INHERITANCE OF MALE STERILITY AND DEVELOPMENT OF NEW MALE STERILE LINE IN RIDGE GOURD (*Luffa acutangula* (L.) Roxb.

By

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(2009 - 12 116)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **'Inheritance of male sterility and development of new male sterile line in ridge gourd (*Luffa acutangula* (L.) Roxb.'** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree diploma fellowship or other similar title of any other university or society

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Dedicated to the evergreen Indian Agriculture

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1 INTRODUCTION

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

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 1965) 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 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 1990) Seeds are reported to possess purgative emetic and anthelmintic properties due to the secondary metabolite *cucurbitacin* (Robinson and Decker Walters 1997)

In ridge gourd (*Luffa acutangula* L (Roxb)) heterosis breeding is a proven method of crop improvement (Mole *et al* 2001 Hedau and Sirohi 2004) The female flowers are solitary whereas male flowers are in racemes Though male sterility was reported in ridge gourd over three decades before (Deshpande *et al* 1979) its utilization in crop improvement programme did not receive much attention Recently a natural mutant line was reported by Pradeepkumar *et al* (2008) which could be used for hybrid seed production programmes In an attempt to elucidate the genetics of male sterility in ridge gourd Hegade (2009) studied the crosses involving male sterile female parent and pollen parents selected from different parts of India Four hybrids out of five were sterile (MS x CO 2 MS x Deepthi MS x IC 92685 MS x IC 92671) and one (MS x Arka Sumeet) was found to be partially fertile This indicate the presence of partial dominant gene action in cytoplasmic genes controlling male sterility which is not yet reported in cucurbits The pattern of inheritance of male sterility and restoration of fertility can only be explained by studying the F_2 and back cross generations and the three way cross involving male sterile hybrids and the pollen parent which restores the fertility A detailed study is required about the genetic mechanism governing the male sterility and the stability of the trait over generations as male sterility may be heritable or transient

Ridge gourd offers great scope for exploitation of hybrid vigour on commercial scale 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 In cross involving *in vitro* regenerated male sterile line MS x Arka Sumeet performed best for yield and yield related traits which points towards the scope of exploiting male sterile lines for heterosis improvement in ridge gourd (Hegade 2009)

Hence the present investigation on male sterility in ridge gourd is undertaken with the objective of investigating the inheritance of male sterility in ridge gourd (*Luffa acutangula* (L) Roxb and to develop new male sterile line in ridge gourd using back cross generations of sterile hybrids Study also aims in evaluating the performance of F_1 hybrid MS x Arka Sumeet for horticultural characters

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

The information available on male sterility inheritance of fertility restoration and exploitation of heterosis using male sterile line in ridge gourd and other relevant crops are reviewed under the following heads

2 1 Male sterility

2 2 Genetics and inheritance of male sterility in cucurbits

2 3 Inheritance of fertility restoration

2 4 Markers linked with male sterility in cucurbits

2 5 Heterosis in ridge gourd

2 1 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 (Table1) on availability characterization mechanism and utilization aspects of male sterility in vegetable crops are available (Kalloo 1988 Kalloo and Berg 1993) Onion crop provides one of the rare example of very early recognition of male sterility (Jones and emsweller 1936) since then male sterility has been reported in several vegetables These male sterile plants were either isolated in natural population or were artificially induced through mutagenesis (Kaul 1988) In recent past male sterility systems have been also developed through genetic engineering (Williams *et al* 1997 Kumar *et al* 2000) and protoplast fusion (Pelletier *et al* 1995)

2 1 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

2 1 1 1 NUCLEAR OR GENIC MALE STERILITY (GMS)

As the name suggests nuclear male sterility (earlier termed as GMS) is controlled by the gene(s) from the nuclear compartment Most of the naturally occurring or induced male sterile mutants are recessive in nature with few exceptions in cole vegetables (e g cabbage and broccoli) and genetically transformed male sterile lines (Kaul 1988 Williams *et al* 1997) Certain mutants which although produce functional pollen pollen fail to self fertilize either due to non dehiscence of pollen or their special flower morphology e g positional sterility in tomato (Atanassova 1999) and functional male sterility in eggplant (Phatak and Jaworski 1989)

The occurrence of predominantly recessive male sterility clearly indicates that GMS is the result of mutation in any gene(s) controlling microsporogenesis (pollen development process) stamen development or microgametogenesis (male gamete development process) *EGMS Line* Certain GMS lines are conditional mutants meaning thereby in a particular environment male sterile mutant plants turn into male fertile After determination of critical environment (usually temperature or photoperiod) for sterility and fertility expression such GMS mutants are classified under environmental sensitive genic male sterile (EGMS) lines In vegetable crops mostly temperature sensitive EGMS lines have been reported (Table 3) From practical application view point it is necessary to

Table 1 Nuclear and cytoplasmic male sterility in selected vegetable species

Vegetable spp	Salient features of GMS	Salient features of CMS
Tomato (*Lycopersicon esculentum*)	More than 55 recessive genes have been reported (Kaul 1988 Georgiev 1991) *sl* 2 *ms* 13 and *ms* 15 are temperate sensitive (Sawhney 1997) *ps* 2 gene has been exploited at commercial scale (Atanassova 1999) YAC clone containing *ms* 14 gene has been cloned (Gorman *et al* 1996)	Sterile cytoplasm has been derived from the distinct species through protoplast fusion (Melchers *et al* 1992 Petrova *et al* 1999) restorer gene (*Rf*) is not available (Petrova *et al* 1999)
Egg plant (*Solanum melongena*)	Monogenic recessive gene has been reported (Kaul 1988) monogenic recessive functional sterility available (Phatak and Jaworski 1989)	Not reported
Pepper (*Capsicum annuum*)	More than 12 recessive genes have been reported (Shifriss 1997) MS 12 (*ms* 509/*ms* 10) and *ms* 3 genes are commercially utilized in India and hungry respectively (Kumar *et al* 2000) The *ms* 10 gene is linked with taller plant height erect growth and dark purple anther (Dash *et al* 2001)	First reported in a Indian accession (Peterson 1958) most of the CMS lines are temperature sensitive (Shifriss 1997) occurrence of *Rf* allele is common in small fruited (usually hot *pepper*) and *rf* in large fruited (usually sweet pepper) lines (Shifriss 1997) RAPD markers lined to *Rf* gene have identified (Boaxi *et al* 2000)
Cole vegetables (*Brassica Spp*)		
Cauliflower (*B Ole var Botrytis)*	Both recessive and dominant genes have been reported (Kaul 1988 Kumar *et al* 2000)	In Cole crops vegetables sterile cytoplasm derived from *Brassica* (Pearson 1972) and *Raphanus sativus* Ogura tupe (Ogura 1968) problem of seedling yellowing (at low temperature) associated with Ogura based CMS lines of broccoli cauliflower cabbage brussels sprout has been solved using protoplast fusion
Cabbage (*B Ole var Capitata)*	Both recessive (Nieuwhof 1961) and dominant (Fang *et al* 1997) genes have been reported RAPD markers lined to dominant gene has been identified (Wang *et al* 1998) and its use in hybrid seed production has been proposed	

Table 1 Contd

Vegetable spp	Salient features of GMS	Salient features of CMS
Brussels sprout (*B Ole var Gemmifera)* Broccoli (*B Ole var Italica)*	Recessive male sterile mutant has been reported (Nieuwhof 1968 Kaul 1988) Six recessive non allelic genes have been reported (Dickson 1970) linkage of ms gene with bright green hypocotyle (Sampson 1966)	Cybrid CMS lines of cabbage and cauliflower are being utilized by seed companies in France (Pelletier *et al* 1995) protoplast fusion has been utilized to transfer Ogura cytoplasm from broccoli into cabbage (Sigareva and Earle 1997)
Muskmelon (*Cucumis melo*)	Five recessive non allelic genes have been reported (McCreight, 1983) ms 1 is commercially utilized in India (Kumar *et al* 2000)	Not reported
Cucumber (*Cucumis sativus*)	Monogenic recessive gene has been reported (Barnes 1961 Kaul 1988) limited scope of utilization because of the availability of gynocious lines (Kumar *et al* 2000)	Not reported
Summer squash (*Cucurbita moschata*)	Monogenic recessive gene has been reported (Eisa and Munger 1968 Kaul 1988) very limited scope of utilization because of the availability of sex regulating mechanism using certain chemicals	Not reported
Onion (*Allium sativum*)	Monogenic recessive gene has been reported (Kaul 1988)	Two types of sterile cytoplasms *viz* S (Jones and Clarke 1943) and T (Berninger 1965) have been reported S cytoplasm is most widely exploited

		(Pelletier *et al.*, 1995).
Carrot (*Daccus carota*)	Recessive male sterile genes have been reported (Welch and Grimball, 1947; Kaul, 1988); utilized at commercial scale because of the availability of cms lines (Singh, 2005).	Two types (petaloid and brown anther) of male sterile lines are available (Welch and Grimball, 1947; Morelock, 1974); genetics of fertility restoration is complex (Peterson and Simon, 1986) because structural variants of mt DNA are numerous (Ranfort *et al.*, 1995).
Radish (*Raphanus sativus*)	Three recessive mutants have been reported (Tokumasu, 1951; Kaul, 1988); commercially utilized (Singh, 2005).	Sterile cytoplasm widely distributed in wild radish; occurrence of *Rf* allele is frequent in Europian and Chinese cultivars and *rf* in Japanese cultivars (Yamagishi and Terachi, 1996).

Table 2. 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 noticable 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; Banga *et al.*, 2005; Haihe *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; McCreight and Elmstorm, 1984a; 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 F_1 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.

identify critical temperature or photoperiod for the fertility/sterility expression in temperature and photoperiod sensitive genetic male sterility, respectively.

2.1.1.1.1 Development of GMS

The monogenic recessive gene controlling male sterility is transferred in the desirable genotype through backcrossing programme. After the identification of recessive male sterile mutant plant, the first step is to cross it with plant of the same variety. In the F_2 and subsequent generations, male sterile plants are pollinated with the bulk pollen from all the male fertile segregants and seeds from only male sterile plants are harvested to raise next generation. Thus after four generations, homozygous male fertile (*MsMs*) plants are eliminated from the population and only heterozygous male fertile (*Msms*) and male sterile (*msms*) plants remain in the population. Thereafter, GMS plants (*msms*) are maintained by back crossing it with heterozygous isogenic line (*Msms*) for male sterility.

2.1.1.1.2 Utilization of GMS

Since GMS is maintained through backcrossing, in hybrid seed production field, 50% male fertile segregants (*Msms*) need to be identified and removed before they shed pollen. In some GMS lines, *ms* genes are tightly linked with the recessive phenotypic marker genes (Table 4). Such marker genes, especially which expresses at seedling stage, are good proposition for the identification of sterile/fertile plants at seedling stage. Hybrid seed production using EGMS line is more attractive because of the ease in seed multiplication of male sterile line.

Seeds of EGMS line can be multiplied in an environment where it expresses male fertility trait while hybrid seeds can be produced in other environment, where it expresses male sterility. Because of more tedious maintenance process and non-availability of suitable marker gene among the vegetable crops, utilization of GMS is restricted only in few vegetables .The identification of fertilizing cytoplasm for specific nuclear male sterile gene (Horner and Palmer, 1995), is an interesting research area, which upon success, may provide opportunity for most efficient utilization of GMS lines, like CMS line.

2.1.1.2 CYTOPLASMIC MALE STERILITY (CMS)

The expression of male sterility in CMS plants is the result of incompatibility between recessive nuclear gene (called maintainer gene; *rf*) and male sterile specific cytoplasmic genome. Now it is well documented that specific open reading frame (ORFs) in mitochondrial genome (mt-genome) are responsible for the expression of male sterile trait (Kumar *et al.*, 2000). Once dominant restorer (*Rf*) gene (located in nuclear genome) responsible for pollen fertility of a cytoplasmic male sterile line is identified, it is commonly known as cytoplasmic- genic male sterility (CMS).

Therefore, those cytoplasmic male sterile lines for which *Rf* gene(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, both CMS and G-CMS can be described under common head (*i.e.* CMS) because of the fact that in both these systems, expression of male sterility is due to the defect in the cytoplasm (mt-genome). Based on mode of action of the pollen fertility restorer (*Rf*) and mainainer (*rf*) alleles, CMS are of two types, *viz.*, (i) gametophytic and (ii) sporophytic. In gametophytic system, expression of restorer allele is pollen specific, thus the pollen grains are the responding elements (e.g. S-cytoplasm in corn, abortive cytoplasm in rice etc.). Therefore, a plant heterozygous for maintainer-restorer locus (*Rfrf*) produces 50% aborted (*rf*) and 50% normal (fertile) pollen (*Rf*). Pollen from such plant (*Rfrf*) crossed on to a sterile plant (*rfrf*), will again produce plants with 50% each of aborted and normal pollen. In contrast, all pollen are either fertile or sterile in sporophytic system, which is most common (Pearson, 1981).

A heterozygous restorer line (*Rfrf*) in this system produces all fertile pollen and when crossed on to a sterile plant (*rfrf*), produces 50% absolute male sterile and 50% absolute male fertile plants. Cytoplasmic male sterility may originate from inter-generic or inter-specific crosses and may be artificially induced through mutagenesis or antibiotic effects on cytoplasmic genes (Kaul, 1988). Cytoplasmic male sterile plants have also been developed in several vegetables through protoplast fusion (Pelletier *et al.*, 1995). In near future, genetically engineered cytoplasmic male sterility may be available after standardization of transformation technique for organelle genome.

GCMS is best exploited for commercial F_1 hybrid-seed production in onion, and the sterile genotype (a line) is characterized by the nuclear gene in recessive condition *i.e.* msms while presence of dominant allele either in homozygous(MsMs) or heterozygous condition (Msms) ensure male fertility(Jones and Mann, 1963). There is an extra nucleus factor controlling fertility and here S denotes sterile cytoplasm and N the normal cytoplasm and recessive nucler GMS ensure male sterility in onion. Change in the constituton of any of the factors restore fertility. Hence genotypes like 'SMsMS', 'SMsms', 'NMsMs', 'NMsms' and 'Nmsms' are all male fertile.

Three parential lines, A, B and C are used for hybrid-seed production. The female parent (A line) has a genetic constitution of 'Smsms' and line B is 'Nmsms'. Since fertile cytoplasm factor is not transferred to female, A line remain male sterile when crossed with B line, which differs only by its sterile cytoplasmic factor. The B line is used to maintain seed supply of male-sterile A line. Both genetic sterility and cytoplasmic sterility factors must be present for the plant to be male sterile. Line C is fertile male-parent, an inbred, ideally diverse from line A or B for ensuring heterotic cross.

2.1.1.2.1 Utilization of CMS

The CMS system is the most commonly utilized male sterility to produce commercial hybrid seeds of several vegetables. The CMS based hybrid development is often term as three line method of hybrid breeding involving A line (male sterile; S-*rfrf*), B line (maintainer; N-*rfrf*) and C or R line (restorer; S-or N-*RfRf*). As mentioned, CMS line without restorer male parent cannot be utilized in fruit producing vegetables (e.g. chilli), but it can be utilized in vegetables where vegetative part is of economic value (e.g. onion, cole vegetables, carrot, radish, leafy vegetables etc.). The CMS system though is the most commonly utilized, its utilization is restricted in specific species because of the following limitations:

- Non-availability of CMS in many crops and their wild relatives.

• Need of fertility restorer allele in fruit producing vegetables.

• Undesirable pleiotropic effect of sterile cytoplasm on horticultural qualities.

• Highly unstable sterile cytoplasm in several cases.

• Poor cross pollination ability of flowers of plants with sterile cytoplasm due to altered morphology.

• Technical complexity involved in seed production and maintenance of parental lines.

Besides, vulnerability of sterile cytoplasm to specific diseases is a major risk due to monopolistic cultivation of hybrids derived from single source of sterile cytoplasm. The devastation of corn hybrids derived from T-cytoplasm by *Helminthosporium* blight in USA during 1970's (Levings, 1990), is a well known example of such risk.

2.1.1.3 TRANSGENIC MALE STERILITY SYSTEMS

From the beginning of 1990's, new genetic approaches have been proposed and implemented to develop male sterility systems through genetic transformation (Mariani *et al.,* 1992). The ability to design new molecular strategies and their successful execution has been possible because of the isolation, cloning and characterization of anther or pollen specific genes and promoter sequences. These genes are expressed in pollen themselves (gametophytic expression) or cells and tissues (sporophytic expression) that directly or indirectly support pollen development, such as tapetum, filament, anther wall. Williams *et al.* (1997) reviewed the reports on genetically engineered male sterility systems under dominant male sterility, recessive male sterility, targeted gametocide and dual method.

