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REVIEW ARTICLE

Bitter Gourd: Breeding and Genomics

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Abstract

Bitter gourd (*Momordica charantia* L.) is known tropical cucurbitaceous vegetable for its nutritional, medicinal and curative properties. The immature fruit is valued for its bitter taste and richness in calcium, phosphorous, iron, copper, potassium and vitamins A, B1, B2 and C. The genus *Momordica* consist of 60 species worldwide and out of them 7 species (*Momordica charantia, M. balsamina*, *M. dioica*, *M. cochinchinensis*, *M. tuberosa*, *M. subangulata M. macrophylla*) are found in Indian sub-continent which expressed large variability with respect to fruit shape, size, coulour due to varied edaphic and climatic conditions. Breeding efforts for emergence of early pistillate flower at earlier nodes, high female to male sex ratio, earliness, fruit colour, firm fruit with narrow seed cavity, less seed development, desirable fruit shape, size, non-ridge fruits, thick flesh, thick flesh, resistant to leaf mosaic and fruit fly and suitability for export, canning and dehydration. Considering the above traits, several varieties and hybrids in different segments has been developed by public and private seed companies. Due to monoecism and expression of gynoecism has played a vital role in development of high yielding varieties, hybrids and seed production. The major limitations of molecular markers are limited in number, and their association with few economically important traits in bitter gourd. Generation of high-density genetic maps is the best way to identify the closely associated or functional markers for marker assisted selection and map-based cloning for fruit-related traits, gynoecium sex and yield. Crop wild relative can play an important role as a source of stress tolerance. The availability of whole genome information, selection of biotic and abiotic stress tolerant genes along with heterosis related alleles can be easily accomplished in breeding programmes.

Keywords: Bitter gourd, Breeding, Genomics, Biotic stress, Abiotic stress, Quality.

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Introduction

Bitter gourd or bitter cucumber or balsam pear or La-Kwa, or Chinese bitter melon (*Momordica charantia* L *syn. M. muricata* Terra.) is an important tropical monoecious crop originated in China or India (Indo-Burma regions). The regions of eastern India and southern China are suggested possible centers of domestication (Sands, 1928). The fruit set and yield was poor, seed viability and germination decreased. Bitter gourd is widely grown in India, Indonesia, Sri Lanka, Malaysia, the Philippines, China, tropical Africa, North & South America and the Caribbean. According to Press information of bureau (PIB) Govt. of India, the total acreage of bitter gourd in the country is 10.9 mt ha and production is 133.0 mt in the year 2020-21. The smaller (or more elongate) and greener variety is more bitter than the larger (or plumper) pale green ones that are usually found in Asian-American markets. The bitter gourd is a common vegetable cultivated extensively all over India. The alkaloid momordicasoides imparts the bitter taste to the fruit. Bitter gourd has rich nutritional and medicinal value. The bitter gourd is cooked and eaten as a vegetable in India and the Far East. The immature fruit is valued for its bitter taste and richness in vitamins

A, B1, B2 and C. The bitterness is reduced by steeping the peeled fruit in salt water before cooking. It is usually eaten fresh (stuffed and/or sliced) but can also be pickled, and has been canned in brine. Young immature bitter gourds are the best for cooking when the skin is bright green in color, the flesh inside is white, and the seeds are small and tender. It is a rich source of phosphorous and iron. It also contains minerals like calcium, phosphorous, iron, copper and potassium. Young shoots and leaves are an excellent source of Vitamin A, and a fair source of protein, thiamin, calcium and phosphorus (Vinning, 1995). It has twice the amount of beta-carotene than in broccoli and twice the calcium content of spinach. The bitter gourd has excellent medicinal virtues as many native herbolarios practice. It helps in purifying blood, enhances digestion, and stimulates the liver and activates spleen. It is a purgative, appetizer, digestive, anti-inflammatory and has healing capacity. It has antidotal, antipyretic tonic, appetizing, stomachic, antibilious and laxative properties. The bitter gourd is also used in native medicines of Asia and Africa. From the Ayurvedic perspective, bitter gourd is excellent for balancing Kapha. Leaf juice is used for curing cough, as a purgative and antihelminthic to expel intestinal parasites and for healing wounds. It became popular in the news recently because of its putative medicinal value especially against HIV/AIDS (compound Q). In folk medicine, the bitter this vegetable is the more medicinal value it has. The crude extract of bitter melon and its components prevent many types of cancers by enhancing reactive oxygen species generation; inhibiting cancer cell cycle, cell signaling, cancer stem cells, glucose and lipid metabolism, invasion, metastasis, hypoxia, and angiogenesis; inducing apoptosis and autophagy cell death, and enhancing the immune defense. Thus, bitter melon may serve as a promising cancer preventive and therapeutic agent (Sur and Ray, 2020).

Genetic resources and Diversity

The genus *Momordica* consists of 60 species worldwide and out of them 7 species (*Momordica charantia*, *M. balsamina*, *M. dioica*, *M. cochinchinensis*, *M. tuberosa*, *M. subangulata M. macrophylla*) is available in the Indian continent. The related species of this genus are *M. dioica* Roxb. ex Wild., M. *cochinchinensis* Spreng., *M. tuberosa* (Roxb) Cogm. (syn. *M. cymbalaria*), *M. balsamina* L. and *M*. cabriei, and among them first 2 species i.e. (*M. dioica* Roxb. Ex. Wild. and *M. cochinchinensis* Spreng.) are edible in nature and cultivated largely. Other wild species like *M. subangulata* Bl., *M. denudata* and *M. macrophylla.* Among them, monoecious (*M. charantia* and *M. balsamina*) and dioecious (*M. dioica* and *M. cochinchinesis*) exhibit divergence in morphological characters like growth habit, maturity, and fruit shape and size, sex expression, leaf, root and seed characters (Shukla *et al*., 2017). In the findings one cluster separates two distinct groups each accessions of *M. charantia* and *M. cochinchinesis* and *M. balsamina* formed distinct group within a major cluster while the second major cluster consisted of all accession of *M. dioica*. Further they revealed that molecular markers are more efficient in differentiating the accessions at and within species level than morphological markers. A natural triploid (2n = 33) of the cultivated *M. charantia* was also reported from India (Roy, 1985). Based on karyological and morphological studies on *M. charantia* (n = 11) and *M. tuberosa* (n = 11), a possible origin of the cultivated *M. charantia* from the wild *Momordica tuberosa* through karyotype alterations including deletion of satellites in the first pair and a pericentric inversion in the 11th pair of chromosomes is visualized (Varghese, 1973). *M. charantia* var. *abbreviata,* a native of Asia, may be the progenitor to the domesticate (Degener, 1974).

In India, bitter gourd is categorized in two botanical forms based on fruit shape, size, color, skin texture: **(1)** *M. charantia* var. *charantia* bears large fusiform fruits having triangular tubercles with non-tapering ends. **(2)** *M. charantia* var. *muricata* (Wild), bears small and round fruits having tubercles, with tapering ends (Chakravarty, 1990). Yang and Walters, 1992 also classified bitter gourd into three horticultural groups or types viz. small-fruited type (10–20 cm long, dark green, and very bitter in nature), long-fruited type (30-60 cm long, light green and slightly bitter), triangular-fruited type (9–12 cm long, light to dark green with moderately to strongly bitterness. Some accessions/ lines possess potential important (unique) horticultural trait reported from different parts of India have been conserved and registered in national germplasm collections of ICAR-National Bureau of Plant Genetic Resources, New Delhi, India for utilization in future breeding programmes. Few examples, IC12285 and VRBT-39 (Pandey and Singh 2001); IC256185, IC248256, IC213311, IC248282, IC256110 and IC248281 (Dhillon *et al*., 2005; resistant to fruit fly); resistant to downy mildew); TCR 404 (high yield and white-fruited type); IC 202195 (high yield and long-fruited type); INGR 03037 (gynoecious sex with high yield) and EC399808 (high yield and greater number of fruits) are available for breeding activities.

