



RESEARCH ARTICLE

Assessment of breeding potential of crosses involving different numbers of parents for green fruit yield in chilli (*Capsicum annuum* L.)

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Abstract

Cultivars derived from bi-parental crosses not only have a limited genetic base but also often lack the desired combination of traits, making them genetically vulnerable. Our hypothesis postulates that cultivars derived from populations of multiple parents are more likely to have greater trait means, genetic variability and thus a broader genetic base. To validate this hypothesis, the breeding potential of nine segregating populations derived from crosses with different numbers of parents of unequal population size ranging from 56 to 270 was field-tested for green fruit yield per plant during the rabi-summer season of 2020–21 at the experimental fields of the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Bengaluru. The breeding potential of these nine segregating F_2 populations was evaluated based on the mean values of quantitative traits, absolute range, standardised range (SR), transgressive segregation index (TSI) and the usefulness criterion (UC). Recognising that different statistical parameters can identify different populations with good breeding potential, UC served as an objective measure to identify populations with greater breeding potential. In particular, the segregating population resulting from a three-way cross hybrid (Gouribidanur (GB) $\times$ CMS 7B) $\times$ CMS 8B) showed increased estimates for trait means (379.24 g), SR (5.22), TSI (30.74) and UC (50906.4) for green fruit yield per plant. This segregating population could be advanced for the isolation of advanced breeding lines. In light of this study, the optimal number of parental lines required to generate potential segregating populations in future chilli breeding programme would be set at three.

Keywords: recombinants; selection; transgression; usefulness; variability; yield

Introduction

Chilli (*Capsicum annuum* L.) is a prominent commercial and export-oriented crop in India, which is the second most traded commodity in the world after black pepper. Within the genus *Capsicum*, the cultivated species encompass *annuum*, *frutescens*, *baccatum*, *chinense* and *pubescens* (1). Among these, *C. annuum* has special significance in India, as it is widely used for both hot (hot pepper) and non-hot (sweet pepper) varieties (2). Hot pepper is valued for its green, ripened and dried fruits, all of which are important to consumers. Although high-yielding hybrids and pure lines are available for both product categories, certain challenges still persist. Biotic stress factors such as pests and diseases as well as abiotic stress factors such as unfavourable climatic conditions and improper agronomic practices are major obstacles. In response to this challenge, a solution has emerged in the form of diversifying the genetic background by including multiple parents in breeding programmes (3, 4). This approach aims to increase the likelihood of capturing favourable trait combinations that would be difficult to capture in individual populations derived from crosses (5). By bringing together different genetic resources, this strategy promotes the development of more resilient and adaptable plant varieties and ultimately contributes to the sustainability and success of chilli breeding.

Hot pepper, a staple ingredient in global cuisines, exhibits diverse phenotypic traits influenced by its genetic makeup. The utilisation of multiple parents in breeding programs allows for the integration of desirable traits from different sources, creating a broader genetic pool that increases the chances of discovering superior segregates (6–10). Despite the increasing interest in multi-parent breeding approaches, studies comparing segregating populations derived from two-, three-, four- and multi-parent crosses in chilli are limited. Although increasing the number of parents in a cross is expected to introduce a greater diversity of favourable alleles and enhance the recovery of superior transgressive segregants (11), evidence of its effectiveness in improving genetic variability and overall breeding potential for green fruit yield remains scarce. Consequently, breeders lack clear guidance on the optimum number of parents required to develop efficient base populations for selection.

Therefore, the present study evaluated nine segregating populations derived from di-, tri-, tetra- and multi-parent crosses using descriptive, variability and genetic parameters, including the usefulness criterion and transgressive segregation index. The objective of this study was to compare the breeding potential of these populations and identify the optimum number of parents required for developing superior breeding populations for green fruit yield in chilli.

Materials and Methods

Experimental material

Nine segregating populations derived from crosses involving different numbers of parents in chilli were used in this study. These populations were developed and maintained between 2018 and 2020 at the Hot Pepper Improvement Unit, Department of Genetics and Plant Breeding, University of Agricultural Sciences (UAS), Bengaluru, using 12 parental lines (Table 1). The field experiment was conducted during the rabi-summer season (2020–21) at the experimental farm of the Department of Genetics and Plant Breeding, UAS, Bengaluru. During the crop growing period, the experimental site received a total rainfall of 268.6 mm, with a mean air temperature of 21.9 °C. The experimental site is located at 13°05' N latitude and 77°34' E longitude, at an altitude of 924 m above mean sea level. Details of the parental lines, crosses and population combinations are presented in Table 2.

