Review Article

Biotechnological aspects and genetic diversity assessment in Zingiber *species*

Abstract

Ginger (Zingiber officinale) is a zingerbiaceae family member. It is a very important old-world crop with a Mediterranean provenance. Its origins may be traced back to Southeast Asia.. It was first grown in India and China. Later it reached Asia Minor. It arrived in Europe in the early Middle Ages. The tropics now cultivate it widely. It is India's most important horticulture crop, used in culinary, ayurvedic medicine, and pharmaceutical industries. India has many types of ginger species. So, it's vital to research the various ginger species and kinds. It is grown in Nepal, Thailand, India, China, and throughout Asia. RAPD and ISSR are utilized as molecular markers for this purpose. For their protection, to avoid cross-species germplasm fusion, and to conserve their vital species. The growing demand for ginger in the food and beverage industry is driving market expansion. The growing use of ginger in food products such as alcoholic and non-alcoholic drinks, soups and sauces is likely to drive the worldwide ginger market growth. Global consumer awareness of ginger's health advantages is positively fueling the ginger market. The global ginger market is likely to benefit from rising consumer demand for bakery and convenience foods. This paper aims to inspire scientists/researchers to develop efficient techniques for molecularly authenticating powerful zingiber species.

Keywords

Zingiber officinale, Genetic diversity, Biosynthesis, Somatic embryogenesis, RAPD, ISSR.

Introduction

Ginger, which is scientifically known as Zingiber officinale, has a wide range of distribution throughout the tropical regions of the planet, and it is propagated vegetatively through the rhizome of the plant. Because it is used as a flavoring agent in food, used in medicines because of their antimicrobial, anti-inflammatory, and cancer-fighting properties as well as for the extraction of various ginger oils such as gingerol, Shagoal zingiberene, and zingiberal as well as chavicol, cineole and graniol, as well as for the extraction of d-camphene and other compounds, it is considered the most valuable crop on the planet. This spice is widely valued for its distinctive odour and extremely strong taste, which distinguishes it from other spices. It is utilized as a source of income by the underprivileged to supplement their income.

In 2012, India produced 703 000 tonnes of ginger, out of a global output of over 2 million tonnes. Indians grow, consume, and export ginger. In India, ginger is grown in Kerala, Odisha, Karnataka, Assam, Arunachal Pradesh and Madhya Pradesh.The Indian ginger cultivars known as Colchine ginger and Calicut ginger are the most widely consumed in the rest of the globe.

In the world, the majority of ginger gene pool research is conducted either by RAPD markers (Jatoi et al., 2006) or through ISSR markers (Jatoi et al2006). (Kizhakkayiland Sasikumar, 2010). Genetic diversity studies have been published from the southern portion of the country using RAPD (Harisaranrajetal, 2009), ISSR (Kizhakkayiland Sasikumar, 2010), rice-SSR as RAPD (Jatoietal., 2008), and AFLP markers (Kavithaetal., 2010) markers among the Indian genetic pool. It is necessary to investigate the genetic variety of ginger by employing several types of molecular markers, such as RAPD and ISSR, to determine the efficiency of various primers in order to achieve this goal.

For the most part, ginger gene pool research use either RAPD marker (Jatoi et al., 2006) or ISSR markers (KizhakkayilandSasikumar, 2010). Many studies of the Indian genetic pool have used RAPD, ISSR, rice-SSR as RAPD (Jatoietal., 2008), and AFLP markers to examine genetic diversity. As a result, molecular

markers like RAPD and ISSR must be used to investigate the genetic diversity of ginger. Different primers can be tested using these methods.

Botanical description

Distribution of genus Zingiber

Zingiber officinale is a plant that is native to South-East Asia and has been used by the Chinese and Hindus since the beginning of recorded history. It was one of the first eastern spices to become recognised in Europe, having been brought to the continent by Arab merchants who sold it to the Greeks and the Romans.
The commonly consumed ginger comes from tropical Asia. It is now grown as a cash crop in Latin America, Africa, and South East Asia, as well as in other parts of the world where it is used. Fifty percent of the world's ginger is grown in India. Jamaica has the best ginger, and it comes from there.
It was first grown in the New World and brought back to Europe in 1585. Jamaican ginger was the first of its kind to be grown there.
There are major top six ginger growing countries in the world. Out of which india produces 703,000 tonnes of ginger in the total world production . Then the china is the second most important produce of ginger in the world which produces about 425,000 of ginger of total world production . Then there is nepal (255,208 tonnes), then Nigeria (156,000), then Thailand (150,000), then Indonesia (113,851 tonnes) according to 2012 census.
Growing ginger is best accomplished in moist, rich, and well-drained soil. The great bulk of carbon and critical nutrients are stored in tropical rainforests by living plants, dead wood, and decaying leaves. Because the decomposable organic matter is recycled so quickly, little nutrients reach the soil, resulting in nutrient-depleted soil.

Morphology of Zingiber Officinalis

In the botanical world, the zingiber officinale falls in the zingiberaceae family, which is well-known for its therapeutic effects and also for its usage as a spice. Zingiber officinale is a perennial plant that grows in an upright position. It has a rhizome that is strong and robust, with palmately branching stems. The stem is covered with tiny scale leaves and small fibrous roots, which provide it a scaly look due to the presence of these scales.. The stems grow to a maximum height of 3 ft. and are encircled by a base of leaves that act as a sheathing. In the inside, the rhizome is light yellow, while the outside is greenish yellow. The leafy branches are annual and grow in an upright position. The linear lanceolate alternating leaves are 20cm long and have a rounded shape. The leafy branch is an annual upright stem that grows to 60-90 cm in height and the sheathing leaf bases provide a deep layer of protection.Blooming at the summit of the stem, floriets are formed in a thick spikes with green yellow petals that surround a yellow flower with only an emerald tip and are borne in groups of three. The rhizome is rich in starch, gums, and oleoresin, as well as an essential oil, among other things. A piece of rhizome is propagated by a component of the plant when every piece of rhizome has at minimum one functional bud on it.

