# SSR polymorphism and genetics and inheritance of yield and yield-attributing traits in bitter gourd (*Momordica charantia* L.)

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**The present study was conducted to ascertain the gene action and inheritance of yield and various yield-attributing traits and to screen the simple sequence repeat (SSR) markers for parental polymorphism in bitter gourd (*Momordica charantia* L.). Two contrasting parents (S-2 and Pusa Purvi) were used for hybridization. Parental polymorphism was analysed using 100 SSR markers. DNA amplicon of size around 150–250 bp in both the parents was obtained for the 10 polymorphic SSR markers. These were used to ascertain the hybridity of $F_1$ progeny, from which the subsequent generations were developed. Generation mean analysis, ABCD scaling test and joint scaling test were performed using the observations from six generations (parent-1, parent-2, $F_1$, $F_2$, back cross 1 and back cross 2). The additive effect of the genes was predominant for the quantitative traits like fruit length, diameter, weight, etc. Duplicate epistasis was observed for the majority of traits. The yield per plant was positively correlated with fruit length, diameter, weight and fruit number per plant. Chi-square test revealed the monogenic inheritance of qualitative traits (fruit tubercles and ridgeness, and shape at apex), in which 'conspicuous tubercles', 'discontinuous ridgeness' and 'acute fruit shape at apex' were dominant. These results reveal the complexity and polygenic nature of the yield. The findings obtained from the present study can be used for yield improvement in the future. For this, the polymorphic SSRs identified can also be used in genetic diversity studies, DNA fingerprinting, genetic mapping, genomics analysis, etc.**

**Keywords:** Epistasis, gene interaction, *Momordica charantia* L., monogenic inheritance, polymorphism.

BITTER gourd (*Momordica charantia* L.), a cucurbit that originated from the Indo-Burma region, has a huge economic potential due to its ethno-botanical and medicinal attributes[1]. It is grown in the tropical areas of Asia, East Africa, South America and the Caribbean[2]. Bitter gourd is consumed regularly as part of several Asian cuisines and has been used for centuries in traditional Indian, Chinese and African pharmacopoeia. Apart from the minerals, the charantin and saponin present act as hypoglycaemic, dechlosterizing and immune modulators, while the red pulpy aril of ripened fruits has high amounts of $\beta$-carotene and riboflavin. Bitter gourd has been used as a folk remedy for a range of ailments, including type-2 diabetes. In countries like China and Thailand, the less bitter, white-coloured, longer fruits are preferred. In India, the southern part prefers less bitter, white-coloured fruits, the northern part long and slender fruits, and the eastern part small oval fruits. In spite of its beneficial attributes and high demand, the productivity of bitter gourd is low in India and other parts of the world. This necessitates the development of improved bitter gourd genotypes having high-yielding capacity along with improved quality attributes suitable to regional preferences. For this, there is a need to understand the genetics and inheritance of yield and yield-attributing characters.

Bitter gourd is a highly cross-pollinated crop owing to its monoecious nature. From the breeder's point of view, exploitation of heterosis with regard to yield, earliness and uniformity is a major concern for its improvement. There is high genetic diversity (maturity, growth habit, fruit size, shape, colour, sex expression and surface texture) among the Indian bitter gourd varieties[1,3]. To improve the yield, the genetics and inheritance of all characters should be thoroughly studied. Adoption of appropriate breeding and selection strategies mainly relies on gene action and gene dosage effects in a breeding population. Generation mean analysis provides information about the additive, dominant or epistatic effect of a gene for any type of complex trait, which can be used by the breeder for the adoption of appropriate breeding methods for bitter gourd improvement. However, there is scanty information available about the gene action and inheritance of various yield-attributing traits in the crop. This is a major hindrance to its genetic improvement. The present study aims to ascertain gene action and inheritance of yield and yield-attributing traits, which can be used to improve bitter gourd.

In the bitter gourd hybridization programme, there is an utmost necessity for the confirmation of hybridity before

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further advancing the generation, as the crop is mostly cross-pollinated. Molecular markers are highly reliable in ascertaining hybridity, being free from environmental effects, growth stage specificity, etc. Simple sequence repeat (SSR) markers are generally preferred among the molecular markers, but the dearth of reliable polymorphic SSRs in bitter gourd due to limited studies necessitates the identifycation of new polymorphic SSRs[2,4]. In this study, we screened 100 SSR markers for parental polymorphism, and the polymorphic SSRs obtained were used for hybridity analysis.

