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THE INDUCTION OF MALE-STERILITY IN SUNFLOWER (*Helianthus annuus L.*) BY USING GIBBERELLIC ACID

Bülent SAMANCI
Akdeniz Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü
Antalya/Türkiye

Abstract: In this review, the use of gibberellic acid in sunflower breeding was discussed. The hand emasculation in sunflower is difficult task and after obtaining many inbred lines they need to be crossed easily to find F_1 hybrids. For this reason, When 75-100 ppm GA_3 in H_2O is applied to sunflower heads (at star stage), 100 % sterility can be obtained easily without emasculation. Today's sunflower breeders use this chemical to get sterility, before the lines converted into Cytoplasmic Male Sterility.

Ayçiçeğinde (*Helianthus annuus L*) Giberelik Asid kullanarak Erkek Kısırlığı Oluşturulması

Özet: Bu derlemede ayçiçek ıslahında giberelik asid kullanımı tartışıldı. Ayçiçeklerinde emasküIasyon zor bir görevdir ve bir çok kendilenmiş hat elde ediltikten sonra F1 hibridlerin bulunabilmesi için kolaylılıkla çaprazlanmaları gerekmektedir. Bu nedenle, suda 75-100 ppm GA_3 ayçiçek tablalarına (yıldız safhası) kullanıldığında emasküIasyona ihtiyaç duymadan % 100 sterilite elde edilebilir. Bugünün ayçiçek ıslahçıları steriliteyi elde etmek için bu kimyasalı kendilenmiş hatları sitoplazmik erkek kısırlığına dönüştürmeden kulanmaktadırlar.

Introduction

Sunflower is a highly cross-pollinated crop, with pollination occurring primarily by insects and only to a limited degree by wind. A system of genetic self-incompatibility exists in certain lines, but generally a wide range of self-fertility occurs among individual plants in breeding populations. Field production of hybrid seed has been achieved in sunflower by the discovery of cytoplasmic male sterility (CMS) in 1969 by Leclercq (1). The use of CMS is aimed entirely at field production. A breeder utilizes several generations to perform the crossing and backcrossing techniques required to incorporate the CMS character into a line. It is easy to understand why the breeder chooses to commit only the most promising parental combinations to CMS conversion.

For the breeder to develop the genetic combinations used in identifying parents for CMS

conversion, he must have a method which easily and effectively renders a plant male-sterile. Hand emasculation is used quite often and although effective, it is a very tedious and time consuming approach. Emasculation should be carried out early in the morning. Undeveloped central florets are removed, usually by cutting them off with a knife at a point just above the ovaries. A few flowers closely adjacent to those emasculated can not be cut off with a knife without danger of damaging the emasculated flowers. These can be removed with forcepts. The stigmas remain receptive for up to 4 or 5 days, but normally pollen is best applied the day after emasculation when stigmatic lobes have separated and the receptive surfaces are exposed. Therefore, many breeders need to use a chemical application procedure which effectively induces sterility. Male Sterility can be induced chemically by gibberellic acid to facilitate backcrosses, testing of inbred lines for combining ability and even hybridization of parental lines for producing hybrid seeds in isolated crossing plots. In the latter case, chemical emasculation of female rows allows the utilization of B lines directly in hybrid combinations, without conversion into CMS or pollen fertility restorer lines facilitating the breeding works and enlarging the possibility of obtaining superior F1 hybrids. The use of Giberellins (specifically) in sunflower breeding programs is a valuable asset for the breeder interested in inducing non-genetical, single generation male sterility to aid his development of hybrid sunflower populations.

Chemistry

Gibberellins in the plant are hormones which are thought to aid the performance of many physiological and biosynthetic activities. Many different gibberellins have been and are continually being found in plants today (2). The identification of these different types is a slow process. This can be related to the fact that the concentrations of gibberellins present in plant tissue are extremely low. To give an idea of how low, consider that combined buds of 100 sunflower seedlings yielded only 0.001 gr of gibberellin (3).