Forty-day-old seedlings of the 12 parental lines were transplanted in a randomised complete block design (RCBD) with 3 replications. The nine F₂ populations derived from di-, tri-, tetra- and multi-parent crosses were transplanted in separate contiguous blocks consisting of 3–5 rows, depending on the population size available at transplanting. The blocks were arranged sequentially in the field to maintain population identity during evaluation and selection, with a spacing of 75 cm between rows and 45 cm between plants within rows. As the objective of the study was to characterise segregating populations rather than compare replicated plot treatments, the F₂ populations were not randomised or subjected to spatial adjustment. All populations were grown under identical agronomic practices in a relatively uniform field to minimise environmental variation. Owing to differences in seed availability, germination and plant survival, the number of plants evaluated per population ranged from 56–270. Data on green fruit yield per plant (g) were recorded from five randomly selected plants in each replication of the parental lines and from all surviving plants in each F₂ population. Green fruit yield per plant (g) was recorded as the cumulative weight of green fruits harvested from each plant over two harvests.

Table 1. Key attributes of hot pepper parental lines employed in developing single, three-way, double and multiparent derived segregating populations

Sl. No.	Parental lines	Salient features
1	Byadgi Dabbi (BD)	High oleoresin/colour
2	Gouribidanur (GB)	High pungency
3	Tiwari (Ti)	Tolerant to sucking pest
4	Byadgi Kaddi (BK)	High oleoresin
5	Japani Long (JL)	Tolerant to sucking pest
6	Vangara (Vang)	High yield with good combining ability
7	CMS 7B (7B)	High yield with good combining ability
8	CMS 8B (8B)	High yield with good combining ability
9	CMS 10B (10B)	High yield with good combining ability
10	Pant C1 (PC1)	Tolerant to sucking pest and chilli leaf curl virus
11	CA 14	High yielding
12	LG 174	Tolerant to <i>Colletotrichum</i> infection (Anthracnose)

Table 2. Parentage of segregating populations derived from crosses with different numbers of hot pepper lines

Segregating populations derived from			
Single crosses (involving two parents)	Three-way crosses (involving three parents)	Double crosses (involving four parents)	8-way crosses (involving eight parents)
GB × Ti (109)	(BK × CMS 8B) × Ti (77)	(PC1 × CMS 10B) × (JL × CMS 8B) (60)	D1 × D2 (60)
PC1 × BK (270)	(GB × CMS 7B) × CMS 8B (74)	(CMS 8B × GB) × (Ti × CMS 7B) (123)	D3 × D2 (56)
CMS 8B × GB (135)			

D1 × D2 = ((LG174 × BD) × (vang × CA14)) × ((PC1 × CMS 10B) × (JL × CMS 8B));

D3 × D2 = ((LG174 × BK) × (vang × CA14)) × ((PC1 × CMS 10B) × (JL × CMS 8B))

Numbers indicated in the parenthesis represent the population size.

Statistical analysis

The mean green fruit yield per plant from each of the 3 replicates of the parental lines was calculated using a total of 5 plants. In addition, observations were recorded for individual plants within each of the nine segregating populations. These data were used for subsequent statistical analysis.

Mean was estimated as

$$\text{Mean } (\bar{x}) = \frac{\text{Sum of observations of 5 plants of parental lines or all plants in segregating population}}{\text{Number of plants in each population}}$$

Standardised range (SR) was estimated as

$$\text{SR} = \frac{\text{Highest trait value} - \text{lowest trait value}}{\text{Mean}}$$

Genetic Advance

The extent of expected genetic advance of each trait at 5 per cent selection intensity was computed using the formula suggested previously (12).

$$GA = k \times h^2 \times \sqrt{V_p}$$

where, k = selection differential which is equal to 2.06 at 5 per cent selection intensity respectively.

h^2 = broad sense heritability

$\sqrt{V_p}$ = phenotypic standard deviation

Levene's test for homogeneity of variances

Levene's test (13) is used to examine if segregated populations derived from crosses involving varying number of parents have equal variances.

