DEVELOPMENT OF INBREDS IN BITTER GOURD (*Momordica charantia* L.) THROUGH CONVENTIONAL AND BIOTECHNOLOGICAL APPROACHES

By

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(2015- 22-006)

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2020

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THESIS

Submitted in partial fulfilment of the

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Kerala Agricultural University

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF HORTICULTURE

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DECLARATION

I, hereby declare that this thesis entitled **"DEVELOPMENT OF INBREDS IN BITTER GOURD (*Momordica charantia* L.) THROUGH CONVENTIONAL AND BIOTECHNOLOGICAL APPROACHES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara,

Date: 18/09/2020

RESHMIKA P. K.

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CERTIFICATE

Certified that this thesis entitled **"DEVELOPMENT OF INBREDS IN BITTER GOURD (*Momordica charantia* L.) THROUGH CONVENTIONAL AND BIOTECHNOLOGICAL APPROACHES"** is a record of research work done independently by Ms. RESHMIKA P. K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara,

Date: 18.09.2020

Dr. Pradeepkumar, T.

(Major advisor, Advisory Committee)
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Professor & Head, Dept. of Vegetable Science, College of Horticulture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. RESHMIKA, P. K., a candidate for the degree of **Doctor of Philosophy in Horticulture** with major in Vegetable Science, agree that the thesis entitled **"DEVELOPMENT OF INBREDS IN BITTER GOURD (*Momordica charantia* L.) THROUGH CONVENTIONAL AND BIOTECHNOLOGICAL APPROACHES"** may be submitted by Ms. RESHMIKA P.K, in partial fulfillment of the requirement for the degree.

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EXTERNAL EXAMINER

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Reshmika P. K.

Affectionately dedicated to my beloved family and Major advisor Dr. Pradeepkumar, T.

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LIST OF ABBREVIATIONS

%	-	Per cent
^{0}C	-	Degree Celcius
ANOVA	-	Analysis of Variance
C.D	-	Critical Difference
et al.	-	Co workers
g	-	Gram
GA	-	Genetic advance
GAM	-	Genetic advance as per cent of mean
GCV	-	Genotypic Co efficient of Variation
Gy	-	Grey
$HgCl_2$	-	Mercuric chloride
IAA	-	Indole Acetic Acid
IARI	-	Indian Agricultural Research Institute
KAU	-	Kerala Agricultural University
kg	-	Kilogram
mg	-	Milligram
PCV	-	Phenotypic Co efficient of Variation
WASP	-	Web Agricultural Statistical Package

Introduction

1. INTRODUCTION

Momordica charantia L. commonly known as bitter gourd, bitter melon, balsam pear, bitter cucumber, or *karela* is a popular vegetable throughout the tropics and subtropics of Asia. It belongs to family *Cucurbitaceae* (2n=2x=22). The crop originated probably in India and China was considered as the secondary centre of diversity (Grubben, 1977). Fruits of bitter gourd are good source of carbohydrate, protein, vitamins and minerals. Antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic properties are reported. They also exhibit the ability to reduce blood sugar (Raman and Lau, 1996). In India, bitter gourd covers an area of 97 thousand hectares with an annual production of 1137 thousand tonnes (NHB, 2019).

F_1 hybrids are popular in bitter gourd. Hybrids in most of the vegetable crops offer the opportunity of earliness, high yield, and quality improvement besides better capacity to face biotic and abiotic stresses. For achieving uniformity in the hybrids, it is essential to develop inbreds and the degree of inbreeding required will be determined by the extent of uniformity desired. In bitter gourd, inbreds are maintained in pure form by selfing without loss of vigor (Behera, 2005). When inbreds are to be produced, the selection is done among the inbred lines to obtain promising inbreds.

In Kerala, though bitter gourd is a popular vegetable, F_1 hybrid development is limping due to the lack of quality inbreds. F_1 hybrids from private sector are popular among farmers. Dependence of farmers on private sector F_1 hybrid is a matter of concern since it involves escalation in seed price. Advantage of these hybrids can be exploited, by directly using them in inbred development programme.

Most of the superior plants, mainly cross-pollinated, exhibit a depression in larger or smaller degree as a consequence of the inbreeding. However, some species that are naturally self-pollinated do not show inbreeding depression. Cucurbits are cross-pollinated, but they are examples of species in which some lines seem to lose little vigor due to inbreeding (Whitaker and Robinson, 1986).

Hayes and Immer (1942) indicated that self pollination of squash resulted in generation of new lines with desirable characteristics. Halsey (1978) confirmed that selfing for several generations achieved homogeneity for some traits in few lines of cantaloupe. Inbreeding with selection was effective in recovering desirable lines in pumpkin (Unander and Ramirez, 1988; Damarany, 1989).

Another approach for inbred development in bitter gourd is through exploitation of *in situ* induction of maternal haploids initiated by pollination with irradiated pollen. Embryo development is stimulated by pollen germination on the stigma and growth of the pollen tube within the style, although irradiated pollen is unable to fertilize the egg cell. Though this technique has been used successfully in several species (Dong *et al.,* 2016), no inbred line has been developed in bitter gourd. The dose of irradiation is the main factor controlling *in situ* haploid production. At lower doses, the generative nucleus is partly damaged and therefore maintains its capacity to fertilize the egg cell. It results in large number of embryos but all are of hybrid origin and abnormal (mutant) phenotype. An increase in the irradiation dose causes a decrease in the total number of developed embryos but the obtained regenerants are mostly of haploid origin. For most plant species, *in vitro* embryo rescue is necessary to recover haploid plants. Inbred development through embryo culture of haploids has not been exploited in bitter gourd so far. Bitter gourd, being the most important vegetable crop in Kerala, it is essential to exploit all opportunities for evolving superior inbred lines which is the prerequisite for F_1 hybrid development programme.

Hence, the present study is undertaken to develop superior inbred lines in bitter gourd through advance generation selection of F_1 hybrids and biotechnological approaches through pollination of irradiated pollen and embryo culture.

Review of literature

2. REVIEW OF LITERATURE

The primary objective of vegetable breeders is the improvement of quantitative and qualitative plant characters. Adequate knowledge of genetics of various characters is essential for the successful implementation of various breeding programmes. Available literature on the development of inbreds in bitter gourd through conventional and biotechnological approaches has been reviewed and presented under the following sub headings.

2.1 Genetic variability, heritability and genetic advance in bitter gourd

Information on the nature and magnitude of variability of plant characters is useful as a basis for selection of desirable parents in plant breeding programmes. The coefficient of genotypic and phenotypic variation is helpful to measure the extent of variability present in a particular trait, whereas, the estimate of heritability provides an index of transmissibility of characters. The variability for any character is the result of interaction of hereditary effects of concerned genes and the influence of environment. Heritable variation can be effectively used with a greater degree of accuracy when heritability is studied in conjunction with genetic advance. Review of literature on mean, range, components of variance, heritability and genetic advance in bitter gourd and related crops are presented in Table 1.

2.2 Performance of hybrids and developments of inbreds in bitter gourd

The main objective of most of the plant breeding programme is to increase the yield potential of the crop plants. Hybridization is one of the means of obtaining increased yield. Being a cross pollinated crop, it is easier to realize the heterosis as practically feasible phenomena in bitter gourd. Heterosis breeding needs identification of potential inbred lines with substantial diversity which is the prerequisite endeavour. For achieving uniformity in the hybrids, it is essential to develop inbreds and the degree of inbreeding required will be determined by the extent of uniformity desired. In bitter gourd, inbreds are maintained in pure form by selfing/sibmating without loss of vigor. When inbreds are to be produced,

Table1. Review of literature on mean, range, components of variance, heritability and genetic advance in bitter gourd and related vegetable crops

Sl. No.	Characters	Crop	Mean	Range	PV	PCV (%)	GV	GCV (%)	h2 (%)	GA	GAM (%)	References
1	Days to first male flower anthesis	Bitter gourd				5.03		4.12	76.57 (ns)	3.76	8.89	Rani *et al*. (2014)
			56.59	53.77-61.22	6.74	34.54	3.44	24.68				Khan *et al*. (2015)
			63.84	57.75- 71.25		5.68		4.76	70.22 (bs)	4.48	7.02	Gowda (2017)
			33.15	28.6- 37.03	8.61	8.85	4.44	6.36	0.52 (bs)	3.12	9.41	Kumar *et al* .(2018)
						11.83		9.55	65.14(bs)	6.60	15.88	Maurya *et al*. (2018)
			53.90	50.00-60.00	10.78	6.09	4.39	3.89	40.7 (bs)	2.75	5.11	Talukder *et al*. (2018)
				35.00- 50.66		12.91		9.07	49.24 (bs)	5.42	13.14	Tyagi *et al*. (2018)
			38.88	32.64- 46.13	18.85	11.16	11.42	8.69	60.60 (bs)	5.40	13.93	Sahoo *et al*. (2017)
2	Nodes to first male flower	Bitter gourd				20.69		18.26	77.92 (bs)	3.18	33.21	Maurya *et al*. (2018)
						9.45		7.08	73.85 (ns)	1.20	14.01	Rani *et al*. (2014)
			11.37	6.18- 18.41		28.66		27.77	93.92 (bs)	5.39	47.37	Gowda (2017)
				6.33 - 13.00		20.17		17.61	76.3 (bs)	2.91	31.70	Tyagi *et al*. (2018)
			5.47	3.40- 7.70	1.42	21.81	1.10	19.17	0.77 (bs)	1.90	34.71	Kumar *et al* .(2018)
3	Days to first female flower anthesis	Bitter gourd	56.41	53.03- 58.25		5.19		5.05	94.60 (bs)	5.71	10.13	Rajput (2012)
			55.27	50.76- 58.29		4.84		4.81	98.80 (bs)	5.45	9.86	Rajput (2012)
						4.95		4.3	82.13 (ns)	5.11	9.84	Rani *et al*. (2014)
			66.29	62.9- 71.43	9.13	37.14	7.37	33.37				Khan *et al*. (2015)
			55.33	52.86- 59.33		5.40		4.91	82.71 (bs)	5.09	9.21	Nandkumar (2016)
			56.15	51.70- 60.68		5.31		4.79	81.4 (bs)	5.00	8.90	Nandkumar (2016)
			55.33	52.86-59.33		5.40		4.91	82.71 (bs)	5.09	9.21	Nandkumar (2016)
			55.11	50.79- 57.19		5.87		4.98	72.02 (bs)	4.80	8.71	Nandkumar (2016)
						11.27		9.33	68.62 (bs)	7.49	15.93	Maurya *et al*. (2018)
			73.77	63.66- 83.2		4.94		3.68	55.54 (bs)	3.56	4.83	Gowda (2017)
4	Nodes to first female flower	Bitter gourd	27.29	25.53- 30.73		10.91		10.74	97.00 (bs)	5.95	21.81	Rajput (2012)
			26.91	24.42- 30.68		7.21		7.14	98.10 (bs)	3.92	14.58	Rajput (2012)
						9.05		7.29	59.54 (ns)	2.33	15.52	Rani *et al*. (2014)
			24.40	21.30- 27.21		9.51		8.38	77.6 (bs)	3.65	15.20	Nandkumar (2016)
			25.49	22.46-28.79		7.51		6.49	74.68 (bs)	2.94	11.55	Nandkumar (2016)
			23.67	22.17- 25.82		8.81		4.88	70.61 (bs)	2.00	8.46	Nandkumar (2016)
			24.15	22.10- 26.84		7.94		6.81	73.69 (bs)	2.91	12.05	Nandkumar (2016)

	Nodes to first female flower	Bitter gourd	16.66	9.92- 24.63		27.24		26.27	93.04 (bs)	7.43	44.60	Gowda (2017)
						15.93		12.84	64.97 (bs)	2.79	21.33	Maurya *et al*. (2018)
5	Days to first harvest	Bitter gourd	64.12	62.86- 64.83		1.71		1.09	41.20 (bs)	0.93	1.45	Rajput (2012)
			62.76	59.53- 65.21		3.05		2.97	95.10 (bs)	3.75	5.97	Rajput (2012)
			60.16	57.08- 64.95		3.38		3.05	81.25 (bs)	3.40	5.67	Nandkumar (2016)
			61.75	58.13-66.16		4.71		4.02	72.78 (bs)	4.36	7.07	Nandkumar (2016)
			61.12	58.46- 64.44		4.64		4.12	78.79 (bs)	4.60	7.53	Nandkumar (2016)
			60.89	57.55-63.20		5.1		4.14	66.06 (bs)	4.23	6.94	Nandkumar (2016)
			85.70	78.33- 95.20		3.78		2.97	61.91 (bs)	3.53	4.11	Gowda (2017)
						9.02		7.05	61.07 (bs)	6.91	11.35	Maurya *et al*. (2018)
6	Relative early yield	Cucumber							24.70 (bs)		97.90	Rastogi and Aryadeep (1990)
											low to moderate	Wehner and Cramer (1996)
									86.92 (bs)			Gu-Xing Fang *et al*. (2004)
		Chilli	234.54	101.50-510.10	9702. 11	42.00	5549.01	31.76	57.20 (bs)	116.50	49.47	Krishna *et al*. (2007)
7	Number of days from anthesis to fruit maturity	Bitter gourd				11.85		5.70	23.16 (bs)	5.65		Pathak *et al*. (2014)
		Spine gourd	15.58	10.00-18.33		13.06		12.26	88.11 (bs)	3.96	23.72	Prabhakar (2014)
		Pumpkin				3.08		1.44	22.00 (bs)			Sampath and Krishnamoorthy (2017)
8	Average fruit weight	Bitter gourd	63.25	51.40- 71.70		16.52		16.45	99.10 (bs)	21.34	33.74	Rajput (2012)
			67.00	52.32- 71.28		9.29		9.27	99.60 (bs)	12.79	19.70	Rajput (2012)
						11.08		10.29	38.54 (ns)	16.75	25.21	Rani *et al*. (2014)
			115.30	102.70 - 130.20	67.69	76.67	36.75	56.49				Khan *et al*. (2015)
			61.09	52.29- 68.02		9.99		9.25	85.76 (bs)	10.79	17.66	Nandkumar (2016)
			62.18	56.79- 71.30		10.82		10.53	94.59 (bs)	13.12	21.10	Nandkumar (2016)

	Average fruit weight	Bitter gourd	64.56	54.84- 68.39		5.78		5.09	77.37 (bs)	5.95	9.22	Nandkumar (2016)
			61.19	52.06- 67.48		10.95		10.28	88.11 (bs)	12.17	19.88	Nandkumar (2016)
			50.68	17.59- 82.59		27.21		27.01	98.54 (bs)	23.92	47.19	Gowda (2017)
						16.00		14.34	80.30 (bs)	28.19	26.47	Maurya *et al*. (2018)
9	Fruit length	Bitter gourd	20.65	20.3- 21.28		2.65		2.07	61.10 (bs)	0.68	3.33	Rajput (2012)
			19.86	18.28- 21.05		5.41		1.15	94.60 (bs)	2.09	10.55	Rajput (2012)
						9.34		8.49	53.14 (ns)	3.12	20.38	Rani *et al*. (2014)
			20.28	15.5-21.99	5.91	53.70	5.56	52.09				Khan *et al*. (2015)
			20.21	17.53- 23.93		7.89		6.99	78.56 (bs)	2.58	12.77	Nandkumar (2016)
			20.81	18.55- 23.57		10.05		8.54	72.25 (bs)	3.11	14.96	Nandkumar (2016)
			21.15	17.64-24.02		10.18		8.37	67.67 (bs)	3.00	14.19	Nandkumar (2016)
			19.32	17.26- 21.88		11.59		10.10	75.96 (bs)	3.50	18.14	Nandkumar (2016)
			11.55	4.89- 17.37		21.11		18.98	89.56 (bs)	3.84	33.28	Gowda (2017)
						20.73		18.57	80.20 (bs)	5.62	34.25	Maurya *et al*. (2018)
10	Fruit diameter	Bitter gourd	2.99	2.71- 3.27		9.37		9.16	95.70 (bs)	0.55	18.47	Rajput (2012)
			3.02	2.72- 3.26		4.90		0.02	97.50 (bs)	0.29	9.85	Rajput (2012)
			10.74	9.86-11.84	0.40	19.31	0.25	15.26				Khan *et al*. (2015)
			3.49	3.26- 3.68		4.75		4.62	94.56 (bs)	0.32	9.27	Nandkumar (2016)
			3.48	3.03-3.66		6.45		5.80	80.86 (bs)	0.37	10.74	Nandkumar (2016)
			3.54	3.36-3.63		3.61		3.55	96.50 (bs)	0.25	7.19	Nandkumar (2016)
			3.51	3.28-3.70		3.53		3.48	97.30 (bs)	0.24	7.09	Nandkumar (2016)
						12.25		9.05	54.59 (bs)	0.50	13.78	Maurya *et al*. (2018)
			13.23	11.30-15.37	1.86	10.30	0.49	5.32	26.61 (bs)	0.75	5.65	Talukder *et al*. (2018)
11	Fruit girth	Bitter gourd	15.08	13.01 - 17.64	1.59	8.37	0.92	6.37	57.90 (bs)	1.50	9.99	Raja *et al.* (2007)
						31.96		31.7	98.35 (bs)		64.76	Praveena (2010)
			12.07			14.56		14.43	98.22 (bs)	3.55	29.47	Chakraborty *et al*. (2013)
						5.91		4.67	88.3 (ns)	1.18	9.73	Rani *et al*. (2014)
			12.01	9.42- 14.80		9.41		7.81	68.88 (bs)	1.37	11.41	Gowda (2017)
12	Flesh thickness	Bitter gourd	5.84	3.96 - 7.92	0.98	16.94	0.99	16.23	91.80 (bs)	1.80	32.05	Raja *et al.* (2007)
						15.57		15.46	98.58 (bs)		31.62	Praveena (2010)
			4.29	4.13- 4.51		4.68		4.26	82.70 (bs)	0.34	7.98	Rajput (2012)

	Flesh thickness	Bitter gourd	4.27	4.10- 4.60		3.83		3.70	93.30 (bs)	0.31	7.37	Rajput (2012)
			0.44			26.19		26.10	99.32 (bs)	0.23	53.59	Chakraborty *et al*. (2013)
		Bitter gourd (Pulp thickness)				5.61		5.20	37.8 (ns)	0.44	12.74	Rani *et al*. (2014)
13	Number of fruits per plant	Bitter gourd	51.5	45.79- 62.26		18.09		18.01	99.00 (bs)	19.01	36.92	Rajput (2012)
			61.85	45.54- 68.04		14.91		14.90	99.90 (bs)	18.97	30.67	Rajput (2012)
						16.61		16.06	53.16 (ns)	7.99	41.02	Rani *et al*. (2014)
			25.26	19.67- 30.00	13.83	73.92	8.75	58.79				Khan *et al*. (2015)
			42.05	37.93- 49.64		12.30		11.43	86.47 (bs)	9.21	21.91	Nandkumar (2016)
			42.37	31.93-50.15		15.81		15.08	90.98 (bs)	12.55	29.63	Nandkumar (2016)
			38.73	30.99- 44.06		17.66		16.80	90.48 (bs)	12.75	32.92	Nandkumar (2016)
			35.77	29.68- 40.45		20.76		18.03	75.36 (bs)	11.53	35.24	Nandkumar (2016)
			28.63	19.75- 45.54		18.05		17.28	91.66 (bs)	8.34	29.12	Gowda (2017)
						23.88		22.71	90.52 (bs)	8.19	44.52	Maurya *et al*. (2018)
14	Yield per plant	Bitter gourd	3.31	2.40- 4.43		31.08		30.05	98.60 (bs)	2.09	63.11	Rajput (2012)
			4.50	2.53- 5.32		22.02		22.01	99.90 (bs)	2.04	45.32	Rajput (2012)
						23.59		23.14	44.30 (ns)	0.78	59.94	Rani *et al*. (2014)
			2.76	2.2 - 3.42	0.18	25.52	0.12	20.83				Khan *et al*. (2015)
			2.64	1.96- 3.57		21.47		20.90	94.79 (bs)	1.11	41.94	Nandkumar (2016)
			2.49	1.85-2.75		16.64		15.39	85.54 (bs)	0.73	29.33	Nandkumar (2016)
			2.17	1.62- 2.72		21.06		18.99	81.26 (bs)	0.76	35.26	Nandkumar (2016)
			1.37	0.57- 2.18		27.89		27.31	95.89 (bs)	0.64	47.06	Gowda (2017)
						22.88		21.60	89.09 (bs)	0.68	42.00	Maurya *et al*. (2018)
15	Number of harvests	Cucumber	9.21	4.52 - 16.60		36.96		31.52	72.80 (bs)	5.10	55.37	Mekap (2016)
		Bell pepper	3.66	2.00- 4.66	0.83	24.92	0.23	13.18	27.99 (bs)	0.52	14.20	Sharma *et al*. (2010)
		Okra	12.23		1.25	9.14	0.43	5.39	34.77 (bs)	0.80	6.54	Chandramouli *et al*. (2016)
		Cowpea	7.94	6.83 - 9.83		11.49		9.53	68.81 (bs)	1.29	16.28	Diwaker *et al*. (2017)

16	Number of seeds per fruit	Bitter gourd	23.22	8.60 - 29.63	36.10	25.87	26.84	22.31	74.30 (bs)	9.20	39.62	Raja *et al.* (2007)
			11.41			34.23		34.15	99.52 (bs)	8.01	70.18	Chakraborty *et al*. (2013)
			8.93	4.50-17.50		40.07		26.75	44.00 (bs)		2.20	Sidhu (2013)
						8.63		5.26	19.26 (ns)	1.66	8.47	Rani *et al*. (2014)
			11.79	6.61- 18.19		26.15		26.13	99.90 (bs)	6.34	53.77	Singh *et al*. (2014)
			14.83	5.33- 20.65	13.47	24.74	11.06	22.42	82.10 (bs)	6.21	41.87	Sahoo *et al*. (2017)
			10.76	6.00- 18.50		34.13		32.70	91.80 (bs)	6.95	64.59	Yadagiri *et al*. (2017)
17	100 seed weight	Bitter gourd	20.26	13.86 - 24.15	10.05	15.60	6.86	12.93	68.60 (bs)	4.47	22.07	Raja *et al.* (2007)
			18.35									Resmi and sreelathakumary (2017)
			17.43	10.44-21.03	7.50	15.73	6.29	14.39	83.70 (bs)	4.73	27.14	Sahoo *et al*. (2017)
		Culinary melon	7.37	4.90-16.75		31.57		31.26	98.09 (bs)		63.79	Rakhi and Rajamony (2005)
		Bottle gourd			9.85	26.06	9.54	25.63	96.83 (bs)	6.26	51.95	Deepthi *et al*. (2016)
18	Fruit seed ratio	Bitter gourd (Seed flesh ratio)	0.08	0.03-0.26	0.003	67.64	0.003	65.306	93.20 (bs)	0.07	93.87	Sahoo *et al*. (2017)
		Pointed gourd (Pulp seed ratio)	13.24	7.76 - 20.37	6.90	52.35	6.70	51.23				Khan *et al*. (2009)

bs-broad sense , ns- narrow sense

selection is done among the inbred lines to obtain promising inbreds. Another approach of inbred development in bitter gourd is the exploitation of *in situ* induction of maternal haploids initiated by pollination with irradiated pollen. Bitter gourd being the most important vegetable crop in Kerala, it is essential to exploit all opportunities for evolving superior inbred lines which is the prerequisite in F_1 hybrid development programme.

2.2.1 Performance of hybrids of bitter gourd

2.2.1.1 Days to first male flower anthesis

Earliness is an important trait in a vegetable like bitter gourd. It is required for realizing the potential economic yield in as less time as possible, which is an important consideration for a farmer. Among 28 hybrids of bitter gourd studied days to first male flower opening varied from 38.87 (IC-044438 × IC-045339) to 45.53 (IC-044417 × IC-470550) days (Rani *et al*., 2014). Sureshkumara *et al*. (2017) reported that Coimbatore Long x Panurthy recorded the lowest mean value of 37.32 days for days to opening of first male flower among the 24 bitter gourd hybrids studied.

2.2.1.2 Nodes to first male flower anthesis

The appearance of node to first male flower varied from 6.93 (IC-044417 × IC-470560) to 10.13 (IC-044417 × IC-045339) among 28 F_1 hybrids of bitter gourd (Rani *et al*., 2014).

2.2.1.3 Days to first female flower anthesis

Days to female flower anthesis varied from 47.67 (DVBTG-5 x Hirkani) to 61.4 days (Delhi Local x Hirkani) in an investigation conducted among 28 F_1 hybrids of bitter gourd (Jadhav *et al*., 2009). Sundram (2009) observed that the hybrid combination MDU 1 x Vadipatti Local (31.78 days) was found to be the earliest to open female flower among the top performing hybrids and was followed by Bikaneer 3 x IC 85643 (32.55 days) and Paravai Local x IC 85643 (32.55 days). Among the nine bitter gourd hybrids studied, the minimum days for first female flower appearance was observed in the hybrid Preethi X MC- 30 (Thangamani *et al*., 2011). Rani *et al.* (2014) reported that range of days to first

female flower appearance varied from 46.27 (IC-044438 × IC-045339) to 57.60 (IC-470550 × IC-470560) days in bitter gourd hybrids. Among the 28 F_1 hybrids studied, days to first female flower anthesis ranged from 29.67 to 49 (Alhariri *et al*., 2018).

2.2.1.4 Nodes to first female flower anthesis

Among the different hybrids studied, female flower at lowest (seventh) node was observed in monoecious X monoecious hybrid and Pusa Vishesh X NDBT-12 (Behera *et al*., 2009). Sundarm (2009) reported that the first female flower on the lowest node (12.89) was observed in the hybrid Bikaneer 1 x IC 85643. The range of node to first female flower appearance was from 23.07 (Co. White Long x DVBTG-7) to 30.65 (Delhi Local x MC-84) in a study conducted among 28 F_1 hybrids of bitter gourd (Jadhav *et al*., 2009). First female flower at third node was observed in gynoecious × monoecious hybrid, DBGy- 201 x S54 followed by DBGy- 201 x DBG 34 at 5^{th} node, whereas monoecious × monoecious hybrids like VNR 22 showed the first female flower at the 11^{th} node and Pusa Hybrid 2 at 9^{th} node (Khan and Behera, 2011). The range of node number at first female flower appeared was 12.00 (IC-044417 × IC-470560) to 17.60 (IC-470550 × IC-470560) in a study conducted among 28 hybrids of bitter gourd (Rani *et al*., 2014). Among the 28 F_1 hybrids studied node number to first female flower varied from 6 to 19.67 (Alhariri *et al*., 2018).

2.2.1.5 Days to first fruit harvest

Jadhav *et al*. (2009) observed a range varied from 52.30 (DVBTG-5 x Hirkani) to 67.9 (DVBTG-5 x Co. White Long) for days to first harvest in an investigation conducted among 28 F_1 hybrids of bitter gourd. The gynoecious hybrids exhibited shorter period for first picking and two best hybrids were DBGY-201 X Pusa Vishesh and DBGY- 201 X Pusa Do Mausami. DBGY-201 X Arka Harit took only 50 days for first fruit picking (Behera *et al*., 2009). Minimum numbers of days for first fruit harvest were taken by DBGy- 201 x S54 with 41 days followed by DBGy- 201 x DBG 34 with 42 days. VNR 22 took a maximum number of days of 51 for first fruit picking and Pusa Hybrid 2

(monoecious × monoecious) took 48 days (Khan and Behera, 2011). Among the 28 F_1 hybrids studied, days to first fruit harvest varied from 51.67 to 74.67 (Alhariri *et al*., 2018). Days to to first fruit harvest was recorded earliest in BRBTL × Pusa Aushadhi (41.48) followed by BRBTL × Gangajalee Small (50.39) in a study conducted among 6 hybrids of bitter gourd (Adarsh *et al*., 2018).

2.2.1.6 Relative early yield

Wehner and Kumar (2012) studied the effect of pollinizer in hybrid cucumber NC- Sunshine. When 'Poinsett 76' cucumber was used as pollinizer, it could give an early yield of 16.3 tons/acre under oxford- North conditions. Garg *et al*, (2017) recorded that the hybrids TH-21 had the maximum early-yield of 3.73 tonnes/hectare among the 7 tomato hybrids studied. Al-juboori *et al*. (2018) stated that the hybrid F17 gave the highest early of 7.64 tonnes per hectare among the 27 F_1 hybrids of cucumber under greenhouse condition.

2.2.1.7 Number of days from anthesis to fruit maturity

Arka Harit took 12-14 days from anthesis to fruit maturity (Anon, 2018). Stage of harvest of pumpkin hybrid Pusa Hybrid 1 was 10 to 15 days after anthesis (Vishwanath and Tomar, 2013). Cucumber reaches edible maturity in 6-7 days after anthesis (Gopalakrishnan, 2007). Fruits of cucumber and sponge gourd attain marketable maturity in 5 to 7 days after anthesis and bottle gourd fruit took 10 to 12 days after anthesis to fruit maturity (Rattan and Kumar, 2016).

2.2.1.8 Average fruit weight

Jadhav *et al*. (2009) conducted an investigation in 28 F_1 hybrids of bitter gourd to study the performance and observed that average fruit weight ranged from 47.44 (Hirkan x NDBT-12) to 77.74 g (Delhi Local x Co.White Long). The individual fruit weight among the top three hybrids varied from 46.50 to 108.60 g (Behera *et al*., 2009). Sundaram (2009) reported that average fruit weight was the highest for Bikaneer 1 x CO 1 (57.82 g), followed by CO 1 x Bikaneer 1 (45.29g). Bitter gourd hybrid, Pusa Hybrid 1 and Pusa Hybrid 2 and CO (Bgo) H I recorded

average fruit weight of 100, 110 and 300g, respectively (Behera *et al*., 2010a). Hybrid Gy x S54 (39.18 g) recorded the maximum fruit weight and was at par with the check VNR 22 (39.43 g). DBGy- 201 x Pusa Vishesh hybrid also showed high fruit weight (38.43 g) while the least was in DBGy- 201 x S29 (23.77 g) and the monoecious × monoecious hybrid, US 33 produced the smallest fruit of 15.07 g (Khan and Behera, 2011). The hybrid MC 84 recorded increased fruit weight of 122.2 g among the 5 hybrids evaluated (Aruna and Swaminathan, 2012). Rani *et al*. (2014) stated that average fruit weight of 28 bitter gourd hybrids varied from 58.82 (IC-044417 × IC-045339) to 98.57 g (IC-044438 × IC-045339). Gynoecious bitter gourd hybrid, Pusa Hybrid 4 recorded average fruit weight of 60 g (IARI, 2017). Alhariri *et al*. (2018) reported that average fruit weight ranged from 56.33 to 78.57 g in 28 F_1 hybrids of bitter gourd. Among six hybrids, the fruit weight was the highest in BRBTL × Gangajalee Small (143.88 g) (Adarsh *et al*., 2018).

2.2.1.9 Fruit length

Agasimani *et al*. (2008) reported that the bitter gourd hybrids, GLA 1 x CGL and GLA 1 x Pusa Vishesh exhibited desirable fruit length (19.69 and 19.15 cm), respectively. Fruit length varied from 15.80 (DVBTG-5 x Hirkani) to 25.74 cm (Co.White Long x NDBT-12) in an investigation conducted among 28 F_1 hybrids of bitter gourd (Jadhav *et al*., 2009). Fruit length of the hybrids ranged from 7.8 to 17.9 cm (Behera *et al*., 2009). Sundaram (2009) reported that the hybrids, Bikaneer 1 x CO 1 (17.59 cm), CO 1 x MDU 1 (15.64 cm) and Bikaneer 1 x MDU 1 (15.30 cm) produced long fruits. The maximum fruit length was observed in DBGy- 201 x S54 (11.07 cm) followed by DBGy- 201 x Pusa Vishesh with 10.87 cm (Khan and Behera, 2011). Among the five hybrids evaluated, fruit length was the highest in 09/BIGHY B7 (38.10 cm) followed by MC84 which recorded the fruit length of 17.18 cm (Aruna and Swaminathan, 2012). Rani *et al*. (2014) stated that fruit length of 28 bitter gourd hybrids varied from 13.45 (IC-033227 × IC-470550) to 20.99 cm (IC-044438 × IC-045339). Pusa Hybrid 4 of gynoecious bitter gourd hybrid recorded average fruit length of

16 cm (IARI, 2017). Among the 28 F_1 hybrids studied, fruit length ranged from 9.93 to 17.67 cm (Alhariri *et al*., 2018).

2.2.1.10 Fruit diameter

Among the top three bitter gourd F_1 hybrids, (PBIG-44-3 X DVBTG-5-5, DVBTG-5-5 X Nakhara and NDBT-12 X DVBTG-5-5) diameter varied from 2.6 to 5.27 cm (Behera *et al*., 2009). Fruit diameter ranged from 2.71 (Delhi Local x - DVBTG-5) to 3.63 cm (Phule Green gold x Hirkani) in an investigation conducted among 28 F_1 hybrids of bitter gourd (Jadhav *et al*., 2009). Hybrids DBGy- 201 x Pusa Vishesh and DBGy- 201 x Nakhara Local recorded the maximum fruit diameter of 11.87 and 11.30 cm, respectively. Among the checks, VNR 22 showed the maximum fruit diameter of 12.20 cm (Khan and Behera, 2011). Average fruit diameter of Pusa Hybrid 4 was 5.5 to 6.5 cm (IARI, 2017). Among the 28 F_1 hybrids studied, fruit diameter ranged from 3.10 to 5.10 cm (Alhariri *et al*., 2018). Highest fruit diameter was observed in BRBTL × Gangajalee Small (15.63 cm) in a study conducted among 6 hybrids of bitter gourd (Adarsh *et al*., 2018).

2.2.1.11 Fruit girth

Sundaram (2009) reported that the girth of fruit ranged from 8.83 (MDU 1 x Vadipatti Local) to 13.89 cm (Bikaneer 1 x Bikaneer 3) among the top bitter gourd hybrids. Rani *et al*. (2014) recorded that fruit girth of bitter gourd hybrids varied from 10.98 (IC-033227 × IC-045339) to 13.89 cm (IC-045339 × IC-470560).

2.2.1.12 Flesh thickness

Fruit flesh thickness is an important fruit quality trait and an essential determinant of yield in bitter gourd. The thicker the fruit flesh, the higher the edible portion of the fruit. Flesh thickness varied from 0.38 (DWD-2 x BLG-1) to 0.89(Green long x PRD-5) mm in bitter gourd hybrids (Mohan, 2005). Jadhav *et al*. (2009) conducted an investigation in 28 F_1 hybrids of bitter gourd to study the performance and observed that fruit flesh thickness ranged from 3.11 (Hirkani x

NDBT-12) to 6.16 mm (Delhi Local x Co. White Long). Among the 28 F_1 hybrids studied flesh thickness varied from 7.03 to 11.10 mm (Alhariri *et al*., 2018).

2.2.1.13 Number of fruits per plant

Agasimani *et al*. (2008) reported that the hybrids, GLA 1 x CGL and GLA 1 x Pusa Vishesh showed the highest number of fruits per plant (3.77 and 32.75, respectively). Jadhav *et al*. (2009) conducted an investigation in 28 F_1 hybrids to study the performance and observed that number of fruits per plant ranged from 41.37 (Co. White Long x NDBT-12) to 66.5 (Phule Green gold x DVBTG-5). Behera *et al*. (2009) reported that among the bitter gourd hybrids evaluated, number of fruits per plant ranged from 11.85 to 42.90. Sundaram (2009) recorded that out of the twenty top performing hybrids, Vadipatti Local x IC 85643 (192.22) had produced the highest number of fruits/vine, followed by Bikaneer 3 x Paravai Local (175.89). Number of fruits per plant was maximum in the hybrid DBGy- 201 x S54 (12.79) followed by DBGy- 201 x DBG 34 (8.85) and hybrid PH2 produced only 3.55 fruits (Khan and Behera, 2011). The hybrid, Preethi x MC-30 registered as the top performing hybrid with respect to the number of fruits per plant (Thangamani *et al*., 2011b). Among the 28 F_1 hybrids studied, Gadag local x Coimbatore Green Long exhibited the highest number of fruits per plant (Laxuman *et al*., 2012). A study conducted in 28 hybrids of bitter gourd, Rani *et al*. (2014) reported that number of fruits per plant ranged from 13.40 (IC-085622 × IC-470550) to 25.40 (IC-044438 × IC-045339). Alhariri *et al*. (2018) reported that the number of fruits per plant ranged from 30.33 to 44 in a study conducted with 28 F_1 hybrids of bitter gourd. Numbers of fruits/vine were observed the highest in Konkan Tara × Pusa Rasdar (45.18) among 6 hybrids of bitter gourd (Adarsh *et al*., 2018)

2.2.1.14 Yield per plant

Pusa Hybrid 1 and CO Bg H1 recorded a yield of 20 and 44.4 tonnes per hectare, respectively (Gopalakrishnan, 2007). The yield of Pusa Hybrid 2 was

observed as 180 q/ha. (IARI, 2016). Agasimani *et al*. (2008) stated that the hybrids, GLA 1 x CGL and GLA 1 x Pusa Vishesh showed the highest performance for fruit yield (3.04 and 2.97 kg per plant), respectively. Gynoecious hybrids DBGY-201 X Priya and DBGY-201 X Arka Harit were promising for higher yield and the range of yield per plant for the hybrids studied was 0.88–3.64 kg (Behera *et al*., 2009). Jadhav *et al*. (2009) conducted an investigation in 28 F_1 hybrids to study the performance and observed that yield per plant ranged from 2.03 (DVBTG-7 x NDBT-12) to 4.90 kg (Phule Green gold x DVBTG). Sundarm (2009) reported that the yield among the top twenty hybrids ranged from 1733.67 g (Bikaneer 3 X IC 85643) to 2777.22 g (Bikaneer 1 X CO 1). The highest yield per plant was obtained in hybrid DBGy- 201 x Pusa Vishesh followed by DBGy-201 x MC 84 (Khan and Behera, 2011). Among the nine hybrids studied the highest yield of 3.42 kg per plant was reported in the hybrid Preethi X MC- 30 (Thangamani *et al*., 2011a). The hybrids Preethi x MC-30, KR x USL, MC-105 x MC-10 and Priyanka x CO-1 can be well exploited through heterosis breeding to obtain higher yield with quality fruits (Thangamani *et al*., 2011b). According to Aruna and Saminathan (2012), the highest yield per plot was recorded for MC 84 among the five hybrids studied. Rani *et al*. (2014) stated that yield per plant of 28 bitter gourd hybrids varied from 0.84 (IC-085622 × IC-470550) to 2.50 kg (IC-044438 × IC-045339). Three hybrids, Gadag local x Coimbatore Green Long, Gadag local x Pusa Vishesh and Gadag local x IC-68310 recorded high mean value for total yield per plant (Laxuman *et al*., 2012). The average yield of gynoecious bitter gourd hybrid, Pusa Hybrid 4 was recorded as 22.2 tonnes per hectare (IARI, 2017). Sureshkumara *et al*. (2017) observed that the top three hybrids for yield per plant are Coimbatore Long x Panurthy (2.32 kg), VRBT-100 x Panurthy (2.19 kg) and Green Long x Panurthy (2.06 kg). Alhariri *et al*. (2018) reported that yield per plant ranged from 2.09 to 3.03 kg in a study conducted in 28 F_1 hybrids of bitter gourd. The fruit yield per plant was observed maximum in BRBTL × BRBTW (5.63) in a study conducted among 6 hybrids of bitter gourd (Adarsh *et al*., 2018).

2.2.1.15 Number of harvests

The number of pickings was found as seven in cucumber hybrid Malini (Sharma and Bhattarai, 2006). From a good crop of bitter gourd, 15 to 20 harvests are possible (Gopalakrishnan, 2007).

2.2.1.16 Number of seeds per fruit

Less number of seeds/fruit in bitter gourd makes it more acceptable to the consumer. Mohan (2005) reported minimum number of seeds/fruit in bitter gourd hybrid Green long x BLG-1 (14.83). Rani *et al*. (2014) stated that number of seeds/fruit in 28 bitter gourd hybrids varied from 15.00 (IC-033227 × IC-470550) to 20.67 (IC-033227 × IC-470558). Jat *et al*. (2016) recorded 23.8 seeds/fruit for Pusa Hybrid 1. Hybrid, Arka Harit x Chidambaram Small with the mean number of seeds per fruit (12.30) may be considered as best for the number of seeds per fruit among the 24 hybrids of bitter gourd (Sureshkumara *et al*., 2017).

2.2.1.17 Hundred seed weight

The hundred seed weight of Pusa Hybrid 1 and Pusa Hybrid 2 of bitter gourd hybrids were observed as 13.8 and 12.5 g, respectively (Jat *et al.* (2016); Shivappa, 2013). Cucumber hybrid, Pant Shankar Khira-1 was found to have 100 seed weight of 2.59 g (Mukulkumar *et al*., 2018).

2.2.1.18 Fruit seed ratio

Cocozelle squash had the lowest ratio of seed yield-to-fruit weight (Nerson *et al*., 2000). Nerson and Paris (2000) stated that there is no any relationship between fruit weight and mean seed weight in Poinsett 76 cucumber.

