Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 03, Issue: 04 (JULY-AUGUST, 2023)
Available online at http://www.agriarticles.com

Sex Determination in Plants

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Sex differentiation in plants is governed by various factors like chromosomal, genic variation. When sex differentiation is governed by chromosomal variation, sex differentiation is not affected by environmental factor. Confirmation of sex in early stage is crucial in the perennial dioecious plants for maintaining sex ratio. Sex determination is a process that utilized to decide whether a particular individual will develop into male or female. At seedling stage plant appear similar in morphology and it is very difficult to identify as male or female. Several efforts have been directed recently to establish for the early detection of seedling gender before their plantation in field. Availability of molecular techniques increase the accuracy of sex determination. Chromosomal banding/amplification patterns is useful for mapping of Y-chromosome as well as sex determination in dioecious plants very effective (Charlesworth, 2002).

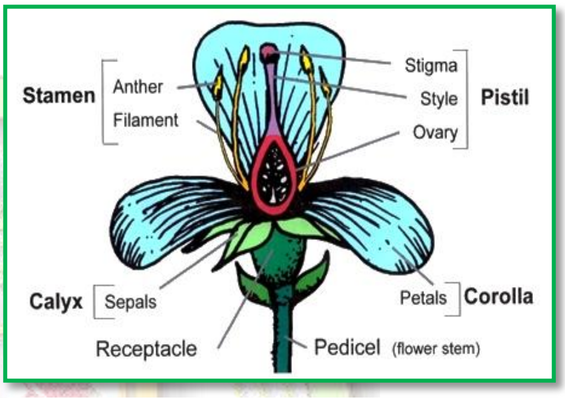


Figure 1: Basic flower structure

Importance of sexuality in plant

Sexual reproduction is the process in which male and female gamete fuse to form zygote through a process called fertilization and it is important for plants as it provides variation to the progeny that helps in the better survival and help it to gain its own uniqueness within the species and remove the unwanted genes . The flower is the characteristic structure concerned with sexual reproduction in flowering plants (angiosperms). The angiosperms which promote sexual reproduction are required to have different sex forms *viz.*, male and female organs. Basic flower contains calyx (sepals), corolla (petals), androecium (stamens) and gynoecium (carpel). The breeding system, or how the sperm from one plant fertilizes the ovum of another, depends on the reproductive morphology and is the single most important determinant of the genetic structure of nonclone plant populations. Also, it is important for the pollination mechanism, development of new lines and increase or decrease the chances of out cross.