However, based on mechanism of male sterility induction and fertility restoration, all transgenic male sterility systems developed so far can be described under five classes, *viz.*, (i) abolition-restoration system, (ii) abolition-reversible system, (iii) constitutive-

reversible system, (iv) complementary-gene system and (v) gametocide-targeted system. Although in transgenic(s) developed within one system, mode of action of trans-gene(s) remains the same, there can be variations in trans-gene constructs including promoter, targeted site (depending upon the promoter used) and methodology adopted within one system. All the transgenic male sterile lines developed till date are GMS, since they have been developed through transformation of male sterility causing gene construct(s) inside the nuclear genome. Regardless of the crop, all transgenic male sterility systems (except constitutive- reversible) with the same trans-gene construct(s) may be utilized to develop transgenic male sterile lines in those vegetables, where transformation and regeneration protocols have been standardized.

2.1.2 Male Sterility and its Commercial Utilization

In tomato (*Lycopersicon esculentum*; $2n = 24$), approximately 19 male sterile based hybrids have been released, 17 of them are based on functional sterility controlled by gene positional sterility (*ps-2*). The use of *ps-2* mutant has become applicable at commercial scale in few countries like Czech Republic, Moldova and Bulgaria (Atanassova, 1999). The *ms-1035* gene is linked with a recessive marker gene (*a*) responsible for absence of anthocyanin. Hence, *ms-1035* sterile plant can be identified at seedling stage and fertile plant can be rouged out in the nursery itself (Georgiev, 1991). Atanassova and Georgiev (1986) suggested that genes *ms-1035*, *ms-1526* and *ps-2* combined with short styles are most promising for hybrid development. In pepper (*Capsicum annuum*; $2n = 24$), the induced male sterile gene in France (*mc-509*; Pochard, 1970; renamed *ms-10* by Daskalov and Poulos, 1994) was found allelic to *msk* allele isolated spontaneously in Korea (Shifriss, 1997).

The *ms-2* line identified by Shifriss and Rylski (1972), was found non-allelic to *ms-1* isolated by Shifriss and Frankel (1969). The *ms-509* line (bell pepper type) of Pochard was introduced in India at Punjab Agricultural University (PAU) and *ms-10* was introgressed in three chilli, genotypes, *viz.*, MS-12, MS-13 and MS-41 (Singh and Kaur, 1986). The MS-12 (*ms-10ms-10*) line is being utilized to produce hybrid seeds of CH-1 and CH-3 hybrids of hot pepper in Punjab state (Hundal, personal communication). In Hungary, male sterile lines possessing *ms-3* gene are being utilized to produce hybrid seeds (Kumar *et al.*, 2000). In the

recent past, development of stable CMS lines of hot pepper has led to its increased utilization in hybrid development.

In South Korea (Shifriss, 1997), China (Boaxi *et al.,* 2000) and India, CMS lines are being utilized to develop hybrids of hot pepper. However, non-availability of *Rf* genes in most of the sweet pepper genotypes is still a handicap in developing CMS based commercial sweet pepper hybrids. Among cole vegetables (*Brassica spp.* $2n = 18$), although GMS based experimental crosses have been developed, it has not been commercially utilized mainly because of the difficulty in multiplication of male sterile seeds and availability of self-incompatibility and CMS systems.

Pelletier *et al.* (1995) developed CMS cybrid plants in cabbage and cauliflower and they were of normal flower morphology, with good nectar production and found highly stable. These promising cybrids, contained genomes resembling more to the *B. oleracea* type than the Ogura and being utilized by seed companies in France to develop hybrids of cabbage and cauliflower. Seed companies in India are also developing CMS based hybrids of cabbage and cauliflower (Singh 2005). On the basis of floral morphology, radish (*Raphanus sativus*; $2n = 18$) CMS lines are generally classified into three types: (i) degenerative corolla, (ii) shrivelled stamen and (iii) abortive pollen. Success has been achieved to identify maintainer of Ogura cytoplasm and transfer of sterile cytoplasm in the new genetic background (Nieuwhof, 1961; Hawaldar *et al.,* 1997). Ogura cytoplasm has been found widely distributed among the wild Japanese radish plants and most of the early European radish populations, in which availability of maintainer allele is more frequent. Where as, most of the Asian radish cultivars including Japanese cultivars possess normal cytoplasm except few cultivars from Tibet and Taiwan (Nieuwhof, 1968; Yamagishi and Terachi, 1994 ; 1996). In a study by Yamagishi (1998), restorer allele was found to be widely distributed in wild radish, European and Chinese cultivars, while occurrence of maintainer allele was more frequent in the Japanese cultivars.

The male sterility in radish is being utilized by seed companies in Taiwan, China, Korea and Japan. In India also seed companies are using CMS system for hybrid development (Singh, 2005). Carrot (*Daucus carota*; $2n = 18$) is one of the few crops in which male sterility was documented very early *i.e.* in the year 1885 (Kaul, 1988). Several

other genic male sterile mutants have been described (Kaul, 1988), however, none of them have been utilized for commercial seed production due to the availability of more efficient CMS system in carrot. Two types of sterile cytoplasm have been reported, namely, (i) petaloid and (ii) brown anther (Welch and Grimball, 1947; Morelock, 1974). Taki Seed Company of Japan developed the first F_1 hybrid variety in 1982 using CMS (Pelletier, *et al.*, 1995). In USA, majority of hybrids are produced from one cytoplasm *i.e.*, Cornell cytoplasm. Considering the risk of disease vulnerability of hybrid variety due to the monopolistic use of Cornell sterile cytoplasm, USDA has released a petaloid type new CMS line derived from sterile cytoplasm of Wisconsin (Morelock *et al.*, 1996).

In onion (*Allium cepa*; $2n = 16$), first male sterile plant was reported within the progenies of an onion cultivar Italian Red (Jones and Emsweller, 1936), which was cytoplasmically inherited and male sterility was under the control of single recessive nuclear restorer locus (Jones and Clarke, 1943). World wide more than 50% onion varieties currently cultivated are F_1 hybrids derived from S-cytoplasm (Pelletier *et al.*, 1995). In India, public sector bred commercial hybrid has not been recommended so far, however, CMS based hybrids are developed at several seed companies and institutes like as Indian Institute of Horticultural Research(IIHR), Indian Agricultural Research Institutes(IARI) etc. In muskmelon (*Cucumis melo*; $2n = 22$), first recessive *ms* gene was reported by Bohn and Whitaker (1949), since then at least four additional male sterile recessive alleles, *viz.*, *ms-2* (Bohn and Principe, 1964), *ms-3* (McCreight and Elmstrom, 1984b), *ms-4* (Pilrat, 1990) and *ms-5* (Lecouviour *et al.* 1990) have been identified. The *ms-1* line has been successfully utilized in India, to develop first commercial hybrid (Punjab Hybrid-1) in vegetable crops, through the exploitation of male sterility at PAU, Ludhiana (Kumar *et al.*, 2000).

2.1.3 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 was 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 Hegade (2009) collected fourteen ridge gourd genotypes from different parts of the country and 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 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 Male sterility in ridge gourd is a form of sporogenous male sterility where stamens form but viable pollens are absent (Pradeepkumar *et al* 2010) Pollen from male sterile lines was poorly stained with acetocarmine (1% solution) as compared to the pollen from fertile line Meiotic analysis of pollen mother cells (PMCs) in the male sterile line revealed normal meiosis but the microspores of the male sterile line did not take up stain in later stages and showed abnormality in morphology such as shrunken nature reduction in size and sterility compared to those from normal fertile flowers

2 1 4 Male sterility in other cucurbits

Single recessive genes for male sterility are known for cucumber melon squash and watermelon (Robinson *et al* 1976) Since no cytoplasmic factor is known in cucurbits to interact with any of the male sterile genes male sterile lines are maintained by crossing plants heterozygous with those homozygous for the male sterile gene using the latter as the female parent The progeny segregates 1 1 for male sterility and must be rogued to remove the fertile plants when used to produce hybrid seed In watermelon the *GMS* gene for male sterility is associated with glabrous foliage and male sterile plants can be identified in the seedling stage

Kalloo (1988) proposed that hybrid melon seed could be produced by open pollination of monoecious male sterile plants He determined that male fertile monoecious plants produced 58% hybrid seed when grown with normal melon plants He calculated that if the monoecious maternal parent segregated 1 1 for male sterility then the population should produce a proportion of hybrid seed intermediate to that amount and the nearly 100%

hybrid seed that male sterile plants produce This assumption was correct for he determined that a monoecious line segregating 1 1 for male sterility produced 78% hybrid seed

The use of male sterility in hybrid seed production for cucurbits has been limited because of the labor required to rogue segregating male sterile lines the possibility of some selfing of the maternal parent of a hybrid if some male fertile plants are not rouged before they flower and the reduced seed yield when seed is harvested from only 50% of the plants Many male sterile mutants cannot be identified until flower buds are visible Rogueing in this late stage of development may result in some male fertile plants flowering and providing pollen to produce nonhybrid seed before they are removed In addition detrimental effects are associated with some male sterile genes

Male sterile cucumber plants can have reduced seed production (Shifriss 1945) Zhang *et al* (1994) concluded that the use of a pollen sterile cucumber mutant in F hybrid seed production is not practical Bhattacharya Kato and Jodo (1970) observed that male sterile melons had smaller fruit than normal in the winter greenhouse More and Seshadri (1998) concluded that the practicality of large scale seed production of hybrid melons by using male sterility is not yet worked out In the U S the principle use of male sterility in hybrid cucurbit seed production has been for winter squash *Cucurbita maxima* Male sterility has been used in China for producing hybrid watermelon seed (Zhang and Rhodes 1993)

2 2 Genetics and inheritance of male sterility in cucurbits

Many workers reported the presence of male sterility in cucurbitaceous crops and they also reported the inheritance pattern 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 hybrids between male sterile and male fertile plants were fertile and m

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

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 1984a 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 1984b) 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)

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 and BC 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 F_2 and back cross progenies revealed that the trait is governed by single recessive gene

2 3 Inheritance of fertility restoration

2 3 1 Inheritance of fertility restoration in ridge gourd

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 Hegade (2009) reported fertility in the hybrid MS x Arka Sumeet and observed both fertile and sterile pollen He hypothised gametophytic control of fertility restoration in Arka Sumeet Nuclear factor of Arka Sumeet may be governing the restoration of fertility He assumed a partial dominant gene action involving more than one gene governing male fertility sterility and fertility restoration in ridge gourd

2 3 2 Inheritance of fertility restoration in other vegetable crop

Roundy and Theurer (1974) in sugarbeet suggested that in the mutant studied a single recessive gene designated *yl* controlled the yellow leaf character and the pollen fertility restoring factor (*Rf3*) was inherited as a single dominant gene There was no linkage between *yl* and the monogerm (m) annual growth habit (B) and *Rf3* genes

Peterson (1958) reported that a major dominant gene governs fertility restoration in pepper However in several testcrosses a hybrid ratio for male sterile and fertile plants was also observed suggesting thereby two independent loci controlling the fertility restoration (Peterson 1958) Contrasting results were reported by Novak *et al* (1971) where in one segregation ratio was in agreement with 3 1 ratio and other 9 7 suggesting complementary gene action of fertility restoration During this study F_2 and test cross segregation ratio revealed presence of single dominant gene for fertility restoration in Pant C 1 These discrepancies with respect to results on inheritance of fertility restoration may be explained on the basis of differences in the utilized parental genotypes at fertility restoration locus Since Pant C 1 is a strong restorer with single gene it should preferably be used in restorer breeding especially in case of sweet pepper lines most of which restorer gene

Kumar *et al* (2000) raised F_2 and test cross population of CCA 4261 x PantC 1 during two consecutive season and individual plant were evaluated along with F_1 plants The pant C 1 was confirmed to be a strong restorer line

2 4 Markers linked with male sterility in cucurbits

2 4 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 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 Heterosis in ridge gourd

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 vigour 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 5 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 per cent (LA 44 x LA 83) Maximum negative relative heterosis was observed for LA 81 x LA 86 (3 57 per cent) 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 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 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 per cent 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 per cent followed by VRST 62 x VRST 61 (14 55%) Hegade *et al* (2009) reported maximum heterobeltiosis (35 56%) and maximum standard heterosis (35 56%) for days to emerging first female flower in the cross (MS x CO2)

2 5 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 per cent (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 42 26 per cent over standard check in LA 43 x LA 37 for this trait Hegade *et al* (2009) reported maximum heterobeltiosis (57 45%) and maximum standard heterosis (11 90%) for node at first female flower in the cross (MS x IC92671)

2 5 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 per cent over the better parent and 0 04 to 23 35 per cent 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 per cent over better parent and DRG 1 x AAUJ 3 gave 23 35 per cent heterosis over top parent Shankarnag and madalageri (2006) reported highest economic heterosis was expressed by the hybrid L5 x T14 (24 59%) Kaddi Girwani *et al* (2008) reported the heterosis for number of fruit per plant range from 14 45 to 127 02 in the cross CO1 x CLN363 Hegade *et al* (2009) reported maximum heterobeltiosis (13 19%) and maximum standard heterosis (37 38%) for number of fruits per plant in the cross (MS x IC92671)

2 5 4 Average fruit weight

Mole *et al* (2001) 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 standard 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 Girwani *et al* (2008) reported the heterosis for average fruit weight range from 11 39 to 175 21% in the cross Cul no110 x L232 Hegade *et al* (2009) reported maximum heterobeltiosis (23 29%) and maximum standard heterosis (23 03%) for average fruit weight in the cross (MS x Deepthi)

2 5 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 heteiosis for average fruit length in ridge gourd (Mole *et al* 2001) Shankarnag and Madalageri (2006) reported heterobeltiosis and economic heterosis ranged from 36 07 to 9 16 and 20 70 to 35 50 per cent in the cross L3 x T12 Hegade *et al* (2009) reported maximum heterobeltiosis (19 79%) and maximum standard heterosis (19 79%) for fruit length in the cross (MS x CO2)

2 5 6 Fruit girth

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 heterosis 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 Hegade *et al* (2009) reported

maximum heterobeltiosis (1 08%) and maximum standard heterosis (13 66%) for fruit girth in the cross (MS x Arka Sumeet)

2 5 7 Days to first harvest

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 per cent Hegade *et al* (2009) reported maximum heterobeltiosis (2 80%) and maximum standard heterosis (10 33%) for days to first harvest in the cross (MS x Deepthi) Earliness one of the most important parameter in hybrids is indicated by number of days for first fruit harvest

2 5 8 Yield per plant

Mole *et al* (2001) 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 Satputia 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 Girwani *et al* (2008) reported the heterosis for fruit yield per plant range from 30 93 to 218 97% in the cross CO1 x L 232 Hegade *et al* (2009) reported maximum heterbeltiosis (10 14%) and maximum standard heterosis (77 47%) for yield per plant in the cross (MS x Arka Sumeet)

MATERIALS AND METHODS

3 MATERIALS AND METHODS

The study was conducted in the Vegetable Research Farm of the Department of Olericulture 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 July to November 2010 and January to April 2011

The project consisted of the experiments written hereunder

3 1 Analysis of F_2 population of the cross MS x Arka Sumeet and back cross generations and the three way crosses involving male sterile hybrids and the pollen parent which restores the fertility (Arka Sumeet)

3 2 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters along with parent Arka Sumeet and popular KAU varieties *viz* Deepthi and Haritam

3 1 Analysis of F_2 population of the cross MS x Arka Sumeet and back cross generations and the three way crosses involving male sterile hybrids and the pollen parent which restores the fertility (Arka Sumeet)

F_2 population of the cross MS x Arka Sumeet was raised during July to November 2010 A total of 106 plants were grown keeping one plant per pit BC_1 (MSx Arka Sumeet) x Arka Sumeet was also raised during the season keeping a population of 42 plants Crop was maintained as per the package of practices recommendations (KAU 2007)

In the same season backcrosses three way crosses and parents were grown in three replications adopting randomized block design

Three way crosses

1 (MS x IC 92685) x Arka Sumeet
2 (MS x Deepthi) x Arka Sumeet
3 (MS x IC 92671) x Arka Sumeet
4 (MS x CO2) x Arka Sumeet

Back crosses
1 (MS x CO 2) x CO2
2 (MS x Arka Sumeet) x Arka Sumeet
3 (MS x Deepthi) x Deepthi
4 (MS x IC 92671) x IC 92671
5 (MS x IC 92685) x IC 92685

