Research Paper

Transferring of Genetic Male Sterility Trait into the Female Parent of Local Chilli (*Capsicum annuum* L.) Hybrid, MICH HY 1

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Abstract

Increasing the F_1 hybrid chilli seed production is an important current need in view of the high demand in Sri Lanka for hybrid chilli seeds. Male sterile systems in chilli are utilized in commercial hybrid seed production to reduce seed production costs. In this study, the most commonly utilized male-sterile gene called *ms* 10 was transferred into *Galkiriyagama* inbred line, the female parent of the first local chilli hybrid, MICH HY1, through backcross breeding strategy, thereby developing the seed parent (*msms*). Non-discriminated segregation of the *ms* 10 gene was further confirmed using the Chi-square test. The seed parent was observed to be a stable line free from the influence of environmental changes. The maintainer line (*Msms*) that is utilized in commercial hybrid production was developed using the seed parent (*msms*) and pollen parent (*MsMs*). The developed lines, seed parent and maintainer line would be utilized in F_1 chilli seed production of MICH HY 1. Further, the developed male-sterile phenotype can be utilized to develop new chilli hybrids with the potential for commercial cultivation.

Keywords: **Backcross breeding, Chilli, Genetic male sterility, Maintainer line, Seed parent, Seed production**

Introduction

Chilli is an important spice and vegetable crop in Sri Lanka. At, present Sri Lankan farmers prefer to cultivate F1 hybrid chilli varieties due to their high yielding ability, tolerance to viral diseases and other fruit quality traits including high pungency (Herath *et al*, 2016; Herath *et al*, 2017). Commercial production of F_1 hybrids is done manually or by exploiting Male Sterility (*MS*) trait. Manual F_1 hybrid seeds production is time-consuming with high cost of labour. It results in high cost of production of F_1 seeds. But the exploitation of male sterility reduces the seed production cost by about 50 % (Dhaliwal & Jindal, 2014; Mishra & Kumari, 2018; Jindal & Dhaliwal, 2020). The concept of male sterility in *Capsicum annuum* L was first time reported by Martin and Grawford (1951). Failure of crops to produce functional pollen results in male sterility. Application of male sterility in F_1 hybrid production is very economical and it helps to avoid the cumbersome task of emasculation and hand pollination (Mol *et al*., 2020).

Both Genetic Male Sterility (GMS) and Cytoplasmic Male Sterility (CMS) have been utilized in commercial hybrid seed production worldwide (Meena *et al*., 2018). However, the application of CMS in chilli hybrid production is influenced by environmental fluctuations and the genetic background of the parents (Dhaliwal & Jindal, 2014; Meena *et al*., 2018). In GMS, nuclear genes are responsible for the male-sterile phenotype and it gives more stable male sterility without influence of environmental fluctuations (Dhaliwal & Jindal, 2014). According to Wang and Bosland, (2006), 20 genes have been identified to govern GMS. Conventional plant breeding approach can be followed to transfer GMS allowing self-pollination after each backcross. Plants with the male sterility gene (*ms*) can be easily identified by phenotypical observation of flowers (Dhaliwal & Jindal, 2014).

Among the male-sterile chilli lines, *MS* 12 line has been utilized by many researchers to develop chilli hybrids (Dhaliwal *et al*., 2014; Aulakh *et al*., 2016). A recessive gene called as *ms* 10 is responsible for the male-sterile trait in *MS* 12 chilli line (Aulakh *et al*., 2016). Punjab Agriculture University developed two chilli hybrid CH-1 and CH-3 utilizing this MS12 line (Aulakh *et al*., 2016). Other than that, this line was commercially exploited by the Indian Institute of Horticulture Research, the World Vegetable Centre and other private chilli hybrid producing organizations (Mishra & Kumari, 2018).

Manual F_1 seed production is currently practiced in hybrid seed production of MICH HY 1, the first local chilli hybrid in Sri Lanka. MICH HY 1 is a high yielding chilli hybrid with the potential yield of 32 t ha^{-1} as green chilli. Female parent of this chilli hybrid is *Galkiriyagam*a inbred line whereas male parent is MI *Waraniya* 1 inbred line (DoA, 2018). Manual F_1 seed production procedure leads to high production cost. Therefore, it is needed to transfer the genetic male-sterile trait into the female parent of this local chilli hybrid to increase the efficiency of F_1 seeds production by minimizing production

costs. This experiment was conducted to transfer genetic male-sterile trait into the *Galkiriyagama* inbred line using the male-sterile chilli line MS12.

Materials and Methods

Location

This experiment was conducted at the Field Crop Research and Development Institute (FCRDI), Mahailuppallama (DL 1b Argo ecological region) in Sri Lanka from 2012 *Yala* to 2018/19 *Maha*.

