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Gender determination mechanisms in plants: foundation for unisexuality

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ABSTRACT

Knowledge of mechanisms determining and maintaining unisexuality in the flowering plants is of ecological, evolutionary and commercial importance. Among the flowering plants sexuality is manifested to various extents. Among these, the dioecious and monoecious plants provide an excellent opportunity to study the gender determination mechanism. Empirical studies carried on dioecious plant species provided a sequence of stages/events that represent the evolution of sex chromosomes in plants. These events include origin of sex determination genes/loci, suppression of recombination at sex determination loci, its extension to adjacent regions, accumulation of random mutations and finally the degeneration of Y chromosome that lead to the origin of heteromorphic pair of sex chromosome. Recent advances in molecular tools have enabled researchers to decipher the role of hormones and micro RNAs. Emerging information from recent studies on various aspects of gender expression in plants suggest that all these components might be interrelated and could be linked to sex chromosomes in future studies. In the present chapter we provide the highlights of current understanding on the sex determination mechanism prevalent in flowering plants with special reference to dioecious and monoecious sexual systems.

Keywords : sexual system, sex chromosomes, micro RNAs, dioecy, monoecy,

INTRODUCTION

The complexity in mating patterns among the flowering plants has generated from the diversity in their sexual systems. Although a great majority of plants bear bisexual flowers (hermaphrodite), unisexuality is widespread across many unrelated taxonomic groups. A recent estimate accounts that dioecy is present in nearly 7% of the genera and 43% of the angiosperm families (Renner 2014). It is now well-established that dioecy (separate male and female flowering bearing plants) has independently evolved from cosexuality with ~100 transitions in various lineages of plants (Ross 1982, Renner 2014). The transition is believed to have been driven under unfavourable environmental conditions to provide certain extent of reproductive assurance to plants (Westergaard 1958, Ross 1982).

The role of gender determination mechanism is crucial in regulating and establishing the stable sexual system in a species. The mechanism may physically separate the male (stamens) and female (carpel) reproductive organs on the same plants but different flowers (monoecy) or on different plants (dioecy). Besides these two extremes, there are many sexual systems (androdioecy, gynodioecy, trioecy etc.) that are often believed to represent the transitory stages of the process (Geber *et al*. 1999, Tanurdzic & Banks 2004). In order to characterize the gender determination mechanism, the route to dioecy followed by a species requires an in-depth investigation. In nature the pathways are usually represented by species populations

containing both the imperfectly differentiated plants (with flowers of separate or combined sexes) and the strictly unisexual plants (Westergaard 1958, Ross 1982, Charlesworth 1991, Geber *et al*. 1999, Charlesworth 2013).

The development of the four key floral organs (calyx, corolla, stamens and carpel) is under the regulation of regulatory genes, thus a single mutation could lead to sterility or abort the function of carpel or anthers at any stage of development (Wellmer *et al*. 2004). Thus, the main emphasis has been on studying the genetic control of sex determination which encompasses the role of sterility mutations in regulating sex expression (Ming *et al.* 2007). In general, unisexuality could be achieved from cosexuality by two ways: (i) the floral meristem of one of the genders is not differentiated and thus strict unisexual flowers are formed and (ii) by the suppression of an opposite gender within a flower at a specific stage, thereby leaving traces of vestigial organs of the opposite sex. The first condition is known as *unisexuallity by inception* and the latter one *unisexualilty by abortion* (Mitchell & Diggle 2005). The monoecious species, more or less, exhibit the first condition and dioecious systems may represent the strict unisexual forms as well as vestiges of opposite sex (cryptic dioecy).

It is well-established that flower development is under a complex network of gene functions, however, the genes regulating dioecy and monoecy are not the same or identical. Moreover, only the regulatory genes are not involved in sex expression (Charlesworth 2013). In this context, dioecious plants offer an excellent opportunity to study the genetic control of differentiation of separate sexes. As dioecy is generally accompanied with hermaphrodite relatives or imperfectly differentiated plants, it is easy to compare the evolution of sex determination mechanisms or genes in unisexual plants with their hermaphrodite relatives or progenitors (Charlesworth B & Charlesworth D 1978, Charlesworth D & Charlesworth B 1978, Charlesworth & Charlesworth 1979, Ross 1982).