Parents

1 IC 92671
2 CO 2
3 IC 92685
4 Deepthi
5 Arka Sumeet

There were four pits per replication and one plant per pit Crop was raised as per the package of practices recommendations (KAU 2007) Plants were evaluated for pollen fertility and other biometric characters

3 1 1 Observations

Following observations were recorded during evaluation of F_2 backcross generations and three way crosses

3 1 1 1 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

3 1 1 2 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

3 1 1 3 Nodes to first fertile male flower

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

3 1 1 4 Nodes to first female flower

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

3 1 1 5 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

3 1 1 6 Number of fruits per plant

The total number of fruits produced per plant was observed

3 1 1 7 Fruit weight (g)

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

3 1 1 8 Fruit length (cm)

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

3 1 1 9 Fruit girth (cm)

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

3 1 1 10 Days to first harvest

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

3 1 1 11 Number of harvest

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

3 1 1 12 Yield per plant (kg/plant)

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

3 1 1 13 Number of seeds per fruit

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

3 1 1 14 Seed yield per fruit (g)

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

3 1 2 Statistical analysis

Statistical parameters *viz* range mean variance were calculated

3 1 2 1 Range

The statistical range is the difference between the lowest and highest valued numbers in a set of numbers

3 1 2 2 Mean

The *arithmetic mean* is the standard average often simply called the mean

$$x = \frac{1}{n} \sum_{i=1}^{n} x_i$$

3 1 2 3 Variance

The square of the standard deviation A measure of the degree of spread among a set of values a measure of the tendency of individual values to vary from the mean value If a random variable X has the expected value (mean) μ E[X] then the variance of X is given by

$$\mathrm{Var}(X) = \mathrm{E}[(X - \mu)^2]$$

3 1 2 5 Estimation of chi square test in the F_2 generation

The value of the test statistic is

$$\mathrm{X}^2 = \sum_{i\ 1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where

X^2 Pearson s cumulative test statistic which asymptotically approaches a χ^2 distribution

O – an observed frequency

E = an expected (theoretical) frequency asserted by the null hypothesis

n the number of cells in the table

3 2 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters along with parent, Arka Sumeet, popular KAU varieties *Viz*, Deepthi and Haritam and IC 92671

F_1 hybrid MS x Arka Sumeet and four parental lines *viz* Arka Sumeet Deepthi and Haritam and IC 92671 were grown during the period January to April 2011 in five replications adopting randomized block design There were four pits per replication and one plant per pit Crop was maintained as per the package of practices recommendation (KAU 2007)

3 2 1 Observation

Observations detailed during the earlier section were taken during the evaluation of F_1 hybrid MS x Arka Sumeet and four parental lines *viz* Arka Sumeet Deepthi Haritam and IC 92671

3 2 2 Statistical analysis

3 2 2 1 Estimation of ANOVA

Correction factor $(GT)^2/N$

Total sum of square – $\sum y_{ij}^2$ cf

Replication sum of square $\sum R_{ij}^2$ cf

Treatment sum of square – $\sum t_i^2/R$

Error sum of square TSS SSR SST

3 2 2 2 Estimation of heterosis

The mean values of parents and hybrid of those five replications for each character were taken for the estimation of heterosis in terms of two parameters 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) and for calculation of heterobeltiosis Arka Sumeet was taken as the better parent

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

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

Heterosis over standard parent (SP) standard heterosis

$$SH \quad \frac{\overline{F1} \quad \overline{SP}}{\overline{SP}} \times 100$$

3 2 2 2 Test of significance

Signficance for heterobeltiosis and Standard heterosis were calculated as follows

T value for HB F_1 BP/SE

T value for SH F_1 SV/SE

Standard error for heterobeltiosis and standard heterosis – $\sqrt{2/r\ EMS}$

Where

r Number of replication

EMS Error mean sum of square

3 3 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

Original male sterile stock plant is now maintained through tissue culture As there is no maintainer line developed so for the male sterile line is maintained under *in vitro* conditions at Centre for Plant Biotechnology and Molecular Biology (CPBMB) College of Horticulture Kerala Agriculture University Vellanikkara

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 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) and BA (2 mg l) 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})

In vitro rooted plants were transferred to disposable polythene cup (250ml) filled with vermicompost fine sand and soil in 3 2 1 ratio They were transferred to polythene cover (20 x 14 cm) after 14 days and kept there for hardening for 24 days Primary and secondary hardening took 38 days Hardened tissue cultured plants were transplanted to the field

RESULTS

4 RESULTS

Results obtained from the experiments are presented under the following headings

4 1 Regeneration of *in vitro* maintained male sterile genotype and evaluation for stability of male sterility

4 2 Analysis of F_2 cross MS x Arka Sumeet along with F_1 and back cross generation (MS x Arka Sumeet) x Arka Sumeet

4 3 Analysis of gene action governing male fertility and male sterility in F_2

4 4 Evaluation of back cross and three way cross for studying the expression of male sterility and restoration of male fertility

4 5 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters

4 1 *In vitro* maintenance and regeneration of male sterile genotype and evaluation for stability of male sterility

Orginal male sterile stock plant is now maintained through tissue culture 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}) Cuttings with 2 3 nodes from *in vitro* shoots were inoculated in multiplication medium (MS + BA 1 mg l^{-1}) 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}

In vitro rooted plants were transferred to disposable polythene cup (250 ml) filled with vermicompost fine sand and soil in 3 2 1 ratio They were transferred to polythene cover (20 x 14 cm) after 14 days and kept there for hardening for 24 days Primary and secondary hardening took 38 days Survival rate during

hardening was observed to be seventy per cent. Hardened tissue cultured plants were transplanted to the field (Plate 2). All the *in vitro* regenerated male sterile plants had a stable expression of male sterility throughout the growing period. The male buds failed to open and fell down 12 days after they attained the size visible to naked eye. Average bud length at this stage was 8.6 mm. There was a marked difference in the appearance of anther lobes and pollen grains. In the male sterile plant, the anther lobes were not properly developed. The pollen grains were small and shrunken. When these pollen grains were mounted in a drop of acetocarmine stain, they did not attain the stain. Pollen fertility, based on the stainability was found to be zero.

4.2 Analysis of F_2 cross, MS x Arka Sumeet along with F_1 and back cross generation, (MS x Arka Sumeet) x Arka Sumeet

4.2.1 Days to emergence of first fertile male flower

F_2 population exhibited a wide range (0-97) for days to male flower production (Table 3). Fourty six point two per cent of the F_2 plants produced fertile male flower between 52^{nd} to 64.9^{th} days after sowing (Table 4 and Plate 3). Two point eight three per cent of the F_2 population reverted to male fertility on final stage of crop growth and produced fertile male flower after 97 days. BC_1 exhibited a narrow range for this character (42-47). Sixty four point three per cent of BC_1 plants produced fertile male flower from 39-51.9 days after sowing (Fig.1).

4.2.2 Days to emerge first female flower

F_2 exhibited wide range in the days to female flower (54-64) production when compared to BC_1 population (Table 3). BC_1 was early in production of female flower. Thirty two per cent of the F_2 plants produced female flower between 50-52 days after sowing while fourtine point two per cent of the BC_1 plants produced female flower between 32-33 days after sowing (Table 4).

Plate 1 Field layout

Table 3. Range, mean, variance values of ridge gourd F_2, BC_1generation involving male sterile line (MS) and Arka Sumeet.

Character	Population	Range	Mean	Variance
Days to emergence of first fertile male flower	F_2	0.0 - 97.0	32.01	987.70
	BC_1	42.0 - 47.0	44.66	6.33
Days to emergence of first female flower	F_2	54.0 - 64.0	59.481	5.20
	BC_1	41.0 - 46.0	43.33	6.33
Node to first fertile male flower	F_2	0.0 - 42.0	7.29	70.05
	BC_1	13.0 -16.0	14.33	2.33
Node to first female flower	F_2	8.0 - 41.0	20.50	36.90
	BC_1	30.0 - 34.0	32.00	4.00
Pollen fertility (%)	F_2	0.0 - 87.0	30.63	1021.05
	BC_1	71.40 - 92.0	83.46	115.40

BC_1- Backcross1, F_2 (MS x Arka Sumeet)

Table 3 Contd

Character	Population	Range	Mean	Variance
Average fruit weight (kg)	F_2	0 03 0 16	0 06	0 001
	BC_1	0 16 0 24	0 20	0 002
Average fruit length (cm)	F_2	10 20 30 20	14 27	14 484
	BC_1	29 00 38 00	32 33	24 330
Average fruit girth (cm)	F_2	7 60 15 80	11 67	2 397
	BC_1	12 00 15 00	13 33	2 330
Days to first harvest	F_2	58 00 64 00	61 88	3 797
	BC_1	56 00 58 00	57 00	1 000
Number of harvests	F_2	19 00 19 00	19 00	0 000
	BC_1	7 00 10 00	8 33	2 330

BC_1 Backcross1 F_2 (MS x Arka Sumeet)

Table 3 Contd

Character	Population	Range	Mean	Variance
Number of fruit per plant	F_2	37 00 99 00	74 53	169 80
	BC_1	16 00 29 00	22 66	42 33
Yield/plant (kg)	F_2	2 59 9 00	5 07	1 97
	BC_1	1 92 2 90	2 37	0 24
Number of seed per fruit	F_2	152 00 – 197 00	175 40	206 20
	BC_1	143 00 159 00	152 66	72 33

BC Backcross1 F_2 (MS x Arka Sumeet)

Table 4 Frequency and distribution of range involving male sterile line (MS) and Arka Sumeet m ridge gourd F_2 and BC_1

Character	Population		1	2	3	4	5	6	7	8	9	10	11	12
Days to emergence of first fertile male flower	Range		0 12 9	13 25 9	26 38 9	39 51 9	52 64 9	65 77 9	78 90 9	91 103				
	F_2	F	46				49	5	3	3				
		%	43 39				46 24	4 71	2 83	2 83				
	BC_1	F			12	27	3							
		%			28 5	64 36	7 14							
Days to emergence of first female flower	Range		32 33	34 35	36 37	38 39	40 41	42 43	44 46	47 49	50 52	53 55	56 58	59 61
	F_2	F							4	15	34	32	17	4
		%							3 77	14 15	32 07	30 18	16 06	3 77
	BC_1	F	6	6		5	1	7	4	9		1	3	
		%	14 2	14 2		11 9	2 38	16 6	9 52	21 68		2 38	7 14	

F_2 (MS x Arka Sumeet) BC_1 – Backcross1 F frequency % percentage

Table 4 Contd

Character	Population		1	2	3	4	5	6	7	8
	Range		0 5 9	6 11 9	12 17 9	18 23 9	24 29 9	30 35 9	36 41 9	42 47 9
	F_2	F	43	22	24	4	3	5	0	5
Node to first fertile male flower		%	40 56	20 78	22 64	3 77	2 83	4 71		4 71
		F	3	12	21	6				
	BC_1	%	7 30	28 5	50	14 2				
	Range		8 12 99	13 17 9	18 22 9	23 27 9	28 32 9	33 37 9	38 42 9	43 47 9
	F_2	F	28	30	27	6	4	5		
Node to first female flower		%	26 41	28 30	25 49	5 66	3 77	4 71		
	BC	F	6		3	12	15	6		
		%	14 2		7 14	28 76	35 7	14 2		

F_2 (MS x Arka Sumeet) BC_1 – Back cross1 F – frequency % percentage

Table 4 Contd

Character	Population		1	2	3	4	5	6	7	8	9	10	11	12	13
Pollen fertility (%)	Range		0 10	11 21	22 32	33 43	44 54	55 65	66 76	77 87	88 98				
	F_2	F	50	0	6	8	10	10	10	12					
		%	47 16		5 66	7 57	9 43	9 43	9 43	11 32					
	BC_1	F					2	3	10	20	7				
		%					4 76	7 86	23 58	47 20	16 6				
Average fruit weight (kg)	Range		0 07 0 08	0 09 0 10	0 11 0 12	0 13 0 14	0 15 0 16	0 17 0 18	0 19 0 20	0 21 0 22	0 23 0 24	0 25 0 26	0 27 0 28	0 29 0 30	0 31 0 32
	F_2	F	45	14	10	4	4	10	5						
		%	42 49	13 20	9 43	3 77	3 77	9 43	4 71						
	BC	F	4	6	4	2	6	2	2	2	3	3	4	1	3
		%	9 52	14 2	9 52	4 76	14 4	4 76	4 76	4 76	7 14	7 14	9 52	2 38	7 14

F_2 (MS x Arka Sumeet) BC_1 Back cross1 F frequency % percentage

Character	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

	Range		8 10	11 13	14 16	17 19	20 22	23 25	26 28	29 31	32 34	35 37	38 40	41 43	44 46	47 49	50 52	53 55
Average fruit length (cm)	F_2	F	13	17	16	13	12	10	10	10	5							
		%	12 26	16 07	15 09	12 26	11 32	9 43	9 43	9 43	4 71							
	BC_1	F	1	1	2	2	2	4		6	10	4	3	3	3			1
		%	2 38	2 38	4 76	4 76	4 76	9 52		14 2	23 92	9 52	7 14	7 52	7 14			2 38
	Range		5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24						
Average fruit girth (cm)	F_2	F	5	9	40	37	7	3	5									
		%	4 71	8 49	37 73	34 93	6 60	2 83	4 71									
	BC_1	F			5	6	6	10	5	4	3	3						
		%			11 9	14 2	14 4	23 8	11 9	9 52	7 14	7 14						

Table 4 Contd

F_2 (MS x Arka Sumeet) BC_1 – Back cross1 F frequency % percentage

Table 4 Contd

Character	Population		1	2	3	4	5	6	7	8	9	10
Character	Range	Population	45 46 1	47 48 2	49 50 3	50 51 4	52 53 5	54 55 6	56 57 7	58 59 8	60 61 9	62 63
Days to first harvest	F_2	F							20	10	36	40
		%							18 86	9 43	33 96	37 75
	BC_1	F			3	11	7	6	7	6	1	1
		%			7 14	26 1	16 6	14 6	16 6	14 2	2 38	2 38
Number of fruit per plant	Range		11 20	21 30	31 40	41 50	51 60	61 70	71 80	81 90	91 100	
	F_2	F			10	18	12	25	19	12	10	
		%			9 43	16 98	11 32	23 58	17 94	11 32	9 43	
	BC	F	2	13	13	8	4	2				
		%	4 76	30 9	30 9	19 0	9 68	4 76				

F_2 (MS x Arka Sumeet) BC_1 Back cross1 F frequency % percentage

Table 4 Contd

	Range		2 3	3 4	4 5	5 6	6 7	7 8	8 9	9 10	10 11
Yield/plant (kg)	F_2	F	14	11	22	14	12	14	19		
		%	13 24	10 37	20 75	13 20	11 32	13 20	17 92		
	BC_1	F	15	14	7	6					
		%	35 7	33 3	16 6	14 4					
	Range		40 50	60 70	80 90	100 110	120 130	140 150	160 170	180 190	200 210
Number of seed per fruit	F_2	F						6	51	24	25
		%						5 66	48 12	22 64	23 58
	BC_1	F			9	8	11	9	5		
		%			21 4	19 0	26 3	21 4	11 9		

F_2 (MS x Arka Sumeet) BC_1 Back cross1 F – frequency % percentage

Plate 2a

Plate 2b

Plate 2c

Plate 2d

Plate 2e

Plate 2f

Plate 2g

Plate 2h

Plate 2a: Male sterile female parent (MS line) regenerated through tissue culture plant, Plate 2b and 2c : Transplanted tissue culture plant, Plate 2d : 14days after transplanting, Plate 2e : 37days after transplanting, 2e and 2f : Tissue culture plants at flowering stage, 2g and 2h : male sterile racemes.

