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Influence of Ethrel on sex expression in cucurbitaceous vegetables

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Abstract
Crops belonging to family Cucurbitaceae are generally known as 'Cucurbits'. It consists of a wide range of vegetables either used for salad purpose. Majority of cucurbits are monoecious and sex ratio (male: female) ranges from 25-30:1 to 15:1. Sex ratio is influenced by environmental factors. High N content in the soil, long days and high temperature favour maleness. Besides environmental factors, endogenous levels of auxins, gibberellins, ethylene and abscisic acid also determine sex ratio and sequence of flowering. High ethylene level induces female sex and is suggested to increase female flowers in cucumber, musk melon, summer squash and pumpkin. This review enumerates the detailed information on the effect of ethrel, a ripening hormone, on sex expression in cucurbitaceous vegetables.

Keywords: Cucurbits, sex expression, Ethrel, ripening Harmone, sex modification

Introduction
Crops belonging to family Cucurbitaceae are generally known as 'Cucurbits'. It consists of a wide range of vegetables either used for salad purpose (cucumber) or for cooking (all gourds), pickling (West Indian gherkin) or as dessert fruit (muskmelon, water melon) or candied or preserved (ash gourd). As a group, cucurbits occupy the largest area in India and in other tropical countries. Its use is not primarily because of calories, minerals or vitamins since they are generally low to moderate in nutrients with a few exceptions like bitter gourd (rich in vitamin C-96 mg/100g), parwal and chow chow (calcium – 531 mg and 140 mg / 100 g respectively). From the nutritional point of view, cucurbit seeds are valued for their high oil and protein contents. Seed proteins of cucurbits are comparable to that of legumes and are richer in methionine. Ashok *et al.*, 2019 [6].

General features
Cucurbit vegetables have the following common features:

1. Long tap root system – Tap root may grow up to 175-180 cm and laterals are confined to top 60 cm. Hence crops like bottle gourd, ash gourd and parwal are largely utilized in river bed cultivation (Diara system).
2. Branched stem-Stem is 3-8 branched and prostrate / climbing and spread up to 9-10 m in *Cucurbita* and *Lagenaria.* Crops like *Cucurbita pepo* have short internodes and are bushy. Nodes usually produce roots by touching on soil
3. Leaves are simple, mostly 3-5 lobed, palmate and rarely pinnately lobed (*Citrullus* sp.).
4. Tendrils on axils of leaves are simple in *Cucumis,* simple or bifid in others and absent in bush types.
5. Cucurbits are highly cross pollinated and pollination is done by honey bees and bumble bees. Flowers are born in axils of leaves and are solitary or in racemose clusters. Individual flowers are unisexual, large and showy.
6. Fruit is essentially an inferior berry and is called as "pepo" due to hard rind when mature. Fruits can be stored for long period in ash gourd, pumpkin, oriental pickling melon, etc. while keeping quality is less in cucumber, snake gourd, bitter gourd, etc. The fruits of all cucurbits except chow chow are many seeded.
7. Seeds are borne in parietal placentation-Placenta is the edible portion in water melon while in ash gourd, ridge gourd and smooth gourd it is endocarp. In muskmelon, edible portion is mostly pericarp with a little mesocarp.

8. Cucurbits are mostly seed propagated. A few are vegetative propagated like parwal and coccinia.
9. Most cucurbits are annuals except chow chow and coccinia, both having perennial habit.
10. Cucurbitacins – Majority of cucurbits are characterized by presence of bitter principles, cucurbitacins at some portions of plant and at some stages of development. Cucurbitacins are tetracyclic triterpins having extensive oxidation levels. It's highest concentration is in fruits and roots and is less in leaves. Pollen grain also carries fairly good amount of bitter principles. This is a common problem in oriental pickling melon, cucumber and bottle gourd and is rarely noticed in ridge gourd and snake gourd. The consumers usually remove fruit tips during conception to avoid possible chance of bitterness in fruits.
11. Sex forms – A wide range of sex forms like monoecious, andro monoecious, gynandro monoecious and dioecious forms are noticed in the family (Robinson and Decker-Walters, 1999).
    - *Hermaphrodite form* - This is the most primitive form and bisexual flowers only are produced in a plant. This is noticed in Satputia variety of ridge gourd and in a few lines of cucumber and mush melon.
    - *Monoecious form* - This is the advanced form and plants produce both male and female flowers in a plant. Majority of the cucurbits exhibit monoecious condition.
    - *Andromonoecious form* – Muskmelon and some cultivars of water melon produce both male and bisexual flowers in a plant. However, non dessert forms like oriental pickling melon, *Cucumis melo* are monoecious.
    - *Gynomonoecious* - This is noticed in cucumber and the plants produce female and bisexual flowers.
    - *Gynoecious form* - Lines producing female flowers alone are rarely noticed in cucumber and have got great potential for commercial F_1 production.
    - *Trimonoecious form*- This is a condition wherein, the male, female and bisexual flowers are produced in a single plant
    - *Dioecious form* – Male and female flowers are produced on separate plants in parwal, coccinia and kakrol.