The Levene test is defined as:

$$H_0: \sigma_1^2 = \sigma_2^2 = \dots = \sigma_k^2$$

$$H_a: \sigma_i^2 \neq \sigma_j^2 \text{ for at least one pair } (i, j).$$

Where, k represents segregating populations.

Usefulness criteria and transgressive segregation index

Usefulness criteria (UC) (14) which covers both trait mean and variance and transgressive segregation index (TSI) were yet other important parameters for identifying the potential population among the segregating populations.

UC was estimated as,

$$UC = \frac{\text{trait grand mean}}{\text{genetic advance}}$$

However, TSI was estimated as

$$TSI = \frac{\text{absolute range of a trait in segregating population}}{\text{absolute parental range of a trait}}$$

Assessing the breeding potential of segregating populations

The segregating population with high trait means, absolute range, standardised range, TSI and UC was considered as those with better breeding potential. The analysis of data and parameter estimation in the present experiment were conducted using R statistical software (version 4.3.1) and Microsoft Excel 2010.

Results and Discussion

Arithmetic mean

The arithmetic means serves as a pivotal measure of central tendency and plays a crucial role in summarising data. It effectively represents the tendency of individuals within a population to cluster around the central value within the distribution. The three-way cross (GB × CMS 7B) × CMS 8B recorded the highest mean green fruit yield per plant (379.2 g), whereas the single cross Gouribidanur (GB) × Tiwari (Ti) had the lowest mean (103.2 g) (Supplementary Tables 1 and 2, Fig. 1). The superior performance of the three-way cross is likely due to the accumulation of favourable additive alleles contributed by GB and CMS 7B, both identified as good general combiners from our previous experiments (Table 1). The mean performance of segregating populations depends on the frequency and distribution of favourable alleles contributed by the parents (15–17). Similar findings were reported in previous studies, that populations with higher mean performance offer greater potential for genetic gain through selection (7). However, the eight-parent populations did not outperform the three-way cross, indicating that breeding potential depends more on the genetic merit and complementarity of the selected parents than on the number of parents involved in the cross, consistent with the theoretical framework (18).

Variability among the populations

In addition to the mean, quantifying the variability of the target trait is crucial for a more informed assessment of the breeding potential of segregating populations. (7, 19–23). Levene's test revealed significant heterogeneity of phenotypic variances among the nine

segregating populations ($F = 7.80$, $P < 0.01$), confirming that the extent of variability differed significantly across populations. The detailed results are presented in Table 3. Among the populations, the three-way cross (GB × CMS 7B) × CMS 8B exhibited the highest phenotypic variance (67138.38), followed by the double cross (CMS 8B × GB) × (Ti × CMS 7B) (50343.89), whereas the single cross GB × Ti recorded one of the lowest variances (13663.98). The greater variability observed in the three- and four-parent populations is likely due to the incorporation of favourable alleles from diverse parental backgrounds and their subsequent reshuffling through recombination, thereby generating a broader range of genotypes. Similar trends have been reported indicating that populations derived from multiple parents generally capture greater genetic variability than biparental populations (23).

Variability was further assessed using absolute and standardised ranges. The single cross PC1 × Byadgi Kaddi (BK) exhibited the largest absolute range (1244 g), followed by the double cross (CMS 8B × GB) × (Ti × CMS 7B) (1058 g) (Table 4). The wider range observed in Pant C1 (PC1) × BK was partly attributable to its larger population size (270 plants), which increased the probability of recovering rare recombinants (24). In contrast, the highest standardised range was recorded in the single cross GB × Ti (6.47), followed by CMS 8B × GB (5.68), whereas the three-way cross (GB × CMS 7B) × CMS 8B recorded a standardised range of 5.22 (Supplementary Table 1, Fig. 2). This apparent discrepancy reflects the fact that standardised range is expressed relative to the population mean; therefore, populations with lower means may exhibit higher standardised ranges. A similar trend was observed in the previous study wherein, the population with lower mean value recorded higher variability (25). These findings indicate that both parental diversity and population size influence the expression of variability, while the genetic merit and complementarity of the parents ultimately determine the usefulness of the segregating population for selection (7, 18, 23).

