ISBN: 978-81-974990-4-3

2. Breeding and Genetic Resistance in Zinger Crops

Vijay Kumar, Harish Kumar,
Ravi Singh Thapa, Jyoti

School of Agricultural Sciences,
IIMT University, Meerut U.P.

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 micro-propagation techniques, somatic embryogenesis, somatic hybridization, Germplasm conservation, transgenic 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, Crop Improvement, Genetic Resistance, Breeding.

2.1 Introduction:

Ginger (*Zingiber officinale* Rosc.) is cultivated as an annual for its underground stem called 'rhizome' which is used as a spice. It 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 (Bhagyalakshmi *et al.*, 1993). It is stimulative in nature and helps in relieving the indigestion, stomachache, 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. It is sold as fresh ginger or, more frequently, in a peeled and split dried form. Ginger is widely used in pickles, candies and such other preparations and as a medicinal herb. Dry ginger is used for

preparing ginger powder, extracting ginger oil, oleoresin etc. 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 (O'Hara M *et al*., 1998). Wide studies have been taken up involving ginger to cure complex diseases such as cancers to the chronic conditions of migraines.

The major ginger producing countries are India, China, Nigeria, Indonesia, Bangladesh, Thailand, Philippines, and Jamaica. Nigeria ranks first with respect to area under ginger covering about 56.23 percent of total world area followed by India (23.60%), China (4.47%), Indonesia (3.37%) and Bangladesh (2.32%). India ranks first with respect to ginger production contributing about 32.75 percent of world's production followed by China (21.41%), Nigeria (12.54%) and Bangladesh (10.80%). Ginger production in India during 2004-05 was at 3.98 lakh tons from an area of around one lakh ha. The production scenario in India from 1990-91 to 2004-05. The World average ginger productivity is 3025 kg/ha. In India, Kerala, Karnataka, Sikkim, Himachal Pradesh, Meghalaya, Assam and other north eastern states cultivate ginger extensively. United Kingdom, United States and Saudi Arabia import large quantities of ginger. High global demand for Indian ginger is due to its lemony flavour. India has capabilities to meet the quality and quantity demands of importing countries matching to international standards (Tamil Selvan and Manoj, 2002; Peter and Nybe, 2002). India earned a foreign exchange of around Rs AO crores during 2005-06 through ginger export. The export scenario of ginger from India from 1990-91 to 2005-06. The crop exhibited an annual growth rate of 4.6 per cent in area, 7.4 per cent in production and 2.7 per cent in productivity (Manjunath, 2004). Finest quality of ginger i.e. Co chin Ginger which has extensive demand in world market is cultivated in Kerala. In this paper an attempt has been made to analyse problems of ginger cultivation in India such as genetic diversity, climate, planting material, input use efficiency, pests and diseases, global trade and value addition along with the future thrust.

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 especially colorectal and ovarian and antiemetic anti-platelet activity (Mowrey DB *et al*., 1982).

Ginger is carminative, pungent, stimulant, used widely for indigestion, stomachache, 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 (Mustafa T and Srivastava KC 1990).

2.2 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 (Nirmal Babu *et al*., 2013). Although traditional mutation breeding has lost its preeminent 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).

2.3 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 involve 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. 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 Maurya *et al*., (2023 a). 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 (Nirmal Babu *et al*., 2013).

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 (Sumaet *et al.,* 2008).

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 is 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.

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 VM0 and VM1 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.

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 (Prasanth *et al*., 2015).

2.4 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 (Prasannakumar *et al.*, 2016). 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 (Shivakumar *et al.*, 2018). 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 (Ravinderan *et al*., 2005 and Nayak *et al*., 2005). Thus, it has been proving that RAPD markers can be used for identification and classification of ginger species (Ravishankar *et al*., 2023).

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 (Das *et al*., 2006).

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 (Donipati *et al*., 2015).

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* (Kumar *et al*., 2009). 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 (Kaewsri *et al.*, 2007). 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 Maurya *et al.*, (2023 b).

Inter-Simple sequence repeats (ISSRs) are semi-arbitrary markers amplified by PCR in the presence of one primer corresponding to a target microsatellite (Das *et al*., 2006). 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 (Sigrist *et al*., 2010).

