Pollen Characteristics of Diploid and Tetraploid Sugarbeet[1]

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ABSTRACT

Pollen characteristics of diploid (2x) and autotetraploid (4x) inbred and heterozygous sugarbeet (*Beta vulgaris* L.) strains were measured to provide information necessary for the use of pollen as an experimental tissue. The viability of pollen from 2x and 4x plants was not different, as measured by a vital stain and by in vitro germination. The diameter of pollen from 2x plants (20.8 μm) was significantly smaller than from 4x plants (25.9 μm). There were significant differences among the seven strains which ranged from 19.3 to 22.5 μm for 2x strains and 23.4 to 27.4 for 4x strains. The average volume of x and 2x pollen (from 2x and 4x plants) was 4708 and 9090 μm^3, respectively. There were 413 X 10^3 x pollen grains per mg and 228 X 10^3 2x pollen grains per mg. The density of x and 2x pollen was 563 X 10^{-12} and 518 X 10^{-12} mg/μm^3, respectively. Pollen grains per anther varied significantly within flowers in most 2x and 4x strains, but the average number of pollen grains per anther was relatively constant from plant to plant within a strain. In five of seven strains, 2x plants had fewer pollen grains per anther than 4x plants. It was estimated that multigerm 2x and 4x plants grown in the field without competition produced about 3.9 X 10^9 and 4.9 x 10^9 pollen grains, respectively. Monogerm 2x and 4x plants produced about 1.5 X 10^9 and 1.9 X 10^9 pollen grains per plant, respectively. When 2x male sterile plants had equal opportunity to be fertilized by x and 2x pollen, the x pollen effected fertilization 89% of the time. This study provides new information about sugarbeet pollen that may facilitate research on population dynamics and in vitro pollen studies.

Additional Key Words: *Beta vulgaris* L., anther, fertilization

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Pollen, which transmits half the nuclear genetic material in sexual reproduction, is being recognized as a potentially important research tissue in genetics, breeding, physiology, and germplasm preservation. In some instances, pollen can be manipulated and utilized somewhat like microorganisms. Assays of pollen characteristics potentially can provide genetic information about the plant that produced the pollen (10, 11, 14). Challenge and selection in pollen has been demonstrated to effect change in the sporophyte (15). Pollen is an ideal tissue for some physiological studies, and can be stored for long periods as a means of preserving plant genetic resources (6, 12).

Relatively little research has been done with sugarbeet (*Beta vulgaris* L.) pollen as an experimental tissue. Consequently, knowledge about sugarbeet pollen and techniques for utilizing it are limited. Artschwager and Starrett in 1933 (2) described sugarbeet pollen as having three nuclei, a tube nucleus and two generative nuclei, each with a plasma membrane that encloses cytoplasm and some cytoplasmic inclusions. Hence, sugarbeet pollen is best described as being tricellular. Sugarbeet pollen is relatively short-lived in nature (1), however, it can be stored frozen for long periods (6). Various techniques for in vitro germination of sugarbeet pollen have been reported by a number of authors, most recently reviewed and expanded by Hecker and McClintock (5). They reported their best germinations in a sucrose, boron, and calcium liquid medium. Fresh humidified pollen germinated as much as 82%, but germinations were usually 30 to 50% for pollen collected in the greenhouse in mass from anthers that had dehisced during the previous 1 to 5 days.

The large scale commercial usage of triploid (3x) sugarbeet hybrids, usually produced by pollinating cytoplasmic male sterile (CMS) diploid (2x) plants with autotetraploids (4x), creates a need for pollen information about tetraploids. Jassem (7) reported no difference in percentage germination between pollen from diploid ($2n = 2x = 18$) and tetraploid ($2n = 4x = 36$) plants, but he reported that more rapid tube growth occurred from 2x pollen produced by tetraploid plants.

The purpose of this research was to develop additional information about pollen parameters of 2x and equivalent autotetraploid 4x sugarbeets, and to compare pollen parameters of homozygous and heterozygous strains of 2x and 4x sugarbeet.