Monoecious plants such as the cucurbits and maize have proved to be useful systems to study sex determination mechanisms, as the mechanisms are more precise in them and have to simultaneously work in an antagonistic manner. This has been hypothesized on the basis of the studies on hormonal regulation in gender reversal (Dellaporta & Urrea 1993, Aryal & Ming 2014). Similarly in some plants, environmental cues are also known to involve in regulating the gender (Chailakhyan & Khryanin 1977, Irish & Nelson 1989). With increasing incidences, the role of hormones and epigenetic regulation is also considered crucial to understand that how unisexuality is conferred in plants. Once the regulatory mechanisms of sex expression are well-understood, the knowledge may be useful in enhancing the expression of desired gender in commercially important plants. The approach to study sex determination mechanisms has become dynamic in evolutionary, economic and developmental contexts in recent years. In the present chapter, we briefly highlight the present understanding of the gender conferring mechanisms in flowering plants.

Sex Chromosomes and Their Evolution— Dioecious plants are ideal systems to study the genetics of evolution of combined versus separate sexes for two reasons (Westergaard 1958, Ming *et al*. 2007). First, different dioecious plant species that have been characterized at various stages of sex chromosome evolution provide a sequence of evolution of heteromorphic chromosomes (Negrutiu *et al*. 2001, Charlesworth 2002, Ming *et al*. 2011, Charlesworth 2013). Second, dioecy and gender polymorphism is widespread in angiosperm lineages (Renner & Ricklefs 1995); thus it is easy to compare the mechanisms among various taxa. Among the angiosperms, heteromorphic sex chromosomes are reported in 19 species from 4 families and homomorphic sex chromosomes are reported in 20 species from 13 families (Ming *et al*. 2011). The reason for a few numbers of cases could be that sex chromosomes have been characterized only for a tiny fraction of the known dioecious taxa and the remaining plants are yet to be investigated.

Both the ecological and evolutionary forces exert selection on hermaphrodites or cosexuals to develop unisexual forms (Charlesworth B & Charlesworth D 1978, Charlesworth D & Charlesworth B 1978, Charlesworth & Charlesworth 1979, Ross 1982). Experimental data on dioecious plant species provide a series of events representing the evolution of sex

chromosomes in plants. These events include origin of sex determination genes/loci, suppression of recombination at sex determination loci, its extension to the adjacent regions in the chromosomes, accumulation of random mutations and finally the degeneration of Y chromosome.

