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1) Soybean linkage and crossover tests

A previous test (Buzzell, 1977) indicated a possible linkage (39.8 ± 3.0%) between Fg_1 and Dt_1. An additional test with F_2 plants of a cross of two 'Harosoy' isolines ($fg_1\ dt_1$ x $Fg_1\ Dt_1$) indicates that the two genes are independent (Table 1).

In reporting on the genetics of flavonol classes 9T to 16T, Buzzell and Buttery (1974) indicated that the 9t to 16t classes which involve fg_4t had not been observed. A close linkage between Fg_4 and T was apparent. In the early generation of a cross between OX 936 ($fg_4\ T$) and 'Beeson' ($Fg_4\ t$), the crossover type was not found among gray pubescent plants. Without a crossover to break the linkage, the following segregations were expected from 8T ($T\ fg_4/t\ Fg_4$) plants:

Class	Linked genotype		Ratio	Segregation without crossover
16T	$T\ fg_4$	$T\ fg_4$	1	All brown
8T	$T\ fg_4$	$t\ Fg_4$	2	Brown and gray
8t	$t\ fg_4$	$t\ fg_4$	1	All gray

On this basis, the following procedure was used. In rows segregating for brown and gray pubescence, the gray plants were discarded and the brown plants were sampled for thin layer chromatography (TLC) and harvested for seed. The plants were separated into 16T and 8T classes and progeny were grown in rows. The 16T rows were checked for pubescence color; when no segregation was observed, the rows were discarded and the cycle was repeated with the 8T rows. In a population of 299 rows derived from F_8 16T plants, several rows were segregating brown and gray pubescence. TLC of gray plants showed that they were 16t.

Table 1. Soybean F_2 linkage test

Genes	a	b	c	d	Sum	R%	SE
$Fg_1\ fg_1$ $Dt_1\ dt_1$	165	58	49	13	285	54.0	4.6

References

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2) Inheritance of presence/absense of flavonoid compounds in soybean seed coats

In soybean plants carrying the gene *T* and having black or brown pigmentation of seed coats, there are numerous compounds that can be detected by thin layer chromatography (TLC). There are four spots (A, B, C and D) that appear to be related; they are yellow-orange under visible light and they fluoresce yellow-orange (duller than quercetin) under UV light after spraying with flavone reagent. Phenotypic positions of A and D on 2-way plates are given in Table 1. A is present in all material tested, but D varies in presence/absence (B and C also vary but have not been studied). Twenty-nine cultivars, two lines, 15 genetic types, and 78 plant introductions were evaluated for leaf flavonol class and for seed coat compounds. The TLC technique followed that of Buttery and Buzzell (1973). The presence of D was associated with Fg_2 in all of the entries tested (Table 2). Segregations support the association (Table 3). Either there is a gene closely linked with Fg_2 or else there is a "pleiotropic effect" of Fg_2 on D. Fg_2 is a flavonol glycoside gene for adding rhamnose with an $\alpha(1\text{-}6)$ linkage (Buttery and Buzzell, 1975). If Fg_2 is involved, the D could be a rhamnose glycoside of a flavonoid compound.

There is another group of compounds that appear to be related to the A-D compounds. They fluoresce a dull orange. We have labeled them *theta* (θ) spots. When present, they occur just to the right of A, B, C and D on the TLC plate. Segregation for Aθ indicates that a dominant gene is involved in its presence (Table 4). When this gene and Fg_2 are present, Dθ occurs.

Table 1. Phenotypic positions of two seed coat compounds on 2-way TLC cellulose plates

Run	Spot A		Spot D	
	Rear	Front	Rear	Front
	mm[a]			
First[b]	21	39	50	58
Second[c]	95	104	93	100

[a]From point of spotting, with runs 140 mm; averages of measurements on 10 cellulose plates.

[b]2% Formic acid.

[c]Amyl alcohol (20): acetic acid (12): water (10).