MATERIALS AND METHODS

Seven diploid sugarbeet strains, consisting of two self-fertile inbreds (52-305 and NB1) and five heterogeneous strains, and their colchicine-converted 4x equivalent strains, were photothermally induced and grown through flowering in the greenhouse from September through February at Fort Collins, Colorado. The 2x and 4x lines within strains were grown at the same time and under the same conditions. Strains were grown at different times. Genetic equivalence of the 2x and 4x lines within each strain was the goal when the conversions from 2x to 4x were made. At least 25 plants were used to generate

each 4x strain. Phenotypically the 2x and the 4x lines within each strain appeared the same, except for the typically rounded leaf shape of the 4x plants. All the 4x strains used were three or more generations after colchicine treatment.

Sixteen plants of each 2x and 4x strain (16 X 7 X 2 = 224 total plants) were used as sources for pollen and anther measurements. Pollen viability comparisons were made using 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT) as a viability indicator (4). In vitro pollen germinations were made in the liquid medium described by Hecker and McClintock (5), except that the pollen was not hydrated prior to germination. The benefits of hydration had not been determined at the time this study was conducted. Pollen was considered germinated if the tube length was at least one pollen diameter. A mix of pollen from 16 plants was used for vital stain tests, in vitro germination, diameter, and weight determinations of each 2x and 4x line within each strain.

After pollen viability determinations, pollen from each 2x-4x pair of the seven sources was used to pollinate four to eight 2x CMS plants from each of two strains (52-305 CMS and FC 607 CMS). Pollen from 2x and 4x plants within a strain was weighed and then quantitatively mixed in proportion to the in vitro viability of each pollen source. The mixed pollen was dispensed onto bagged CMS plants with an air bulb dry atomizing apparatus. The number of CMS plants used (four to eight) and the number of times each CMS plant was pollinated (one to five) was a function of the available pollen quantity and its quality.

The number of pollen grains per anther was determined by macerating hemocytometer. Pollen per anther was determined in five anthers from four to eight flowers on three plants of each of the 14 sources. Pollen diameter was measured with an internal microscope scale at X594. Pollen density is the reciprocal of number of pollen grains per mg divided by volume. Chromosome counts were made by the acetocarmine squash method (13) in random root tips of about 100 germinated seeds that resulted from each of the 2x CMS X 2x and 4x crosses. Completely random designs with two replications were used for all experiments, except chromosome counts. The number of pollen grains counted or measured per replication was 300 for MTT stain, 300 for germination, and 50 for pollen diameter. For pollen per anther, the counts from 25 hemocytometer grids included a total of 16 to 684 pollen grains. For pollen per mg, the 25 grids included 98 to 1348 pollen grains.

RESULTS AND DISCUSSION

Analyses of variance of four pollen parameters are shown in Table 1. The two viability measures, MTT stain and in vitro germination, showed no differences between 2x and 4x plants and no strain differences for in vitro germination, but there were strain differences for amount of MTT-stained pollen. FC 703 produced the least MTT-stained pollen (35%), while FC 607 pollen was 86%

stained (Table 2). The differences among strains may have been due to the strain or culture, or both, since the strains were not grown at the same time, although they were grown in similar wintertime greenhouse conditions. Previous research has shown that pollen viability is affected by season and cultural condition (3, 5). Viability differences among strains were not detected by the pollen germination tests. This may have been due to incomplete germination, because the pollen was not humidified, or lack of a close relationship between germination and vital staining, or both. The significant strain ploidy interactions resulted from staining and germination differences between ploidy levels within specific strains. However, across all strains there was no evidence that pollen quality was related to ploidy level.

Pollen diameter was different among ploidy levels and strains. The mean for x pollen from 2x plants was 20.8 μm and for 2x pollen from 4x plants was 25.9 (Table 2). The mean diameter range of the x pollen was between 19.3 μm for FC 701/4 and 22.5 μm for NB1 (Table 2). The average diameter of the smallest 2x pollen, strain FC 701/4 (4x), at 23.4 μm was not significantly larger than the largest x pollen from NB1 (2x) at 22.5 μm. However, the diameter difference of x and 2x pollen within strains was significant in every case ($P < 0.05$), the pollen of 4x plants being larger. Hence, pollen diameter apparently differentiates diploids and tetraploids within strains grown under the same cultural conditions, but diameter cannot be used to positively identify genetically different 2x and 4x sugarbeets of different strains grown under different conditions.

Table 1. Analyses of variance of pollen parameters for diploid and tetraploid plants within seven sugarbeet strains.