Flowering

Majority of cucurbits start flowering 30-45 days after sowing and it follows a definite sequence. An alternate sequence of male and female flowers follows upto fruit set. The first 4-6 flowering nodes bear male flowers and alter female flowers. Developing fruits in a vine determine production of further female flowers further down in the vine. In crops where immature fruits are harvested at tender stage, this kind of inhibiting mechanism will not be perceptible. But in melon, pumpkin, ash gourd etc. even if perfect or female flowers are produced in the vine, fruits may not set or develop fully or shed in immature condition. That is why number of fruits / vine in a seed crop will be less (4-5) than in a vegetable crop (12-15) in bottle gourd, ash gourd and cucumber.

Pollination

Pollination takes place early in morning between 6-8 am in cucumber, pumpkin, muskmelon and watermelon. Pollination is altered in the day when temperature is high in bottle gourd and ridge gourd. In snake gourd and pointed gourd, anthesis takes place during night and pollination early in the morning. In pumpkin, pollen production is more while in muskmelon, pollen production is scanty and pollen grains are sticky due to oily film surrounding them.

Extent of cross pollination in cucurbits is 60-80%. They are entomophilous and bees, beetles and moths cause pollination.

Sex modification

Majority of cucurbits are monoecious and sex ratio (male: female) ranges from 25-30:1 to 15:1. Sex ratio is influenced by environmental factors. High N content in the soil, long days and high temperature favour maleness. Besides environmental factors, endogenous levels of auxins, gibberellins, ethylene and abscisic acid also determine sex ratio and sequence of flowering. A primordium can form either a female or a male flower and it can be manipulated by addition or deletion of auxins. Endogenous application of plant growth regulators can alter sex form, if applied at 2-4 leaf stage. High ethylene level induces female sex and is suggested to increase female flowers in cucumber, musk melon, summer squash and pumpkin. In cucumber, Ethrel 150-200 ppm induce female flowers.

The principle in sex modification in cucurbits lies in altering the sequence of flowering and sex ratio. Besides the environmental factors, endogenous levels of auxins, gibberellins, ethylene and abscisic acid, at the time and the seat of ontogeny determine the sex ratio and sequence of flowering (Leopold and Kriedemann, 1975) [26].

Application of ethrel may suppress production of male flowers and increase number of female flowers in vegetable crops under cucurbitaceae family (Kohinoor and Mian, 2005).

Table 1: Different sex forms in cucurbits

ex forms	Cucurbits
Monoecious (♀ and ♂)	Cucumber, Musk melon, Pumpkin, Summer squash, Winter squash, Water melon, Sponge gourd, Round melon, Bottle gourd, Bitter gourd.
Gynoecious (♀)	Cucumber, Bitter gourd, Musk melon, Watermelon, Ridge gourd
Androecious (♂)	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)

Megharaj *et al.*, 2017 [31].

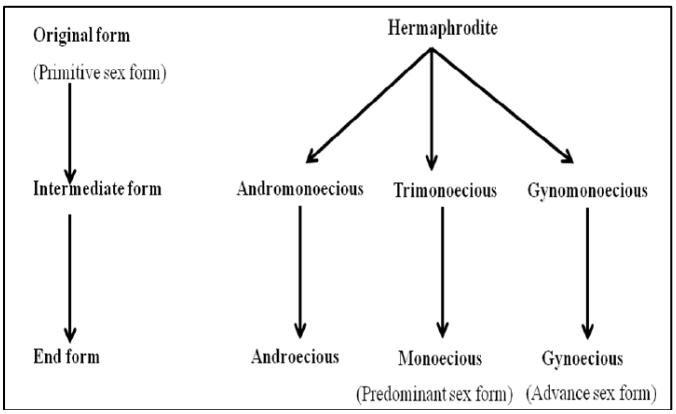

Fig 1: Evolution of sex forms in cucurbits (Whitakar, 1931) [52].