2.2.2 Developments of inbreds in bitter gourd

2.2.2.1 Development of inbreds in bitter gourd through conventional approaches

An inbred line is a ‘pure line’ developed by continued self pollination /sib mating of plants selected on the basis of their superiority from open pollinated population (Dabholkar, 2006). Inbreeding and selection are efficient ways to

improve yield and quality characters. Purpose of inbreeding is to fix characters in homozygous condition so that line is maintained in genetically pure form without any change. Inbred lines can be either used as a new cultivar or they can be used for further breeding programs to produce hybrids in cross pollinated crops.

The source of the population is important for isolating superior plants for developing inbreds. Degree of improvement depends on the magnitude of available desirable genetic variability present in a crop. It can be open pollinated varieties, composites, synthetics, germplasm complexes or any other heterozygous population. Elite hybrid varieties or F_1 hybrids can be used as a reservoir of superior genes in breeding programmes (Van der Have, 1979) and selection has to be done in F_2 and the subsequent generations for desirable characters.

In bitter gourd, inbreds are maintained in pure form by selfing without loss of vigor (Behera, 2005). When inbreds are to be produced, selection is done between the inbred lines to obtain promising inbreds. These inbred lines are tested for their combining ability and most promising inbred lines are used in the production of F_1 hybrids. There are examples in cucurbits where even low-vigour inbred progenies generated vigorous hybrids after heterozygosity recovery. These hybrids will be expected to perform higher than their parents and the cultivar.

Bitter gourd gynoecious inbred DBGy-201 was developed with improved yield and earliness in bitter gourd (Dey *et al*., 2009). Gynoecious inbred line of bitter gourd was improved from cv. Aochu-naga (Iwamoto and Ishida, 2006). Early staminate flowering monoecious lines in bitter gourd was developed and have potential to use as pollenizers for optimizing early and total yield of gynoecious hybrids and gynoecious open-pollinated cultivars (Dhillon *et al*., 2017). Both parental lines of early and high yielding hybrid bitter gourd 'NBH-FIGO' recently released by Noble Seeds were bred by the World Vegetable Center (Dhillon, 2017)**.** Bitter gourd populations for further selection through inbreeding for improved yield, fruit quality, and disease resistance have been developed at the World Vegetable Centre, AVRDC by crossing genetically unrelated bitter gourd varieties and gene bank accessions (Dhillon *et al*., 2016).

Gynoeciuos cucumber inbred line, GBS-1 holds immense potential for exploitation of hybrid vigor with respect to yield and earliness (Pati *et al*., 2015). The F_2 population was obtained from a commercial Japanese cucumber hybrid (Natsu suzumi), which was considered as S0 population. S1, S2, S3, S4 and S5 progenies were obtained by the 'Single Seed Descent' method. There was no loss of vigor due to inbreeding for most of the traits in this population. It shows the potential of developing superior inbreds from commercial hybrids. Robinson (1999) stated that inbred lines have been developed in cucumber, melon, watermelon and pumpkin without loss of vigor. The absence of loss of vigor with the successive self-pollinations was noticed in several reports of cucumber (Jenkins, 1942; Rubino & Wehner, 1986; Cramer & Wehner, 1999). According to Jenkins (1942), many cucumber cultivars which were inbred for five generations were as vigorous as commercial open pollinated stocks. Abd El- Rahman *et al*. (2005) stated that inbreeding and selection program was very efficient to give desirable new inbred lines in gurma melon.

Very few attempts have been made in the past for development of inbred lines in tomato through the exploitation of genetic variability present in the exotic hybrids. F_2 generation obtained from the selfing of F_1 hybrid is the base population providing all possible variations. Gosh *et al*. (2010) evaluated F_2 segregating population of different commercial hybrids of tomato (*Solanum lycopersicum* L.) to develop inbred lines with high yield potential.

Another approach to inbred development in bitter gourd is the exploitation of *in situ* induction of maternal haploids. There are several processes for obtaining haploid and doubled haploid cucurbits (Galazka and Katarzyno, 2013). These are primarily haploid parthenogenesis (induced primarily by pollination with irradiated pollen), *in vitro* gynogenesis (*in vitro* culture of ovules and ovaries) and *in vitro* androgenesis (*in vitro* culture of microspores and anthers). Pollination with irradiated pollen is the most popular method of haploid induction in cucurbits. It is reviewed in detail in section 2.2.2.2

2.2.2.1.1 Inbreeding depression

There is a reduction in mean phenotypic value of various fitness related traits in a given population as a result of inbreeding. There is a decreased heterozygosity due to fixation of unfavourable recessive genes in F_2 and subsequent generations. Cucurbits being monoecious crops, they are essentially cross pollinated. However, they do not suffer from inbreeding depression. Their population structure is similar to that of inbreeders than outbreeders (Allard, 1960).

In flowering plants, evolution continues in sex form to adopt cross fertilization that avoids the harmful effects of inbreeding depression and helps in higher survival and fitness of the species. Most of the flowering plants were hermaphrodite in their primitive sex form. Bitter gourd (*Momordica charantia* L.), a member of gourd family is an example of this gradual evolution of new sex form, during which the earliest sex form was hermaphrodite then monoecious sex form evolved and recently gynoecious sex form has been reported by Ram *et al.* (2002) and Behera *et al.* (2006) from India and Zhou *et al.* (1998) from China. It has been also remarked that small population size used by farmers during the domestication of cucurbitaceous species can be the reason of lack of inbreeding depression (Prohens and Nuez, 2008).

Some of the crosses showed significant negative inbreeding depression for days to first male flower anthesis in bitter gourd (Kumar, 2018). Days to first male flower was significant and negative for most of the crosses in cucumber (Singh *et al*, 2015).

Some of the crosses showed significant negative inbreeding depression for node number to first male flower in bitter gourd (Kumar, 2018). Most of the crosses exhibited significant negative inbreeding depression for node of first male flower in cucumber (Singh *et al*., 2015).

A significant inbreeding depression of -3.96 and -2.71 per cent was observed in bitter gourd for days required to first female flower opening in the crosses, IC- 033227 x IC – 470550 and IC- 033227 x IC-085622, respectively (Rani, 2012). Rani *et al*. (2015) observed that days to first female flower appearance had no inbreeding depression in the cross IC-044438× IC-045339 of bitter gourd. Anant (2018) remarked that significant negative inbreeding depression was noticed in all crosses of bitter gourd for days required to first female flower and varied from -9.90 (Hirkani x Konkan Tara) to -12.87 (Arka Harit X CO White Long). Some of the crosses showed significant negative inbreeding depression for days to first female flower anthesis in bitter gourd (Kumar, 2018). Inbreeding depression for days to first female flower varied from 7.28 to 7.55 in cucumber (Singh *et al*., 2015).

Among the crosses, IC-033227×IC-470550 and IC- 044417×IC-470560 recorded significant inbreeding depression (-25.00 and -20.56% respectively) for node to first female flower appearance. However, few crosses showed low inbreeding depression (Rani *et al*., 2015). Some of the crosses showed significant negative inbreeding depression for node to first female flower in bitter gourd (Kumar, 2018). Inbreeding depression was not observed for flowering traits in pumpkin (Chandrakumar, 2006).

Seven crosses exhibited significant inbreeding depression for sex ratio in bitter gourd ranging from -12.36 to -24.32%. The crosses, IC-044438×IC-045339 and IC-045339×IC-470560 had no inbreeding depression for this trait (Rani *et al*., 2015). Vasudeo (2016) reported negative significant inbreeding depression in sex ratio.

Anant (2018) observed that inbreeding depression in bitter gourd for days to first harvest was negatively significant in all crosses and ranged from -9.8 (Arka Harit x CO White Long) to -20.79 (Phule Green Gold x Preethi). Both positive and negative significant inbreeding depression for days to first harvest was

noticed in sponge gourd. But the cross JSGL – 71 x JSGL – 46 exhibited no inbreeding depression (Thakarshi, 2006).

Inbreeding depression was not observed for earliness in cucumber in most of the crosses. Only 'Addis' × 'Wis. SMR 18' exhibited inbreeding depression (Cramer and Wehner, 1999).

Inbreeding depression was significant in bitter gourd for fruit weight in 12 crosses, ranging from 5.75 (IC- 045339 x IC-470588) to 13.66 per cent (IC-033227 x IC-47060) whereas it was absent in 4 crosses (Rani, 2012). The cross combinations *viz*., Ujjawala x HABG – 22, Preethi x HABG- 22, Preethi x HABG – 21 and K. Baramasi x HABG – 21 1 in F_2 recorded low inbreeding depression for fruit weight in bitter gourd (Hiranand, 2012). Significant positive inbreeding depression was noticed in bitter gourd for fruit weight (Kumar, 2018; Anant, 2018). Inbreeding depression was not observed for fruit weight in cucumber in most of the crosses., Only 'Addis' × 'Wis. SMR 18' exhibited inbreeding depression (Cramer and Wehner, 1999). However, Bhardwaj *et al*. (2009) observed high inbreeding depression for fruit weight in cucumber.

Significant positive inbreeding depression was observed for fruit length in bitter gourd which ranged from 3.79 (IC-470558 x IC-470560) to 13.06 per cent (IC- 470550 X IC-470558) (Rani, 2012). Significant and positive inbreeding depression was also noticed by other authors for fruit length in bitter gourd (Hiranand, 2012; Kumar, 2018; Anant, 2018).

Rani (2012) reported significant inbreeding depression in bitter gourd for fruit diameter in ten crosses, ranging from 2.74 in cross IC-470550x 470558 to 9.65 per cent in cross IC-033227x IC-085622. Positive significant inbreeding depression was noticed in fruit diameter and it varied from 8.03 to 15.03 (Anant, 2018)

Low inbreeding depression in F_2 was recorded for fruit girth in the four cross combinations *viz*., Ujjawala x HABG – 22, Preethi x HABG- 22, Preethi x HABG – 21 and K. Baramasi x HABG – 21 of bitter gourd (Hiranand, 2012).

Inbreeding depression for flesh thickness in pumpkin ranged from -21.69 to 22.86 (Doijode, 1979). According to chandrakumar (2006), inbreeding depression for flesh thickness in pumpkin varied from 0.36 to 3.61.

Significant positive inbreeding depression was reported by Rani (2012) for number of fruits per plant in bitter gourd for 11 crosses, ranging from 5.20 (IC-044417 x IC- 470560) to 16.14 per cent (IC- 033227 x IC-045339). The cross combination Preethi x HABG -21 in F2 recorded low inbreeding depression for number of fruits per plant in bitter gourd (Hiranand, 2012). Kumar (2018) and Anant (2018) also reported positive significant inbreeding depression for this trait. Bhardwaj *et al*. (2009) observed high inbreeding depression for number of fruits per plant in cucumber.

According to Rani (2012), fruit yield in bitter gourd recorded an inbreeding depression, ranged from 9.21 (IC-044417 x 470558) to 21.42 per cent (IC-033227 X IC-045339). The magnitude of inbreeding depression varied from 19.30 % 29.44 for fruit yield per vine (Hiranand, 2012) in bitter gourd. Kumar (2018) and Anant (2018) also reported positive significant inbreeding depression for this trait in bitter gourd. Inbreeding depression was not observed for yield in cucumber in most of the crosses (Cramer and Wehner, 1999). Jenkins (1942) and Rubino and Wehner (1986) observed no inbreeding depression for yield in cucumber lines when self-pollinated for many generations. Significant inbreeding depression was observed for most of the yield traits in pumpkin (Chandrakumar, 2006). Inbreeding depression for yield plant was recorded for most of the crosses in sponge gourd (Thakarshi, 2006).

Inbreeding depression for number of pickings varied from -13.30 to 22.99 per cent (Thakarshi, 2006).

Inbreeding depression for number of seeds/fruit in pumpkin ranged from -41.70 to 39.73 (Doijode and Sulladmath, 1982), 132.00 to 242.00 (Cardoso, 2004) and 1.92 to 7.57 (Chandrakumar, 2006). Negative significant inbreeding depression was observed in bottle gourd for number of seeds per fruit (Vasudeo, 2016). Inbreeding depression affects seed weight in cucumber (Edwards and Lower, 1983).

2.2.2.1.2 Estimate of inbreeding depression derived from regression studies in successive generation

Most of the superior plants, mainly cross-pollinated, exhibit a depression in larger or smaller degree as a consequence of the inbreeding. However, some species that are naturally self-pollinated do not show this depression. Cucurbits are cross-pollinated, but they are examples of species in which some lines seem to lose little vigor due to inbreeding (Whitaker and Robinson, 1986). Experiments conducted by Abdel al *et al*. (1973) proved that cucurbits exhibit no inbreeding depression. In *Cucurbita maxima* and *C. pepo* only some degree of inbreeding depression was observed in $I_{2.}$ Vigor or reproductive capacity was not affected in *Cucurbita maxima* after 10 generations of self pollination (Cummings and Jenkings, 1928). Bushnell (1922) studied the impact of inbreeding for *Cucurbita pepo* and remarked that vigor loss during self pollination did not essentially occur. Loss in vigor for fruit number and weight of muskmelon did not always occur after inbreeding for four to seven generations (Scott, 1933). Regression analysis was carried out using the Wright's inbreeding coefficient in cucumber and none of the slopes were significantly negative for earliness, total yield and the fruit quality traits like fruit colour and shape. In fact, total yield in the spring test resulted in significant positive slope which indicated a yield improvement during the inbreeding process. Yield improvement was not noticed in summer test. Inbreeding depression was not important in affecting the performance of these traits (Rubino and Wehner, 1986). Oviedo *et al*. (2008) generated an F_2 population from a commercial hybrid (Natsu suzumi) in cucumber, which was considered as S_0 population. S_1, S_2, S_3, S_4 and S_5 progenies were obtained by the

'Single Seed Descent' methodology. There was no loss of vigor due to inbreeding in advance generation for the characters, number of commercial fruits, total weight, commercial weight per plant, per cent of commercial fruits and average weight of commercial fruits. It shows the potential of developing superior inbreds from commercial hybrids. Robinson (1999) stated that inbred lines have been developed in cucumber, melon, watermelon and pumpkin without loss of vigor. The absence of loss of vigor with the successive self-pollinations was noticed in several reports of cucumber (Jenkins, 1942; Rubino & Wehner, 1986; Cramer & Wehner, 1999; Ghaderi and Lower (1979). According to Jenkins (1942), many cucumber cultivars which were inbred for five generations were as vigorous as commercial open pollinated stocks.

Inbred lines developed in S6 generation of gurma melon (*Citrullus colocynthoides*) were significantly increased in comparison with the commercial cultivar for number of fruits per plant, 100 seed weight and yield per plant. While the commercial cultivar had the highest values of fruit weight. Selection program was very efficient to obtain desirable new inbred lines for gurma melon (Abd El-Rahman *et al*., 2005).

Hayes and Immer (1942) indicated that self pollination of squash resulted in generation of new lines with desirable characteristics. Halsey (1978) confirmed that selfing for several generations achieved homogeneity for some traits in few lines of cantaloupe. Inbreeding with selection was effective in recovering desirable lines in pumpkin (Unander and Ranirez, 1988; Damarany, 1989). Abd El-Hadi *et al*. (2001) noticed a decrease in ranges from S3 to S5 generation in the traits of new inbred lines of sweet melon which was associated with uniformity for fruit weight, thickness and yield per plant.

Although many researchers assume the hypothesis of reduced inbreeding depression in genus *Cucurbita*, some have reported inbreeding depression for several characteristics of *C. pepo* and *C. maxima* (Borghi *et al*., 1973). Reduction in fruit weight was reported in *Cucurbita pepo* and *Cucurbita maxima* after three generations of self pollination (Chekalina, 1976). Mahzabin *et al.* (2008) stated

that abrupt inbreeding depression after two generations of selfing was observed in *Cucurbita maxima*. Cardoso (2004) also reported a reduction in fruit weight, fruit length and number of seeds per fruit after four generations of self pollination in *Cucurbita moschata*. Regression of performance on Wright's coefficient of inbreeding (F) resulted in a significant negative slope for these characters.

The generation of selfing depends upon the degree of inbreeding depression in the crop. After four generations of selfing, most plants still gave a good seed yield, which indicates a low degree of inbreeding depression (Nieuwhof, 1985). Inbreeding depression depends on the allelic frequency and on the dominance effects (Falconer, 1989). In bitter gourd, the parents/inbreds are maintained in pure form by selfing without loss of vigor (Behera, 2005). However, the selfing precedes selection and the first one or two female flowers on the plants must be selfed, otherwise selfing in later stages often fails to set fruits. It is not necessary to produce inbred lines and homozygous varieties can be used directly as inbreds as in self-pollinated crops like tomato, eggplant, and sweet pepper (Swarup, 1991). Effects of inbreeding on female function and pollen performance both *in vitro* and *in vivo* were studied using *Cucurbita texana*, a wild gourd. It was found that inbreeding affects the performance of the resulting sporophytic generation and the microgametophytes they produce (Johansson *et al*., 1998). There is a difference in response of various species of cucurbita to inbreeding (Whitaker and Robinson, 1986).

2.2.2.1.3 Identification of economically important segregants

Selection of segregants having economic value is done after fixing differential yield levels. Desirable economic segregants were chosen on the grounds of horticultural characters, performance and yielding capacity. Ravishankar (2007) carried out analysis of segregants of economic value in F_2 with regard to important characters in brinjal. The study revealed that there was a higher frequency of economic segregants for fruit weight followed by number of fruits and yield per plant. All the selected segregants were superior in performance over the commercial check and they can be fixed through inbreeding

and selection. Segregants having superior performance were isolated for further utility previously by many researchers in different crops *viz*,; Kale *et al.* (1989), Naidu (1993), Patil (1998), Karaganni (2003), Mallangoud (2005) and Naik (2007) in brinjal and Somashakhar (2006) in chilli.

2.2.2.1.4 Selection differential

Selection differential is the phenotypic superiority of selected individuals, compared to the population from which they were selected. The magnitude of selection differential is governed by the variability present in the initial population and the proportion of individuals selected as parents. Selection differential for mean yield in F_3 generation of rice was 4.64g for Suraksha x Jalanidhi, 4.81g for Suraksha x Phalguna, 4.70g for Suraksha x Mahamaya and 5.37g for Suraksha x RP Bio-226 (Jayaprakash *et al*., 2017). Study conducted in an F_2 segregating population of rice showed that selection differential was positive for the traits, plant height, number of productive tillers per plant, number of grains/panicle and single plant yield. But thousand grain weight was a negative value (Govintharaj *et al*., 2017).

2.2.2.1.5 Selection response

It is the difference between mean phenotypic value of offspring of selected parents and the whole of the initial generation before selection. Abd El-Rahman *et al*. (2005) observed an increase in mean value of hundred seed weight from S_4 to S_6 selfing generations in gurma melon (*Citrullus colocynthoides*). Behera *et al*. (2010b) reported a positive selection response in cucumber for long fruited type and yield after one cycle of marker assisted selection from F_3 to F_4. But, this was not maintained from F_4 to F_5. Comparatively high genetic gain for yield was achieved beyond the base population (F_3), but advanced generations showed a reduction in phenotypic values of yield. The bred population with an average of 39.82 fruits per plant was 37.73 percent improved in comparison to average of the F_3 population (28.91 fruits per plant) in cucumber as reported by Kalvandi *et al.* (2016). Rodriguez and Patta (2006) studied selection response in recombinant

lines obtained from interspecific cross between of *Lycopersicon* species. Response to selection for the fruit weight was 7.71 g for the first group I, -3.75 g for group 2 and -3.14 g for group 3. No response to selection was observed for fruit shelf life. Since response to selection was significant only for fruit weight, It indicates that additive genetic variance was present only this trait. Study conducted in an F_2 segregating population of rice revealed that *s*election response was high for number of grains, thus indicating the effectiveness of selection for this character (Govintharaj *et al*., 2017).

2.2.2.1.6 Selection index

To make an effective selection for higher yield, it is necessary to determine the selection index. It helps to select suitable genotypes from germplasm based on reliable and effective characters. Selection index was prepared in the collection of 13 bitter gourd genotypes based on major components of yield including 100 seed weight, number of seeds/ fruit and yield per plant (Parhi *et al*., 1993). Ram *et al*. (2006) stated that emphasis was given for the number of fruits per plant and average fruit weight in selecting high yielding genotypes in bitter gourd. A selection index was worked out in 24 watermelon genotypes using the characters yield/plant, number of fruits/plant, fruit weight and total soluble solids (Shibukumar, 1995). Node to first female flower, days to first harvest, fruits per plant, average fruit weight, length, girth, diameter and yield per plant were considered as selection criteria in cucumber genotypes (Gayathri, 1997). Varghese (2003) reported that the number of fruits per plant and average fruit weight were the important characters based on which selection index was prepared in ivy gourd. Yield, earliness parameters, fruit quality, sex ratio and mosaic resistance were taken as important characters to work out selection index in 25 ash gourd landraces (Resmi, 2004). Vine length, number of branches per plant, total number of harvests, fruit length, fruit girth and number of fruits per plant in spine gourd was taken to determine selection index (Archana, 2013).

2.2.2.1.7 Incidence of pest and disease

Bactrocera cucurbitae, commonly known as fruit fly is the major pest of bitter gourd and causes more than 50% yield loss in bitter gourd (Narayan and Batra, 1960; Rabindranath and Pillai, 1986). More prickly variety 'Phule BG 4' was comparatively resistant to fruit fly (Peter, 1998). Satpathy *et al*. (2005) observed that the level of infestation varied between 21 and 29% and it did not significantly differ. Preethi was less susceptible to fruit fly infestation (Rajan and Prameela, 2004). Rajput (2012) observed low incidence of fruit fly in segregating populations of bitter gourd. Nandkumar (2016) recorded that among the three crosses, the cross Hirkani x Phule Ujwala showed the minimum infestation of fruit fly (10.12 and 137 8.47 %) whereas, the Hirkani x Kokan Tara recorded the highest infestation of fruit fly (14.22 and 12.23 %) in both F_3 and F_4 generations respectively.

Downy mildew is one of the important foliar diseases of bitter gourd. Rajput (2012) stated that the incidence of downy mildew was negligible in F_2 and F_3 generations of bitter gourd. Nandkumar (2016) reported highest incidence of downy mildew (28.92 and 23.54 %) in most of the progenies of the cross Hirkani x Phule Ujwala and least incidence (23.25 and 19.24 %) in F_3 and F_4 segregating generations of cross Phule Green Gold x Hirkani.

2.2.2.2 Development of inbreds in bitter gourd through biotechnological approaches

Haploids are cells or organisms that contain a gametic chromosome number (n). Haploid technique provides full homozygosity in one step and has opened new perspectives to shorten the time required for the development of homozygous lines (Jia *et al*., 2014). They originate from meiotically reduced tissue that undergoes embryogenesis without fertilisation. They can originate spontaneously in nature or as a result of various induction techniques (Galazka and Katarzyna, 2013). Doubled haploids can facilitate the selection of desirable genotypes for further breeding. Different protocols are employed for generating haploid and

double haploid cucurbits (Galazka and Katarzyna, 2013). These are primarily haploid parthenogenesis (induced by pollination with irradiated pollen), *in vitro* gynogenesis (*in vitro* culture of ovules and ovaries) and *in vitro* androgenesis (*in vitro* culture of microspores and anthers). The most common and efficient method of obtaining haploid cucurbit is through induced parthenogenesis via pollination with irradiated pollen. The results of *in vitro* ovule and ovary culture indicate a low efficacy and need further optimisation. Anther culture is comparable in efficiency to the irradiated pollen technique. Centromere mediated genome elimination technique is not yet tried in cucurbits for haploid induction.

2.2.2.2.1 Haploid parthenogenesis

It is the induction of parthenogenetic development of the egg cell by pollination with irradiated pollen followed by haploid embryo rescue. Embryo development is stimulated by germination of pollen on the stigma and growth of the pollen tube within the style, although irradiated pollen is not able to fertilize the egg cell. Haploid parthenogenesis was first successfully used to obtain haploid embryos of melon and cucumber over 20 years ago (Sauton and Dumas de Vaulx, 1987). Pollination with irradiated pollen is the leading method for the induction of haploids in cucumber and melon. Efficiency of haploid recovery depends on several factors, such as irradiation dose, pollination, genotype of the mother plants, embryo detection, excision and culture (Kurtar and Balkaya, 2010). Time of the year and the month during which pollination with irradiated pollen takes place also determine the outcome. Review of haploid induction in cucurbits through irradiated pollen technique is shown in Table 2.

Table 2. Review of haploid induction in cucurbits through irradiated pollen technique

Crop	Dose of gamma radiation (Gy)	Stage of development	Induction medium (mg/l)	Ploidy identification	Result	References
Bitter gourd	150	10 days		Chromosome counting		Xiao-feng, *et al*. (2016)
Cuccumber	300	3- 5 weeks	E20A	Cytological analysis and chromosome counting	Haploid , diplod and mixoploid plantlets	Przyborowski and Niemirowicz- Szczytt (1994)
	200	4 weeks	MS + 3 % sucrose + 0.01 mg/l IAA	Chromosome counting	Haploid and diploid plantlets	Deunff and Sauton (1994)
	300		E20A		Haploid and diploid plantlets	Caglar and Abak (1999a, b, c)
	100 and 200	3–5 weeks	E20A	Chromosome counting	Haploid and diploid plantlets	Faris *et al*. (1999)
	250 and 500	3–5 weeks	E20H8 + 0.011 mg/l IAA + 2.54 mg/l AgNO3	Flow cytometry	Haploid and diploid plantlets	Claveria *et al*. (2005)
	100–400	2-5 weeks	MS	Morphological observations, pollen counts and seed germination	Haploid , diplod and mixoploid plantlets	Lei *et al*. (2006)
	300	3–5 weeks	E20A		Haploid and diploid plantlets	Smiech *et al*. (2008)
	250	21–23 days	E20A			Lofti and Salehi (2008)
Melon	300	3–5 weeks	E20A + 0.011 mg/l IAA		Haploid plantlets	Sauton and Dumas de Vaulx (1987), Sauton (1988)
	150–2500			Flow cytometry		Cuny *et al*. (1992, 1993)
	300	3–4 weeks	E20A + 0.01 mg/l IAA		Haploid , diplod and mixoploid plantlets	Ari *et al*. (2010)
	250, 500	3–5 weeks	E20H8	Flow cytometry	Haploid and diploid plantlets	Gonzalo *et al*. (2011)
	250	21 days	E20A + 0.011 mg/l IAA	Cytological analysis and flow cytometry	Haploid and diploid plantlets	Godbole and Murthy (2012a, b)

Pumpkin	50, 100	3–6 weeks	E20A	Cytological analysis and chromosome counting	Haploid and diploid plantlets	Kurtar *et al*. (2009)
Squash	25, 50	4–5 weeks	E20A	Cytological analysis, chromosome counting and morphological observations	Haploid and diploid plantlets	Kurtar *et al*. (2002)
	150	35 days	CP + 3 % sucrose + 0.02 mg/l IAA		Haploid and diploid plantlets	Baktemur *et al*. (2014)
Watermelon	200 and 300	2–4 weeks	E20A + 0.107 mg/l IAA	Flow cytometry	Haploid, diploid and tetraploid plantlets	Sari *et al*. (1994)
	275	25 days	CP + 3 % sucrose + 0.02 mg/l IAA		Haploid plantlets	Taskın *et al*. (2013)
Winter squash	50 and 100	3–4 weeks	E20A	Cytological analysis and chromosome counting	Haploid and diploid plants	Kurtar and Balkaya (2010)

2.2.2.2.2 Genotype of the donor plant

When inbred cucumber lines and their hybrids were used, as mother plants, the highest number of haploid embryos was obtained for hybrids as compared to the inbred lines (Niemirowicz-Szczytt *et al*., 1995). Study conducted by Sari *et al*. (1994) among four watermelon cultivars proved that cultivar Halep Karasi was better than the other genotypes. Effect of genotype on the success of haploid induction was studied in different cucurbits by different scientists (Kurtar *et al*., 2002; Nasertorabi *et al*., 2012). Genotypic difference was observed and the highest number of haploid embryos (6.25 haploid embryos per 100 seeds) of watermelon was obtained from Genotype 1 pollinated with 275 Gy irradiation dose (Taskin *et al*., 2013). Baktemur *et al*. (2014) found genotypic difference among squash genotypes in obtaining embryos per 100 seeds. Plant vigor also plays an important role in haploid induction (Cuny *et al*., 1993).

2.2.2.2.3 Source of irradiation

Cobalt (^{60}Co) is most commonly used as the source of gamma rays for inducing haploid parthenogenesis. Gamma radiation is easy to apply and have good tissue penetration, high mutation rate and low lethality (Kurtar and Balkaya, 2010). It has played a major role in the advancement in haploid parthenogenesis. It has been successfully used in many cucurbits.

The X-ray also has been used successfully in cucumber (Antos *et al*., 2001), melon (Yashiro *et al*., 2002), and pumpkin (Kosmrlj *et al*., 2013, 2014).

2.2.2.2.4 Irradiation dose and pollination

Usually, radiation is done to flowers (frequently after the removal of sepals and petals) or to isolated anthers. A large amount of irradiated pollen applied to stigmas can stimulate a parthenogenetic response by increasing the number of pollen tubes reaching the egg cell as observed in melon (Sauton and Dumas de Vaulx, 1987).

The dose of irradiation is the major factor controlling *in situ* haploid production. At lower doses, the generative nucleus is partly damaged and

therefore maintains its capacity to fertilize the egg cell. It results in a large number of embryos but all of hybrid origin and abnormal (mutant) phenotype. An increase in the dose of irradiation results in a decrease in the total number of developed embryos but the obtained regenerants are mostly of haploid origin. Dosage should not be so high as to inhibit pollen tube germination, but also must be high enough to disturb normal fertilization and avoid generation of diploid hybrid embryo. Irradiation dose is specific to each experiment and explained in the table 2.

2.2.2.2.5 Embryo detection, rescue and culture

After pollination with irradiated pollen, the endosperm does not develop (Sauton and Dumas de Vaulx, 1987). The embryo grows at first even without the presence of endosperm, but its development is arrested at an early stage that necessitates embryo rescue and *in vitro* culture. Otherwise, the embryo dies. The medium most commonly used for the rescue is the E20A medium, which was developed by Sauton and Dumas de Vaulx (1987) for melon haploid production. One of the most common methods used for the detection and isolation of embryos consists of the physical opening of all seeds (Sari *et al*., 2010, Godbole and Murthy, 2012b).

2.2.2.2.6 Stage of development

The tenth day after pollination was the best embryo rescue period for female gametogenesis in bitter gourd (Xiao-feng *et al*., 2016). Fruit developmental stage of embryo rescue in various cucurbits is illustrated in table 2. Regeneration rate is influenced by the embryonic development stage. Godbole and Murthy (2012b) studied the effect of stage of embryo development on plant regeneration in *Cucumis melo* var. *momordica.* Among these various embryos cultured, only cotyledonary embryos germinated into plantlets. The highest number of immature embryos (globular and heart-shaped) resulted in a low frequency of haploid induction (Sauton and Dumas de Vaulx, 1987).

2.2.2.2.7 Evaluation of ploidy level

Ploidy level of obtained plants can be evaluated through cytological analysis (including chloroplast number in guard cell, dimensions of guard cell and

stomata etc) morphological observations, chromosome counting, flow cytometry, pollen counts, seed germination etc.

Studies conducted in winter squash by Kurtar and Balkaya (2010) revealed larger stomatal size in diploids compared to haploids. Average stomata length X width was 21.11 X 17.37 and 30.51 X 21.82 µm in haploids and diploids, respectively. Average chloroplast numbers in the guard cells of haploid plants were 7.21 and 11.17 chloroplasts were observed in diploid plants. Godbole and Murthy (2012b) observed that stomatal length, diameter and chloroplast number in guard cells differed between haploid and diploid plants of *Cucumis melo* var *momordica*. The average stomatal length X diameter was 15.15 X 12.3 µm in haploid plants, while in control plants stomatal length and diameter was 23.17 X 15.69 µm. Average number of chloroplasts in the guard cells of haploid and diploid plants were 4.37 and 12.25, respectively. Stomatal size and chloroplast number of guard cells can be taken as alternative criteria to determine the ploidy level in haploid or diploid plants.

Experiments, where ploidy level confirmed through various techniques, are detailed in Table 2.

Materials and Methods

III. MATERIALS AND METHODS

The investigation on development of inbreds in bitter gourd (*Momordica charantia* L.) through conventional and biotechnological approaches was undertaken during the year 2016-2018. The details of the experiment, materials used and techniques adopted in the present investigation are presented in this chapter.

3.1 Experimental site

The experiment on development of inbreds in bitter gourd (*Momordica charantia* L.) through conventional and biotechnological approaches was conducted in the field of Department of Vegetable Science, College of Horticulture, Vellanikkara (Kerala Agricultural University).

3.2 Location and climate

The experimental site is located at an altitude of 23 m above M.S. L. and between $10^0$32" N latitude and $76^0$16"E longitude.

3.3 Experimental details

3.3.1 Development of inbreds in bitter gourd through conventional approaches

Evaluation of commercial F_1 hybrids in bitter gourd

Popular high yielding 16 F_1 hybrids from public and private sector were evaluated and promising 5 F_1 hybrids were (based on characters like, node to 1st female flower appearance, days to 1st picking, relative early yield (weight of immature fruits harvested during the first 3 harvests), total yield (kg per plant), fruit weight (g), fruit length (cm) and fruit diameter (cm)) selfed to produce F_2 population. Details of genotypes used in the experiment are presented in Table 3. General view of the experimental plot is given in Plate 1.

Plate 1. General view of the experimental plot

A. Evaluation of F_1 hybrid, B. Evaluation of F_2 population, C. Evaluation of F_3 families

Table 3. Details of bitter gourd genotypes used in the experiment with their source of collection

Sl. No.	Code	Hybrids	Source
1	MC-131	PH-1	IARI, New Delhi
2	MC-132	PH-2	IARI, New Delhi
3	MC-133	Monalisa	Sakata
4	MC-134	Euro	Rizwan Seed
5	MC-135	US 33	US Agri seeds
6	MC-136	Aakash	VNR
7	MC-137	VNR 22	VNR
8	MC-138	Palee	East West Seed International
9	MC-139	Maya	East West Seed International
10	MC-140	Prachi	East West Seed International
11	MC-141	Racer	Bayer Nunhems
12	MC-142	Aman Shree	Nunhems
13	MC-143	Super Katai	Denmark Agri Sciences
14	MC-144	Chottu	Fito
15	MC-145	Indam Taj	Indo American Hybrid Seeds
16	MC-146	Shiva	Keyonic Seeds
	Control		
17	MC-147	Pusa Rasdar	IARI, New Delhi
18	MC-148	Pusa Ausadhi	IARI, New Delhi
19	Preethi		KAU, Thrissur
20	Priya		KAU, Thrissur
21	Priyanka		KAU, Thrissur

Evaluation of F_2 population

F_2 population was raised from the selfed fruits of F_1 hybrids. All seeds from a single fruit were planted. A selection intensity of 5 % was applied based on the characters mentioned above for identifying superior lines. General view of the experimental plot is given in Plate 1.

Evaluation of F_3 families

F_3 families were raised from the selected plants of F_2 population. A selection intensity of 5 % applied based on the characters mentioned above for

identifying superior lines. General view of the experimental plot is given in Plate 1.

The initial evaluation of F_1 hybrids was carried out in randomized block design with 2 replications. F_2 population and F_3 families were raised in the field without replication. Recommended cultivation practices were followed as per the Package of practices, KAU (KAU, 2016).

3.3.1.1 Observations recorded

Following observations were recorded during the evaluation of F_1, F_2 and F_3 generation**.**

1. Nodes to first female flower
2. Days to first female flower anthesis
3. Nodes to first male flower
4. Days to first male flower anthesis
5. Days to first harvest
6. Relative early yield (kg)
7. Number of days from anthesis to fruit maturity
8. Mean fruit weight (g)
9. Fruit length (cm)
10. Fruit diameter (cm)
11. Fruit girth (cm)
12. Flesh thickness (cm)
13. Number of fruits per plant
14. Yield per plant (kg)
15. Number of harvests
16. Number of seeds/fruit
17. 1000 seed weight (g)
18. Fruit seed ratio
19. Incidence of pest and diseases

Procedures followed for recording observations are furnished below. Fruit characters were recorded in five randomly selected fruits in the single plant.

1. Days to first female flower anthesis: Number of days was counted from the date of sowing to the date of opening of the first female flower

2. Nodes to first female flower: Nodes were counted from the lowest to the one at which the first female flower emerged.

3. Days to first male flower anthesis: Number of days was counted from the date of sowing to the date of opening of the first male flower.

4. Node to first male flower: Nodes were counted from the lowest to the one at which the first male flower emerged.

5. Days to first harvest: Number of days taken from sowing to the harvest of first formed fruit at tender in each plant was recorded.

6. Relative early yield: Weight of immature fruits harvested during the first 3 harvests was recorded.

7. Number of days from anthesis to fruit maturity: Number of days taken from female flower opening to fruit maturity was recorded.

8. Average fruit weight (g): Weight of five fruits from selected plants was recorded and mean was calculated.

9. Fruit length (cm): Length of five fruits from selected plants after harvest was recorded separately and average was calculated.

10. Fruit diameter (cm): Diameter of five fruits from selected plants after harvest was recorded separately and average was calculated

11. Fruit girth (cm): Girth of five fruits from selected plants after harvest was recorded separately and average was calculated.

12. Flesh thickness: Flesh thickness of five fruits from selected plants after harvest was recorded separately and average was calculated.

13. Number of harvests: total number of harvests made from each plant till the end of the crop.

14. Fruits per plant: Total number of tender fruits in each plant was counted at different harvest and added to get total fruits per plant.

15. Yield per plant (kg): Weight of fruits harvested from each plant at different dates was recorded separately and these were added to get total yield/plant.

16. Number of seeds per fruit: Number of seeds per fruit of five fruits from selected plants after harvest was counted.

17. 1000 seed weight (g): Thousand seeds were counted and weight recorded in gram.

18. Fruit seed ratio: Ratio of fruit weight to seed weight

19. Incidence of pest and diseases: Various diseases and pests like mosaic, fruit fly, etc. and their occurrence (severe/ moderate/ low/ absent) was recorded.

3.3.1.2 Statistical analysis

The initial screening of 16 hybrids along with 5 control varieties for various parameters was carried out using randomised complete block design. The data collected on the quantitative characters were subjected for statistical analysis and following different statistical parameters were worked out.

3.3.1.2.1 Analysis of variance (ANOVA)

The model of analysis of variance is given below.

Source of variation	D.F.	SS	MSS	Cal.F
Replications	r-1	RSS	RSS/(r-1)	TrMSS/EMSS
Treatments	t-1	TrSS	TrSS/(t-1)	
Error	(r-1) (t-1)	ESS	ESS/(r-1)(t-1)	
Total	(rt-1)	TSS		

Where,

t = Number of treatments (genotypes)

r = Number of replications

SS = Sum of square

MSS = Mean sum of square

D.F. = Degrees of freedom

The standard error was calculated as,

SEm = √ EMSS/r

The significance of treatments mean squares and replication mean squares were tested by comparing with error mean squares referring to 'F' table values at 5 and 1 per cent level of probabilities.

3.3.1.2.2 Ranking of hybrids, F_2 and F_3 generations based on cumulative index

Ranking of hybrids was done based on the cumulative index. Post hoc test was performed where ever necessary using DMRT for evolving a unique selection criterion based on the vector of characters under consideration, the method of Arunachalam and Bandyopadhyay (1984) was co opted for this study. Selection among F_2 and F_3 generations were also carried out using simple rank order criterion.

3.3.1.2.3 Descriptive statistics

The descriptive statistics namely mean, standard error of mean, standard deviation, variance and range for the three generations were worked out for all the characteristics under study. The comparison of pair wise means for the various characteristics of F_2 and F_3 was done using the standard procedure of two sample case t test after checking the homogeneity of variance through F test.

3.3.1.2.4 Response to selection

It is the difference between mean phenotypic value of offspring of selected parents and the mean of whole of the initial generation before selection.

Selection response $R = \mu_2 - \mu_0$

μ_0 = Mean of initial population

μ_1 = Mean of individuals selected as parents

μ_2 = Mean of offspring of selected parents

3.3.1.2.5 Selection differential

It is the difference between the mean phenotypic value of the individuals selected to be the parents and whole of the initial generation before selection.

Selection differential (S) = $\mu_1 - \mu_0$

μ_0 = Mean of initial population

μ_1 = Mean of individuals selected as parents

It depends upon the phenotypic standard deviation (σp) of the population and the proportion of plants selected for raising next generation (Falconer, 1989).

3.3.1.2.6 Cumulative inbreeding depression

To study the inbreeding depression in subsequent generations,

the estimate of inbreeding depression from F_1 to F_2 is given as:

$[(F_1-F_2) / F_1] \times 100$

Parallel to the said computation inbreeding depression over the subsequent generation from F_2 to F_3 is also defined. The inbreeding chain coefficient over generations may be defined as cumulative summation.