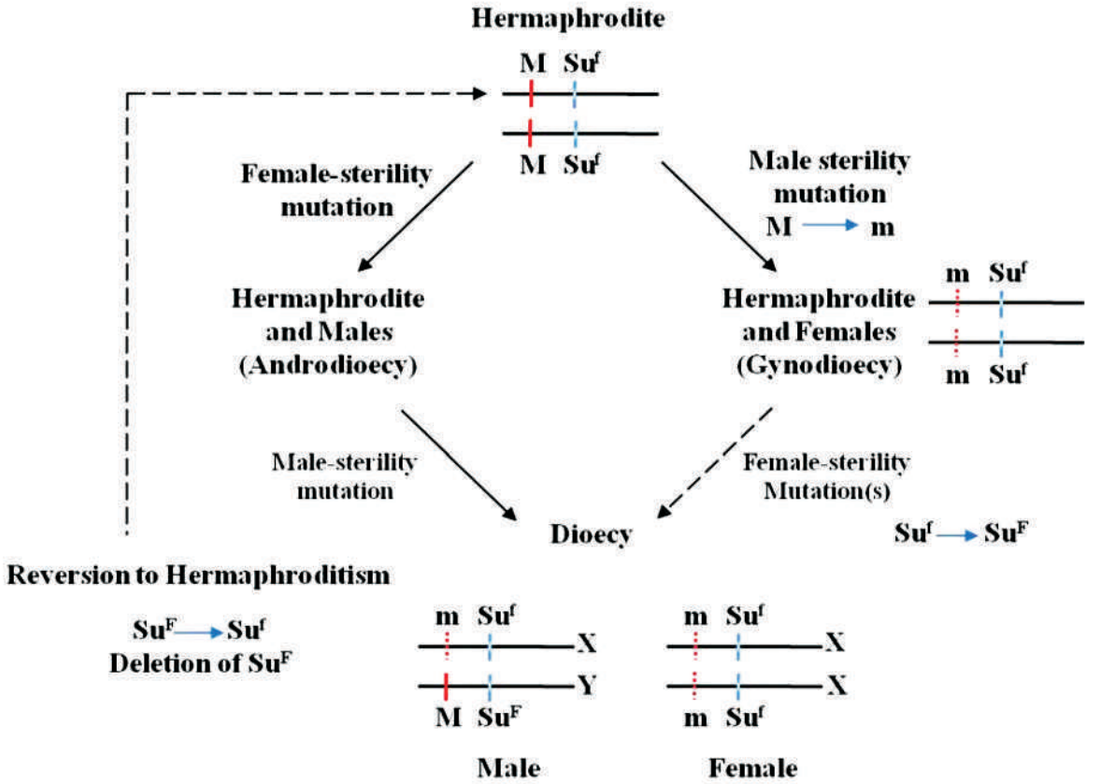
The first unified step for the evolution of unisexuality is the occurrence of sterility mutations on autosomes of hermaphrodite ancestor. Two mutation events are essential for the establishment of dioecy: one that aborts the male function (anther sterility/forming females) and second, that aborts the female function (female sterility/forming males). This is known as 'two mutation model' for the evolution of dioecy and hence the sex chromosomes (Charlesworth B & Charlesworth D 1978). This model provides a most plausible and best supported explanation till date for the evolution of dioecy through different pathways along with stable genetic sex determination mechanism i.e. sex chromosomes (Ming *et al*. 2011, Charlesworth 2013). This model proposes that the sex chromosomes in plants can evolve only when the two sex determining sterility mutations are closely linked on the same chromosome and have complementary dominance (Westergaard 1958, Charlesworth B & Charlesworth D 1978, Bachtrog 2006, Ming *et al*. 2011). In addition to these linked mutations, there are some modifier genes at these loci, which are sex-limited in their expression but makes cosexes more male-like and simultaneously reduce female fertility or may convert female into neuter (Fig. 1) (Charlesworth 2002, Charlesworth 2013). The linkage that appear in male sterility genes and in some modifier genes generally evolve/appear as a cluster. The linkage between these unlinked loci may be created by inversions and/or translocations (Lewis 1942, Charlesworth 2002, Charlesworth 2013). This cluster possesses beneficial effects for the evolution of complete dioecy and for the coalition of proto-X and proto-Y chromosomes (Charlesworth B & Charlesworth D 1978, Ming *et al*. 2011).

Most of the random mutations are deleterious and result in loss-of-function (recessives), while only few mutations lead to gain-of-function and are represented in dominants (Charlesworth 1991, Ming *et al.* 2011). Generally, male sterile mutations are loss-of-function (M to m, recessive) and female sterility mutations are gain-of-function (Su^r to Su^r, Dominant) (Fig. 1A). These mutations have different probabilities to get fixed in a self fertilizing cosexual population. This could be explained easily as that the male sterile mutations (and formation of new females) provide a high reproductive assurance through greater seed set (cross) in a population (gynodioecious pathway; Charlesworth B & Charlesworth D 1978, Charlesworth D & Charlesworth B 1978) while female sterility mutations (and formation of new males, androdioecy pathway) may lead to extinction of species. The above proposal is also true and supportive in explaining the greater preponderance of gynodioecy than androdioecy among the flowering plants.

Consequently with recessive mutation (loss-of-function; M to m), the phenotype would appear only in the homozygous form (on autosome pair) and possibly at low frequency in a population. This results in hermaphrodites and some females (Fig. 1). At the later stages of evolution, female sterility mutations in cosexuals would be advantageous in a gynodioecious population. The reason being the presence of females imposes a selection pressure on hermaphrodites for high pollen output and reduces their female function. These complementary mutations on a chromosomes pair results in a proto-sex chromosomes. At this stage, the proto-X chromosome would carry genes for female fertility and male sterility while proto-Y chromosome, the genes for female sterility and male fertility (Fig. 1A and B, Charlesworth 1991, Charlesworth 2002).

