Plant Archives Vol. 25, No. 1, 2025 pp. 275-288 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.042

A ROADMAP FOR SUSTAINABLE BITTER GOURD (*MOMORDICA CHARANTIA* L.) BREEDING: LEVERAGING GENETIC DIVERSITY AND EXPLOITING HETEROSIS

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(Date of Receiving-12-11-2024; Date of Acceptance-30-01-2025)

ABSTRACT

Hybrid breeding in bitter gourd offers significant potential for yield enhancement but relies heavily on understanding and effectively utilizing genetic diversity. Bitter gourd, a highly cross-pollinated crop with a wide range of genetic variability, envisages ample scope for improvement through heterosis breeding. This study reviewed research on the genetic diversity of bitter gourd germplasm and highlighted strategies for effective hybrid breeding. Our findings revealed that Bitter Gourd populations exhibit substantial genetic diversity, influenced by geographic origin and fruit characteristics. However, studies with limited geographic scope may not always reflect clear geographical clustering patterns. Furthermore, due to the high demand for preferred shape and size by customers, narrowing of the genetic base in commercial breeding programs has been observed. To optimize breeding efficiency and address this challenge, we propose a strategy modelled after successful programs in crops such as rice and maize, focusing on the formation of an extensive heterotic pool. This involves validating the heterosis of bitter gourd germplasm developed by the World Veg program to identify the most promising heterotic pools. Furthermore, each company can utilize this framework to assign their unique sets of lines from their breeding programs into defined pools using the HSGCA (combination of both specific and general combining ability effects) method of pooling. Such collaboration would enable private companies to leverage identified heterotic groups and high general combining ability (GCA) line, facilitating the development of a structured and efficient breeding program that sustainably delivers high-yielding hybrids with improved fruit quality. The inclusion of Gynoecious lines from diverse sources in these heterotic pool studies holds significant potential for further enhancing yield potential and overall breeding efficiency.

Key words : Bitter Gourd, Genetic Diversity, Gynoecious line, Heterosis, Hybrid breeding.

Introduction

Bitter gourd, renowned for its health benefits, is experiencing increased demand in Asian countries. However, the growing demand coupled with limited land resources poses a significant challenge. Hybrid breeding, which capitalizes on the superior performance of offspring from diverse parents, holds significant potential for increasing crop yields to meet growing global demands. Heterosis breeding has been long utilized in many crops, especially Corn. However, successful hybrid breeding necessitates a deep understanding of the genetic diversity within the target crop. While genetic diversity is the required base, a successful breeding program hinges on its operational efficiency and sustained improvement**.** To achieve this, breeders must adopt a multi-faceted approach. This includes assessing genetic diversity, understanding market demands, delving into the genetic basis of desired traits, focusing on improving Good General Combining Ability (GCA) of lines. Strategies such as dividing the diverse lines into “heterotic pools” – identifying 2-3 groups that can be designated as male and female pools – can significantly enhance the efficiency and sustainability of a breeding program. By focusing on intragroup crosses to improve GCA lines within each pool and strategically utilizing intergroup crosses for hybrid development, breeders can achieve a more targeted and resource-efficient approach. This is crucial because, a lack of such strategies can lead to rapid erosion of genetic variability within elite bitter gourd cultivars (Dhillon *et al*., 2016). This low genetic diversity renders the crop vulnerable to biotic and abiotic stresses and severely limits long-term yield improvements. Recognizing this critical issue, Dhillon *et al.* (2020) at the WorldVeg centre have undertaken significant efforts to bridge this gap by introducing new variability into bitter gourd germplasm. These efforts have had a tangible impact, with this enhanced germplasm currently being utilized by 35-40 private companies. However, successfully integrating this new variability into existing breeding programs while simultaneously identifying heterotic pools within the enhanced diversity remains crucial for optimizing breeding efforts and ensuring the long-term sustainability of breeding programs.

This review highlights key findings from various studies on the genetic diversity of bitter gourd accessions, which is subdivided into headings like, different methods employed by researchers to group the genotypes into different clusters based on similarity and dissimilarity, Relationship between distance between genotype and their Geographical distribution, Key Traits contributing to Diversity among germplasm, Gynoecious line forming separate group, Intra cluster and Inter Cluster distance and Distribution pattern of genotypes into different Cluster and Molecular analysis of Diversity using SSR markers. Following this, the review delves into the critical aspect of utilizing this identified genetic diversity to form effective heterotic pools in bitter gourd breeding programs. It explores and compares different approaches for establishing heterotic pools, providing valuable insights for breeders seeking to maximize hybrid vigour.

Centre of Origin and Diversity for Bitter gourd

Understanding the centre of origin and centre of diversity of a crop is paramount for successful plant breeding programs. These regions represent critical hotspots of genetic diversity, harbouring a wealth of genetic resources essential for developing improved cultivars. This includes developing cultivars with enhanced agronomic traits, increased disease resistance and improved adaptability to changing environmental conditions. This knowledge is crucial for developing effective breeding strategies to address the challenges of a changing world. Earlier studies in bitter gourd pointed towards eastern Asia, possibly eastern India or Southern China, as the most likely birthplace of the *Momordica* genus (Walters and Decker-Walters, 1988). However, the advancements in molecular research suggest Africa as the primary centre of origin for the entire *Momordica* genus. As per the recent research by Schaefer *et al*. (2009), *Momordica* is a monophyletic group (all species share a recent common ancestor) that originated in tropical Africa. As per the research, Asian species within the genus, including bitter gourd, are believed to have resulted from a single long-distance dispersal event approximately 19 million years ago.