## Materials and methods

### *Planting materials*

The experimental materials consisted of six generations, including two parental lines ($P_1$ i.e. S-2 (DBGS-2) and $P_2$ i.e. Pusa Purvi), $F_1$, $F_2$, $B_1$ ((S-2 × Pusa Purvi) × S-2) and $B_2$ ((S-2 × Pusa Purvi) × Pusa Purvi). The parental seeds were obtained from the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Pusa Purvi is botanically *Momordica charantia* var. *muricata* producing dark green-coloured, small-sized fruits (4–5 cm long and 3–4 cm diameter), having higher mineral content and antioxidant activity. S-2 (DBGS-2) is botanically *Momordica charantia* var. *charantia* producing green, longer fruits (22–28 cm long, 4.0–4.5 cm diameter) curved at the harvesting stage. The $F_1$s from S-2 × Pusa Purvi were developed during September–January 2019–20 under polyhouse conditions. Leaves of both the parents and $F_1$s were collected for the parental polymorphism study and confirmation of hybridity. Seeds of $F_1$ (in which hybridity was confirmed using SSR markers) were sown to develop $F_2$ and backcross progenies ($B_1$ and $B_2$) during March–July 2020.

### *DNA isolation, parental polymorphism and hybridity confirmation*

Genomic DNA was extracted using the method of Doyle and Doyle[5] from the collected leaves (parents and $F_1$) for the parental polymorphism study and confirmation of hybridity using SSR molecular markers. Amplification reactions (10 μl final volume) contained 2.0 μl genomic DNA (10 ng/μl), 1.0 μl of primer (forward and reverse; 50 ng/μl; Sigma Aldrich, USA), 5.0 μl PCR master mix (GoTaq® Master Mixes, Promega's) and 2 μl nuclease-free double-distilled water. PCR-optimized amplification reactions were performed using a thermocycler (Mastercycler® Nexus-Eppendorf India) with the standard program. To determine the size range of DNA fragments, the 1 kb ladder (Geno Technology, Inc, USA) was used. PCR products were resolved by electrophoresis using 3% agarose gel. Gel photographs were documented in a bio-imaging system (Alpha Imager Pvt Ltd, India). A total of 100 SSR primers were used for studying parental polymorphism.

### *Field evaluation and data collection*

All six generations were evaluated under open field conditions during August–December 2020 in a randomized block design with five replications at the Research Farm of the Division of Vegetable Science, ICAR-IARI, New Delhi. Seedlings were raised in 50-cell plug trays filled with artificial growing media (cocopeat, vermiculite and perlite) in a polyhouse and transplanted at two-true-leaves stage (20–25 days after sowing) with a spacing of 2.0 m between rows and 0.5 m within rows. All the recommended package practices were adopted for the growing of ideal crops. Data were recorded from 10 plants for each parent, 20 for $F_1$, 40 for $B_1$ and $B_2$; and 120 for $F_2$ generation for various traits to determine the gene effect. Various quantitative and qualitative traits were recorded at the proper vegetative and reproductive growth stages of plants of all six generations.

### *Statistical and genetic analyses*

*Quantitative traits:* Generation mean analysis (GMA) and ABCD scaling test were performed according to Hayman[6], and Hayman and Mather[7] respectively, while statistical analysis was carried out using OP Stat software (http://14.139.232.166/opstat/). The joint scaling test was also performed, as the scaling test is not always able to depict the additive-dominance model. The six parameters considered were mean effect ($m$), genetic effects comprising additive ($d$) and dominance ($h$), and epistatic effects comprising additive × additive ($i$), additive × dominance ($j$) and dominance × dominance ($l$). The significance of the corresponding gene effects was determined using the Student $t$-test.

The type of epistatic effect was ascertained using Kearsey and Pooni[8] as follows: (i) complementary gene action – when dominance effect ($h$) and dominance × dominance effect ($l$) were significant and had the same sign and (ii) duplicate epistasis – different signs for these gene effects.

A normal distribution curve with a histogram (bars for frequency distribution) was drawn for inheritance studies. Pearson's simple correlation (SPSS® 16.0) and regression equation analysis were done using $F_2$ means for studying the relationships between yield/plant and other yield-attributing parameters.

