Chapter

Biotechnology and Crop Improvement of Ginger (*Zingiber officinale* Rosc.)

Neeta Shivakumar

Abstract

Ginger is the third most important spice used for its medicinal properties in day to day life. Ginger is one of the widely studied plants for its biochemical and medicinal properties. Biotechnological tools have played a pivotal role in the improvement of this plant species. Many in vitro techniques namely micropropagation techniques, somatic embryogenesis, somatic hybridization, germplasm conservation, transgenics and mutation breeding have been widely studied whereas less studied for haploid production, and cryogenic in ginger. Many of these have been used in the recent times for the improvement of ginger mainly because of the vegetative mode of propagation. Most varietal improvement programs of this species are confined to evaluation and selection of naturally occurring clonal variations. Problems faced in ginger breeding have so far been the very low genetic variation in ginger plant. Wide genetic variation is needed in plant breeding in order to search ideal plant types during the process of selection. Although traditional mutation breeding has lost its preeminent position, induced mutations continue to be in great demand with the assistance of various biotechnological tools. In vitro culture techniques provide an alternative means of plant propagation and a tool for varietal improvement. Here, is an attempt made to collect the information on the studies made in this regard and present the current status of research in ginger.

Keywords: ginger, *Zingiber officinale*, *in vitro* culture, biotechnological tools, crop improvement

1. Introduction

Ginger (*Zingiber officinale* Rosc.) belongs to the family Zingiberaceae, is an important tropical horticultural plant and an important spice crop used in various medicinal and culinary preparations. Ginger consumption is known for its health benefits and widely known to be used in Ayurvedic formulations and Chinese medicine. It is rich in secondary metabolites namely the oleoresins and shogaols contributing widely the pungency and flavors [1]. It is stimulative in nature and helps in relieving the indigestion, stomach ache, diarrhea and nausea. It is widely used as to cure common cold, cough and congestion. The clinical studies have demonstrated it to be anti-emetic, anti-ulcer, anti-platelet, anti-inflammatory, and antioxidant in nature. Ginger has many uses in the home remedies and can be used to help arthritis, diarrhea, flu, headache, heart and menstrual problems, diabetes, stomach upset

and motion sickness [2]. Wide studies have been taken up involving ginger to cure complex diseases such as cancers to the chronic conditions of migraines.

Ginger has been widely used in various medicines viz., Ayurveda, Unani and Chinese medicines to cure many of the health problems. It has been used in various ways either in the food directly as or as a part of the formulations in medicines to cure many of the ailments such as sore throat, muscle strains, chronic cough, asthma, headaches, diabetes, relief of nausea and flatulence. Studies have proved ginger to have anti-inflammatory effects, anti-cancerous specially colorectal and ovarian and antiemetic anti-platelet activity [3].

Ginger is carminative, pungent, stimulant, used widely for indigestion, stomach ache, malaria and fever. It is chiefly used to cure diseases due to morbidity of Kapha and Vata. Ginger with lime juice and rock salt increases appetite and stimulates the secretion of gastric juices. It is said to be used for abdominal pain, anorexia, arthritis, atonic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhea, difficulty in breathing, dropsy, fever, flatulent, indigestion, disorders of gall bladder, hyperacidity, hypercholesterolemia, hyperglycemia, indigestion, morning sickness, nausea, rheumatism, sore throat, throat ache, stomach ache and vomiting. Ginger forms an important constituent of many pharmacopoeial Ayurveda formulations [4].

1.1 Botany of the ginger plant

The ginger plant is an erect herbaceous perennial growing from one to three feet in height. The stem is surrounded by the sheathing bases of the two-ranked leaves. The plant is erect, aerial shoots (pseudostem) with leaves, and the underground stem (rhizome). The fleshy and fibrous roots of ginger have indefinite growth from the base of the sprouts. These are the fibrous roots, and the number of such roots keeps on increasing with the growth of tillers [5] (**Figure 1**).

A club-like spike of yellowish, purple-lipped flowers have showy greenish yellow bracts beneath. Unfortunately, ginger rarely flowers in cultivation [5] (**Figure 2**).

