BREEDING FOR GYNOECY IN BITTER GOURD
(*Momordica charantia* L.)

By
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(2017-12-001)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Breeding for gynoecy in bitter gourd (*Momordica charantia* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Breeding for gynoecy in bitter gourd (*Momordica charantia* L.)"** is a bonafide record of research work done independently by **Ms. Minnu Ann Jose (2017-12-001)** 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.

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Introduction

1. INTRODUCTION

Cucurbits form a predominant and a large group of extensively cultivated vegetables in India and other tropical and sub-tropical countries. They are of enormous economic importance as food crops like edible vegetables, fruits or seeds and as medicinal plants especially in the indigenous medicinal systems.

Momordica charantia L. commonly known as bitter gourd, balsam pear and bitter melon, is a popular cucurbitaceous vegetable throughout tropics and subtropics of Asia. The crop is originated in India (Indo-Burma centre of origin) and the regions of eastern India and Southern China are regarded as possible centres of domestication. In India, bitter gourd covers an area of 99 thousand hectares with an annual production of 1204 thousand tonnes (NHB 2018-19).

The fruits of bitter gourd serve as a good source of carbohydrates, proteins, vitamins, and minerals and have the highest nutritive value among cucurbitaceous vegetables. Furthermore the crude protein content (11.4 - 20.9 g kg^{-1}) of bitter gourd fruits is higher than that of tomato and cucumber. Since the fruit extract possesses antioxidant, antimicrobial, antiviral, antihepatotoxic, antiulcerogenic and hypoglycemic properties, bitter gourd has been used for centuries in traditional medicines of India (Behera *et al.*, 2010).

A wide range of variation in sex forms ranging from hermaphrodite to monoecious forms is noticed in cucurbitaceous vegetable crops (Robinson and Decker-Walters 1999). Among these the gynoecious sex form has been commercially exploited for breeding programme. The predominant sex form in bitter gourd is monoecious, however, gynoecious sex form has been reported lately from India and China (Zhou *et al.*, 1998; Ram *et al.*, 2002 b). Like cucumber, in bitter gourd also gynoecy can be exploited for developing hybrids with high sex ratio and economizing F_1 hybrid seed production. It is already reported in bitter gourd that, by using gynoecious line as one parent, high percentage of pistillate flowers can be realized with high yield potential (Dey *et al.*, 2010). Development of hybrids in any crop is expensive. However, the utilisation of gynoecy is economical and easier for exploiting hybrid vigour in many cucurbitaceous crops including bitter gourd that have high

male:female sex ratio and require hand pollination. Thus, isolation and characterisation of gynoecism in bitter gourd has great significance.

F_1 hybrids from private sector are popular in bitter gourd. When gynoecious line was used as one parent in hybrid breeding, it showed positive impact in terms of yield and earliness (Rao *et al.*, 2018). All gynoecious hybrids showed significant heterosis in a desirable direction for traits like sex ratio, days to first picking, number of fruits per plant, yield per plant, and vine length (Behera *et al.*, 2009). However very little attempt has been made to exploit heterosis in bitter gourd in Kerala. Heterosis breeding needs identification of potential inbred lines with substantial diversity and gynoecious inbred line as female parents to improve the yield plateau substantially. In bitter gourd, inbreds are maintained in pure form by selfing without loss of vigor. Since the availability and commercial utilisation of gynoecious lines are very meagre in bitter gourd, more emphasis has to be given on introgression of gynoecy into commercial inbreds.

Gynoecious types of bitter gourd are now identified from the experimental field of Department of Vegetable Science, Vellanikkara. Although the gene action controlling gynoecism trait in bitter gourd was reported to be monogenic recessive (Mishra *et al.*, 2015 b), the genetics of gynoecious types identified from Kerala is not yet confirmed. Unlike the gynoecious types reported from IARI, Delhi and IIVR, Varanasi, type identified from Vellanikkara is characterized by medium sized dark green fruits. Population generated through sib mating between gynoecious plants and malesibs as well as those with selected monoecious line need to be evaluated for isolating gynoecious line. Identified genotype can be fixed through micropropagation for further studies as it is a potential tool for maintaining gynoecy.

Hence, the present study is undertaken with the objective to develop gynoecious inbred lines in bitter gourd through evaluation of sib mated and crossed population generated from gynoecious plants and maintain gynoecy through hormonal regulation and tissue culture.

Review of Literature

2. REVIEW OF LITERATURE

Bitter gourd is one of the most important vegetable in Kerala, yet the breeding and improvement in this crop is at a slow pace. Heterosis breeding needs identification of potential inbred lines with substantial diversity and using gynoecious inbred line as female parents will improve the yield plateau substantially. In bitter gourd, exploitation of gynoecy is a recent approach for hybrid seed production and now more emphasis is given on introgression of this gene into commercial inbreds. The available literature concerning the research topic 'Breeding for gynoecy in bitter gourd' is presented under the following headings:

2.1 Gynoecy in bitter gourd

2.2 Maintenance of gynoecious lines through hormonal regulation

- 2.2.1 Induction of maleness by the exogenous application of hormones
- 2.2.2 Role of hormones in altering the sex expression of cucurbits

2.3 Maintenance of elite breeding stocks in cucurbits through micropropagation

- 2.3.1 Explant standardization
- 2.3.2 Manipulation of culture media

2.1 Gynoecy in bitter gourd

Gynoecy is the condition where all the flowering nodes produce only pistillate flowers. Though bitter gourd is a typical monoecious cucurbit, gynoecious sex form also has been reported from China (Zhou *et al.,* 1998) and India. The first report on the preliminary characterization of gynoecism in bitter gourd, from India was presented by Ram *et al.* (2002 b). Three gynoecious plants, *viz.,* Gy23, Gy63 and Gy263B were identified from the experimental field during the summer season of 2000. The F_1 generation obtained by sib mating and crossing the gynoecious plants with monoecious plants of other population showed segregation for gynoecious and monoecious plants. The segregation data implicated the existence of heterozygous gene(s) for gynoecism in the utilized male plants. Hence it was concluded that the gynoecism trait in identified plants was heritable and under the control of certain major recessive gene(s) (Ram *et al.*, 2002 a).

Behera *et al.* (2006) in an experiment during spring-summer seasons of 2004 and 2005, observed two gynoecious plants namely DBGy-201 and DBGy-202 from the Research Farm of the Division of Vegetable Science, Indian Agricultural Research Institute (IARI), New Delhi. The identified gynoecious plants were sib mated and crossed with monoecious plants of another population. Progenies were raised and F_1 plants were evaluated for sex expression. Since gynoeciuos plants were recovered in a very small F_1 population, it suggests that gynoecy in the isolated plants is heritable and may have been governed by certain major gene(s).

Iwamoto and Ishida (2006) improved and developed gynoecious inbred lines of bitter gourd from cv. Aochu-naga. Generations were advanced from a breeding line (LCJ980120) which has high percentage of pistillate flowers, and gynoecy was maintained by spraying silver nitrate. The data obtained from the generation mean analysis exposed that the gynoecious sex expression is partially dominant in hybrid plants between the gynoecious pedigree and the monoecious line (KBP1), which in turn suggests that gynoecious inbred lines are promising seed parents for high-female F_1 hybrids.

Ram *et al.* (2006) performed a genetic analysis of the inheritance of gynoecism in bitter gourd by studying a 100 per cent gynoecious line (Gy263B). The F_1 population was monoecious, while the F_2 and testcross populations segregated for gynoecism. The segregation ratio of F_2 and test cross progenies showed a good fit to 3 monoecious: 1 gynoecious plants and 1 monoecious: 1 gynoecious plant respectively. This segregation data revealed that gynoecism in Gy263B is under the control of a single, recessive gene (*gy-1*).

Two gynoecious lines in bitter gourd, DBGY-201 and DBGY-202 were crossed with two inbreds derived from improved monoecious cultivars, Pusa Do Mausami and Pusa Vishesh. The F_1 population was monoecious while the F_2 generation of the crosses segregated 3:1 (monoecious *vs.* gynoecious) which suggests gynoecium in the lines DBGY-202 and DBGY-202 is under the control of single recessive gene (Behera *et al.*, 2009).

Inheritance of gynoecism and genetics of yield and yield contributing traits in bitter gourd was studied by Mishra *et al.* in 2015 (b). The crosses involving a gynoecious line and two monoecious lines demonstrated that the trait gynoecism is under the control of a recessive gene (*gy 1*) in both the crosses but gene effects obtained by generation mean analysis differed with the different genetic backgrounds of the inbred crosses. Investigation on gene action in the crosses by generation mean analysis revealed duplicate epistasis for most of the traits, suggesting the possibilities of obtaining transgressive segregants in later generations.

Pati *et al.* (2015) evaluated the inheritance pattern of gynoecious sex expression in cucumber by utilizing a gynoecious line (GBS-1) and two monoecious lines (Pusa Uday and Punjab Naveen). The F_1 population generated through crossing between the gynoecious line and monoecious lines and F_2 population along with parental lines were examined. The experiment data showed that, all the F_1 plants of the cross were gynoecious and the F_2 population segregated as 3 (gynocious plant): 1 (monoecious plant) which perfectly fitted to the expected mendelian ratio. The segregation of plant sex types evinced monogenic dominant control of gynoecious sex form in cucumber using genotype GBS-1.

2.2 Maintenance of gynoecious lines through hormonal regulation

Sexual differentiation of floral primordia depends on hormonal balance in primordial tissue and by exogenous application of hormones (More and Sheshadri, 1998). Sex expression in different plants including cucurbits can be altered by the exogenous applications of plant growth regulators.

2.2.1 Induction of maleness by the exogenous application of hormones

Pike and Peterson (1969) compared, Gibberellin A_4/A_7 with GA_3 for staminate flower induction on an inbred gynoecious cucumber line (*Cucumis sativus* L.) grown under field conditions. Significantly greater numbers of staminate flowers were induced by Gibberellin A_4/A_7 spray applications at 50 ppm than GA_3 at 1000 ppm. Also high dosage of GA_3 caused excessive stem elongation or brittleness.

Den Nijs and Visser (1980) investigated the effect of silver ions in inducing male flowers in cucumber (*Cucumis sativus* L.). A single spraying of gynoecious cucumber plants with 3 mM Ag + as $Ag_2S_2O_3$ or $AgNO_3$ (500 ppm) in the first true leaf stage produced more staminate flowers than GA_3 (1500 ppm) and almost as many as 3 consecutive sprayings of $GA_{4/7}$ (50 ppm). Male flowering started about 3 weeks after treatment and lasted for a period of up to 4 weeks thereafter. $Ag_2S_2O_3$ never showed any phytotoxic effects, while $AgNO_3$ proved some deleterious effects in poor growing conditions.

Owens *et al.* (1980) opined that, $AgNO_3$ (100, 200, 400 ppm) was the best treatment for inducing maximum number of perfect flowers in gynoecious MSU-1G, muskmelon plants compared to AVG (50, 100, and 200 ppm) and $GA_{4/7}$ (100 ppm) treatments. All the chemicals were applied at twice, either the three-leaf or five-leaf stage with one week interval. $AgNO_3$ treatment, resulted in approximately 12 out of 20 nodes bearing perfect flowers.

Effect of some growth regulators on sex expression of bitter gourd was studied by Ghosh and Basu (1982). They observed that high concentrations of CCC had a masculinizing effect on bitter gourd, possibly by its antigibberellin nature, but low concentrations of BA and CCC, all concentrations of GA and a high concentration of MH induced femaleness.

More and Munger (1986) suggested that twice application of $AgNO_3$ at 250 ppm at two-true-leaf stage produced the maximum number of male flowers in gynoecious cucumber lines. The one-true-leaf stage and one spray of 250 ppm $AgNO_3$ showed more gynoecious stability in the F_1.

Staub and Crubaugh (1987), reported the concentration and dosage of silver thiosulphate to produce staminate flowers in gynoecious genotypes of cucumber. A dosage of either 10 or 20 µl silver thiosulfate was given at concentrations of 6, 3, 1.5, and 0.75 mM to cotyledons of 7-day-old seedlings of the gynoecious inbred line WI 1701 and the data suggested that 20 µl was the effective dosage for induction of maleness, except at 6 mM.

Alteration of sex expression of two inbred cucumber lines by foliar application of different silver nitrate concentrations (0.01 %, 0.02 %, 0.03 % and 0.04 %) during two different sowing seasons (spring and summer) was examined by Stankovic and Prodanovic (2000). The lines used were; PMS which is gynoecious line and PKTZ, monoecious line. With increase in concentration of $AgNO_3$, the number of male flowers increased in the PMS line. The optimal sex conversion of PMS line was observed by the application of 0.02 per cent $AgNO_3$ solution in the spring sowing season whereas in the summer season this was achieved by the 0.03 per cent silver nitrate solution. Silver nitrate had no effect in the development of male flowers of monoecious PKTZ cucumber line.

Chaudhary *et al.* (2001) reported that, $AgNO_3$ was superior over gibberellic acid (GA_3) and silver thiosulphate for effective induction of staminate flower for the maintenance of gynoecious lines in cucumber (*Cucumis sativus* L.). Spraying silver nitrate twice, at concentration 300 and 400 ppm was found to be ideal for maintaining gynoecious lines.

The effect of $AgNO_3$ concentration and number of sprays on sex expression of gynoecious, parthenocarpic, cucumbers was studied by Hallidri in 2002. The treatment started at the first true leaf stage of growth, and subsequent sprays were given at weekly intervals. The maximum number of staminate flowers was inducted on the plants sprayed twice or thrice with 400 to 500 ppm $AgNO_3$, while the treatments with 100 ppm failed to induce staminate flowers. It was concluded that, for earlier possible and the greatest number of staminate flowers in gynoecious cucumber, two foliar sprays of 400 ppm $AgNO_3$ solution, at first true leaf stage and subsequent spray at weekly interval should be applied.

Sharma *et al.* (2004) compared the effect of $AgNO_3$ and GA_3 for inducing male flowers in gynoecious cucumber lines and the best treatment for the same was found to be two sprays of $AgNO_3$ at 250 ppm at 2-3 and 4-6 leaf stages. GA_3 at 1500 and 2500 ppm before flowering failed to produce male flowers in the gynoecious line.

Zhang *et al.* (2007) experimented different concentration of $AgNO_3$ *viz.,* 0, 100, 200, 300, and 400 mgl^{-1} on gynoecious cucumber inbred line, S 17 for sex modification, and the results showed that, two successive sprays of $AgNO_3$ at 300 mgl^{-1} at the two-leaf stage was the best for inducing staminate flowers. The same treatment exhibited lowest node position of the first male flower and minimum rate of mortality.

Susaj and Susaj (2010) revealed that application of $AgNO_3$ at 400-500 ppm twice or thrice in an interval of seven days, produced highest number of staminate flowers in gynoecious (parthenocarpic) cucumber lines. Concentration of $AgNO_3$ and number of sprays significantly influenced, the first node at which staminate flower appeared in different plants. All treatments used once were ineffective for inducing male flowers after tenth node.

Silver thiosulphate at 6 M and 3 M, silver nitrate at 250 ppm and 200 ppm and GA_3 at 1500 ppm and 1000 ppm were sprayed on gynoecious lines of bitter gourd after appearance of 1st female flower to study the induction of hermaphrodite flowers. The observations were noted on node at which 1st female flower appear, ovary length of female flower (cm) and hermaphrodite flower (cm) and number of female flower and hermaphrodite flower per plant during the growing period. The data revealed that silver thiosulfate 6 M was the most effective treatment for sex modification in bitter gourd and silver nitrate did not show any response for the same in both concentrations (Behera *et al*., 2011).

