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Review

Genetic diversity and utilization of ginger (*Zingiber officinale*) for varietal improvement: A review

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Abstract: Ginger is widely cultivated globally and considered the third most important spice crop due to its medicinal properties. It is cultivated for its therapeutic potential in treating different medical conditions and has been extensively researched for its pharmacological and biochemical properties. Despite its significant value, the potential for genetic improvement and sustainable cultivation has been largely ignored compared to other crop species. Similarly, ginger cultivation is affected by various biotic stresses such as viral, bacterial, and fungal infections, leading to a significant reduction in its potential yields. Several techniques, such as micropropagation, germplasm conservation, mutation breeding, and transgenic have been extensively researched in enhancing sustainable ginger production. These techniques have been utilized to enhance the quality of ginger, primarily due to its vegetative propagation mode. However, the ginger breeding program has encountered challenges due to the limited genetic diversity. In the selection process, it is imperative to have a broad range of genetic variations to allow for an efficient search for the most effective plant types. Despite a decline in the prominence of traditional mutation breeding, induced mutations remain extremely important, aided by a range of biotechnological tools. The utilization of in vitro culture techniques serves as a viable alternative for the propagation of plants and as a mechanism for enhancing varietal improvement. This

review synthesizes knowledge on limitations to ginger cultivation, conservation, utilization of cultivated ginger, and the prospects for varietal improvement.

Keywords: ginger; crop improvement; biotechnological tools; breeding; variability; conservation

1. Introduction

Zingiber officinale Rosc., commonly referred to as ginger, is a member of the Zingiberaceae family and the Zingiber genus, comprising approximately 150 species extensively cultivated worldwide. This particular spice holds significant historical and cultural value, ranking as the third most prominent spice globally, and is highly regarded for its distinct flavor and potential health benefits. The genus comprises several important taxa, including large cardamom, cardamom, and turmeric, alongside various other species that hold varying significance in terms of their medicinal and economic value. Due to its widespread popularity, ginger is common in the cuisine of subtropical and tropical countries, including India and China, where it has been cultivated for centuries. The species is not typically observed in its natural habitat and is thought to have originated in the Indo-Malayan region [1]. Nonetheless, conclusive data regarding its domestication or primary point of origin is lacking. The earliest known indications of its domestication can be traced back to the Austronesian communities, who have been cultivating and utilizing various species of ginger, such as turmeric (*Curcuma longa*), bitter ginger (*Zingiber zerumbet*), and white turmeric (*Curcuma zedoaria*). The earliest documented mention of ginger can be traced back to the Analects of Confucius, wherein it is noted that Confucius consumed ginger as a regular dietary component [1]. According to the monk Fa Xian's account in 406 AD, ginger was cultivated in containers and transported on Chinese vessels as a preventive measures against scurvy. As reported by Ravindran et al. [2], ginger was introduced to East Africa in the 13th century CE by the Arabs, and subsequently, the Portuguese facilitated its commercial cultivation in West Africa and the Pacific.

The plant can produce a wide range of natural compounds that exhibit significant nutritional benefits. Its rhizomes are utilized as a spice and alternative medicine for a variety of ailments [1]. The medicinal properties of ginger were highly esteemed by ancient societies, and ginger held a significant position in primary healthcare practices in ancient China and India [1]. Ginger held significant value as a mild carminative in European medicine and was frequently incorporated into various pharmaceutical preparations. The principal commodities derived from ginger are fresh ginger, dried ginger, and preserved ginger [2]. The predominant mode of ginger consumption is using fresh, unripe, and ripe ginger as a culinary ingredient. The immature rhizomes are utilized for culinary purposes in addition to manufacturing processed foods, e.g. marmalades, jams, confectionery, and cakes, in preserved ginger. Dried ginger is commonly used as a spice in its whole, split, or ground form and is widely employed in flavoring processed food products. Value-added products such as ginger oils and oleoresins are derived from dried rhizomes through steam distillation or solvent extraction. Ginger comprises approximately 12.3% carbohydrate, 2.4% fibe, 2–3% protein, 1.2% minerals, and 0.9% fat. It is an important source of vitamins, iron, phosphorous, and calcium. The volatile oils (camphenes, lisabolene, zingiberene, cinol, zingiberol, phellandrene, limoline, citrol, borneol, linaloal, cibonellol, and geranial), oleoresin (shogaol and gingerol), phenol (zingerone and gingerol), proteolytic enzyme (zingibain), vitamin C, vitamin B6, magnesium, phosphorus, calcium, linoleic acid, and potassium are

important constituents of ginger [3]. The characteristic pungency of ginger can be ascribed to the presence of gingerol, whereas the aroma is primarily derived from the volatile oils lisabolene, zingiberol, and zingiberene. According to Kiyama et al. [3], ginger contains all the necessary components for promoting good health and enhancing the nutritional value of food.

India, China, Taiwan, Jamaica, Nigeria, Sierra Leone, Fiji, Indonesia, Mauritius, Brazil, Ghana, Costa Rica, Bangladesh, Japan, the Philippines, Malaysia, Sri Lanka, Thailand, the Solomon Islands, Uganda, Hawaii, Trinidad, Guatemala, and numerous other Pacific Ocean islands are recognized as the major countries that cultivate ginger. Table 1 summaries the global production of ginger across various nations [4]. Due to its predominant usage as a spice and condiment, the global production of ginger cannot be sustained solely by its per capita consumption. This is further compounded by the increasing number of countries producing ginger and engaging in international trade of the crop [5]. The available market data suggests the existence of a popular trend in the United States market, characterized by increasing demand for various spices such as black pepper, chillies, and ginger [5]. This phenomenon is a conspicuous manifestation of evolving dietary preferences across the globe. This trend may also be attributed to the recent changes in the ethnic composition of the populace, where there is an increase in the consumption of Chinese cuisine and Indian curry, which prominently feature ginger as a key component [5]. According to Nair and Nair [5], a noteworthy trend in the ginger industry is the growing utilization of processed ginger oleoresins and oils in several nations, particularly Europe and the USA.

Table 1. Production and area of ginger in the world (2021).

Area	Area harvested (ha)	Yield (hg/ha)	Production (tonnes)
India	205,000	108,537	2,225,000
China	122,593	105,448.3	1,299,431
Nigeria	86,911	88,402	768,304.9
Indonesia	10,610	289,578	307,242
Nepal	21,912	127,422	279,206
Thailand	10,060	168,020	169,035.6
Bangladesh	10,276	79,520	81,715
Cameroon	6891	96,696	66,633.15
Sri Lanka	6139	92,594	56,841.9
Peru	6423	74,417	47,795.98
Japan	1729	24,9579	43,147.62
Guyana	2996	117,137	35,097.62
Republic of Korea	3129	106,591	33,356.22
Mali	3526	91,108	32,123.84
Philippines	3980	70,710	28,144.5
China, Taiwan Province	851	261,304	22,237
Fiji	553	250,000	13,815.1
Ethiopia	3633	28,612	10,394.28
Malaysia	706	123,462	8,718.61
Others	3657	143,146.5	8,1638.47

Source: FOA [4].

Quality is a significant determinant of a commodity's export and demand potential. The quality parameters for ginger include its fiber contents, volatile oil, and non-volatile ether extract. The intrinsic properties and processing suitability of ginger cultivated in different regions of the country exhibit significant variations. This aspect holds greater significance in the context of processing dried ginger as compared to preserved ginger. The size of the rhizome is a pertinent factor, particularly in the context of dried ginger processing, where medium-sized rhizomes are typically deemed optimal. Certain regions cultivate varieties of ginger that produce exceptionally large rhizomes, which are sold fresh because they are not suitable for processing into dried spice due to their higher moisture levels. This phenomenon poses challenges in drying out, leading to yield loss, while the proportion of volatile oil is low. Despite its significant value, ginger has been neglected in terms of its potential for genetic improvement and capacity for sustainable agricultural cultivation. Worldwide, considerably less attention has been given to ginger cultivation than other crop species, as evidenced by the limited research on the subject. Several challenges persist regarding this significant yet overlooked crop, such as its utilization, preservation of genetic resources, and evaluations [6]. Diversifying agricultural production and food sources is imperative to enhance the quality of human nutrition and food security. This study consolidates information on cultivated ginger's utilization, characterization, conservation, and genetic diversity. Additionally, we explore the potential for breeding and varietal enhancement of ginger crops.