Biology and Breeding behavior

The plant is fast growing, trailing or climbing vine with thin stems and tendrils. The leaves are heart-shaped, 5-10 cm in diameter, cut into 5 to 7 lobes. Male and female flowers are borne separately on the same plant and require insects' pollination. In this crop flowering start 45 to 55 days after sowing, if conditions are optimal, and it continues through out a season of usually 6 months (Reyes *et al*., 1994). Flowering behavior varies among cultivars and climatic conditions (Deshpande *et al*, 1979). Flowers are borne singly in the leaf axils. Male flowers appear first and usually exceed the number of female flowers by about 20:1. Anthesis starts by 4 AM and gets completed by 9 AM and remains opens for

only one day. Another dehiscence starts by 5 AM and gets completed by 7.30 AM. Stigma is receptive 24 hr before to 24 hr after anthesis (Deshpande *et al*., 1979) and later on turns brown and dries (Rasco and Castillo, 1990). Pollination takes place by bees. Pollen loses viability as the day advances and may be fully in- viable by midday (Desai and Musmade, 1998). The fruits are characterized by continuous or discontinuous ridges throughout fruit length. Immature fruits are light green, oblong, pointed at the blossom end and have white flesh and become red when they are ripe. As the fruits begin to mature, the surface gradually turns yellow or orange. At maturity, it tends to split open, revealing orange flesh and bright red placenta to which the seeds are attached. Seeds are tan and oval, with a rough etched surface; there are 5 to 7 seeds per gram but it may vary between 6 000 ((Desai and Musmade, 1998) and 17 000 seeds/kg (Reyes *et al*., 1994). For seed production hand pollination can be avoided either by introducing beehives or by blowing pollen around with an unloaded mister. Bees don't work in prolonged wet overcast conditions, and pollen can only be blown when it is dry (Peter McLaughlin, 1998). The polyploids were inferior in economic characters to the diploids, with fewer female flowers, larger flowers and petals and flowers later than diploid. The fruit set and yield was poor, seed viability and germination decreased. Long days cause the male flowers to bloom up to 2 weeks before the female flowers, while short days have the reverse effect (Huyskens *et al*., 1992). Spraying with 50 mg/L Dikegulac at the 6-8 leaf stage increases female flower numbers and may double the number of fruit (Basu *et al*., 1994). Spraying with Gibberellic acid at 25 to 100 mg/L at the 2 to 4 leaf stage increases female flower number and can last for up to 80 days (Wang and Zeng, 1996). Treatment with 2,4-D, maleic hydrazide and Cycocel (Chlormequat) has also increased female flowers and yields, but reduced vine length and leaf area (Kabir *et al*., 1989) Pruning lower laterals increases the total number of flowers per plant by increasing the number of flowers on higher laterals (Rasco and Castillo, 1990).

Breeding objectives, Methodology and Varietal development

Breeding objective of bitter gourd is to improve fruit size and shape with resistant attributes. In addition, following breeding objectives should be considered as given below:

- Appearance of pistillate flower at earlier nodes results into Early fruiting
- Non-bitter cultivars with medicinal benefits such as proteins (charantin), polypeptides (polypeptide-K), glycoalkaloids, phenolics and antioxidants
- High female to male sex ratio or gynoecious based hybrids leading to high yield.
- Fruit color (whitish green to lighter green)
- Firm fruit with narrow seed cavity and without excessive seed development
- Desirable fruit shape, medium long (8-10 cm), non-ridge fruits, thick flesh
- Pest and disease-resistant (virus, powdery & downy mildew, red pumpkin beetle and fruit fly)
- Suitable for international export standards for fresh, canned and dehydration (fruits must be green, 20–25cm long, and possess a short neck)
- Cultivars should possess characteristics that enhance nutrition, such as high vitamin (carotenoids and ascorbic acid) and mineral (iron and calcium) content.

Open-pollinated varieties/cultivars (6) and hybrids (5) have been bred by public and private seed companies and identified by AICRP (VC) & notified by CVRC for bitter gourd cultivation in India (Tables 1 and 2). There are several cultivars and hybrids which are released at state level for commercial cultivation. Today, about 80% of the improved bitter gourd cultivars are hybrids and popular among growers.

Genetic improvement through traditional breeding

The knowledge of traditional breeding and genetics has improved our understanding of crop nature and make possible the breeders to develop improved varieties and hybrids. Earlier the breeding procedure was very slow without the knowledge of inheritance of traits. After the discovery of mendellian genetics, breeders are able to understand how different genes affect the number of traits and their interactions among each other. Conventional breeding has depended directly or indirectly on phenotypic appearance to discriminate the trait of desire for selection in a segregation population which ultimately advances the discovery of new genes and their inheritance (Bhardwaj & Singh, 2022). Traditional breeding includes direct observation of phenotype (fruit shape, color, sex ratio) or a relationship of one phenotype with other (greener leaves with bitter fruits) which has been productive for the improvement of qualitative and quantitative traits (Behera *et al*., 2020). In any breeding strategies, the primary goal is the development of high yielding varieties or hybrids with high quality fruits. In bitter gourd, Major characters that affect directly yield component are the number of fruits per plant, fruit size and fruit weight. Due to monoecious nature of crop, there was a limitation for improving these traits due to presence of high male-to-female ratio. Since the discovery of gynoecious trait, it will become easy for breeder to improve yield by altering plant architecture with better sex ratio. By utilizing gynoecious trait, the development of cultivars and hybrids with early and more female-to-male flowers ratio is another important goal of breeders. Along with high yield, breeders having their own priorities for developing quality traits in bitter gourd. For improving quality traits like shape, size, color, tubercles, phytochemical, shelf life and nutrient content, bitter gourd has tremendous potential for alteration of these traits due to rich diversity in Indian sub-continent. Recently, Traditional breeding

Table 1: Varieties/hybrids identified by AICRP (VC) and notified by CVRC in bitter gourd

Name of varieties/ hybrids	*Year of release*	*Source*	*Special features*	*Recommended for the area*
Arka Harit	1986	ICAR-IIHR, Bengaluru	It is a pure line selection from the local collection (IIHR-4) from Rajasthan. Vine is thin with light green deeply lobed foliage. Fruits are spindle shaped attractive, glossy green with smooth regular ribs and thick flesh and skin without tubercles. Average yield is 12.5-13.5 t/ha. Crop duration is 100-110 days.	VIII
Priya (VK-1)	1992	KAU, Vellanikara (Kerala)	It is selection from the local material. Bears long green spiny fruits tinged white at tip. The fruits are (40 cm) and 17 cm in girth with flesh thickness of 5-9 mm, average fruit weight 225 g; and each fruit contains 24 seeds weighing 6 g (100 seeds weight is 24 g). Average yield is 30.0-32.5 t/ha. It can be cultivated during January-August and September-December. It has been recommended for cultivation in the states of West Bengal, Assam, Madhya Pradesh, Maharashtra, Karnataka, Tamil Nadu and Kerala.	II,VII,VIII
Pant Karela-1 (PBIG-1)	2001	GBPUAT & Tech. Pantnagar (Uttarakhand)	This is pure line selection from inbreds of indigenous germplasm. It has been released by the Uttar Pradesh State Variety Release Committee in the year 1999 and identified in the year 2001 through All India Coordinated Project (Vegetable Crops). Plants are 2.0 m long and fruits are 15.0 cm long with tapering ends. It takes 55 days to first picking and the yield potential is 15.0 t/ha. It is suitable for cultivation in hills.	IV
Phule Priyanka (RHRBGH-1)	2001	MPKV., Rahuri (Maharashtra)	This hybrid derived from a cross of RHRBG-5 and RHRBG-4. Fruits are dark green, highly prickled surface and 20 cm long. This hybrid is suitable for cultivation under rainy and summer seasons. It is tolerant to downy mildew. It gives an average yield of 28.27 t/ha in 180 days of crop duration. It has been recommended for cultivation in Maharashtra.	All
Pusa Hybrid-2	2002	ICAR-IARI, New Delhi	This hybrid has been developed by heterosis breeding utilizing inbred Pusa Vishesh and Pusa Do Mausami. Fruits are medium thick and long, glossy green, suitable for picking and dehydration. First pickling starts at 55-60 days after seed sowing. It is suitable for growing in spring summer season in the states of Punjab, Uttar Pradesh, Bihar, Jharkhand, Chhattisgarh, Odisha Andhra Pradesh, Rajasthan, Gujarat, Haryana and Delhi and has yield potential of 20.00 t/ha. It gives 42% and 48% higher yield than Pusa Vishesh and Pusa Do Mausami, respectively.	IV, V, VI
NBGH-167 (Hybrid)	2004	Nirmal Seeds Co.	1st picking is done 55-60 days after seeding. Dark shiny green, spindle shape, sharp prickled medium long fruits. Uniform fruit size and attractive and better keeping quality. Tolerant to Powdery mildew, downy mildew.	IV
Vivek (Hybrid)	2008	Sungro Seeds Co.	Fruits are ready for harvest in 55 to 60 days after sowing. Length of the fruits is 22 cm and girth is 16 cm. Average weight of fruits is 80 to 85 gm.	VIII
Pusa Aushadhi (Sel.-I)	2013	ICAR-IARI, New Delhi	Its fruits are light green, medium long (16.5 cm) and medium thick. Fruits have 7-8 continues narrow ridges and mature in 48-52 days. The average fruit weight 85 g with an average yield of 12.80 t/ha.	VI

programmes are targeted on improving yield along with quality, maturity time, fruit characteristics, economic traits, phytonutrients content (momordicin, minerals) etc. by utilizing local landraces. In present scenario, disease and pest resistance breeding is under supreme priority for mostly gourd breeding programmes. Studies also being carried out for the incorporation of disease resistance traits without compromising yield and implementation of DNA markers parallelly with traditional breeding to fasten the process.