The impact of silver nitrate applications on the flower quantity of cucumber was studied by Karakaya and Padem (2011). Silver nitrate was sprayed on cucumber plants at concentrations; 0, 250, 500, 750, and 1000 ppm. It was determined that the number of male flowers increased depending on the increased $AgNO_3$ doses.

Nagar (2013) in a study, observed that, silver thiosulphate (STS) performed better over silver nitrate for induction of staminate flowers in gynoecious cucumber cv. 'Infinity' and 'Hilton'. There were eight treatments including two chemicals (silver nitrate and STS), their two doses (2 & 4 mM of STS and 200 & 400 ppm of

silver nitrate) and two times of application (once and twice). The findings indicated that, STS spray at 2 mM concentration, twice followed by silver nitrate at 400 ppm twice was better for maintenance of gynoecious lines.

Production of staminate flower in gynoecious cucumber through silver ion containing compounds was investigated by Nagar *et al.* (2014). Effect of silver nitrate (SN) and silver thiosulphate (STS) concentration, number of sprays and method of applications on induction of male flowers was noted and found that higher dose of $AgNO_3$ (400 ppm) and lower dose of STS (2 mM) was better. Further, twice application over once was found more useful for influencing all the characters in desired direction. Greatest number of staminate flowers (151.0), early staminate flowering (27.83 days) and more number of staminate nodes (20), were obtained from foliar application of STS at 2 mM twice (at 2-3 true leaf stage and thereafter 7 days) followed by SN at 400 ppm twice.

Mishra *et al.* (2015 a) in an experiment, determined the effects of concentrations of silver nitrate (SN), gibberellic acid (GA_3), and silver thiosulfate (STS) for induction of male flowers in the gynoecious variety of bitter gourd, DBGy-201 and the results delineated that, silver thiosulphate at 6 mM was the best treatment to produce highest percentage (57.63%) of hermaphrodite flowers, whereas silver nitrate at 1.2 and 1.5 mM had no effect.

Parthenocarpic gynoecious plants of cucumber cv. Infinity and Hilton were sprayed with two chemical treatments of silver nitrate (200 and 400 ppm) and silver thiosulphate (2 and 4 Mm) once (at 2-3 true leaf stage) and twice (at 2-3 true leaf stage, and 7 days after first application) to study their effect in male flower induction. The experimental data revealed that silver thiosulphate spray at 2 Mm followed by silver nitrate at 400 ppm was superior for the production of staminate flowers (%), node number upto which staminate flower appered and number of pollen per flower in the plants studied (Nagar *et al.*, 2015)

Golabadi *et al.* (2018) carried out a study on modification of sex expression in gynoecious cucumber plants using different concentrations of chemicals *viz.*,

Gibberllic acid (GA_3) (1000 and 1500 ppm), silver thiosulphate ($Ag_2S_2O_3$) (200, 300, and 500 ppm), and silver nitrate ($AgNO_3$) (200 and 300 ppm) at 5, 10, and 15-leaf stages with single and double sprays. Double sprays of $Ag_2S_2O_3$ and $AgNO_3$ at the highest doses applied at the 5-leaf stage were identified as the best treatments for staminate flower induction.

Verma *et al.* (2018) studied modification of sex expression in gynoecious varieties (GYNO-1 and GYNO-2) of cucumber, by use of chemicals *viz.*, gibberellic acid (500, 1000 and 1500 ppm), silver nitrate (250, 500 and 750 ppm) and silver thiosulphate (250, 500 and 750 ppm). The treatments were applied at 2-3 true leaf stage at five days interval till 10-15 leaf stage. Silver thiosulphate exhibited superiority over both gibberellic acid and silver nitrate in mean number of male flowers induced, and it showed less phytotoxicity compared to silver nitrate.

2.2.2 Role of hormones in altering the sex expression of cucurbits

Several works have been conducted in cucurbits to study the effect of growth regulators in altering the sex expression of plants.

Table 2.1: Role of hormones in altering the sex expression of cucurbits

Crop	Hormone / Chemical	Modified trait	Reference
Cucumber (*Cucumis sativus* L.)	GA (10 ppm), MH (100, 200 ppm), NAA (50 ppm)	Staminate	Choudhary and Phatak (1959)
	IAA (200 ppm)	Staminate	Choudhary and Phatak (1960)
	$GA_{4/7}$ (50 ppm)	Staminate	Pike and Peterson (1969)
	Silver nitrate (250 - 300 ppm)	Staminate	Kalloo and Franken (1978); More and Munger, (1986)

	Silver thiosulphate	Staminate	Nagar (2013); Nagar *et al.* (2014); Bharadwaj *et al.* (2017); Golabadi *et al.* (2018)
	GA (1500 -2000 ppm)	Pistillate	Peterson and Anhder (1960)
	NAA (50 ppm), IAA (200 ppm)	Pistillate	Choudhary and Patil (1962)
	MH (100 ppm) and GA (10 ppm)	Pistillate	Singh and Choudhary (1977)
	Ethrel (50 - 200 ppm)	Pistillate	Bhonde (1978); Verma and Choudhary (1980)
	Ethrel (400 ppm)	Pistillate	Bhandary *et al.* (1974); Patil *et al.* (1983)
	ABA + Ethephone	Pistillate	Rudich and Halevy (1974)
	MH (450 μMl^{-1})	Pistillate	Hidayatullah *et al.* (2009)
Bitter gourd (*Momordica charantia* L.)	CCC (50-200 mgl^{-1})	Staminate	Wang and Zeng (1997)
	STS (3 mM - 6 mM) and GA_3 (1000 -1500 ppm)	Hermaphodite	Behera *et al.* (2011); Mishra *et al.* (2015 a)
	B-9 (500 - 5000 ppm) and CCC (500- 2000 ppm)	Pistillate	Bose and Gosh (1968); Gosh and Bose (1970)
	NAA (100 ppm)	Pistillate	Bisaria (1974)
	Boron (4 ppm)	Pistillate	Verma *et al.* (1984)
	IAA (10 ppm)	Pistillate	Akter and Rahman (2010)

	GA_3 (50 - 75 ppm), NAA (100 - 200 ppm)	Pistillate	Ghani *et al.* (2013); Nagamani *et al.* (2015)
Water melon (*Citrullus lanatus* (Thumb) Mansf)	B-9 (250 ppm), GA (50 ppm), Morphactin (20 ppm)	Staminate Staminate	Bhandari and Sen (1973) Bhandari and Sen (1973)
	IAA (50 ppm), Ethrel (250 ppm), Kinetin (50 ppm), CCC (50ppm)	Pistillate	Bhandari and Sen (1973)
	Boron (3 ppm), GA (5 ppm), MH (100 ppm)	Pistillate	Bhonde (1978)
	GA (25 ppm, 50 ppm, 100 ppm)	Pistillate	Gopalkrishnan and Choudhury (1978)
	TIBA (50 ppm)	Pistillate	Alikhan *et al.* (1985)
Pumpkin (*Cucurbita moschata* Duch.)	CEPA (200 - 250 ppm)	Pistillate	Splittstoesser (1970); Swamy *et al.* (1977)
	Ethrel (100 - 200 ppm)	Pistillate	Hopping and Hawthorne (1979); Verma *et al.* (1985)
Snake gourd (*Trichosanthes anguina* L.)	MH (200 - 500 ppm)	Pistillate	Venkatram (1967)
	CCC (2000 ppm)	Pistillate	Ghosh and Bose (1970)
	B-9 (1250 - 5000 ppm)	Pistillate	Ghosh and Bose (1972)
	Ethrel (25 - 100 ppm)	Pistillate	Kohinoor and Mian (2005)

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl)	B-9 (1250 ppm)	Pistillate	Ghosh and Bose (1972)
	NAA (25 ppm), CCC (40, 60 ppm), MH (200 - 300 ppm)	Pistillate	Randhawa and Singh (1976)
	MH (50 ppm, 150 ppm)	Pistillate	Arora *et al.* (1982)
	IAA (100 - 250 ppm), TIBA (10 - 50 ppm)	Pistillate	Rahman *et al.* (1992)
	Ethrel (150 ppm)	Pistillate	Kooner *et al.* (2000)
	GA (5 ppm)	Pistillate	Gaurav *et al.* (2008)
	CCC 250 ppm PBZ (25 ppm), MH (200ppm)	Pistillate	Desai *et al.* (2011)
	Ethrel (600 ppm)	Pistillate	Patel *et al.* (2017)
Ridge gourd (*Luffa acutangula* (R oxb.) L)	CCC (500 - 2000 ppm)	Pistillate	Ghosh and Bose (1970)
	Ethrel or CCC (500 - 2000 ppm)	Pistillate	Patnaik *et al.* (1974)
	B-9, CCC, Phosphon-D (250 - 1000 ppm)	Pistillate	Krishnamoorthy and Bhatia (1976)
	Morphactin (100 ppm)	Pistillate	Bisaria (1977)
	Ethrel (50 ppm, 100 ppm)	Pistillate	Sadhu and Das (1978)

2.3 Maintenance of elite breeding stocks in cucurbits through micropropagation

Micropropagation has been successfully applied for the maintenance of elite plant types in cucurbits (Barnes *et al.*, 1978).

2.3.1 Explant standardization

Agarwal and Kamal (2004) experimented *in vitro* clonal propagation of bitter gourd by using shoot tip, nodal, internodal and leaf explants. Although regeneration was observed from all types of explants, the best response was given by shoot tip explant.

Verma *et al.* (2014) suggested that culturing axillary bud explant gave better response for shoot initiation and number shoots produced per explant compared to apical bud explant, during micropropagation of gynoecious lines in bitter gourd.

Saha and Behera (2015) opined that for *in vitro* multiplication of gynoecious line in bitter gourd, the shoot tip explant out performed nodal explants in all respects. The shoot tip explants took minimum days (5.26 days) for bud sprouting and gave maximum *in vitro* sprouting percentage compared to nodal explant.

An experiment was conducted by Saha *et al.* (2016) to standardize a viable protocol for *in vitro* mass multiplication and maintenance of bitter gourd gynoecious line. Apical bud and nodal segments were used as explants, among which apical bud gave better response for culture initiation. Apical bud took minimum number of days (5.53) for bud sprouting as well as it produced maximum number of shoots per explant (4.77) compared to nodal segment.

2.3.2 Manipulation of culture media

Agarwal and Kamal (2004) used shoot tips, nodal and intermodal and leaf explants from *in vitro* grown seedling of bitter gourd and cultured in modified MS medium, and the same medium supplemented with BAP, IBA and 2, 4-D showed shoot, root and callus differentiation respectively. Shoot and root differentiation was obtained on MS medium combined with BAP + IBA/NAA. MS medium combined with 2.0 mgl^{-1} BAP was the earliest to produce shoot initiation from shoot tip explants.

Moreover maximum number of multiple shoots from shoot tip explant was produced by higher concentration of BAP (2.0 mgl^{-1}).

Maximum response for growth of apical meristem was seen on semi-solid MS medium containing, Kn (0.05 mgl^{-1}) + GA_3 (0.1 mgl^{-1}). For shoot elongation with rooting, the media composed of MS + BA (1.0 mgl^{-1}) + IBA (0.1 mgl^{-1}) + GA_3 (0.3 mgl^{-1}) was found the best among various treatments. In case of root formation the medium containing NAA (0.1 mgl^{-1}) + IBA (0.5 mgl^{-1}) performed the best (Huda and Sikdar, 2006).

Al Munsur *et al.* (2007) opined that, leaf segments and root tips when used as explants produced equal response (65.0) in shoot regeneration, upon culture of the calli on medium with 2.0 or 2.5 mgl^{-1} BAP + 0.2 mgl^{-1} IAA. The MS medium combined with 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} IBA + 0.2 mgl^{-1} GA_3 produced maximum shoot elongation response (3.95 cm) from leaf segments, whereas MS medium containing 3.0 mgl^{-1} BAP with 0.1 mgl^{-1} NAA induced highest number of roots (6.75) and root length (2.45 cm) in root tips.

An efficient protocol for the maintenance and multiplication of male sterile ridge gourd plants was introduced by Pradeepkumar *et al.* (2007). Shoot tip and nodal segements were used as explants. Highest explant response was observed on MS medium supplemented with 1.5 mgl^{-1} IAA + 2.0 mgl^{-1} BAP. Maximum shoot elongation (9.0 cm.) was achieved on MS medium combined with 0.5 mgl^{-1} BAP. The shoots from the multiplication stage were transferred to rooting medium and highest percentage of rooting (95%) was obtained on MS medium (half strength) supplemented with 1.0 mgl^{-1} IBA and 200 mgl^{-1} charcoal.

Al Munsur *et al.* (2009) used nodal and root segments of bitter gourd as explants and cultured on MS medium supplemented with BAP in combination with either 2,4-D or NAA. Highest percentage of callus (93.75 %) within minimum number of days (7.75) in nodal segments was produced by a combination of 1 mgl^{-1} 2,4-D and 1 mgl^{-1} BAP, whereas in root segments 2.5 mgl^{-1} BAP combined with 0.6 mgl^{-1} NAA resulted in highest callus percentage (85.0%). Highest percentage of shoot regeneration (75.0%) from nodal segments, was exhibited by a combination of 1.0 mgl^{-1} 2,4-

D and 1.0 mgl^{-1} BAP. However root segments did not show any sign of regeneration in any of the combinations.

Thiruvengadam *et al.* (2010) proposed a viable protocol for *in vitro* organogenesis from callus-derived immature and mature leaf explants of bitter gourd. Immature leaf explants excised *in vitro* (15-day-old seedlings) and mature leaf explants of *in vivo* plants (45 days old) were used for callus induction. MS medium composed of 5.5 µM TDZ, 2.2 µM NAA, and 3.3 µM silver nitrate ($AgNO_3$) produced high regeneration of adventitious shoots from callus and the medium fortified with 4.0 µM IBA gave best response for root formation in elongated shoots.

Liu *et al.* (2011) developed a technique for *in vitro* multiplication of new variety of bitter gourd, Hualien No 1. Shoot apex and nodal segment were used as explants and cultured on MS medium combined with various concentrations of growth hormones *viz.*, BA, Kinetin, NAA and TDZ. MS medium added with 1.0 mgl^{-1} BA was found to give higher number of shoots per explant compared to other treatments. Successful callus induction of cent per cent was observed from internodal segments in the medium consisted of 2.0 mgl^{-1} NAA and 0.2 mgl^{-1} TDZ and high rooting percentage obtained from half strength MS medium supplemented with 1.0 mgl^{-1} IBA.

Tang *et al.* (2011) studied the role of additives in promoting adventitious bud induction from stem segments of bitter gourd. Initially the explants were inoculated on MS medium combined with IBA 1.0 mgl^{-1} and BA 2.0 mgl^{-1}, and then newly formed callus were transferred to subculture medium containing various concentrations of additives. The results displayed that TDZ at high concentration (0.5 mgl^{-1}) effectively induced adventitious buds and $AgNO_3$ and triacontanol significantly affected the bud formation.

In vitro induction of multiple buds from cotyledonary nodes of bitter gourd was studied by Ma *et al.* (2012). The best results for multiple buds regeneration was obtained from, eight-day old seedling, when cultured on MS medium supplemented with BAP and IBA. The optimum combination for multiple shoot induction was found to be MS medium with 2.5 mgl^{-1} of BAP and 0.1 mgl^{-1} of IBA.

When internodal explants were used, induction of organogenic callus and high frequency shoot regeneration was noticed by Thiruvengadam *et al.* (2012). The medium which induced organogenic callus, was MS + B5 combined with 2,4-D (5.0 µM) and TDZ (2 µM). The callus when transferred to MS medium containing 4.0 µM TDZ, 1.5 µM 2,4-D and 0.07 mM L-glutamine augmented the production of adventitious shoots, with a shoot induction frequency of 96.5 per cent and a high regeneration percentage (48 shoots per explant). Rooting of the elongated shoots was influenced by the hormone IBA, and the maximum percentage of rooting was gained from MS medium with 3.0 µM IBA.