Cytology of Zingiber Officinalis

The three Zingiber species identified were Z. officinale, Z. zerumbet, and Z. cassumunar, all with 2n = 22. Metaphase plates are shown in Figures 6, 7 & 8. Figures 9-11 illustrate their ideograms. While all three species have the same chromosomal number, their morphology differs. Compared to the other two species, Z. officinal's chromosomes are thin and slender. The length of the chromosomes may be characterized as long, medium, or short, with the long and medium being the most distinct. The chromosomes are separated nnmmmminto five pairs: five long, four medium, and two short. 3 pairings of G'G', H'H', and IT (Text-Fig. 9),

based on the letter V arrangement (these are long chromosomes), and 2 pairs of 'crescents'. As demonstrated in Text-Fig. 9, the two 'crescent' pairs are the shortest. Sub- or median constriction may be seen on all chromosomes.

Z. cassumunar has 11 sets of somatic chromosomes (Text-Figs. 8 and 11). The chromosomes are somewhat big and have a diameter that is comparable to that of the Costus species investigated. With the exception of A'A' and IT, almost all of them exhibit median or submedian constrictions (Text-Fig. 11). In these two pairs, a subterminal kinetochore is present. Rod-type chromosomes make up seven of the eleven chromosome pairs. The four remaining pairs, G'G' and H'H' (Text-Fig. 11), are half - moon short chromosome with median constriction, while the others are half - moon short chromosomes with median constriction. The pair H 'H' indicates the complement's longest chromosomes (Text-Fig. 11). Only the short chromosomal pair IT (Text-Fig. 11) exhibits subterminal constriction, while the rest have median constriction. There were no trabants discovered on any of the discs that were examined.

Zingiber zerurnbet.--This species' somatic number is 22 (Text Fig. 7). The chromosomal morphology is quite similar to Z. cassumunar. Compare their ideograms in Text-Figs. 10 and 11. One chromosome in pair E'E' (Text-Fig. 10) has a satellite at its proximal end. But the satellite in its equivalent couldn't be seen.

As can be seen in Text-Figs. 9, 10, and 11, the chromosomes of Zingiber officinale are unique from the others, not only in size but also in shape, when compared to the other Zingiber species under consideration. Zingiber offcinale has three long pairs of V-shaped chromosomes, while Zingiber zerurnbet and Zingiber cassumunar have just two long pairs of V-shaped chromosomes. In Z. zerumbet and Z. cassumunar, on the other hand, there are three pairs of short chromosomes (two crescents with median constriction and one rod with sub-terminal constriction). According to the text-figures 10 and 1I, the chromosomal shape of Z. zerumbet and Z. cassumunar is virtually same, despite the fact that the two species are genetically distinct.

Fig (1-11):Chromosome no and morphology of different zingiber species.

Genetic variety is the result of exchange of genetic material during the inheritance process. With the passage of time and space, things changes. Sexual reproduction is important for genetic diversity preservation because it generates unique offspring by combining the DNA of both parents. Gene mutations, genetic drift, and gene flow all contribute to genetic diversity.
Self-incompatibility and high rates of sterility make ginger (Zingiber officinale Roscoe) a difficult plant to cultivate; its genetic variety can only be achieved by mutation and natural selection. Understanding

ginger's variety may help both conservation and breeding efforts, since it is used both as a flavouring and as a herbal treatment.
The efficiency of various primers may be studied using various types of molecular markers, including as RAPD and ISSR, in order to better understand ginger's genetic diversity. For this plant genotype, direct control of genetic material is believed to be superior to the use of molecular markers such as RAPD, ISSR, etc. (Paterson et al., 1991). In order to research the genetic diversity of various crop species, such as ginger, we employ a variety of markers.

Different markers employed by researchers for germplasm characterisation are listed below.

Morphological markers

In breeding programs, markers that are connected to variation in form, size, color and surface of different plant components are termed morphological markers. Such indicators relate to accessible gene loci that have clear influence on morphology of plant.. With the help of these marker we can study the genetic diversity assessment of Zingiber officinalis by camparing the different species of the same genus.
Ginger (Zingiber officinale Rosc.) is grown for its fresh and dried rhizomes. Ginger never reproduces sexually and never sets seed. Cultivars are named for the place where they originated. It is also vegetatively propagated, making genotype identification difficult. Eight quantitative and two qualitative DUS features were used to describe 27 ginger genotypes. Four characteristics were monomorphic, four dimorphic, and two polymorphic. Most morphological features showed modest genotype variability, whereas rhizome characters showed considerable diversity.
It has been reported that ginger yield, quality traits, and rhizome characteristics can vary (Khan, 1959; Thomas, 1966; Krishnamurthy et al., 1972; Muralidharan and Kamalam, 1973; Mohanty and Sharma, 1979; Nybe and Nair, 1979; Nybe et al., 1980; Sreekumar et al., 1980; Kumar et al., 1980; Mohanty et al (Fig.1). Obsolete cultivars have short rhizomes, limited yields, and better quality than cultivars/improved varieties. Table S1 lists ginger cultivars/varieties suitable for different applications. Genes, soil types, locales, seasons and cultural techniques all affect production and quality in ginger.
The ginger plant (Zingiber officinale) differs from the other ginger species (Z. zerumbet, Z. roseum, and Z. macrostachyum) in that it lacks periderm, has a short-lived functional cambium, and possesses xylem vessels with scalariform thickening in the rhizome (Ravindran et al., 1998).