*Qualitative traits:* The chi-square ($\chi^2$) test was done according to Panse and Sukhatme[9] to study the inheritance of three qualitative traits using the following formula

$$\chi^2 = \frac{(\text{Observed number} - \text{Expected number})^2}{\text{Expected number}}.$$

## Results and discussion

### *Parental polymorphism and hybridity confirmation using SSR markers*

A total of 100 SSR primers were used for parental (S-2 and Pusa Purvi) polymorphism analysis, of which ten (MCSSR_226, MCSSR_235, MCSSR_215, MCSSR_185, MCSSR_217, MCSSR_188, MCSSR_281, MCSSR_283, MCSSR_285 and MCSSR_287) (Table 1) were observed as polymorphic. These polymorphic primers mostly formed a DNA amplicon of size around 150–250 bp in both parents (Figure 1). However, in every marker, one allele was amplified in each parent; thus, the polymorphic information content was zero. These 10 polymorphic SSR primer pairs (forward and reverse) with genomic DNA from both parents and their hybrid ($F_1$) were run on 3.0% agarose gel. The hybridity was confirmed by having two amplicons in $F_1$; whereas the parents had alternate amplicons (Figure 1). The $F_1$ plants with ascertained hybridity were further selfed to get the $F_2$ generation. Very low SSR polymorphism among the parental genotypes can be attributed to the fact that both parents belong to the same *M. charantia* L. Moreover, another factor is the lack of wider genetic diversity among common bitter.

It is necessary for bitter gourd breeding programmes to ascertain the hybridity ($F_1$) before further advancement of the generation. It is also tedious to identify the hybrids if they have resulted from a cross between diverse parents, among which either has a dominant trait. Molecular markers not only reduce the breeding cycle but are also free from environmental effects. They are not plant growth stage-specific and thus more reliable than morphological and biochemical markers. Hence they are being widely used for hybridity confirmation. SSR markers being co-dominant and multi-allelic in nature with higher genomic abundance, are widely used in various crops. However, there is a dearth of polymorphic SSRs in bitter gourd due to limited studies[2,4]. Therefore, the 10 polymorphic SSR markers identified in the present study can be further used for genetic diversity studies, DNA fingerprinting, genetic mapping, genomics analysis, etc., in bitter gourd and also can be tested in wild relatives or other commercial close relatives within the Cucurbitaceae family.

**Table 1.** Primer sequences for the 10 polymorphic SSR markers

| Primer ID | Primer sequence (5′–3′) | Length (nt) |
|---|---|---|
| MCSSR_226 | F: GACAATTAACAACAAACAGCGGCG | 24 |
| | R: CTCCTTCCTCCCTCTCGCGCCGCG | 24 |
| MCSSR_235 | F: AGGTTGAGTAACCGGGGGCT | 20 |
| | R: TCCTATTTTGGCTCTCTTCG | 20 |
| MCSSR_215 | F: TATTTTCAATGTGTACCCGAGG | 22 |
| | R: TTCCATCTTTCTCTCGGTATCCG | 23 |
| MCSSR_185 | F: TACTGCTATCCTCGCTTCTAC | 21 |
| | R: GTGTGTTAATACACGTGAGTGC | 22 |
| MCSSR_217 | F: CATTATGTTGTGTCGACATT | 20 |
| | R: TGATAACAAAGCCGTCACGC | 20 |
| MCSSR_188 | F: CCTTATCTCCCCTTTCACACCC | 22 |
| | R: ATCATGTGATAGGACGTACACC | 22 |
| MCSSR_281 | F: GTCTATCATGCGTCTTGGTG | 20 |
| | R:TGGTGTGTGGTGTGGGAGTG | 20 |
| MCSSR_283 | F: CAGCAGAGCACTTCCACTCT | 20 |
| | R:TGTTTACTTCTCACCCTTTCTTT | 23 |
| MCSSR_285 | F: AAGAGCGATGGACCAACCTG | 20 |
| | R: AGCATCACCATCACCAACTCG | 21 |
| MCSSR_287 | F: GGATCGGGTTCCCATGAGG | 19 |
| | R: CCTCCCCGCCTTGCATTTTT | 20 |

### *Genetic studies using generation mean analysis*

The estimated means of the six generations were calculated to compare the performance of various generations with respect to the traits (Table 2 and Supplementary Figure 1).

