Effectiveness of Selection for Tetraploid Plants in C_0 Generation on the Basis of the Number of Chloroplasts in Stomata

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Introduction

Study of some characteristics of leaves such as chromosome number (7,11,19)[2], size of stomata cells (2,12,14,16), number of nucleoli chromocenters (15), and number of chloroplasts (3,4,5, 7,10,12) is widely used at present time for determination of ploidy levels. Except for examination of chromosome number, the most used method involves determination of the number of chloroplasts in the guard cells of stomata.

Mochizuki and Sueoka (13) first indicated that diploid, triploid and tetraploid sugar beets differ in the number of chloroplasts in the stomata cells of leaf epidermis (Table 1).

Table 1.—Average number of chloroplasts per stoma.

Ploidy levels		According to				
		Mochizuki and Sucoka	Butterfass	Graf	Margara and Touvin	Savitsky
Diploids	14	(12-16)	14.23± 0.10	17.09± 0.25	14.74± 0.32	14.57± 0.28
Triploids	20	(17-22)	20.34± 0.07	20.74± 0.19	18.97± 0.36	20.87± 0.37
Tetraploids	25	(22-28)	25.36± 0.17	24.42± 0.36	25.98± 0.54	25.90± 0.57

Further investigations by Butterfass (3,4,5), comprising more than 900 plants, lent more definition to this method. The kind and age of the leaves that are best for examination were indicated. Although the average number of chloroplasts is different for the diploid, triploid and tetraploid populations, the variation in the number of chloroplasts in the individual plants makes it difficult, in some cases, to determine the ploidy grade. The limits of the number of chloroplasts between diploids and triploids and between triploids and tetraploids are overlapping.

Modification of the number of chloroplasts at different ploidy levels was the main subject of the investigations of Butterfass (5). He indicated that the limits of variation between diploids, triploids and tetraploids should be established for every plot studied, by using the frequency curve. He concluded that counts of chloroplasts in 10 stomata cells permitted determination of ploidy grades for the majority of plants; however, this determina-

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[2] Numbers in parentheses refer to literature cited.

tion was wrong for 1 to 1.5% of the plants and could not be performed for 16 to 18% of the plants. According to Graf (10) determination of ploidy grades in individual plants was wrong for 1.4% of diploids, for 7.0% of triploids and for 5.6% of tetraploids. Dudley (6), basing on study of chloroplasts in one guard cell of stomata in eight diploid and eight tetraploid sugar beets, concluded that diploids and tetraploids can be accurately separated by counting chloroplasts in the guard cells. Because of a certain convenience which the chloroplast method offers, it may be used (but with some limitations) and its applicability varies, depending on the objectives. Especially difficult is identification of triploid plants, because the limits for chloroplast number in triploids also comprise some diploids and tetraploids.

In inducing polyploidy in sugar beets, a study of leaf characteristics (one of which is number of chloroplasts) is a common method for selection of tetraploid C_0 plants after colchicine treatment. The results of a study of reliability of this method for selection of tetraploid plants in the mixoploid C_0 generation are presented in this report.

Materials and Methods

Seed of the following sugar beet strains were exposed to colchicine treatment: 2 self-sterile monogerm populations (573 mm high in curly-top resistance and 202 mm N-type strain); 4 self-fertile monogerm inbred lines (171 mm E-type, 127 mm Z-type, 200 mm leaf-spot resistant, and 537 mm which is highly leaf-spot resistant); and 1 self-fertile multigerm inbred line (509 MM) which is curly-top resistant.

After treatment, seed was planted in soil in a greenhouse. Seedlings coming up were classified as affected (with short, thickened hypocotyls, thick and dissected leaves), or unaffected. All unaffected seedlings were discarded and the affected ones were transplanted into cylinders and kept in the cold frame during the winter for thermal induction. In the spring they were transplanted to the field for further growth and selection for tetraploid C_0 plants.

