

Sex Manipulation in Cucurbitaceous Vegetables

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ABSTRACT

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Sex manipulation is attributed to alter the ratio of male to female flowers within the individuals. A wide range of variation in sex forms ranging from primitive hermaphrodite to gynoecious advanced sex form is observed in cucurbitaceous vegetable crops. Sex expression in cucurbitaceae family is regulated by environmental, genetic and hormonal factors. In general, female sex expression is promoted by low temperature and short photoperiod, which may influence the level of endogeneous hormones (ethylene, auxin and GA) which in turn influence the sex expression. Sex inheritance plays an important role in breeding programme. The reduction in sex ratio, stabilizing the gynoecious character and development of stable gynoecious inbred parents is the main objective of cucurbit breeding programs. Identification of sex expression in the initial stage of the crop with the help of biotechnological tools like marker assisted selection (MAS) may be employed. The molecular breeding will also be helpful for studying the mechanism affecting sex expression and to identify the genes governing the sex character in cucurbits.

Introduction

The cucurbit vegetables are the largest and diverse group comprised of 900 species classified under 130 genera (Jeffrey, 1964; 1980) belongs to the family Cucurbitaceae. The members of Cucurbitaceae family exhibit a fascinating range of sex (Table 1) namely staminate, pistillate and hermaphrodite flowers resulted in evolution of several types of sex forms like monoecious (staminate and pistillate flowers produces in the same plant), gynoecious (pistillate flowers producing plants), andromonoecious (staminate and hermaphrodite flowers in the same plant), gynomonocious (pistillate and

hermaphrodite flowers produces in the same plant), androecious (plant with staminate flowers only), trimonoecious (staminate, pistillate and hermaphrodite flowers produces in the same plant), gynodioecious (pistillate and hermaphrodite plants segregate separately from base population), dioecious (staminate and pistillate flowers are produced in separate plant). A wide range of variation in sex forms owed to evolve from primitive sex form hermaphrodite could lead to evolution of predominant sex form *i.e.* monoecious and advanced sex form *i.e.* gynoecious (Robinson and Decker-Walters, 1999). Sex manipulation

is the process of altering male and female flower ratio within the individuals of a species, the sex modification leads to alteration in the sequence of flowering phenology by modifying beneficial sex ratio in order to enhance the economic yield. Sex inheritance plays an important role in cucurbit breeding, sex expression mainly influenced by genetical and environmental factors. The monoecious plants are characterized by three phases of flowering, *i.e.* an initial period where only male flowers are produced, secondly equal proportionate of male and female flowers are produced for maximum period and finally predominate in female flowers production.

Evolution of sex expression in cucurbitaceae

The original and primitive sex form of cucurbits are assumed to be hermaphrodite nature, and evolutionary changes had been occurred in later generations might be due to the vagaries of environmental factors, the dominant mutation effect may lead to the modification of sex from hermaphrodite to the intermediate sex forms like andromonoecious, trimonoecious and gynomoecious (Fig. 1), furthermore advancement in the evolutionary genetic and environmental changes might cause for modification of androecious, monoecious and gynoecious (Robinson and Decker-Walters, 1999). Sex expression in cucurbits is easily manipulated by controlling the environmental conditions as well as genetic factors, hence these two factors are major effect on sex manipulation. The non-genetic factors like environmental effect, cultural practices, plant growth regulators, and genetic factors like breeding behaviour and genetic constitution plays a significant role in altering the sex expression of cucurbits with the aid of conventional breeding methods and intervention of biotechnological approaches.