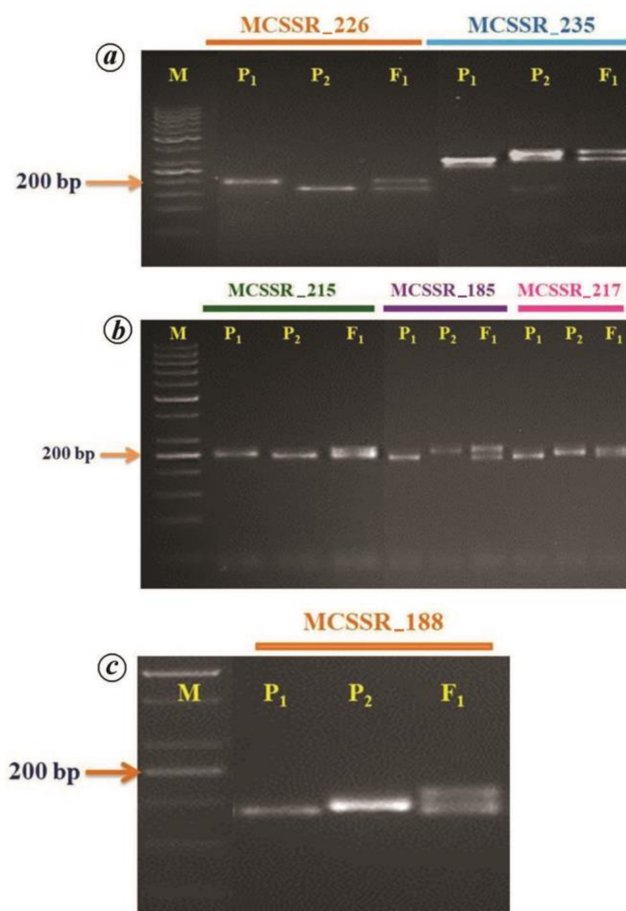


**Figure 1.** Simple sequence repeats (SSR) marker polymorphisms between the parental (S-2 and Pusa Purvi) genotypes and hybridity testing of $F_1$ progeny using the polymorphic SSRs. Lane M, 50 bp DNA ladder; lane $P_1$, S-2; lane $P_2$, Pusa Purvi and lane $F_1$, hybrid (S-2 × Pusa Purvi). Arrow shows the 200 bp fragment of the ladder. Six polymorphic markers: (***a***) MCSSR_226 and MCSSR_235, (***b***) MCSSR_215, MCSSR_185 and MCSSR_217 and (***c***) MCSSR_188 are underlined with different colours and each polymorphic marker represents three lanes ($P_1$, $P_2$ and $F_1$).

**Table 2.** Generation mean for different traits in the cross S-2 × Pusa Purvi of bitter gourd

| Parameter | $P_1$ | $P_2$ | MP | $F_1$ | $F_2$ | $B_1$ | $B_2$ |
|---|---|---|---|---|---|---|---|
| DFFF | 24.00 ± 3.67 | 26.60 ± 1.52 | 25.30 | 30.20 ± 2.59 | 30.35 ± 0.54 | 27.83 ± 1.00 | 35.05 ± 1.14 |
| DFMF | 27.00 ± 3.08 | 21.20 ± 1.31 | 24.10 | 22.80 ± 3.84 | 30.06 ± 0.54 | 31.44 ± 0.88 | 27.64 ± 0.96 |
| NFFF | 11.80 ± 2.38 | 13.40 ± 2.41 | 12.60 | 10.60 ± 3.29 | 13.90 ± 0.55 | 12.16 ± 0.46 | 17.92 ± 0.91 |
| NFMF | 23.00 ± 3.23 | 16.20 ± 2.58 | 19.60 | 12.40 ± 1.52 | 10.95 ± 0.28 | 13.27 ± 0.67 | 10.75 ± 0.78 |
| VL45 DAT | 1.83 ± 0.11 | 0.58 ± 0.03 | 1.21 | 1.32 ± 0.10 | 0.80 ± 0.19 | 1.81 ± 0.16 | 0.96 ± 0.15 |
| VL90 DAT | 3.76 ± 0.88 | 2.37 ± 0.61 | 3.07 | 3.40 ± 0.75 | 2.83 ± 0.08 | 4.22 ± 0.19 | 2.07 ± 0.15 |
| DFFH | 45.60 ± 4.28 | 37.80 ± 1.92 | 41.70 | 31.60 ± 1.52 | 45.15 ± 0.63 | 41.36 ± 1.18 | 42.11 ± 1.02 |
| FL | 16.76 ± 1.98 | 5.76 ± 0.71 | 11.26 | 10.53 ± 1.67 | 8.34 ± 0.16 | 10.51 ± 0.36 | 5.37 ± 0.33 |
| FD | 4.42 ± 0.68 | 2.40 ± 0.65 | 3.41 | 3.70 ± 0.51 | 2.87 ± 1.03 | 3.51 ± 0.13 | 2.08 ± 1.64 |
| FL/D | 3.86 ± 1.20 | 2.60 ± 0.99 | 3.23 | 2.87 ± 0.31 | 3.44 ± 0.16 | 3.08 ± 0.15 | 3.00 ± 0.18 |
| FW | 86.11 ± 9.44 | 20.36 ± 3.29 | 53.23 | 37.49 ± 8.55 | 45.96 ± 1.35 | 52.03 ± 2.09 | 20.16 ± 1.75 |
| FNP | 13.80 ± 2.38 | 20.60 ± 1.82 | 17.20 | 14.60 ± 1.95 | 14.03 ± 0.39 | 14.44 ± 0.81 | 30.50 ± 0.91 |
| YP | 3.05 ± 0.52 | 1.02 ± 0.39 | 2.04 | 1.27 ± 0.42 | 2.58 ± 0.11 | 4.07 ± 0.22 | 1.75 ± 0.14 |