Breeding Methods

Bitter gourd is cross pollinate crop with very less inbreeding depression hence providing better breeding options for its improvement. Breeding methods commonly used in bitter gourd are single plant selection, inbreeding, pure line selection, mass selection, pedigree selection, recurrent selection, bulk population improvement and heterosis breeding (Sirohi, 1997). Earlier, an improved selection from a local landrace has been a most frequently acquired

Table 2: Characteristics of some other popular varieties/hybrids available in bitter gourd

Kashi Urvashi	Develop from ICAR-IIVR, Varanasi and suitable for summer and rainy season cultivation. The fruits are dark green and have good keeping quality. Average yield is 22.0-24.0 t/ha.
Pant Karela-2 (PBIG-2)	This variety has been developed through selection from a germplasm PBIG-1 at GBPUA&T, Pantnagar (Uttarakhand). Its fruits are thin, 25 cm long, dark green with tapering ends. First marketable fruit picking starts at 50 days after seed sowing. It has been recommended for cultivation in the states of Uttar Pradesh, Uttarakhand, Bihar and Chhattisgarh and has yield potential of 15.0-16.0 t/ha.
Coimbatore Long	This variety is released from TNAU, Coimbatore (Tamil Nadu). Fruit is long, tender, white, and suitable for rainy season cultivation. Average yield is 20.0 t/ha at proper cultural practices.
Coimbatore Green	It is selection from the local material and developed by TNAU, Coimbatore (Tamil Nadu). The fruits are extra-long (up to 60 cm) dark green, and each weighing 300-400 g. Yield is 15.0-20.0 t/ha.
CO-1	This variety has been developed through selection from a local material collected from Thudiyaur and released from TNAU, Coimbatore. Vines are medium long (130.0 cm) with 5-7 branches. First flowering starts in 45-50 days and marketable fruit is harvested in 55-60 days after sowing. A total of 6-8 picking can be done. Fruits are dark green, 25.0-30.0 cm long with prominent protuberance. Duration of the crop is 115 days with average yield of 14.0-15.0 t/ha.
CO-2	This variety is developed from TNAU, Coimbatore through induced mutation. It is early in flowering. Fruits are 35.0-40.0 cm long and thick. Each fruit weighs 40.0 g. It gives an average yield of 30.0 t/ha.
Pusa Do Mausmi	It is selection from the local material and released from ICAR-IARI, New Delhi. Suitable for both spring-summer and rainy season sowing. First picking starts after 55-60 days of sowing. Fruit long medium, dark green, thick, club shaped with 7-8 continuous ridges. Fruit length at edible stage is about 15.0-18.0 cm, average fruit weight 110 g. The average fruit yield is 13.0 t/ha.
Pusa Vishes	It is selection from the local material of Uttar Pradesh and released from ICAR-IARI, New Delhi. Fruits are glossy green, thick, medium long in size. Vines short hence more number of plant can be accommodated per unit area. Average fruit weight is 120 g. Average yield is 15.0 t/ha. This variety is released from ICAR-IARI, New Delhi. Suitable for spring summer season; first picking in 55-60 days after sowing.
Pusa Hybrid-1	This high yielding hybrid has been developed by ICAR-IARI, New Delhi. The fruits are attractive green medium long (13.5 cm), medium thick (5.0 cm diameter) with regular, smooth tubercles on the surface. First picking is possible in 50-55 days after sowing. Average fruit weight is 100 g and yield is 22.0 t/ha. It is suitable for pickling and dehydration.
Punjab-14	It is selection from the local material and developed by PAU, Ludhiana (Punjab). Suitable for both rainy and summer season cultivation. Bushy plant habit and average fruit weight is about 35 g. Fruit colour is light green. Average yield is 12.513.0 t/ha.
Kalyanpur Sona	It is released by CSAUA&T, Kalyanpur, Kanpur (U.P.). Its vines are medium in growth. Fruits medium sized, green, and average yield is 12.0-13.0 t/ha in 160 days of crop duration. This variety has been recommended for cultivation in Uttar Pradesh.
Kalyanpur Baramasi	This has been developed at CSAUA&T Vegetable Research Station, Kalyanpur, Kanpur (U.P.). It is vigorous creeper. Fruits are long (30-50 cm) light green, thin and tapering. Yield potential is about 20.0 t/ha in about 120 days. It is reported to be tolerant to fruit fly and mosaic virus. Suitable for *kharif* season cultivation in the state of Uttar Pradesh.
Phule Green Gold (RHRBG-5)	This variety has been developed from the cross between Green Long and Delhi Local followed by pedigree selection at MPKV, Rahuri (Maharashtra). The fruits are dark green, 25-30 cm long and dented (raised tubercles). Average yield is 23.0t/ha. It is tolerant to downy mildew.
Phule Ujwala	This variety has been developed from MPKV, Rahuri (Maharashtra). Fruits are dark green, medium in length and prickled, better shelf-life and suitable for long distance market and tolerant to Downey mildew. Suitable for Kharif (June-July first week) and Summer (January-February)
Hirkani	This variety has been developed from MPKV, Rahuri (Maharashtra) through selection from the local germplasm. Fruits are dark green and 15.0-20.0 cm long. Each vine produces 20-22 fruits, each weighing 100-120 g. duration of the crop is 160 days with average yield of 20.0 t/ha.
Konkan Tara (DPLBG-2)	This variety has been developed from BSKKV, Dapoli (Maharashtra). Its fruits are green, medium long (15-16 cm) and spindle shaped with raised tubercles and good keeping quality with a shelf-life of 7-8 days at ambient temperature. It has been recommended for cultivation in Konkan region of Maharashtra and has yield potential of 23.0-25.0 t/ha.
NS-1024	This early maturing hybrid has been developed from Namdhari Seeds Co., Bengaluru (Karnataka). First picking is possible 45-50 days after sowing. The plants are vigorous with prolific bearing habit. The fruits are 25-30 cm long, 2.5-3.0 cm thick with dark green shining skin and sharp dentation. It is a heavy yielder with good transportation qualities. Average fruit weight is 150-200 g.

MDU-1	This variety has been developed through mutation breeding (gamma irradiation of local cultivar MC-103) at Coimbatore (T. Nadu). Flowering starts at 60 days after sowing with a sex ratio of 1:20 of female and male flowers. Fruits are long with an average length of 40.34 cm and a girth of 17-54 cm having average fruit weight of 410 g. Each vine bears on an average of 16-18 fruits. It has been recommended for cultivation in Tamil Nadu and has yield potential of 30.0-32.5 t/ha.
Preethi (MC-84)	This variety has been developed at KAU, Vellanikara (Kerala). It has light green vine of 7.0 m length. Fruits are white, spiny, and medium in size with 310 g. It has been recommended for cultivation in states of Madhya Pradesh, Maharashtra, Karnataka, Tamil Nadu and Kerala and has yield potential of 14.0-15.0 t/ha in 150 days of crop duration.
Kashi Mayuri (VRBTG-5)	This variety has been developed from ICAR-Indian Institute of Vegetable Research, Varanasi (U.P.). Its fruits are dark green, 20-25 cm long and 80-100 g fruit weight. Tolerant to powdery and downy mildew diseases & fruit fly under field condition. Suitable for *Kharif* and Zaid both the season. It has been recommended for cultivation in Uttar Pradesh and has yield potential of 23-25 t/ha.
Kashi Pratiksha (VRBTG-10)	This variety has been developed from ICAR-Indian Institute of Vegetable Research, Varanasi (U.P.). Its fruits are green, 25-30 cm long and 90-110 g fruit weight. Tolerant to powdery and downy mildew diseases & fruit fly under field condition. Suitable for *Kharif* and Zaid both the season. It has been recommended for cultivation in Uttar Pradesh and has yield potential of 28-30 t/ha.
Priyanka	Priyanka is a high yielding hybrid variety of bitter gourd developed by the Kerala Agricultural University, Vellanikara (Kerala). Fruits are greenish-white in colour and average yield is 20 tonnes per hectare. This particular variety is recommended for the acidic alluvial soil of Kerala.
COBgH 1	This hybrid has been developed between MC 84 x MDU1 by TNAU, Coimbatore (T. Nadu.). It is rich in momordicin (2.99 mg per 100g). Fruits are light green in colour, plumpy with more warts; each weighs 200g.-300g. Yields 44.40 t/ha in 115-120 days.