Non-genetic factors

Influence of environmental effect on sex expression

Sex expression mainly influenced by environmental factors, attributing female sex expression is promoted by low temperature, short photoperiod and high moisture availability, (Atsmon and Galun, 1962; Atsmon, 1968), which intern encourages the buildup of carbohydrates. The high temperature and long photoperiod results in male flower production (Cantliffe, 1981). The phenomenon of sex expression is influenced by environmental factors on production of endogenous hormones especially ethylene, auxin, gibberellic acid and chemical composition, may cause for different expression of sex forms, the ratio of staminate to pistillate flowers greatly varies when the monoecious plants are grown under different environmental conditions (Robinson and Decker-Walters, 1997; Achakzai; Kayani, 2002; Achakzai, 2012). Positive correlation between ethylene release and percentage of nodes and number of female flowers were recorded highest at 28°C/ (6h 18°C+6h 12°C) day/night temperatures. The enhanced female flowers in monoecious cucurbits is not directly regulated by low temperature or low night temperature, but these conditions lead to accumulation of the glucose and sucrose in the shoot apex under certain temperatures, through hexokinase-mediated sugar signalling pathway (Miao *et al.*, 2011). The female flower producing nodes increased over sevenfold in monoecious cucumber and three-folds in andromonoecious cucumber for the production of bisexual flowers due to endogenous ethylene production under short-day (8 h photoperiod). Ethylene evolution from the shoot apices of monoecious and andromonoecious plants at the four-leaf stage indicated the maximum ethylene peak in monoecious and andromonoecious cucumber

plants grown under 8h photoperiod approximately 1.5 times higher than the 16h photoperiod (Yamasaki *et al.*, 2003).

Influence of cultural practice on sex expression

Cultural practices like irrigation, nutrient application and season of planting plays a minor role in sex manipulation, unfavourable growing conditions such as lack of water can cause a slowdown in flower production, higher application of nitrogen results in more vegetative growth intern reduces reproductive stage and lead to the delayed flowering. Mineral nutrients might alter sex expression in plants through effects on hormonal balance (Salisbury and Ross, 1969). The season of planting during higher rainfall results in lesser flower production, whereas planting during hot seasons produces more male flowers and less female flower production. Sub-optimal application of nitrogen, phosphorus, potassium nutrients has been reported to depress flowers and encourage male flowers rather than female flowers (Kraup *et al.*, 2002). The modification of sex ratio in favour of female flowers using different factors such as mineral nutrients, most especially high levels of nitrogen has been most clearly demonstrated in monoecious cucurbits (Lau *et al.*, 1993; 1995). Mineral nutrients like boron also influence sex expression in many cucurbits (Table 2).

Application of potassium fertilizers in potassium deficit soils increased the female flowers and subsequently enhanced fruit yield in squash (Abduljabbar and Ghurbat, 2010), application of bio-fertilizers significantly enhanced the induction of female flowers and reduced male flowers in squash plant (Abd El-Fattah and Sorial, 2000). The application of 100 kg/ha of NPK 15:15:15 induced the increased male to female flowers in pumpkin (Agbaje *et al.*, 2012).

Role of growth regulators on sex expression

Growth regulators have tremendous effects on sex expression and flowering in various cucurbits lead to suppression of male flowers or an increased number of female flowers (Al-Masoum and Al-Masri, 1999), without imposing any deleterious effect on environment and human health. Growth regulators can alter the sex ratio and sequence if applied at the two- or four-leaf stage, which is the critical stage at which the suppression or promotion of either sex is possible (Hossain *et al.*, 2006). The male to female flower ratio of cucumber plants is also regulated by plant hormones, ethylene and auxin promote the formation of female flowers, whereas gibberellins promote the formation of male flowers (Table 3). The first report in any plant species of alteration of sex expression via exogeneous chemicals was done by the application of auxin shifted sex expression in cucumber towards femaleness (Laibach *et al.*, 1949). The foliar application of gibberllic acid promoted male flowers in a monoecious cultivars at nodes where female flowers would have normally occurred (Wittwer and Bukovac, 1958).

Gynoecious lines are obtained with the use of chemicals to induce staminate flower for genetically selfing in a cucumber population, cucumber variety 'Bingo' expressed gynoecious sex of 5%, Whereas Seminis-1, Seminis-3, Micro-c and long green were found predominantly gynoecious sex form and are stabalized gynoecious sex form in cucumber (*Cucumis sativus* L.) performed upon repeated selfing, further cent per cent gynoecious populations in S₃ generation were obtained as SE₁-G and SE₃-G lines, gynoecious plants of these lines were maintained by selfing, bulked seeds of each S₃ line used for the experiment on gynoecious line maintenance (Chaudhary *et al.*, 2001).