Verma *et al.* (2014) described a technique for micro propagation of gynoecious bitter gourd by culturing axillary bud and apical bud of identified gynoecious lines on MS medium for shoot initiation. Maximum shoot length was obtained in half strength MS + 0.5 mgl^{-1} BAP. The best rooting media was found to be half strength MS medium supplemented with 1.0 mgl^{-1} IBA.

Saha and Behera (2015) suggested a technique for rapid *in vitro* multiplication of gynoecious line in bitter gourd. MS media in combination with BAP (2 mgl^{-1}) + NAA (0.2 mgl^{-1}) was the best treatment for culture initiation. In bitter gourd BAP + GA_3 + IBA combination was found to enhance the shoot multiplication rate and MS + BAP (1 mgl^{-1}) +IBA (0.1 mgl^{-1}) + GA_3 (0.3 mgl^{-1}) was the best combination for the same. The experimental data also indicated that elongation media itself served as the rooting media but with little addition of charcoal to it (100 mgl^{-1}); MS + GA_3 (1 mgl^{-1}) + 100 mgl^{-1} charcoal. It was noted that overall rooting capacity of explants improved by the addition of activated charcoal in the rooting medium.

Resmi and Sreelathakumary, (2015) successfully cultured shoot tip explants of bitter gourd either on the MS medium supplemented with IBA (4.00 mgl^{-1}) + BA (0.50 mgl^{-1}) + 2,4- D (2.00 mgl^{-1}) or NAA (2.00 mgl^{-1}) + BA (0.50 mgl^{-1}) + 2,4- D (2.00 mgl^{-1}) and observed induction of potentially organogenic callus and better proliferation. The best response for shoot regeneration from, shoot tip derived callus was noticed on MS medium fortified with 0.05 % AC + 1.00 mgl^{-1} BA with earlier

regeneration, highest regeneration percentage, and more number of leaves. MS fortified with IBA (1.00 mgl^{-1}) showed the best rooting response.

Micropropagation has been attempted to maintain gynoecy in bitter gourd. An efficient protocol for *in vitro* mass multiplication and maintenance of bitter gourd gynoecious line was standardized by Saha *et al.* (2016). Apical bud and nodal segments were used as explants. MS basal medium supplemented with BAP (2.0 mgl^{-1}) + NAA (0.2 mgl^{-1}) was found best for *in vitro* survival (81.3%) and medium combination MS + BAP (1.0 mgl^{-1}) + IBA (0.1 mgl^{-1}) + GA_3 (0.3 mgl^{-1}) was found best for early shoot proliferation. Maximum elongation of microshoots was achieved with MS + GA_3 (1.0 mgl^{-1}) and rooting of micro-shoots was highest in MS + GA_3 (1.0 mgl^{-1}) + activated charcoal (100 mgl^{-1}).

In vitro maintenance and multiplication of parthenocarpic cucumber (*Cucumis sativus* L.) was studied by Bharadwaj *et al.* (2017). *In vitro* germinated cotyledonary leaf explants were used as explants. For shoot initiation and response (%), half strength MS + 0.5 mgl^{-1} IAA + 2 mgl^{-1} BAP) was found to be superior with respect to number of days taken. Better rooting response was exhibited by the treatment half strength MS + 0.25 mgl^{-1} IAA.

Kumar *et al.* (2018) described an efficient protocol for the *in vitro* propagation of bitter gourd from nodal, and root explants. Highest percentage (95 %) of callus induction from nodal explants was observed, when cultured on MS medium fortified with 1.0 mgl^{-1} BAP and 1.0 mgl^{-1} 2,4-D, while for root segments a combination of 2.5 mgl^{-1} BAP and 0.6 mgl^{-1} NAA produced the best (85 %) callus response. Shoot regeneration was highest (77 %) from nodal segments when cultured on medium containing 1.0 mgl^{-1} BAP and 1.0 mgl^{-1} 2, 4-D. They also suggested that for rooting, the best treatment turned out to be MS combined with 2.0 mgl^{-1} BAP, 0.5 mgl^{-1} IBA and 0.2 mgl^{-1} GA_3. No shoot regeneration was observed from the root explants.

Materials and Methods

3. MATERIALS AND METHODS

The present study 'Breeding for gynoecy in bitter gourd (*Momordica charantia* L.)' was carried out at Department of Vegetable Science, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, during the period of 2017-2019. Field experiments were conducted within polyhouse and rain shelter of the department and the laboratory experiments were conducted in the biotechnology laboratory, under Department of Vegetable Science, KAU, Thrissur.

Experiment site was located at an altitude of 22.5m above MSL between 10°32'N latitude and 75°16' longitude. The location experienced warm humid climate. Soil of experimental site was textured class of sandy loam and was acidic in pH (5.7).

3.1 EXPERIMENTAL MATERIALS AND METHODS

The experimental materials consisted of gynoecious type (KAU-MCGy-101) of bitter gourd, (*Momordica charantia* L.) identified from the experimental field of Department of Vegetable Science, Vellanikkara (Plate 1) and monoecious variety 'Preethi' released by Kerala Agricultural University, Thrissur.

3.1.1 Experimental methods

3.1.1.1 Evaluation of sib mated and crossed population involving gynoecious female parents.

During the first season, seeds from the identified gynoecious plants (sib mated) were sown in the polyhouse along with the monoecious variety 'Preethi'.

a. Sib mating of gynoecious plants

Selected plants of the gynoecious population were sprayed with silver thiosulphate at 200 ppm just after flowering, for the induction of male flowers. Well developed female buds of gynoecious plants were selected and covered with butter paper bags in the evening hours on the day before anthesis. In the same way induced male buds of male sibs of gynoecious plants were also selected and covered. Anthesis typically occurs between 3:30 and 7:30 a.m. and the pollen viability is lost relatively

rapidly. The stigma is usually receptive for one day before or after flower opening, after which it dries and turns brown. Pollination was conducted within two hours after anthesis. During this time the pollen collected from the covered male buds were brushed over the stigma of the covered female flowers, and then labelled and tagged. The sib mated female flowers were kept covered for two more days till the fruit developed to avoid pollen contamination. The developed fruits were covered with paper covers to protect from fruit fly attack. The sib mated seeds (I_2) of gynoecious plants were collected at seed maturity and stored.

b. Crossing of gynoecious plants with monoecious variety 'Preethi'

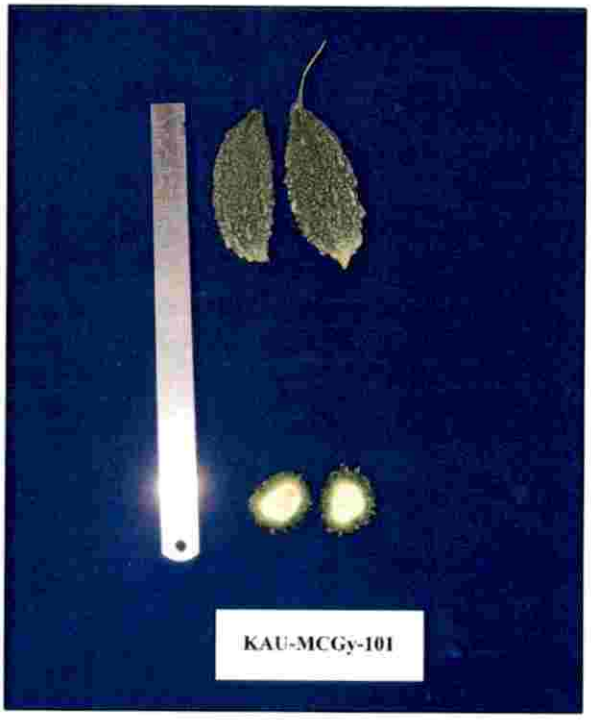
Crossing was done between monoecious plants of 'Preethi' and gynoecious plants in such a way that gynoecious plants served as the female parent. On the day before anthesis well developed female buds of gynoecious plants and male buds of 'Preethi' were selected and covered using butter paper cover in the evening hours. On the next day morning pollination was carried out within two hours after anthesis. The pollen collected from the male flowers were brushed over the selected female flowers of gynoecious plants. The flowers were then tagged and labelled and kept covered for two more days to avoid contamination from foreign pollen. When the fruits started developing, they were covered with paper covers in order to protect from the fruit fly attack. The F_1 (KAU-MCGy-101 × Preethi) seeds were extracted from the matured fruits and stored. A general view of gynoecious inbred and 'Preethi' in the polyhouse is given in the Plate 2.

c. Evaluation of gynoecious inbred (KAU-MCGy-101) and F_1 (KAU-MCGy-101 × Preethi)

The population generated from sib mating between gynoecious plants and male sibs as well as those with selected monoecious line, 'Preethi' (F_1 plants) were raised in the rain shelter in the second season and evaluated for sex expression and fruit quality (Plate 3). There were a total of 124 plants including 64 gynoecious plants (I_2) and 60 F_1 plants in the field. The crop was maintained as per the package of practices recommendation (KAU, 2016).

1.a. KAU-MCGy-101

1.b. Field view of fruits of KAU-MCGy-101



1.c. Fruits of KAU-MCGy-101

Plate 1. General view of identified gynoecious inbred KAU-MCGy-101

2.a. General view of gynoecious inbred and 'Preethi' in the polyhouse

2.b. Fruits of gynoecious inbred

2.c. Enlarged view of fruit of gynoecious inbred

Plate 2. General view of gynoecious inbred and 'Preethi' in the polyhouse

Plate 3. Field layout during evaluation of gynoecious inbred (KAU-MCGy-101) and F_1 hybrid (KAU-MCGy-101 × Preethi)

3.2 PLANT CHARACTERS STUDIED

Observations were recorded on important vegetative, fruit and yield characters in 14 randomly selected plants, in both gynoecious and F_1 plants. Procedures followed for recording observations are furnished below.

3.2.1 Quantitative characters

Fruit characters were recorded in five randomly selected fruits in the 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. **Node at which first female flower emerged**: 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 at which first male flower emerged**: Nodes were counted from the lowest to the one at which the first male flower emerged.

5. **Average fruit weight (g)**: Weight of five fruits from selected plants was recorded and average was calculated.

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

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

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

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

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

11. **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.

12. **Incidence of pest and diseases**: Various diseases and pests like mosaic, fruit fly, etc. and their occurrence (severe/ moderate/ mild/ very low/ nil) was recorded.

3.2.2 Qualitative characters

Five fruits were randomly selected from plants and recorded the following fruit characters based on minimal descriptors of agri-horticultutral crops, NBPGR (2001) and DUS test guidelines (2009).

1. **Fruit colour of skin:** Colour of fruit skin at marketable stage (white/ creamy white/ light green/ green/ dark green/ glossy green

2. **Fruit skin lusture:** Fruit skin lusture recorded at the marketable stage (matt/ intermediate/ glossy)

3. **Fruit: shape of base at peduncle end:** Fruit shape at peduncle end recorded during 8 -14 days after anthesis (acute/ obtuse/ rounded/ flattened)

4. **Fruit: shape of apex at blossom end:** Fruit shape at blossom end recorded during 8 -14 days after anthesis (acute/ obtuse/ rounded/ flattened)

5. **Fruit: shape in longitudinal section:** Fruit shape in longitudinal section recorded during 8-14 days after anthesis (oblong/ ovate/ spindle shaped/ club shaped/ triangular)

6. **Fruit surface:** Nature of tubercles present on the surface of fruits, recorded in cross-section at marketable stage (smooth/ light tubercle/ deep tubercle)

7. **Fruit: tubercles:** Presence or absence or density of tubercles on the fruit surface at harvestable stage (absent/ few/ medium/ many)

8. **Fruit: tubercles prominence:** Prominence of fruit tubercles on the fruit surface at harvestable maturity (conspicuous/ non- conspicuous)

9. **Fruit: ridge:** continuous/ discontinuous

10. **Fruit bitterness:** fruit bitterness observed by tasting the flesh of the middle part of the fruit at marketable maturity (mild/ moderate/ strong)

3.3 Micropropagation of gynoecious genotypes through shoot tip culture

3.3.1 Medium composition

In the present study, the most commonly used MS medium (Murashige and Skoog, 1962) with certain modifications was used. The nutrient medium included inorganic salts, growth regulators and a carbon source. The details of the media composition are given in the appendix I.

3.3.2 Culture medium preparation

For the preparation of MS plant tissue culture media, standard procedures were followed. Initially stock solutions of major and minor nutrients and growth regulators were prepared, by dissolving the required quantities of chemicals in double distilled water and stored under refrigerated conditions. Specific quantities of the stock solutions were pipetted out into 1000 ml beaker. Sucrose (30 gl^{-1}) and inositol were freshly added, after making up the volume upto 1000 ml, using double distilled water. The pH of the media was adjusted to 5.8 ± 0.1 with 1N NaCl or 1N HCl and then solidified with agar (8 gl^{-1}) and autoclaved at 121°C at 15 *psi* (1.06 kg cm^{-2}) for 15-20 minutes. The medium was allowed to cool to room temperature, after sterilization and stored in the culture room until used.

3.3.3 Source of explant

Vigorous, pest and disease free gynoecious plants grown in polyhouse were selected as stock plants. Very young fresh shoot tips were carefully excised from the selected plants and used as explants for the present study.

3.3.4 Collection and preparation of explant

The shoot tip explants were collected from the gynoecious plants in the polyhouse during early morning hours using clean sterilized scissors. The plants were sprayed with Bavistin @ 1g/l at 24 hours before taking the young shoot tip explants. The explants were excised to 2.0-2.5 cm after removing immature leaves and leaf primordia and washed thoroughly in running tap water for 10 minutes and then washed repeatedly in double distilled water. The explants were then soaked in mild detergent (Tween 20) and 0.1 per cent Bavistin for 10 minutes and thoroughly rinsed five to six times with distilled water.

3.3.5 Surface sterilization

The pre-treated explants 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. After sterilization, the explants were allowed to dry by transferring them onto sterile tissue paper on a sterile petri dish.

3.3.6 Inoculation and incubation of explants

All the vessels and tools (beakers, petri-plates, blade, forceps *etc.*) used in the laminar air flow chamber were flame sterilized just before inoculation using a spirit lamp inside the chamber.

The explants were inoculated on MS medium fortified with various concentrations and combinations of BA and NAA for shoot induction, in the test tubes containing 15 ml of the medium. All the cultures were maintained at 27 ± 1 °C in an air conditioned culture room under 16 hour's photoperiod.

3.3.7 Explant Establishment

3.3.7.1 Standardization of shoot initiation and multiple shoot induction media

The MS medium supplemented with different levels of BA and NAA was tested for its efficiency in shoot initiation and multiple shoot induction from young shoot tip explants of gynoecious bitter gourd. The basal media was modified as following treatments (Table 3.1).

Table 3.1: Details of media composition for shoot induction

Treatment	Media composition
T_0	MS + No hormone (Control)
T_1	MS + 0.5 mgl^{-1} BA + 0.1 mgl^{-1} NAA
T_2	MS + 0.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA
T_3	MS + 1.0 mgl^{-1} BA + 0.1 mgl^{-1} NAA
T_4	MS + 1.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA
T_5	MS + 1.5 mgl^{-1} BA + 0.1 mgl^{-1} NAA
T_6	MS + 1.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA
T_7	MS + 2.0 mgl^{-1} BA + 0.1 mgl^{-1}NAA
T_8	MS + 2.0 mgl^{-1} BA + 0.2 mgl^{-1}NAA
T_9	MS + 0.1 mgl^{-1}NAA
T_{10}	MS + 0.2 mgl^{-1}NAA
T_{11}	MS + 0.5 mgl^{-1} BA
T_{12}	MS + 1.0 mgl^{-1} BA
T_{13}	MS + 1.5 mgl^{-1} BA
T_{14}	MS + 2.0 mgl^{-1} BA

Five explants cultured in test tubes were taken as a single replication. The explants were transferred to fresh media at two weeks interval until the formation of shoot buds. Observations on days taken for callusing and shoot initiation, per cent of cultures developing shoot and number of shoots per explant were recorded.