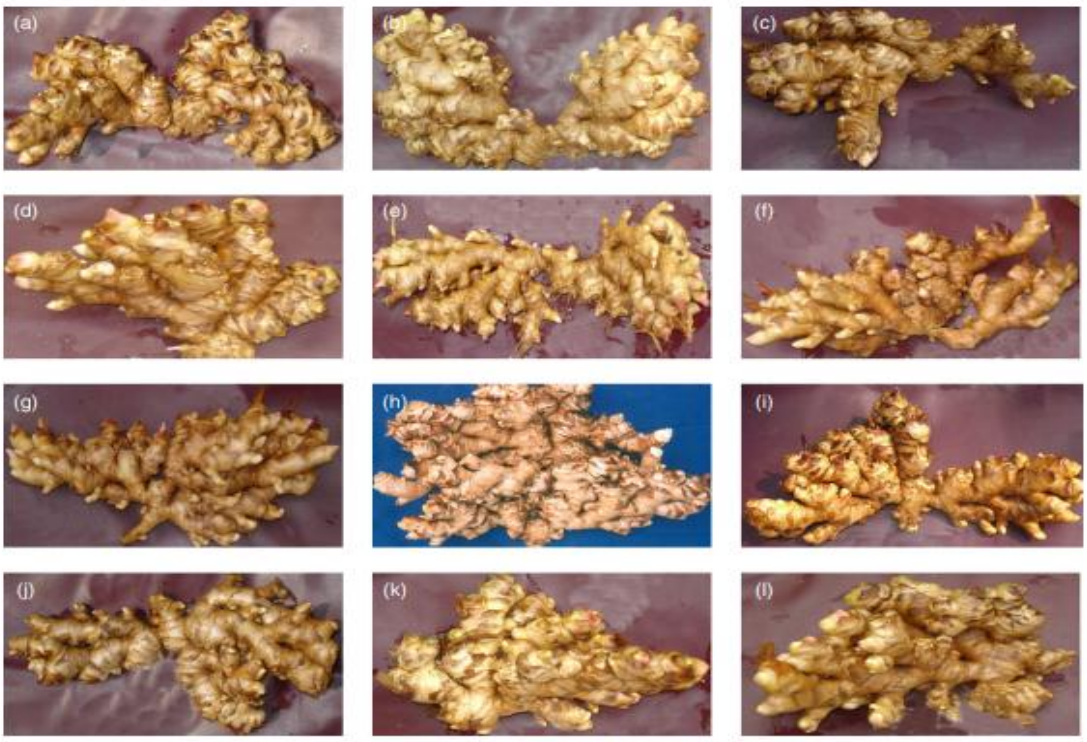


Fig. 1. Variability for rhizome features in Indian ginger germplasm (a) 'Varada', (b) 'Mahima', (c) 'Rejatha', (d) 'Suprabha', (e) 'Sabarimala', (f) 'Kozhikkalan', (g) 'Kakakalan', (h) 'Ellakallan', (i) 'Nadia', (j) 'Rio-de-Janeiro', (k) 'Silent valley' and (l) 'Himachal' (A colour version of this figure can be found online at journals.cambridge.org/pgr).

Phytochemical markers

There are important chemical components in the rhizome of Zingiber officinale that have valuable pharmacological qualities that might be isolated and utilised as alternatives to manufactured medications in the treatment of specific illnesses such as arthritis, rheumatism, and other musculoskeletal disorders. After conducting an extensive review of peer-reviewed journals,We discovered that the fresh rhizome of ginger includes phenolic compounds, essential oil, flavonoids, carbohydrates, glycosides, proteins, alkaloids, steroids, saponins, terpenoids, and tannins.. These phytochemicals are vital in the therapeutic properties of ginger, and they have a variety of functions. The following is a list of chemicals

Table 1: list of chemicals

S.No	*Name of chemical*	*S.No*	*Name of chemical*	*S.No*	*Name of chemical*
1	**[6]- gingerols**	**12**	**1Dehydro-3-dihydro- [10]-ginger-di-one**	**23**	**Methyl di acetoxy- [10]-gingerdiol**
2	**[8]- gingerols**	**13**	**Acetoxy-6-dihydroparadol**	**24**	**1-Dehydro- [3]-gingerdione**
3	**1,7-bis-(40-Hydroxy-30-methoxyphenyl)-3,5-heptadione**	**14**	**[4]-Iso gingerol**	**25**	**Acetoxy- [4] gingerol**
4	**5-Methoxy- [6]-gingerol**	**15**	**[8]-Shogaol**	**26**	**[8]-Paradol**
5	**Methyl di acetoxy- [4]-gingerdiol**	**16**	**[10]-Shogaol**	**27**	**[9]-Paradol**
6	**[4]-Shogaol**	**17**	**[12]-Shogaol**	**28**	**[10]-Paradol**
7	**[6]-Shogaol**	**18**	**[6]-Paradol**	**29**	**[11]-Paradol**
8	**1-(40-Hydroxy-30-methoxyphenyl)- 7-octen-3-one**	**19**	**[7]-Paradol**	**30**	**[13]-Paradol**
9	**1-(40Hydroxy-30-methoxyphenyl)-7-decen-3-one**	**20**	**beta-sitosterol palmitate**	**31**	**Hexacosanoic acid 2,3-dihydroxypropyl ester**

10	**1-(40-Hydroxy-30methoxyphenyl)-7-dodecen-3-one**	**21**	**isovanillin**	**32**	**p-hydroxybenzaldehyde**
11	**1-(omega-ferulyloxyceratyl) glycerols**	**22**	**glycol monopalmitate**	**33**	**emaleimide-5oxim**

While the majority of these chemicals have a variety of medicinal applications, the pungent flavour of fresh ginger is a result of a class of phenols called gingerols, the most abundant of which is [6]-gingerol. Additionally,According to some sources, fresh ginger contains paradol, which is a 5-deoxy derivative of ginger, as well as cytotoxic diarylheptanoids and zingerone, all of which are thought to contribute to ginger's alleged health advantages. Gingerol and shogaol seem to be responsible for the majority of the pharmacological action of ginger. Ginger is rich in phenylalkylketones, including 6- and 8-gingerol. Ginger has an abundance of active chemicals. The principal pungent component of ginger, [6]-gingerol, has strong antiangiogenic activity, and [6]-gingerol's antiangiogenic activity may help prevent tumour formation and spread.

