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Characterization, inheritance of male sterility and development of male sterile and maintainer lines in ridge gourd (*Luffa acutangula* (Roxb.) L.)

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ABSTRACT

Two male sterile mutants IIHRRG-12MS (long fruited) and IIHRRG-28MS (medium long fruited) were identified from the ridge gourd germplasm IIHR-12 and IIHR-28 respectively at ICAR-IIHR, Bengaluru. These two male-sterile (*ms*) sources were characterized by the production of rudimentary male flowers in the racemes in contrast to the bright yellow flowers with fertile pollen and healthy anthers in male fertile, monoecious plants. Using these *ms* lines the inheritance of male sterility was worked out, which is cytoplasmic genic male sterility (CGMS) type, with single dominant gene either in homozygous or heterozygous condition restoring male fertility in the presence of sterile cytoplasm. In order to develop F_1 hybrids using male sterility, several male sterile and maintainer lines were developed in different genetic back grounds such as green/dark green fruit colour and short/medium long/long fruit length.

Keywords: CGMS, gene action, inheritance, maintainer lines, male sterility and ridge gourd

INTRODUCTION

Ridge gourd (*Luffa acutangular* (Roxb.)L.) is an important cucurbitaceous vegetable crop grown in tropical and subtropical countries, especially in Asia and India (Jansen *et al*., 1993). It is a crop grown for immature fruit rich in dietary fibre and minerals (Sheshadri, 1990). In addition to culinary properties, it has numerous medicinal properties which traditionally used for the treatment of stomach ailments and fever (Burkill, 1985; Chakravarty,1990).

Though cultivars of ridge gourd are monoecious, diverse sex forms were reported *viz.,* androecious, gynoecious, gynomonoecious, andromonoecious and hermaphrodite types (Choudhary and Thakur, 1965). The female flowers are solitary whereas male flowers are in racemes. Principally 2 genes are involved in production of various sex forms (Richaria, 1948). Male sterility is of practical importance in vegetable breeding as it facilitates F_1 hybrid seed production without hand pollination. Male sterility in ridge gourd was first reported from India by Deshpande *et al*. (1979) and then by Pradeepkumar *et al*. (2007). Male sterility is governed by single recessive nuclear gene in water melon (Hexun *et al*., 1998; Ping *et al*., 2010); musk melon (Dhatt and Gill, 2000; Park *et al*., 2009), cucumber (Zhang *et al*., 1994) and for the first time, cytoplasmic male sterility (CMS) with two dominant restorer genes has been reported in ridge gourd by Pradeepkumar *et al*. (2012). At ICAR-IIHR, Bengaluru also male sterile mutants were identified in ridge gourd germplasm (Varalakshmi and Deepak, 2017).

Present study was conducted to characterize that male sterility observed, to work out the genetics of its inheritance and to develop male sterile and maintainer lines in different genetic backgrounds of ridge gourd.

MATERIALS AND METHODS

The work was undertaken in the experimental field of Division of Vegetable crops, ICAR-IIHR, Bengaluru. Initially two male sterile mutant plants viz.,IIHRRG-12MS and IIHRRG-28MS in different genetic backgrounds have been identified during kharif, 2015-16 and maintained in the division ever since. Morphological characters of these *male sterile* mutants were recorded *viz*., days for emergence of first fertile male flower, days for emergence of ûrst female flower, node at which first fertile male flower appeared, node at which first female flower appeared, male bud length

