Effect of Low Colchicine Concentrations On Inducing Autotetraploidy In Sugarbeets

HELEN SAVITSKY[1]

Received for publication November 24, 1967

Introduction

The most effective method of inducing tetraploidy in sugarbeets involves treatment of seeds with colchicine solutions. The best results have been obtained by treatment of pregerminated seeds. These have produced more tetraploid plants than have treated dry, or germinated seeds (7)[2]. The colchicine concentrations usually applied ranged from 0.1% to 0.8% (1, 2, 3, 4, 5, 6, 7, 8, 9).

The higher doses (0.5%-0.8%) often produce malformed seedlings and decrease the number of tetraploid plants obtained. To induce autotetraploidy it is desirable to treat the seeds with the lowest colchicine concentrations possible. Effectiveness of treatment of sugarbeet seeds with the low colchicine concentrations is the subject of the present report.

Materials and Methods

Effectivenss of the low colchicine concentrations, 0.05% and 0.1% was compared with that of higher concentration of 0.4%, which is often used for inducing tetraploidy in sugarbeets. Sugarbeet seed rinsed in water during 3 hours was kept for 36-40 hours at 25° C in cuvettes lined with wet blotting paper. During this time the seed swelled, but the root tips did not emerge. Seeds of five self-sterile multigerm populations and of five self-fertile monogerm inbreds ready for germination (pregerminated seed) were treated for 16 hours with colchicine solutions. Treated seeds of each strain were planted in beds in the greenhouse in two replications. Two hundred seeds of each strain were planted (100 seeds per replication). Selection of tetraploid C_0 plants was based on the presence of diploid gametes, not on the leaf characteristics. Plants on which the main inflorescense carried exclusively large pollen grains (diploid gametes) were selected as tetraploids and intercrossed. The young C_1 plants thus obtained have been checked for chromosome number and those

[1] Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California.

[2] Numbers in parentheses refer to literature cited.

having 36 chromosomes were selected. Methods of seed treatment and of producing tetraploid plants, used in this experiment, have been previously reported (6, 7).

Experimental Results

The average effect of colchicine concentrations 0.05%, 0.1% and 0.4% by treatment of all strains is shown in the Table 1 (summarized data). Seed germination decreased from 80.30% to 57.00% with increased colchicine concentrations. In contrast to seed germination, percent of seedlings affected rose from 25.34 to 63.42 when higher concentrations of colchicine were applied. Only 33 tetraploid plants (8.38%) were obtained in all strains treated by the low concentration (0.05%). A concentration 0.1% increased the number of tetraploid plants to 104 (21.85%). A concentration 0.4% produced 344 tetraploid plants (52.92%).

Table 1.—Average effect of seed treatment with different colchicine concentrations on all 10 sugarbeet strains.

Colchicine concentration	Seedlings emerged		Seedlings affected		Plants examined for size of pollen	Tetraploids obtained	
%	No.	%	No.	%	No.	No.	%
0.05	1,606	80.30	407	25.34	394	33	8.38
0.1	1,490	74.50	509	34.16	476	104	21.85
0.4	1,140	57.00	723	63.42	650	344	52.92

In spite of the obviously more effective results from the treatment with the 0.4% concentration, the individual sugarbeet strains responded differently to colchicine concentrations (Table 2).

Seedling affection

Seedlings affected by colchicine are short with thickened hypocotyls and thickened, lobed leaves.

The lowest colchicine concentration (0.05%) affected seedlings in 7 of 10 strains. Percent of seedlings affected varied in these strains from 23.26 to 46.67.

The higher concentration (0.1%) increased percent of affected seedlings in the same strains. For strains 661, 772 and 19, both low concentrations (0.05% and 0.1%) were ineffective and did not produce affected seedlings.

The strongest concentration (0.4%) produced the highest percent (44.79 to 72.34) of seedlings affected in all strains, with the exception of strain 33, for which the 0.1% concentration was more effective.

Tetraploid plants obtained

Treatment of seed with a concentration 0.05% produced tetraploids in three strains only. In two of these strains (126 and 520) the percent of tetraploids was low--17.14 and 19.35, respectively. In the third strain (33), which responded better to the low concentration, the percent of tetraploids increased to 25.71.

