MALE STERILITY AND ITS UTILIZATION FOR CROP IMPROVEMENT IN RIDGE GOURD *Luffa acutangula* (L.) Roxb.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

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2009

DECLARATION

I, hereby declare that this thesis entitled "Male sterility and its utilization for crop improvement in ridge gourd *Luffa acutangula* (L.) Roxb." is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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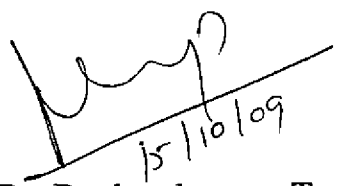
Certified that this thesis entitled "Male sterility and its utilization for crop improvement in ridge gourd *Luffa acutangula* (L.) Roxb." is a bonafide record research work done independently by Mr. Vijeeth C. Hegade under my guidance and supervision and that it has not formed the basis of the award of any degree, diploma fellowship or associateship to him.



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ACKNOWLEDGEMENT

The foremost person to be thanked is Dr. Pradeepkumar, T. Associate Professor, Department of Olericulture and the chairmen of my advisory committee. A source of inspiration but also a humble modest man with mettle words, who's pragmatic suggestion genuine counselling and meticulous care helped me to spawn original efforts into an acceptable final form. His encouragements as a persistent friendly teacher, helped immeasurably and intimidated to do the best.

It is my pleasant privilege to oblige Dr. George T. E., Professor and Head, Department of Olericulture and member of my advisory committee, for his ardent interest, valuable suggestions and critical scrutiny of the manuscript and ever willing help which has helped a lot for the refinement of this work.

No words can truly represent my profound gratitude and indebtedness to Dr. Nirmaladevi, S. Professor, Department of Olericulture and member of my advisory committee for her expert counsel, invaluable guidance, untiring interest, constructive suggestions, esteemed advice and immense help rendered throughout the course of this investigation.

I place a deep sense of obligations to Dr. Sujatha, R. Assistant Professor, Sr. Scale, CPBMB, College of Horticulture, Vellanikkara and member of advisory committee, for her valuable guidance in tissue culture works and cytology. I really consider it my fortune in having her guidance for the thesis work.

I feel immense pleasure to express my deep sense of gratitude to Dr. Laly John, Professor, Department of Agricultural Statistics for her guidance in the statistical analysis. She in spite of a busy schedule has offered great help for the betterment of the investigation.

I am thankful to the teaching and non-teaching staff of the Department of Olericulture for co-operation and help extended to me during the study.

I am thankful to Dr. P. A. Nazeem, Professor and Head, CPBMB, College of Horticulture, Vellanikkara for permitting me for the in vitro studies in the department.

I wish to record my deep and heartfelt gratitude to my family members for the ungrudging co-operation and for keeping me in high spirit during my stay in KAU.

The encouragement rendered by my friends at various stages of this investigation was invaluable and I thank Dhinesh, Srividhya, Kanimozhi, Sivaji, Kiran, Giri, Guru, Lamina, Mittu, Saranya, Bindhya, Jisha, Kavitha, Saritha form the bottom of my heart. My thanks also to my juniors Arya, Prathamesh, Sunil, Gajanan and Ambily. I also acknowledge the help offered by Koteshwar Rao.

The financial help received in the form of Junior Research Fellowship from Kerala Agricultural University is gratefully acknowledged.

(Vijeeth C. Hegade)

15/10/09

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Dedicated to the Scientist and Farming Community of India

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INTRODUCTION

1. INTRODUCTION

Keeping in view of the alarmingly increasing population in the world, the scientists undertake earnest efforts to break the yield plateau in different crops by adopting various conventional and non-conventional breeding procedures *viz.,* introduction of exotic varieties to new agroclimatic zones, heterosis breeding, improvement of existing cultivars for one/few characters, identification and utilization of resistant- lines for biotic and abiotic stresses, application of biotechnology *etc.* Among the agricultural crops, vegetables are the most extensively utilized ones for exploitation of heterosis through creation of F_1 hybrids. Presently hybrids predominate in several vegetable crops. The advantages of hybrid vegetables are uniformity, increased vigour, earliness, higher yield and resistance to specific pests and pathogens. Besides, hybrids provide opportunities for rapid deployment of desirable dominant genes.

If heterosis breeding is combined with some pollination control mechanism such as male sterility, it will certainly economize commercial hybrid seed production. Male sterility is defined as the failure of plants to produce functional anthers, pollen or male gametes (Kaul, 1988). Male sterility has been reported in fairly large number of crops including vegetables. These male sterile plants were either isolated in natural population or were artificially induced through mutagenesis. This phenomenon has always been of long term interest for the researchers of various disciplines of applied, strategic and basic sciences. It is of special interest of the plant breeders to produce more efficient and economic hybrid seed. In cucurbits (musk melon), Nandpuri *et al.* (1982) were the pioneers for its commercial utilization in India.

Public sector in most of the developed countries has successfully utilized male sterility system in several vegetable crops to produce hybrid seeds on commercial scale. In contrast, in the public sector of developing country like India, though the public sector has bred hybrids in several vegetables, genetic

emasculation through male sterility has not been utilized efficiently (Kalloo *et al.*, 1998). Nevertheless, the crops like muskmelon present very successful examples of utilization of male sterility system in India (Kalloo *et al.*, 1998).

The phenomenon of male sterility had already been reported in ridge gourd by Deshpande *et al.* (1979) and Pradeepkumar *et al.*, (2008). Various workers had already reported the scope of heterosis for improvement in this crop (Mole, 2000; Niyaria and Bhalala, 2001; Mole *et al.*, 2001; Hedau and Sirohi, 2004; Ram *et al.*, 2004). The high number of hybrid seeds per cross makes F_1 seed production more economical in ridge gourd. Further, the crop being cultivated at wider spacing, the hybrid seed rate per hectare for commercial vegetable crop would be low and cost effective. Therefore, ridge gourd offers great scope for exploitation of hybrid vigour on commercial scale to increase the production and productivity of this otherwise under exploited cucurbit vegetable. In order to reduce cost of hybrid seed production, it would be appropriate to utilize the available genetic mechanism of male sterility for hybrid seed production in ridge gourd. Like musk melon, male sterility can be exploited in heterosis breeding programme in ridge gourd also. The cost of F_1 hybrid seed production can be reduced by employing genetically emasculated male sterile lines as female parent. Transgene escape can be prevented using male sterile lines and although it is not reported yet, naturally occurring dominant source of male sterility has wide application in this background. However, genetics of male sterility needs to be elucidated for its successful exploitation. The male sterile plant has to be crossed with different male fertile cultivars to verify the inheritance pattern of male sterility and also to track down the occurrence of the fertility restorer gene, if it is a cytoplasmic gene causing the male sterility.

Micropropagation can be employed effectively for maintenance of male sterile lines and *in vitro* propagation via shoot tip/nodal culture is a preferred route for clonal multiplication. The protocol for the clonal propagation of ridge gourd has been already standardized (Pradeepkumar *et. al.*, 2008) and male sterile line is being maintained by this method.

Ridge 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, andromonoecious and hermaphrodite plants are also reported (Choudhary and Thakur, 1966). The male flowers with 5 stamens (synandry) are born in 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.

Tender fruits which are green in colour with shallow ridges are used in soups and curries or as a cooked vegetable. The nutritional estimates as per 100 grams of ridge gourd are as follows: edible protein 82%, moisture 95.2g, protein 0.5g, fat 0.1g, fibre 0.5g, carbohydrate 3.4g, energy 17 k cal, calcium 18mg, vitamin C 5mg, riboflavin 0.01mg, phosphorus 26mg, iron 0.5mg and carotene 33µg (Sheshadri and Parthasarathy, 1980). Beside their use as vegetables, these gourds are utilized for various purposes. The fibre obtained from the mature dry fruit is used in industry for filters of various sorts, good pot holders, table mats, bath room mats, slipper and shoe soles. Also the fibre has proved to be a good insulator for various purposes. Sometimes the dry fruits which gave good storage capacity are used for ornamental purposes also. It is emetic and traditionally used for the treatment of stomach ailment and fever (Chakravarty, 1959). Seeds are reported to possess purgative, emetic and antihelmintic properties due to the secondary metabolite *cucurbitacin* (Robinson and Decker-Walters, 1997). Isolation of Ribosome Inactivating Proteins (RIPs) and luffaculin from ridge gourd seeds and its crystallographic studies (Hou, *et. al.*, 2006) shows the potential of this species in therapeutic use. RIPs have received wide attention for their potential applications in medicine as they posses various pharmacological activities including abortifacient, antifungal, antitumor, antivirus and HIV-1 integrase inhibitory properties.

The present investigation on male sterility and its utilization for crop improvement in ridge gourd is undertaken with the objective of investigating the stability of male sterility in ridge gourd *Luffa acutangula* (L.) Roxb. and expression of male sterility on combinations with different pollen parents of diverse group.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In this chapter available information on various aspects of hybrid breeding in ridge gourd and male sterility in cucurbits is reviewed under the following headings.

2.1 Variability

The success of any crop breeding programme depends purely upon the extent and magnitude of genetic variability that exists in the available germplasm. The knowledge of the estimates of genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance would help the breeder in selecting an appropriate breeding method. Further the estimation of genetic advance helps in deducing the genetic facts.

2.1.1 Days to emergence of first fertile male flower

The number of days to the emergence of first fertile male flower is important. The genotypes varied significantly high for days to emergence of first fertile male flower and the mean value reported to be 13.82 (Ahmed *et al.*, 2006).

2.1.2 Days to emergence of first female flower

Number of days to the emergence of first female flower is an index of earliness. Many workers have reported variability with respect to this character. In the analysis of 56 accessions of ridge gourd, LA 37 (21.00 days) and LA 89 (27.65 days) were the earliest to produce female flower in the first and second season respectively (Anitha (1998). The gcv and pcv were 19.40 and 19.18 in the first season and 16.83 and 15.86 in the second season. There was significant variability between the genotypes and the gcv and pcv were almost same signifying the greater role of genotypic differences for total variability. Ahmed *et al.* (2006) also reported highly significant variation with respect to the character and the mean value reported as 29.26.

2.1.3 Node to first fertile male flower

Ahmed *et al.* (2006) studied 27 genotypes of ridge gourd and the analysis of variance revealed that the genotypes varied significantly for the trait and the mean value was emerged to be 1.01.

2.1.4 Node to first female flower

Genotypes varied for the expression of this character and the value ranged from 6.75 (LA 62) to 23.40 (LA 20) in the first season and from 8.15 (LA 89) to 18.05 (LA 5) in the second season (Anitha, 1998). The gcv and pcv were reported to be 37.97 and 37.39 respectively in the first season and 22.52 and 22.02 respectively in the second season. Narrow difference between gcv and pcv showed less effect of environment on the expression of the trait. All the 27 genotypes showed highly significant variation for node to first female flower and the mean value was 12.76 (Ahmed *et al.*, 2006).

2.1.5 Number of fruits per plant

Anitha (1998) reported that the genotypes varied significantly for number if fruits per plant and the value ranged from 1.00 to 12.00 and from 2.25 to 11.00 in first and second season respectively. The gcv and pcv reported in the first season were 61.58 and 56.58 in the first season and 37.49 and 35.66 in the second season. Ahmed *et al.* (2006) reported a highly significant variation for the trait in their study of 27 ridge gourd genotypes and the mean value found to be 3.28.

2.1.6 Average fruit weight

Anitha (1998) reported significant difference of the fruit size over two seasons. It ranged from 55.0 g (LA 54) to 202.20 g (LA 30) in the first season and 44.45 g (LA 54) to 229.95 g (LA 72) in the second season. The pcv and gcv for the first season were 30.30 and 29.00 respectively. In a study reported by Ahmed *et al.* (2006), all the 27 genotypes of ridge gourd varied significantly for the trait and the value was 600.83g.

2.1.7 Average fruit length

Anitha (1998) reported that the accession LA 86 produced the longest fruits (47.88 cm) over the seasons and shortest fruits were produced by LA 51 (12.80 cm). The pcv and gcv value for the first season reported to be 34.67 and 34.11 respectively and in the second season pcv and gcv were 34.47 and 34.15. Ahmed *et al.* (2006) reported a highly significant variability (21.90) among the 27 genotypes studied.

2.1.8 Average fruit girth

Anitha (1998) observed significant variation and the girth of the fruit ranged from 8.67 cm (LA 52) to 19.52 cm (LA 5). The pcv and gcv for the first season were 21.75 and 21.10 and for the second season it varied 20.38 and 17.98 respectively. Highly significant variability was reported by Ahmed *et al.* (2006) in their study of 27 genotypes and the mean value reported was 10.58 cm.

2.1.9 Days to first harvest

Days to first harvest ranged from 31.65 (LA 89) to 73.90 days (LA 7) in the first season and it ranged from 37.1 (LA 89) to 65.40 days (LA 87) in the second season (Anitha, 1998). During the first season pcv and gcv were 15.90 and 15.51 and during second the season the pcv and gcv were 13.09 and 12.86. Ahmed *et al.* (2006) reported highly significant variability for the trait (35.31 days) in 27 genotypes of ridge gourd.

2.1.10 Yield per plant

Ridge gourd genotypes differed significantly for this trait and the mean value ranged from 70.0g (LA 43) to 1570.0g (LA 12) in the first season and 108.35 g (LA 54) to 2331.25 g (LA 7) in the second season (Anitha, 1998). The pcv and gcv were high with values 67.16 and 64.54 in the first season and 57.62 and 652.76 respectively in the second season.

2.1.11 Seeds per fruit

Anitha (1998) reported significant difference for the trait, where LA 91 (29.10) produced minimum number of seeds and LA 12 (215.45) produced the maximum. The pcv and gcv were 47.70 and 47.51 respectively in the first season and 46.76 and 46.73 respectively in the second season.

2.1.12 Seed yield per fruit

The 100 seed weight ranged from 9.50 g (LA 54) to 17.38 g (LA 37) as reported by Anitha (1998). In the first season, the pcv and gcv were 17.30 and 16.00 respectively and in the second season the values were 16.48 and 16.40 respectively.

2.2 Genetic divergence among ridge gourd genotypes

The divergence analysis helps in the selection of parents for crop improvement works which are of diverse origin. Anitha (1998) grouped 57 ridge gourd genotypes into 9 clusters. The intra-cluster distances were more than the inter-cluster distances.

2.3 Heterosis

Among the agricultural crops, vegetables are most extensively utilized for the exploitation of heterosis through hybrid varieties. Ridge gourd, being predominantly monoecious, is a cross-pollinated crop and provides ample scope for utilization of the hybrid vigor. The estimation of heterosis for yield and its component characters would, therefore, be useful to judge the best hybrid combinations for exploitation of superior hybrids. The work on this aspect in ridge gourd is reviewed hereunder.

2.3.1 Days to first female flower emergence

Abusaleha and Dutta (1994) reported the heterosis in the range of -2.07 to -20.7 per cent over the better parent for days to first female flower emergence. According to Mole (2000), F_1 mean ranged from 36.33 (LA 43 x LA 44) to 40.83

percent (LA 44 x LA 83). Maximum negative relative heterosis was observed for LA 81 x LA 86 (-3.57 percent). Niyaria and Bhalala (2001) recorded heterosis of -1.49 to 12.20 per cent over better parent for the trait. Significant negative (-9.52%) standard heterosis and heterobeltiosis in F_1 hybrid of ridge gourd LA 43 x LA 86 were reported by Mole *et al.* (2001). Hedau and Sirohi (2004) reported that out of 45 F_1 hybrids, 23 and 13 crosses exhibited negative heterosis over better and top parents, respectively for the trait. The best F_1 hybrid CHRG-1 x KRG-5 exhibited -15.22 percent heterosis over better parent and -13.89% over top parent (DRG-2). Ram *et al.* (2004) reported significant heterosis in crosses involving 10 parents and in all combinations, VRST-57 x VRST-53 recorded the highest value of -16.36 percent followed by VRST-62 x VRST-61 (-14.55%).

2.3.2 Node at first female flower

Kadam *et al.* (1995) reported significant heterobeltiosis of -25.17 per cent and standard heterosis of -25.45 per cent in P8 x P9 for the first female flowering node. The F_1 mean ranged from 5.00 (LA43 x LA 87) to 8.50 percent (LA 87 x LA 86) for this trait (Mole, 2000). Similarly, Mole *et al.* (2001) observed highly significant heterosis of -41.36 per cent over better parent and of -42.26 per cent over standard check in LA 43 x LA 37 for this trait.

2.3.3 Number of fruits per plant

Abusaleha and Dutta (1994) reported heterobeltiosis in the range of 3.67 to 93.96 per cent for number of fruits per vine in ridge gourd. Kadam *et al.* (1995) observed significant heterosis over better parent and top parent for this trait. Mole *et al.* (2001) also recorded significant heterobeltiosis and standard heterosis of 75.44 and 120.99 per cent respectively for this trait. Niyaria and Bhalala (2001) also reported the heterobeltiosis of 55.48 per cent in PRG-7 x JRG-5 cross which was 154.63 per cent increase over standard check for number of fruits per plant. The extent of heterosis for number of fruits per plant varied from 0.38 to 75.86 percent over the better parent and 0.04 to 23.35 percent over top parent BRG3-1 (Hedau and Sirohi, 2004). They reported that the F_1 hybrid, CHRG-1 x AAUJ-3

exhibited maximum heterosis of 75.86 percent over better parent and DRG-1 x AAUJ-3 gave 23.35 percent heterosis over top parent.

2.3.4 Average fruit weight

Abusaleha and Dutta (1994) revealed heterobeltiosis for average fruit weight in the range of 3.67 to 3.96 per cent. Kadam *et al.* (1995) reported maximum of 51.58 per cent heterosis over better parent for this trait. Mole *et al.* (2001) also reported 19.96 per cent heterobeltiosis in LA 43 x LA 44 cross for this trait. Niyaria and Bhalala (2001) reported the heterosis in the range of -38.3 to 30.39 per cent over better parent and the stand over the check heterosis in the range of -35.35 to 13.7 per cent. Hedau and Sirohi (2004) reported that the heterosis for fruit weight varied from 1.20 to 16.15 per cent over better parent. Out of 45 crosses, 13 were found to be heterotic over better parent and only one cross was noticed superior than top parent, Pusa Nasdar. The foremost F_1 hybrid KRG-5 x AAUJ-3 exhibited maximum heterosis (16.15%) over better parent and a single hybrid DRG-1 x Pusa Nasdar showed heterosis (2.57%) over top parent.

2.3.5 Fruit length

Abusaleha and Dutta (1994) reported heterobeltiosis for average fruit length which ranged from 3.6 to 36.1 per cent. Kadam *et al.* (1995) and Mole *et al.* (2001) also reported heterobeltiosis to the extent of 7.05 per cent and 15.56 per cent respectively. However, none of the hybrids recorded significant standard heterosis for average fruit length in ridge gourd (Mole *et al.*, 2001). In respect of fruit length, six crosses exhibited heterobeltiosis and only single cross (DRG-1 X Pusa Nasdar) showed heterosis (8.16 %) over top parent Pusa Nasdar (Hedau and Sirohi, 2004). Significant heterosis values were reported in 14 cross combinations of Satputia, the cross combinations VRST-2 X VRST-66 (115.79 %) and VRST-2 X VRST-16 (100 %) recorded the highest heterosis values (Ram *et al.*, 2004).