In another condition, if the two recessive mutations for female sterility appear on two different chromosomes, it would result in similar frequencies of the female plants. This is mainly because the regulatory genes for floral whorls are randomly distributed in the genome (Wellmer *et al*. 2004). On the other hand, if second recessive mutation is located on same the chromosome and becomes homozygous, such plants would be neuters (Charlesworth *et al.* 2005, Ming *et al.* 2011) (Fig.1B). Considerations of above conditions explains the necessity of first requisition for evolution of sex chromosomes i.e. the presence of closely linked, two sex determining sterility mutations on the same

A



B

	Genotype at first locus	
	M/M or M/m	**m/m**
Genotype at modifier locus		
Su^f/Su^f	Hermaphrodite	Female
Su^FSu^f or Su^F/Su^F	Male	Neuter
Effects of Change		
On fertility	Hermaphrodite/male fertility Increases	Female fertility decreases
On fitness	Increases	Decreases

Fig. 1— Transition to dioecy from a hermaphrodite progenitor with sterility mutations and modifiers. **A**. Diagram showing the required minimum requirement of two sterility mutations for a co-sexual plant to evolve into a population with unisexual males and females. The male-sterility mutation is shown by ***M→m***.Gynoecium-suppressing mutation is indicated by $f \rightarrow Su^F$. **B.** Effects of a female-sterility 'modifier' allele on hermaphrodite and female phenotypes; and fitness due to trade-off between male and female function (adapted from Charlesworth 2002, Charlesworth 2013).

chromosome. In a different condition, if a second gain-of-function dominant mutation occurs on a different chromosome in heterozygous condition, it would produce male:female/hermaphrodite in 3:1 ratio. If the second gain-of-function dominant mutation is homozygous, only males would be formed which drives the populations towards extinction (Fig. 1B). Thus, second female sterility mutation (gain-of-function dominant mutation) causing carpel sterility, must occur on the same chromosome, to establish dioecy (Charlesworth B & Charlesworth D 1978) and set the stage for evolution of sex chromosomes (XY system, Fig. 1A and B). The sex chromosomes evolved in plant species by the above mutation pattern (through gynodioecy), are male heterogametic *i.e.* with XY system. The dioecious plant species evolved through androdioecious pathway are female heterogametic *i.e.* 'ZW' system (Ming *et al.* 2011) where the mutation pattern and complementary dominance is reverse to the case of gynodioecy.

The mechanism which maintains the linkage at the sex determining loci is the suppression of recombination between the proto-X and proto-Y chromosome. This idea also explains the lack of recombination between X and Y chromosomes (Nei 1969). If the mechanism for the establishment of complementary dominance between the two kinds of sterility genes and chromosomal rearrangements is lacking, then indeed the intermediate sex phenotypes (subdioecious) will be present in the population (Charlesworth B & Charlesworth D 1978, Charlesworth 2002). Such a population exhibits the intergradations between males and cosexuals, with probable loss of male fertility. Thus, it seems that the lack of recombination between proto-X and proto-Y chromosomes has probably evolved to prevent formation of maladaptive phenotypes/hermaphroditic/neuter plants (Charlesworth & Guttman 1999, Charlesworth 2002, Charlesworth *et al*. 2005, Ming *et al.* 2011).

Suppression of recombination in the specific chromosomal regions is a well-studied phenomenon in animals and humans but least understood in plants. Generally, recombination is suppressed by chromosomal rearrangements such as deletions, inversions, translocations and duplications. For example, two pericentric inversions on human chromosomes 1 and 8 results in suppressed recombination (Jaarola *et al*. 1998). DNA methylation is another epigenetic mechanism that is reported to suppress of recombination in a fungus, *Ascobolus immerses* (Maloisel & Rossignol 1998). Lack of recombination may lead to genetic degeneration of heterozygous sex chromosomes (Charlesworth & Charlesworth 2000), which is an important feature of the evolution of morphologically distinct sex chromosomes. In XY system, X chromosomes in females recombine normally while suppression of recombination and DNA sequence degeneration occurs on Y chromosome. Suppression of recombination spreads in the male-specific region (MSY) or the female-specific regions (FSW, in ZW system). In the suppressed male-specific region of Y chromosome, multiple genes may be specialized to take up the male-specific functions. For example, in *Silene latifolia* (White Champion) maleness is controlled by three dispersed loci: two are present on one arm controlling carpel suppression and early stamen promotion and the third on another arm that controls late anther fertility (Lebel-Hardenack *et al*. 2002).