Yin and Quinin, (1995) elaborated mechanistic model of hormone regulation of sexes in cucurbits. Ethylene plays a major role for inducing female flowers by suppressing gibberellin, a hormone for male flower production (Fig. 2). The internal ethylene level influences on expression of sex phenotypes *i.e.*, gynoeocious lines produce two to threefold higher ethylene level than monoecious or andromonoecious ones.

GA₃ acts as an ethylene biosynthesis blocker which blocks the ethylene precursor due to which the ethylene production is hindered. Similarly, AgNO₃ acts as a ethylene action blocker avoids the ethylene action and increases male flower production (Fig. 3). CEPA 150 ppm and NAA 50 ppm increased the total number of female flowers by 40 and 29 %, respectively in bittergourd by first female flower at 10 to 12 days earlier than control at lower nodes (Baset *et al.*, 2014). The AgNO₃ at rate of 500 mg/l induced highest hermaphrodite flowers (15) on female sweet gourd plants, proportionately hermaphrodite flower production sharply decline at 700 mg/l AgNO₃ (about 10) with plant senescence and wilting of vines. However, the maximum number of female

flowers (39) found in untreated vines (Sanwal *et al.*, 2011).

Genetic factors

Breeding for sex manipulation traits

A wide range of variation in sex forms ranging from hermaphrodite to monoecious forms is observed in cucurbitaceous vegetable crops (Robinson and Decker-Walters 1997). Among these the gynoeocious sex (only female flowers) form has been commercially exploited worldwide for cucurbit breeding programme. Development of hybrids in any crop is expensive (Behera, 2004). However, the utilisation of gynoeocy is economical and easier for exploiting hybrid vigour in many cucurbitaceous crops. Hybrid varieties of cucurbits are predominantly used in the production system, the proportion of hybrid varieties is continuously increasing and thus, gynoeocious lines in cucurbits are important for economic production. Development of hybrids in cucurbits is expensive because of hand pollination. However, it can be made inexpensive by the utilisation of gynoeocy which is economical and easier for exploiting hybrid vigour.

Table.1 Different sex forms in cucurbits

Sex forms	Cucurbits
Monoecious (♀ and ♂)	Cucumber, Musk melon, Pumpkin, Summer squash, Winter squash, Water melon, Sponge gourd, Round melon, Bottle gourd, Bitter gourd.
Gynoeocious (♀)	Cucumber, Bitter gourd, Musk melon, Watermelon, Ridge gourd
Androeocious (♂)	Cucumber, Musk melon
Dioecious (♀ and ♂ in separate plant)	Pointed gourd, Ivy gourd,
Andromonoecious (♂ and ♀ in same plant)	Muskmelon, Water melon, Cucumber
Gynomonoecious (♀ and ♂ in same plant)	Cucumber, Musk melon, Ridge gourd
Trimonoecious (♂, ♀ and ♂ in same plant)	Cucumber
Hermaphrodite (♀)	Ridge gourd (Satputia)

Table.2 Effect of boron on sex expression in different crops

Crop	Dosage	Effect	Reference
Muskmelon	6 ppm	Increased ratio of hermaphrodite: staminate flowers.	Randhawa and Singh, 1974
Cucumber cv. Poona Khira	3-4 ppm	Increased pistillate flowers.	Verma and Choudhary, 1980
	2-4 ppm	Decreased number of days to first female flower, early node to first female flower, lower male: female flower ratio.	Singh and Choudhary, 1988
Cucumber cv. Moti Tionh	0.25%	Low ratio of male:female flowers.	Maurya, 1987
Cucumber	4 ppm	Reduces the number of days to first male and female flower production, number of nodes to first female flower appearance and increases total number of female flowers.	Singh and Chaudhury, 1988
Watermelon cv. Sugar baby	3 ppm	Increased the total number of female flowers per vine and decreased ratio of male: female flowers	Ali <i>et al.</i> , 1985
	2-4 ppm	Decreased number of days to first female flower production and number of nodes to first female flower appearance, more number of female flowers.	Singh and Chaudhury, 1988
Watermelon	0.56 kg/ha	Increased pollen germination percentage	Phatak <i>et al.</i> , 1990
	4 g per plant	Decreased the days to first male and female flower appearance, reduced sex ratio in both kharif and rabi season.	Patel, 2008
Bitter gourd	4 ppm	Increased female flowers per plant	Verma <i>et al.</i> , 1984
	4 ppm	Decreased number of days to first male flower production, reduced male: female flower ratio.	Gedam <i>et al.</i> , 1998