Thus cumulative inbreeding depression from F_1 to F_3 is computed by using the following formula.

Cumulative inbreeding depression = $[(F_1-F_2)/F_1] \times 100 + [(F_2-F_3)/F_2] \times 100$

Test of significance of inbreeding depression

The test statistic for testing the significance of inbreeding depression at each stage is as follows.

Let S_1^2 be the mean square for F_1 at (n_1-1) degrees of freedom

S_2^2 be the mean square for F_2 at (n_2-1) degrees of freedom

S_3^2 be the mean square for F_3 at (n_3-1) degrees of freedom

Case I (F_1- F_2)

To test the significance of inbreeding depression from F_1 to F_2,

The statistic t = Inbreeding depression (%) / Standard error of the difference of means

Where, standard error of the difference of means = $\sqrt{[(n_1-1)\ S_1^2 + (n_2-1)\ S_2^2]/\sqrt{n_1+n_2-2}}$

Case II (F_2 –F_3)

To test the significance of inbreeding depression from F_2 to F_3,

The statistic t = Inbreeding depression (%) / Standard error of the difference of means

Where, standard error of difference of means = $\sqrt{[(n_2-1)\ S_2^2 + (n_3-1)\ S_3^2] / \sqrt{n_2+n_3-2}}$

Case III (F_1-F_3)

The standard error and t value for test of significance for progressive inbreeding depression were estimated as

t = Cumulative inbreeding depression (%) / Standard error of difference of means

Where,

standard error of difference of means = $\sqrt{[\ (n_1-1)\ S_1^2 + (n_2-1)\ S_2^2 + (n_3-1)\ S_3^2] / \sqrt{n_1+n_2+n_3-3}}$

3.3.1.2.7 Pair wise comparison of inbreeding depression

The test statistic for pair wise comparison of inbreeding depression (or coefficient) is given below.

t = (ID H_1 . ID H_2)/ SE (ID H_1 - ID H_2)

Where, H_1- Hybrid 1

H_2- Hybrid 2

ID- Inbreeding depression

3.3.1.2.8 Estimate of inbreeding depression derived from regression analysis

For the characteristics with significant difference among the treatments (inbred populations), regression analysis was carried out using the Wright's inbreeding coefficient (F) (Rubino and Wehner, 1986).

3.3.1.2.9 Genetic parameters

3.3.1.2.9.1 Genotypic, phenotypic and environmental variances

The variance due to genotype, phenotype and environment were computed as follows.

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MS due to genotypes} - \text{MS due to error}}{\text{r (replication)}}$$

Environmental variance ($\sigma^2 e$) = Error mean sum of squares

Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + (\sigma^2 e)$

Where, 'r' is number of replications.

3.3.1.2.9.2 Heritability (h^2)

The broad sense heritability ($h^2 bs$) was estimated by following the procedure suggested by Weber and Moorthy (1952) as indicated here below.

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

h^2 (%) = Heritability (Broad sense)

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

3.3.1.2.9.3 Expected genetic advance

Genetic advance for each character was predicted by the formula given by Johnson *et al*. (1955).

$GA = h^2 \times \sigma p \times k$

Where k = selection differential (2.06) at 5 per cent selection intensity

h^2 = Heritability in broad sense

σp = Phenotypic standard deviation

3.3.1.2.9.4 Genetic advance as per cent of mean (GAM)

Genetic advance as per cent over mean was worked out as suggested by Johnson *et al*. (1955).

$$\text{Genetic advance as per cent over mean (GAM)} = \frac{GA}{\overline{X}} \times 100$$

Where, GA = Genetic advance

$\overline{X}$ = General mean

3.3.1.2.9 Identification of economically important segregants

Observations were recorded on individual plant basis. Average fruit weight, number of fruits per plant, yield per plant and days to first fruit harvest were considered to select economic segregants in F_2 and F_3 generations of hybrids. A plant in F_2 and F_3 generations was judged as an economic segregant for a character after comparing its performance with the bitter gourd variety Preethi raised in the same season. For the character average fruit weight, plant in F2 and F3 generation is considered as economic segregant if it is higher than 140 and 145 g respectively. Similarly, for number of fruits per plant and yield, it is 27 and 28 and 3.5 and 3.7 kg respectively in F_2 and F_3 generation. All those plants having

days to first harvest below 58 and 60.50 in F_2 and F_3 respectively were chosen as segregants of economic value for this character.

The per cent economic segregant was calculated as follows (Ravishankar, 2007).

$$\text{Economic segregants (\%)} = \frac{\text{No. of economic segregants}}{\text{Total no. of plants}} \times 100$$

3.3.2 In situ induction of maternal haploids initiated by pollination with irradiated pollen and development of haploid inbred lines through embryo culture

The crop was raised in polyhouse and the laboratory experiments were conducted in the biotechnology laboratory, under Department of vegetable science, college of horticulture, Vellanikkara (Kerala Agricultural University).

3.3.2.1Medium composition

In the present study, the most commonly used E20A medium, for embryo rescue in cucurbits developed by Sauton and Dumas de Vaulx (1987) was used. The details of the media composition are given in Annexure II.

3.3.2.2 Plant material

In the present study of evaluation of F_1 hybrids, MC-139 was found promising among the top five F_1 hybrids. MC-139 was raised in polyhouse for embryo culture experiment (Plate 2).

3.3.2.3 Parthenogenetic induction with irradiated pollen

Male flowers were collected from, MC-139 on the day before anthesis. After removing petals and partially sepals, they were placed in glass petri dishes for irradiation. Irradiation was performed at the Radio Tracer Lab, Kerala Agricultural University and Meat processing unit under Kerala Veterinary and Animal Sciences University at Thrissur. They were exposed to gamma rays ($Cobalt^{60}$) at doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy (Gray). Irradiated male flowers were stored at room temperature. Pollen grain production was not observed when they were kept at 4°C. Female flowers bagged on the previous day were artificially pollinated by rubbing the anthers of stored irradiated flowers during morning hours between 7 and 8 A.M. Pollinated female

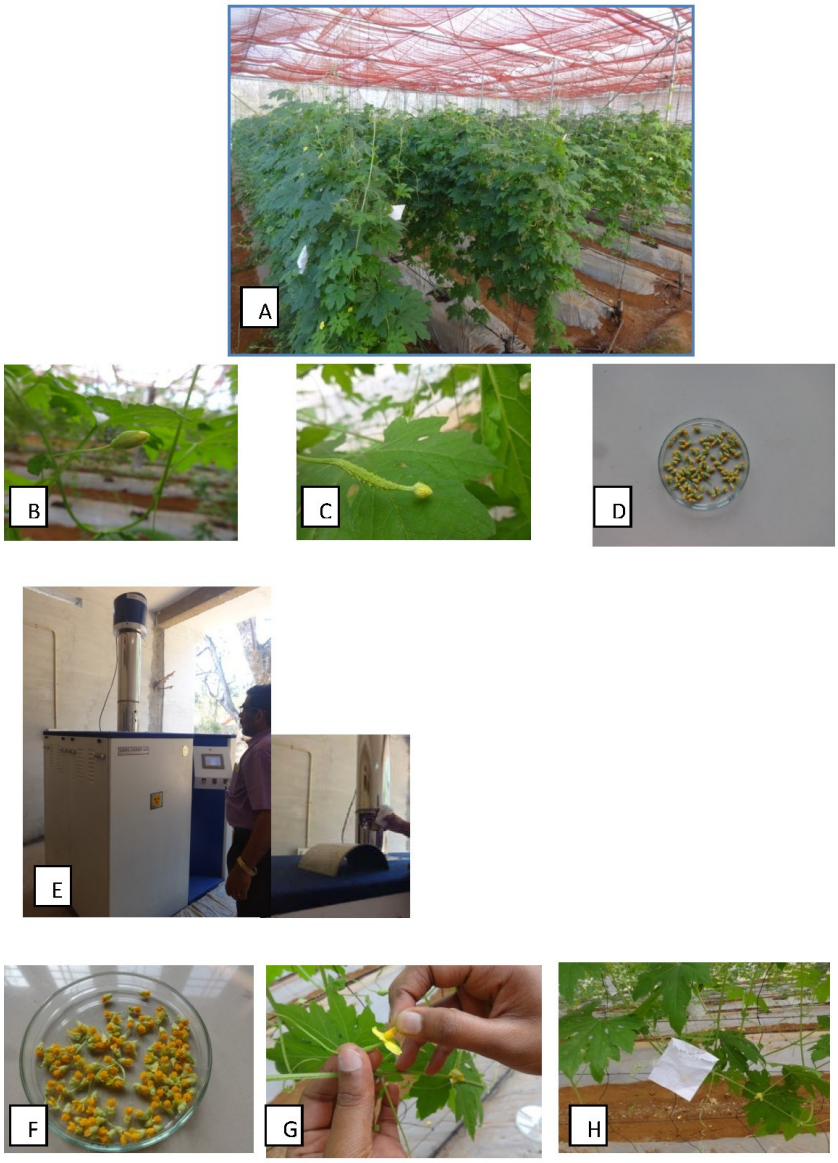


Plate 2. Parthenogenetic fruit production

A. Hybrid MC-139 raised in poly house, B & C .Male and female flower bud one day before anthesis, D. Male flowers collected for irradiation, E. Irradiation of male flowers in gamma chamber, F-H. Artificial pollination using irradiated pollen.

flowers were again enclosed within butter paper bags to prevent pollen contamination until fruit setting. Minimum of 20 flowers were pollinated for each treatment (Plate 2). Observations were recorded on fruit set (%) and no. of seeds and embryos per fruit after 15 days of pollination. Pollen irradiation was also attempted with the above doses of gamma rays.

3.3.2.4 Stage of embryo rescue

Seven to seventeen days old fruits developed through pollinating with unirradiated pollen were harvested and the number of embryos per fruit was found maximum in fruit harvested 15 days after pollination. After 15 days there was no significant difference in number of embryos per fruit (Table 4). So, all fruits developed through pollinating with irradiated pollen were harvested 15 days after pollination.

Table 4. No. of developed seeds per fruit in control

Sl .No.	Days after pollination	No. of developed seeds per fruit
1	7	0 (0.71)^{e}
2	8	0(0.71) ^{e}
3	9	0(0.71) ^{e}
4	10	0(0.71) ^{e}
5	11	0(0.71) ^{e}
6	12	5.40(2.42)±0.99^{d}
7	13	14.60(3.89)±1.45^{c}
8	14	21.66(4.70)±2.19^{b}
9	15	29.13(5.44)±2.53^{a}
10	16	29.06(5.43)±2.60 ^{a}
11	17	30.13(5.53)±1.96 ^{a}
	CD(0.05)	0.113
	CV	5.59

Data are Mean ± Standard deviation, n=15, Value in parentheses are square root transformed

3.3.2.5 Collection and preparation of explant

Harvested fruits were washed with tap water. After soaking in mild detergent (Tween 20) for 10 minutes they were thoroughly rinsed five to six times with distilled water and allowed to dry. Extracted seeds were surface-sterilized

with 0.05 per cent mercuric chloride ($HgCl_2$) for five minutes and were rinsed five times with double-distilled autoclaved water, to remove traces of sterilizing agent immediately after treatment in the laminar air flow cabinet (Plate 3). After sterilization, the explants were allowed to dry by transferring them onto sterile tissue paper on a sterile petri dish.

3.3.2.6 Standardization of induction media

Seeds and embryos were inoculated and cultured in test tubes containing in E20A supplemented with IAA (0.01mg/l) (control) (TSM_1) and E20A supplemented with IAA (0.1mg/l) and BAP (5 mg/l) (TSM_2).

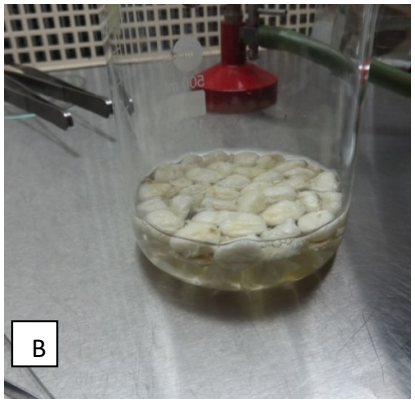
3.3.2.7 Inoculation and incubation of explants

Embryos and normal seeds were placed in glass culture tubes containing E 20A medium supplemented with IAA (0.01mg/l) (Plate 3). Cultured embryos were incubated at 25^0C under a photoperiod of 8 hours of dark and 16 hours of light. Observations on germination per cent of embryo, abnormal culture and callus formation, days for leaf emergence and visible root formation was recorded.

Plantlets showed elongated hypocotyl after germination. Since this growth would not be suitable for subsequent development, hypocotyl was cut at 3 to 3.5 cm below cotyledon and transferred to E20A supplemented with IAA (0.01mg/l) and activated charcoal (3g/lit) for rooting. Days for root initiation and number of roots after three weeks were counted.

3.3.2.8 Hardening and acclimatization

The plantlets were taken out of the test tubes using forceps after soaking the culture in water. The plantlets were thoroughly washed in running tap water to remove the adhering solid medium. Subsequently, the roots were dipped in a solution of bavistin (0.1 %) for 10 minutes to prevent possible contamination in the course of acclimatization. The plants were then planted in polythene bags filled with sterilized cocopeat: soil: sand mixture (1:1:1) and placed in mist chamber for hardening for 20 days in high humidity conditions. After hardening,



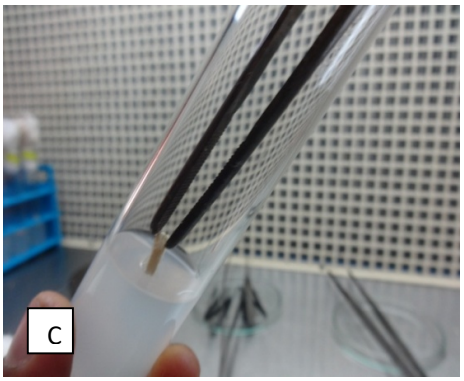


Plate 3. Rescue and culture of embryos

A. Cleaning harvested fruits for embryo rescue, B. Sterilization of extracted seeds, C. Seed inoculation

the plants were transferred to polyhouse. Per cent of plants successfully hardened was found out. Observations on days to 1st male and female flower emergence were taken in regenerated plants.

3.3.2.9 Evaluation of ploidy level

Guard cell size, numbers of chloroplasts per guard cell and pollen grain characteristics were taken as criteria to determine the ploidy level in plants. The 4th or 5th leaves from shoot apex were used to measure guard cell size (width and length) and chloroplast number. Lower epidermal strips of leaves were placed onto a microscope slide for taking observations. Pollen was collected from freshly opened male flowers and viability estimated using acetocarmine stain. Viable pollen stains pink to deep red, while sterile pollen does not stain. Regenerated plants were self pollinated to check the seed set.

The data obtained on various parameters studied in the embryo culture experiment were subjected to analysis of variance, using the online software 'Wasp 2.0'

Results and discussion

4. RESULTS AND DISCUSSION

The present investigation was undertaken to develop superior inbred lines in bitter gourd through advance generation selection of F_1 hybrids and biotechnological approaches through pollination of irradiated pollen and embryo culture. The study was undertaken in five parts.

(i) Evaluation of commercial F_1 hybrids of bitter gourd

(ii) Evaluation of F_2 population and F_3 families

(iii) *In situ* induction of maternal haploids initiated by pollination with irradiated pollen

(iv) Development of haploid inbred lines through embryo rescue and culture

4.1 Evaluation of F_1 hybrids of bitter gourd

F_1 hybrids are popular in bitter gourd. Hybrids in most of the vegetable crops offer the opportunity of earliness, high yield, and quality improvement besides the better capacity to face biotic and abiotic stresses. Being a cross pollinated crop, it is easier to realize the heterosis as a practically feasible phenomena in bitter gourd. F_1 hybrids from the private sector are popular among farmers and white, long fruited types are ruling the market in Kerala. Popular high yielding F_1 hybrids of bitter gourd from public and private sectors were evaluated to assess their performance in Kerala (Plate 4).

Analysis of variance revealed that genotypes were significantly different for all characters (Table 5). Mean performance of 16 hybrids and 5 varieties (control) are given in Table 6. Earliness is an important character in bitter gourd. It is required for realizing the potential economic yield in less time as possible, which is an important consideration for a farmer. Minimum number of days for first male flower appearance was observed in variety MC-147 (36.17) followed by the hybrids, MC-134(38.75), MC-140 (39.16) and MC-144 (39.50). Male flowering was delayed in variety Priyanka (50.75 days) and the hybrid MC-133 (49.67 days). Rani *et al*. (2014) reported similar range for days to first male flower appearance in bitter gourd hybrids. The male flower appeared in the lowest node

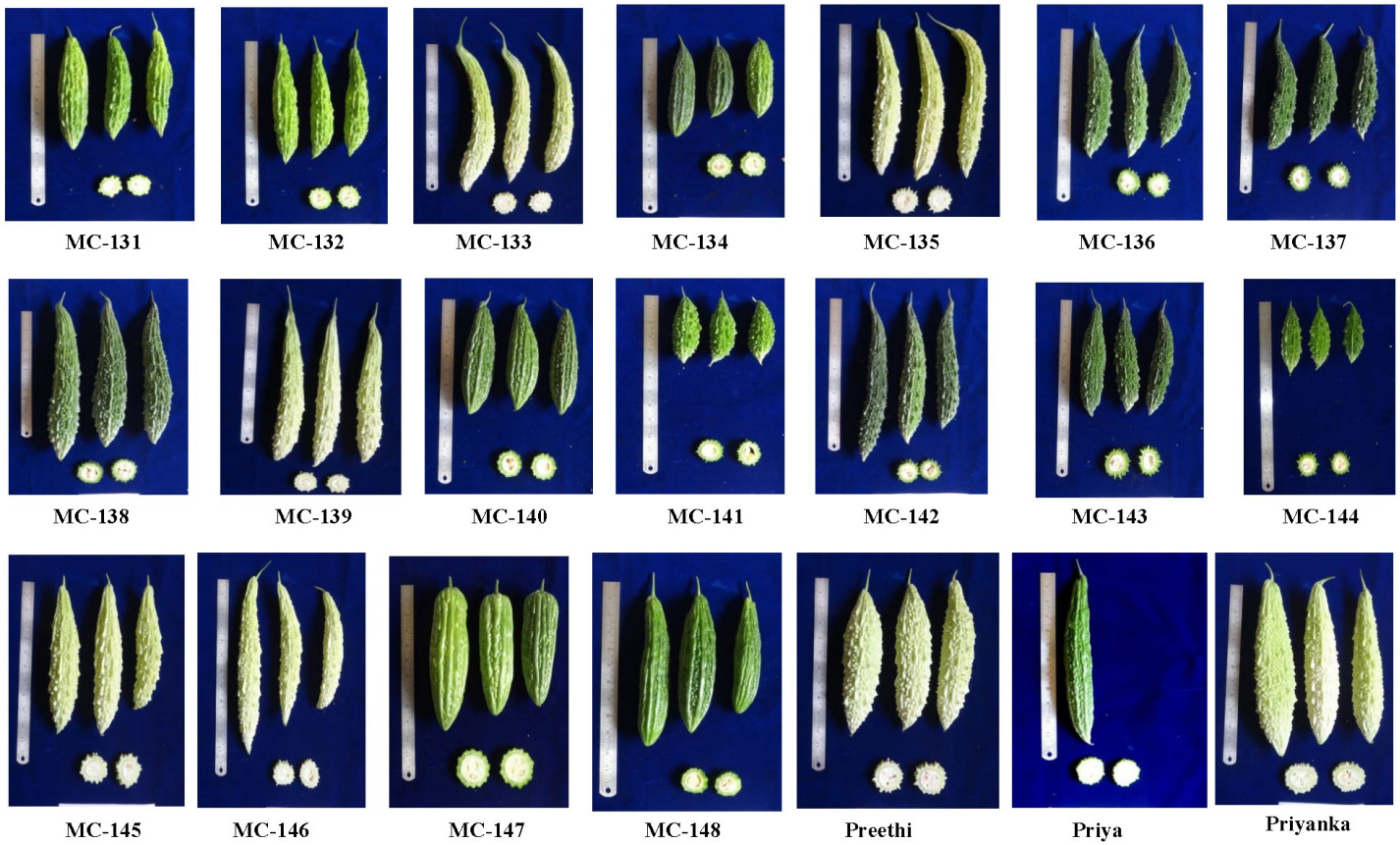


Plate 4. Fruits of bitter gourd genotypes used for evaluation

in variety MC-147 (4.83) followed by hybrid MC-131 (7.58) and the highest node was observed in hybrid MC-135 (17.50) followed by variety Priya (17.00). MC-144 was found to be the earliest to open first female flower (37.75 days) and first harvest (48.00 days) followed by MC-141 which took 38.83 and 48.50 days respectively. Female flowering and days to first harvest were delayed in MC-145 (54.83 and 68.50 respectively) and Priyanka (54.33and 67.50 respectively). Jadhav *et al*. (2009) recorded similar range for days to first harvest in bitter gourd hybrids. Lowest node number to first female flower was recorded in variety MC-148 (7.00) followed by MC-147 (13.00) and hybrid MC-142 (15.83). Earliness in bitter gourd is judged through the appearance of first female flower at lower node and minimum days required for first female flower opening and first harvest (Khan and Behera, 2011).

Table 5. Analysis of variance for different characters in bitter gourd genotypes

Sl. No.	Characters	Replication mean sum of squares	Genotype mean sum of squares	Error mean sum of squares
1	Days to first male flower anthesis	23.455	31.44*	10.919
2	Nodes to first male flower	2.065	28.388**	2.912
3	Days to first female flower anthesis	20.931	38.63*	16.994
4	Nodes to first female flower	9.524	47.88**	6.579
5	Days to first harvest	18.229	53.12**	12.864
6	Relative early yield	0.808	0.48**	0.142
7	Number of days from anthesis to fruit maturity	5.82	1.71**	0.52
8	Average fruit weight	243.154	6723.99**	56.681
9	Fruit length	8.95	71.51**	0.695
10	Fruit diameter	0.001	1.006**	0.037
11	Fruit girth	1.081	10.61**	0.358
12	Flesh thickness	0.002	0.03**	0.001
13	Number of fruits per plant	38.222	1473.77**	57.815
14	Yield per plant	0.659	9.14**	0.519
15	Number of harvests	0.081	3.92**	0.148
16	Number of seeds per fruit	47.078	76.842**	14.749
17	1000 seed weight	95.23	3097.404	98.909

* Significant at 5 % level
** Significant at 1 % level

Table 6. Mean performance of hybrids

Sl. No.	Hybrids	Days to first male flower anthesis	Nodes to first male flower	Days to first female flower anthesis	Nodes to first female flower	Days to first harvest	Relative early yield (kg)	Number of days from anthesis to fruit maturity	Mean fruit weight (g)	Fruit length (cm)
1	MC-131	41.00	7.58	42.75	17.33	56.00	1.47	12.50	122.25	19.58
2	MC-132	41.67	10.17	45.25	20.17	57.00	1.57	13.00	120.40	20.72
3	MC-133	49.67	14.75	49.00	20.10	61.17	2.62	13.50	182.50	29.02
4	MC-134	38.75	9.17	42.50	23.50	53.00	2.10	12.50	92.50	16.75
5	MC-135	44.83	17.50	45.99	24.17	58.50	2.50	13.50	151.00	28.03
6	MC-136	44.99	16.50	41.00	18.83	52.50	2.88	12.65	120.67	22.24
7	MC-137	48.83	16.50	46.00	18.50	58.50	1.93	12.50	125.50	20.54
8	MC-138	44.30	16.33	50.00	28.17	60.33	3.51	13.50	311.67	33.60
9	MC-139	47.45	13.84	47.65	22.17	61.34	2.66	13.50	219.83	32.42
10	MC-140	39.16	10.50	45.50	24.83	57.50	2.30	13.00	130.00	18.81
11	MC-141	41.66	15.17	38.83	20.33	48.50	2.30	11.00	54.50	12.10
12	MC-142	41.17	8.17	42.99	15.83	54.67	2.80	12.50	168.00	29.50
13	MC-143	46.67	16.00	48.33	23.00	62.00	1.72	12.50	120.00	19.96
14	MC-144	39.50	11.00	37.75	19.00	48.00	2.75	11.00	50.23	11.15
15	MC-145	48.67	14.00	54.83	28.00	68.50	2.35	11.50	160.00	25.85
16	MC-146	45.83	16.16	47.83	21.33	61.00	1.90	12.50	124.50	24.10
Control										
17	MC-147	36.17	4.83	41.50	13.00	57.50	2.55	14.00	152.50	19.29
18	MC-148	41.67	7.67	44.75	7.00	56.00	1.80	12.50	100.30	18.19
19	Preethi	44.82	13.83	47.83	23.17	61.50	2.35	11.00	174.25	23.40
20	Priya	43.50	17.00	44.50	21.17	57.50	2.15	13.50	121.35	21.28
21	Priyanka	50.75	14.50	54.33	25.67	67.50	2.10	14.00	215.28	27.09
	Mean	43.86	12.91	45.67	20.73	58.02	2.30	12.67	143.67	22.55
	C.D. (5%)	6.89	3.56	8.59	5.35	7.48	0.79	1.51	15.71	1.74
	C.D. (1%)		4.86		7.29	10.2	1.07	2.06	21.42	2.37
	CV (%)	7.53	13.21	9.02	12.38	6.18	16.39	5.71	5.24	3.69

Table 6. Mean performance of hybrids (Continued)

Sl. No.	Hybrids	Fruit diameter (cm)	Fruit girth (cm)	Flesh thickness (cm)	Number of fruits per plant	Yield per plant (kg)	Number of harvests	Number of seeds/fruit	Thousand seed weight	Fruit seed ratio
1	MC-131	4.65	14.67	0.77	29.00	2.50	7.00	27.92	241.00	14.17
2	MC-132	4.52	13.79	0.79	29.00	2.45	6.63	29.67	241.50	13.13
3	MC-133	5.08	14.91	0.80	42.67	7.08	9.83	16.83	232.83	33.93
4	MC-134	4.33	13.52	0.75	51.10	3.40	8.50	31.50	139.00	14.19
5	MC-135	4.64	14.41	0.76	44.00	5.95	8.83	21.00	230.50	22.73
6	MC-136	4.34	13.57	0.85	84.83	9.00	12.33	16.67	284.43	21.34
7	MC-137	4.81	15.21	0.70	55.50	6.15	9.66	17.67	266.00	22.36
8	MC-138	6.43	20.51	0.95	36.83	10.03	10.00	31.67	226.85	44.15
9	MC-139	5.07	15.72	0.71	41.33	8.06	9.33	24.00	227.17	27.30
10	MC-140	5.00	15.79	1.00	52.30	6.05	9.10	34.08	208.00	16.78
11	MC-141	4.21	12.89	0.68	121.50	5.70	8.50	15.50	224.50	12.30
12	MC-142	4.38	14.43	0.86	53.17	8.49	9.50	28.17	225.57	23.32
13	MC-143	4.91	15.18	0.82	57.50	6.15	9.67	18.75	247.00	26.56
14	MC-144	3.84	11.94	0.56	123.50	5.40	9.00	22.75	221.00	7.78
15	MC-145	5.24	15.48	0.75	42.15	5.85	8.67	28.50	205.50	23.56
16	MC-146	4.60	15.00	0.76	57.50	5.90	9.33	27.25	283.00	16.45
Control										
17	MC-147	5.99	19.06	0.95	29.00	4.15	8.00	31.50	136.00	26.50
18	MC-148	4.34	13.90	0.55	34.50	2.69	5.50	12.75	167.00	16.27
19	Preethi	6.10	19.41	0.82	31.10	4.15	9.27	27.92	258.50	23.45
20	Priya	4.19	13.19	0.55	37.25	3.30	7.63	25.85	240.50	13.35
21	Priyanka	6.01	18.94	0.95	29.50	5.20	9.32	26.50	249.50	25.38
	Mean	4.89	15.31	0.78	51.58	5.60	8.84	24.59	226.44	
	C.D. (5%)	0.40	1.25	0.07	15.86	1.50	0.80	8.01	20.74	
	C.D. (1%)	0.55	1.70	0.09	21.63	2.05	1.09	10.93	28.29	
	CV (%)	3.96	3.91	4.39	14.74	12.86	4.35	15.62	4.39	

The first female flower appeared in the highest node number in MC-138 (28.17) and MC-145 (28.00). Sundaram (2009) observed the first female flower on the lowest position in the bitter gourd hybrid, Bikaneer 1 x IC 85643 (12.89). In the previous reports, first female flower at 3rd node was appeared in Gynoecious × monoecious hybrids**,** DBGy- 201 x S54 followed by DBGy- 201 x DBG 34 at 5th node. Monoecious × monoecious hybrids like VNR 22 had first female flower at 11th node and Pusa Hybrid 2 at 9th node (Khan and Behera, 2011). The highest relative early yield was recorded by MC-138 (3.5 kg) and the least was in MC-131 (1.47 kg). The number of days from anthesis to fruit maturity varied from 11 (MC-141, MC-144 and Preethi) to 14 days (MC-147 and Priyanka). Similar range for days from anthesis to fruit maturity was previously reported in bitter gourd variety Arka Harit which took 12-14 days (Anon, 2018). Stage of harvest of pumpkin hybrid Pusa Hybrid 1 was 10 to 15 days after anthesis (Vishwanath and Tomar, 2013). Cucumber reached edible maturity in 6-7 days after anthesis (Gopalakrishnan, 2007). Fruits of cucumber and sponge gourd attain marketable maturity in 5 to 7 days after anthesis and bottle gourd fruit took 10 to 12 days after anthesis to fruit maturity (Rattan and Kumar, 2016).

Fruit length, fruit diameter and fruit girth are important yield contributing traits. There was a wide range in the hybrid mean value for fruit length from 11.15 (MC-144) to 33.60 cm (MC-138). MC-139 was the second best hybrid for fruit length. MC- 144 and MC-141 produced small fruits. Similar range for fruit length was reported by Aruna and Swaminathan (2012) and Rani *et al*. (2014) in bitter gourd. The maximum fruit diameter was observed in the hybrid MC-138 (6.43cm) and the minimum in MC-144 (3.84 cm). Behera *et al*. (2009) and Alhariri *et al*. (2018) also reported similar range for fruit diameter in bittergourd. Fruit girth was also observed as the highest in MC-138 (20.53 cm) and minimum in MC-144 (11.94 cm). Sundaram (2009) recorded that the girth of fruit ranged from 8.83 cm (MDU 1 x Vadipatti Local) to 13.89 cm (Bikaneer 1 x Bikaneer 3) among the bitter gourd hybrids. Rani *et al*. (2014) observed that fruit girth of bitter gourd hybrids varied from 10.98 (IC-033227 × IC-045339) to (IC-045339 × IC-470560)

13.89 cm. Fruit girth in the present study is high compared to previous studies in bitter gourd.

Fruit flesh thickness is an important fruit quality trait and an essential determinant of yield in bitter gourd. The thicker the fruit flesh, the higher the edible portion of the fruit. Flesh thickness varied from 0.55 (MC-148 and Priya) to 1.00 (MC-140) cm. Similar range for flesh thickness was observed in findings of Mohan (2005) and Alhariri *et al*. (2018) in bitter gourd hybrids.

Yield per plant is highly dependent on average fruit weight and the number of fruits per plant. The highest average fruit weight was exhibited by MC-138 (311.67 g) followed by MC-139 (219.83 g). These hybrids performed extremely well in fruit weight. The next best genotype for fruit weight was the variety Priyanka (215.28 g) followed by hybrid MC-133 (182.50g) and variety Preethi (174.25 g). Priya (121.35 g) and MC-148 (100.30g) produced light weight fruits. Reduced fruit weight was observed in hybrid MC-144 (50.23 g) followed by MC-141 (54.50 g). Rani *et al*. (2014) and Alhariri *et al*. (2018) observed that the average fruit weight ranged from 58.82 to 98.57g and 56.33 to 78.57 g respectively in a study conducted among 28 F_1 hybrids of bitter gourd. The highest number of fruits per plant was observed in the hybrid MC-144 (123.50) followed by MC-141 (121.50). Reduced number of fruits per plant was reported in MC-131, MC-132 and the control MC-147 (29).

Yield per plant is the ultimate and the most important trait. Top five hybrids recording highest *per se* performance were MC-138 (10.03 kg), MC-136 (9.00 kg), MC- 142 (8.49 kg), MC-139 (8.06 kg) and MC-133 (7.08 kg). Majority of the hybrids showed considerably higher performance compared to the control varieties. Number of harvests varied from 5.50 (MC-148) to 12.33 (MC-136).

Less number of seeds per fruit in bitter gourd makes it more acceptable to the consumer. Number of seeds per fruit in the present study ranged from 12.75 (MC-148) to 34.08 (MC-140). Mohan (2005) reported the minimum number of seeds per fruit in bitter gourd hybrid Green long x BLG-1 (14.83). Rani *et al*.

(2014) stated that number of seeds/fruit in 28 bitter gourd hybrids varied from 15.00 (IC-033227 × IC-470550) to 20.67 (IC-033227 × IC-470558). Jat *et al*. (2016) recorded 23.8 number of seeds per fruit for Pusa Hybrid 1. Hybrid, Arka Harit x Chidambaram Small with the mean number of seeds per fruit of 12.30 may be considered as best for the number of seeds per fruit among the 24 hybrids of bitter gourd (Sureshkumara *et al*., 2017). In the present study, the maximum thousand seed weight was observed in the hybrid MC-136 (284.43g) followed by MC-146 (283.00g). The least was reported in MC-147 (136g). According to Jat *et al.* (2016) hundred seed weight of Pusa Hybrid 1 and Pusa Hybrid 2 of bitter gourd hybrids were reported as 13.8 and 12.5 g, respectively. Cucumber hybrid, Pant Shankar Khira-1 was found to have 100 seed weight of 2.59 g (Mukulkumar *et al*., 2018). Fruit seed ratio in the current experiment ranged from 7.78 (MC-144) to 44.15 (MC-138). Nerson *et al*. (2000) reported that cocozelle squash had the lowest ratio of seed yield-to-fruit weight. Nerson and Paris (2000) stated that there is no relationship between fruit and mean seed weight in Poinsett 76 cucumber.

4.1.1 Ranking of hybrids based on cumulative index

To make an effective ranking for higher yield, it is necessary to determine the cumulative index. It helps to sift out suitable genotypes from germplasm based on reliable and effective traits. Ranking of hybrids was done based on cumulative index worked out for characters like, node to 1st female flower appearance, days to 1st picking, fruit weight (g), fruit length (cm), fruit diameter (cm), relative early yield (kg), yield per plant (kg) and number of fruits per plant (Table 7). Top 5 F_1 hybrids ranked based on the cumulative index were MC-142, MC-136, MC-139, MC-138 and MC-133 (Plate 5). In bitter gourd, selection index prepared on the basis of major yield components is effective in ranking of genotypes which was followed in an earlier study of 13 bitter gourd genotypes (Parhi *et al*., 1993). Ram *et al*. (2006) stated that emphasis was given for the number of fruits per plant and average fruit weight in selecting high yielding genotypes in bitter gourd.

MC-133

MC-136

MC-138

MC-139

MC-142

Plate 5: Superior hybrids selected

Thus the study revealed that MC-142, MC-136, MC-139, MC-138 and MC-133 were the most superior hybrids with respect to yield and other economic characters. These hybrids were selfed to produce five F_2 populations. A selection intensity of 5 % was applied for identifying superior lines in F_2. F_3 families raised from the selected plants of F_2 population were evaluated. A selection intensity of 5 % was applied for identifying superior lines.

4.2 Evaluation of F_2 population and F_3 families

4.2.1 Descriptive statistics

The descriptive statistics, namely mean, standard error of mean, standard deviation, variance and range for the three generations were worked out for all the characteristics under study (Table 8). The comparison of pair wise means for the various characteristics of F_2 and F_3 was done using the standard procedure of two sample case t test after assessing the homogeneity of variance using F test.

