.33 | 3.94 | 2.15 | 12.55 | 2.48 | 1.42 | 5.05 | 2.74 |
| CUR 56 | 66.13 | 3.10 | 5.58 | 35.52 | 7.32 | 161.74 | 190.83 | 9.88 | 3.09 | 2.02 | 9.77 | 2.62 | 1.29 | 4.92 | 2.56 |
| CUR 57 | 73.76 | 2.77 | 6.18 | 37.28 | 7.61 | 175.60 | 168.33 | 8.77 | 3.25 | 1.84 | 5.88 | 2.18 | 1.17 | 4.60 | 2.23 |
| CUR 58 | 67.14 | 2.10 | 6.54 | 34.48 | 7.2 | 157.80 | 158.88 | 6.88 | 2.46 | 1.46 | 6.33 | 1.57 | 0.94 | 3.38 | 1.92 |
| CUR 59 | 66.68 | 2.88 | 6.95 | 36.06 | 7.32 | 163.46 | 226.66 | 10.11 | 3.14 | 1.85 | 11.99 | 2.24 | 1.21 | 4.30 | 2.47 |
| CUR 60 | 76.44 | 2.99 | 7.68 | 40.84 | 8.13 | 202.90 | 312.77 | 10.22 | 3.20 | 1.65 | 11.99 | 1.94 | 1.03 | 4.14 | 2.25 |
| CUR 61 | 85.39 | 3.11 | 9.17 | 43.45 | 9.01 | 238.03 | 363.33 | 8.66 | 4.67 | 2.32 | 14.55 | 4.15 | 1.81 | 4.82 | 2.54 |
| CUR 62 | 71.02 | 3.00 | 6.34 | 36.34 | 7.29 | 165.73 | 273.88 | 6.22 | 3.62 | 2.08 | 11.16 | 2.7 | 1.30 | 4.57 | 2.55 |
| CUR 63 | 80.40 | 1.94 | 8.01 | 45.28 | 9.08 | 252.05 | 252.77 | 10.88 | 3.96 | 1.90 | 10.38 | 2.22 | 1.07 | 5.17 | 2.82 |
| CUR 64 | 70.86 | 3.44 | 7.06 | 38.85 | 8.64 | 207.78 | 401.10 | 9.99 | 4.61 | 2.76 | 20.77 | 3.77 | 1.85 | 5.30 | 3.11 |
| CUR 65 | 52.11 | 3.00 | 6.11 | 28.94 | 7.60 | 138.28 | 120.07 | 8.00 | 3.70 | 1.80 | 9.55 | 2.37 | 1.18 | 3.76 | 2.10 |
| CUR 66 | 83.79 | 2.83 | 8.15 | 44.04 | 7.95 | 191.75 | 243.88 | 7.55 | 3.28 | 2.00 | 10.05 | 2.73 | 1.3 | 4.71 | 2.50 |
| CUR 67 | 67.64 | 2.55 | 7.57 | 36.3 | 7.99 | 179.74 | 321.66 | 8.77 | 4.19 | 2.13 | 14.44 | 3.24 | 1.34 | 5.00 | 2.51 |
| CUR 68 | 73.75 | 2.66 | 6.62 | 40.12 | 8.54 | 213.43 | 239.99 | 7.66 | 4.09 | 2.10 | 10.11 | 2.70 | 1.22 | 4.95 | 2.54 |

1: Plant height (cm); 2: Number of tillers; 3: Number of leaves per tiller; 4: Leaf length (cm); 5: Leaf breadth (cm); 6: Leaf area ($cm^2$); 7: Yield per plant (g); 8: Number of primary fingers; 9: Length of primary finger (cm); 10: Diameter of primary finger (cm); 11: Number of secondary fingers; 12: Length of secondary finger (cm); 13: Diameter of secondary finger (cm); 14: Length of mother rhizome (cm); 15: Diameter of mother rhizome (cm)

Table 4.26. Performance analysis of the different genotypes of *Kaempferia rotunda* studied – Performance indices

