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NEW SOURCE OF CYTOPLASMIC GENIC MALE STERILITY (CGMS) AND RESTORATION OF FERTILITY GENE IN RIDGE GOURD

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ABSTRACT

Ridge gourd [*Luffa acutangula* (Roxb.) L.] Male sterile (MS) mutant, regenerated through *in vitro* culture was crossed with five pollen parents. Four hybrids out of five were sterile (MS x CO 2, MS x Deepthi, MS x IC-92685, MS x IC-92671) and one (MS x Arka Sumeet) was found to be partially fertile. Male sterility in the sterile hybrids was stable throughout the flowering season. In partially fertile (MS x Arka Sumeet) hybrid was selfed to made F_2 population. In this F_2 population of the cross (MS × Arka Sumeet) exhibited a good fit to the 9:7 (fertile:sterile) modified ratio indicating the role of complementary genes. Two dominant fertility restorer genes (*Rf1* and *Rf2*) either in homozygous dominant or heterozygous dominant condition restores the male fertility in presence of sterile cytoplasm. All the four three way crosses and seven F_2 population regained fertility indicating the presence of dominant fertility restorer genes in Arka Sumeet. This is the first report of cytoplasmically controlled male sterility (CMS) in cucurbits where two dominant male fertility restorer nuclear genes with complementary gene action governed the restoration of male fertility.

INTRODUCTION

Ridge gourd or ribbed gourd or smooth gourd is a large climber with long tap root system and palmate leaves with 5-7 lobes. Though cultivated varieties are monoecious in nature, different sex forms *viz.*, androecious, gynoecious, gynomonoecious and romonoecious and hermaphrodite plants are also reported (Choudhary and Thakur, 1965). The male flowers with 5 stamens are born 10-20 flowered racemes and the female flowers are solitary on the same axis as that of male flower. The fruits are about 15-30 cm long, cylindrical or club shaped with 10 prominent, almost wing like ridges. The seeds are much compressed, 10-12 mm long, slightly corrugated on the edges and black when ripe. It is emetic and traditionally used for the treatment of stomach ailment and fever (Chakravarty, 1990). Seeds are reported to possess purgative, emetic and antihelmintic properties due to the secondary metabolite *cucurbitacin* (Robinson and Walters, 1997).

Male sterile plants were either isolated from natural population or were artificially induced through mutagenesis. Male sterile system is now commercially exploited in many vegetable crops like chilli, water melon, squash and muskmelon for producing F_1 hybrid seeds (Kumar, 2000; Zang *et al.*, 1996). The specific mechanisms causing male sterility in vegetables vary from species to species and are subject to change in environment, and nuclear and cytoplasmic genes. Apart from the use in commercial seed production programs, current application of male sterility also includes the possibility of gene containment in transgenic plants (Daniel, 2002). Male sterility is of special interest of the plant breeders to produce more efficient and economic hybrid seed as it ensures genetic emasculation. Crops like chilli and musk melon are successful example of utilization of male sterility system in India.

In ridge gourd (*Luffa acutangula* L (Roxb).) heterosis breeding is a proven method of crop improvement (Mole *et al.*, 2001; Hedau and Sirohi, 2004). The female flowers are solitary whereas male flowers are in racemes. Though male sterility was reported in ridge gourd over three decades before its utilization in crop improvement program did not receive much attention (Deshpande *et al.*, 1979). Ridge gourd offers greater scope for exploitation of hybrid vigour (Reddy *et al.*, 2013) on commercial scale to increase the productivity and production. The most common, easy and effective means for developing/ identifying new hybrids or line is by utilizing cytoplasmic genetic male sterility (CMS) technique in the hybrid breeding program (Pratap *et al.*, 2013) is fruitful. The development and use of hybrid rice varieties on commercial scale utilizing CMS fertility restoration system has now proved to be one of the plausible milestones in the history of rice improvement. Hence the present investigation on male sterility in ridge gourd is undertaken with the objective of investigating the inheritance of male sterility in ridge gourd and to develop new male sterile line in ridge gourd using back cross generations of sterile hybrids.