Plant Material

The chilli genotype MS 12 with male-sterile trait was utilized as the donor of male-sterile gene (*ms*) to the female parent (*Galkiriyagama* inbred line).

Transferring of the male-sterile trait into *Galkiriyagama* inbred line using back cross breeding method

Nurseries were maintained for the seed mixture of seed parent and maintainer parent of MS 12 chilli genotype and thirty-five days old seedlings were planted in the field during 2012 *Yala.* At the same time, thirty-five days old seedlings of the *Galkiriyagama* inbred line were planted in 35 × 35 cm polybags filled with a soil mixture of topsoil: cow dung: sand: partially burned paddy husk with the ratio of 2:2:1:1. At the time of flowering of MS 12 genotype, anthers of the flowers were observed visually during anthesis and the plants having anthers without pollen (100 % male sterile) were selected. Twenty plants with male-sterile trait were transplanted into poly bags without damaging the root. Polybags with plants having male-sterile trait were moved to the plant house and crossing was started with the female parent (*Galkiriyagama* inbred line) and continued the backcrossing as explained in Figure 1. Five back crosses were completed using the Galkiriyagama inbred line as recurrent parent and MS12 genotype as donor parent to retain the genetic background of Galkiriyagama inbred line with male sterile trait.

Thirty-five days old seedlings of the F_1 progeny were field planted and allowed to self-pollinate by covering the individual plants with insect proof cages during 2012/13 *Maha*. At the time of fruit ripening, seeds were extracted and allowed to dry. Seedlings of these seeds were planted in the field during 2013 *Yala* and plants with male-sterile trait were selected. At the same time, 20 seedlings of *Galkiriyagama* inbred line were planted in poly bags inside the plant house. Selected 20 plants with male-sterile traits were transplanted in poly bags and moved to plant house after uprooting and transplanting in poly bags. During 2013 *Yala*, 1st backcross was done and seeds were extracted from ripened fruits. Thirty-five days old seedlings of the 1st backcross were planted in the field during 2013/14 *Maha*. The plants were covered using cages with insect-proof nets to allow self-pollination. Seeds were extracted after fruit ripening and allowed to dry.

Seedlings of these seeds were planted in the field during 2014 *Yala* and plants with male-sterile trait were selected.

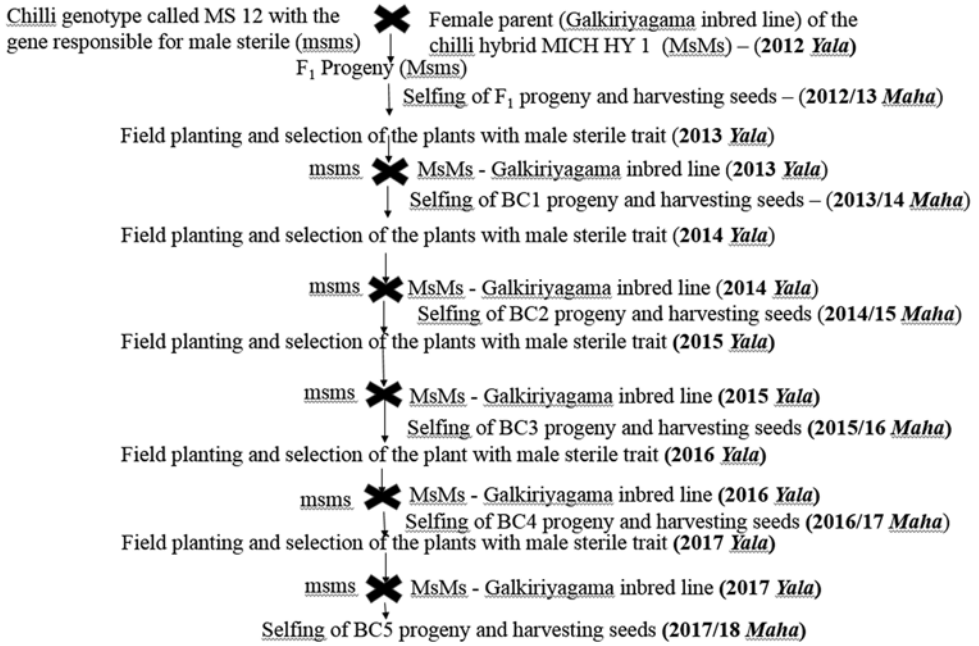

Figure 1. The process of transferring genetic male-sterile gene (msms) into the female parent, Galkiriyagama inbred line

Twenty selected plants were transplanted in poly bags after uprooting and moved to the plant house to complete 2nd backcross with *Galkiriyagama* inbred lines that were planted in poly bags inside the plant house. Selfing of 2nd back cross progenies was done during 2014/15 *Maha* covering the individual plants using insect proof cages. Seeds were extracted from red ripened fruits of self-pollinated plants and dried. Nurseries were maintained for these seeds and planted in the field during 2015 *Yala*. Plants with male-sterile trait were identified and moved to plant house after planting in poly bags for 3rd back cross.