3.3.8 Standardization of shoot elongation media

In vitro shoots from the multiple shoot induction media were cultured for shoot elongation in MS medium fortified with different combinations of NAA and IAA. A total of 12 replications were laid out for each treatment. The details of combinations of growth hormones are given in the Table 3.2.

Table 3.2: Composition of shoot elongation media

Treatment	Media composition
EM_1	MS + No hormone (Control)
EM_2	MS + 0.5 mgl^{-1} NAA
EM_3	MS + 1.0 mgl^{-1} NAA
EM_4	MS + 0.5 mgl^{-1} IAA
EM_5	MS + 0.5 mgl^{-1} IAA + 0.5 mgl^{-1} NAA
EM_6	MS + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} NAA
EM_7	MS + 1.0 mgl^{-1} IAA
EM_8	MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1} NAA
EM_9	MS + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} NAA

Observation on length of microshoots in elongation media after 1 month (cm) was recorded.

3.3.9 Standardization of *in vitro* root induction media

Full MS and half MS media were used for studying *in vitro* rooting. The elongated shoots (>5.0 cm height) were excised and transferred to root induction media. The details of the media supplemented with different combinations of IBA, and activated charcoal are given below (Table 3.3).

Table 3.3: Details of rooting media composition

Treatment	Media composition
RM_1	½ MS + No hormone
RM_2	½ MS + 0.5 mgl^{-1} IBA
RM_3	½ MS + 1.0 mgl^{-1} IBA
RM_4	MS + No hormone
RM_5	MS + 0.5 mgl^{-1} IBA
RM_6	MS + 1.0 mgl^{-1}IBA
RM_7	½ MS + 1.0 mgl^{-1}IBA + 3.0 gl^{-1} Activated charcoal

Observations on number of days taken for root initiation, number of roots and percentage of rooting after every 2 weeks were recorded.

3.3.10 Hardening and acclimatization

The *in vitro* rooted 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. The regenerated plants were then planted in polythene bags containing cocopeat: soil: sand mixture (1:1:1) and placed in mist chamber for hardening for 20 days in high humidity conditions. After that they were transferred to rain shelter for further observations on sex expression.

3.4 Induction of male flowers in gynoecious lines through hormonal regulation and development of inbreds.

During the second season, male flowers were induced in gynoecious lines raised in rain shelter using various treatments of silver thiosulphate (STS) and inbreds were developed through selfing. The effect of various concentrations of silver thiosulphate *viz.*, 150, 200 and 250 ppm, number of sprays and stage of application for induction of male flowers was studied in the experiment.

The details of the treatments are given in the Table 3.4.

Table 3.4: Various treatments of silver thiosulphate (STS) for inducing male flowers

Treatment No.	Silver thiosulphate (STS) concentration and no. of spray
T_1	150 ppm, double spray
T_2	150 ppm, single spray
T_3	200 ppm, double spray
T_4	200 ppm, single spray
T_5	250 ppm, double spray
T_6	250 ppm, single spray

Among the six treatments, the double spray of STS started at four leaf stage of the plant and subsequent spray was given with the appearance of first pistillate flower, while the single spray treatments were applied only after the appearance of first pistillate flower.

3.4.1 Observations

Following Observations were recorded during the experiment.

1. **Days to first male flower anthesis:** Number of days taken for the appearance of first male flower after spraying with silver thiosulphate solution was counted
2. **Node at which first male flower formed:** Nodes were counted from the lowest to the one at which the first male flower emerged
3. **Branch on which first male flower emerged:** Observation was recorded on whether the first male flower was formed on primary, secondary or tertiary branch.
4. **Total number of male flowers:** Total number of male flowers produced was counted and average was taken for each treatment

5. **Duration of male flowering phase:** Total number of days of male flowering phase was counted from day at which first male flower emerged to the day at which last male flower formed.

3.4.2 Preparation of silver thiosulphate (STS) solution

The different concentrations of silver thiosulphate solution *viz.,* 150, 200 and 250 ppm were prepared from 1000 ppm freshly made stock solution as follows.

Materials required:

Sodium thiosulphate ($Na_2S_2O_3$. $5H_2O$) – 59.525 g
Silver nitrate ($AgNO_3$) – 5.1 g
Double distilled water – 1L

Procedure: $Na_2S_2O_3$. $5H_2O$ was dissolved in 250 ml double distilled water in a volumetric flask and then poured into a 1L container. The flask was rinsed with 250 ml double distilled water and poured into the container. $AgNO_3$ was dissolved in 250 ml double distilled water in a separate volumetric flask and was poured into the jar containing $Na_2S_2O_3$. $5H_2O$ solution. The flask was rinsed with 250 ml double distilled water and poured into the container. The working solution of desired strength was prepared by taking appropriate quantities of stock solution, and diluting the same with distilled water to get required concentration of STS solution *i.e.,* 150, 200 and 250 ppm. The appropriate quantity of stock solution to be taken was worked out using the normality equation, $N_1V_1 = N_2V_2$

Application: Before spraying, the solution was diluted with double distilled water in the ratio 1:1. There were 5 replications for each treatment and spraying was done according to the treatments.

3.5 Statistical analysis

Statistical parameters *viz.,* range, mean, variance, standard deviation, standard error, coefficient of variation *etc.* were calculated using the online software 'Wasp 2.0' developed by, ICAR- Central Coastal Agricultural Research Institute for Goa.

3.5.1 Range

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

3.5.2 Mean

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

$$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} xi$$

3.5.3 Variance

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

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

3.5.4 Standard deviation

$$SD = \sqrt{var}$$

3.5.5 Standard error

$$SE = \frac{SD}{\sqrt{n}}$$

Where n = number of plants

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3.5.6 Coefficient of variation

The formula for C.V. was suggested by Snecdecor and Cochran (1967)

$$C.V. = \frac{SD}{Mean} \times 100$$

The data obtained on various parameters studied in the third experiment 'induction of male flowers through hormonal regulation' were subjected to analysis of variance, using the online software 'Wasp 2.0'

Results

4. RESULTS

The present investigation entitled 'Breeding for gynoecy in bitter gourd' was accomplished with an objective to develop stable gynoecious inbred lines in bitter gourd through selection from sib mated and crossed population involving gynoecious female parents and to maintain gynoecy through hormonal regulation and tissue culture. The results obtained from the various experiments are furnished below under following heads.

4.1 Evaluation of sib mated population and F_1 hybrid involving gynoecious female parents

- Estimation of range, mean, standard error, variance and coefficient of variation, for various biometric characters of sib mated gynoecious inbred and F_1 hybrid
- Analysis of qualitative characters of gynoecious inbred and F_1 hybrid
- Genetic analysis of sib mated gynoecious inbred and F_1 hybrid for sex expression

4.2 Micropropagation of gynoecious genotypes through shoot tip culture

- Standardization of culture initiation and multiple shoot induction media
- Standardization of shoot elongation media
- Standardization of *in vitro* rooting media

4.3 Effect of various concentrations of silver thiosulphate (STS), number of sprays and stage of application for induction of male flowers in gynoecious lines.

4.1 Evaluation of sib mated population and F_1 hybrid involving gynoecious female parents

4.1.1 Estimation of range, mean, standard error, variance and coefficient of variation

The range, mean, standard error, variance and coefficient of variation (CV) of different parameters of the sib mated gynoecious inbred (KAU-MCGy-101) and F_1 hybrid (KAU-MCGy-101 × Preethi) was estimated and presented in Table 4.1. The salient findings are given below.

a. Days to first female flower

The sib mated gynoecious inbred (KAU-MCGy-101) exhibited early flowering as it took less number of days (32.78) to form the female flower compared to F_1 hybrid (KAU-MCGy-101 × Preethi) (36.28). The range was more for inbred (21 - 40 days) than the F_1 hybrid (31 - 43 days). As expected, hybrid exhibited low variance and CV when compared to gynoecious inbred. The variance and CV observed for hybrid and inbred were 10.53, 8.94 and 38.64, 18.96 respectively.

b. Node at 1st female flower

Node to first female flower exhibited a similar trend as that of days to form the first female flower. Inbred showed a wide range (15 - 27) for this character when compared to F_1 hybrid (19 - 26). The first female flower was noticed at a lower node (20.07) in inbred than the hybrid (22.36). Higher variation in node at 1st female flower anthesis was observed in the gynoecious inbred with a variance and CV of 13.00 and 17.96 respectively, where as in F_1 hybrid variation was comparatively lesser with a variance and CV of 6.71 and 11.58 respectively.

c. Days to 1st male flower

The gynoecious inbred was found to be stable and pure with regard to sex expression, as none of the plants (64) produced male flowers during the growth phase of 116 days. However the hybrid involving gynoecious inbred produced male flowers on 39th day after sowing. The hybrid showed low variance and CV as expected, which were 2.62 and 4.15 respectively.

d. Node at 1st male flower

The lowest node at which 1st male flower emerged was 16 and the highest node was 23 in the F_1 hybrid. The mean value for node at 1st male flower emergence was 18.50. Less variation was exhibited by the hybrid for this character, with a variance and CV of 5.19 and 12.32.

e. Days to 1st harvest

The female flowers borne on the gynoecious inbred were pollinated with male flowers from the monoecious plants. The gynoecious inbred took more number of days (65.25) to attain harvestable maturity when compared to hybrid (55.93). The character showed a wide range (55 - 73 days) in gynoecious inbred, whereas, it ranged between 51 - 66 days in F_1 hybrid. In both the genotypes relatively lesser variation was noticed for this character with a variance and CV of 23.61 and 8.68 respectively in F_1 hybrid and 34.06 and 8.94 respectively in gynoecious inbred.

f. Number of harvest

With respect to number of harvest, the mean value was found to be higher in the F_1 hybrid (7.14) than the gynoecious inbred (3.64). This character ranged from 6 to 10 in F_1 hybrid and 3 to 5 in gynoecious inbred. The variation observed for number of harvest in both the genotypes was comparable with a CV of 16.34 and 17.38 in F_1 hybrid and inbred respectively.

g. Total no.of fruits

F_1 hybrid (32.64) produced more number of fruits when compared to inbred (15.57). A wide range (28 - 45) was exhibited by F_1 for this character, whereas it ranged between 12 - 25 for gynoecious inbred. Variance and CV were found to be 22.25 and 14.45 respectively in hybrid and 12.72 and 22.91 in inbred. This indicated a lesser variation for total number of fruits in F_1 hybrid compared to gynoecious inbred.

h. Total yield (kg)

High yield was exhibited by F_1 hybrid (2.61 kg) compared to gynoecious inbred (1.25 kg). Relatively broader range was exhibited for this character by both the genotypes, as the total yield ranged from 1.93 to 3.38 kg in F_1 hybrid and 0.87 to 2.08 kg in gynoecious inbred. F_1 hybrid showed low variance and CV (0.26 and 19.64 respectively) when compared to inbred (0.12 and 27.20 respectively).

i. Average fruit weight (g)

F_1 hybrid produced heavy fruits (91.28 g) when compared to gynoecious inbred (76.36 g). Both the genotypes showed a wide range for the character *viz.*, 85 - 124 g in F_1 and 60 - 102 g in inbred. In the case of variation, a similar trend was followed as seen in the above characters, where the hybrid exhibited low variance (106.37) and CV (11.29) compared to the inbred which showed 118.25 and 14.24 variance and CV respectively.

j. Average fruit length (cm)

There was not much variation for fruit length between gynoecious inbred and F_1 hybrid. The fruit length was observed to be 13.55 and 14.73 cm in inbred and hybrid respectively. The character ranged from 12.04 to 15.38 cm in gynoecious inbred, whereas in F1 hybrid it showed a range of 13.5 - 17.58 cm. Low variation was noticed in both genotypes for this character, with a CV of 7.83 and 7.77 in inbred and hybrid respectively.

k. Average fruit girth (cm)

Similar trend was noticed for this character as that of fruit length. Fruit girth was observed to be 16.04 and 16.15 cm respectively in gynoecious inbred and F_1 hybrid. A closer range was exhibited for this character by both the genotypes. In gynoecious inbred fruit girth was ranged from 14.75 to 18.24 cm, whereas in, F1 hybrid it ranged from 15.12 cm to 18.02 cm. Variation with respect to this character was lesser in both the gynoecious inbred and F_1 hybrid, with a CV of 7.00 and 6.61 respectively.

General view of gynoecious inbred and F_1 hybrid in the rain shelter during the evaluation is given in the Plate 4.

Table 4.1: Range, mean, standard error, variance and coefficient of variation for different parameters of gynoecious inbred and F_1 hybrid

Observations	Genotype	Range		Mean ± SE	Variance	CV %
		Min.	Max.			
Days to first female flower	KAU-MCGy-101	21	40	32.78 ± 1.66	38.64	18.96
	KAU-MCGy-101 × Preethi	31	43	36.28 ± 0.87	10.53	8.94
Node at 1st female flower	KAU-MCGy-101	15	27	20.07 ± 0.96	13.00	17.96
	KAU-MCGy-101 × Preethi	19	26	22.36 ± 0.69	6.71	11.58
Days to 1st male flower	KAU-MCGy-101	-	-	-	-	-
	KAU-MCGy-101 × Preethi	37	42	39.00 ± 0.43	2.62	4.15
Node at 1st male flower	KAU-MCGy-101	-	-	-	-	-
	KAU-MCGy-101 × Preethi	16	23	18.50 ± 0.61	5.19	12.32
Days to 1st harvest	KAU-MCGy-101	55	73	65.25 ± 1.56	34.06	8.94
	KAU-MCGy-101 × Preethi	51	66	55.93 ± 1.29	23.61	8.68

Observations	Genotype	Range		Mean ± SE	Variance	CV %
		Min.	Max.			
No. of harvest	KAU-MCGy-101	3	5	3.64 ± 0.17	0.40	17.38
	KAU-MCGy-101 × Preethi	6	10	7.14 ± 0.31	1.36	16.34
Total no.of fruits	KAU-MCGy-101	12	25	15.57 ± 0.95	12.72	22.91
	KAU-MCGy-101 × Preethi	28	45	32.64 ± 1.26	22.25	14.45
Total yield (kg)	KAU-MCGy-101	0.87	2.08	1.25 ± 0.09	0.12	27.20
	KAU-MCGy-101 × Preethi	1.93	3.38	2.61 ± 0.14	0.26	19.64
Average fruit weight (g)	KAU-MCGy-101	60	102	76.36 ± 2.91	118.25	14.24
	KAU-MCGy-101 × Preethi	85	124	91.28 ± 2.76	106.37	11.29
Average fruit length (cm)	KAU-MCGy-101	12.04	15.38	13.55 ± 0.28	1.13	7.83
	KAU-MCGy-101 × Preethi	13.5	17.58	14.73 ± 0.31	1.31	7.77
Average fruit girth (cm)	KAU-MCGy-101	14.75	18.24	16.04 ± 0.3	1.26	7.00
	KAU-MCGy-101 × Preethi	15.12	18.02	16.15 ± 0.28	1.14	6.61

4.a. KAU-MCGy-101 × Preethi (F_1 hybrid)

4.b. Enlarged view of fruit of F_1 hybrid

4.c. KAU-MCGy-101 (Gynoecious inbred)

4.d. Enlarged view of fruit of inbred

Plate 4. General view of F_1 hybrid and gynoecious inbred in the rain shelter

4.1.2 Analysis of qualitative characters

Important qualitative characters of gynoecious inbred and F_1 hybrid were recorded based on minimal descriptors of agri-horticultutral crops, NBPGR, (2001) and DUS test guidelines (2009). The data are given in the Table 4.2. Among the characters studied, the foremost important characters were colour of fruit skin at marketable stage and the fruit bitterness. The gynoecious inbred and the F_1 hybrid differed for these two characters, and the inbred exhibited dark green fruit skin colour, whereas all the F_1 hybrid fruits exhibited light green fruit skin colour. Fruit bitterness was found to be moderate in F_1 hybrid, while fruits of gynoecious inbred were strong bitter in nature.