| Accession number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 1 | 0.90 | 1.07 | 0.89 | 0.87 | 0.92 | 0.80 | 0.75 | 1.20 | 1.17 | 1.01 | 1.49 | 1.39 | 1.08 | 1.13 | 1.07 | 15.74 | 22 |
| CUR 2 | 0.93 | 1.19 | 0.85 | 0.91 | 0.94 | 0.85 | 0.57 | 1.16 | 1.16 | 1.15 | 1.10 | 1.11 | 1.25 | 1.31 | 1.14 | 15.62 | 25 |
| CUR 3 | 0.95 | 1.15 | 1.13 | 0.92 | 0.91 | 0.85 | 0.83 | 1.23 | 1.05 | 1.13 | 1.45 | 1.09 | 1.18 | 1.01 | 1.02 | 15.9 | 19 |
| CUR 4 | 0.85 | 0.86 | 1.08 | 0.82 | 0.81 | 0.70 | 0.95 | 0.66 | 0.82 | 0.69 | 0.51 | 0.84 | 0.74 | 0.67 | 0.73 | 11.73 | 64 |
| CUR 5 | 0.93 | 0.76 | 1.02 | 0.98 | 0.87 | 0.86 | 0.52 | 0.74 | 0.98 | 0.89 | 0.83 | 0.94 | 1.00 | 0.79 | 0.93 | 13.04 | 57 |
| CUR 6 | 0.76 | 0.84 | 0.94 | 0.82 | 0.76 | 0.64 | 0.51 | 1.23 | 0.79 | 0.77 | 0.79 | 0.84 | 0.78 | 0.95 | 0.92 | 12.34 | 61 |
| **CUR 7** | **1.03** | **1.15** | **0.95** | **1.00** | **1.06** | **1.07** | **0.85** | **1.08** | **1.23** | **1.32** | **1.30** | **1.36** | **1.21** | **1.18** | **1.21** | **17.00** | **7** |
| CUR 8 | 0.68 | 0.92 | 0.87 | 0.74 | 1.07 | 0.79 | 0.71 | 1.03 | 1.11 | 1.02 | 1.59 | 0.84 | 0.98 | 1.04 | 0.98 | 14.37 | 40 |
| CUR 9 | 0.65 | 0.95 | 1.02 | 0.77 | 0.96 | 0.76 | 0.76 | 1.20 | 0.96 | 0.93 | 1.26 | 0.84 | 0.85 | 0.97 | 0.95 | 13.83 | 51 |
| CUR 10 | 0.65 | 1.11 | 0.80 | 0.74 | 1.12 | 0.83 | 0.71 | 1.02 | 1.05 | 1.04 | 0.99 | 0.97 | 1.04 | 1.06 | 1.07 | 14.20 | 45 |
| CUR 11 | 0.71 | 1.15 | 0.73 | 0.80 | 1.11 | 0.89 | 0.78 | 1.03 | 0.94 | 0.93 | 0.94 | 1.02 | 0.95 | 1.03 | 1.01 | 14.02 | 48 |
| CUR 12 | 0.72 | 1.03 | 0.88 | 0.77 | 1.05 | 0.80 | 0.59 | 1.06 | 0.91 | 0.96 | 1.53 | 0.98 | 0.85 | 0.97 | 0.97 | 14.07 | 47 |
| CUR 13 | 0.87 | 1.05 | 0.84 | 0.93 | 1.07 | 0.99 | 0.91 | 0.97 | 0.96 | 1.20 | 1.03 | 0.87 | 1.05 | 0.92 | 1.03 | 14.69 | 36 |
| CUR 14 | 0.99 | 1.03 | 0.91 | 1.00 | 0.99 | 0.99 | 0.71 | 0.90 | 1.18 | 1.30 | 1.16 | 1.19 | 1.27 | 1.16 | 1.20 | 15.98 | 18 |
| **CUR 15** | **0.99** | **1.15** | **0.92** | **1.03** | **1.12** | **1.14** | **1.01** | **1.20** | **1.15** | **1.14** | **1.36** | **1.10** | **1.17** | **1.11** | **1.12** | **16.71** | **10** |
| CUR 16 | 1.04 | 1.19 | 0.80 | 1.07 | 1.06 | 1.13 | 0.79 | 1.16 | 1.09 | 1.09 | 1.12 | 1.05 | 1.10 | 1.17 | 1.12 | 15.98 | 18 |
| CUR 17 | 0.95 | 1.15 | 0.72 | 1.04 | 1.15 | 1.18 | 0.64 | 0.95 | 1.08 | 1.29 | 1.13 | 0.99 | 1.22 | 1.05 | 1.13 | 15.67 | 23 |
| CUR 18 | 0.92 | 0.99 | 0.91 | 0.97 | 1.06 | 1.03 | 0.88 | 1.04 | 0.59 | 1.05 | 1.40 | 1.14 | 1.02 | 0.99 | 1.05 | 15.04 | 29 |
| CUR 19 | 1.07 | 0.92 | 0.91 | 1.12 | 1.10 | 1.22 | 0.74 | 0.86 | 1.03 | 1.07 | 0.70 | 0.98 | 0.85 | 1.11 | 1.04 | 14.72 | 35 |
| CUR 20 | 0.99 | 0.99 | 0.69 | 1.08 | 1.16 | 1.27 | 0.69 | 1.02 | 1.05 | 1.24 | 0.98 | 1.16 | 1.22 | 1.11 | 1.17 | 15.82 | 20 |
| **CUR 21** | **1.06** | **1.07** | **0.84** | **1.07** | **1.25** | **1.32** | **0.96** | **1.33** | **1.25** | **1.42** | **1.41** | **1.21** | **1.21** | **1.15** | **1.23** | **17.78** | **4** |
| CUR 22 | 1.07 | 0.84 | 1.10 | 1.03 | 1.00 | 1.02 | 0.70 | 0.97 | 1.08 | 0.97 | 0.95 | 1.02 | 1.08 | 1.05 | 1.04 | 14.92 | 32 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 23 | 1.11 | 0.84 | 0.89 | 1.08 | 1.01 | 1.09 | 0.61 | 0.79 | 0.88 | 0.84 | 0.61 | 0.81 | 0.71 | 0.88 | 0.97 | 13.12 | 55 |
| CUR 24 | 0.96 | 0.92 | 1.03 | 0.94 | 0.94 | 0.93 | 0.46 | 0.82 | 1.07 | 0.96 | 0.64 | 1.16 | 0.97 | 0.97 | 1.01 | 13.78 | 52 |
| CUR 25 | 1.12 | 1.19 | 0.87 | 1.05 | 1.04 | 1.08 | 0.89 | 1.04 | 1.38 | 1.12 | 1.16 | 1.29 | 1.10 | 1.16 | 1.07 | 16.56 | 13 |
| CUR 26 | 1.04 | 1.15 | 0.80 | 1.05 | 1.13 | 1.18 | 0.63 | 1.16 | 1.20 | 1.25 | 1.18 | 1.17 | 1.35 | 1.14 | 1.25 | 16.68 | 11 |
| CUR 27 | 1.08 | 1.07 | 0.96 | 1.12 | 1.01 | 1.12 | 0.57 | 0.77 | 1.00 | 0.96 | 0.71 | 0.92 | 0.98 | 1.05 | 0.95 | 14.27 | 41 |
| **CUR 28** | **1.07** | **1.19** | **0.93** | **1.10** | **1.12** | **1.22** | **0.90** | **0.89** | **1.08** | **1.16** | **1.21** | **1.34** | **1.38** | **1.00** | **1.14** | **16.73** | **9** |
| CUR 29 | 1.04 | 0.99 | 0.78 | 1.06 | 1.15 | 1.23 | 0.54 | 0.48 | 0.71 | 0.73 | 0.36 | 0.80 | 0.77 | 0.97 | 0.89 | 12.50 | 60 |
| CUR 30 | 1.22 | 0.99 | 1.11 | 1.32 | 1.11 | 1.39 | 0.74 | 0.86 | 1.16 | 1.04 | 0.79 | 1.17 | 1.01 | 1.10 | 1.13 | 16.14 | 17 |
| CUR 31 | 0.96 | 1.22 | 0.75 | 1.02 | 0.99 | 1.10 | 1.07 | 0.62 | 1.01 | 1.00 | 0.54 | 0.94 | 0.93 | 1.10 | 1.00 | 14.25 | 43 |
| **CUR 32** | **1.03** | **1.26** | **0.88** | **1.14** | **1.04** | **1.18** | **0.80** | **1.07** | **1.21** | **1.32** | **1.33** | **1.20** | **1.39** | **1.19** | **1.13** | **17.17** | **6** |
| CUR 33 | 0.74 | 1.11 | 0.84 | 0.78 | 0.97 | 0.76 | 0.68 | 0.93 | 0.96 | 0.93 | 0.89 | 0.87 | 0.88 | 0.89 | 0.82 | 13.05 | 56 |
| **CUR 34** | **1.13** | **1.49** | **1.13** | **1.06** | **1.02** | **1.08** | **1.00** | **1.14** | **1.08** | **1.25** | **1.62** | **1.42** | **1.31** | **1.09** | **1.15** | **17.97** | **3** |
| CUR 35 | 1.24 | 1.16 | 1.07 | 1.13 | 1.03 | 1.16 | 0.64 | 1.16 | 1.01 | 1.11 | 1.10 | 1.31 | 1.31 | 1.09 | 1.10 | 16.62 | 12 |
| CUR 36 | 0.98 | 0.84 | 1.11 | 0.99 | 0.92 | 0.90 | 0.82 | 0.85 | 1.01 | 0.98 | 0.89 | 0.97 | 1.08 | 0.89 | 0.99 | 14.22 | 44 |
| CUR 37 | 1.02 | 0.92 | 1.17 | 1.00 | 0.85 | 0.85 | 1.16 | 0.45 | 0.91 | 0.94 | 0.41 | 0.57 | 0.81 | 0.90 | 0.88 | 12.84 | 58 |
| CUR 38 | 0.92 | 0.92 | 0.83 | 0.98 | 0.98 | 0.96 | 0.72 | 1.12 | 1.04 | 0.99 | 1.15 | 0.93 | 1.09 | 1.16 | 1.17 | 14.96 | 31 |
| CUR 39 | 0.90 | 0.80 | 1.03 | 0.95 | 0.89 | 0.84 | 0.80 | 0.93 | 0.91 | 1.00 | 1.20 | 0.92 | 0.97 | 0.98 | 1.05 | 14.17 | 46 |
| CUR 40 | 0.91 | 0.95 | 1.00 | 0.96 | 0.94 | 0.91 | 0.81 | 0.94 | 0.89 | 0.96 | 0.70 | 0.89 | 0.88 | 0.90 | 1.13 | 13.77 | 53 |
| CUR 41 | 1.13 | 0.65 | 1.18 | 1.02 | 0.83 | 0.85 | 0.81 | 1.07 | 1.00 | 0.89 | 1.12 | 0.91 | 0.94 | 1.04 | 0.94 | 14.38 | 39 |
| CUR 42 | 0.92 | 0.73 | 1.23 | 0.99 | 1.02 | 1.03 | 1.01 | 1.15 | 1.09 | 1.03 | 0.82 | 0.91 | 0.94 | 1.03 | 0.99 | 14.89 | 33 |
| CUR 43 | 1.02 | 0.80 | 1.08 | 0.95 | 0.86 | 0.82 | 0.78 | 0.85 | 0.69 | 0.65 | 0.41 | 0.79 | 0.62 | 0.89 | 0.81 | 12.02 | 62 |
| CUR 44 | 1.18 | 0.99 | 1.26 | 1.01 | 0.96 | 1.00 | 1.70 | 0.96 | 1.03 | 0.92 | 1.00 | 0.93 | 1.00 | 0.86 | 0.96 | 15.76 | 21 |
| **CUR 45** | **1.19** | **0.99** | **1.23** | **1.11** | **1.11** | **1.22** | **2.14** | **1.04** | **1.08** | **0.90** | **1.12** | **0.95** | **0.91** | **0.92** | **0.89** | **16.80** | **8** |
| CUR 46 | 1.16 | 0.88 | 1.05 | 1.09 | 0.98 | 1.04 | 1.21 | 0.83 | 0.92 | 0.94 | 0.78 | 0.95 | 0.84 | 0.83 | 0.76 | 14.26 | 42 |
| **CUR 47** | **1.29** | **0.92** | **1.31** | **1.24** | **1.11** | **1.36** | **1.43** | **1.35** | **1.01** | **0.99** | **1.35** | **0.97** | **0.91** | **1.05** | **1.08** | **17.37** | **5** |
| CUR 48 | 1.10 | 1.03 | 0.91 | 0.97 | 0.87 | 0.87 | 1.21 | 1.00 | 1.05 | 1.00 | 0.99 | 1.05 | 0.96 | 0.87 | 0.95 | 14.83 | 34 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CUR 49 | 1.00 | 0.92 | 1.12 | 1.02 | 1.01 | 1.03 | 1.52 | 0.95 | 1.05 | 0.96 | 0.99 | 0.95 | 1.01 | 0.95 | 1.02 | 15.50 | 28 |
| CUR 50 | 0.98 | 0.88 | 1.05 | 0.94 | 0.90 | 0.85 | 1.15 | 1.14 | 0.95 | 1.06 | 1.24 | 1.12 | 1.15 | 1.14 | 1.07 | 15.62 | 25 |
| CUR 51 | 0.95 | 0.88 | 0.93 | 0.95 | 0.90 | 0.87 | 0.95 | 0.91 | 1.00 | 1.00 | 0.60 | 0.99 | 1.00 | 0.98 | 1.02 | 13.93 | 50 |
| CUR 52 | 0.97 | 1.20 | 0.93 | 0.89 | 0.87 | 0.78 | 0.68 | 1.13 | 1.04 | 0.96 | 0.83 | 0.90 | 0.88 | 0.96 | 0.98 | 14.00 | 49 |
| CUR 53 | 0.77 | 0.92 | 0.94 | 0.75 | 0.80 | 0.60 | 0.49 | 0.77 | 0.67 | 0.60 | 0.55 | 0.62 | 0.59 | 0.81 | 0.76 | 10.64 | 66 |
| CUR 54 | 0.86 | 0.99 | 1.06 | 0.82 | 0.82 | 0.68 | 0.60 | 0.98 | 0.61 | 0.66 | 0.72 | 0.48 | 0.64 | 0.74 | 0.72 | 11.38 | 65 |
| CUR 55 | 1.10 | 0.92 | 1.21 | 1.14 | 0.95 | 1.11 | 1.58 | 1.10 | 1.04 | 1.01 | 1.07 | 0.91 | 1.05 | 1.03 | 1.01 | 16.23 | 15 |
| CUR 56 | 0.98 | 1.07 | 0.86 | 0.97 | 0.92 | 0.89 | 1.16 | 1.16 | 0.81 | 0.95 | 0.84 | 0.97 | 0.95 | 1.01 | 0.95 | 14.49 | 38 |
| CUR 57 | 1.10 | 0.95 | 0.95 | 1.01 | 0.95 | 0.97 | 1.03 | 1.03 | 0.85 | 0.86 | 0.50 | 0.80 | 0.86 | 0.94 | 0.82 | 13.62 | 54 |
| CUR 58 | 1.00 | 0.72 | 1.01 | 0.94 | 0.90 | 0.87 | 0.97 | 0.81 | 0.65 | 0.68 | 0.54 | 0.58 | 0.69 | 0.69 | 0.71 | 11.76 | 63 |
| CUR 59 | 0.99 | 0.99 | 1.07 | 0.98 | 0.92 | 0.90 | 1.38 | 1.19 | 0.83 | 0.87 | 1.03 | 0.82 | 0.89 | 0.88 | 0.91 | 14.65 | 37 |
| CUR 60 | 1.14 | 1.03 | 1.19 | 1.11 | 1.02 | 1.12 | 1.91 | 1.20 | 0.84 | 0.77 | 1.03 | 0.71 | 0.76 | 0.85 | 0.83 | 15.51 | 27 |
| **CUR 61** | **1.27** | **1.07** | **1.42** | **1.18** | **1.13** | **1.31** | **2.22** | **1.02** | **1.23** | **1.09** | **1.25** | **1.53** | **1.34** | **0.99** | **0.94** | **18.99** | **2** |
| CUR 62 | 1.06 | 1.03 | 0.98 | 0.99 | 0.91 | 0.91 | 1.67 | 0.73 | 0.95 | 0.98 | 0.95 | 1.00 | 0.96 | 0.94 | 0.94 | 15.00 | 30 |
| CUR 63 | 1.20 | 0.67 | 1.24 | 1.23 | 1.14 | 1.39 | 1.54 | 1.28 | 1.04 | 0.89 | 0.89 | 0.82 | 0.79 | 1.06 | 1.04 | 16.22 | 16 |
| **CUR 64** | **1.05** | **1.19** | **1.09** | **1.06** | **1.08** | **1.15** | **2.45** | **1.17** | **1.21** | **1.30** | **1.78** | **1.39** | **1.37** | **1.09** | **1.15** | **19.53** | **1** |
| CUR 65 | 0.77 | 1.03 | 0.94 | 0.79 | 0.95 | 0.76 | 0.73 | 0.94 | 0.97 | 0.84 | 0.82 | 0.87 | 0.87 | 0.77 | 0.78 | 12.83 | 59 |
| CUR 66 | 1.25 | 0.97 | 1.26 | 1.20 | 1.00 | 1.06 | 1.49 | 0.89 | 0.86 | 0.94 | 0.86 | 1.01 | 0.96 | 0.96 | 0.92 | 15.63 | 24 |
| CUR 67 | 1.00 | 0.88 | 1.17 | 0.99 | 1.00 | 0.99 | 1.97 | 1.03 | 1.10 | 1.00 | 1.24 | 1.20 | 0.99 | 1.02 | 0.93 | 16.51 | 14 |
| CUR 68 | 1.10 | 0.92 | 1.02 | 1.09 | 1.07 | 1.18 | 1.47 | 0.90 | 1.08 | 0.99 | 0.86 | 1.00 | 0.90 | 1.01 | 0.94 | 15.53 | 26 |