However, when it comes to centre of Diversity, the exact place of bitter gourd domestication is unknown or unclear due to lack of archaeological evidence supporting its domestication in one region, but proposed areas by various researchers include southern China, eastern India, and southwestern India (Sands, 1928; Degner, 1947; Walters and Decker-Walters, 1988; Raj *et al*., 1993; Robinson and Decker-Walters, 1997; Joseph, 2005). India is widely considered the likely place of origin for bitter gourd cultivation. This belief is supported by several lines of evidence. Firstly, the earliest written records of bitter gourd cultivation originate from India, dating back to 2000-200 BCE (Decker-Walters, 1999) and its use in Ayurvedic medicines and mention in several Sanskrit literature (Sivarajan and Balachandran, 1994). Secondly, the presence of both domesticated and wild bitter gourd progenitors within India suggests a long history of cultivation and evolution within the region. In contrast, the earliest reference to bitter gourd in China is from northern China and was reported in Dian nam Pen Tsao in 1370 (Yang and Walters, 1992), suggesting that it was domesticated in India and subsequently introduced to China. Recent attempts by Marr *et al*. (2004) on investigation of domestication history of bitter gourd using genetic and morphological data also remain inconclusive due to limited samples representing some potential geographies like Eastern India, Southern China and Southeast Asia. Further research with broader geographic sampling is crucial to pinpoint the exact origin of bitter gourd.

Recent research indicates that Africa can be considered the Centre of Origin for the *Momordica* genus. The Centre of Diversity for bitter gourd is a contentious issue between China and India. Population structure analyses conducted by various researchers indicate that bitter gourd commercially cultivated in South Asian countries, such as India, Sri Lanka, Pakistan, and Bangladesh, is genetically distinct from those grown in North Asia and Southeast Asia, including China, Thailand, Vietnam, Indonesia and Philippines. Bitter gourd requirement in South Asia is characterized by spiny fruits ranging from 5 cm to 25 cm in length while in North and Southeast Asia preference is for smooth, elongated fruits measuring 20 to 35 cm. From a breeding perspective, it is recommended to classify samples from South Asia as a separate population from that of North and Southeast Asia to maintain healthy genetic distance. When considering germplasm collection for breeding programs in India, prioritizing regions in East India and Southwest India is essential.

Methods for Grouping Genotypes based on Genetic Diversity

Researchers have employed various multivariate statistical techniques to evaluate genetic diversity in bitter gourd germplasm. One prevalent method is Mahalanobis D^2 analysis, which calculates genetic distances among genotypes (Tyagi *et al.*, 2017; Maurya *et al.*, 2018; Jatav *et al.*, 2019; Kumar *et al.*, 2020). Bhati *et al.* (2023) used Mahalanobis D^2 analysis to classify 20 bitter gourd genotypes into distinct clusters based on their genetic divergence. In addition to Mahalanobis D^2 analysis, Principal Component Analysis (PCA) is another method available for grouping genotypes. Mallikarjun *et al.* (2023) effectively utilized PCA to cluster genotypes. PCA is a valuable multivariate technique for assessing genetic diversity and can complement the findings from Mahalanobis D^2 analysis, as it helps to reveal patterns of variability among genotypes based on multiple traits.

Relationship between distance between genotypes and their geographical distribution

The relationship between the distance between genotypes and their geographical distribution in Bitter Gourd reveals a complex interplay between genetic diversity and geographic factors. Singh and Kandasamy (2020) conducted a study on the genetic diversity of Bitter Gourd using 40 genotypes and observed significant variation among them. The research involved grouping these genotypes into six clusters through a clustering technique, highlighting the genetic divergence present within the species. Interestingly, their study revealed that genotypes from different geographical regions could cluster together, while genotypes originating from the same location might be placed in separate clusters. This suggests that genetic diversity arises from a combination of factors beyond just geographical separation.

Similar findings regarding clustering patterns that are independent of geographical origin are reported by several researchers (Devmore *et al.*, 2007; Dey *et al.*, 2007; Islam *et al.*, 2010; Resmi and Sreelathakumary, 2012; Singh, 2013b; Singh *et al.*, 2014; Tyagi *et al.*, 2017; Angadi and Mulge, 2018; Maurya *et al.*, 2018; Jatav *et al.*, 2019). These collective observations emphasize the importance of considering genetic diversity as a primary criterion for selecting genotypes for hybridization programs in bitter gourd, rather than relying solely on geographical origin. According to Resmi and Sreelathakumary (2012), the lack of correlation between genetic diversity and geographical distance suggests that factors other than geographical origin—such as genetic stock exchange, genetic drift, natural mutation, spontaneous variation, or both natural and artificial selection—may contribute to the observed genetic diversity. A critical observation across these studies is the narrow scope of germplasm utilized, often comprising fewer than 50 accessions collected from a limited geographical area.

Key traits contributing to Diversity among germplasm

High genetic diversity within a population is a vital resource for enhancing the genetic base of any crop breeding program. This diversity enables breeders to select parents with complementary traits, resulting in the development of superior offspring (Dhillon *et al.*, 2016; Pandey *et al.*, 2019; Alhariri *et al.*, 2021). The significant variation observed in morphological traits such as days to flowering, number of fruits per plant, fruit size and shape and yield per plant underscores the abundant genetic diversity present within bitter gourd germplasm.

Numerous studies have explored the specific traits that significantly contribute to genetic divergence in bitter gourd (*Momordica charantia*) (Angadi and Mulge, 2018 and Maurya *et al.* 2018). The results indicated that fruit weight is the primary contributor, followed by fruit yield and fruit length. Additionally, Jatav *et al.* (2019) and Singh *et al.* (2013b) both recognized fruit weight as a crucial factor, with Jatav *et al.* (2019) also identifying vine length as another important trait. Conversely, Singh *et al.* (2013b) found that vine length and yield per plant had minimal effects on genetic divergence. In contrast, Tyagi *et al.* (2017) highlighted vine length as the most significant trait influencing divergence, followed by fruit diameter and average fruit weight. While these studies differ slightly in the ranking of specific traits, they all emphasize the importance of considering genetic diversity when selecting parents for breeding programs in bitter gourd. By leveraging this rich genetic diversity and focusing on key traits like fruit weight, fruit length and vine length, bitter gourd breeders can advance the development of superior varieties with improved yield and other desirable agronomic characteristics.