No significant variation was seen for the character, fruit skin lustre, as both the inbred and hybrid presented intermediate skin lustre. With respect to the shape of fruit at peduncle end, no variation was observed among them and it was found to be acute in both gynoecious inbred and F_1 hybrid. Fruit shape of apex at blossom end was obtuse in both the gynoecious inbred and F_1 hybrid. All the fruits of inbred and F_1 hybrid were spindle shaped in longitudinal section, hence no variation was observed for this character among the two. Fruit surface was characterized with numerous deep triangular tubercles in both the genotypes. Gynoecious inbred and F_1 hybrid did not exhibit variation in tubercles characteristics. There were many tubercles which are non-conspicuous in nature on the fruit surface of inbred and F_1 hybrid. Fruit ridge character appeared similar in both gynoecious inbred and F_1 hybrid, and it was found to be broken or discontinuous surface ridges on fruits (Plate 5).

Table 4.2: Qualitative characters of sib mated gynoecious inbred and F_1 hybrid

Characters	Genotype	
	KAU-MCGy-101	**KAU-MCGy-101 ×Preethi**
Fruit colour of skin	Dark green	Light green
Fruit skin lustre	Intermediate	Intermediate
Fruit: shape of base at peduncle end	Acute	Acute
Fruit: shape of apex at blossom end	Obtuse	Obtuse
Fruit: shape in longitudinal section	Spindle shaped	Spindle shaped
Fruit surface	Deep tubercle	Deep tubercle
Fruit: tubercles	Many	Many
Fruit: tubercles prominence	Non-conspicuous	Non-conspicuous
Fruit: ridge	Discontinuous	Discontinuous
Fruit bitterness	Strong	Moderate

5.a. Fruits of gynoecious inbred

5.b. Fruits of F_1 hybrid

5.c. Enlarged view of fruits of gynoecious inbred

5.d. Enlarged view of fruits of F_1

Plate 5. Fruits of gynoecious inbred and F_1 hybrid

4.1.3 Genetic analysis of sib mated gynoecious inbred and F_1 hybrid for sex expression

As there was no segregation for the gynoecious character in the sib mated gynoecious inbred and F_1 hybrid, chi-square test was not carried out. It is clear from the observation that the gynoecious character is recessive in nature as all the F_1 plants (60) were monoecious in sex expression. The homozygosity of gynoecious sex expression is also proved in the present investigation as all the sib mated plants studied (64) were observed to be gynoecious in sex expression (Table 4.3).

4.1.4 Incidence of pest and diseases

Incidence of pest and diseases for gynoecious inbred and F_1 hybrid was recorded and presented in the Table 4.4. Moderate incidence of cucumber mosaic disease was observed in both inbred and F_1. Fruit fly (*Bactrocera cucurbitae*) attack was also moderate in both the genotypes. Mild infestation of stem gall fly (*Neolasioptera falcata*) was seen gynoecious inbred and F_1 hybrid. The symptom appeared as formation of galls in the distal shoot and later drying of shoot tip was observed. At the later stage of the crop, mild infestation by epilachna beetle (*Henosepilachna vigintioctopunctata*) was noticed in both the genotypes. Very low infestation of anthracnose (*Colletotrichum lagenarium*) was also observed in the later stage of the crop in both gynoecious inbred and F_1 hybrid.

Table 4.3: Genetic analysis of sib mated gynoecious inbred and F_1 hybrid for sex expression

Population	Number of plants		Total
	Gynoecious plants	Monoecious plants	
KAU-MCGy-101	64	0	64
KAU-MCGy-101 × Preethi	0	60	60

Table 4.4: Incidence of pest and diseases in F_1 hybrid and gynoecious inbred

Genotype	Cucumber mosaic disease	Fruit fly (*Bactrocera cucurbitae*)	Stem gall fly (*Neolasioptera falcata*)	Epilachna beetle(*Henosepilachna vigintioctopunctata*)	Anthracnose (*Colletotrichum lagenarium*)
KAU-MCGy-101	Moderate	Moderate	Mild	Mild	Very low
KAU-MCGy-101 × Preethi	Moderate	Moderate	Mild	Mild	Very low

4.2 Micropropagation of gynoecious genotypes through shoot tip culture

Occurance of gynoecy is a rare event in nature and it is generally maintained by application of sex modifying chemicals. Micropropagation is a viable and potential tool that can be adopted for the maintenance of gynoecious genotypes and to fix gynoecy without any genetic change. Hence, micropropagation was carried out to maintain the gynoecious line, identified in the experimental field.

The gynoecious plants raised in the polyhouse were selected as the stock plant for micropropagation and the shoot tips were cultured in MS medium containing various concentrations of BA and NAA. The data on *in vitro* regeneration of gynoecious genotype of bitter gourd from the shoot tip explants are presented below.

4.2.1 Standardization of culture initiation and multiple shoot induction media

A total of 15 treatments with various combinations of hormones, BA and NAA including control were tried to appraise its effect on callusing, shoot initiation and multiplication. The results obtained from the experiment are given in the Tables 4.5, 4.6 and 4.7.

4.2.1.a Callus initiation from shoot tip explants

Callus initiation was observed in all the treatments except T_0, T_9 and T_{10} (Table 4.5). Wide variation was noticed in the case of number of days taken for callus initiation (3.33 to 11.16). The treatment T_4 (MS + 1.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA) took minimum number of days (3.33) for callus initiation followed by T_7 (3.58), whereas maximum days for the same was exhibited by the treatment T_{11} (MS + 0.5 mgl-1 BA) *i.e.,* 11.16 days. Maximum callus response (100 %) was observed for the treatment T_8 (MS + 2.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA) (Plate 6) followed by T_2, T_6, and T_4 which gave good response *viz.*, 91.66, 83.33 and 83.33 per cent respectively for callus initiation and was on par with T_8. Among the 15 treatments tried, T_9 and T_{10} exhibited rhizogenesis without callusing, and they produced thick and fleshy roots.

4.2.1.b Shoot initiation response from gynoecious plants

The effect of BA and NAA for shoot initiation from shoot tip explants of the gynoecious genotype are presented in the Table 4.6. Out of the 15 treatments

attempted, only six treatments showed shoot initiation response. Significant difference was observed for this character between the treatments with a maximum of 66.66 per cent response exhibited by the treatment T_{14}, (MS + 2.0 mgl^{-1} BA) (Plate 7). Comparable response for shoot initiation was seen in the treatment T_{13} (58.33 %). Other treatments *viz.*, T_{12}, T_{11}, T_8 and T_6 exhibited 33.33, 25.00, 25.00 and 16.66 per cent response respectively. All the remaining treatments failed to respond for shooting under *in vitro* condition. The treatment T_8 (MS + 2.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA) was the earliest (19.33 days) to produce shoot initiation followed by T_{12} (19.75 days), while T_{11} (29.33) took maximum number of days for the shoot initiation.

4.2.1.c Multiple shoot induction

Multiple shoot initiation was observed in shoot induction media. Maximum number of shoots per explant (5.50) was obtained with the treatment T_{14} (MS + 2.0 mgl^{-1} BA) (Plate 8) which was on par with T_8 (MS + 2.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA) and T_{12} (MS + 1.0 mgl^{-1} BA), whereas the minimum number of multiple shoots was observed with the treatment T_6 (2.5). The results are given in the Table 4.7.

4.2.2 Standardization of shoot elongation media

In vitro sprouts were transferred to MS media fortified with various combinations of NAA and IAA. Data on shoot elongation are presented in the Table 4.8.

4.2.2.a Length of microshoots in elongation media after one month

Among the nine treatments experimented, *in vitro* shoot elongation was observed only in three treatments. Maximum length of shoot (9.03 cm) after one month was observed with the treatment EM_5 (MS + 0.5 mgl^{-1} IAA + 0.5 mgl^{-1} NAA), which was significantly superior to other treatments (Plate 9). The length of shoot was found to be 6.66 cm and 6.24 cm in other two treatments EM_9 (MS + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} NAA) and EM_8 (MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1} NAA) respectively, which were on par. All other treatments failed to give shoot elongation response.

Table 4.5: Effect of BA and NAA for callus initiation from shoot tip explants

Treatment	Days taken for callus initiation*	Callus initiation response (%)
T_0	NR	0.00^{c} (1.43)
T_1	4.25 ± 0.32^{d}	75.00^{ab} (64.52)
T_2	4.17 ± 0.38^{d}	91.66^{ab} (79.05)
T_3	4.08 ± 0.39^{d}	75.00^{ab} (60.00)
T_4	3.33 ± 0.14^{d}	83.33^{ab} (69.52)
T_5	4.00 ± 0.42^{d}	58.33^{b} (50.00)
T_6	3.83 ± 0.29^{d}	83.33^{ab} (74.05)
T_7	3.58 ± 0.35^{d}	75.00^{ab} (60.00)
T_8	3.67 ± 0.26^{d}	100.00^{a} (88.57)
T_9	NR	0.00^{c} (1.43)
T_{10}	NR	0.00^{c} (1.43)
T_{11}	11.16 ± 0.64^{a}	75.00^{ab} (60.00)
T_{12}	9.58 ± 0.58^{b}	66.66^{ab} (59.52)
T_{13}	8.33 ± 0.39^{c}	58.33^{b} (50.00)
T_{14}	8.42 ± 0.51^{c}	58.33^{b} (50.00)
CD (0.05)	1.15	32.75
CV (%)	25.22	37.85

* Data are Mean ± Standard error, n=12, NR – No Response, Value in parentheses are arc sin transformed

T0 = MS + No hormone (Control); T1 = MS + 0.5 mgl^{-1} BA + 0.1 mgl^{-1} NAA; T2 = MS + 0.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA; T3 = MS + 1.0 mgl^{-1} BA + 0.1 mgl^{-1} NAA; T4 = MS + 1.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA; T5 = MS + 1.5 mgl^{-1} BA + 0.1 mgl^{-1} NAA; T6 = MS + 1.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA; T7 = MS + 2.0 mgl^{-1} BA + 0.1 mgl^{-1}NAA;T8 = MS + 2.0 mgl^{-1} BA + 0.2 mgl^{-1}NAA; T9 = MS + 0.1 mgl^{-1}NAA; T10 = MS + 0.2 mgl^{-1}NAA; T11 = MS + 0.5 mgl^{-1} BA; T12 = MS + 1.0 mgl^{-1} BA; T13 = MS + 1.5 mgl^{-1} BA; T14 = MS + 2.0 mgl^{-1} BA.

Table 4.6: Effect of BA and NAA for sprouting from shoot tip explants (Shoot initiation response %)

Treatment	Days taken for shoot initiation*	Shoot initiation response (%)
T_0	NR	0.00^{d} (1.43)
T_1	NR	0.00^{d} (1.43)
T_2	NR	0.00^{d} (1.43)
T_3	NR	0.00^{d} (1.43)
T_4	NR	0.00^{d} (1.43)
T_5	NR	0.00^{d} (1.43)
T_6	20.50 ± 1.50^{b}	16.66^{cd} (8.9)
T_7	NR	0.00^{d} (1.43)
T_8	19.33 ± 0.88^{b}	25.00^{bcd} (25.48)
T_9	NR	0.00^{d} (1.43)
T_{10}	NR	0.00^{d} (1.43)
T_{11}	29.33 ± 2.03^{a}	25.00^{bcd} (25.48)
T_{12}	19.75 ± 2.01^{b}	33.33^{abc} (35)
T_{13}	28.71 ± 2.62^{a}	58.33^{ab} (50)
T_{14}	20.75 ± 1.73^{b}	66.66^{a} (59.52)
CD (0.05)	5.26	26.63

* Data are Mean ± Standard error, n=12, NR – No Response, Value in parentheses are arc sin transformed

Table 4.7: Effect of NAA and BA for multiple shoot induction

Treatment	Shoots per explant (No.)*
T_6	2.5 ± 0.50^{c}
T_8	4.3 ± 0.33^{ab}
T_{11}	2.66 ± 0.33^{c}
T_{12}	4.25 ± 0.47^{ab}
T_{13}	3.71 ± 0.42^{bc}
T_{14}	5.50 ± 0.62^{a}
CD (0.05)	1.32
CV (%)	30.40

*Data are Mean ± Standard error, n=12, NR – No Response

Table 4.8: Effect of NAA and IAA for shoot elongation

Treatment	Length of microshoots in elongation media after one month (cm)*
EM_1	NR
EM_2	NR
EM_3	NR
EM_4	NR
EM_5	9.03 ± 0.29^{a}
EM_6	NR
EM_7	NR
EM_8	6.24 ± 0.27^{b}
EM_9	6.66 ± 0.34^{b}
CD (0.05)	0.86
CV (%)	14.30

*Data are Mean ± Standard error, n=12, NR – No Response

EM1 = MS + No hormone (Control); EM2 = MS + 0.5 mgl^{-1} NAA; EM3 = MS + 1.0 mgl^{-1} NAA; EM4 = MS + 0.5 mgl^{-1} IAA; EM5 = MS + 0.5 mgl^{-1} IAA + 0.5 mgl^{-1} NAA; EM6 = MS + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} NAA; EM7 = MS + 1.0 mgl^{-1} IAA; EM8 = MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1} NAA; EM9 = MS + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} NAA.

6.a. Shoot tip explants from gynoecious inbred

6.b. Callusing - initial stage

6.c. Callusing - later stage

Plate 6. Shoot tip explants and stages of callusing response

7.a. Shoot initiation - stage I

7.b. Shoot initiation - stage II

7.c. Shoot initiation - Stage III

7.d. Shoot initiation - stage IV

Plate 7. Stages of shoot initiation

8.a. Multiple shoot induction - stage I

8.b. Multiple shoot induction - stage II

8.c. Multiple shoot induction - stage III

Plate 8. Multiple shoot induction stages

4.2.3 Standardization of *in vitro* rooting media

Well developed *in vitro* shoots with length ≥ 5 cm from the elongation media were transferred to root induction media (MS and ½ MS) supplemented with different concentrations of IBA and activated charcoal. The results obtained from different treatments are presented in the Table 4.9.

Out of the seven treatments compared, *in vitro* rooting was obtained from only two treatments *viz.*, RM_7 and RM_3. Among these two, RM_7 (½ MS + 1.0 mgl^{-1} IBA + 3 gl^{-1} activated charcoal) took minimum number of days (12.0) for root initiation compared to RM_3 (½ MS + 1.0 mgl^{-1} IBA) which took 16 days for the same. Maximum rooting percentage was also recorded by RM_7 (100%) when compared to RM_3 which exhibited only 33.33 per cent rooting response. Significant difference was observed between the treatments with respect to number of roots produced, and RM_7, (Plate 9) recorded highest value of 7.00 compared to RM_3 which produced only 3.66 roots. All the other treatments failed to respond *in vitro* rooting.

4.2.4 Evaluation of regenerated plants in polyhouse

All the *in vitro* rooted plantlets were transferred to sterilized potting medium and kept inside the mist chamber for hardening for 20 days (Plate 10). Survival per cent was found to be 70.58 during the hardening stage. The hardened regenerated plants were then transferred to rain shelter and biometric characters were studied. The data are given in the Table 4.10.

