Recurrent parent phenome recovery analysis in six different populations with the introgression of genetic male sterile gene ms10 from hot pepper to heat tolerant bell pepper

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Abstract

Backcrossing is one of the most commonly used breeding methods to transfer target trait(s) into a bell pepper inbreds. We attempted for the first time to transfer genetic male sterile (GMS) ms10 gene from the donor parent MS-12, popular GMS line in India, into the genetic background of six heat tolerant bell pepper inbreds, in restricted generations through the marker-assisted selection (MAS) and backcross breeding method, to develop new GMS lines in bell pepper. In this project, the recurrent parent phenome (RPP) recovery was analyzed in each generations of backcrossing using economic phenotypic markers. Phenotypic background analysis revealed that the extent of RPP recovery ranged from 43.59% (MSSM-1) to 66.26% (MSSM-21) and 70.89% (MSSM-17) to 92.09% (MSSM-21) for fruit weight, from 19.0% (MSSM-21) to 55.14% (MSSM-3) and 61.0% (MSSM-21) to 98.75% (MSSM-1) for number of lobes fruit⁻¹, from 55.77% (MSSM-17) to 94.25% (MSSM-2) and 69.83% (MSSM-17) to 98.26% (MSSM-3) for total fruit yield plant⁻¹ in BC₁F₁ and BC₂F₁ populations, respectively. Based on a paired 't' test analysis in BC₁F₁ population, out of six, the MSSM-1 and MSSM-2 generation was shown to be non-significant for all 19 studied traits followed by MSSM-3 (18), indicating a successful recovery of the recipient genome through marker-assisted backcross breeding after successful introgression of the ms10 gene. The GMS lines developed from this project will be utilized in bell pepper breeding programs aimed to identify the best cross-combination for their cultivation under north-Indian plains.

Introduction

Pepper is a globally important and often cross-pollinated vegetable crop that exhibits significant amount of heterosis, the fruit yield of F₁ hybrids is up to 50% greater than that of their parental lines (Meena et al. 2020a). In spite of, one of the major factors that limit the application of heterosis is hand emasculation, which is not only time consuming and costly, but also unaffordable for pepper crop. Incidentally, the use of a male sterile (MS) line, which does not need emasculation during hybrid F₁ production, is a best solution for overcoming this problem (Meena et al. 2020b). Both the genetic male sterility (GMS) and the nuclear-cytoplasmic male sterility (C-GMS or CMS) have been reported and utilized for hybrid development in Capsicum (Dhaliwal and Jindal 2014; Meena 2017). The expression of MS trait in most of the CMS lines reported so far is incomplete (partial) and unstable due to the various reasons in Capsicum, influenced by the temperature fluctuations and the genetic background, and till date, gene(s) controlling this trait are still not clear (Meena et al. 2018). Therefore, GMS is an important in various crops including Capsicum for hybrid F₁ seed production in many countries; one GMS line, MS-12, is being utilized to produce hybrid seed of chilli pepper in India. GMS in Capsicum was first reported by Martin and Crawford (1951) in C. frutescens. Up to date, approximately twenty such independently inherited male sterile (MS) genes have been documented, namely ms1 (allelic with ms2), ms2 to ms9 (mc9), ms10 (mc509), ms11 (mc705), ms12 to ms15, msc-1, msc-2 and mscx (Jindal et al. 2020). Except one gene, Dms (mutation of ms5; dominant) (Daskalov and Poulos 1994), the MS trait is reported to be inherited by a single recessive gene (Rani 2020).

The molecular marker(s) linked to ms gene has been developed and reported by different research groups. Recently, Aulakh et al. (2016) mapped GMS gene ms10 in hot pepper (C. annuum), and a SSR marker ‘AVRDC-PP12’ linked to MS gene was identified. Backcrossing is one of the most commonly used breeding methods to transfer target trait(s) into a bell pepper inbreds. By using marker-assisted backcross breeding (MABB) strategy, we attempted for the first time at Punjab Agricultural University (PAU), Ludhiana to transfer ms10 gene from hot pepper to bell pepper background to develop new genetic male sterile (GMS) lines suitable for hybrid development (Rani et al. 2021). Backcross breeding is typically performed with the specific intent to keep the target gene (ms10) and reduce the amount of donor segment as much as possible. With every successive backcrossing the amount of donor segment decreases and the amount of recurrent parent segment increases (Lewis and Kernodle 2009). The stringent phenotypic background selection for the recurrent parent phenome recovery is a very feasible option for the maximization of recurrent parent genome recovery (Joseph et al. 2004; Gopalakrishnan et al. 2008). This approach is save the cost and the time involved in the recovery of recurrent parent genome (Ahmed et al. 2016), marker assisted selection (MAS) for background selection is still a distant dream for resource-limited laboratories (Singh et al. 2013).

The molecular marker assisted foreground selection for ms10 gene along with stringent phenotypic selection in every backcrossed generation ensure selection of individual plant(s) with desirable gene(s), which has highest recurrent parent phenome (RPP) recovery (Singh et al. 2013; Miah et al. 2015). The main goal of the phenotypic background selection was to speed up the return to the recurrent parent trait outside the target gene. The main objective of present research was to determine the amount of RPP recovery from new GMS lines that were developed by crossing hot pepper and heat tolerant bell pepper.

Materials And Methods
Plant materials and breeding strategy

Hot pepper GMS line, MS-12 was used as the donor parent to transfer the *ms10* gene into six heat tolerant bell pepper inbreds namely, PAU SM-1, PAU SM-2, PAU SM-3, PAU SM-9, PAU SM-17, and PAU SM-21. In first cycle, the bell pepper recurrent parents were crossed as the male parent with the donor, MS-12. The selected F₁ plants of six crosses were backcrossed with respective bell pepper inbreds to generate BC₁F₁ seed. Foreground selection in BC₁F₁ populations for *ms10* gene was performed using tightly linked SSR marker, AVRDC-PP12 (Rani et al. 2021). The gene positive individual plant(s) with the maximum phenotypic similarity to the respective recurrent parent were backcrossed to generate BC₂F₁ seed of six population’s viz., MS-12/PAU SM-1//PAU SM-1 (MSSM-1), MS-12/PAU SM-2//PAU SM-2 (MSSM-2), MS-12/PAU SM-3//PAU SM-3 (MSSM-3), MS-12/PAU SM-9//PAU SM-9 (MSSM-9), MS-12/PAU SM-17//PAU SM-17 (MSSM-17), and MS-12/PAU SM-21//PAU SM-21 (MSSM-21). The foreground and phenotypic background selections were carried out to select elite plants from each backcross series (Rani et al. 2021). Research trial of present work was carried out in a randomized complete block design (RCBD) with two replicates for each parent and population.

Phenotypic selection

Stringent phenotypic selection was carried out over the entire population of BC₁F₁ and BC₂F₁ populations after foreground selection. The phenotypic traits included plant height (cm), plant spread (cm), number of primary branches plant⁻¹, fruit weight (g), fruit length (cm), fruit width (cm), pericarp thickness (mm), number of fruits plant⁻¹, number of seed fruit⁻¹, 1000 seed fresh weight (g), 1000 seed dry weight (g), dry matter (%), ascorbic acid content (mg.100g⁻¹), capsaicin content (%), chlorophyll a (mg.100g⁻¹), chlorophyll b (mg.100g⁻¹), total chlorophyll content (mg.100g⁻¹), total fruit yield plant⁻¹ (g), fruit color, fruit orientation and fruit shape.

Statistical analysis

To validate the successful revival of the recurrent parent genome (RPG) in the BC₂F₁ populations, the horticultural and morphological performances of the improved lines carrying the male sterility (*ms10*) gene together with the adaptable recurrent parent were compared by paired ‘t’ test analysis by using SPSS software Version 22.0 (IBM Corp 2013).