Source of variation	df	MTT stained	In vitro germination	Pollen diameter	Pollen grains per mg
Strain	6	*	NS	**	NS
Ploidy	1	NS	NS	**	*
S X P	6	**	**	—	**

*, ** P = 0.05 and 0.01, respectively.

The number of pollen grains per mg was different for the two ploidy levels, averaging 413,179 for 2x plants and 228,107 for 4x, but there were no differences among strains within ploidy groups. The strain X ploidy interaction, although significant, resulted from differences in relative magnitude, not from any switches of 2x and 4x means within strains. This character was measured with less precision than diameter.

Pollen volume was a function of diameter, hence, volume data were not analyzed, but the means are listed in Table 2. The pollen of the seven 4x strains had 77 to 116% larger volumes than pollen from the 2x strains. The two inbreds in this study (52-305 and NB1) had significantly larger pollen ($P < 0.01$) than the five heterogeneous strains for both 2x and 4x. The reduced

plant vigor due to inbreeding obviously did not reduce pollen size. However, from these limited data it cannot be concluded that inbreeding increases pollen size.

Table 2. Means for vital staining (MTT), germination in vitro, diameter, volume, grains/mg, and density of pollen for seven strains of diploid (2x) and tetraploid (4x) sugarbeet.

Sugarbeet line or group mean	MTT stained %	Germinated %	Diameter μm	Volume μm^3	Number per mg (X 103)	Pollen density mg/μm^3 (X 10^{-12})
FC 702/4 (2x)	58	4	19.9	4124	606	400
FC 702/4 (4x)	39	8	25.6	8780	278	410
FC 703 (2x)	37	1	20.8	4709	564	377
C 703 (4x)	33	5	26.3	9520	466	225
52-305 (2x)	56	27	21.4	5129	418	466
52-305 (4x)	54	12	26.5	9739	215	478
FC 607 (2x)	92	9	20.1	4250	390	603
FC 607 (4x)	80	12	26.0	9198	153	718
NB1 (2x)	44	8	22.5	5961	212	791
NB1 (4x)	58	6	27.4	10765	156	595
FC 701/4 (2x)	48	2	19.3	3762	374	711
FC 701/4 (4x)	62	3	23.4	6705	227	657
FC 606 (2x)	85	8	21.4	5129	328	594
FC 606 (4x)	73	15	25.9	9092	202	544
Inbreds (2x)	50	18	21.9	5497	315	628
Inbreds (4x)	56	9	56.9	10187	186	536
Heterozygotes (2x)	64	5	20.8	4378	452	537
Heterozygotes (4x)	57	9	25.9	8576	265	511
Mean (2x)	60	8	20.8	4708	413	563
Mean (4x)	57	9	25.9	9090	242	518

Pollen density was a function of mass and volume, and was not included among the analyses of variance. Hence, there were no errors to test the slightly higher densities of the 2x and inbred plants (Table 2).

The determinations of pollen grains per anther within flower and plant were analyzed (Table 3). Within flowers there were significant differences for the number of pollen grains from anther to anther in six of the seven 2x strains and five of the seven 4x strains. Hence, within most beet flowers the pollen grains per anther varied a great deal. However, at the flower level, this variability was reduced so that only three 2x strains and one 4x strain showed significant differences from flower to flower. At the plant level there were even fewer differences within strains. Hence, using photothermally induced seedlings, brought to flower in the greenhouse, the average number of pollen grains per anther was relatively constant from plant to plant within a strain. This was true in both diploids and tetraploids, and in inbreds and heterogeneous strains. From strain to strain, however, there were considerable differences among pollen grains per anther (Table 4). It is unlikely that these were cultural differences, even though the strains flowered at different times. In four strains, tetraploids produced more pollen per anther than diploids, in two strains diploids produced more, and in one strain there was no difference. Since pollen from 4x plants is almost twice as large volumetrically as that from 2x plants, the anthers of some 4x strains must be more than twice as large as anthers of the diploid. It is unlikely that pollen in the 4x anthers is packed more densely.

Table 3. Analyses of variance of pollen grains per anther, within anther, flower, and plant for diploids (2x) and tetraploids (4x) within seven sugarbeet strains.