Table 7. Ranking of hybrids based on cumulative index

Sl. No.	Hybrid	Cumulative index	Rank
1	MC-142	3.00	1
2	MC-136	3.16	2
3	MC-139	3.50	3
4	MC-138	3.84	4
5	MC-133	3.87	5
6	MC-144	4.09	6
7	MC-141	4.18	7
8	MC-135	4.20	8
9	MC-137	4.59	9
10	MC-147	4.99	10
11	MC-146	5.16	11
12	MC-145	5.25	12
13	MC-143	5.30	13
14	MC-134	5.32	14
15	MC-140	5.37	15
16	Priyanka	5.68	16
17	Preethi	5.71	17
18	MC-148	5.86	18
19	Priya	5.91	19
20	MC-131	6.14	20
21	MC-132	6.18	21

Table 8. Descriptive statistics of F_2 and F_3 generations of bitter gourd

Sl. No.	Parameters	Mean			t value	Std. Error of Mean		Std. Deviation		Variance		F value	Range	
			F_2	F_3		F_2	F_3	F_2	F_3	F_2	F_3		F_2	F_3
1	Days to first male flower anthesis	MC-142	41.63	42.75	NS	1.18	0.57	5.12	4.44	26.25	19.72	NS	36.00-59.00	28.00-69.00
		MC-136	47.23	46.35	NS	1.38	0.40	4.97	2.64	24.69	6.95	6.66*	42.00-59.00	42.00-54.00
		MC-139	43.75	44.84	NS	0.93	0.52	4.53	3.18	20.54	10.14	NS	37.00- 52.00	38.00-53.00
		MC-138	48.18	44.94	NS	1.85	0.38	9.78	2.63	95.56	6.93	31.41**	37.00-74.00	40.00-54.00
		MC-133	43.53	42.00	NS	0.82	0.55	4.66	3.15	21.74	9.94	4.54*	37.00-53.00	36.00-52.00
2	Nodes to first male flower	MC-142	8.26	6.82	2.95**	0.47	0.23	2.05	1.80	4.20	3.24	NS	6.00-14.00	4.00-12.00
		MC-136	14.54	12.77	NS	1.12	0.37	4.03	2.46	16.27	6.04	6.82*	7.00-22.00	6.00-17.00
		MC-139	14.75	11.58	5.2**	0.39	0.42	1.89	2.57	3.59	6.63	NS	12.00-18.00	7.00-17.00
		MC-138	12.96	11.62	2.95**	0.37	0.27	1.97	1.87	3.89	3.5	NS	7.00-16.00	9.00-19.00
		MC-133	12.41	10.03	3.17**	0.58	0.48	3.27	2.76	10.70	7.59	NS	6.00-19.00	6.00-16.00
3	Days to first female flower anthesis	MC-142	46.84	46.57	NS	1.17	0.49	5.08	3.76	25.81	14.15	NS	35.00-59.00	38.00-56.00
		MC-136	46.12	45.09	NS	1.44	0.46	5.93	3.07	35.11	9.43	13.48**	39.00-59.00	37.00-50.00
		MC-139	47.92	46.47	NS	1.21	0.65	5.91	4.00	34.95	16.04	8.76**	37.00-59.00	39.00-58.00
		MC-138	53.82	49.13	2.35*	1.93	0.52	10.19	3.58	103.78	12.81	21.91**	41.00-79.00	41.00-57.00
		MC-133	51.22	47.06	2.99**	1.15	0.78	6.51	4.46	42.43	19.87	8.45**	41.00-64.00	36.00-57.00
4	Nodes to first female flower	MC-142	16.53	13.55	2.49*	1.06	0.58	4.61	4.50	21.26	20.22	NS	9.00-26.00	5.00-22.00
		MC-136	20.53	13.84	7.68**	0.41	0.77	1.70	5.09	2.89	25.90	22.45**	18.00-23.00	5.00-24.00
		MC-139	19.62	18.08	NS	0.71	0.56	3.48	3.45	12.15	11.91	NS	10.00-24.00	12.00-27.00
		MC-138	21.57	18.57	2.42*	1.12	0.53	5.92	3.63	35.07	13.16	7.70**	13.00-35.00	11.00-28.00
		MC-133	19.59	18.64	NS	0.83	0.64	4.69	3.69	21.99	13.61	NS	11.00-33.00	11.00-27.00
5	Days to first harvest	MC-142	59.63	57.00	2.42*	1.14	0.49	4.96	3.83	24.58	14.64	NS	50.00-73.00	51.00-67.00
		MC-136	56.71	54.61	NS	1.50	0.44	6.17	2.94	38.10	8.61	19.80**	48.00-69.00	46.00-59.00
		MC-139	61.50	57.68	2.8**	1.19	0.59	5.84	3.63	34.09	13.14	9.26**	53.00-74.00	52.00-69.00
		MC-138	66.79	60.94	2.80**	2.03	0.5	10.72	3.45	114.92	11.93	27.27**	54.00-94.00	55.00-68.00
		MC-133	63.19	56.52	3.57**	1.16	0.77	6.55	4.44	42.87	19.70	5.60*	52.00-77.00	46.00-66.00
6	Relative early yield	MC-142	0.85	1.01	NS	0.12	0.06	0.51	0.44	0.26	0.19	NS	0.23-2.16	0.41-2.26
		MC-136	1.44	0.88	3.42**	0.15	0.08	0.64	0.55	0.41	0.30	NS	0.09-2.60	0.42-2.73
		MC-139	0.81	1.01	NS	0.12	0.10	0.60	0.60	0.36	0.36	NS	0.20-1.81	0.31-2.44
		MC-138	0.98	1.37	2.92**	0.11	0.08	0.56	0.54	0.32	0.3	NS	0.27 - 2.06	0.51 - 2.63

		MC-133	0.79	0.70	NS	0.06	0.07	0.36	0.43	0.13	0.18	NS	0.41-1.76	0.22-1.60
7	Number of days from anthesis to fruit maturity	MC-142	12.00	11.72	NS	0.33	0.15	1.45	1.15	2.11	1.32	NS	10.00-14.00	10.00-14.00
		MC-136	11.76	12.00	NS	0.38	0.20	1.56	1.32	2.44	1.74	NS	7.00-13.50	8.00-14.00
		MC-139	11.58	12.22	NS	0.29	0.17	1.44	1.06	2.08	1.13	6.8*	10.00-14.00	9.50-15
		MC-138	12.31	12.81	NS	0.24	0.09	1.29	0.63	1.68	0.39	20.97**	4.00 - 7.00	4.00-8.00
		MC-133	11.75	10.50	4.23**	0.23	0.19	1.28	1.10	1.64	1.20	NS	9.00-14.00	9.00-13.00
8	Mean fruit weight	MC-142	84.45	101.08	2.39*	6.08	3.39	26.52	26.29	703.26	691.18	NS	50.69-136.75	58.00-199.50
		MC-136	99.94	93.37	NS	6.66	3.87	27.47	25.67	754.76	658.85	NS	8.71-128.11	55.23-186.80
		MC-139	94.33	130.40	4.26**	6.84	5.16	33.53	31.83	1124.00	1013.00	NS	42.00-139.35	85-214.33
		MC-138	149.84	189.88	3.59**	8.87	6.79	46.92	46.57	2202	2169	NS	62.34 - 231.50	94.32 -330.10
		MC-133	96.27	95.10	NS	4.72	4.43	26.69	25.47	712.45	648.80	NS	56.00-161.14	60.20-149.50
9	Fruit length	MC-142	19.27	20.89	NS	0.80	0.43	3.47	3.33	12.03	11.11	NS	9.50-23.00	12.00-26.90
		MC-136	16.91	14.37	3.33**	0.88	0.33	3.62	2.22	13.10	4.92	NS	6.55-25.30	8.20-18.55
		MC-139	20.70	22.06	NS	0.96	0.64	4.72	3.96	22.24	15.70	NS	12.00-30.75	14.00- 30.47
		MC-138	21.63	25.77	3.59**	0.91	0.7	4.83	4.81	23.31	23.18	NS	13.30 - 37.00	16.00 -36.43.00
		MC-133	19.98	16.79	4.63**	0.50	0.48	2.83	2.74	8.00	7.53	NS	11.50-24.24	12.65-22.85
10	Fruit diameter	MC-142	3.59	3.85	NS	0.12	0.07	0.51	0.52	0.26	0.27	NS	3.10-4.60	3.30-5.60
		MC-136	4.11	4.19	NS	0.14	0.07	0.57	0.43	0.33	0.19	NS	2.50-4.86	3.20-4.80
		MC-139	3.50	4.15	4.17**	0.13	0.10	0.62	0.60	0.38	0.36	NS	2.40-4.40	3.20-5.60
		MC-138	4.41	4.88	4.26**	0.09	0.06	0.5	0.43	0.25	0.18	NS	3.50 to 6.00	4.00 to 6.00
		MC-133	3.75	3.76	NS	0.11	0.10	0.60	0.58	0.36	0.34	NS	2.60-5.20	2.60-4.80
11	Fruit girth	MC-142	11.70	12.10	NS	0.43	0.24	1.89	1.82	3.57	3.32	NS	9.80-16.35	9.80-17.70
		MC-136	13.98	14.15	NS	0.52	0.19	2.14	1.26	4.59	1.59	NS	7.55-17.40	10.34-16.86
		MC-139	11.32	13.43	4.6**	0.36	0.28	1.77	1.75	3.12	3.08	NS	7.80-13.60	11.00-18.10
		MC-138	14.03	16.08	5.87**	0.31	0.2	1.63	1.35	2.65	1.83	NS	11.15 -18.50	13.00 -18.95
		MC-133	12.32	11.93	NS	0.33	0.32	1.85	1.84	3.42	3.40	NS	8.90-17.50	8.79-15.20
12	Flesh thickness	MC-142	0.49	0.59	4.09**	0.02	0.01	0.10	0.10	0.01	0.01	NS	0.38-0.75	0.41-0.83
		MC-136	0.57	0.56	NS	0.03	0.01	0.10	0.07	0.01	0.01	NS	0.30-0.73	0.43-0.80
		MC-139	0.44	0.57	6.89**	0.02	0.01	0.08	0.08	0.01	0.01	NS	0.30-0.55	0.40-0.75
		MC-138	0.55	0.62	4.26**	0.02	0.01	0.09	0.07	0.01	0.01	NS	0.40 to 0.70	0.48 - 0.75
		MC-133	0.50	0.54	2.02*	0.02	0.02	0.09	0.10	0.01	0.01	NS	0.30-0.60	0.40-0.80

13	Number of fruits per plant	MC-142	32.11	34.82	NS	2.06	1.16	8.98	8.98	80.65	80.56	NS	13.00-47.00	16.00-62.00
		MC-136	44.47	33.75	3.59**	2.65	1.55	10.92	10.29	119.26	105.87	NS	24.00-67.00	11.00-59.00
		MC-139	22.37	26.50	NS	2.02	1.50	9.90	9.23	98.07	85.28	NS	6.00-41.00	13.00-52.00
		MC-138	23.07	25.44	NS	1.44	0.89	7.61	6.14	57.85	37.69	NS	7.00- 36.00	12.00- 39.00
		MC-133	24.00	25.45	NS	1.34	1.31	7.59	7.52	57.68	56.51	NS	14.00-43.00	12.00-39.00
14	Yield per plant	MC-142	2.39	2.84	NS	0.27	0.14	1.19	1.09	1.42	1.20	NS	0.77-5.40	0.99-5.95
		MC-136	3.70	2.36	3.38**	0.35	0.20	1.46	1.35	2.14	1.81	NS	0.13-6.50	0.73-6.82
		MC-139	2.05	2.72	NS	0.30	0.23	1.46	1.44	2.14	2.09	NS	0.30-4.52	0.97-6.09
		MC-138	2.76	3.87	3.46**	0.26	0.19	1.35	1.36	1.83	1.83	NS	0.90 -5.16	1.43 -6.57
		MC-133	2.12	1.96	NS	0.19	0.18	1.05	1.03	1.10	1.07	NS	0.60-4.50	0.70-4.14
15	Number of harvests	MC-142	8.74	8.48	NS	0.57	0.21	2.47	1.63	6.09	2.66	4.69*	5.00-15.00	5.00-11.00
		MC-136	12.12	8.00	8.21**	0.61	0.21	2.52	1.36	6.36	1.86	NS	5.00-17.00	6.00-11.00
		MC-139	7.54	7.53	NS	0.43	0.27	2.13	1.69	4.52	2.85	NS	4.00-10.00	5.00-12.00
		MC-138	5.54	6.04	2.36*	0.17	0.13	0.92	0.88	0.85	0.78	NS	4.00 - 7.00	4.00-8.00
		MC-133	8.31	7.09	3.01**	0.35	0.20	1.99	1.16	3.96	1.34	4.71*	5.00-14.00	5.00-10.00
16	Number of seeds per fruit	MC-142	21.60	16.48	4.36**	1.07	0.57	4.67	4.39	21.77	19.24	NS	13.00-27.00	10.00-28.67
		MC-136	19.63	18.97	NS	1.65	0.76	6.82	5.04	46.53	25.38	NS	3.00-33.00	7.00-31.00
		MC-139	20.16	18.37	NS	1.51	0.92	7.42	5.66	55.08	32.06	NS	8.50-35.33	7.00-29.33
		MC-138	21.93	24.66	2.06*	1.12	0.77	5.92	5.29	34.99	28.03	NS	10.60-33.00	11.80-36.20
		MC-133	16.68	16.15	NS	0.96	0.87	5.46	4.99	29.78	24.91	NS	9.30-30.00	8.00-26.00
17	1000 seed weight	MC-142	193.86	193.57	NS	5.39	1.85	23.48	14.31	551.12	204.92	13.93**	165.00-242.11	160.97-221.00
		MC-136	220.48	239.05	NS	12.82	5.36	52.86	35.58	2794.02	1265.89	NS	107.23-290.93	164.59-310.76
		MC-139	173.89	177.25	NS	6.20	2.81	30.38	17.31	923.16	299.51	NS	117.10-237.33	152.88-221.13
		MC-138	145.58	162.96	4.65**	3.73	1.84	19.75	12.64	389.89	159.77	NS	101.70-187.90	120.68-183.23
		MC-133	199.26	197.25	NS	4.92	3.06	27.85	17.56	775.49	308.52	7.04*	152.07-277.00	167.40-278.00

4.2.1.2 Nodes to first male flower

Nodes to first male flower became nonsignificant between F_2 and F_3 generations of MC-136. It was significantly reduced in F_3 generations of MC-142 (6.82), MC-139 (11.58), MC-138 (11.62) and MC-133 (10.03). Variance significantly reduced from F_2 (16.27) to F_3 (6.04) in case of MC-136. Variance between F_2 and F_3 of the rest of the hybrids was observed as non significant. The range became equal or reduced in subsequent generations (F_2 and F_3) of all hybrids except MC-139 and MC-138.The lower range values and variance indicated the uniformity of F_3 population with respect to this character.

4.2.1.3 Days to first female flower anthesis

F_3 generation plants of MC-138 and MC-133 showed earliness to initiate female flowering (49.13 and 47.06) compared to its F_2. Significant difference was not found between F_2 and F_3 of MC-142, MC-136 and MC-139. Variance was significantly reduced in F_3 generations of MC-136 (9.43), MC-139 (16.04), MC-138 (12.81) and MC-133 (19.87) compared to its F_2 generation. The range was reduced in subsequent generations (F_2 and F_3) of all hybrids. The difference between genotypes with respect to its response to selection was apparent for this character.

4.2.1.4 Nodes to first female flower

Plants in the F_3 generation of MC-142, MC-136 and MC-138 produced the first female flower at the lowest node (13.55, 13.84and 18.57) compared to its F_2 generation. A significant difference was not found between F_2 and F_3 of MC-139 and MC-133. Variance was significantly reduced in F_3-MC-138 compared to its F_2 generation. Variance between F_2 and F_3 of the hybrids MC-142, MC-139 and MC-133 were found as non significant. Whereas F_3 generation of MC-136 showed a higher variance than its F2 generation. A wide range was observed in F_3 compared to its F_2 in MC-136 and the range reduced in subsequent generations (F_2 and F_3) of all other

hybrids. Production of female flower in lower nodes is an indication of earliness and inbreds in F_3 of majority of hybrids produced female flowers in lower nodes.

4.2.1.5 Days to first harvest

Days to first harvest reduced significantly in F_3 generations of hybrids MC-142 (57 days), MC-139 (57.68 days), MC-138 (60.94 days) and MC-133 (56.52 days) compared to its F_2 generation. There was no significant difference between F_2 and F_3 of MC-136. According to Oviedo *et al*. (2008) days to first harvest was on par at different inbreeding levels (S_0 to S_5) of hybrid Japanese cucumber. Variance was significantly reduced in F_3 generations of MC-136 (8.61), MC-139 (13.14), MC-138 (11.93) and MC-133 (19.70) compared to its F_2 generation. Variance showed non significant difference between segregating generations of MC-142. The range was reduced in subsequent generations (F_2 and F_3) of all hybrids. Lower range in subsequent generation is an indication of attainment of uniformity for this character.

4.2.1.6 Relative early yield

Relative early yield increased significantly from F_2 to F_3 generation in MC-138 (0.98 to 1.37 kg) and decreased in MC-136 (1.44 to 0.88 kg). Significant difference was not found between F_2 and F_3 of MC-142, MC-139 and MC-133. Significant difference in variance was not observed between F_2 and F_3 of all hybrids. The wider range was observed in F_3 compared to F_2 generation in hybrids, MC-139, MC-138 and MC-133 and reduced in MC-142 and MC-136.

4.2.1.7 Number of days from anthesis to fruit maturity

There was no significant difference in the number of days from anthesis to fruit maturity between F_2 and F_3 of all hybrids except MC-133 where it reduced significantly from 11.75 to 10.50 days. Variance was significantly reduced in F_3 generations of MC-139 (1.13) and MC-138 (0.39) compared to its F_2 generation. Variance showed non significant difference between F_2 and F_3 of MC-142, MC-136

and MC-133. The range became equal or reduced in subsequent generations (F_2 and F_3) of all hybrids except for MC-139 and MC-138.

4.2.1.8 Average fruit weight

A significant increase of average fruit weight from F_2 to F_3 generation was recorded in MC-142 (84.45 to 101.08 g), MC-139 (94.33 to 130.4 g) and MC-138 (149.84 to 189.88 g). Significant difference was not found between F_2 and F_3 generation of MC-136 and MC-133. Abd El-Rahman *et al*. (2005) remarked that fruit weight decreased from S4 to S6 generation and the range for the character became smaller in the S6 generation than those in the S4 generation in gurma melon (*Citrullus colocynthoides*). According to Oviedo *et al*. (2008) average fruit weight was on par at different inbreeding levels (S_0 to S_5) of hybrid Japanese cucumber. Non significant difference of variance between F_2 and F_3 generations and a wide range from F_2 to F_3 was observed for all hybrids. Abd El-Hadi *et al*. (2001) on sweet melon (*Cucumis melo var. aegyptiacus*) noticed a decrease in ranges from S3 to S5 generation for fruit weight

4.2.1.9 Fruit length

Fruit length from F_2 to F_3 generations was significantly increased in MC-138 (21.63 to 25.77 cm) and decreased in MC-136 (16.91 to 14.37 cm) and MC-133 (19.98 to 16.79 cm). Significant difference was not observed between F_2 and F_3 generations of MC-142 and MC-139. Nonsignificant difference of variance between F_2 and F_3 generations was shown by all hybrids. The range was reduced in subsequent generations (F_2 and F_3) of all other hybrids except MC-142. Behera *et al*. (2010b) reported an increase in fruit length in cucumber after one cycle of marker assisted selection from F_3 to F_4.

4.2.1.10 Fruit diameter

Fruit diameter from F_2 to F_3 generations was significantly increased in MC-139 (3.50 to 4.15 cm) and MC-138 (4.41 to 4.88 cm). Significant difference was not found between F_2 and F_3 of MC-142, MC-136 and MC-133. Nonsignificant difference of variance between F_2 and F_3 generations was shown by all hybrids. The range was decreased in subsequent generations (F_2 and F_3) of all other hybrids except MC-142 and MC-139.

4.2.1.11 Fruit girth

A significant increase of fruit girth from F_2 to F_3 generation was recorded in MC-139 (11.32 to 13.43 cm) and MC-138 (14.03 to 16.08 cm). Significant difference was not found between F_2 and F_3 generation of MC-142, MC-136 and MC-133. Nonsignificant difference of variance between F_2 and F_3 generations was observed for all hybrids. The range was decreased in subsequent generations (F_2 and F_3) of all other hybrids except MC-142 and MC-139.

4.2.1.12 Flesh thickness

Flesh thickness of F_3 fruits was the highest in MC-142 (0.59 cm), MC-139 (0.57 cm), MC-138 (0.62 cm) and MC - 133 (0.54 cm) compared to its F_2 except in MC-136. Non significant difference of variance was present between F_2 and F_3 generations of all hybrids. The range was decreased in subsequent generations (F_2 and F_3) of all other hybrids except MC-142, MC-139 and MC-133.

4.2.1.13 Number of fruits per plant

There was no significant difference in the values of number of fruits per plant in F_2 and F_3 of all hybrids except in MC-136 where it reduced significantly from 44.47 to 33.75. According to Oviedo *et al*. (2008) number of commercial fruits was on par at different inbreeding levels (S_0 to S_5) of hybrid Japanese cucumber. The

variance for the trait was non significant among segregating generations. Range decreased in F_3 generations of MC-138 and MC133 compared to its F_2.

Abd El-Rahman *et al*. (2005) remarked that number of fruits per plant decreased from S4 to S6 generation and the range for the character became narrower in the S6 generation than those in the S4 generation in gurma melon (*Citrullus colocynthoides*).

4.2.1.14 Yield per plant

Plants of F_3 generation of MC-138 were found to be high yielders (3.87 kg) compared to its F_2. Significant difference was not found between F_2 and F_3 generations of the hybrids MC-142, MC-139 and MC-133. Yield per plant reduced significantly from F_2 (3.7 kg) to F_3 (2.36 kg) in MC-136. Yield improvement during inbreeding process was reported in cucumber by Rubino and Wehner (1986). According to Oviedo *et al*. (2008) yield per plant was on par at different inbreeding levels (S_0 to S_5) of hybrid Japanese cucumber. Behera *et al*. (2010b) reported an increase in yield of cucumber after one cycle of marker assisted selection from F_3 to F_4. Variance between F_2 and F_3 of all hybrids were non significant. The range was decreased from F_2 to F_3 in hybrids, MC-136 and MC-133. Abd El-Hadi *et al*. (2001) on sweet melon (*Cucumis melo var. aegyptiacus*) noticed a decrease in ranges from S3 to S5 generation for yield per plant.

4.2.1.15 Number of harvests

Number of harvests from F_2 to F_3 was increased in MC-138 (5.54 to 6.04) and decreased in MC-136 (12.12 to 8) and MC- 133 (8.31 to 7.09). Significant difference was not found among segregating generations of MC-142 and MC-139. Variance was significantly decreased from F_2 to F_3 in MC-142 (6.36 to 2.66) and MC-133 (3.96 to 1.34). Non significant difference was observed among F_2 and F_3 of MC-136, MC-139 and MC-138. The range was decreased from F_2 to F_3 in hybrids, MC-142, MC-136 and MC-133.

4.2.1.16 Number of seeds per fruit

Number of seeds per fruit from F_2 to F_3 was reduced significantly (21.60 to 16.48) in MC-142 and increased (21.93 to 24.66) in MC-138. Significant difference was not found in segregating generations of other hybrids. Variance became non significant among F_2 and F_3 generations of all hybrids. The range was decreased from F_2 to F_3 in hybrids, MC-136, MC-139 and MC-133.

4.2.1.17 1000 seed weight

Thousand seed weight increased significantly from F_2 to F_3 (145.58 to 162.96 g) in MC-138 and recorded non significant difference in other hybrids. Variance was significantly decreased from F_2 to F_3 in MC-142 (551.12 to 204.92) and MC-133 (775.40 to 308.52). Non significant variance was observed for other hybrids. The range became narrow from F_2 to F_3 of all hybrids.

4.2.1.18 Incidence of pest and diseases

Incidence of pest and diseases in F_1, F_2 and F_3 generations of bitter gourd genotypes are given in Annexure 1.

4.2.2 Ranking of genotypes in F_2 and F_3 generations based on cumulative index

Ranking of genotypes in F_2 and F_3 generations were done based on cumulative index worked out for characters like, nodes to 1st female flower appearance, days to 1st picking, fruit weight (g), fruit length (cm), fruit diameter (cm), relative early yield (kg), yield per plant (kg) and number of fruits per plant (Table 9 to table 18). It helps to make an effective selection for superior genotypes (Plate 6, 7, 8, 9 and 10). Selection was carried out in F_2 and the subsequent generation (F_3) for desirable characters.

Ram *et al*. (2006) stated that emphasis was given for the number of fruits per plant and average fruit weight in selecting high yielding genotypes in bitter gourd.

Varghese (2003) reported that the number of fruits per plant and average fruit weight were the important characters based on which selection index was prepared in ivy gourd. Yield, earliness parameters, fruit quality, sex ratio and mosaic resistance were taken as important characters to work out selection index in 25 ash gourd landraces (Resmi, 2004). Vine length, number of branches per plant, total number of harvests, fruit length, fruit girth and number of fruits per plant in spine gourd was taken to determine selection index (Archana, 2013).

Table 9. Prominent ten genotypes in F_2 population of the hybrid MC-142

Sl. No.	Genotype	Cumulative index	Rank
1	MC-142-5	44.50	1
2	MC-142-2	50.50	2
3	MC-142-19	51.50	3
4	MC-142-4	54.00	4
5	MC-142-14	67.00	5
6	MC-142-9	69.00	6
7	MC-142-12	70.50	7
8	MC-142-10	71.00	8
9	MC-142-17	72.00	9
10	MC-142-13	73.00	10

Table 10. Prominent ten genotypes in F_3 population of the hybrid MC-142

Sl. No.	Genotype	Cumulative index	Rank
1	MC-142-19-9	107.00	1
2	MC-142-5-16	112.50	2
3	MC-142-5-11	127.00	3
4	MC-142-5-9	144.00	4
5	MC-142-5-3	145.50	5
6	MC-142-19-13	160.50	6
7	MC-142-5-31	165.00	7
8	MC-142-5-5	172.00	8
9	MC-142-2-8	173.00	9
10	MC-142-5-22	174.00	10

MC-142-5

MC-142-2

MC-142-19

(A) Selected genotypes in F_2 generation of MC-142

MC-142-5-11

(B) Promising genotype in F_3 generation of MC-142

Plate 6. Superior genotypes in F_2 and F_3 generations of MC-142
(A) Selected genotypes in F_2 generation of MC-142, (B) Promising genotype in F_3 generation of MC-142

Table 11. Prominent ten genotypes in F_2 population of the hybrid MC-136

Sl. No.	Genotype	Cumulative index	Rank
1	MC-136-10	34.50	1
2	MC-136 -6	44.00	2
3	MC-136 -1	48.00	3
4	MC-136 -2	50.00	4
5	MC-136-7	57.00	5
6	MC-136-11	61.00	6
7	MC-136-14	63.00	7
8	MC-136-4	70.00	8
9	MC-136-13	71.50	9
10	MC-136-3	78.00	10

Table 12. Prominent ten genotypes in F_3 population of the hybrid MC-136

Sl. No.	Genotype	Cumulative index	Rank
1	MC136-10-25	31.00	1
2	MC136-10-22	72.00	2
3	MC136-10-12	78.50	3
4	MC136-10-21	81.00	4
5	MC136-6-5	90.50	5
6	MC136-10-7	91.50	6
7	MC136-10-3	92.50	7
8	MC136-10-28	137.00	8
9	MC136-10-9	138.50	9
10	MC136-10-23	143.00	10

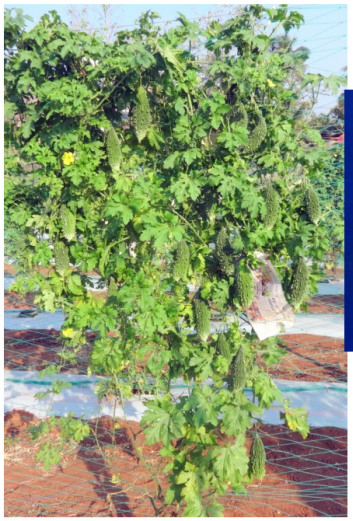
Table 13. Prominent ten genotypes in F_2 population of the hybrid MC-139

Sl. No.	Genotype	Cumulative index	Rank
1	MC-139-2	56.00	1
2	MC-139-7	59.50	2
3	MC-139-1	61.50	3
4	MC-139-13	62.50	4
5	MC-139-17	63.00	5
6	MC-139-4	63.50	6
7	MC-139-15	66.50	7
8	MC-139-8	69.00	8
9	MC-139-22	81.50	9
10	MC-139-3	95.50	10

MC-136-10

MC-136-6

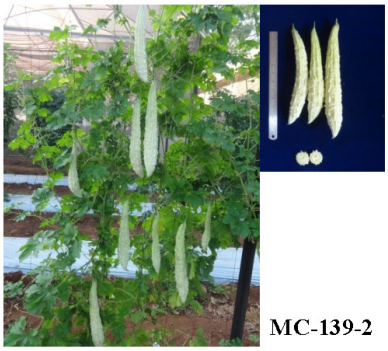
(A) Selected genotypes in F_2 generation of MC-136



MC-136-6-5

(B) Promising genotype in F_3 generation of MC-136

Plate 7. Superior genotypes in F_2 and F_3 generations of MC-136
(A) Selected genotypes in F_2 generation of MC-136, (B) Promising genotype in F_3 generation of MC-136



MC-139-2

MC-139-7

(A) Selected genotypes in F_2 generation of MC-139

MC-139-7-12

(B) Promising genotype in F_3 generation of MC-139

Plate 8. Superior genotypes in F_2 and F_3 generations of MC-139
(A) Selected genotypes in F_2 generation of MC-139, (B) Promising genotype in F_3 generation of MC-139

Table 14. Prominent ten genotypes in F_3 population of the hybrid MC-139

Sl. No.	Genotype	Cumulative index	Rank
1	MC-139- 7-12	69.50	1
2	MC-139-2-10	75.00	2
3	MC-139-7-17	82.50	3
4	MC-139 -7-4	93.00	4
5	MC-139 -2-6	94.00	5
6	MC-139 -7-5	95.00	6
7	MC-139 -7-9	98.00	7
8	MC-139 -2-13	98.50	8
9	MC-139 -7-18	108.50	9
10	MC-139 -2-11	110.00	10

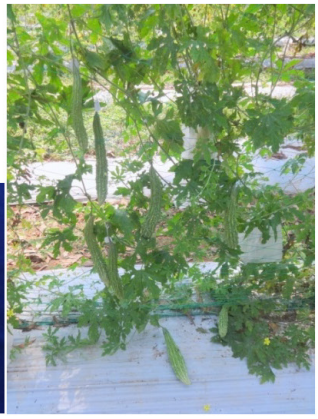
Table 15. Prominent ten genotypes in F_2 population of the hybrid MC-138

Sl. No.	Genotype	Cumulative index	Rank
1	MC-138-15	56.00	1
2	MC-138-25	58.50	2
3	MC-138-17	62.00	3
4	MC-138-9	65.00	4
5	MC-138-8	75.00	5
6	MC-138-19	94.00	6
7	MC-138-14	95.00	7
8	MC-138-18	97.00	8
9	MC-138-20	98.50	9
10	MC-138-13	103.00	10

Table16. Prominent ten genotypes in F_3 population of the hybrid MC-138

Sl. No.	Genotype	Cumulative index	Rank
1	MC-138- 25-3	67.00	1
2	MC-138- 25-18	83.50	2
3	MC-138- 25-13	90.00	3
4	MC-138- 25-15	102.50	4
5	MC-138- 25-7	109.00	5
6	MC-138- 15-16	118.00	6
7	MC-138- 25-4	120.50	7
8	MC-138- 25-25	131.00	8
9	MC-138- 25-17	136.50	9
10	MC-138- 25-9	146.50	10

MC-138-15



MC-138-25

(A) Selected genotypes in F_2 generation of MC-138

MC-138-25-3

(B) Promising genotype in F_3 generation of MC-138

Plate 9. Superior genotypes in F_2 and F_3 generations of MC-138
(A) Selected genotypes in F_2 generation of MC-138, (B) Promising genotype in F_3 generation of MC-138

Table 17. Prominent ten genotypes in F_2 population of the hybrid MC-133

Sl. No.	Genotype	Cumulative index	Rank
1	MC-133-16	61.00	1
2	MC-133-8	81.50	2
3	MC-133-17	82.00	3
4	MC-133-13	83.50	4
5	MC-133-22	87.50	5
6	MC-133-10	91.50	6
7	$MC\text{-}133_2$-26	96.00	7
8	$MC\text{-}133_2$ -24	100.50	8
9	MC-133-18	104.00	9
10	MC-133-27	106.50	10

Table18. Prominent ten genotypes in F_3 population of the hybrid MC-133

Sl. No.	Genotype	Cumulative index	Rank
1	MC-133-16-10	53.50	1
2	MC-133 -16-7	66.50	2
3	MC-133 -16-20	68.00	3
4	MC-133 -16-6	70.50	4
5	MC-133 -16-17	74.50	5
6	MC-133 -16-26	77.00	6
7	MC-133 -16-11	80.00	7
8	MC-133 -16-19	81.00	8
9	MC-133 -16-15	95.00	9
10	MC-133 -16-18	97.00	10

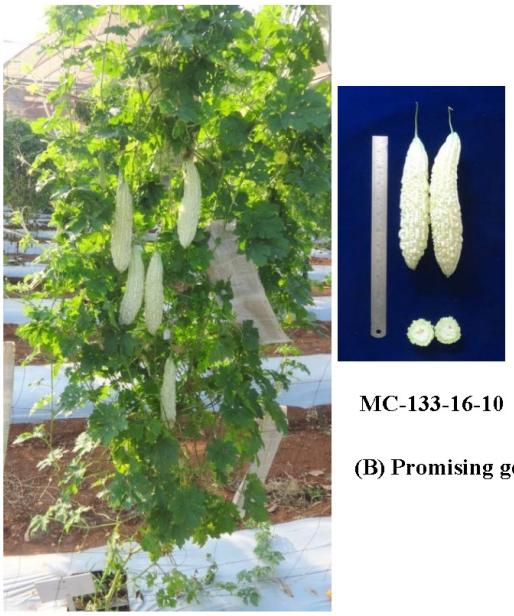
MC-142-5, MC-142-2 and MC-142-19 were observed as the superior F_2 genotypes of the hybrid MC-142. Top five selected F_3 genotypes of the hybrid were MC-142-19-9, MC-142-5-16, MC-142-5-11, MC-142-5-9 and MC-142-5-3.

Cumulative index worked out in F_2 population of the hybrid MC-136 recorded MC-136-10 and MC-136-6 as the promising genotypes. Promising genotypes registered in F_3 generation were MC136-10-25, MC136-10-22, MC136-10-12, MC136-10-21 and MC136-6-5.

MC-133-16

MC-133-8

(A) Selected genotypes in F_2 generation of MC-133



MC-133-16-10

(B) Promising genotype in F_3 generation of MC-133

Plate 10. Superior genotypes in F_2 and F_3 generations of MC-133
(A) Selected genotypes in F_2 generation of MC-133, (B) Promising genotype in F_3 generation of MC-133

MC-139-2 and MC-139-7 were identified and selected as the promising F_2 genotypes of the hybrid MC-139. Top five selected F_3 genotypes of the hybrid were MC-139-7-12, MC-139- 2-10, MC-139- 7-17, MC-139-7-4 and MC-139 -2-6.

Based on cumulative index, MC-138-15 and MC-138-25 were found to be superior genotypes among the F_2 population of the hybrid MC-138. They were selfed to produce F_3 families. Promising genotypes selected in F_3 generation were MC-138-25-3, MC-138-25-18, MC-138- 25-13, MC-138-25-15 and MC-138-25-7.

Comparison of different genotypes in F_2 population of the hybrid MC-133 revealed the superiority of MC-133-16 followed by MC-133-8. MC-133-16-10, MC-133-16-7, MC-133-16-20, MC-133-16-6 and MC-133-16-17 were the promising genotypes in F_3 generation raised from the selfed fruits of selected plants in F_2.

4.2.3 Response to selection and selection differential

Response to selection measures the change in the population mean on account of selection. It has an important role in determining the success of the breeding programme. Increase in mean results in the positive value of selection response in next generation. The selection differential is the difference between the mean of the selected plants and the mean of the population from which they were selected.

Hayes and Immer (1942) reported that self pollination of squash caused an improvement and new lines with desirable characteristics were obtained. Inbreeding with selection was efficient in recovering desirable lines from pumpkin (Damarany, 1989).

Selections were made in F_2 generation to raise F_3 and were evaluated for selection response and selection differential for the following characters (Table 19).

4.2.3.1 Days to first male flower anthesis

Selection differential was negative only in F_2 generation of MC-138. Selection differential was negative for all the F_3 generations of hybrids except in F_3-MC-133.

Response to selection for days to first male flower anthesis was negative in F_3generation of MC-136, MC-138 and MC-133 indicating an improvement in earliness in this generation through selection in F_2 generation.

4.2.3.2 Nodes to first male flower

A negative value of selection differential for nodes to first female flower in F_2 generation of MC-142 and MC-136 and F_3 generation of MC-139 was observed. A negative value for response to selection in F_3 generations of all hybrids shows the appearance of male flower at early nodes.

4.2.3.3 Days to first female flower anthesis

Negative selection differential was observed for days to first female flower anthesis in F_2 generations of MC-138 and MC-133 and F_3 generation of all hybrids. Response to selection in negative direction in F_3 generation of all hybrids indicates the effectiveness of the selection for this trait.

4.2.3.4 Nodes to first female flower

Selection differential for nodes to first female flower was negative in F_2 generation of MC-136 and MC-133 and F_3 generation of MC-142, MC-139 and MC-138. A negative value for response to selection in F_3 generations of all hybrids shows the appearance of female flower at early nodes.

4.2.3.5 Days to first harvest

F_2 generation of MC-138 and MC-133 and F_3 generation of all hybrids exhibited a negative value of days to first harvest for selection differential. Response to selection also showed negative value for the trait in F_3 generations of all the hybrids. This clearly explains the improvement in earliness in F_3 generation for days to first harvest through selection.

Table 19. Response to selection and selection differential

Sl. No.	Characters		Response to selection	Selection differential	
				F2	F3
1	Days to first male flower anthesis	MC-142	1.12	1.04	-1.15
		MC-136	-0.88	0.77	-0.55
		MC-139	1.09	1.75	-2.64
		MC-138	-3.24	-2.68	-1.94
		MC-133	-1.53	0.47	0.40
2	Nodes to first male flower	MC-142	-1.45	-0.60	0.58
		MC-136	-1.77	-0.54	0.03
		MC-139	-3.17	0.25	-2.18
		MC-138	-1.35	0.54	0.98
		MC-133	-2.38	2.09	1.17
3	Days to first female flower anthesis	MC-142	-0.28	3.16	-4.37
		MC-136	-1.03	0.38	-2.29
		MC-139	-1.44	6.08	-2.87
		MC-138	-4.69	-3.82	-4.53
		MC-133	-4.16	-0.22	-1.46
4	Nodes to first female flower	MC-142	-2.98	2.47	-1.35
		MC-136	-6.69	-0.03	0.36
		MC-139	-1.55	0.38	-4.08
		MC-138	-3.00	2.43	-1.77
		MC-133	-0.96	-1.59	0.56
5	Days to first harvest	MC-142	-2.63	3.37	-3.80
		MC-136	-2.09	1.29	-1.61
		MC-139	-3.82	8.00	-2.28
		MC-138	-5.85	-5.79	-4.34
		MC-133	-6.67	-1.69	-1.92
6	Relative early yield	MC-142	0.16	0.98	0.59
		MC-136	-0.56	1.13	1.30
		MC-139	0.20	0.96	1.01
		MC-138	0.38	2.09	0.75
		MC-133	-0.09	0.79	0.72
7	Number of days from anthesis to fruit maturity	MC-142	-0.28	-0.67	0.78
		MC-136	0.24	0.24	0.40
		MC-139	0.64	1.42	0.08
		MC-138	0.50	-0.81	-0.21
		MC-133	-1.25	0.00	0.30
8	Average fruit weight	MC-142	16.64	40.66	14.62
		MC-136	-6.57	18.22	41.15
		MC-139	1.36	1.45	1.99
		MC-138	40.04	50.22	35.31
		MC-133	-1.17	17.23	43.26
9	Fruit length	MC-142	1.62	3.34	0.71
		MC-136	-2.54	0.24	3.07
		MC-139	1.36	1.45	1.99
		MC-138	4.14	5.19	4.15
		MC-133	-3.20	1.62	4.04

10	Fruit diameter	MC-142	0.26	0.32	0.24
		MC-136	0.08	0.64	0.44
		MC-139	0.66	0.73	0.34
		MC-138	0.46	0.70	0.24
		MC-133	0.02	0.08	0.65
11	Fruit girth	MC-142	0.40	0.73	0.77
		MC-136	0.16	2.20	0.71
		MC-139	2.11	1.75	1.27
		MC-138	2.05	2.12	0.42
		MC-133	-0.39	0.00	2.14
12	Flesh thickness	MC-142	0.11	0.12	0.06
		MC-136	-0.01	0.06	0.07
		MC-139	0.09	0.11	0.02
		MC-138	0.08	0.10	0.05
		MC-133	0.05	0.04	0.11
13	Number of fruits per plant	MC-142	2.71	12.23	8.58
		MC-136	-10.72	19.03	16.05
		MC-139	4.13	16.63	11.90
		MC-138	2.38	8.43	4.35
		MC-133	1.45	15.00	4.55
14	Yield per plant	MC-142	0.44	2.18	1.12
		MC-136	-1.33	2.40	3.11
		MC-139	0.68	2.38	2.19
		MC-138	1.12	2.37	1.85
		MC-133	-0.16	1.88	1.66
15	Number of harvests	MC-142	-0.25	3.93	0.92
		MC-136	-4.12	3.88	2.60
		MC-139	-0.02	2.46	1.87
		MC-138	0.51	1.46	0.56
		MC-133	-1.22	3.69	1.51

4.2.3.6 Relative early yield

Selection differential for relative early yield in F_2 and F_3 generations of all hybrids were positive. Response to selection was positive in F_3 generation of hybrids MC-142, MC-139 and MC-138 indicating the effectiveness of selection for relative early yield.

4.2.3.7 Number of days from anthesis to fruit maturity

A negative value of selection differential for number of days from anthesis to fruit maturity was shown in F_2 generation of MC-142 and F_2 and F_3 generations of

MC-138. A negative value of response to selection in F_3 generation of MC-142 and MC-133 was observed. This indicates the requirement of less number of days from anthesis to fruit maturity in these generations of hybrids as a result of effective selection.

4.2.3.8 Average fruit weight

Selection differential for average fruit weight was positive in F_2 and F_3 generations of all hybrids. Estimated response to selection in F_3 generation was positive for MC-142, MC-139 and MC-138. An increase in change in mean from the previous generation to next generation indicates the effectiveness of the selection for this trait. Chhonkar *et al*. (1979) indicated that selection in muskmelon could be effective for fruit weight. Similar findings were observed in selection response in recombinant lines obtained from an interspecific cross between of *Lycopersicon* species where the response to selection for the fruit weight was positive for the first group I and negative for group 2 and group 3 (Rodriguez and Patta, 2006).

4.2.3.9 Fruit length

Fruit length of all hybrids showed positive value for selection differential in F_2 and F_3 generations. Judicious selection for fruit length led to the improvement of fruit length in F_3 generations of MC-142, MC-139 and MC-138 where the response to selection gave positive value. This is in accordance with the finding of Behera *et al*. (2010b) who reported a positive selection response in cucumber for long fruited type after one cycle of marker assisted selection from F_3 to F_4.

4.2.3.10 Fruit diameter

Selection differential for fruit diameter was positive in F_2 and F_3 generations of all hybrids. Response to selection was also positive for fruit diameter in F_3 generation all hybrids. Selection increased the population genotypic mean for fruit diameter.

4.2.3.11 Fruit girth

Selection differential for fruit girth was positive in F_2 and F_3 generations of all hybrids. While proceeding from F_2 to F_3 generation, response to selection was found positive for fruit diameter in F_3 generation of all hybrids except MC-133.

4.2.3.12 Flesh thickness

Selection differential for flesh thickness showed positive in F_2 and F_3 generations of all hybrids. Estimated response to selection in F_3 generation was positive for MC-142, MC-139, MC-138 and MC-133. An increase in change in flesh thickness from F_2 to F_3 generation indicates the effectiveness of the selection for this character.

4.2.3.13 Number of fruits per plant

Number of fruits per plant showed positive value of selection differential in F_2 and F_3 generations. Similar reports were observed in a study conducted in F_2 segregating population of rice where the selection differential was positive for number of productive tillers per plant (Govintharaj *et al*., 2017). Response to selection was positive in F_3 of all hybrids except in MC-136. Selection based on genotypic values may lead to an increase in mean of selected population in next generation resulting in the positive value of response to selection. Abd El-Rahman (2005) stated that inbreeding and selection was very efficient to give desirable new lines of gurma melon for number of fruits per plant. Similar observations were reported in the bred population of cucumber where number of fruits per plant was improved in comparison to the average of the F_3 population (Kalvandi *et al.,* 2016).

4.2.3.14 Yield per plant

Positive selection differential for yield per plant was noticed in F_2 and F_3 generations of all hybrids. This is in agreement with previous findings. According to Jayaprakash *et al*. (2017) selection differential for mean yield in F_3 generation of rice

was 4.64g for Suraksha x Jalanidhi, 4.81g for Suraksha x Phalguna, 4.70g for Suraksha x Mahamaya and 5.37g for Suraksha x RP Bio-226. Study conducted in an F_2 segregating population of rice showed that selection differential was positive for single plant yield (Govintharaj *et al*., 2017). Yield per plant showed positive response to selection from F_2 generation to F_3 generation in MC-142, MC-139 and MC-138. The result indicated a positive improvement in F_3 mean for yield per plant in these hybrids through selection in F_2 generation. Abd El-Rahman (2005) stated that inbreeding and selection was very efficient to give desirable new lines of gurma melon for yield per plant. Behera *et al*. (2010b) reported a positive selection response in cucumber for yield after one cycle of marker assisted selection from F_3 to F_4.