2.3.6 Fruit girth

Abusaleha and Dutta (1994) reported heterosis in the range of 1.76 to 30.16 per cent over better parent for the average fruit girth in ridge gourd. In another study, best cross showed maximum of 9.69 per cent heterobeltiosis (Kadam *et al.*, 1995). The maximum and significant heterobeltiosis and standard heterosis were reported in LA 81 X LA 44 cross of ridge gourd by Mole *et al.* (2001). Niyaria and Bhalala (2001) observed heterosis in the range of -35. 35 to 13.70 per cent over standard check . Hedau and Sirohi (2004) reported the highest and positive heterosis over better parent for fruit diameter in the cross KRG-5 X AAUJ-3 of ridge gourd.

2.3.7 Days to first harvest

Earliness, one of the most important parameter in hybrids, is indicated by number of days for first fruit harvest. The F_1 mean ranged from 50.94 (LA 87 x LA 86) to 53.44 (LA 43 x LA 86) for this trait (Mole, 2000). Among 45 crosses, 18 and 12 crosses were noted superiors than their respective better and top parents for the traits (Hedau and Sirohi, 2004). They also reported that the best F_1 hybrid CHRG-1 X KRG-5 exhibited -13.03 and -9.67 percent heterosis over better and top parent (DRG-2). Ram *et al.* (2004) reported significant heterosis in 12 cross combinations of *L. hermaphrodita* for days to first harvest. The highest values of heterosis were observed in the cross, VRST-2 X VRST-66 (-16.2%) followed by VRST-2 X VRST-53 with heterosis value of -10.99 percent.

2.3.8 Yield per plant

Kolhe (1972) reported maximum of 51.2 per cent heterobeltiosis in cross B-24 X M-33 of ridge gourd for yield per plant. Heterosis for fruit yield per plant in the range of 23.70 to 85.00 pre cent over better parent was reported by Abusaleha and Dutta (1994). Significant heterobeltiosis of 72.94 per cent for total yield per plant was reported by Kadam *et al.* (1995). Mole (2000) reported that the F_1 means ranged from 1359.14 g (LA 43 x LA 86) to 2838.33 g (LA 81 x LA 44). Mole *et al.* (2001) also reported significant heterosis for fruit yield per plant over

better parent and standard parent. The range of heterosis for fruit yield per plant varied from 2.05 to 93.09 per cent and 2.49 to 93.09 per cent over better and top parent respectively. It was in the range of -46.05 to 67.88 per cent over better parent and of -18.11 to 121.50 per cent over standard check in the studies of Niyaria and Bhalala (2001). The three best performing F_1 hybrids for yield were DRG-1 X Pusa Nasdar, DRG-1 X PRG-7 and DRG-1 X AAUJ-3 with heterosis value of 93.09, 68.51 and 66.50 per cent respectively over top parent DRG-1 (Hedau and Sirohi, 2004). Ram *et al.* (2004) observed that 13 cross combinations of Sathputia had significant heterosis values and crosses, VRST-2 X VRST-16 (121.74%) and VRST-62 X VRST-16 (108.26%) recorded the highest values. There is no separate gene system for yield *per se* and the yield is an end product of the multiplicative interaction between various yield components.

2.4 Male sterility

Male sterility is defined as the failure of plants to produce functional anthers, pollen or male gametes (Kaul, 1988). Nevertheless, crops like chilli and musk melon present a very successful example of utilization of male sterility system in India (Kalloo *et al.,* 1998). Several compiled reports (Table 1 and 2) on availability, characterization, mechanism and utilization aspects of male sterility in vegetable crops are available (Kalloo, 1988; Kalloo and Berg, 1993)

2.4.1 Classifications of male sterility

Kaul (1988) has classified male sterility in two major groups, *viz.* genetic (spontaneous or induced) and non-genetic (induced) male sterility. On phenotypic basis, genetic male sterility has been classified in three classes *ie.* sporogenous, structural and functional. Similarly, non-genetic male sterility has been classified as chemical, physiological and ecological male sterility. Further, on genotypic basis genetic male sterility was grouped as genic, cytoplasmic and genic-cytoplasmic male sterility.

Table 1 An overview of important reports on GMS in some vegetable crops

Vegetable spp.	References	Remark
Tomato (*Lycopersicon esculentum* Mill.)	Rick, 1944; Georgiev, 1991; Karbinskaya *et al.*, 1985; Kaul, 1988; Sawhney, 1997; Masuda *et al.*, 1998.	More than40 male sterile alleles have been reported. Functional sterility also reported. *sl*-2, *ms*-13 and *ms*-15 are temperature sensitive. Several *ms* alleles have been reported to be linked with seedling marker gene.
Brinjal (*Solanum melongena* L.)	Jasmin, 1954; Nuttall, 1963; Chauhan, 1986; Kaul, 1988; Phatak and Jaworski, 1989.	Two recessive genes controlling male sterility have been reported. Functional sterility is available.
Chilli/bell pepper (*Capsicum annuum*L.)	Daskalov, 1971; 1972; Shifriss and Frankel, 1969; Shifriss and Rylsky, 1972; Shifriss, 1973; Meshram and Narkhede, 1982; Kaul, 1988; Daskalov and Poulos, 1994; Shifriss, 1997.	More than 12 recessive male sterility alleles are reported to be highly stable. The MS12 (*ms*-509) and *ms*-3, are commercially utilized in India and Hungry, respectively.
Cauliflower (*Brassica oleracea* var. *botrytis*)	Nieuwhof, 1961; Kaul, 1988.	Dominant alleles for male sterility also reported.
Cabbage (*Brassica oleracea* var. *capitata*)	Nieuwhof, 1961; Kaul, 1988; Fang *et al.*, 1997.	Dominant alleles for male sterility also reported.
Broccoli (*Brassica oleracea* var. *italica*)	Cole, 1957; Dickson, 1970; Kaul, 1988.	Six recessive male sterility alleles have been reported. Linkage of *ms* gene with bright green hypocotyle has been reported.
Watermelon (*Citrullus lanatus* Schrad)	Watts, 1962; Kaul, 1988; Zhang *et al.*, 1996.	Linkage of *ms* gene with delayed-green seedling marker gene (*dg*) has been reported.

Muskmelon (*Cucumis melo* L.)	Bohn and Whitaker, 1949; Bohn and Principe, 1964; Pitrat, 1990; McCreight and Elmstrom, 1984; Kaul, 1988; McCreight, 1983.	Five recessive male sterile alleles have been reported. The *ms-1* is commercially utilized in India.
Cucumber (*Cucumis sativus* L.)	Barnes, 1961; Kaul, 1988.	Limited scope to utilize male sterility due to availability of gynoecious lines.
Chive (*A. schoenoprasum* L.)	Kaul, 1988; Engelke and Tatlioglu, 1996.	Male sterility genes have been studied to test allelism with maintainer gene of sterile cytoplasm.

Table 2 An overview of important reports on CMS in some vegetable crops

Vegetable spp.	References	Remark
Tomato (*Lycopersicon esculentum* Mill.)	Kaul, 1988; Melchers *et al.*, 1992; Petrova *et al.*, 1999.	Restorer allele has not been reported. Sterile cytoplasm has been derived from the distinct species.
Chilli/bell pepper (*Capsicum annuum*L.)	Peterson, 1958; Shifriss and Frankel, 1971; Novak *et al.*, 1971; Kaul, 1988; Csillery, 1989; Yoo, 1990; Shifriss, 1997.	Sterile cytoplasm has been derived from three sources, but all were found identical and majority of them is temperature sensitive. Occurrence of *R* alleles is common in hot type and *r* in sweet type lines.
Onion (*Allium ceepa* L.)	Jones and Emsweller, 1936; Jones and Clarke, 1943; Devis, 1957; Cohan and Weigle, 1966; Meer and Bennekom, 1969; 1971; Kaul, 1988.	Two sterile cytoplasms (S and T) have been reported. S-cytoplasm is widely used to produce hybrids.
Cole crops (*Brassica oleracea* L.)	Pearson, 1972; Bannerot *et al.*, 1974; McCollum, 1981; Hoser-Krause and Antosik, 1987; Kaul, 1988; Crisp and Tapsell, 1993; Pelleriter *et al.*, 1995; Sigareva and Earle, 1997.	Sterile cytoplasm has been incorporated from *B. nigra* and Ogura of radish. Cybrid CMS is being utilized by several seed companies in France to produce hybrids.

Chive (*A.schoenoprasum* L.)	Kaul, 1988; Engelke and Tatlioglu, 1996.	GMS allele has been found non-allelic to maintainer allele of CMS.
Corrot (*Daucus carota* L.)	Welch and Grimball, 1947; Thompson, 1961; Banga *et al.*, 1964; Erickson and Peterson, 1979; Kaul, 1988; Morelock *et al.*, 1996.	Two types of CMS *viz.*, petaloid and brown anthers have been reported. Genetics of restoration is complex.
Radish (*Raphanus sativus* L.)	Ogura, 1968; Kaul, 1988; Zhou and Zhang, 1994; Yamagishi and Terachi, 1994 a, b; 1996; Su *et al.*, 1995; Hawaldar *et al.*, 1997; Yamagishi, 1998.	Sterile cytoplasm is widely distributed in wild radish. Occurrence of *R* alleles is frequent in European and Chinese cultivars and *r* in Japanese cultivars.

Table 3 Overview on reports on male sterility in cucurbitaceous crops

Crop	Reference	Remark
Ridge gourd (*Luffa acutangula*)	Deshpande *et al.*, 1979; Pradeepkumar *et al.*, 2007.	Male sterile mutant reported with rudimentary male buds in racemes which fail to open. No noticeable difference between male sterile and normal except in male inflorescence and male flower. No fruit set on selfing but fruit sets when fertilized with pollen from a fertile pollen parent. Genetics of male sterility is unclear.
Water melon (*Citrullus lanatus*)	Watts, 1962; Kaul, 1988; Dyutin and Sokolov, 1990; Zhang and Wang, 1990; Zhang *et al.*, 1996; Xun *et al.*, 1998; Rhodes and Zhang, 1999; Bang *et al.*., 2005; Haihé *et al.*, 2006; Yinhua and Ping, 2006.	Male sterility controlled by a pair of recessive genes. Pollen aborted completely in male sterile plants. In male sterile flower buds, the IAA and GA content were lower whereas zeatin riboside content was higher. Male sterility gene was successfully transferred by Agrobacterium-mediated transformation. Male sterile mutant expressed unique morphological features. Linkage of *ms* gene with delayed-green seedling marker gene (*dg*) has been

		reported.
Musk melon (*Cucumis melo*)	Bohn and Whitaker, 1949; Bohn and Principe, 1964; Nandpuri *et al.*, 1982; Mc Creight and Elmstorm, 1984; Lecouvior *et al.*, 1990; Park and Crosby., 2004;	Five male sterile genes *viz.*, *ms*-1, *ms*-2, *ms*-3, *ms*-4 and *ms*-5 were reported. *ms*-1 characterized by indehiscent anthers with empty pollen walls in tetrads. In *ms*-2 anthers were indehiscent, containing mostly empty pollen walls. *ms*-3 found superior to *ms*-1 and *ms*-2 being very easy to identify and possessing superior horticultural traits. In *ms*-5 lines, male flower buds abort prematurely. Sterility in all these male sterile mutants was controlled by monogenic recessive gene action. Magnitude of heterosis and combining ability also assessed. Punjab Hybrid, a F1 hybrid between *ms*-1 x Hara Madhu was released in Punjab state and subsequently at the national level in 1985.
Squash (*Cucurbita pepo*)	Eisa and Munger, 1968; Kaul, 1988; Dyutin and Puchkov, 1996; Carle, 1997; Dyutin *et al.*, 2007	Sterility is governed by a single recessive nuclear gene, designated by *s*2. Male flower abort before anthesis. The mutant crosses readily with other fertile lines and can be successfully utilized in heterosis breeding programme. Recessive alleles *ms*-1 and *ms*-2 reported to govern male sterility. Male flowers abort before anthesis. Limited scope to utilize male sterility due to availability of gynoecious lines.
Cucumber (*Cucumis sativus*)	Barnes, 1961; Kaul, 1988; Litinskaya *et al.*, 1998;	Recessive alleles *ms*-1 and *ms*-2 reported to govern male sterility. Male flowers abort before anthesis. Limited scope to utilize male sterility due to availability of gynoecious lines

Based on the location of gene(s) controlling genetic male sterility, spontaneously isolated, artificially induced through mutagenesis, artificially incorporated through protoplast fusion or genetically engineered male sterility systems can be classified as (i) Genic male sterility (GMS; more appropriately nuclear male sterility) and (ii) Cytoplasmic male sterility (CMS; more appropriately nuclear-cytoplasmic male sterility). In most of cytoplasmic male sterile plants, pollen fertility is usually restored by certain dominant nuclear allele (called restorer gene; *R* allele). Hence, the expression of male sterility in such cases is the result of incompatibility between nuclear allele *r* and cytoplasmic genome. Therefore, those cytoplasmic male sterile lines for which restorer allele(s) have been identified, are widely known as genic-cytoplasmic male sterility (G-CMS) and more often treated as a separate class of male sterility system. However, in both the CMS and G-CMS, location of male sterility gene(s) is mitochondrial genome (*mt*-genome) and it is the nuclear dominant restorer allele (*R*), which restores fertility in the cytoplasm.

2.4.2 Male sterility in Cucurbits

Cucurbits form an important and a big group of vegetable crops cultivated extensively in this country. There are several reports on the mechanism of male sterility in cucurbitaceous crops which are reviewed as under (Table 3).

2.4.3 Male sterility in ridge gourd

Male sterility in ridge gourd was first reported by Deshpande *et al.* (1979). They reported that there were no observable differences in germination and vegetative growth between male sterile plant and normal plants except in inflorescence and male flower. Buds failed to open and had rudimentary androecium, shriveled and ill-developed anthers. They also reported that no viable pollen was developed by the mutant. Similarly male sterility in this crop was also reported by Pradeepkumar *et al.* (2008). They reported that no fruit formed on selfing but there was fruiting when the male sterile mutant is pollinated using

pollen from a fertile plant. They also standardized the media for *in vitro* regeneration and maintenance of male sterile ridge gourd plant.

2.4.4 Inheritance of male sterility in cucurbits

Many workers reported the presence of male sterility in cucurbitaceous crops and they also reported the inheritance pattern. These reports are reviewed hereunder.

A male sterile mutant was discovered in the muskmelon (Bohn and Principe, 1964). The progeny tests demonstrated that the male sterility in musk melon, LJ 40460, is governed by a single recessive gene. They also reported that the two genes (*ms 1* and *ms 2*) governing male sterility in musk melon inherited independently from each other, or, if linked, only loosely so. Similarly, Lecouviour *et al.* (1990) also reported the inheritance pattern of male sterile genes in musk melon (*ms 5*). The F_1 hybrids between male sterile and male fertile plants were fertile and in F_2 progenies these segregated in the ratio of 3:1 (fertile: sterile). This result also supports the monogenic recessive inheritance of male sterility in musk melon.

Pollen aborted completely in male sterile plants. Zhang and Wang (1990) found two male sterile watermelon plants with small, shrunken anthers and aborted pollen, from among the selfed progeny of cv. Nongmi No. 100. The study for inheritance pattern of this trait revealed monogenic recessive control of male sterility. The segregation ratios obtained from F_1 and BC_1 F_2 population suggested that the male sterile and delayed green traits are inherited independently and that delayed-green is inherited as a single recessive nuclear gene (Zhang *et al.*, 1996). Preliminary studies of a dwarf, male sterile watermelon (DMSW) showed that male sterility was controlled by a pair of recessive nuclear genes (Xun *et al.*, 1998).

In summer squash of marrow and custard types, Dyutin and Puchkov (1996) reported male sterility and its inheritance was governed by single recessive nuclear gene. Carle (1997) studied F_8 plants of *Cucurbita pepo* line YSN531PMR and reported that one third of plants were male sterile with a rudimentary androecium. The analysis of F_8 and F_9 data indicated that this form of sterility is controlled by a single recessive gene, designated by *s2*. Dyutin *et al.* (2007) discovered male sterile plants in summer squash. They crossed these with fertile ones and the study over F_1, F_2 and back cross progenies revealed that the trait is governed by single recessive gene.

2.4.5 Genetics of male sterility in cucurbits

Five single recessive genes for male sterility including *ms-1* to *ms-5* have been identified in musk melon (Bohn and Whitaker, 1949; Bohn and Principe, 1964; McCreight and Elmstorm, 1984; Lecouviour *et al.* 1990; Pitrat, 1991, 2002). Genetic studies of male sterility in musk melon indicated that the locus of *ms-2* is different from that of *ms-1* and the two genes were probably not linked. No allelism was found between different male sterile genes (Bohn and Principe, 1964; Lecouviour *et al.,* 1990; McCreight and Elmstorm, 1984). The results were confirmed by Pitrat (1991), who reported that these sterility genes were located on five different linkage groups (LGs) of classical melon map. McCreight (1983) and Pitrat (1991) reported loose linkages between red stem (*r*) and the *ms-1* gene, and between yellow green leaves (*yg*) and the *ms-2* gene, respectively.

Analysis of data of F_1 hybrid between watermelon lines containing the single recessive gene (*ms*) and glabrous male sterile (*gms*) revealed that *ms* and *gms* were non allelic (Murdock *et al.*, 1990).

2.4.6 Phenotypic expression of male sterile mutants in cucurbits

Musk melon plants homozygous for *ms-2* were slightly retarded in growth rate; they often flowered later than their normal sibs (Bohn and Principe, 1964).

Staminate flowers were normal in size, shape and all parts except anthers. They were small and failed to dehisce. contained empty microspores.

Zhang and Wang (1990) reported a GMS watermelon line G17AB, which showed no major morphological differences from male fertile plants; flowers were small, opened late and had shrunken inviable pollen. A new male sterile mutant indentified by Bang *et al.* (2005) showed multiple unique morphological features. The number of leaf lobes of the mutant was much fewer than normal plants. Seedlings appeared to grow much slower and had a spindly appearance compared to their non-male sterile counterparts. The male sterile mutants also had much longer internodes than non-male sterile plants, and the growth rate of the male sterile mutants appeared to be much slower than normal segregants. The leaf lobing was much less on mutant plants and appeared to be lesser than that was reported for the dwarf male-sterile watermelon (ms-dw). The curvature of the leaf was also more convex compared to non-male sterile plants. The stem above the first node exhibited a mild fasciation in mature plants, which gradually returned to the normal angular stem above the second node. Microscopic evaluation of anthers from the male sterile mutants revealed that the pollen sac did not dehisce. The female flowering pattern appeared normal.

Male sterility was observed in ridge gourd for the first time by Deshpande *et al.* (1979) in one of the local accession at University of Agricultural Sciences, Dharwad. They reported no noticeable differences in germination and vegetative growth between male sterile plant and normal plant except the male flower. The inflorescence was observed to be normal but male buds, which were slightly smaller than the normal one, failed to open. No viable pollen produced in the male sterile mutant. Similarly, Pradeepkumar *et al.* (2008) reported male sterile mutant in the crop. Rudimentary male buds were produced 70-75 days after germination and no fruit was observed on selfing. However, fruit set was observed when pollination was carried out with male flower of monoecious variety 'Haritham'. Anther lobes of the male sterile mutant were more flat and pubescent when

compared with normal male fertile plant. The microspores were small, shrunken and sterile.