1: Plant height (cm); 2: Number of tillers; 3: Number of leaves per tiller; 4: Leaf length (cm); 5: Leaf breadth (cm); 6: Leaf area ($cm^2$); 7: Yield per plant (g); 8: Number of primary fingers; 9: Length of primary finger (cm); 10: Diameter of primary finger (cm); 11: Number of secondary fingers; 12: Length of secondary finger (cm); 13: Diameter of secondary finger (cm); 14: Length of mother rhizome (cm); 15: Diameter of mother rhizome (cm)

**Fig. 4.17. Rhizome of *Kaempferia rotunda*, Rank No. 1, Accession No. CUR 64**


| | | |
|---|---|---|
| Plant height (cm) | : | 70.86 |
| Number of tillers | : | 3.44 |
| Number of leaves per tiller | : | 7.06 |
| Leaf area ($cm^2$) | : | 207.78 |
| Number of primary fingers | : | 9.99 |
| Number of secondary fingers | : | 20.77 |
| Yield per plant (g) | : | 401.10 |

**Fig. 4.18. Rhizome of *Kaempferia rotunda* Rank No. 2, Accession No. CUR 61**


| | | |
|---|---|---|
| Plant height (cm) | : | 85.39 |
| Number of tillers | : | 3.11 |
| Number of leaves per tiller | : | 9.17 |
| Leaf area ($cm^2$) | : | 238.03 |
| Number of primary fingers | : | 8.66 |
| Number of secondary fingers | : | 14.55 |
| Yield per plant (g) | : | 363.33 |

**Fig. 4.19. Rhizome of *Kaempferia rotunda*, Rank No. 3, Accession No. CUR 34**


| | | |
|---|---|---|
| Plant height (cm) | : | 76.13 |
| Number of tillers | : | 4.33 |
| Number of leaves per tiller | : | 7.32 |
| Leaf area ($cm^2$) | : | 195.04 |
| Number of primary fingers | : | 9.66 |
| Number of secondary fingers | : | 18.88 |
| Yield per plant (g) | : | 164.44 |

**Fig. 4. 20. Rhizome of *Kaempferia rotunda*, Rank No. 4, Accession No. CUR 21**


| | | |
|---|---|---|
| Plant height (cm) | : | 71.28 |
| Number of tillers | : | 3.10 |
| Number of leaves per tiller | : | 5.44 |
| Leaf area ($cm^2$) | : | 239.55 |
| Number of primary fingers | : | 11.33 |
| Number of secondary fingers | : | 16.44 |
| Yield per plant (g) | : | 157.77 |

**Fig. 4. 21. Rhizome of *Kaempferia rotunda* Rank No. 5, Accession No. CUR 47**


| | | |
|---|---|---|
| Plant height (cm) | : | 86.72 |
| Number of tillers | : | 2.66 |
| Number of leaves per tiller | : | 8.49 |
| Leaf area ($cm^2$) | : | 246.31 |
| Number of primary fingers | : | 11.44 |
| Number of secondary fingers | : | 15.77 |
| Yield per plant (g) | : | 233.88 |