2. Characterization and diversity of ginger

Zingiber is categorized within the Zingibereae tribe, an integral part of the Zingiberales order and the broader Zingiberaceae family. The Zingiberaceae family encompasses three additional tribes: Globbeae, Alpinieae, and Hedychieae. Within the Zingibereae tribe are seven notable genera—Curcuma, Hedychium, Amomum, Kaempferia, Roscoea, Camptandra, and Boesenbergia. The Zingiber genus comprises 150 diverse species organized into four divisions distributed across tropical regions in Asia and Australia. Table 2 illustrates the extensive economic significance of various species within the Zingiber genus, extending beyond the well-known Zingiber officinale. The geographical dispersion of ginger clones, coupled with selective breeding and mutations, is believed to be the driving force behind the rich diversity observed in ginger cultivars [1]. Ginger's genetic potential has been explored only to the extent that it has been used for germplasm selection in the pursuit of higher yields and improved quality, in addition to some mutation and ploidy breeding. Anatomical and morphological characterization of ginger yield, rhizome features, and quality traits has been reported [1]. The obsolete cultivars generally have a low yield, small rhizomes, and higher quality. In contrast, the improved elite variety has appealing bold rhizomes, a high yield, and a mixture of quality attributes. However, the yield of ginger can vary depending on the climatic conditions, cultural methods, soil types, and genotypes. Other factors influencing yield and quality levels include regions, seasons, and locations. In comparison to *Z. macrostachyum*, *Z. roseum*, and *Z. zerumbet*, Ginger (*Z. officinale*) possesses unique anatomical characteristics such as short-lived functional cambium, absence of periderm, and the presence of xylem vessels with scalariform thickening in the rhizome. Ginger is also known for its pungent flavor [1].

The characterization has successfully identified ginger cultivars with distinct biochemical profiles, encompassing elements such as crude fiber, essential oil, and oleoresin contents. In the analysis of dried ginger samples, crude fiber content ranged from 4.8% to 9% [2]. The ginger rhizome's origin and status were found to have an impact on the essential oil level, which ranged from 0.2% to 3% [2]. The

oleoresin concentration of ginger varied significantly, ranging from 3% to 11%, and was impacted by various parameters, including genotype, solvent extraction conditions, rhizome state, area of origin, and harvest season [3]. The non-pungent and pungent components, gingerol and shogaol, showed variations across different Indian ginger cultivars, while Australian ginger exhibited minimal variance in their levels [1]. Sri Lankan ginger, when dried, had high quantities of ar-curcumene and beta-bisabolene, coupled with significant levels of citral isomers, though zingiberene levels were notably low [1]. The environment and the rhizome's state significantly influenced ginger's production of non-volatile and volatile compounds. Despite this general trend, some varieties and early forms of ginger were notable for their exceptional performance in specific chemical compounds, especially the more intense components [1].

Table 2. Significant economically important of Zingiber species.

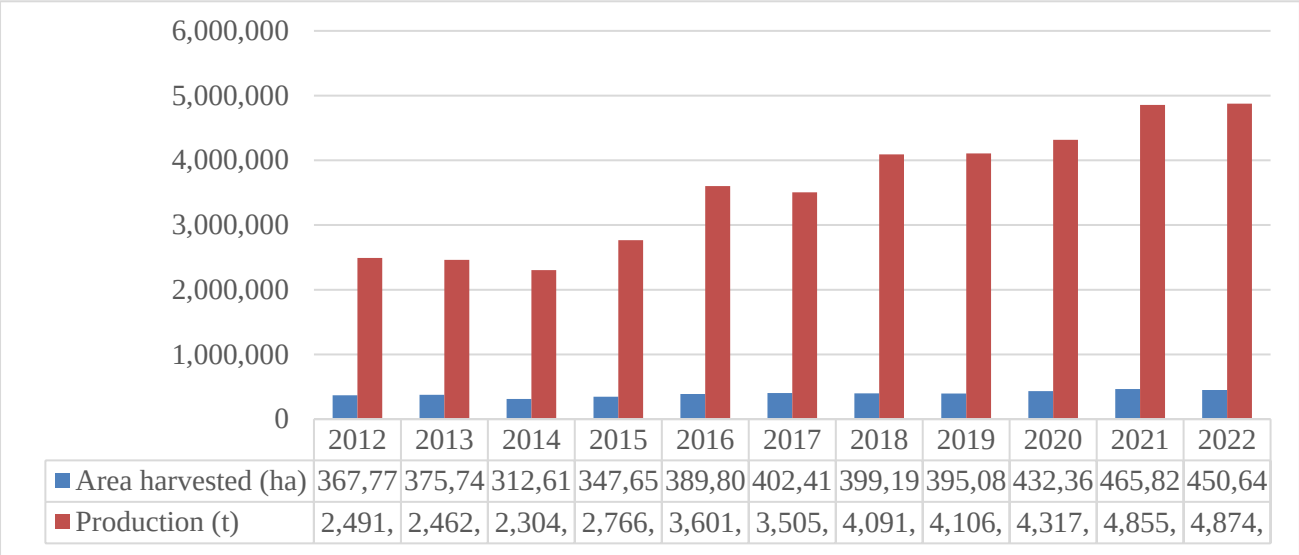
Species/subspecies	Use	Occurrence
Z. officinale Roscoe	Spice, condiment, medicinal	Tropical countries, China, USA
Z. officinale var. rubra	Medicinal, spice	Malaysia
Z. zerumbet (L.) Smith	Medicinal, ornamental	Tropical Asia
Z. clarkii King	Ornamental	Sikkim Himalayas
Z. *rubens* Roxb	Medicinal, ornamental	Indo-Malaya
Z. ottensii Valet	Medicinal, ornamental	South East Asia
Z. americanus Bl.	Medicinal, vegetable	South East Asia
Z. officinale var. rubrum	Medicinal, spice	Malaysia
Z. mioga Roscoe	Vegetable. Shoot and flower are edible	Japan
Z. montanum (Koenig) Link ex Dietr	Used in traditional medicine	India, Malaysia, Sri Lanka, Java
Z. aromaticum Val	Ornamental, medicinal, flavoring	Tropical Asia
Z. griffithii Baker	Ornamental	Malaysia
Z. corallinum Hance	Chinese medicine, ornamental	South East Asia
Z. argenteum (J. Mood and I. Theilade)	Ornamental	Sarawak, Malaysia

Source: Kizhakkayil and Sasikumar [1].

3. The potential of ginger

Cultivating cash crops, such as ginger, can provide farmers with an additional source of income, enabling them to raise their standard of living. The crop species possess significant commercial value in the global market owing to its widespread consumption across various regions worldwide [1]. For centuries, ginger has been utilized globally as a spice supplement and in alternative medicine [7]. This particular spice is highly valued for its subtle aroma and flavor, promoting its use in cuisine and confectionery products. Ginger is a widely utilized flavoring agent in various culinary preparations, including baked goods such as cakes, cookies, gingerbread and beverages like tea, ale, juice, and ginger beer. These applications have garnered significant recognition in the global food industry, as reported by Sangwan et al. [8]. The gustatory attributes of ginger are contingent upon the presence of both volatile and non-volatile constituents. Fresh young ginger, which has a low fiber content and a succulent texture, is commonly used in producing ginger candy, crushed fresh ginger, salted, and

preserved ginger. In contrast, mature fresh ginger is typically employed in ginger shreds, ethnic ginger chutney, dry ginger, ginger oil, ginger powder, ginger curry, and ginger tea. Ginger is essential in food items such as curry powder, sauces, gingerbread, and carbonated beverages with ginger flavor. It is also utilized in pickles, biscuits, and other dietary preparations [1]. In the last decade, there has been a consistent growth in global ginger production, as illustrated in Figure 1 [4]. The demand for ginger is driven by several factors, including the increasing global population, export opportunities, crop rotation and diversification, research and innovation, culinary applications, and its expanding role in medicinal and nutraceutical applications. This surge in demand can be attributed to consumers' growing preference for natural and nutritious ingredients. As individuals actively search for remedies and functional foods to improve their overall well-being, ginger aligns well with these preferences and is experiencing a significant increase in demand. Moreover, there is a growing inclination towards natural and clean-label ingredients, emphasizing minimal processing and excluding artificial additives.