Strain	Anthers w/in fl. w/in pl.		Flow. w/in plant		Plant	
	2x	4x	2x	4x	2x	4x
FC 702/4	NS	**	**	NS	NS	NS
FC 703	**	NS	*	*	NS	NS
52-305	**	**	NS	NS	NS	NS
FC 607	*	NS	NS	NS	NS	NS
NB1	**	**	NS	NS	**	NS
FC 701/4	**	**	*	NS	NS	**
FC 606	**	**	NS	NS	NS	NS

*, ** P = 0.05 and 0.01, respectively.

Table 4. Mean number of pollen grains per anther in seven strains of 2x and 4x sugarbeet.

Sugarbeet strain	Pollen grains per anther		sig. of 2x-4x difference
	2x	4x	
FC 702/4	39,889 ± 1665	46,100 ± 1286	**
FC 703	50,759 ± 1456	46,567 ± 1484	*
52-305	48,636 ± 1430	51,811 ± 1812	NS
FC 607	63,113 ± 2476	97,442 ± 3924	**
NB1	16,700 ± 571	14,619 ± 521	**
FC 701/4	36,472 ± 913	58,969 ± 1934	**
FC 606	66,972 ± 1970	90,534 ± 2206	**
Mean	46,077 ± 593	58,006 ± 765	**

*, ** P = 0.05 and 0.01, respectively.

In a separate study it was determined that the typical densely flowered heterozygous field-grown plant in Colorado, from a transplanted mother root, has about 17,000 flowers, if multigerm (two or more flowers per cluster), and 6,600 flowers if it is a densely flowered monogerm (one flower per cluster). Among large plants, grown without competition, no consistent differences in flower number were found between 2x and 4x plants. Using these data, the data from Table 4, and assuming five anthers per flower, the average multigerm plant grown without competition under relatively ideal field conditions should produce about 3.9×10^9 pollen grains as a diploid and 4.9×10^9 as a tetraploid. This assumes pollen per anther is the same in the field and greenhouse. Monogerms should produce about 1.5×10^9 and 1.9×10^9 pollen grains per diploid and tetraploid plant, respectively. Magassay (9) had previously estimated that each plant, presumably field-grown multigerm, produced about 10^9 pollen grains. My calculations indicate that four or five times that many may be produced. Photothermally induced multigerm seedlings of heterogeneous lines grown in a greenhouse produced about 850 flowers per plant, hence, about 2×10^8 pollen grains per diploid plant and 2.5×10^8 per tetraploid plant.

The frequencies of 2x and 3x progeny from 2x CMS plants pollinated by x and 2x pollen are shown in Table 5. Among six strains the frequency of 2x progeny ranged from 70 to 98%. The frequency across all six averaged 89% 2x progeny. NB1 failed to effect any fertilization, either as a diploid or a tetraploid. NB1 is a very low vigor S_{20} inbred. Pollen production was poor but the total absence of fertilization is unexplained.

The deviation from an expected frequency of 50% 2x progeny cannot be explained from the evidence presented here, namely, that tetraploids produce as much or more pollen as diploids, that pollen from each is of approximately equal viability, and, from Jassem (7), that pollen from 2x and 4x plants has the same distribution frequency and wind-borne range, in spite of differences in weight and volume. The frequency of successful fertilization by x pollen (89%) is about the same as reported in a field study by Lasa et al. (8) where they found that x pollen in competition with 2x pollen was successful in fertilizing 2x females 89.5% of the time and 4x females 89%. Haploid pollen, the natural condition in this normally diploid species, apparently has an advantage in effecting fertilization. It is unlikely that embryo abortion caused the large deviation from 50% because there were very few flowers, open at pollination, that failed to develop a seed. This advantage means that a 4x population would be converted to 2x in a few generations in the presence of even a small percentage of contamination with pollen from diploids.

Table 5. Numbers of diploid (2x) and triploid (3x) progeny that resulted from pollinations of 2x CMS plants with pollen from 2x and 4x plants, pollen mixed in equal proportions based on in vitro viability tests.

Pollinator	Number and (%) of progeny	
	2x	3x
FC 702/4	85 (85)	15 (15)
FC 703	97 (97)	3 (3)
52-305	93 (79)	24
(21)		
FC 607	70 (81)	16 (19)
NB1	0	0
FC 701/4	94 (94)	6 (6)
FC 606	98 (98)	2 (2)
Mean	(89)	(11)

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