

HETEROSIS AND MUTATION BREEDING APPROACHES FOR GENETIC IMPROVEMENT OF CUCURBITACEOUS VEGETABLE CROPS

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ABSTRACT

Cucurbitaceous vegetable crops constitute an important group of horticultural species widely cultivated for their nutritional, economic, and medicinal value. Enhancing productivity, quality, and resilience to biotic and abiotic stresses in these crops remains a major objective of plant breeding programs. Heterosis (hybrid vigor) has been extensively exploited in cucurbits to improve traits such as fruit yield, size, earliness, uniformity, and resistance to diseases and environmental stresses. Development of high-performing hybrids through intra-specific and inter-specific hybridization has significantly contributed to commercial vegetable production worldwide. Mutation breeding represents another powerful approach for generating novel genetic variability through the use of physical and chemical mutagens such as gamma rays and ethyl methane sulfonate (EMS). Induced mutations have facilitated the development of improved genotypes possessing desirable traits including dwarf growth habit, altered sex expression, improved fruit characteristics, and enhanced tolerance to stresses. Recent advancements in molecular breeding and genome editing tools such as CRISPR/Cas9 have further increased the precision and efficiency of mutation breeding, enabling targeted modification of genes controlling economically important traits. The integration of heterosis breeding with induced mutagenesis and modern genomic tools offers significant potential for accelerating genetic improvement in cucurbitaceous vegetable crops. These combined approaches can contribute to the development of high-yielding, climate-resilient, and nutritionally improved cultivars, thereby supporting sustainable vegetable production and food security.

KEYWORDS: Cucurbitaceae, Heterosis, Hybrid breeding, Mutation breeding, Genetic variability, EMS, Gamma rays, CRISPR/Cas9, Yield improvement, Disease resistance, Vegetable breeding.

INTRODUCTION

The Cucurbitaceae family is regarded as the second most important vegetable crop family after Solanaceae, encompassing a wide range of economically valuable edible species cultivated across tropical and subtropical regions of the world (1,2). There are about 115 genera and 960 species in the family, most of which are herbaceous annual vines or perennial lianas, frequently with tendrils. They are mostly found in tropical and subtropical regions, with very few found in temperate ones. They can be either monoecious or dioecious (sometimes hermaphrodite). Bicollateral vascular bundles, which have phloem on both the inner and outer sides of the xylem, are a distinctive property of the Cucurbitaceae (3). According to cucurbitacin, the primary ingredient responsible for the bitter flavor, is commonly found in cucurbits (4). The family Cucurbitaceae contains a variety of vegetables or fruit crops, which are of great significance to the global or local economy.

Breeding efforts for cucurbits have been transformed by the use of heterosis, or hybrid vigor, which has made it possible to create high-performing hybrid varieties with improved yield potential, disease resistance, and fruit quality attributes. The phenomenon known as heterosis occurs when the hybrid offspring show characteristics that are superior to those of their parents, which helps them be more productive and adaptable in a variety of agroecological settings. Complementary alleles, dominance effects, overdominance, and epistatic interactions are all complex aspects of the genetic underpinning of heterosis that work together to give hybrids their higher performance. Utilizing heterosis in cucurbitaceous vegetables through breeding involves a variety of methods, such as creating homozygous inbred lines, locating and taking use of heterotic groups, and incorporating molecular markers for hybrid prediction and selection. By utilizing the genetic diversity seen in cucurbit germplasm pools, these tactics hope to promote the creation of hybrid combinations with desired characteristics (5). Utilizing heterosis in cucurbitaceous vegetable crops has many practical benefits, including increased yield potential, better fruit quality features, increased resistance to disease, and longer shelf life, all of which help farmers

and consumers satisfy their needs. The preservation of parental purity, the control of heterotic groups, and the preservation of genetic variation within breeding populations are some of the issues that must be resolved for heterosis breeding programs to be implemented successfully.

The creation of new mutant alleles is the essential and distinctive characteristic of mutation breeding. Mutation breeding is the process of using induced mutations to improve crops. Induced mutagenesis was extensively studied in the US, Europe, Japan, and China in the 1950s. A significant effort on crop plant mutagenesis was started in India by Swaminathan and his group at the Indian Agricultural Research Institute in New Delhi. In addition to changing linkage groups, mutations can change alleles at known and unknown loci alike, causing both qualitative and quantitative variation rather quickly (6). Direct mutants can be obtained using induced mutagenesis, and these mutants can then be used in hybridization to break through yield plateaus and provide desired horticultural features (7).