	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Area harvested (ha)	367,77	375,74	312,61	347,65	389,80	402,41	399,19	395,08	432,36	465,82	450,64
Production (t)	2,491,	2,462,	2,304,	2,766,	3,601,	3,505,	4,091,	4,106,	4,317,	4,855,	4,874,

Source: FOA [4].

Figure 1. Global production and yield quantities of raw ginger 2012–2022.

3.1. Pharmacological benefits and use of ginger in modern medicine

Ginger is recognized as a botanical species with medicinal properties that offer abundant health advantages. The plant is widely recognized for its medicinal properties, including anti-nausea, antidiabetic, antimicrobial, anti-inflammatory, antioxidant, and antiemetic, effects. These properties are attributed to its abundance of bioactive compounds, such as paradols, gingerols, shogaols, and essential oil, thereby promoting the extensive use of ginger as medicine [9,10]. The plant is a commonly recommended treatment for various ailments such as headaches, colds, coughs, and constipation. Ginger can be consumed as herbal teas, capsules, powder, or syrup, alone or alongside other species [7]. Furthermore, ginger has been reported for its aphrodisiac and tonic properties and has been found to have the potential to enhance sperm quality [11]. In addition, empirical research has demonstrated that extracts derived from ginger possess beneficial properties that mitigate oxidative stress, hyperglycemia, and hyperlipidemia. According to Wang et al. [12], the consumption of ginger

can potentially reduce obesity by increasing the utilization of fat as an energy source aside from catabolism in skeletal muscles. Additionally, ginger oil use on the skin has been found to have analgesic properties. Although ginger is believed to possess various other beneficial properties, the current scientific evidence is inadequate to support these claims.

The medicinal value of ginger has been confirmed by recent pharmacological studies [13,14]. Ginger has been considered highly valued in Chinese medicine since the 4th century BC. According to Grant and Lutz [15], ginger is utilized by the Chinese for treating a diverse range of medical issues, including toothaches, respiratory problems, heart disease, headaches, stomach discomfort, diarrhea, cholera, nausea, asthma, rheumatoid arthritis, migraine, and hypercholesterolemia. As reported by Kizhakkayil and Sasikumar [1], ginger is utilized by Africans and West Indians to treat various health conditions. Similarly, ginger has been used in the Mediterranean region to treat arthritis-related complications, muscular discomfort and rheumatological problems. Ginger is a traditional remedy for various ailments such as convulsions, pain, rheumatism, constipation, scabies, indigestion, cholera, prolepsis, fistula, throat pain, cold, tuberculosis, cough, and fever [1]. Ginger is a potential remedy for various livestock diseases in veterinary medicine. The anthelmintic activity of dried powdered ginger in sheep infected with gastrointestinal nematodes was reported by Iqbal et al. [16]. The medicinal properties attributed to ginger as a spice are related to its volatile and non-volatile compounds, which are responsible for the pungency and aroma of ginger. The primary volatile compounds in ginger comprise sesqui- and mono-terpenes, sesqui-phellandrene, camphene, cineole, curcumene, phellandrene, geranyl acetate, borneol, terpenes, terphineol, geraniol, zingiberene, linalool, limonene, farnesene, and bisabolene. El-Baroty et al. [17] and Hsu et al. [18] reported that most of the above-listed compounds have been researched to have therapeutic properties. Ginger contains significant amounts of gingerols and shogaols, which are non-volatile pungent compounds. These compounds have been studied to possess various beneficial properties, such as analgesic, antipyretic, cardiotonic, antioxidant, and anti-inflammatory effects. Additionally, they have been observed to suppress cytokine formation and promote angiogenesis [19,20]. According to Yang et al. [21], gingerol has the highest biological activity, specifically 6-gingerol. According to Kizhakkayil and Sasikumar [1], the growth of Proteus spp., Streptococci, *Escherichia coli*, Salmonella, and Staphylococci can be inhibited by ginger.

4. Limitation to ginger cultivation

The cultivation of ginger is subject to a range of abiotic and biotic factors that can impact its production. According to Paret et al. [22] and Sharma et al. [23], the biotic factors influencing ginger production include viruses, bacteria, fungi, and nematodes. Bacteria are the most significant biotic factor that can cause wilt and soft rot, followed by fungi; which are responsible for causing various plant diseases of ginger such as soft rot, rhizome rot, yellows disease, and Sclerotium rot. The presence of nematodes in ginger plants is known to result in root-knot disease, while viruses are responsible for mosaic and chlorotic fleck diseases. These conditions lead to economic loss because they affect the yield of the rhizome. Ginger crops are susceptible to infestation by various insects, including *Aspidiella hartii*, *Conogethes punctiferalis*, rhizome fly, thrips, and rhizome scale. Abiotic factors such as high light intensity can result in sunburn, while excessive liming of the soil can lead to lime-induced chlorosis in ginger crops. Understanding the disease symptoms, the causative agent, and the preventive measures is paramount. Understanding the significant nature of ginger diseases through the causative agent, host resistance, and symptoms of these diseases is highly important to enhance ginger production.

4.1. Viral disease

Ginger is susceptible to viral infections such as Mosaic and Chlorotic fleck. Insect vectors, including *Rhopalosiphum insertum*, *Macrosiphum euphorbiae*, *Myzus persicae*, *M. humuli*, and *M. certus* facilitate the mosaic vir*us* transmission. The viruses exhibit higher efficiency when transmitted by *Myzus certus* and *M. persicae* than other vectors [24]. According to So [24], the cucumber mosaic virus is believed to be transmitted through the sap to various plants that hosts the virus. The evaluation of viral concentration in different components of ginger indicated that the flower and leaves exhibit a greater viral concentration than the stem, rhizome, and other plant parts. The ginger infected by the virus is characterized by spherical particles measuring 23 to 38 nanometers in diameter. The virus particle that underwent purification exhibited a positive response in serological testing when exposed to cucumber mosaic virus antiserum. The electron microscopic observation and serological relationship of the virus led to the suggestion that the ginger mosaic virus belongs to the cucumber mosaic virus group. The identification of the cause of mosaic disease in ginger was supported by a Malaysian research group, which determined that the causal agent was the cucumber mosaic virus, based on analysis of the partial nucleic acid sequence of the coat protein (GenBank: MH355647.1). The Ginger Mosaic Virus is identifiable by the presence of a dark-green and yellowish mosaic pattern on the leaves of ginger during the initial stages of infection. As the infection progresses, the rhizomes and leaves become stunted. The viral infection on ginger has been observed to decrease the yield of rhizomes significantly.

The manifestation of chlorotic fleck in ginger is attributed to the presence of the ginger chlorotic fleck virus (GCFV). This particle comprises a prominent protein coat having a molecular weight of 29 kDa and a single-stranded RNA with a molecular weight of 1.5 × 106 Da. The dissimilarities between mosaic virus and chlorotic fleck virus are evident in serology, host range, and particle properties, with the latter's diameter varying between 28 to 33 nm. The salt-labile nature of particles, ssRNA, limited host range, and other properties observed in the chlorotic fleck virus are reminiscent of the sobemovirus group [25]. However, despite these similarities, this virus is serologically distinct from several sobemoviruses, including turnip rosette virus, velvet tobacco mottle virus, lucerne transient streak virus, southern bean mosaic virus, cocksfoot mottle virus, *Solanum nodiflorum* mottle virus, and sowbane mosaic virus. The foliage of affected plants exhibits chlorotic flecks, ranging from 1 to 10 mm in length, that are oriented in a parallel and centred fashion with respect to the veins. Symptoms were observed in the juvenile foliage approximately 3–4 weeks post-infection, followed by their manifestation in other leaves. No noticeable symptoms manifest on the rhizomes. The GCFV pathogen is exclusively transmitted through mechanical means to ginger, unlike the ginger mosaic virus, which is transmitted by *Pentalonia nigronervosa*, *Rhopalosiphum padi*, or *R. maidis* and *Myzus persicae* [25].

