A STUDY OF THE INHERITANCE AND EXPRESSIVITY OF SOME SEEDLING CHARACTERS IN THE SUNFLOWER, HELIANTHUS ANNUUS L.

by

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ABSTRACT

First generation hybrids of sunflowers have been in use for a number of years in Manitoba. In order to make possible a rapid estimate of the percentage of hybrids in seedlots, seedling markers would be valuable. The inheritance and applicability of six seedling characters were studied with this in view.

Red hypocotyl was found to be controlled by a single dominant gene but fairly strong light was necessary for its expression. Spoon-shaped cotyledon, dwarf plant type and two virescents were all found to be recessive to their normal alleles and inherited on a mono-factorial basis. The two virescents were shown to be conditioned by separate genes. Leaf serration was found to be controlled by two or more genes which lacked dominance.

Red hypocotyl showed the most promise for use as a seedling marker. Both spoon-shaped cotyledon and dwarf plant type could probably also be used successfully. These characters are simply inherited, distinctive and apparently not detrimental to the yield of the plant.

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INTRODUCTION

The cultivated sunflower, Helianthus annuus ssp. annuus var. macrocarpus (DC) CKll, (3) is an important crop plant in many parts of the world. Russia was the first country to grow sunflowers on a large scale and the U.S.S.R. is at present the world's largest producer. (4). Argentine, the second largest producer, has grown sunflowers since 1870. Other countries producing sunflowers on a considerable scale are: Canada, Uruguay, Peru, United States, Rhodesia, Great Britain, several countries of central Europe and a number of countries in the Far East. Sunflower production is still spreading rapidly because of the increasing demand for the type of edible oil it furnishes and because it is adaptable to many ecological environments.

Production of sunflowers in Manitoba began in 1943 when about 2500 acres were grown. The annual average acreage for the period 1947-1956 was 22,500 acres (10). Most of this acreage was seeded to Advance, a first generation hybrid variety. It has been replaced to some extent by Beacon, a rust resistant synthetic variety, first grown commercially in 1955.

The Advance hybrid is a cross of an inbred line S-37-388, and an open pollinated variety, Sunrise. The crossing blocks for commercial seed production are seeded so that two adjacent rows of female (S-37-388) alternate with two adjacent rows of male (Sunrise). Only S-37-388 is harvested to provide the "hybrid" seed. Cross pollination is effected mainly by insects with bees responsible for most of the pollinating. Emasculation of the female rows is not practicable. Poor coincidence of flowering, adverse weather conditions for insects and other factors of

environment often result in seedlots in which the percentage of hybrid seed is low (sometimes nearly zero). Therefore, an appropriate seedling character which could be incorporated readily into one of the parents of future first generation single or top cross hybrids would be valuable in indicating the proportion of hybridization in specific seedlots.

A number of distinctive seedling characters have been isolated. The purpose of this investigation is to determine the inheritance of these seedling characters; to obtain information concerning any linkage which may exist between the genes conditioning their inheritance and to study their applicability as seedling markers in hybrid sunflower production.

LITERATURE REVIEW

The basic chromosome number of the genus Helianthus is n = 17. Most of the species which have been described are diploids with 17 pairs of chromosomes, but H. grosseserratus has apparently only 16 pairs. A number of tetraploid and hexaploid species also occur in the Helianthus genus. The cultivated variety is a diploid with 17 pairs of chromosomes (2).

Relatively little is known about the inheritance of characteristics in the sunflower. One of the earliest reported cases of inheritance in this crop was by Shull (12) in 1908. Since this was shortly after the rediscovery of Mendel's laws of inheritance this type of information was of special interest. Shull studied branched stem in the sunflower versus unbranched stem. The branched plant had a corymbed appearance with a branch in the axil of each leaf on the main stem. His results showed that branched stem was dominant to unbranched stem and that it was inherited on a monofactorial basis. To designate the characters, he used DD for dominant and RR for recessive.

Shull (12) also studied disk color of the sunflower. A Giant Russian plant with a yellow disk was crossed to a "Western plant" with a purple disk. The F_2 segregated in a 3:1 ratio of purple disk to yellow disk and the backcross of the F_1 to a Giant Russian plant resulted in a 1:1 ratio. He concluded, therefore, that one gene with purple disk dominant explained these ratios.

The pigmentation of the seed coat of the sunflower was first studied by Satsyperov (11). He distinguished three genes controlling achene color: P controlling the development of a dark layer between the sclerenchyma and the subepidermal tissue; T controlling the presence of a water soluble black-violet pigment in the subepidermal tissue; and S controlling the presence of

pigment in the epidermis. Satsyperov concluded that the color of each layer was controlled by a single dominant gene and that these three genes were not linked.

Kuptsov reported a unisexual female sunflower in 1935. (5). Bagged inflorescences of this plant did not set seed but seed was produced when pollen from other plants was applied. The pollen sacs did not swell and the stamen filaments elongated less than in normal plants. Kuptsov found that this character was controlled by one recessive gene. He concluded that this character could be of value in crossing sunflowers since emasculation was not necessary.

Putt (7) in 1940 studied the inheritance of branched stem and the color of the third layer of the seed coat, the latter corresponding to Satsyperov's P gene. The branched inbred line had branches in the axils of the leaves all the way up the stem except for the top two or three axils, quite similar to the type of branching studied by Shull (12). Frost occurred while the segregating generations were in the field so that segregation for this character was also observed. An inbred line having the characteristics Single stem (si), Black and White striped seed (BW) and Frost resistant (FR) was crossed to one with a branched stem (Br), Black and gray striped seed (Bg) and showing Frost susceptibility. The Black stripes occurred in the epidermal layer and the white or gray color occurred in the third layer showing through the upper two layers. The pigmentation of the third layer was shown to be controlled by one gene with gray being dominant to white. Putt's work also showed that branched stem was dominant to single stem and gave a one-factor ratio in F_2. Frost resistance was shown to be dominant to frost susceptibility and this character also showed monogenic inheritance. No linkage was found to exist between the genes controlling the three characters studied.