breeding program. Pedigree selection is typically used for the incorporation of traits like high and early yield with nutritionally rich [high vitamin C and A] and disease resistance after crossing two parents. However, strategies for the incorporation for disease and pest resistance and improved yield needs strategic execution since there is presence of a negative correlation with yield and linkage drag, as observed in cucumber (Staub and Grumet, 1993; Singh *et al*., 2014; Bhardwaj & Singh, 2022). It is an indigenous and cross-pollinated crop; rich diversity/variation is noticed in quantitative and qualitative characters. Hence, exploitation of hybrid vigor is preferable due to ample heterosis for earliness and yield-related traits. Lately, in bitter gourd, many investigations on heterosis for yield, quality traits and earliness have been achieved across the globe including WVC, Taiwan, ICAR-Indian Institute of Vegetable Research, Varanasi and ICAR-Indian Agricultural Research Institute, New Delhi. Presently, many open-pollinated (often landraces) cultivars and hybrids have been developed for commercial cultivation (Sirohi, 1997) and nearly 80-85% of the crop area is from established F_1 hybrids released from both public and private sectors. Hybrids are usually expensive (due to its development cost) but provide higher yields and uniformity than open-pollinated cultivars, which leads it to be the first choice of farmers. In India, cultivar choice is mainly depends on regional consumer preference and nearby market demand for fruit shape, size, color, presence and absence of tubercles, thickness and extent of bitterness. Here we are going to discuss some popular methods of breeding

Heterosis Breeding

As a cross pollinated crop, bitter gourd has a wide scope for heterosis exploitation. Divergence between parents for yield-related traits is the key factor that influences the degree of heterosis. Hence, selection of superior parent's based on a number of fruits, fruit weight, fruit length, yield, pedigree and combining ability will be useful for proper exploitation of heterosis. The heterotic effect is solely depending on dominance variation and complementary gene action for yield traits (Mishra *et al*., 1998; Kumar *et al*., 2020; Ram *et al*., 1997). In the evaluation of 25 diverse genotypes of bitter gourd, wide variation recorded for most of the horticultural traits and clustering resolved into two major clusters indicated trait base breeding (Singh *et al*., 2014). Before utilizing the parents, it is essential to achieve inbreeding to achieve uniformity in hybrids. Parental inbreeds are generally maintained and multiplied by selfing without inbreeding depression (Behera, 2004). Nevertheless, selfing for 6 to 7 generations is advisable because in later stages plants often produce poor fruit set. Finally, before making crosses information regarding general and specific combining ability through diallel analyses and/or North Carolina I or II mating design will facilitate the breeder for developing superior hybrids (Haripriya, 1991). On the basis of general and specific combining ability, the potential inbred lines are chosen for the production of F_1 hybrid (Acharya *et al*., 2019; Ram *et al*., 2007 Brar and Sidhu, 1977).

Heterosis in biter gourd was first studied in 1943, at the ICAR-Indian Agricultural Research Institute, New Delhi (Pal and Singh, 1946). Heterosis for yield per plant depends on genotype ranges from 27 to 86% (Behera, 2004). Pusa Hybrid-1 was first public sector hybrid followed by Pusa Hybrid-2, which gives 42% heterosis and 75% heterosis for yield/plant over better parent and check Pusa Do Mausami, respectively (Sirohi, 2000). Appreciably amount of significant heterosis was reported in desirable direction for days to

first female flower appearance, node number of first female flower, average fruit weight, fruit diameter, fruit length, number of fruits per plant, number of primary branches, vine length (Jadhav *et al*., 2009; Lawande and Patil 1989; Bhatt *et al*., 2017; Moharana *et al*., 2018), fruit length and node at which first female flower appeared (Thangmani and Pugalendhi, 2013), vine length, days to first female flowering, fruit length and fruit number (Celine and Sirohi, 1996), number of fruits per plants and days to first harvest (Al-Mamun *et al*., 2015), number of fruit/plants, days to first harvest, fruit diameter, fruit length, fruit weight, and fruit yield/plant (Pal and Singh, 1946; Singh and Joshi, 1980; Vahab, 1989; Lawande and Patil, 1989). Positive heterosis was observed over superior parent for carotene, vitamin C and total sugar content in bitter gourd (Adarsh *et al*., 2018). A wide range of variation in the parents and heterosis (mid parent and better parent) observed positive (yield) and negative (earliness) which expressed usefulness of parents in the development of hybrids. Days to early emergence of male and female flower at lower node should be considered for yield improvement. The parents which contributed significantly in expression of heterosis in expression are VR-10, VR-103, EC381654, VR-11 and VR-156 for all the traits under study (Bhardwaj and Kumar 2021). Initially, gynoecious flowering trait have been observed in nature "Gy-333" (Ram *et al*., 2002). Later on, two gynoecious lines (DBGy-201, DBGy-202) were distinct from wild inland source (*M. charantia* var. *muricata* L.) (Behera *et al*., 2006). Further, two more gynoecious lines (IIHRBTGy-491 and IIHRBTGy-492) were identified at ICAR-IIHR, Bengaluru, India (Varalakshmi *et al*., 2014). Other gynoecious lines widely used in the hybrid breeding program as parental material are OHB61-5 in Japan (Matsumura *et al*., 2014), K44 (Cui *et al*., 2018), X Hei-d-d (Wang *et al*., 2010a), Z-1-4 (Wang and Xiang, 2013), and Yuqiang-2 (Li *et al*., 2011) in China. Predominately gynoecious character is also reported in line PreGy-1 (IC-0591254; INGR12014) at ICAR-IARI, New Delhi (Behera *et al*., 2012). By using this trait, superior hybrids were developed for earliness and yield characters (Dey *et al*., 2012; Rao *et al*. 2018; Alhariri *et al*., 2018; Moharana *et al*., 2022; Shukla *et al*., 204) while monoecious hybrids were superior for fruit length, flesh thickness, average fruit weight, fruit diameter and yield per plant (Rao *et al*., 2018; Alhariri *et al*., 2018). In bitter gourd, combining ability studies were carried out using gynoecious population and findings revealed that mean square due to gca and sca were highly significant for all the traits (Shukla *et al*., 2014). Finding also suggests the importance of both additive and non-additive gene effects. In the study Gynoecious line Gy323 was the best combiner for all the traits except fruit diameter and seeds/fruit, whereas monoecious line DRAL-41 was best general combiner for first flower anthesis and intermodal length as they have made significant contribution in yield contributing characters. Reports indicate that the gynoecious combinations Gy333 x DRAL-41, Gy323 x DVBTG-7 and Gy323 x VRBT-904 exhibits high sca effect for all horticultural traits in desired direction. In bitter gourd inbreeding depression results due to the fixation of unfavorable recessive genes in F_2. The high inbreeding depression indicates the presence of non-additive gene action (dominance and epistasis) for the traits like first male and female flower anthesis, fruit weight, plant height and number of fruits/plant. The minimum inbreeding depression was observed in cross VRBT-32 x VRBT-6 and VRBT-94 x VRBT-49 (Bhardwaj and Singh, 2022).

Mutation and Polyploidy Breeding

Mutation breeding also known as variation breeding in which spontaneous genetic variation is induced by using physical (y-rays, X-rays) (Beyaz and Yildiz, 2017) and chemical mutagens (ethyl methane sulfonate (EMS) (Ahloowalia and Malugzgnslia, 2001). It involves alteration in genetic material, leads to developmental changes within organism. Among the mutant population, mostly mutations are recessive in nature and need to fixed by selfing (Miniraj *et al*., 1993). A bitter gourd variety, MDU 1, developed from landrace MC-103, through gamma radiation treatment (seed) which improved in yield, tolerance to fruit fly, pumpkin beetle and leaf spot diseases (Rajasekharan and Shanmugavelu, 1984). Likewise, a white fruit color mutant developed from spontaneous mutation of green type Pusa Do mausami natural population (Behera *et al*., 2010). A treatment of the seedlings at the cotyledon stage with 0.2% colchicine results in production of polyploids in bitter gourd. Treatment of seeds with Amiprophos-methyl also resulted in octoploid plants with better germination. Seed treatment with 0.003% amiprophos-methyl or 0.2 and 0.4% colchicine was effective for doubling chromosomes, out of which the treatment with 0.4% colchicine was most productive with moderate mortality (Yasuhiro Cho *et al*., 2006). Parameters like guard cell size were bigger, and ratio of leaf length and leaf width was lower in octoploid in comparison with tetraploid plants. As per Saito (1957), Seedless triploid plants of bitter gourd were produced by crossing the tetraploid plants (colchicine induced) with diploid plants. In seedlings, Colchicine treatment to the shoot tip at 0.2% concentration for 18 h was sufficient to produced tetraploids (Kadir and Zahoor, 1965). However, Reyes and Rasco (1994) reported that tetraploids were inferior in terms of economic traits than diploids.