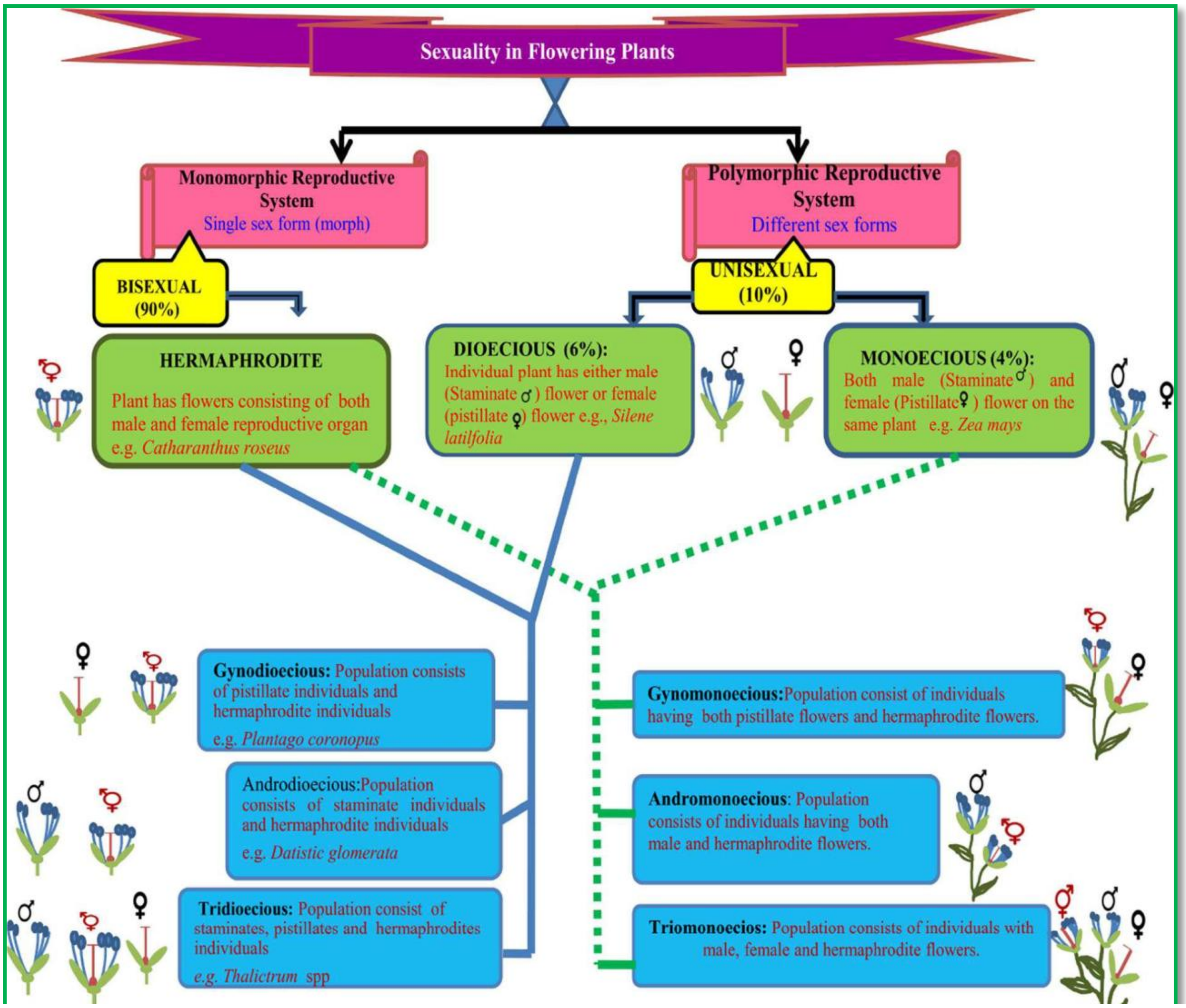


Figure 2: Various reproductive systems in plant kingdom (Heikrujam *et al.*, 2015)

- Sexual dimorphism is a fundamental mechanism occurring widely amongst the multicellular organisms that delineates male individuals from female individuals which leads to differences in the form or traits of the respective sex organs. Significance of such studies lies in the fact that they are helpful in the comprehensive study of evolutionary and developmental pathways to dioecy.

ABC model of flower development

This model proposed that different organ-identity genes act alone and in various combinations to specify each of the four types of floral organs. Model of flower development was first formulated by George Haughn and Chris Somerville in 1988.

A CLASS GENE

- In Arabidopsis thaliana, function A is mainly represented by two genes APETALA I (AP I) and APETALA 2 (AP 2). Mutations in the class A gene AP 2 result in C activity spreading into whorls one and two and consequently homeotic transformations in organ identity with carpels replacing sepals in whorl one and stamens replacing petals in whorl two.

B CLASS GENES

- In Arabidopsis thaliana the type- B function mainly arises from two genes, APETALA 3 (MP 3) and PISTILLATA (P I). A mutation of either of these genes causes the homeotic conversion of petals into sepals and of stamens into carpels.