DFFF, Days to first female flower; DFMF, Days to first male flower; NFFF, Node to first female flower; NFMF, Node to first female flower; VL45DAT, Vine length (m) at 45 days after transplanting; VL90DAT, Vine length (m) at 90 days after transplanting; DFFH, Days to first fruit harvest; FL, Fruit length (cm); FD, Fruit diameter (cm); FL/D, Fruit length/diameter (cm); FW, Fruit weight (g); FNP, Fruit number per plant and YP, Yield per plant (kg).

Among the contrasting parents, a higher yield was obtained from the female parent, i.e. S-2 (3.05 kg), compared to the male, i.e. Pusa Purvi (1.02 kg). However, the fruit number/plant was higher in Pusa Purvi (20.60) compared to S-2 (13.80). Irrespective of the lower fruit number/plant, the higher yield of S-2 can be explained by its higher fruit weight (86.11 g), which is nearly four times that of Pusa Purvi (20.36 g). Similarly, the fruit length (16.76 cm) and diameter (4.42 cm) of S-2 were higher than Pusa Purvi (fruit length – 5.76 cm, diameter – 2.40 cm). $P_1$ (S-2) was found to be a better parent for most yield-attributing traits.

It was observed that the $F_1$ means of all the parameters (except the days to the first female flower) were intermediate of the individual parental means ($P_1$ and $P_2$; Table 2). The $F_2$ means varied for different yield-related parameters, but $B_1$ and $B_2$ showed similarity to their recurrent parent. The $F_1$ means of days to the first female flower, vine length at 45 DAT (days after transplanting) and 90 DAT, and fruit diameter were higher than the mid-parent (MP) values. This superiority of $F_1$ over MP indicates the overdominance of these traits, and thus, these can be improvized through heterosis breeding. The presence of heterosis in bitter gourd hybrids was also reported by Al-Mamuna *et al.*[10] for traits like earliness, vine length, yield and quality characters.

In the present study, inbreeding depression was observed for node to first male flower, vine length at 45 DAT and 90 DAT, fruit length and diameter as evident from the reduction of mean performance of the $F_2$ population compared to $F_1$. Rathod *et al.*[11] also reported the inbreeding depression influence for traits like vine length and fruit weight in bitter gourd.

### *Gene-interaction studies using scaling and joint scaling test*

ABCD scaling test was performed to study the genic interaction for various traits (Table 3). The A-scale was significant for the node to first male flower, fruit length, fruit diameter and fruit weight, while the B-scale was highly significant for node to first male flower, vine length at 90 DAT, fruit length, fruit diameter and fruit weight. However, the C-scale was observed as highly significant for node to first male flower, vine length at 45 DAT, fruit length, fruit diameter and fruit number/plant, while the D-scale was highly significant for days to first fruit harvest, fruit length, fruit length/diameter and fruit weight. The mean effect ($m$) was statistically significant for all the traits, indicating that all these traits were quantitatively inherited. Rathod *et al.*[11] also reported that vine length, days to the first female flower, fruit weight, fruit number/vine and yield/vine are quantitatively inherited in bitter gourd. In the present study, the scaling and joint scaling tests were significant for most of the traits. This can be attributed to the presence of inter-allelic interaction, affecting the expression of a trait. Thus, additive dominance alone may not be sufficient, considering such traits and their improvement may become cumbersome. Hence the six-parameter model was also used to estimate the six components of genetic variation ($m$, $d$, $h$, $i$, $j$ and $l$)[12]. Previous reports showed that non-allelic gene interactions were involved in the expression of quantitative characters in bitter gourd[13,14].

In the present study, the additive effects of the genes were predominant, as evidenced by the presence of positive and significant additive effect ($d$) for most of the traits (Table 3) like days to first male flower, node to first male flower, vine length at 45 DAT and 90 DAT, fruit length, fruit diameter, fruit length/diameter, fruit weight and yield/plant. Thus, the selection of these traits should be delayed to later generations. However, the traits like days to the first female flower, days to first fruit harvest, and fruit number/plant had positive and significant values of $h$, indicating the predominance of dominant gene effects. Thus, it is advisable to delay the selection for these traits until heterozygosity is reduced and homozygosity is achieved in the population.