**Fig. 4. 22. Rhizome of *Kaempferia rotunda*, Rank No. 6, Accession No. CUR 32**


| | | |
|---|---|---|
| Plant height (cm) | : | 69.06 |
| Number of tillers | : | 3.66 |
| Number of leaves per tiller | : | 5.71 |
| Leaf area ($cm^2$) | : | 213.87 |
| Number of primary fingers | : | 9.11 |
| Number of secondary fingers | : | 15.55 |
| Yield per plant (g) | : | 131.66 |

**Fig. 4. 23. Rhizome of *Kaempferia rotunda*, Rank No. 7, Accession No. CUR 7**


| | | |
|---|---|---|
| Plant height (cm) | : | 69.06 |
| Number of tillers | : | 3.33 |
| Number of leaves per tiller | : | 6.18 |
| Leaf area ($cm^2$) | : | 193.70 |
| Number of primary fingers | : | 9.21 |
| Number of secondary fingers | : | 15.55 |
| Yield per plant (g) | : | 139.88 |

**Fig. 4. 24. Rhizome of *Kaempferia rotunda*, Rank No. 8, Accession No. CUR 45**


| | | |
|---|---|---|
| Plant height (cm) | : | 80.37 |
| Number of tillers | : | 2.88 |
| Number of leaves per tiller | : | 7.99 |
| Leaf area ($cm^2$) | : | 220.4 |
| Number of primary fingers | : | 8.88 |
| Number of secondary fingers | : | 13.10 |
| Yield per plant (g) | : | 349.99 |

**Fig. 4. 25. Rhizome of *Kaempferia rotunda* Rank No. 9, Accession No. CUR 28**


| | | |
|---|---|---|
| Plant height (cm) | : | 71.78 |
| Number of tillers | : | 3.44 |
| Number of leaves per tiller | : | 6.01 |
| Leaf area ($cm^2$) | : | 221.05 |
| Number of primary fingers | : | 7.55 |
| Number of secondary fingers | : | 14.11 |
| Yield per plant (g) | : | 147.21 |

**Fig. 4. 26. Rhizome of *Kaempferia rotunda*, Rank No. 10, Accession No. CUR 15**


| | | |
|---|---|---|
| Plant height (cm) | : | 66.88 |
| Number of tillers | : | 3.33 |
| Number of leaves per tiller | : | 5.96 |
| Leaf area ($cm^2$) | : | 206.3 |
| Number of primary fingers | : | 10.21 |
| Number of secondary fingers | : | 15.88 |
| Yield per plant (g) | : | 165.55 |

## 4.6. Performance of *Kaempferia rotunda* based on the status of the planting material used

*K. rotunda* is a valued aromatic medicinal plant that faces acute narrowing of natural populations due to human activities. Moreover, inappropriate cultivation practices, habitat destruction, deforestation and the high demand from pharmaceutical industries on wild sources make this plant threatened. Scarcity of planting material is a major problem in popularizing the crop. Under these circumstances, the present experiment has been designed to explore the possibility of using the mother rhizomes, primary fingers and secondary fingers of the rhizome as planting material so that the crop can be popularized in a better way.

Agronomic characters were examined during the present study. Among the fifteen, only four characters such as the number of tillers, number of leaves per tiller, yield per plant and length of mother rhizome exhibited statistically significant variations based on the status of the planting material (Table 4.27). Plants raised from primary fingers showed significantly higher number of leaves per tiller (5.30) and it was the lowest in plants raised from secondary finger (4.73). Number of tillers and yield were found to be higher in mother rhizome raised plants (4.07, 221.91 g). Length of mother rhizome was found to be significantly higher in plants raised from secondary fingers (4.42 cm) and it was the lowest in plants raised from primary fingers (4.11 cm). In the case of yield, plants developed using mother rhizome as planting material performed better (221.91 g) followed by plants developed from primary finger (213.75 g) and plants developed from secondary fingers (167.65 g). However, these variations were statistically significant. Hence it could be concluded that there is significant reduction in yield when rhizomes with different statuses are used as planting material and the farmers are free to use mother rhizome as seed material for the cultivation of *K. rotunda* based on the availability of the planting material.

Table 4.27. Observations on the growth and yield characters of *Kaempferia rotunda* in relation to the status of the planting material used.

| Sl.No. | Characters | Mother rhizome | | Primary finger | | Secondary finger | | CD 5% |
|---|---|---|---|---|---|---|---|---|
| | | Mean ±SE | CV | Mean ±SE | CV | Mean ±SE | CV | |
| 1 | Plant height | 48.76±1.38 | 23.36 | 50.13±1.35 | 22.26 | 47.69±1.31 | 22.58 | NS |
| 2 | Number of tillers | 4.07±0.14 | 27.52 | 3.43±0.14 | 34.69 | 2.32±0.09 | 32.76 | 0.35 |
| 3 | Number of leaves per tiller | 5.17±0.11 | 17.41 | 5.30±0.11 | 17.36 | 4.73±0.12 | 21.78 | 0.34 |
| 4 | Leaf length | 28.38±0.87 | 25.26 | 28.38±0.84 | 24.52 | 27.94±0.79 | 23.30 | NS |
| 5 | Leaf breadth | 7.49±0.16 | 18.16 | 7.50±0.18 | 20.13 | 7.46±0.20 | 21.58 | NS |
| 6 | Leaf area | 130.27±6.24 | 39.49 | 133.28±6.19 | 38.31 | 128.43±6.25 | 40.15 | NS |
| 7 | Yield per plant | 221.91±15.29 | 56.83 | 213.75±12.49 | 48.21 | 167.65±10.79 | 53.12 | 36.02 |
| 8 | Number of primary fingers | 7.40±0.31 | 34.73 | 7.38±0.24 | 27.24 | 6.99±0.23 | 26.75 | NS |
| 9 | Length of primary finger | 3.62±0.10 | 22.10 | 3.66±0.08 | 18.31 | 3.71±0.09 | 21.02 | NS |
| 10 | Diameter of primary finger | 2.02±.05 | 21.29 | 2.07±.0.04 | 17.73 | 2.10±.54 | 21.14 | NS |
| 11 | Number of secondary fingers | 13.21±0.84 | 52.46 | 13.09±0.78 | 49.04 | 10.97±0.63 | 47.40 | NS |
| 12 | Length of secondary finger | 2.80±0.09 | 26.79 | 2.77±0.07 | 22.18 | 2.76±0.08 | 22.46 | NS |
| 13 | Diameter of secondary finger | 1.47±.0.04 | 24.69 | 1.46±0.04 | 22.81 | 1.40±0.04 | 25.71 | NS |
| 14 | Length of mother rhizome | 4.32±0.09 | 17.13 | 4.11±0.08 | 16.06 | 4.42±0.09 | 16.74 | 0.09 |
| 15 | Diameter of mother rhizome | 2.38±0.05 | 18.49 | 2.38±0.06 | 19.33 | 2.37±0.06 | 20.25 | NS |

Studies in some other rhizomatous crops have showed different types of results in relation to the status of planting material used. Studies of Kumar and Gill (2010) in *Curcuma longa* noticed that mother rhizome derived plants showed higher number and weight of total rhizomes per plant and the highest fresh, dry and processed turmeric yield as compared to the plants raised from primary and secondary fingers. Manhas *et al*. (2010) also recommends the use of mother rhizome as it provides maximum yield over primary and secondary finger planting in turmeric. Similar observations have been reported in *Curcuma amada* also, where the mother rhizome produced plants showed 35-50% higher yield per plant suggesting the benefits of using mother rhizome as planting material (Jayasree *et al*., 2014a). Maheswarappa *et al*. (1999) observed significantly higher yield of *Kaempferia galanga* when using mother rhizome as the planting material as compared to finger rhizome.

### 4.7. Pharmacognostic standardization of *Kaempferia rotunda*

With the immense increase in the use of medicinal plants, several concerns regarding the quality and safety of herbal medicines have developed. Hence, it has become necessary to standardize quality assurance measures so as to safeguard supply of medicinal plants of good quality. It is fundamental to innovate and develop tools that can help in the proper identification of the medicinal plant and also the collected raw materials. Pharmacognosy is one such method adopted in Ayurveda to ensure the quality and safety of medicinal plants. Pharmacognostic standardization of plant materials includes its morphological, anatomical and biochemical characteristics (Mahesh *et al*., 2013).