Results And Discussion

Analysis of variance (ANOVA) for parents and backcross populations

The ANOVA for experimental design for 19 plant growth, yield, seed and biochemical traits is presented in Table 1 and 2. The ANOVA showed greater significant mean squares (MS) due to parents for all the studied 19 traits, it indicating that the presence of sufficient amount of inherent variability among the parents which could be exploited via selection. The MS due to developed BC₂F₁ populations were non-significant for plant spread, 1000 seed fresh weight and chlorophyll-a content, and significant for rest of the traits studied, which depicted the differential response of different BC₂F₁ population to studied traits. This is in agreement with the finding of Usman et al. (2014).

Recovery of recurrent parent traits in BC₂F₁ populations

To validate the successful revival of the recurrent parent genome (RPG) in the BC₂F₁ populations, the horticultural and morphological performances of the improved lines carrying the male sterility (*ms10*) gene together with the adaptable recurrent parent were compared by paired ‘t’ test analysis. The results are furnished in the Table 3-7.

Evaluation of BC₂F₁ population for plant growth traits

Plant height is considered as an important yield attributing trait, because it leads to more number of branches and prolonged harvest duration, ultimately results in higher productivity. The taller plants have less disease incidence than the dwarf ones (Sood and Kumar 2013). The mean performance of six recipient parental lines for plant height ranged from 67.67 cm (PAU SM-21) to 103.70 cm (PAU SM-17). On the other hand, plant height of the BC₂F₁ population, varied from 55.25 cm to 106.20 cm. The generation MS-12/PAU SM-17//PAU SM-17 (106.20 cm) showed maximum plant height followed by MS-12/PAU SM-3//PAU SM-3 (86.51 cm), and the minimum was recorded in MS-12/PAU SM-21//PAU SM-21 (55.25 cm) and MS-12/PAU SM-9//PAU SM-9 (63.47 cm). Plant spread for six parental lines ranged from 44.71 cm to 58.01 cm, and the maximum plant spread was recorded for PAU SM-1 followed by PAU SM-3, while, the
minimum was observed for PAU SM-9 followed by PAU SM-17. The BC\textsubscript{2}F\textsubscript{1} population means for plant spread varied from 48.94 cm in MS-12/PAU SM-9//PAU SM-9 to 61.93 cm in MS-12/PAU SM-3//PAU SM-3. Number of primary branches plant\textsuperscript{1} is an essential yield contributing trait, having a direct effect on yield. The plant having more number of branches results increased productivity. Number of primary branches plant\textsuperscript{1} of parental lines ranged from 3.04 to 4.53. The maximum number of primary branches plant\textsuperscript{1} was recorded by PAU SM-3 followed by PAU SM-1, while, the minimum was observed in PAU SM-9 and PAU SM-21. Among the six developed BC\textsubscript{2}F\textsubscript{1} populations, the maximum number of primary branches plant\textsuperscript{1} was recorded by MS-12/PAU SM-3//PAU SM-3 (5.21) followed by MS-12/PAU SM-2//PAU SM-2 (4.72) and MS-12/PAU SM-1//PAU SM-1 (4.39), whereas, the minimum was recorded by MS-12/PAU SM-21//PAU SM-21 (3.24), MS-12/PAU SM-9//PAU SM-9 (3.73) and MS-12/PAU SM-17//PAU SM-17 (3.97). From the six BC\textsubscript{2}F\textsubscript{1} populations, the paired t test was non-significant for mean values of five developed populations and their recurrent parent for plant growth traits except MS-12/PAU SM-9//PAU SM-9 for plant height and MS-12/PAU SM-17//PAU SM-17 for plant spread and number of primary branches plant\textsuperscript{1} which showed significant differences with their respective recurrent parent.

Evaluation of BC\textsubscript{2}F\textsubscript{1} population for fruit and yield traits

Development of high yielding cultivars along-with good fruit quality is important to enhance the crop productivity per unit area as well as the farmers’ income. Therefore, the total fruit yield and fruit traits are the most important economic characters concerned by most breeders. Fruit weight is one of the most important traits which have a direct effect on fruit yield plant\textsuperscript{1}. It is vital to clarify that the average weight of fruit can be altered according to the fruit yield plant\textsuperscript{1}. Under Indian marketing system, medium sized fruits (50-80 g) are generally preferred. The per se performance for fruit weight among the developed BC\textsubscript{2}F\textsubscript{1} populations ranged from 33.33 g (MS-12/PAU SM-9//PAU SM-9) to 71.83 g (MS-12/PAU SM-3//PAU SM-3) in comparison to 43.98 g (PAU SM-21) to 84.88 g (PAU SM-3) of parental lines. For fruit length, the lowest and the highest mean values were recorded by the backcross generation MS-12/PAU SM-9//PAU SM-9 and MS-12/PAU SM-3//PAU SM-3 in BC\textsubscript{2}F\textsubscript{1} populations, while by PAU SM-21 and PAU SM-3 in parental lines. Fruit length of backcrossed populations ranged from 5.66 cm to 7.50 cm, on the contrary, fruit length of recurrent parents to be 4.82 cm to 7.38 cm. Per se performance for fruit width of parental lines ranged from 4.56 cm to 7.34 cm, on the other hand, of the BC\textsubscript{2}F\textsubscript{1} population varied from 3.58 cm to 6.74 cm. Among the recurrent parents, the maximum fruit width was recorded by PAU SM-3 followed by PAU SM-1, while, the minimum fruit width was observed by PAU SM-21 followed by PAU SM-17. In the backcrossed populations, the maximum fruit width was recorded by MS-12/PAU SM-3//PAU SM-3 and MS-12/PAU SM-2//PAU SM-2, while, the minimum fruit width was recorded by MS-12/PAU SM-17//PAU SM-17 and MS-12/PAU SM-21//PAU SM-21. Pericarp thickness is a very important character which decides the firmness of fruit. Thick fleshed bell pepper can endure long shipping distance. Pericarp thickness of backcrossed population ranged from 3.14 mm in MS-12/PAU SM-17//PAU SM-17 to 3.93 mm in MS-12/PAU SM-3//PAU SM-3 whereas pericarp thickness of recurrent parents was varied from 3.29 mm in PAU SM-17 to 4.83 mm in PAU SM-3.

