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Mechanisms of Sex Determination in Plants

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Abstract: Mechanisms of sex determination in plants are popular research topic. Research studies are initiated to describe sex determination mechanisms and develop molecular methods for sex differentiation in plant species where sexual dimorphism is weakly expressed or absent at the early stages of development. This study presents different mechanisms of sex determination in plants, identifies unexplored areas and discusses problems which limit our knowledge of the subject.

Key words: sex determination, sexual dimorphism, dioecious plants

Introduction

Whereas sexual dimorphism is common in the animal kingdom, most plant species are bisexual by nature. Angiosperms have two types of reproductive structures: a plant may produce flowers containing both stamens and styles, alternatively, one individual can develop both male flowers (with stamina) and female flowers (with pistils). Only around 6% of angiosperms are dioecious, i.e. male flowers and female flowers grow on separate plants (Renner and Ricklefs 1995). It could seem that dioecy poses a barrier to reproduction, but in fact, it plays a very important evolutionary role. The exchange of genetic material between two individuals leads to new combinations of genes which enable a plant to easily adapt to a changing environment. Dioecy also prevents self-fertilization. Plants fertilized with foreign pollen produce healthier and more abundant offspring than individuals fertilized with own pollen (Malinowski 1978). This correlation is clearly visible in *Cannabis sativa* where dioecy contributes to greater yield and supports crossbreeding to produce offspring containing more valuable fiber (Mandolino et al. 1999).

How comes it to sex expression? What is the basis of this process? So far, there are a few known mechanisms of sex determination in plants. This paper is a review of this methods and also draws attention to what is still unexplored.

Development of reproductive organs in plants

There are no universal models supporting sex determination in plants. The results of studies into the development of sex organs already reveal a variety of hidden mechanisms. The discussed investigations analyzed the time and character of sexual development inhibition (Ainsworth 2000). The following trends are observed in the majority of plants: male and female organs are formed and developed simultaneously, but only up to a point when the growth of either set of sex organs is inhibited. The above is noted in *Melandrium album* (Grant et al. 1994), *Rumex acetosa* (Ainsworth et al. 1995) and *Pistacia vera* (Hormaza and Polito 1996). Species such as *Mercurialis annua* (Durand and Durand 1991), *Cannabis sativa* (Mohan Ram and Nath, 1964), *Spinacia oleracea* (Sherry et al. 1993) and taxa of the genus *Humulus* (Shephard 1999b) develop at the very beginning organs that are specific for the given sex. This mechanism points to an early divergence of paths that lead to sexual differentiation. The reverse is observed in *Actinidia deliciosa* (Schmid 1978) and *Asparagus officinalis* (Galli et al. 1993, Caporali et al. 1994) where sexual differentiation takes place very late, and in consequence, male and female flowers appear to be identical at first glance. In addition to differences in timing, the inhibition of sexual development can also vary in character. In most cases, sexual development is inhibited by the absence of cell division, for example in *Rumex acetosa* (Ainsworth et al. 1995) and *Melandrium album* (Farbos et al.

1997), or necrosis of sexual organ cells, which is noted in *Asparagus officinalis* (Caporali et. al. 1994) and *Actinidia deliciosa* (Harvey and Fraser 1988).

Molecular basis of sex determination

Despite a large body of research investigating different species, the molecular mechanisms of sex determination in plants remain relatively unknown. A number of generalized hypotheses have been proposed to explain this process.

It is generally believed that metabolic processes and the sexual determination pathway have genetic origins. Attempts are made to identify and estimate the number of genes which determine sex in various plant species. This is a highly complex task. An identified gene is often not the primary element in the sex determination mechanism (Ainsworth 2000), but only one of the many genes in the mechanism's cascade. Researchers often find it difficult to ascertain whether this intermediary gene is activated through the expression of the primary gene or through environmental factors. Frankel and Galun (1977) proposed the key gene theory to explain the sex determination mechanisms in plants. In 1991, the same theory was used by Truong et al. in reference to the animal kingdom. When triggered (gene activation), the key gene activates a cascade of other genes whose expression leads to the development of the respective sex organs. The above implies that the genes conditioning the development of male and female sex organs are present simultaneously in every plant, and those sets of loci are activated subject to the allelic architecture of the key gene. A single-gene mechanism controls sex determination in plants such as *Asparagus officinalis* (Gao et al. 2007), *Ecballium elaterium* (Ainsworth 2000), *Pistacia vera* (Hormaza 1994) and *Carica papaya* (Storey 1953). A multiple-loci system which is responsible for sex distinction in *Mercurialis annua* (Louis 1989) seems to defy the above hypothesis.

Sex chromosomes

Like other types of genetic information, sex-determining sequences are located on chromosomes. In plants, they could be sex chromosomes or autosomes. In animals, sex chromosomes are responsible for sex differentiation, but in plants, they are a relatively young evolutionary achievement. Sex chromosomes have been identified in selected plant species, and their existence is merely suspected in other taxa. The expression of sex chromosomes seems to be biologically justified. It supports the accumulation of sequences whose nucleotide arrangement should not be modified in a single location. In general, the closer the genetic distances, the lower the recombination frequency. A stable structure preserves the existing expression and sequence function, which is of utmost importance in sex determination.