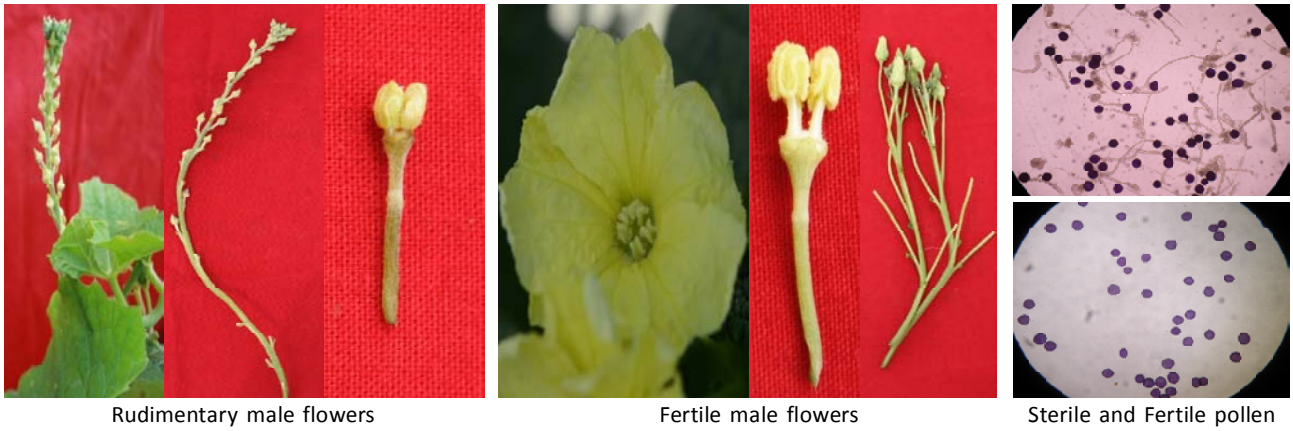
and pollen fertility (%). Pollen fertility percentage was assessed from ten randomly selected male ûower buds in each line at anthesis on the basis of stainability in acetocarmine and the counts were taken from ten fields under microscope for each flower bud. Well filled, uniformly and darkly stained pollen grains were considered as fertile and the rest as sterile. Simultaneously, these *ms* plants were crossed with 22 monoecious lines *viz*.,IIHR-1, IIHR-7, IIHR-10-2, IIHR-11, IIHR-12, IIHR-17-2-1-6, IIHR -19, IIHR-23, IIHR-26, IIHR-27, IIHR-29, IIHR-31, IIHR-34, IIHR-35, IIHR-39, IIHR-40, IIHR-41, IIHR-43, IIHR-46, IIHR-47, IIHR-49 and IIHR-72-2 to study the inheritance of male sterility and fertility restoration in ridge gourd during kharif season of 2015-16. All the 22 F_1 hybrids and parental lines were grown with recommended package of practices during Rabi-summer season of 2016-17. Observations pertaining to male and female fertility were recorded from 15 plants in each line/hybrid. Among the 22 hybrids, 10 fertile hybrids (IIHRRG-28MS x IIHR-10-2, IIHRRG-28MS × IIHR-72-2, IIHRRG-12MS × IIHR -17-2-1-6, IIHRRG-12MS x IIHR-1, IIHRRG-12MS x IIHR-12, IIHRRG-12MS x IIHR-40, IIHRRG-12MSx IIHR-41, IIHRRG-12MS x IIHR-43, IIHRRG-12MS x IIHR-47 and IIHRRG-12MS x IIHR-49) were selfed to generate F_2 population as well as back crossed with respective male parent to produce BC_1 generation. Five hybrids were male sterile *viz*.,IIHRRG-12MSxIIHR-19, IIHRRG-12MSxIIHR-27, IIHRRG-12MSxIIHR-31, IIHRRG-12MSx IIHR-34 and IIHRRG-12MSxIIHR-39. Remaining seven hybrids were not uniform with respect to fertility (IIHRRG-12MSxIIHR-7, IIHRRG-12MSxIIHR-11, IIHRRG-12MSxIIHR-23, IIHRRG-12MSxIIHR-26, IIHRRG-12MSxIIHR-29, IIHRRG-12MSxIIHR-35 and IIHRRG-12MSxIIHR-46) and were not considered further in the study. F_2 population (200 plants), BC_1 generation (50 plants) were raised during the kharif season, 2017-18 and evaluated for male sterility and restoration of fertility. Chi-square (χ^2) goodness-of-fit analysis (Russell, 1996) was conducted for segregation of male fertility and sterility in F_2 populations of two crosses viz., IIHRRG-12*ms*x IIHR-17-2-1-6 and IIHRRG-28*ms*x IIHR-72-2.