4.2. Bacterial diseases

The predominant bacterial diseases that impact cultivated ginger are Bacterial wilt and Bacterial soft rot. The bacterial pathogen *Ralstonia solanacearum* Yabuuchi is accountable for causing bacterial wilt disease in ginger and is currently recognized as the second most significant destructive bacterial pathogen. The pathogen *R. solanacearum* is transmitted through various means such as soil-borne dissemination via vehicle tyres, tools, boots, hands, and field equipment, as well as through water during irrigation and rainfall. The disease can also spread through infected ginger rhizomes [26].

According to Swanson et al. [27], the bacterium gains entry into the ginger plant via the rhizomes and roots through wounds incurred during handling or through openings where lateral roots emerge, as well as through parasitic insects or root-knot nematodes, following its presence in the soil. The bacterial pathogens are capable of persisting within the affected plant detritus, as well as existing independently within the soil environment. Bacterial wilt disease of ginger is known to thrive in warm temperate, tropical, and subtropical regions across the globe, and its impact on ginger production is highly detrimental, resulting in severe economic loss. The infection of ginger by bacterium results in a rapid wilt within 5 to 10 days. The pathogenicity of the disease is attributed to its rapid dissemination, which is facilitated by favorable environmental factors such as high temperatures and increased precipitation. The initial noticeable symptom following infection is the mild drooping and curling of the leaf margins on the lower leaves, followed by progression to the upper leaves. During the advanced stage of infection, ginger plants display severe symptoms such as wilting and yellowish discoloration. The plant infected with the disease exhibits a persistent stance and does not collapse. Upon gentle pressure, the vascular strands of the infected pseudo stem and rhizome discharge a foul odor and discharge a milky ooze.

Although bacterial soft rot is not typically regarded as a significant threat in ginger, however, sporadic occurrences have been observed when the plant is cultivated in waterlogged soil [28]. The prevalence of the disease is higher in rhizomes developed at greater depths within the soil. The presence of bacterial soft rot is not typically observed in adequately drained soils. *Erwinia chrysanthemi* is the only species of Erwinia responsible for soft-rot occurrence in ginger [28]. The disease is often aggravated by injuries incurred during seed preparation, as well as by saturated soils and high temperatures. The disease induces a progressive deterioration of the rhizome tissue concomitant with a malodorous scent. Bacteria in ginger can be attributed to direct inoculation, infected seed, or natural or wound openings. The bacteria initiated the process of consuming fluids that were discharged from damaged cells, leading to their proliferation. Bacterial organisms can secrete pectolytic enzymes, which facilitate the breakdown and degradation of cellular structures, thereby augmenting the nutrient availability for the bacterial population [28]. Frequently, the epidermis remains intact, confining the putrefying tissue within until a fissure permits the discharge of its purulence, thereby spreading contagion to adjacent individuals. The transmission of bacteria from infected plants to adjacent ones occurs through direct and indirect contact, such as insect vectors. Maintaining hygienic growing practices is the most efficient approach to prevent the onset of this disease. The recommended practice for maintaining the quality of stored plants involves eliminating plant debris from storage facilities and disinfection of the storage unit using copper sulphate or formaldehyde after each harvest. It is also important to ensure proper ventilation and temperature control within the storage facility while maintaining low humidity levels. Bacterial soft rot can also be prevented by implementing cultivation practices in well-drained soils and the rotation of non-susceptible plants with susceptible plants [28].

4.3. Fungal diseases

The cultivation of ginger is significantly impacted by the deuteromycetous fungal group, which induces a range of symptoms [29]. According to Johsi and Sharma [30], fungal diseases significantly impact the potential yield across various stages, including market values, storage, and field, resulting in losses exceeding 50%. Ginger is susceptible to various fungal diseases, including soft rot/rhizome

rot, yellows/wet rot, and storage rots. The prevalence of soft rot in ginger-growing regions is widely regarded as the most potentially dangerous and detrimental disease with the potential yield loss of 50–90 under higher humidity and temperature. Soft rot disease in ginger exhibits a high incidence rate during its growth phase. The susceptibility to infection is significantly high in sprouts, roots, developing rhizomes, and the pseudostem collar region. The initial manifestation of symptoms is observed on the above-ground structures of the plant. The pathogen induces the formation of lesions characterized by a watery consistency and a brown coloration in the collar area of the pseudostem. Subsequently, the lesion undergoes enlargement, coalescence, and consequent stem rot before collapsing [31]. Chlorosis symptoms, characterized by yellowing, manifest initially in the tips of older leaves and subsequently propagate along the margin to encompass the entirety of the leaf sheath and leaf blade. According to ISPS [32], the chlorosis originating from the older leaves gradually spreads to the younger leaves, resulting in the death of the entire plant. In contrast to bacterial rot, the soft rot induced by fungal infection does not generate malodorous emissions. Eleven distinct species of Pythium cause soft rot diseases in ginger. Among these, *P. aphanidermatum* and *P. myriotylum* are particularly destructive in regions with warm climates. The fungal pathogen Fusarium has been reported as a causative agent of ginger soft rot. According to Moreira et al. [33], the rot of ginger rhizomes are caused by *F. oxysporum* among various species of Fusarium, and the disease is transmitted through rhizomes and soil. Favorable conditions that influence the oospore development include soil temperature, high soil moisture, and wet soil conditions [34]. The severity of the disease is impacted by rainfall intensity and the cultivation of rhizomes in soil with high clay content and inadequate drainage. According to Le et al. [35], implementing cultural practices such as appropriate drainage, tillage, organic amendment, crop rotation, seed selection, and quarantine measures can effectively manage and restrict the dissemination of Pythium spp. The identification of Pythium-resistant ginger is crucial for the efficient management of soft rot disease. In a study by Bhai et al. [36], 650 ginger accessions were screened, revealing that a mere 7% of the accessions exhibited relative resistance to Pythium sp.

Ginger rhizome and stem rot caused by yellow disease is a significant issue of grave concern. It is commonly observed and prevalent in high humidity and temperature environments. According to Yang et al. [37], *Fusarium oxysporum* f.sp. *zingiberi* Trujillo is responsible for the development of yellow disease. Meenu and Jebasingh [28] reported that various species of Fusarium, including *F. equiseti* (Corda) Sacc., *F. moniliforme* Sheld, *F. graminearum* Schwabe, *F. solani* (Mart.) Sacc., and several other *Fusarium* spp., have been linked to the occurrence of the yellow disease. The disease exhibits chlorosis symptoms on the lower leaf margins, which progressively expand and encompass the entirety of the leaves. Plants that have been infected may exhibit symptoms such as stunted growth, wilting, premature drooping, and drying in patches or entirely. According to Meenu and Jebasingh [28], the favorable temperature range favoring the proliferation of the yellows disease is between 15–30 °C, with the optimum temperature being between 23–29 °C. Additionally, high humidity and rainfall are conducive to the development of this disease. The highest occurrence of disease was observed within the range of 24 to 25 °C soil temperature and 25 to 30% soil moisture [28]. The transmission of the disease primarily occurs via infected rhizomes. Therefore, cultivating healthy seed rhizomes is the optimal approach for managing the yellow disease, while developing a Fusarium-resistant strain would be an ideal solution. During storage, rhizomes soft are affected by bacteria and fungi [28].

Post-harvest losses caused by various abiotic and biotic stresses experienced in ginger production presents a major challenge. In storage, several fungi, including *Verticillium chlamydosporium* Goddard,

Thanatephorus cucumeris (Frank) donk, *Stachybotrys sansevieriae, Mucor racemosus* Fresen., *Graphium album* (Corda) Sacc., *Gliocladium roseum* Bainer, *Cladosporium tenuissimum, Aspergillus flavus*, *Geotrichum candidum*, *F. oxysporum* Schlechtend ex Fr., *P. myriotylum* Drechs., and *P. deliense* Meurs affect the rhizome soft of ginger [28]. *Verticillium chlamydosporium, Fusarium oxysporum*, and *Pythium ultimum,* bring about storage rot characterized by discoloration and subsequent dry rotting and decaying of the rhizome. According to Jadhav et al. [39], the disease incidence is controlled by immersing ginger in garlic extract (20% w/v) for 30 minutes. Similarly, dipping rhizomes in a suspension of *T. harzianum* and *P. fluorescens (*0.5% for 30 min) or immersing the rhizomes in the *Allium sativum* extract before storage can reduce the disease incidence [38].