4.2.3.15 Number of harvests

Selection differential was positive for number of harvests in F_2 and F_3 generations of all hybrids. F_3 generation of MC-138 only showed a positive response to selection for number of harvests.

4.2.4 Inbreeding depression

Inbreeding is a cumulative phenomenon and in the course of successive generations, it increases homozygosity by 50 per cent (Chattopadhyay, 2016). In the present study, the performance of subsequent generations of five hybrids in bitter gourd for inbreeding depression for various characters was estimated. The character wise results and discussion are highlighted here as under.

4.2.4.1 Inbreeding depression in F_2 and F_3 generations and cumulative inbreeding depression (Table 20)

4.2.4.1.1 Days to first male flower anthesis

Significant negative inbreeding depression in F_2 generation (-13.72 %) and cumulative inbreeding depression (-16.40 %) was observed in the hybrid MC-142.

Table 20: Estimation of inbreeding depression in bitter gourd

Characters	Hybrid	Inbreeding depression (%)		Cumulative inbreeding depression (%)
		F_2	F_3	
Days to first male flower anthesis	MC-142	-13.72*	-2.69	-16.40**
	MC-136	-6.80	1.87	-4.94
	MC-139	5.58	-2.50	3.08
	MC-138	-12.92	6.72	-6.20
	MC-133	2.54	3.52	6.06
Nodes to first male flower	MC-142	-13.54**	17.51**	3.97*
	MC-136	-1.43	12.18**	10.75**
	MC-139	-21.23**	21.50**	0.27
	MC-138	12.63**	10.34**	22.97**
	MC-133	3.74	19.15**	22.90**
Days to first female flower anthesis	MC-142	-21.14**	0.59	-20.56**
	MC-136	-12.94*	2.23	-10.71*
	MC-139	-3.29	3.01	-0.28
	MC-138	-17.28*	8.71	-8.57
	MC-133	-16.70*	8.12	-8.58
Nodes to first female flower	MC-142	-13.11**	18.01**	4.90
	MC-136	-18.06**	32.58**	14.52**
	MC-139	6.30*	7.90*	14.20**
	MC-138	3.66	13.91**	17.57**
	MC-133	-16.38**	4.87	-11.51*
Days to first harvest	MC-142	-18.74**	4.41	-14.32**
	MC-136	-9.17	3.69	-5.48
	MC-139	-3.46	6.20	2.75
	MC-138	-16.27	8.76	-7.51
	MC-133	-12.83*	10.56	-2.28
Relative early yield	MC-142	64.08**	-18.37**	45.71**
	MC-136	37.59**	39.08**	76.67**
	MC-139	59.67**	-24.36**	35.31**
	MC-138	68.24**	-39.80**	28.45**
	MC-133	65.45**	11.78**	77.23**
Number of days from anthesis to fruit maturity	MC-142	3.14*	2.34	5.48**
	MC-136	8.33**	-2.03	6.30**
	MC-139	12.40**	-5.53**	6.87**
	MC-138	6.90**	-4.06**	2.84**
	MC-133	5.97**	10.66**	16.63**
Average fruit weight	MC-142	44.97*	-19.70	25.27
	MC-136	12.35	6.57	18.92
	MC-139	52.32	-38.23	14.09
	MC-138	47.41	-26.72	20.68
	MC-133	40.89	1.22	42.12
Fruit length	MC-142	25.06**	-8.41*	16.65**
	MC-136	21.36**	15.02**	36.38**
	MC-139	29.22**	-6.57	22.65**
	MC-138	30.42**	-19.14**	11.28*
	MC-133	29.11**	16.03**	45.14**

Fruit diameter	MC-142	15.74**	-7.10**	8.64**
	MC-136	2.55**	-1.94**	0.61
	MC-139	22.73**	-18.84**	3.89**
	MC-138	19.94**	-10.66**	9.28**
	MC-133	14.68**	-0.41	14.27**
Fruit girth	MC-142	17.65**	-3.40	14.25**
	MC-136	-4.39**	-1.15	-5.54**
	MC-139	22.57**	-18.67**	3.90*
	MC-138	18.09**	-14.61**	3.47*
	MC-133	11.04**	3.18	14.23**
Flesh thickness	MC-142	36.34**	-21.64**	14.70**
	MC-136	17.24**	2.01**	19.25**
	MC-139	30.88**	-23.91**	6.97**
	MC-138	34.44**	-12.73**	21.71**
	MC-133	30.29**	-9.56**	20.73**
Number of fruits per plant	MC-142	36.07**	-8.45	27.63**
	MC-136	38.52*	24.11*	62.63**
	MC-139	43.53**	-18.46	25.06**
	MC-138	35.62**	-10.27	25.35**
	MC-133	41.78**	-6.06	35.72**
Yield per plant	MC-142	66.77**	-18.55**	48.22**
	MC-136	48.79**	36.08**	84.87**
	MC-139	69.25**	-32.84**	36.40**
	MC-138	68.70**	-40.22**	28.48**
	MC-133	65.71**	7.17**	72.88**
Number of harvests	MC-142	6.94**	2.90	9.85**
	MC-136	-10.72**	33.98**	23.26**
	MC-139	17.22**	0.20	17.42**
	MC-138	35.25**	-9.03**	26.22**
	MC-133	14.01**	14.70**	28.70**

*, ** Significant at 5 % and 1% levels, respectively

Generation F_3 of MC-142 exhibited non significant value for this trait. Inbreeding depression in F_2 and F_3 generations and cumulative inbreeding depression was non significant for other hybrids. Significant negative inbreeding depression in F_2 generation for days to first male flower anthesis in some of the crosses of bitter gourd was previously reported (Kumar, 2018). According to Singh *et al* (2015) days to first male flower was significant and negative for F_2 generations of most of the crosses in cucumber.

4.2.4.1.2 Nodes to first male flower

Generation F_2 showed significant positive inbreeding depression for nodes to first male flower in MC-138 (12.63%) and significant negative inbreeding depression in MC-142 (-13.54) and MC-139 (21.23). Generation F_3 of all hybrids recorded highly significant positive inbreeding depression (MC-142 (17.51%), MC-136 (12.18%), MC-139 (21.50%), MC-138 (10.34%) and MC-133 (19.15%)) for this trait. Cumulative inbreeding depression was also registered as a significant positive value for all hybrids except MC-139 (MC-142 (3.97%), MC-136 (10.75%), MC-138 (22.97%) and MC-133 (22.90%). Significant positive inbreeding depression observed for this trait is in a desirable direction. Cumulative inbreeding depression was non significant in MC-139. Kumar (2018) remarked that some of the crosses in F_2 generation showed significant negative inbreeding depression for node number to first male flower in bitter gourd. Significant negative inbreeding depression in F_2 of most of the crosses for node of first male flower is reported in cucumber (Singh *et al*., 2015).

4.2.4.1.3 Days to first female flower anthesis

Significant negative inbreeding depression in F_2 generation was observed in MC-142 (-21.14%), MC-136 (-12.94%), MC-138 (-17.28%) and MC-133 (-16.70%) for days to first female flower anthesis. This is in accordance with the findings of Anant (2018) who remarked that significant negative inbreeding depression was noticed in all crosses of bitter gourd in F_2 generation for days required to first female flower and varied from -9.90 (Hirkani x Konkan Tara) to -12.87 (Arka Harit X CO White Long). Whereas, positive significant inbreeding depression for days to first female flower was reported in some of the F_2 generations of cucumber which varied from 7.28 to 7.55 (Singh *et al*., 2015). In the present study, inbreeding depression was found nonsignificant in all generations of MC-139. Generation F_3 of all hybrids of MC-139, MC-138 and MC-133 exhibited non significant inbreeding depression for

this trait. Similar results were previously reported by Rani *et al*. (2015) in F_2 generation of the cross IC-044438× IC-045339 in bitter gourd. Significant negative cumulative inbreeding depression was observed in MC-142 (-20.56) and MC-136 (-10.71).

4.2.4.1.4 Nodes to first female flower

Significant negative inbreeding depression for node to first female flower was observed in F_2 generation of MC-142 (-13.11%), MC-136 (-18.06%) and MC-133 (-16.38%). F_2 generation of MC-139 (6.30%) and MC-138 showed positive significant and non significant inbreeding depression respectively. Similar findings were previously reported by Kumar (2018) and Rani *et al*. (2015) who remarked that some of the crosses in F_2 generation showed significant negative inbreeding depression for node number to first female flower in bitter gourd. Significant positive inbreeding depression in F_3 generation was observed in MC-142 (18.01%), MC-136(32.58 %), MC-139 (7.90%) and MC-138 (13.91%) and inbreeding depression became non significant in F_3 generation of MC-133. Positive significant cumulative inbreeding depression was noticed in MC-136(14.52%), MC-139 (14.20) and MC-138 (17.57%). Positive significant inbreeding depression for nodes to first female flower indicates that the trait is in a desirable direction giving female flower at earlier nodes. Significant negative and non significant cumulative inbreeding depression were observed in MC-133 (-11.51%) and MC-142 respectively.

4.2.4.1.5 Days to first harvest

Generation F_2 showed significant negative inbreeding depression for days to first harvest in MC-142 (-18.74%) and MC-133 (-12.83%) and non significant values in other hybrids. Similar observations could find in earlier reports. Anant (2018) noticed negatively significant inbreeding depression for days to first harvest in F_2 generation of all crosses in bitter gourd. In the present study, inbreeding depression was absent in F_3 generations of all hybrids. Cumulative inbreeding depression was

significantly negative only in MC-142 (-14.32%). And all other hybrids showed no cumulative inbreeding depression. Thakarshi, (2006) reported similar findings in sponge gourd where both positive and negative significant inbreeding depression for days to first harvest was noticed and the cross JSGL – 71 x JSGL – 46 exhibited no inbreeding depression. Oviedo *et al*. (2008) reported that there was no difference between hybrid and populations at different inbreeding levels (F_2-S_5 progenies) for days to first harvest in cucumber.

4.2.4.1.6 Relative early yield

Significant positive inbreeding depression for relative early yield was observed in F_2 generation of all hybrids, which varied from 37.59 (MC-136) to 68.24% (MC-138). Inbreeding depression in F_3 generation was positively significant in MC-136 (39.08%) and MC-133 (11.78%) and negatively significant in MC-142 (-18.37), MC-139 (-24.36%) and MC-138 (-39.80%). Negative value of inbreeding depression in the F_3 generation indicates an increase in *per se* performance of F_3 over its F_2 for relative early yield. Significant positive cumulative inbreeding depression was observed for all hybrids, which varied from 28.45 (MC-138) to 77.23% (MC-133). This shows a decrease in relative early yield in F_3 over its F_1 generation. In earlier reports, inbreeding depression was not observed for early yield in cucumber in most of the crosses. Only 'Addis' × 'Wis. SMR 18' exhibited inbreeding depression (Cramer and Wehner, 1999).

4.2.4.1.7 Number of days from anthesis to fruit maturity

Inbreeding depression in F_2 generation of all hybrids was positively significant and varied from 3.14 (MC-142) to 12.40% (MC-139). Inbreeding depression in F_3 generation was positively significant in MC-133 (10.66%), negatively significant in MC-139 (-5.53%) and MC-138 (-4.06%) and absent in MC-142 and MC-136. Cumulative inbreeding depression was significantly positive for all hybrids and

varied from 2.84 (MC-138) to 16.63 % (MC-133). Improvement in the number of days from anthesis to fruit maturity is in a desirable direction.

4.2.4.1.8 Average fruit weight

Inbreeding depression for average fruit weight was non significant in F_2 generation of all hybrids except in MC-142 where a significant positive value (44.97%) was observed. Inbreeding depression in F_3 generation and cumulative inbreeding depression were non significant for all hybrids. This is in agreement with findings of Cramer and Wehner, (1999) who observed no inbreeding depression for fruit weight in cucumber in most of the crosses., Only 'Addis' × 'Wis. SMR 18' exhibited inbreeding depression. Loss in vigor for fruit weight of muskmelon did not always occur after inbreeding for four to seven generations (Scott, 1933). Oviedo *et al*. (2008) reported that there was no difference between hybrid and populations at different inbreeding levels (F_2-S_5 progenies) for the average weight of commercial fruits in cucumber.

Although many researchers confirmed reduced inbreeding depression for fruit weight, some have reported inbreeding depression in *Cucurbita pepo* and *Cucurbita maxima* after three generations of self pollination (Chekalina, 1976). Cardoso (2004) reported a reduction in fruit weight after four generations of self pollination in *Cucurbita moschata*. Abd El-Rahman *et al*. (2005) observed that commercial cultivar had the highest values of fruit weight in comparison with the inbred lines developed in S6 generation of gurma melon (*Citrullus colocynthoides*).

4.2.4.1.9 Fruit length

Significant positive inbreeding depression was observed in F_2 generation of all hybrids, which varied from 21.36 (MC-136) to 30.42% (MC-138). It clearly indicates a reduction in fruit length in F_2 over F_1 generation. Similar results were noticed in earlier reports for fruit length in bitter gourd (Hiranand, 2012; Kumar, 2018; Anant, 2018). F_3 generation of MC-136 (15.02%) and MC-133 (16.03%) recorded positive

significant inbreeding depression. It was negatively significant in MC-142 (16.03%) and MC-138 (-19.14%) and non significant in MC-139. Cumulative inbreeding depression was significantly positive for all hybrids and varied from 11.28 (MC-138) to 45.14% (MC-133) and noticed a decrease in fruit length in the F_3 generation over its F_1. It is in accordance with findings of Cardoso (2004) who reported a reduction in fruit length after four generations of self pollination in *Cucurbita moschata*.

4.2.4.1.10 Fruit diameter

Inbreeding depression for fruit diameter was significantly positive in F_2 generation of all hybrids and varied from 2.55 (MC-136) to 22.73 % (MC-139). A reduction in diameter in F_2 population over its F_1 was noticed due to this inbreeding depression. Similar results were reported by Rani (2012) and (Anant, 2018) in bitter gourd F_2 population. Negatively significant inbreeding depression in F3 generation was observed in MC-142 (-7.10%), MC-136 (-1.94%), MC-139 (-18.84%) and MC-138 (-10.66%). It indicates an increase in fruit diameter in F_3 generation over its F_2. Non significant inbreeding depression in F_3 generation was observed in MC-133. Positive significant cumulative inbreeding depression in MC-142 (8.64%), MC-139 (3.89%), MC-138 (9.28%) and MC-133 (14.27%) clearly indicate a reduction in fruit diameter in the F_3 generation over its F_1. Cumulative inbreeding depression was a non significant value for MC-136.

4.2.4.1.11 Fruit girth

Fruit girth in F_2 generation of all hybrids except MC-136 exhibited significant positive inbreeding depression ranged from 11.04 (MC-133) to 22.57% (MC-139). Negatively significant inbreeding depression was observed in MC-136 (-4.39%). Significant negative inbreeding depression in F_3 was noticed in MC-139 (-18.67%) and MC-138(-14.61%) and other hybrids showed non significant value. All hybrids except MC-136 showed significant positive cumulative inbreeding depression varied

from 3.47 (MC-138) to 14.25% (MC-142%). Negatively significant cumulative inbreeding depression was recorded in MC-136 (-5.54%).

4.2.4.1.12 Flesh thickness

Inbreeding depression for flesh thickness was significantly positive in F_2 generation of all hybrids and varied from 17.24 (MC-136) to 36.34% (MC-142). Similar results were previously reported by Chandrakumar (2006) in pumpkin. All hybrids except MC-136 showed significant negative inbreeding depression in F_3 ranged from -9.56 (MC-133) to -23.91% (MC-139). Positively significant inbreeding depression was observed in MC-136 (2.01%). Significant positive cumulative inbreeding depression recorded in all hybrids varied from 6.97 (MC-139) to 21.71% (MC-138). There is a reduction in flesh thickness in F_3 generation over its F_1.

4.2.4.1.13 Number of fruits per plant

Significant positive inbreeding depression for number of fruits per plant in F_2 generation was recorded by all hybrids which ranged from 35.62 (MC-138) to 43.53% (MC-139). Earlier reports of Kumar (2018) and Anant (2018) suggested positive significant inbreeding depression for this trait. Bhardwaj *et al*. (2009) observed high inbreeding depression for number of fruits per plant in cucumber. Significant positive inbreeding depression in F_3 was observed only in MC-136(24.11%) and others have shown non significant value for this trait. Cumulative inbreeding depression was positively significant in all hybrids and varied from 25.06 (MC-139) to 62.63 % (MC-136). There is a reduction in number of fruits per plant in F_3 generation over its F_1. According to Scott (1933), loss in vigor for fruit number of muskmelon did not always occur after inbreeding for four to seven generations. Oviedo *et al*. (2008) reported that there was no difference between hybrid and populations at different inbreeding levels (F_2-S_5 progenies) for number of commercial fruits in cucumber. Inbred lines developed in S6 generation of gurma melon

(*Citrullus colocynthoides*) were significantly increased in comparison with the commercial cultivar for number of fruits per plant (Abd El-Rahman *et al*., 2005).

4.2.4.1.14 Yield per plant

Yield per plant showed significant positive inbreeding depression in F_2 generation of all hybrids which ranged from 48.79 (MC-136) to 69.25% (MC-139). This is in accordance with results of Rani (2012), Hiranand, (2012), Kumar (2018) and Anant (2018) in bitter gourd. Positively significant inbreeding depression in F_3 generation was noticed in MC-136 (36.08%) and MC-133(7.17%). Significant negative inbreeding depression in F_3 generation was observed in MC-142 (-18.55%), MC-139 (-32.84%) and MC-138 (-40.22%). This clearly suggests an increase in yield per plant in F_3 generation over its F_2. It shows the potential of developing superior inbreds from commercial hybrids. Jenkins (1942) and Rubino and Wehner (1986) observed no inbreeding depression for yield in cucumber lines when self-pollinated for many generations. Inbreeding depression was not observed for yield in cucumber in most of the crosses (Cramer and Wehner, 1999). Inbred lines developed in S6 generation of gurma melon (*Citrullus colocynthoides*) were significantly increased in comparison with the commercial cultivar for yield per plant (Abd El-Rahman *et al*., 2005). In the present investigation cumulative inbreeding depression of all hybrids exhibited significant positive value which varied from 28.48 (MC-138) to 84.87% (MC-136). It indicated a reduction in yield per plant in F_3 generation over its F_1. But significant negative inbreeding depression in F_3 generation over its F_2 indicate that the selection programme was very efficient to obtain desirable F_3.

4.2.4.1.15 Number of harvests

Number of harvest in F_2 generation of all hybrids except MC-136 exhibited significant positive inbreeding depression which ranged from 6.94 (MC-142) to 35.25% (MC-138). Negatively significant inbreeding depression was observed in MC-136 (-10.72%). Similar findings were previously reported by Thakarshi, (2006)

in sponge gourd. MC-136 (33.98%) and MC-133 (14.70%) showed positively significant inbreeding depression in F_3 generation. MC-138 (-9.03%) showed negatively significant inbreeding which indicate an increase in number of harvests in F_3 generation compared to its F_2. Cumulative inbreeding depression of all hybrids exhibited significant positive value which varied from 9.85 (MC-142) to 28.70% (MC-133). It suggests a decrease in number of harvests in F_3 generation over its F_1.

4.2.4.2 Pair wise comparison of inbreeding depression

Pair wise comparison of inbreeding depression was worked out (Table 21 (a) to table 21(o)).

- All the diagonal elements are inbreeding depressions and off diagonal elements show significant difference among any pair of inbreeding depressions.
- '-'indicates no significant inbreeding depression / negative inbreeding depression for the characteristics other than earliness and positive inbreeding depression for the aforesaid parameters.
- Inbreeding depression of F_2 over F_1- regular font
- Inbreeding depression of F_3 over F_2- italic font
- Cumulative inbreeding depression-bold font

Table 21(a): Pair wise comparison of inbreeding depression of days to first male flower

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- - -	- - -	- - -	- - -	- - -
MC-139		- - -	- - -	- - -	- - -
MC-142			-13.72 - **-16.4**	- - -	- - -
MC-133				- - -	- - -
MC-136					- - -

Table 21 (b): Pair wise comparison of inbreeding depression of nodes to first male flower

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- - -	- - -	- - -	- - -	- - -
MC-139		-21.23 - -	4.28** - -	- - -	- - -
MC-142			-13.54 - -	- - -	- - -
MC-133				- - -	- - -
MC-136					- - -

Table 21 (c): Pair wise comparison of inbreeding depression of days to first female flower anthesis

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	-17.28 - -	- - -	NS - -	NS - -	NS - **NS**
MC-139		- - -	- - -	- - -	- - -
MC-142			-21.14 - **-20.56**	NS - **-**	NS - **2.28***
MC-133				-16.7 - -	NS - -
MC-136					-12.94 - **-10.71**

Table 21 (d): Pair wise comparison of inbreeding depression of nodes to first female flower

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- - -	- - -	- - -	- - -	- - -
MC-139		- - -	- - -	- - -	- - -
MC-142			-13.11 - -	NS - -	NS - -
MC-133				-16.38 - **-11.51**	NS - -
MC-136					-18.06 - -

Table 21 (e): Pair wise comparison of inbreeding depression of days to first harvest

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- - -	- - -	- - -	- - -	- - -
MC-139		- - -	- - -	- - -	- - -
MC-142			-18.74 - **-14.32**	NS - -	- - -
MC-133				-12.83 - -	- - -
MC-136					- - -

Table 21 (f): Pair wise comparison of inbreeding depression of relative early yield

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	68.24 - **28.45**	16.52** - **12.65****	8.39** - **35.16****	6.51** - **105.10****	53.22** - **85.82****
MC-139		59.67 - **35.31**	8.68** - **20.67****	13.18** - **88.07****	37.34** - **71.38****
MC-142			64.08 - **45.71**	3.36** - **74.98****	46.29** - **59.17****
MC-133				65.45 *11.78* **77.23**	56.32** *55.41*** **NS**
MC-136					37.59 *39.08* **76.67**

Table 21 (g): Pair wise comparison of inbreeding depression of number of days from anthesis to fruit maturity

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- *-4.06* **-**	- *NS* **-**	- - **-**	- - **-**	- - **-**
MC-139		- -5.53 **-**	- - **-**	- - **-**	- - **-**
MC-142			- - **-**	- - **-**	- - **-**
MC-133				- - **-**	- - **-**
MC-136					- - **-**

Table 21 (h): Pair wise comparison of inbreeding depression of Average fruit weight

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	- - -	- - -	- - -	- - -	- - -
MC-139		- - -	- - -	- - -	- - -
MC-142			44.97 - -	- - -	- - -
MC-133				- - -	- - -
MC-136					- - -

Table 21 (i): Pair wise comparison of inbreeding depression of fruit length

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	30.42 - **11.28**	NS - **2.68****	NS - **NS**	NS - **13.82****	2.57* - **6.85****
MC-139		29.22 - **22.65**	NS - **NS**	NS - **6.66****	2.22* - **4.16****
MC-142			25.06 - **16.65**	NS - **9.41****	NS - **6.69****
MC-133				29.11 *16.03* **45.14**	2.94** *NS* **3.44****
MC-136					21.36 *15.02* **36.38**

Table 21 (j): Pair wise comparison of inbreeding depression of fruit diameter

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	19.94 - **9.28**	4.67** - **9.79****	7.70** - **NS**	8.76** - **9.00****	31.50** - **-**
MC-139		22.73 - **3.89**	13.37** - **8.91****	13.74** - **17.80****	38.11** - **-**
MC-142			15.74 - **8.64**	1.99* - **10.47****	29.05** - **-**
MC-133				14.68 - **14.27**	22.41** - **-**
MC-136					2.55 - -

Table 21 (k): Pair wise comparison of inbreeding depression of fruit girth

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	18.09 - **3.47**	2.48* - **NS**	NS - **6.34****	3.88** - **6.30****	- - **-**
MC-139		22.57 - **3.90**	3.3** - **6.24****	7.34** - **6.20****	- - -
MC-142			17.65 - **14.25**	4.34** - **NS**	- - -
MC-133				11.04 - **14.23**	- - -
MC-136					- - -

Table 21 (l): Pair wise comparison of inbreeding depression of flesh thickness

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	34.44 - **21.71**	41.41** - **174.99****	18.65** - **70.14****	40.95** - **9.80****	146.56** - **24.02****
MC-139		30.88 - **6.97**	76.30** - **98.72****	7.8** - **181.12****	147.76** - **155.54****
MC-142			36.34 - **14.7**	65.50** - **63.71****	173.37** - **46.79****
MC-133				30.29 - **20.73**	120.79** - **15.30****
MC-136					17.24 *2.01* **19.25**

Table 21 (m): Pair wise comparison of inbreeding depression of number of fruits per plant

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	35.62 - **25.35**	NS - **NS**	NS - **NS**	NS - **NS**	NS - **3.91****
MC-139		43.53 - **25.06**	NS - **NS**	NS - **NS**	NS - **3.55****
MC-142			36.07 - **27.63**	NS - **NS**	NS - **3.35****
MC-133				41.78 - **35.72**	NS - **2.66****
MC-136					38.52 *24.11* **62.63**

Table 21 (n): Pair wise comparison of inbreeding depression of yield per plant

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	68.7 - **28.48**	NS - **5.89****	NS - **15.92****	2.65* - **37.77****	13.00** - **39.25****
MC-139		69.25 - **36.40**	NS - **9.33****	3.05** - **30.40****	12.94** - **32.81****
MC-142			66.77 - **48.22**	NS - **22.62****	11.51** - **26.94****
MC-133				65.71 *7.17* **72.88**	12.26** *23.75*** **9.16****
MC-136					48.79 *36.08* **84.87**

Table 21 (o): Pair wise comparison of inbreeding depression of number of harvests

	MC-138	MC-139	MC-142	MC-133	MC-136
MC-138	35.25 - **26.22**	12.86** - **6.56****	19.8** - **11.80****	15.34** - **2.02***	- - **2.18***
MC-139		17.22 - **17.42**	5.84** - **4.48****	NS - **7.17****	- - **3.44****
MC-142			6.94 - **9.85**	4.12** - **11.82****	- - **7.87****
MC-133				14.01 *14.70* **28.70**	- *11.42*** **3.42****
MC-136					- *33.98* **23.26**

There was no significant difference among any pair of inbreeding depressions with respect to the characters *viz*, days to first male flower, nodes to first female flower, days to first harvest, number of days from anthesis to fruit maturity and average fruit weight. Inbreeding depression of F_2 over F_1 for nodes to first male flower between MC-139 and MC-142 was significantly different. The cumulative inbreeding depression between MC-142 and MC-136 showed significant difference

for days to first female flower anthesis. Inbreeding depression of F_2 over F_1 for early yield between MC-138 and other hybrids *viz*, MC-139, MC-142, MC-133 and MC-136 was found to be significantly different. A similar trend could observe in case of all other four hybrids. Inbreeding depression of F_3 over F_2 found significantly different only between MC-133 and MC-136. Cumulative inbreeding depression was significant between most of the hybrids. But it showed a non significant value between MC-133 and MC-136. Fruit length showed significant difference for inbreeding depression of F_2 over F_1 between MC-136 and MC-138, MC-136 and MC-139 and MC-136 and MC-133. Cumulative inbreeding depression for fruit length was significant between MC-138 and MC-139, MC-138 and MC-133, MC-138 and MC-136, MC-139 and MC-133, MC-139 and MC-136, MC-142 and MC-133 and MC-142 and MC-136. Inbreeding depression of F_2 over F_1 for fruit diameter between hybrids recorded significant differences. Cumulative inbreeding depression for fruit diameter was significant between MC-138 and MC-139, MC-138 and MC-133, MC-139 and MC-142, MC-139 and MC-133 and MC-142 and MC-133. Fruit girth registered significant difference for inbreeding depression of F_2 over F_1 between MC-138 and MC-139, MC-138 and MC-133, MC-139 and MC-142, MC-139 and MC-133 and MC-142 and MC-133. Cumulative inbreeding depression for fruit girth was significant between MC-138 and MC-142, MC-138 and MC-133, MC-139 and MC-142 and MC-139 and MC-133. Inbreeding depression of F_2 over F_1 and cumulative inbreeding depression for flesh thickness recorded significant differences between hybrids. There was non significant difference for inbreeding depression of F_2 over F_1 for number of fruits per plant. Significant cumulative inbreeding depression was recorded between MC-138 and MC-136, MC-139 and MC-136, MC-142 and MC-136 and MC-133 and MC-136. Significant inbreeding depression of F_2 over F_1 for yield per plant was observed between MC-138 and MC-133, MC-138 and MC-136, MC-139 and MC-133, MC-139 and MC-136, MC-142 and MC-136 and MC-133 and MC-136. Inbreeding depression of F_3 over F_1 was found significantly different

between MC-133 and MC-136. Significant difference in cumulative inbreeding depression was observed between MC-138 and MC-133, MC-138 and MC-136, MC-139 and MC-142, MC-139 and MC-133, MC-139 and MC-136, MC-142 and MC-133, MC-142 and MC-136 and MC-133 and MC-136. Inbreeding depression for number of harvests in F_2 over F_1 exhibited significant difference between MC-138 and MC-139, MC-138 and MC-142, MC-138 and MC-133, MC-139 and MC-142 and MC-142 and MC-133. Significantly different inbreeding depression in F_3 over F_2 was observed between MC-133 and MC-136. Cumulative inbreeding depression was found significantly different between MC-138 and MC-139, MC-138 and MC-142, MC-138 and MC-133, MC-138 and MC-136, MC-139 and MC-142, MC-139 and MC-133, MC-139 and MC-136, MC-142 and MC-133, MC-142 and MC-136 and MC-133 and MC-136.

4.2.4.3 Estimate of inbreeding depression derived from regression analysis in successive generation

Regression analysis was attempted using the Wright's inbreeding coefficient (F) (Rubino and Wehner, 1986) for the characteristics with statistical difference among the treatments. But none of the regression equations gave a good fit as evidenced by very poor R^2. Hence no further inferences were drawn through regression analysis as was conceived of.

4.2.5 Estimates of heritability, genetic advance (GA) and genetic advance as percent of mean (GAM)

The estimation of heritability has a greater role to play in determining the effectiveness of selection of a character provided it is considered in conjunction with the predicted genetic advance as suggested by Johnson *et al.* (1955). Results of heritability and genetic advance in F_2 and F_3 generations of selected hybrids in bitter gourd are presented in Table22.

4.2.5.1 Average fruit weight

High heritability coupled with high genetic advance as percent of mean was observed in F_2 generations of MC-142 (89.59 % and 60.49 %), MC-136 (81.25% and 49.86%) MC-139(87.95% and 67.65%), MC-138 (91.76% and 61.17%) and MC-133 (84.22% and 51.55%). F_3 generations of all hybrids exhibited a similar pattern of high heritability and high GAM. Thus, there is ample scope for improving the character through direct selection. This indicates that selection may be effective for this character. This is in confirmation with findings of Rani *et al*. (2014), Rajput (2012) in segregating population of bitter gourd. Nandkumar (2016) also reported similar results in segregating population of bitter gourd (Hirkani x Phule Ujwala).

Table 22. Estimates of heritability and genetic advance of fruit characteristics

Characters	Hybrid	h2 (%)		GA		GAM (%)	
		F_2	F_3	F_2	F_3	F_2	F_3
Average fruit weight	MC-142	89.59	87.42	51.10	49.95	60.49	49.41
	MC-136	81.25	89.1	49.85	49.34	49.87	52.84
	MC-139	87.95	90.09	63.87	61.56	67.65	47.19
	MC-138	91.76	86.55	91.68	87.90	61.18	46.29
	MC-133	84.22	90.82	49.64	49.55	51.55	52.09
Fruit length	MC-142	87.08	89.35	6.58	6.41	34.15	30.70
	MC-136	89.44	90.42	6.97	4.28	41.21	29.78
	MC-139	87.41	85.89	8.92	7.38	43.03	33.46
	MC-138	95.09	93.43	9.65	9.52	44.62	36.96
	MC-133	72.71	86.11	4.79	5.16	23.96	30.76
Fruit diameter	MC-142	86.4	83.48	0.96	0.96	26.78	24.82
	MC-136	82.44	83.27	1.04	0.80	25.48	19.06
	MC-139	84.18	85.68	1.15	1.13	32.72	27.18
	MC-138	79.74	68.29	0.90	0.69	20.39	14.21
	MC-133	82.68	84.98	1.09	1.08	29.23	28.68
Fruit girth	MC-142	86.07	83.42	3.56	3.36	30.45	27.81
	MC-136	91.07	78.21	4.17	2.23	29.83	15.79
	MC-139	87.92	80.62	3.36	3.11	29.69	23.11
	MC-138	73.46	69.84	2.78	2.24	19.78	13.90
	MC-133	86.03	90.63	3.48	3.58	28.23	30.02
Flesh thickness	MC-142	86.39	83.41	0.19	0.18	38.45	31.08
	MC-136	78.53	71.83	0.18	0.13	31.73	22.41
	MC-139	75.6	67.39	0.14	0.13	32.13	24.72
	MC-138	72.67	50.97	0.14	0.09	25.77	14.40
	MC-133	74.24	80.35	0.16	0.17	32.27	31.59

4.2.5.2 Fruit length

High heritability accompanied by high genetic advance as percent of mean was noticed in F_2 of MC-142 (87.08 % and 34.14%) MC-136 (89.44 % and 41.21 %) MC-139 (87.41% and 43.03%), MC 138 (95.09% and 44.61%) and MC-133(72.71% and 23.95%). F_3 generations of all hybrids exhibited a similar pattern of high heritability and high GAM. This indicates that selection may be effective for improving fruit length. This is in agreement with findings of Gowda (2017). According to Rajput (2012) and Nandkumar (2016) high heritability and low to moderate GAM was observed in bitter gourd among segregating population.

4.2.5.3 Fruit diameter

High heritability and high GAM was observed in F_2 generations of MC-142 (86.4% and 26.78%), MC-136 (82.44% and 25.48%), MC-139 (84.18% and 32.72%), MC-138 (79.74% and 20.39 %) and MC-133 (82.68% and 29.23%). MC-142, MC-139 and MC-133 exhibited high heritability percentage and GAM in F_3 generations also. So, the character can be improved by selection. F_3 generations of MC-136 and MC-138 showed high heritability with moderate GAM. Rajput (2012) and Nandkumar (2016) reported high heritability percentage along with low to moderate values for GAM in bitter gourd. According to Maurya *et al*. (2018) fruit diameter recorded moderate heritability percentage and moderate GAM in bitter gourd, where as Talukder *et al*. (2018) observed low heritability percentage and low GAM for this trait.

4.2.5.4 Fruit girth

High heritability accompanied by high GAM was observed in F_2 generations of MC-142 (86.07% and 30.45%), MC-136 (91.07% and 29.83%), MC-139 (87.92% and 29.69%) and MC-133 (86.03% and 28.23%). F_3 generations of the hybrids MC-142, MC-139 and MC-133 also followed similar pattern. Hence selection can be

recommended for improvement of the character. Praveena (2010) and Chakraborty *et al*. (2013) reported similar results in bitter gourd. High heritability with moderate GAM in F_3 generation was observed in the hybrids MC-138 and MC-136 which is in agreement with findings of Gowda (2017) in bitter gourd.

4.2.5.5 Flesh thickness

High heritability coupled with high genetic advance as percent of mean was observed in F_2 generations of MC-142 (86.39% and 38.45%), MC-136 (78.53% and 31.73%) MC-139 (75.60% and 32.13%), MC-138 (72.67% and 25.76%) and MC-133 (74.24% and 32.27%). High heritability with high genetic advance as per cent of mean was also observed in F_3 generations of all hybrids where the character can be improved through selection. F_3 generation of MC-138 showed moderate heritability and moderate GAM. Raja *et al.* (2007), Praveena (2010) and Chakraborty *et al*. (2013) confirmed high heritability with high GAM in bitter gourd for flesh thickness. Rani *et al*. (2014) observed moderate heritability and GAM in flesh thickness of bitter gourd fruit.

4.2.6 Identification of economically important segregants

Selection of segregants having economic value is done after fixing the differential yield levels. Desirable economic segregants were chosen on the grounds of earliness and yield contributing characters. Average fruit weight, number of fruits per plant, yield per plant and days to first fruit harvest were considered to select economic segregants in F_2 and F_3 generations of hybrids. A plant in F_2 and F_3 generations was judged as an economic segregant for a character after comparing its performance with the variety Preethi raised in the same season. Results of per cent economic segregants in F_2 and F_3 generations of selected hybrids are presented in Table 23.

4.2.6.1 Days to first harvest

Per cent of economic segregants ranged from 17.86 (MC-138) to 64.71 % (48.00-56.00 days) (MC-136) in F_2 and 48.94 (MC-138) to 100.00 % (46.00-59.00 days) (MC-136) in F_3 generations, respectively.

4.2.6.2 Average fruit weight

The maximum per cent of economic segregants for average fruit weight was observed in MC-138 (64.29%) (144.00-231.50 g) followed by MC-133 (3.13%) in F_2 generation. Economic segregants were not found in F_2 generations of hybrids, MC-142, MC-136 and MC-139. Per cent of economic segregants in F_3 ranged from 4.55 (MC-136) to 85.11% (MC-138) (150.00-330.10g) for this character.

Table 23. Per cent economic segregants in F_2 and F_3 generations of selected hybrids

Character	Hybrid	Economic segregants in F_2 generation (%)	Range	Economic segregants in F_3 generation (%)	Range
Days to first harvest	MC-142	36.84	50.00-57.00	83.33	51.00-60.00
	MC-136	64.71	48.00-56.00	100.00	46.00-59.00
	MC-139	29.17	53.00-57.00	81.58	52.00-60.00
	MC-138	17.86	54.00 -57.00	48.94	55.00-60.00
	MC-133	25.00	52.00-57.00	90.91	47.0-60.00
Average fruit weight (g)	MC-142	0.00	-	5.00	145.11-199.50
	MC-136	0.00	-	4.55	155.80-186.80
	MC-139	0.00	-	31.58	148.75-214.33
	MC-138	64.29	144.00-231.50	85.11	150.00-330.10
	MC-133	3.13	158.00-161.14	6.06	145.30-149.50
Number of fruits per plant	MC-142	68.42	28.00-47.00	75.00	29.00-62.00
	MC-136	94.12	29.00-67.00	68.18	29.00-59.00
	MC-139	37.50	28.00-41.00	34.21	29.00-52.00
	MC-138	32.14	28.00-36.00	27.66	30.00-39.00
	MC-133	28.13	28.00-43.00	39.39	30.00-39.00
Yield per plant (kg)	MC-142	15.79	3.56-5.40	23.33	3.77-5.95
	MC-136	58.82	3.54-6.50	13.64	4.42-6.82
	MC-139	16.67	3.90-4.52	28.95	3.80-6.09
	MC-138	28.57	4.00-5.16	53.19	3.83-6.09
	MC-133	6.25	4.30-4.50	12.12	3.72-4.14

4.2.6.3 Number of fruits per plant

MC-136 showed the highest per cent of economic segregants (94.12%) (29.00-67.00) for number of fruits per plant in F_2 generation. The least was reported in MC-133 (28.13%). Per cent of economic segregants in F_3 ranged from 27.66 (MC-138) to 75 % (MC-142) (29.00-62.00) for this character.

4.2.6.4 Yield per plant

Per cent of economic segregants for yield per plant ranged from 6.25 (MC-133) to 58.82% (3.54-6.50 kg per plant) (MC-136) in F_2 generation. The maximum per cent of economic segregants for yield per plant in F_3 generation was observed in MC-138 (53.19%) (3.83 – 6.57) kg per plant followed by MC-139 (28.95%), MC-42(23.33%), MC-136 (13.64%) and MC-133(12.12%).

All the selected segregants were superior in performance when compared to Preethi and they can be fixed through inbreeding and selection. Ravishankar (2007) carried out analysis of segregants of economic value in F_2 with regard to important characters in brinjal. The study revealed that there was a higher frequency of economic segregants for fruit weight followed by number of fruit per plant and yield per plant. Segregants having superior performance were isolated for further utility previously by many researchers in different crops viz,; Kale *et al.* (1989), Naidu (1993), Patil (1998), Karaganni (2003), Mallangoud (2005) and Naik (2007) in brinjal and Somashakhar (2006) in chilli.

4.3 *In situ* induction of maternal haploids initiated by pollination with irradiated pollen (haploid parthenogenesis)

Haploid parthenogenesis is the induction of parthenogenetic development of the egg cell by pollination with irradiated pollen followed by haploid embryo rescue. Embryo development is stimulated by germination of pollen on the stigma and growth of the pollen tube within the style, although irradiated nuclei is not able to

fertilize the egg cell. Haploid technique provides full homozygosity in one step and has opened new perspectives to shorten the time required for the development of homozygous lines (Jia *et al*., 2014). An efficient protocol for haploid embryo culture has been established in bitter gourd.