Table.3 Effect of growth regulators on different crops

Crop	Growth regulator	Dosage	Effect	Reference
Muskmelon	GA ₃	10 mg/l	Increased fruit yield per hectare	Randhava and Kirtisingh, 1973
	GA ₃	400 mg/l (Seed soaking)	Increased number of fruits and yield	Ram <i>et al.</i> , 2012
Cucumber	Silver nitrate	400 ppm	Increased total number of staminate flowers/plant, early days to flowering in main axis, early node of first male flowering in main axis.	Badri <i>et al.</i> , 2001
	Ethephon	100 and 200 mg/l	Increased yield	Thappa, 2011
	MH + Ethephon	100 ppm each	Earliness, increased sex ratio and reduced plant expansion	Thappa, 2011
	Ethrel	500 ppm	Increased female flowers and reduced male flowers	Bhandary <i>et al.</i> , 1974
	Ethephon (ethrel)	400 ppm	Maximal suppression of staminate flowers	Mishra <i>et al.</i> , 1976
Watermelon	GA ₃	10 ppm	Produced more number of female flowers	Gopalkrishna and Choudhary, 1978
	TIBA	50-200 ppm	Producing a favourable female to male ratio and increased number of fruits.	Gopalkrishna and Choudhary, 1978
Bitter gourd	GA ₃	10 ppm	Effective in improving the yield components	Islam, 1995
	Ethrel (2-Chloroethyl phosphonic acid)	200 to 600 ppm	Stunting growth and significant reduced production of male flowers.	Ravindran, 1971
Pumpkin	Ethephon	300 mg/l	Increased number of female flowers	Sure <i>et al.</i> , 2012
Pointed gourd	Ethrel	150 ppm.	Pistillate flowers, fruit numbers/plants, fruit size and fruit weight were increased.	Ramaswamy <i>et al.</i> , 1976
Snake gourd	Ethephon (ethrel)	250 ppm	Altered sex expression and increased fruit yield	Cantliffe, 1976

Table.4 Report of different inheritance pattern for sex expression in cucurbits

Crops	Mode of inheritance	Gene symbol	Investigators
Cucumber	Three majoe genes	F/f, M/m, A/a	Kubicki, 1969a; Galun, 1961
Musk melon	Two recessive genes	<i>A-ggmm</i>	Kenigsbuch and Cohen, 1990
Bitter gourd	Single recessive gene	<i>gy-1</i>	Ram <i>et al.</i> , 2006
Water melon	Single recessive gene	<i>gy</i>	Jiang and Lin, 2007
Ridge gourd	Major recessive gene	-	Singh <i>et al.</i> , 2012

Fig.1 Evolution of sex forms in cucurbits (Whitaker, 1931)