Usefulness criterion

The usefulness criterion (UC) provides a more reliable estimate of breeding potential than population mean or variance alone because it predicts the expected performance of selected progenies by integrating population mean with the genetic variation available for selection (19–22). Consequently, populations with a high UC are expected to produce greater genetic gain following selection. Among the nine segregating populations, the three-way cross (GB × CMS 7B) × CMS 8B recorded the highest UC (50906.4), despite having a relatively small population size (74 plants), whereas the single cross GB × Ti exhibited the lowest value (18289.1) (Supplementary Table 1, Fig. 3). The superior UC of the three-way cross can be attributed to its combination of the highest population mean (379.2 g) and substantial phenotypic variance (67138.38),

Table 3. Levene's test for homogeneity of variances for green fruit yield per plant among F_2 mimicking segregating populations derived from crosses with different numbers of parents in chilli

Segregating populations derived from	Parentage	Number of parents involved	Degrees of freedom	Phenotypic variance	F value	p value
Single cross hybrids	GB × Ti	Two		13663.98	7.80	< 0.01
	PC1 × BK			35574.78		
	CMS 8B × GB			15691.85		
Three-way cross hybrids	(BK × CMS 8B) × Ti	Three		29836.96		
	(GB × CMS 7B) × CMS 8B			67138.38		
Double cross hybrids	(CMS 8B × GB) × (Ti × CMS 7B)	Four		50343.89		
	(PC1 × CMS 10B) × (JL × CMS 8B)			34976.56		
Multiparent cross	D1 × D2	Eight	8	45668.50		
	D3 × D2			46003.57		

Table 4. Estimates of absolute range for green fruit yield per plant among F₂ mimicking segregating populations derived from crosses with different numbers of parents in chilli

Segregating populations derived from	Parentage	Number of parents involved	Maximum trait value	Minimum trait value	Absolute range
Single cross hybrids	GB × Ti	Two	678	10	668
	PC1 × BK		1254	10	1244
	CMS 8B × GB		754	10	744
Three-way cross hybrids	(BK × CMS 8B) × Ti	Three	826	20	806
	(GB × CMS 7B) × CMS 8B		1006	16	990
	(CMS 8B × GB) × (Ti × CMS 7B)		1088	30	1058
Double cross hybrids	(PC1 × CMS 10B) × (JL × CMS 8B)	Four	852	12	840
Multiparent cross	D1 × D2	Eight	892	36	856
	D3 × D2		920	30	890

Double cross Hybrids: D1 = ((LG174 × Byadgi Dabbi) × (Vangara × CA14))
D2 = ((Pant C1 × CMS 10B) × (Japani Long × CMS 8B))
D3 = ((LG174 × Byadgi Kaddi) × (Vangara × CA14))
GB: Gouribidanur; Ti: Tiwari; PC 1: Pant C1; BK: Byadgi Kaddi; JL: Japani Long; 7B: CMS 7B; 8B: CMS 8B; 10B: CMS 10B.