2.4.7 Cytological expression of male sterile mutants in cucurbits

Meiosis and pollen development were observed in male sterile mutant of musk melon by Bohn and Principe (1964) in acetocarmine and acid fusion lactophenol smears. Meiosis appeared to be normal in early stages. No laggards or bridges were observed. The diad cells were often odd-shaped rather spherical. Anaphase and telophase II proceeded normally without laggards. Spindle orientation at second meiotic metaphase was irregular. Microspore cell size, shape and spatial arrangement were also irregular. Disorganization of cell contents occurred shortly after the formation of microspore cells.

The cytological study of a spontaneous male sterile mutant of watermelon revealed that the male sterility was expressed in androecium development, disrupting male-flower function. Female fertility of the male-sterile phenotype was normal. Comparative histological analysis between sterile (*msms*) and fertile (*MsMs*, *Msms*) genotypes showed that the lack of tapetum and the abnormal deposition of fluorescing proteins precluded meiocyte development after telophase II and caused degeneration of meiocytes in sterile anthers. The ms mutation altered the pattern of differentiation of anther wall and resulted in the persistence of five to seven cell layers of locule wall throughout meiosis (Zhang *et al.*, 1994).

2.4.8 Markers linked with male sterility in cucurbits

2.4.8.1 Morphological markers

Hybrid seed production can be facilitated by using male sterility coupled with a seedling marker. Zhang *et al.* (1996) reported male sterile mutant of watermelon with yellow cotyledons and pale-green, newly developed, true leaves. Bang *et al.* (2005) reported a new male sterile mutant in watermelon with multiple unique morphological features. The number of leaf lobes of the mutant was much

fewer than normal plants. Seedlings appeared to grow much slower and had a spindly appearance compared to their non-male sterile counterparts. The male sterile mutants also had much longer internodes than non-male sterile plants, and the growth rate of the male sterile mutants appeared to be much slower than normal segregants. The leaf lobing was much less on mutant plants and appeared to be less than that reported for the dwarf male-sterile watermelon (*ms-dw*). The curvature of the leaf was also more convex compared to non-male sterile plants. The stem above the first node exhibited a mild fasciation in mature plants, which gradually returned to the normal angular stem above the second node.

2.4.8.2 Molecular markers

Molecular marker studies using near-isogenic lines (NILs) or bulked seggregant analysis (BSA) accelerated the mapping of many genes in different plant species (Staub *et al.*, 1996). Molecular markers linked to male sterile gene would be useful in transferring male sterile genes to elite cultivars and breeding lines. Marker assisted breeding is now at the verge of becoming a standard application in modern plant breeding. Park and Crosby (2004) identified RAPD markers linked to *ms-3* gene controlling male sterility using bulked seggregant analysis (BSA) in an F_2 population from the melon cross of line *ms-3* (male sterile) x TAM Dulce (male fertile). RAPD marker OAM08.650 was closely linked to the *ms-3* gene at 2.1cM. SCAR marker SOAM08.644 was developed on the basis of the specific primer designed from the sequence of RAPD marker OAM08.650. Park and Crosby (2004) were the first to report development of SCAR marker linked to *ms-3* gene in melon. The linked RAPD and SCAR markers were confirmed in F2 populations from the cross of line ms-3 x Mission to be consistently linked to the *ms-3* gene at 5.2cM. These markers were also present in 22 heterozygous fertile F_1 plants having *ms-3* gene. They also reported that these markers could be utilized for backcrossing of male sterility into elite melon cultivars and lines for use as parents of F_1 hybrid seed production.

2.5 *In Vitro* maintenance

In vitro multiplication of elite clones will be an attractive approach in order to meet the requirement of quality propagules at large scale for commercial cultivation. Development of efficient and reproducible regeneration protocol from cells/tissues is a pre-requisite for the successful application of recent cellular manipulation techniques for the improvement of crop plants.

2.5.1. Cucurbits

Jain and More (1992) regenerated *Cucumis melo* c.v. Pusa Madhuras *in vitro* and reported that hormones such as IAA (1.0 mg/l) + Kinetin (5.0 mg/l) with MS basal medium are highly effective in inducing regeneration of shoot buds in epicotyl explant callus obtained on MS + BAP (0.5 mg/l) medium, whereas GA3 (0.5 mg/l) with MS basal medium is effective for inducing regeneration in cotyledonary leaf explant callus obtained on MS + BAP (0.5 mg/l) medium.

Sapountzakis and Tsaftaris (1994) reported that the presence of auxin (NAA) in the culture media decreased the shoot propagation rate of cucumber hybrids 'Brunex and 'Bambina'. The cytokinin (BA) level in the culture media affected the number of shoots produced. The optimum level was 0.5 mg/l and 1.0 mg/l for the hybrids 'Brunex' and 'Bambina' respectively. They also reported that the presence of gibberellin (GA_3) in high levels (5.0-10 mg/l) increased the number of shoots produced. In muskmelon Spetsidis *et al.* (1996) reported that the presence of cytokinin (BA) in the culture media increased the shoot proliferation rate, and the optimum level was 2.0 mg/l. Mythili and Thomas (1999) developed protocol for rapid *in-vitro* multiplication of 2 female cultivars (Swarna Alaukik and Swarna Rekha) and one male line of pointed gourd by culturing shoot tip and nodal explants on Murashige and Skoog (MS) medium containing IAA (1.0 µM) and IBA (0.2 µM). Single shoots with short internodes (1.0-1.15 cm) accompanied by rooting were obtained in all genotypes within four weeks. In a study on improving culture efficiency of *Cucumis metuliferus* protoplasts, McCarthy *et al.* (2001) reported that the densities between 1×10^3 and 1×10^4

protoplasts per ml with agarose nurse culture was an efficient technique for both *C. metuliferus* and *C. sativus* protoplast isolation and culture. The importance of weekly replenishment of limiting nutrients and plant-growth regulators should not be ignored as a possible advantage of agarose over liquid culture. In dioecious vegetable crops like pointed gourd, tissue culture technique has proved useful for rapid multiplication of superior types.

2.5.2. Ridge gourd

Protocol for the clonal propagation is standardized by Pradeepkumar *et al.* (2008). MS medium supplemented with BA 0.5 mg/l and MS medium fortified with IAA 1.5 mg/l and BA 2 mg/l were used for inoculating nodal cuttings of male sterile lines from field. MS + BA 1 mg/l was the promising establishment media reported by them. The *in vitro* shoots were successfully rooted in MS medium (half strength) fortified with IBA 1 mg/l and charcoal 200 mg/l.

2.6 Exploitation of male sterility for commercial hybrid seed production in vegetables

Male sterility is an important component and is being widely used in hybrid seed production of vegetables. High cost of hybrid seeds is attributed to labour involved for emasculation and pollination processes. Emasculation can be avoided and labour saved if male sterile line is used as the female parent (Dhaliwal and Cheema, 2008). Among the available sources of male sterility, cytoplasmic male sterility (CMS) system is commonly used. However, in the event of non-availability of CMS source, efforts are being made to use genic male sterility (GMS) with a condition to remove male fertile plants at an appropriate time (Dhatt and Gill, 2000). The reports on commercial utilization of male sterility in hybrid seed production of vegetables are reviewed hereunder.

2.6.1 Genic male sterility

Genetic male sterility has huge potential for hybrid seed production. Five recessive nuclear genes (*ms-1, ms-2, ms-3, ms-4, ms-5*) are already known. The

genetic males sterility gene *ms-1* was introduced to India more than 25 years ago and is in use in hybrid seed production in muskmelon (Nandpuri *et al.,* 1982). The development of first commercial hybrid muskmelon cultivar Punjab Hybrid (ms 1 x Hara Madhu) is one such example. Genetically Hara Madhu is *Ms 1 Ms 1* and the sterile plants are *ms 1 ms 1* and thus the hybrid seed produced is *Ms 1 ms1* which is fully fertile. In Punjab, at many places even farmers are producing hybrid seeds utilizing *ms-1* male sterile line (Kalloo *et al.,* 1998). Despite the complex maintenance process and additional labour requirement to remove fertile segregants in hybrid seed production field, production of male sterile based hybrid seeds is economical than the seeds produced by manual emasculation (Kumar *et al.,* 2000).

More than 55 male sterile (*ms*) alleles causing sporogenous, structural and functional sterility have been reported (Kaul, 1988). A report in tomato indicated that cost price of hybrid seed was reduced by 20 per cent when *ms-10* was used as d female parent. The *ms* gene in this line is linked with a recessive marker gene (*a*) responsible for absence of anthocyanin . Hence, *ms-10* sterile plant can be identified at seedling stage and fertile plant can be rouged out in the nursery itself (Georgiev, 1991). Dhaliwal and Cheema (2008), in tomato, reported that 54.4 per cent time required for hybrid seed production can be saved if male sterile line is used as a female parent. This clearly indicates the potential of exploiting male sterility in tomato.

Most of the previously reported GMS lines were in the sweet pepper (bell pepper). However, recently incorporation of male sterility gene has also been accomplished in the hot types (chilli) and is being utilized to produce hybrid seeds economically (Kalloo, 1998). The *ms-509* line (bell pepper type) of Dr. Pochard was introduced in India at Punjab Agricultural University and recessive male sterility allele was introgressed in three chilli genotypes *viz.,* MS 12, MS 13 and MS 41 (Singh and Kaur, 1986). All the reported *ms* alleles were highly stable, hence found to be promising for hybrid seed production.

Carrot is one of the few species in which male sterility was documented very early (Kaul, 1988). Welch and Grimball (1947) reported isolation of genic male sterile plant within the commercial variety Tendersweet.

2.6.2 Cytoplasmic male sterility

In carrot, two types of sterile cytoplasm have been reported *viz.,* petaloid and brown anther (Welch and Grimball, 1947; Morelock, 1974). Taki seed Company of Japan developed the first F_1 hybrid variety in 1982 using CMS (Pelleriter *et al.,* 1995). In USA, vast majorities of hybrids are produced from one cytoplasm *i.e.,* Cornell cytoplasm (Morelock *et al.,* 1996).

Worldwide more than 50% onion varieties currently cultivated are F1 hybrids derived from S-cytoplasm (Pelleriter *et al.,* 1995). In India, public sector bred commercial hybrid has not been recommended so far, however, in few areas farmers are cultivating male sterile based hybrids developed by seed companies (Kumar *et al.,* 2000).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study was conducted in the vegetable research farm of the Department of Olericulture and male sterile line was maintained under *in vitro* condition at Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agricultural University, Vellanikkara which is located at an altitude of 23 m above MSL and between 10° 32'' and 76° 16'' East longitude. The experiment was conducted in two seasons, May to August 2008 and January to April 2009. Important weather parameters during the cropping season is given in the Annexure I

The project consisted of the experiments written hereunder.

3.1 *In vitro* maintenance and regeneration of male sterile genotype of ridge gourd and evaluation for stability of male sterility

3.2 Collection and evaluation of ridge gourd accessions from different parts of the country and hybridization of selected pollen parents with male sterile genotype

3.3 Evaluation of F_1s along with male sterile female parent and male fertile parents and studying the expression of male sterility in different genotypic combinations

3.1 *In vitro* maintenance and regeneration of male sterile plants and evaluation for stability of male sterility

The protocol for the clonal propagation of male sterile ridge gourd plant as standardized by Pradeepkumar *et al.* (2008) was followed for maintenance and generation of male sterile plants in the experiment. *In vitro* regeneration and multiplication of male sterile plant was undertaken in tissue culture laboratory of

Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agricultural University, Vellanikkara.

Single noded cuttings (2 cm) of the field grown male sterile plants were taken as explants for the *in vitro* regeneration. Explants were washed with tap water for 10 minutes, kept in 0.1% Bavistin solution for 20 minutes and rinsed in distilled water for 3 minutes. Surface sterilization was done using $HgCl_2$ (0.05%) for 10 minutes followed by washing in sterile water for 3-4 minutes in laminar air flow chamber. The explants were inoculated in MS medium fortified with IAA (1.5 mg l^{-1}) and BA (2 mg l^{-1}). Cuttings (2-3 nodes) from *in vitro* shoots were used for inoculation in the multiplication medium (MS + 1 mg l^{-1} BA). The shoots from multiplication stage were used for rooting in MS medium (half strength) fortified with IBA (1 mg l^{-1}) and charcoal (200 mg l^{-1}). The rooted plants were transferred to polybags with sterile sand and kept in mist chamber for hardening. After one month these plants were transferred to field.

Male flower buds of 2 mm size were collected at 12.00 to 12.30 pm for cytological analysis. The study was conducted as suggested by Sain *et al.* (2002). For meiotic studies, the collected male flower buds were fixed at room temperature in Carnoy's fluid (1 part acetic acid : 3 part chloroform : 6 part ethyl alcohol) with a drop of ferric chloride (5%). Buds were fixed for 24 hrs and then transferred to alcohol (70%) for storage until the studies were made by the acetocarmine (1%) squash technique. The slides were prepared, sealed and screened for different stages of meiosis under Image Analyzing System (Digi Pro 2).

Pollen fertility of the male sterile line was assessed throughout the flowering season to study the stability of male sterility. The observations were recorded from 10 randomly selected male buds. Pollen grains were collected from matured male flower buds were used for pollen studies. Anthesis time in ridge gourd is in the evening, which starts at 4.00 pm and continues upto 7.30 pm. The

pollen grains for study were collected at the time of anthesis. Delayed anthesis was observed on cloudy and rainy days. Fertility was assessed on the basis of stainability of pollen grains in acetocarmine stain. Pollen grains were mounted in a drop of acetocarmine stain on a clean slide. The counts were taken after an hour from ten fields for each male bud. Well filled and uniformly stained pollen grains were considered as fertile and the rest as sterile.

3.2 Collection and evaluation of ridge gourd accessions from different parts of the country and hybridization with male sterile parent

The experimental materials consisted of the male sterile mutant and fourteen accessions collected from different parts of the country and maintained in the Department of Olericulture, College of Horticulture, Vellanikkara. The genotypes collected were – CO 1, CO 2, Arka Sumeet, Arka Sujat, Deepthi, MUR-RG-VG, Satputia, IC-92685, IC-92671, IC-93393, IC-339224, IC-23247, IC-385911 and IC-392334.

The experiment was laid out in a randomized block design with two replications. There were two pits per replication and two plants per pit. Crop was raised during May to August, 2008. Crop was maintained as per package of practices recommendations (KAU, 2007). Observations were recorded for the assessment of variability in the genotypes.

3.2.1 Observations

Following observations were recorded in both replications on four plants.

Days to emergence of first fertile male flower

The days were counted from the date of sowing to the date of opening of the first male flower.

Days to emergence of first female flower

The days were counted from the date of sowing to the date of opening of the first female flower.

Nodes to first fertile male flower

The nodes were counted from the lowest to the one at which the first fertile male flower emerged.

Nodes to first female flower

The nodes were counted from the lowest to the one at which the first female flower emerged.

Pollen fertility (%)

The pollen grains for the study were collected at the time of anthesis. Fertility was assessed on the basis of stainability of pollen grains in acetocarmine stain. Pollen grains were mounted in a drop of acetocarmine stain on a clean slide. The counts were taken after an hour from ten fields for each male bud. Well filled and uniformly stained pollen grains were considered as fertile and the rest as sterile.

Number of fruits per plant

The total number of fruits produced per plant was observed.

Fruit weight (g)

The weights of five fruits were recorded and average was worked out.

Fruit length (cm)

The lengths of five fruits were recorded from each plant separately after harvest and average was worked out.

Fruit girth (cm)

The girth at the middle of five fruits was recorded separately after harvest and average was worked out.

Days to first harvest

The days were counted from the date of sowing to the date of first harvest.

Number of harvest

The number of harvests was counted from the very first harvest to the last harvest from each plant.

Yield per plant (kg/plant)

The weights of fruits harvested from each plant were recorded separately and the average was worked out.

Number of seeds per fruit

The seeds per fruit were counted in five fruit each and average was worked out.

Seed yield per fruit (g)

The weights of seeds harvested from each fruit were recorded separately and average was worked out.

3.2.3 Estimation of variability among the genotypes

The mean of the values observed on four plants in the two replications were taken for statistical analysis. The data thus obtained were processed for analysis of variance, genotypic and phenotypic variance, genotypic and phenotypic coefficient of variance, genetic advance, genetic gain, genetic divergence, heritability and heterosis.

Phenotypic, genotypic and environmental variance

The variance components were estimated using the formula suggested by Burton (1952).

Phenotypic variance (V_P) = $V_g + V_e$

Where,

V_g – genotypic variance

V_e – environmental variance

Genotypic variance (V_g) = $(V_T - V_E)/N$

Where,

V_T - mean sum of squares due to treatments

V_E - mean sum of squares due to error

N - number of replication

Environmental variance (V_e) = V_E

Phenotypic and genotypic coefficient of variation

The phenotypic and genotypic coefficients of variation were calculated by the formula suggested by Burton and Devane (1953).

Phenotypic coefficient of variation (pcv) = $(V_P^{1/2} / \overline{X}) \times 100$

Where,

V_P = Phenotypic variance

$\overline{X}$ =Mean of character under study

Genotypic coefficient of variation (gcv) = $(V_g^{1/2} / \overline{X}) \times 100$

Where,

V_g = Genotypic variance

$\overline{X}$ = Mean of characters under study

Heritability

Heritability in broad sense is estimated by the formula suggested by Burton and Devane (1953). Heritability in broad sense,

$$H = (V_g / V_p) \times 100$$

Where,

V_g – Genotypic variance

V_p – Phenotypic variance

Expected genetic advance

The genetic advance expected for the genotype at five percent selection pressure was calculated using the formula by Lush (1949) and Johnson *et al.* (1955) with the value of constant K as 2.06 as given by Allard (1960).

Expected genetic advance $GA = (V_g / V_P) \times K$

Where,

V_g – Genotypic variance

V_P – Phenotypic variance

Genetic gain (genetic advance as percentage of mean)

Genetic advance (GA) calculated using the above method was used for the estimation of genetic gain.

Genetic gain (GG) = $(GA/\overline{X}) \times 100$

Where,

GA – Genetic advance

$\overline{X}$ – Mean of character under study

3.2.3 Estimation of genetic divergence among genotypes

The genetic divergence was calculated according to the method suggested by Mahalanobis (1928). Clustering of accessions was done using Tocher's method (Rao, 1952).

3.2.4 Selection of pollen parents and hybridization

Pollen parents were selected from different clusters based on the genetic divergence. The *in vitro* regenerated male sterile female parent is crossed with these selected male parents and hybrid seeds were produced.

Well developed female buds were selected and covered with butter paper bags on the day of opening and tagged. In the same way, the male buds on selected male parents were also covered and tagged. Stigma receptivity is maximum at the time of anthesis (4.00 pm to 7.30 pm) as reported by Deshpande *et al.* (1980). So, at this time, the pollen from covered male flowers were collected and brushed over the covered female flowers of male sterile parent. The crossed female flowers were kept covered for 2 more days till the fruit developed to avoid pollen contamination. The developed fruits were covered with perforated polyethene bags to protect from the fruit fly damage. The hybrid seeds were collected from matured fruits and stored.