Number of lobes fruit\textsuperscript{1} affects the shape of fruit in bell pepper. Generally, a medium sized, blocky and four lobed fruit are acceptable in the market. The backcross populations means for number of lobes fruit\textsuperscript{1} ranged from 1.83 to 3.17, and that of recurrent parents varied from 2.90 to 3.50. Based on per se performance, the population namely MS-12/PAU SM-2//PAU SM-2, MS-12/PAU SM-1//PAU SM-1 and MS-12/PAU SM-3//PAU SM-3, and the parental lines namely PAU SM-3, PAU SM-2 and PAU SM-1 were identified with higher number of lobes fruit\textsuperscript{1}. Number of fruits plant\textsuperscript{1} is the most important component trait, which is directly related with increased fruit yield plant\textsuperscript{1}. Number of fruit plant\textsuperscript{1} of backcrossed populations varied from 28.59 to 39.18, which was higher than their recurrent parents i.e., 25.14 to 33.02. Among the parental lines, the maximum number of fruit plant\textsuperscript{1} was recorded by PAU SM-17 followed by PAU SM-3 and PAU SM-21, while, the minimum number of fruit plant\textsuperscript{1} was observed by PAU SM-2 followed by PAU SM-1. Among the backcrossed populations, the maximum number of fruit plant\textsuperscript{1} was recorded by MS-12/PAU SM-9//PAU SM-9 followed by MS-12/PAU SM-3//PAU SM-3, while, the minimum was observed by MS-12/PAU SM-21//PAU SM-21 followed by MS-12/PAU SM-17//PAU SM-17. The main focus of cultivating a crop is to have the maximum yield per unit area for better returns. Moreover, high fruit yield is the ultimate goal of any breeding program; hence, it requires the highest consideration. It is the key factor in adaptation of variety by farmers. In the parental lines, the mean values of total fruit yield plant\textsuperscript{1} varied from 1109.50 g to 2028.40 g. The maximum total fruit yield plant\textsuperscript{1} was recorded by PAU SM-3 followed by PAU SM-1, while, the minimum was depicted by PAU SM-9 followed by PAU SM-21. On the contrary, total fruit yield plant\textsuperscript{1} of the BC\textsubscript{2}F\textsubscript{1} population was ranged from 1024.32 g to 2060.27 g. The maximum total fruit yield plant\textsuperscript{1} was recorded by MS-12/PAU SM-1//PAU SM-1 followed by MS-12/PAU SM-3//PAU SM-3, while, the minimum was recorded by MS-12/PAU SM-17//PAU SM-17 followed by MS-12/PAU SM-9//PAU SM-9.
For studied fruit and yield traits all the developed populations depicted non-significant differences with their respective recurrent parents based on paired $t$ test analysis, except MS-12/PAU SM-21//PAU SM-21 for fruit length and number of lobes fruit$^{-1}$, and MS-12/PAU SM-9//PAU SM-9 for number of lobes fruit$^{-1}$ revealed significant differences with their recurrent parent. This suggested that fruit traits of individuals of the developed $BC_2F_1$ populations were in the range of the recurrent parent value. It indicated that the complete phenome of recurrent parent has been transfer or recovered in respective backcrossed population or genetic background of the backcrossed populations and their respective recurrent parent became identical for these traits. Into the genetic background of widely cultivated Malaysian variety of rice (MR219), Miah et al. (2015) successfully introgressed the blast resistant $P$ gene (putative $Piz$) and newly developed improved line depicted similar phenotypic traits when compared with recurrent parent MR219, which proves it was similar to the MR219 genetic backgrounds.

To the significant $t$ test populations, continued backcross with recurrent parent will be performed up-to the degree of similarity of the backcrossing progeny is greatly close to the recurrent parent. The final last backcross population will be self-pollinated to develop new GMS lines of bell pepper, which is almost isogenic to their recurrent parent in the future.

*Evaluation of $BC_2F_1$ population for seed traits*

Apart from the male sterility, GMS lines should be possessed the normal female fertility and higher number of seed fruit$^{-1}$ (Meena et al. 2018). Number of seed fruit$^{-1}$ ranged from 43.15 to 69.28 in parental lines. The maximum number of seed fruit$^{-1}$ was recorded by PAU SM-3 (69.28) followed by PAU SM-21 (67.11), whereas, the minimum was recorded by PAU SM-17 (43.15) followed by PAU SM-1 (59.41). Among the $BC_2F_1$ populations, number of seed fruit$^{-1}$ ranged from 40.93 to 60.47. The backcross population, MS-12/PAU SM-3//PAU SM-3 (60.47) showed maximum number of seed fruit$^{-1}$ followed by MS-12/PAU SM-1//PAU SM-1 (58.57), and the minimum was recorded by MS-12/PAU SM-17//PAU SM-17 (40.93) and MS-12/PAU SM-2//PAU SM-2 (48.87). Test weight of fresh seed among the developed $BC_2F_1$ populations ranged from 8.00 g (MS-12/PAU SM-9//PAU SM-9) to 12.31 g (MS-12/PAU SM-3//PAU SM-3) in comparison to 9.20 g (PAU SM-9) to 13.48 g (PAU SM-3) in parental lines. Therefore, the performance of tested populations was statistically on par with that recorded for recurrent parents. Based on paired $t$ test analysis, it was observed that the developed breeding backcross populations had similar test (1000) weight of dry seed that of recurrent parent. Mean performance of 1000 seed dry weight for parental lines ranged from 5.94 g to 9.35 g. The maximum weight were recorded by PAU SM-2 followed by PAU SM-21 and PAU SM-3, whereas, weight were recorded by PAU SM-17 followed by PAU SM-9. Among the $BC_2F_1$ populations, it ranged from 6.74 to 8.18. The progeny MS-12/PAU SM-2//PAU SM-2 showed maximum weight followed by MS-12/PAU SM-17//PAU SM-17, and the minimum was recorded by MS-12/PAU SM-9//PAU SM-9 followed by MS-12/PAU SM-3//PAU SM-3. For the studied seed traits, the mean values of developed backcrossed population possessing $ms10$ gene was depicted statistically non-significant differences to their recurrent parents, on the basis of paired $t$ test analysis, it indicated that with the male sterility traits the developed progenies have good seed setting ability as their parental lines of bell pepper.

*Evaluation of $BC_2F_1$ population for quality traits*

Higher content of dry matter means higher processed product yield. Therefore, fruit dry matter is very important trait for breeding pepper for processing industry, since a large proportion of the pepper produced in the world is used in the form of powder (Lannes et al. 2018). The parental lines mean for dry matter content ranged from 5.55 to 8.87%. The parent line PAU SM-3 (8.87%) expressed the highest mean value followed by PAU SM-21 (7.78%), while, the minimum by PAU SM-17 (5.55%) and PAU SM-2 (6.68%). *Per se* performance of $BC_2F_1$ population for dry matter content ranged from 6.99 to 10.91%. In $BC_2F_1$ populations, the progeny MS-12/PAU SM-3//PAU SM-3 (9.56%) had maximum dry matter content followed by MS-12/PAU SM-1//PAU SM-1 (8.91%), and the minimum was recorded by MS-12/PAU SM-17//PAU SM-17 and MS-12/PAU SM-2//PAU SM-2. Ascorbic acid is the major component of the nutritional quality in bell pepper. Ascorbic acid content (mg.100g$^{-1}$) for all the six developed backcross populations ranged from 80.56 mg.100g$^{-1}$ to 115.01 mg.100g$^{-1}$, that of the recurrent parents varied from 61.53 mg.100g$^{-1}$ to 104.73 mg.100g$^{-1}$. Among the parental lines, the maximum ascorbic acid content was observed by PAU SM-9 and PAU SM-3, whereas, the minimum was recorded by PAU SM-17 and PAU SM-2. In $BC_2F_1$ populations, the progeny MS-12/PAU SM-9//PAU SM-9 showed maximum ascorbic acid content followed by MS-12/PAU SM-3//PAU SM-3, and the minimum was recorded by MS-12/PAU SM-17//PAU SM-17 followed by MS-12/PAU SM-2//PAU SM-2. For the traits, dry matter and ascorbic acid, all the developed populations were highly similar to their recurrent parent, confirmed by non-significant values of the paired $t$ test analysis; this suggested that the developed populations had maximum recurrent parent phenome recovery for mentioned traits.
The backcrossed populations mean for capsaicin content ranged from 0.25 to 0.41%, on the other hand, the parental lines mean varied from 0.18 to 0.30%. The progeny MS-12//PAU SM-9//PAU SM-9 depicted the highest mean value for capsaicin content followed by MS-12//PAU SM-17//PAU SM-17, while the lowest capsaicin content was recorded by MS-12//PAU SM-2//PAU SM-2 and MS-12//PAU SM-3//PAU SM-3. This variation for pungency and dry matter can be used in Capsicum breeding programs for the production of spice market oriented cultivars. Therefore, the developed GMS lines of bell pepper from this project could be utilized as maternal parent in hybrid breeding program to identify best cross-combination(s) with non-pungent high powder yield for industrial purposes. Among the parental lines, the maximum capsaicin content was recorded by PAU SM-21 followed by PAU SM-9, although, the minimum was observed by PAU SM-1 followed by PAU SM-3. Out of six BC$_2$F$_1$ populations, four populations showed non-significance difference with their respective recurrent parents which signified that developed populations had maximum recurrent parent genome recovery. Two populations namely MS-12//PAU SM-9//PAU SM-9 and MS-12//PAU SM-21//PAU SM-21 expressed significant differences with their recipient parent for capsaicin content.