Ginger is the modified rhizome stem having nodes with scale leaves and internodes. Except for the first few nodes, all the nodes have axillary buds. Generally, the seed rhizome bit usually termed as setts is used for planting. There may be one or more apical buds on it. Normally only one bud becomes active. When large pieces are

Figure 1.
Ginger cultivation. Source: https://in.images.search.yahoo.com/.

Figure 2.
Flowering in ginger. Source: https://in.images.search.yahoo.com.

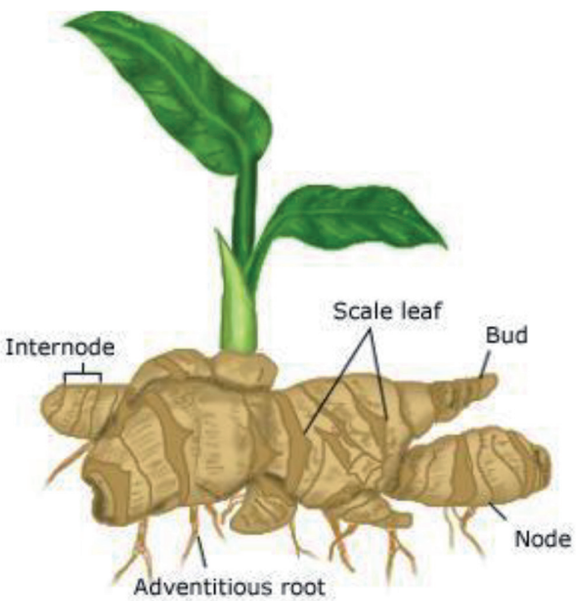

Figure 3.
Botany of ginger plant. Source: https://in.images.search.yahoo.com/.

used, more than one bud may develop simultaneously. If more than one branch from the parent rhizome is responsible for the ultimate growth and development of the adult rhizome, the branches of the mature rhizome lie in the same plane (**Figure 3**).

1.2 Color and appearance

Ginger can be found in Yellowish brown or light brown colors. Scraped rhizome with buff external surface showing longitudinal striations and occasional loose fibers, outer surface dark brown [1] and more or less covered with cork which shows conspicuous, narrow, longitudinal and transverse ridges (**Figure 4**).

1.3 Nutrient composition

Nutrient composition of ginger includes 80% moisture, 2.3%protein, 0.9%fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin,

Figure 4.
Modified rhizome stem. Source: https://in.images.search.yahoo.com.

niacin and vitamin C. The composition varies with the type variety, agronomic conditions, curing methods, drying and storage conditions. The rhizome, which is valued for its flavor, contains two constituents such as the essential oils and oleoresins [5].

1.4 Ginger varieties and its constituents

1.4.1 Varieties of ginger

Zingiberus family includes about 50 genera and 1300 species of ginger are known to exit worldwide. They occur in different parts of the world namely Japan, Australia, Haiti, Bangladesh, Jamaica, Sri Lanka, Nigeria. Several cultivars of ginger grown in different ginger growing areas in India and they are generally named after the localities where they are grown. Some of the prominent indigenous cultivars of ginger grown in India are Himachal, Maran, Kuruppampadi, Wayanad, Varada, etc. Exotic cultivars such as Rio-de Janeiro have also become very popular among cultivars. Maran, Nadia, Karakkal, Rigodi are suited for high dry ginger. Varieties like Ernad Chernad, China and Rio-de Janeiro provide high oleoresin content. Sleeve local, Narasapattam, Himachal are suited for high volatile oil. Rio de Janeiro, China, Wayanad, Maran, Varada are suited for green ginger [6].

2. Biotechnology of ginger

Biotechnological tools have played a pivotal role in the improvement of plant species. There are many techniques specially the micropropagation techniques, production of enhanced secondary metabolite production using cell suspension cultures [7]. *In vitro* production of haploids, somatic embryogenesis, somatic hybridization [8], germplasm conservation and storage, recombinant DNA technology and transgenic [9]. Many of these have been used in the recent times for the improvement of ginger mainly because of the vegetative mode of propagation and those techniques have been discussed below.