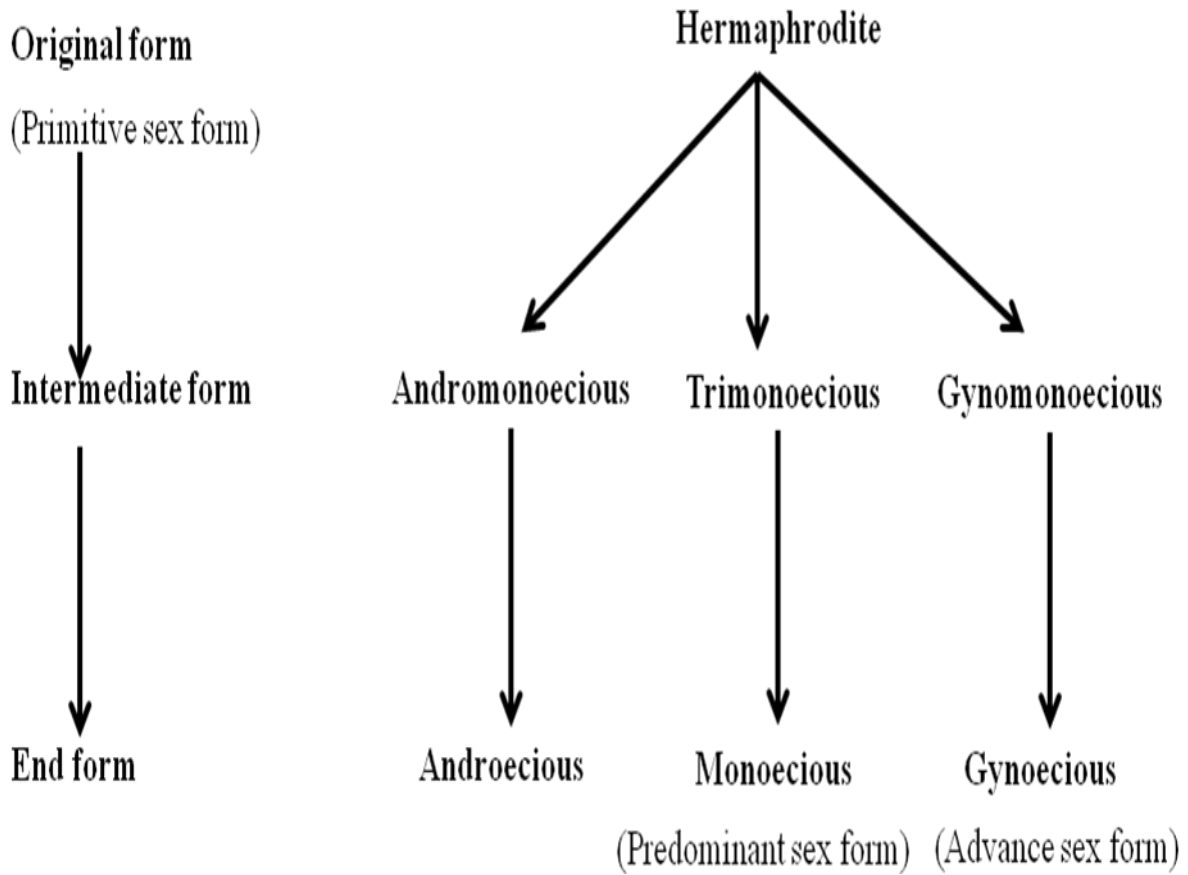


Fig.2 Effect of ethylene on flower manipulation

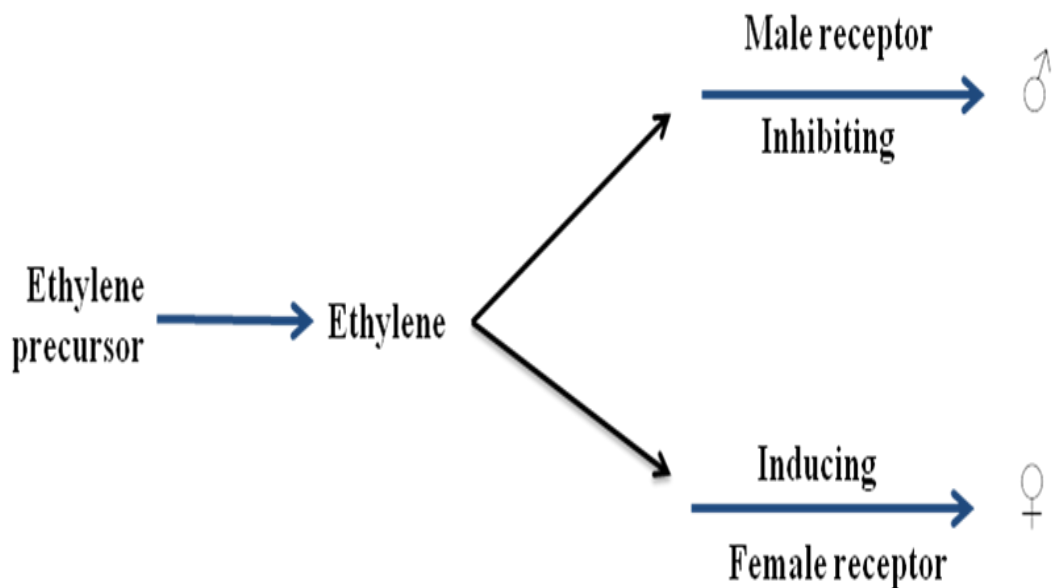