Molecular markers

Plant genotypes may be identified and characterised using molecular markers, which provide full access to genetic materials in the case of plants. When it comes to horticultural crops, molecular markers are used in a variety of ways and are particularly effective in breeding efforts. Generic variation and close relationships between varieties, accessions, and species are often determined in ginger by the use of molecular markers, which are regularly found in the plant. As a result, it contributes significantly to the development of imaginative management techniques for ginger improvement projects. Instead, a molecular identifier might serve as a unifying tool for species documentation, allowing them to be more easily identified. In this review, he talks about how to use molecular markers to study the genetic diversity of ginger species. Isozyme markers are the most common. Other markers like RAPD, AFLP, SSR, ISSR, and others like RFLP, SCAR, NBS, and SNP are also used..There are many different forms of molecular genetic markers that may be used to identify and characterise genetic diversity in different populations.
Molecular markers each have their own strengths, so they must be chosen carefully based on the information they give and how easy it is to genotype them.

Table 2: Characteristics of some widely used molecular markers

	Abundance	**Reproducibility**	**Degree of polymorphism**	**Locus specificity**	**Technical requirement**	**Quantity of IJNA required**	**Major application**
RFLP	**High**	**High**	**Medium**	**Yes**	**High**	**High**	**Physical mapping**
RAPD	**High**	**Low**	**Medium**	**No**	**Low**	**Low**	**Gene tagging**
SSR	**Medium**	**Medium**	**Medium**	**No**	**Medium**	**Low**	**Genetic diversity**
SSCP	**Low**	**Medium**	**Low**	**Yes**	**Medium**	**Low**	**mapping**
CAPS	**Low**	**High**	**Low**	**Yes**	**High**	**Low**	**Allelic diversity**
SCAR	**High**	**High**	**Medium**	**Yes**	**Medium**	**Low**	**Gene tagging and physical mapping**
AFLP	**$^{Hi}K^{h}$**	**$^{Hi}S^{h}$**	**Medium**	**No**	**Medium**	**Medium**	**Gene tagging**

Table 3: Advantages and disadvantages of some widely used markers

Type of markers	Advantages	Disadvantages
Restriction Fragment Length Polymorphism (RFLP)	• High genomic abundance • Codominant markers • Highly reproducible • Can use filters many times • Good genome coverage • Can be used across species • No sequence information • Can be used in plants reliably (welltested) • Needed for mapbased cloning	• Need a large amount of good quality DNA • Laborious (compared to RAPD) • Difficult to automate • Need radioactive labeling • Cloning and characterization of the probe are required
Randomly Amplified Polymorphic DNA (RAPD)	• High genomic abundance • Good genome coverage • No sequence information • Ideal for automation • Less amount of DNA (poor DNA acceptable) • No radioactive labeling • Relatively faster	• No probe or primer information • Dominant markers • Not reproducible • Cannot be used across species • Not very well-tested
Simple Sequence Repeat (SSR)	• High genomic abundance • Highly reproducible • Fairly good genome coverage • High polymorphism • No radioactive labeling • Easy to automate • Multiple alleles	• Cannot be used across species • Need sequence information • Not well-tested
Amplified Fragment Length Polymorphism (AFLP)	• High genomic abundance • High polymorphism • No need for sequence information • Can be used across species • Work with smaller RFLP fragments • Useful in preparing contig maps	• Very tricky due to changes in patterns concerning materials used • Cannot get a consistent map (not reproducible) • Need to have very good primers

SequenceTagged Site (STS)	• **Useful in preparing contig maps** • **No radioactive labeling** • **Fairly good genome coverage** • **Highly reproducible** • **Can use filters many times**	• **Laborious** • **Cannot detect mutations out of the target sites** • **Need sequence information** • **Cloning and characterization of probe are required**
ISOZYMES	• **Useful for evolutionary studies**	• **Laborious**

Isozymes and allozymes

Isozyme markers are rapid and simple to employ since they do not need DNA extraction, sequence information, primers, or probes, all of which are required by other types of markers. Electrophoretic protein polymorphisms are detected by these biochemical markers, which are used to identify variances.
It is estimated that around 90 isozyme mechanisms have been employed in plants. The isozyme loci have been identified in a number of investigations.
Use of isozyme markers in Zingiberaceae species such as Curcuma alismatiflrifolia, Siphonochilus aethiopicus has proved effective.Natural populations of ginger species have more variety than farmed populations, according to the results of the study conducted using the markers. Polymorphisms ranged from 4.5 to 100 percent in farmed populations, but they ranged from 50 to 100 percent in wild populations, according to the research.
Ginger species have also been studied for their links among taxa using isozyme markers, according to the researchers.This approach has shown to be effective in supporting taxonomical categorization.

Random amplified polymorphic DNA (RAPD)

Random amplification of polymorphic DNA (RAPD) is a kind of polymerase chain reaction (PCR) in which the DNA segments amplified are chosen at random. Assuming that pieces of genomic DNA would amplify, the scientist performs RAPD by creating random, short (8–12 nucleotide) primers. An RAPD response yields a semi-unique profile by resolving the patterns.
The primers will bind to a specific position in the sequence, but where is unknown. It is thus beneficial for contrasting DNA in living organisms that have not received considerable attention from the scientific community, or when just a small number of DNA sequences are matched (it is not suited for establishing a cDNA databank. Degraded DNA samples cannot be used since it depends on a large intact DNA template sequence. Its resolving capacity is inferior to focused DNA comparison approaches like short tandem repeats. Many plant and animal species have recently had their phylogeny characterised and traced using RAPD.
Samples are put onto an agarose or polyacrylamide gel for electrophoresis after PCR amplification. Depending on the sample source, the varying siznot be created if the template DNA contains a mutation at the primer location, as a consequence of which the arrangement of amplified DNA strands on the gel is different.
Depending on where the samples were gathered, RAPD markers may potentially be utilised to identify across different ginger populations. There were two groups of Z. zedoaria and Z. officinale populations,
Zingiber officinale and Curcuma longa, two ginger species, have polymorphic microsatellite markers that Different varieties of ginger may be distinguished .As of right now, there are 56 genomic SSR markers for C. longa and 17 EST-SSR markers for Z. officinale. Only eight genomic SSR markers have been found for Z. officinale so far.one in hill regions and the other in plain areas, whereas farm collections showed a larger genetic diversity than GenBank and market samples.