The maximum mean values for chlorophyll 'a' (mg.100g$^{-1}$) was expressed by PAU SM-21 followed by PAU SM-9, though, the minimum by PAU SM-17 followed by PAU SM-3, among the parental lines. On the other hand, among the BC$_2$F$_1$ population, the progeny MS-12//PAU SM-2//PAU SM-2 exhibited the highest mean values followed by MS-12//PAU SM-1//PAU SM-1, while, the minimum was recorded by MS-12//PAU SM-9//PAU SM-9 and MS-12//PAU SM-21//PAU SM-21. Chlorophyll 'a' of BC$_2$F$_1$ populations ranged from 0.17 mg to 0.29 mg.100g$^{-1}$ whereas chlorophyll 'a' of recurrent parent was varied from 0.16 mg to 0.37 mg.100g$^{-1}$. Among the parental lines, chlorophyll 'b' (mg.100g$^{-1}$) content was ranged from 0.11 to 0.32 mg.100g$^{-1}$. The maximum chlorophyll 'b' was recorded by PAU SM-2 followed by PAU SM-1, however, the minimum chlorophyll 'b' was observed in PAU SM-21 followed by PAU SM-17. The mean values of chlorophyll 'b' for BC$_2$F$_1$ populations ranged from 0.10 to 0.30 mg.100g$^{-1}$. In the BC$_2$F$_1$ populations, the progeny MS-12//PAU SM-2//PAU SM-2 showed maximum chlorophyll 'b' followed by MS-12//PAU SM-1//PAU SM-1, and minimum was recorded by MS-12//PAU SM-17//PAU SM-17 followed by MS-12//PAU SM-21//PAU SM-21. For this trait, one population namely MS-12//PAU SM-9//PAU SM-9 revealed significant differences to the recurrent parent, while rest of the populations showed non-significance differences with their recurrent parents. The paired t-test analysis demonstrated similar mean values of total chlorophyll content (mg.100g$^{-1}$) between the developed backcrossed population and their parental lines except for MS-12//PAU SM-9//PAU SM-9 and MS-12//PAU SM-21//PAU SM-21, this suggested that total chlorophyll content of individuals of four BC$_2$F$_1$ populations was in the range of chlorophyll content of recurrent parent, and it depicted that developed populations had maximum RPG recovery. For total chlorophyll content (mg.100g$^{-1}$), the parental means varied from 0.15 to 0.32, on the contrary, the backcross population means ranged from 0.14 to 0.29 mg.100g$^{-1}$. Among the six BC$_2$F$_1$ populations, MS-12//PAU SM-2//PAU SM-2 registered the highest per se performance followed by MS-12//PAU SM-1//PAU SM-1, while, the lowest was observed by MS-12//PAU SM-21//PAU SM-21 and MS-12//PAU SM-17//PAU SM-17.

**Evaluation of BC$_2$F$_1$ populations for morphological traits**

The color of the fruit determines the marketable acceptability of bell pepper. Generally uniform, non-blotchy fruits are desirable in the market. The green color of the fruit is principally due to the presence of chlorophyll which includes chlorophyll 'a', chlorophyll 'b' and total chlorophyll. It is evident from the Table 8, all developed BC$_2$F$_1$ populations had green fruit color as similar as parents. This indicated that all the populations had utmost recovered recurrent parent phenome. All the parental lines showed pendent fruit orientation except PAU SM-9 which showed erect fruit orientation. Likewise, all BC$_2$F$_1$ populations had pendent fruit orientation as their respective parents except MS-12//PAU SM-9//PAU SM-9 which had erect fruiting as that of recurrent parent. Fruit shape of bell pepper is a vital trait which determines the acceptability in the market. The standard shape for bell pepper is blocky, which is mostly preferred by the consumers. The fruit shape of all BC$_2$F$_1$ populations was blocky which is quite similar to their recurrent parents.

**Assessment of fruit traits across the generations**

The morphological traits of the donor parent (DP), hot pepper line namely MS-12, recurrent parent (RP), bell pepper lines namely PAU SM-1, PAU SM-2, PAU SM-3, PAU SM-9, PAU SM-17 and PAU SM-21, their F$_1$ hybrids, BC$_1$F$_1$ and BC$_2$F$_1$ generations were evaluated to know the trend of recovery of recurrent parent phenome (RPP). The mean trait values of recurrent parents, reference F$_1$, BC$_1$F$_1$ and BC$_2$F$_1$ populations were examined by LSD 5% and presented in Figure 1 (a-h). In PAU SM-1 generation, for number of lobes fruit$^{-1}$, the perusal of means value of donor parent, recurrent parent, F$_1$ cross, BC$_1$F$_1$ and BC$_2$F$_1$ were 0, 3.2, 0, 1.14 and 3.16, respectively. Assessment of RPP content among BC$_1$F$_1$ and BC$_2$F$_1$ population and recurrent parent showed that average RPP content in BC$_1$F$_1$ was 35.63 and in BC$_2$F$_1$ was 98.75%. In PAU SM-3 generation, for fruit width (cm) the per se performance of F$_1$ hybrid was 2.53 cm, BC$_1$F$_1$ and BC$_2$F$_1$ was 4.10 cm and 6.74 cm, respectively. Evaluation of RPP content between BC$_2$F$_1$, BC$_2$F$_1$ population and recurrent parent depicted that average
RPP content was 55.86 and 91.83%. In PAU SM-17 generation, for pericarp thickness (mm), F₁ cross performance was 1.57 mm while, presentation of BC₁F₁ and BC₂F₁ were 2.06 mm and 3.14 mm, respectively. The average RPP content was 62.61 and 95.44% in BC₁F₁ and BC₂F₁, respectively. In case of PAU SM-21 generation, the BC₂F₁ (1044.46 g) progeny was gave close resemblance to the recurrent parent (1117.18 g) in contrast donor parent (445.50 g) for total fruit yield plant⁻¹. In PAU SM-2 generation, the findings revealed that total fruit yield plant⁻¹ of F₁ hybrid was 1573.83 g, whereas BC₁F₁ and BC₂F₁ progeny was 1731.73 g and 1871.28 g. In other studies conducted by different research group, the RPG recovery was found up to 91.6% in BC₂F₁ generation for pyramiding blast resistance genes Piz5 and Pl54 into an elite Basmati rice restorer line 'PRR78' using MAS (Singh et al. 2013). In BC₂F₁ populations, the genetic background recovery was observed 95.0% in case of Swarna and Samba Mashuri (Prigge et al. 2008). Our findings were similar to the findings of Ahmed et al. (2016), who achieved a recovery of 65.55 to 77.8 and 78.79 to 95.5 % in BC₁F₁ and BC₂F₁ populations, respectively. In BC₂F₁ generation, RPG recovery from 96.6% to 98.8% was recorded among CMS-A lines by Meena et al. (2019), which is similar to present findings.