Phytochemical standardization for the identification of the plant material can be accomplished by obtaining chemical fingerprint through chromatographic technique or bioassays. Chromatographic as well as spectroscopic techniques have proved very useful in isolation and proper

identification of active constituents in the plant extracts. Hence, standardization measures involve the quality control of various factors affecting the therapeutic property of plant right from selection of plant species to formulation of drugs so as to minimize variation and meet standards of quality, safety and efficacy. In this context, an experiment was designed and carried out for the pharmacognostic standardization of rhizomes of *K. rotunda* in order to authorize its macro and microscopic standards, powder characteristics and also for preliminary phytochemical investigation as suggested by Sahil *et al*. (2011).

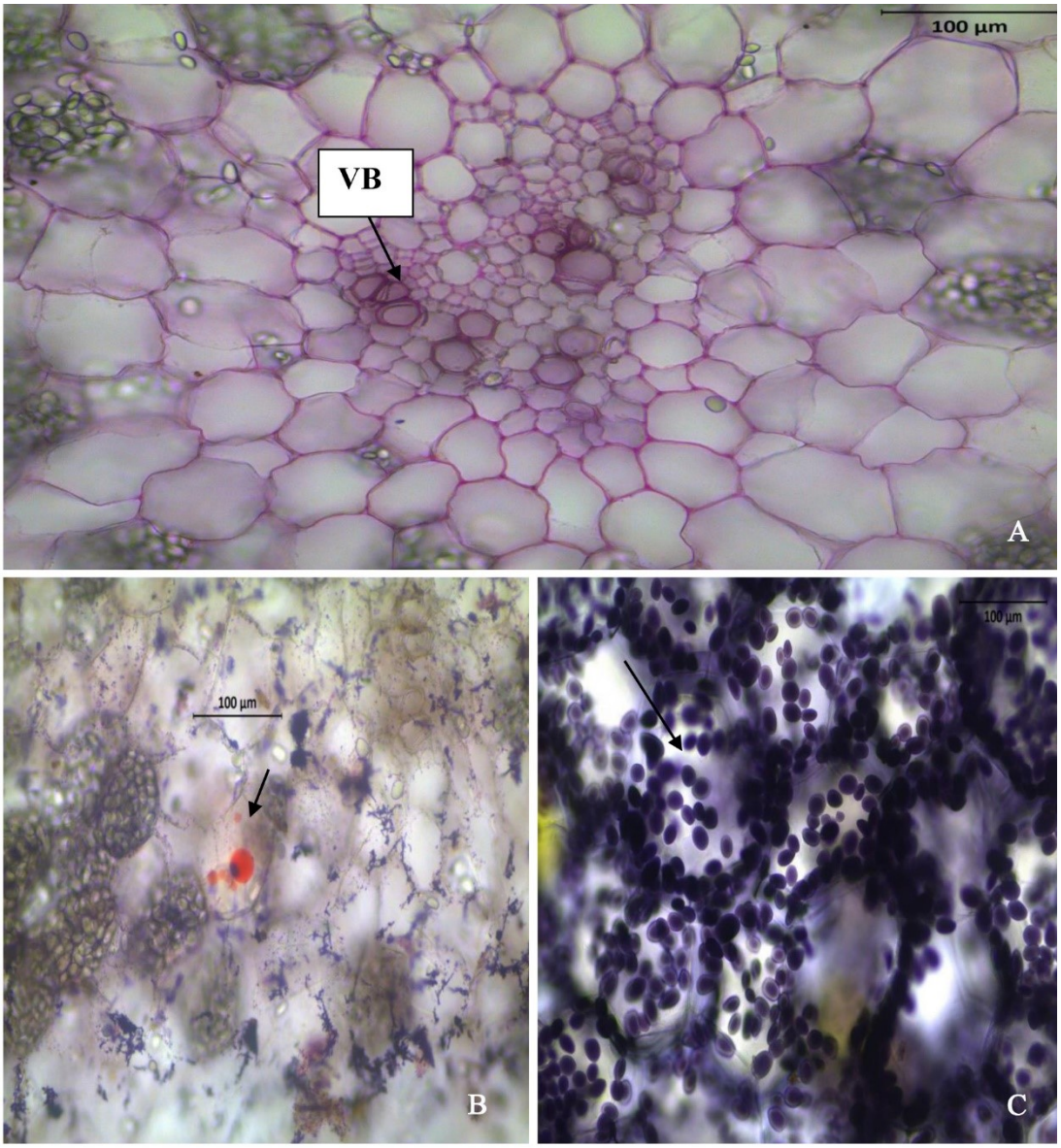
### 4.7.1. Microscopic studies

Detailed anatomical study of *K. rotunda* rhizome showed the presence of epidermal cells followed by an array of parenchyma cells. Vascular bundles were found scattered in the parenchymatous ground tissue. (Fig. 4.27A). These parenchyma cells were filled with oval shaped starch granules (SG) and oil globules (OG) (Fig. 4.27 B-C).

### 4.7.2. Powder analysis

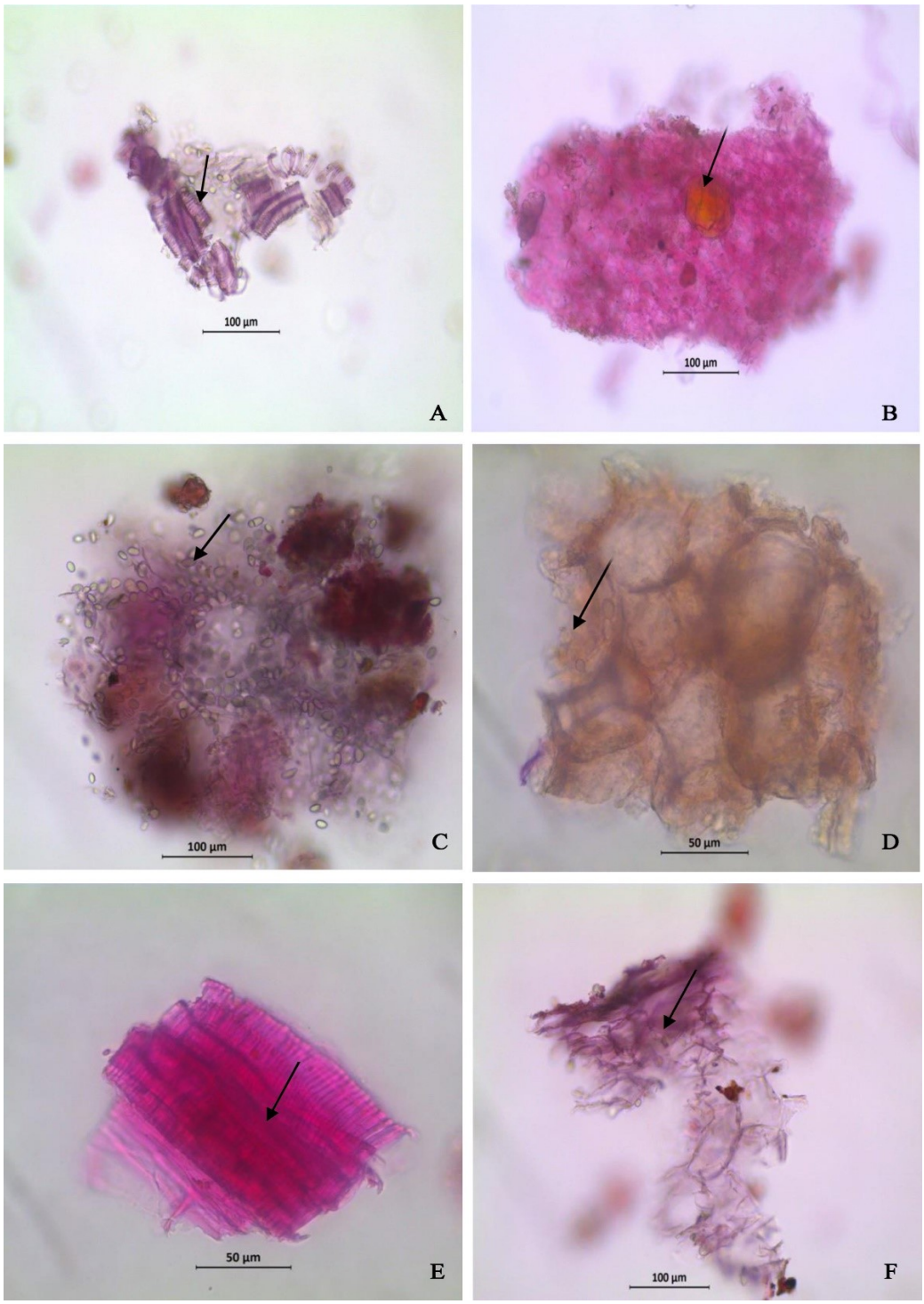
Powder microscopic studies are very important in Ayurveda for the proper identification and authentication of plant materials during drug standardization (Sreedhar *et al*., 2013). Powder analysis of *K. rotunda* displayed fragments of vessel with spiral thickening, cortical cells with oleoresin, parenchyma cells and cortical cells with starch grains, fragments of cork cells with underlying cortical cells (Fig. 4.28 A-F).