5. Breeding for varietal improvement

Enhancing ginger types through breeding involves the deliberate selection and crossbreeding of parent plants exhibiting desirable characteristics, resulting in new ginger cultivars exhibiting improved traits. The primary characteristics in ginger breeding include yield, disease resistance, rhizome quality, flavor, and adaptation to diverse growth conditions (Figure 2). Ginger's restricted capacity to produce flowers and seeds substantially hinders its breeding activities. The primary emphasis of crop improvement programmes involving this species centres on selecting and assessing naturally existing clonal variations. Until recent advancements, breeders had a significant challenge due to the limited genetic diversity in ginger plants. A substantial pool of genetic variability is essential for the selection process in plant breeding [40]. Although conventional mutation breeding is no longer the prevailing practice, induced mutations are in great demand and can be produced using many biotechnology techniques. In recent years, significant changes have occurred in the methods used to cause mutations and study mutated lines. Plant propagation by in vitro culture techniques offers an extra means of plant reproduction and serves as a tool for improving crop quality. Tissue culture technology has advanced to treating and maintaining tiny tissue samples, such as stems, tips or growing callus, by placing them on a standard nutrient medium. After treatment, the explants are permitted to reach maturity and then evaluated for beneficial mutations. To overcome the issue of chimeras, which often arises when inducing mutations in vegetatively propagated plants, advanced in vitro methods such as single-cell cultures and somatic embryogenesis can be employed [41]. These technological improvements play a crucial role in overcoming the challenges in ginger breeding, offering new opportunities for genetic enhancement.

5.1. Varietal improvement through selection process

Despite numerous constraints associated with producing and preserving high-quality ginger seed rhizomes, breeding efforts should concentrate on selecting genotypes that can solve the limitations specified as selection objectives characteristic. In this approach, breeding operations need to contribute to the production of cultivars that contain significant qualities such as resistance to pests and diseases, productivity, essential oil, fiber, and oleoresin content. Additionally, the scope of such research should be expanded to include efforts to improve the genetic makeup of cultivars, both in terms of their capacity to adapt to a larger production area and the qualities that make them valuable commercially [42]. The development of new cultivars should result in the creation of new varieties that aligns with the preferences of customers and market demands. Hence, the greater the genetic diversity of a population is, the greater the likelihood that it will contain alleles favorable to the many qualities being selected [42].

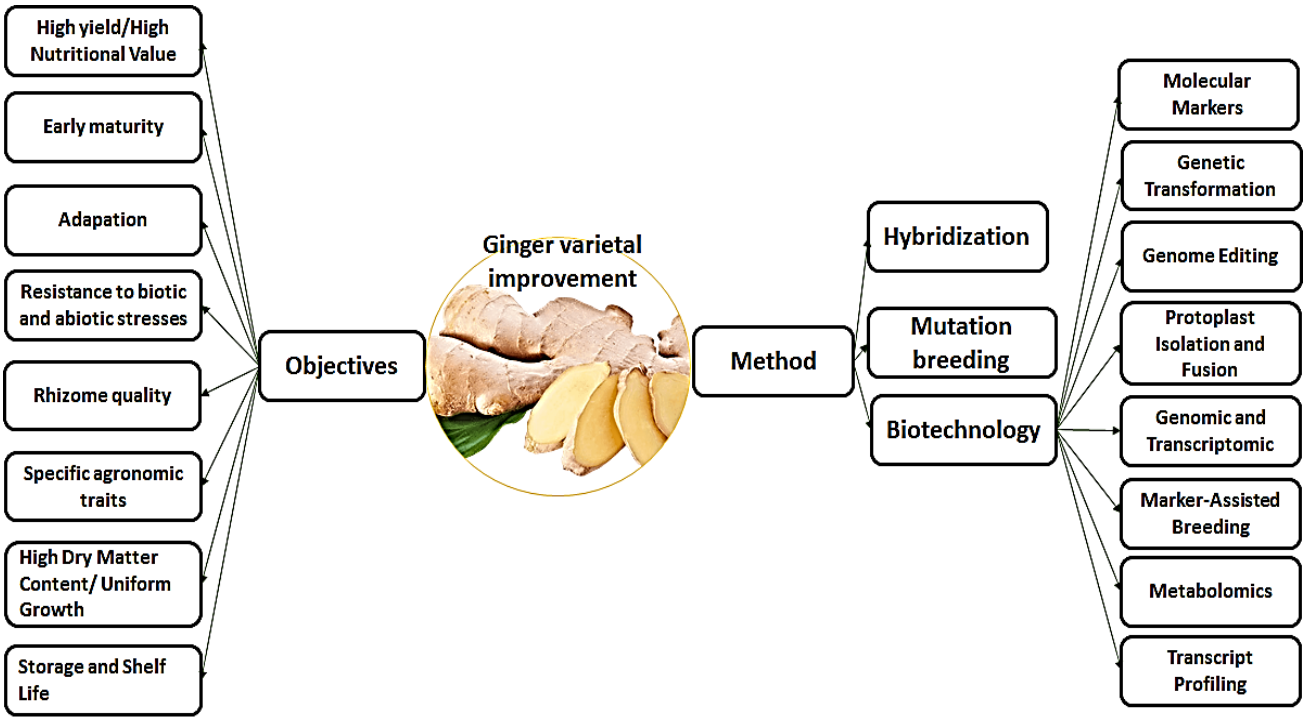


Figure 2. Varietal improvement in ginger.

The process of assessing the amount of genetic variation within or between a population is referred to as genetic diversity analysis. Quantitative data and a wide variety of different variables are included in the content. Numerous data groups such as morphological, passport, pedigree, and biochemical data have been utilized to analyze crop genetic diversity. Alternately, data-based on DNA markers have been utilized to access a variety of more reliable genotypes [43]. Molecular-based methods, e.g. isozyme or DNA analysis can be used to evaluate the degree of variation directly among the population. The evaluation and discovery of an organism's genetic variety can assist us in comprehending the molecular underpinnings of various processes that occur inside the organism [44]. Identifying and characterizing ginger germplasm is a crucial link in the chain that connects the conservation of ginger genetic resources with their commercial use. It is now essential to characterize the genetic diversity within native populations, advanced selections, and cultivars of ginger [45], before starting a judicious breeding program. This is one of the methods of obtaining reliable genetic information for further breeding and germplasm collection [46]. Plants can be identified using numerous marker-based methods, among these PCR-based molecular markers have seen widespread application across a diverse range of plant taxa. This is mostly due to their reliability and ease of use for population studies, species identification, phylogenetic research and genetic linkage mapping [45].

Genotypic evaluation and molecular studies can be used to examine genetic diversity. These tools enable advanced knowledge of the variety and assist in decision-making when selecting the most effective variations for the trait(s) of interest [42]. It is only possible to perform varietal improvement of vegetatively propagated plants with the availability of clones that differ genetically from one another. Therefore, maintaining a high level of biodiversity is essential to achieve diversified and sustainable production of the plant as well as the success of the improvement program. Agronomic evaluation is vital in plant breeding because it allows one to determine the variability in the available genetic

material [47]. As a result, agronomic experiments have been carried out in large ginger-producing countries with high value on the commodities, such as Japan, India, China, Brazil, and Thailand [47,48]. Studies of this kind indicated varying degrees of genetic variation in ginger. Trials, in addition to demonstrating the genetic variety, make it possible to quantify the correlations between different qualities [47,49]. Therefore, data on agronomic and commercial features of the plant, such as yields, essential oils, and aesthetics of the rhizome, has been reported by Das et al., [48]. However, despite its characteristics and vegetative propagation paths, which minimize variability, this crop appears to demonstrate, on average, a low genetic variability.