Bottazzi (1) studied the distribution of anthocyanin pigments in the sunflower plant. He showed that there was a positive correlation between the presence of pigment in the petioles and in the parts of the reproductive organs. He explained his results by postulating one gene subject to the action of modifying factors.

Wallace, Bushnell and Newcomer (14) showed that mutations could be induced by ultrasonic waves. This method was used by Wallace and Schwarting (15) to treat two-day-old sunflower seedlings. The treated plants when crossed produced normal F_1 plants. One of the F_2 families contained a number of yellow plants. These plants were bright yellow and contained little or no chlorophyll. The yellow plants bred true until the F_5 when a pure white mutant was found. This second mutant also bred true, was recessive to normal green and the expression of this character was controlled by a single gene. The white mutant produced green color under controlled conditions. The production of chlorophyll was found to be greatest between 0.5 and 0.25 foot candles. At light intensities greater than 0.5 foot candles it produced progressively less chlorophyll until at 10 foot candles the plants were pure white. Below 0.25 foot candles the plants also produced less chlorophyll.

The white mutant contained no carotinoids and at 0.5 foot candles produced less protochlorophyll and chlorophyll a and b than the normal sib, although the relative proportions of each type of chlorophyll were the same. These plants were self-fertile and exhibited no dormancy. The mutant could be propagated quite readily by grafting it into normal stocks at the one-day-old stage. The authors suggested it would be valuable in chlorophyll studies.

The inheritance of resistance to sunflower rust (Puccinia helianthil.)

was studied by Putt and Rojas M. (9). The source of resistance used was an inbred line, 953-102-1-1-22, derived from the Texas Wild Annual sunflower. Six inbred lines showing varying degrees of susceptibility were crossed with the rust resistant line. The F_2 and F_4 populations were grown in the field at Morden, Manitoba, and the F_3 at La Molina, Peru, also in the field. Local collections of urediospores, from the respective countries, were used for inoculum. The F_1 plants were resistant, showing that rust resistance was dominant to susceptibility. The segregating progenies gave monofactorial ratios. Therefore, the resistance was controlled by a single dominant gene.

Stefansson (13) discovered a method for estimating the percentage of hybrids in seedlots of first generation Advance sunflowers based on a seedling character. Advance is a hybrid produced by a cross of the inbred line, S-37-388, by the variety Sunrise, with the inbred being used as the female. The leaf venation of S-37-388 is considerably more reticulate than that of the variety Sunrise. Advance or the F_1 has venation approximately midway between the two parents. Using the venation as a guide it was relatively simple and fast to pick out the crossed plants grown from a seedlot, thus determining the percentage of hybrids. But since neither parent had been selected for uniform type of venation, the variability within the parents prevented complete accuracy of classification. However, in four different seed lots classified by the seedling character and on the basis of mature plant characters the discrepancy between the two systems was never greater than 4.4%.

MATERIALS and METHODS

The seedling markers used in this study were obtained from B. R. Stefansson, who selected them out of large populations of sunflower lines and varieties. The material selected had been selfed to get true-breeding lines which expressed the characters used in this study.

The inheritance study of the six characters selected was begun at the Canada Agriculture, Experimental Farm, Morden, Manitoba, in 1956. The lines procured from Mr. Stefansson had been selfed for four generations and the fifth generation was used to make the crosses for the genetic study. Approximately 100 lines of this material were sown in flats in the greenhouse in April to check the uniformity of the lines and the clarity of character expression. The lines which appeared true-breeding and best expressed the character concerned were selected and remnant seed from these lines was used for the field crossing plot. Several inbred lines from the Morden breeding program were examined in the greenhouse to provide a "normal" line in use in crosses with the seedling marker lines. Inbred CM39 was selected because it best expressed the opposite character of each of the seedling markers.

Table 1 gives the source from which the lines used were selected. A description of the seedling markers follows:

Red hypocotyl - The hypocotyl is a deep reddish color as opposed to the green color of normal plants, (Figure 1).

Serrated leaves - The first true leaves and succeeding ones have deeply serrated edges compared to nearly entire edges of some other lines, (Figure II).

Spoon-shaped - The cotyledons of this line appear spoon-shaped due to the fact that their edges are curled upwards, (Figure III).

Dwarf Plant - The internodes of this line are shortened to about half the length of normal internodes, which gives the plants a dwarf appearance, (Figure IV).

Virescent-1 - The cotyledons and leaves of this line are a yellowish green with small flecks of darker green in the leaves. The plants were reduced in size, (Figures V and VII).

Virescent-2 - The cotyledons and young leaves are light green with yellowish green in the basal portion of the newly developing leaves forming a yellowish rosette around the top of the young plant. No flecking is present, (Figures VI and VIII).

Normal - This rust resistant line has dark green color, flat cotyledons, nearly entire leaves, green hypocotyl and is about twice as tall as the dwarf line.

Table 1. Source of the selected seedling markers.

Character Expressed	Manitoba Variety Number	Source
Red hypocotyl	75-4	C.V.O. 10 x Rust resistant
Serrated leaves	110-2	47/154
Spoon-shaped cotyledon	170-2	317/4-21-1
Dwarf Plant	77AB	Rust resistant x Sunrise[2]
Virescent-1	34-1	C.V.O. 77-2-3
Virescent-2	163	260/vir.277
Normal	CM39	Rust resistant hybrid

Figure 1. Comparison of red versus green hypocotyls in the cotyledon and two-leaf stages. On left, line 75-4; on right, line CM39.