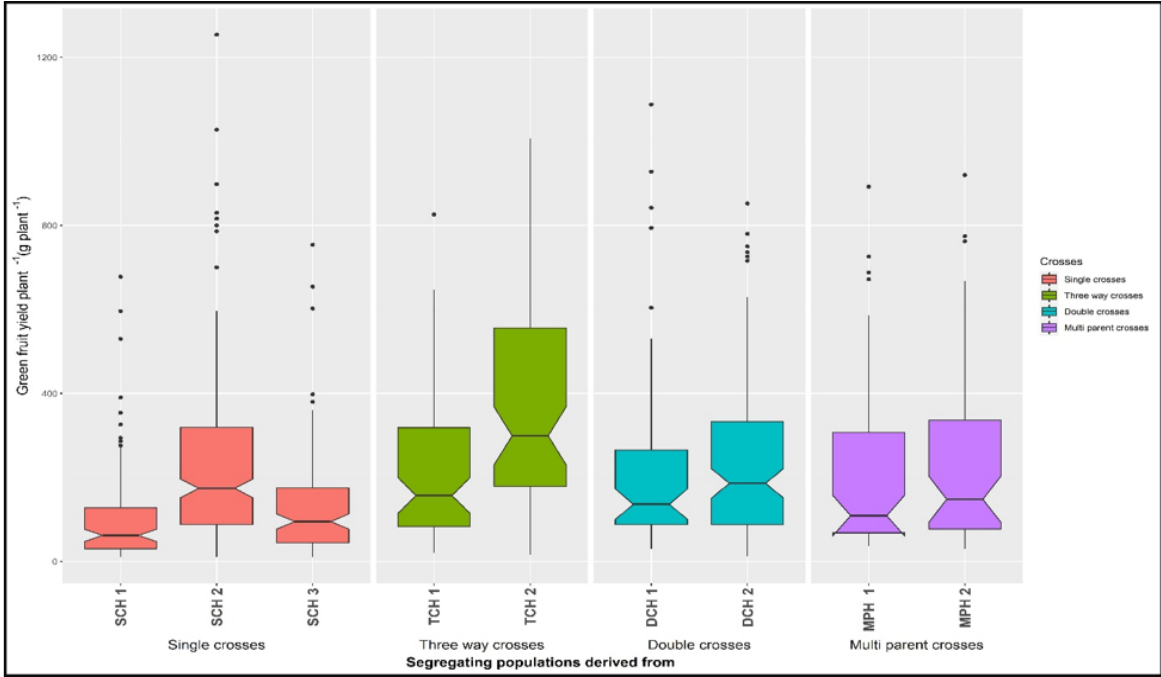


Fig. 1. Box-Whisker plots depicting distribution of mean green fruit yield per plant across nine F₂mimicking segregating populations derived from crosses with different numbers of parents in chilli. SCH: single cross hybrid; TCH: three-way cross hybrid;DCH: double cross hybrid; MPH: multiparent cross.

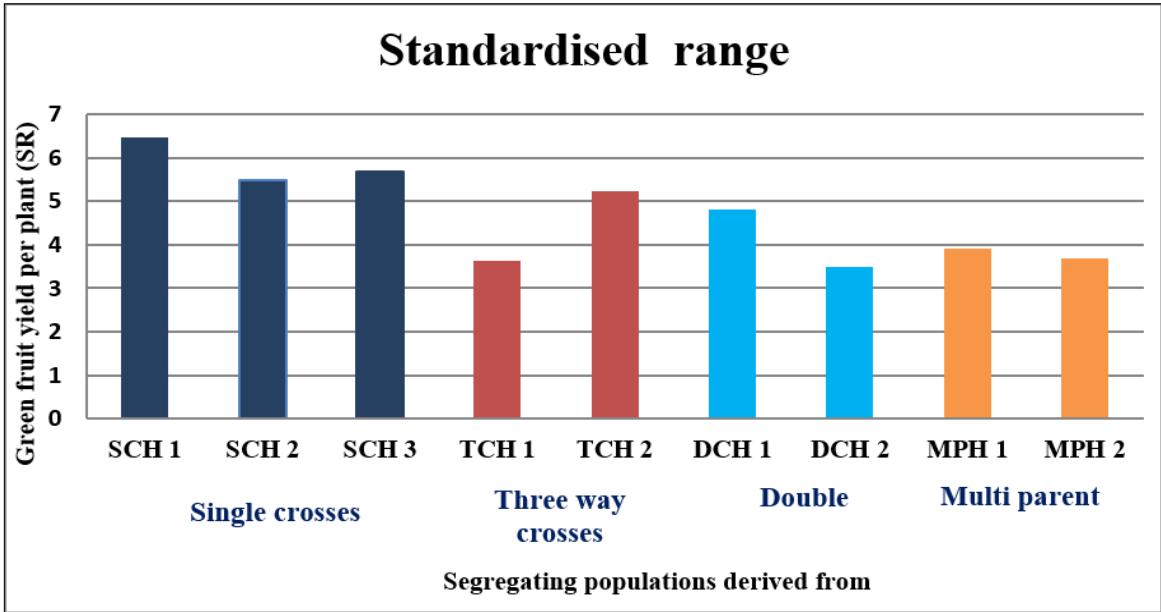


Fig. 2. Distribution of standardised range for green fruit yield per plant across nine F₂mimicking segregating populations derived from crosses with different numbers of parents in chilli.