Gynoecious line forming separate group

Several studies have predominantly gynoecious lines in their diversity analysis experiment to investigate the clustering patterns of gynoecious (predominantly female flowering) lines in bitter gourd. In some instances, gynoecious lines clustered with monoecious genotypes—those that produce both male and female flowers—likely due to shared traits such as earliness, sex ratio and fruit characteristics (Alhariri *et al*., 2021; Mallikarjun *et al*., 2023). Two specific gynoecious lines, PVGy-1 and PDMGy-201, along with a predominantly gynoecious variety, Pusa Aushadhi, were identified as valuable resources for breeding early-flowering and early-fruiting bitter gourd cultivars (Alhariri *et al*., 2021). However, contrasting findings were also reported, indicating that gynoecious lines sometimes formed distinct clusters (Jatav *et al*., 2019). Additionally, an exceptional sub-gynoecious line, PBIG-22, was noted for its highly female-biased flower ratio (1 male to 5.5 females) and desirable agronomic traits.

Despite these variations in clustering patterns, researchers like Alhariri *et al*. (2021); Jatav *et al*. (2019); Ram *et al*. (2002) and Khan and Behera (2011) all acknowledge the potential of gynoecious lines. These lines, which produce only female flowers, can mature earlier and potentially yield more fruit. This suggests that using gynoecious lines as parents in breeding programs could enhance hybrid vigour, leading to significantly improved yields. Many researchers have conducted further research to validate the contribution of gynoecious lines to yield in progeny; the results from such studies are presented in the heterosis section.

Intra-cluster and Inter-cluster distances in Diversity analysis and Distribution of Lines Across clusters

Intra-cluster and inter-cluster distances are crucial metrics in diversity analysis, particularly in the context of genetic studies. These distances help assess the genetic variation among different genotypes and make informed breeding strategies. Numerous researchers have noted that intra-cluster distance, which quantifies the genetic differences between genotypes within a cluster, is typically lower than inter-cluster distance. This indicates that the variability among genotypes from different clusters is greater than that among those within the same cluster (Tyagi *et al*., 2017; Maurya *et al*., 2018; Jatav *et al*., 2019; Kumar *et al*., 2020; Bhati *et al*., 2023). This pattern indicates that crosses between genotypes from different clusters are likely to exhibit greater genetic diversity, which can potentially lead to the development of superior offspring. Resmi and Sreelathakumary (2012) noted that all short-type genotypes clustered together, highlighting a high degree of genetic similarity within that specific group. However, some researchers have observed exceptions to this trend. Angadi and Mulge (2018) and Singh *et al*. (2013b) reported instances where intra-cluster distance was higher than inter-cluster distance. For example, Singh *et al*. (2013b) found the maximum intra-cluster distance in cluster 6. Similarly, Kumar *et al*. 2020) observed an inter-cluster distance lower than some intra-cluster distances. These findings suggest that genetic diversity can be present within clusters as well. Importantly, high intra-cluster distance can also be informative. It indicates sufficient diversity within a group for further breeding population development, even if crosses between clusters might still be generally preferred. Additionally, studies by Alhariri *et al*. (2021) and Mallikarjun *et al*. (2023) have shown that distinct lines can sometimes group together within the same cluster. For example, they observed gynoecious lines (predominantly female flowering) co-existing with monoecious lines (both male and female flowers) in the same cluster. Mallikarjun *et al*., 2023) also reported some genotypes of *Momordica charantia* var. muricata clustering with cultivated *M. charantia* var. *charantia*, possibly due to similar fruit types. In a separate study, Kumar *et al*. (2020) also observed that genotypes Katahi and Jhalari were grouped together in both multivariate analysis and molecular characterization despite exhibiting significant phenotypic differences: Katahi is spiny with a 15 cm length, while Jhalari has smooth skin and exceeds 20 cm in length.

Distribution of the number of lines to each cluster is influenced by factors such as sample size and the level of genetic variation within the germplasm. Several studies consistently observed the presence of mono-genotypic clusters within bitter gourd germplasm, indicating a varying degree of genetic similarity among different lines. Kumar *et al*. (2020) identified two major clusters and several mono-genotypic clusters among 20 genotypes. Similarly, various studies also observed a significant number of mono-genotypic clusters (Resmi and Sreelathakumary, 2012; Kumari *et al*., 2017; Jatav *et al*., 2019; Bhati *et al*., 2023). Singh *et al*. (2013b) and Maurya *et al*. (2018) independently categorized 30 genotypes into six distinct clusters, observing notable variations in the sizes of these clusters. Angadi and Mulge (2018) further corroborated this trend by analysing 55 genotypes, which resulted in the formation of 10 clusters, eight of which contained only two genotypes each. These studies collectively underscore the genetic diversity found within bitter gourd germplasm and highlight the significance of clustering in understanding the genetic relationships among various lines. The classification of lines into different clusters and their respective quantities can vary considerably based on the number of traits or markers and the specific germplasm used in the analysis. It is essential to recognize that, due to the high level of genetic diversity, distinct lines may sometimes be grouped together, while similar lines may be separated. A key to success in any breeding program is to simplify the analysis by identifying patterns, particularly by focusing on the performance of key lines, such as the parents of successful hybrids.