3.3 Evaluation of F_1s along with male sterile female parent and male fertile parents

In the second season, the F_1 and the parental lines were grown in four replications in randomized block design. Each replication consisted of one pit and there were two plants per pit. Crop was raised during January to April, 2009. Crop was maintained as per the package of practices recommendations (KAU, 2007). Observations were recorded as in the first season.

3.3.1 Expression of male sterility in hybrids

The expression of male sterility was assessed by evaluating pollen fertility percentage in the hybrids. The observations were recorded from 10 randomly selected male flowers from each hybrid. Pollen grains were collected from flower at the time of anthesis. Fertility was assessed on the basis of stainability of pollen grains in acetocarmine stain. Pollen grains were mounted in a drop of acetocarmine stain on a clean slide. The counts were taken after an hour from ten

fields for each flower. Well filled and uniformly stained pollen grains were considered as fertile and the rest as sterile.

3.3.2 Estimation of heterosis

The mean values of parents and hybrids of those four replications for each character were taken for the estimation of heterosis in terms of three parameters, heterosis over mid parent (relative heterosis, RH), heterosis over the better parent (heterobeltiosis, HB) and heterosis over the standard parent (standard heterosis, SH) and these were worked out as suggested by Briggle (1963) and Hayes *et al.* (1965). For calculation of standard heterosis, the genotype Deepthi was taken as standard parent (SP).

For each character, the average value of the two parents in each cross was taken as the mid parental value (MP) and that of superior parent as better parent (BP) value.

Relative heterosis is estimated over the mid parent (MP) *i.e.,* mean value or average of the two parents.

$$RH = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

Heterobeltiosis is the deviation of hybrid mean from better parent (BP) values.

$$HB = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Heterosis over standard parent (SP), standard heterosis,

$$SH = \frac{\overline{F_1} - \overline{SP}}{\overline{SP}} \times 100$$

To test significance of difference of F_1 mean over mid and better parents, critical difference (CD) was worked out. Critical difference was calculated from the standard error of difference as given bellow (Briggle, 1963).

To test the significance of heterosis

$$CD = te' (0.05) X \sqrt{\frac{2MSe}{r}}$$

Where,

te – Critical value of t statistic at 5% level of significance

MSe – Error mean square

r – Number of replications

SE – Standard error of difference between two means

RESULTS

4. RESULTS

Results obtained from the experiments are presented under the following headings

4.1 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

4.2 Collection and evaluation of ridge gourd accessions from different parts of the country and hybridization of selected pollen parents with male sterile genotype

4.3 Evaluation of F_1s along with male sterile female parent and male fertile parents and studying the expression of male sterility in different genotypic combinations

4.1 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

Maintenance of male sterile line is a major challenge and for genetic dissection of male sterility, it has to be crossed with different pollen parents for identifying fertility restorer genes if any in the natural population. Micropropagation is the only viable means for maintaining this unique source as the genotype can be fixed without any genetic change.

The field grown male sterile plants were established *in vitro* in MS medium fortified with IAA (1 mg l^{-1}) and BAP (2 mg l^{-1}). Single shoots with close nodes were observed (Table 4). About 60% establishment rate was observed (Plate 1a). Cuttings with 2-3 nodes from *in vitro* shoots were inoculated in multiplication medium (MS + BA 1 mg l^{-1}). On an average 4-5 multiple shoots were observed in the medium. (Plate1b). The shoots from multiplication stage were used for rooting

Table 4 Period taken for *in vitro* regeneration and multiplication of male sterile genotype in ridge gourd

Stages in tissue culture	Days taken	Remarks
Establishment	25	Callus formation in the base. Single shoots with close nodes were produced. About 60% establishment rate observed.
Multiplication	35	Callus formation in the base. 4-5 multiple shoots with length of 7.00 cm were formed. Closer nodes were produced
Rooting	35	High amount of rooting. Shoot growth continued.
Hardening	30	Tissue cultured plants transferred to mist house in polybags. 70 % survival in sterilized sand.

in MS medium (half strength) fortified with IBA 1 mg l^{-1} and charcoal 200 mg l^{-1}. High percentage of rooting (95%) and continued shoot growth were observed in this medium (Plate1c). The rooted plantlets were transferred to sterilized sand polybags and kept in mist house for one month (Plate 2). Survival rate during hardening observed to be 70 percent. 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 season. The pollen fertility percent was observed to be zero. There were no observable differences in vegetative or growth characters as compared with other male fertile plants except in male sterility (Plate 3). These plants produced rudimentary male buds in racemes (Plate 4a) when compared to normal male fertile cultivar. The male buds were failed to open and fell down 12 days after they attained the size visible to naked eye. Average bud length at this stage was 8.79 mm whereas the average bud length of fertile male bud prior to the day of opening was 10.04 mm. There was a marked difference in the appearance of anther lobes (Plate 4b) and pollen grains (Plate 4c) of the male sterile plant when compared with the fertile line. 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. Pollen fertility, based on the stainability was found to be zero. Cytological analysis of PMCs (Pollen Mother Cell) revealed normal meiosis (Plate 5a, b, c, d, e). Gradual degradation of microspores was observed in the post meiotic stage (Plate 5f). Female flowers were normal and opened normally. The female fertility was normal and there was fruit setting on pollination with pollen from other selected male fertile parents. But no fruit set observed on selfing. Seed setting also found to be normal.

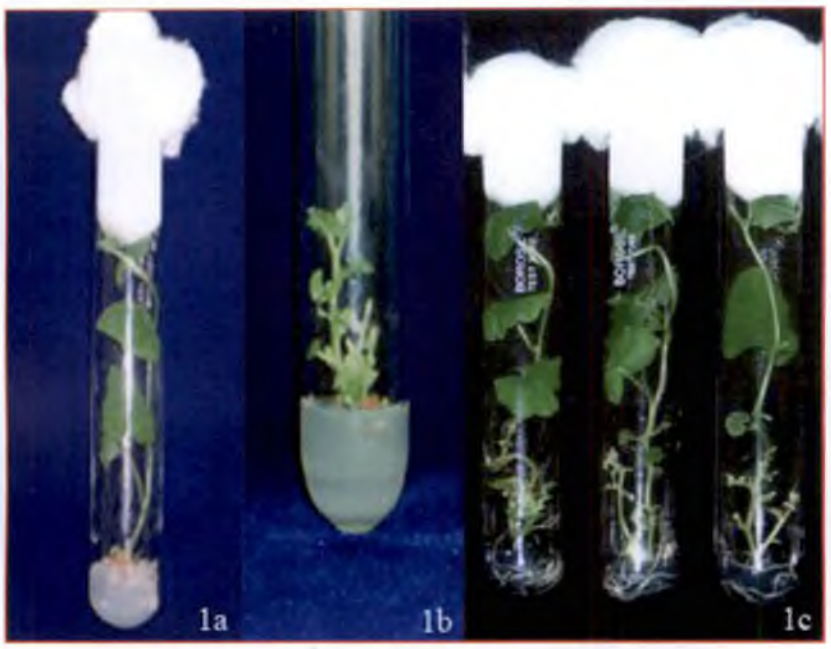

Plate 1 *In vitro* maintenance of mule sterile ridge gourd line

Plate 2 Hardening of *in vitro* rooted plantlets

Plate 3 Male fertile and male sterile plats at the time of anthesis

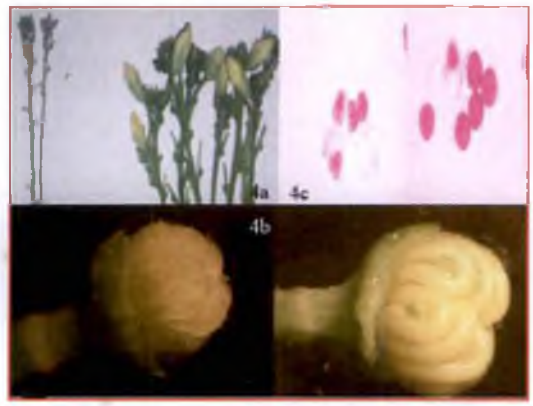

Plate 4 Comparison for male buds, anther lobes and pollen grains in male sterile and fertile lines

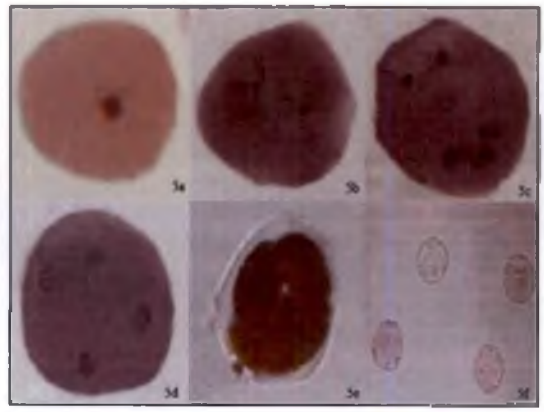

Plate 5 Cytological analysis of meiosis in male sterile line

4.2 Collection and evaluation of ridge gourd accessions from different parts of the country and hybridization of selected pollen parents with male sterile genotype

Fourteen ridge gourd genotypes were collected from different parts of the country *viz.*, CO 2 (Plate 6), CO 1 (Plate 7), Arka Sumeet (Plate 8), Arka Sujat (Plate 9), Deepthi (Plate 10), MUR-RG-VG (Plate 11), IC-92685 (Plate 12), IC-92671 (Plate 13), IC-93393 (Plate 14), IC-339224 (Plate 15), IC-23247 (Plate 16), IC-385911 (Plate 17), Satputia (Plate 18) and IC-392334 (Plate 19). The ridge gourd genotypes were raised in research field of Department of Olericulture (Plate 20). These genotypes were evaluated for gynoecious sex expression and variability.

4.2.1. Estimation of variability among the ridge gourd genotypes

The success of any crop breeding programme purely depends upon the magnitude of genetic variability that exists in the available germplasm. Analysis of variance is given in Table 5. Mean values of all the genotypes for the characters studied are given in Table 6. The values of range, mean, standard deviation, standard error, genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance and genetic gain are given in Table 7.

4.2.1.1 Days to emergence of first fertile male flower

Days to emergence of first fertile male flower among the genotypes studied varied from 28.13 to 45.25 in the first season. The accession Satputia was the first to produce male flower (28.13 days) and CO 2 (45.25 days) produced the flower very late. The genotypic coefficient of variation (gcv) and phenotypic coefficient of variation (pcv) were 11.88 and 12.13 respectively. Heritability was found to be 0.98. Genetic advance and genetic gain were 2.02 and 6.07 correspondingly.

Plate 6 CO 2

Plate 7 CO 1

Plate 8 Arka Sumeet

Plate 9 Arka Sujat

Plate 10 Deepthi

Plate 11 MUR-RG-VG

Plate 12 IC-92685

Plate 13 IC-92671

Plate 14 IC-93393

Plate 15 IC-339224

Plate 16 IC-23247

Plate 17 IC-385911

Plate 18 Satputia

Plate 19 IC-392334

Plate 20 Field layout

Table 5 Analysis of variance for 14 characters in 14 accessions of ridge gourd

Source of variation	*df*	Days to emergence of first fertile male flower	Days to emergence of first female flower	Node to first male flower	Node to first female flower	Pollen fertility (%)	No. of fruits / plant	Fruit wt. (g)	Fruit length (cm)	Fruit girth (cm)	Days to first harvest	No. of harvests	Yield / plant (Kg)	Number of seeds/fruit	Seed yield/fruit (g)
Replication	1	0.002	0.056	0.056	0.143	13.553	1.181	0.08	0.009	0.056	0.009	0.002	0.002	58.58	0.155
Treatment	13	66.094	134.315	19.676	160.209	206.307	331.858	29048.48	854.47	26.732	200.61	1.796	3.495	6905.888	72.508
Error	13	0.113	0.07	0.061	0.052	6.733	0.421	0.931	0.264	0.056	0.115	0.031	0.004	27.518	0.388
$CD_{(0.05)}$		2.231	3.179	1.218	3.471	4.002	4.998	46.736	8.017	1.419	3.885	0.371	0.513	22.833	2.341

Table 6 Mean values of the genotypes

Lines/character	Days for emergence of first fertile male flower	Days for emergence of first female flower	Node at first male flower	Node at first female flower	No. of fruits per plant	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Days for first harvest	No. of harvests	Yield per plant (Kg)	Pollen Fertility (%)	No. of seeds per fruit	Seed yield per fruit (g)
CO 2	45.25	56.50	14.75	32.13	17.00	410.25	73.50	18.25	81.75	4.13	4.91	99.75	196.50	28.23
CO 1	44.13	52.00	11.38	38.63	21.00	393.88	63.63	19.38	66.38	3.88	4.96	96.63	184.00	25.08
Arka Sumeet	38.13	44.00	8.13	17.50	41.88	375.75	57.88	25.13	51.88	5.00	6.04	91.75	171.38	27.67
Arka Sujat	38.50	47.63	8.13	22.13	31.38	425.50	47.50	27.00	68.25	4.50	5.19	90.74	176.75	25.98
Deepthi	29.50	37.50	6.63	14.50	18.50	234.63	28.38	21.38	54.50	3.50	5.15	90.38	184.38	26.52
MUR-RG-VG	31.50	38.38	5.75	18.75	33.38	174.13	18.75	23.38	62.50	5.13	4.19	85.63	118.00	20.58
Satputia	28.13	28.13	5.13	5.13	50.63	110.13	9.75	19.25	50.25	5.63	7.21	79.13	38.50	10.12
IC-92685	30.38	37.88	3.88	12.50	39.00	140.75	10.50	17.00	49.38	6.38	4.54	85.38	44.88	12.68
IC-92671	29.25	35.25	3.25	15.88	38.25	192.25	23.63	17.38	50.50	5.50	7.73	61.00	50.00	14.00
IC-93393	30.50	34.63	5.63	11.88	44.00	136.88	28.13	17.50	49.13	6.00	4.96	76.13	62.13	13.60
IC-339224	29.38	34.50	6.63	10.13	41.25	80.00	10.13	14.13	50.50	4.88	5.19	92.25	67.25	16.51
IC-23247	32.50	31.38	3.50	9.63	25.63	145.13	19.88	16.13	50.38	5.88	3.05	98.38	85.25	16.83
IC-385911	28.63	32.00	4.13	12.38	41.00	176.13	27.63	17.88	49.88	6.75	7.61	86.63	76.00	19.38
IC-392334	30.13	34.50	5.13	18.88	34.25	278.88	21.38	16.25	47.00	5.88	6.31	72.75	92.25	18.28
CD	2.231	3.179	1.218	3.471	4.002	4.998	46.736	8.017	1.419	3.885	0.371	0.513	22.833	2.341

Table 7 Range, mean, standard deviation, standard error, genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance and genetic gain values of ridge gourd genotypes

Chraracters	Range	Mean	S. D.	S. E.	gcv	pcv	Heritability	Genetic advance	Genetic gain
Days for emergence of first male flower	28.13 - 45.25	33.26*	5.64	1.06	11.88	12.13	0.98	2.02	6.07
Days for emergence of first female flower	28.13 - 56.50	38.87*	8.04	1.52	14.56	14.71	0.99	2.04	5.25
Node at first male flower	3.25 - 14.75	6.58*	3.08	0.58	31.97	34.25	0.93	1.92	29.22
Node at first female flower	9.63-38.63	18.93*	11.04	2.08	41.14	41.36	0.99	2.05	10.82
Pollen fertility (%)	61.00 - 99.75	86.43*	10.15	1.91	8.28	8.33	0.99	2.05	2.37
No. of fruits per plant	17.00 -50.63	33.99*	12.65	2.39	24.13	24.23	1.00	2.05	5.55
Average fruit weight	80.00 - 425.50	233.86*	118.26	22.35	35.76	35.76	1.00	2.06	0.88
Average fruit length	9.75 - 73.50	31.45*	20.28	3.83	45.58	45.65	1.00	2.06	6.54
Average fruit girth	14.13-27.00	19.28*	3.59	0.67	12.84	13.51	0.95	1.96	10.16
Days to first harvest	47.00 -81.75	55.89*	9.83	1.85	12.39	12.48	0.99	2.05	3.66
No. of harvests	3.50 - 6.75	5.21*	0.93	0.17	6.44	16.83	0.38	0.79	15.13
Yield per plant (Kg)	3.05 - 7.73	5.50*	1.29	0.24	13.03	19.65	0.66	1.37	24.82
No. seeds per fruit	38.50 - 196.50	110.32*	57.79	10.92	37.04	37.05	1.00	2.06	1.87
Seed yield per fruti (g)	10.12 - 28.23	19.67*	5.92	1.11	21.10	21.49	0.98	2.02	10.28

* Significant at 5% level of significance

4.2.1.2 Days to emergence of first female flower

Accessions varied significantly for the character and the range was 28.13 to 56.50 days. Satputia was the first to produce female flower (28.13 days) and CO 2 (56.50 days) was very late to produce female flower. The accession Arka Sumeet produced the flower first in 33.00 days and the male sterile female parent was very late to produce the flower (66.50 days). The gcv and pcv values for the character were 14.56 and 14.71 respectively. Genetic advance and genetic gain values were found to be 2.04 and 5.25 respectively with high heritability values of 0.99.

4.2.1.3 Node to first fertile male flower

All the accessions varied significantly for the trait and range was 3.25 to 14.75. IC-92671 produced male flower at the lowest node (3.25) and CO 2 produced at the highest node (14.75). The gcv and pcv values were 31.97 and 34.25 respectively. Heritability was found to be 0.93 and the values of genetic advance and genetic gain were 1.92 and 29.22 respectively.

4.2.1.4 Node to first female flower

The accession, IC-23247 produced the female flower in the lowest node (9.63) and the CO 1 produced the flower in the highest node (38.63). The gcv and pcv values were 41.14 and 41.36 respectively. Heritability was 0.99 for the character with an expected genetic advance of 2.05 and genetic gain, 10.82.

4.2.1.5 Pollen fertility

Pollen fertility percentage among the accessions varied from 61.00 to 99.75 percent. The accession CO 2 exhibited maximum percentage of pollen fertility (99.75%) and IC-92671 had minimum fertile pollens (61.00 %). The gcv and pcv values for the character were 8.28 and 8.33. Heritability was 0.99 and genetic advance and genetic gain were 2.05 and 2.37 respectively.

4.2.1.6 Number of fruits per plant

The CO 2 produced minimum number of fruits (17.00) and Satputia produced the maximum (50.63). The gcv and pcv values were 24.13 and 24.23 respectively. Heritability was found to be 1.00. Genetic advance and genetic gain values were 2.05 and 5.55 respectively.

4.2.1.7 Fruit weight

The genotype IC-339224 produced fruits with the lowest weight (80.00 g) and Arka Sujat produced the heaviest fruits (425.00g). Both the gcv and pcv values were 35.76. Heritability value was 1.00. Genetic advance and genetic gain values were found to be 2.06 and 0.88 respectively.