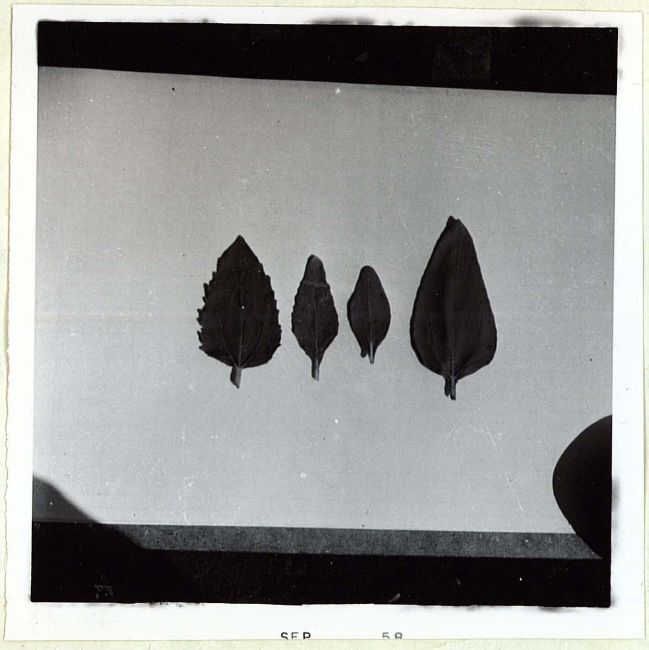


Figure 11. Comparison of serrated versus entire leaves showing the first and second true leaves. On left, line 110-2; on right, line CM39.

Figure III. Plants from line 170-2 showing the spoon-shaped appearance of the cotyledons.

Figure IV. Comparison of normal versus dwarf plants in the cotyledon and two-leaf stages. On left, line CM39; on right line 77AB.

Figure V. Comparison of normal versus virescent-1 cotyledons under greenhouse conditions. Top row, line CM39; bottom row, line 34-1.

Figure VI. Comparison of normal versus virescent-2 cotyledons under greenhouse conditions. Top row, line CM39; bottom row, line 163.

Figure VII. Comparison of normal versus virescent-1 plants under field conditions. On left, line CM39; on right, line 34-1.

Figure VIII. Comparison of normal versus virescent-2 plants under field conditions. Extreme left, line CM39; extreme right, line 163.

The crosses planned were as follows:

1. A cross of line 75-4 (red hypocotyl) and line CM 39 (green hypocotyl).

2. Diallel crosses among the following lines:

110-2	Serrated leaves
170-2	Spoon-shaped cotyledons
77AB	Dwarf Plant
34-1	Virescent-1
163	Virescent-2
CM39	Normal

3. Backcrosses of the F_1 to the recessive parent wherever this was possible.

The original crosses were all made in the field. The parent lines were seeded in single rows of fifteen hills each at weekly intervals on three successive dates. All rows were thinned to one plant per hill, leaving the plants which best expressed the desired character. Based on prior information from preliminary crosses by Mr. Stefansson it was assumed that all the characters, except red hypocotyl, were recessive to their normal counterparts. The recessive line was, therefore, used as the female in each case. Pollen for each cross was taken from a single plant only. Emasculation was not done in the original crosses because it was felt that hybrid F_1 plants would be easy to identify using the criteria of the seedling markers and hybrid vigour.

All the crosses planned were attempted. Due to poor coincidence of flowering the numbers crossed were small and the proportion of selfed seed was high. Some crossed heads were lost due to damage by 2,4-D, insects and fungal diseases. The result was failure to obtain seed of some of the crosses planned.

Crosses virescent-1 x virescent-2 and spoon-shaped cotyledon x virescent -1 were made in the greenhouse at Morden in the winter of 1956-57 because the field crosses were destroyed.

The F_1 generation was grown in the field at Morden in single rows in 1957. Parental material of each character was seeded on four different dates at

at weekly intervals to provide material for checks and to make the backcrosses. All backcrosses were made with the aid of emasculation. The technique of emasculation employed was similar to that described by Putt in 1941 (8). In most cases an F_1 plant was used as the male parent to conserve F_2 progenies. In the cross 77AB x CM39 each of the F_1 plants had several large branches which developed quite large heads. The heads on the branches were used as the female in the backcross so that the same plant produced both backcross and F_2 generation seed.

In order to effect selfing in the F_1 and parental material the heads were covered with paper or fine mesh cotton bags just before the flowers opened. For corssing, only paper bags were used.

The F_2 progenies were grown in the greenhouse at the University of Manitoba in the winter of 1957-58 and also in the field at Morden in 1958. In the greenhouse the plants were grown in flats containing 3-4 inches of soil. Parental material was grown with all the F_2 progenies to provide material for checking. The seedlings were allowed to grow for one or two weeks and then they were classified. A number of recessive plants from the F_2 generation were grown to maturity and the F_3 progenies grown in the field at Morden in 1958 to ascertain whether or not further segregation would occur.

The only artificial light used in the greenhouse at the University was from three 200-watt bulbs ten feet apart and five feet above the seedlings. This intensity of light would have very little effect so that it would be more accurate to say that no artificial light was used.

To determine if light materially affected the red hypocotyl character a small experiment was set up in the greenhouse at Morden in July of 1958. Red hypocotyl (75-4) and green hypocotyl (CM39) were used. The sunflowers were seeded in four-inch pots at the rate of ten seeds per pot. One pot of CM39 and two pots of 75-4 were used in each treatment. Four treatments were employed as follows:

1. The pots were kept covered constantly so that the plants received no light.

2. The pots were kept uncovered from 8.00 a.m. to 12.00 noon to give the plants a four-hour day.
3. The pots were uncovered from 8.00 a.m. to 5.00 p.m. to give the plants a nine-hour day.
4. No covers were used so that these plants had a daylength of approximately sixteen hours of good light.

The covers used to shut out light were four-inch pots with the drain hole plugged with cork stoppers, and inverted on the pot containing the seedlings. A negligible amount of light may have seeped in at the juncture of the pots. Pictures of the hypoctyls were taken on the fifth day after emergence (Figure IX). The covers were then all removed and pictures again taken three days later (Figure X).