Molecular approach for Diversity analysis

Molecular markers, particularly Simple Sequence Repeats (SSRs), have proven to be valuable tools for assessing genetic diversity in multiple crops. SSR markers offer several advantages, including multi-allelic nature, co-dominant inheritance, and high levels of polymorphism. Recent studies have utilized SSR markers to characterize genetic diversity within and among bitter gourd populations. These markers have been instrumental in identifying genetic relationships, understanding population structure and selecting superior parents for breeding programs. Given the importance of SSR markers in understanding genetic diversity and structure, this review focuses on their application in bitter gourd research.

Saxena *et al*. (2015) pioneered the development of SSR markers for *Momordica* by sequencing a small insert genomic library enriched for ten different repeat motifs. This ground breaking work resulted in the identification of 160 novel microsatellite markers for bitter gourd (*M. charantia*). These markers have significant potential for genetic diversity studies, breeding programs, and conservation efforts. To demonstrate their utility, Saxena *et al*. (2015) used a subset of these markers to assess genetic diversity among 54 bitter gourd genotypes. UPGMA-based clustering analysis revealed three major clusters and several smaller clusters. Interestingly, exotic and indigenous accessions tended to group separately. Furthermore, the analysis of 15 Indian-released varieties highlighted the discriminatory power of these 28 polymorphic markers, suggesting their potential for genetic mapping, variety protection, and hybrid seed purity testing. In an independent study, Dhillon, *et al*. (2016) used a publicly available transcriptome dataset to develop SSR markers for bitter gourd. After cleaning and assembling the sequence reads, they identified potential SSR regions. Primers were designed for 100 of these SSRs and tested on a diverse set of bitter gourds and *Momordica* balsamina accessions. Out of these 100 markers, 50 markers with high polymorphism were selected for further analysis of genetic diversity in 114 bitter gourd accessions collected across South and South Asian countries. Results revealed distinct clusters largely reflecting geographic origins. South Asian accessions formed a cohesive group, while Southeast Asian and Taiwanese accessions were genetically distinct. Interestingly, fruit colour and shape were associated with specific genetic clusters, emphasizing the importance of considering both genetic and morphological traits in breeding programs. A comprehensive analysis of four cucurbit genomes identified a substantial number of SSR markers, particularly in bitter gourd (Cui *et al*., 2017). The majority of these SSR motifs were A/T-rich. From the identified SSRs, over 138,000 unique primer pairs were designed for bitter gourd. A subset of 71 primer pairs was successfully validated, demonstrating their utility for genetic analysis. They further expanded the study using a subset of 21 polymorphic SSR markers on 211 bitter gourd accessions from diverse geographic origins including North Asia unlike earlier study. Notably, South Asian and Chinese bitter gourd populations formed distinct clusters, corroborating Dhillon’s findings. This emphasizes the significant role of geographic origin in shaping genetic diversity. These results contrast with earlier diversity studies that were primarily based on phenotypic characteristics and relied on small sample sizes collected from limited geographical regions.

Alhariri *et al*. (2021) and Mallikarjun *et al*. (2023) conducted independent studies to assess genetic diversity in bitter gourd using SSR markers. Both studies identified distinct clusters based on fruit size, with small and medium-fruited genotypes grouping together, and long and extra-long fruited genotypes forming another cluster. These findings align with Dhillon *et al*. (2016), who emphasized the significance of morphological traits in shaping bitter gourd genetic diversity. Building on these findings, Meghashree *et al*. (2024) conducted a deeper investigation into genetic diversity by incorporating nutritional traits alongside markers. Their analysis revealed significant variations in nutritional traits, such as ascorbic acid and mineral content, in addition to genetic diversity assessed using InDel and SSR markers. The study identified two main genetic groups and four subgroups, further supporting the complex genetic structure of bitter gourd.

A key difference between Alhariri’s study and previous research lies in the diversity of the germplasm used. Alhariri’s study included a broader range of accessions, encompassing most of the Gynoecious lines and commercially cultivated varieties from India. In contrast, earlier studies often focused on a wider geographic range, potentially leading to different clustering patterns due to the varying genetic makeup of the accessions. This makes the markers used in Alhariri's study particularly valuable for fingerprinting, genetic mapping, and association analysis of bitter gourd varieties, especially those of Indian origin. However, for studies involving a wider geographic range, including Southeast and North Asia, the marker sets developed by Saxena *et al.* (2015); Dhillon *et al.* (2016) and Cui *et al.* (2017) would be more suitable.

These studies collectively underscore the critical role of understanding genetic diversity in advancing bitter gourd breeding through the use of advanced molecular techniques. Employing molecular markers provides deeper insights into genomic variation and offers a more reliable assessment compared to phenotypic diversity analysis, as it is not influenced by environmental factors. However, the results obtained from molecular marker analysis are dynamic and can vary significantly depending on the number and distribution of polymorphic markers used in the study.

Population structure of bitter gourd genotypes

Until recently, the genetic diversity of bitter gourd using genomic information was not well understood due to limited germplasm resources and the availability of molecular markers. Only a few studies have explored the population structure of bitter gourd genotypes.

A model-based approach (STRUCTURE HARVESTER) was utilized to investigate the population structure of bitter gourd (Dhillon *et al.*, 2016; Cui *et al.*, 2017; Alhariri *et al.*, 2021; Mallikarjuna *et al.*, 2023). While three studies identified three main population groups (with value of 3), Mallikarjuna's study revealed six (K=6) distinct groups. This difference in the number of groups may be attributed to factors such as sample size, geographic origin, and genetic diversity within the studied populations. These studies have consistently highlighted the influence of geographic origin, morphological traits, and genetic factors on the formation of distinct genetic groups. Dhillon and Cui's studies revealed that genetic diversity in bitter gourd is often structured along geographic lines, with South Asian and Southeast Asian accessions forming distinct clusters (Dhillon *et al.*, 2016; Cui *et al.*, 2017). This suggests that geographic isolation has played a significant role in shaping the genetic diversity of bitter gourd. Alhariri and Mallikarjuna's studies further delineated the genetic diversity within bitter gourd populations. They identified distinct subpopulations based on fruit size, shape and maturity. These studies also revealed evidence of gene flow between different varieties, particularly between *M. charantia* var. muricata and *M. charantia* var. charantia.