2.1 Micropropagation of ginger

Ginger is one of the important spice crops in India under the family Zingiberaceae. This plant is a highly important horticultural crop and plays a very important role in pharmaceuticals, food and beverage industries. In a vegetatively propagated plant like ginger, risk of systemic Infections by root knot nematodes, bacterial wilt from the

propagules is very high. Heavy losses in ginger productions is due to diseases caused by bacterial wilt (P-50), soft rot (Pythium) and rhizome rot (E-Oxy). Therefore, the diseases are mainly transmitted by rhizome propagules; production of disease free clones is necessary. Micropropagation using tissue culture technique can be the alternative method. *In vitro* culture technique provides an alternative means of plant propagation and a tool for crop improvement [10]. Clonal multiplication of ginger through shoot multiplication also has been reported. Clonal propagation is achieved through direct organogenesis or indirect organogenesis [11]. Establishment of the explants plays a very important role in micropropagation. The explants usually used are the rhizome buds. Leaf explants, internodes and roots. However, the adventitious buds have been widely used. The buds have the preformed primordia for the direct organogenesis. Nutrients are provided for the development of the shoots from these buds. In indirect organogenesis the explants is subjected to enter the callus phase and then dedifferentiate into plantlets. Callus culture is proposed for rapid proliferation of plant cells [12]. However, contamination plays a very important role in the establishment. In order to achieve contamination free cultures the buds thoroughly washed with running water and with the detergents namely Tween 20 under running water. These dust free/soil free buds are later subjected with surface sterilants such as mercuric chloride and ethanol, Followed by washing with distilled water and inoculated. The following are the established cultures seen in the author's lab (**Figure 5**).

These shoots are subjected to multiple shoot formation steps which needs the specific media for multiplication. This requires the lower concentration of auxins and cytokinins with 2 mg/l NAA and 0.1 mg/l BAP was used for the multiplication [7]. Following are the established cultures seen in the author's lab (**Figure 6**).

After multiplication is the elongation step where the gibberllic acid is added. However since this step was not required the shoots are transferred to rooting media (**Figure 7**).

Multiple shoots transferred for rooting with MS medium supplemented with 2 mg/l NAA and 0.1 mg/l BAP. Many other researchers have tried different concentrations growth enhancers. After rooting the shoots have been primarily acclimatized by transferring to the netted pots filled with sterilized peat mixture and kept in the growth chamber by maintaining a humidity of 80% and the light period 16 ± 8 hrs. After 2 weeks transferred to green house for secondary acclimatization of the plants in netted pots [7].

2.2 Enhanced secondary metabolite production

There are various studies conducted in ginger to compare the secondary metabolite production both *in vitro* and i*n vivo*. The In vitro culture did not enhance the production of the gingerols and gingiberene. However, the in vivo produced the same amount of secondary metabolites [11]. In another study the influence of mevalonic acid (MVA) increased the production of 6-gingerol in in vitro grown callus cultures compared to other precursors and biotic elicitors. Other active substances did not influence the production of 6-shogaol, 8-gingerol and 10-gingerol.30 μg/100 mg callus FW was recorded with 1 mg/l MVA [12]. Many elicitation studies need to be conducted for different varieties of ginger.

2.3 *In vitro* production of haploids

Studies have not been done on haploid production in ginger. The probable reason may be the poor flower setting which makes androgenic or gynogenic studies nearly impossible.

Figure 5.
Stages of micropropagation through callus (source: author's research work).

Figure 6.
Fully grown ginger explants via micropropagation (source: author's research work).

Figure 7.
Stages in hardening of ginger explants (source: author's research).

2.4 Somatic embryogenesis

It has been studied in ginger using leaf explants The embryogenic callus was maintained using MS media with dicamba as the best growth regulator [13]. Somatic embryogenic studies were taken using meristematic explants to produce pathogen free seedling [14]. Not many studies have been taken up with respect to different varieties of ginger for somatic embryogenesis.

2.5 Somatic hybridization

Plays a pivotal role in the improvement of the crop species specially the vegetatively propagated ones. Different methods of somatic hybridization have been

proposed namely the physical and chemical method of fusion techniques. Chemical method is commonly used technique using polyethylene glycol [14]. In this study, two different species of ginger ['Lushan Zhangliangjiang' + 'Chenggu Huang Jiang' (LZ + CH)] were fused to obtain a hybrid and later regenerated after 15 months. This technique could be adapted to create many other hybrids were the varieties are low yielding in terms of quantity and quality of rhizomes.