Bitter gourd mutants, the suitable concentration and duration of ethyl methanesulfonate (EMS) mutagenesis have not been determined. In this study, mutant collection was conducted to create new germplasms and widen genetic diversity. By employing the seeds of the inbred line Y52 as the mutagenic material, EMS as the mutagen, and the suitable mutagenic conditions for bitter gourd seeds (EMS concentration 0.2%, mutagenic time 10 h), we mutated 10,000 seeds and acquired 3223 independent M1 lines. For

Table 3: Inheritance of morphological and physiological traits in bitter gourd

S. No.	*Trait*	*Inheritance*	*Reference*
1.	Seed coat	Light brown seed (lbs) coat color is recessive to dark brown	Srivastava and Nath, 1972; Ram *et al*., 2006; Kole *et al*., 2012
2.	Seed size	Large seed (ls) size is recessive to small seed size	Srivastava and Nath, 1972
3.	Epicarp	White epicarp (w) is recessive to green	Suribabu *et al*., 1986; Vahab, 1989
4.	Fruit colour	Green is dominant to white	Miniraj *et al*., 1993; Hu *et al*., 2002; Liou *et al*., 2002; Liu *et al*., 2005 [Digenic]; Srivastava and Nath, 1972; Hu *et al*., 2002; Dalamu *et al*., 2012; Suribabu *et al*., 1986; Vahab, 1989; Esquinas-Alcazar and Gulick, 1983 [Monogenic] and Dalamu *et al*., 2012 and Huang and Hsieh, 2017 [polygenic/quantitative]
5.	Tubercles	Spiny (triangular tubercles) fruit to be dominant over smooth	Vahab, 1989
6.	Fruit length	Short fruit length is partially dominant over long fruit length, and tubercles and curviness of fruits	Kumari *et al*., 2015
7.	Fruit surface (Ridgeness)	Discontinuous ridge governed by a single dominant gene	Dalamu *et al*., 2012
8.	Fruit Size	Small fruit was partially dominant over large fruit Incompletely dominant and is controlled by a minimum of five genes	(Kim *et al*. 1990) (Zhang *et al*., 2006)
9.	Bitterness (higher content of glycosides)	Monogenic inheritance with more bitterness dominant to less	Suribabu *et al*., 1986

the randomly selected 1000 M_2 lines, 199 M_2 lines with visible phenotypes were found, and 167 M2 lines were mutants of fruit shape, size, and tubercles. Furthermore, fourteen dwarf, eleven-leaf color, five-leaf shape, and eight meristem defect mutants were discovered in this mutant collection. In addition, three lines of 1253, 2284, and 3269 represented recessive mutants crossed with Y52. Furthermore, the yellow leaf lines of 2284 and 3269 were not mutated at the same gene locus (Yu *et al*., 2022).

Traits and their Inheritance

Fruit and seed characters

In Asian meals, bitter gourd fruits are used in the immature stage hence, internal and external qualities relating to fruit shape, color, size and firmness are desirable. High heritability of fruit color controlled digenic where green is dominant to white (Liu *et al*., 2005). Similarly, the inheritance of other morphological traits is mentioned in Table 3. Some traits like fruit color, fruit size and presence and absence of tubercules are mostly governing its marketability with regional preferences. For example, green-fruited have more demand in southern China (Light} and Northern India (dark green to glossy with prominent tubercles) while white color fruits are most preferred in central China and Southern India (Dhillon *et al*., 2020). Days to first harvest and fruits/plant are largely controlled by additive and additive x additive components (Sirohi and Choudhury, 1979). Studies also indicated that green fruit color, dark brown seeds and seed size are monogenically inherited. White epicarp is controlled by a single gene 'w', white being recessive to green (Alcazar and Gulick 1973). Vahab (1989) observed that inheritance of fruit colour and surface are monogenic, green and spiny fruits being dominant over white and smooth fruits respectively. Alcazan and Gulick (1993) reported that white epicarp is controlled by a single gene 'w', white being recessive to green Vahab (1989) found that inheritance of fruit colour and surface are monogenic, green and spiny fruits being dominant over white and smooth fruits, respectively.

Sex expression

Bitter gourd is a predominately monoecious crop and sex expression has played a vital role in development of high-yielding varieties and seed production. Flowering traits like days to first pistillate flower, male: female (♂:♀) sex ratio and node at first pistillate flower appearance are directly correlated to earliness and fruit yield. Iwamoto and Ishida (2006) observed that gynoecious sex expression was partially dominant in Japanese germplasm (LCJ 980120; predominantly female) while Ram *et al*. 2006, Behera *et al*., 2009 and Mishra *et al*., 2015 reported that it is governed by a single recessive gene gy-1 in Indian accessions. Another study showed that gynoecious trait is governed by two pairs of genes (Cui *et al*., 2018) Regardless of Inheritance, these studies justified that gynoecious or predominantly female flowering lines bear immense potential for the development of gynoecious based F_1 hybrids (Matsumara *et al*., 2020).

Yield

The most genetic determinate of yield are complementary epistasis and dominance-dominance interactions (Singh and Ram, 2005). For most of the traits, genotypic correlation coefficients are greater than phenotypic coefficients (Dey *et al*., 2009). However, phenotypic evaluation of yield and related traits studied in path coefficient analysis suggested that fruit weight had the greatest direct effect on yield, followed by number of fruits per plant and node number of first pistillate flower (Moharana *et al*., 2018). Yield per plant had highly positive significant correlation with number of nodes per vine (Islam *et al*., 2009), number of fruits per plant (Ramachandran and Gopalkrishnan, 1979; Kole *et al*., 2012; Srivastava and Srivastava, 1976), fruit length, fruit weight and number of flowers per plant (Parhi *et al*., 1995; Ramachandran and Gopalkrishnan, 1979). Average fruit weight, fruit length and number of fruits per plant are controlled by additive factors; and have direct positive effects on yield (Sharma and Bhutani, 2001). Thus, simple selection strategies targeted on average fruit weight, number of fruits per plant, days to harvesting, fruit length and diameter and harvest index could be utilized to improve the yield. Bio-chemical traits like ascorbic acid content and total carotenoid content had a strong negative but indirect effect on marketable yield through fruit weight, fruit length, and diameter. Hence, the selection of small-fruited cultivars should be done to improve ascorbic acid and total carotenoid content. The ANOVA for sums and differences were significant for all earliness and yield characters, indicating the value of additive and dominant components of variation for these characters (Ram *et al*., 2002). Traits like days to 50% flowering, days to first harvesting, number of fruits per plant, fruit diameter, fruit length and yield per plant unveiled a predominance of non-additive gene action, whereas over-dominance gene action for most of the yield-related traits were observed (Rao *et al*., 2018). Inheritance of traits like carotene, vitamin C, total sugar, and reducing sugar was governed by the non-additive gene effect reported by Sundharaiya and Shakila (2011); Kumara *et al*., (2011) and Kumar and Pathak (2018). Considering genetic dominance and complementary gene action associated with some of the yield-affecting traits indicate that for yield improvement, heterosis breeding would be an preferred strategy (Mishra *et al*., 1998; Celine and Sirohi, 1998). In bitter gourd 38 monoecious lines and 6 gynoecious lines were evaluated pertaining to 9 important quantitative morphological traits and results indicate that VRBTG-5 was found to be the best performer among the monoecious lines, whereas, Gy-323 was the best performing gynoecious line concerning the yield/plant (g). moreover, for all other major yield-contributing traits, both of these lines have exhibited superior performance over all other accessions across the seasons and can be exploited further in crop improvement (Moharana *et al*., 2022; Bhardwaj *et al*., 2023).