All four studies found a strong correlation between the results from Structure analysis and UPGMA clustering. The distribution of genotypes into different groups was consistent across both methods.

These findings provide valuable insights into the genetic diversity and population structure of bitter gourd, which can be leveraged to inform and optimize breeding strategies. For example, a crucial question for any breeding program is whether South and Southeast Asian populations should be considered as two separate breeding pools, leading to crosses between them for heterosis exploitation, or if they should be treated as distinct populations with subsequent identification of breeding pools and heterotic patterns within each population. This consideration is crucial for maintaining a healthy level of genetic diversity and maximizing the potential for heterosis. Studies on the relationship between genetic distance and heterosis can provide valuable data to inform this crucial decision.

Relationship between Genetic distance and Heterosis

The relationship between genetic distance and heterosis in bitter gourd (*M. charantia*) has been explored in various studies, revealing significant insights into how genetic diversity influences hybrid vigour. Laxuman *et al.* (2012) explored the connection between parental genetic diversity and heterosis in bitter gourd. Their study involved the analysis of 28 hybrids derived from eight genetically diverse parental lines. The parents were categorized into four divergence classes based on genetic distance: high, low, and two intermediate levels. Notably, hybrids from parents with moderate genetic divergence (DC2 and DC3) demonstrated higher levels of heterosis compared to those from parents with either high or low divergence. The study highlights the importance of balancing genetic diversity in parent selection. While moderate divergence seems to be optimal for maximizing heterosis, extreme divergence can lead to negative effects. Similarly, Singh and Gupta (2019) investigated the relationship between genetic distance (GD) and heterosis in pearl millet. Their findings revealed a low to moderate, but statistically significant, positive correlation between GD and heterosis for grain yield. Likewise attempts to establish a relationship between genetic distance and Heterosis is also conducted in vegetable crops. Brinjal hybrids derived from crossings between parents with moderate genetic distance exhibited more heterosis than those resulting from parents with a very high genetic distance (Rajan *et al.*, 2023). Similarly, the genetic distance in Cucumber doesn't have a significant linear correlation with heterosis for yield-related traits (Olfati *et al*., 2012; Liu *et al*., 2022).

These findings suggest that genetic distance can serve as a reliable predictor of heterosis in parents, but only up to a certain threshold. Genetic distance has limitations for heterosis prediction, as the genetic distance between parents of hybrids is calculated by their divergent genetic locus (Yu *et al*., 2005), but these divergent genetic loci do not have a linear relationship with heterosis related QTLs. Besides, the contribution of these divergent genetic loci to heterosis is not equal, with some even having no contributions. Moreover, among various traits, the cucumber yield traits are only related to a few major QTLs (Yuan *et al*., 2008; Pan *et al*., 2020). Dias *et al*., 2004) further highlighted the complexity of this relationship by reviewing 54 studies across various crops. They found that 28 studies showed a positive correlation between GD and hybrid performance, while the remaining 26 reported negative correlations or inconclusive results. Therefore, whether the genetic distance is effective for heterosis prediction depends upon the proportion of divergent genetic locus having a contribution to heterosis.

Heterosis in Bitter Gourd

Heterosis has been extensively studied to enhance yield and quality traits in bitter gourd. Several studies indicate that various hybrids exhibit significant heterosis for different morphological and yield-related characteristics. Kumari *et al*. (2020) found that several bitter gourd hybrids exhibited significant heterosis for fruit yield, surpassing both their parents and standard check varieties. The extent of heterosis varied among different crosses, with some hybrids showing substantial improvements in yield. The mid parent heterosis for yield of marketable fruit per plant ranged from -18.11% to 58.77% and heterobeltiosis ranged from -39.34 percent to 45.65 percent and standard heterosis ranged from -14.35 to 45.65 percent. A similar study identified promising hybrid combinations, such as Katheri × BGCV-2, Katheri × OBGCS-1, and Katheri × OBGCS-2, which exhibited significant heterosis for total fruit yield (Kumar *et al*., 2020). Both studies highlighted the potential of heterosis breeding to develop high-yielding Bitter Gourd hybrids. Similarly, Bhardwaj and Singh (2022), evaluated 45 bitter gourd hybrids to assess the extent of heterosis for yield and related traits. Significant genetic variability was observed among the hybrids, with most showing positive heterosis for fruit characters and yield and expressed high heterosis for yield components, indicating the additive or synergistic effect on the fruit yield. Ten specific cross combinations were identified as promising due to their high heterosis for both yield and yield components. Thangamani and Pugalendhi (2013) evaluated 90 bitter gourd hybrids, identifying significant variation in traits like earliness, yield, and fruit quality. Some hybrids, such as 'CO-1' x 'MC-105', showed improved earliness, while others, like 'UB' x 'GL', exhibited a favourable shift in sex ratio. The hybrid 'CO-1' x 'MC-105' displayed the highest positive heterosis for fruit size, and 'Preethi' x 'MC-30' exhibited the highest positive heterosis for fruit yield. Based on these findings, four promising hybrids were selected for further testing across multiple locations. These combinations have the potential to be exploited for commercial hybrid development.

Preliminary studies quantifying heterosis in bitter gourds provide strong evidence that strategically exploiting this phenomenon can yield significant advantages, a cornerstone of successful breeding programs. This strategic approach necessitates integrating market demands with a deep understanding of genetic diversity and the formation of effective heterotic pools within each breeding program. By capitalizing on the inherent genetic diversity within bitter gourd germplasm, breeders can develop superior hybrid varieties that exhibit increased yield, enhanced fruit quality, and improved disease resistance.

