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*Allium'*larda Erkek Kısırlığı Uygulamaları

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Özet

Allium grubu içerisinde ekonomik açıdan önemli, soğan (*Allium cepa* L.) ve pırasa (*A. ampeloprasum* var. porrum L.) gibi ülkemizde de yoğun olarak tüketilen türler yer almaktadır. Bu türler çoğunlukla 2 yıllık olup açık tozlanma göstermektedirler ve toplu çiçek yapısına sahiptirler. Bu yapı yüzlerce küçük çiçekten meydana gelmektedir. Çiçekler elle yapılacak yapay tozlanma çalışmalarında kullanılamayacak kadar küçük ve narin oldukları için, hibrit çeşit geliştirme ve hibrit tohum üretim çalışmalarında sitoplazmik-genik erkek kısırlık (CMS) özelliğinden yoğun olarak faydalanılmaktadır. Erkek kısır olarak adlandırılan bitkiler fonksiyonel polene sahip olmadıkları için kendilenme riskleri yoktur. Bu nedenle bu bitkilerden ancak melezleme sayesinde tohum alınabilmektedir. *Alliumlarda* hibrit çeşit geliştirebilmek için, erkek kısır, idameci ve tozlayıcı hatların önceden belirlenmiş olması ve bu hatların birbiri ile uyumunun iyi olması gerekmektedir. Bu çalışma ile *Alliumlarda* ve özellikle soğanda hibrit çeşit geliştirmek için önemli olan erkek kısırlık konusu değerlendirilmiştir.

Anahtar Kelimeler: *Alliumlar*, erkek kısırlık, hibrit, tohum üretimi, soğan, *Allium cepa* L. pırasa

Male Sterility Applications in *Alliums*

Abstract

The subgenus *Allium* section *Allium* includes economically important species such as onion (*Allium cepa* L.), leek (*A. ampeloprasum* var. porrum L.) and chives (*A. schoenoprasum* L.). These species have a biennial habit and many cultivars are open pollinated; and high levels of heterozygosity must be maintained. The *Alliums*' umbel consists of a few, to hundreds of perfect flowers of per umbel. These flowers are too small to synthetic pollination studies due to the large-scale emasculation is not practical so the development of hybrid varieties is economically feasible using systems of cytoplasmic-genic male sterility (CMS). Male sterile plants, which haven't got any fertile pollen, incapable of self-pollination so any seed produced must result from cross-pollination. *Alliums* hybrid production requires the development of male-sterile, maintainer and pollinator lines with good specific combination ability. This review outlines the using male sterility feature in hybrid breeding program for *Alliums* especially for onions.

Key Words: *Alliums*, male sterility, hybrid, seed production, onion, *Allium cepa* L., leek

INTRODUCTION

The genus *Allium* comprises around 750 species [1] which are almost entirely distributed on the Northern hemisphere. The latest classification divides the genus *Allium* into 15 subgenara and 72 sections [2]. The subgenus *Allium* is the largest comprising around 280 species [3]. The edible *Alliums* are of major economic and dietary importance in all parts of the world. They can be divided into two groups according to the method used for breeding. The group of garlic (*Allium sativum*), great headed garlic (*Allium ampeloprasum* L.), rakkyo (*Allium chinense)* and chinese chives (*Allium tuberosum*) can't flower normally so they are asexually propagated. The other group includes onion (*Allium cepa* L.), Japanese bunching onion (*Allium fistulosum* L.), leek (*Allium ampeloprasum* var. *porrum* L.), and chives (*Allium schoenoprasum* L.), all produce fertile flowers thus they can be used in conventional breeding methods [4].

Onion (*Allium cepa* L.) is the most important species of the *Allium* subgenus belonging to family *Alliaceae.* It is since ancient times a valuable vegetable crop for all people over the world. Because, onions rank seventh after tomatoes and watermelons [5] it can be regarded as the important vegetable species. Onion is a biennial predominantly cross-fertilizing diploid species (2n=2x=16) in which strong inbreeding depression is present [6]. Due to the biennial nature of this out crossing species onion breeding is a slow process. Out crossing among onion plants is encouraged by protoandri [7] and system of cytoplasmic-genic male sterility (CMS) [8, 9, 10].

CMS, is defined as inability to produce viable pollen grain in plants, has been identified in many plant species [11] such as maize, carrot, petunia, rice, sugar beet and sunflower. It is commonly used to produce hybrid seed. Two different CMS systems have been described in onion, termed S- and T-cytoplasm [8, 10]. S- cytoplasm was discovered in the cultivar 'Italian Red' and is conditioned by the interaction of the cytoplasm with a single nuclear gene; fertility is restored by a dominant (*Ms*) allele [9]. Male-sterile plants possess S-cytoplasm and are homozygous recessive at the restorer locus (S *msms*). T-cytoplasm was discovered in the French cultivar 'Jaune paille des Vertus', and restoration of fertility is conditioned by three independently segregating loci [12]. Commercial onion hybrid breeding requires the development of selected male-sterile lines, inbred maintainers for them and inbred pollinator lines, with good specific combination ability.