**Table 3.** Scaling test, gene effects and standard error for different traits in the cross S-2 × Pusa Purvi of bitter gourd

| Parameter | *A* | *B* | *C* | *D* | *m* | *d* | *h* | *i* | *j* | *l* | Epistasis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DFFF | –1.46 ± 2.00 | –13.30 ± 1.69 | –10.39 ± 3.07 | –2.18 ± 0.84 | 30. 35** ± 0.24 | –7.11 ± 0.68 | 9.27** ± 2.21 | 4.37** ± 1.67 | –11.84 ± 2.24 | –19.13 ± 4.11 | D |
| DFMF | –13.08 ± 2.34 | –11.28 ± 2.00 | –26.45 ± 3.86 | 1.04 ± 0.76 | 30.06** ± 0.24 | 3.80** ± 0.59 | 3.38 ± 2.40 | –2.08 ± 1.51 | 1.80 ± 1.89 | –22. 27 ± 4.45 | C |
| NFFF | –1.92 ± 1.86 | –11.84 ± 1.99 | –9.19 ± 3.45 | –2.28 ± 0.67 | 13.89** ± 0.25 | –5.76 ± 0.45 | 2.57 ± 2.11 | 4.57** ± 1.34 | –9.92 ± 1.77 | –18.33 ± 3.90 | D |
| NFMF | 8.86** ± 1.71 | 7.10** ± 1.51 | 20.21** ± 2.35 | –2.13 ± 0.53 | 10.95** ± 0.13 | 2.52** ± 0.46 | –2.95 ± 1.56 | 4.25** ± 1.06 | –1.76 ± 2.07 | 11.71** ± 2.99 | D |
| VL45 DAT | –0.46 ± 0.21 | –0.01 ± 0.18 | 1.87** ± 0.34 | –1.17 ± 0.07 | 0.79** ± 0.01 | 0.85** ± 0.05 | 2.45** ± 0.21 | 2.34** ± 0.13 | 0.45 ± 0.21 | –2.80 ± 0.41 | D |
| VL90 DAT | –1.28 ± 0.54 | 1.62** ± 0.45 | 1.63 ± 0.84 | –0.64 ± 0.13 | 2.82** ± 0.04 | 2.15** ± 0.11 | 1.63** ± 0.49 | 1.29** ± 0.27 | 2.91** ± 0.52 | –0.96 ± 0.94 | D |
| DFFH | –5.52 ± 2.28 | –14.82 ± 1.43 | –33.98 ± 2.74 | 6.82** ± 0.89 | 45.14** ± 0.28 | –0.75 ± 0.69 | 23.74** ± 2.18 | –13.64 ± 1.79 | –9.30 ± 2.51 | –6.70 ± 3.91 | D |
| FL | 6.27* ± 1.07 | 5.54** ± 0.67 | 10.22** ± 1.43 | 0.79** ± 0.26 | 8.34** ± 0.07 | 5.13** ± 0.21 | –2.32 ± 0.88 | –1.58 ± 0.53 | –0.72 ± 1.04 | 13.39** ± 1.67 | D |
| FD | 1.11* ± 0.40 | 1.95** ± 0.39 | 2.74** ± 0.64 | 0.16 ± 0.13 | 2.87** ± 0.04 | 1.43** ± 0.09 | –0.03 ± 0.40 | –0.32 ± 0.26 | 0.85 ± 0.46 | 3.37** ± 0.75 | D |
| FL/D | 0.56 ± 0.57 | –0.54 ± 0.49 | –1.56 ± 0.80 | 0.79** ± 0.18 | 3.44** ± 0.07 | 7.37** ± 0.10 | –1.94 ± 0.52 | –1.57 ± 0.36 | –1.11 ± 0.73 | 1.59 ± 0.91 | D |
| FW | 19.53** ± 5.99 | 17.53** ± 4.39 | –2.41 ± 9.19 | 19.74** ± 1.72 | 45.96** ± 0.60 | 31.87** ± 1.22 | –55.22 ± 5.61 | –39.47 ± 3.44 | –2.00 ± 5.09 | 76.54** ± 10.40 | D |
| FNP | –0.48 ± 1.55 | –25.80 ± 1.44 | 7.47** ± 2.31 | –16.87 ± 0.65 | 14.03** ± 0.17 | –16.06 ± 0.54 | 31.15** ± 1.70 | 33.75** ± 1.30 | –25.32 ± 1.72 | 60.03 ± 3.17 | C |
| YP | –3.82 ± 0.36 | –1.21 ± 0.28 | –3.71 ± 0.51 | 0.65 ± 0.15 | 2.58** ± 0.05 | 2.32** ± 0.11 | 0.55 ± 0.39 | 1.31** ± 0.31 | 2.61** ± 0.37 | –6.33 ± 0.70 | D |

C, Complementary epistasis and D, Duplicate epistasis; **Significant at $P \leq 0.01\%$; *Significant at $P \leq 0.05\%$.