**Fig. 4.27. Microscopic studies of *Kaempferia rotunda***




**(A-C) T.S. of *Kaempferia rotunda* rhizome showing Vascular bundle (A), Oil globules (B) and Starch grains (C)**

**Fig. 4.28. Powder characteristics of *Kaempferia rotunda***




**(A) Vessel with spiral thickening (B) Cortical cells with oleoresin (C) Parenchyma cells with starch grains (D) Cortical cells with Starch grains (E) Fragments of Vessels (F) Fragments of corkcells and underlying cortical cells**

#### 4.7.2.1. SEM analysis of rhizome powder of *Kaempferia rotunda*

The surface morphology of the rhizome powder of *K. rotunda* was investigated using Scanning Electron Microscope (SEM). SEM micrographs of rhizome powder particles taken at room temperature with different magnifications are shown in Fig. 4.29. Powder particles of different sizes such as 19.68 µm, 16.13 µm, 12.32 µm, 15.36 µm and 8.72 µm are observed under 10 µm magnifications. Powder particles are more or less elliptical in shape with rough surface.

### 4.7.3. Phytochemical analysis of *Kaempferia rotunda* using GC-MS

The result of methanolic extract of *K. rotunda* rhizome revealed 9 peaks (Fig. 4.30), with 9 compounds identified (Table 4.28) representing 100% of the entire extract. The major among them were camphor (1.88%) with retention time (RT) of 9.157 minute, bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-acetate (5.24%) with retention time of 13.054 minute, pentadecane (11.89%) with retention time of 18.765 minute, eicosane (1.90%) with retention time of 23.450 minute, phytol acetate (3.57%) with retention time of 26.196 minute, retinol (4.85%) with retention time of 32.056 minute, (1R-1α,2β,4α,5β,6α,7α))-3,8-dioxa tricycle (5.1.0.$0^{(2,4)}$)octane-5,6-diyl diacetate,4-benzoyl oxy methyl (50.23%) with retention time of 39.041 minute, cholest-5-en-3-yl benzoate (2.40%) with retention time of 46.971 minute and 2β,9α-dihydroxyverrucosane (18.03%) with retention time of 48.731 minute. Identification of these compounds in the rhizome extract serves as the basis in determining the possible health benefits of the plant leading to further pharmacological study.

**Fig. 4.29 Scanning electron micrographs obtained from rhizome powder of *Kaempferia rotunda* under different magnifications**

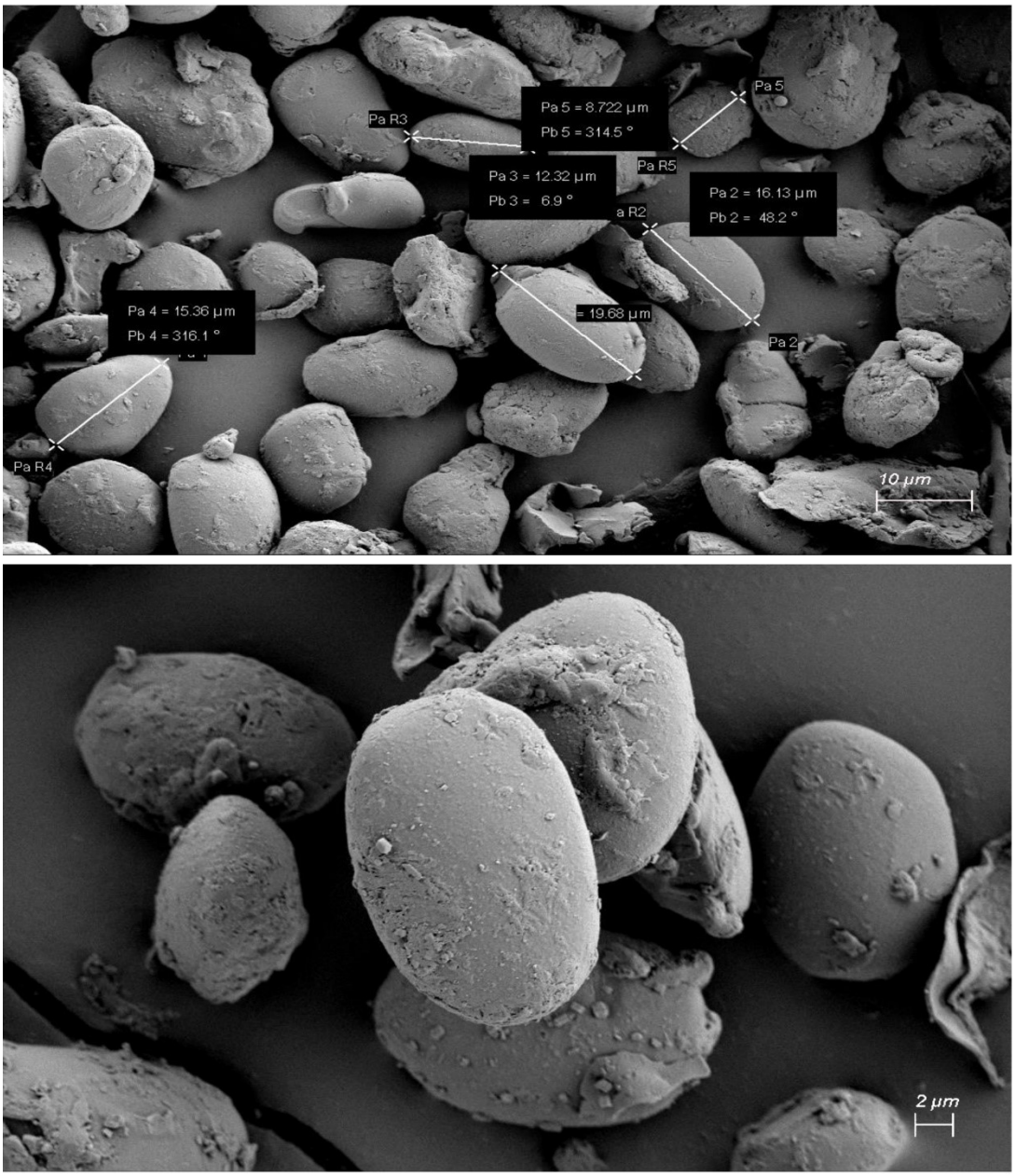
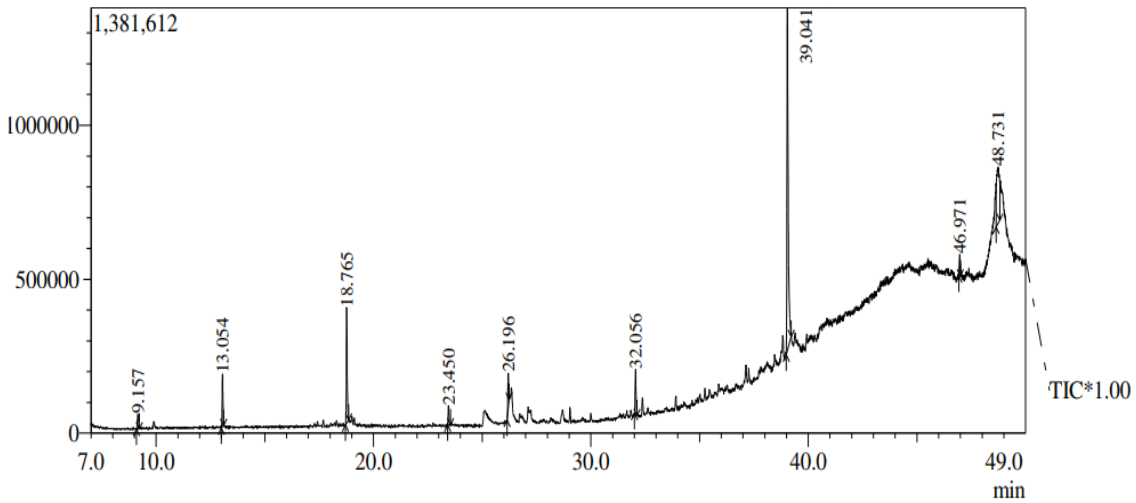