2.6 Germplasm conservation and storage

Germplasm conservation is an important technique for conservation of the plant diversity for any of the country. The plant species can be conserved mainly through in situ and ex situ methods. In situ methods have many disadvantages mainly being the land requirement for cultivation, seasonal changes which may lead to destruction of the plant material and high cost incurred during cultivation. However Ex situ conservation in recent days has gained more prominence and importance utilizes the in vitro techniques for the conservation of the species. In vitro techniques are the widely used techniques for ex situ conservation and utilizes the principle of the reduced amount of nutrients supplied to the explants and in turn would reduce the growth of plant and less attention can be given for a certain amount of time. Studies on the ginger germplasm conservation have shown that the cultures can be maintained on half strength MS with reduced amount of sucrose/carbohydrate source and maintain the cultures at ambient temperature of 22 ± 2oC. The cultures can be maintained for 200–240 days with 75–81 percentage of survival. Half strength MS with 20 g/l of sucrose extended the culture span to 360 days [14]. Usage of polypropylene caps is also reported to maintain the cultures for 7 months. Around 100 accessions have been maintained at Indian Spice Research Institute (IISR) and In vitro conservation provides a promising tool for maintaining the ginger cultivars. However, there are no reports available for the successful cryopreservation strategy.

2.7 Recombinant DNA technology

2.7.1 Transgenics

Nowadays, researches are taking place in bringing out transgenic varieties of ginger. Trasgenics have been developed to overcome crop loss due to disease, lack of resistance, and post-harvest losses. Since ginger is vegetatively propagated, the genetic variability is limited. When compared to other spices, transgenic developments are very few in number in case of ginger.

Transient expression of GUS was successfully induced in ginger embryogenic callus bombarded with plasmid vector pAHC 25 and promoter Ubi-1(maize ubiquitin) callus tissue [15]. Helium bombarded ginger embryo-genic calli with microprojectiles (1.6 μm gold particles) using 'BioRad' PDS-1000/He gene gun at 900 and 1100 psi helium pressure with the target distance of either 6 or 9 cm. The pAHC 25 vector used contained GUS (β-glucuronidase) and BAR (phosphinothricin - acetyl transferase) as reporter and selectable marker genes respectively. The best GUS score was obtained when the target distance was 9 cm with 900 psi helium pressure. The GUS score of 133 blue spots per cm^2 indicated not only the optimization and efficiency of the biolistic process, but also the ability of the ubiquitin promoter to drive the expression of the reporter gene. [16].

It was reported that the genetic transformation of ginger buds through somatic embryogenesis. They found that young buds had very good embryogenic potential and were superior to other explants. The transformation protocol included 3 day

pre-culture of explants on callus induction medium, bacterial (*Agrobacterium tumefaciens* strainEHA105/p35SG) dilution of 1:20 (v/v) as the initial inoculum, an infection time of 5 minutes, 2 day co-cultivation with Agrobacterium and post cultivation on callus induction medium with 100 mgL^{-1} kanamycin and 300 mg L^{-1} cefotaxime under darkness for 2 weeks, followed by a 16/8 h photoperiod regime. Acetosyringone was effective at a concentration of 200 μm for vir induction. With young bud as explant, a transformation frequency of 1.1 to 2.2% was noticed. The callus growth was very slow in the presence of antibiotics [17].

3. Breeding of ginger

Breeding of ginger is seriously handicapped by poor flowering and seed set. Most crop improvement programs of this species are confined to evaluation and selection of naturally occurring clonal variations. Problems faced in ginger breeding have so far been the very low genetic variation in ginger plant. Wide genetic variation is needed in plant breeding in order to search ideal plant types during the process of selection [18]. Although traditional mutation breeding has lost its pre-eminent position, induced mutations continue to be in great demand with various biotechnological tools. The methods of mutation induction and analyses of mutants have witnessed great changes in recent years. *In vitro* culture techniques provide an alternative means of plant propagation and a tool for crop improvement (Vasil, 1988). The advent of tissue culture technology enables small and easily handled amount of tissue to be treated. Excised stem tips or callus growing on standard nutrient medium could be treated and the explants are grown to maturity and evaluated for useful mutations. Advanced *in vitro* manipulations such as somatic embryogenesis and single cell cultures also reduce the problem of chimerism often encountered in the induction of mutations on vegetatively propagated plants (Chopra, 2005).