EXPERIMENTAL RESULTS

Red Hypocotyl

The F_1 plants of the cross of plants with green hypocotyls by the plants with red hypocotyls all had red hypocotyls showing that red was dominant to green color.

The coloration of the plants with red hypocotyls showed considerable variation in F_2. Some were quite dark while others had only a trace of color. All the plants which had any red color were placed in the red class.

Seven F_1 plants were used to produce the F_2 populations. Table 2 shows the results obtained with 608 plants grown in the greenhouse at the University in November. The segregation of red and green hypocotyl resulted in a close fit to a 3:1 ratio respectively. The data also showed good homogeneity according to the chi-square test for heterogeneity.

Table 3 shows the results obtained with 625 plants from the same seven families as shown in Table 2 but grown in December. Here the data gave a poor fit to a 3:1 ratio of red and green hypocotyl respectively although the homogeneity was satisfactory. The recessive class was considerably larger than required for a 3:1 ratio.

A third sample from the same seven families was grown in the field in June of 1958. Table 4 shows that the 753 plants classified gave a good fit to a 3:1 ratio of red to green hypocotyls. The chi-square test for heterogeneity showed a high probability value for uniformity.

Table 5 shows the backcross data from the cross CM39 x (CM39 x 75-4). The data showed a satisfactory fit to a 1:1 ratio and the variation between families was not serious as shown by the test for heterogeneity.

Since a 13:3 ratio in F_2 could be mistaken for a 3:1 ratio several recessive F_2 plants were selfed to check whether further segregation would occur. Four F_3 progenies ranging from 27-49 plants were produced from F_2 green hypocotyl plants. Absence of segregation provided support for the one-

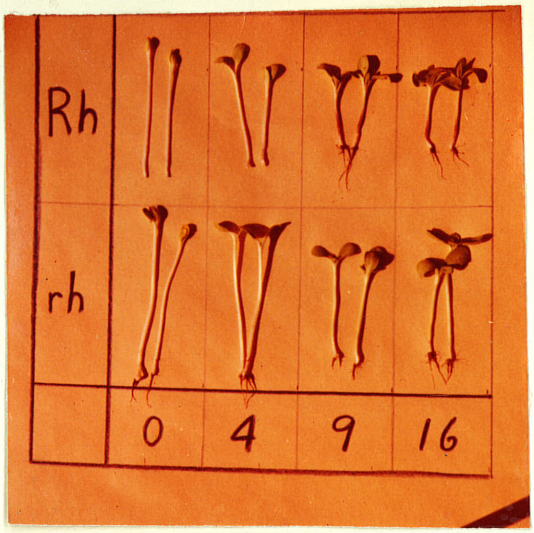
factor hypothesis.

In the study of the effect of light on red hypocotyl, treatment (1) with no light produced etiolated seedlings with no red color in the 75-4 plants and no green color in the CM39 plants. The four-hour light treatment produced a trace of red color around the base of the red hypocotyl plants and pale green coloration of the green hypocotyl line. The nine-hour light treatment produced plants in which the red color developed to an intensity just detectably less than that produced by the plants which were uncovered. Development of the dark green color in the CM39 plants in the nine and sixteen-hour treatments was also approximately equal while the four-hour treatment produced only pale green hypocotyls (Figure IX).

Three days after the covers had all been removed the 75-4 plants of the zero and four-hour light treatment had also produced red color. In these plants the red color was as dark as that produced by the sixteen-hour light treatment and it extended up the stem to the base of the cotyledons (see Figure X).

75-4

CM39



Hours of light per day

Figure IX. Effect of various amounts of light on red (75-4) and green (CM39) hypocotyls. Picture taken five days after emergence.

75-4

CM39

Hours of light per day

Figure X. Effect of 16 hours of light per day, after three days, on plants which had been treated as in Figure IX for five days. Picture taken eight days after emergence.

Table 2. Summary of data from the F_2 generation of the cross CM39 x 75-4 showing segregation for red versus green hypocotyl. November, 1957.

Progeny	Total	Phenotype		Chi-square 3:1	Probability
		Red	Green		
3050-1	102	78	24	0.117	.70-.80
" -2	86	63	23	0.387	.50-.70
" -3	92	66	26	0.522	.30-.50
" -4	86	65	21	0.015	.90-.95
" -5	98	79	19	1.648	.10-.20
" -6	68	51	17	0.000	1.0
" -8	76	63	13	2.526	.10-.20
Totals	608	465	143	0.071	.70-.80

Chi-square for heterogeneity = 5.144

P value for heterogeneity = .50-.70

Table 3. Summary of data from the F_2 generation of the segregation for red versus green hypocotyl. December, 1957

Progeny	Total	Phenotype		Chi-square 3:1	Probability
		Red	Green		
3050-1	103	71	32	2.023	.10-.20
" -2	90	59	31	4.2804	.02-.05
" -3	90	68	22	0.0148	.90-.95
" -4	81	61	20	0.0041	.90-.95
" -5	75	46	29	7.4707	<.01
" -6	89	64	25	0.4532	.50-.70
" -8	97	69	28	0.7732	.30-.50
Totals	625	438	187	7.215	<.01

Chi-square for heterogeneity = 6.9403

P value for heterogeneity = .30-.50

Table 4. Summary of data from the F_2 generation of the cross CM39 x 75-4 showing segregation for red versus green hypocotyl. June, 1958.

Progeny	Total	Phenotype		Chi-square 3:1	Probability
		Red	Green		
3050-1	116	87	29	0.000	1.0
" -2	106	74	32	1.522	.20-.30
" -3	107	81	26	0.028	.70-.80
" -4	97	77	20	0.993	.30-.50
" -5	98	78	20	1.102	.20-.30
" -6	108	79	29	0.198	.50-.70
" -8	121	96	25	1.215	.20-.30
Totals	753	572	181	0.372	.50-.70

Chi-square for heterogeneity = 4.685

P value for heterogeneity = .50-.70

Table 5. Summary of data from the backcross CM39 x (CM39 x 75-4) Showing segregation for red versus green hypocotyl. December 1957.