Fig.3 Role of different hormones in flower modification

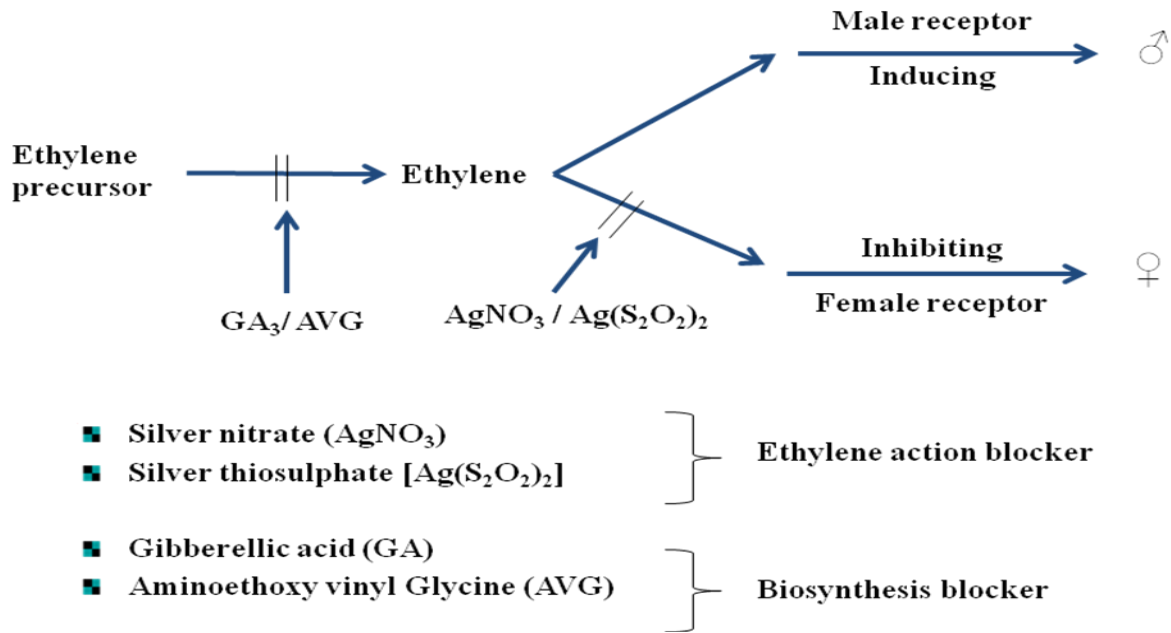
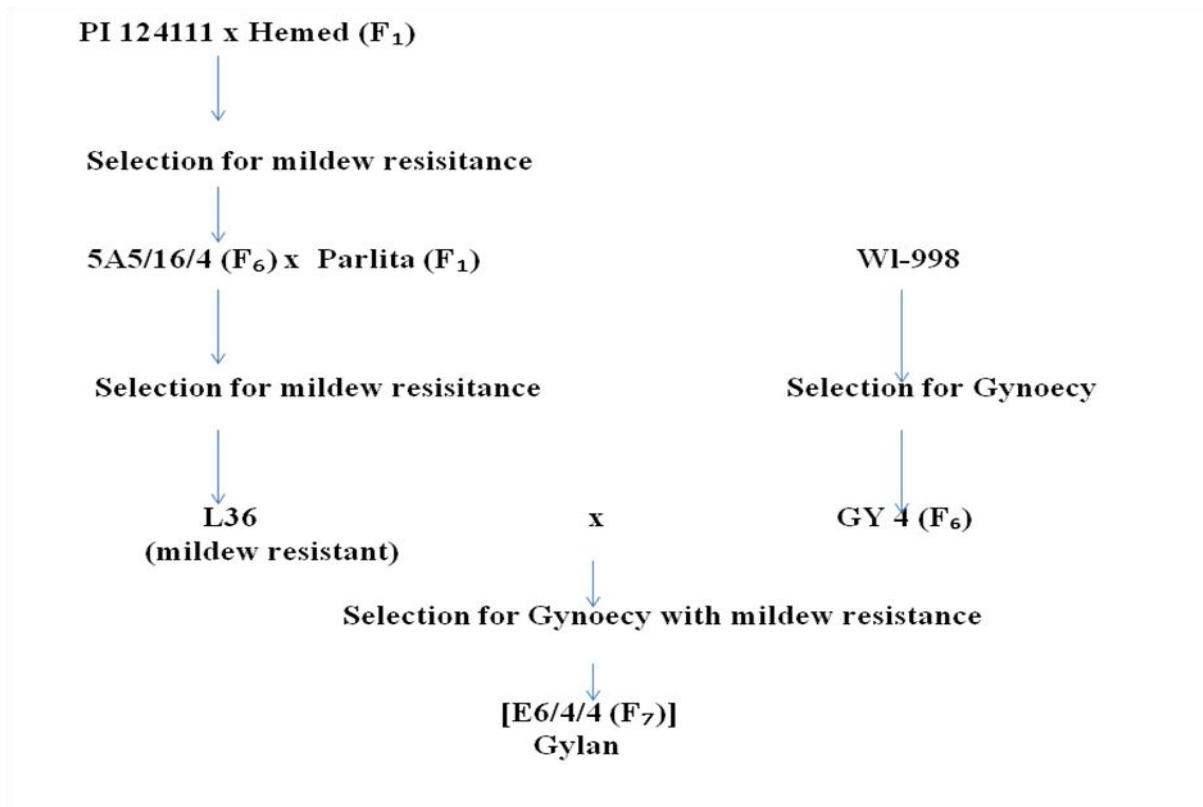