5.2. Markers in ginger breeding

The term "marker-assisted selection" (MAS) refers to the process of identifying desirable parental lines and genes of interest in breeding populations through the use of molecular markers [50]. This method has enjoyed wide adoption owing to its high reliability and cost-effectiveness. Due to the botanical characteristics of ginger which can only reproduce through the process of vegetative growth, varietal improvement efforts have been very scarce [50]. As a result, clone selection efforts take up significant time in research programmes devoted to this crop, while hybridizations are performed infrequently. Additionally, limited genotypic variability in ginger poses enormous challenges to developing genetic gain through sexual means. This is especially true when compared to other species with more genetic diversity. Hence, mutant breeding and tissue culture are among the options for obtaining allelic variability to provide new genotypic variants in ginger. Past investigations of embryological, physiological, anatomical, and morphological characterization have been utilized in genetic analysis to estimate variation in a species or population. Analysis of morphological or biochemical characteristics has typically been used in the past as a method for researching plants' genetic diversity [51]. The influence of environmental factors on gene expression can affect the reliability of phenotype evaluation as a means of estimating genetic diversity. Due to the relative simplicity of their production and the absence of any influence from the surrounding environment, molecular markers are central to genetic variability and varietal research. The analysis of genetic variation and patterns of genetic diversity in species and populations might provide useful information for developing breeding approaches that promote rapid conservation and adaptability. Genotype information (allele and gene frequencies) and single nucleotide polymorphisms (SNPs), as well as the measurement of population structure and genetic differences, are examples of the types of information that can be obtained from molecular markers [52].

Various molecular genetic markers can be utilized to characterize and identify genetic diversity. Each molecular marker possesses unique qualities; therefore, selecting the appropriate molecular markers based on the information they provide and their ease of genotyping is essential. [53]. In recent years, markers for determining genetic diversity, known as arbitrarily amplified dominant (ADD), have been replaced by more sophisticated ones e.g. gene-targeted functional markers. Phylogenetic, evolutionary, ecological, taxonomic, and genetic diversity investigations of plants use popular methods, such as AFLP, SSR, RAPD, and ISSR. It is common knowledge that these classic genetic approaches have their benefits and drawbacks. The various methods of molecular evaluation differ in their primary characteristics, such as the cost per assay, required amount and quality of DNA, polymorphism, the specificity of the locus, the technical requirements, the ease of replication, and the level of reliability [52]. Unfortunately, efforts to apply molecular markers in ginger species have been scarce. Only a handful

of published researchhas documented the use of biochemical or molecular markers to differentiate ginger species. The application of molecular markers for detecting and exploiting DNA polymorphisms is often regarded as the most significant achievement in molecular genetics [54,55]. Curcuma species and *Zingiber officinale* were the most common targets for the application of these methods.

Due to the lack of precise information regarding the genome, random amplified polymorphic DNA markers (RAPD Markers) are utilized in ginger plants. The genome of ginger is inadequately researched and has limited available information. A study employed metabolic profiling and phylogenetic analysis to examine the diversity within and between ginger species. Zingiber samples from various geographic locations were indistinguishable [56]. RAPD markers exhibit a high resolving power value, enabling differentiation of cultivars, clones, accessions, genotypes, or varieties. RAPD generate reproducible polymorphic bands, making it a useful marker without specific genome information. In the early stages of the plant's development, the variation among the induced mutant clones may assist in the selection process. Populations of *Z. officinale* and *Z. zedoaria* were found to be separated into two distinct groups, one of which was found in hill areas and the other in plain areas while cultivated samples displayed a high level of genetic diversity. Numerous studies have demonstrated that RAPD is an efficient method for identifying genetic variation on both the intraspecific and interspecific levels [45,57]. Consequently, it has been demonstrated that RAPD markers are suitable for identifying and characterizing ginger species.

Amplified fragment length polymorphism (AFLP) markers can more polymorphisms in a single reaction than other markers. As a result, this marker has emerged as the most important in the field of genetic marker technology [58]. AFLP markers are useful for determining the genetic connection between members of the same species or genus. The degree of polymorphism between individuals of the same species was relatively low. AFLP analysis revealed a correlation between the phylogenetic relationships of ginger and their morphological characteristics and reproductive modes. It has been demonstrated that the reproduction mechanism affects the patterns of genetic diversity found within populations of Curcuma with varying numbers of genome copies [41]. Markers based on ALFP sequences have the potential to give species-specific identification for analyzed species, as well as provide a large number of repeatable markers that may be used to evaluate the diversity of the nuclear genome. Utilizing this DNA fingerprinting marker, three different species of Zingiber: *Z. zerumbet*, *Z. montanum*, and *Z. officinale* were identified. This demonstrates that ALFP is a reliable measure that can be utilized for the species identification [59].

Microsatellites, also simple sequence repeats, are co-dominant DNA markers of short tandem repeats of 2 to 6 bp of nucleotides. These microsatellites are widely distributed across the genomes of a wide variety of different species. There are two different kinds of microsatellites *viz*: Expressed-sequence-tag-based/genic SSR (EST-SSR) and genomic SSR. SSR markers had advantages over other markers because of their co-dominant inheritance pattern. These advantages include a high level of polymorphism, high specificity, and repeatability. Because of this, SSR markers are utilized extensively in the research of germplasm characterization, marker-assisted selection, linkage mapping, and quantitative trait loci mapping [60]. Molecular characterization of ginger has been done in the past using several different molecular markers, even though there is very little molecular information available on ginger. Since EST-SSRs are closely related to the genes that govern distinct phenotypes, they are more able than other neutral markers to disclose the extent of local adaptation and environmental variation. In addition, it is possible to transfer it to other related species even if the

markers are developed for a particular crop because their locations are in conserved regions. The identification of microsatellite loci has been made more accessible due to advancements in next-generation sequencing technologies, such as low-cost de novo transcriptome sequencing [50].

ISSRs, or Inter-Simple Sequence Repeats, are semi-arbitrary markers that can be amplified using PCR with one primer that corresponds to a target microsatellite. ISSRs can differentiate between closely related organisms and are widely dispersed across the whole genome. ISSRs have numerous advantages, the most prominent of which is that their amplification does not require information about the genome sequence and is highly polymorphic. ISSR is found to be an effective marker for differentiating the relationships between ginger cultivars that are genetically close, as reported by Sigrist et al. [61]. When evaluated on Curcuma species, it was discovered that ISSR marker exhibited higher polymorphism level than RAPD and AFLP markers [62]. However, it was found in the research carried out by Kaewsri et al. [60] that AFLP markers supplied more information than ISSR markers in the case of wild ginger species *Z. moran* and cultivars of Northwest Himalayan ginger. The capacity of each of these markers to resolve variations between individuals varies to some degree since each of these markers targets a distinct portion of the genome. It is vital to apply a range of different molecular markers in assessing the genetic diversity of any crop to get accurate results.

The combined use of RAPD and ISSR techniques is employed for ginger species identification. RAPD and ISSR have been utilized in conducting studies on the genetic diversity of various plant species, including ginger. According to some reports, RAPD markers are better suited to conducting analysis of genetic diversity. Compared to RAPD markers, ISSR markers have a higher level of reproducibility. Consequently, the percentage of polymorphisms found using ISSR markers was significantly higher than that found using RAPD markers. Integrating data from both markers would enhance species differentiation outcomes. The genetic diversity of micro-propagated and cloned ginger species was evaluated using RAPD and ISSR techniques [63]. Other markers, such as single nucleotide polymorphism (SNP), nucleotide binding site (NBS), sequence characteristic amplified region (SCAR), and restriction fragment length polymorphism (RFLP), have been used to identify and characterize ginger species [63].