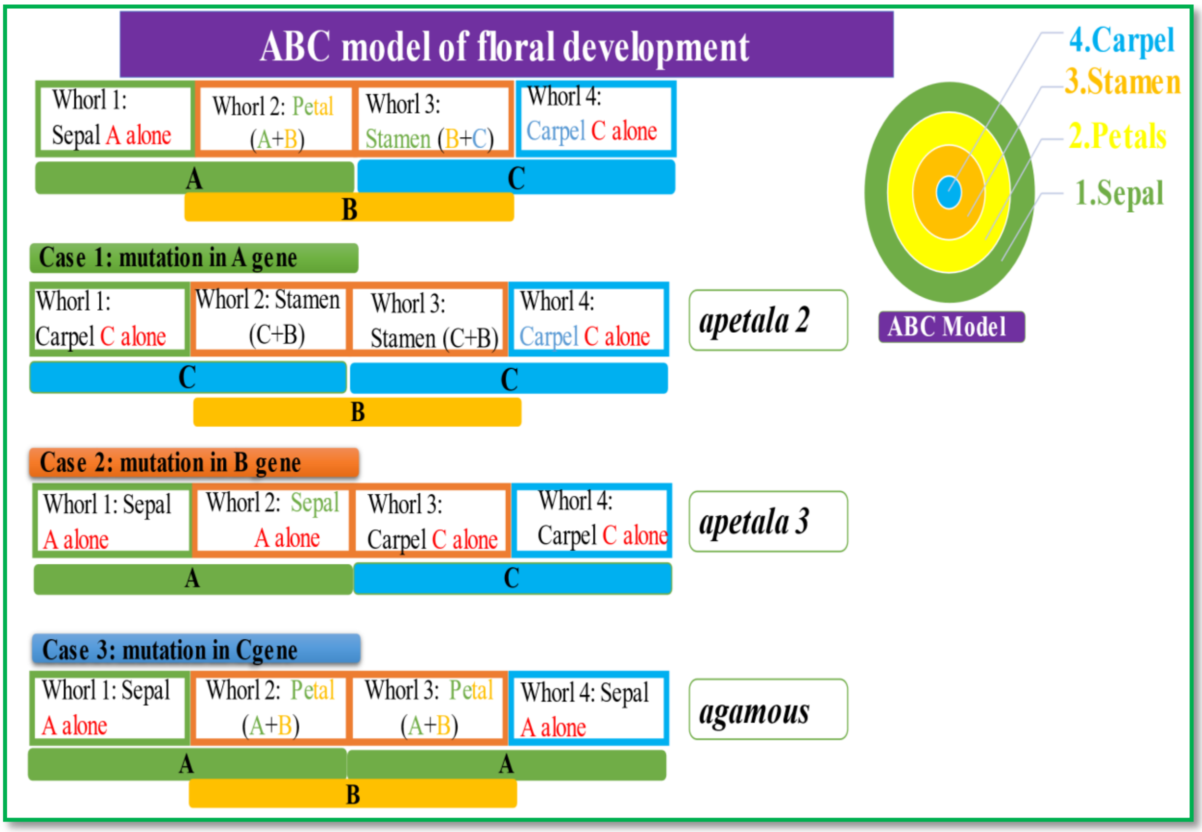


Figure 3: ABC model of flower development

Source:- https://youtu.be/In-MpS29LMU?si=LzEKKwKbfylNbTkI

C CLASS GENES

- In Arabidopsis thaliana, the C function is derived from one gene called AGAMOUS (AG). Mutations in the class C gene AG result in class A activity in all four whorls. The AG mutants produced indeterminate flowers repeating the pattern of organs (Se Pe Pe). According to improved findings, MADS-box genes also identify the flower development (Zhao *et al.*, 2011)

Sex determination, Sex differentiation and Sex identification

- Sex determination is a process of utilizing various genetical concepts to decide whether a particular individual will develop into male or female.
- Sex identification is a process of prediction that developing individual is male or female.
- Sex differentiation is a process of development of differences between male and female.

Use of sex determination in crop plant

1. To identify ideal seed production requirements of crop.
2. Exploitation of different sex for development of new lines and varieties.
3. To study expression of character associated with sex chromosomes.
4. Early detection of sex in dioecious plants for maintaining sex ratio.

Classification of mechanisms of sex determination in plants

Chromosomal sex determination: Those chromosomes, which differ in morphology and number in male and female sex and contain genes responsible for the determination of sex are known as allosomes or sex chromosomes. Homomorphic sex chromosome *e.g.* asparagus, spinach. Heteromorphic sex chromosome e.g. *canabis sativa*, *coccinia indica.*

Genic Sex Determination: Sex determination in some plant species *e.g.* papaya, spinach, *vitis cinerea*, *asparagus etc.*, it is postulated to be governed by genes. Genes which determine sex may be single or several *e.g.* papaya, *vitis cinenria*.

Mechanisms of sex determination in plants

1. Chromosomal sex determination
    - Homomorphic sex chromosome *e.g.* Asparagus, Spinach
    - Heteromorphic sex chromosome *e.g. Canabis sativa, Coccinia indica*
2. Genic sex determination *e.g.* Papaya, *Vitis cinenria*
3. Environmental sex determination *e.g.* Cucumber, Cannabis
4. Epigenetic inheritance of sex determination *e.g.* Melon

Environmental sex determination: Environmental factors such as temperature, photoperiod, nutrition and water availability can determine sex expression in many species. The sex of the flowers is affected by many environmental factors like temperature, day-length, ethylene, GA_3, some ions (Ca^{+2} , Mg^{+2}) *etc*. In general treatment with **ethylene or GA_3** promotes the production of **female flowers.** Cytokinin may trigger the female flower development and gibberellin acid may control the male flower development. Temperature and photoperiodism influence the male sterility governed by genes. *e.g*. cucumber, cannabis

Epigenetic inheritance of sex determination: The difference in the sexual phenotype of flowers without any difference in genetic loci directly involved in the development of the sex organs is termed as epigenetic regulation of sex determination. It can occur at the transcription level by modification of genomic loci in males and females, histone modification or DNA modification, or at post-transcriptional level by RNA mediated regulation. e.g.- in melon, female promoting gene *CmACS11* represses the expression of male promoting gene *CmWIP1* via deposition of *H3K27me3*.