Progeny	Total	Phenotype		Chi-square 1:1	Probability
		Red	Green		
3036-3	21	13	8	1.190	.20-.30
" -8	18	9	9	0.000	1.0
" -6	60	31	29	0.666	.30-.50
" -7	62	27	35	1.032	.30-.50
Totals	161	80	81	0.006	.90-.95

Chi-square for heterogeneity = 2.873

P value for heterogeneity = .30-.50

Dwarf Plant

Information regarding the dwarf plant character was derived from crosses 77AB x CM39 and 77AB x 170-2 (spoon-shaped) and the backcross of 77AB x CM39 to 77AB.

The F_1 plants in both crosses were tall, showing that dwarf was recessive to the tall plant type.

Table 6 presents the data from the F_2 generation of the cross 77AB x CM39 showing that the plants segregated for a good fit to a ratio of three tall plants to one dwarf. The data from the various families were quite uniform as shown by the P value for heterogeneity. Apparently dwarf was controlled by one gene.

The data from the backcross are shown in Table 7. The variation of individual progenies was not serious and the fit to a 1:1 ratio of tall to dwarf plants was excellent.

Table 8 shows the segregation of the dwarf character in a cross with the spoon-shaped cotyledon line. Considering the segregation for the dwarf only, there were 226 plants of the tall phenotype and 86 plants of the dwarf phenotype. The chi-square value for this cross shows an acceptable fit to a 3:1 ratio.

Several dwarf plants in the F_2 and backcross population were selfed to check the possibility of further segregation in the recessive class. Four F_3 progenies were produced from F_2 plants and four progenies from the backcross population. The F_3 populations ranged from 9 - 36 plants with the exception of one progeny in which only one seed germinated. No segregation occurred in any of the progenies, thus giving further evidence to support the conclusion that one gene controlled the inheritance of dwarf plant type.

Table 6. Summary of data from the F_2 generation of the cross 77AB x CM39 showing segregation for tall versus dwarf plant type.

Progeny	Total	Phenotype		Chi-square	Probability
		Tall	Dwarf	3:1	
3033-1	38	28	10	0.035	.80-.90
" -2	35	27	8	0.086	.70-.80
" -3	44	34	10	0.121	.70-.80
" -4	38	29	9	0.035	.80-.90
" -5	30	21	9	0.040	.80-.90
" -6	27	20	7	0.012	.90-.95
" -7	25	16	9	1.613	.20-.30
" -8	42	32	10	0.031	.80-.90
" -9	50	38	12	0.027	.80-.90
" -10	34	26	8	0.039	.80-.90
" -12	27	18	9	1.000	.30-.50
" -13	40	31	9	0.133	.70-.80
Totals	430	320	110	0.077	.70-.80

Chi-square for heterogeneity = 3.095

P value for heterogeneity = >.99

Table 7. Summary of data from the backcross (77AB x CM39) x 77AB showing segregation for tall versus dwarf plant type.

Progeny	Total	Phenotype		Chi-square 1:1	Probability
		Tall	Dwarf		
3033-2A	29	13	16	0.310	.50-.70
" -6A	26	13	13	0	1.0
" -7A	39	19	20	0.026	.90-.95
" -8A	5	3	2	0.200	.50-.70
" -10A	12	7	5	0.333	.50-.70
Totals	111	55	56	0.009	.95-.98

Chi-square of heterogeneity = .860

P value of heterogeneity = .95-.90

Table 8. Summary of data from the F_2 generation of the cross 77AB x 170-2 showing segregation for tall versus dwarf plant type and flat versus spoon-shaped cotyledons. November 1957.

	Phenotype						
	Tall		Dwarf			Chi-square	P
Progeny	Flat	Spoon	Flat	Spoon	Total	9:3:3:1	value
3043-1	47	11	11	4	72	2.025	.50-.70
" -2	41	3	17	3	64	6.777	.05-.10
" -3	41	7	15	3	66	9.542	.02-.05
" -4	30	10	16	5	61	2.913	.30-.50
" -5	33	3	12	0	48	8.250	.02-.05
	192	34	71	15	312	14.533	$<.01$
	226		86				

Chi-square for heterogeneity = 14.974

P value for heterogeneity = $<.01$

Chi-square for tall vs. dwarf = 1.094

P value for tall vs. dwarf = .20-.30

Chi-square for flat vs. spoon = 14.376

P value for flat vs. spoon = $<.01$

Chi-square for independence of spoon vs. dwarf = 0.119

P value for independence = .70 -.80

Spoon-shaped Cotyledons

The spoon-shaped cotyledon line was involved in three successful crosses and two backcrosses which were 170-2 x 163, 170-2 x 34-1, 170-2 x 110-2, (170-2 x 34-1) x 170-2 and 170-2 x (163 x 170-2). However, the F_2 generation of the cross, 163 x 170-2 was so badly damaged by hail that most of the cotyledons were destroyed or damaged so that classification for spoon-shaped was unreliable. The data for spoon-shaped are presented in Tables 8 to 11.

The F_1 plants in all crosses had flat cotyledons showing that spoon-shaped cotyledon was recessive to flat cotyledon.

Table 8 shows that F_2 data from the cross of dwarf by spoon-shaped lines. Two of the progenies showed a good fit to a 9:3:3:1 ration. The probability of a fit to a 9:3:3:1 ratio for the other three progenies was rather low. A low number of spoon-shaped plants was the main reason for the poor fit obtained for the totals. Classifying the data for normal versus spoon-shaped revealed that the data did not fit a 3:1 ratio. No evidence of linkage was revealed, as indicated by the Chi-square test for independance.