All the regenerated plants exhibited gynoecious sex expression (Plate 11 and 12), and the plants took 50.16 days for first female flower emergence. The node at which first pistillate flower appeared was found to be 22.33.

9.a. Shoot elongation - initial stage

9.b. Shoot elongation - later stage

9.c. Rooting - initial stage

9.d. Rooting - later stage

Plate 9. Stages of *in vitro* shoot elongation and rooting

Table 4.9: Effect of different media composition, including IBA and activated charcoal for root initiation

Treatment	Days taken for root initiation*	No. of Roots (after 4 weeks)*	Rooting %	
			(after 2 weeks)	(after 4 weeks)
RM_1	NR	0.00	0.00	0.00
RM_2	NR	0.00	0.00	0.00
RM_3	16.0 ± 0.57	3.66 ± 0.33	0.00	33.33
RM_4	NR	0.00	0.00	0.00
RM_5	NR	0.00	0.00	0.00
RM_6	NR	0.00	0.00	0.00
RM_7	12.0 ± 0.89	7.00 ± 0.36	50.00	100.00
CV %	14.45	13.86		

*Data are Mean ± Standard error, n=6, NR – No Response

RM1 = ½ MS + No hormone (control); RM2 = ½ MS + 0.5 mgl^{-1} IBA; RM3 = ½ MS + 1.0 mgl^{-1} IBA; RM4 = MS + No hormone; RM5 = MS + 0.5 mgl^{-1} IBA; RM6 = MS + 0.5 mgl^{-1} IBA; RM7 = ½ MS + 1.0 mgl^{-1} IBA + 3 gl^{-1} activated charcoal.

Table 4.10: Biometric parameters of hardened regenerated plants

Biometric parameters	Observations *
Survival % (after 20 days)	70.58 ± 0.16
Days taken for 1st female flower emergence	50.16 ± 0.89
Node at 1st female flower	22.33 ± 0.93

* Data are Mean ± Standard error, n=12

10.a. Hardening stage

10.b. Regenerated plant after hardening

10.c. Regenerated plant after transplanting

10.d. Regenerated plant after one week

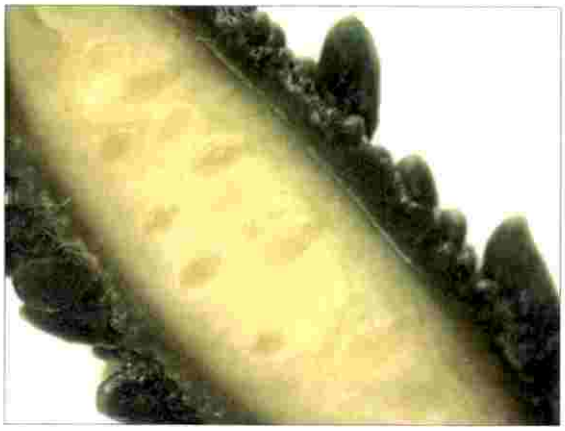
Plate 10. Hardening and transplanting stages of *in vitro* regenerated plants

11.a. General view of regenerated plants in rain shelter

11.b. female flower of regenerated plant

11.c. Fruit view of regenerated plant

Plate 11. General view of tissue cultured regenerated gynoecious plants



12.a. Ovary of female flower of regenerated gynoecious plant

12.b. Ovary of female flower of normal gynoecious plant

Plate 12. Microscopic view of ovary of female flowers of regenerated gynoecious plant and seed germinated gynoecious plant

4.3 Effect of various concentrations of silver thiosulphate (STS), number of sprays and stage of application for induction of male flowers in gynoecious lines

Silver thiosulphate (STS) at different concentrations was sprayed to study their effect in inducing male flowers in gynoecious lines. Concentrations ranged from 150, 200 and 250 ppm were sprayed on gynoecious plants once or twice according to the treatment starting from four leaf stage. Out of six, only the double spray treatments *viz.*, T_1, T_3 and T_5 were started at the four leaf stage. Young plants exhibited phytotoxic effects of silver ions with burning symptoms on leaves and stunted growth. Subsequent spray of these treatments was given after the appearance of first pistillate flower. The single spray treatments were applied only after the first female flower emergence. The data regarding the effect of various STS treatments on gynoecious line for male flower induction are presented in the Table 4.11.

It was observed that, none of the treatment induced male flower in gynoecious line. However, all the six treatments altered sex expression of gynoecious genotype from, pistillate to hermaphrodite within two weeks of application of STS. There was no significant difference between the treatments for days taken for the occurance of first hermaphrodite flower. The results revealed that, application of STS at four leaf stage in gynoecious bitter gourd, has no significant effect in alteration of sex. Induced hermaphrodite flowers were larger than the normal female flowers and characterised with large showy yellow coloured petals (pentamerous). They were actinomorphic, bracteate, pedicellate and pentamerous with calyx lobes alternating with corolla tubes. The flowers were distinguished with the presence of stamens (3-5) with free filament and fused anther lobes and epigynous ovary, thick and short style with a green three-lobed stigma (Plate 13 and 14).

The pollen grains from the stamen were crushed out and subjected to staining using acetocarmine (1.0 %) and was observed under microscope. Pollen grains were plump and fertile in all the treatments indicating the efficacy of silver thiosulphate treatment in inducing male fertility (Plate 15).

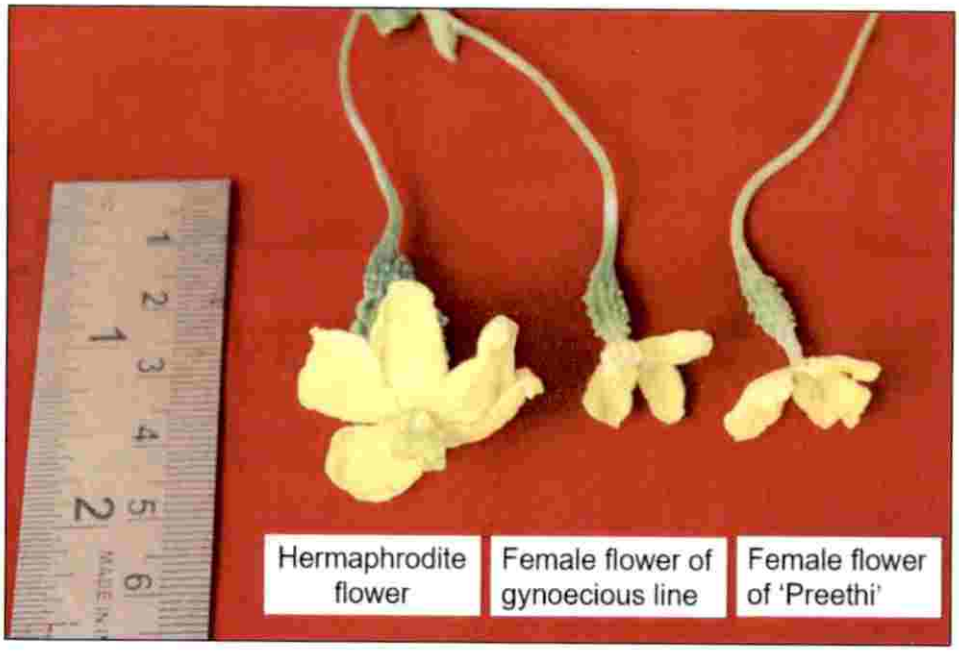
Node at 1st hermaphrodite flower emergence ranged from 22.4 to 29.6 and no significant variation was observed between treatments. Similarly the treatments did not differ significantly for the character, 'branch on 1st hermaphrodite flower formed'. All the treatments exhibited significant variation for only one character *i.e.*, total number of hermaphrodite flowers produced. Maximum number of hermaphrodite flowers were formed in the treatment T_4 (72.6) *i.e.*, 200 ppm STS single spray followed by T_3 (68.4) *i.e.*, 200 ppm double spray. While minimum number of bisexual flowers where produced in T_2 (32.4) *i.e.*, 150 ppm single spray. The range for this character was found to be 32.4 to 72.6. Duration of appearance of the hermaphrodite flower was also studied. The duration of bisexual flowering phase was observed to be approximately three weeks after the spray. The range for this character lies between 17.20 - 20.80, for the treatments, with no significant variation among the treatments.

Table 4.11: Effect of different treatments of STS on gynoecious inbred for hermaphrodite flower induction

Treatment	Days to 1st hermaphrodite flower anthesis	Node at which 1st hermaphrodite flower emerged	Branch on which 1st hermaphrodite flower emerged	Total number of hermaphrodite flowers	Duration of hermaphrodite flowering phase
T_1	13.0	22.4	2.4	38.6[b]	18.2
T_2	13.6	27.4	2.2	32.4[b]	18.2
T_3	13	26	2.2	68.4[a]	18.8
T_4	13.4	29.6	1.6	72.6[a]	20.8
T_5	13	28	2.2	47.8[b]	19.8
T_6	13.2	25.2	2.2	44.4[b]	17.2
CD 0.05	NS	NS	NS	19.12	NS
CV (%)	2.76	16.27	26.37	28.90	19.17

13.a. Hermaphrodite flower

13.b. Hermaphrodite flower and male flower



13.c. Hermaphrodite flower and female flowers

Plate 13. Hermaphrodite flower with male flower and female flowers

14.a. STS- 150 ppm
double spray

14.b. STS- 150 ppm
single spray

14.c. STS- 200 ppm
double spray

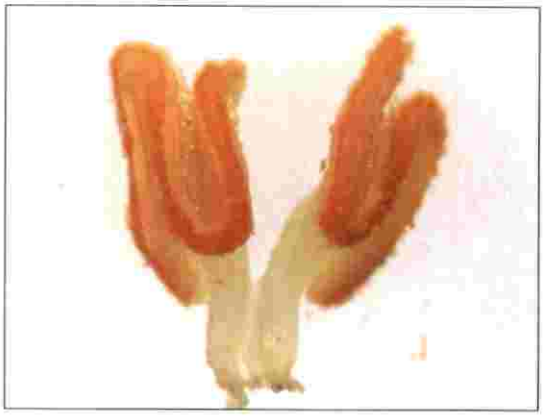
14.d. STS- 200 ppm
single spray

14.e. STS- 250 ppm
double spray

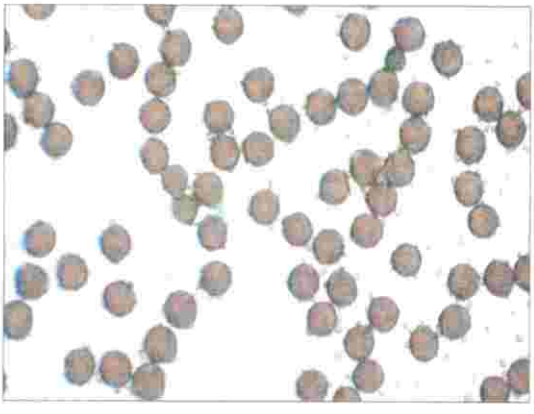
14.f. STS- 250 ppm
single spray

Plate 14. Induction of hermaphrodite flowers by various treatments of STS

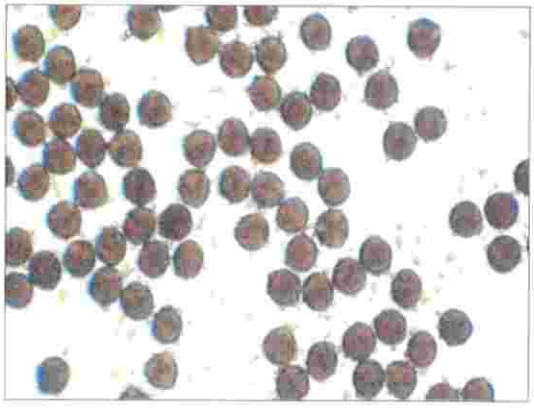
15.a. Hermaphrodite flower without petals and sepals



15.b. Anther lobe of hermaphrodite flower



15.c. Pollen grains of hermaphrodite flower after staining with acetocarmine



15.d. Pollen grains of male flower of monoecious variety 'Preethi' after staining with acetocarmine

Plate 15. Microscopic view of hermaphrodite flower and pollen grains

Discussion

5. DISCUSSION

Cucurbits are known to be monoecious in nature. However, sex expression in cucurbits varied from primitive hermaphrodite to andromonoecious, gynomonoecious, trimonoecious, dioecious, androecious, gynoecious, and monoecious, sex forms (Sheshadri, 1986). Of the different sex forms, gynoecious trait assumes more significance for the breeders as the trait can be manipulated for economizing F_1 hybrid seed production in bitter gourd. Utility of the gynoecious sex expression depends heavily on the agronomic superiority of the gynoecious inbreds. Though one hybrid has been developed from IARI, it has not been popularized in south India, due to the above limitation.

In the present investigation, the gynoecious line (KAU-MCGy-101) identified from the Kerala Agricultural University, was tested for stability of the gynoecious sex expression. The inbred was also crossed with the monoecious line 'Preethi' and the F_1 hybrid was evaluated for various biometric characters along with the sib mated population of the gynoecious lines. The salient findings from the various experiments are discussed below.

5.1 Evaluation of sib mated population and F_1 hybrid involving gynoecious female parent

The results revealed that gynoecious inbred plants developed through sib mating produced female flowers throughout the growth period, indicating the stability of the gynoecious sex expression. In India Ram *et al.* (2002 a,b) and Behera *et al.* (2006) also reported stable gynoecious lines in bitter gourd. Though there was no variation in the sex expression, variability was observed for the biometric characters in the gynoecious lines. Gynoecious inbred exhibited a wide range for all the characters studied compared to the F_1 hybrid (KAU-MCGy-101 × Preethi), *viz.*, days to first female flower anthesis, node number of first female flower, days to first harvest, number of harvest, total number of fruits per plant, and yield per plant. In cucurbits, days to first female flower anthesis and node number of first female flower is an index of earliness (More and Sheshadri, 1998). The gynoecious inbred exhibited early bearing of female flowers at lower nodes compared to the F_1 hybrid. Hence the

gynoecious inbred, KAU-MCGy-101 holds enormous potential for future breeding programme for earliness in bitter gourd. Hermaphrodite flowers were induced in the gynoecious line through STS application and sib mating was carried out between gynoecious lines to study their fruit characters. The inbred produced fruits with an average weight of 76.36 g which is higher than the earlier reported lines from India (Behera *et al.*, 2006). The early bearing habit as well as the high fruit weight points towards the scope of the present inbred lines in future breeding programme. The inbred recorded an average fruit length and girth of 13.55 cm and 16.05 cm respectively. The range of these biometric characters observed for the gynoecious inbred was found to be higher than the early reported gynoecious lines (Fruit weight = 68.93 g, Fruit length = 8.87 cm) (Behera *et al.*, 2006).

F_1 hybrid involving gynoecious inbred as the female parent and monoecious stable variety (Preethi) from Kerala Agricultural University as male parent was grown along with the gynoecious inbred lines to compare the biometric characters and to study the genetics of sex expression. Hybrid outperformed the inbred with respect to fruit and yield characters. This is the preliminary indication of the potential of present inbred line which need to be confirmed by making a series of crosses with selected monoecious lines.

With respect to qualitative characters, all the sib mated gynoecious plants produced dark green colour spindle shaped fruits, whereas the fruits of F_1 hybrid were light green in colour with spindle shape. Generally North Indian consumers prefer long or medium long and spindle shaped glossy green fruits whereas South Indians require long but white fruits (Behera *et al.*, 2008; Dey *et al.*, 2010). The fruits of gynoecious inbred were strong bitter in nature, while moderate bitterness was exhibited by F_1 hybrid fruits. Fruit surface in both inbred and hybrid was characterized with the presence of numerous triangular deep tubercles. Discontinuous or broken surface ridges was found in both gynoecious inbred and F_1 hybrid.