In order to transfer the male sterility in to different genetic backgrounds, crosses were made between male sterile lines and ten different advanced breeding lines with different genetic backgrounds to convert them into *ms* lines as well as maintainer lines viz., IIHR-6-2(long, green), IIHR-5-1-2 (Medium long, green), IIHR-37-4-1, IIHR-23-5-4, IIHR-34-2-2, IIHR-49-3-1, IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 and IIHR-11-1-2. Male sterile progeny was repeatedly backcrossed with the male parents (maintainer lines) for six generations to develop the male sterile (A line) and maintainer lines (B line).

RESULTS AND DISCUSSION

Characterization of male sterility in ridge gourd

Male sterility is defined as failure of plant to produce the functional anthers, pollen or male gametes. At ICAR-IIHR, two male sterile mutants were identified in IIHRRG-12 (long fruited) and IIHRRG-28 (medium long fruited) germplasm lines. These two *ms* sources viz., IIHRRG-12MS and IIHRRG-28MS were characterized by the production of rudimentary male flowers in the racemes in contrast to the bright yellow flowers with fertile pollen and healthy anthers in male fertile, monoecious plants (Fig.1 and Fig. 2). Rudimentary male buds remained unopened and fell down 12–16 days after the emergence. Similar



Rudimentary male flowers | Fertile male flowers | Sterile and Fertile pollen

Fig. 1. Characterization of male sterility in Ridge gourd

Fig. 2. Male flower production in monoecious line (left) and absence of male flowers in male sterile line (right)

characteristics of the male sterile line were reported by Pradeepkumar *et al.* (2010) in ridge gourd.

Expression of male sterility and restoration of fertility in F_1 hybrids

Hybrids have expressed different fertility status, viz., complete sterile, complete fertile and some hybrids with both fertile and sterile plants. If male sterility was controlled by dominant gene, which was a rare phenomenon in cucurbits, all the hybrids should have expressed complete sterility in F_1 generation, then as all the individuals carrying *Ms* allele are sterile and do not produce progenies as pollen parents. If it is controlled by recessive nuclear gene as in musk melon (Park *et al*., 2009), water melon (Ping *et al*., 2010), squash (Carle, 1997) and cucumber (Zhang *et al*., 1994), then F_1 should have segregated into 1:1 fertile and sterile plants based on the homozygosity/ heterozygosity of the locus controlling the sterility. But here in this case, male sterility expression of F_1 hybrids indicates the role of CMS genes. CMS is maternally inherited and is associated with a specific (mitochondrial) gene whose expression impairs the production of viable pollen without otherwise affecting the plant (Kempken and Pring, 1999; Budar and Pelletier, 2001). Premature degeneration of the tapetum at the early to mid uni-nucleate microspore stage leads to the development of non-viable pollen (Roberts *et al*., 1995). General theory about the phenotype of CMS plants which usually appear normal, vigorous, and undistinguishable from the fertile analogue (Hanson and Conde, 1985) proved true in the present study also.

There are nuclear genes that can restore fertility, termed nuclear restorer (Rf) or fertility restorer (Fr) genes, which are specific for each studied CMS system (Popova *et al*., 2007). The restorer of fertility (Rf) genes in the nucleus function to suppress the CMS phenotype and restore the male fertility. Dominant nuclear fertility restorer gene in 'IIHR-1, IIHR-10-2, IIHR-12, IIHR -17-2-1-6, IIHR-40, IIHR-41, IIHR-43, IIHR-47, IIHR-49, IIHR-72-2' out of 22 genotypes is responsible for regaining male fertility of hybrids with *ms* mutant line. All these ten lines could be possible restorer lines.

Seven other crosses (IIHRRG-12MSxIIHR-7, IIHRRG-12MSxIIHR-11, IIHRRG-12MSxIIHR-23, IIHRRG-12MSxIIHR-26, IIHRRG-12MSxIIHR-29, IIHRRG-12MSxIIHR-35 and IIHRRG-12MSxIIHR-46) had both male sterile and male fertile plants in different ratios indicating that the fertility restorer genes might be in heterozygous condition in these inbred lines which can be used to develop either maintainer lines or restorer lines after progeny evaluation and back crossing.