Table 9 presents the data from the cross of the spoon-shaped cotyledon line by the virescent-1 line classified for flat versus spoon-shaped cotyledon. The March data agreed well with a 3:1 ratio of flat to spoon-shaped cotyledons. However, a deficiency of spoon-shaped plants in the June data resulted in a probability for a fit, to a 3:1 ratio, of less than .01. The probability of a fit to a 3:1 ratio for the combined data was also less than .01 although only three progenies had a P value of less than .20.

The data from the backcross, shown in Table 10 were in close

agreement with the expected 1:1 ratio if a single gene for spoon-shaped were postulated.

Table 11 gives the results of the backcross with virescent-2 and the cross of spoon-shaped with the serrated-leaf line. The backcross data agreed closely with a 1:1 ratio of flat to spoon-shaped cotyledon. The F_2 data of spoon-shaped with the serrated line did not fit a 3:1 ratio. This can, however, be explained by the fact that the cotyledons of the serated line tend to curl upwards which would explain the higher proportion of spoon-shaped plants.

Table 9. Summary of data from the F_2 generation of the cross 170-2 x 34-1 showing segregation for flat versus spoon-shaped cotyledons. 1958.

Progeny	Phenotype: Flat March	Flat June	Flat Total	Spoon March	Spoon June	Spoon Total	Total: Chi-square 3:1	Total: P Value
9-2	8	13	25	4	2	8	0.010	.90-.95
9-3	3	17	20	2	0	2	2.970	.05-.10
9-5	21	31	52	2	6	8	4.355	.02-.05
9-6	5	20	25	3	2	5	1.111	.20-.30
9-7	20	7	27	8	4	12	0.692	.30-.50
9-8	25	13	38	9	6	15	0.308	.50-.70
9-9	22	25	47	8	3	11	0.873	.30-.50
10-2	34	24	58	6	7	13	1.604	.20-.30
10-3	10	3	13	6	2	8	1.921	.10-.20
10-4	32	20	52	8	4	12	1.333	.20-.30
10-5	13	8	25	4	2	6	0.527	.30-.50
11-3	28	18	47	8	4	12	0.565	.30-.50
March	222			68			0.342	.50-.70
June		199			36		11.746	< .01
Total			421			104	7.543	< .01
				525				

Chi-square for heterogeneity = 8.726 P = .50-.70

Table 10. Summary of data from the backcross (170-2 x 34-1) x 170-2 showing segregation for flat versus spoon-shaped cotyledons. 1958.

Progeny	Phenotype					
	Flat		Spoon		Total	
	March	June	March	June	March	June
10-1	10	9	9	8	19	17
10-6	-	10	-	8	-	18
11-2	7	10	11	13	18	23
12-1	2	7	3	7	5	14
12-2	7	5	10	3	17	8
12-3	10	5	10	4	20	9
	36	46	43	43	79	89
	82		86		168	

Chi-square for 1:1 ratio = 0.095

P value = .70-.80

Table 11.

Summary of data from the backcross 170-2 x (163 x 170-2) and from the F_2 generation of the cross 170-2 x 110-2 showing segregation of flat versus spoon-shaped cotyledons. June 1958.

Cross	Progeny	Phenotype		Total
		Flat	Spoon	
170-2 x (163 x 170-2)	3052-5	8	10	18
"	3052-14	13	10	23
"	3059-10	14	24	38
		35	44	79
170-2 x 110-2	3028-14	59	36	95

Chi-square for 1:1 ratio in the backcross = 1.025

P value = .30-.50

Virescent-1 and Virescent-2

Virescent-1 was involved in successful crosses with the spoon-shaped line and with virescent-2. In both crosses the F_1 plants were normal dark green showing that virescent-1 and virescent-2 are both recessive to normal green color. The fact that the F_1 of the intercross of the two virescents was green also showed that the two characters were controlled by separate genes.

The virescent-2 line was also involved in a cross with the spoon-shaped line. The F_1 of this cross was also dark green, again showing that virescent-2 is recessive to normal dark green.

Table 12 shows the data from the cross of the spoon-shaped line by virescent-1 classified for green versus virescent. The probability values for a fit to a 3:1 ratio for both the March and June data were quite low. A one gene hypothesis for virescent-1 cannot, however, be ruled out on the basis of these results. Only one of the individual progenies had a probability value of less than .20.

No evidence of linkage was found to exist in the data from the cross of spoon-shaped by virescent-1. The F_2 plants from this cross were classified as, 351 green with flat cotyledons, 70 green with spoon-shaped cotyledons, 70 virescent with flat cotyledons and 34 virescent with spoon-shaped cotyledons. The Chi-square value for a fit to a 9:3:3:1 ratio was 26.985 with a P value of less than .01. The ratio for each of these genes considered separately was 421:104 which did not fit a 3:1 ratio, indicating that some other factor besides a single gene was influencing their expression. Since the cross was made in the repulsion phase, linkage of the two genes involved should make the two central classes larger than expected for independent assortment. However, the opposite was the case so that these genes cannot

be linked.

Table 13 shows the data from the cross of the two virescents, 34-1 x 163. The totals and the data for March did not fit a 9:7 ratio of green to virescent plants although two of the progenies gave a good fit. The June data, however, agreed closely with a 9:7 ratio.

Table 14 shows the data from the cross of virescent-2 x the spoon-shaped line. The population was small but agreed closely with a one factor ratio of three green plants to one virescent plant.

Virescent plants of both virescent-1 and virescent-2 were selfed in F_2 to check on the possibility of further segregation in F_3. From five virescent-1 plants selfed only three F_3 progenies resulted with 19 - 44 plants in each. Seven virescent-2 plants were selfed but only three F_3 progenies could be produced, with 1, 1 and 49 plants respectively. Self-sterility apparently accounted for the failure of some of the plants to set seed and germination also was poor. The F_3 of both virescents bred true for virescent. The fact that the F_3 bred true for virescent also supports a hypothesis for one factor for these two characters but the sample was too small to be entirely conclusive.