Effect of ethylene in cucurbits

The ethrel has been found to be the best effective to increase the number of female flowers and fruit yield in many cucurbits. The hormone induces production of female flowers an suppress male flowers (Verma *et al.*, 1984) [49, 50]; Kumar and Rao, 1988; Sarkar *et al.*, 1989; Mandal *et al.*, 1990;1991; Kalia and Dhillon, 1964) [24, 41, 30, 29].

Ethrel is used for more number of female flowers due to its property of better development of gynoecium, fruit ripening, stress induction, lateral cell expansion (Taiz and Zeiger, 2002) [46].

Ethylene plays a major role for inducing female flowers by suppressing gibberllin, a hormone for male flower production (Fig. 1). The internal ethylene level influences on expression of sex phenotypes *i.e.*, gynoecious lines produce two to threefold higher ethylene level than monoecious or andromonoecious ones (Megharaj *et al.*, 2017) [31].

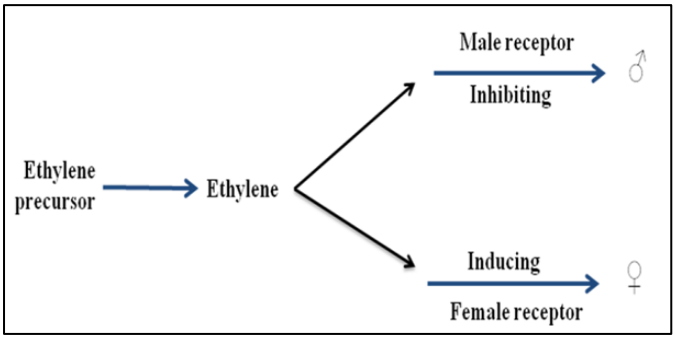

Fig 2: Effect of ethylene on flower manipulation

Snake gourd

Snake gourd (*Tricosanthes anguina* L.). Snake gourd is usually grown under kitchen garden as a summer vegetable. But at present, it is also being grown as commercial crop near the urban areas. Moreover, it can also be grown in any type of soil having good drainage system. Snake gourd contains considerable amount of protein, fat, minerals, fibre and carbohydrates (Gopalan *et al.*, 1982) [15]. Ripe fruits are rich in vitamin A. Due to high keeping quality (Banerjee and Mangal, 1986) [7], it has also export potentiality. But one of the main problems of snake gourd is flower dropping. It can be minimized by application of plant growth regulators.

Bitter gourd

Bitter gourd (*Momordica charantia* L.) is also known as balsam pear, bitter melon, bitter cucumber, and African cucumber (Heiser, 1979) [16]. It is a medicinal plant, belonging to cucurbita-ceous family is one of the most popular vegetables in Bangladesh and also in other Asian countries namely China, Taiwan, Malaysia, Vietnam, Thailand, India and the Philippines. It is adapted to a wide range of environments and can be grown in tropical and subtropical climates (Lim, 1998; Reyes *et al.*, 1994) [28, 39]. This vegetable is a different nature's bountiful gifts to mankind, which does not only have fabulous digestional properties, it is a storehouse of remedies for many common ailment. The fruits, leaves and even the roots of *M. charantia* have been used in Ayurveda for a number of diseases such as a bitter stomachic, laxative and anathematic. A compound known as 'charantin' present in the bitter gourd is used in the treatment of diabetes to lower blood sugar level (Anunciado and Masangkay, 2002) [3]. The fruit accumulates bitterness with time due to build up of three pentacyclic triterpenes momordicin, momordicinin and momordicilin, and then loses the bitterness during ripening (Cantwell *et al.*, 1996) [11]. The whole extract of the fruit is also advocated in diseases of spleen, liver, rheumatism and gout. The immature fruit of bitter gourd is valued for its bitter flavor, considered to bring out the flavor in other ingredients. It is usually eaten fresh (stuffed and/or sliced) but can also be pickled and has been canned in brine (Vinning, 1995) [51]. The plant also has a rich amount of vitamin C, iron, phosphorus and carbohydrates (Behera, 2004) [8]. The small type bitter gourd, 'uchja' fruits contained relatively more protein (2.1%), lipid (1.0%), ash (1.33%) and iron (9.4 mg/100 g) than the large type fruit 'karala', as well as good levels of carbohydrate (7.2%), sugars (0.42%), phosphorous (140 mg/100 g) and ascorbic acid (74 mg/100 g), and was therefore considered to have the best nutritional value (Choudhury, 1990; Kale *et al.*,1991; Kore *et al.*, 2003) [12, 20, 22]. To have the higher yield, the male and female flower ratio needed to be synchronized. Maleness and femaleness can usually be altered by environmental variables such as temperature, photoperiod and nutrition or by the appli-cation of plant growth regulators (Krishnamoorthy, 1981) [23]. Like other cucurbits, maleness is one of the major obstacles in bitter gourd which significantly reduces the fruit and seed yields.

