Obtaining Tetraploid Monogerm Self Fertile, Self Sterile, and Male Sterile Beets

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Introduction

Insofar as inbreds and male sterile lines have been included in breeding work in recent years, it has become important to study the behavior of these breeding stocks in the tetraploid condition. The following subjects aroused special interest:

a. How easily can tetraploid inbred lines be propagated? Is their degree of self-fertility the same as the original diploid lines?

b. Will the manifestation of male sterility remain the same *in* tetraploid male sterile lines as in diploid lines or is the degree of male sterility increased in the tetraploid condition?

c. What will be the level of heterosis in tetraploid hybrids compared to diploids?

Data concerning the behavior of 4n inbred lines are not numerous for different crops. Randolph (5)[2] described a pronounced decrease in vigor and fertility in inbred strains of maize, while tetraploids derived from stocks which had not been inbred were as vigorous or more vigorous than the diploid parents and were highly fertile. Tetraploid plants produced directly from inbred lines were consistently much shorter and less robust than their diploid sibs. They produced few viable seeds, but abundant pollen and well developed ears. The doubling of chromosomes *in* heterozygous open-pollinated varieties or in hybrids derived from inbred lines produced vigorous, robust and highly fertile tetraploids.

Abegg (1) reported a striking decrease in vigor and fertility in *4n* self fertile strains of sugar beets. Seeds collected from unbagged branches of colchicine-treated self fertile plants with a high degree of self fertility were assumed to be tetraploid and were compared with related diploid lines in field tests. The second generation of tetraploid progeny from this plant was also tested. A total of 193 tetraploid plants yielded approximately only half as much as the closely related diploid plants. The majority of greenhouse-grown tetraploid plants derived from the highly self fertile diploid strain produced only a small amount of seed under paper bags.

Lundquist (3, 4) indicated a lower inbreeding depression in tetraploids than in the corresponding diploid variety of rye. The inbreeding material was obtained by selfing elite diploid and tetraploid populations of Steel rye. Plant height, number of spikelets and seed fertility were studied. Reaction of diploids and tetraploids to inbreeding was similar for different characters. However, the depression was less in tetraploids. The values for the tetraploids were in all characters and generations beyond those of the diploids.

Material and Method of Treatment

The following sugar beet breeding stocks were exposed to colchicine treatment:

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[2] Numbers in parentheses refer to literature cited.

1. S_3 monogerm self fertile inbred lines NN 509, 600, 602, 610 and 5891.

2. Male sterile monogerm lines N 9563/602, N 24/610, and N 9333/600. Line 9563/602 had male sterile plasma from a mangel variety and was pollinated by the inbred line N 602 during the last two generations. Line N 24/610 had male sterile plasma from curly top-resistant sugar beet variety U. S. 56 and was crossed back in the last generation to inbred line 610. Line N 9333/600 had male sterile plasma from the old high sugar Polish variety Janash; the two last backcrosses were made with inbred line N 600.

3. The first monogerm self sterile beets which were selected in hybrids between the self fertile monogerm SL 101 and self sterile multigerm beets.

Young seedlings were treated with a colchicine solution in the fall of 1952. The cotyledons and the upper part of the hypocotyl were treated by the method described in a previous paper (7) . Treatment lasted 6-7 hours. Concentration of colchicine applied was 0.4-0.5 percent.

The seeds were germinated on filter paper. When the tips of the radicles had scarcely appeared the seeds were plunged into an 0.5 percent colchicine solution for five hours. Treated seeds were rinsed in water and planted into pots.

All treated seedlings were grown for two months in a greenhouse and then transferred to a cold frame for thermal induction. Thev were transplanted into the field in the spring. The inbreds and male sterile beets were grown in one isolation plot, the self steriles in another.

The tetraploid plants in self fertile and self sterile beets were selected according to size of pollen grains. All attempts to distinguish tetraploid plants in male sterile beets by determination of the size of pollen mother cells did not give reliable results. Although pollen mother cells of diploid and tetraploid plants were measured at the same stage of meiosis, authentic data were not obtained because of variability and insufficient differences in cell size. Therefore, male sterile tetraploid plants were selected by the determination of chromosome number at meiosis of the treated plants. The aceto-carmin smear method was used for this purpose.