Microsatellite or simple sequence repeat (SSR)

A microsatellite is a segment of DNA that contains repetitive DNA motifs (lengths ranging from one to six base pairs). Microsatellites are found throughout an organism's genome. A higher mutation rate than other DNA regions results in considerable genetic diversity. Plant geneticists refer to microsatellites as simple sequence repeats (SSRs) or short tandem repeats (STRs) in the context of plants and animals.
Microsatellites and minisatellites are both DNA with VNTR (variable number of tandem repeats) repetitions in their sequences. When genomic DNA is centrifuged in a test tube, it is discovered that a distinct bulk DNA layer is formed, which is distinguished from a "satellite" DNA layer. This finding led to the name "satellite" DNA.
They are often used for DNA profiling in forensic investigations and kinship analysis (especially paternity testing), among other applications. Genetic linkage analysis is used to identify the gene or mutation that is responsible for a feature or condition. Microsatellites are used in population genetics to analyze subspecies, groups, and individuals, and they are becoming more popular.
Zingiber officinale and Curcuma longa, two ginger species, have polymorphic microsatellite markers that can be used to identify different types of ginger.As of right now, there are 56 genomic SSR markers for C. longa and 17 EST-SSR markers for Z. officinale. Only eight genomic SSR markers have been found for Z. officinale so far.

Inter-simple sequence repeats

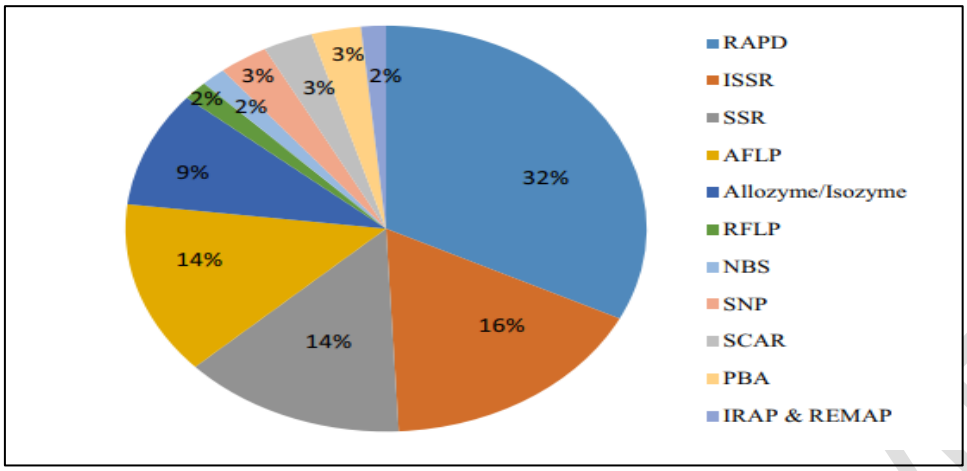
Inter-simple sequence repeats (ISSRs) are microsatellite-bound areas in the genome. Using a single primer to amplify these areas provides several amplification products that can be used to examine genetic variation in distinct organisms. As a result, ISSR markers are appropriate genetic markers for novices and species with less genetic information. We discuss some of the nuances commonly neglected in ISSR experiment design, dispel certain myths, and make recommendations for employing ISSR markers in genetic variation investigations.
ISSR also demonstrated substantial polymorphism (77%) amongst Curcuma alismatifolia variants . In the example of wild ginger Z. moran and Northwest Himalayan cultivars (ISSR, 66.7 percent polymorphism compared to SSR, 77.8 percent), ISSR markers were shown to be less informative (34.61 percent) than AFLP (95.06 percent).

Amplified fragment length polymorphism

The AFLP-PCR technique has the potential to be used in a variety of applications in genetics research and gene editing, including DNA fingerprinting and gene editing. Following the digestion of genomic DNA using restriction enzymes, the process is completed by ligating connectors to the sticky ends of the restriction fragments. Following that, an amplified copy of a choice of restriction fragments is generated. Primers are made up of the adaptor sequence, the restriction site sequence, and a few nucleotides that are found inside the restriction site fragments of the restriction site (as described in detail below). A gel electrophoresis, autoradiography, fluorescence, or automated capillary sequencing are all used to separate and examine the sample, which is then preserved in liquid nitrogen.AFLP is an abbreviation for "Amplified fragment length polymorphism," which isn't really an acronym. The data are classified as presence-absence polymorphisms then instead of length polymorphisms.
AFLP-PCR detects DNA polymorphisms with great sensitivity. In 1993, Vos and Zabeau published the first description of it. The following are the three steps in this technique:

1. *Using restriction enzymes, the total amount of cellular DNA is digested, and then ligated to all of the restriction fragments.*

2. *The use of adapter and restriction site specific primers allowed for the selective amplification of certain of these fragments during PCR.*
3. *The stages entail separating amplicons on a gel matrix and visualising the band pattern.*



Graph 1: Percentage of ginger diversity studies utilizing different types of molecular markers

Achievements made in Zingiber officinalis through modern biotechnological tools

This plant (Zingiber officinale Rosco) belongs to the Zingiberaceae family of plants, which includes the ginger plant. A rhizome that is both powerful and aromatic is produced by this plant, making it one of the most important spices in the world. According to some accounts, ginger rhizomes are widely regarded all throughout the world, not just as a spice, but also as a natural treatment for a variety of ailments. The use of ginger has been mentioned in a number of publications as being severely limited. The following are some instances of situations in which ginger is prohibited:

- *Since the rhizome is the most important useful component of the plant, it reduces the number of ginger seeds necessary for production.*
- *Because ginger grows in the soil, it's easy for it to be infected by soil-borne pathogens, like bacterial wilt, soft rot, and nematodes. These can cause the crop a lot of damage.*
- *ginger rhizomes change and degenerate when they are grown through vegetative propagation for a long time.*
- *Because of insufficient flowering and seed set, conventional ginger breeding is a major challenge.*

Advanced tissue culture, cell and molecular biology, and gene conservation might help alleviate the challenges of over-exploitation of this highly endangered species. These approaches could be used to produce significant secondary metabolites while also conserving germplasm. In recent years, biotechnologically based technologies have shown considerable promise in the production of key plant resources as well as the safeguarding of these resources (Wilson and Roberts 2014).There are many methods to achieve high yielding tissue or organ cultures such as metabolic engineering, cell suspension culture and bioreactors. From this we examined somatic embryogenesis, artificial seed production, cell suspension cultures, direct or indirect organogenesis, and suspension cultures cultures in Zingiber species.