5.2 Genetic analysis of sib mated gynoecious inbred and F_1 hybrid for sex expression

Sex inheritance pattern of gynoecy varies in different cucurbitaceous crops (More and Sheshadri, 1998). In the present investigation inheritance of gynoecious sex expression was analysed and the results revealed that there was no segregation for the gynoecious character in the sib mated gynoecious inbred and F_1 hybrid. Since all the F_1 plants (60) were monoecious in sex expression, it is evident that gynoecious character in bitter gourd is recessive in nature. Moreover gynoecious sex expression was found to be homozygous, as all the sib mated plants studied (64) were gynoecious in sex expression. The present findings were in agreement with the previous reports that, gynoecism in bitter gourd is under the control of recessive gene (*gy-1*) (Ram *et al.*, 2006; Dey *et al.*, 2008; Behera *et al.*, 2009; Mishra *et al.*, 2015 b).

5.3 Micropropagation of gynoecious genotypes through shoot tip culture

Standardization of protocol for rapid *in vitro* multiplication of gynoecious lines in bitter gourd could be exploited for hybrid seed production (Saha and Behera, 2015). Utilization of micropropagation techniques may augment the ability to develop homozygosity in a short period particularly for gynoecious trait.

Different types of explants were used for *in vitro* propagation of bitter gourd *viz.*, shoot tip, nodal, internodal and leaf explants. Although regeneration was observed from all types of explant, best response was given by the shoot tip explant (Agarwal and Kamal, 2004; Saha and Behera, 2015; Saha *et al.*, 2016). Due to apical dominance the shoot tip explant has advantage of fast sprouting compared to other explants. Micropropagation using shoot tip explant is preferable, as it is the growing point which is considered to be free from virus.

On account of these facts, the present investigation was carried out in order to standardize a micropropagation protocol for the maintenance and mass multiplication of gynoecious inbred (KAU-MCGy-101) of bitter gourd identified from the experimental field of Department of Vegetable Science, College of Horticulture, Thrissur. The plant regeneration was achieved through direct organogenesis via

initiation of adventitious buds in the shoot tip explants. The results obtained from the experiment are discussed under following headings.

5.3.1 Standardization of culture initiation and multiple shoot induction media

The *in vitro* mophogenesis depends on the endogenous plant hormones produced in the tissue as well as their interactions with exogenously applied growth hormones. The hormones auxins, cytokinins, and auxin-cytokinin interactions are considered to be the most important for regulating growth and organized development in plant tissue cultures (Gasper *et al.*, 1996). Use of different growth regulators were found to stimulate cell division in the cultures. *In vitro* callus initiation could be obtained by using auxin alone or in combination with cytokinin. The results of the present study indicated that the MS medium supplemented with different combinations of BA and NAA exhibited positive response for callus initiation and the explants initiated calli within 12 days of inoculation.

Maximum callus response (100%) from shoot tip explants was obtained on the medium supplemented with 2.0 mgl^{-1} BA and 0.2 mgl^{-1} NAA. The medium combined with 0.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA, 1.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA and 1.5 mgl^{-1} BA + 0.2 mgl^{-1} NAA were also found to give good callusing response. Al Munsur *et al.* (2007) obtained similar result when leaf segments of bitter gourd cultured on medium supplemented with 2.0 mgl^{-1} BAP and 0.30 mgl^{-1} NAA and produced the highest percentage of callus (68.75%) within the minimum number of days (9.75). Srivastava *et al.* (1989) noticed initiation of organogenic calli in the medium supplemented with a combination of NAA and BA. Moreover, Kumar *et al.* (2018) reported that a combination of 2.50 mgl^{-1} BAP and 0.6 mgl^{-1} NAA produced the highest per cent of callus in minimum days from the nodal segments of bitter gourd. Bharadwaj *et al.* (2017) opined that half strength MS medium supplemented with 0.25 mgl^{-1} IAA + 2 mgl^{-1} BAP gave best callusing response from cotyledonary leaf explants of parthenocarpic cucumber (*Cucumis sativus* L.).

Direct organogenesis is the preferred route for micropropagation rather than callus mediated organogenesis. In the present study direct organogenesis was observed from the treatments supplemented with BA alone and in combination with NAA.

Culture establishment and direct shoot proliferation of bitter gourd explants was found to be significantly influenced by the addition of BA in the medium. When the percentage of shoot initiation response and the number of days taken for the shoot initiation were taken into account, the best treatment was found to be MS medium fortified with 2.0 mgl^{-1} of BA followed by the treatment supplemented with 1.5 mgl^{-1} BA. The treatment with 2.0 mgl^{-1} BA exhibited highest percentage (66.66) of shoot initiation within 21 days of inoculation. This is in accordance with the findings of Agarwal and Kamal (2004) in bitter gourd, where best response for shoot initiation from shoot tip explants was observed on the media containing 2.0 mgl^{-1} BAP which took 20 days for shooting response. Vasudevean *et al.* (2001) observed shoot initiation from shoot tip explants of *Cucumis sativus* L. on individual treatments of either BAP or kinetin. Highest percentage of shoot initiation (62) with an average of eight shoots per explant was noticed from shoot tip explants of cucumber when cultured on MS medium supplemented with BA alone (Vasudevan *et al.,* 2008). In contrast to these finding, Saha *et al.* (2016) reported that 2.0 mgl^{-1} BAP in combination with 0.2 mgl^{-1} NAA gave best response for explant survival and successful bud sprouting in bitter gourd gynoecious lines.

In the present investigation multiple shoot initiation was noticed in the shoot induction media. Earlier reports suggested that a medium with combination of BA and NAA was the best to induce multiple shoots in bitter gourd (Sultana *et al.*, 2003; Agarwal and Kamal, 2004; Guo-Li, 2008; Al Munsur *et al.,* 2009) However, present study revealed that, addition of BA alone favoured multiple shoot induction from the shoot tip explants of bitter gourd. Maximum number of shoots per explant was obtained with higher concentration BA (2 mgl^{-1}). This is in consonance with the findings of Liu *et al.* (2011) where, higher shoot multiplication was obtained from apical segments of bitter gourd in the medium supplemented with BA (1.0 mgl^{-1}) alone. BA is also known to induce multiple shoots from axillary buds of cucumber (Aziz and McCown, 1985). Adventitious bud proliferation was promoted by cytokinin in the *in vitro* cultures of bitter gourd (Song and Gao, 2006). The efficiency of cytokinin in inducing shoot regeneration and multiple shoot induction in *Momordica dioica* (Roxb.) has also been reported by Devendra *et al.* (2009).

A balance between auxin and cytokinin is most often required for the formation of adventitious shoots. However, the present study revealed that optimum concentration of cytokinin enhanced axillary branches and multiple shoot formation from the shoot tip explants of bitter gourd.

5.3.2 Effect of hormones on elongation of microshoots

Among the various treatments tried for *in vitro* shoot elongation, MS medium supplemented with 0.5 mgl^{-1} IAA and 0.5 mgl^{-1} NAA was significantly superior to all other treatments. Maximum shoot length for microshoots after one month was obtained from this treatment combination *i.e.,* 9.03 cm. Various combinations of IAA and BA were also used to achieve shoot elongation in bitter gourd (Al Munsur *et al.,* 2009; Kumar *et al*., 2018). Auxin favoured elongation under *in vitro* condition, and the role of auxin is manifested in the present study for promoting *in vitro* shoot elongation.

5.3.3 *In vitro* rooting response

Efficiency of IBA in root induction in bitter gourd has been reported (Huda and Sikdar, 2006; Al Munsur, 2009; Thiruvengadam *et al.,* 2012). Addition of activated charcoal in the rooting medium along with IBA was found to enhance root initiation in the *in vitro* shoots of bitter gourd, in the present study. *In vitro* regeneration of adventitious roots was the best in half strength MS medium augmented with 1.0 mgl^{-1} IBA and 3.0 gl^{-1} activated charcoal. Maximum rooting response (100%) and early root initiation (12 days) was observed with this treatment combination. IBA and charcoal combination was found to be the best for inducing maximum number of roots (7.0). The half strength MS medium supplemented with IBA (1.0 mgl^{-1}) alone took more number of days for root initiation and exhibited only 33.33 per cent rooting response. These results corroborate the findings of Saha and Behera (2015) in bitter gourd, as the addition of activated charcoal in the rooting medium improved overall rooting capacity of mature explants. Pradeepkumar *et al.* (2007) obtained a similar result from *in vitro* culturing of male sterile ridge gourd plants, as highest percentage of rooting (95 %) was observed on half strength MS medium supplemented with 1.0 mgl^{-1} IBA and 200 mgl^{-1} charcoal. Augmentation of rooting medium with activated charcoal also proved to be excellent in terms of earliness in root induction, root number, and length

as well as response to rooting of *in vitro* generated microshoots (Gantait *et al.*, 2009). The promotary effect of activated charcoal on rooting can be interpreted considering two facts; the adsorption of inhibitory substances in the culture medium (Fridborg and Eriksson, 1975; Fridborg *et al.,* 1978) and establishment of a darkened environment in medium which is conducive to the accumulation of photosensitive auxin or cofactors (Druart *et al,* 1982; Dumas and Monteuuis, 1995).

Full strength MS medium was mostly used for rooting in bitter gourd, but Liu *et al.* (2011) observed excellent rooting in medium with half concentration of macronutrients.

5.3.4 Hardening of *in vitro* cultured plants and evaluation of regenerated plants for sex expression

The well rooted plantlets were transferred to polythene bags containing sterilized cocopeat: 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 regenerated plants were transferred to rain shelter. The survival per cent was observed to be 70.58 during the hardening stage. This is in agreement with the earlier reports of micropropagation in bitter gourd (Saha *et al.*, 2016). The regenerated plants become fully established and grew well and all of them exhibited gynoecious sex expression. Variation was observed in the biometric characteristics of regenerated plants and the parental plants. The regenerated plants took more number of days (51.8) to produce first female flower compared to the parental plants (32.78). Similar trend was observed for node to first female flower emergence. The regenerated plants formed first female flower at a higher node (22.33) compared to the parental plants (20.07).

Unlike earlier studies where the micropropagation protocol was standardized upto the initial survival percentage in the field, present investigation evaluated the gynoecious sex expression of the *in vitro* regenerated plants. It was found that earlier exposure of the explant to high dose of cytokinin or auxin had in no way influenced the sex expression of the gynoecious line which proved the efficacy of the protocol and stability of the gynoecious sex expression.

The present study demonstrated that micropropagation can be successfully adopted for the maintenance and multiplication of gynoecious line in bitter gourd. The tissue culture protocol developed was found to be successful for fixing the gynoecious sex expression in the regenerated plants. Use of shoot tip as explant for *in vitro* culturing ensured virus free plants. Since the protocol was found to be viable and efficient, it can be used for the maintenance of gynoecious inbred in bitter gourd which can be further exploited for genetic improvement of gynoecious inbred lines and developing F_1 hybrids.

5.4 Effect of various concentrations of silver thiosulphate (STS), for induction of male flowers in gynoecious lines

Occurrence of gynoecy in bitter gourd is a boon, as it can be exploited for improving yield and economizing hybrid seed production. Maintenance of gynoecious line is an important aspect to be considered in this context. Application of growth regulators for sex modification has been exploited for easy maintenance of gynoecious lines in many crops (Risser and Rode, 1979; More and Munger, 1986; More and Sheshadri, 1987; Choudhary *et al.*, 2001). Sex modification and production of staminate flowers in gynoecious line of cucumber (*Cucumis sativus* L.) by the application of growth hormones has been reported by many authors (Peterson and Anhder, 1960; Tolla and Peterson, 1979; More and Munger, 1986).

However, not much work has been reported in gynoecious lines of bitter gourd to standardize the concentration of STS and stage of application for inducing maleness. Hence the present investigation was carried out to study the effect of various concentrations of silver thiosulphate, stage of application, and number of sprays in inducing maleness in the gynoecious line of bitter gourd. Friedlander *et al.* (1977) opined that hormonal treatments, may influence the flowering pattern of cucumber, either by affecting the sexual differentiation of the floral bud or by a selective promotive or inhibitory effect on later development of the differentiated floral bud towards anthesis. Among the various hormones, GA_3, silver nitrate and silver thiosulphate are generally recommended for inducing maleness in cucurbits. Ethylene, one of the growth regulators in plants, has been found to induce feminization. Most of

the effects of ethylene can be antagonized by specific ethylene inhibitors such as GA_3, silver nitrate and silver thiosulphate. Silver ions (Ag^+) applied as silver thiosulphate are capable of generating ethylene insensitivity in plants by replacing copper ions (Cu^+) which are part of ethylene receptor and thus reducing ethylene sensitivity and enhancing staminate sex expression (Zhao *et al.*, 2002).

STS in general induces male flowers through the activity of silver ions. However, the present study revealed that in bitter gourd, instead of male flowers STS application induced hermaphrodite within two weeks of application (Figure 1). This is in line with the findings of Mishra *et al.* (2015 a), where in gynoecious bitter gourd, bisexual flowers appeared rather than male flowers after application of various concentrations of STS and GA_3. The induced hermaphrodite flowers were larger than the normal female flowers, but not much variation was observed for the flower size between the treatments. Pollen grains when subjected to acetocarmine (1.0 %) test, found completely stained with pinkish red colour, which is an indication of male fertility, thus proving the efficacy of silver thiosulfate in inducing male fertility.

Among the six treatments of silver thiosulphate with different concentrations ranged from, 150, 200 and 250 ppm, single spray of STS at 200 ppm after the first female flower emergence was found to be the best (Figure 2), as it was superior to all other treatments in terms of total number of hermaphrodite flowers produced (72.6). Higher concentration of STS (500 ppm) at two- four leaf stage was found to be the best treatment for inducing maleness in gynoecious cucumber plants as reported by Verma *et al.* (2018), whereas Bharadwaj (2017) observed, 300 ppm STS double spray started at two to four leaf stage as the best to induce more number of staminate flowers in parthenocarpic and gynoecious cucumber genotypes. In bitter gourd STS application, when sprayed twice at a concentration of 6 mM resulted in highest percentage (57.63) of hermaphrodite flower, and it remained effective up to 15 days from application (Mishra *et al.*, 2015 a). In spite of that, in the present study all the treatments produced hermaphrodite flowers for a period of approximately three weeks (17.20 - 20.80 days) and then reverted back to original sex expression *i.e.*, pistillate.

Normally sex differentiation in cucurbits occurred at two to four leaf stage (More and Sheshadri, 1998) and growth hormones for altering the sex expression are recommended during two to four leaf stage. Nevertheless present study revealed that application of STS at the later stage after the emergence of first female flower is equally effective in inducing hermaphrodite flowers when compared to double application, one at two-four leaf stage and other at later stage. Thus breeder got the choice of altering the sex expression of the identified gynoecious line at the later stage of crop growth which holds huge potential in bitter gourd crop improvement.

