

Attempts to Induce Parthenogenesis in Sugar Beets¹

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Introduction

It has long been known that some plants produce seed by parthenogenesis, the development of an embryo from an unfertilized egg. The study reported here is concerned with attempted parthenogenesis by stimulating the stigma of male-sterile sugar beet flowers with different compounds, following the suggestion made by Tschermak (13)³.

In the case of sugar beets, a desirable character may be introduced by crossing to a plant carrying this character. If parthenogenesis could then be introduced and a haploid could be obtained, some progeny would carry the new character. Changing these haploid plants to diploid by the use of colchicine would develop homozygous plants without several generations of self pollinating. Thus, seed production by parthenogenesis may be helpful in producing homozygous parent strains suitable for breeding stock.

Review of Literature

According to Sharp (12) we have two types of parthenogenesis.

1. Reduced parthenogenesis: In this case the developing gamete has the reduced number of chromosomes.

2. Unreduced parthenogenesis: In this case the developing gamete has the unreduced (zygotic) number of chromosomes.

The reduced parthenogenesis has been demonstrated in datura, tomato, wheat, tobacco, maize, pepper and many other plants. The first reduced parthenogenesis in plants was recorded by Blakeslee (1) in 1922 in datura and developed as a sport. Since then many workers have obtained haploid plants by crossing different species together (3), x-raying the young spikes (8), by heat treatment of the pollinated but unfertilized flowers (9), and from twin seedlings (4).

In recent work with corn, Chase (2) crossed a purple plumule-colored plant (pollen parent) with a colorless plumule plant (female). The progeny were all purple (the purple color is dominant) except a few that were white. Those with a colorless plumule were tested for haploidy by morphological characters and by counting the chromosomes of the root tips.

The incidence of haploidy was affected by both parents and varied from 0 in 4,500 to a high of 1 in 145 seedlings. The haploid plants could be recognized since, in comparison with diploids, they were shorter, less vigorous with more slender stalks, narrower leaves, smaller and sterile panicles and smaller glumes.

Unreduced parthenogenesis occurs frequently in vascular plants as a standard reproductive phenomenon and the resulting plant is not necessarily homozygous since it is like the mother plant genetically. Chararinita, Marsilea, Thalictum are examples. Artificial parthenogenesis has been induced in Spirogyra and Chlamydomonas (5) by growing them in six percent solution of cane sugar.

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³ Numbers in parentheses refer to literature cited.

Recently Tschermak (13) reported the formation of seed by dusting the carefully emasculated flowers with different compounds such as road dust, dead pollen, maize powder, crushed betaxin, cebione tablets, oat meal, wheat flour, chalk, etc. He repeated the dusting until the stigma was visibly dried. He states that the seed obtained by stimulating with different compounds are not haploid but, rather, diploid. The explanation for diploid parthenogenesis, as translated from Tschermak, is that the development of the unfertilized egg cell was stimulated by the treatment and then was changed to the diploid condition by a single skipping of cell division after nuclear division.

According to Mendel's fundamental law, in a single factor hybrid the F_1 generation has two kinds of gametes, A and a, in equal numbers which when selfed produce a ratio of 1 AA:2Aa: 1 aa. If A is dominant a phenotypic ratio of 3:1 is obtained. If the F_1 is stimulated to produce seed parthenogenetically, we would get the A and a types to develop in equal numbers, giving a ratio of 1:1 homozygous for each factor.

Overpeck, Conklin and Blakeslee (10) explain the process of seed setting and fruit ripening which normally takes place after pollination in three separate steps.

1. The preparation of the medium in which the seed has to develop:

This consists in the prevention of abscission of the fruit and the stimulation of the development of the ovary, the ovules with their integuments and, in some cases, the nucellus after pollination but before actual fertilization. This can be shown in *Meilandrums* where, after pollination, the ovary and ovules are enlarged considerably before the pollen tube has reached the embryo sac.

Pavolachko (11) reported that, if the style of *Nicotiana* are removed three hours after pollination, the pollen caused the development of the ovaries and delayed dropping.

2. The stage of embryo production:

It consists of the division of the polar nucleus and the egg cell and these initial divisions are not necessarily connected with fertilization (in the sense of fusion of nuclei). Ferguson (6) observed that after pollination of *petunia* the polar nucleus may divide many times before the sperm nuclei have left the pollen tube. The mere presence of the pollen tube in the embryo sac seems to be sufficient to cause the initial division of the endosperm. Jorgensen (7) observed that in the cross of *Solarium nigrum* by *Solarium luteum* the sperm nucleus never fuses with the egg nucleus but the sperm nucleus degenerates. Its presence, however, is sufficient to start the division in the egg cell which ultimately develops into a completely maternal plant. (Eighty percent of such plants are diploid while the other 20 percent are haploid).

The observation of twin embryos indicates that a haploid embryo can start development under the influence of the fertilization of an adjacent ovule. This indicates that, if a diffusible substance stimulates the development of the egg, its area of activity may be limited. This might be due to either a large sized molecule which permeates membranes with difficulty or to a more readily diffusible substance with a high rate of destruction.