Heterosis breeding in cucurbitaceous vegetable

The edible fruits and culinary diversity of cucurbitaceous vegetable crops, such as cucumber (*Cucumis sativus*), melon (*Cucumis melo*), squash (*Cucurbita pepo*) and pumpkin (*Cucurbita maxima*), make them commercially significant crops that are farmed all over the world (8). However, improving these crops' quality, production, and resistance to biotic and abiotic stressors continues to be a top priority for agricultural scientists and breeders. By utilizing genetic variety and fusing the skills of parental lines, heterosis, or hybrid vigor, offers a viable way to accomplish these goals and produce superior hybrid varieties with improved performance and market appeal.

By using heterosis in cucurbitaceous vegetable crops, high-yielding hybrid cultivars with enhanced fruit quality, shelf life, and disease and insect resistance have been created. Cytoplasmic male sterility (CMS) and double haploid (DH) technologies are two examples of hybrid seed production techniques that have made it easier to commercially produce and distribute hybrid seeds to growers, increasing agricultural productivity and profitability. The use of heterosis in breeding programs is still limited, though, by issues including heterosis loss, inbreeding depression.

Cucumber

Studies on heterosis in melon hybrids revealed promising results evaluated 18 crosses and identified significant heterosis in combinations like LC- × K-75 and LC-2-2 × Poinsette (9). Specifically, LC- × K-75 showed 104.93% heterosis over the better parent and 52.43% over the standard check. LC-2-2 × Poinsette exhibited 67.50% residual heterobeltiosis and 24.47% economic heterosis. Two cross combinations, LC-1-1 × K75 and LC-2-2 × Poinsette, performed well in both F1 and F2 generations for yield and yield-contributing traits.

In another study, Kaur et al. (2022) assessed heterosis in 45 crosses of musk melon and found that hybrids KP4HM-15×MM Sel-103 and KP4HM15×MM-1831 recorded high standard heterosis for fruit yield and TSS. Additionally, landrace-derived inbred lines Kajri, MM Sel-103, and KP4HM-15 produced hybrids with moderate to high resistance to FW. These findings can be useful for developing high-yielding and disease-resistant melon varieties (10).

Bitter gourd

Several studies have reported significant heterosis for yield and yield-related traits in bitter gourd found notable heterotic effects in traits such as node number, fruit length, and yield. The highest negative standard heterosis was observed in S-54 × Pusa Aushadhi (-51.34%) for node number of 1st pistillate flower, while S-2 × Pusa Aushadhi showed a 68.33% increase in yield plant⁻¹ (11). Heterosis has also been reported for quality traits such as flesh thickness, vitamin C, and TSS. Found significant heterobeltiosis in Phule Green Gold × Pusa Do Mausami for flesh thickness (26.87%) and vitamin C (24.75%) (12). Thusi × Pusa Do Mausami exhibited high heterobeltiosis for fruit yield vine⁻¹ (98.75%). Studied heterosis and combining ability in 45 F1 hybrids and reported significant positive heterobeltiosis in traits such as yield vine⁻¹, fruit length, and fruit weight. IC-85605 × IC45346 and MDU-1 × IC-85605 exhibited high heterobeltiosis for yield vine⁻¹, with gains of 43.85% and 41.38%, respectively (13).

Bottle gourd

Heterosis for yield and yield-related traits has been reported in several studies. Evaluated 21 cross combinations and found significant positive better parent heterosis in 17 crosses, ranging from 6.27% to 49.72%. The hybrid P3 × P4 exhibited the highest significant positive better parent heterosis (49.72%), followed by P3 × P5 (44.97%) and P4 × P5 (39.47%). Significant heterosis for fruit yield per vine has also been reported (14). Found that cross combinations ABGS 11-23 × DBG 5, ABG 1 × Arka Bahar, and DBG 6 × DBG 5 showed highly positive significant heterosis over the standard check (15). In another study, heterosis in 36 F1 hybrids was investigated by fruit yield per vine ranged from -42.02% to 47.92% over the better parent and -44.85% to 54.74% over the standard parent Pusa Naveen. The top heterotic F1 hybrids for fruit yield were P7 × P9 (54.74%), P1 × P7 (47.43%), and P3 × P7 (46.55%), which also exhibited high estimates of significant heterosis for number of fruits per vine (16).