4.3.1 Irradiation dose and pollination

Female flowers collected from the best performing F_1 hybrid MC-139 on the day before anthesis were exposed to gamma rays ($Cobalt^{60}$) at doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy (Gray). Female flowers bagged on the previous day were artificially pollinated using nonirradiated (control) and irradiated pollen. No differences in fruit set were detected between pollen irradiated with gamma rays (10 to 100 Gy) and unirradiated pollen (control). All irradiation doses from 10 to 100 Gy were efficient for hundred per cent fruit set (Table 24) (Plate 11). This is in agreement with observations of Deunff and Sauton (1994) in cucumber where pollination with irradiated pollen induced fruit set was similar to that seen with normal pollination. Moussa and Salem (2009) reported similar findings in watermelon where pollen irradiated with gamma rays (600 Gy) and unirradiated pollen was used. According to Kurtar *et al.* (2002) fruit set was induced at all gamma ray doses (25 to 200 Gy) in squash, but in general, was reduced when the level of gamma ray doses increased. Zhang *et al.* (2006) found average fruit setting as 50% and 10% in melon when the gamma radiation doses were 300 and 600 Gy, respectively. Kurtar (2009) reported that fruit set decreased at high doses gamma irradiation (300 Gy) in pumpkin and winter squash. A gradual decrease in fruit set was observed at increased irradiation doses (Kosmrlj *et al.,* 2014). According to Guler *et al*., (2017) the highest fruit set was recorded in lower (50 and 75 Gy) doses while the fruit set decreased by increased doses of irradiation in bottle gourd. Different doses affect the fruit set and formation of haploid embryos in various cucurbit crops (Gursoz *et al.,* 1991; Sari *et al*., 1994; Kurtar *et al.,* 2002).

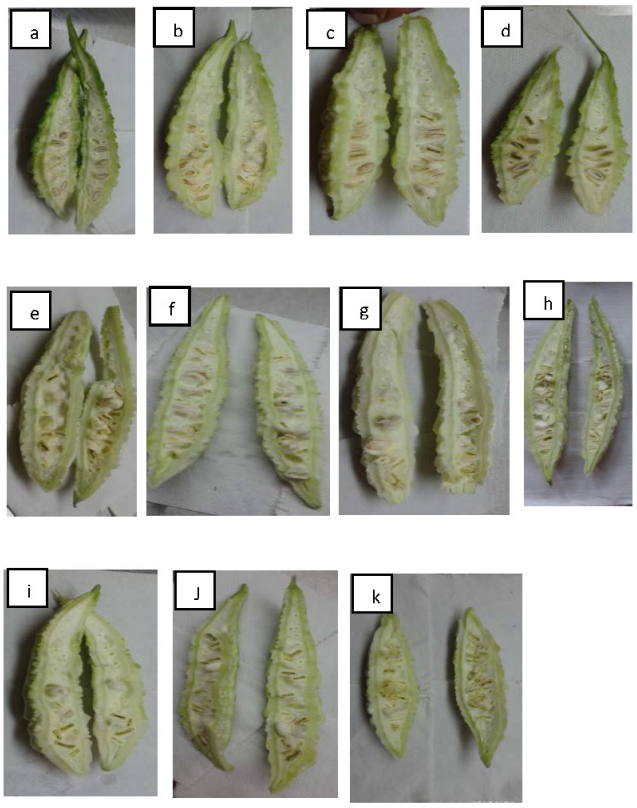


Plate 11. Fruits harvested 15 days after pollination with irradiated pollen at various doses of 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g), 70 (h), 80 (i) , 90 (j) and 100 gray (k).

Table 24. Effect of irradiation on fruit set, number of seeds per fruit and number of embryos per fruit

Irradiation treatment (Gy)	Fruit set (%)	Number of seeds per fruit	Number of embryos per fruit
T_0 (0Gy-control)	100	29.13 ± 2.53^{a}	$29.13(5.44)\pm2.53^{a}$
T_1(10 Gy)	100	25.13 ± 1.77^{b}	$5.8(2.51)\pm0.56^{b}$
T_2 (20 Gy)	100	24.93 ± 1.33^{bc}	$4.13(2.14)\pm0.83^{c}$
T_3 (30 Gy)	100	24.8 ± 2.88^{bc}	$3.67(2.03)\pm0.72^{c}$
T_4 (40 Gy)	100	23.93 ± 1.39^{bcd}	$2.87(1.83)\pm0.52^{d}$
T_5 (50 gy)	100	23.33 ± 2.02^{cde}	$1.4(1.36)\pm0.63^{e}$
T_6 (60 Gy)	100	22.8 ± 2.27^{def}	$0.93(1.16)\pm0.70^{f}$
T_7 (70 Gy)	100	22.47 ± 4.6^{def}	$0.61(1.00)\pm0.63^{fg}$
T_8 (80 Gy)	100	22.13 ± 2.5^{ef}	$0.53(0.98)\pm0.52^{gh}$
T_9 (90Gy)	100	21.33 ± 2.09^{f}	$0.27(0.84)\pm0.46^{hi}$
T_{10} (100Gy)	100	18.67 ± 1.84^{g}	$0\ (0.71)^{i}$
CD(0.05)		1.75	0.16
CV		10.41	11.97

Data are Mean ± Standard deviation, n=15, values in parenthesis are square root transformed

4.3.2 Embryo detection

Seeds per fruit and embryos per fruit were counted 15 days after pollination with irradiated pollen. Effect of irradiation on number of seeds and embryos per fruit is illustrated in Table 24. A drastic effect of irradiation was recorded for the number of seeds and embryos per fruit. The number of seeds and embryos per fruit decreased significantly as the irradiation dose increased. The highest number of seeds per fruit (29.13) was recorded in control (0 Gy) and the lowest (18.67) was in fruit developed through pollinating irradiated pollen of 100 Gy. Difference was non significant between fruits developed through pollination of irradiated pollen with dosages 20 Gy and 30 Gy. Similarly, fruit developed through pollinating irradiated pollen of 60 and 70 Gy also exhibited non significant difference in the number of seeds per fruit.

Fruits obtained in control exhibited the highest number of embryos per fruit (29.13). A drastic reduction in number of embryos was observed in fruits obtained through pollination with irradiated pollen (10 to 100 Gy). Among the fruits which obtained after pollinating with irradiated pollen at 10 different doses, treatment T_1 (5.8) showed the highest number of embryos per fruit followed by T_2 (4.13), T_3

(3.67), T_4 (2.87) and T_5 (1.4). Treatment T_9 (90 Gy) showed only 0.27 embryos per fruit and ploidy level of regenerated plant was confirmed as haploid through cytological analysis (Table 32). An increase in the dose of irradiation results in a decrease in the number of developed embryos but the resultant plants were mostly of haploid origin. Treatment T_{10} (100 Gy) failed to produce embryos and all seeds were empty. The abortion of embryos after pollination with pollen irradiated with gamma rays may be due to chromosomal abnormalities induced by gamma rays in the generative nucleus. Number of embryos per fruit significantly reduced as the irradiation dose increased from 10 to 100 Gy. This is in accordance with findings of Faris *et al* (1999) where the lowest (0.1kGy) dose of irradiation gave the highest number of embryos while the highest (0.3 kGy) dose produced the lowest number of embryos. The number of normal seeds was significantly reduced when female flowers were pollinated with irradiated pollen and only empty seeds were observed at 600–800 Gy in watermelon (Moussa and Salem, 2009). Kurtar *et al*. (2002) suggested that seeds with differently formed embryos in fruit were obtained with lower doses of only 25–50 Gy. A decrease in fruit set and mean number of embryos per 100 seeds was noticed at increased irradiation doses (Kosmrlj *et al*., 2014). Percentage of embryos per hundred seeds decreased from 2.8 to 0.4 as the irradiation dose increased from 50 to 200Gy in bottle gourd (Guler *et al*., (2017). Hence it can be concluded that as the irradiation dose increases, there is a significant reduction in the number of seeds and embryos per fruit in bitter gourd.

4.3.3 Irradiation dose and haploid recovery

In the present study, 90 Gy was chosen as the best irradiation dose for haploid recovery and ploidy level of germinated plants were confirmed (Table 32). The dose of irradiation is the major factor controlling *in situ* haploid production. At lower doses, the generative nucleus is partly damaged and therefore maintains its capacity to fertilize the egg cell. It results in a large number of embryos but all of hybrid origin

and abnormal (mutant) phenotype. Dosage should not be so high as to inhibit pollen tube germination, but also must be high enough to disturb normal fertilization and avoid generation of diploid hybrid embryo. Irradiation dose is specific to each experiment.

The results of different studies revealed that the preferred radiation dose is specific to each particular experiment. Studies have shown that higher irradiation doses result in lower for haploid induction; 100–300 Gy, specifically, is effective for cucumber (Przyborowski and Niemirowicz-Szczytt,1994; Lei *et al*., 2006; Lotfi and Salehi, 2008; Smiech *et al*., 2008). Haploid plants were obtained in melon when irradiation dose of 250-300 Gy was used (Ari *et al*. , 2010; Gonzalo *et al*., 2011 and Godbole and Murthy, 2012a, b). Baktemur *et al*. (2014) suggested that irradiation dose of 150 Gy was the best to induce haploid plants in squash. Taskın *et al*. (2013) recommended irradiation dose of 275Gy in watermelon for haploid production. On the other hand, haploid plants was successfully obtained with lower irradiation doses in squash (25 and 50 Gy) (Kurtar *et al*. 2002), pumpkin (50 and 100 Gy) (Kurtar *et al*. 2009) winter squash (50 and 100 Gy) (Kurtar and Balkaya 2010) and bottle gourd (50and 75 Gy) (Guler *et al*., 2017).

4.4 Development of haploid inbred lines through embryo rescue and culture

4.4.1 Stage of embryo rescue

After pollination with irradiated pollen, the endosperm does not develop (Sauton and Dumas de Vaulx 1987). The embryo grows at first even without the presence of endosperm, but its development is arrested at an early stage that necessitates embryo rescue and *in vitro* culture. Without embryo rescue, embryo failed to survive and dies.

Embryos were rescued from fruit obtained after 15 days of pollination with irradiated pollen. There was no significant increase in embryos per fruit after 15 days

of pollination with pollen irradiated at different levels (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy) (Table 25). According to Xiao-feng, *et al*. (2016) tenth day after pollination was the best embryo rescue period for female gametogenesis in bitter gourd. In cucumber Smiech *et al*. (2008) recorded 3-5 weeks after pollination as the best stage of embryo rescue. According to Lofti and Salehi (2008) it was 21 to 23 days in watermelon. Three to five weeks after pollination was the best embryo rescue stage in melon as reported by Gonzalo *et al*. (2011). It took 35 days after pollination in squash (Baktemur *et al*., 2014). Taskın *et al*. (2013) suggested embryo rescue after 25 days of pollination in watermelon.

Table 25. Number of embryos per fruit

Sl. No.	Irradiation dose (Gy)	Days after pollination (No.)	No. of embryos per fruit
1	0 (Control)	14	22.07(4.75)±2.01[b]
		15	29.13(5.44)±2.53[a]
		16	28.53(5.37)±3.44[a]
		CD (0.05)	0.19
		CV	5.08
2	10	14	3.47(1.86)±0.64[b]
		15	5.8(2.41)±0.56[a]
		16	5.93(2.43)±0.8[a]
		CD (0.05)	0.11
		CV	6.69
3	20	14	3.4(1.83)±0.74[b]
		15	4.13(2.02)±0.83[a]
		16	4.33(2.07)±0.72[a]
		CD (0.05)	0.15
		CV	9.94
4	30	14	3.27(1.80)±0.7[b]
		15	3.67(1.91)±0.72[ab]
		16	3.93(1.98)±0.7[a]
		CD (0.05)	0.14
		CV	9.98
5	40	14	1.87(1.36)±0.35[b]
		15	2.87(1.69)±0.52[a]
		16	3(1.73)±0.53[a]
		CD (0.05)	0.11
		CV	9.63
6	50	14	0.67(1.02)±0.82[b]
		15	1.4(1.36)±0.63[a]
		16	1.6(1.43)±0.63[a]
		CD (0.05)	0.2
		CV	21
7	60	14	0.4(.91)±0.51[b]
		15	0.93(1.16)±0.7[a]
		16	1.2(1.28)±0.68[a]
		CD (0.05)	0.21
		CV	25.8
8	70	14	0.33(0.88)±0.49
		15	0.6(1.01)±0.63
		16	0.67(1.03)±0.72
		CD (0.05)	NS
		CV	30.72
9	80	14	0.27(0.85)±0.46
		15	0.53(0.98)±0.52
		16	0.6(1.02)±0.51
		CD (0.05)	NS
		CV	26.97

10	90	14	0.13(0.78)±0.35
		15	0.27(0.85)±0.46
		16	0.33(0.88)±0.49
		CD (0.05)	NS
		CV	27.1
11	100	14	No embryo
		15	No embryo
		16	No embryo

Data are Mean ± Standard deviation, n=15, Value in parentheses are square root transformed

4.4.2 Standardization of induction media

Seeds and embryos were inoculated and cultured in test tubes containing in E20A supplemented with IAA (0.01mg/l) (control) (TSM_1) and E20A supplemented with IAA (0.1mg/l) and BAP (5 mg/l) (TSM_2). They were incubated at 25^0c under eight hours of dark and sixteen hours of light photoperiod conditions (Baktemur *et al*. 2013; Taskin *et al*. 2013 and Baktemur *et al*. 2014). The response of seed and embryo in different media composition is given in Table 26 and 27, respectively.

Significant difference was observed for the percentage of seed response to *in vitro* germinated plants in E20A supplemented with IAA (0.01mg/l) (84.2 %) and E20A supplemented with IAA (0.1mg/l) and BAP (5 mg/l) (22.97 %) media for *in vitro* seed germination. Percentage of abnormal seedlings was significantly higher in TSM_2 (77.03%). Significant difference was not observed for days to leaf emergence.

Table 26. Response of seed in different media composition

Treatment	**Seed response to *in vitro* germinated plants (%)**	**Abnormal seedlings (%)**	**Days for leaf emergence**
TSM_1 (E20A+ IAA (0.01mg/l))	84.2**	15.8	43.33±5.23
TSM_2(E20A+IAA (0.1mg/l)+BAP(5 mg/l))	22.97	77.03**	46.26±4.46

** Significant at 1 % level (t test)

Table 27. Response of embryo in different media composition

Treatment	Embryo response to *in vitro* germinated plants (%)	Abnormal seedlings (%)	Days for leaf emergence
TEM_1 (E20A+ IAA (0.01mg/l))	68.67**	31.33	31.87±2.75
TEM_2 (E20A+IAA (0.1mg/l)+BAP(5 mg/l))	35.33	64.67**	37.27±2.84**

** Significant at 1 % level (t test)

Embryos were excised from seed and cultured in test tubes containing E20A supplemented with IAA (0.01mg/l) (control) (TEM_1) and E20A supplemented with IAA (0.1mg/l) and BAP (5 mg/l) (TEM_2). Treatment TEM_1 showed significantly the highest percentage of embryo response to *in vitro* germinated plants (68.67%) and the minimum days for leaf emergence (31.87). It showed lesser percentage of abnormal seedlings compared to TEM_2. Hence the preferred media for embryo induction is E20A supplemented with IAA (0.01mg/l). In cucurbits, the medium most commonly used for embryo rescue is the E20A medium, which was developed by Sauton and Dumas de Vaulx (1987) for melon haploid production. Godbole and Murthy (2012a, b) and Ari *et al*. (2010) suggested E20A + 0.011 mg/l IAA media for haploid recovery in melon. Faris *et al*. (1999), Smiech *et al*. (2008) and Guler *et al*. (2017) obtained watermelon, cucumber and bottle gourd haploid embryos, respectively in E20A media.

4.4.3 Embryo culture

Both seeds and embryo were cultured in test tubes containing E20A medium supplemented with 0.01 mg/lit IAA. The data pertaining to the response of seed and embryo is presented in Table 28 and 29 respectively (Plate 12, 13 and 14).

In seed culture experiment, the highest percentage of seed response to *in vitro* germinated plants (88.89%) was observed in irradiation treatment, TS_0 (control) followed by TS_1 (77.77%), TS_2 (68.89%) and TS_3 (66.67%) which gave a good response. They exhibited low percentage of abnormal seedlings *viz.,* 11.11, 22.23,

Plate 12. Plantlets developed from embryo through pollination with irradiated pollen at various doses of 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g), 70 (h), 80 (i) and 90 gray(j).

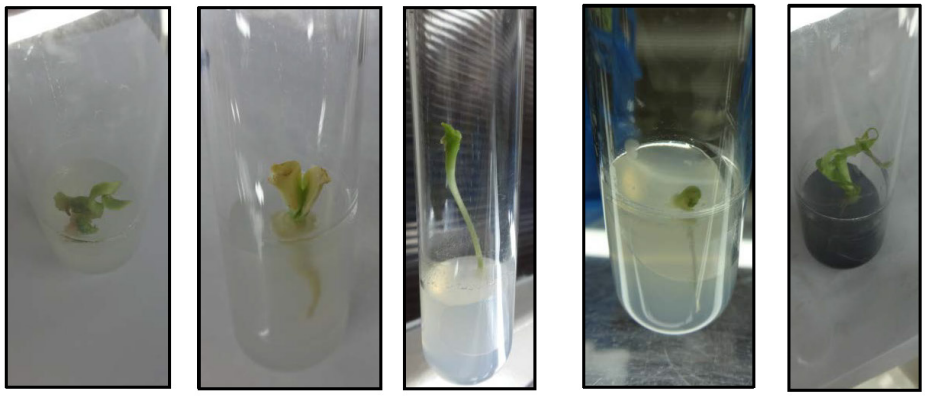


Plate 13. Abnormal cultures observed during embryo rescue and culture

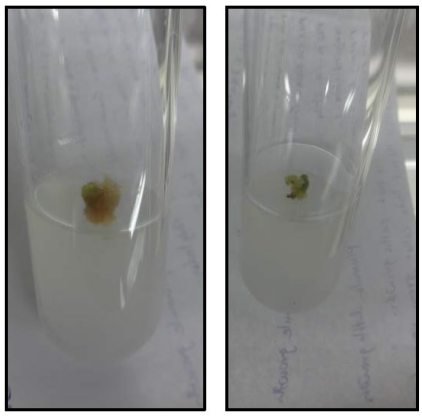


Plate 14. Callus formation observed during embryo rescue and culture

31.11 and 33.33 per cent respectively. The lowest percentage of seed response to *in vitro* germinated plants (22.22 %) and maximum percentage of abnormal seedlings were noticed in TS_9 (90 Gy). Significant reduction in seed response to *in vitro* germinated plants and increase in percentage of abnormal seedlings was observed as the irradiation doses increases. The minimum days for leaf emergence was recorded in TS_0 (43.00) (control) followed by TS_1 (51.67), TS_2 (52.33) and TS_3 (56.33). There was no significant difference between TS_4 and TS_5 for the character. The maximum days for leaf emergence were observed in TS_8 (86.33) which were on par with TS_6, TS_7 and TS_9. The treatment TS_0 exhibited the minimum days for visible root (14.66) followed by TS_1 (20.33). The treatment TS_9 (90 Gy) took maximum days for visible root.

Table 28. Response of seed in E20A medium supplemented with 0.01mg/l IAA

Irradiation dose	Seed response to *in vitro* germinated plants (%)	Abnormal seedlings (%)	Days for leaf emergence (No.)	Days for visible root (No.)
TS_0 (0Gy-control)	88.89 (70.73)^{a}	11.11 (19.26)^{h}	43^{e}	14.66^{g}
TS_1 (10 Gy)	77.77 (61.93) ^{b}	22.23 (28.07)^{g}	51.67^{d}	20.33^{f}
TS_2 (20 Gy)	68.89 (56.13)^{c}	31.11 (33.87)^{f}	52.33^{cd}	23.16^{e}
TS_3 (30Gy)	66.67 (54.80)^{c}	33.33 (35.19)^{f}	56.33^{c}	25.5^{d}
TS_4 (40 Gy)	53.33 (46.92)^{d}	46.67 (43.07)^{e}	64.66^{b}	28^{c}
TS_5 (50 Gy)	42.22 (40.52)^{e}	57.78 (49.48)^{d}	66^{b}	28.33^{c}
TS_6 (60 Gy)	35.56 (36.59)^{ef}	64.44 (53.41)^{cd}	84^{a}	30.15^{bc}
TS_7 (70 Gy)	31.11 (33.87)^{fg}	68.89 (56.12)^{bc}	85.67^{a}	30.83^{b}
TS_8 (80 Gy)	26.67 (31.09)^{gh}	73.33 (58.91)^{ab}	86.33^{a}	31^{b}
TS_9 (90 Gy)	22.22 (28.07)^{h}	77.78 (61.93)^{a}	86^{a}	35^{a}
TS_{10} (100 Gy)	0(0.74) ^{i}	0(0.74) ^{i}	-	-
CD(0.05)	4.84	4.84	4.38	2.18
CV	6.17	6.46	3.8	4.79

Value in parentheses are arc sine transformed

When embryos were cultured, the highest percentage embryo response to *in vitro* germinated plants (80.00 %) was noticed in TE_0 (control) followed by TE_1 (71.11 %). Treatments TE_8 and TE_9 failed to produce plantlets. A decrease in percentage of embryo response to *in vitro* germinated plants was noticed as the irradiation doses increase. Out of the ten treatments compared, callus formation was observed in TE_9 (11.11), TE_8 (13.33%) and TE_7 (8.89) and they were on par. Embryo

response to callus formation was noticed only at higher doses of irradiation. Percentage of abnormal seedlings was maximum in the treatment TE_9 (88.89%) which is on par with TE_8 (86.67%). The minimum percentage of abnormal seedlings was recorded in control (TS_0). Days for leaf emergence ranged from 31.67 (TS_0) to 74.67 days (TE_7). Days for visible root varied from 9.66 to (TS_0) 15.83.00 days (TE_7).

Table 29. Response of embryo in E20A medium supplemented with IAA (0.01 mg/lit)

Irradiation treatment (Gy)	*Percentage of embryo response to *in vitro* germinated plants	*Percentage of abnormal seedlings	**Percentage of embryo response to callus formation	Days for leaf emergence (No.)	Days for visible root (No.)
TE_0 (0Gy- control)	80 (64.29)^{a}	20 (25.72)^{e}	0(0.71)^{b}	31.67 ^{g}	9.66^{e}
TE_1 (10 Gy)	71.11 (57.70)^{ab}	28.89 (32.29)^{de}	0(0.71)^{b}	39.50 ^{f}	12.16^{d}
TE_2 (20 Gy)	62.22 (52.19)^{bc}	37.78 (37.80)^{cd}	0(0.71)^{b}	40.50 ^{f}	12.50 ^{cd}
TE_3 (30 Gy)	55.56 (48.19)^{c}	44.44 (41.80)^{c}	0(0.71)^{b}	46.33 ^{e}	13.66 ^{bcd}
TE_4 (40 Gy)	40 (39.19)^{d}	60.00 (50.81)^{b}	0(0.71)^{b}	53.83 ^{d}	14.00^{bc}
TE_5 (50 Gy)	35.56 (36.59)^{de}	64.44 (53.41)^{b}	0(0.71)^{b}	59.50 ^{c}	14.83 ^{ab}
TE_6 (60 Gy)	26.67 (30.97)^{ef}	73.33 (59.03) ^{b}	0(0.71)^{b}	72.00 ^{b}	15.00 ^{ab}
TE_7 (70 Gy)	20 (26.56)^{f}	71.11 (57.52)^{b}	8.89 (3.02)^{a}	74.67 ^{a}	15.83 ^{a}
TE_8 (80 Gy)	0 (0.74)^{g}	86.67 (69.02)^{a}	13.33 (3.64)^{a}	-	-
TE_9 (90 Gy)	0 (0.74)^{g}	88.89 (70.73)^{a}	11.11 (3.37)^{a}	-	-
CD(0.05)	7.8	8.74	0.678	2.58	1.58
CV	12.83	10.3	26.56	2.86	6.78

*Value in parentheses are arc sine transformed

** Value in parentheses are square root transformed

4.4.3.1 Effect of charcoal on rooting

In vitro rooting was obtained from E20A+ IAA (0.01 mg/lit) medium supplemented with activated charcoal (3g/lit) which took 7.2 days for root initiation and six number of roots were produced after 3 weeks of culture. Root initiation was not observed in control (E20A+ IAA (0.01 mg/lit)). Addition of activated charcoal in the rooting medium was found to enhance root initiation. Maximum rooting response (100%) was observed with this treatment (Table 30).

These results confirm the findings of Saha *et al*. (2016) in bitter gourd, as the addition of activated charcoal in the rooting medium improved overall rooting capacity of explants. Pradeepkumar *et al.* (2008) obtained a similar result from *in vitro* culturing of male sterile ridge gourd plants. The promotary effect of activated charcoal on rooting can be interpreted by considering two facts; the adsorption of inhibitory substances in the culture medium and establishment of a darkened environment in medium which is conducive to the accumulation of photosensitive auxin or cofactors (Druart *et al.,* 1982).

Table 30. Effect of charcoal on rooting

Treatments	Days taken for root initiation	Number of roots after 3 weeks	Rooting (%) (after 3 weeks)
E20A+ IAA (0.01 mg/l) (Control)	NR	0	0
E20A + IAA (0.01 mg/l)+Charcoal (3g/l)	7.2±0.94*	6±0.65*	100

*Mean ± Standard deviation, n=15. NR- No Response

4.4.4 Hardening of *in vitro* cultured plants and evaluation

The well rooted plantlets were transferred to polythene bags containing sterilized coco peat: soil: sand mixture (1:1:1). Hardening for 20 days in mist chamber was found to be essential for survival in the field. After hardening, the plants were transferred to polyhouse. Variation was observed in the biometric characteristics of plants (Table 31). The highest percentage of plants successfully hardened after 20 days was noticed in control (81.57%) followed by T_1 (79.1%), T_2 (71.18%), T_3 (70.9%) and T_4 (69.04%). The least per cent was observed in T_9 (90Gy). According to Faris *et al* (1999) number of plants regenerated was similar for the entire irradiation dose (0.1, 0.2 and 0.3kGy). The treatment T_0 (control), T_1 and T_2 took lesser number of days to first male flower appearance and they were on par (Plate 16). Higher number of days to first male flower emergence was noticed in T_4 (46.9), T5 (46.6), T6 (46.8) T_7 (47.5), T_8 (47.20). There was no significant difference between these treatments. T_9 took 47 days to first male flower emergence. The treatments T_0 (42), T_1 (41.8) and T_2 (40.9) took less days for days to female flower

appearance and they were on par. Higher number of days to first female flower emergence was noticed for treatments T_4, T_5, T_6, T_7 and T_8 and no significant difference was found between them. T_9 took 49 days for first female flower appearance. Different developmental stages of haploid and diploid plants are shown in Plate 15.

Table 31. Biometric parameters of hardened plants

Irradiation treatment (Gy)	Plants successfully hardened (after 20 days) (%)	Days taken for 1st male flower emergence	Days taken for 1st female flower emergence
T_0 (0Gy-control)	81.57	37.5±1.35[c]	42±1.25[c]
T_1(10 Gy)	79.1	37.8±1.4[c]	41.8±1.40[c]
T_2 (20 Gy)	71.18	38.1±0.74[c]	40.9±3.21[c]
T_3 (30 Gy)	70.9	42.6±1.71[b]	45.2±1.40[b]
T_4 (40 Gy)	69.04	46.9±1.37[a]	48.8±1.14[a]
T_5 (50 gy)	57.14	46.6±0.84[a]	48.1±1.37[a]
T_6 (60 Gy)	53.57	46.8±1.23[a]	49±1.15[a]
T_7 (70 Gy)	47.82	47.5±1.51[a]	49±1.25[a]
T_8 (80 Gy)	41.67	47.2±1.09[a]	48.80±1.48[a]
T_9 (90Gy)	10.00	47.00	49.00
*CD (0.05%)		1.63	2.08
CV		2.99	3.62

* Based on 9 no. of treatments (except T_9)

Mean ± Standard deviation, n=10 except for treatment T_8 (n=5) and T_9 (n=1)

4.4.5 Evaluation of ploidy level

Guard cell size, number of chloroplasts per guard cell and pollen grain characteristics can be taken as criteria to determine the ploidy level in plants. The results of the study are presented in Table 32 (Plate 17 and 18).

A significant difference in size, number of chloroplast per guard cell and pollen grain size were noticed between plants regenerated through pollination with irradiated pollens of 0 to 80 Gy and 90 Gy. T_9 (90 Gy) exhibited significantly lower values for all these traits. T_0 to T_8 plants showed a larger guard cell where the guard cell length and width ranged from 19.50 to 20.20 and 5.38 to 5.73 micron respectively while in T_9, guard cells of smaller size 16.23 and 4.66 micron length and width respectively were noticed. As for guard cell dimension, the difference between

Plate 15. Different developmental stages of haploid (a) and diploid (b) plants

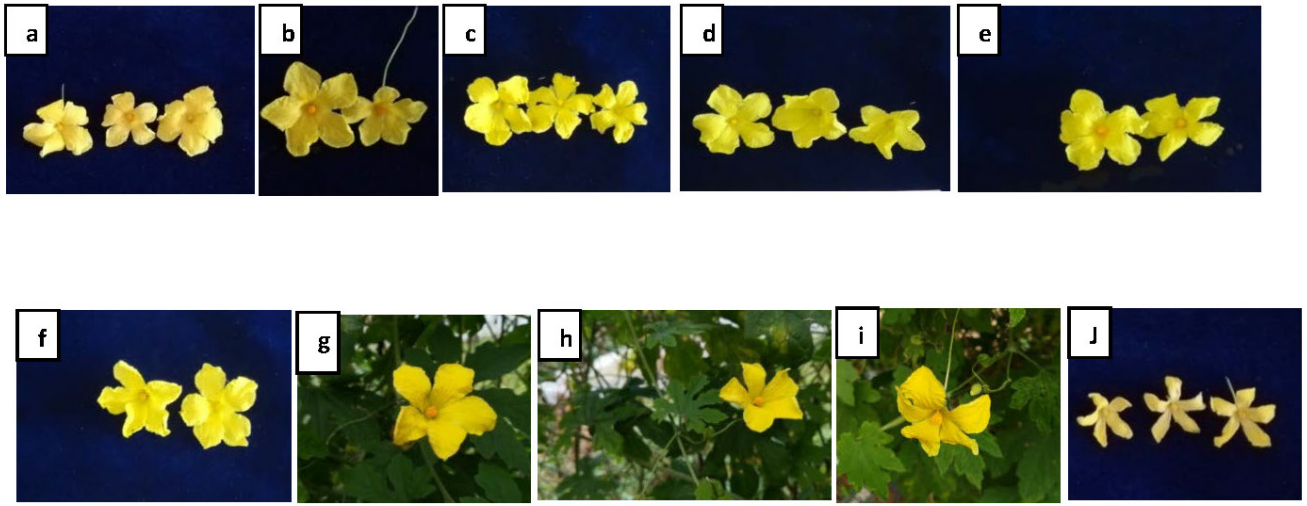


Plate 16. Male flowers produced by plants which are developed from embryo through pollination with irradiated pollen at various doses of 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g), 70 (h), 80 (i) and 90 gray(j).

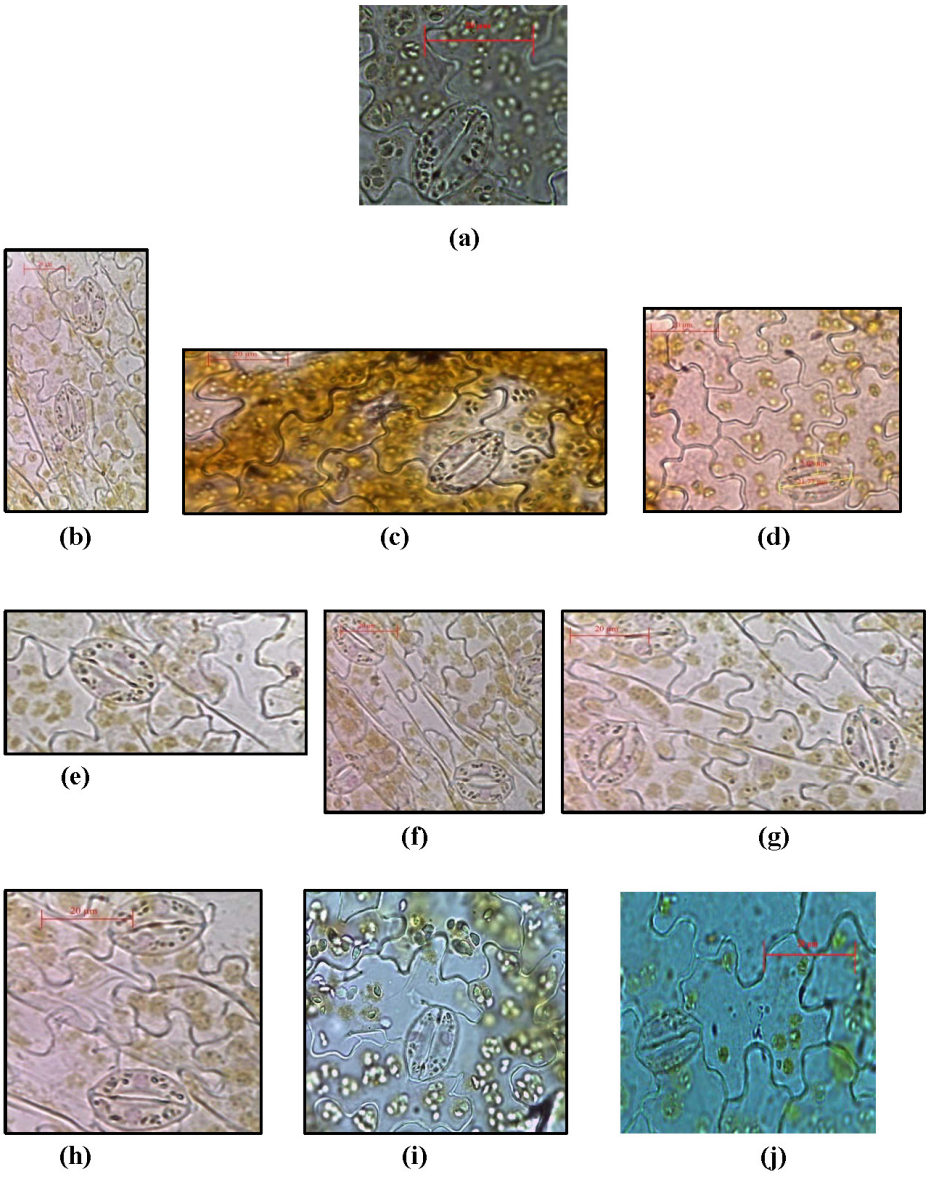
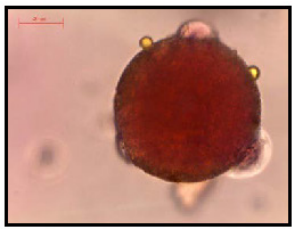


Plate 17. Stomatal guard cell (100 X) of plants which are developed from embryo through pollination with irradiated pollen at various doses of 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g), 70 (h), 80 (i) and 90 gray(j).

(a)



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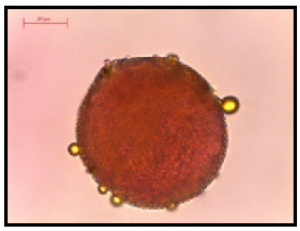
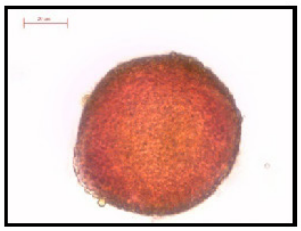
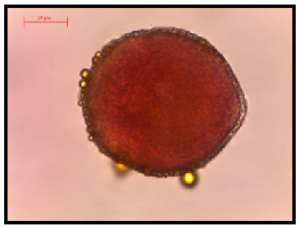


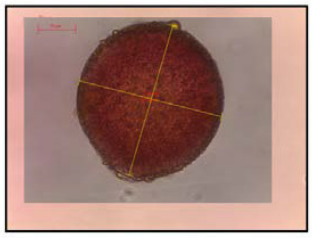
(c)



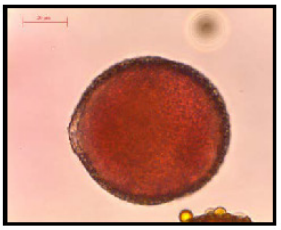
(d)



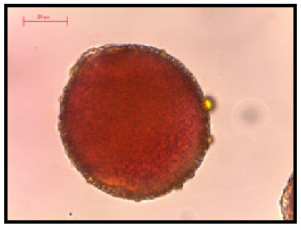
(e)



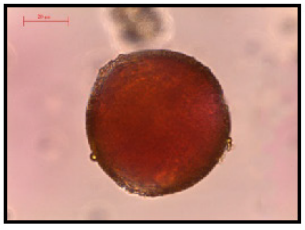
(f)



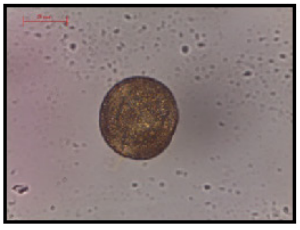
(g)



(h)



(i)



(j)

Plate 18. Pollen grains (100 X) produced by plants which are developed from embryo through pollination with irradiated pollen at various doses of 0 (a), 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), 60 (g), 70 (h), 80 (i) and 90 gray(j).

Table 32. Characteristics of leaf guard cell, pollen grain and number of chloroplasts per guard cell

Irradiation treatment (Gy)	Leaf guard cell			Diameter of pollen grain (Micron)	Stainable pollen (%)
	Length (Micron)	Width (Micron)	Number of chloroplast/cell		
T_0 (0Gy-control)	19.62±1.33[a]	5.38±0.74[a]	12.17±1.19[a]	67.98±1.51[a]	100
T_1(10 Gy)	19.5±0.79[a]	5.64±0.84[a]	12±1.21[a]	68.04±1.49[a]	100
T_2 (20 Gy)	19.52±1.17[a]	5.53±0.71[a]	11.75±0.97[a]	68.44±3.46[a]	100
T_3 (30 Gy)	20.14±0.8[a]	5.52±0.53[a]	11.66±1.55[a]	67.76±4.65[a]	100
T_4 (40 Gy)	20.2±1.51[a]	5.55±0.2[a]	12.17±1.7[a]	69.62±3.52[a]	100
T_5 (50 gy)	19.82±1.51[a]	5.71±0.68[a]	11.58±1[a]	68.36±1.63[a]	100
T_6 (60 Gy)	19.65±1.08[a]	5.63±0.49[a]	11.92±0.67[a]	68.39±1.85[a]	100
T_7 (70 Gy)	20.19±0.48[a]	5.73±0.62[a]	12.33±0.49[a]	68.15±1.82[a]	100
T_8 (80 Gy)	19.94±0.49[a]	5.68±0.47[a]	12.42±0.51[a]	67.41±1.93[a]	100
T_9 (90Gy)	16.23±0.48[b]	4.66±0.2[b]	6.83±0.58[b]	37.34±4.42[b]	0
CD(0.05)	0.83	0.48	0.85	2.31	
CV	5.34	10.86	9.29	4.42	

Mean ± Standard deviation, n=12

T_0 to T_8 and T_9 plants in number of chloroplasts per guard cell was significant. Number of chloroplasts per guard cell in T_0 to T_8 ranged from 11.58 to 12.42. Whereas in T_9, only 6.83 chloroplasts per guard cell were found. In T_0 to T_8, the pollen grain diameter ranged from 67.41 to 69.62 micron. While T_9 recorded the least values of 37.34 micron for the same. All treatments except T_9 (90 Gy) produced stainable pollen which indicated the normal diploid nature of regenerated plants. However, plant regenerated through pollination with irradiated pollen of 90 Gy produced only sterile pollen. Moreover male and female flowers were smaller in T_9 compared to other treatments. It indicated a haploid (n) ploidy level in plant regenerated through pollination with irradiated pollen of 90 Gy. Others were showing diploid ploidy level (T_0 to T_8). Haploid plants were less vigorous and had smaller leaves and flowers with sterile pollen grains when compared to diploid plants. Seed set was not observed when plant regenerated through pollination with irradiated pollen of 90 Gy was self pollinated (Plate 19). Findings in the experiment are in agreement with studies conducted in winter squash by Kurtar and Balkaya (2010) which revealed larger stomatal size in diploids compared to haploids. Average stomata length X width was 21.11 X 17.37 and 30.51 X 21.82 µm in haploids and

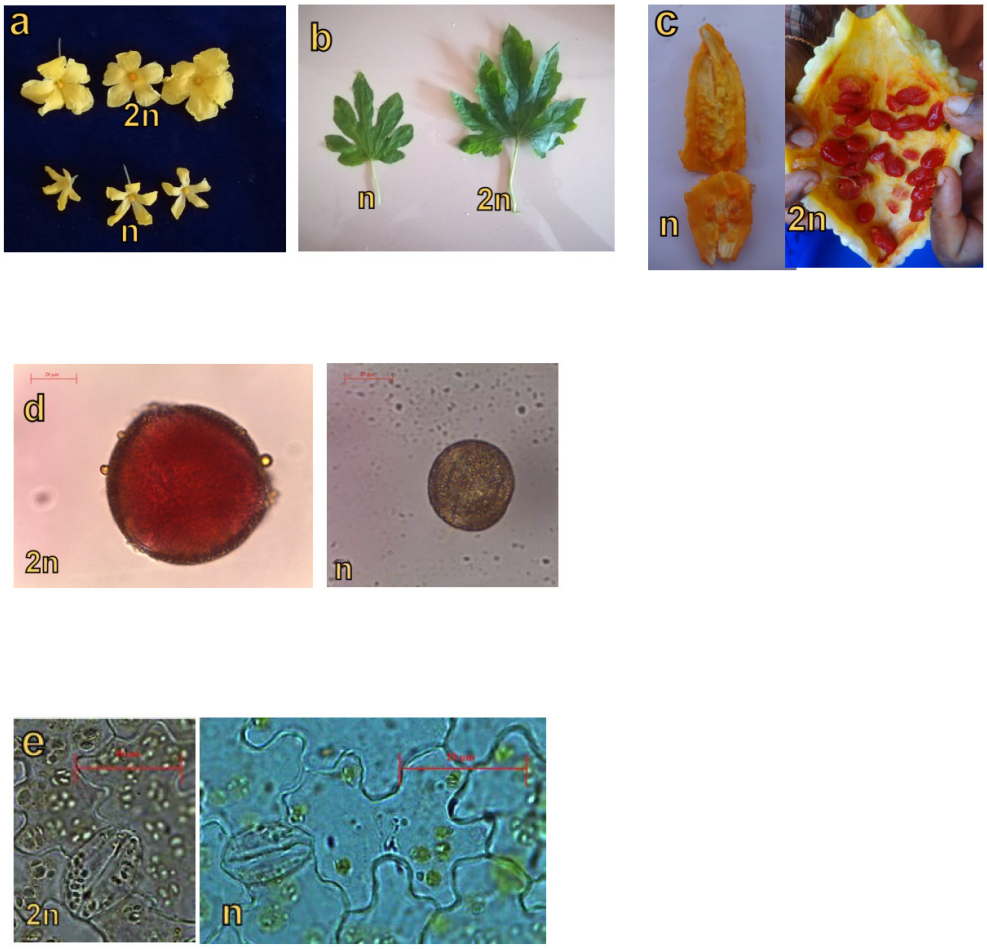


Plate 19. Comparison of diploid and haploid plants.

a - Male flower, b- Leaf, c- Selfed fruits, d - Pollengrain (100 X) , e- Stomatal guard cell (100 X)

diploids, respectively. Average chloroplast numbers in the guard cells of haploid plants were 7.21 and 11.17 chloroplasts were observed in diploid plants.