Table 2. Numbers of black- and brown-pigmented entries by leaf-flavonol classes that have either Fg_2 or fg_2 and either presence or absence of compound D in seed coats

	Fg_2 present and Spot D present				
Seed coat color	1T	2T	4T	6T	14T
Black	2	7	11	38	0
Brown	0	4	9	18	1
	Fg_2 absent and Spot D absent				
	3T	5T	7T	8T	
Black	0	1	2	2	
Brown	0	0	29	0	

Table 3. Segregation of Fg_2/fg_2 and numbers of plants with and without spot D in the F_2 of Lincoln-*i* x Beeson *i*

	Spot D	
Leaf flavonol class	Present	Absent
6T (Fg_2)	50	0
8T (fg_2)	0	17

Table 4. Segregation for a flavonoid compound of soybean seed coats

	Aθ		3:1 test	
T31 x 0X936	Present	Absent	Chi-square	P
F_2-91 (Aθ absent) F_3	0	29	--	--
F_2-93 (Aθ present) F_3	31	14	0.60	0.50-0.40

References

Buttery, B. R. and R. I. Buzzell. 1973. Varietal differences in leaf flavonoids of soybeans. Crop Sci. 13:103-106.

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3) Genetics of black pigmentation of soybean seed coats/hila

Gene *T* is involved in black pigmentation and W_1 in the presence of *t* is involved in imperfect black pigmentation (Bernard and Weiss, 1973). *T* is a phenolase gene resulting in quercetin formation and brown pubescence (Buttery and Buzzell, 1973); W_1 is a flower- and hypocotyl-color gene (Hartwig and Hinson, 1962). Thin layer chromatography shows two major 'spots' involved in black pigmentation of soybean seed coats. Phenotypic descriptions are given in Table 1. Evaluation of the seed coats of 23 cultivars, 2 lines, 11 genetic types, and 36 plant introductions having black pigmentation indicated that spot #1 is associated with the presence of W_1 and that the presence of spot #2 is associated with *T* (Table 2). This association is supported by the segregations listed in Table 3. Preliminary analyses at Harrow suggest that spot #1 is a delphinidin and spot #2 a cyanidin glycoside. Yoshikura and Hàmaguchi (1969) have identified delphinidin 3-monoglucoside and cyanidin 3-monoglucoside as anthocyanins of black soybean.

Other genes were tested for possible effects upon seed coat anthocyanins (Table 4). The genes w_3 and w_4 (Hartwig and Hinson, 1962) did not affect the presence of spots #1 and #2. The gene *wm* (Buzzell et al., 1977) did not affect the presence of spot #2 (the W_1 *wm* combination was not tested). The gene *td* (Bernard, 1975) did not affect the presence of spots #1 and #2. In addition, spot A (Buzzell and Buttery, 1982) was not affected by any of these genes.

Table 1. Phenotypic description of anthocyanins of soybean seed coats on 2-way TLC plates

Run[a]	Spot #1[c]		Spot #2[d]	
	Rear	Front	Rear	Front
	mm[b]			
Imperfect black seed - Purple flowers - Gray pubescence				
First	14	20	--	--
Second	46	54	--	--
Black seed - White flowers - Brown pubescence				
First	--	--	20	45
Second	--	--	68	87
Black seed - Purple flowers - Brown pubescence				
First	15	37	20	49
Second	45	53	71	86

[a]First -- 2% formic acid; Second -- amyl alcohol (20): acetic acid (12): water (10).

[b]From pointing of spotting, with 140-mm runs; averages of measurements of each type on 5 cellulose plates.

[c]Unsprayed: red-purple in visible light. Sprayed with flavone reagent: blue in visible light, fluoresces red-purple.

[d]Unsprayed: red-purple in visible light. Sprayed with flavone reagent: varies in intensity within the spot from blue to violet-blue in visible light, fluoresces red-purple.

Table 2. Association of anthocyanins of soybean seed coats with genes W_1 and T

Seedcoat color	No. of entries	Flower color	Spot #1	Pubescence color	Spot #2
Black	30	Purple (W_1)	Present	Brown (T)	Present
Black	2	Dilute purple ($W_1W_3w_4$)	Present	Brown (T)	Present
Black	35	White (w_1)	Absent[a]	Brown (T)	Present
Imperfect-black	4	Purple (W_1)	Present	Grey (t)	Absent[a]

[a]Not detectable with technique used but may be present in trace or low amounts.