Bottle gourd

Bottle gourd (*Langeraria sicenaria* (monlina) standl.) is a photo-insensitive crop but sensitive to thermo periodism. Thus, most of the existing bottle gourd varieties are season specific. It is rich in vitamin 'B' and source of minerals *viz.*, P, Ca and Fe. It is also important for medicinal use as ion case of headache, urines trouble and jaundice.

Research findings in cucurbits

Ravindran (1971) [38] reported that the exogenous application of ethrel (2-Chlorothyl phosphonic acid) at concentrations ranging from 200 to 600 ppm induced stunting growth, retardation and male sterility and the production of male flowers significantly reduced in snake gourd.

In ridge gourd (*Luffa acutangula)* seedlings treated with 500, 1000 or 2000 ppm of ethrel, Patnaik *et al.* (1974) [35, 36] reported that ethephon treated plants produced pistillate flowers only, but the number of fruits and total yield were inferior to those of untreated plants.

In cucumber (*Cucumis sativus* L.), application of ethral up to 500 ppm (Bhandary *et al.*, 1974) [9] increased the female flowers. They stated that ethrel concentration up to 500 ppm delayed male flowering up to 14 days and advanced female flowering by up to 9 days, while number of male flower were also reduced and female flowers increased by the application of same treatments.

Mishra *et al*. (1976) [33] reported that in cucumber maximal suppression of staminate flowers was obtained by the application of 400 ppm of ethrel.

In case of fruit yield and yield components like number of pistillate flowers, fruit numbers plants-1, fruit size and fruit weight were increased in *Trichosanthes anguina* plants by the application of ethrel at 50 to 150 ppm. The best result was obtained with ethrel at 150 ppm (Ramaswamy *et al*., 1976) [37].

Ethrel reduced the growth which might be due to decrease in level of gibberellins as also reported by Rudich *et al.,* (1970) [40]. Application of ethrel caused reduction of vine length and induction of dwarfism had resulted in the increased number of branches. This result was in accordance with the finding of Arora and Pratap (1989) with ethrel in Pumpkin.

Verma *et al*. (1980) [48] reported that ethrel treatments (50, 100, 150 and 200 ppm) were the most effective in increasing the number of female flowers, producing the largest number of fruits and greatest fruit weight plant-1 in bitter gourd.

Li (1983) [27] reported that ethrel at 200 or 300 ppm could lower the site of the first female flower, promote the appearance of female flowers, increase the number of fruits and leaf area, reduce the number of male flower, fruit setting and increase yields of three cucumbers. The ethylene-releasing chemical, ethrel enhanced the development of pistillate flowers and delays development of staminate flowers of monoecious cucurbits (Sheshadri, 1986) [42].

Application of ethrel caused reduction of days for first female flower appearance. The results obtained are in agreement with Verma *et al.,* (1984) [49, 50] in bitter gourd.

Singh and Choudhury (1988) [43] stated that ethrel at 50 and 100 ppm induced the first pistillate flowers earlier and at lower nodes in cucumber and bottle gourd, but delayed the appearance of female flowers in water melon.

Karim *et al*. (1990) [21] treated hybrid seedlings of cucumber with water, ethephon (250 and 350 ppm) at 1, 2, 3 and 4 leaf stage. Seedlings treated with ethephon at any stage produced more female flowers than water treated plants. The maximum increase in the number of female flowers occurred with 250 ppm ethephon applied at the 2-leaf stage.