MATERIALS AND METHODS

There was no suitable natural maintainer line, male sterile (MS) mutant was being maintained under *in vitro* conditions at Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, KAU, Kerala and was regenerated using the standardized protocol (Pradeepkumar *et al.*, 2007 and 2010). Phenotypic characters of in vitro regenerated Male Sterile mutant were evaluated by growing them in the research field of Department of Olericulture. Observations for days for emergence of first fertile male flower, days for emergence of first female flower, nodes to first fertile male flower, nodes to first female flower, male bud length and pollen fertility (%) were recorded from 20 *in vitro* regenerated MS mutants. Pollen fertility percentage was assessed from 10 randomly selected male flower buds in each line at anthesis on the basis of stainability in acetocarmine and the counts were taken from 10x fields under microscope for each plant line flower bud. Well filled, uniformly stained pollen grains were considered as fertile and the rest as sterile. Five ridge gourd accessions viz., 'Arka Sumeet', 'IC-92685', 'Deepthi', 'IC-92671' and 'CO-2' were crossed as male parents with MS mutant to produce five F_1 hybrids viz., MS × Arka Sumeet, MS × IC-92685, MS × Deepthi, MS × IC-92671 and MS × CO-2. F_1 hybrids and parental lines were grown during January to April, 2009 as per the package of practices recommendations (KAU, 2007). Observations pertaining to male and female fertility noted for MS mutant were recorded from 12 plants in each line/hybrid. The fertile hybrid (MS × Arka Sumeet) was selfed to generate F_2 population as well as back crossed with Arka Sumeet to produce BC_1 generation. Remaining male sterile hybrids viz., (MS × IC-92685), (MS × Deepthi), (MS × IC-92671) and (MS × CO-2) were crossed with 'Arka Sumeet' to generate three way cross hybrids. F_2 population (106 plants), BC_1 generation (24 plants), three way cross hybrids (24 plants each) were raised during 2010–2011 and evaluated for male sterility and restoration of fertility. Chi-square goodness-of-fit analysis was conducted for segregation of male fertility and sterility in F_2 and BC (Back cross) populations (Russell, 1996).

RESULTS AND DISCUSSION

Morphological characterization of tissue culture regenerated male sterile mutant plants

In ridge gourd tissue culture survival rate during hardening was observed to be 70 per cent. Hardened tissue cultured plants were transplanted to the field. All the *in vitro* regenerated male sterile plants had a stable expression of male sterility throughout the growing period. The male buds failed to open and fell down 12 days after they attained the size visible to naked eye (Fig. 1). Average bud length at this stage was 8.6 mm. There was a marked difference in the appearance of anther lobes and pollen grains. In the male sterile plant, the anther lobes were not properly developed. The pollen grains were small and shrunken. When these pollen grains were mounted in a drop of acetocarmine stain, they did not attain the stain (Fig. 1). Pollen fertility, based on the stainability was found to be zero.

Expression of male sterility and restoration of fertility in F_1 cross, MS × Arka Sumeet

Recently, a natural mutant line was reported by Pradeepkumar *et al.* (2008), which could be used for hybrid seed production programs. In an attempt to elucidate the genetics of male sterility in ridge gourd, studied the crosses involving male sterile female parent and pollen parents selected from different parts of India (Hegade, 2009). Four hybrids out of five were sterile (MS x CO 2, MS x Deepthi, MS x IC-92685, MS x IC-92671) and one (MS x Arka Sumeet) was found to be partially fertile. This indicates the presence of partial dominant gene

Table 1: Range, mean, variance values of ridge gourd BC_1 generation and three way cross hybrids involving male sterile lines (MS)