Plate 3 F_2 Field view for male fertile and male sterile plants during anthesis

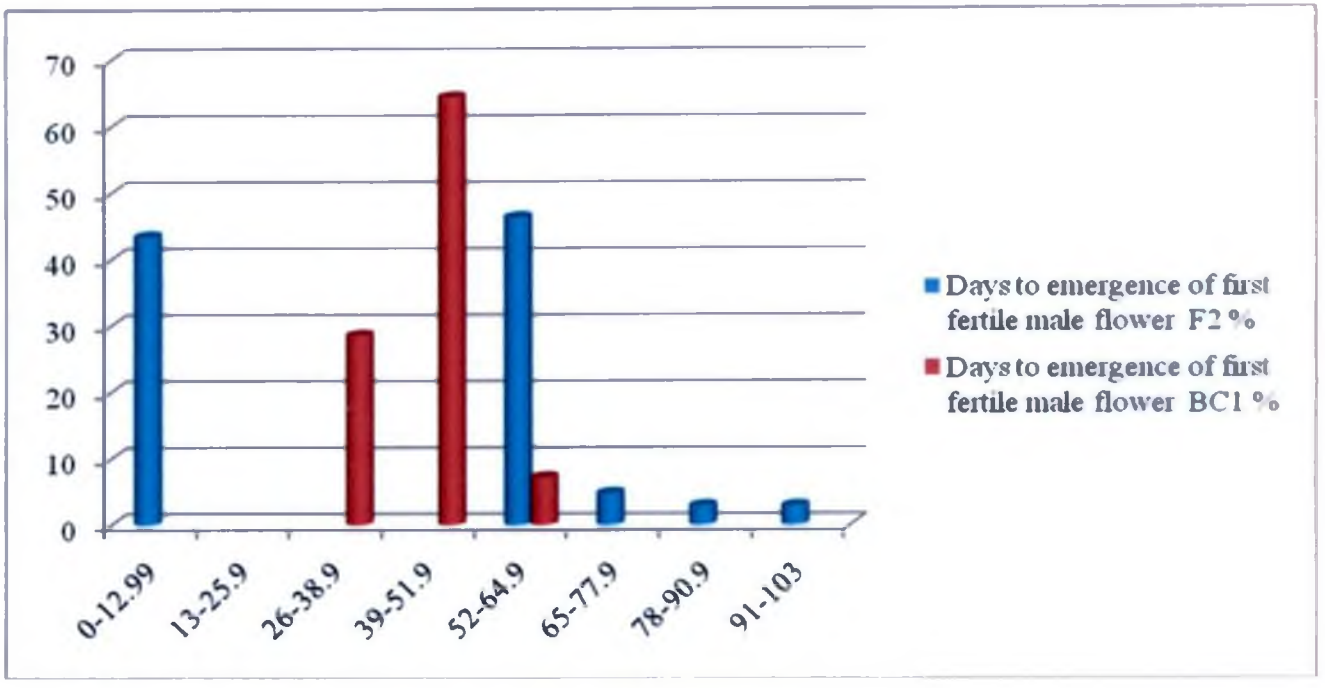


Fig.1. Days to emergence of first fertile male flower for F_2 and BC_1

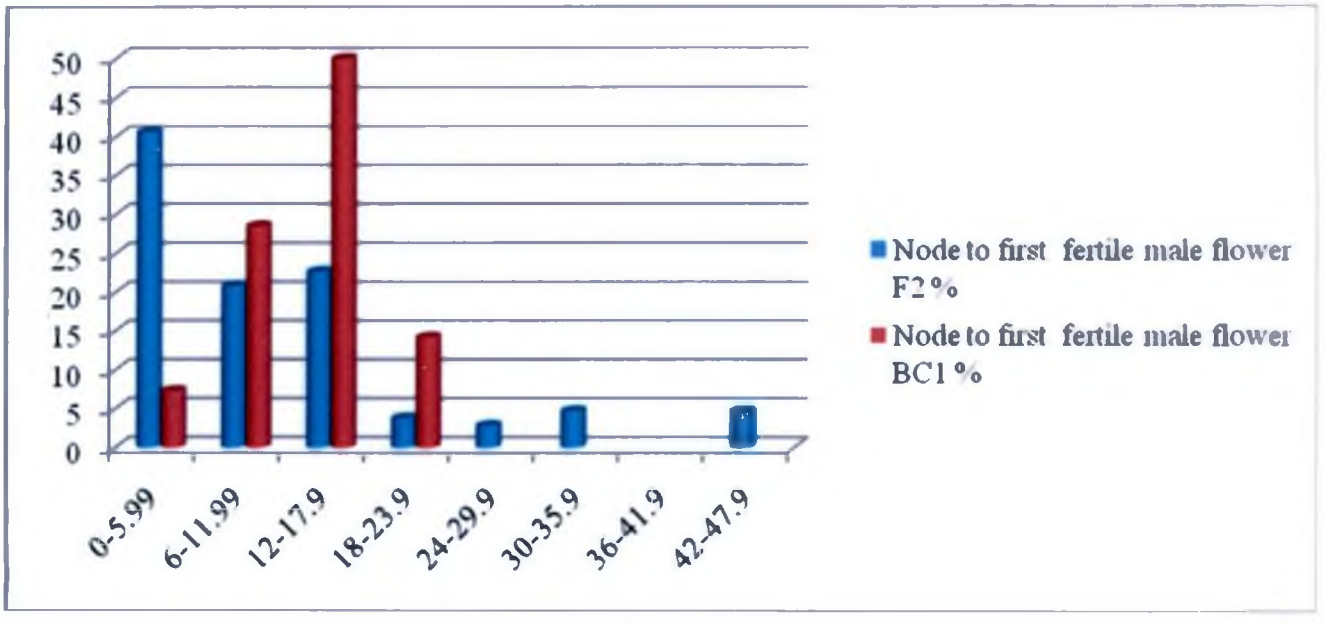


Fig.2. Node to first fertile male flower for F_2 and BC_1

4.2.3 Node to first fertile male flower

F_2 population widely segregated for male fertility with a range of 0-42 while BC_1 expressed a close range for this character (13-16). In fourty point five six per cent of the F_2 plants, male flower emerged at nodes below 5.99^{th} node and fifty per cent of the BC_1 produced male flower between 12 to 17.9^{th} node (Fig.2).

4.2.4 Node to first female flower

Though F_2 exhibited a wide range in female flower producing node (7-42), mean value was lower compared to BC_1 generation. Twenty eight point thirty per cent of F_2 population produced female flower between 13-17.9^{th} node while in thirty five point seven per cent of the BC_1 plants, female flower was produced between 28-32.9^{th} node.

4.2.5 Pollen fertility

Fourty two per cent of F_2 population exhibited 0 pollen fertility, BC_1 population exhibited high pollen fertility (Plate. 4, 5 and 6) and fourty seven point two per cent of the BC_1 population exhibited pollen fertility in the range of seventy seven to eighty seven per cent (Table 4). Only leaven point thirty two per cent of the F_2 population exhibited pollen fertility in the range of seventy seven to eighty seven per cent (Fig.3). F_2 population exhibited high variance for pollen fertility compared to BC_1 generation (Table 3).

4.2.6 Average fruit weight

F_2 population exhibited a wide range for average fruit weight (0.01-0.16 g). BC_1 plants produced bigger fruits (0.203 g) when compared to F_2 plants (0.065). Fourty two point two of the F_2 plants produced small fruits (0.07-0.08g) while fourteen point four of the F_2 plants produced fruits in the range of 0.15 to 0.16 g (Fig.4).

Plate 5 F_2 male fertile plants

Plate 6 F_2 male sterile plants

Plate 4a F_2 sterile pollen

Plate 4b F_2 sterile pollen

Plate 4c F_2 Partial fertile pollen

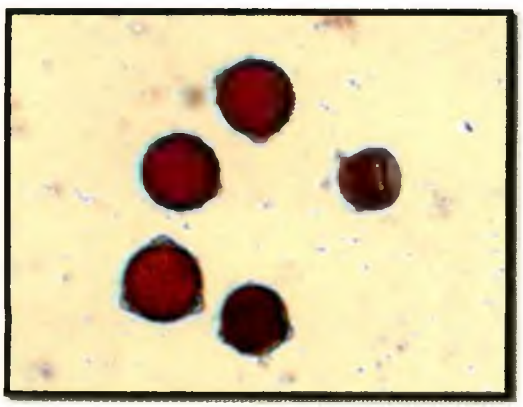


Plate 4d F_2fertile polllen

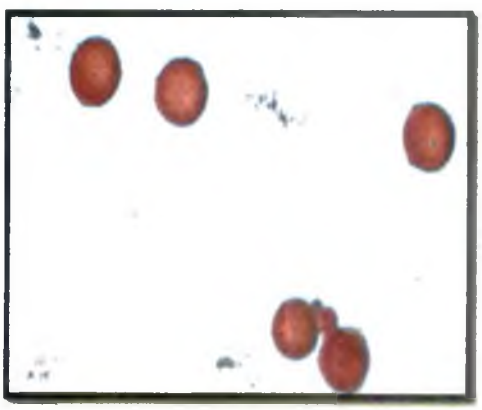


Plate 4e F_2 fertile pollen

Plate 4 F_2 pollen fertility

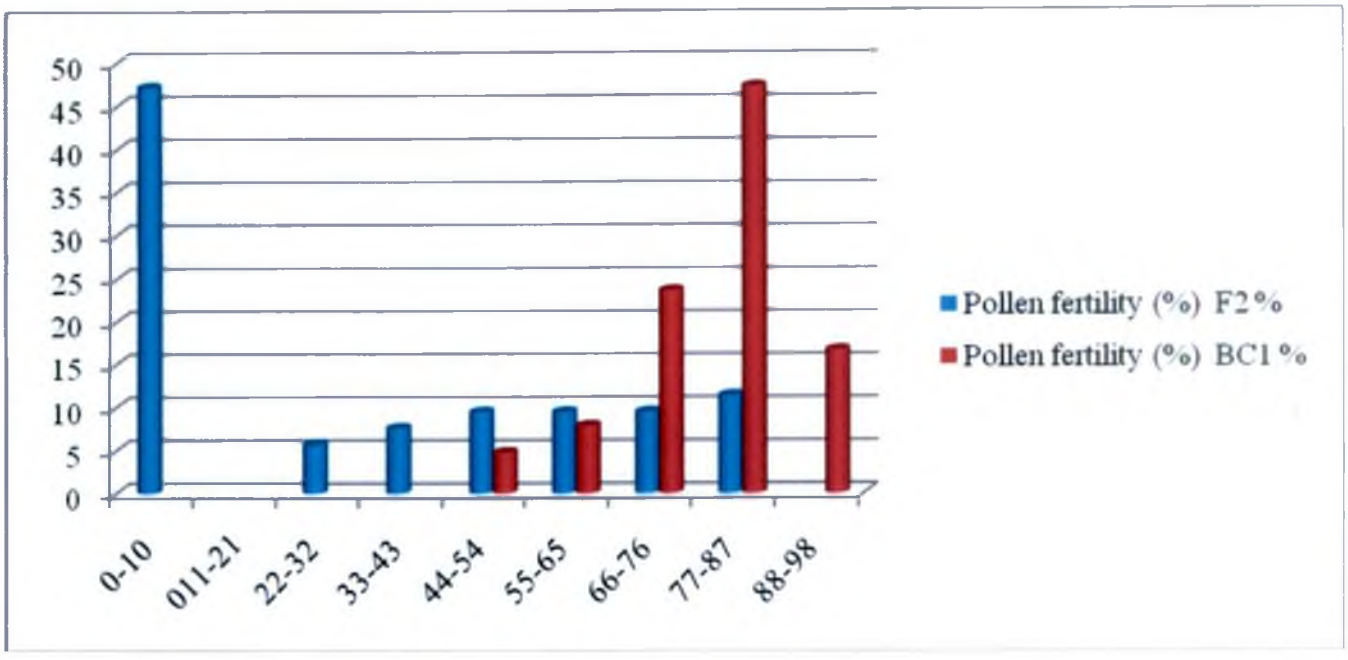


Fig.3. Pollen fertility for F_2 and BC_1

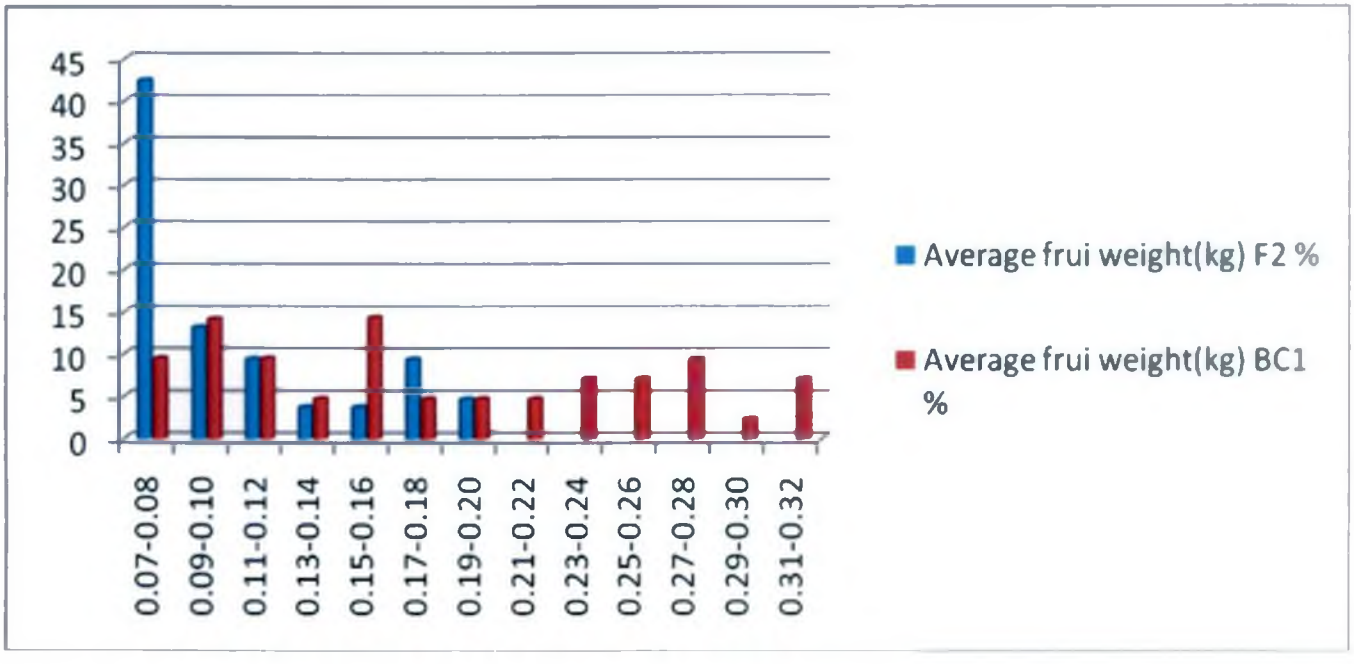


Fig.4. Average fruit weight for F_2 and BC_1

4.2.7 Average fruit length

As in the case of fruit weight F_2 plants exhibited low value for fruit length (10.22-30.22 cm). BC_1 produced longer fruit (32.33 cm) compared to plants of F_2 population (14.27 cm). F_2 population exhibited almost uniform distribution between different groups (Table 4).

4.2.8 Average fruit girth

F_2 population exhibited wide range for average fruit girth (7.6-15.8cm) compared to BC_1 generation. Thirty seven point seven three of the F_2 plants produced fruits with a girth of 9-10 cm while 23.8% of the BC_1 plants produced thicker fruit with a girth of 15-16 cm (Table 4).

4.2.9 Days to first harvest

F_2 plants took more days to attain harvest stage with a wide range (58-64 days after sowing) compared to BC_1 (56.58) plants (Table 3). Thirty seven point seven five per cent of the F_2 plants attain harvest stage between 62-63rd days after sowing, while twenty five point one of the BC_1 plants produced marketable fruits between 50-51st days after sowing(Fig.5).

4.2.10 Number of fruit per plant

F_2 population exhibited a wide range for number of fruits per plant (37-99) compared to BC_1 (16.0-29.0). Twenty three point five eight per cent of F_2 plants produced 61-70 fruits. Sixty one point eight per cent of the BC_1 plants produced 47-50 fruits per plant (Table 4).

4.2.11 Yield per plant (kg)

Some of the F_2 plants were heavy yielders (9 kg/plant) and population exhibited a wide range for yield per plant (2.59-9 kg). F_2 exhibited a high mean (5.078 kg) over BC_1 (2.37 kg) (Table 3). 20.75% of the F_2 plants produced fruit in the range of 4-5 kg/plant (Fig.6).