All selected tetraploid plants were bagged with white paper bags. Several bags were put on every plant. On each *An* plant derived from self fertile lines one bag remained for selfing, while all other bags were used for intercrosses between different 4*n* inbred plants and for pollination of 4*n* plants by the pollen of 4*n* self sterile multigerm beets. Male sterile *An* plants were crossed by changing bags with 4*n* inbred plants and with *An* self sterile multigerm beets. The 4N self sterile plants were crossed inter se by exchanging bags and also pollinated by pollen of *An* self sterile multigerm beets.

Experimental Results and Discussion

Tetraploid Inbred Lines

Twenty-two tetraploid plants were obtained from inbred lines. All these tetraploids developed normally; many of them showed typical tetraploid characteristics: thick, round, dark green leaves, robust stems and large buds. No dwarf or defective plants were observed among them, but they were much inferior in pollen production and self fertility compared with plants growing in the same field from the same inbred lines which remained diploid after colchicine treatment.

All tetraploid plants produced from inbred lines could be distributed into three groups according to their fertility characteristics: male sterile, self sterile and plants showing some degree of self fertility. Of these tetraploid plants, 22.7 percent were male sterile (Table 1). Some of them developed small transparent anthers. Others had yellow or brown anthers of normal size which did not dehisce. Examination of the anthers showed that they were practically empty. In some plants no pollen grains in the anthers were observed. The anthers of other plants in this group contained small aborted pollen (9-12μ), together with fragments of pollen grains and among them a few large diploid pollen grains. The destruction of pollen occurred after meiosis because meiosis in these male sterile plants took its normal course as in otther tetraploids. Determination of chromosome number at meiosis showed that these plants were tetraploid.

Table 1.—Tetraploids Produced from Self Fertile Inbred Lines.

2n parental line number	4n plant number	Anther de-hiscence	Pollen accumu-lation	Percent 2n pollen	Fertility characteristics: Seed setting after pollination by: Selfing	4n from inbreds	4n self steriles	General behavior
600	12:35	no	none	0	none	good	good	Male sterile
600	13-29	no	none	0	none	....	good	do.
5891	14-29	no	none	0	none	poor	inter	do.
5891	14-12	no	none	0	none	......	poor	do.
610	9-29	no	none	0	none	poor	good	do.
610	7-38	yes	small	45	none	inter.	good	Self sterile
602	10-27	yes	small	30	none	inter.	poor	do.
602	10-14	yes	small	70	none	good	good	do.
5891	14-24	yes	small	50	none	....	good	do.
5891	14-26	yes	none	50	none	....	inter.	do.
5891	14-7	yes	small	75	none	....	inter.	do.
5891	14-10	yes	small	80	none	......	inter.	do.
5891	14-9	yes	small	70	none	....	inter.	do.
5891	14-4	yes	small	45	none	....	good	do.
509	No. 1	yes	small	80	none	....	good	do.
509	No. 2	yes	none	80	none	....	inter.	do.
610	7-31	yes	small	40	poor	poor	good	Low or semi-self-fertile
610	8-33	yes	small	90	poor	poor	good	do.
602	9-33	yes	inter.	90	inter.	.	good	do.
602	11-14	yes	large	90	fair	good	good	do.
600	11-12	yes	large	90	good	good	good	do.
5891	14-2	yes	inter.	40	inter.	....	good	do.

The largest group (50 percent) of tetraploids produced from inbred lines consisted of plants which developed a sufficient quantity of good diploid pollen grains but remained self sterile. The quantity of well developed diploid pollen grains varied in different individual plants from 30 to 80 percent; at any rate, the quantity of pollen was sufficient for self fertilization if it could pollinate or the pollen tubes could have grown through the pistils.