Al-Masoum and Al-Masri (1999) [1] reported that cucumber cv. Beit Alpha grown in a greenhouse in 1996 to 1997 was treated with ethephon at 250, 350 and 450 ppm at the seedling stage (2 to 4 true leaves). They obtained positive effect of ethephon on the early and total yield, late number of female flowers, number of male flowers, days to the first female flowers, number of nodes to the first female flower, number of nodes to the first male flower and plant height. Ethephon induced femaleness (pistillate flowers) on the main stem that led to greater fruit production. 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) [53].

Negi *et al*. (2003) [34] studied the effect of ethephon and row spacing on the growth and yield of bitter gourd. Treatments comprised: two ethephon levels (0 and 250 ppm) and three row spacing (1.0, 1.25 and 1.50 m). Ethephon (250 ppm) reduced the length of main vine and number of branches and delayed the appearance of the first male and female flowers. Increasing row spacing increased the total number of female flowers per plant and appearance of the first female flower at the lower nodes. The fruit number as well as total fruit yield per plant increased with increased in spacing and decreased with ethephon application.

Kohinoor and Mian, 2005 studied in snake gourd, significant increase in female flowers was obtained with ethrel treatment, an appreciable increase in fruit yield was not obtained. This might be due to delaying in the production of male flowers, which is necessary for pollination and fruit setting. The findings are in agreement with other worker on ridge gourd (Patnaik *et al.,* 1974) [35, 36] and pumpkin (Hopping and Hawthorne, 1979) [18]. Application of Ethrel delayed flowering in bitter gourd reported by Ghani *et al.,* 2013 [14].

Ethophon 150 ppm proved to be superior to ethophon 300 ppm in including high number of female flowers in bitter gourd reported by Mia *et al.,* 2014 [32].

Exogenous application of ethrel 500 ppm were observed lowest node number (22.13) on which first female flower appered as compared to control in bitter gourd cv. Phule Green Gold reported by Hirpara *et al.,* 2015 [17].

Sureshkumar *et al.,* 2016 [46] reported in bitter gourd exogenous application ethrel, the fruit quality characters like total soluble solids and ascorbic acid which showed the maximum values in ethrel @ 250 ppm. The increased content of total soluble solids and ascorbic acid might be due to the stimulation and balancing of the growth hormones with application of ethrel which also enhances the accumulation and translocation of photosynthates from source to sink (fruits). These findings are in conformity with the results of Arora *et al*. (1995) [5] in ridge gourd, Elizabeth *et al*. (1999) [13] in snake gourd.

Kumari *et al.,* 2019 [25] studied in bottle gourd the significant increase in growth and fruit characters would be obtained by the spraying of ethrel 200 ppm at 2 and 4 true leaves.

Table 2: Effect of Ethrel on different crops

Crop	Growth regulator	Dosage	Effect	Reference
Cucumber	Ethephon	100 and 200 mg/l	Increased yield	Thappa, 2011 [47]
	Ethrel	500 ppm	Increased female flowers and reduced male flowers	Bhandary *et al.,* 1974 [9]
	Ethephon (ethrel)	400 ppm	Maximal suppression of staminate flowers	Mishra *et al.,* 1976 [33]
Bitter gourd	Ethrel (2-Chlorothyl phosphonic acid)	200 to 600 ppm	Stunting growth and significant reduced production of male flowers.	Ravindran, 1971 [38]
	Ethrel	100 ppm	Delayed the appearance of first male and female flowers	Verma *et al.,* 1984 [49, 50]
Pumpkin	Ethephon	300 mg/l	Increased number of female flowers	Sure *et al.,* 2012
Pointed gourd	Ethrel	150 ppm.	Pistillate flowers, fruit numbers/plants, fruit size and fruit weight were increased.	Ramaswamy *et al.,* 1976 [37]
Snake gourd	Ethephon (ethrel)	250 ppm	Altered sex expression and increased fruit yield	Cantliffe, 1976 [10]

Snake gourd	Ethrel	100ppm(4 times 10 –15 days after sowing at weekly intervals)	Increase	Jeyakumar, 2015 [19]
Bitter gourd	Ethrel	100ppm 4 times 10 –15 days after sowing at weekly intervals	Increase in yield	
Pumpkin	Ethrel	250ppm 4 times 10-15 days after sowing at weekly intervals	Increase in yield	
Ash gourd	Ethrel	250 ppm (2.5 ml/10 lit of water) four times at weekly intervals commencing from 15th day after sowing.	Increase in yield	Anon, 2106

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