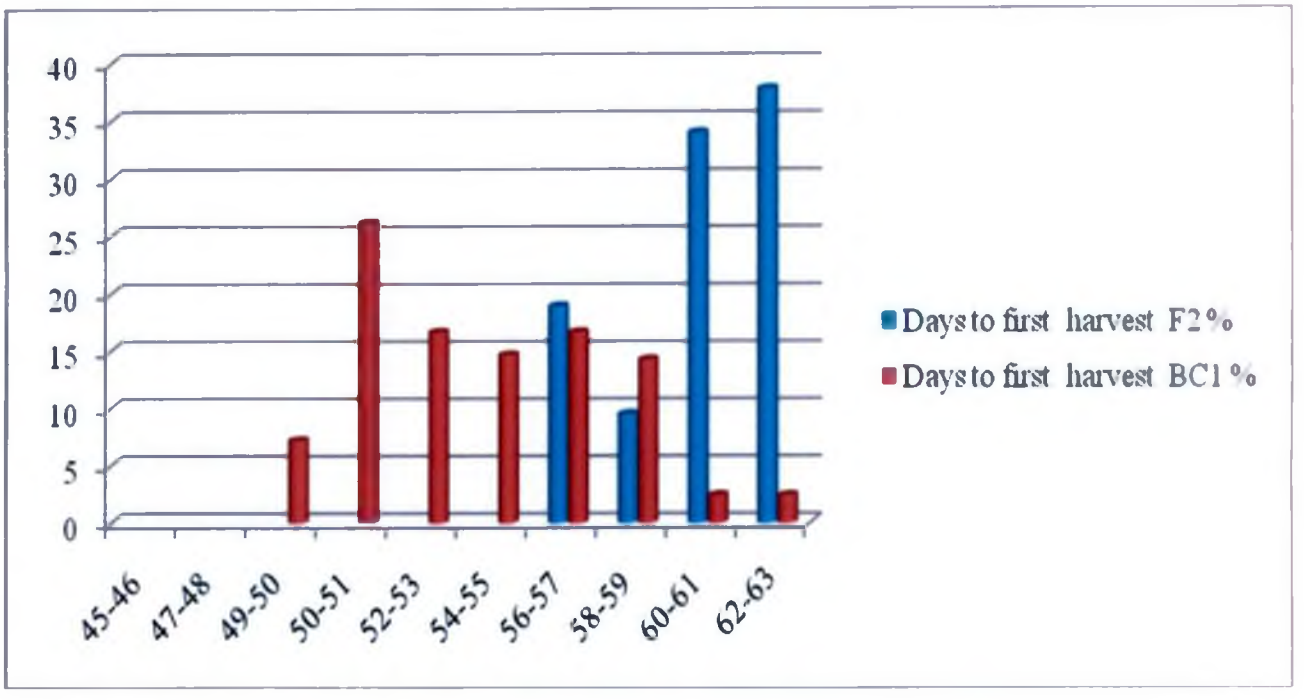


Fig.5. Days to first harvest for F_2 and BC_1

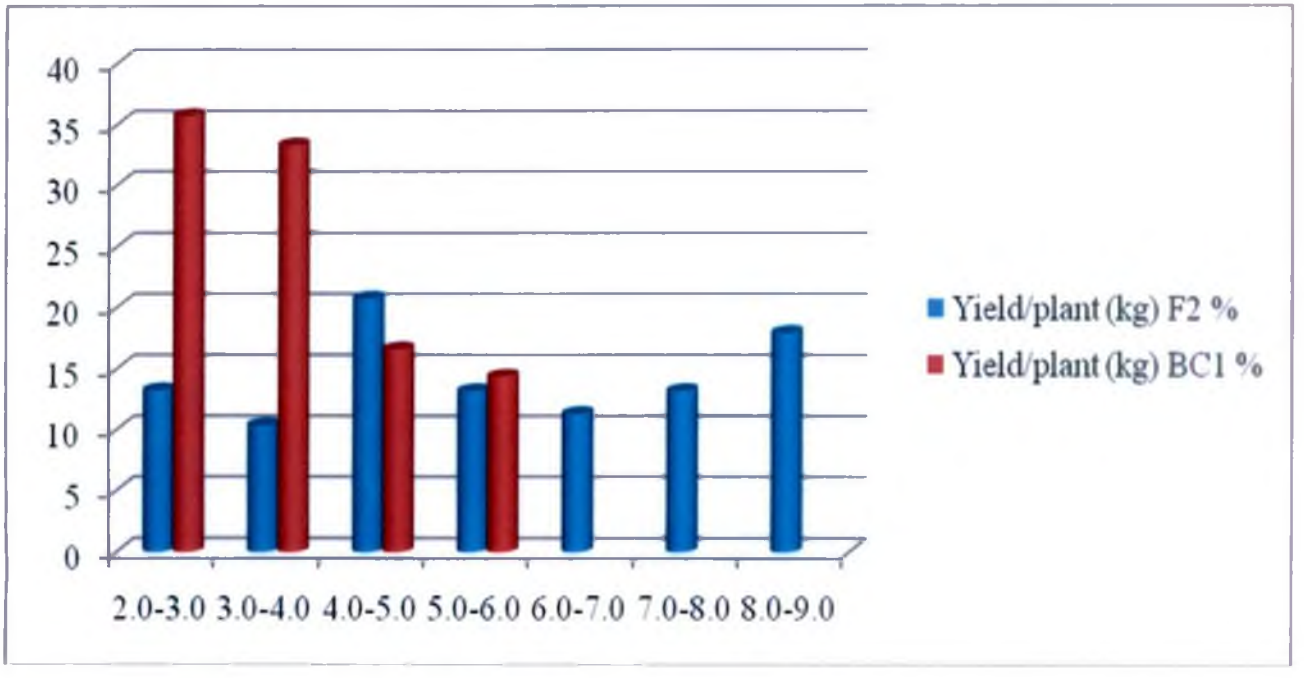


Fig.6. Yield per plant for F_2 and BC_1

4.2.12 Number of seed per fruit

All the F_2 plants produced seed under open pollination and sib mating was resorted for producing fruits in the case of fertile F_2 plants. F_2 plants produced seed in the range 152-197 seed per fruit. Fourty eight point one per cent of the F_2 plants produced seed in the range of 160-170 seed per fruit. BC_1 exhibited a low mean value (152.66) compared to F_2 (175.40)

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

Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile (Plate 5, 6). Few plants produced fertile male flower in the last stage of crop growth. As F_2 population segregated in to two classes, monohybrid ratio, 3 : 1 and modified dihybrid ratio *viz*, 13 : 3 and 9 : 7 were tested for significance using χ^2 test. Presence of single gene either of dominant or of recessive nature governing male fertility is ruled out as the monohybrid ratio, 3 :1 was found to be significantly deviated from the expected ratio. The F_2 data indicate that this may be case of cytoplasmic genic male sterility (CGMS) with probably more than one gene involved in the fertility restoration (Table 5). The χ^2 test suggested that the fertility restoration is governed by two dominant genes with interaction. The F_2 ratio doesn't fit in to inhibitory gene action (13:3) in governing male fertility restoration. The χ^2 value for the 9:7 (fertile: sterile) complementary gene action ratio was non significant and the observed ratio exhibit a good fit to the expected ratio (P=0.05) (Table 10). If two genes are involved in a specific pathway and functional products from both are required for expression, then one recessive allelic pair at either loci would result in the mutant phenotype. As both genes are required for the correct phenotype, this interaction is called complementary gene action. Result indicated the role of two fertility restorer genes in ridge gourd which is explained in the discussion section.

Table 5. χ^2 analysis of F_2 population.

Ratio	Observed Value	Expected Value	D=O-E	$\chi^2 = D^2/E$	Total	(P=0.05)	Remarks
	Fertile-56	106*3/4=79.5	-23.5	6.94			
3:1	Sterile-50	106*1/4=26.5	23.5	20.8	27.74	0.988	significant
	Fertile-56	106*13/16=80	-24	7.2			
13:3	Sterile-50	106*3/16=20	30	45.0	52.2	0.988	significant
	Fertile-56	106*9/16=60	-4	0.26			Non-significant
9:7	Sterile-50	106*7/16=46	4	0.34	0.60	0.988	

4.4 Evaluation of back cross and three way cross for studying the expression of male sterility and restoration of male fertility

The male sterile F_1 hybrids *viz.* (MS x Deepthi), (MS x IC-92685), (MS x IC-92671) and (MS x CO2) were crossed with respective pollen parents and four back crosses *viz.,* (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2 were evaluated for the expression of male sterility and restoration of male fertility.

There was significant difference between backcrosses, three way crosses with respect to days to emergence of first male flower (Table 6). Among backcrosses, back cross (MS x Arka Sumeet) x Arka Sumeet was the earliest to form male flower (38.66). Backcross (MS x Deepthi) x Deepthi produced fertile male flower at a late stage (58 days) when compared to other backcrosses (Fig.7). There was significant difference between backcrosses, three way crosses with respect to node to first fertile male flower. In general three way crosses were early to form fertile male flowers compared to back crosses (Table 6).

All the male sterile hybrids on crossing with Arka Sumeet produced fertile male flowers early and took only few nodes for generating fertile male racemes (Fig. 8). Three way cross (MS x IC-92685) x Arka Sumeet and back crosses, *viz* (MS x IC-92685) x IC-92685 and (MS x IC-92671) x IC-92671 were early and on par with regard to the node taken for producing fertile male flower.

There was significant difference between backcrosses and three way crosses with respect to pollen fertility. Back cross (MS x Arka Sumeet) x Arka Sumeet and three way crosses *viz,* (MS x Deepthi) x Arka Sumeet, (MS x IC-92671) x Arka Sumeet and), (MS x IC-92685) x Arka Sumeet expressed high pollen fertility and were on par (Table 6). In general three way crosses exhibited high pollen fertility (Plate 8).

Table 6. Evaluation of backcrosses and three way crosses for male fertility.

Back cross	Days to emergence of first male flower	Node to first male flower	Pollen fertility (%)
(MS x CO2) x CO2	48.3	16.3	72.80
(MS x ArkaSumeet) x ArkaSumeet	38.6	14.3	83.46
(MS x Deepthi) x Deepthi	58.0	25.0	63.66
(MS x IC-92671) x IC-92671	41.0	11.6	80.10
(MS x IC-92685) x IC-92685	40.6	11.3	64.46
Three way cross			
(MS x Deepthi) x ArkaSumeet	38.3	12.6	87.33
(MS x IC-92671) x ArkaSumeet	37.6	13.0	85.36
(MS x IC-92685) x ArkaSumeet	38.0	11.0	83.33
(MS x CO2) x Arka Sumeet	37.6	13.0	79.66
F test	*	*	*
CD	0.513	0.678	5.388

*Significant @ 0.05 level

Table 7. Analysis of back cross, three way crosses for important biometric characters

Backcross/3Waycross/Parents	Days to first harvest	Average fruit weight (cm)	Average fruit length (cm)	Average fruit girth (cm)
IC-92671	50.66	0.06	12.33	10.66
IC-92685	59.33	0.08	32.00	12.66
CO2	59.00	0.12	31.33	12.66
Deepthi	56.66	0.23	38.66	17.00
Arka Sumeet	50.33	0.22	44.33	21.33
(MS x CO2) x CO2	52.00	0.37	31.33	21.33
(MS x Arka Sumeet) x Arka Sumeet	57.00	0.20	32.33	13.33
(MS x Deepthi) x Deepthi	59.66	0.10	35.33	12.66
(MS x IC-92671) x IC-92671	54.66	0.17	22.00	18.00
(MS x IC-92685) x IC-92685	49.00	0.19	17.00	18.00
(MS x Deepthi) x ArkaSumeet	52.66	0.24	33.00	16.66
(MS x IC-92671) x ArkaSumeet	56.66	0.18	23.66	14.66
(MS x IC-92685) x ArkaSumeet	53.66	0.11	37.66	11.66
(MS x CO2) x Arka Sumeet	62.33	0.11	33.00	14.00
F test	*	*	*	*
CD	0.90	0.022	2.29	1.18

*Significant @ 0.05 level

Table 7. Contd...

Back Cross/3 waycross/Parents	Number of harvests	Number of fruit per plant	Number of seed per fruit	Yield/plant (kg)
IC-92671	8.66	34.33	91.33	2.74
IC-92685	10.6	52.66	117.33	4.74
CO2	8.33	39.00	100.00	4.29
Deepthi	8.66	46.33	101.00	6.95
Arka Sumeet	5.00	26.00	113.00	4.16
(MS x CO2) x CO2	9.00	36.66	134.00	5.34
(MS x ArkaSumeet) x Arka Sumeet	8.33	22.66	152.66	2.37
(MS x Deepthi) x Deepthi	6.66	25.33	138.33	2.46
(MS x IC-92671) x IC-92671	7.33	44.33	159.66	5.20
(MS x IC-92685) x IC-92685	8.33	44.33	149.66	6.18
(MS x Deepthi) x ArkaSumeet	9.66	34.33	141.66	7.55
(MS x IC-92671) x ArkaSumeet	10.0	41.00	102.66	7.79
(MS x IC-92685) x ArkaSumeet	7.00	27.33	137.33	3.28
(MS x CO2) x Arka Sumeet	13.6	35.00	130.33	6.70
F test	*	*	*	*
CD	0.9687	3.6979	8.0052	0.7399