Table 4.28. Phytochemical compounds identified in the methanolic extract of *Kaempferia rotunda* rhizome using GC-MS analysis

| Peak Number | RT (min) | Name of identified compounds | Area % | Class of compound | Base m/z value |
|---|---|---|---|---|---|
| 1 | 9.157 | Camphor | 1.88 | Terpenoid | 95.20 |
| 2 | 13.054 | Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-acetate | 5.24 | Monoterpene | 95.10 |
| 3 | 18.765 | Pentadecane | 11.89 | Acyclic alkanes | 57.10 |
| 4 | 23.450 | Eicosane | 1.90 | Acyclic alkanes | 57.05 |
| 5 | 26.196 | Phytol acetate | 3.57 | Diterpene | 57.05 |
| 6 | 32.056 | Retinol | 4.85 | Vitamin A | 123.15 |
| 7 | 39.041 | (1R-1α,2β,4α,5β,6α,7α))-3,8-dioxa tricycle (5.1.0.0$^{(2,4)}$) octane-5,6-diyl diacetate,4-benzoyl oxy methyl | 50.23 | Diterpene | 105.05 |
| 8 | 46.971 | Cholest-5-en-3-yl benzoate | 2.40 | Ester of cholesterol and benzoic acid | 147.10 |
| 9 | 48.731 | 2β,9α-dihydroxyverrucosane | 18.03 | Diterpene | 187.10 |

**Fig. 4.30. GC chromatogram of methanolic extract of *Kaempferia rotunda* rhizome**



# Chapter V
# SUMMARY AND CONCLUSION

*Kaempferia rotunda* L. belonging to the family Zingiberaceae is an important and rare medicinal plant that faces acute narrowing of natural populations due to various anthropogenic activities. It is necessary to conserve the plant in order to meet pharmaceutical needs and also to prevent the species from becoming extinct. Selection for genotypes with the accumulation of potential dominant factors controlling agronomic characters is essential for the development of improved varieties useful to cultivars so that the crop yield is improved and the crop is more widely accepted both for pharmaceutical as well as economic reasons.

The plant is popularly known as peacock ginger. It is possibly a native of Indo-china and is distributed throughout India from Eastern Himalaya to Sri Lanka and the Malay Peninsula to Malay Island. This plant is grown as under growth in mixed forests or in open grassy areas. There is not so much of published information on the agronomical aspects of *K. rotunda*. The medicinal properties of *K. rotunda* are associated with various phytochemical constituents present in it.

Exploring and conserving the genetic variability of *K. rotunda* is very important since its natural habitats are getting deteriorated due to various anthropogenic activities and the displacement of peasantry farming by industrial agriculture. Hence, the present experiments were designed and carried out to study the genetic variability of the genotypes of *K. rotunda* in the study area and the interrelationships and association of growth and yield characters of it. An experiment has also been carried out to optimize the nature of rhizomes that could be used as planting materials.

The present experiments were conducted in the experimental plot of the Genetics and Plant breeding division of the Department of Botany, University of Calicut, Kerala from 2016 to 2018. Sixty eight genotypes of *K. rotunda* collected from different locations of Kerala State formed the experimental material. Fresh and healthy rhizomes collected during October to December 2016 were planted for preliminary screening and multiplication of the planting material in the experimental plot. The evaluation trials and other experiments were started in the first cropping season of 2017 in the first week of May before the onset of South-West monsoon. The plants were allowed to grow for six months to reach full maturity and harvested simultaneously.

Genetic diversity is essential for genetic improvement of crops. If the information on genetic diversity is not available, collection and analysis of the diversity is essential. The survival value of a population is directly related to the genetic variability prevailing in it. It is established that genetic variability is a prerequisite for selection to evolve superior genotypes. Genetic variability of *K. rotunda* was studied presently using standard tools of analysis based on the germplasm collected for the purpose. Genetic control of growth and yield characters and phenotypic and genotypic variability, heritability and genetic advance of the characters were analysed to evaluate the genetic variability of the populations raised from the genotypes collected.

Study of frequency distribution gives a fundamental idea of the genetic control of characters and the nature of distribution of dominant and recessive alleles in the gene pools of the characters. Among the growth characters, plant height, leaf length and leaf breadth showed continuous frequency distribution with skewness of distribution towards the distal side showing the accumulation of higher number of dominant alleles. This shows that gene pools of these characters show accumulation of higher number of dominant

alleles even when maintaining good genetic base ranging from comparatively lower to higher values. Selecting the promising genotypes with maximum accumulation of dominant contributing alleles is necessary for the development of superior genotypes and for maintaining good genetic base. In the case of growth characters, number of tillers and number of leaves per tiller showed skewness of the distribution towards the proximal side thus indicating the accumulation of higher number of recessive contributing factors. Hence, more scientific selection studies are to be carried out to develop promising varieties with higher accumulation of dominant alleles of these characters. However, in the case of these characters also, the frequency distributions were with comparatively broad genetic bases indicating the existence of genotypes with different levels of allelic combinations ensuring the genetic diversity of the plant populations under study.

Among the yield characters, yield per plant, length of primary finger, number of secondary fingers and length of secondary finger showed skewness of the distribution towards the proximal side thus indicating the presence of higher number of recessive alleles. Diameter of mother rhizome and diameter of secondary finger showed skewness of the distribution towards the distal end of the distribution curve indicating the contribution of dominant alleles for this character. It also confirms the essentiality of selection for better phenotypes and genotypes with higher number of dominant contributing alleles so as to develop superior varieties. This is a desirable phenomenon since both the characters directly contribute towards the rhizome yield of plants.

Selection for yield and yield contributing traits is very important in the genetic stock of *K. rotunda* occurring in Kerala. The above analysis shows that the genetic base of *K. rotunda* in the study area is comparatively broad and there exists no threat of narrowing of genetic diversity threatening the

existence of *K. rotunda* in natural and cultivated habitats. However, due to utilization of agricultural land for other purposes and changes in cropping pattern, *K. rotunda* faces acute threat in its natural habitats and traditional homesteads. Hence, steps should be taken to conserve the species in its natural and homestead habitats so that the diversity of the species is maintained potentially and the unexplored potential of this crop is accessible to the future generations to study and explore plant based drugs that improve the quality of human life considerably.

Study of the significance of genetic variability of agronomic characters revealed that the growth characters studied showed statistically significant variation at 1% level of significance except for number of tillers. The highest coefficient of variation was observed for yield per plant followed by number of secondary fingers, length of secondary finger, diameter of secondary finger and leaf area in that order. The lowest coefficient of variation was observed for leaf breadth. Yield per plant is the most variable character and is less stable. Statistically significant level of variability in the case of all the characters studied indicates the possibility of selection of promising genotypes based on the above character in further improvement programmes of this species.

Study of variability using phenotypic coefficient of variation and genotypic coefficient of variation unveiled the existence of high phenotypic coefficient of variation (PCV) over genotypic coefficient of variation (GCV) for all the characters studied. Higher value of phenotypic coefficient of variation over genotypic coefficient of variation indicated the positive influence of environment on these characters. In the case of growth characters, the highest PCV was shown by number of tillers followed by leaf area. The lowest PCVs were exhibited by leaf length and leaf breadth. GCV was found to be the maximum for leaf area. Among the yield characters, the

highest PCV and GCV were shown by yield per plant. The differences between PCV and the corresponding GCV were higher in the case of number of tillers and number of secondary fingers indicating comparatively higher influence of environment on the phenotypic expression of these characters compared to the remaining characters. Characters with lesser difference between PCV and GCV show the limited role of environment on these characters.

Fifteen growth and yield characters of *K. rotunda* have been studied presently for broad sense heritability. Growth characters showed heritability varying from 13.04% in the case of number of tillers to 62.57% in the case of plant height. Heritability of yield characters ranged from 36.36% in the case of diameter of secondary finger to 62.88% in the case of yield per plant. This indicates the occurrence of comparatively higher heritability in the case of yield characters as compared to growth characters. The characters such as plant height, yield per plant, diameter of primary finger, diameter of mother rhizome, leaf length and leaf area showed heritability above 50% and these characters would respond to selection in a better way and the improvement of these parameters could be achieved through direct selection.