Formation of Heterotic Groups

This study has underscored the considerable genetic diversity found within bitter gourd germplasm, which serves as a valuable resource for increasing genetic variability in breeding populations. The formation of heterotic groups is essential for improving hybrid breeding programs, especially in crops like bitter gourd. By identifying and classifying these groups, breeders can maximize hybrid vigour through the selection of suitable parental lines that possess complementary traits. Studies utilizing both phenotypic and molecular markers consistently demonstrate that bitter gourd germplasm can be clustered into 3-8 distinct groups. However, as Dias *et al*. (2004) reported in his review paper, a variable relationship exists between genetic distance and hybrid performance across various crops, hence the effectiveness of genetic distance in predicting heterosis needs further validation. This can be achieved by conducting crosses both within and between clusters and subsequently evaluating economic heterosis. Such systematic approach helps in leveraging genetic diversity and developing high-yielding hybrids with optimized resources. When working with multiple diverse groups, identifying the most promising groups is crucial. A heterosis validation experiment can effectively identify the top-performing groups based on yield and other desirable traits. Fan *et al.* (2009) noted that no method for classifying heterotic groups is flawless, largely due to the extensive array of potential genetic combinations. Despite this, implementing a well-structured system can greatly enhance breeding efficiency. The primary objective is to identify heterotic groups that reliably generate superior hybrids through inter-group crosses. This approach enables breeders to streamline the hybrid development process, focusing on combinations that maximize hybrid vigour while minimizing unnecessary trials.

By understanding the genetic relationships among groups, breeders can prioritize specific groups for further development, potentially classifying them as male and female groups. This strategic approach, long utilized in crops like maize with well-established heterotic pools, accelerates the development of improved cultivars with desirable traits such as high yield, disease resistance, and superior fruit quality. Recent efforts have extended this approach to other crops, including rice and pearl millet. For example, Singh and Gupta (2019) classified 147 pearl millet lines into eight distinct groups based on molecular marker analysis. Representative lines were selected from each group, considering both the number of parents within the group and their genetic distances. These representative lines were then crossed with each other to identify the heterotic pattern among the groups. Based on the analysis, eight groups were reclassified into four heterotic pools: two for seed parents (HPB1 and HPB2) and two for restorer parents (HPR3 and HPR4). Among these groups, the cross between HPB1 and HPR3 exhibited the highest level of heterosis, demonstrating significant potential for developing higher-yielding pearl millet hybrids. Similarly, Xie *et al.* (2014) investigated heterotic patterns in tropical hybrid rice breeding, an area lacking clear information despite over 30 years of commercialization. Using molecular markers, 168 parental lines were divided into two Restorer groups and four Maintainer groups. Out of these 168 parental lines, 18 were selected for further heterosis experiments. Based on SSR molecular data and field trial results, four groups were identified as potential heterotic groups within the IRRI hybrid rice germplasm for developing tropical hybrids. Conversely, parents in two marker-based groups were identified with limited breeding value due to their lowest contribution to heterotic hybrids. Upon correlating the placement of parental lines with commercially released hybrids in the Philippines, it was found that 95% of the parental lines belonged to the identified heterotic groups, further supporting the validity of the proposed heterotic groups. However, in vegetable crops, such as bitter gourd, this area requires further research. A study by Laxuman *et al.* (2012) selected eight lines, which were classified into four groups with two lines in each group. Half-diallel crosses were made between these lines, and the F_1 progeny were evaluated to estimate heterosis. The data revealed that the average mid-parent heterosis of crosses made between Group IV and Group II was significantly higher than that of other groups. All four crosses between these two groups exhibited positive mid-parent heterosis, while three of them were significant at the 1% level. Conversely, all intragroup crosses, except one in Group IV, exhibited highly significant negative heterosis. This finding further supports the observation that lines within the same group are genetically similar and, therefore, less likely to exhibit heterosis. Consequently, focusing on intergroup crosses, particularly those involving Group II and Group IV, is more likely to yield successful hybrid combinations.

In summary, from the above examples, it is evident that conducting validation experiments is crucial for identifying the most promising heterotic groups. Prioritizing these groups with most breeding resources will significantly enhance the efficiency and success of hybrid development programs.

Use of Gynoecious lines for Heterosis Exploration in Bitter gourd

The potential of using gynoecious or predominantly gynoecious lines to improve yield based on them per se performance has been recognized by numerous researchers. Subsequent sections of this review will highlight studies that have validated the potential of gynoecious lines in increasing yield and demonstrating significant heterosis in their progeny. Sunny (2022) research investigated the effectiveness of using gynoecious lines as female parents in bitter gourd breeding for heterosis (hybrid vigour). His findings demonstrated that hybrids developed with Gynoecious lines displayed significant heterosis for traits related to earliness. These traits included days to first female flower opening, node bearing the first female flower, and days to first harvest. The hybrids also exhibited a lower sex ratio (fewer male flowers) and a higher number of fruits per plant, contributing to increased yield. Similar observations were reported by previous researchers who utilized different gynoecious lines in their heterosis breeding programs (Behera *et al.*, 2009; Dey *et al.*, 2012; Alhariri *et al.*, 2018). Behera (2009) even identified promising gynoecious lines (DGy201 and DGy202) that were subsequently used in crosses with monoecious lines.

Studies published in 2011 (Khan and Behera) demonstrated that these hybrids significantly outperformed control varieties in terms of yield. This highlights the significant potential of gynoecious lines for exploiting heterosis (hybrid vigour) to achieve superior yield and earliness in bitter gourd breeding programs. In analogy to CMS-based crops such as rice and pearl millet, Gynoecious and Monecious lines can be classified as two broad genetic groups in bitter gourd.