4.2.1.8 Fruit length

All the accessions varied significantly for the trait and the gcv and pcv values were 45.58 and 45.65 respectively. Satputia produced the shortest fruits (9.75cm) and CO 2 produced the longest fruits (73.50 cm). The values of heritability, genetic advance and genetic gain were 1.00, 2.06 and 6.54 respectively.

4.2.1.9 Fruit girth

The genotype IC-339224 produced fruits with less girth (14.13cm) whereas Arka Sujat produced fruits with more girth (27.00 cm). The gcv and pcv values were 12.84 and 13.51 respectively. The values of heritability, genetic advance and genetic gain were 0.95, 1.96 and 10.16 respectively.

4.2.1.10 Days to first harvest

All the accessions varied significantly for the trait and number of days for first harvest ranged from 47.00 to 81.75. IC-392334 produced marketable fruits early (47.00 days) while CO 2 was the last to harvest (81.75). The gcv and pcv values were 12.39 and 12.48 respectively. Heritability was 0.99 and genetic advance and genetic gain values were 2.05 and 3.66 respectively.

4.2.1.11 Number of harvests

Number of harvests was more in IC-385911 (6.75) and less in Deepthi (3.50). The gcv and pcv values were 6.44 and 16.83 respectively among the genotypes and the trait exhibited very low heritability (0.38). The genetic advance and genetic gain values were 0.79 and 15.13 respectively.

4.2.1.12 Yield per plant

The genotype IC-92671 gave the maximum yield (7.73 Kg) followed by IC-385911 which yielded on an average 7.61 Kg/plant. The genotype IC-92685 has given minimum yield of 4.54 Kg/plant. The gcv and pcv values were 13.05 and 19.65 respectively. Heritability was found to be 0.66. Genetic advance and genetic advance were 1.37 and 24.82 respectively.

4.2.1.13 Number of seeds per fruit

The genotype CO 2 produced maximum number of seeds per fruit (196.50) in the first season followed by Deepthi (184.38). Satputia produced minimum number of seeds (38.50) per fruit. The gcv and pcv values were 37.04 and 37.05 and heritability was 1.00. Genetic advance and genetic gain values were 2.06 and 1.87 respectively.

4.2.1.14 Seed yield per fruit

CO 2 exhibited high seed yield (28.23 g/fruit) followed by Arka Sumeet (27.67 g/fruit). Satputia produced less seed (10.12g/fruit). The gcv and pcv values were 21.10 and 21.49 respectively. Heritability, genetic advance and genetic gain values were 0.98, 2.02 and 10.28 respectively.

4.2.2 Estimation of genetic divergence and selection of male parents for hybridization

In order to identify the pollen parents, the 14 accessions of ridge gourd were grouped into 5 clusters based on 11 characters (Table 8) resorting D^2 analysis.

Intra and inter-cluster distances and cluster mean for 11 characters are given in Table 9. Cluster mean values are given in Table 10.

Cluster I had maximum number of genotypes (6) and all the other groups had 2 genotypes each. Inter-cluster distance was low between cluster III and V (36113.72) whereas it was high between cluster I and II (627897.70). Intra-cluster distance was low in cluster II (15245.68) and it was high in cluster IV (45211.33).

The genotypes in cluster I were Satputia, IC-92685, IC-93393, IC-339224, IC-23247, IC-385911. The intra-cluster distance was found to be 20802.86. Cluster mean for number of harvest was the maximum among all clusters (5.90). The genotype IC-92685 was selected from this cluster as male parent (Plate 12).

The genotypes in cluster II were CO 2 and CO 1. The intra-cluster distance found to be 15245.68, which was the minimum among all clusters. The genotypes in this cluster produced both male and female flowers very late. Cluster mean for average fruit weight was the maximum among all the clusters (402.06 g) but that for number of harvest was minimum (4.00). CO 2 was selected for hybridization with male sterile female parent (Plate 6).

Deepthi and MUR-RG-VG were the genotypes in cluster III. The intra-cluster distance was 18505.50. Cluster mean for yield per plant in this cluster was 4.67. Deepthi was selected as the male parent in this cluster (Plate 10).

Arka Sumeet and Arka Sujat were the genotypes in cluster IV. The intra-cluster distance was 45211.33, the highest among all the clusters. Cluster mean for average fruit girth was 26.00 cm which was the maximum among all the clusters. Arak Sumeet was selected as pollen parent for hybridization from this cluster (Plate 8).

Table 8 Clustering pattern of ridge gourd genotypes

Clusters	No. of genotypes	Names of genotypes
I	6	Satputia, IC-92685, IC-93393, IC-339224, IC-23247, IC-385911
II	2	CO 2, CO 1
III	2	Deepthi, MUR-RG-VG
IV	2	Arka Sumeet, Arka Sujat
V	2	IC-92671, IC-392334

Table 9 Inter-cluster and intra-cluster distances for five clusters

Clusters	I	II	III	IV	V
I	20802.86	627897.70	70828.83	342750.20	64263.69
II		15245.68	319695.00	82472.64	375328.30
III			18505.50	143081.30	36113.72
IV				45211.33	159884.50
V					31536.62

Table 10 Cluster means of five clusters of ridge gourd genotypes

Characters / Groups	Days to emergence of first fertile male flower	Days to emergence of first female flower	Node to first fertile male flower	Node to first female flower	Number of fruits per plant	Average fruit weight (g)	Average fruit length (cm)	Average fruit girth (cm)	Days to first harvest	Number of harvests	Yield/ plant (Kg)
I	29.92	33.08	4.81	10.27	43.54	131.56	17.71	16.98	49.92	5.90	5.44
II	44.56	54.19	12.94	35.38	19.50	402.06	68.25	18.81	74.00	4.00	4.94
III	30.50	37.94	6.19	16.63	25.94	204.38	23.56	22.38	58.50	4.31	4.67
IV	38.31	45.81	8.13	19.81	36.63	400.31	52.69	26.00	60.06	4.75	5.61
V	29.69	34.88	4.38	17.38	46.25	235.56	22.50	16.81	48.94	5.69	7.00

Cluster V contained IC-92671 and IC-392334. The intra-cluster distance was 31536.62. The genotypes in this cluster produced male flower earlier than other clusters. The cluster mean for yield per plant was 7.00 kg, which was highest. The genotype IC-92671 was selected for hybridization male sterile plant from this cluster (Plate 13).

4.3 Estimation of heterosis

Ridge gourd being predominantly monoecious is a cross-pollinated crop and provides ample scope for utilization of the hybrid vigour. The estimation of heterosis for yield and its component characters would, therefore, be useful to judge the best hybrid combination for superior hybrids. Five hybrids were produced by crossing the male sterile plant with selected pollen parents. The hybrids are MS x CO 2 (Plate 21), MS x Arka Sumeet (Plate 22), MS x Deepthi (Plate 23), MS x IC-92685 (Plate 24) and MS x IC-92671 (Plate 25). Heterosis values were estimated in three different ways, heterosis over mid parent (relative heterosis), heterosis over better parent (heterobeltiosis) and heterosis over standard parent (standard heterosis). For calculating standard heterosis values, variety Deepthi is taken as the standard parent. Table 11 contains the estimated values of relative heterosis, heterobeltiosis and standard heterosis and Table 12 depicts the values of range, mean in parents, F_1 hybrids, heterosis and percentage superiority.

Earliness, which is one of the most important parameter in hybrids, is indicated by number of days for the opening of first female flower. Parent range was 33.75 to 66.00 and hybrid range was found to be 39.50 to 45.75. All the five F_1 hybrids exhibited highly significant negative heterosis over mid parent with respect to this trait. But not a single cross showed negative heterosis over better or standard parent. The best cross MS x IC-92671 exhibited -26.17 % heterosis over mid parent, followed by MS x CO 2 (-23.91%). Range of heterosis over better parent and standard parent was found to be 17.04 to 35.56.

Plate 21 MS x CO 2

Plate 22 MS x Arka Sumeet

Plate 23 MS x Deepthi

Plate 24 MS x IC-92671

Plate 25 MS x IC-92671

Table 11 Estimated heterosis values

Lines	Days for the emergence first female flower				Node at first female flower				Number of fruits/plant			
	Mean	RH	HB	SH	Mean	RH	HB	SH	Mean	RH	HB	SH
MS	66.00	-	-	-	31.50	-	-	-	22.13	-	-	-
CO 2	54.25	-	-	-	33.13	-	-	-	15.75	-	-	-
Arka Sumeet	33.75	-	-	-	10.50	-	-	-	25.75	-	-	-
Deepthi	34.63	-	-	-	11.50	-	-	-	22.25	-	-	-
IC-92685	36.13	-	-	-	5.88	-	-	-	31.75	-	-	-
IC-92671	41.00	-	-	-	6.88	-	-	-	40.75	-	-	-
MS X CO 2	45.75	-23.91**	35.56**	35.56**	24.50	-24.18**	317.02**	133.33**	25.88	36.63**	-36.50**	0.49
MS X Arka Sumeet	42.88	-14.04**	27.04**	27.04**	22.25	5.95**	278.72**	111.90**	24.88	3.92**	-38.96**	-3.40*
MS X Deepthi	41.25	-18.01**	22.22**	22.22**	10.63	-50.58**	80.85**	1.19	31.00	39.72**	-23.93**	20.39**
MS X IC-92685	43.13	-15.54**	27.78**	27.78**	13.38	-28.43**	127.66**	27.38**	25.25	-6.26**	-38.04**	-1.94
MS X IC-92671	39.50	-26.17**	17.04**	17.04**	9.25	-51.79**	57.45**	-11.90**	35.38	12.52**	-13.19**	37.38**
SE	1.37				1.42				1.02			
CD @ 0.05	2.79				2.90				2.08			
CD @ 0.01	5.13				5.33				3.82			

* Significant @ 0.05 level of significance

** Significant @ 0.05 level of significance

Table 11 Contd...

Lines	Average fruit weight				Average fruit length				Average fruit girth			
	Mean	RH	HB	SH	Mean	RH	HB	SH	Mean	RH	HB	SH
MS	152.63	-	-	-	24.40	-	-	-	18.50	-	-	-
CO 2	293.00	-	-	-	46.95	-	-	-	18.16	-	-	-
Arka Sumeet	292.00	-	-	-	47.25	-	-	-	16.10	-	-	-
Deepthi	173.25	-	-	-	32.85	-	-	-	15.45	-	-	-
IC-92685	124.00	-	-	-	20.40	-	-	-	13.80	-	-	-
IC-92671	109.88	-	-	-	15.70	-	-	-	15.15	-	-	-
MS X CO 2	193.38	-13.21	-34.00*	-33.78*	37.90	6.24*	-19.79**	-19.79**	18.30	-0.16	-1.08*	13.66**
MS X Arka Sumeet	193.00	-13.19	-34.13*	-0.34	35.40	-1.19	-25.08**	-25.08**	17.65	2.02**	-4.59**	9.63**
MS X Deepthi	224.75	37.94*	-23.29	-23.03	34.00	18.78**	-28.04**	-28.04**	15.65	-7.81**	-15.41**	-2.80**
MS X IC-92685	67.50	-51.20**	-76.96**	-76.88**	23.05	2.90*	-51.22**	-51.22**	14.15	-12.38**	-23.51**	-12.11**
MS X IC-92671	79.38	-39.52*	-72.91**	-72.82**	15.30	-23.69**	-67.62**	-67.62**	12.60	-25.11**	-31.89**	-21.74**
SE	12.04				1.88				0.30			
CD @ 0.05	24.58				3.83				0.61			
CD @ 0.01	45.15				7.05				1.12			

* Significant @ 0.05 level of significance

** Significant @ 0.05 level of significance

Table 11 Contd...

Lines	Days for first harvest				Number of harvests				Yield/plant			
	Mean	RH	HB	SH	Mean	RH	HB	SH	Mean	RH	HB	SH
MS	84.88	-	-	-	8.25	-	-	-	6.51	-	-	-
CO 2	73.38	-	-	-	5.88	-	-	-	5.46	-	-	-
Arka Sumeet	46.00	-	-	-	8.25	-	-	-	5.25	-	-	-
Deepthi	45.88	-	-	-	10.50	-	-	-	5.51	-	-	-
IC-92685	48.13	-	-	-	8.25	-	-	-	8.46	-	-	-
IC-92671	40.13	-	-	-	7.63	-	-	-	3.53	-	-	-
MS X CO 2	55.75	-29.54**	38.94**	21.20**	7.88	11.50**	-25.00**	-4.55**	7.05	17.91**	-16.60**	34.37**
MS X Arka Sumeet	46.63	-28.75**	16.20**	1.36	9.13	10.61**	-13.10**	10.61**	9.31	58.48**	10.14**	77.47**
MS X Deepthi	41.25	-36.90**	2.80	-10.33**	8.75	-6.67**	-16.67**	6.06**	6.64	10.49**	-21.50**	26.49**
MS X IC-92685	46.25	-30.45**	15.26**	0.54	9.88	19.70**	-5.95**	19.70**	5.47	-26.91**	-35.33**	4.19**
MS X IC-92671	41.75	-33.20**	4.05	-9.24**	9.88	-5.95**	19.70**	19.70**	4.41	-12.01**	-47.81**	-15.91**
SE	2.30				0.21				0.25			
CD @0.05	4.69				0.42				0.51			
CD @0.01	8.62				0.78				0.93			

* Significant @ 0.05 level of significance

** Significant @ 0.05 level of significance

Table 11 Contd...

Lines	Number of seeds/fruit				Seed yield/fruit			
	Mean	RH	HB	SH	Mean	RH	HB	SH
MS	72.64	-	-	-	15.29	-	-	-
CO 2	186.27	-	-	-	23.05	-	-	-
Arka Sumeet	175.79	-	-	-	24.55	-	-	-
Deepthi	184.12	-	-	-	28.39	-	-	-
IC-92685	48.87	-	-	-	12.11	-	-	-
IC-92671	45.88	-	-	-	13.48	-	-	-
MS X CO 2	211	62.99**	13.28	20.03	24.81	29.42**	-12.61**	1.07**
MS X Arka Sumeet	204.2	64.39**	9.62	16.16	29.28	46.99**	3.13*	19.27**
MS X Deepthi.	198.72	54.80**	6.68	13.05	25.69	17.62**	-9.52**	4.64**
MS X IC-92685	88.52	45.70**	-52.48**	-49.65**	14.47	5.62**	-49.03**	-41.05**
MS X IC-92671	44.44	-0.25	-76.14**	-74.72**	18.19	26.47**	-35.93**	-25.9**
SE	10.37				1.11			
CD @0.05	21.17				2.26			
CD @0.01	38.88				4.16			

* Significant @ 0.05 level of significance

** Significant @ 0.05 level of significance

Table 12 Range, mean in parents, F1 hybrids, heterosis and percentage superiority

Particulars	Days to the emergence of first female flower	Node at first female flower	Number of fruits/plant	Average fruit weight (g/fruit)
Parent range	33.75 to 66.00	5.88 to 33.13	15.75 to 40.75	109.88 to 293.00
Hybrid range	39.50 to 45.75	9.25 to 24.50	24.88 to 35.38	67.50 to 224.75
Best performing parent	Arka Sumeet (33.75)	IC-92685 (5.88)	IC-92671 (40.75)	CO 2 (293.00)
Best performing hybrid	MS x IC-92671 (39.50)	MS x IC-92671 (9.25)	MS x IC-92671 (35.38)	MS x Deepthi (224.75)
Number of heterotic hybrids over better parent	0	0	0	0
Number of heterotic hybrids over standard parent	0	2	5	3
Range of heterosis percentage over better parent	17.04 to 35.56	57.45 to 317.02	-38.96 to -13.19	-76.96 to -23.29
Range of percentage superiority over standard parent	17.04 to 35.56	-11.90 to 133.33	-3.40 to 37.38	-76.88 to -0.34
Hybrids with highest percentage superiority over better parent	MS x IC-92671 (17.04)	MS x IC-92671 (57.45)	MS x IC-92671 (-13.19)	MS x Deepthi (-23.29)
Hybrids with highest percentage superiority over standard parent	MS x IC-92671 (17.04)	MS x IC-92671 (-11.9)	MS x Deepthi (20.39)	MS x Deepthi (-23.29)

Table 12 Contd..

Particulars	Fruit length (cm)	Fruit girth (cm)	Days to first harvest	Number of harvest
Parent range	15.70 to 47.25	13.80 to 18.50	40.13 to 84.88	5.88 to 10.50
Hybrid range	15.30 to 37.90	12.60 to 18.30	41.25 to 55.75	7.88 to 9.88
Best performing parent	Arka Sumeet (47.35)	CO 2 (18.50)	IC-92671 (40.13)	Deepthi (10.50)
Best performing hybrid	MS x CO 2 (37.90)	MS x CO 2 (18.30)	MS x Deepthi (41.25)	MS x IC-92685 & MS X IC-92671 (9.88)
Number of heterotic hybrids over better parent	0	0	0	0
Number of heterotic hybrids over standard parent	3	3	2	0
Range of heterosis percentage over better parent	-67.62 to -19.79	-31.89 to -1.08	2.80 to 38.94	-25.00 to 19.70
Range of percentage superiority over standard parent	-67.62 to -19.79	-21.74 to 13.66	-10.33 to 21.20	-4.55 to19.70
Hybrids with highest percentage superiority over better parent	MS x CO 2 (-19.79)	MS x CO 2 (-1.08)	MS x IC-92685 (15.26)	MS x IC-92685 (-5.95)
Hybrids with highest percentage superiority over standard parent	MS x CO 2 (-19.79)	MS x Arka Sumeet (9.63)	MS x Deepthi (-10.33)	MS x IC-92685 & MS X IC-92671 (19.70)

Table 12 Contd..

Particulars	Yield per plant (kg)	Number of seeds per fruit	Seed yield per fruit (g)
Parent range	3.53 to 8.46	45.88 to 186.27	12.11 to 28.39
Hybrid range	4.41 to 9.31	44.44 to 204.20	14.47 to 29.28
Best performing parent	IC-92685 (8.46)	CO 2 (186.27)	Deepthi (28.39)
Best performing hybrid	MS x Arka Sumeet (9.31)	MS x Arka Sumeet (204.20)	MS x Arka Sumeet (29.28)
Number of heterotic hybrids over better parent	1	3	1
Number of heterotic hybrids over standard parent	3	3	1
Range of heterosis percentage over better parent	-47.81 to 10.14	-76.14 to 13.28	-49.03 to 3.13
Range of percentage superiority over standard parent	-15.91 to 77.47	-74.72 to 20.03	-41.05 to 19.27
Hybrids with highest percentage superiority over better parent	MS x Arka Sumeet (10.14)	- -	MS x Arka Sumeet (3.13)
Hybrids with highest percentage superiority over standard parent	MS x Arka Sumeet (77.47)	- -	MS x Arka Sumeet (19.27)

Out of five F_1 hybrids, four exhibited highly significant negative heterosis over mid parent for node to first female flower. MS x IC-92671 exhibited maximum negative heterosis over mid parent (-51.79%). All the crosses were significantly superior over better parent in positive direction. The only hybrid which exhibited highly significant negative heterosis over standard parent (Deepthi) was MS x IC-92671 (-11.90%).