Table 3. Segregations for W_1/w_1 and T/t showing association with anthocyanins for black pigmentation of soybean seed coats

Cross	Generation	Gene	Spot #1		Spot #2	
			Present	Absent[a]	Present	Absent[a]
T31 (T) x OX 936 (T)	F_3	W_1	53	0	53	0
		w_1	0	18	18	0
Lincoln-i x Beeson-i	F_2	W_1	70	0	53	0
		w_1	0	24	15	0
		T	53	0	68	0
		t	17	0	0	26

[a]Not detectable with technique used but may be present in trace or low amounts.

Table 4. Tests for effects of other genes on seed coat compounds

		Spot A	Spot #1	Spot #2
w_3 and w_4				
L67-3469[a]	*T* W_1 w_3 W_4 *i R*	Present	Present	Present
OX 326[b]	*T* W_1 W_3 w_4 *i R*	Present	Present	Present
OX 327[c]	*T* W_1 w_3 w_4 *i R*	Present	Present	Present
wm				
OX 694[d]	*T* w_1 *wm i R*	Present	Absent*	Present
td				
Cloud	*T td* w_1	Present	Absent*	Present
T 69	*T td* w_1	Present	Absent*	Present
Kingwa	*T td* W_1	Present	Present	Present
Sooty	*T td* W_1	Present	Present	Present

[a]Clark isoline from Dr. R. L. Bernard.

[b]From L70-4422 (W_1 W_3 w_4 i^i) x L67-3469.

[c]From L68-1774 (W_1 w_3 w_4 i^i) x L67-3469.

[d]From OX 281 (w_1 *wm I t r*) x Harman (W_1 W^m *i TR*).

*Not detectable with technique used but may be present in trace or low amounts.

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4) Soybean emergence in clay soil and tolerance to phytophthora rot

In 1979 at the Woodslee Soil Substation, 19 F_2 populations were planted May 23 in single-row plots, 100 seeds per row, with 12 replications. These populations consisted of 'Williams' x 'Wells', and Williams and Wells each by 'Adams', 'Beeson', 'Bonus', 'Calland', 'Corsoy', 'Cutler 71', 'Hark', 'Kanrich' and 'Prize'. The field had been in continuous soybeans since 1973 and is known to be infested predominantly with race 7 of *Phytophthora megasperma* f. sp. *glycinea* (Pmg).

Emergence was slow as a result of heavy rainfall (7 cm in the week after planting followed by 12 cm during the next three weeks) and soil compaction/crusting. Emergence counts were taken June 21. Diseased plants were recorded and removed from the plots biweekly until maturity.

The amount and occurrence of phytophthora rot was small and variable, thus comparisons of each cross could not be made; and, although some rows of parents were planted, results are not presented because seed of similar quality/viability was not available for all of them. Each of the varieties is susceptible to race 7 of Pmg with hypocotyl inoculation.

Wells is moderately susceptible to Pmg in the field (Buzzell and Anderson, 1982); Williams is moderately tolerant (Schmitthenner and Walker, 1979). Averages were run over the nine progenies for Wells and for Williams (Table 1). Williams progenies had higher percentage of emergence and less plant loss than did Wells progenies. The Williams x Wells cross was intermediate, suggesting polygenic inheritance.

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Table 1. Seedling emergence in clay soil and plant loss from phytophthora rot

	Emergence (%)	Plant loss (%)
Williams progenies*	46 b	2 a
Williams x Wells	41 ab	6 b
Wells progenies*	38 a	8 b
SE	1.8	1.0
C.V. %	15	66

*Average of nine crosses.

a-b Means in columns followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

References

Buzzell, R. I. and T. R. Anderson. 1982. Plant loss response of soybean cultivars to *Phytophthora megasperma* f. sp. *glycinea* under field conditions. Plant Dis. (In press).

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5) Greenhouse determination of soybean tolerance to phytophthora rot

One method of determing field tolerance of soybeans to *Phytophthora megasperma* f. sp. *glycinea* (Hildeb.) Kaun & Erwin (Pmg) is to determine the percent of plant loss from emergence to maturity for cultivars grown in an infested field under conditions favorable for the disease (Buzzell and Anderson, 1982). Drawing upon the field results and the results of two unpublished greenhouse experiments (given below), a greenhouse technique similar to the field test was developed.