Five hybrids viz.,IIHRRG-12MSxIIHR-19, IIHRRG-12MSxIIHR-27,IIHRRG-12MSxIIHR-31, IIHRRG-12MSxIIHR-34 and IIHRRG-12MSxIIHR-39 were male sterile indicating the maintenance of sterility and these advanced breeding lines could be possible maintainer lines. Though the five male parents exhibited high pollen fertility (52-83%), they failed to transmit this character to F_1 hybrids indicating the cytoplasmic inheritance of male sterility in ridge gourd. The average bud length of male buds of male sterile hybrids at full development stage was found to be 0.6±0.01cm which was significantly different from the average bud length of male fertile parents (1.7± 0.05cm) (Supplementary Data Table S1). These rudimentary male buds in racemes of male sterile hybrids remained unopened and fell down 12–16 days after the emergence. The anther lobes were undeveloped and pollen grains were small, shrunken and poorly stained in these hybrids throughout the crop growth indicating a stable sterility mechanism. Male fertile hybrids had high mean pollen fertility (47±6.57%) throughout the crop growth.

In the male sterile hybrids node for the first female flower was earlier (9.6th node) compared to the male fertile hybrids (10.2nd node) and also the days taken for the emergence of first female flower is less in male

sterile hybrids (41.2 days) compared to male fertile hybrids (43.4days) (Supplementary data Table S2). Similarly mean female bud length was more (94.8 cm) in male sterile hybrids than male fertile hybrids (4.6cm) and also the fruit length was more in sterile hybrids (24.8cm) than in fertile hybrids (20.2cm)

Analysis of F_2 population from the crosses, IIHRRG-12MSxIIHR-17-2-1-6 and IIHRRG - 28MSx IIHR-72-2 for male sterility and restoration of fertility:

Out of the 239 F_2 plants of the cross IIHRRG-12MS x IIHR-17-2-1-6, 182 were male fertile and 57 were male sterile till the end of the season. There were observable differences between the male sterile and male fertile plants with respect to male flower production though female flowers in both types were similar. Node for the first fertile male flower ranged from 2-14th node with the mean of 4.92 and the days taken for the first male flower ranged from 29-51 days with a mean of 42.08 days. Average male flower bud length was less in male sterile plants (0.61cm) compared to the male fertile plants (1.89 cm) (Supplementary data able S3). Mean pollen fertility of these male fertile plants was 24.95% as against zero fertility of male sterile plants. With respect to female flower traits, there were slight differences between male sterile and male fertile plants. Node for first female flower was earlier in sterile plants (9.4) compared to male fertile plants (10.18), similarly even the number of days taken for first female flower appearance was less in male sterile plants (43.3 days) compared to male fertile plants (45.99). However, the average female flower bud length and fruit length were almost same in both male sterile and male fertile plants.

In another F_2 population of the cross, IIHRRG-28MSx IIHR-72-2, out of 235 F_2 plants, 175 were male fertile and 60 were male sterile. In this cross also there were differences between male sterile as well as male fertile plants with respect to male flower production. Node for the first fertile male flower ranged from 2-8th node with the mean of 4.21 and the days taken for the first male flower ranged from 39-55 days with a mean of 42.84 days. Average male flower bud length was less in male sterile plants (0.63cm) compared to the male fertile plants (1.85 cm). Mean pollen fertility of these male fertile plants was 7.56% as against zero fertility of male sterile plants. With respect to female flower traits, there were slight differences between male sterile and male fertile plants. Node for first female flower was earlier in sterile plants (8.52) compared to male fertile plants (9.82), similarly even the number of days taken for first female flower appearance was less in male sterile plants (42.8 days) compared to male fertile plants (44.38)(Supplementary data Table S3). However, the average female flower bud length and fruit length were almost same in both male sterile and male fertile plants.