Several evolutionary factors for Y chromosome degeneration have been proposed in plant systems. These are: (1) After the suppression of recombination at sex determining loci, the proto-Y chromosome gradually accumulates deleterious mutations, primarily by 'Muller's ratchet' (Muller 1964, Felsenstein 1974) *i.e.* through random mutations, insertion of transposable elements and chromosomal rearrangements that disrupt the functions without a mechanism to repair or replace the loss-of-function mutations. (2) 'Hitchhiking', a genetic process which selects the favourable mutations along with linked deleterious mutations because there is no recombination in this region (Rice 1987). (3) The other factors include the background selection, which accelerate the fixation of weakly deleterious mutations (Charlesworth 1994); Hill-Robertson effect, which hinders the spread of favourable alleles and elimination of deleterious alleles under selection (McVean & Charlesworth 2000). (4) Another mechanism is the lack of adaptation on the non-recombining Y chromosome which assumes that even positive selection for the beneficial mutations is less beneficial on Y (Peck 1994,

Bachtrog 2006). Accumulation of deleterious mutations and lack of recombination in MSY region may cause Y chromosome to degenerate in both size and gene content (Charlesworth & Charlesworth 2000). This degeneration of Y chromosome content and accumulation of mutations leads to precocious separation of Y with its homolog (X chromosome) (McVean & Charlesworth 2000, Bachtrog 2006).

Sex chromosomes evolution in different plant systems: stages and features-In an earlier model, evolution of sex chromosomes was grouped into three classes (Westergaard 1958) which represented the different evolutionary stages. According to this hypothesis, the earlier stages of sex chromosomes are characterized by the viability of YY genotype and Y differs from X only in sex determining loci. This condition exists in *Asparagus officinalis*. The second stage is represented by papaya and *Silene latifolia* where YY genotype is non-viable and the Y chromosome plays a vital role in sex determination. At the third stage, Y chromosome is completely non-functional and X:autosome ratio determines the sex e.g. in *Rumex*. However, the recent knowledge gathered form extensive genetic and molecular studies have redefined the model (Charlesworth *et al*. 2005, Jamilena *et al*. 2008) and now six evolutionary stages of sex chromosomes have been recognized (Fig. 2 , Ming *et al.* 2011).