Heterosis has also been reported for earliness and vegetative traits. found significant differences among genotypes for all characters, with the hybrid Pusa Naveen × Punjab Komal showing significantly higher negative standard heterosis for earliness, while the hybrid KBG-16 × Pusa Samridhi exhibited highly significant heterosis for vegetative traits (17).

Ridge gourd

To investigated eight different parental line to developed 28 F1 hybrids of ridge gourd through diallel method of analysis (excluding reciprocals) to assess the heterosis for earliness and vegetative characters. They resulted the appreciable heterosis in desirable direction was found over better parent and check parent for the characters viz. days to first female flower, node number to first female flower, vine length (m), number of primary branches and days taken to first fruit harvesting. Crosses PCPGR 7256 × PRG 142, PRG 117 × PRG 142, PRG 117 × PRG 131, PRG 117 × PRG 132 and PRG 117 × PRG 120 were found promising for earliness. Crosses PCPGR 7256 × PRG 117, PCPGR 7256 × PRG 131 and PRG 132 × PRG 120 were recorded promising for vegetative traits (18).

Sets of 28 F1s crosses developed by using 8 diverse parents of ridge gourd by through half diallel analysis method to estimated heterosis for growth and yield of ridge gourd and resulted, the analysis of variance indicated highly significant differences for growth and yields characters, which suggesting presence of wide genetic variability. Which provided the opportunity to exploit heterosis in ridge gourd; the positive heterosis exhibited for most of growth and yield characters viz., vine length, number of leaves, fruit yield per vine and fruit yield per hectare. The present study showed that the crosses DMRG-36 × DMRG-25, DMRG-25 × Arka Sumeet and DMRG-25 × DMRG-22 exhibited high heterosis for yield characters (19).

Pumpkins

A study on standard heterosis in pumpkin evaluated 15 diverse genotypes and their 36 F1 hybrids, revealing promising results for earliness and yield contributing characters (20). The parents Kasi Harit, Vadhalagundu Local, and CO-2 were identified as top performers for earliness and yield traits. Notably, the hybrids Kasi Harit x Avinashi Local and Vadhalagundu Local x CO-2 exhibited significant standard heterosis for earliness and yield characters, making them potential candidates for improving pumpkin productivity. These findings highlight the potential of heterosis breeding for developing high-yielding and early-maturing pumpkin varieties.

Melon

A study by evaluated the extent of heterosis in 15 intra-specific hybrids of muskmelon, developed from four selected genotypes and seven Mangalore melons. The results showed that 11 hybrids exhibited significant positive heterosis over the mid-parent, while four hybrids outperformed the better parent for fruit yield per vine. Notably, the hybrid Hara Madhu × MS 78 showed the highest heterosis over both mid-parent (53.20%) and better parent (42.02%), followed by Hara Madhu × MS 28 and Hara Madhu × MS 30. Additionally, specific hybrids exhibited superior performance for various traits, such as Arka Siri × MS 79 for fruit length, fruit diameter, and shelf life, and Arka Siri × MS 17 for flesh thickness, vine length, and TSS content (22).

Another study by evaluated heterosis for fruit, yield, and quality parameters in 15 hybrids of muskmelon. The analysis of variance revealed significant variation for most characters, and four crosses (C10, C11, C14, and C15) showed superior performance for yield and quality traits. These findings highlight the potential of heterosis breeding for improving muskmelon productivity and quality (23).

Mutation breeding

Mutation breeding, which uses physical, chemical, or biological agents to intentionally induce genetic differences, is a potent and adaptable method of crop improvement. Breeders can create cultivars with higher yield, better quality, greater resilience to biotic and abiotic stressors, and improved adaptability to changing environments by creating new mutations that unlock novel features and increase the genetic diversity accessible for selection. In addition to changing linkage groups, mutations can change alleles at known and unknown loci alike, causing both qualitative and quantitative variation rather quickly (6). Direct mutants can be obtained using induced mutagenesis, and these mutants can then be employed in hybridization (7).