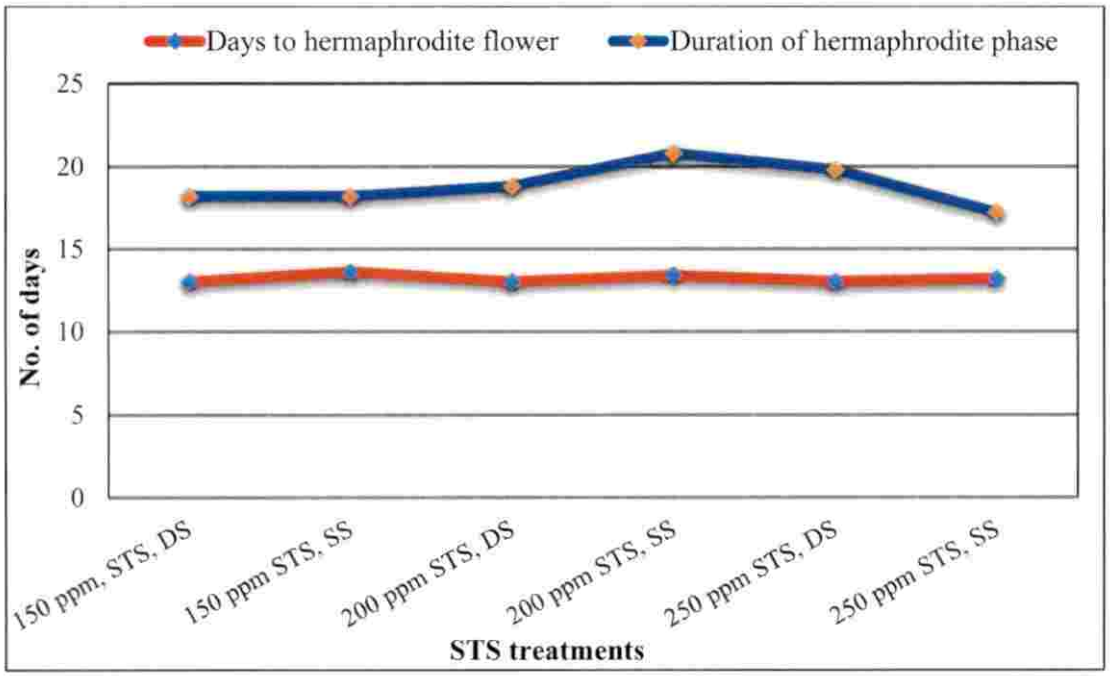


Figure 1: Effect of silver thiosulphate treatments on days to first hermaphrodite flower emergence and duration of flowering phase

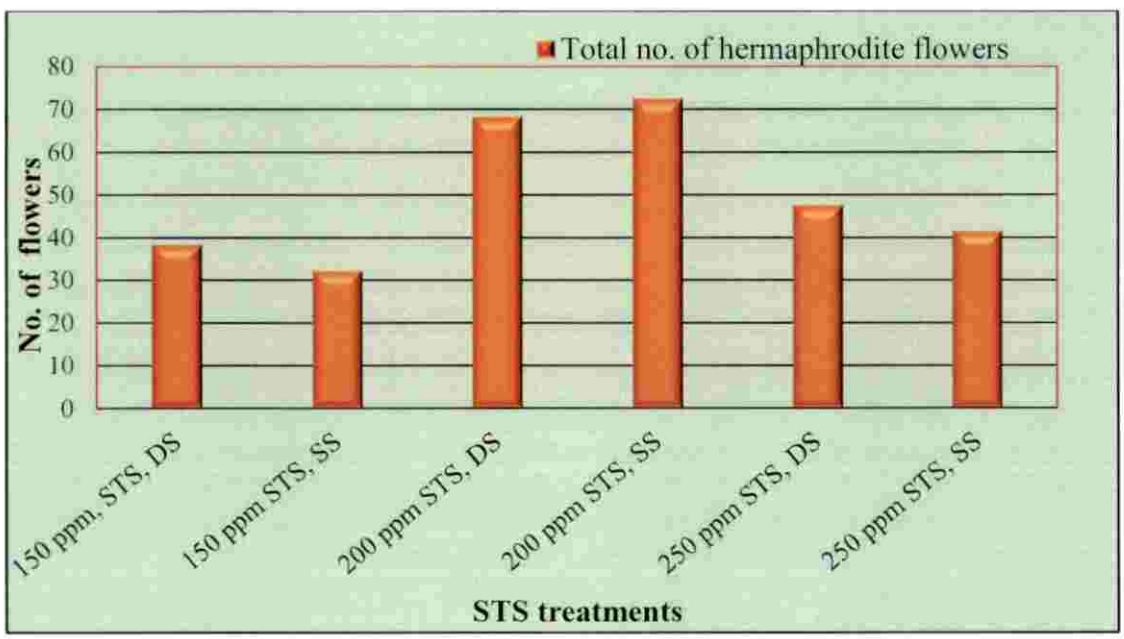


Figure 2: Effect of silver thiosulphate treatments on total number of hermaphrodite flowers produced

Summary

6. SUMMARY

Sex expression varied between species in cucurbitaceae family. Bitter gourd, one of the most popular cucurbitaceous vegetable, is predominantly monoecious in sex expression. However gynoecious sex form has been reported in bitter gourd from India and China. Since the pure gynoecious inbred parent produces only pistillate flowers at every node, gynoecy can be exploited for easy and economic hybrid seed production. Thus isolation and characterisation of gynoecious lines in bitter gourd has a great significance in breeding programme. In Bitter gourd, only a few works utilizing gynoecious lines in breeding have been reported. Considering this aspect the present investigation was undertaken to develop stable gynoecious inbred lines in bitter gourd through selection from sib mated and crossed population involving gynoecious female parents and maintain gynoecy through hormonal regulation and tissue culture.

The present study was carried out at Department of Vegetable Science, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, during the period of 2017 - 2019. The experimental materials consisted of gynoecious type (KAU-MCGy-101) of bitter gourd (*Momordica charantia* L.) identified from the experimental field of Department of Vegetable Science, Vellanikkara and monoecious variety 'Preethi' developed by Kerala Agricultural University, Thrissur. During the first season, the gynoecious plants were raised in the polyhouse along with the monoecious line 'Preethi'. The gynoecious plants were sib mated with the male sibs of the same population as well as crossed with the monoecious plants of 'Preethi', in such a way that gynoecious plants served as female parent. The gynoecious line was maintained by spraying silver thiosulphate at 200 ppm just after flowering.

In the second season, the sib mated gynoecious inbred (KAU-MCGy-101) was grown along with the F_1 hybrid (KAU-MCGy-101 × Preethi) generated by crossing the gynoecious plants with monoecious variety 'Preethi' under rain shelter conditions to compare the biometric characters and to study the genetics of sex expression. The gynoecious inbred exhibited early bearing of female flowers at lower nodes compared to the F_1 hybrid. Moreover the inbred exhibited higher average fruit weight, fruit length

and girth compared to early reported gynoecious lines. Hence the gynoecious inbred, KAU-MCGy-101 holds enormous potential for future breeding programme for earliness and yield in bitter gourd. When comparing the gynoecious inbred and F_1, the hybrid outperformed the inbred with respect to fruit and yield characters.

Sib mated gynoecious inbred as well as F_1 hybrid were evaluated for qualitative characters. Colour of fruit skin and bitterness are most important qualitative parameters with regard to bitter gourd. All the sib mated gynoecious plants produced dark green colour spindle shaped fruits, which is not the preferred colour of south Indian consumers whereas, the fruits of F_1 hybrid were light green in colour with spindle shape. Gynoecious inbred fruits exhibited strong bitterness while, the fruits of F_1 hybrid were moderate bitter in nature. Presence of tubercles and its characteristics were found to be similar in both inbred and hybrid. There were numerous, triangular deep tubercles on the fruit surface of both inbred and hybrid.

Genetic analysis of sib mated gynoecious inbred and F_1 hybrid for sex expression revealed that there was no segregation for the gynoecious character in the sib mated gynoecious inbred and F_1 hybrid. The experiment confirmed the recessive nature of gynoecious character. Moreover gynoecious sex expression was found to be homozygous, as all the sib mated plants studied were gynoecious in sex expression.

Experiments were undertaken to standardize the protocol for maintenance of gynoecious bitter gourd lines through *in vitro* culture. The most commonly used MS medium with certain modifications was used for culturing the shoot tip explants of gynoecious bitter gourd. Maximum callus response (100 %) was obtained on the medium supplemented with 2.0 mgl^{-1} BA and 0.2 mgl^{-1} NAA. In the present study direct organogenesis was observed from the treatments supplemented with BA alone and in combination with NAA. The best treatment with respect to shoot initiation percentage and number of days taken for shooting was observed to be MS medium fortified with with 2.0 mgl^{-1} of BA. The shoot initiation medium added with BA alone was found to induce multiple shoots from the shoot tip explants and maximum number of shoots per explant was achieved with higher concentration of BA (2 mgl^{-1}). For *in*

vitro shoot elongation, MS medium supplemented with 0.5 mgl^{-1} IAA and 0.5 mgl^{-1} NAA performed best and was significantly superior to all other treatments compared. Augmentation of activated charcoal in the rooting medium along with IBA was found to enhance root initiation in the *in vitro* shoots of bitter gourd. Half strength MS medium combined with 1.0 mgl^{-1} IBA and 3.0 gl^{-1} activated charcoal produced maximum rooting response (100 %) and early root initiation. IBA and activated charcoal combination proved to be the best with respect to number of roots produced also. While evaluating the regenerated plants after hardening, 70.58 per cent survival per cent was recorded. Sex expression of tissue cultured, regenerated plants was studied and it was noticed that, the earlier exposure of the explant to high dose of cytokinin or auxin did not influence the character, as all of the regenerated plants were gynoecious in sex expression. Hence the tissue culture protocol developed was found to be successful for fixing the gynoecious sex expression and can be adopted for maintenance of gynoecious lines in bitter gourd.

In another experiment, effect of various concentrations of silver thiosulphate (STS) and stage of application in inducing maleness in gynoecious bitter gourd line was studied. The treatment concentrations varied from, 150, 200 and 250 ppm of STS were applied as foliar spray once or twice starting from four leaf stage and on appearance of female flowers. STS in general induces male flowers through the activity of silver ions. However in the present study, all the treatments of STS induced hermaphrodite flowers instead of male flowers. The pollen grains, when subjected to acetocarmine (1.0 %) test was found to be viable and fertile, thus proving the efficacy of STS in inducing male fertility in gynoecious bitter gourd lines. Among the six treatments compared, single spray of STS at 200 ppm after the first female flower emergence was found to be the best, as it was significantly superior to all other treatments in terms of total number of hermaphrodite flowers produced. Application of STS (single spray) at the later stage after the emergence of first female flower is equally effective in inducing hermaphrodite flowers when compared to double application, one at two-four leaf stage and other at later stage. Thus the above finding

disproves the theory that, growth regulator should be applied during early growth phase (2-4 leaf stage) for altering sex expression in cucurbits.

The present investigation provided an indication of the potential of gynoecious inbred line identified from KAU, which need to be confirmed by making a series of crosses with selected monoecious lines. Since the tissue culture protocol developed was found to be successful for fixing the gynoecious sex expression in the regenerated plants, it can be used for the maintenance of gynoecy in bitter gourd which can be further exploited for genetic improvement of gynoecious inbred lines and developing F_1 hybrids.

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BREEDING FOR GYNOECY IN BITTER GOURD

(*Momordica charantia* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Bitter gourd (*Momordica charantia* L.), is one of the most popular vegetable from cucurbitaceae family. Though monecy is the predominant sex form in bittergourd, gynoecy has been reported in bitter gourd from India. The present investigation entitled 'Breeding for gynoecy in bitter gourd (*Momordica charantia* L.) was undertaken with an objective to develop stable gynoecious inbred lines in bitter gourd through selection from sib mated and crossed population involving gynoecious female parents and maintain gynoecy through hormonal regulation and micropropagation. The study was carried out at Department of Vegetable Science, College of Horticulture, Vellanikkara, during the period of 2017-2019. The gynoecious line (KAU-MCGy-101) identified from the Kerala Agricultural University was tested for stability of the gynoecious sex expression. The sib mated gynoecious inbred along with the F_1 hybrid (KAU-MCGy-101 × Preethi) developed by crossing with monoecious variety 'Preethi' were subjected to morphological characterization and evaluated for biometric characters and genetics of sex expression. For maintaining and fixing the gynoecious trait, micropropagation was attempted through shoot tip culture. Hormonal regulation for the maintenance of gynoecy using various concentrations of silver thiosulphate (STS) was also studied.

The results revealed the stability of gynoecious sex expression in the inbred (KAU-MCGy-101), as all the plants resulted from sib mated population produced only female flowers throughout the growth phase. The gynoecious inbred exhibited early bearing of female flowers at lower nodes compared to the F_1 hybrid. Moreover the inbred exhibited higher average fruit weight, fruit length and girth compared to early reported gynoecious lines. Hence the gynoecious inbred, KAU-MCGy-101 holds enormous potential for future breeding programme for earliness and yield in bitter gourd. The sib mated gynoecious inbred and F_1 hybrid were evaluated for qualitative characters and they differed for colour of fruit skin and fruit bitterness. All the sib mated gynoecious plants produced dark green colour spindle shaped fruits which were strong bitter in nature, whereas, the fruits of F_1 hybrid were light green in colour with spindle shape and moderate bitterness.

Genetic analysis for sex expression confirmed the recessive nature of gynoecy in bitter gourd since there was no segregation for the gynoecious character in the sib mated gynoecious inbred and F_1 hybrid.

Micropropagation protocol for maintenance of gynoecious bitter gourd lines through *in vitro* shoot tip culture was standardized. Direct organogenesis was observed in the MS medium supplemented with BA alone and in combination with NAA. MS medium fortified with 2.0 mgl^{-1} of BA alone was found to be suitable for better and faster shoot initiation. Multiple shoot induction was observed in shoot initiation medium added with BA alone and maximum number of shoots per explant was achieved with higher concentration of BA (2 mgl^{-1}). For *in vitro* shoot elongation, MS medium supplemented with 0.5 mgl^{-1} IAA and 0.5 mgl^{-1} NAA performed best. *In vitro* rooting response was found to be enhanced with the addition of activated charcoal in the medium along with IBA. Half strength MS medium combined with 1.0 mgl^{-1} IBA and 3.0 gl^{-1} activated charcoal exhibited early rooting, maximum rooting response (100%) and more number of roots. While evaluating the regenerated plants after hardening, 70.58 per cent survival per cent was recorded. All the regenerated plants were gynoecious in sex expression.

Effect of various concentrations of STS and stage of application in inducing maleness in gynoecious bitter gourd was studied. STS in general induces male flowers in cucurbits through the activity of silver ions. However in the present study, all the treatments of STS induced hermaphrodite flowers instead of male flowers. Single spray of STS at 200 ppm after the first female flower emergence was found to be the best, as it was superior in terms of total number of hermaphrodite flowers produced. Application of STS at the later stage after the emergence of first female flower is equally effective in inducing hermaphrodite flowers when compared to double application, one at two-four leaf stage and other at later stage, which has enormous significance in the exploitation of gynoecy for crop improvement in bitter gourd.

Appendices

APPENDIX I

Composition of Murashige and Skoog Medium (1962)

Major elements	**mgl^{-1}**
$CaCl_2.2H_2O$	440.0
$FeSO_4.H_2O$	27.8
KNO_3	1900.0
KH_2PO_4	170.0
$MgSO_4.7H_2O$	370.0
NH_4NO_3	1650.0
Na_2.EDTA	37.5
Minor elements	
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
H_3BO_3	6.20
KI	0.83
$MnSO_4$	22.30
$NaMoO_4.2H_2O$	0.25
$ZnSO_4$	8.6
Organic constituents	
Glycine	2.0
Myo-inositol	100.0
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Sucrose	30.0 g
Agar	7.0 g

APPENDIX II

ABBREVIATIONS

AC	Activated charcoal
BA	Benzyl adenine
DUS	Distinctness, Uniformity and Stability
gl^{-1}	Gram per litre
$HgCl_2$	Mercuric chloride
HCl	Hydrochloric acid
IAA	Indole aceic acid
IBA	Indole butyric acid
Kn	Kinetin
µl	Micro litre
mgl^{-1}	Milligram per litre
MS	Murashige and Skoog medium
MSL	Mean Sea Level
NAA	Naphthalene acetic acid
NBPGR	National Bureau of Plant Genetic Resources
NHB	National Hortiulture Board
NaOH	Sodium hydroxide
ppm	Parts per million
STS	Silver thiosulphate
TDZ	Thidiazuron