Table 12. Summary of data from the F_2 generation of the cross 170-2 x 34-1 showing segregation for green versus virescent. 1958.

Progeny	Phenotype: Green March	Green June	Green Total	Virescent March	Virescent June	Virescent Total	Total: Chi-square 3:1	Total: P Value
9-2	9	15	24	3	2	5	1.150	.20-.30
9-3	4	16	20	1	4	5	0.333	.50-.70
9-5	18	34	52	5	5	10	1.612	.20-.30
9-6	7	18	25	1	8	9	0.039	.80-.90
9-7	22	7	29	6	3	9	0.035	.80-.90
9-8	27	13	40	7	3	10	0.667	.30-.50
9-9	26	20	46	4	8	12	0.575	.30-.50
10-2	33	21	54	7	7	14	0.706	.30-.50
10-3	12	3	15	4	0	4	0.158	.50-.70
10-4	33	14	47	7	6	13	0.355	.50-.70
10-5	15	8	23	2	2	4	1.494	.20-.30
11-3	30	16	46	7	2	9	2.188	.10-.20
March	236			54			6.294	.01-.02
June		185			50		1.737	.10-.20
Total			421			104	7.543	$<.01$
				525				

Chi-square for heterogeneity = 1.769 P = $>.99$

Table 13. Summary of data from the F_2 generation of the cross 34-1 x 163 showing segregation for green versus virescent. 1958.

Progeny	Phenotype: Green March	Green June	Green Total	Virescent March	Virescent June	Virescent Total	Chi-square 9:7	P value
1	35	4	39	14	2	16	4.803	.02-.05
2	39	18	57	17	23	40	0.249	.50-.70
3	33	11	44	21	5	26	1.243	.20-.30
4	39	18	57	14	14	28	4.035	.02-.05
March	146			66			13.715	< .01
June		51			44		0.254	.50-.70
Total			197			110	7.824	< .01

Chi-square for heterogeneity = 2.506

P value for heterogeneity = .30-.50

Table 14. Summary of data from the F_2 generation of the cross 163 x 170-2 showing segregation for green versus virescent. June 1958.

Progeny	Phenotype		Chi-square		
	Green	Virescent	Total	3:1	Probability
3045-3	14	6	20	0.533	.30-.50

Because of hail damage these seedlings could not be classified for the spoon-shaped character.

Serrated Leaves

The serrated leaves character did not show complete dominance. The F_1 appeared to be roughly intermediate between the serrated and non-serrated parents. Most of the F_2 plants also were in the medium serration class with only a few plants showing the parental characters.

Table 15 shows the results obtained with the F_2 populations of two crosses involving serrated leaves. The 107 progenies of cross 170-2 x 110-2 came from the cross made in 1957 and the 3028 progeny came from the same cross made in 1956. The F_2 population of cross 110-2 x CM39 was grown in the greenhouse in February and also in the field in June. The greenhouse data showed more parental types than the field data. The differences in serration between plants of the F_2 were not as striking in the greenhouse as they were in the field sample.

The two crosses involving the spoon-shaped line and the serrated one (Table 15) also showed that most of the F_2 plants were of intermediate serration. Only a few plants showed the parental characters defined as well as in the parental checks. The variation in serration between F_2 plants was noticeable but not objective enough to allow classification into precise groups.

Table 15. Summary of data from the F_2 generation from two different crosses showing segregation for serrated versus entire leaves.

Cross	Progeny	Phenotype			Total	Date of Experiment
		Entire*	Intermediate	Serrated**		
110-2 x CM39	3026-5	3	21	2	26	Feb. 1958
"	3026-6	2	20	3	25	"
"	3026-10	3	24	2	29	"
"	3026-14	2	24	1	27	"
110-2 x CM39	3026-5	0	39	0	39	July 1958
"	3026-6	0	31	1	32	"
"	3026-10	0	29	2	31	"
"	3026-14	0	24	0	24	"
170-2 x 110-2	107-1	1	50	1	52	"
"	107-2	0	44	3	47	"
"	3028-14	0	35	1	36	"
Total		11	341	16	368	

* as entire as or nearly as entire as the entire parent.

** as serrated as or nearly as serrated as the serrated parent.

DISCUSSION

Red Hypocotyl

The ratios obtained in the F_2 and backcross progenies involving red hypocotyl were explained by assuming red hypocotyl to be controlled by one gene dominant to its allele which produced green hypocotyl. Absence of segregation among the progenies grown in F_3 supported the hypothesis of monogenic inheritance postulated from the F_2 data. The symbols Rh for red hypocotyl and rh for green hypocotyl are proposed to designate the alleles of this gene. The monogenic inheritance found for this color feature agrees with the previous work of Shull (12), Bottazzi (1) and Satsyperov (11) on what probably is the same color character but expressed in other parts of the plant.

The deficiency of red hypocotyl plants in the samples grown in winter can probably be explained by inadequate light. Meyer and Anderson (6) stated that high light intensity, drought, low temperature and low nitrogen supply favoured anthocyanin synthesis in plants. The light experiment on red hypocotyl showed that light was also important for anthocyanin synthesis in the hypocotyls of the sunflowers used.

Red hypocotyl is a distinctive, easily recognizable character and has, as far as is known, no detrimental effect on seed production. It is also simply inherited and would as a result serve well as a seedling marker that could easily be incorporated into the male parent of a hybrid variety. However, to ensure the expression of the Rh gene the environmental conditions favouring anthocyanin synthesis would have to be provided.

Dwarf Plant

In heritance of the dwarf plant type was controlled by a single recessive gene as indicated by the two crosses and one backcross in which this character was involved. The gene symbol proposed to designate the allele for dwarf is d

with D representing the dominant allele for normal plant height.

Since the F_3 progenies produced bred true for dwarf plant type the genotype of the F_2 plants which were selfed to produce the F_3 must have been dd.