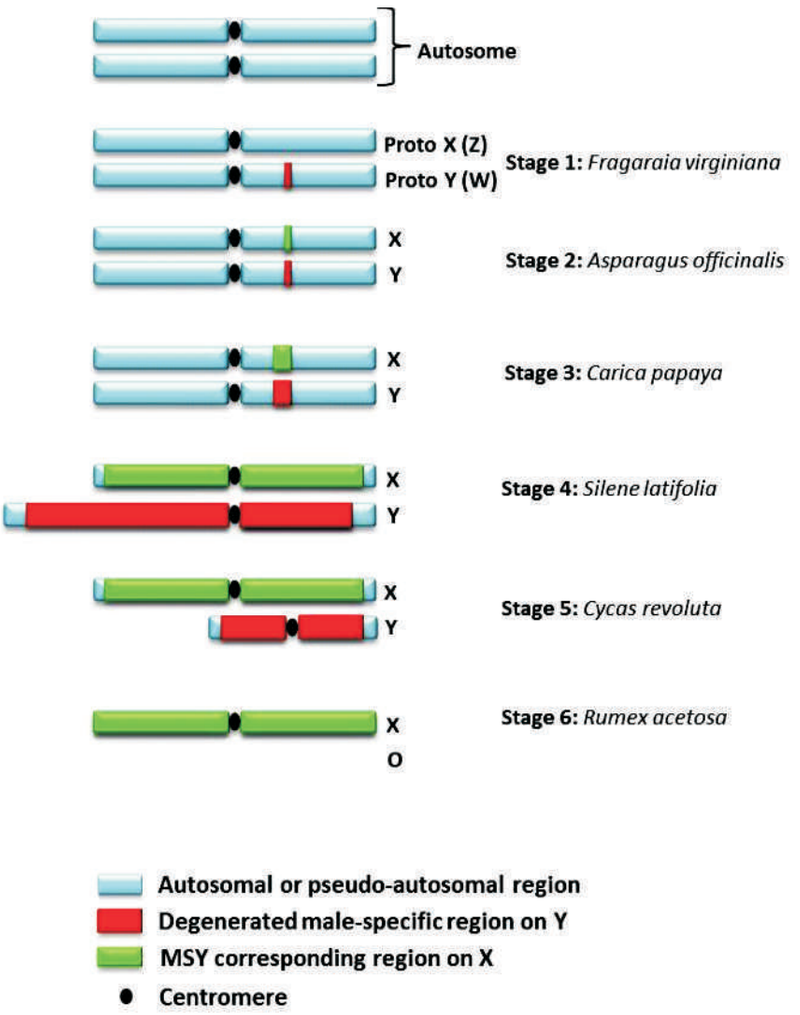


Fig. 2-- Stages of sex chromosome evolution in the plants (Adapted from Ming *et al.* 2011).

Stage 1: At this the male and female sterility mutations with complementary dominance appear on a chromosome, in close proximity (Fig. 2). Recombination between the loci is not suppressed and the hermaphrodite and neuter plants co-occur in the population. *Fragaria virginiana* is a well-studied taxon representing this stage. The sex chromosomes of *F. virginiana* represents the ZW system, where the males are homogametic ZZ and the females heterogametic ZW.

Stage 2: Suppression of recombination is present between the two sex determining loci and in immediate neighboring regions (Fig. 2). The YY genotype is viable. As seen in, *Asparagus officinalis*, the hermaphrodites and male plants present in population while sterile plants are also seldom present in populations (Marks 1973). A small MSY region also appears.

Stage 3: The suppression is extended to the neighboring regions and allows a Y-linked gene to degenerate. This creates a nascent Y chromosome with a male-specific region. The region starts expanding owing to the accumulation of retrotransposons and chromosomal rearrangements. Here, the YY genotype is lethal. Papaya sex chromosomes are known to occur at this stage. At the cytological level they are homomorphic but functionally they are heteromorphic (Fig. 2, Charlesworth & Charlesworth 2000). Papaya is a commercial crop and exhibits trioecious system thus it has received a worldwide attention for the development of gender-linked markers and characterization of genes. Now it is evident that sex determination in the species is controlled by a recently evolved XY chromosome pair, with slightly different two Y chromosomes controlling the development of males (Y) and hermaphrodites (Y^h). The hermaphrodite-specific region of the Y^h chromosome differs from the X counterpart by two large-scale inversions and numerous additional chromosomal rearrangements (Wang *et al*. 2012).

Stage 4: The male-specific region expands onto the majority of the Y chromosome and further degeneration occurs. There is considerable expansion in size and DNA content of Y chromosome due to the accumulation of transposable elements and duplications. At cytological level sex chromosomes are heteromorphic (Fig. 2). For example, sex chromosomes in *Silene latifiolia* are of this type (Delph *et al*. 2010). Many functional Y-linked genes have been isolated, which provide a clue that the degeneration of Y-linked genes is at a very early stage and the Y chromosome appears euchromatic. Though, Y chromosome of the species does not recombine with X chromosome. Another case may be *Cannabis sativa* where heteromorphic XY system with larger Y has been characterized recently (Divashuk *et al*. 2014).

Stage 5 : Extensive degeneration and loss-of-function in most of the genes located on Y chromosome causes its shrinkage. In angiosperms, sex chromosomes at this stage are not characterized yet (Ming *et al.* 2011) but are known in a gymnosperm, *Cycas revoluta,* where the chromosomes are heteromorphic with a smaller Y chromosome (Fig. 2).

Stage 6: Due to the spread of suppression of recombination, complete degeneration of Y chromosome occurs and the Y chromosome is totally absent (Fig. 2). Thus, a new sex determination system evolves *i.e.* X to autosome ratio. In flowering plants such a stage is represented by *Rumex* spp.