Analogous systems of male sterility, conditioned by the combination of a cytoplasmic factor and a major gene factor have been found in Japanese bunching onion [13] and chives [14]; in both these crops the system of male sterility has been exploited to develop hybrid cultivars but their consumption is more less than onion so publications of male sterility applications' works are also reduced as those onion.

As the other hybrid alliums species, hybrid leek is more uniform and significantly higher yielding than open-pollinated populations. Male sterility has been reported in leek; however, genetic studies revealed a genic male-sterility system that requires rouging of male fertile segregates in hybrid production fields [12]. Although asexual propagation of a genic male-sterile plant may be used to produce hybrid-leek seed [15], CMS would be a cheaper alternative. Toward this goal, [16] generated an interspecific hybrid between S-cytoplasmic onion and leek as an initial step to transfer CMS from onion to leek.

CMS is one of the most important materials to produce hybrid seeds of many crops especially *Alliums*. Hybrid alliums offer numerous advantages to growers, including significant heterosis over the inbred parent or open-pollinated source of the inbred, greater uniformity, and the possibility to combine dominantly inherited disease resistances [17, 6]. Various experiments have been published in recent years on this subject. In these studies, different local varieties and conditions have been tested by researchers. Some of the improvements of CMS-based hybrid breeding systems for *Alliums* are given to in this paper.

Male Sterility

The most important qualitative genes in the edible *Alliums* are those that cause male sterility which was first exploited in 1925 by Jones and Clarke using a male sterile specimen of cv. Italian Red onion. In male-sterile plants pollen fails to develop therefore they are incapable of self-pollination so, every seed produced is the result of cross-pollination. This trait has been used to produce hybrid cultivars. Although this technique is primarily designed for onions, has been used in several other important crops as mentioned above.

The *Alliums'* umbel consists of a few, to hundreds of perfect flowers of per umbel. In addition to this its flowers are too small to synthetic pollination studies. Therefore male sterility is the most desired feature for develop the hybrids. Since [9] have published a paper describing the genetics of male sterility and how it could be used to produce hybrid cultivars, many studies have been made by researchers from all over the world. In later sections of this review have previous study examples.

In 1989, some researchers from France published an article about characterization of the wild *Allium ampeloprasum* complex. According to this report all species were considered useful sources (for breeding cultivated *Allium*) of male sterility [18].

In a research, male sterile and male fertile plants of cv.Wilau 35 were crossed, and male-fertile plants were also selfed. mtDNA was digested using *HindIII* and DNA hybridization performed using clones containing maize mitochondrial genes for 26S rRNA and cytochrome-c oxidase (*coxII*). The selected male sterile phenotypes of Wilau had smaller darker brown anthers than those of the known (S) male sterile genotypes. This phenotype was also noted in the S_1 progenies of fertile Wilau plants and in F_1 progenies where a Wilau male sterile plant was used as a female parent. Prior to first pollen mitosis the cytoplasm in microspores of (S) male sterile genotypes degenerated, but in Wilau male sterile selections microsporogenesis was interrupted earlier, at late metaphase II. No differences were noted in mtDNA restriction endonuclease patterns and fragment location of mitochondrial genes was observed by varying the nuclear background of the two male sterile cytoplasm S and M [19].

The mitochondria of chive plants with normal N or male-sterile S cytoplasms have been examined by restriction fragment analysis and Southern hybridizations of mitochondrial DNA (mtDNA) and in organelle protein biosynthesis. Restriction fragment patterns of the mtDNA differed extensively between N-and S-cytoplasms. The percentage of fragments with different mobility varied between 44–48% depending on the restriction enzyme used. In contrast to mtDNA, the restriction fragment patterns of the chloroplast DNA from N- and S-cytoplasms were identical. The organization of the analyzed mitochondrial genes *cox*II, *cox*III, *nad*1 and *nad*3 was different in N- and S-cytoplasms. Comparison of mitochondrial proteins analyzed by in organelle translation revealed an 18-kDa protein present only in S-cytoplasm. The restorer gene *X* suppressed the synthesis of that protein in S-cytoplasm. Thus, the 18-kDa protein seems to be associated with the cytoplasmic male-sterile phenotype [20].

Another research about the identification of cytoplasm using the polymerase chain reaction (PCR) to aid in the extraction of maintainer lines from open-pollinated populations of onion is conducted by the researchers. According to research amplification by the PCR of a fragment that carries an autapomorphic 100-bp insertion in the chloroplast DNA of N-cytoplasm offers a significantly quicker and cheaper alternative to crossing or Southern analysis [21].