This technique has been confirmed to show higher polymorphisms compared to RAPD and AFLP markers when tested on *Curcuma* species (Pandotra *et al*., 2013). 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 (Kaewsri *et al*., 2007). 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 (Jatoi *et al*., 2006). 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) (Jatoi *et al.*, 2006) have been used to identify and characterize ginger species.

2.5 Ginger Varieties and Its Constituents:

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.

Genetic Resistance:

Plant genetic resistance to disease may be based on major or minor genes. Major gene or gene-for-gene resistance works by the product of the resistance (R) gene recognising the product of an avirulence (Avr) gene in the pathogen and initiating the plant defences known as the hypersensitive response. Marker assisted selection (MAS) of resistant varieties is a reliable and faster method of selecting the right varieties for cultivation. The aim of the present study is to find the genes responsible for resistance in highly resistant varieties. Some of the best examples of genetic resistance involve the tolerance of certain bacteria to antibiotics and of certain pests to pesticides.

2.6 Conclusion:

Good diversity coupled with scientific advancement in ginger would augment the ginger production to meet the projected demand. High yielding types have been well accepted by farmers across the states and ginger growing regions. The challenging task in future ginger research would be on development of varieties with high yield, bacterial wilt & soft rot resistance and high quality besides suitable to all major ginger growing areas of the country. Location specific soft rot and bacterial wilt diseases integrated disease management strategy need to be developed. Biocontrol research needs to be strengthened. Technology should be developed for repeated cultivation of ginger in the same field. Indian dry ginger (Cochin ginger) is highly valued in world market. GI registration would boost the ginger trade at intemational level. Though India is the major producer of ginger, the quantity exported is very less. Export earnings can be enhanced through innovative value addition mainly as ginger confectioneries, soups, beverages, syrups and marmalades etc.

2.7 References:

1. Bhagyalakshmi, B., Narasimhan, S. and Narendra, S. (1993). The yield and quality of ginger produced by micro-propagated plants as compared with conventionally propagated plants. *Journal of Horticultural Science*. 4:645-651.
2. Das, A., Kesari, V. and Satyanarayana, V. M. (2011). Genetic relationship of curcuma species from Northeast India using PCR-based markers. *Molecular Biotechnology*. 49:65-76.
3. Donipati, P. and Sreeramulu, S. H. (2015). Relationships among six medicinal species of curcuma assessed by RAPD markers. International Journal of Recent Scientific Research. 6(8):5909-5912.
4. Jatoi, S. A., Kikuchi, A. and Yi, S. S. (2006). Use of rice SSR markers as RAPD markers for genetic diversity analysis in Zingiberaceae. *Breeding Science.* 56:107-111.
5. Kaewsri, W., Paisooksantivatana, Y., Veesommai, U., Eiadthong, W. and Vadrodaya, S. (2007). Phylogenetic analysis of Thai Amomum (Alpinioideae: Zingiberaceae) using AFLP markers. *Kasetsart Journal (Natural Science)*. 41:213-222.
6. Kumar, P., Gupta, V.K. and Misra, A.K. (2009). Potential of molecular markers in plant biotechnology. *Plant Omics.* 2(4):141-162.
7. Manjunath, K. (2004). Production and export of ginger in India accessed on 17th January 2004. http:// www.toenre.ac.Inlseminar!seml-manjuk.htm.