**Conclusion**

In the current research, based on phenotypic background analysis, the recurrent parent phenotype content in the BC₂F₁ generations ranged from 62.07 (capsaicin content) to 98.75% (number of lobes fruit⁻¹) in MSSM-1, from 74.58 (number of primary branches plant⁻¹) to 96.0% (fruit width) in MSSM-2, from 77.78 (capsaicin content) to 98.40% (fruit length) in MSSM-3, from 50.0 (chlorophyll a) to 96.37% (total fruit yield plant⁻¹) in MSSM-9, from 69.83 (total fruit yield plant⁻¹) to 100% (total chlorophyll) in MSSM-17, and from 45.95 (chlorophyll a) to 98.85% (plant spread) in MSSM-21. The greater recovery in the present study was majorly due to the contribution of recurrent parent alleles from the backcross derivatives, which was made possible through stringent phenotypic selections. Phenotypic selection reduced both the cost and the time involved in the recovery of RPG. The results of paired t-test analysis showed that out of six BC₂F₁ populations, the populations namely MS-12/PAU SM-2//PAU SM-2 and MS-12/PAU SM-2//PAU SM-1 showed non-significance difference for all the studied traits, MS-12/PAU SM-3//PAU SM-3 exhibited non-significant differences with the recurrent parent for eighteen traits, MS-12/PAU SM-17//PAU SM-17 for sixteen traits, MS-12/PAU SM-21//PAU SM-21 for fifteen traits, MS-12/PAU SM-9//PAU SM-9 for thirteen traits. It indicated that after successful introgression of ms10 gene through MABB, the utmost complete genome of recurrent parent has been transfer or recovered in these developed population for plant growth, fruit yield and quality traits or genetic background of these BC₂F₁ population that of recurrent parent became identical.

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**Tables**

Table 1. Analysis of variance for plant growth, yield and quality traits of parental lines
### Table 1 (Cont’d.)

| Source of variation | df | Number of seed fruit⁻¹ | 1000 seed fresh weight (g) | 1000 seed dry weight (g) | Dry matter content (%) | Ascorbic acid content (mg.100g⁻¹) | Capsaicin content (%) | Chlorophyll a (mg.100g⁻¹) | Chlorophyll b (mg.100g⁻¹) | Total chlorophyll (mg.100g⁻¹) | Total fruit yield plant⁻¹ (g) |
|---------------------|----|------------------------|----------------------------|--------------------------|-----------------------|----------------------------------|----------------------|--------------------------|---------------------------|-------------------------------|-------------------------------|
| Replication         | 2  | 25.98                  | 0.84                       | 1.64                     | 0.33                  | 94.94                            | 0.0002               | 0.0001                  | 0.0007                    | 0.0001                        | 54740.73                      |
| Genotypes           | 6  | 239.76**               | 11.69**                    | 4.34**                   | 92.02**              | 1883.38**                     | 0.0474**             | 0.0260**                | 0.03**                    | 0.02**                       | 900932.35**                   |
| Error               | 12 | 30.47                  | 1.42                       | 0.19                     | 0.45                  | 18.89                           | 0.0003               | 0.0003                  | 0.0001                    | 0.0001                        | 8042.05                       |

**Note:** Data’s are mean sums of squares; *significant at p = 0.05; **significant at p = 0.01; ***non-significant

### Table 2. Analysis of variance for plant growth, yield and quality traits of BC₂F₁ population of bell pepper

| Source of variation | df | Plant height (cm) | Plant spread (cm) | Number of primary branches plant⁻¹ | Fruit weight (g) | Fruit length (cm) | Fruit width (cm) | Pericarp thickness (mm) | Number of lobes fruit⁻¹ | Number of fruits plant⁻¹ |
|---------------------|----|-------------------|-------------------|------------------------------------|-----------------|------------------|---------------------|--------------------------|------------------------|--------------------------|
| Replication         | 2  | 11.95             | 22.41             | 0.13                                | 19.39           | 0.07             | 0.20                | 0.35                     | 0.01                   | 2.37                     |
| Genotypes           | 5  | 990.74**          | 70.31             | 1.51***                            | 988.49**        | 1.36*            | 4.28***            | 0.26*                    | 1.09**                 | 37.90*                   |
| Error               | 10 | 60.51             | 36.20             | 0.12                                | 29.38           | 0.39             | 0.16                | 0.06                     | 0.09                   | 14.29                    |

**Note:** Data’s are mean sums of squares; *significant at p = 0.05; **significant at p = 0.01; ***non-significant

### Table 2 (Cont’d.)

| Source of variation | df | Number of seed fruit⁻¹ | 1000 seed fresh weight (g) | 1000 seed dry weight (g) | Dry matter content (%) | Ascorbic acid content (mg.100g⁻¹) | Capsaicin content (%) | Chlorophyll a (mg.100g⁻¹) | Chlorophyll b (mg.100g⁻¹) | Total chlorophyll (mg.100g⁻¹) | Total fruit yield plant⁻¹ (g) |
|---------------------|----|------------------------|----------------------------|--------------------------|-----------------------|----------------------------------|----------------------|--------------------------|---------------------------|-------------------------------|-------------------------------|
| Replication         | 2  | 16.6343               | 0.29                       | 0.05                    | 0.95                  | 73.74                            | 0.001                | 0.00002                 | 0.0002                    | 0.00007                       | 1948.07                       |
| Genotypes           | 5  | 222.27***             | 2.54                       | 2.46***                 | 6.84***              | 499.26***                       | 0.012***             | 0.0013                  | 0.0037***                | 0.00205***                   | 40431.91***                   |
| Error               | 10 | 28.0626               | 0.90                       | 0.40                    | 0.61                  | 29.31                            | 0.000                | 0.0004                  | 0.0002                    | 0.00015                       | 3365.98                       |

**Note:** Data’s are mean sums of squares; *significant at p = 0.05; **significant at p = 0.01; ***non-significant

### Table 3. Recovery of recurrent parent traits in BC₂F₁ population of bell pepper for plant growth traits (appraise similarity through paired t-test)
| Pairs | Individuals | Plant height (cm) | Plant spread (cm) | Number of primary branches plant<sup>1</sup> |
|-------|-------------|-------------------|-------------------|-------------------------------------|
|       |             | Mean     | SEM±     | t   | p-value | Mean     | SEM±     | t   | p-value | Mean     | SEM±     | t   | p-value | RPP<sub>R</sub> (%) | RPP<sub>R</sub> (%) |
| 1     | PAU SM-1    | 83.23    | 3.58     | 1.32<sup>ns</sup> | 0.317 | 98.54    | 58.01    | 3.33     | 0.24<sup>ns</sup> | 0.836 | 97.63    | 4.14     | 0.26     | 1.04<sup>ns</sup> | 0.409 | 94.31    |
|       | MSSM-1     | 84.46    | 4.26     |              |      |          | 59.42    | 3.14     |              |      | 4.39     | 0.27     |          |            |        |
| 2     | PAU SM-2    | 69.50    | 3.34     | 1.81<sup>ns</sup> | 0.213 | 93.59    | 56.71    | 3.42     | 0.62<sup>ns</sup> | 0.597 | 93.21    | 3.52     | 0.21     | 3.00<sup>ns</sup> | 0.095 | 74.58    |
|       | MSSM-2     | 74.26    | 3.60     |              |      |          | 60.84    | 3.69     |              |      | 4.72     | 0.20     |          |            |        |
| 3     | PAU SM-3    | 81.22    | 4.85     | 4.15<sup>ns</sup> | 0.053 | 93.89    | 57.33    | 3.63     | 0.69<sup>ns</sup> | 0.561 | 92.57    | 4.53     | 0.21     | 2.57<sup>ns</sup> | 0.124 | 86.95    |
|       | MSSM-3     | 86.51    | 3.58     |              |      |          | 61.93    | 3.26     |              |      | 5.21     | 0.24     |          |            |        |
| 4     | PAU SM-9    | 82.51    | 3.92     | 9.26*       | 0.011 | 76.92    | 44.71    | 2.51     | 1.21<sup>ns</sup> | 0.351 | 91.36    | 3.04     | 0.11     | 2.98<sup>ns</sup> | 0.097 | 81.50    |
|       | MSSM-9     | 63.47    | 3.45     |              |      |          | 48.94    | 3.26     |              |      | 3.73     | 0.15     |          |            |        |
| 5     | PAU SM-17   | 103.70   | 5.86     | 0.24<sup>ns</sup> | 0.830 | 97.65    | 49.89    | 3.26     | 11.55*     | 0.007 | 91.84    | 3.90     | 0.13     | 6.06*     | 0.026 | 98.24    |
|       | MSSM-17    | 106.20   | 6.50     |              |      |          | 54.32    | 3.22     |              |      | 3.97     | 0.14     |          |            |        |
| 6     | PAU SM-21   | 67.67    | 3.44     | 2.78<sup>ns</sup> | 0.109 | 81.65    | 56.97    | 3.39     | 0.10<sup>ns</sup> | 0.930 | 98.85    | 3.14     | 0.19     | 0.69<sup>ns</sup> | 0.562 | 96.91    |
|       | MSSM-21    | 55.25    | 2.56     |              |      |          | 57.63    | 3.56     |              |      | 3.24     | 0.20     |          |            |        |