History of vegetable mutation

Mutation breeding, which uses physical, chemical, or biological agents to intentionally induce genetic differences, is a potent and adaptable method of crop improvement. Breeders can create cultivars with higher yield, better quality, greater resilience to biotic and abiotic stressors, and improved adaptability to changing environments by creating new mutations that unlock novel features and increase the genetic diversity accessible for selection. In addition to changing linkage groups, mutations can change alleles at known and unknown loci alike, causing both qualitative and quantitative variation rather quickly (6). Direct mutants can be obtained using induced mutagenesis, and these mutants can then be employed in hybridization (7). A crucial factor in any mutagenesis program is the amount of mutagen used. An indication of the ideal dose of the mutagens is provided by the lethal dose-50 (LD50). The maximum mutations are produced with the fewest risks at the optimal dose. While a modest amount of mutagens will result in a too-low mutation spectrum and frequency, an overdose will kill too many plants. The concentration, length of treatment, and treatment temperature all have a major impact on the mutagenic dose. The modifying parameters for the mutagenic effect include pre-soaking, solution pH, metallic ions, carrier agents, post-washing, post-drying, and seed storage. Three different kinds of impacts are caused by physical and chemical mutagens: chromosomal abnormalities, gene mutations, and physiological harm.

Physiological consequences are often limited to the M1 generation, however gene and chromosomal alterations can be passed down from the M1 to subsequent generations. Errors in DNA replication lead to spontaneous gene mutations. The majority of these mistakes are fixed, but some might be carried over to the next cell cycle and manifest in plant progeny. Since the early 1900s, ionizing radiation has been used to artificially induce mutations. However, it took a long time to prove that these kinds of alterations could be advantageous for plant breeding. Because of this, crop improvement through conventional mutagenesis is now widely established, and novel radiation treatment techniques and chemical agents with mutagenic qualities are therefore useful instruments for increasing crop genetic variation in order to get over bottleneck circumstances.

The process of treating plant propagules (seed, cutting, or plant tissue) with chemical or physical mutagens is known as mutation induction. Selection for appealing (wanted) alterations in the resulting mutants comes next. Mutation induction is a technique used by plant breeders to increase the genetic basis of germplasm and employ the mutant lines directly as new varieties or sources of new diversity. According to mutation breeding has emerged as a fantastic method for producing novel plant genotypes. Selecting individuals from a sizable altered population who satisfy specific selection criteria is known as mutation screening, and mutant affirmation is reevaluated (24,25).

Mutation breeding in cucurbits

In plant breeding, mutagenesis enables the screening and selection of mutant genotypes with enhanced characteristics. When crops have limited genetic resources, this approach may be employed. Individual target genes can be silenced or interrupted to investigate gene function that connects gene variation sequences of particular symptoms (24). Superior features originate as a result of X-rays, biological processes, physical activities, chemicals, or the employment of agents that cause mutations (26). Commonly utilized are protons, γ -, α -, or β -particles, neutrons, and the chemicals sodium azide, hydrogen fluoride, and ethyl methane sulfonate (EMS), which cause mutations. Through EMS-mediated mutagenesis, a *C. sativus* mutant was created (27). This resulted in a detectable mutant phenotype with few spines and long or short, pale green fruit, increasing phenotypic variability. Cucurbitaceae mutants created with EMS also affect *C. pepo* plant vigor, fruit set, and development (28); *C. sativus* short fruit and yellow-green fruit peel (29); and *C. lanatus* seed coat color and changes in the location of fruit set in the central stem (30). Advances in molecular genetics have enabled the identification of several genes associated with important agronomic traits in cucurbits, including genes regulating leaf development, flower differentiation, fruit morphology, and stress tolerance (Fig. 1) (31). These gene-based insights provide valuable information for functional genomics studies and assist breeders in developing improved cultivars through mutation breeding.

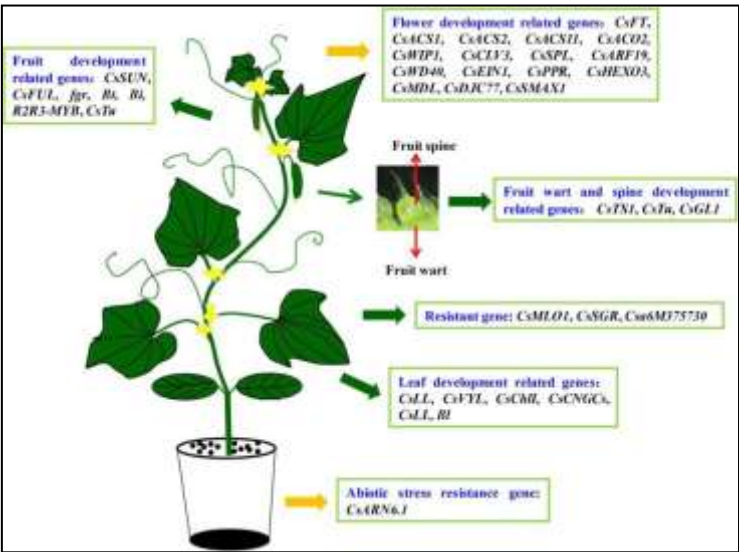


Fig. 1. Illustration of the agronomic traits-related genes in cucumber. Genes regulating leaf development, flower differentiation, optimal fruit production and stress resistance (31)