3. Fusion of egg polar nuclei with sperm nuclei:

A definite step in typical seed development is the fusion of the sperm nuclei with egg and

polar nuclei which makes hybridization possible. It has been shown that the nuclear fusion is neither necessary for growth and enlargement of the ovary and ovules nor for development of the egg into an embryo. Polyembryony, common in many plants, presents the best example of embryos formed from somatic maternal tissue. This occurs, however, in the presence of an embryo resulting from fertilization. Sporophyte polyembryony among citrus shows true embryos which are developed from the nucellus. A seed of an orange regularly contains several viable embryos, only one of which may be the result of fertilization. The nonsexual seeds will produce seedlings each with a genetic constitution like that of the female parent.

Experimental Procedure

Six male-sterile sugar beets were isolated in the greenhouse in March, 1950. On a portion of a branch, the stigmas were treated with different chemicals and labels were tied at both ends of the treated sections. One of the beets was untreated. The chemicals were applied with a camel hair brush to the stigmas. The time of application was not regular but an attempt was made to treat the flowers at different stages from bud to mature flowers.

The treatments were: 1—chalk, 2—wheat flour, 3—maize extract, 4—flour, 5—soap stone, 6—maize pollen, 7—sodium nucleate, one percent, 8—sodium nucleate solution two percent, 9—sodium nucleate powder, 10—clipping the tip of the branches, and 11—control (nothing applied).

On May 15 the greenhouse temperature rose excessively and the windows were opened. This may have led to pollination of some of the flowers from sugar beet plants from a nearby section of the greenhouse. In order to better analyze the results of this experiment the seed balls produced were divided into three classes.

1. From early flowers which produced seed balls before the windows were opened.

2. From mid-early flowers which produced seed balls which may be cross-pollinated.

3. From late flowers which initiated seed balls after the day when the windows were opened.

Three of the male-sterile plants had white hypocotyl and the other three had red hypocotyl. The seeds from white hypocotyl plants were tested since they should have given all white hypocotyl progeny if the seed were produced by parthenogenesis. This germination test showed that a high percentage of the seedlings were red and indicated that the red seedlings, at least, were the result of fertilization. A count of the beets in nearby greenhouses showed 33 white and 77 red. These white plants very likely were the parents of the white seedlings. Even the seed produced early gave a high percentage of red seedlings. Apparently there was considerable contamination by outside pollen both before and after the windows were opened. Evidently a much more positive protection from pollen source must be provided for sugar beets.

Chromosome counts were made from root tips of about 15 of the white hypocotyl progeny and all were diploid, $2n = 18$. The plants appeared normal in growth habit. From this experiment, the results of which are given in Table 1, no proper conclusion can be drawn on the occurrence of parthenogenesis.

A few male-sterile annual beets were planted in the greenhouse in the summer of 1950. During the fall three plants bolted but because of the temperature most of the growth was vegetative.

One plant was treated with vitamin B₁, another with vitamin C and the third was kept as a control. The vitamins were applied by pepper shaker on alternate days until the stigma was dried. This required six applications. Eleven branches, some on each of the three plants, were bagged and no other treatment applied. No seed was formed on any of the plants, but it was observed that by application of these vitamins, in the form of powder, the ovaries were stimulated and were larger in size than untreated ovaries.

Table 1.—Number of Seeds Obtained on Each Sugar Beet Plant.
Experiment 1.

Beet No.	Stage of flower	Treatment	Color of Bud	Number of seeds	Color of Hypocotyl Red	White
I	Early flower	None	Red	3	1	2
	Late flower	None	Red	1	1
				4	2	2
II	Early flower	None	Red	12	9	3
	Mid early flower	None	Red	9	7	2
	Late flower	None	Red	55	37	18
				78	53	25
III	Early flower	None	Red	3	1	2
	Mid early flower	Sodium nucle- ate powder	Red	2	2
	Late flower	None	Red	8	3	5
				13	6	7
IV	Early flower	None	White	7	6	1
	Mid early flower	None	White	7	2	5
	Late flower	None	White	26	14	12
				40	22	18
V	Early flower	None	White	9	2	7
	Mid early flower	Sodium nucle- ate powder	White	2	...	2
	Mid early flower	None	White	2	1	1
	Late flower	None	White	8	3	5
				21	6	15
VI	Early flower	None	White	41	28	13
Control	Late flower	None	White	40	30	10
				81	58	23

Two male-sterile plants were isolated in a greenhouse where there was no possibility of stray pollen. The flowers were treated with different chemicals from bud stage to mature flowers and at different times of the day.

The 611 seed obtained with the different treatments shown in Table 2, when planted, did not germinate. Each seed ball appeared normal but when cut open neither embryo or endosperm was present. The hormones caused the formation of parthenocarpic fruits.

Summary

An attempt was made to induce by parthenogenesis homozygous lines

of male-sterile sugar beets by stimulating the stigma of the flowers with various compounds, following the suggestion of Tschermak.

Substances in the form of powder, vitamin compounds, chalk, etc., applied to the stigma of sugar beet flowers did stimulate the growth of the ovary but no seed was obtained.

Hormones, in the form of dust or liquid, stimulated growth of the ovary and embryo sac. The seed ball and embryo sac were normal in size and appearance but no embryo was formed.

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