Mid parental heterosis was found to be highly significant with respect number of fruits per plant. All the F_1 hybrids exhibited heterosis in positive direction except MS x IC-92685(-6.26). MS x Deepthi exhibited maximum mid parental heterosis of 39.72%. Heterobeltiosis was found to be in negative direction in all crosses. MS x IC-92671 exhibited maximum heterosis over standard parent (37.38%).

MS x Deepthi was the only F_1 hybrid which exhibited heterosis in positive direction with respect to fruit weight (37.94%). All the crosses exhibited heterobeltiosis and standard heterosis in negative direction.

In respect to fruit length, a highly significant relative heterosis was exhibited by MS x Deepthi (18.78%). Heterobeltiosis and standard heterosis were in negative direction in all crosses.

The only F_1 hybrid which exhibited relative heterosis for fruit girth in positive direction was MS x Arka Sumeet (2.02%) and the value was highly significant. MS x IC-92671 exhibited maximum relative heterosis (-25.11%) but it was in negative direction. Not a single F_1 hybrid exhibited heterobeltiosis in positive direction. Out of five crosses only two exhibited positive standard heterosis. MS x CO 2 and MS x Arka Sumeet exhibited highly significant standard heterosis of 13.66% and 9.63% respectively.

Earliness is indicated by the number of days for first harvest. All the F_1 hybrids exhibited negative relative heterosis. MS x Deepthi exhibited the highest superior heterosis over mid parent (-36.90%) and the value was highly significant. Not even a single F_1 hybrid exhibited heterobeltiosis in negative direction. Only two F_1 hybrids exhibited negative standard heterosis. The most superior hybrid over standard parent was MS x Deepthi (-10.33%) followed by MS x IC-92671 (-9.24 %).

With respect to number of harvests, three crosses exhibited relative heterosis in positive direction. The maximum positive relative heterosis was exhibited by MS x IC-92685 (19.70%), the value was highly significant. Only MS x IC-92671 exhibited positive heterobeltiosis (19.70%) and the value being highly significant. MS x IC-92685 and MS x IC-92671 both exhibited highly significant standard heterosis (19.70%).

All the hybrids showed highly significant heterosis over mid, better and standard parent for yield per plant, which is the ultimate aim of hybridization programme. Three hybrids showed relative heterosis in positive direction. MS x Arka Sumeet exhibited maximum values for relative heterosis, heterobeltiosis and standard heterosis (58.48, 10.14 and 77.47% respectively) and the values were highly significant.

Number of seeds per fruit is an important trait in seed production. Four of the F_1 hybrids exhibited relative heterosis in positive direction. MS x Arka Sumeet exhibited the maximum relative heterosis of 64.39% followed by MS x CO 2 (62.99%). Not a single hybrid exhibited significant heterobeltiosis or standard heterosis in positive direction.

Maximum seed yield per fruit makes seed production economic. The F_1 hybrid MS x Arka Sumeet was the best with respect to this trait as it exhibited highest values for relative heterosis, heterobeltiosis and standard heterosis (46.99,

3.13, 19.27% respectively). Except MS x Arka Sumeet, all other hybrids exhibited negative heterobeltiosis for the trait.

4.4 Expression and stability of male sterility in hybrid progenies

Among the five hybrids, four were male sterile *viz.*, MS x CO 2, MS x Deepthi, MS x IC-92685, MS x IC-92671. As in the case of male sterile female parent, no difference was observed with respect to any of the vegetative (Plate 26) or female flower characters (Plate 27). The average bud length of male buds of these hybrids at this stage was found to be ranging from 7.56 mm to 9.05 mm (Plate 28a, b, c, d) and these rudimentary male buds in racemes remained unopened and fell down 12-16 days after they attained a size visible to naked eye (Plate 29a, b, c, d,). The anther lobes were undeveloped. Pollen grains were small and shrunken in all these crosses. When the pollen grains from these plants were mounted on acetocarmine stain, they did not attain the stain (Plate 30 a, b, c, d) and pollen fertility was found to be zero throughout the season. Male sterility was stable in these hybrids throughout the season. There was no fruit set on selfing in any of the hybrids. But fruit set was observed when these hybrids were backcrossed with their respective male parents (Plate 31a, b, c, d). Fruit set percentage varied from 60.00 to 86.67 % in back crossed fruits. Highest fruit set percentage (Table 13) was found in MS x CO 2 (73.33 %). The F_1 hybrid MS x IC-92685 exhibited minimum fruit set percentage of 60.00. Normal fruits were observed in the F_1 hybrids on open pollination. Seed set was normal in these crossed fruits. Number of seeds per fruit ranged from 44.44 to 211.00 in back crossed fruits. The F_1 hybrid MS x CO 2 produced maximum number of seeds per fruit 211.00, whereas, MS x IC-92671 produced the minimum (44.44). The observed germination and seedling survival percentage ranged from 65.98 to 98.00% and 51.14 to 83.33% respectively in seeds from back crosses. Among the male sterile hybrids maximum germination percentage (Table 13) was observed in the back crossed seeds of MS x CO 2 (82.55 %), whereas the minimum was in seeds of MS x IC-92671(65.98 %).

Plate 26 Leaf character of hybrids

Plate 27 Female flowers of hybrids

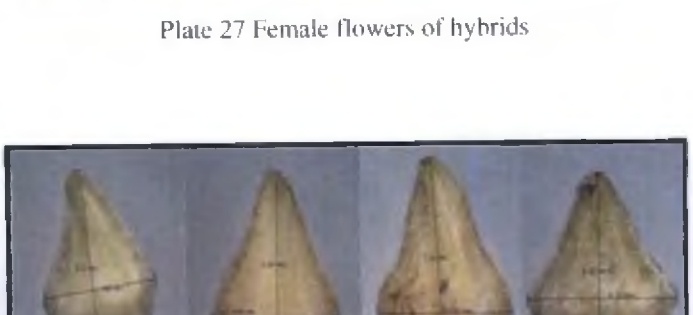

Plate 28 Male flower buds of hybrids

Plate 29 Male racemes of hybrids

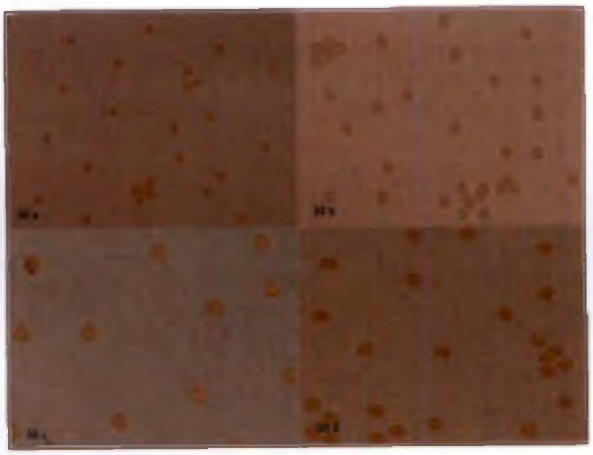

Plate 30 Pollen stainability of hybrids

Plate 31 Fruits of hybrids

Table 13 Fruit set percentage, number of seeds/fruit, germination percentage and seedling survival percentage in F_1 hybrids of ridge gourd using male sterile genotype as female parent

F1 hybrids	Number of flowers		Number of fruits obtained		Fruit set percentage		No. seeds/fruit		Germination percentage		Seedling survival percentage	
	Selfed	Back crossed	Selfed	Back crossed	Selfed	Back crossed	Selfed	Back crossed	Selfed	Back crossed	Selfed	Back crossed
MS x CO 2	15.00	15.00	0.00	11.00	0.00	73.33	-	211.00	-	82.55	-	75.00
MS x Arka Suheet	15.00	15.00	12.00	13.00	80.00	86.67	201.13	207.75	97.45	98.00	84.52	82.36
MS x Deepthi	15.00	15.00	0.00	10.00	0.00	66.67	-	198.72	-	80.57	-	62.50
MS x IC-92685	15.00	15.00	0.00	9.00	0.00	60.00	-	88.52	-	76.82	-	51.14
MS x IC-92671	15.00	15.00	0.00	10.00	0.00	66.67	-	44.44	-	65.98	-	83.33

Restoration of male fertility

MS x Arka Sumeet was found to be male fertile with average pollen fertility of 34 percent throughout the season. In contrast to the male sterile female parent, these plants produced normal male and female flowers which opened normally in evening (Plate 32 a & b). The anthers were found to be normal (Plate 33 a). Both normal and sterile pollens grains were spotted (Plate 33 b) when staining of pollen grain was done using acetocarmine. Fruit set was observed on selfing and backcrossing (Plate 34), with a fruit set percentage of 80.00 and 86.67 respectively. Also, when these plants were allowed for natural open cross pollination, normal fruit set was observed. Seed set was also normal. The average number of seeds per fruit was 201.13 and 207.75 respectively in selfed and back crossed fruits. Seeds from selfed and crossed fruits exhibited high germination percentage (97.45 and 98.00 respectively). Seedling survival percentage of selfed and crossed seeds was found to be 84.52 and 82.36 respectively (Table 13).

Plate 32 Female and male flowers of MS x Arka Sumeet

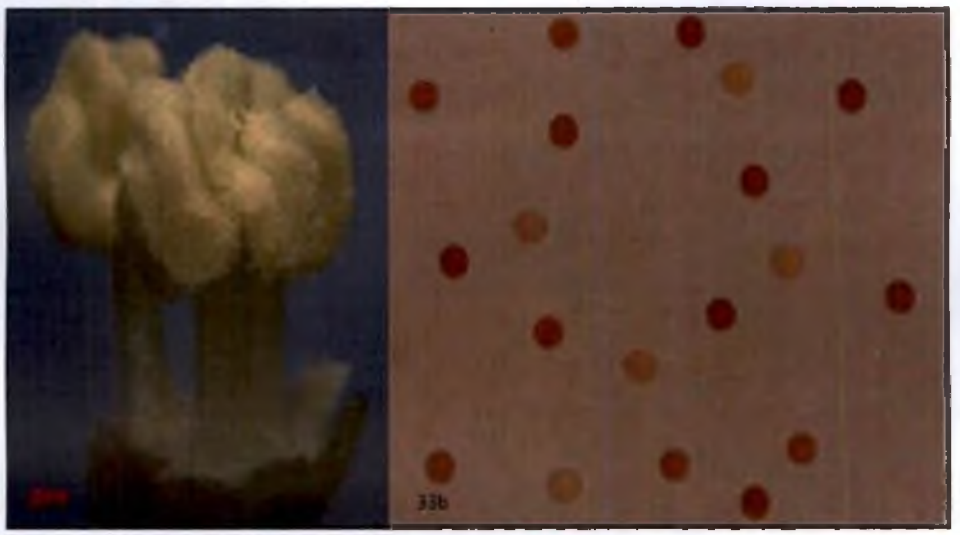

Plate 33 Anther lobe and pollen stainability of MS x Arka Sumeet

Plate 34 Fruit of MS x Arka Sumeet

DISCUSSION

5. DISCUSSION

Male sterility is the failure of plants to produce functional anthers, pollen or male gamete (Kaul, 1988). This phenomenon had already been reported in ridge gourd by Deshpande *et. al.* (1979) and Pradeepkumar *et. al.* (2008). Ridge gourd, though not a major vegetable cultivated in the state on commercial basis, is an important vegetable cultivated in homesteads. It is well known for its nutritional and medicinal properties (Sheshadri and Parthasarathy, 1980; Robinson and Decker-Walters, 1997; Hou, *et. al.,* 2006). As far as crop improvement of ridge gourd is concerned, a limited work has been conducted in Kerala Agricultural University and two varieties were released through selection *viz.,* Deepthi and Haritham.

Till date, there is no report on utilization of male sterility for crop improvement in ridge gourd

Utilization of available genetic mechanism of male sterility in this crop would be appropriate for economizing hybrid seed production. In this context, the present study was conducted to investigate utilization of male sterility for crop improvement in ridge gourd.

The present work is discussed under the following headings

5.1 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

5.2 Evaluation of ridge gourd accessions from different parts of the country and hybridization of selected pollen parents with male sterile genotype

5.3 Evaluation of F_1s along with male sterile female parent and male fertile parents and studying the expression of male sterility in different genotypic combinations

5.1 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

5.1.1 *In vitro* maintenance and regeneration of ridge gourd

Micropropagation was already reported by many workers in cucurbits (Barnes *et al.*, 1978; Jain and More, 1992; Sapountzakis and Tsaftaris, 1994; Spetsidis, 1996; McCarthy *et al.*, 2001; Pradeepkumar *et al.*, 2008) and the standardized protocol for the *in vitro* regeneration and multiplication of ridge gourd by Pradeepkumar *et al.* (2008) was effective in regenerating the field grown male sterile plant. The establishment medium (MS + IAA 1.5 mg l^{-1} + BA 2 mg l^{-1}) was succesful for *in vitro* establishment of field grown plant. Shoots took 25 days to develop and these were inoculated in multiplication media (MS + BA 1 mg l^{-1}). 4-5 multiple shoots were produced in this medium. The shoots took 35 days to produce roots in the rooting medium and these *in vitro* regenerated plants were given hardening treatment for one month before transplanting.

5.1.2 Evaluation of male sterility

Male sterility in ridge gourd is an already reported phenomenon (Deshpande *et al.*, 1979; Pradeepkumar *et al.*, 2008). All the *in vitro* regenerated plants were male sterile. The phenotypic expression of the mutant in the present study was in confirmation with the reported type of male sterility. There were observable differences between the male sterile and a male fertile plant with respect to male flower production though female flowers in both types were similar. The production of rudimentary male buds in racemes was reported by the earlier workers (Deshpande *et al.*, 1979; Pradeepkumar *et al.*, 2008). The cytological study of PMCs in the present investigation revealed normal meiosis. The microspore mother cells appeared to be normal. Meiosis proceeded normally up to the tetrad stage, but just after that the microspores degenerated and no fertile pollen formed. Some male sterile genes delay tapetum degeneration and this could lead to nucleotide starvation of the developing PMC and microspores leading to their degeneration and ultimately male sterility. Anthers and microspores of male sterile plants of almost all species show changes in the content and proportion of

various amino acids, a high deficiency of DNA and RNA , reduced carbohydrate and protein content, significant reduction in the activities of callase. cytochrome oxidase and several other enzymes, lower contents and altered proportions of growth regulators (Singh, 2005). As the meiosis observed to be normal and pollen development is broken down in the post meiotic stage, degeneration of pollen may be due to the faulty timing in the activity of callase enzyme, leading to failure of callose (a specialized pollen wall material composed of β (1-3) - linked glucose polymers). Callose is synthesized in plants during differentiation processes and contributes to the molecular strategies of morphogenesis during reproduction (Bhatia and Malik, 1996; Peel *et al.*, 1997). It has been reported that in most of plants, the deposition and degradation of callose during microsporogensis and gametogensis are indispensable for the formation of functional pollen grains (Bhatia and Malik, 1996; Lu *et al.*, 2003) and faulty timing in the deposition or degradation of callose lead to male sterility (Izhar and Frankel, 1971; Worral *et al.*, 1992). Delayed activity of callase causing male sterility in chilli is reported by Pochard (1970) and Dash *et al.* (2001). On the contrary, meiotic abnormalities were reported by Bohn and Principe (1964) in musk melon male sterile mutant, which belongs to cucurbit family. In GMS mutants, the abnormalities may involve aberrations in any one of the following stages: during meiosis, in the formation of tetrads, during the release of tetrads (*i.e.*, the dissolution of callose), at the vacuolated microspore stage or at mature or near mature pollen stage (Kaul, 1988). Normal meiosis observed in the PMCs of the male sterile genotype and further degeneration of tetrads calls for elaborate cytological studies to confirm the role of callose or other degenerating enzymes in the control of male sterility.

5.2 Evaluation of ridge gourd accessions and hybridization of selected pollen parents with male sterile genotype

Ridge gourd genotypes were collected from all over the country and evaluated for variability.

5.2.1 Genetic variability

The success of any crop improvement programme depends on the precise information available on the genetic variability and divergence of the crop. The analysis of variance revealed presence of significant variability among the genotypes with respect to days to emergence of first fertile male flower, days to emergence of first female flower, node first fertile male flower, node to first female flower, pollen fertility, number of fruits per plant, fruit weight, fruit length, fruit girth, days to first harvest, number of harvest, yield per plant, number of seeds per fruit, seed yield per fruit (Table 5). The existence of high variability had already been reported by many workers (Deshpande *et al.*, 1980; Anitha, 1998; Ahmed *et al.,* 2006).

The study revealed a significant variability with respect to days to the emergence of first fertile male flower. The genotypic coefficient of variation was found to be high and the difference between genotypic coefficient of variation (gcv) and phenotypic coefficient of variation (pcv) was very little. This indicates the variation is due to genotypes and the environmental effect on expression of this trait is very low. The value of heritability in broad sense was high which point outs that though the effect of environment on the expression of this trait is low, selection for improvement of this character may not be rewarding. These results are in accordance with the results of Anitha (1998). Genetic advance was found to be low, which indicates the character under study is governed by non-additive genes and heterosis breeding may be useful. The same trend was observed in case of days for emergence of first female flower.

The genotypes exhibited significant variation with respect to node to first fertile male flower and node to first female flower. Both traits showed narrow difference between gcv and pcv which shows low effect of environment on the expression of this trait. Heritability was found to be high with low genetic advance and high genetic gain. High heritability accompanied with low values of genetic advance is indicative of non-additive gene action and heterosis can be exploited for improvement of this character. Greater role of genotypic difference for total variability along with high heritability values for nodes to first female flower are in conformity with results obtained by Anitha (1998).

Pollen fertility, in the present investigation, showed the signs of low gcv but the difference between gcv and pcv was very low indicating less effect of environment on the expression of this character. Heritability was high confirming the greater role of genotypes in the total variability. The low genetic advance and genetic gain values for the character indicated the supremacy of non-additive gene action.

Number of fruits per plant varied significantly and the gcv and pcv were almost same. Heritability was the highest (1.00) which confirmed that the effect of environment on the expression of this trait was nil. The genetic advance and genetic gain values were low. Average fruit length also showed the same trend. These two traits, in the present investigation, showed non-additive gene action governing the heritability of these traits. The result for number of fruits per plant is in contrast with that obtained by Anitha (1998) where she reported additive gene action for this trait.

Significant variation was observed with respect to fruit weight. This character exhibited high gcv value. The gcv and pcv values were the same with highest heritability value (1.00) which indicates the total variability is due to genotypes alone.

Fruit girth showed very little difference in gcv and pcv values with high gcv. Heritability was found to be high. The genetic advance was found to be low but the genetic gain was high. High heritability with low genetic advance indicates the presence of non-additive gene action. This was also reported by Anitha (1998).

The gcv and pcv for days to first harvest were almost the same in the present investigation, which indicating that the genotype play a major role in the xpression of the trait and high heritability value confirmed it. The genetic advance and genetic gain were low.

In the present study, number of harvests exhibited considerable difference between gcv and pcv which indicates that the environment is playing a greater role in the expression of this character. The heritability was also very low (0.38). The genetic advance was low with high genetic gain. Low heritability coupled with low genetic advance revealed that the character is highly influenced by environment and genetic improvement through selection would be difficult. Yield per plant also exhibited the same results.

Number of seeds per plant varied significantly and there was no difference between gcv and pcv indicating that the effect of environment as nil. Heritability was found to be greatest (1.00) which confirmed the above fact. The genetic advance and genetic gain were low.