3.1 Mutational breeding in ginger

Mutational breeding has a very prominent role to play in breeding a new variety specially the ones which are clonally propagated. The traditional methods of mutation breeding involves the use of the mutagens in creating the mutation and check for the mutated genes to pass from one generation to the other. These trails consume lot of time, laborious and needs the involvement of large population. With the advent of Biotechnology techniques, the process is made easier in mutation breeding wherein the explants are treated with the mutagens and subjected to screening under *in vitro* conditions. The other method of creating variation is through somaclonal variation, somatic hybridization induced under in vitro conditions and recently is through transgenic technology.

Mutation breeding can be taken up by treating large number of rhizomes under in vivo conditions and screen for the phenotypic and genotypic characters. These studies require large number of planting material, cost and space. The methods of mutation induction and analysis of mutants have witnessed a great change in recent years. With the advances in recent biotechnological studies, mutation studies can be taken under in vitro conditions. The explants namely the adventitious buds, leaf explants, stem and roots and generate the plants through direct regeneration or indirect regeneration.

Ginger is severely affected bacterial wilt and rhizome rot diseases. The lack of genetic variability among the genotypes for disease resistance is one of the bottlenecks in ginger genetic improvement. Studies have been done to induce mutability and radio sensitivity of the ginger genotypes using different doses gamma rays.

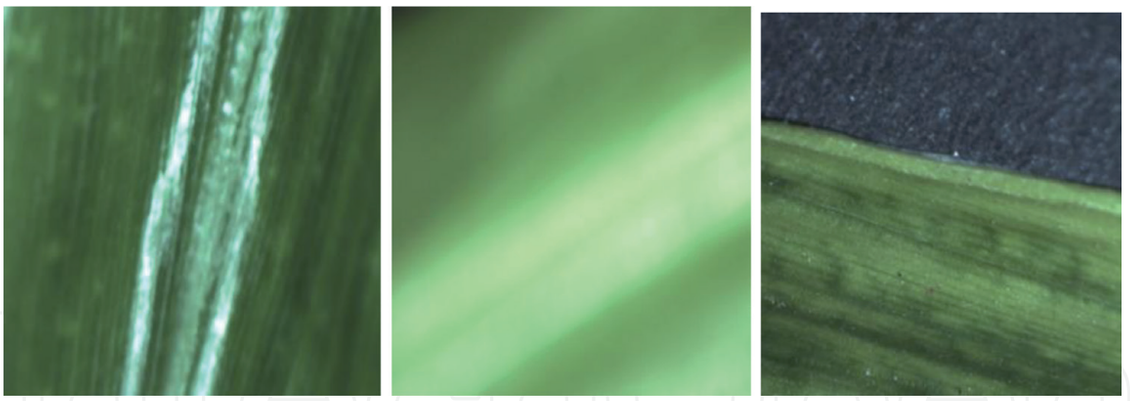

Figure 8.
Stereo microscope images of the mutants.

Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the gamma rays (0.5–1.2kR). High frequency of chlorophyll mutants (5.13%) indicates mutability of ginger. The spectrums of chlorophyll mutations (albino, xantha, and chlorina) were observed and grouped. The overall mutation spectrum for ginger showed that xantha occurred with the highest frequency, followed by chlorina and albino. The mutagenic effectiveness decreased with the increase in dose of mutagen that negative relationship between effectiveness and dose of mutagen. Compared to control, wide variability was recorded for various morphological characters under different doses of gamma rays. Artificial screening of mutants against Ralstonia solanacearum and Pythium sp. led to identification of six potential mutants against these two pathogens [18].

The chlorophyll mutation frequency in mutation generation is the most dependable index for evaluating the genetic effects of mutagenic treatments. The effect of Ethyl Methane Sulfonate (EMS), Sodium Azide and Colchicine on induction of different types of chlorophyll mutants have been widely used to determine their frequency in ginger. There are many mutagens which can be used namely physical viz., UV rays, X rays, Gama rays mutagens etc., chemical EMS, Sodium azide and Choline biological [19] (**Figure 8**).