*Significant @ 0.05 level

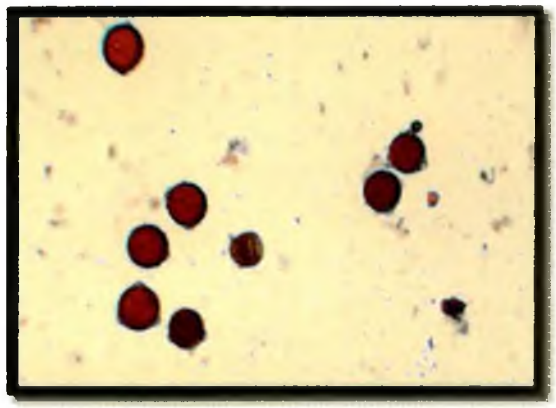


Plate 8a (MS x IC-92671) x Arka Sumeet

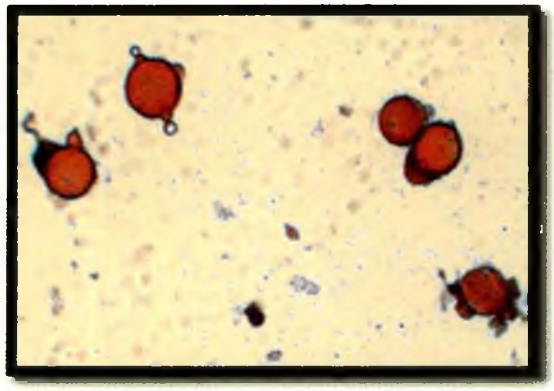


Plate 8b (MS x Deepthi) x Arka Sumeet

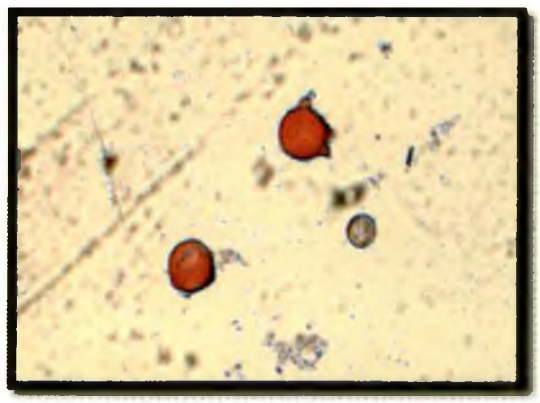


Plate 8c (MS x IC-92685) x Arka Sumeet

Plate 8 Pollen fertility of three way crosses

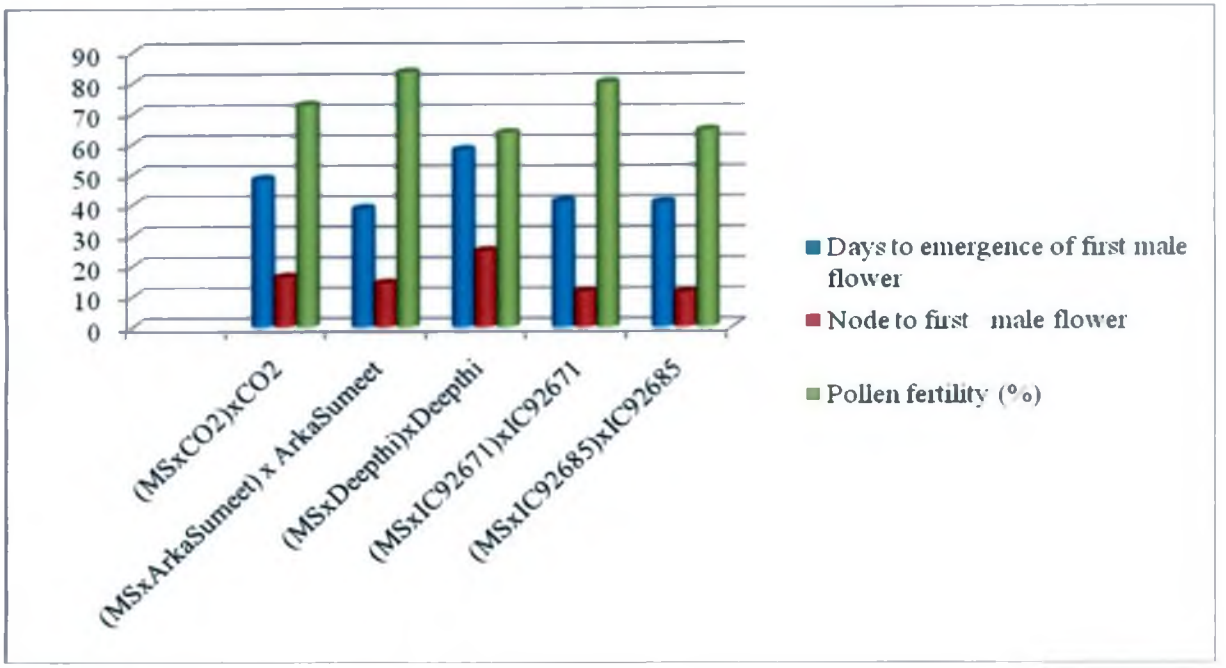


Fig.7. Evaluation of backcrosses for male fertility

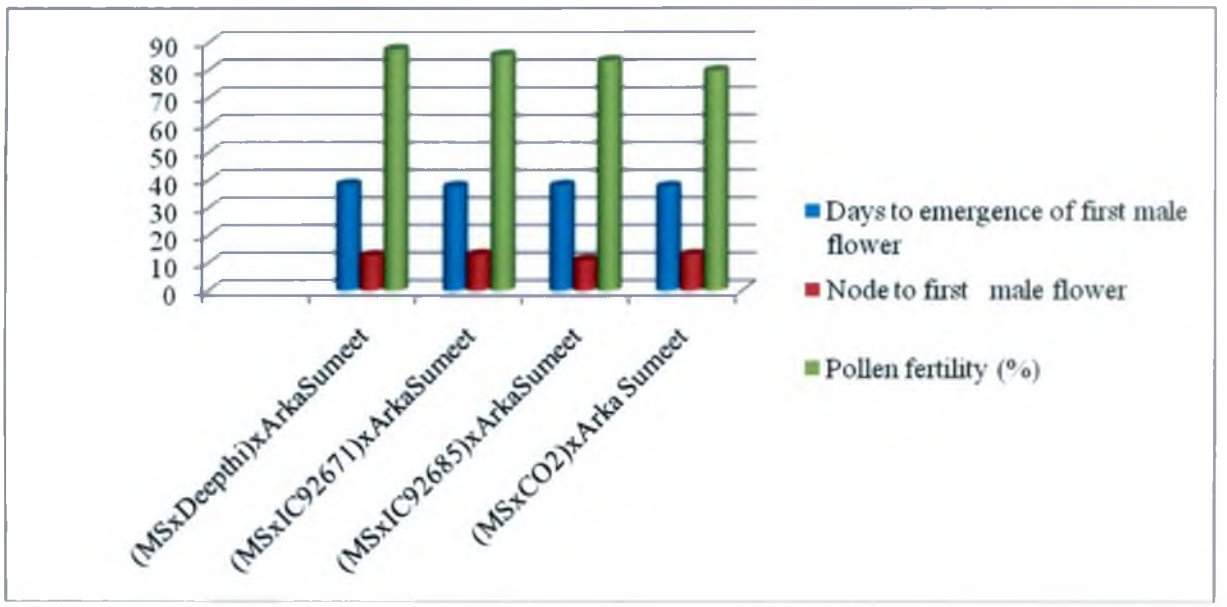
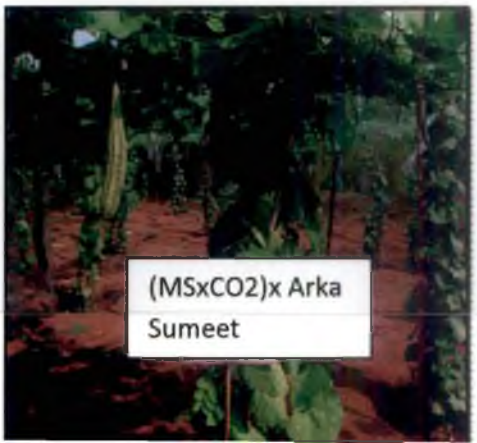


Fig.8. Evaluation of three way crosses for male fertility

Plate 10 (MS x IC-92671) x Arka Sumeet

Plate 11 (MS x IC-92685) x Arka Sumeet



4.4.1 Evaluation of back crosses and three way crosses for other important horticultural characters

There was no significant difference between crosses and genotypes with respect to days to emergence of first fertile male flower (Table 7). However genotypes, backcrosses and three way crosses differed significantly with respect to days to emergence of first female flower. The back cross (MS x IC-92685) x IC-92685 was the earliest to form female flower (31).

There was no significant difference between accessions with respect to days to first harvest and average fruit weight (Table 7). However genotypes, backcrosses, three way crosses differed significantly with respect to average fruit length. The genotype Arka Sumeet produced longest fruit (44.33cm). There was no significant difference between accessions with respect to average fruit girth. However genotypes, backcrosses, three way crosses differed significantly with respect to number of harvests (Table 7). The three way cross (MS x CO2) x Arka Sumeet was harvested for more number of days (13.6). There was significant difference between genotype, backcrosses, three way crosses with respect to number of fruit per plant (Plate 10 to 18). The accession IC-92685 produced highest number of fruit per plant (fifty two point sixty six).

There was no significant difference between accessions with respect to number of seed per fruit. However genotypes, backcrosses, three way crosses differed significantly with respect to yield per plant (Table 7). The three way cross (MS x IC-92671) x Arka Sumeet exhibited highest yield per plant (7.79kg/plant).

4.5 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters

4.5.1 Estimation of heterosis (MS x Arka Sumeet)

Heterosis was estimated for the hybrid (MS x Arka Sumeet) in two different ways, heterosis over better parent (Arka Sumeet) and heterosis over standard parent

Plate 10 (MS x IC-92671) x Arka Sumeet

Plate 11 (MS x IC-92685) x Arka Sumeet

Plate 12 (MS x Deepthi) x Arka Sumeet

Plate 13 (MS x CO2) x Arka Sumeet

Plate 14 (MS x IC-92685) x IC-92685

Plate 15 (MS x IC-92671) x IC-92671

Plate16 (MS x Arka Sumeet) x Arka Sumeet

Plate 17 (MS x Deepthi) x Deepthi

Plate 18 (MS x CO2) x CO2

(Deepthi). Table 8 contains the estimated values of heterobeltiosis and standard heterosis.

The F_1 cross (MS x Arka Sumeet) exhibited significant heterobeltiosis for days to emergence of first female flower, node to first male flower, node to female flower, number of fruits per plant and yield per plant. Significant negative heterosis was observed for pollen fertility (minus twenty nine point fifty two per cent) and average fruit length (minus twenty one point fifty five per cent). Significant negative standard heterosis was also observed for average fruit girth. Hybrid exhibited high heterosis for yield characters and exhibited significant heterobeltiosis (twenty per cent) and standard heterosis (twenty five point eight per cent) for number of fruit per plant. Significant positive heterosis was also exhibited for yield per plant with heterobeltiosis of 48.8% and standard heterosis thirty three point three per cent respectively (Table 8).

4.5.2 Performance evaluation of ridge gourd genotypes and F_1

There was significant difference between genotypes and F_1 for all the characters studied (Table 9). Arka Sumeet produced early male flower (thirty six point two) and female flower (thirty four point eight) indicating the earliness of the genotype (Fig. 9). Accession IC-92671 produced earliest first fertile male node (Four point eight) while the accession IC-92685 was the earliest to produce female flower node (Nine point eight). The F_1 hybrid exhibited lowest pollen fertility (fifty four point one six per cent) while genotype Arka Sumeet was having the highest pollen fertility (Eighty two point three six per cent) (Plate 7, Fig. 9). IC-92671 was the earliest to attain harvest stage (Fig.9). Genotypes and F_1 differed significantly with respect to all fruit characters (Plate 19 to 25), *viz* average fruit length, average fruit weight and fruit girth (Fig.10). The accession Arka Sumeet produced longest and heavy fruits (43.6 cm and 0.243 g respectively). The accession Deepthi recorded highest fruit girth (15.5cm). Accession IC-92684 produced highest number of fruit (Thirty two point two) followed by IC-92671 (31.4) and F_1 hybrid (Thirty one point two) (Plate 9). The F_1 hybrid recorded highest yield per plant (6.4kg/plant) (Fig.10) while the

Table 8. Heterobeltiosis and standard heterosis for F_1 hybrid (MS x Arka Sumeet)

Character	Mean			H.B	S.H	S.E
	Arka Sumeet	Deepthi	MS x Arka Sumeet			
Days to emergence of first fertile male flower	36.2	38.8	40.6	12.15*	4.63*	4.39
Days to emergence of first female flower	34.8	35.4	46.2	32.75	30.50	0.787
Node to first fertile male flower	6.2	6.2	18.6	200.0	200.0	2.49
Node to first female flower	15.6	12.8	22.2	42.30	73.43	0.723
Pollen fertility (%)	82.3	64.2	58.0	-29.52	-9.65*	5.44
Average fruit length (cm)	43.6	33.6	34.2	-21.55	1.78*	0.831
Average fruit weight (kg)	0.243	0.166	0.205	-15.63	23.49	0
Average Fruit girth (cm)	14.7	15.5	13.0	-11.56*	-16.12**	0.956
Number of fruit per plant	26	24.8	31.2	20.0**	25.80	1.89
Yield/plant (kg)	4.3	4.8	6.4	48.8	33.33	0.262
Number of seed per fruit	177.8	174	179	0.67*	2.87*	7.50

*Significant @ 0.05 level, **Significant @ 0.01 level, H.B - Heterobeltiosis, S.H - Standard heterosis, S.E - Standard error

Table 9. Evaluation of F_1 hybrid (MS x Arka Sumeet) and genotypes for biometric characters

Parent-F_1	Days to emergence of first fertile male flower	Node to first fertile male fiower	Node to first female flower	Pollen fertility (%)	Days to first harvest	Average fruit length (cm)	Average fruit weight (g)	Average fruit girth (cm)	Number of fruit per plant	Yield / plant (Kg)	Number of seed per fruit
F_1	40.6	18.6	22.2	58.0	48.2	34.2	0.205	13.0	31.2	6.4	179
Arka Sumeet	36.2	6.2	15.6	82.3	45.7	43.6	0.243	14.7	26	4.3	177.8
IC-92671	42.4	4.8	13.2	75.1	51.0	21.8	0.130	13.7	31.4	3.5	47.2
Haritham	43.8	9.2	13.4	76.5	53.1	36.8	0.122	13.2	22.6	3.2	49
Deepthi	38.8	6.2	12.8	64.2	49.3	33.6	0.166	15.5	24.8	4.8	174
IC-92685	51.2	17.8	9.6	79.6	61.4	34.2	0.132	12.0	32.2	6	45.2
F test	*	*	*	*	*	*	*	*	*	*	**
CD	3.1069	1.7628	0.5119	3.8506	3.4321	0.5881	0.0050	0.6764	1.3392	0.1855	4.8442

*Significant @ 0.05 level, **Significant @ 0.01 level

Plate 19 IC-92671

Plate 20 Deepthi

Plate 21 Arka Sumeet

Plate 22 IC-92685

Plate 23 CO2

Plate 24 F_1 hybrid (MS x Arka Sumeet)

Plate 25 Haritham

Plate 7a Pollen of Arka Sumeet

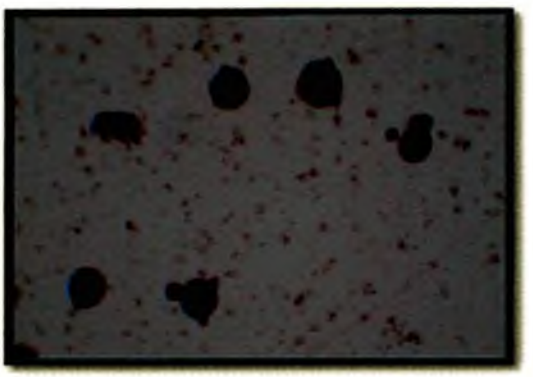


Plate 7b Pollen of MS x Arka Sumeet

Plate 7c Pollen of (MS x Arka Sumeet) x Arka Sumeet

Plate 7 Pollen fertility of Arka Sumeet, F_1 hybrid (MS x Arka Sumeet) and three way cross (MS x Arka sumeet) x Arka Sumeet

Plate 9a IC-92671

Plate 9b F1 bhybrid

Plate 9c Arka Sumeet

Plate 9d Haritham

Plate 9e Deepthi

Plate 9f IC-92685

Plate 9 Fruits of F_1 and lines

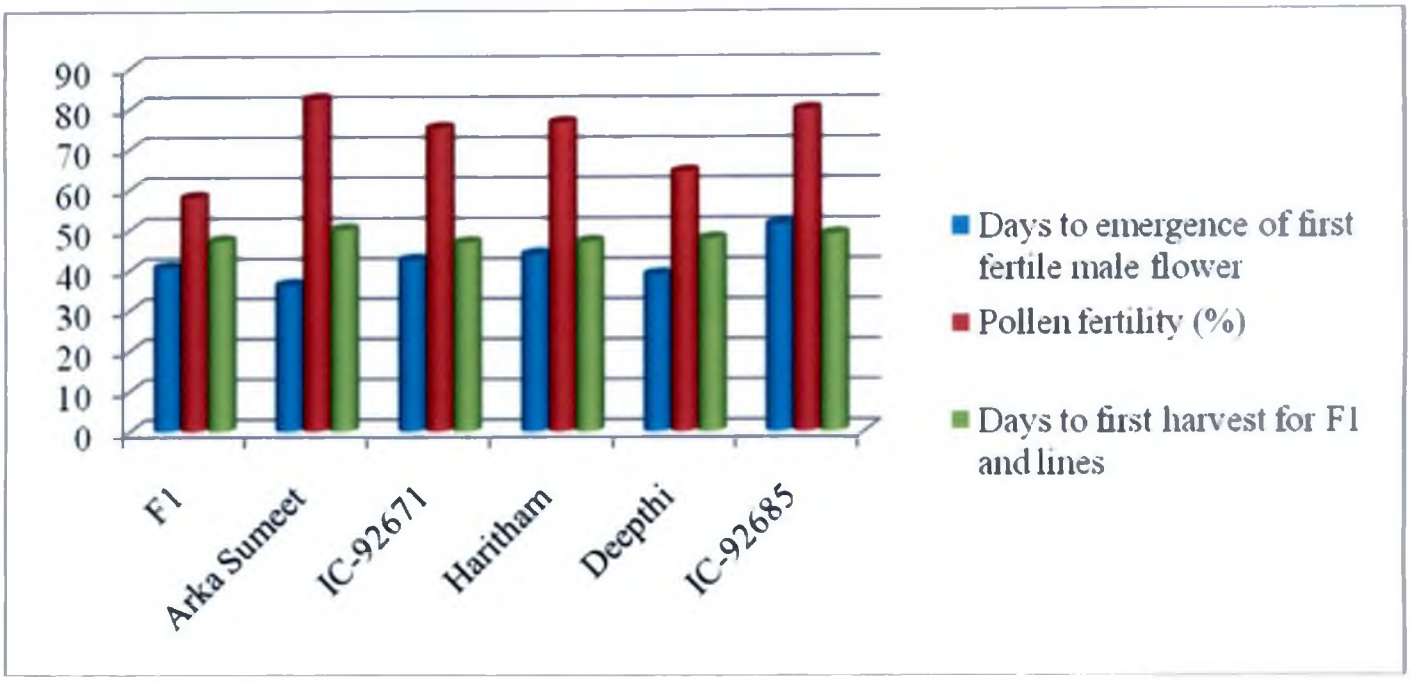


Fig.9. Days to emergence of first fertile male flower, Pollen fertility and Days to first harvest for F_1 and lines