All the F_1 plants of these two *ms* x *mf* crosses and their corresponding back cross populations were male fertile. As the F_2 population segregated into two classes in both the crosses, monohybrid ratio, 3:1 was tested for significance using chi-*square* test. The chi-square value for the 3:1 (fertile: sterile) single dominant gene action exhibited a good fit to the expected ratio (80-90% probability) (Table 1 and 2). The F_2 data indicated the presence of cytoplasmic genic male sterility (CGMS) in ridge gourd with single dominant gene restoring male fertility in the presence of sterile cytoplasm. However, Pradeepkumar *et al*. (2012) earlier reported that two dominant fertility restorer genes are responsible for restoration of fertility in the presence of sterile cytoplasm in ridge gourd using Arka Sumeet variety as restorer line. This could be due to different genetic makeup of different male sterile and restorer lines used in these studies.

Assuming that MS line is having genotype, rf1rf1 and sterile cytoplasm (S) and male parent, IIHR-17-2-1-6/IIHR-72-2 possesses a genotype Rf1Rf1 carrying a fertility restorer gene in homozygous dominant state and normal fertile cytoplasm (N), F_1 will be male fertile as the genotype of F_1 is SRf1rf1. Though F_1 is inheriting a sterile cytoplasm from male sterile female parent, presence of a dominant fertility restorer gene, viz., Rf1 restores the fertility of F_1 (Table 3). In F_2 presence of single dominant fertility restorer gene in either homozygous or heterozygous condition ensures male fertility. The gene action governing male sterility can be explained with the following model.

Evaluation of back crosses made between fertile hybrids with restorers during summer

Three male fertile hybrids were back crossed with restorer lines and all these back cross progenies were male fertile indicating the restoration of male fertility in these lines (restorer lines) (Table 4).

Table 1. Segregation of male sterile and male fertile plants in F_1, Back cross and F_2 generation of the crosses, IIHRRG-28MSx IIHR-72-2 and IIHRRG-12MSxIIHR-17-2-1-6

Cross	F_1's		Back cross		F_2's	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
(IIHRRG-12MSxIIHR-17-2-1-6)	15	0	44	0	182	57
(IIHRRG-28MSxIIHR-72-2)	15	0	37	0	175	60

Table 2. Chi-square test for F_2 population segregating for male sterility and male fertility in ridge gourd

Cross	Genotype	F_2's (3:1)	
		Fertile	Sterile
(IIHRRG-12MSxIIHR-17-2-1-6) F_2 population	Expected	179	60
	Observed	182	57
	Difference	**3**	**-3**
	Chi Square value	**0.169**	
	Probability	**50-70%**	
		F2's (3:1)	
		Fertile	**Sterile**
(IIHRRG-28MSxIIHR-72-2) F_2 population	Expected	176	59
	Observed	175	60
	Difference	**-1**	**1**
	Chi Square value	**0.035**	
	Probability	**80-90%**	

Table 3. Proposed genetic model for Single dominant gene action in ridge gourd

Parents	Male sterile line IIHRRG-12MS/ IIHRRG-28MS	Male fertile line IIHR-17-2-1-6/ IIHR-72-2
	S(rfrf)	**N(RfRf)**
Gametes	S(rf)	N(Rf)
F_1	Male fertile S(Rfrf)	
Gametes	Rf, rf	
Eggs/pollen	Rf	Rf
Rf	SRfRf Male fertile	SRfrfMale fertile
rf	SRfrf Male fertile	SrfrfMale Sterile

BC_1 generation of the cross (IIHRRG-28MS × IIHR-72-2) x IIHR-72-2 exhibited increased male fertility compared to F_1 (IIHRRG-28MS × IIHR-72-2). All three BC_1-populations took little more days to male flower production (45-46) and wide variation was observed among the back cross populations with respect to the node for the first female flower appearance (4-26th node) and days taken for the emergence of first female flower (34-65 days) (Table 5). BC populations exhibited pollen fertility in the range of 40-78%. Wide variation was observed for average female bud length (4-6 cm) and fruit length (20-25cm) among the three back cross populations.