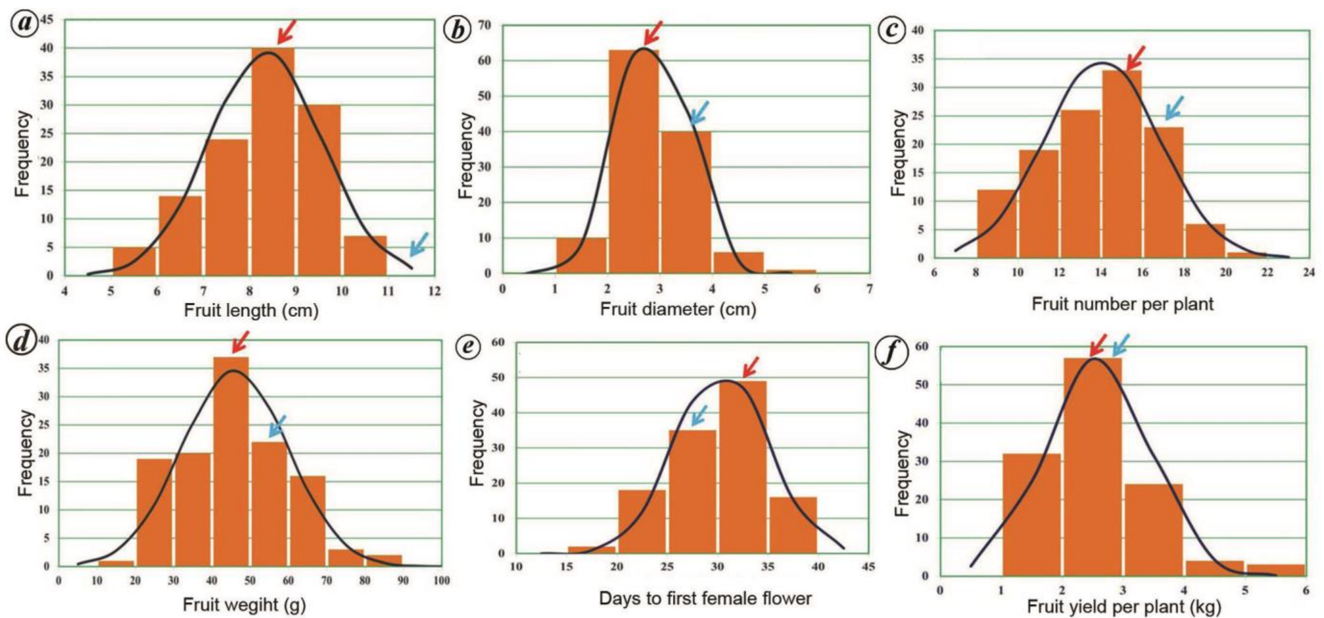


**Figure 2.** Normal distribution curve with histogram (bars representing the frequency of each class) of various parameters in $F_2$ population of S-2 × Pusa Purvi. $N = 120$ (for all parameters). The red arrow indicates the class (interval) to which the mean of $F_2$ belongs, while light blue arrow indicates the class (interval) to which the mid-parent value belongs.

Among the various interaction effects (*i*, *j* and *l*), the additive × additive (*i*) gene effect was highly significant and positive for traits like days to first female flower, node to first female flower, node to first male flower, vine length at 45 DAT and 90 DAT, fruit number/plant and yield/plant. For node to first female flower, additive × additive (i) gene effect was higher than the dominance (*h*) effect, indicating the predominance of additive × additive gene interactions. Thus, a simple selection procedure can be adopted to improve this trait. The dominance × dominance (*l*) gene effect was highly significant and positive for traits like fruit length, diameter and weight. The magnitude of the *l* was also higher than that of *i* and *j*. These findings reveal the greater magnitude of the dominance gene effect compared to the additive effect for most traits. Therefore, it can be advocated that a combination breeding approach based on hybridization followed by selection with moderate selection intensity should be followed to improve these traits. A previous study also showed the involvement of dominance and dominance × dominance gene action for fruit weight, length and fruit number/plant in bitter gourd[15].