Godbole and Murthy (2012b) observed that stomatal length, diameter and chloroplast number in guard cells differed between haploid and diploid plants of *Cucumis melo* var *momordica*. The average stomatal length X diameter was 15.15 X 12.3 µm in haploid plants, while in diploid plants stomatal length and diameter were 23.17 X 15.69 µm. Average number of chloroplasts in the guard cells of haploid and diploid plants were 4.37 and 12.25, respectively. According to Dal *et al*. (2016) haploid plants of melon had smaller flowers without pollen and smaller leaves when compared to diploids. The average chloroplast number in the stomata guard cells of the haploid plants was found to be 4–6, while 10–12 chloroplasts were counted in the stomata of diploid plants. Przyborowski and Niemirowicz-Szczytt (1994), Kurtar *et al*. (2002, 2009), Xie *et al*. (2006), Kurtar and Balkaya (2010) and Min *et al*. (2016) used this method to confirm ploidy level in cucurbits. This study showed that stomata measurements and chloroplast scoring methods could be used in bitter gourd to determine ploidy level.

Summary

5. SUMMARY

The present investigation was undertaken to develop superior inbred lines in bitter gourd through advance generation selection of F_1 hybrids and biotechnological approaches through pollination of irradiated pollen and embryo culture. The experiment was conducted in the Department of Vegetable Science, College of Horticulture, Vellanikkara, Kerala Agricultural University.

Evaluation of F_1 hybrids of bitter gourd

Performance evaluation of bitter gourd hybrids was conducted during the year 2016-2017. The experimental material comprised of 16 hybrids and 5 varieties (control) of bitter gourd collected from public and private sectors. Observations were recorded for eighteen characters and ranking of hybrids was done based on cumulative index estimated for characters like, nodes to 1st female flower appearance, days to 1st picking, fruit weight (g), fruit length (cm), fruit diameter (cm), relative early yield (kg), yield per plant (kg) and number of fruits per plant. Significant difference was observed among hybrids for the selected characters. Promising five F_1 hybrids selected based on the cumulative index were MC-142, MC-136, MC-139, MC-138 and MC-133.

Evaluation of F_2 and F_3 generation

F_2 population raised from the selfed fruits of selected hybrids and F_3 families raised from the selfed fruits of selected plants of F_2 population were evaluated. A selection intensity of 5 % was applied based on the characters mentioned above for identifying superior genotypes in F_2 and F_3. The descriptive statistics, namely mean, standard error of mean, standard deviation, variance and range for these two generations were worked out for all the characteristics under study. F_3 family of the hybrid MC-138 was found early compared to its F_2 population for days to first female flower anthesis and days to first harvest. Plants in the F_3 generation of MC-142, MC-

136 and MC-138 produced the first female flower at the lowest node compared to its F_2 generation. Variance also decreased significantly from F_2 to F_3 generation for these characters in MC-138. Earliness parameters like nodes to first male flower, days to first female flower anthesis and day to first harvest was also found significantly decreased in F_3 generation compared to its F_2 in the hybrid MC-133. Production of female flower in lower nodes is an indication of earliness and inbreds in F_3 of the majority of hybrids produced female flowers in lower nodes.

Relative early yield increased in F_3 compared to its F_2 only in the case of the hybrid MC-138. Yield contributing characters like average fruit weight, fruit diameter, fruit girth, flesh thickness and number of harvests were significantly increased in F_3 generation compared to its F_2 in MC-138 and MC-139. Fruit length and yield per plant showed a significant increase in F_3 compared to its F_2 in MC-138. Fruit length, number of fruits per plant, yield per plant and number of harvests significantly reduced in F_3 generation of MC-136 compared to its F_2 generation. Non significant variance was observed between F_2 and F_3 generations of all hybrids for the characters like relative early yield, average fruit weight, fruit diameter, fruit length, fruit girth, flesh thickness, number of fruits per plant and yield per plant. Lower range in F_3 compared to F_2 generation was observed for most of the hybrids for characters like days to first male and female flower, nodes to first male and female flower, number of days from anthesis to fruit maturity, fruit length, fruit diameter, fruit girth, flesh thickness, number of fruit per plant, yield per plant and number of harvests. Lower range in the subsequent generation is an indication of attainment of uniformity for these characters.

Response to selection and selection differential

Nodes to first male flower, days to first female flower, nodes to first female flower and days to first harvest showed negative value for response to selection for all the hybrids studied indicates improvement in earliness in F_3 generation through selection

in F_2. Judicious selection in F_2 generation led to a positive response to selection for the characters like relative early yield, average fruit weight, fruit length, fruit diameter, fruit girth, flesh thickness, number of fruits per plant and yield per plant for most of the hybrids. Selection based on genotypic values may lead to an increase in mean of selected population in next generation resulting in the positive value of response to selection. The difference between genotypes with respect to its response to selection was apparent. Yield per plant, fruit length, fruit weight and relative early yield showed negative value for response to selection on MC-136 and MC-133. F_2 and F_3 generations of all hybrids showed positive value of selection differential for all the yield contributing characters.

Inbreeding depression

Significant negative inbreeding depression was observed in F_2 generation for most of the earliness parameters. But through effective selection in F_2 generation, nodes to first male and female flower in F_3 generation and cumulative inbreeding depression of most of the hybrids showed positive value for inbreeding depression. This clearly suggests that improvement in earliness parameters are in a desirable direction producing male and female flower at earlier nodes. Inbreeding depression in F_2 generation and cumulative inbreeding depression of all hybrids were positively significant for number of days from anthesis to fruit maturity. Inbreeding led to reduction in days taken for fruit maturity from anthesis.

A significant positive inbreeding depression in F_2 and cumulative inbreeding depression for all hybrids observed for relative early yield, fruit length and yield per plant. But through efficient selection procedure carried out in F_2, negatively significant inbreeding depression was noticed in F_3 generation for these characters for all hybrids except MC-136 and MC-133. Negative value of inbreeding depression in the F_3 generation indicates an increase in *per se* performance of F_3 over its F_2 for these characters. Inbreeding depression for average fruit weight was non significant

in F_2 generation of all hybrids except in MC-142. Inbreeding depression in F_3 generation and cumulative inbreeding depression were non significant for all hybrids. It confirmed reduced inbreeding depression of fruit weight in bitter gourd. There was no difference between hybrid and populations at different inbreeding levels (F_2-F_3 progenies) for the average weight. Even though the number of fruits per plant and flesh thickness exhibited positive significant inbreeding depression in F_2 and positive significant cumulative inbreeding depression in all hybrids, their inbreeding depression was negatively significant in F_2 generation except for MC-136. Fruit diameter and fruit girth showed significant positive inbreeding depression in F_2 and significant positive cumulative inbreeding depression except for MC-136 where fruit diameter and fruit girth was non significant and negatively significant respectively for cumulative inbreeding depression. Other Hybrids exhibited negatively significant or nonsignificant inbreeding depression in F_2 only. Fruit girth of MC-136 was not affected by inbreeding in F_2 and F_3 generations. Number of harvests recorded positively significant inbreeding depression in F_2 and positively significant cumulative inbreeding depression for most the hybrids. But a non significant or negatively significant inbreeding depression in F_2 indicated improvement through selection for this character for all hybrids except MC-136 and MC-133.

Even though inbreeding depression was observed in F_2 generation, an increase in *per se* performance was noticed in F_3 generation of hybrids, MC-142, MC-138 and MC-139 for most of the characters studied. But MC-136 and MC-133 could not regain its potential in F_3 through advance generation selection for most of the characters. Loss in vigour after inbreeding was observed for yield per plant, relative early yield per plant, number of harvests and fruit length in MC-136 and MC-133. MC-136 also showed a reduction in flesh thickness and number of fruits per plant after successive self pollination.

Identification of economically important segregants

Desirable economic segregants were chosen on the grounds of earliness and yield contributing characters. A plant in F_2 and F_3 generations was judged as an economic segregant for a character after comparing its performance with the variety Preethi raised in the same season. Per cent of economic segregants for days to first harvest was maximum in F_2 (64.71 %) (48.00-56.00 days) and F_3 generations (100%) (46.00-59.00 days) of the hybrid MC-136. The maximum per cent of economic segregants for average fruit weight was observed in F_2 (64.29%) (144.00-231.50 g) and F_3 generations (85.11%) (150.00-330.10g) of the hybrid MC-138. Economic segregants were not found in F_2 generations of hybrids, MC-142, MC-136 and MC-139. MC-136 showed the highest per cent of economic segregants (94.12%) (29.00-67.00) for number of fruits per plant in F_2 generation. MC-142 exhibited the maximum per cent of economic segregants in F_3 generation for number of fruits per plant (75%) (29.00-62.00). Per cent of economic segregants for yield per plant was the highest in MC-136 (58.82%) (3.54-6.50 kg per plant) and MC-138 (53.19%) (3.83 – 6.57 kg per plant) in F_2 and F_3 generations, respectively. All the selected segregants were superior in performance when compared to Preethi and they can be fixed through inbreeding and selection.

***In situ* induction of maternal haploids initiated by pollination with irradiated pollen (haploid parthenogenesis)**

An efficient protocol for haploid embryo culture has been established in bitter gourd. Male flowers of bitter gourd hybrid MC-139 were irradiated with varying doses of gamma rays (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy). Then pollen from irradiated flowers was used for pollinating female flowers. All irradiation doses from 10 to 100 Gy induced fruit set. The mean number of seeds and embryo per fruit decreased significantly as the irradiation dose increased. The highest number of seeds per fruit (29.13) was recorded in control (0 Gy) and the lowest (18.67) was in fruit

developed through pollinating irradiated pollen of 100 Gy. An increase in the dose of irradiation resulted in a decrease in the total number of developed embryos and the least was observed in T_9 (90 Gy). Treatment T_{10} (100 Gy) failed to produce embryos and all seeds were empty. Results revealed that 90 Gray of gamma-irradiation was successful for haploid recovery. Low irradiation doses were not effective in inducing haploid embryos. The best stage of embryo rescue is 15 days after pollination. E20A medium supplemented with IAA (0.01mg/l) was found as most suitable for embryo culture.

The highest percentage of *in vitro* germinated plants was obtained when seed was used as explants. When the embryo was used as explant, irradiation doses of 80 and 90 gray failed to produce plantlets. Significant reduction in the percentage of *in vitro* germinated plants and increase in the percentage of abnormal seedlings was observed as the irradiation doses increases. Callus formation was observed only in TE_9 (11.11), TE_8 (13.33%) and TE_7 (8.89%) and they were on par.

When the seed was used as explants, the minimum days for leaf emergence was recorded in TS_0 (43.00) (control). The maximum days for leaf emergence were observed in TS_8 (86.33) which were on par with TS_6, TS_7 and TS_9. Days for visible root ranged from 14.66 (TS_0) to 35 days in TS_9 (90 Gy). In case of embryo, it was ranged from 31.67 (TE_0) to 74.67 days (TE_7) and 9.66 to (TE_0) 15.83 days (TE_7) for days for leaf emergence and days for visible root respectively.

In vitro rooting was obtained from E20A+ IAA (0.01mg/l) medium supplemented with activated charcoal (3g/lit) which took 7.2 days for root initiation and six number of roots were produced after 3 weeks of culture. Addition of activated charcoal in the rooting medium was found to enhance root initiation.

The highest percentage of plants successfully rooted after hardening was noticed in control (81.57%) followed by T_1 (79.10%), T_2 (71.18%), T_3 (70.90%) and T_4 (69.04%). The lowest percentage was observed in T_9 (90Gy). Haploid plant took 47 and 49 days to first male and female flower emergence respectively.

Guard cell size, number of chloroplasts per guard cell and pollen grain characteristics can be taken as criteria to determine the ploidy level. Diploid and haploid plants recorded significant differences for these characters. T_9 (90 Gy) exhibited significantly lower values for all these traits. T_0 to T_8 plants showed a larger guard cell size where the guard cell length and width ranged from 19.50 to 20.20 and 5.38 to 5.73 micron respectively while in T_9, plants produced guard cells of smaller size and it had 16.23 and 4.66 micron length and width respectively. As for guard cell dimension, the difference between T_0 to T_8 and T_9 plants in number of chloroplasts per guard cell was significant. Number of chloroplasts per guard cell in T_0 to T_8 ranged from 11.58 to 12.42. Whereas in T_9, only 6.83 number of chloroplasts per guard cell were found. In T_0 to T_8, the pollen grain diameter ranged from 67.41 to 69.62 micron. While T_9 recorded the least values of 37.34 micron for the same. All treatments except T_9 (90 Gy) produced stainable pollen which indicated the normal diploid nature of regenerated plants. However, plant regenerated through pollination with irradiated pollen of 90 Gy produced only sterile pollen. Moreover male and female flowers were smaller in T_9 compared to other treatments. Seed set was not observed when plant regenerated through pollination with irradiated pollen of 90 Gy was self pollinated. It indicated a haploid (n) ploidy level in plant regenerated through pollination with irradiated pollen of 90 Gy. Others were showing diploid ploidy level (T_0 to T_8). Present protocol can be adopted for generating haploids in bitter gourd.

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Abstract

Development of inbreds in bitter gourd (*Momordica charantia* L.) through conventional and biotechnological approaches

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ABSTRACT

The present investigation was undertaken to develop superior inbred lines in bitter gourd through advance generation selection of F_1 hybrids and biotechnological approaches through pollination of irradiated pollen and embryo rescue. The experiment was conducted at the Department of Vegetable Science, College of Horticulture, Kerala Agricultural University during the year 2016-2018.

Performance evaluation of sixteen hybrids and five varieties (control) of bitter gourd was conducted and five promising hybrids, MC-142, MC-136, MC-139, MC-138 and MC-133, were selected based on the cumulative index. These hybrids were advanced to F_2 and F_3 generation. Yield contributing characters such as average fruit weight, fruit diameter, fruit girth, flesh thickness and number of harvests were significantly increased in F_3 generation compared to its F_2 in MC-138 and MC-139. Fruit length, relative early yield and yield per plant showed a significant increase in F_3 compared to its F_2 in MC-138. Fruit length, number of fruits per plant, yield per plant and number of harvests have significantly reduced in F_3 generation of MC-136 compared to its F_2 generation. Nonsignificant variance was observed between F_2 and F_3 generations of all hybrids for majority of the yield contributing characters. Lower range in the subsequent generation is an indication of attainment of uniformity for these characters.

Negative value of response to selection for earliness parameters have indicated an improvement in the characters in F_3 generation through selection in F_2. Judicious selection in F_2 generation led to a positive response to selection for yield contributing characters in most of the hybrids. The difference between genotypes

with respect to its response to selection was apparent. Yield per plant, fruit length, fruit weight and relative early yield have shown negative value for response to selection on MC-136 and MC-133. F_2 and F_3 generations of all hybrids showed positive value of selection differential for all the yield contributing characters.

Positive value of inbreeding depression for nodes to first male and female flower in F_3 generation and cumulative inbreeding depression for most of the hybrids indicated that the improvement in earliness parameters are in a desirable direction, producing male and female flowers at earlier nodes. Inbreeding led to a reduction in the number of days taken for fruit maturity from anthesis in all hybrids. Even though inbreeding depression was observed in F_2 generation, an increase in *per se* performance was noticed in F_3 generation of hybrids, MC-142, MC-138 and MC-139 for most of the yield contributing characters. But MC-136 and MC-133 could not regain its potential in F_3 through advance generation selection for most of the characters. Loss in vigour after inbreeding was observed for yield per plant, relative early yield per plant, number of harvests and fruit length in MC-136 and MC-133. After successive self pollination, MC-136 also had shown a reduction in flesh thickness and the number of fruits per plant.

Desirable economic segregants were chosen based on earliness and yield contributing characters. Per cent of economic segregants for days to first harvest was the maximum in F_2 (64.71 %) (48.00-56.00 days) and F_3 generations (100%) (46.00-59.00 days) of the hybrid MC-136. Per cent of economic segregants for yield per plant was the highest in MC-136 (58.82%) (3.54-6.50 kg per plant) and MC-138 (53.19%) (3.83-6.57 kg per plant) in F_2 and F_3 generations, respectively. Selected segregants can be fixed through inbreeding and selection.

An efficient protocol for *in vitro* haploid embryo culture was standardized in bitter gourd. Pollen from irradiated flowers was used for pollinating female flowers. All irradiation doses from 10 to 100 Gy induced fruit set in the hybrid MC-139. The mean number of seeds and embryos per fruit decreased significantly as the irradiation

dose increased. Treatment T_{10} (100 Gy) failed to produce embryos and all seeds were empty. The results showed that 90 Gy was the best irradiation dose and 15 days after pollination was the best stage of embryo rescue for haploid recovery. The *in vitro* seed germination in E20A medium was best for embryo culture.Addition of activated charcoal (3g/ L) in E20A medium has enhanced the root initiation.

The highest percentage of successful plants after 20 days of hardening was noticed in control (81.57 %) followed by T_1 (79.10 %), T_2 (71.18 %), T_3 (70.90 %) and T_4 (69.04 %). Significant difference was observed for the plant under the treatment T_9 with respect to size of guard cell, pollen grain diameter and number of chloroplasts per guard cell compared to the plants under the treatments T_0 to $T_{8.}$ The guard cells in haploid plants had 16.23 micron length, 4.66 micron width, 37.34 micron pollen grain diameter, and contained 6.83 chloroplasts whereas guard cells in diploids had 19.50 to 20.20 micron length, 5.38 to 5.73 micron width, 67.41 to 69.62 micron pollen grain diameter and 11.58 to 12.42 chloroplasts. All other treatments except T_9 (90 Gy) produced stainable pollen which indicated the normal diploid nature of plants. Plant developed through pollination with irradiated pollen of 90 Gy (T_9) produced only sterile pollen. Moreover, seed set was not observed when it was self pollinated which can be attributed to the haploid (n) status of the plant. Others were showing diploid ploidy level (T_0 to T_8). The present protocol is a successful strategy for generating haploids in bitter gourd.

In conclusion, results show that inbreeding and selection are efficient to improve the earliness and yield characters in bitter gourd. Pollination by gamma irradiated pollen can induce haploid embryo development in bitter gourd and this is the first successful report in bitter gourd on this aspect.

പരമ്പരാഗത രീതിയിലൂടെയും ജൈവസാങ്കേന്തിക വിദ്യയിലൂടെയും പാവലിലെ മികച്ച നൈസർഗ്ഗിക ഇനങ്ങളെ (inbreds) കണ്ടെത്തുക

സംക്ഷിപ്തം

വംശശുദ്ധീകരണം വഴിയും ഗാമവികിരണത്തിനു വിധേയമായ പരാഗരേണു വഴിയുള്ള കൃത്രിമ പരാഗണവും തുടർന്നുള്ള എബ്രിയോ റെസ്ക്യൂ മുഖാന്തരവും പാവലിലെ മികച്ച നൈസർഗ്ഗിക ഇനങ്ങളെ (inbreds) വികസിപ്പിക്കുന്നതിനായി കേരള കാർഷിക സർവ്വകലാശാലയുടെ കീഴിലുള്ള ഹോർട്ടികൾച്ചർ കോളേജിലെ പച്ചക്കറിശാസ്ത്രവിഭാഗത്തിൽ 2016 - 2018 കാലയളവിൽ പഠനം നടത്തുകയുണ്ടായി.

പാവക്കയുടെ പതിനാറ് സങ്കരയിനങ്ങളും 5 ഇനങ്ങളും പഠനവിധേയമാക്കുകയും ക്യുമുലേറ്റീവ് സൂചികയുടെ അടിസ്ഥാനത്തിൽ 5 മികച്ച സങ്കരയിനങ്ങളായ എംസി-142, എംസി-136, എംസി-139, എംസി-138, എംസി-133 എന്നിവ തിരഞ്ഞെടുക്കുകയും അവയുടെ F_2, F_3 എന്നീ തലമുറകൾ വികസിപ്പിക്കുകയും ചെയ്തു.

എംസി-138 ൻ്റെയും എംസി-139 ൻ്റെയും F_2 തലമുറയെ അപേക്ഷിച്ച് F_3 തലമുറയിൽ കായയുടെ ശരാശരി തൂക്കം, വ്യാസം, മാംസത്തിന്റെ കനം, വിളവെടുപ്പുകളുടെ എണ്ണം എന്നിവയിൽ ഗണ്യമായ വർധനവ് കണ്ടു. കായയുടെ നീളം, ആദ്യകാല വിളവ്, മൊത്തവിളവ് എന്നിവ എംസി-138 ൻ്റെ F_3 തലമുറയിൽ ഗണ്യമായി വർധിച്ചു. കായയുടെ നീളം, ഒരു ചെടിയിലെ മൊത്തം കായകളുടെ എണ്ണം, വിളവ്, വിളവെടുപ്പുകളുടെ എണ്ണം എന്നിവയ്ക്കു എംസി-136 ൻ്റെ F_3 തലമുറയിൽ F_2 തലമുറയെ അപേക്ഷിച്ച് സാരമായ കുറവ് കണ്ടു. അപ്രധാനമായ വൈജാത്യമാണ് F_2, F_3 എന്നീ തലമുറകൾ തമ്മിൽ വിളവുമായി ബന്ധപ്പെട്ട ഭൂരിഭാഗം ഘടകങ്ങൾക്കും കാണാനായത്.

F_2 തലമുറയെ അപേക്ഷിച്ച് F_3 തലമുറയിൽപ്പെട്ട ചെടികൾ നേരത്തെ പുഷ്പിക്കുകയും കായ്ഫലം തരികയും ചെയ്തു. അവയിൽ കായയുടെ തൂക്കം, നീളം, വീതി, വിളവ്, ആദ്യകാല വിളവ്, കായ്കളുടെ എണ്ണം എന്നിവ ഉയർന്നു നിൽക്കുന്നതായി കണ്ടെത്തി. എന്നാൽ എല്ലാ സങ്കരയിനങ്ങളുടെ F_3 തലമുറയിലും സമാനമായ പ്രവണത

പ്രകടമായിരുന്നില്ല. വിളവുമായി ബന്ധപ്പെട്ട എല്ലാ ഘടകങ്ങൾക്കും F_2, F_3 എന്നീ തലമുറകളിൽ സെലക്ഷൻ ഡിഫറെൻഷ്യലിനു പോസിറ്റീവ് മൂല്യമായിരുന്നു.

F_3 തലമുറയിലെ ഇൻബ്രീഡിങ് ഡിപ്രെഷൻ, ക്യുമുലേറ്റീവ് ഇൻബ്രീഡിങ് ഡിപ്രെഷൻ എന്നിവ ആദ്യത്തെ ആൺ-പെൺ പൂക്കളുടെ നോഡുകളുടെ സ്ഥാനത്തിന് പോസിറ്റീവ് മൂല്യം നൽകുക വഴി ഇവയുടെ പ്രകടനം തലമുറ കഴിയുന്തോറും അഭികാമ്യമായ ദിശയിലാണെന്നു മനസിലായി. F_2 തലമുറയിൽ ഇൻബ്രീഡിങ് ഡിപ്രെഷൻ കണ്ടുവെങ്കിലും എംസി-142, എംസി-139, എംസി-138 എന്നീ സങ്കരയിനങ്ങളുടെ F_3 തലമുറയിൽ പ്രകടനമിഴവു കാണുവാൻ സാധിച്ചു. എന്നാൽ എംസി-136, എംസി-133 എന്നീ സങ്കരയിനങ്ങളിൽ ഇത് പ്രകടമായിരുന്നില്ല.

ആദ്യ വിളവെടുപ്പിനുള്ള കാലയളവ് പരിശോധിച്ചാൽ ഇക്കണോമിക് സെഗ്രിഗെന്റ്സ്ൻ്റെ ശതമാനം കൂടുതലായി കാണുന്നത് എംസി-136 എന്ന സങ്കരയിനത്തിൻ്റെ F_2, F_3 എന്നീ തലമുറകളിലാണ്. വിളവിൻ്റെ കാര്യത്തിലാണെങ്കിൽ അധിക ശതമാനം കണ്ടത് എംസി-136 ൻ്റെ F_2 തലമുറയിലും എംസി-138 ൻ്റെ F_3 തലമുറയിലുമാണ്. നല്ല പ്രകടനം കാഴ്ച വെയ്ക്കുന്ന ഇത്തരം നൈസർഗ്ഗിക ഇനങ്ങളെ വംശശുദ്ധീകരണത്തിനു വിധേയമാക്കാവുന്നതാണ്.

പത്തു മുതൽ നൂറ് ഗ്രേ വരെ ഡോസുള്ള ഗാമവികിരണങ്ങൾക്ക് വിധേയമായ ആൺ പൂക്കൾ ഉപയോഗിച്ച് പാവലിൻ്റെ സങ്കരയിനമായ എംസി-139ൽ കൃത്രിമ പരാഗണം നടത്തുകയുണ്ടായി. എല്ലാ റേഡിയേഷൻ ഡോസുകളിലും കായപിടിത്തം ഉണ്ടായി .റേഡിയേഷൻ അളവ് വർധിക്കുന്നതിനനുസരിച്ചു ഓരോ കായയിലും ഉണ്ടായ വിത്തുകളുടെയും ഭ്രൂണങ്ങളുടെയും ശരാശരി എണ്ണത്തിൽ ഗണ്യമായ കുറവ് കാണപ്പെട്ടു. 100 ഗ്രേ വികിരണങ്ങൾക്ക് വിധേയമായ ആൺ പൂക്കൾ പരാഗണം നടത്തിയുണ്ടായ കായയിലെ എല്ലാ വിത്തുകളും ശൂന്യമായിരുന്നു. ഭ്രൂണത്തിന്റെ സാന്നിധ്യം കണ്ടെത്തുവാൻ കഴിഞ്ഞില്ല.

90 ഗ്രേ മികച്ച റേഡിയേഷൻ ഡോസാണെന്നും കൃത്രിമപരഗണത്തിനു 15 ദിവസത്തിനുശേഷമുള്ള ഘട്ടമാണ്

ഹാപ്ലോയ്ഡ് എംബ്രിയോ റെസ്ക്യൂന് അനുയോജ്യമെന്നും പരീക്ഷണത്തിൽ തെളിഞ്ഞു. E20A എന്ന മാധ്യമത്തിൽ വിത്ത് മുളപ്പിക്കുന്ന രീതിയാണ് എംബ്രിയോ കൾച്ചറിനു അനുയോജ്യം .കരി (മൂന്ന് ഗ്രാം ഒരു ലിറ്ററിന്) ഈ മാധ്യമത്തിൽ ചേർക്കുന്നത് വേരുപിടിപ്പിക്കുന്നതിന് ഫലപ്രദമാണെന്ന് കണ്ടെത്തി.

90 ഗ്രേ വികിരണത്തിനു വിധേയമായ ആൺ പൂക്കൾ പരാഗണം നടത്തി എംബ്രിയോ കൾച്ചർ വഴി വികസിപ്പിച്ചെടുത്ത ചെടിയുടെ (T_9) ഗാർഡ് കോശങ്ങളുടെ വലുപ്പം,പരാഗരേണുവിൻറെ വ്യാസം, ഹരിത കണങ്ങളുടെ എണ്ണം എന്നിവ മറ്റു റേഡിയേഷൻ ഡോസുകൾക്ക് വിധേയമായി വികസിപ്പിച്ചെടുത്ത ചെടികളുമായി (T_0 മുതൽ T_8) താരതമ്യം ചെയ്യുമ്പോൾ ഗണ്യമായി കുറഞ്ഞതായി കണ്ടെത്തി. T_9 (90 ഗ്രേ) ഒഴികെയുള്ള മറ്റെല്ലാ ട്രീട്മെന്റുകളിലും പരാഗരേണുവിന് അസെറ്റോക്കർമിനുമായി പ്രവർത്തിച്ചപ്പോൾ ചുവപ്പു നിറം ലഭിക്കുന്നതായി കണ്ടു. എന്നാൽ 90 ഗ്രേ വികിരണത്തിന് വിധേയമായ ആൺ പൂക്കൾ പരാഗണം നടത്തി എംബ്രിയോ കൾച്ചർ വഴി വികസിപ്പിച്ചെടുത്ത ചെടി ഉദ്പാദിപ്പിച്ച പരാഗരേണുക്കൾ എല്ലാം തന്നെ വന്ധ്യമായതായിരുന്നു. മാത്രമല്ല, സ്വയംപരാഗണത്തിലൂടെയുണ്ടായ കായയിൽ വിത്തുകൾ ഉണ്ടായിരുന്നില്ല. ഇവയെല്ലാം തന്നെ ചെടിയുടെ ഹാപ്ലോയ്ഡ് സ്വഭാവത്തെ സൂചിപ്പിക്കുന്നു. പാവക്കയിൽ ഹാപ്ലോയ്ഡ് ചെടികൾ ഈ രീതിയിൽ വിജയകരമായി ഉദ്പാദിപ്പിച്ചെടുക്കാവുന്നതാണ്.

വംശശുദ്ധീകരണം വഴിയും ജൈവസാങ്കേന്തിക വിദ്യയുപയോഗിച്ചും പാവക്കയിൽ മികച്ച നൈസർഗ്ഗിക ഇനങ്ങളെ (inbreds) വാർത്തെടുക്കാം.

Annexure

Annexure 1

Incidence of pest and diseases in F_1, F_2 and F_3 generations of bitter gourd genotypes

Incidence of pest and diseases in F_1 generation of bitter gourd genotypes

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** *(Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-131	Very low	Very low	Moderate	Nil
MC-132	Very low	Mild	Severe	Nil
MC-133	Very low	Mild	Moderate	Nil
MC-134	Very low	Very low	Mild	Nil
MC-135	Very low	Mild	Mild	Nil
MC-136	Very low	Mild	Mild	Nil
MC-137	Very low	Mild	Mild	Nil
MC-138	Very low	Very low	Mild	Nil
MC-139	Very low	Very low	Mild	Nil
MC-140	Very low	Mild	Mild	Nil
MC-141	Very low	Mild	Mild	Nil
MC-142	Very low	Very low	Mild	Nil
MC-143	Very low	Mild	Mild	Nil
MC-144	Very low	Mild	Mild	Very low
MC-145	Very low	Mild	Mild	Nil
MC-146	Very low	Mild	Severe	Nil
Control				
MC-147	Very low	Mild	Mild	Nil
MC-148	Very low	Very low	Mild	Nil
Preethi	Very low	Mild	Mild	Nil
Priya	Very low	Very low	Mild	Nil
Priyanka	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_2 generation of bitter gourd hybrid MC-142

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides)*	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-142-1	Very low	Very low	Mild	Nil
MC-142-2	Very low	Very low	Mild	Nil
MC-142-3	Very low	Very low	Mild	Nil
MC-142-4	Very low	Very low	Mild	Nil
MC-142-5	Very low	Very low	Mild	Nil
MC-142-6	Very low	Very low	Mild	Nil
MC-142-7	Very low	Very low	Mild	Nil
MC-142-8	Very low	Very low	Moderate	Nil
MC-142-9	Very low	Very low	Mild	Nil
MC-142-10	Nil	Very low	Mild	Nil
MC-142-11	Very low	Very low	Very low	Nil
MC-142-12	Very low	Very low	Mild	Nil
MC-142-13	Very low	Very low	Mild	Nil
MC-142-14	Very low	Mild	Moderate	Nil
MC-142-15	Very low	Very low	Moderate	Nil
MC-142-16	Very low	Very low	Mild	Nil
MC-142-17	Very low	Very low	Mild	Nil
MC-142-18	Very low	Very low	Mild	Nil
MC-142-19	Very low	Very low	Mild	Nil
MC-142-20	Mild	Very low	Mild	Nil
MC-142-21	Very low	Mild	Mild	Nil
MC-142-22	Very low	Very low	Moderate	Nil
MC-142-23	Very low	Very low	Mild	Nil
MC-142-24	Very low	Very low	Mild	Nil
MC-142-25	Very low	Very low	Moderate	Nil
MC-142-26	Very low	Very low	Mild	Nil
MC-142-27	Very low	Very low	Moderate	Nil
MC-142-28	Very low	Very low	Mild	Nil
MC-142-29	Very low	Very low	Mild	Nil
MC-142-30	Very low	Very low	Mild	Nil
MC-142-31	Very low	Very low	Mild	Nil
MC-142-32	Very low	Mild	Mild	Nil
MC-142-33	Very low	Very low	Moderate	Nil
MC-142-34	Very low	Very low	Mild	Nil
MC-142-35	Very low	Very low	Mild	Nil
MC-142-36	Very low	Very low	Mild	Nil
MC-142-37	Very low	Very low	Mild	Nil
MC-142-38	Very low	Very low	Mild	Nil
MC-142-39	Very low	Very low	Mild	Nil
MC-142-40	Very low	Very low	Mild	Nil
MC-142-41	Very low	Very low	Mild	Nil
MC-142-42	Very low	Very low	Very low	Nil

MC-142-43	Very low	Very low	Mild	Nil
MC-142-44	Very low	Very low	Moderate	Nil
MC-142-45	Very low	Very low	Mild	Nil
MC-142-46	Very low	Very low	Mild	Nil
MC-142-47	Very low	Very low	Mild	Nil
MC-142-48	Very low	Very low	Mild	Nil
MC-142-49	Very low	Very low	Moderate	Nil
MC-142-50	Very low	Very low	Moderate	Nil
MC-142-51	Very low	Very low	Mild	Nil
MC-142-52	Very low	Very low	Mild	Nil
MC-142-53	Very low	Very low	Very low	Nil
MC-142-54	Very low	Very low	Very low	Nil
MC-142-55	Very low	Very low	Mild	Nil
MC-142-56	Very low	Very low	Mild	Nil
MC-142-57	Very low	Very low	Mild	Nil
MC-142-58	Very low	Very low	Mild	Nil
MC-142-59	Very low	Very low	Mild	Nil
MC-142-60	Very low	Very low	Moderate	Nil

Incidence of pest and diseases in F_3 generation of bitter gourd hybrid MC-142

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-142-2-1	Very low	Very low	Mild	Nil
MC-142-2-2	Very low	Very low	Mild	Nil
MC-142-2-3	Very low	Very low	Mild	Nil
MC-142-2-4	Very low	Very low	Mild	Nil
MC-142-2-5	Very low	Very low	Very low	Nil
MC-142-2-6	Very low	Very low	Mild	Nil
MC-142-2-7	Very low	Very low	Mild	Nil
MC-142-2-8	Very low	Very low	Mild	Nil
MC-142-2-9	Very low	Very low	Mild	Nil
MC-142-2-10	Very low	Very low	Mild	Nil
MC-142-2-11	Very low	Very low	Mild	Nil
MC-142-2-12	Very low	Very low	Mild	Nil
MC-142-2-13	Very low	Very low	Mild	Very low
MC-142-2-14	Very low	Very low	Mild	Very low
MC-142-2-15	Very low	Very low	Mild	Nil
MC-142-2-16	Very low	Very low	Mild	Nil
MC-142-2-17	Very low	Very low	Mild	Nil
MC-142-2-18	Very low	Very low	Mild	Nil
MC-142-2-19	Very low	Very low	Moderate	Nil
MC-142-2-20	Very low	Very low	Moderate	Very low
MC-142-5-1	Very low	Very low	Moderate	Nil
MC-142-5-2	Very low	Very low	Mild	Nil
MC-142-5-3	Very low	Very low	Mild	Nil
MC-142-5-4	Very low	Very low	Moderate	Nil
MC-142-5-5	Very low	Very low	Very low	Nil
MC-142-5-6	Very low	Very low	Moderate	Nil
MC-142-5-7	Very low	Very low	Moderate	Nil
MC-142-5-8	Very low	Very low	Moderate	Very low
MC-142-5-9	Very low	Very low	Mild	Nil
MC-142-5-10	Very low	Very low	Moderate	Nil
MC-142-5-11	Very low	Very low	Mild	Very low
MC-142-5-12	Very low	Very low	Mild	Nil
MC-142-5-13	Very low	Very low	Moderate	Nil
MC-142-5-14	Very low	Very low	Moderate	Nil
MC-142-5-15	Very low	Very low	Mild	Nil
MC-142-5-16	Very low	Very low	Very Low	Nil
MC-142-5-17	Very low	Very low	Mild	Nil
MC-142-5-18	Very low	Very low	Mild	Nil
MC-142-5-19	Very low	Very low	Very Low	Nil
MC-142-5-20	Very low	Very low	Mild	Nil
MC-142-5-21	Very low	Very low	Moderate	Nil
MC-142-5-22	Very low	Very low	Mild	Very low

MC-142-5-23	Very low	Very low	Mild	Nil
MC-142-5-24	Very low	Very low	Modeate	Very low
MC-142-5-25	Very low	Very low	Mild	Nil
MC-142-5-26	Very low	Very low	Mild	Very low
MC-142-5-27	Very low	Very low	Mild	Nil
MC-142-5-28	Very low	Very low	Mild	Nil
MC-142-5-29	Very low	Very low	Mild	Nil
MC-142-5-30	Very low	Very low	Mild	Nil
MC-142-5-31	Very low	Very low	Mild	Nil
MC-142-5-32	Very low	Very low	Mild	Nil
MC-142-5-33	Very low	Very low	Mild	Nil
MC-142-5-34	Very low	Very low	Moderate	Very low
MC-142-5-35	Very low	Very low	Mild	Very low
MC-142-5-36	Very low	Very low	Mild	Nil
MC-142-5-37	Very low	Very low	Modeate	Nil
MC-142-5-38	Very low	Very low	Mild	Nil
MC-142-5-39	Very low	Very low	Moderate	Nil
MC-142-5-40	Very low	Very low	Moderate	Very low
MC-142-5-41	Very low	Very low	Mild	Nil
MC-142-5-42	Very low	Very low	Modeate	Nil
MC-142-5-43	Very low	Very low	Mild	Nil
MC-142-5-44	Very low	Very low	Mild	Nil
MC-142-5-45	Very low	Very low	Mild	Nil
MC-142-5-46	Very low	Very low	Mild	Nil
MC-142-5-47	Very low	Very low	Mild	Nil
MC-142-5-48	Very low	Very low	Mild	Nil
MC-142-5-49	Very low	Very low	Mild	Nil
MC-142-5-50	Very low	Very low	Mild	Nil
MC-142-5-51	Very low	Very low	Moderate	Nil
MC-142-5-52	Very low	Very low	Moderate	Nil
MC-142-5-53	Very low	Very low	Moderate	Nil
MC-142-5-54	Very low	Very low	Mild	Nil
MC-142-5-55	Very low	Very low	Mild	Nil
MC-142-5-56	Very low	Very low	Mild	Nil
MC-142-19-1	Very low	Very low	Mild	Nil
MC-142-19-2	Very low	Very low	Mild	Nil
MC-142-19-3	Very low	Very low	Mild	Nil
MC-142-19-4	Very low	Very low	Mild	Nil
MC-142-19-5	Very low	Very low	Mild	Nil
MC-142-19-6	Very low	Very low	Mild	Nil
MC-142-19-7	Very low	Very low	Mild	Nil
MC-142-19-8	Very low	Very low	Moderate	Nil
MC-142-19-9	Very low	Very low	Very low	Nil
MC-142-19-10	Very low	Very low	Mild	Nil
MC-142-19-11	Very low	Very low	Mild	Nil
MC-142-19-12	Very low	Very low	Mild	Very low
MC-142-19-13	Very low	Very low	Moderate	Nil