Sex determination mechanisms in flowering plants (Dioecious plants)

- It has been postulated that a wide variety of sex determination are prevalent in plant kingdom and genetic control of sex determination is driven by chromosomes and its sex determining genes in the dioecious plants. A vast variability in the mechanism of genetically controlled sex determination has been seen within dioecious plants and it has been mainly attributed to DNA polymorphism in the loci that are directly involved in expression of a particular sexual phenotype.
- Genetic sex determination may be due to a single locus or multiple loci either unlinked or tightly linked on autosomes. The simplest sex determination mechanism involving a single genetic locus, segregating as mendelian genetic system has been seen in ecballium elaterium (*Squirting cucumber*) where allelic constitution (three alleles) at a single locus 'a' localized on a structurally and functionally undifferentiated pairs of autosomes determine whether the plant will be male, female or monoecious. A single locus on a homologue pair of chromosomes determines the sex. A relatively complex sex determination has been found in case of mercurialis annua where the sex is controlled by multiple unlinked loci (Heikrujam *et al.*, 2015).
- Notwithstanding, several genes which are compiled into special linkage groups, found on homomorphic/ heteromorphic sex chromosomes or on the autosomes have been

documented to be associated with sex determination process among several species reinforcing dioecism. Heteromorphic sex chromosomes can be identified cytologically by their size and morphology as well as during meiosis as the sex chromosomes are unable to pair completely like other chromosomes. Presence of plant sex chromosomes was first reported in the liverwort *Spaerocarpus donnellii*.

- The sex chromosomes have been identified in some important dioecious crops such as *Carica papaya*, *Humulus lupulus*, *Humulus japonicus*, *Silene latifolia* and *Rumex acetosa*.

XX/XY system

- Among the dioecious plants, such sex inheritance (XX/XY system) has been documented in *Silene latifolia*, *Cannabis sativa* and *Carica papaya etc*.
- Males are heterogametic having an X and a dominant Y chromosome which are physically different from each other and females are homogametic having two identical X chromosomes. Displaying this type of sexual dimorphism, *Silene latifolia* is one of the well-studied plant.
- The presence of Y chromosome is essential for maleness in such type of sex determination and thus known as active Y chromosome system. Three functionally different regions located on the non-recombining regions of the Y chromosome: region containing carpel development suppression genes, region containing stamen promoting genes and region containing male determining genes have been assigned to be the reason of being male in *Silene latifolia*.
- In case of *Carica papaya* two differen types of Y chromosomes, Y and Y^h have been found to be responsible for maleness and hermaphroditism, respectively and the sex determining locus has been mapped to 14 cM in the papaya genome. The X chromosome also appears to play an essential role in both males and females because only monoploid females could be obtained by in vitro technique.

X: A balance system

- the primary sex determination system is independent of the presence or absence of the Y chromosome and is controlled by X: Autosome's ratio parallel to the sex determination mechanism.
- In *Spinacia oleracea* sex locus has been mapped on autosomal pair 1 and in case of *Asparagus officinalis* male-sex determining region was mapped to 0.25 cM on chromosome 5.

ZZ and ZW system

- In this type of sex determination, female heterogamety (ZZ male and ZW female) has been observed as in case of *Fragaria elatior* that resembles to sex inheritance in birds. Besides, this type of sex determination has also been suspected in *Myrstica fragrance*, Populus spp. In *Fragaria virginiana* (polyploid strawberry), sex determination is regulated by two loci that are linked to each other at a genetic distance of about 5 cM. In Populus, the sex determining locus has been mapped on chromosome 19.

Epigenetic control of sex determination

- Such type of sex inheritance is regulated by switching the genes (even without changing the DNA sequences) at transcriptional level or post-transcriptional level under the regime of various environmental and physiological determinants. It also occurs through altered euchromatin structure, DNA methylation and insertion of transposons in the genome

Hormonal control of sex determination

- Auxins and cytokinins influence and alter the sex expression varyingly with respect to plant species and are considered as mascularizing and feminizing hormones.

SEX DETERMINATION IN PAPAYA

The diagnosis of sex is very difficult in the dioecious plant prior flowering where in sex identification at the seedling stage is of great importance to breeders as well as farmers for crop improvement or production purpose. Papaya is a dioecious plant as the male and female flowers are present on the different plant individuals. Papaya is diploid with 9 pairs of chromosomes. It has a small genome of 372 Mb (Arumuganathan and Earle, 1991). Generation time as short as 9 months. Papaya is some what unusual in that it is trioecious with three basic sex forms: female, male, and hermaphrodite. Cymose inflorescences arise in axils of leaves. The type of inflorescence produced depends on the sex of the tree. Male trees are characterized by long, pendulous, many-flowered inflorescences bearing slender male flowers lacking a pistil, except for occasional pistil-bearing flowers at the distal terminus. Female trees have short inflorescences with few flowers bearing large functional pistils without stamens. Hermaphroditic trees have short inflorescences bearing bisexual flowers that can be sexually variable. Not only are the sex forms morphologically distinct, they are inherited in unexpected ratios that are due to a lethal factor associated with male dominant alleles (Table 1).