Quality traits

Fruits of bitter gourd are a rich source of carbohydrates, proteins, vitamins and minerals (Table 4) and possess the highest nutritive and medicinal value among gourds (Miniraj *et al*., 1993; Desai and Musmade, 1998; Upadhyay *et al*., 2015). Considerable variation in nutrients and phytochemicals has been observed in bitter gourd due to genotypic variation (Yuwai *et al*., 1991). In fact, bitter gourd fruits have higher crude protein content (11.4-20.9 g kg^{-1}) than that of tomato and cucumber (Xiang *et al*., 2000). In phytochemical, bitter gourd mainly consists of proteins, sterols, glycosides, fatty acids and volatile constituents (Haque *et al*., 2011; Lee *et al*., 2009) (Table 5). Breeding for quality traits are typically focusing on accessions with relatively high vitamin C content, and moderate bitterness (Dey *et al*., 2006a). Bitter gourd lines like DBTG-3, DBTG-8, DBTG-6, and DBTG-9 contain>1000 $mg.kg^{-1}$ vitamin C in fruits as compared to 500$mg.kg^{-1}$ in standard cultivars (Dey *et al*., 2006b). Many Indian bitter gourd varieties already reported rich in traits like high total soluble solid content (>3.1_Brix; MC-84, Preethi, RHRBG-5, and PBIG-1), vitamin C (>950$mg.kg^{-1}$; Konkan Tara and Hirkani) and protein (>1.5%; DVBTG-1, Preethi, Hirkani and Konkan Tara) content (Kore *et al*., 2003). These nutritionally rich cultivars /lines are aggressively used in breeding programs for development of cultivars with high quality traits along with yield.

Biotic stress tolerance

Management of biotic stress which cause significant loss not only increases production cost but also increases negative

Table 4: Proximate constituents and nutrient composition of bitter gourd fruits

Constituents	Quantity
Moisture (g/100 g)	83.2
Carbohydrates (g/100 g)	10.6
Proteins (g/100 g)	2.1
Fiber (g/100 g)	1.7
Calcium (mg/100 g)	23
Phosphorus (mg/100 g)	38
Potassium (mg/100 g)	171
Sodium (mg/100 g)	2.4
Iron (mg/100 g)	2
Copper (mg/100 g)	0.19
Manganese (mg/100 g)	0.08
Zinc (mg/100 g)	0.46
B carotene	126
Vitamin C	96

Source: Gopalan *et al*., (1993). Nutritive value of Indian foods. National Institute of Nutrition, ICMR, Hyderabad)

Table 5: Phytochemicals and their uses in bitter gourd (*Momordica charantia* L.)

Phytochemicals	*Plant parts*	*Classes and uses*	*References*
β-momorcharin	Seeds	Glycoprotein that acts as midterm abortifacient	Chan *et al*. (1984)
Vicine	Seeds	Hypoglycemic glycoalkaloid	Dutta *et al*. (1981) and Handa *et al*. (1990)
Charantin	Fruits	Non nitrogenous compound having hypoglycemic activity	Lotlikar and Rao (1962)
Momordicosides A and B	Seeds	Triterpene glycosides that inhibit tumor growth	Okabe *et al*. (1980)
MAP30 (momordica anti-HIV protein of 30 kDa)-anti-HIV plant protein	Seeds, fruits	Basic protein that inhibits Human Immunodeficiency Virus (HIV)	Lee-Huang *et al*. (1990, 1995)
Polypeptide-p	Seeds, fruits	Hypoglycemic peptide, called plant insulin	Khanna and Jain (1981)
Phenols	Seeds	Antioxidants that reduce blood pressure and with anticancer and cardioprotective properties	Horax *et al*. (2005)
Carotenoids	Seeds, fruits	Antioxidants with anticancer and cardioprotective effects	Rodriguez *et al*. (1976)

Source: Behera *et al*., 2010

impact on the environment and ecology. Suitable strategies to avoid these losses are to utilize resistant varieties that are healthier, economical and environmentally friendly. Recently, breeders are using both traditional and modern tools to minimize the time period of varietal development. At the beginning of any programme, the durability of resistance is the foremost important aspect which need knowledge of resistance inheritance, expression and its interaction with other genes and the surrounding environment. Dhillon *et al*., 2005 have found an association between morphological traits and insect resistance which may be helpful for indirect selection during resistant breeding program. In the same study, fruit infestation percentage by fruit fly is directly correlated with rib depth, flesh thickness nevertheless fruit length and diameter are negatively related with fruit toughness. Inheritance of fruit fly resistance is dominant and has additive and dominant gene effects, as well as duplicate epistasis (Tewatia and Dhankhar, 1996). Hence, relative fruit toughness may be used as a selection criterion and reciprocal recurrent selection as a breeding strategy for the development of fruit fly-resistant varieties. Powdery mildew a serious foliar fungal disease of bitter gourd caused by Podosphaera xanthii which affects its production due to premature foliage loss ultimately reduction in growth and yield (Keinath *et al*., 2004). World Veg, Taiwan released five PM-resistant lines in bitter gourd lines (THMC 113, THMC 143, THMC 153, THMC 167, and THMC 170) in 2018 and their resistance is controlled by two independent, recessive genes [Dhillon *et al*., 2018, 2019].

Bitter gourd distortion mosaic virus is a serious disease in which infestation ranges from 41-95% (Rabindranath and Pillai, 1986; Hollingsworth *et al*., 1997). BDMV resistance is governed by polygenes with non-additive gene action and their expressions are highly influenced by the environment (Arunachalam, 2002). Tomato leaf curl New Delhi virus (ToLCNDV) (genus Begomovirus, family Geminiviridae) is a bipartite begomovirus that leads to severe epidemics loss in bitter gourd cultivation (Nagendran *et al*., 2017). A line AVBG1655 (derived from a Bangladeshi landrace) was developed by WorldVeg, and consistently rated as resistant to ToLCNDV in multi-location trials conducted by private seed companies in India (Yadav *et al*., 2019). This line could be use as a potential source for introgression of ToLCNDV resistance in available commercial varieties. Multi-locational trails are undergoing of recently developed two bitter gourd hybrids (NBL 368, NBTH 19156) resistant to ToLCNDV using this resistance source, by Noble Seeds Private Limited India (Bangalore, India) (Dhillon *et al*., 2020). Several resistance hybrids have released in China for commercial cultivation *viz.* for Fusarium wilt and powdery mildew (Cuiyu), virus (Hengza No. 2) and powdery mildew (Chunyu) (Xiao *et al*., 2005; Chang *et al*., 2005). Genetics of several other biotic stresses have not been studied and are very limited. Hence, more studies are required to know the durability of resistance and its sources to apply in resistance breeding programs.

Abiotic stress tolerance

In bitter gourd, abiotic factors limit germination, growth etc. to a great extent. Abiotic stress resistance breeding in bitter gourd is still at the juvenile stage. Very less reports are available regarding genetic inheritance and tolerance. Liou *et al*. (2002) developed and heat-tolerant variety "Pintong Black Seed" also suited for tropical regions. Tao M-Q *et al*. (2020) reported that use of bitter gourd as rootstock improves the heat tolerance of cucumber by reduce the photo-inhibition induced by heat stress and enhancing the antioxidant defense capacity by regulating the changes in H_2O_2 and polyamines in leaves under high-temperature stress. Improvement of stress tolerance capacity in bitter gourd as future targeted areas of research, are still at a starting

stage. The plant with its ability against cold temperatures captured worldwide attention and the anti-cold specie of *Momordica Charantia* L. seedlings was screened out. The core genes and metabolites which related to bitter gourd seedling facing low temperatures and core genes were validated by Q-PCR, including McSOD1 and McERF1. Moreover, the initial metabolites, including amimo acids, polypeptides, sugars, organic acids and nucleobases, were apparently increased in cold resistant species Y54 than cold-susceptible species Y17, indicating that these metabolites might contribute to the cold tolerance**.** Low-temperature stress resulted in increased accumulation of MDA, H_2O_2 and proline content in two species, but less expressions in cold-resistant species Y54. As compared to Y17, cold-resistant species Y54 presented significantly enhanced antioxidant enzyme activities of POD (peroxidase), CAT (cataalase) and SOD (superoxide dismutase). Meanwhile, higher expressed genes encoded antioxidant enzymes and transcription factors when exposure to the low temperature were found in cold-resistant species Y54, and core genes were explored by Q-PCR validation, including McSOD1, McPDC1 and McCHS1. Moreover, plant metabolites containing amino acid, sugar, fatty acid and organic acid in Y54 were higher than Y17, indicating their important roles in cold acclimation. Meanwhile, initial metabolites, including amino acids, polypeptides, sugars, organic acids and nucleobases, were apparently increased in cold-resistant species Y54 than cold susceptible species Y17. Our results demonstrated that the *M. Charantia* L. seedlings achieved cold tolerance might be due to through mobilization of antioxidant systems, adjustment of the transcription factors and accumulation of osmoregulation substance. This work presented meaning information for revealing the anti-cold mechanism of the *M. Charantia* L. seedlings and new sight for further screening of anti-cold species in other plants (Yu *et al*., 2020).