Fig.4 Schematic representation of development of gynoecious line in muskmelon (Yigal, 1993)



Achievements of gynoeic development in cucurbitaceous vegetable crops

Gynoeic line was reported for the first time in cucumber cultivar 'Shogoin' (PI 220860) (Peterson and Anhder, 1960), gylan—a gynoeic muskmelon obtained (Fig. 4) in segregated F₇ population of GY-4 × 36 (Yigal, 1993), Gy263B gynoeic lines was reported in bitter melon (Ram *et al.*, 2006). Gynoeic gene (*gy-1*) in watermelon had been located (Jiang and Lin, 2007).

The development of gynoeic line is mainly due to the involvement of spontaneous or chance segregation of gynomoecious lines lead to the isolation of gynoeic lines in the segregating population. Gynoeic lines are improved by repeated backcrossing and further maintained by selfing, it is possible by using growth regulators by induction of staminate flower for selfing as pollen source.

Hybrid seed production is more effective with the use of gynoeic lines and are mainly used as a female parental line, since gynoeic plants does not produce male flowers as pollen source, all nodes bears pistillate flowers lead to high yielding with addition of parthenocarpic traits. The cross of heterosexual (cross between female and male plant), homosexual (cross between two females by inducing bisexual flowers in any one of the female plants) revealed the highest number of fruits per plant of F₁ heterosexual (32.1) than F₁ homosexual (30.7).

The sex expression in progeny seeds of female homosexual crosses produced 100 % female plants in F₁, indicate the female: male sex ratio of 1:0 whereas in heterosexual cross, is 1:1 segregation of male and female plants indicated that the sex expression is controlled by xx/xY chromosomes. The gynoeic sex expression in sweet melon is governed by a single homozygous recessive gene (Sanwal *et*