MC-142-19-14	Very low	Very low	Modeate	Nil
MC-142-19-15	Very low	Very low	Mild	Nil
MC-142-19-16	Very low	Very low	Mild	Nil
MC-142-19-17	Very low	Very low	Very low	Very low
MC-142-19-18	Very low	Very low	Modeate	Nil
MC-142-19-19	Very low	Very low	Mild	Nil
MC-142-19-20	Very low	Very low	Mild	Nil
MC-142-19-21	Very low	Very low	Very low	Nil
MC-142-19-22	Very low	Very low	Very low	Nil
MC-142-19-23	Very low	Very low	Very low	Nil
MC-142-19-24	Very low	Very low	Very low	Nil

Incidence of pest and diseases in F_2 generation of bitter gourd hybrid MC-136

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-136 -1	Very low	Very low	Mild	Nil
MC-136 -2	Very low	Very low	Moderate	Nil
MC-136 -3	Very low	Very low	Mild	Nil
MC-136 -4	Very low	Very low	Mild	Nil
MC-136 -5	Mild	Mild	Severe	Nil
MC-136 -6	Very low	Very low	Mild	Nil
MC-136 -7	Very low	Very low	Mild	Nil
MC-136 -8	Very low	Very low	Mild	Nil
MC-136 -9	Very low	Very low	Mild	Nil
MC-136 -10	Very low	Very low	Mild	Nil
MC-136 -11	Very low	Very low	Mild	Nil
MC-136 -12	Very low	Very low	Mild	Nil
MC-136 -13	Very low	Very low	Mild	Nil
MC-136 -14	Very low	Very low	Mild	Nil
MC-136 -15	Very low	Very low	Moderate	Nil
MC-136 -16	Very low	Very low	Mild	Nil
MC-136 -17	Very low	Very low	Moderate	Nil
MC-136 -18	Very low	Very low	Moderate	Nil
MC-136 -19	Very low	Very low	Moderate	Nil
MC-136 -20	Very low	Very low	Mild	Nil
MC-136 -21	Very low	Very low	Moderate	Nil
MC-136 -22	Very low	Very low	Moderate	Nil
MC-136 -23	Very low	Very low	Mild	Nil
MC-136 -24	Very low	Very low	Mild	Nil
MC-136 -25	Very low	Very low	Mild	Nil
MC-136 -26	Very low	Very low	Mild	Nil
MC-136 -27	Very low	Very low	Mild	Nil
MC-136 -28	Very low	Very low	Mild	Nil
MC-136 -29	Very low	Very low	Mild	Nil
MC-136 -30	Very low	Very low	Mild	Nil
MC-136 -31	Very low	Very low	Mild	Nil
MC-136 -32	Very low	Very low	Moderate	Nil
MC-136 -33	Very low	Very low	Mild	Nil
MC-136 -34	Very low	Very low	Mild	Nil
MC-136 -35	Very low	Very low	Moderate	Nil
MC-136 -36	Very low	Very low	Mild	Nil
MC-136 -37	Very low	Very low	Mild	Nil
MC-136 -38	Very low	Very low	Mild	Nil
MC-136 -39	Very low	Very low	Mild	Nil
MC-136 -40	Very low	Very low	Mild	Nil
MC-136 -41	Very low	Very low	Mild	Nil
MC-136 -42	Very low	Very low	Moderate	Nil

MC-136 -43	Very low	Very low	Moderate	Nil
MC-136 -44	Very low	Very low	Mild	Nil
MC-136 -45	Very low	Very low	Mild	Nil
MC-136 -46	Very low	Very low	Mild	Nil
MC-136 -47	Very low	Very low	Moderate	Nil
MC-136 -48	Very low	Very low	Mild	Nil
MC-136 -49	Very low	Very low	Mild	Nil
MC-136 -50	Very low	Very low	Moderate	Nil

Incidence of pest and diseases in F_3 generation of bitter gourd hybrid MC-136

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-136-10-1	Very low	Very low	Mild	Nil
MC-136-10-2	Very low	Very low	Mild	Nil
MC-136-10-3	Very low	Very low	Mild	Nil
MC-136-10-4	Very low	Very low	Mild	Nil
MC-136-10-5	Mild	Very low	Mild	Nil
MC-136-10-6	Very low	Very low	Mild	Nil
MC-136-10-7	Very low	Very low	Mild	Nil
MC-136-10-8	Very low	Very low	Mild	Nil
MC-136-10-9	Very low	Very low	Mild	Nil
MC-136-10-10	Very low	Very low	Mild	Nil
MC-136-10-11	Very low	Very low	Mild	Nil
MC-136-10-12	Very low	Very low	Mild	Nil
MC-136-10-13	Very low	Very low	Mild	Nil
MC-136-10-14	Mild	Very low	Mild	Nil
MC-136-10-15	Mild	Very low	Mild	Nil
MC-136-10-16	Very low	Very low	Mild	Nil
MC-136-10-17	Very low	Very low	Mild	Nil
MC-136-10-18	Very low	Very low	Mild	Nil
MC-136-10-19	Very low	Very low	Mild	Nil
MC-136-10-20	Very low	Very low	Mild	Nil
MC-136-10-21	Very low	Very low	Mild	Nil
MC-136-10-22	Very low	Very low	Mild	Nil
MC-136-10-23	Very low	Very low	Mild	Nil
MC-136-10-24	Very low	Very low	Mild	Nil
MC-136-10-25	Very low	Very low	Mild	Nil
MC-136-10-26	Very low	Very low	Mild	Nil
MC-136-10-27	Very low	Very low	Mild	Nil
MC-136-10-28	Very low	Very low	Mild	Nil
MC-136-10-29	Very low	Very low	Mild	Nil
MC-136-10-30	Very low	Very low	Mild	Nil
MC-136-10-31	Very low	Very low	Mild	Nil
MC-136-10-32	Very low	Very low	Mild	Nil
MC-136-10-33	Very low	Very low	Mild	Nil
MC-136-10-34	Very low	Very low	Mild	Nil
MC-136-10-35	Very low	Very low	Mild	Nil
MC-136-10-36	Very low	Very low	Mild	Nil
MC-136-10-37	Very low	Very low	Mild	Nil
MC-136-10-38	Very low	Very low	Mild	Nil
MC-136-10-39	Very low	Very low	Mild	Nil
MC-136-10-40	Very low	Very low	Mild	Nil
MC-136-10-41	Very low	Very low	Mild	Nil
MC-136-10-42	Very low	Very low	Mild	Nil

MC-136-10-43	Very low	Very low	Mild	Nil
MC-136-10-44	Very low	Very low	Mild	Nil
MC-136-10-45	Very low	Very low	Mild	Nil
MC-136-10-46	Very low	Very low	Mild	Nil
MC-136-10-47	Very low	Very low	Mild	Nil
MC-136-10-48	Mild	Very low	Mild	Nil
MC-136-10-49	Very low	Very low	Mild	Nil
MC-136-10-50	Very low	Very low	Mild	Nil
MC-136-10-51	Very low	Very low	Mild	Nil
MC-136-10-52	Very low	Very low	Mild	Nil
MC-136-10-53	Very low	Very low	Mild	Nil
MC-136-10-54	Very low	Very low	Mild	Nil
MC-136-10-55	Very low	Very low	Mild	Nil
MC-136-10-56	Very low	Very low	Mild	Nil
MC-136-10-57	Very low	Very low	Mild	Nil
MC-136-10-58	Very low	Very low	Mild	Nil
MC-136-10-59	Very low	Very low	Mild	Nil
MC-136-10-60	Very low	Very low	Mild	Nil
MC-136-10-61	Very low	Very low	Mild	Nil
MC-136-10-62	Very low	Very low	Mild	Nil
MC-136-10-63	Very low	Very low	Mild	Nil
MC-136-10-64	Very low	Very low	Mild	Nil
MC-136-10-65	Very low	Very low	Mild	Nil
MC-136-10-66	Very low	Very low	Mild	Nil
MC-136-10-67	Very low	Very low	Mild	Nil
MC-136-10-68	Very low	Very low	Mild	Nil
MC-136-10-69	Very low	Very low	Mild	Nil
MC-136-10-70	Very low	Very low	Mild	Nil
MC-136-10-71	Very low	Very low	Mild	Nil
MC-136-10-72	Very low	Very low	Mild	Nil
MC-136-10-73	Very low	Very low	Mild	Nil
MC-136-10-74	Very low	Very low	Mild	Nil
MC-136-10-75	Very low	Very low	Mild	Nil
MC-136-10-76	Very low	Very low	Mild	Nil
MC-136-10-77	Very low	Very low	Mild	Nil
MC-136-10-78	Very low	Very low	Mild	Nil
MC-136-10-79	Very low	Very low	Mild	Nil
MC-136-10-80	Mild	Very low	Mild	Nil
MC-136-6-1	Very low	Very low	Moderate	Nil
MC-136-6-2	Very low	Very low	Mild	Nil
MC-136-6-3	Mild	Very low	Mild	Nil
MC-136-6-4	Very low	Very low	Mild	Nil
MC-136-6-5	Very low	Very low	Mild	Nil
MC-136-6-6	Very low	Very low	Mild	Nil
MC-136-6-7	Very low	Very low	Moderate	Nil
MC-136-6-8	Very low	Very low	Moderate	Nil
MC-136-6-9	Very low	Very low	Mild	Nil

MC-136-6-10	Very low	Very low	Moderate	Nil
MC-136-6-11	Very low	Very low	Mild	Nil
MC-136-6-12	Very low	Very low	Moderate	Nil
MC-136-6-13	Very low	Very low	Mild	Nil
MC-136-6-14	Very low	Very low	Mild	Nil
MC-136-6-15	Very low	Very low	Mild	Nil
MC-136-6-16	Very low	Very low	Mild	Nil
MC-136-6-17	Very low	Very low	Mild	Nil
MC-136-6-18	Very low	Very low	Mild	Nil
MC-136-6-19	Very low	Very low	Mild	Nil
MC-136-6-20	Very low	Very low	Mild	Nil
MC-136-6-21	Very low	Very low	Moderate	Nil
MC-136-6-22	Very low	Very low	Mild	Nil
MC-136-6-23	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_2 generation of bitter gourd hybrid MC-139

Genotype	**Fruit fly** *(Bactrocera cucurbitae)*	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-139-1	Very low	Very low	Mild	Nil
MC-139-2	Very low	Very low	Very low	Nil
MC-139-3	Very low	Very low	Mild	Nil
MC-139-4	Very low	Mild	Severe	Nil
MC-139-5	Very low	Mild	Moderate	Nil
MC-139-6	Very low	Mild	Moderate	Nil
MC-139-7	Very low	Very low	Very low	Nil
MC-139-8	Very low	Very low	Severe	Nil
MC-139-9	Very low	Very low	Moderate	Nil
MC-139-10	Very low	Very low	Mild	Nil
MC-139-11	Very low	Very low	Mild	Nil
MC-139-12	Very low	Very low	Severe	Nil
MC-139-13	Very low	Very low	Very low	Nil
MC-139-14	Very low	Very low	Mild	Nil
MC-139-15	Very low	Very low	Very low	Nil
MC-139-16	Very low	Very low	Mild	Nil
MC-139-17	Very low	Mild	Mild	Nil
MC-139-18	Very low	Very low	Mild	Nil
MC-139-19	Very low	Very low	Mild	Nil
MC-139-20	Very low	Very low	Mild	Nil
MC-139-21	Very low	Very low	Mild	Nil
MC-139-22	Very low	Very low	Mild	Nil
MC-139-23	Very low	Very low	Mild	Nil
MC-139-24	Very low	Very low	Moderate	Nil
MC-139-25	Very low	Very low	Moderate	Nil
MC-139-26	Very low	Very low	Severe	Nil
MC-139-27	Very low	Very low	Mild	Nil
MC-139-28	Very low	Very low	Mild	Nil
MC-139-29	Very low	Very low	Mild	Nil
MC-139-30	Very low	Mild	Mild	Nil
MC-139-31	Very low	Very low	Very low	Nil
MC-139-32	Very low	Very low	Moderate	Nil
MC-139-33	Very low	Mild	Moderate	Nil
MC-139-34	Very low	Very low	Mild	Nil
MC-139-35	Very low	Very low	Mild	Nil
MC-139-36	Very low	Very low	Very low	Nil
MC-139-37	Very low	Very low	Very low	Nil
MC-139-38	Very low	Very low	Mild	Nil
MC-139-39	Very low	Very low	Mild	Nil
MC-139-40	Very low	Very low	Mild	Nil
MC-139-41	Very low	Very low	Mild	Nil
MC-139-42	Very low	Very low	Mild	Nil

MC-139-43	Very low	Very low	Mild	Nil
MC-139-44	Very low	Very low	Moderate	Nil
MC-139-45	Very low	Very low	Mild	Nil
MC-139-46	Very low	Very low	Mild	Nil
MC-139-47	Very low	Very low	Mild	Nil
MC-139-48	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_3 generation of bitter gourd hybrid MC-139

Genotype	**Fruit fly** *(Bactrocera cucurbitae)*	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-139-2-1	Very low	Very low	Mild	Nil
MC-139-2-2	Very low	Very low	Mild	Nil
MC-139-2-3	Very low	Very low	Mild	Nil
MC-139-2-4	Very low	Very low	Mild	Nil
MC-139-2-5	Very low	Very low	Very low	Nil
MC-139-2-6	Very low	Very low	Very low	Nil
MC-139-2-7	Very low	Very low	Mild	Nil
MC-139-2-8	Very low	Very low	Mild	Nil
MC-139-2-9	Very low	Very low	Mild	Nil
MC-139-2-10	Very low	Very low	Mild	Nil
MC-139-2-11	Very low	Very low	Moderate	Nil
MC-139-2-12	Very low	Very low	Mild	Nil
MC-139-2-13	Very low	Very low	Mild	Nil
MC-139-2-14	Very low	Very low	Mild	Nil
MC-139-2-15	Very low	Mild	Severe	Nil
MC-139-2-16	Very low	Mild	Severe	Nil
MC-139-2-17	Very low	Very low	Moderate	Nil
MC-139-2-18	Very low	Very low	Mild	Nil
MC-139-2-19	Very low	Very low	Mild	Nil
MC-139-2-20	Very low	Very low	Mild	Nil
MC-139-2-21	Very low	Very low	Moderate	Nil
MC-139-2-22	Very low	Mild	Mild	Nil
MC-139-2-23	Very low	Very low	Moderate	Nil
MC-139-2-24	Very low	Very low	Mild	Nil
MC-139-2-25	Very low	Very low	Mild	Nil
MC-139-2-26	Very low	Very low	Mild	Nil
MC-139-2-27	Very low	Very low	Moderate	Nil
MC-139-2-28	Very low	Very low	Moderate	Nil
MC-139-2-29	Very low	Very low	Mild	Nil
MC-139-2-30	Very low	Mild	Mild	Nil
MC-139-2-31	Very low	Mild	Mild	Nil
MC-139-2-32	Very low	Very low	Mild	Nil
MC-139-2-33	Very low	Very low	Mild	Nil
MC-139-2-34	Very low	Very low	Mild	Nil
MC-139-2-35	Very low	Very low	Mild	Nil
MC-139-2-36	Very low	Very low	Mild	Nil
MC-139-2-37	Very low	Very low	Mild	Nil
MC-139-2-38	Very low	Very low	Mild	Nil
MC-139-2-39	Very low	Mild	Mild	Nil
MC-139-2-40	Very low	Very low	Mild	Nil
MC-139-2-41	Very low	Very low	Mild	Nil
MC-139-2-42	Very low	Very low	Mild	Nil

MC-139-2-43	Very low	Mild	Moderate	Nil
MC-139-2-44	Very low	Very low	Moderate	Nil
MC-139-2-45	Very low	Very low	Mild	Nil
MC-139-7-1	Very low	Very low	Mild	Nil
MC-139-7-2	Very low	Very low	Mild	Nil
MC-139-7-3	Very low	Very low	Mild	Nil
MC-139-7-4	Very low	Very low	Mild	Nil
MC-139-7-5	Very low	Very low	Mild	Nil
MC-139-7-6	Very low	Very low	Mild	Nil
MC-139-7-7	Very low	Very low	Mild	Nil
MC-139-7-8	Very low	Very low	Mild	Nil
MC-139-7-9	Very low	Very low	Mild	Nil
MC-139-7-10	Very low	Very low	Mild	Nil
MC-139-7-11	Very low	Very low	Mild	Nil
MC-139-7-12	Very low	Very low	Mild	Nil
MC-139-7-13	Very low	Very low	Mild	Nil
MC-139-7-14	Very low	Very low	Mild	Nil
MC-139-7-15	Very low	Very low	Mild	Nil
MC-139-7-16	Very low	Very low	Mild	Nil
MC-139-7-17	Very low	Very low	Mild	Nil
MC-139-7-18	Very low	Very low	Mild	Nil
MC-139-7-19	Very low	Very low	Mild	Nil
MC-139-7-20	Very low	Very low	Mild	Nil
MC-139-7-21	Very low	Very low	Mild	Nil
MC-139-7-22	Very low	Very low	Mild	Nil
MC-139-7-23	Very low	Very low	Mild	Nil
MC-139-7-24	Very low	Very low	Mild	Nil
MC-139-7-25	Very low	Very low	Mild	Nil
MC-139-7-26	Very low	Very low	Mild	Nil
MC-139-7-27	Very low	Very low	Mild	Nil
MC-139-7-28	Very low	Very low	Mild	Nil
MC-139-7-29	Very low	Very low	Mild	Nil
MC-139-7-30	Very low	Very low	Mild	Nil
MC-139-7-31	Very low	Very low	Mild	Nil
MC-139-7-32	Very low	Very low	Mild	Nil
MC-139-7-33	Very low	Very low	Mild	Nil
MC-139-7-34	Very low	Very low	Mild	Nil
MC-139-7-35	Very low	Very low	Mild	Nil
MC-139-7-36	Very low	Very low	Mild	Nil
MC-139-7-37	Very low	Very low	Mild	Nil
MC-139-7-38	Very low	Very low	Mild	Nil
MC-139-7-39	Very low	Very low	Mild	Nil
MC-139-7-40	Very low	Very low	Mild	Nil
MC-139-7-41	Very low	Very low	Mild	Nil
MC-139-7-42	Very low	Very low	Mild	Nil
MC-139-7-43	Very low	Very low	Mild	Nil
MC-139-7-44	Very low	Very low	Mild	Nil

MC-139-7-45	Very low	Very low	Mild	Nil
MC-139-7-46	Very low	Very low	Mild	Nil
MC-139-7-47	Very low	Very low	Mild	Nil
MC-139-7-48	Very low	Very low	Mild	Nil
MC-139-7-49	Very low	Very low	Mild	Nil
MC-139-7-50	Very low	Very low	Mild	Nil
MC-139-7-51	Very low	Very low	Mild	Nil
MC-139-7-52	Very low	Mild	Mild	Nil
MC-139-7-53	Very low	Very low	Mild	Nil
MC-139-7-54	Very low	Mild	Moderate	Nil
MC-139-7-55	Very low	Very low	Moderate	Nil
MC-139-7-56	Very low	Very low	Moderate	Nil
MC-139-7-57	Very low	Very low	Mild	Nil
MC-139-7-58	Very low	Very low	Mild	Nil
MC-139-7-59	Very low	Very low	Mild	Nil
MC-139-7-60	Very low	Very low	Mild	Nil
MC-139-7-61	Very low	Mild	Severe	Nil
MC-139-7-62	Very low	Very low	Mild	Nil
MC-139-7-63	Very low	Very low	Mild	Nil
MC-139-7-64	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_2 generation of bitter gourd hybrid MC-138

Genotype	**Fruit fly** *(Bactrocera cucurbitae)*	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-138-1	Very low	Very low	Very low	Nil
MC-138-2	Very low	Very low	Very low	Nil
Mc-138-3	Very low	Very low	Mild	Nil
MC-138-4	Very low	Severe	Severe	Nil
MC-138-5	Very low	Very low	Mild	Nil
MC-138-6	Very low	Very low	Mild	Very low
MC-138-7	Very low	Very low	Mild	Very low
MC-138-8	Very low	Very low	Mild	Very low
MC-138-9	Very low	Very low	Very low	Very low
MC-138-10	Very low	Very low	Mild	Nil
MC-138-11	Very low	Mild	Moderate	Nil
MC-138-12	Very low	Very low	Mild	Nil
MC-138-13	Very low	Very low	Mild	Very low
MC-138-14	Very low	Very low	Mild	Nil
MC-138-15	Very low	Very low	Very low	Nil
MC-138-16	Very low	Very low	Mild	Nil
MC-138-17	Very low	Very low	Moderate	Nil
MC-138-18	Very low	Very low	Mild	Nil
MC-138-19	Very low	Mild	Mild	Nil
MC-138-20	Very low	Very low	Mild	Nil
MC-138-21	Very low	Severe	Severe	Nil
MC-138-22	Very low	Severe	Severe	Nil
MC-138-23	Very low	Very low	Moderate	Nil
MC-138-24	Very low	Very low	Mild	Nil
MC-138-25	Very low	Very low	Very low	Nil
MC-138-26	Very low	Very low	Mild	Nil
MC-138-27	Very low	Very low	Mild	Nil
MC-138-28	Very low	Very low	Mild	Nil
MC-138-29	Very low	Very low	Mild	Nil
MC-138-30	Very low	Very low	Mild	Nil
MC-138-31	Very low	Moderate	Severe	Nil
MC-138-32	Very low	Very low	Mild	Nil
MC-138-33	Very low	Very low	Mild	Nil
MC-138-34	Very low	Very low	Mild	Nil
MC-138-35	Very low	Very low	Very low	Nil
MC-138-36	Very low	Severe	Moderate	Nil
MC-138-37	Very low	Mild	Severe	Nil
MC-138-38	Very low	Very low	Mild	Nil
MC-138-39	Very low	Very low	Very low	Nil
MC-138-40	Very low	Very low	Very low	Nil
MC-138-41	Very low	Very low	Very low	Nil
MC-138-42	Very low	Mild	Moderate	Nil

MC-138-43	Very low	Mild	Moderate	Nil
MC-138-44	Very low	Severe	Severe	Nil
MC-138-45	Very low	Very low	Mild	Nil
MC-138-46	Very low	Very low	Mild	Nil
MC-138-47	Very low	Very low	Mild	Nil
MC-138-48	Very low	Severe	Severe	Nil
MC-138-49	Very low	Very low	Mild	Nil
MC-138-50	Very low	Very low	Moderate	Nil
MC-138-51	Very low	Severe	Severe	Nil
MC-138-52	Very low	Severe	Severe	Nil

Incidence of pest and diseases in F_3 generation of bitter gourd hybrid MC-138

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-138-15-1	Very low	Very low	Mild	Nil
MC-138-15-2	Very low	Very low	Mild	Nil
MC-138-15-3	Very low	Very low	Mild	Nil
MC-138-15-4	Very low	Very low	Mild	Nil
MC-138-15-5	Very low	Very low	Mild	Nil
MC-138-15-6	Very low	Very low	Very low	Nil
MC-138-15-7	Very low	Very low	Moderate	Nil
MC-138-15-8	Very low	Very low	Mild	Nil
MC-138-15-9	Very low	Very low	Mild	Nil
MC-138-15-10	Very low	Very low	Moderate	Nil
MC-138-15-11	Very low	Very low	Mild	Nil
MC-138-15-12	Very low	Very low	Mild	Nil
MC-138-15-13	Very low	Very low	Mild	Nil
MC-138-15-14	Very low	Very low	Very low	Nil
MC-138-15-15	Very low	Very low	Mild	Nil
MC-138-15-16	Very low	Very low	Mild	Nil
MC-138-15-17	Very low	Very low	Mild	Nil
MC-138-15-18	Very low	Very low	Mild	Nil
MC-138-15-19	Very low	Very low	Moderate	Nil
MC-138-15-20	Very low	Very low	Mild	Nil
MC-138-15-21	Very low	Very low	Moderate	Nil
MC-138-15-22	Very low	Very low	Mild	Nil
MC-138-15-23	Very low	Very low	Mild	Nil
MC-138-15-24	Very low	Very low	Mild	Nil
MC-138-15-25	Very low	Very low	Mild	Nil
MC-138-15-26	Very low	Very low	Mild	Nil
MC-138-15-27	Very low	Very low	Mild	Nil
MC-138-15-28	Very low	Very low	Mild	Nil
MC-138-15-29	Very low	Very low	Mild	Nil
MC-138-15-30	Very low	Very low	Mild	Nil
MC-138-15-31	Very low	Very low	Mild	Nil
MC-138-15-32	Very low	Very low	Mild	Nil
MC-138-15-33	Very low	Very low	Moderate	Nil
MC-138-15-34	Very low	Very low	Mild	Nil
MC-138-15-35	Very low	Very low	Mild	Nil
MC-138-15-36	Very low	Very low	Mild	Nil
MC-138-15-37	Very low	Very low	Mild	Nil
MC-138-15-38	Very low	Very low	Mild	Nil
MC-138-15-39	Very low	Very low	Mild	Nil
MC-138-15-40	Very low	Very low	Very low	Nil
MC-138-15-41	Very low	Very low	Mild	Nil
MC-138-15-42	Very low	Very low	Moderate	Nil

MC-138-15-43	Very low	Very low	Mild	Nil
MC-138-15-44	Very low	Very low	Mild	Nil
MC-138-15-45	Very low	Very low	Mild	Nil
MC-138-25-1	Very low	Very low	Mild	Nil
MC-138-25-2	Very low	Very low	Mild	Nil
MC-138-25-3	Very low	Very low	Mild	Nil
MC-138-25-4	Very low	Very low	Mild	Nil
MC-138-25-5	Very low	Very low	Mild	Nil
MC-138-25-6	Very low	Very low	Mild	Nil
MC-138-25-7	Very low	Very low	Mild	Nil
MC-138-25-8	Very low	Very low	Mild	Nil
MC-138-25-9	Very low	Very low	Mild	Nil
MC-138-25-10	Very low	Very low	Mild	Nil
MC-138-25-11	Very low	Very low	Mild	Nil
MC-138-25-12	Very low	Very low	Mild	Nil
MC-138-25-13	Very low	Very low	Mild	Nil
MC-138-25-14	Very low	Very low	Mild	Nil
MC-138-25-15	Very low	Very low	Mild	Nil
MC-138-25-16	Very low	Very low	Mild	Nil
MC-138-25-17	Very low	Very low	Mild	Nil
MC-138-25-18	Very low	Very low	Mild	Nil
MC-138-25-19	Very low	Very low	Mild	Nil
MC-138-25-20	Very low	Very low	Mild	Nil
MC-138-25-21	Very low	Very low	Mild	Nil
MC-138-25-22	Very low	Very low	Mild	Nil
MC-138-25-23	Very low	Very low	Mild	Nil
MC-138-25-24	Very low	Very low	Mild	Nil
MC-138-25-25	Very low	Very low	Mild	Nil
MC-138-25-26	Very low	Very low	Mild	Nil
MC-138-25-27	Very low	Very low	Mild	Nil
MC-138-25-28	Very low	Very low	Mild	Nil
MC-138-25-29	Very low	Very low	Mild	Nil
MC-138-25-30	Very low	Very low	Mild	Nil
MC-138-25-31	Very low	Very low	Moderate	Nil
MC-138-25-32	Very low	Very low	Moderate	Nil
MC-138-25-33	Very low	Very low	Mild	Nil
MC-138-25-34	Very low	Very low	Mild	Nil
MC-138-25-35	Very low	Very low	Mild	Nil
MC-138-25-36	Very low	Very low	Mild	Nil
MC-138-25-37	Very low	Very low	Mild	Nil
MC-138-25-38	Very low	Very low	Mild	Nil
MC-138-25-39	Very low	Very low	Mild	Nil
MC-138-25-40	Very low	Very low	Mild	Nil
MC-138-25-41	Very low	Very low	Mild	Nil
MC-138-25-42	Very low	Very low	Mild	Nil
MC-138-25-43	Very low	Very low	Mild	Nil
MC-138-25-44	Very low	Very low	Mild	Nil

MC-138-25-45	Very low	Very low	Mild	Nil
MC-138-25-46	Very low	Very low	Mild	Nil
MC-138-25-47	Very low	Very low	Mild	Nil
MC-138-25-48	Very low	Very low	Mild	Nil
MC-138-25-49	Very low	Very low	Mild	Nil
MC-138-25-50	Very low	Very low	Mild	Nil
MC-138-25-51	Very low	Very low	Mild	Nil
MC-138-25-52	Very low	Very low	Mild	Nil
MC-138-25-53	Very low	Very low	Mild	Nil
MC-138-25-54	Very low	Very low	Mild	Nil
MC-138-25-55	Very low	Very low	Mild	Nil
MC-138-25-56	Very low	Very low	Mild	Nil
MC-138-25-57	Very low	Very low	Mild	Nil
MC-138-25-58	Very low	Very low	Mild	Nil
MC-138-25-59	Very low	Very low	Mild	Nil
MC-138-25-60	Very low	Very low	Mild	Nil
MC-138-25-61	Very low	Very low	Mild	Nil
MC-138-25-62	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_2 generation of bitter gourd hybrid MC-133

Genotype	**Fruit fly** *(Bactrocera cucurbitae)*	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-133-1	Very low	Very low	Mild	Nil
MC-133-2	Mild	Very low	Mild	Nil
MC-133-3	Very low	Very low	Moderate	Nil
MC-133-4	Very low	Very low	Moderate	Nil
MC-133-5	Very low	Very low	Mild	Nil
MC-133-6	Very low	Mild	Mild	Nil
MC-133-7	Very low	Very low	Moderate	Nil
MC-133-8	Very low	Very low	Mild	Nil
MC-133-9	Very low	Very low	Moderate	Nil
MC-133-10	Very low	Very low	Severe	Nil
MC-133-11	Mild	Very low	Mild	Nil
MC-133-12	Very low	Very low	Severe	Nil
MC-133-13	Very low	Mild	Moderate	Nil
MC-133-14	Very low	Very low	Mild	Nil
MC-133-15	Very low	Very low	Mild	Nil
MC-133-16	Very low	Very low	Mild	Nil
MC-133-17	Very low	Very low	Mild	Nil
MC-133-18	Very low	Very low	Severe	Nil
MC-133-19	Very low	Very low	Mild	Nil
MC-133-20	Very low	Very low	Mild	Nil
MC-133-21	Very low	Very low	Moderate	Nil
MC-133-22	Very low	Very low	Moderate	Nil
MC-133-23	Mild	Very low	Mild	Nil
MC-133-24	Very low	Very low	Severe	Nil
MC-133-25	Very low	Very low	Mild	Nil
MC-133-26	Very low	Very low	Moderate	Nil
MC-133-27	Mild	Very low	Mild	Nil
MC-133-28	Mild	Very low	Mild	Nil
MC-133-29	Very low	Very low	Severe	Nil
MC-133-30	Very low	Very low	Mild	Nil
MC-133-31	Very low	Very low	Moderate	Nil
MC-133-32	Very low	Mild	Mild	Nil
MC-133-33	Very low	Mild	Mild	Nil
MC-133-34	Very low	Very low	Moderate	Nil
MC-133-35	Very low	Very low	Mild	Nil
MC-133-36	Very low	Very low	Mild	Nil
MC-133-37	Very low	Very low	Severe	Nil
MC-133-38	Very low	Very low	Mild	Nil
MC-133-39	Very low	Very low	Mild	Nil
MC-133-40	Very low	Very low	Severe	Nil
MC-133-41	Very low	Very low	Moderate	Nil
MC-133-42	Very low	Very low	Mild	Nil
MC-133-43	Very low	Very low	Mild	Nil
MC-133-44	Very low	Very low	Severe	Nil
MC-133-45	Very low	Very low	Mild	Nil

Incidence of pest and diseases in F_3 generation of bitter gourd hybrid MC-133

Genotype	**Fruit fly** *(Bactrocera cucurbitae*)	**Anthracnose** (*Colletotrichum gloeosporioides*)	**Downey mildew** (*Pseudoperonospora cubensis*)	**Mosaic disease**
MC-133-8-1	Very low	Very low	Mild	Nil
MC-133-8-2	Very low	Very low	Mild	Nil
MC-133-8-3	Very low	Very low	Moderate	Nil
MC-133-8-4	Very low	Very low	Moderate	Nil
MC-133-8-5	Very low	Very low	Mild	Nil
MC-133-8-6	Very low	Moderate	Mild	Nil
MC-133-8-7	Very low	Mild	Mild	Nil
MC-133-8-8	Very low	Very low	Mild	Nil
MC-133-8-9	Very low	Very low	Mild	Nil
MC-133-8-10	Very low	Mild	Mild	Nil
MC-133-8-11	Very low	Very low	Mild	Nil
MC-133-8-12	Very low	Very low	Mild	Nil
MC-133-8-13	Very low	Very low	Mild	Nil
MC-133-8-14	Very low	Mild	Moderate	Nil
MC-133-8-15	Very low	Very low	Moderate	Nil
MC-133-8-16	Very low	Very low	Moderate	Nil
MC-133-8-17	Very low	Very low	Mild	Nil
MC-133-8-18	Very low	Very low	Mild	Nil
MC-133-8-19	Very low	Very low	Mild	Nil
MC-133-8-20	Very low	Very low	Moderate	Nil
MC-133-8-21	Very low	Very low	Moderate	Nil
MC-133-8-22	Very low	Very low	Moderate	Nil
MC-133-8-23	Very low	Very low	Moderate	Nil
MC-133-16-1	Very low	Very low	Moderate	Nil
MC-133-16-2	Very low	Very low	Moderate	Nil
MC-133-16-3	Very low	Very low	Mild	Nil
MC-133-16-4	Very low	Very low	Mild	Nil
MC-133-16-5	Very low	Very low	Mild	Nil
MC-133-16-6	Very low	Very low	Mild	Nil
MC-133-16-7	Very low	Very low	Mild	Nil
MC-133-16-8	Very low	Very low	Mild	Nil
MC-133-16-9	Very low	Very low	Moderate	Nil
MC-133-16-10	Very low	Very low	Mild	Nil
MC-133-16-11	Very low	Very low	Mild	Nil
MC-133-16-12	Very low	Very low	Mild	Nil
MC-133-16-13	Very low	Very low	Moderate	Nil
MC-133-16-14	Very low	Very low	Mild	Nil
MC-133-16-15	Very low	Very low	Mild	Nil
MC-133-16-16	Very low	Very low	Moderate	Nil
MC-133-16-17	Very low	Very low	Mild	Nil
MC-133-16-18	Very low	Very low	Mild	Nil
MC-133-16-19	Very low	Very low	Mild	Nil

MC-133-16-20	Very low	Very low	Mild	Nil
MC-133-16-21	Very low	Very low	Moderate	Nil
MC-133-16-22	Very low	Very low	Mild	Nil
MC-133-16-23	Very low	Very low	Mild	Nil
MC-133-16-24	Very low	Very low	Moderate	Nil
MC-133-16-25	Very low	Very low	Mild	Nil
MC-133-16-26	Very low	Very low	Mild	Nil
MC-133-16-27	Very low	Very low	Mild	Nil
MC-133-16-28	Very low	Very low	Mild	Nil
MC-133-16-29	Very low	Very low	Mild	Nil
MC-133-16-30	Very low	Very low	Mild	Nil
MC-133-16-31	Very low	Very low	Moderate	Nil
MC-133-16-32	Very low	Very low	Moderate	Nil
MC-133-16-33	Very low	Very low	Mild	Nil
MC-133-16-34	Very low	Very low	Moderate	Nil
MC-133-16-35	Very low	Very low	Moderate	Nil
MC-133-16-36	Very low	Very low	Moderate	Nil
MC-133-16-37	Very low	Very low	Mild	Nil
MC-133-16-38	Very low	Very low	Mild	Nil
MC-133-16-39	Very low	Very low	Mild	Nil
MC-133-16-40	Very low	Very low	Mild	Nil
MC-133-16-41	Very low	Very low	Moderate	Nil
MC-133-16-42	Very low	Very low	Moderate	Nil
MC-133-16-43	Very low	Very low	Mild	Nil
MC-133-16-44	Very low	Very low	Mild	Nil
MC-133-16-45	Very low	Very low	Mild	Nil
MC-133-16-46	Very low	Very low	Mild	Nil
MC-133-16-47	Very low	Very low	Mild	Nil
MC-133-16-48	Very low	Very low	Mild	Nil
MC-133-16-49	Very low	Very low	Mild	Nil
MC-133-16-50	Very low	Very low	Mild	Nil
MC-133-16-51	Very low	Very low	Mild	Nil
MC-133-16-52	Very low	Very low	Mild	Nil
MC-133-16-53	Very low	Very low	Mild	Nil
MC-133-16-54	Very low	Very low	Mild	Nil
MC-133-16-55	Very low	Very low	Mild	Nil
MC-133-16-56	Very low	Very low	Mild	Nil
MC-133-16-57	Very low	Very low	Mild	Nil
MC-133-16-58	Very low	Very low	Mild	Nil
MC-133-16-59	Very low	Very low	Mild	Nil
MC-133-16-60	Very low	Very low	Mild	Nil
MC-133-16-61	Very low	Very low	Mild	Nil
MC-133-16-62	Very low	Very low	Mild	Nil
MC-133-16-63	Very low	Very low	Mild	Nil
MC-133-16-64	Very low	Very low	Mild	Nil
MC-133-16-65	Very low	Very low	Mild	Nil
MC-133-16-66	Very low	Very low	Mild	Nil

MC-133-16-67	Very low	Very low	Mild	Nil
MC-133-16-68	Very low	Very low	Moderate	Nil
MC-133-16-69	Very low	Very low	Moderate	Nil
MC-133-16-70	Very low	Very low	Moderate	Nil
MC-133-16-71	Very low	Very low	Mild	Nil
MC-133-16-72	Very low	Very low	Mild	Nil
MC-133-16-73	Very low	Very low	Mild	Nil
MC-133-16-74	Very low	Very low	Mild	Nil
MC-133-16-75	Very low	Very low	Mild	Nil
MC-133-16-76	Very low	Very low	Mild	Nil
MC-133-16-77	Very low	Very low	Moderate	Nil
MC-133-16-78	Very low	Very low	Moderate	Nil
MC-133-16-79	Very low	Very low	Moderate	Nil
MC-133-16-80	Very low	Very low	Mild	Nil
MC-133-16-81	Very low	Very low	Mild	Nil
MC-133-16-82	Very low	Very low	Mild	Nil
MC-133-16-83	Very low	Very low	Mild	Nil
MC-133-16-84	Very low	Very low	Mild	Nil
MC-133-16-85	Very low	Very low	Mild	Nil
MC-133-16-86	Very low	Very low	Mild	Nil
MC-133-16-87	Very low	Very low	Mild	Nil
MC-133-16-88	Very low	Very low	Mild	Nil
MC-133-16-89	Very low	Very low	Moderate	Nil

Annexure 2

Composition of E20A medium

Macro and micro elements (mg l^{-1})			
KNO_3	1,075	$MnSO_4.7H_2O$	11.065
NH_4NO_3	619	$ZnSO_4.7H_2O$	1.812
$MgSO_4.7H_2O$	206	H_3BO_3	1.575
$CaCl_2.2H_2O$	156.5	KI	0.345
KH_2PO_4	71	$Na_2MgO_4.2H_2O$	0.094
$Ca(NO_3)_2.4H_2O$	25	$CuSO_4.5H_2O$	0.008
$NaH_2PO_4.4H_2O$	19	$CoCl_2.6H_2O$	0.008
$(NH_4)_2SO_4$	17	$FeSO_4.7H_2O$	27.8
KCI	3.5	Na_2EDTA	37.3

Vitamins and amino acids (mg l^{-1})		Others	
Myo-inositol	50.3	Sucrose	20.00 g l^{-1}
Pyridoxine-HCI	5.5	Agar	7.00 g l^{-1}
Nicotinic acid	0.7	IAA	0.01 mg l^{-1}
Thiamine -HCI	0.6	pH	5.9
Calcium pantothenate	0.5		
Glycine	0.1		
Biotine	0.005		