8. Maurya, A.K., Aditya, John, V., Pant, H., Sharma, S. P., El Refaey, D. Z., Sami, R., Helal, M., Fadi Baakdah, and Ahmed, N. (2023 a). Unveiling Oil Seed Cakes Ability to Suppress Fusarium Wilt (*Fusarium udum* Butler) in Pigeonpea (*Cajanus cajan* L. Millsp.). *Journal of Biobased Materials and Bioenergy*. 17(6); 790–796. Doi: doi:10.1166/jbmb.2023.2319
9. Maurya, A.K., John, V., Pant, H., Raghav, R. and Kumar, M. (2023 b). Eco-friendly management of Pigeon pea wilt caused by *Fusarium udum*. *Pest Management Strategies in Pulses and Cereal crops*. 157-166. ISBN: 978-81-19149-06-3.
10. Mowrey, D.B. and Clayson, D.E. (1992). Motion sickness, ginger, and psychophysics. *Clinical Trials.* 1(8273):655-657.
11. Mustafa, T. and Srivastava, K. C. (1990). Ginger (*Zingiber officinale*) in migraine headache. *Journal of Ethnopharmacology*. 29:267-273.
12. Nayak, S., Naik, P.K., Acharya, L., Mukhergee, AK., Panda, PC. and Das, P. (2005). Assessment of genetic diversity among 16 promising cultivars of ginger using cytological and molecular markers. Zeitschrift für Natur for schung. 60c:485-492.
13. NirmalBabu, K., Suraby, E.J., Cissin, J., Minoo, D., Pradeepkumar, T. and Parthasarathy, V.A. (2013). Status of transgenics in Indian spices. *Journal of Tropical Agriculture*. 51(1-2):1-14
14. O'Hara, M., D, Kiefer., K, Farrell. And K, Kemper. (1998). A review of 12 commonly used medicinal herbs. *Archives of Family Medicine.* 8(5):376.
15. Pandotra, P., Gupta, A.P. and Husain, M.K. (2013). Evaluation of genetic diversity and chemical profile of ginger cultivars in the North-Western Himalayas. *Biochemical Systematics and Ecology.* 48:281-287.
16. Peter, K.V. and Nybe, E.V. (2002). Dominating Global Markets. The Hindu Survey of Indian agriculture. pp. 87-97.
17. Prasannakumar, P., Subramanian, S., Suresh, J., Kannan Bapu, J.R. and Gnanam, R. (2016). Effect of gamma rays on induction of chlorophyll mutants in ginger genotypes. *International Journal of Agricultural.* 6:105-110.
18. Prasanth, D., Suseela Bhai, R. and Nair, R. (2015). Induction of Variability in Ginger through Induced Mutation for Disease Resistance, Conference: National Symposium on Spices and Aromatic Crops. pp. 16-18.
19. Ravinderan, P.N., Nirmal, B.K. and Shiva, K.N. (2005). Botany and crop improvement of ginger. In: Ravinderan PN, Nirmal BK, editors. Ginger: The Genus Zingiber. New York: CRC Press; pp. 15-85.
20. Ravishankar, L. V., Pandey, M.K., Dey, T., Singh, A., Rasool, B., Diskit, S., Dar, N.A., Maurya, A.K., John, V., Sami, R., Shami, A. A., Al Kashgry, N. A. T., Althaqafi, M. M. and Algopishi, U. B. (2023). Phenotyping and Molecular Characterization of Durable resistance in Bread Wheat for Stripe Rust (*Puccinia striiformis* f.sp. *tritici*). *Journal of Biobased Materials and Bioenergy*, 18:1-11.
21. Shivakumar, N. and Agrawal, P. (2018). The effect of chemical mutagens upon morphological characters of ginger in M0 generation. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*. 20:126-135.
22. Sigrist, M.S., Pinheiro, J.B. and Azevedo-Filho J.A. (2010). Development and characterization of microsatellite markers for turmeric (Curcuma longa). *Plant Breeding*. 129:570-573
23. Suma, B., Keshavachandran, R. and Nybe, E.V. (2008). Agrobacterium tumefaciens mediated transformation and regeneration of ginger (*Zingiber officinale* Rosc). *Journal of Tropical Agriculture*. 46:38-44

24. Tamil Selvan, M. and Manojkumar, K. (2002). *Indian Journal of Arecanut, Spices and Medicinal Plants.* 4 (3): 109-116.
25. Yun, H.D., Young, K.K., Lok, C.I., Dong, K.S., Soo, P.M., Hyun, D.Y., Kim, K.Y., Choi, I.L., Kim, S.D. and Park, M.S. (1998). Change of stomatal behavior and chlorophyll fluorescence to environmental conditions in ginger (*Zingiber ojjicinale* Rose.). *Journal of the Korean Society for Horticultural Science.* 39 (2):145-148.