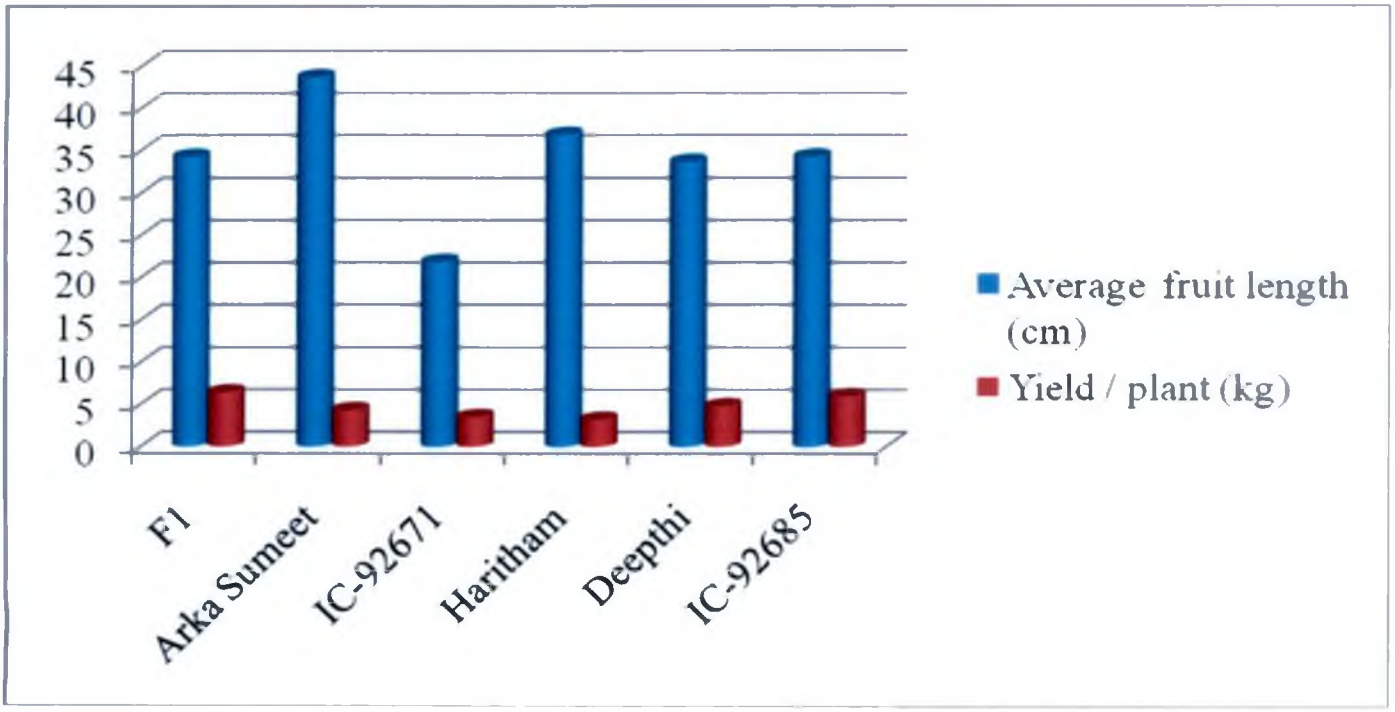


Fig.10. Average fruit length (cm) and Yield / plant (kg)

genotype Haritham produced lowest yield (3.2 kg/plant) (Figure 10). The F_1 hybrid produced highest number of seed per fruit (179) while genotype IC-92671 produced lowest number of seed per fruit (Fourty seven point two).

DISCUSSION

5. DISCUSSION

Male sterility has been reported in fairly large number of crops including vegetables (Kaul, 1988). These male sterile plants were either isolated from natural population or were artificially induced through mutagenesis. Male sterility ensures genetic emasculation and is of special interest to plant breeders. Crops like chilli and musk melon present a very successful example of utilization of male sterility system in India. 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. Hence, ridge gourd offers great scope for exploitation of hybrid vigour on commercial scale.

Novel source of male sterility in ridge gourd was reported from Kerala (Pradeepkumar *et al.*, 2008). They developed the tissue culture protocol for maintenance of male sterile line. Hegade (2009) selected five pollen parents for hybridization with the *in vitro* regenerated male sterile female parent. Four hybrids out of five were sterile (MS x CO2, MS x Deepthi, MS x IC-92685, MS x IC-92671) and one (MS x Arka Sumeet) was found to be partially fertile. 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 (Arka Sumeet) which restores the fertility. Pollen parents generating male sterile hybrid on crossing with MS line can be used for generating new male sterile lines which can be exploited for commercial breeding programme without depending the *in vitro* route for maintaining male sterile line. A detailed study is required about the genetic mechanism governing the male sterility and the stability of the trait over generations as male sterility may be heritable or transient. Hegade (2009) evaluated the horticultural performance of F_1 hybrids using male sterile line (MS). The hybrid, MS x Arka Sumeet performed best for yield and yield related traits

which points towards the scope of exploiting male sterile lines for heterosis breeding programme in ridge gourd.

Hence the present investigation was undertaken with the objective of investigating the inheritance of male sterility in ridge gourd and to develop new male sterile line in ridge gourd using back cross generations of sterile hybrids. Study also aims in analysing the horticultural superiority of F_1 hybrid, MS x Arka Sumeet in comparison with promising varieties/genotypes.

The present work is discussed under the following headings

5.1 Regeneration of *in vitro* maintained male sterile genotype and evaluation for stability of male sterility

5.2 Analysis of F_2 cross MS x Arka Sumeet along with F_1 and back cross generation, (MS x Arka Sumeet) x Arka Sumeet

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

5.4 Evaluation of back cross and three way cross for studying the expression of male sterility and restoration of male fertility

5.5 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters and estimation of heterosis

5.1 Regeneration of *in vitro* maintained male sterile genotype and evaluation for stability of male sterility

Protocol developed for the regeneration of *in vitro* maintained male sterile genotype by Pradeepkumar *et al.* (2008) and Pradeepkumar *et al.* (2010) was effective in hardening and transplanting of *in vitro* maintained male sterile genotype. Primary hardening in disposable polythene cups (250 ml) filled with sand ensures high survival percentage.

All *in vitro* regenerated plants were male sterile. The phenotypic expression of the regenerated plants was in confirmation with the reported type of male sterility (Deshpande *et al.,* 1979; Pradeepkumar *et al.,* 2008) characterized by the production of rudimentary male buds in racemes.

5.2 Analysis of F_2 (MS x Arka Sumeet) along with F_1 and back cross generation, (MS x Arka Sumeet) x Arka Sumeet

The F_2 population segregates for genes and each single plant in F_2 carry different alleles resulted through recombination and independent assortment of genes. F_2 population generated through selfing the fertile F_1 of a cross involving a male sterile female parent is the starting population for genetic analysis to elucidate the inheritance of male sterility and fertility restoration. The F_2 population resulted from the crosses between male sterile source and normal pollen parent exhibited a discontinuous pattern for male fertility which reinforces the recessive genic nature of male sterility in musk melon (McCreight, 1983). In cucurbits, F_2 population resulted from the cross involving male sterile line is extensively studied for elucidating genetics of male sterility in musk melon.

In the present study, the node to fertile male flower, days taken for the emergence of fertile male flower and pollen fertility were critically observed among 106 F_2 plants. Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile. Seven plants produced fertile male flower after 97 days of crop growth. The phenotypic expression of the male sterile F_2 plants in the present study was in confirmation with the reported type of male sterility in ridge gourd. The rudimentary male buds were produced in racemes as reported by earlier workers (Deshpande *et al.,*1979; Pradeepkumar *et al.,* 2008). There were observable differences between the male sterile and male fertile plants with respect to male flower production though female flowers in both types were similar.

The biometric characters of BC_1 generation exhibited a narrow range of variation while F_2s varied widely between extremes. In cross pollinated crops like ridge gourd variability and useful segregants are expected in F_2 population which can be advanced to later generation (Seshadri and More, 2009). F_2 population widely segregated for nodes to produce fertile male raceme with a range of 0-42 while BC_1 expressed a close range for this character. In 50% of the BC_1, male flower emerged between 12 to 17.9th node. F_2 population exhibited a wide range (0-97) for days to male flower production. Fourty six point two per cent of the F_2 plants produced fertile male flower between 52nd to 64.9th days after sowing. Two point eight three per cent of the F_2 population reverted to male fertility on final stage of crop growth and produced fertile male flower after 97days. BC_1 exhibited narrow range for this character (42-47). Sixty four point three per cent of BC_1 plants produced fertile male flower from 39-51.9 days after sowing.

Pollen from male sterile F_2 plants were poorly stained with acetocarmine (1% solution) when compared to the pollen from fertile line. In cucurbits, complete pollen abortion is reported in male sterile plants (Zhang and Wang, 1990). Fourty three point three per cent of the F_2 plants did not produce any fertile male flower while more than half of the F_2 population were male fertile. This clear segregation indicates the role of dominant fertility restorer gene in ridge gourd. The plants exhibiting more than ten per cent pollen fertility were taken as fertile and those exhibiting a pollen fertility in the range of zero to ten per cent as sterile plants in F_2. Fourty seven per cent of the population exhibited pollen fertility in the range of 0 to 10 and considered as a sterile. The fertile plants in F_2 exhibited pollen fertility between twenty two to eight seven per cent. Fourty seven per cent of the BC_1 exhibited pollen fertility between seventy seven to eighty seven indicating the potential of Arka Sumeet in restoration of male fertility.

Fruit characters *viz*, fruit length, fruit girth and fruit weight showed wide variation in F_2 population. The F_2 population exhibited high variance for fruit length, fruit girth and fruit weight. BC_1 attained harvest stage earlier (57.0) compared to F_2 (61.8). Some of the F_2 plants exhibited high yield potential and

nine point four three per cent of F_2 population gave more than 91fruits per plant and seventeen point nine per cent of the F_2 plants yielded 8 to 9 kg per plant. The F_1 hybrid, MS x Arka Sumeet yielded 6.2 kg per plant and high yield potential some of the F_2 segregants should be exploited for crop improvement. Transgressive segregants for yield is reported in cucurbits (Seshadri and More, 2009). High yielding fertile plants in F_2 can be advanced for making selection in the future generation.

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

Hegade (2009) suspected the presence of cytoplasmic male sterility in the MS line of ridge gourd as the crosses involving CO2, Deepthi, IC-92685, IC-92671 as pollen parents were sterile. All the plants in four crosses (MS x CO2, 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. Nuclear factor of Arka Sumeet may be governing the restoration of fertility. Partially fertile F_1 (MS x Arka Sumeet) was selfed to produce F_2 seeds. This F_2 population was studied for analysing gene action governing male fertility/ sterility in ridge gourd.

Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile. Seven plants produced fertile male flower after 97 days. As F_2 population segregated in to two classes, monohybrid ratio, 3 : 1 and modified dihybrid ratio *viz,* 13 : 3 and 9 : 7 were tested for significance using χ^2 test. Presence of single gene either dominant or of recessive nature governing male fertility is ruled out as the χ^2 value for monohybrid ratio, 3 :1 was found to be significant. The F_2 data indicate that more than one gene were probably involved in the restoration of fertility of the cytoplasmic male sterility (CMS). The χ^2 test suggested that two dominant genes which are having a complementary action governs fertility restoration making this a CGMS mechanism. The deviation from 9:7 (fertile: sterile) complementary gene action ratio was non-significant as the calculated χ^2 value is lesser than the table value (P=0.05). If two genes are involved in a specific pathway and functional products from both are

required for expression, then one recessive allelic pair at either loci would result in the mutant phenotype. Because both genes are required for the correct phenotype, this epistatic interaction is called complementary gene action (Duvick, 1956). In a dihybrid cross involving two heterozygous parents, if dominant alleles from both loci complement each other when they are present together (AABB....) producing a particular phenotype, the F_2 ratio 9:3:3:1 is modified as 9:7.

CMS is maternally inherited and is associated with a specific mitochondrial gene whose expression impairs the production of viable pollen without otherwise affecting the plant (Budar and Pelletier, 2001). The restorer of fertility (*Rf*) genes in the nucleus function to suppress the CMS phenotype and restore the male fertility. So far cytoplasmic male sterility has not been reported in cucurbits (Kaul, 1988). So there was no mention of male fertility restorer gene in cucurbits. However dominant restorer alleles have been identified in several hot pepper genotypes (Zhang *et al.*, 2000) with an intermediate phenotype in the F_2 generation, which resulted in partially sterile pollen, presumably due to either interaction with the environment or quantitative genetic control.

Assuming that MS line is having genotype, *rf1rf1 rf2rf2* and sterile cytoplasm (S) and male parent, Arka Sumeet possesses a genotype *Rf1Rf1 Rf2Rf2* carrying both fertility restorer gene in homozygous dominant state and normal fertile cytoplasm (N), F_1 will be male fertile as the genotype of F_1 is *Rf1rf1 Rf2rf2* with sterile cytoplasm. Here though F_1 is inheriting a sterile cytoplasm from male sterile female parent, presence of both complementary dominant fertility restorer genes, *viz*, *Rf1* and *Rf2* restores the fertility of F_1. In F_2 presence of both dominant fertility restorer genes in either homozygous or heterozygous condition ensures male fertility.

The gene action governing male sterility can be explained with the following model (Table 10)

Table 10. Proposed genetic model for complementary gene action

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

The knowledge about inheritance of fertility restoration of male sterile line is of vital importance in improving or transferring fertility restoring genes and quality of restorer line breeding. Fertility restorer gene controlled by complementary gene action is reported in cole crops (Sigareva and Earle, 1997) and in pepper. (Shifriss, 1997). Depending on the type of sterile cytoplasm, interaction of *Rf* allele varies. In carrot two types (petaloid and brown anther) of male sterile lines are available (Welch and Grimball, 1947; Morelock, 1974) depending on the type of cytoplasm and here genetics of fertility restoration is

complex (Peterson and Simon, 1986) because structural variants of mt DNA are numerous (Ranfort *et al.*, 1995). The proposed model, suggest cytoplasmic male sterility in ridge gourd in which sterility is modified by the influence of dominant fertility restorer genes *viz, Rf1* and *Rf2*. This is the first report of cytoplasmic male sterility and fertility restorer gene in cucurbits.

5.4 Evaluation of back cross and three way cross for expression of male sterility and restoration of male fertility

All three way crosses *viz*, (MS x Deepthi) x Arka Sumeet, (MS x IC-92685) x Arka Sumeet, (MS x IC-92671) x Arka Sumeet and (MS x CO2) x Arka Sumeet regained fertility indicating the presence of dominant fertility restorer gene in Arka Sumeet. All the male sterile hybrids on crossing with Arka Sumeet produced fertile male flowers early and took only few nodes for generating fertile male racemes. Hegade (2009) also reported the restoration of fertility when male sterile line (MS) was crossed with Arka Sumeet and suspected the presence of fertility restorer gene in Arka Sumeet. Present results confirmed his finding and the segregation pattern in F_2 indicate the presence of two fertility restorer genes in Arka Sumeet *viz Rf1* and *Rf2*.

Surprisingly the sterile hybrids on back crossing with respective pollen parents also exhibited male fertility at various stages of crop growth. Among the four back crosses *viz,* (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2, only the the back cross, (MSx Deepthi) x Deepthi exhibited male sterility in the initial stages but produced fertile male flower after 58 days. Among backcrosses, back cross (MS x Arka Sumeet) x Arka Sumeet was the earliest to form male flower (38.66) which is again an indication of the presence of fertility restorer genes in Arka Sumeet. Restoration of male fertility in BC_1 generation indicates the unstable nature of sterile cytoplasm which has been reported in pepper and carrot (Kaul, 1988). Among various pollen parents, Deepthi restores the male fertility after a stage in crop growth, indicating the strong interaction between cytoplasm and nuclear genes and dominant effect of fertility restorer genes in the late stage of crop

growth. Since this is the first report of cytoplasmic sterility in cucurbits, more studies are required to find out the exact nature and potential of this cytoplasm before exploiting the same for crop improvement.

5.5 Evaluation of back crosses and three way crosses for other importance biometric characters

Backcrosses and three way crosses differed significantly with respect to average fruit length, number of harvests, number of fruit per plant and yield per plant. Arka Sumeet produced longest fruit (44.33 cm) while the accession, IC-92685 produced highest number of fruits per plant (52.66). The three way cross (MS x IC-92671) x Arka Sumeet exhibited highest yield per plant (7.79 kg/plant). Superiority of three way crosses can be attributed to the accumulation of dominant alleles governing yield characters which has been reported in many cucurbits (Seshadri and More, 2009). This approach was utilized in cucumber and a triple cross hybrid was developed which is high yielding with good fruit shape, color and had good processing qualities both as brinestek and fresh pack. It had broad spectrum resistance to cucumber diseases (Pike, 1974).