In Vitro Micropropagation

In vitro micropropagation is the most effective method of delivering disease-free plant plantlets for commercial use in the laboratory.

Explant source, type, developmental stage, and size are all important factors to consider when developing in vitro technologies. Other factors to consider include disinfection of explants, growth regulator and medium, culture conditions, and other factors that are important for in vitro technological success. These factors are optimised for optimal tissue culture in a variety of plant species, including tobacco (Xiao et al., 1997). Not all tissues or organs are capable of undergoing transformation (Hartamann et al., 1997). For the goal of micropropagation, culture establishment is the process of introducing explants into aseptic culture under in vitro culture conditions in order to promote the production of continuous shoots (Hartamann et al., 1997). All of the requirements mentioned above should be met in order to create an aseptic ginger culture and propagate it by clonal propagation. The primary industrial goal of in vitro cultivation is to produce more plants in months rather than years (Haque et al., 2009).

The establishment of the explants is critical in the process of micropropagation of plants. Rhizome buds are the most often employed explants in this application. Leaf explants, internodes, and roots are all available. The adventitious buds, on the other hand, have been frequently used. The buds include prepared primordia that will allow for direct organogenesis. The growth of the shoots from these buds is aided by the provision of nutrients. In indirect organogenesis, explants are treated to the callus phase and subsequently dedifferentiate into plantlets after entering the callus phase. For the fast multiplication of plant cells, callus culture has been suggested . Contamination, on the other hand, is quite vital in the establishment. It is necessary to thoroughly wash the buds with running water as well as with detergents such as Tween 20 under running water in order to ensure contamination-free cultivation.

These dust- and soil-free buds are next treated to surface sterilants such as mercuric chloride and ethanol, which kill any remaining bacteria. After that, it was washed with deionized water and then inoculated.

They are exposed to a number of shoot development processes, each of which required the use of a particular medium for multiplication. For the multiplication process, a low-concentration of auxins and cytokinins was employed, with 2 mg/l NAA and 0.1 mg/l BAP being used for the multiplication process.

The elongation stage follows after multiplication, and here is where the gibberllic acid is introduced.The shoots are moved to rooting medium, although this step is not necessary since it was not needed.

Shoots were transplanted for roots in MS media supplemented with 2 mg/l NAA and 0.1 mg/l BAP to ensure a successful transplant. Many other scientists have experimented with various concentrations of growth promoters. As part of the acclimatisation process, the shoots were transferred to netted pots packed with sterilised peat mixture and housed in the culture room with a humidity of 80 percent and an illumination time of 16 ± 8 hours after germination. After 2 weeks, the plants in netted pots were relocated to a greenhouse environment for secondary acclimatization.

Somatic embryogenesis

It is a process in which clusters of somatic cells/tissues result in the creation of somatic embryos that are similar in appearance to the zygotic embryos of intact seeds and are capable of growing into seedlings when cultivated in the appropriate medium. It has been shown in a number of medicinal plant species that plant regeneration may occur by somatic embryogenesis from single cells, which can be induced to produce an embryo and, eventually, a full plant.

In ginger, leaf explants have been used in studies, and the findings have been positive. A modified MS medium supplemented with dicamba was used to preserve the embryogenic callus, which was shown to be the most effective growth regulator.

Somatic embryogenic research have been carried out using meristematic explants in order to produce pathogen-free seedlings, and the findings have been reported in scientific journals. Unfortunatelly, there hasn't been much study done on the impact of different types of ginger on somatic embryogenesis, which is disappointing.

Somatic hybridization

It has a crucial role in the improvement of agricultural species, particularly those that are reproduced vegetatively. There have been many ways of fusion techniques suggested, including the physical and chemical ones. The chemical approach, which employs polyethylene glycol, is a widely used technique [14]. This research used the cross-pollination of two separate ginger species ['Lushan Zhangliangjiang' and 'Chenggu Huang Jiang' (LZ + CH)] in order to create a hybrid that was then regenerated after 15 months. This approach might be used to develop many different hybrids in situations where the types are poor yielding in terms of both number and quality of rhizomes, such as in the case of tomatoes.

Germplasm conservation and storage

Germplasm conservation is an essential strategy for conserving plant variety in any nation, and it is particularly vital in developing countries. Currently, the plant species may be preserved primarily via the use of in situ and ex situ techniques. In situ techniques offer a number of drawbacks, the most significant of which are the area requirements for cultivation, seasonal variations that may result in the loss of plant material, and the high costs associated with cultivation. While the relevance of ex situ conservation has increased in recent years, the use of in vitro methods for the conservation of species has acquired increasing popularity and importance. Ex situ conservation strategies in vitro are the most extensively employed because they exploit the notion of restricting food supply to explants , which in turn slows the plant's development and enables it to be neglected for a while.. It has been discovered via research on the preservation of ginger germplasm that the cultures may be maintained on half strength MS with a decreased quantity of sucrose/carbohydrate supply and at an ambient temperature of 22 ± 2 ℃. The cultures can be kept alive for 200–240 days, with a survival rate of 75–81 percent throughout that time. The use of half-strength MS in conjunction with 20 g/l of sucrose increased the culture span to 360 days. It has also been claimed that the use of polypropylene covers may keep cultures alive for up to seven months.