Hormonal control of sex determination—As early as 1937, Chailakhyan suggested that phytohormones could be involved in transition from vegetative to reproductive phase (flowering) and called them sex hormones. The early experimental explanation to the role of phytohormones appeared in literature during 1960s' on the studies carried out on hemp, spinach and cucurbits. It was shown that in Hemp plants, exogenous application of auxins and ethrel (2-chloroethyl-phosphonic acid, source of ethylene) enhances femaleness (Heslop-Harrison 1957, Mohan Ram & Jaiswal 1970 respectively). Similar treatments of hemp plants with gibberellins increased maleness (Atal 1959). Likewise, *in vitro* application of cytokinins on young hemp and spinach plants that were deprived of their roots increased the number of female plants (Chailakhyan & Khryanin, 1977, 1978). In *Mercurialis annua* (Mercury plant), cytokinins and auxins were correlated with conversion of male into females and females into males, respectively (Durand 1967). The other evidences for hormonal regulation of gender determination are from *Kalanchoe crenulata* (Catarino 1964) and *Vitis vinifera* (Negi & Olmo, 1966, 1972), where the exogenous applications of cytokinins led to the suppression of stamens and enlargement of ovary and feminization of

males, respectively. Now, due to a large number of evidences, gibberellins are considered as masculine hormone and ethylene as feminizing hormone (Aryal & Ming 2014). Auxins and cytokinins are thought to interactively regulate sex expression in plants (Irish & Nelson, 1989).

Although various hormones are known to induce alteration in sex expression, reversion by the application of some other hormones is also possible. This has also been shown in hemp plants; the effect of gibberellins can be inhibited by the simultaneous treatment of abscisic acid, though abscisic acid itself does not change the sex (Mohan Ram & Jaiswal 1972). Papaya plants treated with GA_3 inhibitor, chlorflurenol, showed carpel development on male plants, which suggests that gibberellins play a role in maintaining the maleness (Kumar & Jaiswal 1984).

The effect of hormones on sex expression may not be uniform in all the dioecious plant species. For instance, *Rumex acetosella* (Sorrel) does not show any noticeable effect on sex expression after treatment with cytokinins and gibberellic acid (Bavrina *et al*. 1991). In other condition the effect of a hormone might be opposite to other species e.g. gibberellic acid increases femaleness in maize while increases maleness in cucumber. Moreover, the stage at which the hormones induce their effect is a chief determinant for sex determination in plants. At too early stages or too late stages of floral development, the phytohormones fail to regulate sex expression. For example, in *Silene noctiflora*, the gibberellins and cytokinins were effective only when plants were treated before the onset of sex differentiation in floral meristems (Folke & Delph 1997, Khryanin 2002).

Sex reversal by application of hormones and their inhibitor in unisexual plants suggests that the required genetic machinery for the development of stamens or pistil is available in such plants but it is somehow suppressed (Irish & Nelson 1989). Various genes and genetic mechanisms have been identified which primarily supports the above mentioned surmise. Among the monoecious and dioecious systems such as maize, cucurbits, grape etc. sex determination is well-understood. There are several mutants that have been developed in maize e.g. *dwarfs* and *tassel seeds*. The biochemical, physiological and genetic analysis of these mutants suggests that there is an active suppression of organs of the inappropriate sex in the ears and tassels due to endogenous gibberellins and there are epistatic interactions of involved genes (Irish & Nelson, 1989). In cucurbits, different levels (exogenous application or endogenous biosynthesis) of ethylene were associated with sex determination and several *ACS* genes responsible for endogenous biosynthesis have been identified and correlated with sex reversal/change. The *ACS* codes for an enzyme, 1-aminocyclopropane-1-carboxylate synthase (ACS), which is crucial for ethylene biosynthesis (Yamasaki *et al*. 2003, Papadopoulou *et al*. 2005). In andromonoecious *Cucumis melo* mutants (named as *ACS* melon), the constitutive expression of *ACS* gene (high endogenous levels of ethylene production) was correlated with the increased production of female flowers and bisexual flowers (Papadopoulou *et al*. 2005). Later genetic studies on *ACS* genes from melon characterized and named it *CmACS-7*. The coding *CmACS-7* gene suppresses the androecium development on female plants while the gynoecium development is suppressed by *CmWIP1* (Baualem *et al*. 2008). Likewise in *Vitis vinifera,* sex determination is believed to be regulated by a gene with separate alleles for male, female and hermaphrodite. This gene codes for adenine phosphor-ribosyl-transferase, a key enzyme of cytokinin metabolic pathway.