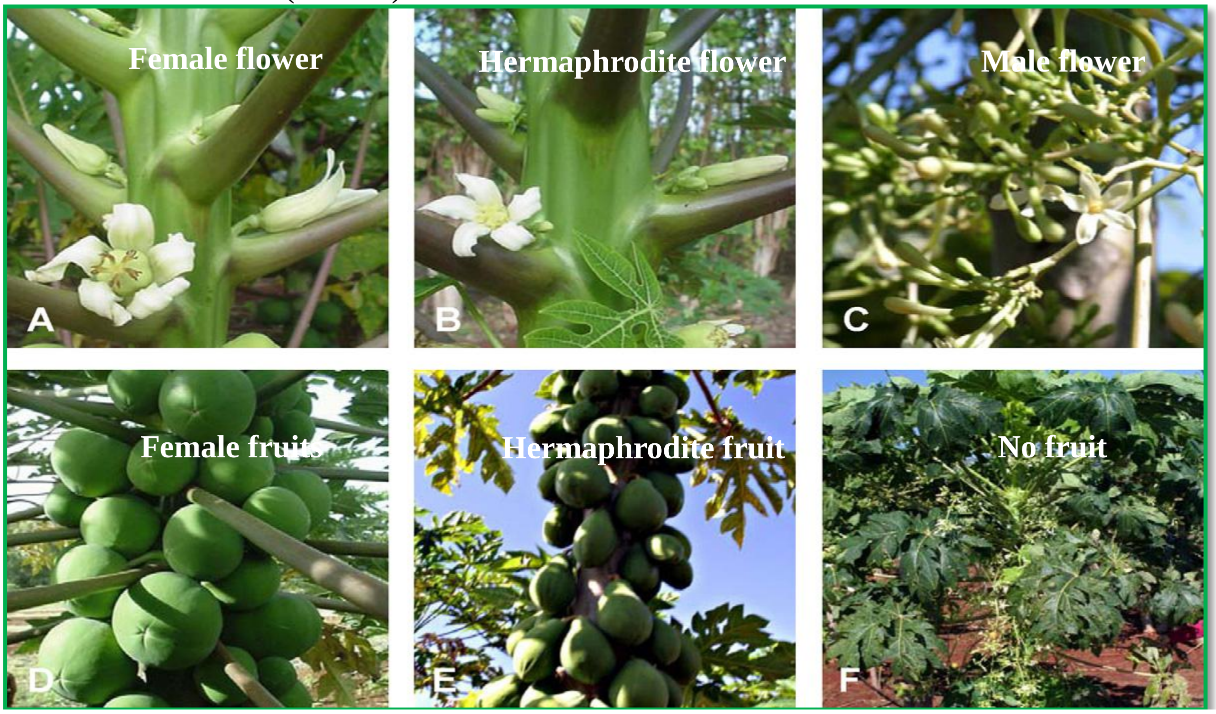


Figure 4: Papaya plants with female, hermaphrodite and male flowers with fruits (Ming *et al*., 2007)

Staminate (Male)

- Male trees are characterized with long inflorescence, bearing dozens of flowers having yellow in color, arise in clustered form and possess ten stamens without ovary (Ming *et al*., 2007).

Pistillate (Female)

- Female trees are characterized with short inflorescence having few flowers, are white or cream in color with large rounded superior ovary without stamens. Fruits are round or oval shape and female plants are important for commercial papain production (Ming *et al*., 2007).

Hermaphrodite (Bisexual)

- Hermaphroditic trees are having short inflorescence, bearing bisexual flowers and functional ovary along with stamens. Fruits are pyriform-shaped that are preferred for consumption (Ming *et al*., 2007).

Hermaphrodite plants are further classified into four types:

1. **Elongate type:**

It having ten stamens in two clusters, smooth and elongated functional ovary.

2. **Intermedia or carpelloid type:**

It possesses two to ten mostly distorted stamens and irregularly ridged ovary.

3. **Pentandria type:**

It is characterized with five stamens attached with the base of rounded ovary.

4. **Barren or sterile type:**

It having ten functional stamens but the ovary aborts.

Why sex determination is necessary in papaya?

- Sex of the papaya plant cannot be predicted morphologically at early seedling stages. As male plant do not have economic importance farmers have to remove the male plants from the field and leave the female or hermaphrodite plants on the basis of floral morphology which can be performed only after three to four months from germination (Ma *et al*., 2004). If the prediction of sex of papaya could be done at early seedling stage, then an expected male and female plants ratios (5% males: 95% females) would be maintained by removing excess male trees. This would save the resources such as space required for planting, fertilizers, water and labor *etc.* otherwise spent on these undesirable male trees.