Exogenous MT enhanced salinity stress tolerance in bitter melon through different mechanisms. Exogenously applied MT (150 µM) in salt-stressed plants improved growth and photosynthetic parameters, increased osmoprotectant through higher proline content, lowered oxidative stress by up-regulating antioxidant enzymatic activity, regulated ionic homeostasis and importantly, resulted in the transcriptional regulation of multiple defense-related genes. Furthermore, MT induced the transcription levels of genes linked to the secondary metabolites. Overall, it can be concluded that MT can be successfully employed as an effective priming agent for the amelioration of salt stress in bitter melon plants (Morteza *et al*., 2022). The fruit transcriptome of bitter gourd using 254.5 million reads (Phred score > 30) from the green rind, white rind, pulp, immature seeds, and mature seeds were studied which consisted of 125,566 transcripts with N50 value 2,751 bp, mean length 960 bp, and 84% completeness. Transcript assembly was validated by RT-PCR and qRT-PCR analysis of a few selected transcripts. The transcripts were annotated against the NCBI non-redundant database using the BLASTX tool (E-value < 1E−05). In gene ontology terms, 99,443, 86,681, and 82,954 transcripts were classified under biological process, molecular function, and cellular component which will serve as an important genomics resource (Kumar *et al*., 2022).

Molecular Breeding Approaches

Conventional breeding mainly depends on the selection of phenotype of desired traits among segregating populations which is extremely helpful for qualitative traits. However, in quantitative characters it is very difficult to select phenotypes among large populations which is time-consuming, more space and cost requirements and is highly influenced by the environment. Disease resistance breeding activities also often challenging mostly due to the quantitative nature and influence of the environment on symptoms that appeared. Hence, molecular markers are the potential tools that helps in overcoming the limitation linked with traditional breeding, since they are not affected by the environment, are non-destructive, evaluated for multiple traits simultaneously. However, there use required mapping population. Although, these are costly but in long term, exploitation in combination with multiple trait they become cost effective.

Molecular markers

Molecular markers are the variations arise due to point mutation/insertion/deletion/repetition in the genomic DNA sequences of different individuals. These can be identified by PCR-based methodology or by sequencing or by using restriction enzymes. The diversity in various morphological traits such as sex expression, fruit shape, size, color, and surface texture and maturity of *M. charantia* could enable DNA markers to assist in diversity analysis (Robinson and Decker-Walters, 1997; Behera *et al*., 2008). Limited studies are available on the use of polymorphic microsatellite markers in diversity analysis of bitter gourd, including random amplified polymorphic DNA (RAPD) (Dey et al., 2006b; Behera *et al*., 2008a,b; Paul *et al*. 2010), amplified fragment length polymorphism (AFLP) (Behera et al., 2008a; Gaikwad *et al*., 2008; Kole et al., 2009), simple sequence repeat (SSR) (Dhillon et al., 2016; Kole *et al*., 2009; Cui *et al*., 2017; Saxena *et al*., 2015; Ji *et al*., 2012), SCAR (Paul *et al*., 2010) and inter-simple sequence repeat (ISSR) (Singh *et al*., 2007; Behera *et al*.. 2008a), for the assessment of genetic diversity and population genetic structure. While the cost of SSR marker development is high, however based on its reproductivity, co- dominance nature, easy transfer once developed make it suitable for various genetic analysis with accurate results with least number of loci (Maughan *et al*., 1995). Among limited information available on SSR markers in bitter gourd, 16 SSR markers have been reported using the FIASCO technique (Guo *et al*., 2012; Ji *et al*., 2012), 11 SSR

markers designed through genomic library enrichment (Xu *et al*., 2011) and 43 SSR markers developed through cross-species transferability from other cucurbits (Chiba *et al*., 2003; Watcharawongpaiboon and Chun wongse 2008; Xu *et al*., 2011).

The major limitations of molecular markers are limited in number, and their association with few economically important traits in bitter gourd. Tang *et al*. (2007) suggested that higher number of markers are necessary to generate a high-density/saturated genetic map and also for fully exploitation of markers for marker-assisted selection. Generation of high-density genetic maps is the best way to identify the closely associated or functional markers for marker-assisted selection (MAS) and map-based cloning for fruit-related traits, gynoecium sex and yield (Rao, 2021). In this regard, first genetic linkage map generated by using 108 AFLP markers (Kole *et al*., 2012), later on many map was generated by using EST-SSR, AFLP and SRAP for gynoecious trait (Wang and Xiang, 2013), RAPD marker (OPZ 13, 700 bp) linked to (*gy-1*) gene (Mishra *et al*., 2014), ISSR marker associated with the gynoecious trait (Gaikwad *et al*., 2014), RAD-seq (restriction-associated DNA tag sequencing) to identify linked SNP for gynoecious trait (Matsumura *et al*., 2014), SNPs using genotyping-by-sequencing (GBS) technology mapping gynoecious trait (Rao *et al*., 2018). Shukla *et al*., (2015) detects the candidate gene and developed the functional markers in bitter gourd gynoecious line (Gy323) and monoecious line (DRAR1) through De novo transcriptome sequencing.

QTL mapping of horticultural traits

Kole *et al*. (2012) develops the genetic linkage map with 108 AFLP markers in the F_2 population developed from a cross between Taiwan White (*Momordica charantia* var. *charantia*) and CBM12 (*M. charantia* var. *muricata*) (Table 6). Results revealed that 12 QTLs for 5 fruit traits like length (2), diameter (1), number (4), weight (1) and yield (4) were detected on 5 linkage groups. Another linkage map was constructed for *M. charantia* using an F_2 population resultant from a cross between gynoecia and monoecious parent (Z-1-4 × 189–4-1). The map was generated by using EST-SSRs, SSRs, AFLP markers and SRAP markers spanned over 1005.9 cM with 12 linkage groups. Out of 43 QTLs, 1 QTL was detected on LG-5, which contained the most important QTLs for yield and yield related traits with high contributions towards phenotypic variance (5.8–25.4%) (Wang and Xiang 2013). A linkage map derived from RAD-seq (restriction associated DNA tag sequencing) analysis in the F_2 mapping population developed from a OHB61–5 (gynoecious) and OHB95-1A (monoecious) using 552 codominant markers. Findings revealed that SNP locus, GTFL-1 (5.46 cM) was the closest to the gynoecious locus (scaffold44_7277273) which transformed to DNA marker using invader assay technology, for further application in marker assisted selection of gynoecious trait in bitter gourd breeding program (Matsumura *et al*., 2014). Cui *et al*., (2018) constructed a restriction site associated DNA (RAD) based genetic map with 1009 SNP markers spanned over 2203.95 cM with 11 LGs using K44 (gynoecious) and Dali-11 (monoecious) parents derived F_2 population. A high-resolution genetic map was constructed by using 2013 SNP markers, spanning over 2329.2 cM on 20 LGs and 22 QTLs were analysed for major traits like node and days at first female flower appearance, gynoecy and sex ratio (Rao *et al*., 2018). To identify genetic loci controlling earliness, fruit, and seed related traits in bitter gourd genotyping-by-sequencing (GBS) approach was used to genotype 101 individuals of F4 population derived from a cross between an elite cultivar Punjab-14 and PAUBG-6. This population was phenotyped under net-house conditions for three years 2018, 2019, and 2021. The linkage map consisting of 15 linkage groups comprising 3,144 single nucleotide polymorphism (SNP) markers was used to detect the QTLs for nine traits. A total of 50 QTLs for these traits were detected which were distributed on 11 chromosomes. The QTLs explained 5.09–29.82% of the phenotypic variance. The highest logarithm of the odds (LOD) score for a single QTL was 8.68 and the lowest was 2.50. For the earliness related traits, a total of 22 QTLs were detected. For the fruit related traits, a total of 16 QTLs and for seed related traits, a total of 12 QTLs were detected. Out of 50 QTLs, 20 QTLs were considered as frequent QTLs (FQ-QTLs) by Kaur *et al*. (2022).