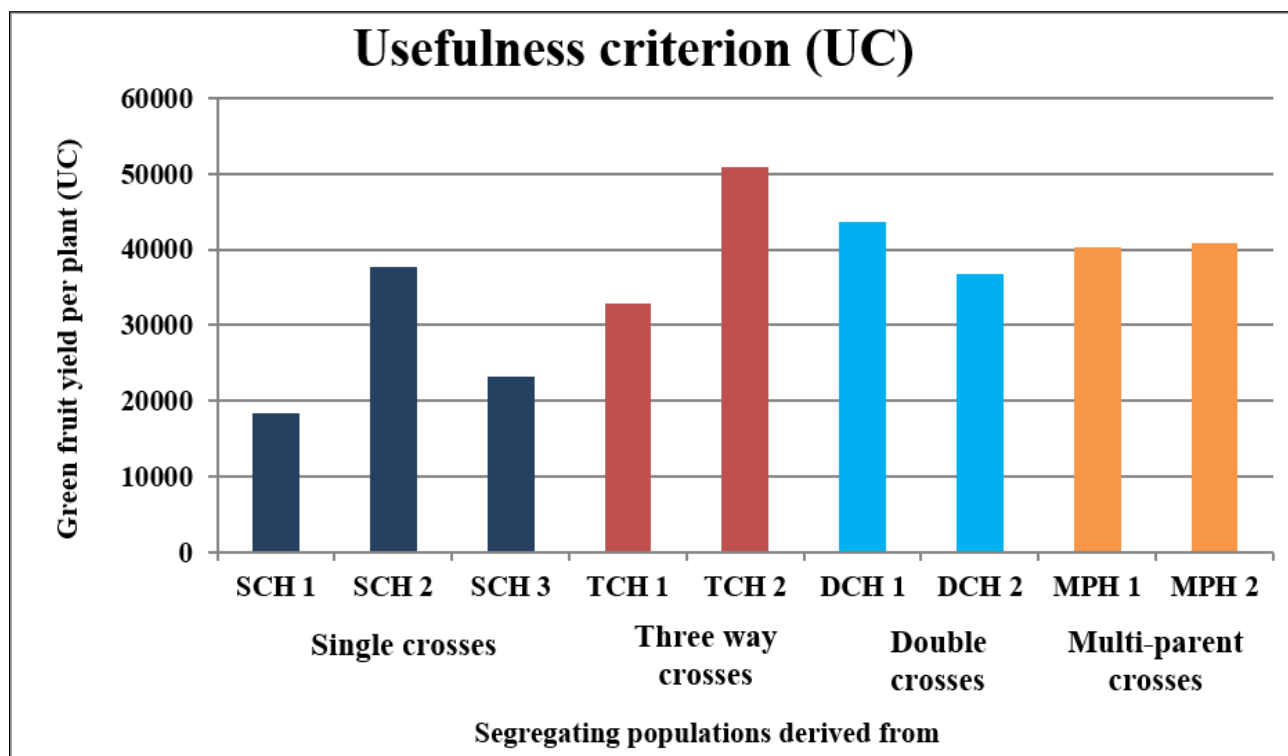


Fig. 3. Usefulness criterion (UC) for green fruit yield per plant across nine F_2 -mimicking segregating populations derived from crosses with different numbers of parents in chilli.

indicating a favourable balance between average performance and genetic variability. Previous studies have shown that the usefulness criterion identifies populations with the greatest expected response to selection by simultaneously considering both parameters rather than either alone (19–22). Likewise, (7) emphasised that populations combining a high mean with sufficient genetic variability provide the greatest opportunity for long-term genetic improvement.

Although the single cross $PC1 \times BK$ recorded a UC (37702.9) comparable to the multi-parent populations, its larger population size (270 plants) likely increased the probability of recovering favourable recombinants. In contrast, the superior performance of $(GB \times CMS\ 7B) \times CMS\ 8B$ despite its smaller population size suggests that parental quality and the accumulation of favourable alleles contributed more to breeding potential than population size alone. These findings highlight that selecting genetically complementary parents capable of generating populations with both high mean performance and adequate genetic variability is more important than simply increasing the number of parents involved in the cross (7, 18–20).

Transgressive segregation index

The transgressive segregation index (TSI) reflects the ability of a segregating population to produce individuals that outperform the superior parent. All populations exhibited positive transgressive segregation index (Fig. 4), with single-cross populations recording higher TSI values than three-, four- and multi-parent populations. Among all populations, $CMS\ 8B \times GB$ recorded the highest TSI (169.09), indicating the greatest frequency of superior recombinants (Supplementary Table 1). Transgressive segregation results from the accumulation of complementary favourable alleles from both parents and their reshuffling through recombination, producing novel gene combinations that exceed the superior parent (7, 26). However, despite its lower TSI, the three-way cross $(GB \times CMS\ 7B) \times CMS\ 8B$ recorded the highest usefulness criterion (50906.4), reflecting greater expected genetic gain because UC

combines population mean with expected genetic advance, which is determined by the heritable genetic variability available for selection (19–22). Thus, while TSI identifies populations producing exceptional recombinants, UC identifies populations with greater overall breeding potential (27). These findings emphasise that parental complementarity and the accumulation of favourable alleles are more important than simply increasing the number of parents for developing superior breeding populations.