Theoretically, the multiple crosses (three way, four way *etc.*) provide an opportunity for recombination of genes from many parents creating large genetic variability and improvement of populations through favorable gene combinations and associations of desired traits (Allard, 1960). Generally in a genetic improvement programme, two parents complimenting each other with respect to desirable characters are crossed to generate variability. However, it is often noticed that all the desirable characters need not be distributed among two parents. Hence, the need for wider gene pool and broad genetic base arises. From this angle it is necessary to involve multiple cross combinations of three parents or more to generate substantial variability and also to improve the chances of obtaining segregants which accumulate the maximum number of desirable genes (distributed between parents). Further, it is contemplated on sound theoretical footing that if the performance of three-way cross hybrid is considerably higher

with all the consumer requirements, then it should be feasible to isolate high yielding segregants in large numbers from the segregating populations. The selected segregants will be advanced through continuous selfing so that, complete homozygosity and homogeneity could be achieved. After attaining homozygosity in these isolated economic segregants from three-way cross populations one could think of population improvement by recurrent selection for combining ability by crossing these selected lines. Though the main objective of developing three way crosses involving male sterile hybrids and Arka Sumeet was to test the fertility restorer character of later, high yielding crosses such as (MS x IC-92671) x Arka Sumeet can be exploited for extraction of breeding lines.

5.5.1 Developing new male sterile line in ridge gourd

As the backcrosses (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2 regained fertility, scope of developing new male sterile lines from the backcross generation is rather limited. Among the various back crosses, (MS x Deepthi) x Deepthi produced fertile male flower only in later stages. This BC_1 can be selfed to isolate new male sterile lines. However, F_2 segregants from the cross MS x Arka Sumeet producing fertile racemes during last stage of crop growth have more potential for developing male sterile lines. Selective mating of these plants with male sterile plants or selfing can be advocated for developing new male sterile lines.

5.6 Evaluation of the F_1 hybrid MS x Arka Sumeet for yield and horticultural characters and estimation of heterosis

Hegade (2009) evaluated the F_1 hybrid, MS x Arka Sumeet and reported the potential yield characters. In the present study, significant difference between genotype and F_1 was observed for all the biometric characters studied. Genotype, Arka Sumeet produced early male flower (36.2) and female flower (34.8) indicating the earliness. Genotypes and F_1 differed significantly with respect to all fruit characters, *viz* average fruit length, average fruit weight and fruit girth. The

accession Arka Sumeet produced longest and heavy fruits (43.6 cm and 0.243 g respectively).The accession Deepthi recorded highest fruit girth (15.5cm). Accession IC-92684 produced highest number of fruits (32.2) followed by IC-92671 (31.4) and F_1 hybrid (31.2). The F_1 hybrid recorded highest yield per plant (6.4kg/plant) while the genotype Haritham produced lowest yield (3.2 kg/plant). The F_1 hybrid produced highest number of seeds per fruit (179) while genotype IC-92671 produced lowest seeds per fruit (47.2). Apart from the hybrid MS x Arka Sumeet, accession IC-92685 also exhibited yield potential and should be advanced for further evaluation.

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). Hegade (2009) reported significant heterosis for yield and related characters in the cross MS x Arka Sumeet.

The F_1 hybrid (MS x Arka Sumeet) exhibited significant heterobeltiosis for days to emergence of first female flower, node to first male flower, node to female flower, number of fruits per plant and yield per plant. Significant negative heterosis was observed for pollen fertility (Minus twenty nine point five two per cent) and average fruit length (Minus twenty one point five five per cent). Significant negative standard heterosis was observed for average fruit girth. Hybrid exhibited high heterosis for yield characters and exhibited significant heterobeltiosis (Twenty per cent) and standard heterosis (Twenty five point eight per cent) for number of fruit per plant. Significant positive heterosis was also exhibited for yield per plant with heterobeltiosis of fourty eight point eight per cent and standard heterosis thirty three point three per cent respectively. Findings

of Hegade (2009) is validated in the present study and the high heterosis exhibited by the F_1 hybrid (MS x Arka Sumeet) indicates the scope of exploiting the male sterile line in heterosis breeding. The F_1 hybrid (MS x Arka Sumeet) can be tested further for commercial cultivation.

This is the first study which attempted find out the inheritance of male sterility and fertility restoration in ridge gourd and is the first report of presence of cytoplasmic male sterility and dominant fertility restorer genes in cucurbits.

SUMMARY

6. SUMMARY

Male sterility is the failure of plants to produce functional anthers, pollen or male gametes. Male sterility is of special interest to the plant breeders to produce more efficient and economic hybrid seed as it ensures genic emasculation.

Novel source of male sterility in ridge gourd was reported from Kerala and tissue culture protocol was developed for maintenance of male sterile line. In an attempt to elucidate the genetics of male sterility in ridge gourd crosses were made involving male sterile female parent and pollen parents selected from different parts of India. 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. 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. Hence, the present investigation on male sterility in ridge gourd is undertaken with the objective of investigating the inheritance of male sterility in ridge gourd and to develop new male sterile line in ridge gourd using back cross generations of sterile hybrids. Study also aims in evaluating the performance of F_1 hybrid MS x Arka Sumeet for horticultural characters.

Micropropagation was effective in maintaining the male sterile lines. 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.

Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile. There were observable differences between the male

sterile and male fertile plants with respect to male flower production though female flowers in both types were similar. Fourty six point two per cent of the F_2 plants produced fertile male flower between 52nd to 64.9th days after sowing. Two point eight three per cent of the F_2 population reverted to male fertility on final stage of crop growth and produced fertile male flower after 97days. BC_1 exhibited narrow range for this character (Fourty two to four seven per cent). Sixty four point three per cent of BC_1 plants produced fertile male flower from 39-51.9 days after sowing. The F_2 population exhibited high variance for fruit length, fruit girth and fruit weight. Some of the F_2 plants exhibited high yield potential and nine point four three per cent of F_2 population produced more than 91 fruit per plant and seventeen point nine per cent of the F_2 plants yielded 8 to 9 kg per plant. Some of the F_2 plants produced more number of fruits and high yield compared to BC_1 generation.

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

are absent in these genotypes. The proposed model, suggests cytoplasmic male sterility in ridge gourd which is modified by the influence of dominant fertility restorer genes viz, *Rf1* and *Rf2*.

All three way crosses *viz*, (MS x Deepthi) x Arka Sumeet, (MS x IC-92685) x Arka Sumeet, (MS x IC-92671) x Arka Sumeet and (MS x CO2) x Arka Sumeet regained fertility indicating the presence of dominant fertility restorer gene in Arka Sumeet. Surprisingly the sterile hybrids on back crossing with respective pollen parents also exhibited male fertility at various stages of crop growth. Among the four back crosses viz, (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2, only the back cross, (MS x Deepthi) x Deepthi exhibited male sterility in the initial stages, but produced fertile male flower after 58 days. Restoration of male fertility in BC_1 generation indicates the unstable nature of sterile cytoplasm which has been also reported in pepper and carrot.

Genotypes, backcrosses and three way crosses differed significantly with respect to average fruit length, number of harvests, number of fruits per plant and yield per plant. The genotype Arka Sumeet produced longest fruit (44.33cm) while the accession IC-92685 produced highest number of fruits per plant (52.66). The three way cross (MS x IC-92671) x Arka Sumeet exhibited highest yield per plant (7.79 kg/plant). Though the main objective of developing three way crosses involving male sterile hybrids and Arka Sumeet was to test the fertility restorer character of later, high yielding crosses such as (MS x IC-92671) x Arka Sumeet can be exploited for extraction of breeding lines.

As the backcrosses (MS x Deepthi) x Deepthi, (MS x IC-92685) x IC-92685, (MS x IC-92671) x IC-92671 and (MS x CO2) x CO2 regained fertility, scope of developing new male sterile lines from the backcross generation is rather limited. Among the various back crosses, (MS x Deepthi) x Deepthi can be selfed to isolate new male sterile lines. However, F_2 segregants producing fertile racemes during last stage of crop growth have more potential for developing male

sterile lines. Selective mating of these plants with male sterile plants or selfing can be advocated for developing new male sterile lines.

F_1 was evaluated with KAU varieties and other accessions to test horticulture characters. Genotypes and F_1 (MS x Arka Sumeet) differed significantly with respect to all fruit characters, *viz* average fruit length, average fruit weight and fruit girth. Arka Sumeet produced longest and heavy fruits (43.6cm and 0.243 g respectively). The Deepthi recorded highest fruit girth (15.5cm). Accession IC-92684 produced highest number of fruit (32.2) while the F_1 hybrid recorded highest yield per plant (6.4 kg/plant).

Heterosis was estimated for the hybrid (MS x Arka Sumeet) in two different ways, heterosis over better parent (Arka Sumeet) and heterosis over standard parent (Deepthi). The F_1 hybrid (MS x Arka Sumeet) exhibited significant heterobeltiosis for days to emergence of first female flower, node to first male flower, node to female flower, number of fruits per plant and yield per plant. Hybrid exhibited high heterosis for yield characters and exhibited significant heterobeltiosis (Twenty per cent) and standard heterosis (Twenty five point eight per cent) for number of fruit per plant. Significant positive heterosis was also exhibited for yield per plant with heterobeltiosis of fourty eight point eight per cent and standard heterosis thirty three point three per cent respectively. High heterosis exhibited by the F_1 hybrid (MS x Arka Sumeet) indicates the scope of exploiting the male sterile line in heterosis breeding. The F_1 hybrid (MS x Arka Sumeet) can be tested further for commercial cultivation.

This is the first study which attempted find out the inheritance of male sterility and fertility restoration in ridge gourd and is the first report of presence of cytoplasmic male sterility and dominant fertility restorer gene in cucurbits.

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APPENDIX I

Data on weather changes in COH, Vellanikkara campus from 04/01/10 to 29/04/11									
Week		Temperature (°C)		Humidity (%)		Wind speed (km/h)	Bright sunshine (hrs/day)	Rainfall (mm)	Evaporation (mm)
No.	Date	Max	Min	I	II				
1	4/4-7/1	30.6	22.7	95	78	5.2	9.8	0	5.3
2	8/1-14/1	29.5	23.3	74	70	10.3	9.9	0	7.3
3	15/1-21/1	28.3	21.9	95	78	10.5	9.4	0	6.8
4	22/1-28/1	29.3	27.3	94	76	5.2	8.5	0	5.9
5	29/1-4/2	30.6	22.2	95	71	5.0	9.5	0	7.3
6	5/2-11/2	30.4	22.3	96	73	5.8	9.7	0	5.2
7	12/2-18/2	31.3	22.5	92	67	4.4	9.6	0	6.7
8	19/2-25/2	30.8	22.5	91	71	6.0	10.0	0	5.6
9	26/2-4/3	28.1	22.8	83	71	3.9	7.8	0	5.0
10	5/3-11/3	31.0	21.3	89	59	9.2	7.6	0	3.8
11	12/3-18/3	31.4	21.5	91	59	10.5	8.4	1.2	4.5
12	19/3-25/3	30.9	22.8	77	55	6.8	7.8	0.3	5.5
13	26/3-1/4	30.7	21.8	76	51	9.9	7.9	0.4	4.8
14	2/4-8/4	31.9	22.3	84	51	9.3	6.8	2.6	5.8
15	9/4-15/4	33.2	22.3	89	45	4.2	4.9	1.3	5.4
16	16/4-22/4	32.9	20.9	73	36	6.7	5.9	2.4	4.6
17	23/4-29/4	32.2	22.9	67	38	8.4	7.3	0.4	6.4

APPENDIX II

Data on weather changes in COH, Vellanikkara campus from 02/07/10 to 15/10/10									
Week		Temperature (°C)		Humidity (%)		Wind speed (km/h)	Bright sunshine (hrs/day)	Rainfall (mm)	Evaporation (mm)
No.	Date	Max	Min	I	II				
1	2/2-9/7	30.2	23.1	96	76	5.2	5.8	28.6	3.3
2	9/2-16/7	28.6	22.8	96	81	6.3	4.9	26.6	5.3
3	16/2-24/7	31.2	24	95	72	6.5	5.4	33.2	6.8
4	24/2-30/7	27.8	30	97	84	5.2	6.5	23.9	5.9
5	30/1-7/8	29.6	22.3	95	81	6.0	6.5	14.8	4.3
6	8/1-14/8	28.6	22.3	96	74	5.8	5.7	19.2	5.2
7	14/1-23/8	30.6	24.1	95	73	4.4	9.6	4.4	6.7
8	23/1-27/8	29.5	23	94	79	6.0	5.0	6.2	5.6
9	27/1-30/8	28.7	23.3	94	83	3.9	5.8	1.9	5.0
10	1/1-11/9	28.6	22.8	95	76	9.2	4.6	9.1	3.8
11	11/1-18/9	29.9	23.1	94	73	8.5	6.4	5.6	4.5
12	18/1-24/9	29.8	23.2	96	72	6.8	6.8	21.2	5.5
13	24/1-30/9	30.2	23	95	72	9.9	5.9	8.1	4.8
14	30/1-8/10	31.9	23	92	68	9.3	6.8	10.8	5.8
15	8/1-11/10	30.6	22.7	95	78	4.2	4.9	41.4	5.4
16	11/1-15/10	29.5	23.3	74	70	6.7	5.9	5.1	4.6

INHERITANCE OF MALE STERILITY AND DEVELOPMENT OF NEW MALE STERILE LINE IN RIDGE GOURD (*Luffa acutangula* (L.) Roxb.

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study entitled "Inheritance of male sterility and development of new male sterile line in ridge gourd *Luffa acutangula* (L.) Roxb." was undertaken in the Department of Olericulture, College of Horticulture, Vellanikkara during 2010-11. The objective of the study was to investigate the inheritance of male sterility in ridge gourd and to develop new male sterile line in ridge gourd using back cross generations of sterile hybrids. Study also aims in evaluating the performance of F_1 hybrid, MS x Arka Sumeet for horticultural characters. Male sterile line is now maintained under *in vitro* condition. All the *in vitro* regenerated plants exhibited stability in the expression of male sterility.

F_2 seeds resulted from the selfing of F_1 hybrid, MS x Arka Sumeet was raised to study the expression of male fertility. Out of 106 plants raised in the F_2 generation, 56 plants were male fertile and 50 plants were male sterile. There were observable differences between the male sterile and male fertile plants with respect to male flower production whereas female flowers in both types were similar. Chi square test was employed to test the goodness of fit and the 9:7 (fertile: sterile) complementary gene action ratio was found to be significant. The Chi square test suggested that two dominant genes might have certain interactions with cytoplasmic male sterility (CMS). Two dominant fertility restorer gene viz., *Rf1* and *Rf2* is now proposed for this model.

Assuming that MS line in ridge gourd is having a genotype, *S (rf1rf1 rf2rf2)* carrying both fertility restorer gene in homozygous recessive state and sterile cytoplasm, S, and Arka Sumeet possess a genotype *N (Rf1Rf1 Rf2Rf2)* carrying both fertility restorer gene in homozygous dominant state and normal fertile cytoplasm, N, F_1 will be male fertile as the genotype of F_1 is *S (Rf1rf1 Rf2rf2)*. Here though F_1 is inheriting a sterile cytoplasm from male sterile female parent, presence of both dominant fertility restorer gene, *viz.*, *Rf1* and *Rf2*

restores the fertility of F_1. In F_2, presence of both dominant fertility restorer gene in either homozygous or heterozygous condition ensures male fertility.

All three way crosses viz, (MS x Deepthi) x Arka Sumeet, (MS x IC-92685) x Arka Sumeet, (MS x IC-92671) x Arka Sumeet and (MS x CO2) x Arka Sumeet regained fertility indicating the presence of dominant fertility restorer gene in Arka Sumeet. The sterile hybrids on back crossing with respective pollen parents also exhibited male fertility at various stages of crop growth. Restoration of male fertility in BC_1 generation indicate the unstable nature of sterile cytoplasm. F_2 segregants producing fertile racemes during last stage of crop growth have more potential for evolving stable male sterile lines. Selective mating of these plants with male sterile plants or selfing can be advocated for developing new male sterile lines.

The F_1 hybrid (MS x Arka Sumeet) exhibited significant heterobeltiosis for days to emergence of first female flower, node to first male flower, node to female flower, number of fruits per plant and yield per plant. High heterosis exhibited by the F_1 hybrid (MS x Arka Sumeet) indicates the scope of exploiting the male sterile line in heterosis breeding. This is the first study which attempted to find out the inheritance of male sterility and fertility restoration in ridge gourd and is the first report of presence of cytoplasmic male sterility and dominant fertility restorer gene in cucurbits.