A characteristic of this group of plants was that in spite of normally developed anthers many of them did not dehisce, or when they did the pollen did not accumulate in the paper bags. The paper bags which covered the branches did not contain pollen at all, or contained very little. Some of these anthers were examined under the microscope. They showed lumps of pollen grains stuck together and located on the split portions of anthers or on their suface. Many pollen grains germinated directly on the anthers and the pollen tubes reached a comparatively good length.

All the plants of this group were self sterile, but when the pollen of self sterile plants N 10-27 and N 10-14 was used for pollination of other *An* plants derived from inbred lines, some seeds were produced.

The third group (27.3 percent) consisted of plants which showed some degree of self fertility. The plants of this group developed a sufficient quantity of good diploid pollen grains. The anthers were yellow and of normal size. The amount of pollen collected from the anthers, as well as the quantity of selfed seed, varied *in* different plants. Four plants set a few seeds after selfing. Two plants (N 11-14 and N 11-12) had much pollen in the paper bags and the number of selfed seed amounted to 30 and 40 percent.

Observation of the behavior of 22 tetraploid plants derived from self fertile parental plants led to the general conclusion that in spite of normal vegetative development of these 4*n* plants their reproductive ability was much lower than in corresponding diploid inbred lines. Only certain *An* plants obtained directly from inbred lines were able to produce selfed progeny. The majority of inbred lines would be lost in the tetraploid condition.

When 4*n* plants derived from inbred lines were crossed *inter se* by exchanging pollinating bags a few seeds were obtained in certain crosses. No difference was observed regarding whether the crosses were made within the limit of the same line or between different lines.

Pollination of some of these *An* plants by pollen from a self sterile *An* population gave much better results. Seeds obtained from these 4*n* plants were planted in the fall. A total of 67 beets grew from selfed seed and 84 plants were derived from crosses between 4*n* plants.

Tetraploid Male Sterile Monogerm Beets

Sixteen tetraploid plants were produced from three monogerm male sterile lines. These diploid male sterile lines were derived from repeated crosses to self fertile inbred lines and those which were not male sterile became self fertile themselves; the semi-male sterile plants which occurred sometimes in such lines produced selfed seed. Therefore it must be expected that the regularities observed in inbred lines could be applied to male sterile lines, too. Only the degree of homozygosity was not so high in these male sterile lines as in the corresponding inbreds which were used for their pollination.

All tetraploid plants derived from male sterile lines were highly male sterile. They all developed small, transparent empty anthers; not one of them had yellow anthers or developed viable pollen. They set a little more seed than 4*n* inbred lines when pollinated by pollen from 4*n* inbred lines. In their progeny 53 plants were obtained from crosses to 4*n* inbreds and 75 plants from hybridization with a self sterile tetraploid population.

Tetraploidy might be very important to produce completely male sterile strains. If it can be confirmed in succeeding generations that polyploidy increases the degree of male sterility, such 4*n* male sterile lines could be successfully involved in hybridization for obtaining tetraploid hybrids. Josefsson (2) indicates a high yielding capacity for hybrids between *An* stocks in Fodder beets. The 4*n* yellow tankard in comprehensive trials produced 23 percent higher yield of dry matter than its mother line, Svalof yellow tankard; 4*n* Sirius outyielded Svalof Bortfelder by 29 percent and Svalof Ostersundom by 15 percent of dry matter. The selection of crosses between *An* families of different origin called "Svalof tetraploid Sirius turnips" will be marketed in the spring of 1953.

Self-sterile Monogerm Tetraploids

Tetraploid plants were obtained from the first self sterile monogerm lines. Production and activity of pollen in these *An* plants was much higher than in 4*n* beets derived from inbreds. All plants produced a sufficient quantity of well developed diploid pollen grains. The anthers were large, yellow, dehisced in a normal way, and the paper bags used for controlled pollination always contained a sufficient quantity of pollen.