The third back cross was completed by crossing with the *Galkiriyagama* inbred line that was planted in poly bags inside the plant house and seeds were extracted from red ripened fruits and allowed to dry. Seedlings of 3rd back cross progenies were planted in the field after maintaining nurseries during 2015/16 *Maha* and allowed to self-pollinate covering the plants using the insect proof cages. After the fruit ripening, seeds were extracted and dried. Nurseries were maintained for these seeds during 2016 *Yala* and planted in the field after 35 days and plants with male-sterile traits were selected. Same procedure was followed through successive back crosses up to BC_5 during 2016/17 *Maha* and 2017 *Yala*.

Extracted seeds after the 5th back cross were field planted and allowed to self-pollinate during 2017/18 *Maha*. Plants with the male-sterile trait (*msms*) were identified during 2018 *Yala* after filed planting of self-pollinated 5th back cross progenies. The number of male-sterile plants and male fertile plants of each

self-pollinated back cross progeny were recorded to confirm the non-discriminated segregation of the ms10 gene responsible for male sterility.

Twenty identified GMS character transferred plants (*msms*) were transplanted without damaging the root and moved to the plant house after planting in the poly bags. Similarly, 20 male fertile seedlings of Galkiriyagama inbred line (*MsMs*) were planted in the poly bags inside the plant house. The cross between *msms* x *MsMs* was completed after flowering and seeds were harvested that contain *Msms* genotypes. Seedlings of this genotype were planted in the poly bags and the cross between *Msms* x *msms* was completed. Seeds were harvested as the maintainer line.

Data collection

Data on the number of male-sterile plants and male fertile plants were collected from a total of 160 individuals of each self-pollinated back cross progenies to perform chi-square test and check whether the ratio fitted well with the Mendelian ratio of 3:1 to confirm the non-discriminated segregation of the ms10 gene that is responsible for the male sterility.

Data analysis

The Chi-square test was applied to determine the goodness of fit for the observed phenotypic segregation ratio of each self-pollinated back cross progenies based on the predicted segregation ratio of 3:1. Probabilities under Chi-square test for goodness of fit were calculated using Microsoft Excel using the following equation.

$$x^2\,(n\text{-}1)\,df = \sum\left\{ \frac{(O\text{-}E)^2}{E} \right\}$$

Where, x^2= Chi square test value,
O= observed value, E = Expected value

Results and Discussion

Identification of genetic male sterile flower and male fertile flower and back cross breeding up to BC_5

Genetic male sterility is one of the most valuable traits in chilli hybrid breeding. Male sterile plants of the MS12 genotype were identified easily by visual observations as presented in Figure 2.

Figure 2. Phenotypic difference of male-sterile and male fertile flowers of chilli

During anthesis, pollen grains were not observed on the anthers of male-sterile flowers. Further, male-sterile flowers exhibited purple colour shrivelled anthers. Similar observations were reported by Rani *et al*., (2021). But, pollens were observed on the green colour anthers of male-fertile flowers at the time of anthesis as shown in Figure 2. Therefore, it was very easy to separate male-sterile and male-fertile plants by visual observations at each back cross progeny from BC_1 to BC_5. The male sterile flower of the Galkiriyagama inbred line observed after selfing of 5th back cross progeny is presented in Figure 3. Further, male-sterile phenotypes after selfing of each back cross progeny were observed to be highly stable under field conditions and clearly distinguishable from the male-fertile phenotypes. Moreover, the male sterile phenotypes after selfing of each back cross progeny produced flowers with abundant pollen during anthesis.

Figure 3. Male sterile flower of the Galkiriyagama inbred line observed after selfing of 5 th back cross progenies

Analysis of the segregation of male-sterile and male-fertile trait

Results of phenotypic segregation of male sterile and male fertile traits in each self-pollinated back cross progeny is presented in Table 1. The recorded Chi-square values for self-pollinated backcross progenies from backcross 1 to backcross 5 were 0.5, 2.1, 2.7, 1.63 and 1.2. These values were less than the tabulated value of 3.84 (P= 0.05). Accordingly, Chi-square test results of each self-pollinated back cross progeny indicated that the ratios well fitted with the Mendelian ratio of 3:1. It confirmed the non-discriminated segregation of the ms10 gene that is responsible for the male sterile trait as observed by Shifriss and Rylsky (1972) and Rani *et al*, (2021).

Genetic nature of the developed seed parent and maintainer line

At the end of the crossing programme seed parent and the maintainer line were developed as explained in the methodology. Genetic nature of the developed seed parent (*msms*) and maintainer line (*Msms*) are presented in Table 2.