Ethyl Methane Sulfonate (EMS) is a mutagenic which is carcinogenic in nature. It produces random mutations in the genetic material. The EMS concentrations used varied from 5 mM to 10 mM. Following are the chlorophyll variations observed and classified as follows:

- Xantha: Leaves with little or no chlorophyll but have carotenoid pigmentation and are yellow.

- Striata: They have yellow or white longitudinal bands alternating with green color.

- Maculata: Have spots where chlorophyll and/or carotene have been destroyed.

The frequency of mutation can be calculated using the below formula:

$$\text{Mutation frequency (\%)} = \frac{\text{No. of mutants}}{\text{Total no. of plants scored}} \times 100 \qquad (1)$$

Mutation studies have been taken up in the indigenous varieties of ginger from India namely Bidar, Himachal and Humnabad. They observed the variation in 3 months plants of the mutant varieties and were studied with respect to various growth parameters like survival percentage, sprouting percentage, plant height,

number of tillers, etc. They found that the treatment with chemical mutagens had significant effect on all the growth and yield parameters. Chlorophyll mutations spectrums were observed and grouped. Mutation frequency was calculated for VM_0 and VM_1 generation, mainly with the occurrence of maculata highest, followed by xantha and striata. More number of mutants was found with the plants treated with EMS, followed by colchicines and sodium azide. The albinism was rarely exhibited by plants [20].

Similarly, Sri Lankan varieties have been studied for using EMS for the yield and quality parameters studied different chlorophyll variants induced with gamma rays and EMS and combination of both the treatments. Physical mutagens are mainly the gamma radiations and UV radiations which have been studied. Most effective being the Gamma radiations and can be used widely not only in ginger but also other crops [20].

3.2 Role of markers in ginger breeding

Ginger (*Zingiber officinale*) is a crop grown widely in tropical and subtropical regions which is of medicinal, economic and horticultural importance. There are more than 150 varieties of ginger. In species like ginger which reproduces mainly through vegetative modes, the chances of crop improvement are very limited. A molecular marker is a molecule contained within a sample taken from an organism or other matter. It can be used to reveal certain characteristics about the respective source. Markers have played a tremendous role in breeding and conservation of species like ginger [21].

Randomly Amplified Polymorphic DNA (RAPD) Markers are used for plant species like ginger because no specific information about the genome is available. Ginger genome is one among the poorly studied genomes and information available is very little. Using phylogenetic analysis and metabolic profiling of diversity within and among *Zingiber* species was investigated. It was found that *Zingiber* from different geographical locations were indistinguishable [22]. Clones, cultivars, varieties, accessions or genotypes can be distinguished by RAPD markers with high resolving power value. RAPD as a marker is well-established in generating reproducible polymorphic bands. Variation among the induced mutant clones could be used to support the selection process at the early stage of the plant. RAPD markers can differentiate ginger populations based on their collection sources. Populations of *Z. zedoaria* and *Z. officinale* were distributed into two groups that exist in hill areas and plain areas, respectively, while farm collections showed wide genetic diversity. RAPD has proven to be an effective tool for detecting genetic diversity at the interspecific and intraspecific levels [23, 24]. Thus, it has been proves that RAPD markers can be used for identification and classification of ginger species.

Amplified fragment length polymorphism (AFLP) markers are capable of disclosing a greater number of polymorphisms with a single reaction. Thus, this marker has become the main tool in genetic marker technologies [25]. The genetic relationship within a species or genus could be determined using AFLP markers. The polymorphic bands were quite low within species. Using AFLP analysis it was found that ginger phylogenetic relationships mostly corresponded to their morphological characteristics and modes of reproduction. Genetic diversity patterns within different genome sizes of Curcuma populations were confirmed to be influenced by the mode of reproduction. ALFP markers could provide species-specific identification for examined species and produce a large number of reproducible markers to assess diversity across the nuclear genome. Three species of *Zingiber*, *Z. officinale, Z. montanum* and *Z. zerumbet*, were identified by using this DNA fingerprinting marker. This shows that ALFP can be used as a reliable parameter for identifying species [26].

