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Sex Linked Molecular Marker Gene in Medicinal Plants - Review

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ABSTRACT

Plants have been used as a medicinal plant since humans have existed. Paleontologists have found the remains of medicinal plants such as Opium poppies, Cannabis, and Ephedra from around 60,000 years ago. Medicinal properties are attributed due to the bioactive compounds obtained from the plant parts whether it is root, bark, shoot, seeds, flower, berry or fruits. The metabolites which are obtained from the reproductive tissues or parts of female plants like flower, fruits and seeds are discussed in this review. To avoid the resources being wasted on nurturing the male plants, it would be beneficial if one can detect and discard the male plants at seedling stage. Many plants species are sexually dimorphic which can be seen by naked eyes. However, determination of sex of a plant is quite difficult at early stage i.e. before flowering. There has been a huge up-rise in plant biotechnology and breeding for the development of molecular genetic data. Development of sex linked molecular marker genes in plants is a great discovery and have been useful in agriculture industries, nurseries etc. Sex linked molecular marker genes are used to identify whether the plant is male or female. The male plants are used for pollination and female plants bear fruits, so if it can be determined that whether the plant is male or female at the seedling stage then it will be helpful in saving our cost, time, and labor. Only one male plant can be used to pollinate many female plants. Our review paper focuses on some sex-linked molecular marker genes in medicinal important plants which have been identified and are employed in determination of sex at early stages of the plant development.

1. Introduction

A molecular marker is a molecule contained within a sample taken from an organism which can be used to study different properties of an organism. Sex discriminating molecular marker genes is used to know the sex of an individual plant. Male plants are used for pollination and female plants bear fruits. In animals it is easy to identify whether it is male or female with naked eyes but in plants it becomes difficult at the previous stage of development before flowering.

Sex determination becomes more complicated in bryophytes where sex determination of an adult individual is difficult or even impossible. In case of papaya and many other plants, female plants bear fruits that have certain medicinal properties. In such cases sex linked molecular marker genes play a crucial role in identification of plants on the basis of their sex. Identification of various plants, for example papaya, date palm, etc. at an early stage is economically useful as it enhances the profit of seed-based cultivation. The individual in the population of sexually reproduced plant species have some variations in their genomic DNA which is caused by mutation, duplication etc. Such variation can be detected or screened by genomic marker that can be a gene/ particular sequence etc. (Maria et al., 2018).

In general, molecular marker genes are genetics loci that can beeasily detected and used to identify many things, for examples

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sex of individual plant and its properties etc. There has been an evolution in sex determination system of plants and sex chromosomes many times. The gene recombination is rare between the different loci and may be there as on for genetic degeneration of chromosome (Kundapura *et al.,* 2015). There are many plant species who see sex linked molecular marker genes were developed. After identification of sex-linked molecular marker gene in some plants like papaya, date palm, etc. it becomes easy to identify plants on basis of their sex. It will reduce cost; labor and time as we know only one male plant can be used for mating with many female plants which then produce fruits with some medicinal properties. The molecular marker genes are used in nursery and in many other places. So this useful technique of identification of sex linked molecular marker gene in plants will be widely used in many cases. There are many techniques which are used to identify marker genes for example ISSR, RAPD, ALFP, and PCR. These are easy and quick methods. These methods are used to identify sex of some plants for example date palm, papaya etc. Date palm is a long and viable plant which is cultivated in dry zone for food, shelter and for earnings. Almost 60% of world's production of date palm is held in Arabic countries with approx. 800 different kinds (V.S.Srivashtav *et al.,* 2013, S.S. Adawy ey al., 2014, El-Juhany, 2004, Al-Abdoulhadi et al., 2011).

Papaya, a family of *caricaceae,* is first cultivated in America. It is most important and widely consumed fruit with medicinal properties in India and its neighboring countries. Papaya is edible fruit which yields a proteolytic enzyme called papain which has many values. Papaya is very useful in medical applications like it aids digestion, helps in reducing swelling, used in the treatment of fever and ulcers (Aravind *et al.,* 2013). The Papaya has three main sex types that are male, female and hermaphrodite. Dioecious is associated with sexual dimorphism, however it is difficult to determine the sex of an individual plant at seedling stage. In the case of bryophytes, sex determination becomes difficult or even impossible. Sometimes biochemical and cytological analyses cannot determine the sex of an individual plant. Here, molecular tools are helpful. A molecular marker (DNA marker) is a DNA sequence which can easily distinguish between male and female plants. After the development of molecular marker gene, it becomes easy to determine the sex of an individual plant for example genetic molecular markers (GMM) are developed for coding sequences EST and are derived from complementary DNA. It can also be developed from characterized genes.