Sex determination in papaya behaves as a single gene with three alleles

- In this crop, the sex is genetically determined by a pair of sexual chromosomes (XX-female, XY-male and XYh-hermaphrodite), and there is a genomic region in the Y and Yh chromosomes where recombination with X is suppressed.
- This region is involved in the determination of the male and hermaphrodite genders, which are designated as the male-specific region on the Y chromosome (MSY) and the specific region for hermaphroditism on the Yh chromosomes (HSY) (Ming *et al*. 2007).
- Based on the segregation ratios from crosses among three sex types (Table 1), Hofmeyr and Storey independently proposed that sex determination in papaya is controlled by a single gene with three alleles, named as *M*1, *M*2, and *m* by Hofmeyr and *M*, *Mh*, and *m*.
- Male individuals (*Mm*) and hermaphrodite individuals (*Mhm*) are heterozygous, whereas female individuals (*mm*) are homozygous recessive. The dominant combinations of *MM*, *MhMh*, and *MMh* are lethal, resulting in a 2:1 segregation of hermaphrodite to female from self-pollinated hermaphrodite seeds and 1:1 segregation of male to female or hermaphrodite to female from cross-pollinated female seeds. Ming *et al*., (2007) proposed that two genes (stamen suppressing gene in female flower and carpel suppressor gene in male flower) plays an important role for determination of sex forms. Stamen-suppressing gene causes abortion of stamen while carpel suppressor gene aborts the carpel at a later developmental stage.

Segregation ratio in papaya

Table 1: Segregation ratio of crosses between different combinations of sex types

Crosses	Genotypes	Segregation ratio			
		Male (Mm)	Female (mm)	Hermaphrodite (M^hm)	Non-viable genotypes
Hermaphrodite (selfed)	M^hm × M^hm	0	1	2	1
Female × Hermaphrodite	mm × M^hm	0	1	1	0
Hermaphrodite × Male	M^hm × Mm	1	1	1	1
Female × Male	mm × Mm	1	1	0	0

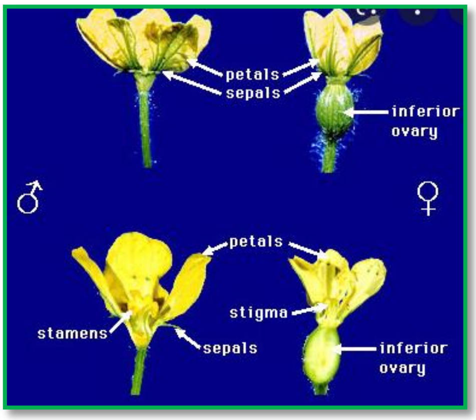
1. Hermaphrodite papaya trees are primarily self-pollinated. However, seeds from selfed hermaphrodite trees always segregate into hermaphrodites and females at the ratio 2:1. The seed should preferably originate from controlled crosses (a) between female and hermaphrodite plants; and (b) between hermaphrodite plants or by self-pollination. In the first case, 50 % of the obtained plants are female and 50% are hermaphrodite. In the second case, however, 2/3 of the generated plants are hermaphrodite and 1/3 are female. In many regions of the world, hermaphrodites are preferred for their higher productivity since every tree will produce fruit, whereas using female trees for fruit production involves the loss of 6-10 % of field space for growing male trees to pollinate the females. One of the difficulties surrounding papaya production is lack of knowledge of sex in young plants, since sexing is only possible three to four months after planting, by observing the flowers. The relevance of sexual determination lies in the greater commercial interest in hermaphrodite plants, as their fruits have a higher market value, both domestically and internationally, because of their elongated shape and smaller internal cavity, which allows for a higher pulp/total fruit volume ratio (Duarte *et al.*2020).
2. However, in subtropical regions with cool winters, female production is preferred because female flowers are stable at low temperature while hermaphrodite flowers tend to fuse anthers to the carpels and produce deformed carpellodic fruit. Farmers using hermaphrodites for production need to germinate a minimum of five seedlings per hill to assure there are no more than 3 % female trees. The five plants in a hill must be grown for 4-6 months until sexes can be determined. Finally, rouging must be practiced to obtain sex ratios conducive to optimal productivity. This process is inefficient of time, labor, water, and nutrients, and also results in delayed production due to competition among the plants in early growth.
3. Female with hermaphrodite to produce ratio of 1:1 segregate ratio. On the other hand, farmers depending on female tree production need to germinate four seedlings per hill to keep 6 to 10 % male trees in the field.
4. Hermaphrodite papaya trees cross with Male to produce all type 1:1:1:1 ratio produce.
5. Female × Male produce only 1:1male to female ratio.

SEX DETERMINATION IN CUCURBITS

Table 2: 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
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

Megharaj *et al.* (2017)



Flower formula:

Male flower - ⊕ ♂ $K_{(5)}\ C_{(5)}\ A_{(2)+(2)+1}\ G_0$

Female flower - ⊕ ♀ $K_{(5)}\ C_{(5)}\ A_{(0)}\ G_{(3)}$

Figure 5: Male and female flower of water melon with flower formula

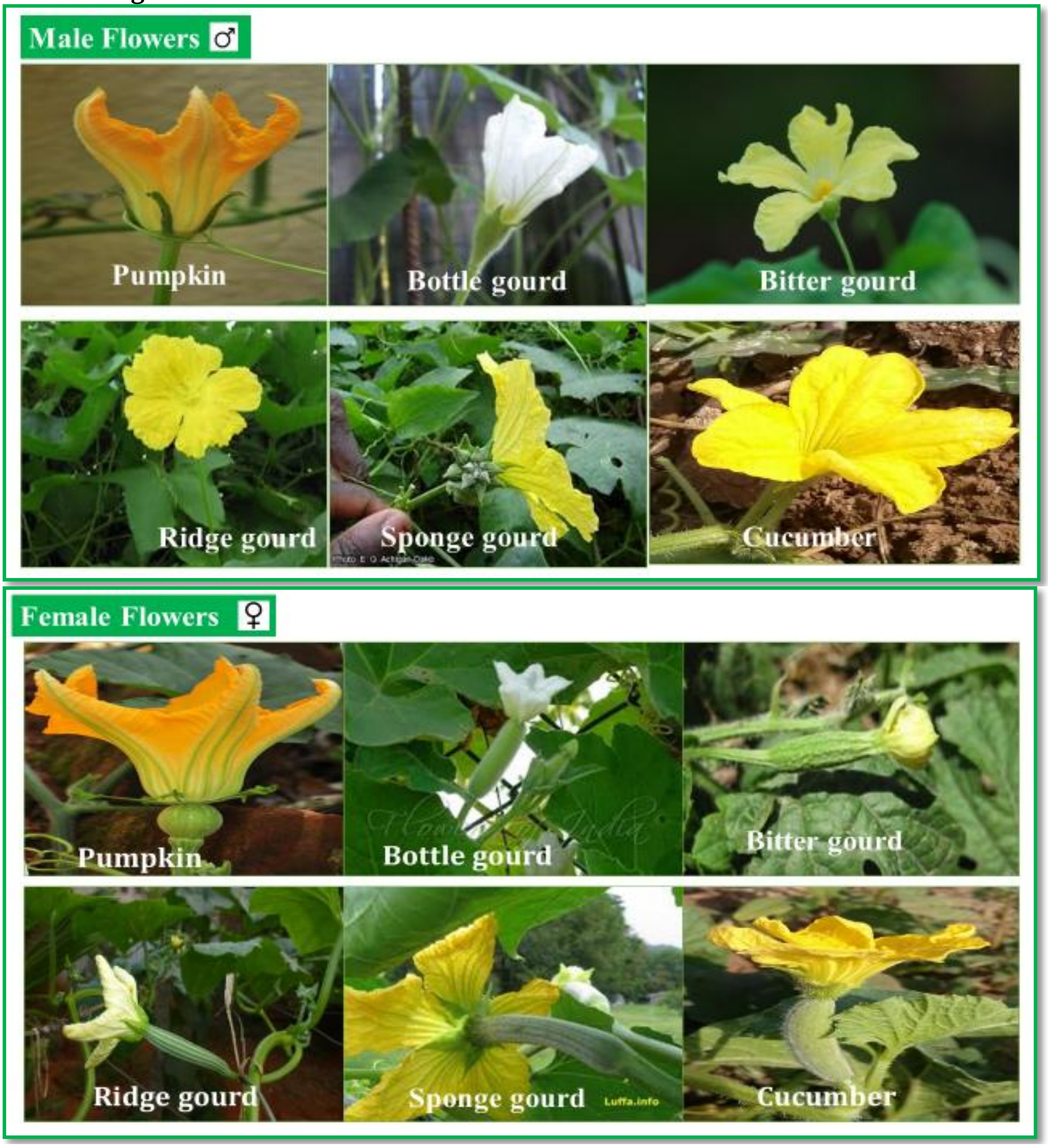


Figure 6: different male and female flower of cucurbits

In the early stage of flower development all floral buds are morphologically hermaphrodite containing stamen and pistillate primordia. Selective arrest of either the staminate or pistillate parts result in female flower or male flower leads to unisexual flower development. If arrest does not happen, bisexual flower formed (Megharaj *et al*. (2017). The plants usually have unisexual flowers which are borne in the leaf axils. Cucurbit flower consisting of five sepals and yellow or white corolla consisting of five petals. Male flowers have up to five anthers, often fused or joined in a complex way and female flowers usually have three carpels. Staminate flowers differentiate at lower nodes and produced in greater numbers than pistillate flowers. The staminate flowers have thinner, longer pedicels than the pistillate flowers. The pistillate flowers have inferior ovaries that usually are round or oval in shape**.**