The genotypic and phenotypic coefficients of variation with respect to seed yield per fruit were found to be almost same indicating little effect of environment on the expression of this trait and revealed that this particular character may be improved by selection. Heritability was high with low genetic advance and high genetic gain. High heritability coupled with high genetic gain also indicated the role of additive gene effects and selection may be effective for improvement of this trait.

5.2.2 Estimation of genetic divergence and selection of parents of hybridization

Since there existed wide variability for the characters studied, Mahalonobis's D^2 statistic was employed for the efficient clustering of genotypes. Estimation of genetic divergence using Mahalonobis's D^2 statistics is a reliable tool for selecting parents of diverse genetic background for hybridization. Anitha (1998) used this technique for the selection parents in crop improvement of ridge gourd.

All the fourteen genotypes were grouped into five groups. Genetic divergence study was found to be effective in selecting pollen parents from different genetic backgrounds as the varieties released by Tamil Nadu Agricultural University, Indian Institute of Horticulture Research and Kerala Agricultural University fell in different groups and all the crosses had good amount of fruit set. Inter-cluster distance was found to be higher than intra-cluster distance which also confirms the effectiveness of this tool. Similar results were reported by Anitha (1998). The pollen parents were selected on the basis of acceptable fruit characters. The selected pollen parents were CO 2, Arka Sumeet, Deepthi, IC-92685 and IC-92671. All these belong to different clusters. Hermaphrodite type was IC-92685.

5.3 Evaluation of F_1 hybrids along with male sterile female parent and male fertile parents and studying the expression of male sterility in different genotypic combinations

The F_1 progenies were evaluated based on the superiority over mid, better and standard parent.

5.3.1 Estimation of heterosis

Exploitation of heterosis has played a significant role in increasing productivity and production of several crops world over. Availability of suitable pollination control system and the extent of outcrossing between female and male

parents, existence of exploitable level of heterosis and feasibility of hybrid seed production on large scale are the key factors determining the success of commercial exploitation of heterosis in any crop. Exploitable level of heterosis has already been reported by many workers in ridge gourd (Niyaria and Bhalala, 2001; Mole *et al.*, 2001; Hedau and Sirohi, 2004; Ram *et al.*, 2004).

All the five hybrids showed significant heterosis for days to the emergence of first female flower. But, not a single hybrid exhibited negative heterosis over either better or standard parent.

For nodes at first female flower, the best hybrid found to be was MS x IC-92671, as it exhibited negative heterosis over standard parent Deepthi.

The values of heterosis, in the present investigation, were not appreciable over better parent as none of the hybrid exhibited positive heterosis for number of fruits per plant (Fig.1). Mean values for number of fruits per plant and yield per plant are compared in Figure 2 and 3 respectively. For this trait also MS x IC-92671 can be considered as good cross as it exhibits maximum positive heterosis over standard parent.

Heterosis values for fruit weight and average fruit length were not appreciable as none of the hybrids expressed positive heterosis over either better or standard parent.

For fruit girth, all the hybrids expressed negative heterosis over better parent. MS x CO 2 proved to be the best cross as it exhibited maximum positive standard heterosis followed by MS x Arka Sumeet.

For days taken for first harvest, which indicate earliness, all the hybrids showed negative relative heterosis. None of the hybrids showed negative heterosis

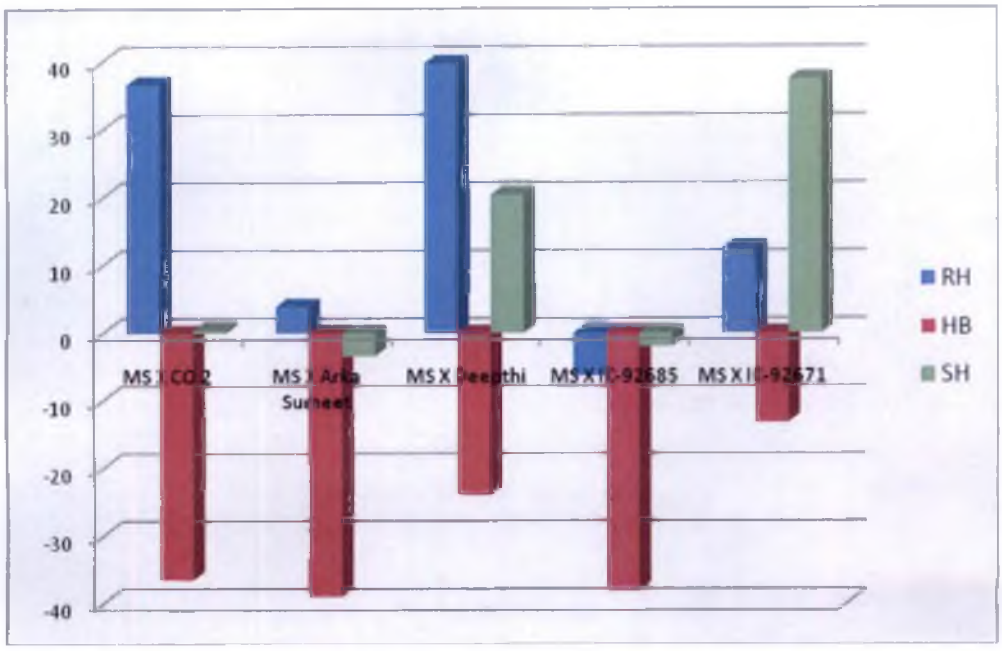

Figure 1 Relative heterosis, heterobeltiosis and standard heterosis for number of fruits per plant

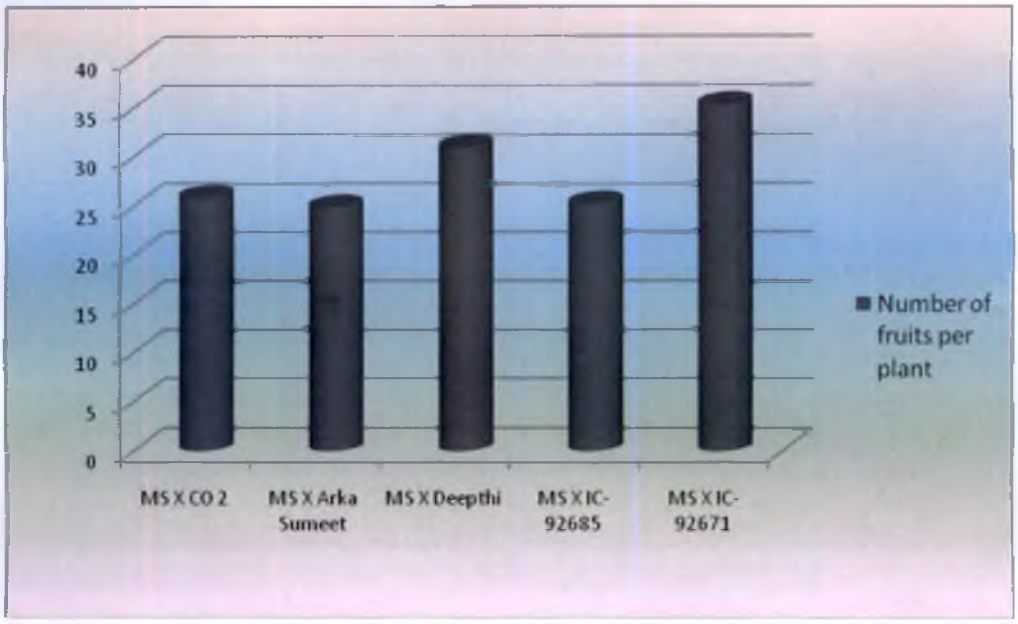

Figure 2 Mean values for number of fruits per plant among hybrids

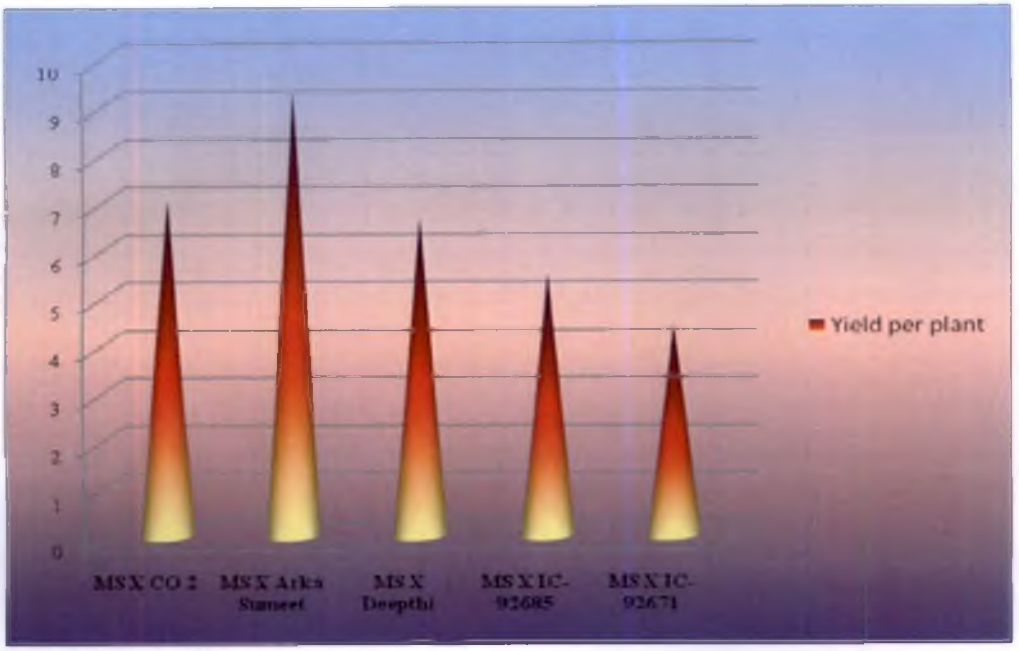

Figure 3 Mean values for yield per plant among hybrids

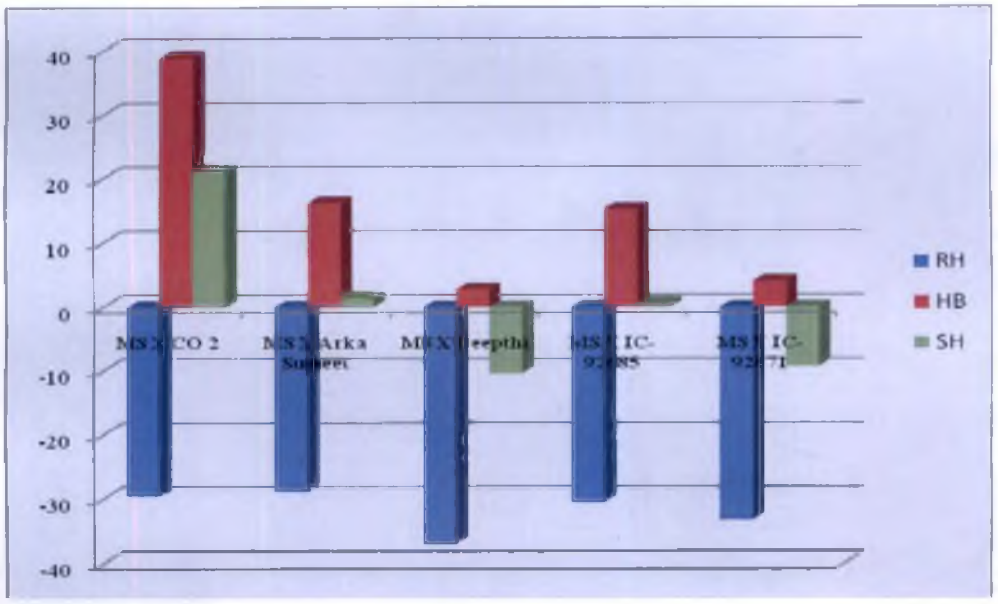

Figure 4 Relative heterosis, heterobeltiosis and standard heterosis for days for first harvest

over better parent (Fig. 4). MS x Deepthi appeared as best hybrid as it showed negative heterosis over standard parent.

Both MS x IC-92685 and MS x IC-92671 proved to be best for number of harvests, which is important from the yield point of view. These hybrids exhibited highest positive standard heterosis which was highly significant.

MS x Arka Sumeet appeared to be the best hybrid being higher in both hetetobeltiosis and standard heterosis for yield and related characters (Fig.5).

Four hybrids *viz.,* MS x CO 2, MS x Arka Sumeet, MS x Deepthi, MS x IC-92685 were positively superior over mid parent for number of seeds per fruit. MS x IC-92671 showed negative midparental heterosis. None of the hybrids showed significant positive heterosis over better parent or standard parent.

MS x Arka Sumeet proved to be the best with respect to seed yield per fruit, as it exhibited highest positive heterosis over both the better and standard parents.

As the hybrid with MS x Arka Sumeet as parentage exhibited best yield per plant and also proved to be the best in seed yield per fruit and also the hybrid restores fertility, it can be concluded as this particular hybrid can be promoted as a commercial hybrid.

5.3.2 Expression of male sterility in different genotypic combinations

In the present study, when crosses were made between male sterile genotype and five different pollen parent, four hybrids (MS x CO 2, MS x Deepthi, MS x IC-92685, IC-92671) were found to be male sterile and one cross (MS x Arka Sumeet) was partially fertile. This situation offers a range of possibilities in explaining the genetic basis of male sterility in the crop. The male sterility observed in present study is not of recessive genic type, which is most predominant in cucurbits, because four out of five hybrids are sterile. If recessive

genes are responsible for control of male sterility, all the F_1 would have been heterozygous fertile (Fig 6). If dominant nuclear gene is responsible for male sterility, all the F_1 hybrids would have been sterile (Fig. 7). So far no dominant gene has been reported in cucurbits. Male sterility controlled by dominant nuclear gene(s) is of rare occurrence. The dominant nuclear gene controlling male sterility (*Ms*) is quickly eliminated from the population, as all the individuals carrying *Ms* allele are sterile and do not produce self progenies. However, if outcrossing rate is high as in other members of cucurbitaceae, these genes can be maintained in the population. On the contrary, recessive genic male sterility can be maintained in the population in the heterozygous (*Ms ms*) state. If recessive homozygous *ms ms* genetic constitution assumed for male sterile female parent and heterozygous *Ms ms* genetic constitution for the pollen parent, 50 % of the F_1 hybrid population would have been sterile and rest 50 % of F_1 would have been fertile (Fig. 8). But in the present investigation, all the plants in four crosses (MS x CO 2, MS x Deepthi, MS x IC-92685, MS x IC-92671) were sterile and all the plants in one cross (MS x Arka Sumeet) were partially fertile. Hence the role of heterozygous recessive gene action in governing male sterility cannot be confirmed. If the male sterility, in present case, is governed by cytoplasmic genes, again all the F_1 hybrids would have been sterile as the cytoplasmic factor is inherited from maternal parent (Fig. 9). Hence, this confirms that there is no sterile cytoplasm in cucurbits. So, the reported pattern of male sterility (Kaul, 1988) is not sufficient to explain the genetic mechanism of male sterility in *Luffa acutangula*. Based on all these, it can be assumed that the inheritance of male sterility and restoration of fertility in ridge gourd may be a complex mechanism.

Some workers had already reported the mechanism of sex inheritance in *Luffa* (Richaria, 1948; Singh *et al.,* 1948 and Chaudhury and Thakur, 1965). They postulated that sex expression in *Luffa* is controlled by two independent genes 'A' and 'G'. 'A' suppresses the male organ in the solitary flowers while 'G' suppresses femaleness in racemes. In the absence of both of these dominant genes, the plant exhibits basic hermaphroditic nature.

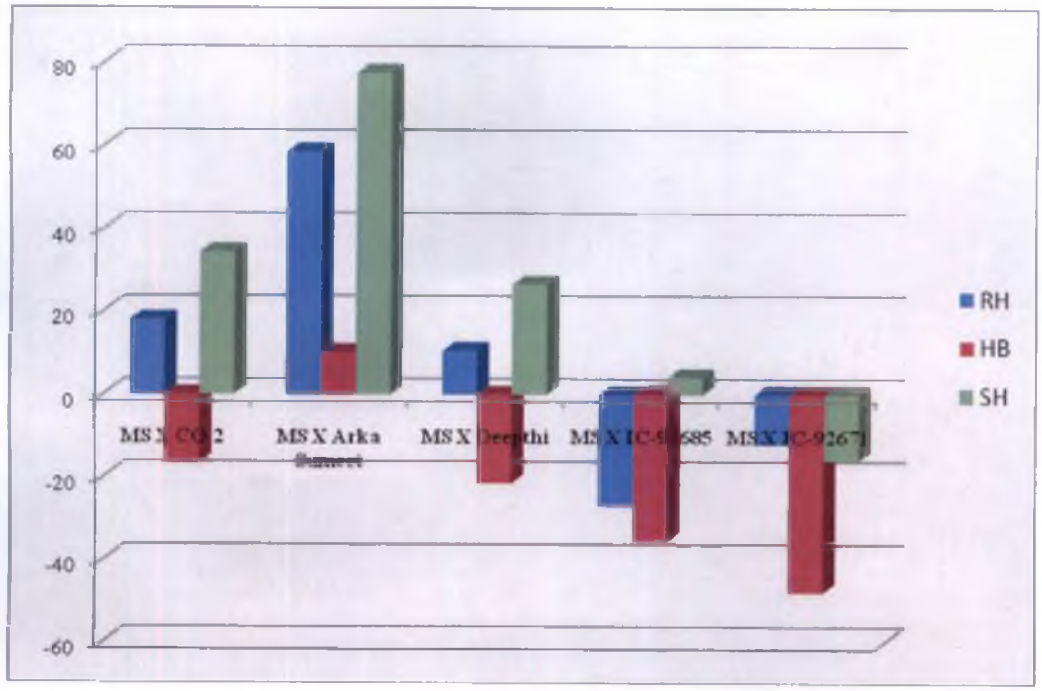

Figure 5 Relative heterosis, heterobeltiosis and standard heterosis for yield per plant

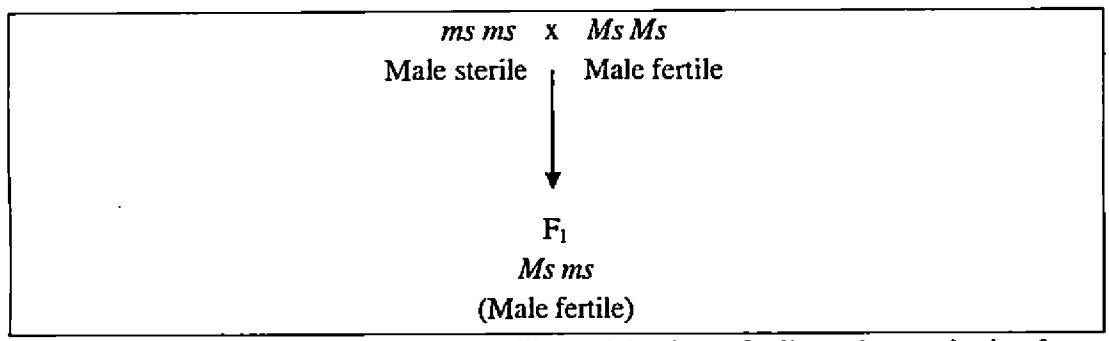

Figure 6 Monogenic recessive male sterility and dominant fertile genic constitution for male fertile parent