Bioreactor

Using seed, somatic embryo, shoots and root of horticultural and medicinal plants, large scale micropropage with bioreactor culture has been accomplished recently (Paek et al, 2005). Micropropagation of disease-free clones is critical for industrial companies to cut culture costs and manpower. Compared to traditional tissue cultivation, bioreactor culture has higher productivity, automation, and shorter growth duration. Trotz all dieser Vorteile, the bioreactor cultivation of disease-free ginger clones is mostly unstudied. Because liquid media in bioreactors may create hyperhydraulic tative clones, and many plants are sensitive to liquid culture, this effort attempts to develop a technology for mass producing disease-free ginger clones. Several crop species grew slowly due to desiccation, lower oxygen tension, and growth regulators such absisic acid in the culture media.. Shoot clusters of Zingier officinal were cultured in Murashige and Skoog medium with 0.1 mg. Shoots per explant were maximum in MS medium with 0.5 mg L-1 BA and 3 percent sucrose after 4 weeks of bioreactor culture utilising adventitious shoots, whereas shoot height was greatly increased in MS medium with 0.1 mg L-1 BA and 3 percent sucrose. Proliferation and development of in vitro shoots were outstanding when sucrose content was raised to 6% in MS medium containing 0.1 mg L-1 BA. The findings may be useful for large-scale in vitro shoot multiplication in bioreactor culture. (Lee Jung Joon, Kim TaiSoo, Kim HyunSoon, Lee Seung Yeob 2007) Explants produced the most plantlets and fresh weight per plant when cultivated in MS liquid medium with 0.3 mg/L NAA and 2.0 mg/L kinetin for 40 days. Using an airlift bioreactor, a quick mass propagation approach for normal ginger production was shown. A 10 L bottle type bubble bioreactor produced 1.5 to 1.6 times more plantlets than a 250 ml Erlenmeyer flask.

Secondary metabolite

Cultured plant cells make a lot of important and secondary metabolites that are worth money used as a choice for a job vegetation is a natural source of food and potentially good metabolites and things compounds that are good for the body.

Gingerol ([6]-gingerol) is the main phenol and pungent ingredient of ginger oil. J. C. Thresh isolated it from the ginger rhizome in 1879. (Zingiber officinale). The principal ginger bioactive components are [6]-shogaol and its dehydrated counterpart. When raw ginger is heated or cooked, shogaol and zingerone are formed. Ginger's main pharmacological component is 6-gingerol. It has anti-cancer, anti-inflammation, and antioxidant properties. 6-Gingerol has anticancer properties through its effects on apoptosis, cell cycle regulation, cytotoxicity, and angiogenesis. In this article, we study the properties, biosynthesis, and biological importance of ginger.

Because of a reverse aldol process, cooking ginger results in the conversion of gingerol to zingerone, which is less aromatic and has a spicy-sweet smell. As a result of drying or mild heating, gingerol dehydrates and decomposes, resulting in shogaols, which are about twice as pungent as gingerol. Thus, dried ginger has a more intense flavour than fresh ginger, as explained above.

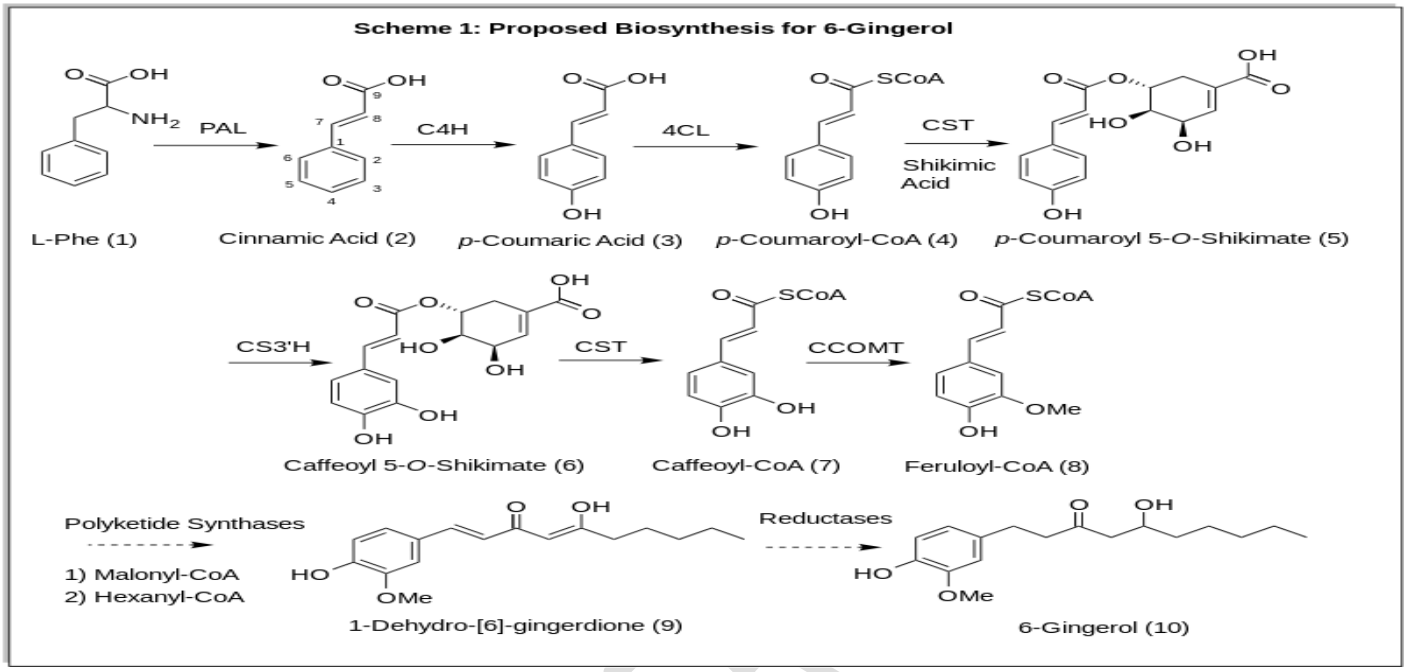
Ginger oil is a versatile important oil that can be used in a variety of industries. Solvent extraction method is used to extract ginger oil from ginger rhizome. Because of its widespread use, it must be extracted and separated at a low cost.