The seed set after exchanging pollinating bags with other tetraploid self sterile monogerm and multigerm plants was much more abundant than from 4*n* inbredls or 4*n* male sterile beets. The seeds were more viable, their germination rate was higher and the offspring were more vigorous. The monogerm self sterile tetraploids were not different from multigerm self sterile tetraploids *in* the production of a sufficient quantity of pollen and in seed setting ability.

Self sterile multigerm tetraploids recently developed by the above described method from strains SL 824 and SL, 92 and Swiss Chard differed very much from the tetraploids obtained at Hilleshog, Sweden. According to Rasmusson (6) , tetraploid sugar beets obtained by dipping young flower stalks in colchicine solution were much inferior in their vitality and productivity *bi* their first generations than parental diploid strains. Every *An* type was usually derived from two first colchicine treated plants.

Using designations c,, c, etc., to indicate generations of propagation after colchicine treatment, the number of c_n plants was very small and their seeds were not comparable to the normal diploid seeds. It was also difficult to produce c_2 seeds. Therefore, no attempts were made to try out any field trials. Only c_3 seeds could be produced under normal conditions and in lots large enough to allow testing of *An* types. Only those strains were used for tests which had at least 15 plants in the c, generation. The leaf color of the first tetraploids was much lighter than in diploids; the difference began to disappear in the c.,-c_4 generation and the old tetraploids had nearly the same color as diploids. The artificial tetraploids started as very inferior types but automatically their original low productivity was improved in later generations (from c_3 to *c.* and c_6) so much that they reached at least the level of comparable diploids.

The effect of hybridization in tetraploid material was considerably greater than in diploids and some of the new tetraploid strains developed from 4*n* bulk crosses were superior to all diploid strains and even to the best diploid bulk crosses.

The tetraploid population of strain SL 824 was developed at Salt Lake City on the basis of 50 c_0 plants and the tetraploid population from strain SL 92 on the basis of more than 40 c_0 plants. The plants of the first generation after treatment (c_r) produced so much seed under open pollination that tetraploids from strain 824 could be tested in 5-6 replications on three different fields in the usual variety tests. The beets in plantings of both tetraploids were well developed, very uniform and showed very pronounced tetraploid characteristics: large, thick, dark-green leaves. Small or dwarf plants could be observed as a rare exception. Both tetraploids were developed from curly top-resistant diploid strains and maintained the same high degree of resistance in the tetraploid condition.

Production of pollen in c_0 plants was inferior to that in diploids, but sufficient, and this improved in the following c_1 and c_2 generations. The quantity of seed set in c_1 and in the next generations under normal field conditions was practically normal. The seedballs were larger than in diploids. The tetraploids showed a little sterility due to abnormalities at meiosis and formation of a certain amount of nonviable gametes, but the quantity of seed set was always abundant, starting from the first generation. The size of tetraploid populations grown was limited by economics only, not by the vitality of tetraploids.

The data for two years' variety tests (Table 2) indicated that tetraploids produced from SL 824, in spite of lower stand, outyielded in different trials its diploid mother strain from 8 to 18 percent. But the loss in sucrose was considerable, especially in the Salt Lake Citv test in 1953. The increase in yielding ability in tetraploids was obvious but the data indicated the necessity of breeding work in tetraploids to increase the sucrose percentage.

Table 2.—Yield Test of 2n SL 824 and *An* 824.

		Twin Falls, Idaho 1953		Salt Lake City, Utah 1953		Salt Lake City, Utah 1952	
		Absolute figures	Percent of 2*n* SL 824	Absolute figures	Percent of 2*n* SL 824	Absolute figures	Percent of 2*n* SL 824
Beets per 100 feet of row	2*n*	106	100	109	100	62	100
	4*n*	100	94	97	89	74	119
Tons beets per acre	2*n*	29.4	100	35.1	100	22.2	100
	4*n*	31.8	108	39.0	111	26.1	118
Sucrose percent	2*n*	17.4	100	14.9	100	14.9	100
	4*n*	16.1	92	13.4	89	14.1	95