Table 4. Evaluation of back crosses made between fertile hybrids and fertility restorers - male flower characters

Male fertile back cross	Node at first fertile male flower		Days for the emergence of first fertile male flower		Average male bud length		Pollen fertility %	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
(IIHRRG-28MS × IIHR-10-2) × IIHR-10-2	2-7	4	39-48	42	1.0-2.6	1.85	19-63	40
(IIHRRG-28MS × IIHR-72-2) × IIHR-72-2	3-12	5	37-45	42	1.1-2.6	1.91	47-100	74
(IIHRRG-12MS × IIHR -17-2-1-6) × IIHR -17-2-1-6	3-16	5	40-48	43	1.0-2.6	1.80	19-88	58
Mean		1.0		0.8		0.1		16.8
SEm±		0.6		0.5		0.0		9.7

Development of *ms* lines (A lines) and maintainer lines (B lines) in different genetic back grounds

The identified cytoplasmic male sterility (*cms* trait) has been transferred to different genetic backgrounds, by crossing ten different advanced breeding lines with different genetic backgrounds viz., IIHR-6-2 (long, green), IIHR-5-1-2 (Medium long, green), IIHR-37-4-1 (short, green) IIHR-23-5-4 (medium, green), IIHR-34-2-2, IIHR-49-3-1(medium, green), IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 (long, dark green) and IIHR-11-1-2 with male sterile line (IIHRRG-28MS/ IIHRRG-12MS maintained through sib mating with maintainer line, IIHRRG-28/IIHRRG-12) to convert them into *ms* lines. All these F_1 populations were male sterile due to cytoplasmic inheritance of male sterility in the identified source. These F_1's were repeatedly back crossed with their respective male parents/ maintainer lines for six generations continuously. The back cross population plants which were having similar fruit attributes of maintainer lines in each generation were selected and back crossed with the maintainer line. In each generation the back cross populations were checked for maintenance of sterility and found that all were maintaining sterility in 100% population. Thus, by BC_6 generation, all these ten populations viz.,IIHR-6-2MS, IIHR-5-1-2MS, IIHR-37-4-1MS, IIHR-23-5-4MS, IIHR-34-2-2MS, IIHR-

Table 5. Evaluation of back crosses made between fertile hybrids and fertility restorers - female flower characters

Male fertile back cross	Node at first fertile flower		Days for the emergence of first female flower		Average female bud length (cm)		Average fruit length (cm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
(IIHRRG-28MS x IIHR-10-2) × IIHR-10-2	4-25	12.1	34-62	46	5-7.5	6	16.5-30	25
(IIHRRG-28MS × IIHR-72-2) x IIHR-72-2	5-15	9.5	34-62	45	5-7.5	6	12.5-30	20
(IIHRRG-12MS × IIHR -17-2-1-6) × IIHR -17-2-1-6	4-26	24.0	34-65	45	4-5.5	4	12-28	20
Mean		15.2		45.1		5.7		21.8
SEm±		4.5		0.3		0.6		1.7

49-3-1MS, IIHR-22-4-2MS, IIHR-26-4-2MS, IIHR-70-1MS and IIHR-11-1-2MS were perfectly male sterile resembling the respective maintainer lines morphologically in different genetic back grounds such as green, dark green, long, medium long, short fruit back grounds (Fig. 3). Thus, these ten maintainer lines IIHR-6-2, IIHR-5-1-2, IIHR-37-4-1, IIHR-23-5-4, IIHR-34-2-2, IIHR-49-3-1, IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 and IIHR-11-1-2 proved to possess fertility restorer gene (Rf) in homozygous recessive condition making them as ideal maintainer lines (Pradeepkumar *et al.*, 2018). These 10 sets of male sterile (A lines) as well as maintainer lines (B lines) in different genetic backgrounds (Fig 3) are now ready for the development of hybrids using fertility restorer lines (C lines). This study confirms the presence of CGMS system in ridge gourd paving way for commercial hybrid seed production in this crop as reported by Pradeepkumar *et al.*, (2018), who for the first time developed CGMS system in ridge gourd by developing MS LA 101 and LA 101, male sterile (A line) and maintainer line (B line) respectively.

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IIHR-70-1 (long, dark green)

IIHR-6-2 (long, green)

IIHR-49-3-1(medium, green)

IIHR-37-4-1 (short, green)

Fig. 3. Fruits of male sterile and maintainer lines in different genetic backgrounds (long/medium/short fruit length and dark green/green fruit color) in ridge gourd

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