al., 2011). Jiang and Lin (2007) discovered the gynoeic gene (*gy*) in watermelon, by crossing gynoeic line (Gynoeic 1) with monoecious line (A123) lead to production of normal monoecious F₁ hybrid. The ratio of monoecious to gynoeic sex form was 86:18 in F₂ and in the progeny backcrosses further lead to the segregation, results of ratio 59:43, could reveal gynoeic in watermelon is controlled by single recessive gene. The inheritance of gynoeic in bitter melon had been studied with the use of GY263B gynoeic line and Pusa-Do-Mousami monoecious line were used as parents and the phenotype of F₁ revealed to be the monoecious and upon examine of F₂ population and testcross (3:1 and 1:1 ratio of monoecious to gynoeic respectively) revealed the gynoeic in bitter melon is controlled by single recessive gene (Ram *et al.*, 2006). 'Gylan'- an improved gynoeic muskmelon variety with resistance to downy mildew disease had been developed (Fig. 4) by crossing with lines 124111F, governing two dominant downy mildew resistance genes *Pc1* and *Pc2*, the stable gynoeic muskmelon breeding line GY-4 isolated from a segregating population (gynoeic: gynomoecious) of Wisconsin 998, carrying a pair of recessive genes for gynoeic sex, *g* and *m* (Yigal 1993).

Biotechnological intervention in sex manipulation

The monoecious (M-ff) cucumbers hypothesized to produce two types of flower buds namely one produces more ethylene and the other produces less ethylene (Yamasaki *et al.*, 2001). This could be due to the 1-aminocyclopropane-1-carboxylic acid (ACC) is an immediate precursor of ethylene (Adams and Yang, 1979) and that the expression of ACC synthase genes (ACS) generally correlates with ethylene biosynthesis in plants.

Development of male and female specific markers at early identification of male and female plants and efficiency in improving of dioecious vegetables (Ivy gourd, Pointed gourd, Spine gourd and Asparagus etc.) as well as transferring the desirable gene governing sex character in to the required plants adapting tissue culture techniques for mass multiplication of gynoecious lines and biotechnological approaches import a crucial role in which RAPD marker associated with gynoecious trait (gy-1 gene) in bitter gourd had been revealed namely, OPZ 13 marker of 700 bp produced specific band in gynoecious lines makes easy identification of gynoecious lines in bitter gourd (Mishra *et al.*, 2014). Male specific RAPD markers in pointed gourd isolated namely, OPC05_{1 0 0 0} of 1000 bp produced specific banding pattern found only in males. Similarly, OPC14_{4 0 0} of 400 bp was the female specific marker produces specific banding pattern only in females (Kumar *et al.*, 2008). Ethylene regulation has been studied and revealed the sex expression genes in cucumber plants namely CS-ETR₂ and CS-ERS genes. CS-ETR₂, CS-ERS, CS-ETR₁ are ethylene receptor genes and CS-AC₂ is a female inducing gene. The effect of ethrel on gene expression of CS-ETR₁, CS-ETR₂, and CS-ERS in the shoot apices of cucumber var. Otone no. 1 at the 4-leaf stage is increased, however the effect of AVG (Amyl-Vinyl-Glycine) on gene expression of CS-ETR₁, CS-ETR₂, and CS-ERS in the shoot apices of Higan-fushinari at the 4-leaf stage found to be low. The results revealed the expression gene is influenced by exogenous or indigenous hormonal concentration (Yamasaki *et al.*, 2001). Gynoecious lines acts as a male sterile line in cucurbitaceous vegetables and predominantly used in heterosis breeding, use of gynoecious lines complicated by isolation and maintenance, less stability due to the variation of gene depending upon environmental effects. Utilization of

gynoecious lines has been commercially exploited in cucumber *viz.*, Pusa Sanyog, Phule Prachi, Phule Champa, and DBGH 12 in bitter gourd.

Sex manipulation in cucurbits plays an important role in the reduction of sex ratio. Gynoecious lines acts as a male sterile line in cucurbitaceous vegetables. Henceforth, stabilization of gynoecious trait and development of stable gynoecious inbred parents will be helpful in hybrid development programs. The mechanism affecting sex expression and identification of the genes governing the sex character is possible through specific molecular markers. In dioecious crops, the molecular markers linked to gynoecious trait are useful in early identification of sex at earlier stage of the crop.

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