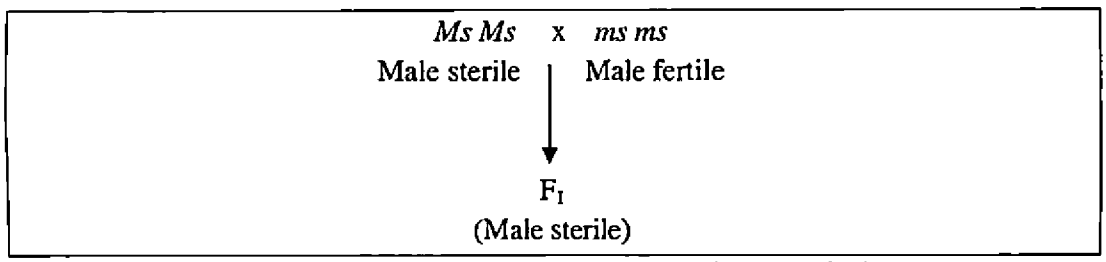

Figure 7 Dominant male sterile parent and recessive male fertile parent

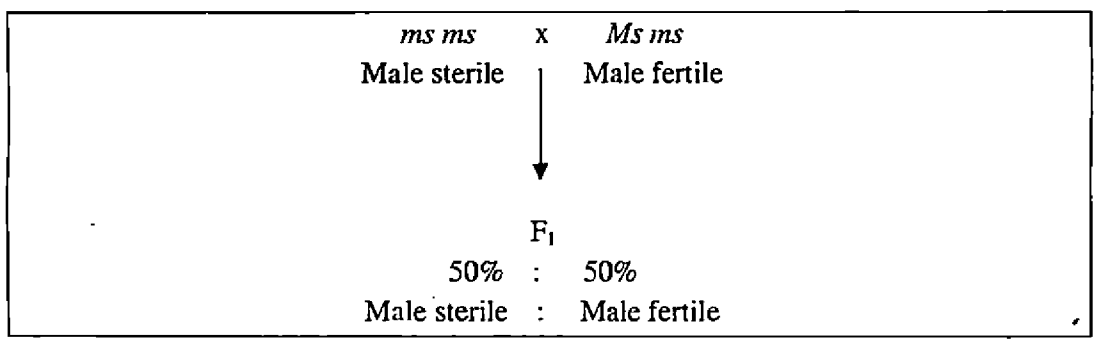

Figure 8 Monogenic recessive male sterility and heterozygous fertility genic constitution in male fertile parent

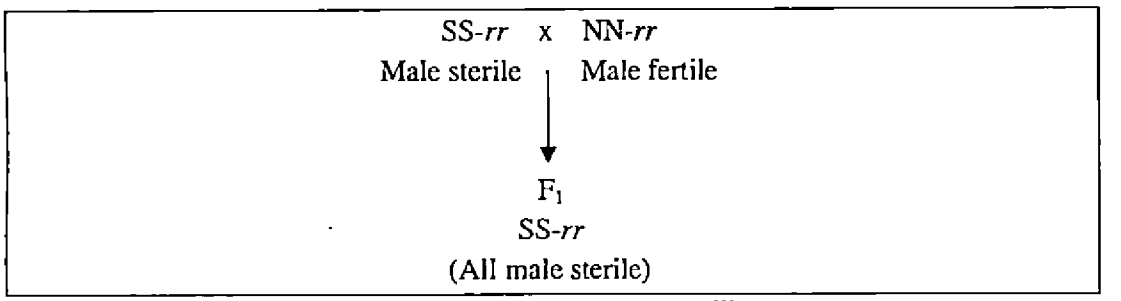

Figure 9 Cytoplasmic male sterility

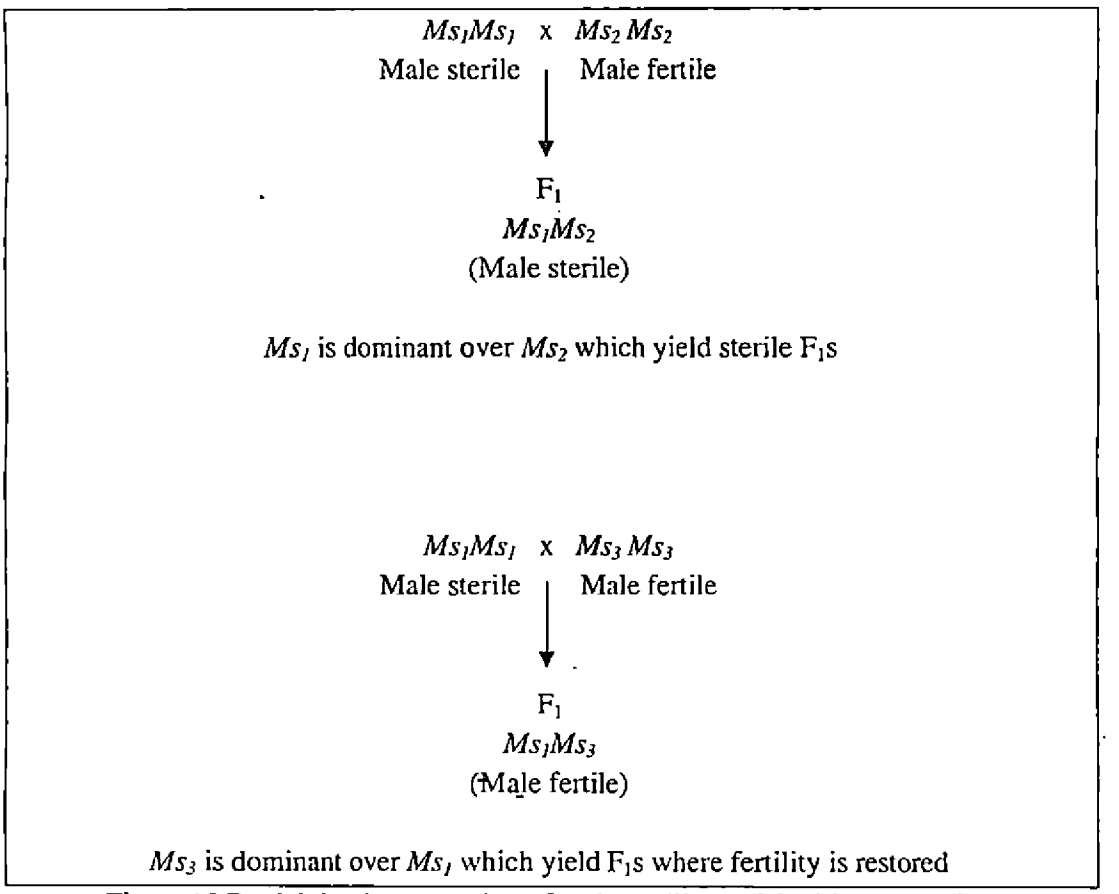

Figure 10 Partial dominance action of male sterility and fertility restoration

5.3.3 Fertility restoration

Nuclear factor of Arka Sumeet may be governing the restoration of fertility. We can assume a partial dominant gene action involving more than one gene governing male fertility, sterility and fertility restoration in ridge gourd. Partial dominant nature of male sterility is evident from the expression of male sterility in four crosses. However Arka Sumit possesses a dominant gene for male fertility which overcomes the partial dominant gene action of male sterility in ridge gourd (Fig. 10). The exact genetic mechanism governing this character can only be explained by studying the F_2 and back cross population of MS x Arka Sumeet and three way cross populations involving male sterile hybrids as female and Arka Sumeet as male parent. The pollen parent Arka Sumeet may contain fertility restorer nuclear gene by virtue of which it is restoring the fertility, to some extent, in the hybrids.

The restorer gene(s) may have sporophytic or gametophytic mode of restoration (Kaul, 1988). In case of sporophytic restoration, the heterozygous F_1 plants produce fully fertile pollen, because fertility of the pollen is governed by genotype of F_1 plant, while in case of gametophytic restoration, both fertile and sterile pollen are produced, because pollen fertility in this case is governed by the gametophyte of pollen itself. As the hybrid, MS x Arka Sumeet is containing both fertile and sterile pollen, the mode of restoration may be of gametophytic in nature.

5.3.4 Developing new source of male sterility and maintainer line

As the crosses involving Deepthi, IC-92685, IC-92671 as pollen parents are sterile, these lines can be used for developing new male sterile lines. Since F_1 hybrid involving IC-92671 is having acceptable fruit characters, IC-92671 would be best suited for development of new male sterile line through series of back crosses (Fig. 11). Once the new male sterile line is developed from IC-92671, it can be maintained through controlled crossing with original IC-92671. Hence all the four genotypes have the potential for using as a source of developing new

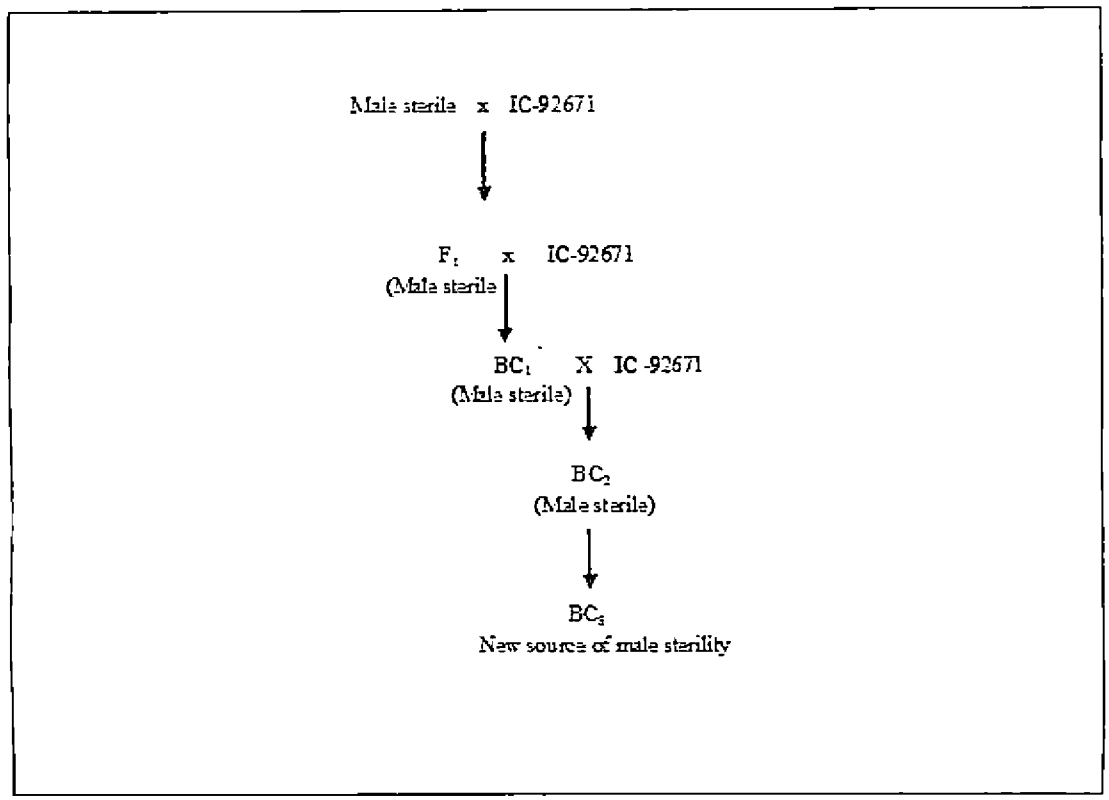

Figure 11 Development of new source of male sterility

male sterile genotypes and maintainer lines in ridge gourd. Whereas the hybrid having MS x Arka Sumeet combination is fertile and it showed good heterotic percentage over both better and standard parents, Arka Sumeet can be used as fertility restorer line as well as pollen parent for commercial hybrid seed production.

Large variability present in the ridge gourd genotypes with respect to yield and related characters can be utilized for crop improvement. Genetic mechanism of male sterility and its restoration have to be elucidated by studying the F_2 and back cross progenies. Further, markers linked with male sterile gene have to be discovered.

This is the first study which attempted to trace out a unique mechanism of male sterility and fertility restoration in ridge gourd and is the first report of fertility restorer line in ridge gourd.

SUMMARY

SUMMARY

Male sterility is the failure of plants to produce functional anthers, pollen or male gametes. If heterosis breeding is combined with some pollination control mechanism such as male sterility, it will certainly economize commercial hybrid seed production. Male sterility is an already reported phenomenon in ridge gourd. But till the date, no work is reported on crop improvement of ridge gourd where male sterility is utilized. Report on genetic dissection of male sterility is also not found. So, the present investigation was undertaken with the objective of investigating the stability of male sterility in ridge gourd *Luffa acutangula* (L.) Roxb. and expression of male sterility on combinations with different pollen parents of diverse groups.

Micropropagation was effective in maintaining the male sterile lines. *In vitro* maintenance, multiplication and multiplication of male sterile plants were undertaken in tissue culture laboratory of Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Vellanikkara. Standardized protocol was used for the experiment. All the *in vitro* regenerated plants exhibited stability in male sterility. Sterility was assessed on the basis of pollen fertility percentage, which was found to be zero throughout the flowering season. There was no observable difference with respect to vegetative growth or production of female flowers between male sterile and fertile plants. These plants produced rudimentary male buds in racemes which failed to open. Cytological analysis of pollen mother cells revealed normal meiosis. Tetrads were formed normally. Post meiotic degradation of pollen grains was observed. No fruit set was observed on selfing but crossing yielded normal fruits. Seed setting was also normal.

Fourteen ridge gourd genotypes were collected from different parts of the country were raised in experimental field of Department of Olericulture, College of Horticulture, Vellanikkara. The extent of genetic variability among these accessions with respect to 14 traits was studied during May to August, 2008.

Analysis of variance revealed significant variability among these genotypes with respect to characters observed.

The genotypes were grouped into 5 cluster based on Mahalonobis's D^2 statistics. Pollen parents from diverse groups were selected for hybridization with male sterile female parent. Hybrid seeds were collected and stored for further studies.

The F_1 hybrids were evaluated along with their respective parents during January to April, 2009. The crop was raised in experimental field of Department of Olericulture. Heterosis over mid, better and standard parent were estimated. All the five hybrids expressed superiority over standard parent for number of fruits per plant. Three hybrids expressed standard heterosis for yield per plant. Hybrid MS x Arka Sumeet performed best for yield and yield related traits.

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. This situation reveals a unique system of male sterility and fertility restoration. It is not a recessive genic type which is predominant in cucurbits. Inheritance of sterility and restoration of fertility may be a complex mechanism. The available information on male sterility is not sufficient to explain this mechanism. Nuclear factor may be governing the male sterility and fertility restoration. This may be result of partial dominant gene action involving more than one gene. The exact genetic mechanism governing this character can only be explained after studying the F_2 and back cross population of MS x Arka Sumeet and three way cross populations involving male sterile hybrids as female and Arka Sumeet as male parent. The restorer gene(s) may have sporophytic or gametophytic mode of restoration. New male sterile lines can be developed by series of back crosses using one of the suitable pollen parents which produced sterile hybrids. The pollen parent Arka Sumeet, which produced partially sterile

hybrid, can be used as restorer line and also pollen parent for commercial hybrid seed production.

This study has attempted to trace out the unique mechanism of male sterility and fertility restoration in ridge gourd and is the first report of the fertility restorer line in ridge gourd.

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APPENDIX I

Data on weather change in COH,Vellanikkara campus from 01/01/09 to 13/05/09									
Week		Temperature (^{0}C)		Humidity (%)		Wind speed (km/h)	Bright sunshine (hrs/day)	Rainfall (mm)	Evaporation (mm)
No.	Date	Max	Min	I	II				
1	1/1-7/1	32.0	20.2	77.0	39.0	5.8	9.6	0.0	5.3
2	8/1/-14/1	32.4	23.5	65.0	40.0	10.4	9.9	0.0	7.8
3	15/1-21/1	31.8	23.0	65.0	37.9	10.9	9.9	0.0	8.0
4	22/1-28/1	33.6	21.5	71.0	38.0	5.9	8.6	0.0	6.3
5	29/1-4/2	35.2	20.2	78.0	27.0	5.0	9.6	0.0	5.9
6	5/2-11/2	34.5	21.8	69.0	28.0	5.9	9.9	0.0	6.9
7	12/2-18/2	34.5	21.8	82.0	35.0	4.5	9.5	0.0	5.7
8	19/2-25/2	35.7	23.2	75.0	39.0	6.0	10.0	0.0	7.1
9	26/2-4/3	35.4	24.6	93.0	56.0	3.6	7.9	0.0	5.2
10	5/3-11/3	35.0	24.3	84.0	53.0	9.4	7.9	0.0	6.3
11	12/3-18/3	35.3	23.9	86.0	49.0	10.5	8.3	3.3	5.6
12	19/3-25/3	35.1	24.3	88.0	51.0	6.8	7.7	0.8	5.4
13	26/3-1/4	35.1	25.2	89.0	56.0	9.7	7.9	0.0	5.0
14	2/4-8/4	36.6	25.3	90.7	62.4	9.1	6.6	2.4	5.3
15	9/4-15/4	33.1	21.5	91.0	60.0	4.8	2.9	0.6	3.6
16	16/4-22/4	29.0	26.0	89.1	65.7	6.6	5.0	0.4	4.5
17	23/4-29/4	34.8	25.2	85.0	52.9	8.0	8.0	0.0	5.5
18	30/4-6/5	30.2	26.0	85.1	56.4	7.4	7.1	0.4	5.4

MALE STERILITY AND ITS UTILIZATION FOR CROP IMPROVEMENT IN RIDGE GOURD *Luffa acutangula* (L.) Roxb.

By

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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ABSTRACT

The present investigation on male sterility and its utilization for crop improvement in ridge gourd is undertaken with the objective of investigating the stability of male sterility in ridge gourd *Luffa acutangula* (L.) Roxb. and expression of male sterility on combinations with different pollen parents of diverse groups.

Micropropagation was effective in maintaining the male sterile line. Standardized protocol was followed for *in vitro* maintenance of male sterile line. *In vitro* regenerated plants exhibited stable male sterility all round the flowering season. Pollen fertility found to be zero in all the male sterile plants. Cytological analysis of pollen mother cells revealed normal meiosis in form of tetrad formation and pollen degradation found to be in post meiotic stage.

Fourteen ridge gourd genotypes were collected from different parts of the country and evaluated for variability with respect fourteen traits. The genotypes exhibited significant variability for the characters studied. Genotypes were grouped into five clusters based on Mahalanobis's D^2 statistics. Five pollen parents from diverse groups were selected for hybridization with the male sterile female parent.

Heterosis values were estimated over mid, better and standard parents. Out of five hybrids, four were male sterile and one was partially fertile. Inheritance of male sterility and restoration of fertility is a complex mechanism and the available information on male sterility is not sufficient to explain this unique mechanism. Available result points towards the presence of partial dominant gene action in controlling male sterility. 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.

This is the first study which attempted to trace out a unique mechanism of male sterility and fertility restoration in ridge gourd. This is the pioneer work in ridge gourd where male sterility is used for crop improvement.