Simple sequence repeat (SSR) marker is believed to be one of the most powerful markers in studying genetic diversity. It can accurately assess the level of genetic diversity within a germplasm of any crop. Available polymorphic microsatellite markers have been found in the ginger species *Zingiber officinale* and *Curcuma longa* [27]. Until now 56 genomic SSR markers and 17 EST-SSR markers have been developed for *Curcuma longa*, while only eight genomic SSR markers have been reported in *Z. officinale*. SSR markers displayed better polymorphism results compared to ISSR and IRAP markers [28]. The development and characterization of microsatellite markers for ginger species would be useful for future studies evaluating genetic diversity and genetic divergence among species. SSR markers could be used for ginger breeding improvement programs.

Inter-Simple sequence repeats (ISSRs) are semi-arbitrary markers amplified by PCR in the presence of one primer corresponding to a target microsatellite [25]. They are randomly distributed throughout the genome. The main advantage of ISSRs is that the amplification does not require genome sequence information and produces high fragments. ISSR is effective for differentiating relationships among closely related ginger cultivars [29].

This technique has been confirmed to show higher polymorphisms compared to RAPD and AFLP markers when tested on Curcuma species [30]. ISSR markers were found to be less informative compared to AFLP in the case of wild the ginger species, *Z. moran* and cultivars of Northwest Himalayan [28]. The differences in resolving power of these markers is due to the difference portions of the genome that are targeted. Thus, it is essential to apply different molecular techniques in diversity studies of any crops.

Combination of RAPD and ISSR is also used in identification of ginger species. Several ginger studies applied both RAPD and ISSR as molecular markers. RAPD and ISSR have been used for genetic diversity studies in various plant species including ginger. RAPD markers are reported to be more suitable for genetic diversity analysis. ISSR markers are more reproducible compared to RAPD markers. Therefore, the percentage of polymorphisms using ISSR markers was quite higher than RAPD markers. Combining data from the two markers would give a better result in terms of species differentiation. RAPD and ISSR have been used to assess genetic diversity among micro-propagated and cloned ginger species [31]. The aim of the studies was to reveal monomorphic bands to confirm genetic stability or uniformity.

Other markers that have been used in ginger species include restriction fragment length polymorphism (RFLP), sequence characterized amplified region (SCAR), nucleotide binding site (NBS) and single nucleotide polymorphism (SNP) [31] have been used to identify and characterize ginger species.

Author details

Neeta Shivakumar
Department of Biotechnology, R V College of Engineering, Bangalore, India

*Address all correspondence to: neeta@rvce.edu.in

References

[1] Bhagyalakshmi B, Narasimhan S, Narendra S. The yield and quality of ginger produced by micropropagated plants as compared with conventionally propagated plants. Journal of Horticultural Science. 1993;**4**:645-651

[2] O'Hara M et al. A review of 12 commonly used medicinal herbs. Archives of Family Medicine. 1998;**8**(5):376

[3] Mowrey DB et al. Motion sickness, ginger, and psychophysics. Clinical Trials. 1982;**1**(8273):655-657

[4] Mustafa T, Srivastava KC. Ginger (*Zingiber officinale*) in migraine headache. Journal of Ethnopharmacology. 1990;**29**:267-273

[5] Bhatt N, Waly MI, Essa MM, Ali A. Ginger: A Functional Herb. Nova Science Publishers; 2013. pp. 51-71

[6] Bag BB. Ginger processing in India (*Zingiber officinale*): A review. International Journal of Current Microbiology and Applied Sciences. 2018;**7**(4):1639-1165

[7] shivakumar N, Agrawal P. Callus induction and regeneration from adventitious buds of *Zingiber officinale*. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2014;**16**(4):881-885

[8] Kackar A, Bhat SR, Chandel KPS, et al. Plant regeneration via somatic embryogenesis in ginger. Plant Cell Tissue Culture. 1993;**32**:289

[9] Baskaran P, Jayabalan N. An efficient micropropagation system for Eclipta, alba—A valuable medicinal herb. In Vitro Cellular & Developmental Biology – Plant. 2005;**41**:532-539

[10] Nirmal Babu K et al. Protocols for in vitro propagation, conservation, synthetic seed production, embryo rescue, microrhizhome production, molecular profiling, and genetic transformation in ginger. Methods in Molecular Biology. 2016;**1391**:403-426

[11] Seran TH. In vitro propagation of ginger (*Zingiber officinale*) through direct organogenesis: A review. Pakistan Journal of Biological Sciences. 2013;**16**(24):1826-1835