The identification of sex chromosomes in plants is problematic because most of them do not differ morphologically from autosomes or from one another (*Spinacia oleracea*, *Asparagus officinalis*) (Michalik 2009). In some species, such as *Actinidia deliciosa var. deliciosa* (Shirkot et al. 2002), X and Y chromosomes are too small to support observations of their distinguishing characteristics. Heteromorphic sex chromosomes have been discovered in *Rumex acetosa* (Kihara and Ono 1923), *Melandrium album, Melandrium rubrum* (Blackburn 1923), three species of the family *Cannabidaceae,* i.e. *Humulus lupulus, Humulus japonicus* and *Cannabis sativa* (Shephard et al. 1999a), as well as in *Rumex nivalis* (Stehlik and Barrett 2005) and three liverwort species: *Sphaerocarpos donnellii, Sphaerocarpos texanus* (Allen 1917) and *Marchantia polymorpha* (Bischler 1986). An occurrence of heteromorphic sex chromosomes is also suspected in mosses of the genus *Macromitrium* (Ramsay 1966).

Sex chromosomes have been observed in a relatively small group of plants, therefore the presence of a diverse chromosomal mechanism of sex differentiation may be surprising. In most cases, it is identical to the mechanism found in animals which relies on the presence of the Y chromosome and its characteristic sequences which act as a maleness enhancer and suppress the development of female sex organs. The above mechanism is present in *Melandrium album*, *Asparagus officinalis* and *Spinacia oleracea*. In some plant species, the sex determination mechanism is independent of the Y chromosome. The ratio of the number of X chromosomes and autosomes is an important bit of information. The above mechanism is responsible for sex determination in *Rumex acetosa* (Ainsworth 2000), *Humulus lupulus* (Shephard 1999a) and *Phoenix dactylifera* (Siljak-Yakovlev et al. 1996). It could seem that the Y chromosome is obsolete in the discussed type of plants, nevertheless, it is present in those species where is plays various roles. Firstly, the Y chromosome is needed for meiotic division (Parker and Clark 1991), and secondly, it contributes to pollen development, for example in *Humulus lupulus* (Parker and Clark 1991). Sorrel and hops have two types of chromosomes Y: XY_1Y_2 (male) and XX (female) (Dellaporta and Calderon-Urrea 1993). In some plant species, such as *Rumex hestatulus* (Parker and Clark 1991), sex is determined by both mechanisms: an active Y chromosome and the ratio of X chromosomes to autosomes. The above is observed in the American *Rumex hestatulus* (Parker and Clark 1991). The presence of an active Y chromosome with a typically female proportion of X chromosomes to autosomes (> 0.5 and < 1.0) will produce a plant with both male and female organs (Smith 1963).

In diploid species with a chromosomal mechanism of sex differentiation, males are generally heterogametic (XY) and females are homogametic (XX). A rare sex determination mechanism is observed in polyploid, dioecious species of the genus *Fragaria* where female individuals are heterogametic (Dellaporta and Calderon-Urrea 1993). A similar mechanism is suspected in *Myristica fragrans* (Flach 1966). Sex determination is much simpler in haploids where males and females have completely different sex chromosomes: Y chromosomes in males and X chromosomes in females (e.g. *Marchantia polymorpha*).

Labile sex

Next to the stable, genetic mechanism of sex determination, some plants possess labile systems. In the latter case, the environmental factors determine a fixed sex expression with switching on specific genes (Korpelainen 1998). Sexual lability is encountered in various plant taxa, but ferns are the only group of plants where the above phenomenon is a rule (Korpelainen 1998). In ferns, sex is determined by a gametophyte's age (older gametophytes produced by rapidly germinating spores are hermaphroditic, while younger gametophytes are male) and size (taller gametophytes are capable of lifting up a zygote and a developing sporophyte), as well as the secretion of antheridiogen, a gibberellin-like substance characteristic of ferns. Male individuals are produced when, at an early stage of development (1-4 cells), a gametophyte is exposed to the above compound secreted by mature hermaphroditic gametophytes (Korpelainen 1998). The antheridiogen-controlled mechanism of sex determination has been described on the example of *Ceratopteris richardii* (Tanurdzic and Banks 2004). Biotic factors, such as population density, also affect sex expression in the above species. Dense populations have a preference for male individuals which contribute to the exchange of genetic material, whereas sparse populations produce hermaphrodites to boost fertilization (Tanurdzic and Banks 2004).