Character	Population	Range	Mean	Variance
Days to emergence of first fertile male flower	BC_1(MS x Arka Sumeet) x Arka Sumeet	42.0-47.0	44.7	6.33
	(MS x Deepthi) x Arka Sumeet	37.0-40.0	38.3	17.25
	(MS x IC-92671) x Arka Sumeet	37.0-38.0	37.6	6.5
	(MS x IC-92685) x Arka Sumeet	37.0-38.0	38	14.25
	(MS x CO 2) x Arka Sumeet	37.0-39.0	37.6	6.8
Node to first fertile male flower	BC_1(MS x Arka Sumeet) x Arka Sumeet	13.0-16.0	14.3	2.33
	(MS x Deepthi) x Arka Sumeet	12.0-14.0	12.6	3.22
	(MS x IC-92671) x Arka Sumeet	12.0-14.0	13	3.32
	(MS x IC-92685) x Arka Sumeet	10.0-13.0	11	2.88
	(MS x CO 2) x Arka Sumeet	12.0-13.0	13	2.85
Pollen fertility (%)	BC_1(MS x Arka Sumeet) x Arka Sumeet	71.4-92.0	83.5	11.4
	(MS x Deepthi) x Arka Sumeet	72.3-91.2	87.3	12.3
	(MS x IC-92671) x Arka Sumeet	74.5-90.2	85.4	11.1
	(MS x IC-92685) x Arka Sumeet	71.2-91.2	83.3	10.33
	(MS x CO 2) x Arka Sumeet	64.5-88.2	79.7	10.52

Table 2: Chi-square test for F_2 families segregating for Male sterility and male fertility in ridge gourd

Population	Male fertile	Male sterile	total	X^2 analysis					
				3:1F:S	p	9:7F:S	P	13:3F:S	p
F2	56	50	106	27.74	0.005	0.60	0.10	55.46	0.005

Table 3: Male fertility traits in parents and hybrids

Parents/hybrid	Days for the emergence of first fertile male flower		Node at first fertile male flower		Average male bud length		Pollen fertility (%)	
MS mutant	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Co2	0	0	07.5-7.60	0	7.52-7.60	7.58	0	97.75
Arka sumeet	45.2-46.1	45.58	10.0-10.3	95.099.7	10.0-10.3	10.12	95.5-92.0	91.41
Deepthi	38.1-39.0	38.71	11.2-11.4	90.5-92.0	11.2-11.4	11.4	90.5-92.0	90.46
IC-92685	29.8-30.5	30.22	10.1-10.2	84.0-88.0	10.1-10.2	10.2	84.0-88.0	85.79
IC-92671	28.7-30.5	29.48	09.3-9.61	61.0-65.0	9.3-9.61	9.5	61.0-65.0	63
MSxArka sumeet	45.2-46.5	46.06	10.0-10.0	32.0-36.2	10.0-10.0	10.03	32.0-36.2	34
MSxDeepthi	0	0	8.15-8.30	0	8.15-8.30	8.25	0	0
MSxIC-92685	0	0	7.36-7.52	0	7.36-7.52	7.45	0	0
MSxIC-92671	0	0	08.0-8.12	0	8.0-8.12	8.05	0	0
MSxC02	0	0	09.05-9.1	0	9.05-9.1	9.08	0	0
LSD(0.05)		3.12		2.05		1.72		13.4

Table 4: Female fertility traits in parents and hybrids

	Days for the emergence of first fertile female flower		Node at first fertile female flower	
	Range	Mean	Range	Mean
MS mutant	65.0-66.0	65.33	29.0-31.0	30.167
Co2	53.0-56.0	54.41	32.0-32.37	32.37
Arka sumeet	33.0-35.0	34.25	10.0-11.0	10.5
Deepthi	33.0-36.0	34.54	11.0-12.0	11.5
IC-92685	36.0-38.0	37.37	5.0-7.0	6.2
IC-92671	41.0-42.0	41.35	6.0-7.0	12.41
MS x Arka sumeet	41.0-42.0	41.62	12.0-13.0	10.5
MS x Deepthi	41.0-42.0	41.62	10.0-11.0	13.4
MSxIC-92685	43.0-44.0	43.37	13.0-14.0	10.08
MSxIC-92671	39.0-40.0	39.5	9.0-11.0	24.5
MSxC02	44.0-45.0	44.91	24.0-25.0	1.32
LSD(0.05)		4.25		

action in cytoplasmic genes controlling male sterility which is not yet reported in cucurbits. The pattern of inheritance of male sterility and restoration of fertility can only be explained by studying the F_2 and back cross generations and the three way cross involving male sterile hybrids and the pollen parent which restores the fertility.