*Significant at the 5% level of significance (p<0.05); ns: non-significant at 5% level of significance; RPP<sub>R</sub>: recurrent parent phenome recovery

**Table 4.** Recovery of recurrent parent traits in BC<sub>2</sub>F<sub>1</sub> population of bell pepper for fruit traits (appraise similarity through paired t-test)
| Pairs | Individuals       | Fruit weight (g) |          | Fruit length (cm) |          | Fruit width (cm) |          |
|-------|-------------------|------------------|----------|-------------------|----------|------------------|----------|
|       |                   | Mean  | SEm± | t    | p-value | RPP_R (%)     | Mean  | SEm± | t    | p-value | RPP_R (%)     | Mean  | SEm± | t    | p-value | RPP_R (%)     |
| 1     | PAU SM-1          | 79.20 | 3.43 | 2.91 | 0.101   | **88.60**   | 6.83  | 0.21 | 2.28 | 0.150   | **94.88**     | 5.66  | 0.35 | 3.51 | 0.073   | **87.28**     |
|       | MSSM-1 (BC2F1)   | 70.17 | 2.90 |       |          |            | 6.48  | 0.33 |       |          |            | 4.94  | 0.15 |       |          |            |
| 2     | PAU SM-2          | 74.97 | 4.26 | 1.34 | 0.312   | **87.60**   | 6.71  | 0.31 | 3.91 | 0.060   | **92.42**     | 5.50  | 0.27 | 2.15 | 0.164   | **96.00**     |
|       | MSSM-2 (BC2F1)   | 65.67 | 4.92 |       |          |            | 7.26  | 0.37 |       |          |            | 5.28  | 0.37 |       |          |            |
| 3     | PAU SM-3          | 84.88 | 4.93 | 3.18 | 0.086   | **84.63**   | 7.38  | 0.31 | 0.32 | 0.777   | **98.40**     | 7.34  | 0.36 | 3.48 | 0.074   | **91.83**     |
|       | MSSM-3 (BC2F1)   | 71.83 | 3.48 |       |          |            | 7.50  | 0.51 |       |          |            | 6.74  | 0.20 |       |          |            |
| 4     | PAU SM-9          | 45.20 | 2.93 | 2.71 | 0.113   | **73.74**   | 4.82  | 0.27 | 1.80 | 0.214   | **85.16**     | 5.09  | 0.30 | 1.62 | 0.247   | **83.10**     |
|       | MSSM-9 (BC2F1)   | 33.33 | 2.52 |       |          |            | 5.66  | 0.21 |       |          |            | 4.23  | 0.24 |       |          |            |
| 5     | PAU SM-17         | 50.54 | 2.67 | 3.25 | 0.083   | **70.89**   | 5.03  | 0.19 | 3.26 | 0.083   | **80.61**     | 4.84  | 0.26 | 3.58 | 0.070   | **73.97**     |
|       | MSSM-17 (BC2F1)  | 35.83 | 1.86 |       |          |            | 6.24  | 0.18 |       |          |            | 3.58  | 0.19 |       |          |            |
| 6     | PAU SM-21         | 43.98 | 2.78 | 1.98 | 0.186   | **92.09**   | 4.89  | 0.28 | 10.44| 0.009   | **73.53**     | 4.56  | 0.29 | 2.63 | 0.119   | **80.04**     |
|       | MSSM-21 (BC2F1)  | 40.50 | 2.52 |       |          |            | 6.65  | 0.22 |       |          |            | 3.65  | 0.20 |       |          |            |

Table 4 (Cont’d.)

| Pairs | Individuals       | Pericarp thickness (mm) |          | Number of lobes fruit⁻¹ |          | Number of fruits plant⁻¹ |          |
|-------|-------------------|-------------------------|----------|--------------------------|----------|--------------------------|----------|
|       |                   | Mean  | SEm± | t    | p-value | RPP_R (%)     | Mean  | SEm± | t    | p-value | RPP_R (%)     | Mean  | SEm± | t    | p-value | RPP_R (%)     |
| 1     | PAU SM-1          | 4.55  | 0.24 | 4.15 | 0.053   | **86.15**   | 3.20  | 0.19 | 0.25 | 0.826   | **98.75**     | 27.22 | 1.42 | 3.00 | 0.096   | **83.52**     |
|       | MSSM-1 (BC2F1)   | 3.92  | 0.12 |       |          |            | 3.16  | 0.13 |       |          |            | 32.59 | 0.94 |       |          |            |
| 2     | PAU SM-2          | 4.05  | 0.21 | 0.87 | 0.476   | **92.10**   | 3.40  | 0.18 | 0.96 | 0.437   | **93.24**     | 25.14 | 1.26 | 2.07 | 0.174   | **79.71**     |
|       | MSSM-2 (BC2F1)   | 3.73  | 0.27 |       |          |            | 3.17  | 0.18 |       |          |            | 31.54 | 1.85 |       |          |            |
| 3     | PAU SM-3          | 4.83  | 0.18 | 2.71 | 0.114   | **81.37**   | 3.50  | 0.21 | 1.27 | 0.331   | **89.43**     | 31.45 | 1.93 | 0.81 | 0.510   | **94.33**     |
|       | MSSM-3 (BC2F1)   | 3.93  | 0.16 |       |          |            | 3.13  | 0.19 |       |          |            | 33.34 | 1.50 |       |          |            |
| 4     | PAU SM-9          | 3.76  | 0.18 | 0.55 | 0.640   | **94.15**   | 2.90  | 0.14 | 6.34*| 0.024   | **73.10**     | 30.62 | 1.60 | 2.62 | 0.120   | **78.15**     |
|       | MSSM-9 (BC2F1)   | 3.54  | 0.23 |       |          |            | 2.12  | 0.11 |       |          |            | 39.18 | 2.58 |       |          |            |
| 5     | PAU SM-17         | 3.29  | 0.20 | 0.78 | 0.519   | **95.44**   | 3.10  | 0.12 | 0.28 | 0.808   | **98.39**     | 33.02 | 1.80 | 0.93 | 0.450   | **94.31**     |
|       | MSSM-17 (BC2F1)  | 3.14  | 0.16 |       |          |            | 3.05  | 0.14 |       |          |            | 31.14 | 1.29 |       |          |            |
| 6     | PAU SM-21         | 4.10  | 0.19 | 1.14 | 0.374   | **91.22**   | 3.00  | 0.11 | 9.36*| 0.011   | **61.00**     | 31.07 | 1.26 | 1.86 | 0.204   | **92.02**     |
|       | MSSM-21 (BC2F1)  | 3.74  | 0.13 |       |          |            | 1.83  | 0.18 |       |          |            | 28.59 | 1.52 |       |          |            |
Note: *Significant at the 5% level of significance (p<0.05); ns: non-significant at 5% level of significance; RPP_R: recurrent parent phenome recovery