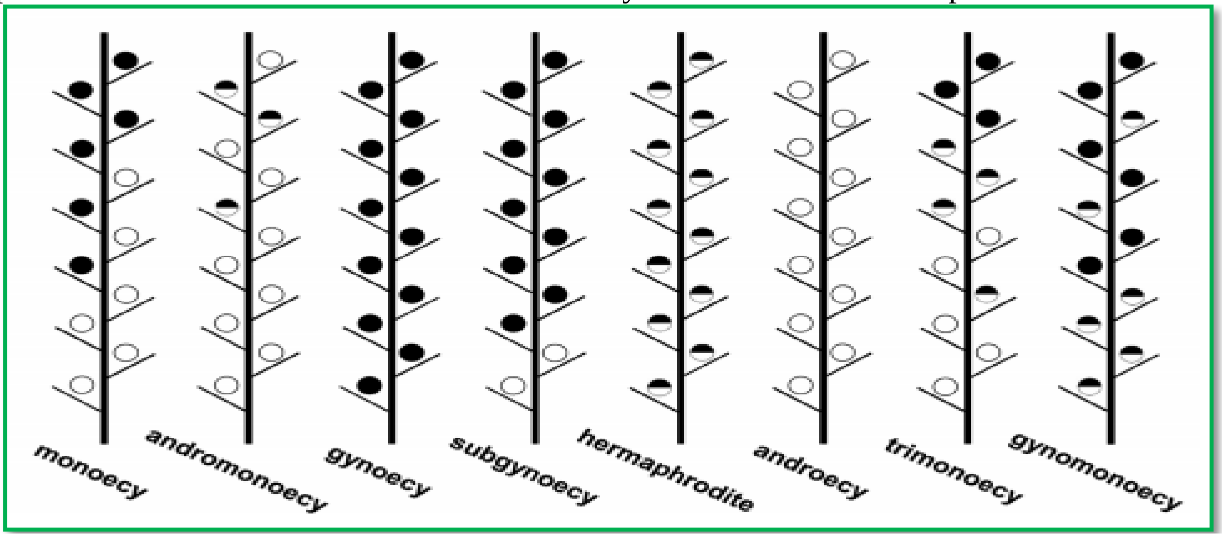


Figure 7: Sex expression in cucurbits. Schematic diagram of sex expression in the plant main vine in cucurbits. Black, blank, and mix circles represent female, male, and bisexual flowers, respectively. Sex type of the first blooming flower was used to defined the sex type of the node arising the flower.

Types of sex expression

- Usually, in a monoecious cucumber plant, male flowers arise in early or lower nodes, followed by a mixture of male and female flowers at the middle nodes, and ending with female flowers only in the higher nodes.
- In cucumber and melon, gynoecious lines produce only female flowers, while androecious plants bear only male flowers.
- Male and bisexual flowers can be found in andromonoecious lines, which can be regarded as bisexual flowers replacing female flowers found in monoecious lines.
- Hermaphroditic plants bear only bisexual flowers.
- Subgynoecious plants, which are found in some watermelon, zucchini, and cucumber lines, produce few male flowers in the beginning nodes and all female flowers in the later nodes.
- Their most obvious difference from the monoecious lines is the lack of the mixed phase comprising male and female flowers.

Sex expression in cucurbits

- Dioecy is codified by divergent chromosomes or gene complexes, while monoecy conferred by a small number of independently segregating genes (Sarkar *et al*. 2017).
- Unisexuality results from specific suppression of either stamen or carpel primordia subsequent to initial whorl formation.
- Numerous studies with exogenous hormone have indicated that ethylene is the primary hormone determinant of sex in cucurbits.

- It has been proposed that sex determination results from a combination of ethylene production and perception by relevant female primordia at critical times in development.

SEX DETERMINATION IN DATE PALM

- Date palm (*Phoenix dactylifera* L.) is dioecious tree with male and female individual (2n=2x=36).
- In Date palm mostly hand pollination done for commercial production. Among the seedlings from seeds, more than 50 % are male.
- Date palm tree start bearing flowers after 5 years so before it, prediction of male or female based phenotype is not accurate (Bekheet and Hanafy, 2011).

Figure 8: Male tree

Figure 9: Female tree

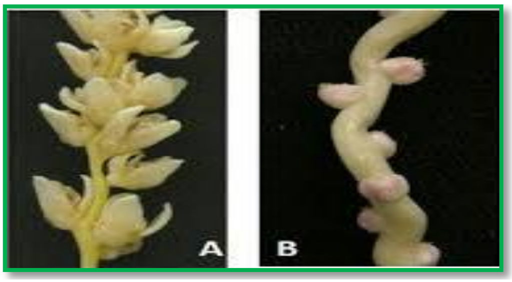


Figure 10: Male and Female strands

Conclusion

- Sex in plant evolved from long phylogenesis process, which could be understood by mutation and evolution of active Y chromosome.
- Early sex determination and elite male genotypes could be helpful in maintaining female to male ratio and identification of suitable male and female combinations.
- The molecular breeding will also be helpful to identify the genes governing the sex development in papaya and datepalm.

Future thrust

- Exact mechanism of sex determination in all the unisexual crops need to be studied for further utilization in crop improvement.
- Genomic studies on sex-linked genes and their expression needs to be studied along with various regulatory pathways.
- Identifying male and female liked molecular marker in seed-bearing plants for differentiation of male and female at the seedling stage, especially prior to propagation.

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