Percentage of genetic advance is an estimate indicating the quantum of improvement that is possible through selection. Heritability values coupled with genetic advance would be more reliable and useful in selection practices. Among the growth characters, the highest genetic advance was shown by leaf area and the lowest genetic advance was shown by number of tillers. Among the yield characters, the highest genetic advance was shown by yield per plant and the lowest genetic advance was shown by length of mother rhizome.

Selection cannot be accomplished on the basis of a single character since most of the agronomic characters are polygenic in nature and they are interrelated. Correlation study is very important in plant breeding because of

it is useful in disclosing the magnitude and direction of the relationship between yield and yield contributing characters. Positive correlation between desirable characters is supposed to be favourable and it helps the breeder in selection whereas negative correlation interrupts the recovery of the combinations in both the characters.

Correlation analysis of fifteen agronomic characters of *K. rotunda* was carried out presently using the data obtained from sixty eight accessions. In the present study yield per plant exhibited significant correlation with plant height, number of leaves per tiller, leaf length, leaf area and number of secondary fingers. It indicates that these characters have some inherent interrelationship with yield, suggesting the possibility of better yield through the improvement of these correlated characters.

The quantitative characters of an organism show different levels of associations between them due to gene sharing. Study of such association of characters is carried out for grouping of variables and data reduction so as to find out the lead characters that can be employed in selection processes in crop improvement programmes while searching for superior genotypes. Factor analysis by means of principal component analysis has been done for the purpose presently based on six growth and nine yield characters of *K. rotunda.*

Character association in *K. rotunda* has been found out presently with the help of factor analysis using fifteen variables. Factor analysis resulted in the grouping of the fifteen characters into three factors based on factor loading. The first factor was found to be associated with ten variables while the second factor group was occupied by four variables and the third factor group by one variable when analysed based on factor loading. The first factor was associated with number of tillers, leaf breadth, leaf area, length of primary finger, diameter of primary finger, number of secondary fingers,

length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome. Characters belonging to the same factor group share common alleles to a considerable extent in their expresssion. Plant height, number of leaves per tiller, leaf length and yield per plant are grouped under the second factor. The third factor group was found to be associated with number of primary fingers. Number of tillers, leaf breadth, leaf area, length of primary finger, diameter of primary finger, number of secondary fingers, length of secondary finger, diameter of secondary finger, length of mother rhizome and diameter of mother rhizome could be used as lead characters in crop improvement programmes in *K. rotunda.*

Genetic divergence studies help the grouping of different accessions into different clusters. Cluster analysis enables the understanding of the genetic behavior of genotypes under consideration. Study of genetic divergence among the sixty eight genotypes of *K. rotunda* was presently carried out by principal component analysis.

Different genotypes of a plant species exhibit varying degrees of genetic divergence due to the similarities and variations of their genetic constitution. Genetic divergence analysis measures the extent of genetic diversity prevailing in the selected genotypes, which further fastens the selection process of diverse genotypes as parents for further breeding programmes. Cluster analysis is an efficient and widely used statistical tool which enables the understanding of the genetic behavior of genotypes under consideration and there by the genetic divergence. Cluster analysis grouped the entire accessions into three clusters. The first cluster is occupied by sixty six accessions from all the thirteen districts, showing maximum accommodation of genotypes which are related. The first cluster at a linkage distance of 0.996 bifurcated again into two sub clusters, the first consisting of

55 genotypes and the second consisting of 11 genotypes. The second cluster is occupied by one accession namely CUR 13 collected from Kottayam district and the third cluster occupied by CUR 58 collected from Wayanad district.

Genotypes from the same locality were placed in separate clusters indicating wide genetic diversity among them. This may be due to frequent exchange of germplasm between different geographical regions. Genotypes from these clusters could be used as parental lines for further breeding programmes. Genotypes belonging to the same clusters show higher levels of similarity and it is generally presumed that they show genetic proximity. Genotypes belonging to different clusters are genetically distant from each other. Being clonally propagated species, diverse accessions of *K. rotunda* could be selected for further breeding programmes based on further assessment of performance and this helps to explore the genetic variability and production of high yielding varieties could be possible.

Comparative performance of the different accessions of *K. rotunda* has been analysed presently based on major growth and yield characters with the help of performance indices calculated for each agronomic character and the cumulative index calculated for each genotype.

Performance analysis revealed that among the sixty eight accessions, accession number CUR 64 ranked first with a cumulative performance index of 19.53, followed by CUR 61 with a cumulative performance index of 18.99, CUR 34 with a cumulative performance index of 17.97, CUR 21 with a cumulative performance index of 17.78 and CUR 47 with a cumulative performance index of 17.37. The accessions CUR 32, CUR 7, CUR 45, CUR 28 and CUR 15 ranked from 6 to 10 with a cumulative performance index of 17.17, 17.00, 16.80, 16.73 and 16.71 in that order. These superior accessions exhibit significantly higher values of agronomic characters over the remaining

accessions and these can be subjected to further breeding programmes. Promising genotypes of *K. rotunda* with better agro-morphological characters can be made available to the farming community in this way.

An experiment was carried out to evaluate the performance of the crop of *K. rotunda* in relation to the status of the planting materials used. The experimental material consisted of seed rhizomes of three different statuses such as mother rhizome, primary fingers and secondary fingers. A crop of 68 plants for each type of planting material was raised from fresh and disease free rhizomes of about 3 cm – 5 cm length and 25 g – 30 g weight and the same agronomic practices as mentioned in the previous experiments were adopted.

The influence of morphological status of the planting material on fifteen morphometric characters of *K. rotunda* was investigated by using mother rhizome, primary finger and secondary finger as planting material. Among the fifteen, only four characters such as the number of tiller, number of leaves per tiller, yield per plant and length of mother rhizome exhibited statistically significant variations based on the status of the planting material. In the case of yield, plants developed using mother rhizome as planting material performed better (221.91 g) followed by plants developed from primary finger (213.75 g) and plants developed from secondary fingers (167.65 g). However, these variations were statistically significant. Number of tillers and yield were found to be higher in mother rhizome raised plants.

Identification of medicinal plants in the manufacture of herbal drugs has been a great challenge in the present scenario due to fall in its availability. This might lead to decrease in the quality of raw materials that are used in the manufacture of drugs. Adulteration has resulted in the decline of quality in the medicinal formulations of Ayurveda. Pharmacognosy is a method which can help in the proper identification of medicinal plant in Ayurveda to ensure

quality. Quality evaluation of medicinal preparation is a fundamental requirement of pharmaceutical industry. The process of standardization can be achieved by pharmacognostic and phytochemical analysis.

Anatomical study of rhizome of *K. rotunda* showed the presence of epidermal cells followed by an array of parenchyma cells. Vascular bundles were found scattered in the parenchymatous ground tissue. These parenchyma cells were filled with starch granules and oil globules. Powder analysis of the rhizome displayed fragments of vessel with spiral thickening, cortical cells with oleoresin, parenchyma cells and cortical cells with starch grains, fragments of cork cells with underlying cortical cells. SEM micrographs of rhizome powder particles of different sizes such as 19.68 µm, 16.13 µm, 12.32 µm, 15.36 µm and 8.72 µm were observed under 10 µm magnification.

The methanolic extract of *K. rotunda* rhizome revealed 9 peaks with 9 compounds. The major compounds identified were camphor (1.88%), bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-acetate (5.24%), pentadecane (11.89%), eicosane (1.90%), phytol acetate (3.57%), retinol (4.85%), (1R-1α,2β,4α,5β,6α,7α))-3,8-dioxa tricycle (5.1.0.$0^{(2,4)}$)octane-5,6-diyl diacetate,4-benzoyl oxy methyl (50.23%), cholest-5-en-3-yl benzoate (2.40%) and 2β,9α-dihydroxyverrucosane (18.03%). Identification of these compounds in the rhizome extract serves as the basis for determining the possible health benefits of the plant leading to further pharmacological study.

Being a marginalized crop, the improvement of yield potential in *K. rotunda* is very important for popularization of the crop as food component and also for product diversification. In Kerala, local cultivars are being used for cultivation and they show high level of variability. There is an urgent need to conduct crop improvement programmes for the development of superior high yielding varieties and also for the conservation of this plant. It

is hoped that the present study has been useful in generating such information and also in selecting superior genotypes from the gene pool of *K. rotunda* collected and conserved for the purpose and the best performing genotypes identified presently could be used for further breeding programmes.

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# PUBLICATIONS

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