All gibberrelins identified are classified into what is referred to as the "A" series (A_1, A_2,... An). The type of gibberellin used to induce male-sterility in sunflower is the third in the "A" series or GA_3. Chemically, it is $C_{10}H_{22}O_6$. In 1970, Piquemal (4) evaluated many studies performed with GA_3 a s a chemo-sterilant to sunflower. He found that obtaining 100 % sterility depended on the stage of flowering, the concentration of GA_3 in solution and the method of application. Today's sunflower breeder generally uses a concentration of 75-100 ppm GA_3 in H_2O. The concentration may be applied directly to the bud at what is commonly refereed to as the "star stage". The bud is about 1.0-1.5 cm in diameter and the involucral bracts are easily visible at this time. This stage tends to occur three days after flower initiation. Application of the concentration may be facilitated by spraying a small amount on the bud general breeding purposes, or, if accuracy is required for a specific research project, 2 ml concentration per flower bud may be applied with a two-way valve syringe (5).

Physiology and Cytology

Seetharim and Kusuma-Kumari (6) researched the effects of GA_3 application to both the female and male organs of sunflower. The effect of GA_3 on the female was determined by comparing seed set of treated plants to that of control plants. Data clearly showed that GA_3 treatment at the stage which induces maximum male-streility does not affect female fertility. Observations of GA_3 effects on male organs icluded shrunken anthers, shortened filaments, lack of pollen release, partially to completely empty pollen grains, failure of pollen tube elongation, and in some plants, anthers were found to be completely empty which indicated failure of microsporogenesis.

It is known that gibberellin exists in a natural ratio is thought to influence the expression of maleness or femaleness for a particular species. It is believed by many researchers that hormones perform on two levels in plants. In the first or enzymatic Regulation, enzyme proteins which are always present are thought to be regulated by hormones. Plants express a fast response to the activity of the regulatory hormones (7). The second level where hormones thought to act is that Gene Expression Regulation. Gibberellins have been shown to work at the genetic level. How gibberellins cause the appearance of alpha-amylase activity which results in the hydrolysis of starch in seeds have been investigated (8). The use of methods such as radioactive labeling of amino acids, inhibitors of protein synthesis (cyclohexamide) and inhibitors of DNA-dependent RNA synthesis (like actinomycin D) showed that gibberellins must be involved in the production of mRNA molecules on the DNA template and hence act as an activator of genes that code for the hydrolytic enzymes.

The investigations of the activity of gibberellins during seed germination should correspond to its effect on inducing male-sterility in flower formation. Since floral buds become flower structures via rapid cell division and cell differentiation for sex, it would be seem that the activity of gibberellins in inducing male-sterility should be similar to the mode of action described for the coding of alpa-amylase. Given this, we should relate the concept of hormones regulating gene expression to the finding of empty anthers in GA_3 treated sunflower plants by Seeharim and Kusuma-Kumari (9). Since the empty anthers indicated the failure of microsporogenesis, and hormones are thought to regulate gene expression, one could theorize that it is a change in the polypeptides via hormonal activity which prevented microsporogenesis from occurring. Therefore, when today's breeder adds GA_3 concentration to a sunflower bud, he may be changing the hormonal ratios in the plant, which in turn effects the synthesis of polypeptides and, thus causes failure of pollen development in the treated flower.

Conclusion

There is little doubt that further investigations could and should be persued concerning the cytological and physiological activity of GA_3 towards the inducement of male sterility in sunflower. Main disadvantage of this type of research is that gibberellins occur at very low levels in sunflower buds, so that exact identification of their action towards inducing male-sterility is very difficult. To know this would aid the research of hormonal activity towards floral initiation a great deal. In sunflower breeding, the use of GA_3's is very common and before the conversion of finished inbred lines to cytoplasmic male sterility, they need to be crossed to know the heterotic patterns and high yielding combinations.

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