5.3. Mutational breeding in ginger

Mutational breeding plays a significant role in developing new varieties, particularly those propagated clonally. The conventional techniques of mutation breeding entail the utilization of mutagens to induce mutations, followed by assessing the heritability of the mutated genes across successive generations [64]. This method requires a significant investment of time and labor, and necessitates evaluating a substantial population. Biotechnology techniques have facilitated mutation breedings by treating explants with mutagens and subjecting them to in vitro screening. An alternative approach to generating variation is via somaclonal variation, which involves the induction of somatic hybridization under in vitro conditions. More recently, transgenic technology has also been utilized; which involves the treatment of a significant quantity of rhizomes under in vivo conditions, followed by screening phenotypic and genotypic characteristics [64]. In recent years, there has been a significant transformation in the techniques employed for inducing mutations and analyzing mutants. The explants, including adventitious buds, leaf, stems, and roots, produce plants via direct or indirect regeneration. One of the limiting factors in the genetic enhancement of ginger is the insufficient genetic diversity observed among the genotypes regarding their disease-resistance capabilities. Research has been

conducted to enhance ginger genotype mutability and radio sensitivity by applying varying doses of gamma rays [65].

Through artificial screening of mutants, six mutants have been identified as potential lines against the *Ralstonia solanacearum* and Pythium sp. Pathogens [40]. A negative relationship was observed between mutagenic effectiveness and mutagen dose, suggesting a decrease in effectiveness with increasing doses. Diverse morphological characteristics showed significant variation across varying levels of gamma-ray radiation compared to the control. Various doses of gamma rays (ranging from 0.5 to 1.2 kR) were utilized to induce broad mutation frequencies and spectra. The prevalence of chlorophyll mutants suggests that ginger exhibits a high degree of mutability. Three categories of chlorophyll mutations which include xantha, chlorina, and albino were observed. The frequency of xantha was the highest among the mutation spectrum of ginger, followed by chlorina and albino. Studies on the effects of Gamma radiations and chemical mutagens on growth and yield parameters, such as sprouting percentage, survival percentage, number of tillers, and plant height have shown significant improvement [66,67].

5.4. Gene transformation

Transgenic innovations in ginger are extremely limited in number because ginger is propagated using vegetative means and it has a low genetic variability unlike other spices. One of the most potentially beneficial approaches is introducing novel resistance factors by genetic transformation. Gene transfer-compatible regeneration techniques have been optimized to introduce novel genes, particularly those involved in disease resistance or tolerance in ginger. Ginger was able to express GUS for a short period successfully. Through the use of a gene gun, the embryogenic callus was subjected to a bombardment of plasmid vector pAHC 25 carries the BAR (phosphinothricin acetyl transferase) and GUS (-glucuronidase) genes were utilized [68]. Standardizing the transformation technique in ginger required using EHA105/p35SGUSInt Agrobacterium tumefaciens strain, which successfully expressed the activity of β-glucuronidase. This was validated by the histochemical GUS test and polymerase chain reaction [69]. Young rhizomes of ginger were analyzed, and their transcripts of the (S)-α-bisabolene synthase gene were discovered. The cDNA had the ability to encode a protein and was given the name ZoTps1. This hypothetical protein had 550 amino acid residues and shared 49–53% identity with the sesquiterpene synthases isolated from Zingiber [70].

5.5. Isolating Resistance and Important Agronomic Candidate Genes

In the event of the lack of sexual reproduction, utilizing genes from alternative sources alongside comparisons of heterologous genomes provides us with the requisite clues for labeling. Sequence information in databases can aid in identifying candidate genes that control pathogenesis. The utilization of degenerate primers and functional genomics presents an efficient methodology for enhancing the quality of ginger. The utilization of data obtained from the Arabidopsis genome, presented by Laurent et al. [71], may be applied towards identifying and isolating genes associated with resistance in ginger.

Aswati Nair and Thomas [72] conducted a study wherein they utilized degenerate primers based on conserved motifs from the NBS (Nucleotide Binding Site) domains of plant resistance to isolate, characterize, and express resistance gene candidates (RGCs) from both wild and cultivated Zingiber

species. Additionally, an assessment was conducted on primer sets specific to resistance genes (R-genes) and the characterization of resistance gene clusters (RGCs) in ginger. Four R-genes cloned were targeted by the design of 14 oligonucleotide primers specific to their conserved regions. The clones obtained from 17 amplicons produced by 12 primers that yielded successful results were subjected to sequence characterization. The clones obtained using three primers exhibited significant homology to cloned R-genes or RGCs from other plant species. Additionally, these clones contained conserved motifs that are characteristic of the non-TIR (Toll Interleukin1 Receptor) subclass of the NBS-LRR (Nucleotide Binding Site-Leucine-Rich Repeat) R-gene superfamily. The ginger RGCs were segregated into two distinct subclasses based on phylogenetic analysis, corresponding to clades 3 and 4 of non-TIR-NBS sequences found in plants. The present research establishes a foundation for further RGC extraction and offers significant perspectives into the phylogenetic and characteristic similarities of non-TIR-NBS-LR R-gene subclass in the plant's genome. Priya and Subramanian [73] isolated and conducted a molecular analysis of the R-gene presents in ginger varieties that exhibit resistance to *F. oxysporum*. The presence of the R-gene was noted exclusively in ginger varieties that exhibit resistance. The cloned R-genes represent a novel pool of molecular markers that can be utilized for marker-assisted selection (MAS) and easy detection of varieties resistant to Fusarium yellow.

According to Kavitha and Thomas [74], *Z. zerumbet*, which is closely related to ginger, has been identified as a promising candidate for conferring resistance to *P. aphanidermatum* in ginger. The researchers utilized mRNA and AFLP markers differential display methodology to detect genes that exhibited changes in expression levels in a *Z. zerumbet* accession that displayed resistance pre- and post-inoculation. Several transcript-derived fragments (TDFs) exhibiting differential expression were isolated, cloned, and subjected to sequencing. The clones were subjected to homology searches and functional categorization, which led to the identification of defense/stress/signaling groups. These groups were found to be homologous to genes that have been previously reported to play a crucial role in pathogenesis-related functions in other plants. Kavitha and Thomas [74] discovered that *Z. zerumbet* exhibited adequate variability in both its DNA composition and its reaction to Pythium. Aswati Nair et al. [72] observed a constituent member of the gene family encoding pathogenesis-related protein group 5 (PR5) in *Z. zerumbet*. The gene was found to be expressed repeatedly, but not upregulated in reaction to *P. aphanidermatum* infection. The extraction of R-genes from closely related genera can facilitate ginger enhancement through transgenic methodologies. In response to the barley stripe mosaic virus discovered to infect two species within the zingiberaceae family, a transient knockdown of gene expression was accomplished in culinary ginger, specipically targeting phytoene desaturase. According to Renner et al. [75], the findings indicate the possibility of utilizing BSMV-VIGS on monocots in addition to cereals, thereby enabling targeted genetic investigations of numerous significant crop species in tropical and temperate regions.

The Initial discovery, characterization, and cloning of a mannose-binding lectin from ginger rhizomes was documented by Chen et al. [76]. The study reports the successful cloning of the complete cDNA sequence (746 bp) of *Zingiber officinale* agglutinin (ZOA) via the rapid amplification of cDNA ends (RACE) method. The cloned sequence encompasses an open reading frame (ORF) of 510 bp, which encodes a precursor lectin consisting of 169 amino acids and a signal peptide. ZOA exhibits three characteristic mannose-binding sites. The results of semi-quantitative RT-PCR analysis indicated that ZOA was present in all the examined tissues of *Z. officinale*, such as rhizome, root, and leaf, indicating its constitutive expression.

Yua et al. [77] analyzed the functional and isolation characteristics of β-eudesmol synthase

derived from *Z. zerumbet* Smith. In the process, a novel sesquiterpene synthase gene (ZSS2) was identified from *Zingiber zerumbet* Smith. The functional expression of ZSS2 in *Escherichia coli* and subsequent in vitro enzyme assay demonstrated that the enzyme encoded by ZSS2 facilitated the production of β-eudesmol along with five other by-products. The seasonal variations in ZSS2 transcript accumulation in rhizomes were found to be significant through quantitative RT-PCR analysis. The researchers incorporated a gene cluster that encodes six enzymes of the mevalonate pathway into *E. coli* and co-expressed it with ZSS2. This was done to verify the activity of ZSS2 and evaluate the feasibility of metabolic engineering for the production of β-eudesmol. Upon addition of mevalonate, the genetically modified *Escherichia coli* exhibited a comparable sesquiterpene profile to that observed in the in vivo enzyme assay, resulting in the production of 100 mg/l of β-eudesmol.

