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1) Variation in water-absorbing capacity of soybean seeds.

Water-absorbing capacity (WAC) of soybean seeds is an important factor in the efficient production of soy food products in Japan and other Asiatic countries. Variation in WAC, therefore, should interest persons in the USA concerned with exporting soybeans to these countries. Soybeans vary in WAC depending upon soaking conditions (e.g., temperature and length of soaking time), initial moisture content of the seed, quality of the seed coat, and probably genotype. We are interested in learning the relative importance of genotype.

We have screened 1,271 soybean genotypes (mostly plant introductions) from maturity groups I, II, III, and IV grown by Dr. R. L. Nelson at Urbana, Illinois, in 1980. We estimated WAC by soaking 10 g of apparently healthy and intact seeds in 30 ml of distilled water for 10 hours at room temperature. This method is not as refined as the more precise but more time-consuming method developed by Cheng (1981). It is approximately the same as the shorter method described by Cheng for screening and ranking large numbers of genotypes and is expressed by the following formula:

$$\text{WAC} = \frac{(\text{weight of soaked seeds} - \text{weight of dry seeds})}{\text{weight of dry seeds}} \times 100$$

The moisture content of the "dry seeds" (before soaking) was not measured. Possible variations in initial water content might reduce the accuracy of our screening in ranking genotypes.

Our estimates of WAC ranged from 18.1 to 155.8 when all 1,271 genotypes were considered (Table 1). Low estimates of WAC were associated with high percentages of hard seed. Genotypes with high WAC estimates had few or no hard seeds. Variations in frequency of hard seeds, however, did not account for all the variation in WAC. Even within populations of samples having only soft seeds, estimates of WAC ranged from 117.0 to 148.8 in genotypes from maturity group I and 116.3 to 155.8 in those from maturity group IV. The range was even greater in group II (90.6) and in group III (50.0).

Lines differing widely in WAC have been crossed to generate F_2 hybrid populations for inheritance studies. Tests of 1982-grown materials are being run to determine the importance of determining initial moisture content of seeds and the effects of years on estimation of WAC.

Reference

Cheng, Shui-Ho. 1983. Variation in some soybean (*Glycine max*) [L.] Merr.) seed characteristics of possible importance in soybean processing. M.S. thesis. University of Illinois, Urbana-Champaign, Illinois, USA 61801.

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Table 1. Variation in frequency of hard seeds and in water absorbing capacity in soybean germplasm (1980 seeds)

Maturity group	— Number of genotypes tested —			——— Water absorbing capacity (WAC) among: ———				
				All genotypes			Genotypes with no hard seeds	
	Total	With no hard seeds	With hard seeds	Low	High	Mean	Low	High
I	217	52	165	18.1	148.8	115.9	117.0	148.8
II	209	72	137	38.4	152.5	114.1	61.9	152.5
III	307	154	153	25.2	150.4	129.1	93.4	143.4
IV	538	282	256	47.3	115.8	133.8	116.3	155.8
	1,271	560	711					

2) Studies in polyploidy in soybeans: A simple and effective colchicine technique of chromosome doubling for soybean (*Glycine max* (L.) Merr.) and its wild relatives.

Tang and Loo (1940) first reported the induction of tetraploid soybeans by soaking day-old seedlings in 0.05 to 0.1% colchicine solution for 24 or 48 h. Oinuma (1952) obtained tetraploids by soaking dry soybean seeds in 0.1% colchicine solution for 24, 48 and 72 h. His results showed that the survival of resulting plants was poor. Sen and Vidyabhusan (1960) reported that polyploidy could be induced either by soaking the seeds in colchicine solution or treating the apical bud of the germinating seedling by a cotton wad saturated with colchicine solution, but they obtained few polyploids with these methods. A more successful colchicine technique for inducing tetraploidy in soybeans was reported by Tang and Lin (1963). They found that treating the apical buds with 0.3% colchicine-lanolin mixture gave about 47% success. Treatment with a colchicine-lanolin mixture, however, may cause continued induction of chromosome doubling because the lanolin mixture can last much longer than an aqueous solution of colchicine. This may reduce the frequency of recovered tetraploids. In the wild relatives of soybean, Palmer and Hadley (1968) obtained tetraploid plants of *Glycine tomentella* (formerly *Glycine tomentosa*) by applying warm 0.5% colchicine-lanolin paste to the axillary buds at the base of young cotyledons just before the cotyledons spread open. We have found the procedures described below to be quite effective in doubling chromosome numbers of several cultivars of *G. max* as well as different wild soybean genotypes. Detailed statistics have not been determined, but the degree of success has been well over 50%.

Procedure 1 - for Subgenus *Soja*, i.e., *Glycine max* and *Glycine soja*

1) Germinate the seeds in pots. When the two single leaves of resulting seedlings spread completely open and the apical bud grows to about 0.5 cm long, but no longer, wrap the three portions of meristematic tissue (i.e., the apical portion plus the axillary portions at the base of both single leaves) with cotton. Completely saturate the resulting cotton wad with a 0.1% aqueous solution of colchicine twice a day by using a dropper. A total of 3 applications is enough, e.g., treatment can be done once in the morning and once in the afternoon of the first day and once in the morning of the next day. The time interval between treatments on the first day should be longer than 4 h.