Bitter gourd genomics

A draft genome of bitter gourd was generated with the Japanese line (OHB3-1) by using Illumina sequencing with a size of 339 Mb provided an opportunity to develop more new molecular markers in bitter gourd genotypes (Urasaki *et al*., 2017). Based on the synteny, association of bitter gourd was more closely related to watermelon as compared to cucumber and melon (Yilmaz and Khawar, 2020). Further, bitter gourd genome was completely assembled to chromosome-level, with a maximum contig N50 close to 10 Mb, based on the Nanopore long-read assembler. This resequencing evident the divergence between wild and South Asian accessions about 6,000 y ago and Southeast Asian accessions about 800 y ago. Existence of large difference between two regional cultivar groups likely due to regional consumer preferences in different countries (Matsumura and Urasaki, 2020). Recently, Cui *et al*., (2020) completed whole-genome sequencing of the cultivar Dali-11 and the wild small-fruited line TR and resequencing of 187 bitter melon germplasm from sixteen countries. Studies showed that Bi clusters responsible for bitter taste (cucurbitane triterpenoids) are highly conserved in melon, cucumber and watermelon and it is absent in bitter gourd. Furthermore, phylogenetic analysis revealed that the TR group showing difference from *M. charantia*, may belong to a new species or subspecies. This study helps to achieve

Table 6: QTL reported for major quantitative traits in bitter gourd

References	*Trait*	*QTLs*	*R^2 (%)*	*Parents*	*Markers*	*Population*
Kole *et al*. (2012)	Fruit length	2	13.4	Taiwan White (*M. charantia* var. *charantia*) × CBM12 (*M. charantia* var. *muricata*)	108 AFLP markers; 11 linkage groups spanning a total of 3060.7 cM	146 F_2
	Fruit number	4	39.7	Taiwan White (*M. charantia* var. *charantia*) × CBM12 (*M. charantia* var. muricata)	108 AFLP markers; 11 linkage groups spanning a total of 3060.7 cM	146 F_2
	Fruit diameter	1	12.9	Taiwan White (*M. charantia* var.*charantia*) × CBM12 (*M. charantia* var. *muricata*)	108 AFLP markers; 11 linkage groups spanning a total of 3060.7 cM	146 F_2
	Fruit yield	4	38.1	Taiwan White (*M. charantia* var. *charantia*) × CBM12 (*M. charantia* var. *muricata*)	108 AFLP markers; 11 linkage groups spanning a total of 3060.7 cM	146 F_2
	Fruit weight	1	11.1	Taiwan White (*M. charantia* var. *charantia*) × CBM12 (*M. charantia* var. *muricata*)	108 AFLP markers; 11 linkage groups spanning a total of 3060.7 cM	146 F_2
Wang and Xiang (2013)	Stem diameter	2	23.6-25.3	Z-1-4 (gynoecios) × 189–41(monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Internode length	2	16.7-18.1	Z-1-4 (gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Female flower ratios	3	6.7-16.1	Z-1-4 (gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	First female flower node	3	12.2-21.4	Z-1-4(gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit length	4	11.2-16.2	Z-1-4 (gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit diameter	5	13.0-18.2	Z-1-4(gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Flesh thickness	2	8.9-12.1	Z-1-4(gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit shape	5	12.9-23.3	Z-1-4 (gynoecios) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit pedicel length	3	10.5-27.0	Z-1-4 (gynoecious) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit pedicel length ratios	5	5.1-33.1	Z-1-4 (gynoecious) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit weight	4	13.1-25.4	Z-1-4 (gynoecious) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Number of fruits	3	18.3-20.1	Z-1-4 (gynoecious) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
	Fruit Yield	2	7.4-15.9	Z-1-4 (gynoecious) × 189–41 (monoecious)	Total: 194 (26 EST-SSR, 28 SSR, 124 AFLP and 16 SRAP)	144 $F_{2:3}$
Gangadhara Rao *et al*. (2018)	Female flower ratios	9	6.35-20.95	DBGy-201 (gynoecious) × Pusa Do Mousami (monoecious)	2013 SNPs	90 F_2 and 65 $F_{2:3}$
	First female flower node	5	2.09-13.94	DBGy-201 (gynoecious) × Pusa Do Mousami (monoecious)	2013 SNPs	90 F_2 and 65 $F_{2:3}$
	Days to first female flower	8	0.05-58.75	DBGy-201 (gynoecious) × Pusa Do Mousami (monoecious)	2013 SNPs	90 F_2 and 65 $F_{2:3}$

Cui *et al*. (2018)	Gynoecy	2	11.2-59.6	K44 (gynoecios) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	First female flower node	2	12.0-32.0	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	Female flower number	2	21.2-52.8	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	Fruit wart	1	52.5-56.7	K44 (gynoecious s) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	Width of fruit ridge	1	17.7-30.9	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	White fruit color (W)	1	70.5-86.1	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	Fruit color	1	66.5-75.9	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2
	Fruit color	1	65.2-73.4	K44 (gynoecious) × Dali-11 (monoecious)	1009 SNPs	423 F_2

new insights into genetic diversity and domestication of bitter gourd and will facilitate the future genomics-enabled improvement program.

Conclusion

Apart from an important vegetable crop bitter gourd is a rich source of bioactive components with many medicinal properties (Horax *et al*., 2005). From last decade, we enhanced our understanding of taxonomy and phylogenetical relationship by using classical genetics. Breeder identified many numbers of genes associated with economic important traits and using them in further improvement of cultivars. However, incorporation of many traits both qualitative and quantitative needs support of molecular markers to reduce the time with more accuracy. For fully exploitation of the marker assisted breeding, it is important to keep the moderation between molecular marker technology and traditional breeding. Focus on development of molecular marker distributed in whole genome is also needed for fine mapping of important traits. Since very scanty information available on biotic stress and abiotic stress, there is need to emphasis on discovery of the trait and identification of closely linked DNA markers associated with resistance or tolerance to these traits. In this regard, crop wild relative can play an important role as a source of stress tolerance. Recently, with the availability of whole genome information, selection of biotic and abiotic stress tolerant genes along with heterosis related alleles can be easily accomplished in breeding programmes. Future breeding in bitter gourd improvement should be focus on development of nutritional rich high yielding varieties with resistance to disease, pest and climatic stresses with consideration of regional and foreign market preferences.

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सारांश

करेला (मोमोर्डिका चारेंटिया एल.) अपने पोषण, औषधीय और उपचारात्मक गुणों के लिए कद्दुवर्गीय की महत्वपूर्ण सब्जी के रूप में जाना जाता है। इसका फल स्वाद में कड़वा और कैल्शियम, फॉस्फोरस, लौह, तांबा, पोटेशियम और विटामिन (ए, बी 1, बी 2 और सी) से भरपूर होता है। दुनिया भर में मोमोर्डिका की लगभग 60 प्रजातियां पायी जाती हैं और उनमें से 7 प्रजातियां भारतीय उपमहाद्वीप में पाई जाती हैं (मोमोर्डिका चारेंटिया, एम. बाल्सामिना, एम. डियोइका, एम. कोचिनचिनेंसिस, एम. ट्यूबरोसा, एम. सुबंगुलाटा तथा एम. मैक्रोफिला), जो विभिन्न भू- और जलवायु-परिस्थितियों के कारण फलों के आकार, रंग, सतह की बनावट, इत्यादि के लिए विभिन्ता प्रदर्षित करता है। करेला में आनुवंषिकीय सुधार के लिए महत्वपूर्ण लक्षणों जैसे प्रारंभिक गांठों पर मादा फूल का शीघ्र आना, उच्च मादाःनर लिंगानुपात, अगेतीपन, फल का रंग, दृढ़ फल, धीमा बीज विकास, वांछनीय फल आकार, कमधारीवाला फल, मोटा गूदा, पर्ण मोजेक और फल मक्खी से प्रतिरोधी और निर्यात, डिब्बाबंदी और निर्जलीकरण के लिए उपयुक्तता पर अनुसंधान कार्य केन्द्रित रहता है। उपरोक्त लक्षणों को ध्यान में रखते हुए, सार्वजनिक और निजी बीज कंपनियों द्वारा विभिन्न उद्देष्यों के लिए कई किस्में और संकर प्रजातियों का विकास हुआ है। मोनोसिज्म एवं गाइनोसिज्म, उच्च उपज देने वाली किस्मों, संकर प्रजातियों और बीज उत्पादन के विकास में महत्वपूर्ण भूमिका निभाई है। मालिक्युलर मार्करों की संख्या में कमी एवं महत्वपूर्ण लक्षणों से उनका कमजोर जुड़ाव मालिक्युलर ब्रीडिंग के उपयोग को सीमित कर देता है। उच्च-घनत्व आनुवंषिक मानचित्रों का सृजन मार्कर चयन और फल-संबंधी लक्षणों, गायनोसिज्म और उपज के लिए तथा मैप-आधारित क्लोनिंग के लिए निकट से जुड़े या कार्यात्मक मार्करों की पहचान करने का सबसे अच्छा तरीका है। फसल संबंधी जंगली प्रजाति तनाव सहनषीलता के स्रोत के रूप में महत्वपूर्ण भूमिका निभा सकते हैं। संपूर्ण जीनोम की जानकारी एवं उपलब्धता, ओज से संबंधित एलील्स के साथ-साथ जैविक और अजैविक तनाव सहिष्णु जीन का चयन करके प्रजनन कार्यक्रमों में आसानी से पूरा किया जा सकता है।