Analysis of F_2 population of the crosses, MS × Arka Sumeet for male sterility and restoration of fertility

Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile. 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. 46.2 per cent of the F_2 plants produced fertile male flower between 52nd to 64.9th days after sowing. 2.83 per cent of the F_2 population reverted to male fertility on final stage of crop growth and produced fertile male flower after 97 days.

As F_2 population segregated into two classes with respect to male fertility, monohybrid ratio, 3:1 and modified dihybrid ratio viz, 13:3 and 9:7 respectively, were tested for significance using $\div^2$ test (Table 2). The 9:7 (fertile: sterile) complementary gene action ratio was found to be a good fit to the observed ratio. Assuming that MS line in ridge gourd is having a genotype, *S (rf1rf1 rf2rf2)* carrying both fertility restorer gene in homozygous recessive state and sterility inducing cytoplasm (S) and Arka Sumeet possess a genotype *N (Rf1Rf1 Rf2Rf2)* carrying both 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 *S (Rf1rf1 Rf2rf2)*. Here though F_1 is inheriting a sterile cytoplasm from male sterile female parent, presence of both complementary dominant alleles of fertility restorer gene (*Rf1* and *Rf2*) restores the fertility. In F_2 presence of dominant alleles of both fertility restorer genes in either homozygous or heterozygous condition ensures male fertility. Thus the genotypes *Rf1Rf1 Rf2Rf2*, *Rf1Rf1 Rf2rf2*, *Rf1rf1 Rf2Rf2*, *Rf1rf1 Rf2rf2* and *Rf1rf1 Rf2Rf2* were male fertile in presence of sterile cytoplasm. Genotypes *Rf1Rf1 rf2rf2*, *Rf1rf1 rf2rf2*, *rf1rf1 Rf2Rf2*, *rf1rf1 Rf2rf2* and *rf1rf1 rf2rf2* were male sterile in presence of sterile cytoplasm as both dominant fertility restorer alleles are absent in these genotypes. The proposed model, suggests cytoplasmic male sterility in ridge gourd which is modified by the influence of dominant fertility restorer genes viz., *Rf1* and *Rf2*.

Evaluation of back cross and three way crosses for male sterility and restoration of male fertility in ridge gourd

All three way crosses *viz.*, (MS x Deepthi) x Arka Sumeet, (MS x IC-92685) x Arka Sumeet, (MS x IC-92671) x Arka Sumeet and (MS x CO2) x Arka Sumeet regained fertility indicating the presence of dominant fertility restorer gene in Arka Sumeet (Table 1). All the male sterile hybrids on crossing with Arka Sumeet produced fertile male flowers early and took only few nodes for generating fertile male racemes. Hegade (2009) also reported the restoration of fertility when male sterile line (MS) was crossed with Arka Sumeet and suspected the presence of fertility restorer gene in Arka Sumeet. Present results confirmed his finding and the segregation pattern in F_2 indicate the

Table 5: Proposed genetic model for complementary gene action

	Male sterile MS line		Male fertile Arka Sumeet	
Parents	S (rf1rf1 rf2rf2)		N (Rf1Rf1 Rf2Rf2)	
Gametes	S (rf1rf2)		Rf1Rf2	
F_1	Male fertile S (Rf1rf1 Rf2rf2)			
Gamets	Rf1Rf2 Rf1rf2 rf1Rf2 rf1rf2			
eggs / pollen	Rf1Rf2	Rf1rf2	rf1Rf2	rf1rf2
Rf1Rf2	S (Rf1Rf1 Rf2Rf2) male fertile	S (Rf1Rf1 Rf2rf2) male fertile	S (Rf1rf1 Rf2Rf2) male fertile	S (Rf1rf1 Rf2rf2) male fertile
Rf1rf2	S (Rf1Rf1 Rf2rf2) male fertile	S (Rf1Rf1 rf2rf2) male sterile	S (Rf1rf1Rf2rf2) male fertile	S (Rf1rf1 rf2rf2) male sterile
rf1Rf2	S (Rf1rf1 Rf2Rf2) male fertile	S (Rf1rf1 Rf2rf2) male fertile	S (rf1rf1 Rf2Rf2) male sterile	S (rf1rf1 Rf2Rf2) male sterile
rf1rf2	S (Rf1rf1 Rf2rf2) male fertile	S (Rf1rf1 rf2rf2) male sterile	S (rf1rf1 Rf2rf2) male sterile	S (rf1rf1 rf2rf2) male sterile