F_1 Hybrids Between a 4*n* Inbred Line and a 4*n* Self-Sterile Beet

Two tetraploid plants, N 1 and N 2 in the monogerm S_3 inbred **line** N 509, were obtained one year earlier. Because of their complete self sterility and the absence of pollen in the paper pollinating bags they were pollinated.

by pollen from 4*n* self sterile beets. The F_1 plants demonstrated striking hybrid vigor. These seed beets were very tall, with large leaves and very thick robust stems. The fruits, like diploid F_1 hybrids between monogerm and multigerm beets, were multigerm (mostly doubles) with a few mono-germ seed on the ends of the branches. Examination of pollen from these F_1 plants showed big differences among different plants. All F_1 plants could be classified on quality of pollen into three groups (Table 3) . The group with poorly developed pollen consisted of three plants. The plants of this group failed to develop normal pollen grains. The anthers contained a few large pollen grains among fragments and small, aborted pollen. The paper pollinating bags which covered the branches had practically no pollen.

Table 3.—Distribution of Fi Tetraploid Plants According ot Quality of Pollen.

Poor pollen group			Intermediate pollen group			Good pollen group		
Plant No.	Amount of pollen accumulated	Percent 2*n* pollen	Plant No.	Amount of pollen accumulated	Percent 2*n* pollen	Plant No.	Amount of pollen accumulated	Percent 2*n* pollen
1-4	none	10	1-1	intermediate	40	1-2	large	90
1-12	small	1	1-5	none	50	1-3	intermediate	90
1-14	small	1	1-7	intermediate	70	1-6	small	90
			1-8	intermediate	50	1-9	large	95
			1-10	intermediate	50	1-17	large	96
			1-11	intermediate	55	1-10	large	95
			1-13	intermediate	60	1-20	intermediate	93
			1-15	small	25	1-21	large	95
			1-16	small	60	2-1	large	95
			1-18	intermediate	50			

Ten plants of the second group showed satisfactory development of pollen. The quantity of well developed diploid pollen grains varied in different plants from 25 to 70 percent. For the majority of plants it approached 50 percent. The remaining pollen consisted of small, mostly aborted pollen grains. Seven of the ten plants in this group had a sufficient amount of pollen in the paper pollinating bags.

The third group consisted of nine plants which developed excellent uniform diploid pollen. The quantity of well developed pollen exceeded 90 percent in all plants. Six of the nine plants in this group had abundant pollen in the paper bags, two plants had a sufficient amount, and only one contained a scarcity of pollen *in* the bag.

Thus, from 22 tetraploid F_1 plants 19 developed a sufficient quantity of good viable pollen grains and 15 of them (four having scant pollen in the bags being excluded) contained much or a sufficient amount of pollen in the paper bags to provide for selfing or cross fertilization. In spite of this, all F_1 plants except one (N 1-3) remained self sterile. The diploid F_1 hybrids between self fertile and self sterile beets were always self fertile. When these F_1 plants were crossed by exchanging paper bags *inter se* they showed almost complete cross incompatibility. Some cross combinations remained completely sterile, while the largest number set 2, 3 or 4 fruits in the large paper pollinating bags and only two combinations set 15 and 20 fruits in several hundred flowers. Six of the F_1 plants were isolated in a separate greenhouse. These six vigorous plants set only 3 grams of seed under open pollination.

Certain F_1 tetraploid plants were pollinated by pollen from self fertile and self sterile diploid plants, the others by pollen from self sterile tetraploid plants. In all cases the quantity of seed set was sufficient or good. This indicates that a sufficient number of female gametes with diploid or close to a diploid number of chromosomes were formed and that the disturbances at meiosis were not so great as to cause an insufficient activity of pollen grains. Rather, some other factors were responsible for the inactivity of male gametes in F_1 hybrids.

The same F_1 tetraploid plants which were self incompatible and cross incompatible with other F_1 tetraploid plants were compatible when pollinated by pollen from unrelated self sterile or sell fertile diploid and tetra-ploin plants. Thus F_1 progeny between self fertile and self sterile tetraploid plants formed a self sterile and inter sterile group.