1.1. Sex Linked Molecular Marker Gene in Some Medicinal Plants

Hippophae, is a medicinal plant and its three species (*Hippophae rhamnoides, Hippophae salicifolia, Hippophae tibetana)* are found in India (Dwivedi S. *et al.,* 2009). These species have many medicinal properties as they contain polyunsaturated fatty acids like omega 3, omega 6 etc (Gupta S.M. *et al*., 2011) *Hippophae salicifolia* have highest amount of vitamin C as compared to other species of sea buckthorn (Gupta and Ahmed, 2010). The berries of *Hippophae salicifolia* are used to make wine in India (Himachal Pradesh). Many nutraceutical companies also use the berries of this plant. These species are found in other parts of India like Ladakh, Uttrakhand, etc (Dwivedi S. et al., 2009). The mode of propagation of these plants are woodcutting, seeds etc (Singh V *et al.,* 2008). The problem arises is that male and female plants looks similar till the time of flowering. They cannot be discriminated on the basis of their sex (Gupta S.M *et al.*, 2012). Only 10 percent of male plants are required for pollination (Dwivedi and Ahmed, 2006). So, sex determination at an early stage is important from commercial as well as research point of view as it will save time, labour, and money. There are various techniques for example RAPD, SSR, SCAR which are used to differentiate the plants on the basis of their sex (Jain *et al*., 2010, Korekar *et al.*, 2012, Sharma *et al*., 2010). Only ten genotypes were used as in the report of (Rana *et al*., 2009) based on sex linked molecular marker genes in *Hippophae tibetana*. Sex linked SCAR markers that is HrX1 and HrX2 were tested on *Hippophae salicifolia* and *Hippophae tibetana* (Korekar *et al*., 2012).

Table 1. *Medicinal Plants With Molecular Markers*

Plant Species	Methods	Determined Sex And Number of Discovered Markers	Medicinal Property of Plants	Reference
Actinidia Chinensis	RAPD	1F, 1M	Reduce blood pressure, prevent heart diseases, and contain antioxidants.	Harley *et al*.,(1997) Parle *et al*.,(2016)
Actinidia deliciosa var deliciosa	RAPD	6F,2M	Along with antioxidant agents, it also has components which helps in reducing blood pressure and helps in preventing cardiovascular diseases.	Shirkot *et al*., (2002) (Mahammad
Asparagus officinalis	RAPD	2F,2M	It contains fibres, vitamin A, vitamin b, and vitamin c.	(Jiang and Sink, 1997) Iqbal *et al*.,(2017)

Encephalartos Natalensis	RAPD	1F	Have many medicinal properties.	(Prakash and Staden, 2006) S. R. Cousins *et al*., (2012)
Ginkgo biloba	RAPD- SCAR AFLP	1F,1M 3F,1M	It have antioxidants, use to cure heart diseases, reduce anxiety, depression and may be improve to use eye vision, use for treatment of Alzheimer diseases	Liao *et al*.,(2009) Oken *et al*.,(1998)
Pseudoallergen Trifolium	ISSR-SCAR	1F	Use to cure lymphoma diseases.	Korpelainen *et al*., (2008)
RumexNivalis	AFLP AFLP-SCAR	1M 1M	Use to treat Alzheimer diseases	(Stehlik and Blattner, 2004)
Trichosanthesdioica	RAPD	1F,1M	Use as antidiabetic and anticancer properties	Kumar *et al*.,(2008) (Maurya and Srivastava, 2011)
Simmondsiachinensis	RAPD	1M	Use to make herbal drugs	Agrawal *et al*., (2007) Kalpana *et al*., (2004)
Rumexacetosa	RFLP	1M	Use to treat breast cancer.	Ruiz *et al*., (1994) Heater *et al*., (2007)
Salix viminalis	RAPD SCAR	2F,	Use to make medicines	D. Hammer *et al*., (2006)

Table 2. *Medicinal plants with molecular markers and gender specificity*

Plant Species	Methods	Size Of Sex Specific Fragments(Bp)	Gender Specificity	Medicinal Properties	References
C.papaya	RAPD	1.7 kb	Male and Hermaphrodite	It is high in fibers and also contains high water content. Papaya also have antioxidant effect, anticancer effect and also help to cure heart diseases	Gunter *et al*., (2003)
		0.4 kb	Male and Hermaphrodite		Krishna *et al*., (2008)
	RAPD	2.18 kb	Female		E. Niroshini *et al*., (2008)
	RAPD	800 bp	Male and Hermaphrodite		Jaime *et al*., (2007)
	RAPD	800 bp	Male		A.S. Parasnis *et al*., (2000)
	RAPD	438 bp	Hermaphrodite		R. Ming *et al*., (2002)
	SCAR	800 bp	Male and Hermaphrodite		N. Urasaki *et al*., (2002)
	SCAR	450 bp	Male and Hermaphrodite		Kanupriya *et al*., (2014)
	SCAR	800 bp	Male in Dioecious and female in Gynodioecious		A.S. Parasnis *et al*., (1999) Fabiane *et al*., (2011)
	ISSR	5 kb	Male		Ali *et al*., (2018)
	ISSR	500 bp	Female and Hermaphroite		