Concentration 0.1% produced tetraploid plants in 7 of 10 strains. Percent of tetraploids in six of these strains was not high, ranging from 10.00 to 29.17. In the seventh strain 33, a high percent (54.29) of tetraploids was obtained after treatment with 0.1% concentration.

Table 2.—Effect of seed treatment of 10 individual sugarbeet strains with different colchicine concentrations.

Strain number	Colchicine concentration	Seedlings emerged		Seedlings affected		Plants examined for size of pollen	Tetraploids obtained	
	%	No.	%	No.	%	No.	No.	%
123 S.St. M_2	0.05	108	54.00	44	40.74	42	0	0
	0.1	104	52.00	46	44.23	46	6	13.04
	0.4	74	37.00	46	62.16	46	24	52.17
112 St.St. M_2	0.05	258	129.00	60	23.26	55	0	0
	0.1	267	133.50	111	41.57	92	14	15.22
	0.4	205	102.50	138	67.32	116	69	59.48
211 St.St. M_2	0.05	184	92.00	84	45.65	84	0	0
	0.1	192	96.00	99	51.56	90	9	10.00
	0.4	140	70.00	92	65.71	92	38	41.30
126 S.Fert. m_2	0.05	150	75.00	70	46.67	70	12	17.14
	0.1	136	68.00	84	61.76	84	18	21.43
	0.4	94	47.00	68	72.34	60	38	63.33
33 S. Fert. m_2	0.05	112	56.00	37	33.04	35	9	25.71
	0.1	92	46.00	70	76.09	70	38	54.29
	0.4	64	32.00	42	65.63	12	7	58.33
716 S. Fert. m_2	0.05	130	65.00	46	35.38	46	0	0
	0.1	98	49.00	45	45.92	40	5	12.50
	0.4	76	38.00	48	63.16	45	20	44.44
520 St.St. M_2	0.05	154	77.00	66	42.86	62	12	19.35
	0.1	98	49.00	48	48.98	48	14	29.17
	0.4	76	38.00	48	63.16	47	24	51.06
661 S.Fert. m_2	0.05	174	87.00	0	0	0	0	0
	0.1	166	83.00	0	0	0	0	0
	0.4	140	70.00	92	65.71	86	43	50.00
772 S.Fert. m_2	0.05	134	67.00	0	0	0	0	0
	0.1	124	62.00	0	0	0	0	0
	0.4	96	48.00	43	44.79	40	20	50.00
19 S. St. M_2	0.05	202	101.00	0	0	0	0	0
	0.1	213	106.50	0	0	0	0	0
	0.4	175	87.50	106	60.57	106	61	57.55

The strongest concentration (0.4%) produced the highest percent of tetraploids (41.30 to 63.33) in all strains. For three strains 661, 772 and 19, both low concentrations (0.05% and 0.1%) were ineffective. Tetraploid plants were obtained in these strains only after treatment with a concentration of 0.4%.

Colchicine concentration was the main factor influencing variability in polyploidy induction in these experiments (Table 3). The value of F calculated (275.92 and 1252.71) for seedling affection and tetraploid plants obtained, respectively, greatly exceeded the tabular value of F (3.55 at 5%, and 6.01 at 1% level). The next factor causing variability was the responsiveness of the individual strains to different colchicine concentrations. F calculated for seedling affection was 57.74 and for tetraploids obtained, 60.46, against a tabular F value of 2.46 at the 5% level, and 3.60 at the 1% level.

Table 3.—F test in analysis of variance for seedlings affected and tetraploid plants obtained.

Source of variance	Seedlings affected F calculated	Tetraploids obtained F calculated	F tabulated for seedlings affected and tetraploids obtained 5%	1%
Strains	57.74	60.46	2.46	3.60
Colchicine Conc.	275.92	1252.71	3.55	6.01
Replications	1.61	< 1	4.41	8.28
Replic. × Concentration	1.52	1.44	3.55	6.01
Strain × Replication	2.79	< 1	2.46	3.60
Strain × Concentration	13.61	20.49	2.25	3.19

Discussion and Conclusions

A colchicine concentration of 0.05% is too low to induce tetraploidy in the majority of sugarbeet strains. An insignificant number of tetraploids was obtained in only three strains after treatment with this concentration.