Researchers investigated the origin and distribution of normal cytoplasm of onion. In this study seventy populations of bulb onion from different part of the world were evaluated for the presence of Normal (N) male-fertile and male-sterile (S) cytoplasm using three polymorphisms in the chloroplast genome so in the end he found that only one accession acquired from Turkey possessed S-cytoplasm. Fifty accessions possessed only N cytoplasm. Nineteen accessions possessed both N and S cytoplasm, of which S predominated in two accessions [22].

Sato [23] identified that unusual transcript pattern for the mitochondrial *cob* gene in S- cytoplasm. According to Sato sequencing of the mitochondrial *cob* gene revealed an insertion of chloroplast DNA sequence into the upstream region of *cob* in the S-cytoplasm and PCR detectable polymorphism based on differences in the sequences of the mtDNA that distinguishes N- and S- cytoplasm allows a quick and confident identification of the cytoplasm of individual plants.

Yamashita and Tashiro [24] investigated that possibility of developing a male sterile line of shallot (*Allium cepa* L. *Agregatum* Group) with cytoplasm from *A.galanthum* Kar.et Kir. In this study for develop a male sterile line of shallot a continuous backcrossing was carried out using *A.galanthum* Kar.et Kir. as a cytoplasm donor the shallot as a nucleus donor. Meiosis and fertility in the B_1, B_2 and B_3 progenies were examined. The F_1 hybrids had low pollen fertility, whereas the B_1 plants were pollen sterile or nearly so; The B_2 and B_3 plants were completely pollen sterile. According to these results there is a realizable possibility of developing male sterile line of shallot using this genetic approach.

Engelke and Tatlioglu [25] published an article named ‘A PCR-marker for the CMS_1 inducing cytoplasm in chives derived from recombination events affecting the mitochondrial gene *atp*9. Accordingly, the complete coding and 3´-flanking region of the mitochondrial gene *atp*9 of chives was determined in order to develop primers that allow the identification of *atp*9-related sequences in subsequent PCR-amplifications. One of these sequences is of a chimerical nature, consisting of *atp*9-homologous regions on its end, interrupted by an insertion that is composed of one *atp*6-homologous part and one part of unknown origin. This PCR-fragment is 762 bp in size and exclusively amplified in the sterility inducing cytoplasm of CMS_1. Thus it can be used as a PCR-marker in order to distinguish this cytoplasm type from the remaining cytoplasm types of chives. The chimerical marker sequence forms a putative open reading frame (*orf*A501), from which CMS_1 might originate.

Gökçe et al. [26] tried to find methods for molecular tagging of the *Ms* locus in onion. These models were consistent with field and other molecular analyses documenting that N-cytoplasm and the dominant Ms allele predominant in OP onion populations.

In the other research the chimerical mitochondrial CMS_1-specific sequence in chives was used to develop a PCR-marker that distinguishes both male-sterility inducing cytoplasm, CMS-(S) and CMS-(T), from the normal cytoplasm in onion. In combination with a previously described marker for CMS-(S), which anchors in the upstream region of the mitochondrial gene *cob*, all of the three known cytoplasm in the onion is distinguishable. In this research the PCR marker system was tested in 361 onion plants, which were selected from F_1-hybrids and different open-pollinated (OP) varieties. The latter are mainly landraces from Turkey, in which all three cytoplasm types were detected [27].

Kim et al. [28] investigated distribution of three cytoplasm types in onion cultivars bred in Korea and Japan. In this study, 116 onion cultivars were tested with molecular markers. So only a few cultivars containing CMS-S cytoplasm were identified in Korean cultivars.

Frequency of male sterile plant of several bunching onion accessions, which collected from Japan, China, Mongolia, Korea and Taiwan, are evaluated by Yamashita et al. [29]. Finally found that, in eight (‘Nogiwa Aigara', ‘Bansei Hanegi', ‘Amarume', ‘Kimnung', ‘Zhangqiu', ‘INT/CHN/1990/GOTOU', ‘Natsunegi' and ‘Guangzhou') of 135 accessions, male sterile plants appeared with varied frequencies from 1.7% (‘Nogiwa Aigara' and ‘Bansei Hanegi') to 24.5% (‘Zhangqiu'). The CMS germplasm described as a useful source for the development of “A” lines to be used in F_1 hybrid seed production of bunching onion. Male fertility in ‘Nogiwa Aigara', ‘Bansei Hanegi', ‘Kimnung', ‘INT/CHN/1990/GOTOU' and ‘Natsunegi' was verified to be controlled by a single fertility restoration locus.

CONCLUSION

Alliums especially onions have great importance in human diet, their commercial production are based on open-pollinated or hybrid cultivars. The production of hybrid onion seed requires a male sterility system. Cytoplasmic-genic male sterility is the most economical and widely used method to produce hybrid-onion seed. *Alliums* are biennial plants and 4 to 8 years are required to determine a cytoplasm type by test-crossings. Distinguish of onion cytoplasm types by molecular markers in individual plant might be helpful to reduce the efforts in a breeding time, when new sterile lines and the corresponding maintainer lines will be developed.

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