As we can see from this table that molecular markers 1F and 1M is determined in Actinidia Chinensis with the help of RADP technique (Harvey *et al*., 1997), markers 6F and 2M are found in Actinidia deliciosa var deliciosa (Parle *et al*., 2016), Asparagos officinalis contain 2F and 2M markers and determined by RADP technique (Jiang and Sink, 1997). Many more molecular markers are determined by RAPD technique for example, Encephalartos Natalensis contain 1F marker (Prakash and Staden, 2006), Trichosan thesdioica contain 1F and 1M markers (Kumar *et al*., 2008), Simmondsia chinensis contain 1M marker (Agrawal *et al*., 2007), salixviminalis contain 2F marker (Hammer *et al*., 2006) are determined by RAPD technique. The technique AFLP is use to determine various markers in many plants for example Ginko biloba contain 1F,1M,3F and 1M, markers (Liao *et al*., 2009). Rumexnivalis contain 1M marker (Korpelainen *et al*., 2008). SCAR technique is also use to determine various markers in plants for example Ginko biloba contain 1F, 1M markers (Liao *et al*., 2009), *Paseudocalliergon Trifarium* contain 1F marker (Korpelainen *et al*., 2008), Salix viminalis contain 2F marker (Hammer *et al*., 2006).

1.2. Medicinal Properties of Plants

*Actinidia Chinensis*is used to reduce blood pressure, prevent heart diseases(Parle *et al.*, 2016).*Asparagus officinalis* contain fibers, vitamin A, vitamin B, and vitamin C (Iqbal *et al.*, 2017). Ginko Biloba has antioxidants, use to cure heart diseases, reduce anxiety, depression and may be improve to use eye vision (Oken *et al.*, 1998). *Paseudocalliegrol Trifarium* contains flavonoids, and helps in wound healing (korpelainen et al., 2008). *Trichosanthesdioica* is used to treat diabetic and have anti-cancer properties (Maurya and Srivastava, 2011). *Simmondsia chinensis* and *Salix viminalis* are used to make herbal drugs (Kalpana *et al*., 2014). *Rumexacetose*is used to treat breast cancer (heater s boon *et al*., 2007). *Pistachio* and *Simmondsia chinensis* have many medicinal properties (Elmira *et al.*, 2016**)**. Eucommia Ulmoides Olive gives relief from back pain and increase stamina (Tarique *et al*., 2016**)**.

1.3. Various Techniques Used to Find Sex Linked Molecular Marker Gene

In RFLP (Restriction Fragment Length Polymorphism) technique, restriction enzymes is used which cut the DNA fragment in unique pattern. After that, separation of DNA is done by agar gel electrophoresis followed by transferring of DNA species to membrane filter. After incubating with cloned and labeled probes, RFLP bands detection is performed (Williams, 1989). In this technique, isolation of the genomic DNA is done followed by denaturing. Annealing of DNA template with primer is performed so that complementary strand synthesis takes place. Then amplification of the product by gel electrophoresis is performed (J. P. N. Singh et al., 2006). A very common, PCR based technique in which selective amplification of subset of restriction enzymes digested DNA is performed, known as AFLP. This technique is widely used for plants and microbes' studies. It is also used for identifying molecular marker genes in medicinal plants. AFLP is a PCR based technique use in DNA fingerprinting, genomic research etc. This technique uses restriction enzymes to digest then adaptors are ligated to sticky ends of the fragments. After that, the subset of digested fragment is amplified and detection is done by agarose gel electrophoresis (Paun and Schönswetter, 2012). SSR are used to discriminate the sex of plants. SNP (single nucleotide polymorphism) represent at difference in nucleotide is use to know the sex of plants. SCAR (sequence characterized amplified region) are DNA fragment amplified by PCR using primers area is used know the sex of plants.

2. Conclusion

Molecular marker genes are used in sex determination, plant breeding, taxonomy, physiology, embryology, genetics, evolution, genetic engineering etc. After identification of sex-linked molecular marker gene in medicinal plants like papaya, date palmare develop then it became easy to identify plants on their basis of the sex. It will be reduced labor and time as we know only one male plant can be used formatting many female plants which then produce fruits. The molecular maker genes are used in nursery, in many other cases. It is clear that Genetic molecular marker and specially the FMS are extremely useful source of markers in plants breeding for marker assisted selection because these markers may represent the genes responsible for expression of target traits. If there will not be any recombination between the markers and thus representing perfect in direct selection tools. It will be more fruitful if a concentrated effort is made to integrate the existing molecular fingerprinting data and to co-ordinate the projects of molecular characterization of medicinal plants. Further, more specific primers can be designed from these generated sequences which could be used for sex identification of Date Palm in a more precise way at seedling stage. The search for sex-related molecular markers paves the way for future scientific discoveries. Gender-related markers alone do not explain the molecular mechanism for sex determination in bipartite plants, but the number of markers, their sequence structure, and their homology between characteristic male and female sequences provide some starting point for studying sex-determining mechanisms. They also have practical applications in the study and study of human behavior, aiming to understand the norms and behaviors underlying sexuality. Bryophytes, which received male and female recognition, such advances may inspire a large number of researchers in future.

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