A concentration of 0.1% affected the majority of strains, but it did not produce a large number of tetraploids. For three strains, a concentration of 0.1% was ineffective.

The 0.4% concentration had a universal effect and produced the largest number of tetraploids in all strains.

In inducing tetraploidy, by seed treatment, not all morphologically affected seedlings produced tetraploid plants. In these experiments, low colchicine concentrations were sufficient in some cases to affect seedlings, but not strong enough to double the chromosomes in the sub-epidermal tissues from which the gametes develop.

In four strains treated with 0.05% concentration, 40.74%, 23.26%, 45.65%, and 35.38% of the seedlings were affected, but no tetraploid plants were obtained from them. Responsiveness of individual sugarbeet strains to different colchicine concentrations has not been previously reported. Although an indication of this was noted in Savitsky (7). Such responsiveness is, however, clearly demonstrated in these experiments. Three of 10 strains did not produce tetraploids at low concentrations of 0.05% or 0.1%, whereas, strain 33 responded very well to these concentrations. After seeds of the strain 33 were treated with concentration 0.05%, 25.71% of the plants were tetraploid. Concentrations 0.1% and 0.4% gave 54.29% and 58.33% tetraploids, respectively. Thus, for strain 33, a concentration of 0.1% was almost as effective as a concentration 0.4%.

Actually, every line or population should be treated with a particular colchicine concentration to obtain the best results. For the lines, which present some difficulties in being converted into tetraploids, the suitable concentrations should be found experimentally. However, for the majority of sugarbeet strains, treatment of pregerminated seed with concentrations from 0.2%, or 0.3 to 0.5%, as established in previous experiments (7), is highly effective.

Summary

The pregerminated seed of 10 sugarbeet strains were treated by 0.05%, 0.1%, and 0.4% colchine concentrations. A colchicine concentration 0.05% is too low to induce tetraploidy in the majority of strains. A higher concentration 0.1% affected seedlings in the majority of strains, but did not produce a large number of tetraploids. The concentration 0.4% produced the largest number of tetraploids in all strains. Individual sugarbeet strains demonstrated different responsiveness to colchicine concentrations. Three of 10 strains did not produce tetraploids at concentrations 0.05% and 0.1%, whereas 1 strain responded well to concentration 0.05%, and the concentration 0.1% was as effective for it as the concentration 0.4%. For the lines which present difficulties in being converted into tetraploids the suitable colchicine concentrations should be found experimentally.

Literature Cited

(1) ABEGG, F. A. 1940. The induction of polyploidy in *Beta vulgaris* L. by colchicine treatment. Am. Soc. Sugar Beet Technol. Proc. 1: 118-119.

(2) ARTSCHWAGER, E. 1940. Indication of polyploidy in sugar beets induced by colchicine. Am. Soc. Sugar Beet Technol. Proc. 1: 120-121.

(3) KLOEN, D. and G. J. SPECKMAN. 1953. The creation of tetraploid beets. Euphytica. 2: 187-196.
(4) KNAPP, E. 1958. *Beta*-Rueben bes. Zuckerrueben. Handbuch der Pflanzenzuechtung Ed. 2 V. 3: 197-284.
(5) MATSUMURA, S. 1953. Improvements of sugar beets by means of triploidy. Nat. Inst. of Genetics and Kihara Inst. Biolog. Res. No. 14, 134 p.
(6) SAVITSKY, H. 1966. Effectiveness of selection for tetraploid plants in C_0 generation on the basis of the number of chloroplasts in stomata. J. Am. Soc. Sugar Beet Technol. 13 (8): 655-661.
(7) SAVITSKY, H. 1966. A method of inducing autopolyploidy in sugar beets by seed treatment. J. Am. Soc. Sugar Beet Technol. 14 (1): 26-47.
(8) SCHLOSSER, L. A. 1940. Untersuchungen an autoploiden Zuckerrueben. Zeitschr. der Wirtschaftsgruppe Zuckerindustrie. 90: 88-106.
(9) SEDLMAYR, K. 1955. Polyploide Zuckerrueben. Die Bodenkultur 8: 235-243.