Treatment of seed is an effective method of inducing polyploidy (17). About 40% of affected seedlings develop completely, or almost completely, tetraploid main inflorescence in which hundreds of flowers carry diploid gametes.

Tetraploid plants were selected on the basis of the size of pollen grains in flowers developed in the main inflorescence. It is well known that tetraploidization increases the size of pollen grains in many crop plants as well as in sugar beets. Abegg (1), Artschwager (2), Schlösser (18) and Von Rosen (19) indicated

larger size of pollen in tetraploid beets in comparison with the diploid. Ernould (8) found that mean size of pollen in diploid beets equals 19.86μ and in tetraploid 26-27μ. Feltz (9) reported that the average size of pollen for diploids was 21.87μ, and for tetraploids 26-27μ. According to Savitsky (16) the size of mature, normally developed pollen varies in diploid beets from 19 to 22μ, and in tetraploid beets from 28 to 33μ.

In several plants studied in this experiment, the chromosome number was determined in meiosis. The buds on branches carrying large pollen grains had 36 chromosomes in the Pollen Mother Cells at prophase, and those on the branches with small pollen grains had 18 chromosomes.

Plants which developed large pollen grains (28-33μ) in all, or in almost all, flowers (some flowers with smaller pollen grains may have been missed) of the main inflorescence were considered to be tetraploids. Plants with smaller pollen grains (19-22μ) were considered to be diploids. Actually, all plants in the treated generation (C_0) are to some extent mixoploids, carrying both diploid and tetraploid tissues, but those plants which develop prevailingly a majority of diploid gametes in flowers of the main inflorescence may be considered tetraploids, for practical purposes, because they produce tetraploid progeny after being intercrossed (17).

The number of chloroplasts was counted in both guard cells of stomata in the epidermis of the lower surface of leaves. Leaves were collected from the same seed stalk from which the anthers were examined for the size of pollen grains. Chloroplasts were counted in 10 stomata cells (20 guard cells) of each plant. Silver nitrate (1%) was used for dyeing.

On the basis of average number of chloroplasts per stoma cell, plants were classified as falling into 1 of 3 groups as follows: 1) plants having the number of chloroplasts corresponding to diploids. Such plants had mostly 13, 14, 15 or 16 chloroplasts per stoma cell; 2) plants with a number of chloroplasts corresponding to tetraploids (24 or higher); and 3) plants with an intermediate number of chloroplasts (19 to 24). The last group might include diploid, as well as tetraploid plants, since the number of chloroplasts in this group does not give a clear indication as to the level of ploidy of individual plants. This group arose partly because of the chimeral nature of the C_0 plants. In some leaves stomata lying side by side were observed with number of plastids corresponding to diploids and tetraploids, or close to their limits.

Data concerning the number of chloroplasts in stomata cells of diploid and tetraploid plants are presented in the Tables 2 and 3. Photomicrographs of chloroplasts in stomata of diploid and tetraploid beets are presented (Figures 1 and 2).

Table 2.—Number of chloroplasts in stomata cells of diploid C_0 sugar beet plants.

Strain designation	Plants with number of chloroplasts		
	Corresponding to		Intermediate-Between Diploids and Tetraploids
	Diploids	Tetraploids	
537	17	4	2
201	5	5	3
171	8	13	3
127	1	15	1
509	3	3	4
573	43	45	7
202	23	19	1
Total	100	104	21
Percent	44.44	46.22	9.33

Table 3.—Number of chloroplasts in stomata cells of tetraploid C_0 sugar beets plants.