1962 Experiment. Cores of soil were obtained from a field known to be infested with Pmg race 1, the field having been used in a previous study (Fulton et al., 1961). In the fall of 1961, each of 40 pieces of aluminum pipe (17 cm diameter by 24 cm) was pressed 20 cm into the ground using a hydraulic jack, dug up containing soil, and moved to a greenhouse. Each was set on a tin plate as a "pot," with pots making up a five-replicate test. Allowing

for volume increases, the soil was tilled with a trowel to a sufficient depth to allow for four planting treatments of 0, 38, 76, and 114 mm of worked soil between the seeds and the untilled soil layer for each of 25 and 50 mm seed depths of planting. Twenty-five seeds of 'Harosoy' were planted per pot January 8-9, 1962. The number of plants with phytophthora rot were recorded from January 28 to March 22.

The depth of seed placement at 25 and 50 mm had no effect on the incidence of phytophthora rot. There was no interaction between depth of seed and distance of seed from the untilled soil layer. The average loss (over depths) of Harosoy plants killed by the disease was 36, 30, 12, and 13% for the 0, 38, 76, and 114 mm distances of seed to untilled layer; the L.S.D. 0.05 was 15%. It was concluded that seed placement at or near the untilled layer increased the amount of phytophthora rot.

1974 Experiment. Soil was collected from around dead soybeans in 31 fields. The soils were placed in 30-cm-diameter fibre pots in a greenhouse. Repetitive plantings of 'Amsoy', Harosoy, and 'OX20-8' soybeans as a trap crop were made in each soil for 4 to 6 times. The number of emerged seedlings and the number of killed seedlings were recorded. Pmg (races 3, 4, and/or 6) was isolated from some of the killed seedlings (Buzzell et al., 1977); Amsoy, Harosoy and OX20-8 are susceptible to each of these races. Each of the cultivars differed significantly (P = 0.05) from the others in the percentage of plants killed by phytophthora rot: Amsoy 4%, Harosoy 10%, and OX20-8 26%.

Tolerance Experiment. Clay soil was obtained from a field being used for phytophthora tolerance tests (Buzzell and Anderson, 1982). Forty-eight fibre pots (20 cm diameter, 23 cm tall) were filled with 18 cm of soil after placing glass wool in the drainage holes. The soil was tamped into the pots with a small weight, then watered. Three plantings of the extremely susceptible OX20-8 were made from January to April, 1978. Interspersed with these plantings were two plantings of a 3-replicate test of 15 cultivars ('Altoona', 'Amsoy 71', 'Dawn', 'Harcor', 'Harlon', 'Harosoy 63', 'Harwood', 'Maple Arrow', 'Nairn', OX20-8, 'Starbuck', 'Steele', 'Viking', 'Wells', 'XK505') covering a range of field tolerance along with 'Toyosuzu' as a resistant check. Twenty-five seeds per pot were placed onto the clay surface and covered with about 3 cm of builder's sand. The pots were watered as required, especially as seedlings were emerging to break up crusted sand and to wash the sand back down around the base of the seedlings. Counts were made of emerged seedlings and of live plants 18 to 26 days from planting. For a few days prior to the final reading, the pots were allowed to dry out sufficiently to place the seedlings under some moisture stress. The sand became dry which meant that if there were seedlings sufficiently diseased to not be rooted into the soil, their death was hastened. The same soil was used in each planting, without being tilled. The seedlings were cut off and removed without disturbing the soil. The dry sand was dumped into tubs and re-used in the next planting.

There was no plant loss in the resistant check. The two later plantings of OX20-8 had more plant loss (60 and 63%) than the first planting (45%), indicating a possible buildup in inoculum. Likewise the second planting of the cultivar test averaged higher plant loss (27%) than the first (14%). Averaged over the two plantings there were significant (P = 0.01) cultivar differences in plant loss; the C.V. was 12%. There was a positive correlation between the greenhouse plant losses and unpublished field plant losses from 1974 and 1975 (r = 0.75**) and field plant losses (Buzzell and Anderson, 1982) from 1977 and 1978 (r = 0.79**).

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