Novel approach for Heterotic Group formation

Given the inherent limitations of existing heterotic pooling methods, the need for innovative approaches to group germplasm effectively has become increasingly apparent. To overcome the limitations of conventional methods, which often rely on analysing diversity followed by validation experiments, researchers have explored the utilization of General Combining Ability (GCA) and Specific Combining Ability (SCA) to classify lines into distinct heterotic groups. The following section will delve into the research conducted in this area and provide a comparative analysis of the efficiency of different approaches.

Analysing high-performing hybrids reveals that both additive and non-additive genetic effects contribute to heterosis. Studies indicate that while additive effects arise from the cumulative influence of multiple genes, non-additive effects, including dominance and epistasis, play a crucial role in the expression of hybrid vigour (Laxuman *et al.*, 2012; Singh *et al.*, 2013a). Interestingly, it was noted that 66% of the crosses exhibiting high heterosis had at least one parent with low general combining ability (GCA) effects, indicating the significance of specific gene combinations (Liu *et al.*, 2022). These findings emphasize the need to consider both GCA and SCA for effective breeding program design. High x low general combining ability combinations are suitable for heterosis breeding. High x high general combining ability combinations can be considered for developing superior variants through pedigree method (Singh *et al.*, 2013).

Fan *et al.* (2009) proposed a novel approach, the Heterotic Group Specific Combining Ability (HSGCA) method, which considers both general combining ability (GCA) and specific combining ability (SCA) effects to assign lines to heterotic groups. The HSGCA method has demonstrated superior performance compared to traditional methods like SSR-based classification and SCA_PY. The HSGCA method significantly improved breeding efficiency compared to traditional methods like SSR-based classification by 16.7% and 23.7% when compared to SCA_PY. By incorporating both GCA and SCA information, HSGCA can more accurately predict the potential of a line to produce high-yielding hybrids. This method has been shown to reduce the number of missed opportunities for identifying superior hybrids, leading to increased breeding efficiency. To effectively implement the HSGCA method, it is crucial to identify representative tester lines for each heterotic group. These tester lines serve as benchmarks for evaluating the combining ability of new inbred lines. In conclusion, the HSGCA method offers a valuable tool for maize breeders to optimize their breeding programs. By considering both GCA and SCA effects, this method can help identify the most promising crosses and accelerate the development of high-yielding maize hybrids.

A major limitation of heterotic grouping methods, such as SCA and HSGCA, is their reliance on a single trait, typically grain yield. This can be problematic as grain yield is a complex trait which is controlled by polygenes and has low heritability especially under stress environments. To address this issue, a new approach, Heterosis based on General combining ability of multiple traits (HGCAMT) (Badu-Apraku *et al.*, 2013) is proposed. As HGCAMT considers the GCA effects of multiple traits which are more heritable and can be improved over selection, it provides a more comprehensive assessment of inbred line potential. By incorporating information on traits like disease resistance or drought tolerance, HGCAMT can help identify lines with desirable combinations of traits and develop superior combinations specially where resistance or tolerance to multiple stresses is a major breeding objective. This approach can lead to more accurate and reliable heterotic group classifications, ultimately improving breeding efficiency and the development of superior hybrids.

Following the proposal of different methods of heterotic grouping many researchers started comparing the efficiency of different methods. In a significant study, researchers conducted a comparative analysis of heterotic grouping that focused on general and specific combining ability effects of grain yield (HSGCA), heterotic grouping based on the GCA effects of multiple traits (HGCAMT), and the use of DArTseq (Diversity Array Technology sequencing) markers (Adewale *et al.*, 2023). Their findings contribute to the ongoing discussion regarding the most effective methods for classifying genotypes into heterotic groups, which is essential for optimizing hybrid breeding strategies. They observed similar groupings under stress and control conditions using HSGCA and HGCAMT. The HSGCA method demonstrated the highest accuracy in classifying lines across different environments, with a success rate of 87%. DArTseq followed closely with a success rate of 76%, while HGCAMT achieved a success rate of 68%. Both HSGCA and DArTseq methods were found to be effective in classifying lines across different environments, with breeding efficiencies of 52.8% and 52.5%, respectively. Interestingly, both methods also exhibited some similarity in placement of lines into different groups. Several studies have reported HSGCA as the most efficient method for classifying inbred lines across different environments (Akinwale *et al*., 2014; Amegbor *et al*., 2017; Annor *et al*., 2020). However, findings from other studies have shown varying levels of efficiency for different methods. For instance, Badu-Apraku *et al*. (2016) found that HGCAMT was the most efficient, followed by HSGCA and then the SNP-GD method, when defining heterotic groups for early-maturing yellow maize inbreds. In contrast, Badu-Apraku *et al*. (2015) reported the highest breeding efficiency for the SNP-based GD method. These discrepancies in findings across studies may be attributed to the unique genetic makeup of the inbred lines used in each respective study. In addition, Oyetunde *et al*. (2020) used a novel approach of discriminant analysis to compare the efficiencies of heterotic grouping methods in the classification of tropical maize inbred. They observed that the effectiveness of different grouping methods varied across different environments. HSGCA and SCA were consistently the most effective methods. However, for specific conditions, such as Striga infestation and drought, HGCAMT and HSGCA, respectively were recommended.