Table 5. Recovery of recurrent parent traits in BC²F₁ population of bell pepper for seed traits (appraise similarity through paired t-test)

| Pairs | Individuals   | Number of seed fruit¹ | 1000 seed fresh weight (g) | 1000 seed dry weight (g) |
|-------|---------------|-----------------------|----------------------------|--------------------------|
|       |               | Mean                  | SEM±| t      | p-value | RPP_R (%) | Mean                  | SEM±| t      | p-value | RPP_R (%) |
| 1     | PAU SM-1      | 59.41                 | 3.01| 0.15ns | 0.892   | 98.59     | 11.34                 | 0.68| 2.12ns | 0.168   | 84.66     |
|       | MSSM-1        | 58.57                 | 2.53|        |         |           | 9.60                  | 0.42|        |         | 6.80      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 2     | PAU SM-2      | 64.62                 | 3.95| 2.83ns | 0.106   | 75.63     | 11.83                 | 0.60| 2.66ns | 0.117   | 79.97     |
|       | MSSM-2        | 48.87                 | 2.37|        |         |           | 9.46                  | 0.55|        |         | 8.18      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 3     | PAU SM-3      | 69.28                 | 3.16| 1.33ns | 0.315   | 87.28     | 13.48                 | 0.79| 3.05ns | 0.093   | 91.32     |
|       | MSSM-3        | 60.47                 | 3.47|        |         |           | 12.31                 | 0.44|        |         | 7.14      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 4     | PAU SM-9      | 61.60                 | 2.59| 2.60ns | 0.122   | 89.01     | 9.20                  | 0.53| 1.37ns | 0.305   | 86.96     |
|       | MSSM-9        | 54.83                 | 3.73|        |         |           | 8.00                  | 0.47|        |         | 6.74      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 5     | PAU SM-17     | 43.15                 | 2.62| 1.90ns | 0.198   | 94.86     | 9.92                  | 0.68| 0.32ns | 0.780   | 96.59     |
|       | MSSM-17       | 40.93                 | 2.73|        |         |           | 10.27                 | 0.61|        |         | 7.81      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 6     | PAU SM-21     | 67.11                 | 3.37| 3.12ns | 0.089   | 78.48     | 12.70                 | 0.89| 2.73ns | 0.112   | 71.50     |
|       | MSSM-21       | 52.67                 | 2.64|        |         |           | 9.08                  | 0.56|        |         | 7.22      |

Note: *Significant at the 5% level of significance (p<0.05); ns: non-significant at 5% level of significance; RPP_R: recurrent parent phenome recovery

Table 6. Recovery of recurrent parent traits in BC²F₁ population of bell pepper for biochemical traits (appraise similarity through paired t-test)

| Pairs | Individuals   | Number of seed fruit¹ | 1000 seed fresh weight (g) | 1000 seed dry weight (g) |
|-------|---------------|-----------------------|----------------------------|--------------------------|
|       |               | Mean                  | SEM±| t      | p-value | RPP_R (%) | Mean                  | SEM±| t      | p-value | RPP_R (%) |
| 1     | PAU SM-1      | 59.41                 | 3.01| 0.15ns | 0.892   | 98.59     | 11.34                 | 0.68| 2.12ns | 0.168   | 84.66     |
|       | MSSM-1        | 58.57                 | 2.53|        |         |           | 9.60                  | 0.42|        |         | 6.80      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 2     | PAU SM-2      | 64.62                 | 3.95| 2.83ns | 0.106   | 75.63     | 11.83                 | 0.60| 2.66ns | 0.117   | 79.97     |
|       | MSSM-2        | 48.87                 | 2.37|        |         |           | 9.46                  | 0.55|        |         | 8.18      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 3     | PAU SM-3      | 69.28                 | 3.16| 1.33ns | 0.315   | 87.28     | 13.48                 | 0.79| 3.05ns | 0.093   | 91.32     |
|       | MSSM-3        | 60.47                 | 3.47|        |         |           | 12.31                 | 0.44|        |         | 7.14      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 4     | PAU SM-9      | 61.60                 | 2.59| 2.60ns | 0.122   | 89.01     | 9.20                  | 0.53| 1.37ns | 0.305   | 86.96     |
|       | MSSM-9        | 54.83                 | 3.73|        |         |           | 8.00                  | 0.47|        |         | 6.74      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 5     | PAU SM-17     | 43.15                 | 2.62| 1.90ns | 0.198   | 94.86     | 9.92                  | 0.68| 0.32ns | 0.780   | 96.59     |
|       | MSSM-17       | 40.93                 | 2.73|        |         |           | 10.27                 | 0.61|        |         | 7.81      |
|       | (BC²F₁)       |                       |     |         |         |           |                       |     |         |         |           |
| 6     | PAU SM-21     | 67.11                 | 3.37| 3.12ns | 0.089   | 78.48     | 12.70                 | 0.89| 2.73ns | 0.112   | 71.50     |
|       | MSSM-21       | 52.67                 | 2.64|        |         |           | 9.08                  | 0.56|        |         | 7.22      |

Note: *Significant at the 5% level of significance (p<0.05); ns: non-significant at 5% level of significance; RPP_R: recurrent parent phenome recovery
| Pairs | Individuals | Dry matter (%) | Ascorbic acid content (mg.100g⁻¹) | Capsaicin content (%) |
|-------|-------------|----------------|-----------------------------------|----------------------|
|       |             | Mean ± SEm | t- | p-value | RPP_R (%) | Mean ± SEm | t- | p-value | RPP_R (%) | Mean ± SEm | t- | p-value | RPP_R (%) |
| 1     | PAU SM-1    | 7.12 ± 0.21 | 2.72 | 0.113 * | 79.91%     | 83.10 ± 2.68 | 2.55 | 0.125 * | 86.59%     | 0.18 ± 0.009 | 6.95 | 0.024 | 62.07%     |
|       | MSSM-1 (BC²F₁) | 8.91 ± 0.57 |         |         |            | 95.97 ± 2.86 |         |         |            | 0.29 ± 0.008 |         |         |            |
| 2     | PAU SM-2    | 6.68 ± 0.29 | 1.06 | 0.400 * | 92.91%     | 71.09 ± 2.76 | 2.84 | 0.105 * | 86.33%     | 0.23 ± 0.01 | 1.07 | 0.398 | 92.00%     |
|       | MSSM-2 (BC²F₁) | 7.19 ± 0.56 |         |         |            | 82.35 ± 3.46 |         |         |            | 0.25 ± 0.01 |         |         |            |
| 3     | PAU SM-3    | 8.87 ± 0.26 | 1.13 | 0.347 * | 92.78%     | 91.30 ± 3.28 | 3.35 | 0.079 * | 92.73%     | 0.21 ± 0.007 | 10.39 | 0.009 | 77.78%     |
|       | MSSM-3 (BC²F₁) | 9.56 ± 0.51 |         |         |            | 98.46 ± 3.32 |         |         |            | 0.27 ± 0.012 |         |         |            |
| 4     | PAU SM-9    | 7.56 ± 0.30 | 3.52 | 0.072 * | 92.42%     | 104.73 ± 3.69 | 2.15 | 0.165 * | 91.06%     | 0.28 ± 0.01 | 6.31 | 0.024 | 68.29%     |
|       | MSSM-9 (BC²F₁) | 8.18 ± 0.48 |         |         |            | 115.01 ± 4.52 |         |         |            | 0.41 ± 0.01 |         |         |            |
| 5     | PAU SM-17   | 5.55 ± 0.28 | 2.93 | 0.100 * | 79.40%     | 61.53 ± 2.48 | 3.22 | 0.084 * | 76.38%     | 0.26 ± 0.009 | 5.13 | 0.036 | 72.22%     |
|       | MSSM-17 (BC²F₁) | 6.99 ± 0.27 |         |         |            | 80.56 ± 3.62 |         |         |            | 0.36 ± 0.014 |         |         |            |
| 6     | PAU SM-21   | 7.78 ± 0.21 | 0.17 | 0.880 * | 98.73%     | 75.62 ± 3.83 | 3.36 | 0.078 * | 87.31%     | 0.30 ± 0.009 | 2.51 | 0.129 | 88.24%     |
|       | MSSM-21 (BC²F₁) | 7.88 ± 0.36 |         |         |            | 86.61 ± 2.96 |         |         |            | 0.34 ± 0.008 |         |         |            |