Biosynthesis of Gingerol

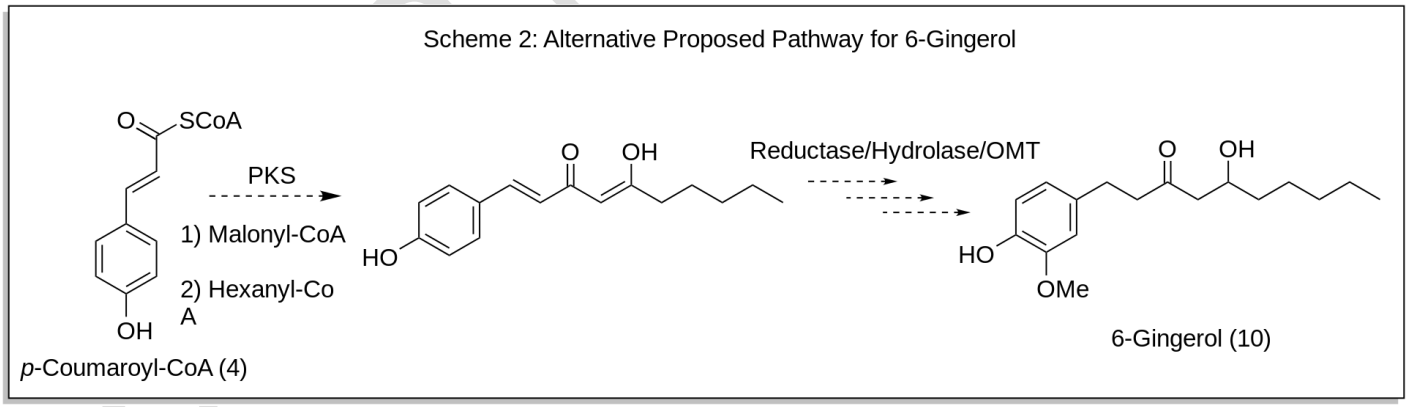
To better understand the biosynthesis of gingerols, early studies were done over 25 years ago (Denniff and Whiting, 1976; Macleod and Whiting, 1979; Denniff et al, 1980). The suggested biosynthetic route includes the following steps:

1. *The starting material is L-Phe (1). Cinnamic acid is formed from it with the use of the enzyme phenylalanine ammonia lyase (PAL)*
2. *Afterwards, with the assistance of an enzyme known as cinnamate 4-hydroxylase, the Cinnamic acid is converted into p-Coumaric acid (C4H).*
3. *Then, with the assistance of an enzyme known as 4-coumarate:CoA ligase, p-Coumaric acid is transformed into p-Coumaroyl-CoA.*
4. *Then The covalent association of shikimic acid and p-Coumaroyl-CoA is catalysed by the enzyme p-Coumaroyl shikimate transferase (CST), which is found in the liver. Then, with the assistance of an enzyme called p-coumaroyl 5-O-shikimate 3'-hydroxylase (CS3'H), the complex produced at the C3 site is oxidised to alcohol.*
5. *One of the activities of CST is the separation of shikimate from this intermediate, which results in the formation of Caffeoyl-CoA.*
6. *Caffeoyl-CoA O-methyltransferase (CCOMT) converts the hydroxyl group at C3 into methoxy, as shown in Feruloyl-CoA, in order to create the needed substitution structure on the phenyl ring.*
7. *According to Ramirez-Ahumada et al., enzyme activity has reached a high level up to this point. Several polyketide synthases and reductases are suspected of being involved in the creation of 6-Gingerol.*

8. *It isn't clear what will happen when the methoxy group is added to the polyketide synthase. An alternative path will be taken. After PKS, the methoxy group is added. Oxylases that use S-adenosyl-L-methionine to make O-methyltransferases are likely to be involved in this other way, as well (OMT). When you use Reductase to do this, you can do it in three different ways. After PKS and Hydroxylase and OMT are finished, it can be done right away or at the end after that.*



Proposed biosynthetic pathway to gingerols in ginger. Enzymes are as follows: PAL = phenylalanine ammonia lyase; C4H = cinnamate 4-hydroxylase; 4CL = 4-coumarate:CoA ligase; CST = p-coumaroyl shikimate transferase; CS3'H = p-coumaroyl 5-O-shikimate 3' -hydroxylase; OMT = O-methyltransferase; CCOMT = caffeoyl-CoA O-methyltransferase.



Conclusions and future prospective

Ginger is used as a flavoring agent in food, used in medicines because of their antimicrobial, anti-inflammatory, and cancer-fighting properties as well as for the extraction of various ginger oils such as gingerol, Shagoal zingiberene, and zingiberal as well as chavicol, cineole and graniol, as well as for the extraction of d-camphene and other compounds, it is considered the most valuable crop on the planet. Due to this reason the demand of this crop is increasing day by day .But due to certain problem like poor flowering , poor seed yield the production of this crop is decreasing. The careful regulation of zingiber species is critical to the biodiversity conservation as well as the sustained production of bioactive chemicals

to fulfil the needs of pharma companies. Identifying and estimating Zingiber species diversity at the morphological, biochemical, and genetic levels is critical for determining the extent to which the species varies. To solve the yield releated issue and to manage properly we employ certain biotechnological techniques. This techniques not only increase the quality and quantity but also help the farmers to produce disease and pest free plant with the help of micropropagation technique . If you use tissue culture-based biotechnological interventions, like in vitro multiplication, callousing, hairy root culture, and so forth, you can come up with effective ways to make bioactive compounds in the lab that don't put a lot of strain on naturally derived populations.

In the intention of providing a foundation for future research in the domain of molecular characterisation of superior Zingiber spp., the authors have written this review to serve as such. Researchers are becoming more aware of the importance of increasing the production of secondary metabolites in the environment as a result of emerging biotechnological technologies such as genetic manipulation and metabolic engineering. For the most part, genetic engineering therapies have opened up the door to several innovative options for genetic modification of this medicinally significant plant.

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