In order to identify plants that have a specific modified gene based on nucleotide sequence data, the development of mutant-based reverse genetics offers a method to produce novel mutant alleles at a chosen locus. To find mutations of vegetative and reproductive features, such as plant vigor, leaf size and shape, sex expression, sex determination, and fruit set and development, a sophisticated technique called Targeting Induced Local Lesions in Genomes (TILLinG) combined with EMS was used (28). Using direct or reverse-genetic methods, these results provide insights into the possible applications of mutants found in novel alleles for functional genomics and cucurbita breeding (28). The TILLinG mutation causes *C. melo* to develop recessive resistance to strains of the Cucumber mosaic virus (CMV) (32). Genome editing techniques like clustered regularly interspaced short palindromic repeat (CRISPR) and associated protein9 (Cas9) can be used to generate agronomically significant features in plants (33,34). By enabling the introduction of clear, predictable changes as well as precise incisions, mutations, and substitutions in plants, genome editing capabilities enhance breeding (35). The ability to modify multiple features at once makes the CRISPR/Cas9 system beneficial (36,37). Base-pairing of designed single-guide RNAs (sgRNAs) to target DNA locations is necessary for targeted mutations caused by CRISPR/Cas9 (38). Melatonin levels in *C. lanatus* and other Cucurbitaceae crops with improved disease resistance against plant diseases can be raised by CRISPR/Cas9 genome editing, which can also act as a natural source of antioxidant compounds (39). By focusing on the Phytoene desaturase gene (CIPDS), the sgRNA guided genome editing system employing type II CRISPR/Cas9 has been shown to produce knockout mutations in *C. lanatus* (40). The CIPDS gene is a crucial enzyme in the production of carotenoid and is necessary for the biosynthesis of chlorophyll (40). Through *Agrobacterium*-mediated transformation, the CRISPR/Cas9 technique can provide virus resistance in nontransgenic *C. sativus* (41). Molecular mapping of important biochemical compounds and quality traits in cucurbitaceous crops has identified several quantitative trait loci (QTLs) associated with carotenoids, β -carotene, α -carotene, lutein, and sugar accumulation (Table 1) (42–44). These mapped genomic regions provide valuable targets for genome editing approaches aimed at improving nutritional quality and functional traits in cucurbits.

Table 1. Molecular mapping of compounds improvement in cucurbits crops

Compound	Plant species	Gene/QTL ID	Genetic position (cM)	Reference
Carotenoid	<i>C. melo</i>	CARQU9.1	64.32	(42)
β -carotene	<i>C. maxima</i>	q β cr2.1	123.0–127.6 222.9	

	<i>C. moschata</i>	qβcr4.1 qβcr14.1 qαcr20-a	144.8 4.69–6.152	(43, 44)
α-Carotene	<i>C. maxima</i>	qαcr2.1 qαcr4.1	120.3–139.5 222.9	(43)
Lutein	<i>C. moschata</i>	qlut20-a	1.17–2.54	(44)
Sugar	<i>C. melo</i> <i>C. lanatus</i>	SSCQU8.3 GCE1.1 1	102.66 4.01	(4,42)

After being exposed to gamma (g) rays, the bitter gourd landrace MC 013 gave rise to the new cultivar MDU 1, which has improved yield, long greenish white fruits, and resistance to leaf spot diseases, pumpkin insects, and fruit flies (45). Additionally, two gynoeccious lines of bitter gourd (*Momordica charantia* L.) known as DBGy-201 and DBGy-202 were isolated from their natural source due to spontaneous mutations (46). Mutation breeding has generally been shown to be a successful method for increasing agricultural trait diversity and producing novel plant genotypes (47,48). In light of these results, the main goal of the current study was to maximize the mutagenic dosages of EMS and gamma rays in bitter gourds. Recent advances in molecular genetics have facilitated the identification of genes and quantitative trait loci (QTLs) associated with important quality and yield traits such as fruit weight, fruit length, fruit shape, sex expression, and ovary length in cucurbit crops (Table 2) (42,49–53). Identification of these genomic regions provides valuable targets for mutation breeding and molecular breeding approaches aimed at improving yield and quality traits in cucurbitaceous vegetables. Based on these findings, optimization of mutagenic treatments such as EMS and gamma rays remains an important objective for enhancing genetic variability and developing improved bitter gourd genotypes.