[12] El-Nabarawya MA, El-Kafafia SH, Hamzab MA, Omara MA. The effect of some factors on stimulating the growth and production of active substances in *Zingiber officinale* callus cultures. Annals of Agricultural Science. 2015;**60**(1):1-9

[13] Faria RT, lllg RD. Micropropagation of *Zingiber spectbile* Griff. Scientia Horticulturae. 1995;**62**:135-137

[14] Rostiana O, Syahid SF. Somatic embryogenesis, from meristem explants of ginger. Biotropia. 2008;**15**(1):12-16

[15] NirmalBabu K, Yamuna G, Praveen K, Minoo D. Cryopreservation of spices genetic resources. INTECH. 2012;**16**:457-484

[16] Abbas MS, Gaber E s I. In vitro propagation of ginger (Zingiber officinale Rosco). Journal, Genetic Engineering & Biotechnology. 2011;**9**:165-172

[17] Babu KN, Minoo D, Geetha SP, Samsudeen K, Rema J, Ravindran PN, et al. Plant biotechnology-its role in improvement of spices. Indian Journal of Agricultural Sciences. 1998;**68**(8):533-547

[18] NirmalBabu K, Suraby EJ, Cissin J, Minoo D, Pradeepkumar T, Parthasarathy VA, et al. Status of transgenics in Indian spices. Journal of Tropical Agriculture. 2013;**51**(1-2):1-14

[19] Suma B, Keshavachandran R, Nybe EV. Agrobacterium tumefaciens mediated transformation and regeneration of ginger (Zingiber officinale Rosc). Journal of Tropical Agriculture. 2008;**46**:38-44

[20] Prasanth D, Suseela Bhai R, Nair R. Induction of Variability in Ginger through Induced Mutation for Disease Resistance, Conference: National Symposium on Spices and Aromatic Crops. 2015. pp. 16-18

[21] Prasannakumar P, Subramanian S, Suresh J, Kannan Bapu JR, Gnanam R. Effect of gamma rays on induction of chlorophyll mutants in ginger genotypes. International Journal of Agricultural. 2016;**6**:105-110

[22] shivakumar N, Agrawal P. The effect of chemical mutagens upon morphological characters of ginger in M0 generation. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2018;**20**:126-135

[23] Ravinderan PN, Nirmal BK, Shiva KN. Botany and crop improvement of ginger. In: Ravinderan PN, Nirmal BK, editors. Ginger: The Genus Zingiber. New York: CRC Press; 2005. pp. 15-85

[24] Nayak S, Naik PK, Acharya L, Mukhergee AK, Panda PC, Das P. Assessment of genetic diversity among 16 promising cultivars of ginger using cytological and molecular markers. Zeitschrift für Naturforschung. 2005;**60c**:485-492

[25] Das A, Kesari V, Satyanarayana VM, et al. Genetic relationship of curcuma species from Northeast India using PCR-based markers. Molecular Biotechnology. 2011;**49**:65-76

[26] Donipati P, Sreeramulu SH. Relationships among six medicinal species of curcuma assessed by RAPD markers. International Journal of Recent Scientific Research. 2015;**6**(8):5909-5912

[27] Kumar P, Gupta VK, Misra AK, et al. Potential of molecular markers in plant biotechnology. Plant Omics. 2009;**2**(4):141-162

[28] Kaewsri W, Paisooksantivatana Y, Veesommai U, Eiadthong W, Vadrodaya S. Phylogenetic analysis of Thai Amomum (Alpinioideae: Zingiberaceae) using AFLP markers. Kasetsart Journal (Natural Science). 2007;**41**:213-222

[29] Sigrist MS, Pinheiro JB, Azevedo-Filho JA, et al. Development and characterization of microsatellite markers for turmeric (Curcuma longa). Plant Breeding. 2010;**129**:570-573

[30] Pandotra P, Gupta AP, Husain MK, et al. Evaluation of genetic diversity and chemical profile of ginger cultivars in the North-Western Himalayas. Biochemical Systematics and Ecology. 2013;**48**:281-287

[31] Jatoi SA, Kikuchi A, Yi SS, et al. Use of rice SSR markers as RAPD markers for genetic diversity analysis in *Zingiberaceae*. Breeding Science. 2006;**56**:107-111