Table 6 (Cont’d.)
| Pairs | Individuals | Chlorophyll a (mg.100g\(^{-1}\)) | Chlorophyll b (mg.100g\(^{-1}\)) | Total chlorophyll content (mg.100g\(^{-1}\)) |
|-------|-------------|-------------------------------|-------------------------------|---------------------------------------------|
|       |             | Mean  | SEm±  | t     | p-value | Mean  | SEm±  | t     | p-value | Mean  | SEm±  | t     | p-value | RPP_R (%) |
| 1     | PAU SM-1    | 0.34  | 0.012 | 2.90\(^{ns}\) 0.101 | 82.35 | 0.25  | 0.009 | 1.75\(^{ns}\) 0.222 | 88.00 | 0.30  | 0.002 | 2.70\(^{ns}\) 0.114 | 83.33 |
|       | MSSM-1 (BC\(_2\)F\(_1\)) | 0.28  | 0.015 | 0.25  | 0.15 | 0.22  | 0.015 | 0.25  | 0.14 |
| 2     | PAU SM-2    | 0.32  | 0.007 | 3.87\(^{ns}\) 0.061 | 90.63 | 0.32  | 0.009 | 3.34\(^{ns}\) 0.312 | 93.75 | 0.32  | 0.008 | 3.54\(^{ns}\) 0.071 | 90.63 |
|       | MSSM-2 (BC\(_2\)F\(_1\)) | 0.29  | 0.014 | 0.30  | 0.24 | 0.30  | 0.024 | 0.29  | 0.14 |
| 3     | PAU SM-3    | 0.22  | 0.012 | 1.38\(^{ns}\) 0.302 | 95.45 | 0.21  | 0.007 | 0.87\(^{ns}\) 0.478 | 95.24 | 0.22  | 0.004 | 1.46\(^{ns}\) 0.282 | 95.45 |
|       | MSSM-3 (BC\(_2\)F\(_1\)) | 0.21  | 0.016 | 0.20  | 0.07 | 0.20  | 0.007 | 0.21  | 0.11 |
| 4     | PAU SM-9    | 0.34  | 0.007 | 26.50\(^*\) 0.001 | 50.00 | 0.25  | 0.012 | 6.44\(^*\) 0.023 | 56.00 | 0.30  | 0.006 | 25.68\(^*\) 0.002 | 53.33 |
|       | MSSM-9 (BC\(_2\)F\(_1\)) | 0.17  | 0.007 | 0.14  | 0.06 | 0.14  | 0.006 | 0.16  | 0.04 |
| 5     | PAU SM-17   | 0.16  | 0.007 | 1.48\(^{ns}\) 0.277 | 88.89 | 0.14  | 0.007 | 3.46\(^{ns}\) 0.074 | 71.43 | 0.15  | 0.003 | 3.15\(^{ns}\) 0.88 | 100.00 |
|       | MSSM-17 (BC\(_2\)F\(_1\)) | 0.18  | 0.008 | 0.10  | 0.07 | 0.10  | 0.007 | 0.15  | 0.01 |
| 6     | PAU SM-21   | 0.37  | 0.007 | 20.00\(^*\) 0.002 | 45.95 | 0.11  | 0.007 | 1.00\(^{ns}\) 0.423 | 91.67 | 0.24  | 0.006 | 22.30\(^*\) 0.002 | 58.33 |
|       | MSSM-21 (BC\(_2\)F\(_1\)) | 0.17  | 0.009 | 0.12  | 0.09 | 0.12  | 0.009 | 0.14  | 0.08 |

**Note:** *Significant at the 5% level of significance (*p*<0.05); ns: non-significant at 5% level of significance; RPP_R: recurrent parent phenotype recovery

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**Table 7.** Recovery of recurrent parent traits in BC\(_2\)F\(_1\) population of bell pepper for total fruit yield (appraise similarity through paired t-test)

| Pairs | Individuals | Total fruit yield plant\(^{-1}\) (g) |
|-------|-------------|-------------------------------------|
|       |             | Mean  | SEm±  | t     | p-value | RPP_R (%) |
| 1     | PAU SM-1    | 1923.44 | 85.57 | 2.44\(^{ns}\) 0.135 | 93.36 |
|       | MSSM-1 (BC\(_2\)F\(_1\)) | 2060.27 | 124.10 |
| 2     | PAU SM-2    | 1632.16 | 87.36 | 4.22\(^{ns}\) 0.052 | 87.22 |
|       | MSSM-2 (BC\(_2\)F\(_1\)) | 1871.28 | 83.35 |
| 3     | PAU SM-3    | 2028.40 | 88.09 | 0.84\(^{ns}\) 0.489 | 98.26 |
|       | MSSM-3 (BC\(_2\)F\(_1\)) | 1993.12 | 128.90 |
| 4     | PAU SM-9    | 1109.50 | 76.55 | 1.11\(^{ns}\) 0.383 | 96.37 |
|       | MSSM-9 (BC\(_2\)F\(_1\)) | 1069.21 | 43.34 |
| 5     | PAU SM-17   | 1466.97 | 54.00 | 4.10\(^{ns}\) 0.055 | 69.83 |
|       | MSSM-17 (BC\(_2\)F\(_1\)) | 1024.32 | 59.45 |
| 6     | PAU SM-21   | 1117.18 | 48.49 | 1.42\(^{ns}\) 0.292 | 93.49 |
|       | MSSM-21 (BC\(_2\)F\(_1\)) | 1044.46 | 79.62 |

**Note:** *Significant at the 5% level of significance (*p*<0.05); ns: non-significant at 5% level of significance; RPP_R: recurrent parent phenotype recovery
Table 8. Morphological evaluation of parents and BC<sub>2</sub>F<sub>1</sub> populations

| Parents and populations | Fruit color | Fruit orientation | Fruit shape |
|-------------------------|-------------|-------------------|------------|
| Parental lines          |             |                   |            |
| MS-12                   | Light green | Erect             | Elongate   |
| PAU SM-1                | Green       | Pendent           | Blocky     |
| PAU SM-2                | Green       | Pendent           | Blocky     |
| PAU SM-3                | Green       | Pendent           | Blocky     |
| PAU SM-9                | Green       | Erect             | Blocky     |
| PAU SM-17               | Green       | Pendent           | Blocky     |
| PAU SM-21               | Green       | Pendent           | Blocky     |
| BC<sub>2</sub>F<sub>1</sub> Populations |             |                   |            |
| MSSM-1                  | Green       | Pendent           | Blocky     |
| MSSM-2                  | Green       | Pendent           | Blocky     |
| MSSM-3                  | Green       | Pendent           | Blocky     |
| MSSM-9                  | Green       | Erect             | Blocky     |
| MSSM-17                 | Green       | Pendent           | Blocky     |
| MSSM-21                 | Green       | Pendent           | Blocky     |

Declarations

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Declarations of interest

We have no conflicts of interest to disclose.

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Authors’ contributions

MR was the leading researcher, including designed and conducts the field and laboratory experiments, organized the database, performed the statistical analysis, wrote and revised the manuscript. SKJ conceived, designed the experiments, and reviewed the manuscript. OPM contributed to field management, helped in recording of database and manuscript writing.