Conclusion

The three-way cross population $(GB \times CMS\ 7B) \times CMS\ 8B$ exhibited the highest breeding potential, with superior performance across descriptive, variability, genetic parameters including usefulness criteria. These results indicate that incorporating three genetically diverse parents enhances genetic variability and the recovery of superior recombinants. Further multi-season and multi-environment evaluation is needed to validate its breeding potential for chilli improvement.

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Authors' contributions

KB and AMR contributed to the conceptualisation and design of the study. KB, GP, GVR, SCB and CB were involved in material preparation, field experiments and data collection. KB carried out the curation and analysis of data and prepared the tabulation of results. KB, AMR and SR wrote the original draft of the manuscript.

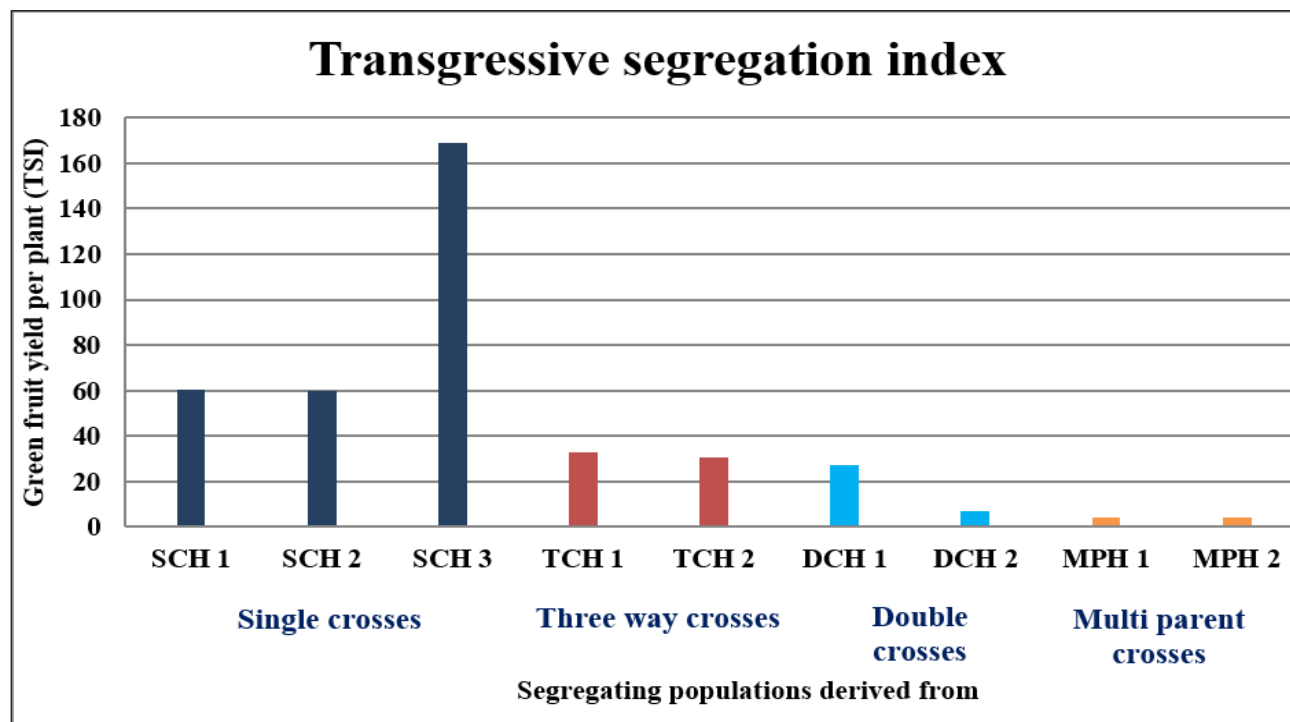


Fig. 4. Transgressive segregation index for green fruit yield per plant across nine F_2 mimicking segregating populations derived from crosses with different numbers of parents in chilli.

KB, AMR, SR, GP, SCB and CB contributed to editing and preparing the communication-ready version of the manuscript. AMR supervised the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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