Maintenance of seed parent and maintainer line in hybrid seeds production

Maintenance of seed parent and maintainer lines in hybrid seeds production could be done by crossing the genotypes, *Msms* and *msms* as explained in Figure 4. The seed lot used as the maintainer line contained 50% of the population of the seed parent and 50 % of the population of the maintainer line.

Table 1. Phenotypic segregation of male sterile and male fertile trait in each self-pollinated back cross progenies

	Trait	Observed	Expected	(O-E)	$(O-E)^2$	$(O-E)^2/E$	chi square value	P value
BC_1	MF	124	120	4	16	0.13	0.53	0.48^{ns}
	MS	36	40	-4	16	0.4		
BC_2	MF	128	120	8	64	0.53	2.13	0.14^{ns}
	MS	32	40	-8	64	1.6		
BC_3	MF	129	120	9	81	0.67	2.7	0.1^{ns}
	MS	31	40	-9	81	2.02		
BC_4	MF	127	120	7	49	0.40	1.63	0.2^{ns}
	MS	33	40	-7	49	1.22		
BC_5	MF	126	120	6	36	0.3	1.2	0.27^{ns}
	MS	34	40	-6	36	0.9		

Table 2. Genetic nature of the developed seed parent and maintainer lines

Line	Expected genotype	Anther morphology
Seed parent	Msms	Sterile
Maintainer	Msms	Fertile

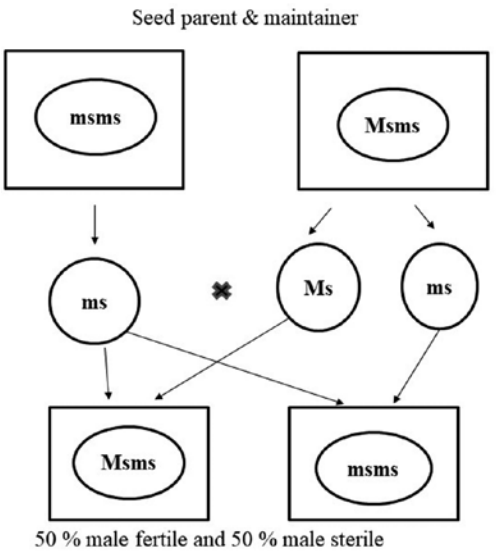

Figure 4. Maintenance of the seed parent and maintainer line

Chilli plant bare large number of fruits within one crop cycle. One chilli fruit contain >60 seeds. Therefore, it is easy to multiply and maintain the maintainer line. As explained by Kumar *et al*., 2014, the cost on labour deployed in emasculation and pollination was high as 78% of the total cost on labour employed in hybrid seed production. Hand eamsculationa and pollination is not needed in hybrid seeds production using GMS lines since it applied natural cross pollination using the pollinators like honeybees.There is a possibility to cut down seed production cost by higher percentage using the GMS line. Therefore, it is economiacl to apply GMS lines in hybrid seed production.

There are several reports on plant growth promoting and biocontrol activity of *P. oxalicum* and it is widely used as a P solubilizing fungus and biocontrol agents in various crops. Biocontrol activity of *P. oxalicum* against Fusarium wilt of tomato was recorded by Larena *et al*. (2002).

Sing *et al*, (2014) developed 18 chilli hybrids using a male-sterile parent, and 2 promising hybrids; MS 12 and 8 were identified. Further, Dhaliwal *et al*., (2014) developed nine promising chilli hybrids using the same genetic male sterile line. Therefore, based on the previous research,

there is a huge potential to utilize the male sterile gene in MS12 chilli line in hybrid seed production. Further, Aulakh *et al*., (2016) reported two Simple Sequence Repeat (SSR) markers linked to ms10 gene of the MS12 chilli line namely, AVRDC-PP12 and AVRDC_MD997. Utilizing these markers for the selection of male-sterile genotypes after each back cross in male-sterile trait transferring in the breeding programme will help reduce the time taken for the production of male-sterile lines.

Conclusions

In this study, the male sterile line (seeds parent) and maintainer line of the female parent; *Galkiriyagama* inbred line were successfully developed for the commercial production of hybrid seeds to minimize the cost of production. The male sterile line that was developed was easily identified by the visual observation of the flower and displayed environmental stability with no environmental influence on the male sterility trait. Further, it would be possible to utilize the male sterile line as the female parent to develop new chilli hybrids. However, it is needed to conduct an economic analysis on the cost of seed production using this male sterile line to confirm the reduction of the cost of seed production by 50% as mentioned by the previous researchers.

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Author Contributions

HMSN: designed and conducted the experiment, wrote the manuscript, KN: provided the MS12 genotype, HMS: assisted in data collection, filed and plant house crop management, TMWRTB: assisted in data collection, filed and plant house crop management, WG: assisted in data collection, filed and plant house crop management, SMNIK: assisted in data collection, filed and plant house crop management including the crossing

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