Duplicate (*D*) epistasis was observed for the majority of the traits (except days to first male flower and fruit number/plant; Table 3), suggesting the possibility of obtaining transgressive segregants in later generations, while the complementary (C) epistasis signified that the parents used for hybridization were diverse for that particular trait. Hence it is possible to realize an enhanced genetic gain in breeding programmes. Kumari *et al.*[14] also reported the presence of duplicate epistasis for the majority of the traits in bitter gourd hybridization programmes.

### *Frequency distribution and normal distribution curve*

For all six traits, the $F_2$ mean belonged to the class having the highest frequency (Figure 2 *a–f*). The higher range value in all the traits ascertained the presence of higher variability in the $F_2$ population (Supplementary Table 1). Continuous classes of frequency distribution and symmetrical bell-shaped normal distribution curve of the segregating $F_2$ population established that these traits were quantitatively governed. It can also be inferred from Figure 2 *b–f* among the five traits (except fruit length), the MP value (Table 2) belongs to the class (indicated by a light blue arrow) which is closest to the highest frequency class, to which the $F_2$ mean also belongs (indicated by red arrow). However, for fruit length (Figure 2 *a*), the MP value (11.26 cm) (Table 2) falls under the 11–12 cm class, which is far away from the 8–9 cm class (having the highest frequency) to which the mean of $F_2$ also belongs. This shows that shorter fruit length is partially dominant over longer fruit length, which is also supported by the study of Kim *et al.*[16].

### *Correlation and regression analysis for quantitative traits*

The correlation coefficient analysis indicated that among the 11 yield-attributing traits studied, yield/plant was positively correlated with vine length at 45 DAT (0.002) and 90 DAT (0.054), fruit length (0.044), fruit diameter (0.097), fruit weight (0.023) and fruit number/plant (0.322; Supplementary Table 2). However, among these, fruit number/plant

(0.322) was highly significant, while the other four were statistically non-significant. Thus, it can be inferred that the higher the fruit number/plant, the higher the yield/plant.

The regression analysis of fruit yield/plant with other yield-attributing traits in the $F_2$ population revealed a lower $R^2$ value (<0.1) for almost all the parameters, indicating the presence of higher diversity in the population (Supplementary Figure 2). Thus, the data points were highly scattered around the regression line.

### *Inheritance pattern of qualitative traits*

The inheritance pattern of qualitative traits like fruit tubercles, ridgeness, and fruit shape at apex was examined using the $\chi^2$ test in the six populations (Supplementary Table 3). All the fruits of S-2 showed non-conspicuous tubercles and continuous ridgeness on the fruit surface with an acute-shaped apex, while Pusa Purvi showed conspicuous tubercles and discontinuous ridgeness on the surface with a round apex. In $F_1$, the fruits had conspicuous tubercles, discontinuous ridgeness and acute-shaped apex, showing the dominance of these traits over their counterparts. The $\chi^2$ and *P*-values in $F_2$ progeny proved that the observed frequency of $F_2$ plants fitted well in the expected ratio of 3 : 1. Similarly, the $B_1$ (tubercle and ridgeness) and $B_2$ (fruit shape at apex) populations also fitted well in the expected ratio (1 : 1), as evident from $\chi^2$ and *P*-values. The dominant traits 'conspicuous tubercle' and 'discontinuous ridgeness' were expressed in all the plants in $B_2$, but the 'acute fruit shape at apex' was expressed only in $B_1$. This is because the dominant traits 'conspicuous tubercles' and 'discontinuous ridgeness' are present in Pusa Purvi, which is the recurrent parent in the development of $B_2$, but the trait 'acute fruit shape at apex' is present in S-2, which is the recurrent parent in $B_1$. All these findings suggested the monogenic inheritance of these three qualitative traits. Kumari *et al.*[14] also suggested the dominant inheritance of tubercles in bitter gourd. However, to our knowledge there are no previous studies regarding the inheritance pattern for fruit ridgeness and shape at the apex in bitter gourd, although these qualitative traits often decide consumer preference and demand.

## Conclusion

From the results of this study, it can be concluded that yield is a complex polygenic trait in bitter gourd. Both additive and non-additive gene interactions operate among the yield-attributing traits, with the additive predominant. Epistasis gene interaction was also observed in the yield-attributing traits, with duplicate epistasis being dominant for majority of the traits. These findings signify that the breeding for high-yielding bitter gourd genotypes can be undertaken by combining the breeding approach based on hybridization followed by selection with moderate selection intensity. The qualitative traits like 'conspicuous tubercles', 'discontinuous ridgeness' and 'acute shaped fruit apex' followed monogenic inheritance and were dominant compared to their counterparts. Moreover, 10 polymorphic SSR markers have been identified, which can be used to improve the bitter gourd breeding programmes.

*Conflict of interest:* The authors declare that they have no conflict of interest.


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