This method of classification is gaining attraction and is being verified by many other researchers in different crops (Kumar *et al*., 2022). Compared the efficiency of specific combining ability (SCA) and heterotic group specific combining ability (HSGCA) method for classifying lines into different heterotic group for baby corn yield without husk (BCY). HSGCA resulted in higher breeding efficiency (50.0%) compared to the SCA method (41.66%). Based on these results, the HSGCA method was more efficient at identifying superior hybrids in intergroup crosses relative to the SCA method. This research stands out as the first to employ this technique for baby corn germplasm, offering a new dimension to breeding strategies useful for crops which have limited to no genomic information available. Author further suggested that using the inbred lines with positive and significant GCA from opposite heterotic groups based on the HSGCA method can result in maximum heterosis. Similarly, Mahato *et al*. (2021) aimed to assess the genetic diversity among 12 sweet corn inbred lines and to classify them into heterotic groups using various methods, including SCA, HSGCA, HGCAMT, and SSR-based GD. All four methods successfully classified the inbred lines into three distinct groups, although with slight variations. In terms of breeding efficiency, the SSR-based GD and HSGCA methods were found to be the most effective. In another effort, Verma *et al*. (2022) conducted a study on sorghum to investigate the effectiveness of heterotic grouping methods. They used HSGCA and SCA-PY to classify 31 inbred lines into four distinct groups. By employing HSGCA and SCA-PY methods, they classified the sorghum lines into four distinct heterotic groups. In their study, both methods were equally effective with respect to placement of the same number of lines into similar groups. They also highlighted the drawback of the HSGCA and SCA-PY grouping methods to classify some lines into any of the heterotic groups represented by three testers.

The findings highlight the potential of GCA based heterotic grouping in improving breeding efficiency and selecting promising parental lines for hybrid development specially for crops where marker availability is not there. The majority of researchers have found HSGCA, which considers both GCA and SCA effects, to be more efficient in identifying superior crosses and classifying lines across different environments. This method is less influenced by genotype-environment interactions compared to SCA-PY. However, in cases where major breeding objectives include resistance to multiple biotic and abiotic stresses, HGCAMT could be a more suitable method as it incorporates information on these traits.

Selecting the Appropriate approach for Heterotic Pool identification

Effective heterotic pool identification is crucial for successful hybrid breeding programs. Several approaches can be employed depending on the specific needs and resources of the breeding program. One approach to heterotic pool identification involves creating a half-diallel and evaluating the crosses in replicated trials. Utilizing methodologies like HSGCA, parental lines can be classified into distinct groups. Disciplined utilization of inter-group crosses for commercial hybrid development, while intra-group crosses are utilized for population improvement, can effectively increase between-group genetic distance, leading to enhanced heterosis in future. This method offers the advantage of directly basing heterotic groups on observed yield performance, making it particularly valuable when marker information is limited or unavailable. However, a significant limitation of this approach is the substantial number of crosses that require evaluation in multiple replications. The number of lines that can be practically evaluated will inevitably depend on the available resources within each breeding program.

When dealing with a large number of inbred lines or populations, as is often the case, conducting a half diallel and producing sufficient F_1 seed for multi-environment field testing becomes impractical. In such scenarios, where numerous germplasm accessions lack established heterotic patterns and polymorphic SSR or SNP markers are available, a multi-stage approach is recommended. This approach involves: 1) Grouping the germplasm based on genetic similarity; 2) Selecting representative genotypes (e.g., two to four lines or one population) from each subgroup; 3) Conducting a half-diallel among the subgroup representatives, including the original parents; and 4) Evaluating these crosses in replicated field trials. Importantly, data analysis should focus on group-level performance rather than individual crosses, identifying between- and within-group heterosis. This approach aims to identify heterotic groups that exhibit high yielding potential across multiple combinations. It's crucial to remember that an ideal heterotic pool should demonstrate consistent high heterosis across a wide range of crosses, rather than relying on the exceptional performance of a few specific combinations.

In situations where well-defined heterotic pools already exist, such as in maize, but the need arises to incorporate new variability (e.g., resistance to emerging diseases), the HSGCA method described by Fan *et al.* (2009) is highly recommended. This method involves crossing testers from established male and female pools with newly generated material. By evaluating the GCA and SCA of these new lines with the testers, they can be assigned to the appropriate male or female group. Utilizing a minimum of 2-3 testers from each group is crucial for accurate group assignment.

It is important to acknowledge that crop-specific factors, such as the availability of cytoplasmic-genetic male sterility (CGMS) systems in crops like pepper and rice, or the presence of gynoecious lines in crops like bitter gourd and cucumber, will necessitate additional considerations when defining heterotic groups. Ultimately, the most suitable method for heterotic pool identification will depend on various factors, including resource availability (ability to conduct large-scale trials, access to marker technologies), and the maturity of the breeding program (availability of established heterotic pools).

Conclusion and Future Perspective

Multiple studies, employing both morphological and molecular markers, have consistently demonstrated significant genetic diversity within bitter gourd populations. Geographic origin and fruit characteristics have emerged as key factors influencing population structure. While studies encompassing wider geographic regions often reveal distinct clustering patterns based on origin, this trend may be less pronounced in studies confined to specific regions. The relationship between accessions is complex and influenced by various factors, including the number of markers used and the diversity of the germplasm sampled. Notably, study at world veg has revealed a concerning trend: the inclusion of commercial hybrids in genetic diversity analyses revealed a close clustering of these hybrids, suggesting a potential narrowing of the genetic base within Asian bitter gourd breeding programs. This trend is likely driven by market demands for uniformity and high yield, which can inadvertently lead to a loss of valuable genetic diversity. The public-private partnership program established at WorldVeg serves as an exemplary model of successful collaboration. Currently, 35-40 companies are utilizing germplasm developed by WorldVeg in their breeding programs to broaden their germplasm base and incorporate necessary variability. As emphasized earlier, establishing defined heterotic pools is crucial for the success and sustainability of any breeding program. Inspired by the successes of institutions like IRRI in rice and ICRISAT in maize, we propose identifying the heterotic pattern among the WorldVeg lines leading to formation of a global heterotic pool for bittergourd. This can significantly enhance the ongoing partnership. This framework would enable private companies to leverage the identified heterotic groups and high-GCA lines within each pool. Furthermore, each company can utilize this framework to assign their unique sets of lines into defined pools using the HSGCA method. The inclusion of Gynoecious lines from diverse sources into these heterotic pool studies would present new opportunities for enhancing yield potential and overall breeding efficiency.

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