The characterization and molecular cloning of violaxanthin de-epoxidase (VDE) in ginger was reported by Huang et al. [78]. This study reported the successful cloning of a violaxanthin de-epoxidase (GVDE) cDNA from ginger, spanning a length of 2000 base pairs. The cloning process involved the use of RT-PCR and Rapid amplification of cDNA ends (RACE) techniques. The obtained cDNA sequence was assigned the accession number AY876286. The study aimed to characterize the expression patterns of GVDE in response to light. The ORF of GVDE spans 1431 base pairs, and the anticipated polypeptide comprises 476 amino acids, with a molecular weight of 53.7 kilodaltons. The expression pattern of GVDE was predominantly observed in leaves as determined by Northern blot analysis. There was an increase in the mRNA level of GVDE following an extended period of illumination under high lighting conditions. The GVDE function was ascertained by the integration of its antisense sequence into tobacco plants through the EHA105 methodology. The integration of antisense GVDE in the tobacco genome was confirmed through PCR-Southern blot analysis. The results of chlorophyll fluorescence measurements indicated that the transgenic plants exhibited maximum efficiency of PSII photochemistry (Fv/Fm) and reduced levels of non-photochemical quenching (NPQ) compared to the untransformed controls when exposed to high light conditions. The findings suggest that the suppression of GVDE occurred in antisense T-VDE tobacco, as evidenced by the lower size and ratio of the xanthophyll cycle pigment pool compared to the control. The findings indicate that VDE plays a significant role in mitigating photoinhibition.

5.6. Micropropagation of ginger

The vegetative propagation of plants such as ginger poses a significant risk of systemic infection from the propagules. The significant reduction in ginger yield can be attributed to the prevalence of bacterial wilt, soft rot, and rhizome rot diseases. Consequently, the transmission of diseases primarily occurs through rhizome propagules, thus emphasizing the need for cultivating clones free from disease. Employing a tissue culture technique for micropropagation may serve as a viable approach. The micropropagation process in ginger is employed to enhance the quality of different varieties by utilizing tissue culture methods to efficiently propagate and identify favorable genetic variations in ginger plants. The method techniques include the generation of a substantial quantity of genetically identical plants originating from specialized fragments of plant tissue. The utilization of this method proves to be highly advantageous in the propagation of plants that pose challenges when propagated through conventional methods. Through micropropagation, it becomes possible to facilitate the advancement of ginger cultivars that exhibit desirable characteristics, including enhanced productivity, resistance against diseases, superior quality, and the ability to thrive in diverse environment [79]. The

propagation of ginger through shoot multiplication has been documented to achieve clonal multiplication. Clonal propagation can be accomplished via direct or indirect organogenesis, as reported by Seran [80]. The proper establishment of explants is a crucial factor in micropropagation. Typically, the explants employed in this context comprise rhizome buds, leaves, internodes, and roots. The utilization of adventitious buds has been extensively employed. The preformed primordia within the buds facilitate direct organogenesis. The buds are a source of nutrients that facilitate the growth and development of the shoots. During indirect organogenesis, the explant undergoes a callus phase and subsequently undergoes dedifferentiation to form plantlets. Based on the findings of El-Nabarawya et al. [81], the utilization of callus culture has been suggested to achieve rapid proliferation of plant cells. Contamination is a crucial factor in the establishment process. To attain cultures free from contamination, the buds were subjected to a rigorous washing process under running water and subsequent washing using the detergent Tween 20 under continuous running water. The buds, free from dust and soil, undergo a process of surface sterilization using substances such as mercuric chloride and ethanol. This is followed by a washing step with distilled water, after which they are inoculated. The shoots undergo a series of steps in forming multiple shoots, which require a specialized medium for their proliferation. According to Shivakumar and Agrawal [82], a low concentration of auxins and cytokinins, specifically 2 mg/L NAA and 0.1 mg/l BAP, respectively, were utilized in the multiplication stage. Afterwards, the elongation phase commenced, during which gibberellic acid was applied. Nevertheless, as this particular stage was not deemed mandatory, the sprouts are subsequently transferred to a rooting substrate. Several shoots were transferred into an MS rooting medium that was supplemented with 2 mg/L of NAA and 0.1 mg/L of BAP. Several other scholars have attempted to utilize varying concentrations of growth enhancers. Following the rooting process, the shoots were predominantly acclimatized via transplant into netted pots containing a sterilized peat mixture. The pots were subsequently placed within a growth chamber, where a humidity level of 80% and a light period of 16 ± 8 hours were maintained. After two weeks, the plants were transferred to a greenhouse for secondary acclimatization in netted pots, as described by Shivakumar and Agrawal [82].

Somatic embryogenesis has been successfully achieved in certain species to generate a large number of seeds. Somatic embryogenesis is a more effective method of generating somatic seeds compared to organogenesis. In addition, somatic embryogenesis is the preferred method for plant genetic improvement through in vitro culture and genetic transformation. This is because plants generated from single cells, such as somatic embryos, are easier to regulate. Typically, somatic embryogenesis and meristem-generated plants exhibit true-to-type characteristics genetically identical to the mother plants [83]. However, variations may occur depending on the specific traits of the plant species. The utilization of leaf explants to study somatic hybridization has been investigated in ginger. According to Nery et al. [83], the maintenance of the embryogenic callus was achieved through MS media, where dicamba was identified as the most effective growth regulator. Pathogen-free seedlings were produced using meristematic explants in somatic embryogenic studies [84]. This methodology has the potential to be modified to generate numerous additional hybrid variations in cases where the cultivars exhibit suboptimal yields concerning both the quantity and quality of their rhizomes.

6. Conclusions

The inefficacy of conventional breeding in ginger is attributed to pollen sterility and the absence of seed set. The creation of polyploidy lines and utilization of in vitro techniques for pollination and

embryo rescue present promising avenues for advancing ginger breeding. These approaches aim to enhance pollen fertility and expand the possibilities for genetic improvement. The identification and selection of advantageous natural and induced mutants are expected to significantly contribute to the enhancement of ginger crops until alternative methods become available. Biotechnological methods, such as somaclonal variation, in vitro selection and mutation, and the creation of disease-resistant transgenic organisms, can serve as complementary strategies to achieve this objective. Due to the relatively low multiplication rate observed in conventional propagation techniques, agronomists have turned to micropropagation technology to ensure a continuous supply of healthy plant propagules suitable for commercial use. Concerning tissue culture of ginger, there have been documented instances of successful micropropagation originating from rhizome buds. The optimal size of sterilized explants and the selection of appropriate culture media are crucial factors for initiating in vitro cultures. The utilization of MS basal media, along with the incorporation of cytokinin (such as kinetin or BAP) either individually or in combination with auxin (such as IBA, IAA, or NAA), is a widely practiced technique for the production of in vitro plantlets in the cultivation of ginger. The shoot multiplication process is primarily influenced by the specific concentration and type of cytokinins nutrient medium. In ginger, the process of microrhizome rooting typically occurs naturally. However, one may consider utilizing auxins such as IBA, IAA, or NAA to promote root formation. Many research has reported successful acclimatization and survival of in vitro ginger plantlets in field conditions. This technique shows promising potential for expediting the clonal propagation of robust ginger plants within a condensed timeframe, thereby facilitating their cultivation on a larger scale for commercial purposes. The process of molecular characterization of germplasm is expected to result in the identification of duplicate entries within the germplasm and facilitate the creation of core collections with a reduced number of entries. The integration of diagnostic markers with metabolic profiling has the potential to facilitate the preservation of genetic purity and the detection of contaminants in this species of significant medicinal value. Furthermore, this practice aids in the preservation of genetic integrity within the cultivated specimens. Identifying and tagging of genes of interest through a genomics approach is crucial for the effective utilizing of marker technology. The utilization of genomic databases and information obtained from conserved regions has the potential to serve as putative markers. Additionally, this information can be employed to authenticate and enhance the specificity of markers for particular traits in the intended crop. This practice also facilitates the identifying and isolating desirable genetic variants, particularly in ginger, a plant species known for its numerous pharmacological properties.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors certify that they do not have any competing interests to declare.

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