Strain designation	Plants with number of chloroplasts		
	Corresponding to		Intermediate-Between Diploids and Tetraploids
	Diploids	Tetraploids	
537	8	42	14
201	2	46	6
171	4	57	10
127	0	30	3
509	1	9	3
573	16	69	7
202	6	22	6
Total	37	275	49
Percent	10.2	76.2	13.6

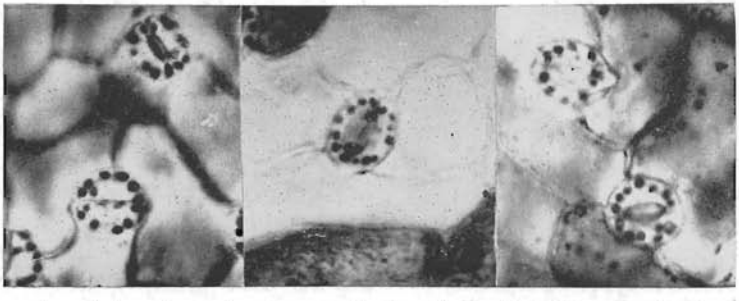

Figure 1.—Chloroplasts in stomata leaf cells in diploid sugar beets × 42.

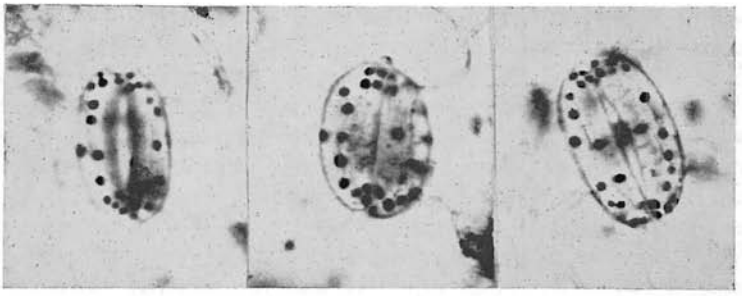

Figure 2.—Chloroplasts in stomata leaf cells in tetraploid sugar beets × 42.

Experimental Results

Only 44.4 percent of diploid plants (with small pollen grains) had the number of chloroplasts characteristic of diploids (Table 2). Forty-six percent (46.2%) of plants in this group showed chloroplast numbers corresponding to tetraploids and in 9.3% of plants the number of chloroplasts exceeded the number of chloroplasts peculiar to diploids. Thus, 55.5% of the diploid population had larger number of chloroplasts in leaves than should be present in diploids. This indicated that the epidermal tissue was affected by colchicine to a greater degree than the subepidermal tissue.

As a consequence, doubling of chromosomes occurred more often in the epidermis than in the tissues which produce sexual cells.

Among tetraploid plants (with large pollen grains) 76.2% of plants had the number of chloroplasts corresponding to tetraploids (Table 3). Only 10.2% had the number of chloroplasts corresponding to diploid plants. Also, 13.6% of the plants showed lower numbers than tetraploids had to have. Therefore, if selection of tetraploids had been made on the base of chloroplasts number, 23.8 percent of the tetraploid plants would have been discarded.

At the same time, the group of tetraploid plants, selected from the whole population (586 plants), would have consisted of 275 true tetraploids and 104 (27.4%) diploids. This group of diploid plants would very highly contaminate the tetraploid population. Instead of obtaining a C_1 progeny with a high percent of tetraploid plants, the C_1 generation will contain many, if not a majority, of diploid and triploid plants which would necessitate extensive screening to eliminate plants with undesirable chromosome numbers.

Leaf characteristics, such as size of stomata cells, number of chloroplasts, and even number of chromosomes, often used for selection of tetraploids, should be used in C_0 generation only for preliminary test and screening, if such a screening is really needed in some circumstances or climates. Final selection for tetraploids in the C_0 generation should be based on direct selection of diploid gametes which will produce a tetraploid progeny. The ploidy level of gametes is determined either by the size of pollen grains or by chromosome number in the pollen mother cells. This gamete selection is highly effective and reduces the work of controlling chromosome numbers in the following generations.

Conclusion

Selection for tetraploid plants in the treated mixoploid C_0 generation based on the leaf characteristics, number of chloroplasts, number of chromosomes, size of stomata, etc. is an unreliable method.

Selection for tetraploids in C_0 generation should be based on selection of diploid gametes (pollen grains) which will produce a tetraploid progeny.

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