Figure 1: Male sterile tissue culture plant during anthesis

Figure 2: Male fertile tissue culture plant during anthesis

Figure 3: Racemes of both male sterile and fertile plant

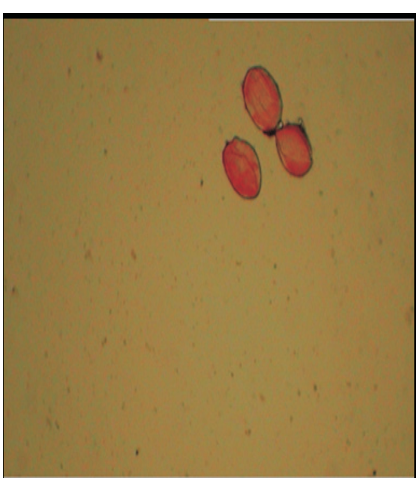

Figure 4: Male sterile pollen during anthesis

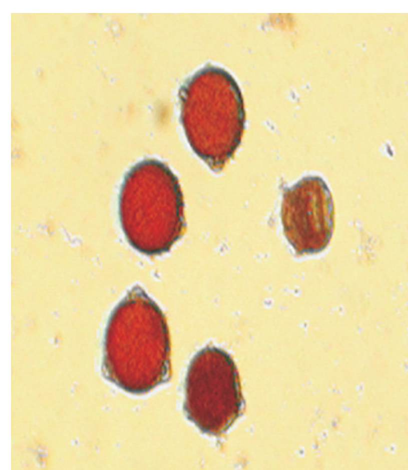

Figure 5: Male fertile pollen during anthesis

presence of two fertility restorer genes in Arka Sumeet *viz Rf1* and *Rf2*.

Surprisingly the sterile hybrids on back crossing with respective pollen parents also exhibited male fertility at various stages of crop growth. Among the four back crosses *viz.,* (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2, only the back cross, (MSx Deepthi) x Deepthi exhibited male sterility in the initial stages but produced fertile male flower after 58 days. Among back crosses, back cross (MS x Arka Sumeet) x Arka Sumeet was the earliest to form male flower (38.66) which is again an indication of the presence of fertility restorer genes in Arka Sumeet.

Restoration of male fertility in BC_1 generation indicates the unstable nature of sterile cytoplasm which has been reported in pepper and carrot (Kaul, 1988). Among various pollen parents, Deepthi restores the male fertility after a stage in crop growth, indicating the strong interaction between cytoplasm and nuclear genes and dominant effect of fertility restorer genes in the late stage of crop growth. Since this is the first report of cytoplasmic sterility in cucurbits, more studies are required to find out the exact nature and potential of this cytoplasm before exploiting the same for crop improvement.

Analysis of gene action governing male fertility and male sterility in F_2

The knowledge about inheritance of fertility restoration of male sterile line is of vital importance in improving or transferring fertility restoring genes and quality of restorer line breeding. Fertility restorer gene controlled by complementary gene action is reported in cole crops (Sigareva and Earle, 1997) and in pepper. (Shifriss, 1997). Depending on the type of sterile cytoplasm, interaction of *Rf* allele varies. In carrot two types (petaloid and brown anther) of male sterile lines are available (Welch and Grimball, 1947; Morelock, 1974) depending on the type of cytoplasm and here genetics of fertility restoration is complex (Peterson and Simon, 1986) because structural variants of mt DNA are numerous (Ranfort *et al.*, 1995). The proposed model, suggest cytoplasmic male sterility in ridge gourd in which sterility is modified by the influence of dominant fertility restorer genes *viz., Rf1* and *Rf2* (Table 5). This is the first report of cytoplasmic male sterility and fertility restorer gene in cucurbits.