Epigenetic regulation of unisexuality—In recent years, new evidences for the regulation of sex determination are reported from epigenetic studies. Epigenetics deals with the study of heritable changes in gene function without any change in the DNA sequence. In this context, the epigenetic regulation of sex determination may be defined as the difference in sexual phenotypes of flowers but without changing the genetic makeup of the loci that are directly related to the development of sex organs (Aryal & Ming 2014). The major epigenetic regulation mechanisms are mediated by small RNAs, histone modification and DNA methylation (Brock & Fisher 2005).

It has also been proposed that 'Muller's ratchet' is driven by differential methylation in nuclear DNA between females and males, so to cause initial

differences between sexes and helps in Y chromosome formation. Methylation of promoters of loci coding for gamete production, leads to conversion of hermaphroditic plants into females or males (Gorelick 2003). The epigenetic regulation of temperature-dependent vernalization in plants (Wakimoto 1998, Brock & Fisher 2005) also suggests that plants might respond to environmental factors through small RNAs or DNA modifications. Although direct evidences of epigenetic regulation of sex determinationare already available in animals e.g. X-chromosome inactivation in mammals and positional-effect variegation (Wakimoto 1998, Brock & Fisher 2005), conclusive studies are lacking in plants. Nevertheless, some examples/studies highlighted below indicate that sex determination in plants could also be under the epigenetic regulation along with genetic regulation.

The role of non-coding RNAs in sex determination has been shown in maize by Chuck *et al*. (2007). The sex determination occurs through abortion of primordia of opposite sex in tassel and ear. A gene named *INDETERMINATE SPIKELET1* (*IDS1*), is a member of the APETALA2 floral homeotic transcription factor family, is responsible for spikelet meristem determinacy. Another gene *TASSELSEED4* (*TS4*) encodes micro RNA 172 (miR-172), which directs at *apetala2* and *ts4* mutations allows pistil development in the tassel. Thus, miR-172 is necessary to maintain maleness in maize. The study proposed that sex determination and acquisition of meristem fate share a common pathway and is certainly under epigenetic regulation. Recently, micro RNAs are proposed to be involved in the development of unisexual flowers in cucumber by selective abortion of carpel under environmental stress (Sun *et al*. 2010). Small RNA hotspots have also been characterized on incipient sex chromosome of *Populus trichocarpa* (Tuskan *et al*. 2012).

Another example of epigenetic regulation is from melon. In the promoter of *CmWIP1* gene, insertion of a transposon, Gyno-hAT leads to epigenetic changes and formation of pistillate flowers (Martin *et al*. 2009). Cytosine methylation is also necessary to maintain the unisexuality of male plants in dioecious *Silene latifolia*. Inhibition of DNA methylation causes reversal of male flowers to hermaphrodite (Janousek *et al*. 1996). Similarly, in *Elaeis guineenis* (Oil palm) the loss of DNA methylation leads to production of epimutant with aberrant sexual phenotypes (Jaligot *et al*. 2011).

Conclusion—Gender determination mechanisms provides evolutionary stability to the unisexual plants. The presented account suggests that all dioecious plants must be possessing sterility mutation/s, with a possibility of sex chromosomes at stage 1 or 2 (homomorphic) (Ming *et al.* 2011). The evidences are increasing and suggest that sex chromosomes are not rare among the plants as previously thought; they need to be investigated across the plant groups. The cytological and molecular investigations in other plants species and families, especially with polymorphic sexual system, might provide new insights to the sequence of evolutionary stages of sex chromosomes recognized so far. The process of hormonal and epigenetic regulation of sex determination is complex. The interference of environmental factors appears to make the regulation more complex. Once the mechanism is deciphered, it may be easy to link this type of regulation with sex chromosomes in plants. Moreover, phylogenetic approaches could highlight the need and importance of multiple sex determination mechanisms in plants.

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