In plants, sex can be modified by external factors, mostly abiotic, as well as physiological factors (phytohormones) and, in infrequent cases, biotic elements. Those factors disturb physiological processes, leading to potential changes in the expression of sex determination genes. Environmental stressors such as drought, low temperature, less than optimal light, low nutrition, less than optimal pH and nitrogen-deficient soils favor maleness in spermatophytes (Korpelainen 1998). The reverse probably applies in bryophytes where males seem to be more susceptible to environmental stressors (Longton 1985, 1988; Cameron and Wyatt 1990; Shaw et al. 1991; Bisang and Hedenäs 2005). The results of scarce research into the determination and expression of sex in bryophytes suggest that abiotic factors are most likely to influence sex determination. There exists modest but highly convincing evidence that physiological factors affect sex expression in bryophytes. Under experimental conditions, auxins and gibberellins in *Bryum argenteum* favored maleness, whereas cytokines showed a clear preference for females (Korpelainen 1998).

In an overview of sex determining factors in plants, Korpelainen (1998) cites experiments where different species were treated with exogenous phytohormones to show that chemical treatment induces sex change in animals. Conversions to hermaphrodites were rarely observed. Korpelainen's observations corroborate the findings of Dellaport and Calderon-Urrea (1993) who demonstrated that the above plants are sexually bipotent, and that genes determine sex expression through hormone control. In woody plants, sex transformation is only partial, alternatively, the process may involve a monoecious form (Matsui et al. 1995; Ushimaru and Matsui 2001; Nanami et al. 2004). *Ilex integra*, a dioecious tree native to East Asia, seems to be an extraordinary plant in view of the above findings. Complete sex change, both from female to male and male to female, is observed in adult individuals of the above species. It remains unknown whether the noted changes are reversible (Takagi and Togashi 2012).

External factors seem to have no effect on the chromosomal mechanism of sex differentiation in the dioecious *Rumex acetosa* and its relative, *Rumex acetosella*. No changes in sex were noted when the above taxa were treated with phytohormones (Culafic 1999). Similar observations were made with regard to *Melandrium album* (Ye et al. 1991). In *Melandrium rubrum*, the stability of sex expression may be disrupted by yeast infections caused by (Audran and Batcho 1981).

Sexual lability could be a manifestation of a plant's inability to preserve its genetically coded sex in a disturbed environment (Korpelainen 1998) or it could be an adaptation mechanism that supports survival in a new habitat (Charnov and Bull 1977). The fact that the majority of plants characterized by labile sexual expression were perennial plants supports the latter observation (Korpelainen 1998). In wooded plants, sex change is also influenced by reproductive strategy (Takagi and Togashi 2012).

Epigenetic inheritance

Since sexual lability serves a specific purpose, it should not be surprising that changes in the expression of sex-linked genes are not heritable. Gene expression in the next generation will once again be determined by external factors. The above could give rise to epigenetic inheritance, a very interesting phenomenon which is based on inheritance unrelated to changes in the DNA sequence (Wierzbicki 2004). Chromatin structure is the carrier of information. Changes in chromatin affect gene expression and may be heritable. Chromatin modifications induced by DNA and histone methylation are exceptionally stable, and may be passed onto the following generations.

Epigenetic inheritance is also responsible for sex determination and sex inheritance in plants. The above has been demonstrated by a study of *Melandrium album*, a plant which changes its sex from male to androhermaphrodite when treated with the nucleoside analog of 5-azacytidine (5-azaC). The pollination of wild females with androhermaphrodite pollen leads to the re-appearance of androhermaphroditic forms in this completely dioecious species. The above phenomenon is undoubtedly related to 5-azaC-induced hypomethylation of DNA. The most plausible theories explaining the above process include the inhibition of Y-lined female-suppressing genes or the activation of autosomal female-promoting genes whose expression, due to genomic imprinting, could be passed onto the next generation (Janoušek et al. 1996). The exact mechanism by which hypomethylation induces sex change in *Melandrium album* remains unknown. A reverse process is responsible for the sex change in melons.

The hypermethylation of the promoter of the *CmWIP1* gene responsible for pistil growth inhibits gene expression. The insertion of transposons is a direct cause of epigenetic change in the promoter region (Martin et al. 2009). Transposons are, however, strongly methylated (Wierzbicki 2004; Slotkin et al. 2007; Weil & Martienssen 2008).

Inherited chromatin states are far less stable in comparison with changes in DNA sequences, but the above makes them reversible. The probability of reversal ranges from three percent in selected plant genes to several dozen percent in mammals (Jacobsen & Mayerowitz 1997; Rakyan et al. 2002). In view of the stability of changes, it could be said that sex changes induced by epigenetic mechanisms are an intermediary process between the chromosomal mechanism and sexual lability which is stimulated by external factors.

Conclusion

The exact mechanism of sex determination in plants, despite the numerous research in this direction, there is still a mystery. Currently, an important signpost on the road to the exploration of these mechanisms seem to be molecular sex-linked markers. Although these markers alone do not explain the molecular basis of sex determination in dioecious plants, but their number, structure of sequence or sequence homology between the typical, male and female sequences represent a foothold in the study of the mechanisms of sex determination. The work on the development of sex-linked markers still enjoy a great popularity.

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