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REFERENCES

Chakravarty, H. H. 1990. Cucurbits in India and their role in development of vegetable crops. In: Bates, D.M., Robinson, R.W., Jeffrey, C. (Eds.), Biology andUtilization of the Cucurbitaceae. *Cornell University Press, Ithaca,* New York, pp. 325-348.

Choudhary, B. and Thakur, M. R. 1965. Inheritance of sex forms in Luffa. *Indian J. Genet.*

Daniell, H. 2002. Molecular strategies for gene containment in transgenic crop. *Nature biotechnology.* **20:** 5581-5586.

Deshpande, A. A. Ravishankar, H. and Bankapur, V. M. 1979. A male sterility mutant i ridge gourd (Luffa acutangula Roxb.). *Curr. Res.* **6:** 97-98.

Hedau, N. K. and Sirohi, P. S. 2004. Heterosis studies in ridge gourd. *Indian J. Hort.* **61(3):** 236-239.

Hegade, V. C. 2009. Male sterility and its utilization for improvement in ridge gourd (Luffa acutangula L.). MSc (Hort) thesis, Kerala Agricultural University, Thrissur. p. 78p.

Karthik reddy, P., Reddy, V. S. K. and Vijaya padma, S. S. 2013. Performance of parents and hybrids for yield and yield attributing characters in ridge gourd. *The Bioscane.* **8(4):** 1461-1465.

KAU 2007. Package of Practices Recommendations: Crops, twelfth ed. Directorateof Extension, Kerala agricultural University, Thrissur.

Kumar, S., Banerjee, M. K. and Kalloo, G. 2000. Male sterility: Mechanisms and current status on identification, characterization and utilization in vegetables. *Veg. Sci.* **27(1):** 1-24.

Pratap, N., Shekhar, R., Singh, P. K. and Soni, S. K. 2013. Combining ability, gene action and heterosis using CMS lines in hybrid rice. *The Bioscane.* **8(4):** 1373-1377.

Pradeepkumar, T., Hegde, V. C., Sujatha, R., George, T. E. 2010. Characterization an maintenance of novel source of male sterility in ridge gourd (Luffa acutang (L.)Roxb.). *Curr. Sci.* **99:** 1326-1327.

Pradeepkumar, T., Krishnaprasad, B. T., Sujatha, R. and Johnkutty, I. 2008. New source of male sterility in ridge gourd (*Luffa acutangula*(L.) Roxb.) and its maintenance through *in vitro* culture. In: Yesodharan, E. P. (ed.), *Proceedings of the 20th Kerala Science Congress*; 28-31 January 2008, Thiruvananthapuram. *Kerala State Committee for Science*, Technology and Environment, Government of Kerala, pp 30-32.

Robinson, R. W. and Walters, D. S. 1997. *Cucurbits. New York Cab International.* p. 226.

Russell, P. J. 1996. Genetics, fourth ed. *Harper Collins College Publ, New York*

Zhang, X. P., Rhodes, B. B., Baird, W. V., Skorupska, H. T. and Bridges, W. C. 1996. Development of genic male sterile watermelon lines with delayed-green seedling marker. *Hort Sci.* **31:** 123-126.

Kaul, M. L. H. 1988. Male sterility in higher plants. Monograph on Theor. Appl. Genet. 10. Springer-Verlag. Berlin. p. 210.

Mole, T. J., Devi, N. S., Rajan, S. and Sadhankumar, P. G. 2001. Heterosis and combining ability in ridge gourd (*Luffa acutangula* Roxb.). *Veg. Sci.* **28(2):** 165-167.

Morelock, T. E. 1974. Influence of cytoplasm source on the expression of male sterility in carrot, *D. carota*. Ph. D. thesis. Wisconsin University, USA.

Peterson, C. E. and Simon, P. W. 1986. Carrot breeding. In: Basset M. J. (Ed.), Breeding vegetable crops. AVI Publishing Co., West Port, Connecticut. pp. 321-356.

Ranfort, J., Saumitou, L. P., Cuguen, J. ad Couvet, D. 1995. Mitochodrial DNA diversity and male sterility in natural populations of *Daucus carota* ssp. Carota. *Theor. Appl. Genet.* **91:** 150-159.