Table 2. Molecular mapping of quality traits improvement in cucurbits crops

Trait	Plant species	Gene/QTL ID	Genetic position (cM)	Referennce
Fruit weight	<i>C. melo</i>	FWQU5.1	40.51	(42)
Fruit weight	<i>C. lanatus</i>	FW8.1 8	18.51	(4)
Sex and earliness	<i>M. charantia</i>	gy-1	3.04	(49)
Femaleness	<i>C. sativus</i>	sg3.1 sg1.1	10.17–15.73 3.01–9.30	(50)
Fruit shape	<i>C. pepo</i>	MFSH_3 MFSH_4	171.0 39.4	(51)
Fruit length	<i>C. pepo</i>	MFLc_3	169.1	(51)
Fruit length	<i>C. sativus</i>	fl7.1	19.091–28.242	(52)
Ovary length	<i>C. sativus</i>	ovl1.1	10.42–12.09	(53)

The selection of an effective and efficient mutagen is crucial for producing a high frequency of desirable mutations in any mutation breeding operation. Induce diversity in Chinese Long cultivars of cucumbers by administering varying dosages of ethyl methyl sulfonate (EMS). In every experiment, germination rate, index, and percent declined as EMS concentration increased (54).

Targeted mutations in the *CIWIP1* gene (Cla008537) were employed to develop gynoeccious watermelon lines using the CRISPR/Cas9 system. Specifically, the target sequences CTACTACTGGACTTAATAAGG and CCTTTGGACATGGCCATGCAGC, corresponding to the ATG and TGA regions, respectively, were targeted for genome editing (55). In addition, CRISPR/Cas9-mediated mutation of the *CIBG1* gene was used to develop transgenic watermelon plants with reduced seed size. The target sequences GAGGAAGATTCGCCACCGTTGG and CCGAGCGGAATTTGTTTTCGGAA were used for targeted modification of *CIBG1*, a gene associated with seed-size regulation (56).

The snake gourd, *Trichosanthes anguina*, showed the highest percentage of seed germination (93.33) and plant survival (86.66%) at 100 Gy, with 200 Gy being the LD50 dose. It was discovered that the lower dosages (100 Gy and 150 Gy) were more efficient and successful, producing mutants with desired characteristics such as shorter vine length, larger fruit

diameter, and short fruit (57). It has also been discovered that EMS therapy enhances pumpkin fruit output and plant growth. According to a study the best treatment for increasing plant height, flowering age, and fruit weight was 0.70% EMS concentration (58). The ideal plant height (185.98 cm), flowering age (28.26 days), fruit weight per sample (0.881 kg), and fruit weight per plot (1.727 kg) were all achieved with this treatment.

CONCLUSION

Heterosis breeding has proven to be an effective strategy for improving yield potential, fruit quality, earliness, and resistance to various biotic and abiotic stresses in cucurbitaceous vegetable crops. Exploitation of hybrid vigor has enabled the development of superior hybrids with enhanced productivity and adaptability across diverse agro-ecological conditions. At the same time, mutation breeding has played an important role in broadening the genetic base of cucurbits by generating novel allelic variations that are not readily available in natural populations. Induced mutations using physical mutagens such as gamma rays and chemical mutagens such as EMS have successfully produced improved genotypes with desirable horticultural traits including improved plant architecture, fruit characteristics, and stress tolerance. Recent advancements in molecular biology and genome editing technologies, particularly CRISPR/Cas9, have further enhanced the scope of mutation breeding by enabling precise and targeted genetic modifications. Integration of conventional heterosis breeding with induced mutagenesis and modern genomic tools provides new opportunities for accelerating genetic improvement in cucurbits. Future research should focus on combining classical breeding approaches with molecular techniques to develop high-yielding, stress-tolerant, and nutritionally superior cultivars suited to changing climatic conditions. These integrated breeding strategies will play a crucial role in ensuring sustainable vegetable production and global food security.

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Author Contribution Information

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Consent for publication

All authors consent the publication of the current article.

Competing interests

The authors declare no competing interests.

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