The dwarf character is very distinctive and therefore would be useful as a seedling marker. Since it is a recessive character it would have to be incorporated into the female parent of the hybrid. It does not appear to be greatly influenced by environment, a feature which would add to its value. On the basis of the visual appearance of the dwarf line this gene probably would not have any adverse effect on seed yield since the internode length alone seems to be affected. Its effect on yield should, however, be tested before use is made of this character in a commercial hybrid.

Spoon-shaped Cotyledons

The results with the spoon-shaped character were somewhat variable in the various F_2 populations. The cotyledons of the dwarf line, for example, showed a slight tendency toward curled edges so that one would expect a greater proportion of spoon-shaped plants in the F_2 of the dwarf by spoon-shaped cross. The fact that less than the required amount of spoon-shaped plants, for a 3:1 ratio, resulted can perhaps be ascribed to environment or to chance. Another source of difficulty showed up in the cross of the spoon-shaped line with the virescent-1 line. Virescent-1 had very small cotyledons and as a result many of the F_2 plants had small cotyledons which tended to mask the expression of the spoon-shaped character.

Spoon-shaped is a recessive character as shown by the F_1 generations of all crosses that were made. The weight of the evidence from the F_2 and backcross generations favours the hypothesis that spoon-shaped is inherited on a monogenic basis. The symbol proposed for the allele controlling spoon-shaped cotyledon is sc with Sc representing the dominant allele responsible for flat cotyledon.

In spite of the variability shown by the F_2 populations, spoon-shaped might, in crosses with some inbred lines, serve as a useful marker gene. In the crosses studied it was shown that hybrid plants could be picked out in the F_1 on the basis of this marker. Since the F_1 generation is used for commercial hybrids the fact that the F_2 might be variable would be of no consequence. Spoon-shaped is recessive and, therefore, would be incorporated into the female parent of a hybrid. Provided the male parent used were selected for flat, fairly large cotyledons sc should provide a distinctive marker gene.

Virescent-1 and Virescent-2

Both virescents were shown to be recessive to normal green color. They were also shown to be controlled by independent genes and not alleles of the same locus. Although the data were not entirely conclusive, a single gene controlling the inheritance of each virescent was indicated.

The gene symbols proposed to designate the virescent lines are: v_1 controlling the virescent-1 character and v_2 controlling virescent-2 with V_1 and V_2 representing the normal alleles of the respective genes. The proposed hypothesis requires that virescent-1 be of the genotype $v_1\ v_1\ V_2\ V_2$ and virescent-2 of the genotype $V_1\ V_1\ v_2\ v_2$. The F_1 from a cross of virescent-1 and virescent-2 would have the genotype $V_1\ v_1\ V_2\ v_2$, resulting in green plants. In the F_2 generation there would be segregation for green and virescent plants to give a 9:7 ratio respectively. The nine green plants would result from the presence of at least one dominant allele from each of the two gene pairs. The seven virescent plants would result from these genotypes which were homozygous recessive for at least one factor pair or homozygous recessive for both factors.

The expression of the virescent characters was considerably influenced by light intensity . For example, a trial sample from the virescent lines

was grown in the greenhouse in November in which the virescent plants were hard to distinguish from normal green plants. By March the light was strong enough to distinguish quite readily between virescent and green plants; however, the distinction between green and virescent was much more apparent in the sample grown in June. For this reason more weight should probably be place on the data from material grown under strong light conditions. The explanation for the lower proportions of virescent plants in the winter samples is probably inability of the genotype to express itself because of low light intensity. Another factor which may have been operating to change the true ratios is the "self-sterility" exhibited by the virescent lines. Pollen grains bearing the recessive alleles may be at a disadvantage compared to pollen grains bearing the dominant allele thus resulting in a decrease of the virescent class.

Virescent-1 would make a good seedling marker under bright light conditions. The virescent plants, however, do not exhibit much vigour so that their use in commercial hybrids would be questionable. The lack of vigour in the virescent plants must be due to the v_1 allele because the green siblings of the virescent plants in the F_2 were all much more vigorous.

As a seedling marker the virescent-2 character would not be as distinctive as virescent-1. It could be used if the seedlings were grown under bright light. The v_2 gene results in plants which, after the seedling stage, are nearly normal green so that the reduction of yield probably would not be very serious. However, it is not as distinctive as the other seedling markers which have been discussed.

Serrated Leaves

The inheritance of the serrated leaves character appears to be controlled by two or more genes judging by the fact that only few parental types were obtained in the F_2 population.

As a seedling marker serrated leaves do not show very much promise. This character is not simply inherited and if it did not already exist in the desired line it would require rather large populations for incorporation by the backcross method. This character is not very distinctive, especially in the two leaf stage. In the four and six leaf stages the differences between serrated and non-serrated lines, becomes more evident and hybrids are consequently easier to distinguish from selfed plants. Another feature which detracts from its value as a seedling marker is that it seems to be environmentally influenced to a considerable extent.

SUMMARY

Six seedling characters which might be applicable as seedling markers in two-line hybrids of sunflowers were selected and the mode of their inheritance studied.

Red hypocotyl was found to be controlled by a single dominant gene. But the gene for anthocyanin production cannot express itself unless adequate light is supplied.

The dwarf condition causing shortened internodes was found to be due to a single recessive gene and was quite stable under different environments.

The spoon-shaped character was recessive to flat cotyledon and was controlled by one gene. Its expression in the F_2 seemed to be more variable than in the inbred line 170-2.

The virescent-1 character appeared to be controlled by one gene recessive to normal green. Fairly strong light was necessary for its expression.

Virescent-2 was found to be recessive to normal green color and monogenic inheritance was indicated. The gene controlling virescent-2 was not allelic with the gene for virescent-1. Strong light was necessary for its expression.

The serrated leaf character appeared to be controlled by two or more genes which lacked dominance. The F_1 generations produced exhibited serration approximately midway between that of the parents.

No evidence was found to suggest that any linkage occurred between the characters studied.

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