The behavior of these F_1 tetraploid plants could be explained on the basis of the oppositional factor hypothesis. The genetype of 4*n* F_1 plants regarding fertility versus sterility factors could be $S^f S^f S^1 S^2$. These F_1 plants would produce four types of gametes in the ratio: 2-$S^f S^f$ 4-$S^f S^1$, 4-$S^f S^2$, 2-$S^1 S^2$. Gametes with $S^1 S^2$ factors could not grow through the pistils of F_1 plants because of the presence of $S^1 S^2$ factors in the pistils.

Gametes $S^f S^1$ and $S^f S^2$ might produce fertilization in some cases if the presence of one factor, S^f, in the pollen grain could compensate for the negative action of the factor S^1 or S^2. In F_1 diploid hybrids between self fertile and self sterile beets, the pollen grains which carry one factor S^f are effective on the pistils with one S^f factor ($S^f S^1$) . Whether two S^f factors in the pistil of F_1 tetraploid plants will permit the pollen grains with one S^f factor to grow through it is not quite clear now, but this possibility may be expected and in such cases a few seeds may be produced sometimes by selfing or in inter-crosses of F_1 4*n* hybrids. However, the experimental data do not indicate that the pollen grains of any genetic structure were successful in selling or in sib crosses in F_1 4*n* plants.

It was obvious that the pollen grains which carried two S^f factors were also not stimulated on the pistils with two S^f factors ($S^f S^f S^1 S^2$) . The experimental data obtained are in agreement with the oppositional factor hypothesis. This hypothesis explains the behavior of all F_1 plants except one which was semi-self-fertile. The most probable explanation of the partial self fertility in this plant may be the assumption that it carried in its genotype some genes independent from the S series which caused an intermediate degree of self fertility of an associated type. There is also the possible assumption of alteration in action of certain genes of the 5 allelic series in consequence of mutation, deletion or duplication.

If the phenomenon of self and cross incompatibility in 4*n* F_1 hybrids between self fertile and self sterile beets can be confirmed, then the possibility arises of using such F_1 4*n* hybrids in double crosses for obtaining 3*n* and 4*n* hybrids.

The self sterility or very low seed production by selfing or inter-cross-mg 4*n* inbred plants was a general observation. With few exceptions all plants were practically self and cross incompatible. It is possible that the growth of pollen tubes with two S^f factors was prevented as a rule on the stigma with four S^f factors. But the existence of rare tetraploid plants which showed self fertility arouses suspicions as to whether self and cross incornpatibility in tetraploid inbred plants were also caused by some other phenomena besides the relation between factors of the S allelic series in the pistil and in pollen grains. Among these other phenomena, the general low vitality of pollen grains carrying two S^f genes or the accumulation of a number of identical homozygous genes in 4*n* inbred lines could be mentioned. Further cytogenetic investigation of tetraploids is necessary to make final conclusions.

Summary

Tetraploids were obtained in mongerm self sterile, self fertile and male sterile sugar beets. The self sterile monogerm tetraploids did not differ from the multigerm self sterile tetraploids obtained previously. They developed more viable pollen than tetraploids obtained from self fertile inbred lines. Seed setting and germination of seeds were better in 4*n* self sterile plants than in 4*n* inbreds or in *4n* male sterile plants.

All 4*n* male sterile beets were highly male sterile. They set some seeds when pollinated with 4*n* self sterile beets and with 4*n* self fertile inbreds.

The quality of pollen in tetraploid inbreds was lower and the ability for self fertilization decreased in comparison with diploid plants from the same in bred lines. Some of the *An* inbred plants became male sterile, while the majority of them were self sterile and only a few set some seeds after selfing.

The first hybrid generation between 4*n* self fertile and 4*n* self sterile plants, contrary to the corresponding self fertility of diploid F_1 hybrids, was self incompatible, and cross incompatibility was observed in sib crosses. The behavior of F^1 4*n* plants might be explained on the basis of the oppositional factor hypothesis.

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