2) On the third day, after the colchicine treatment is finished, remove the cotton from the wrapped plants. Care for the treated plants in the regular way. After 4 to 6 days, the axillary buds at the base of cotyledons will grow out. Be sure to remove them as soon as they appear.

3) When polyploid buds grow out from the three treated parts of the seedling, check them visually and remove any new, rapidly growing shoots that look like the original plant morphologically and are probably diploid.

Procedure 2 - for chromosome doubling of Subgenus *Glycine* such as *Glycine tabacina*, *Glycine tomentella*, etc., and their hybrids either among themselves or with Subgenus *Soja*

1) Seedlings of the Subgenus *Glycine* are very tiny. Furthermore, treatment of one meristematic region of these perennial types simply arrests growth in that area of the plant while growth continues in other areas. There is no forcing of treated tissue to renew growth necessary for recovery of cells with the doubled chromosome number. Thus, a straightforward grafting technique as described by Newell and Hymowitz (1979) is used first to graft the scion of the wild soybean onto stock of the cultivated soybean before colchicine treatment.

2) About 2 to 3 weeks later, when the scion has grown out after grafting, cut out the apical shoot as the second leaf of the wild soybean scion spreads open and the axillary bud grows up about 0.5 cm (no longer than 0.5 cm). Do the same wrapping, treating, and caring work as in Procedure 1 for the Subgenus *Soja*.

References

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3) Studies in polyploidy in soybeans: Cytologically identified tetraploid *Glycine max* and *Glycine soja* and a preliminary observation on seed yields of tetraploid 'Williams' plants.

A partial list of cytologically identified tetraploid *Glycine max* and *Glycine soja* genotypes is presented in Table 1. Some of these genotypes apparently are new at the tetraploid level.

Sixty-three (63) single plants of tetraploid Williams were harvested on the Agronomy South Farm at Urbana in 1982. Five single plants of diploid Williams grown on the same experimental field were sampled as a check. The distribution of single plant seed yield of the tetraploid Williams ranged from 3.7 to 79.6 g per plant (Table 2). Seed yields of diploid Williams

varied from 33.6 to 69.8 g per plant and averaged 52.1 g. Some of the tetraploid plants were comparable to the diploid plants. Tremendous variation in fertility of the tetraploid plants was observed.

Table 1. Tetraploid *Glycine max* and *Glycine soja* genotypes

Glycine max	Chromosome no.	*Glycine max*	Chromosome no.
	4n=		4n=
Williams	80	Blackhawk	80
Beeson	80	Lincoln	80
Wells	80	Harman	80
Century	80	Dunfield	80
Amcor	80	Manchukota	80
Gnome	80	Dunn	80
Harosoy	80	*Glycine soja*	80
Richland	80	PI 378702	80
Chippewa	80		

Table 2. Distribution of seed yields of 63 tetraploid Williams plants

Seed yield/plant (g)	Number of plants
1-10	7
11-20	16
21-30	22
31-40	9
41-50	7
51-60	0
61-70	1
> 70	1

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4) Evaluation of chlorophyll-retention near-isogenic lines of soybeans.

The phenomenon of chlorophyll retention in soybeans [*Glycine max* (L.) Merrill] is worthy of investigation for several different reasons: 1) it may have a physiological impact upon yield, 2) it may be useful in helping to explain the process of senescence, and 3) it causes production of green seeds, which may differ from normal yellow seeds in chemical composition, size, germination, nutritional qualities, and/or potential usefulness as vegetable types.

Different genetic systems control the retention of chlorophyll, which results in green seed color (1). Ten near-isogenic lines in both 'Clark' and 'Harosoy' backgrounds are currently being studied to characterize and compare the different chlorophyll-retention types with their normal counterparts. Differences among lines involve two types of cytoplasm and variations at three nuclear loci (Table 1). All lines, with the exception of the "normal" forms, were derived by the backcross method by R. L. Bernard, from whom our material was obtained.

Agronomic as well as physiological traits were determined for all genotypes, and some preliminary results from our first year's evaluation (1981) are reported here.

Table 1. Listing of soybean isolines of 'Clark' (C) and 'Harosoy' (H) background

Designation	Genetic constitution	Phenotypes	
		Seedcoat	Embryo
C-"normal"	$gg\text{-}D_1D_1D_2D_2$	Yellow	Yellow
C-Gd_1	$GG\text{-}d_1d_1D_2D_2$	Green	Yellow
C-Gd_2	$GG\text{-}D_1D_1d_2d_2$	Green	Yellow
C-Gd_1d_2	$GG\text{-}d_1d_1d_2d_2$	Green	Green
C-Cyt *G*	cytoplasmic	Green	Green
H-"normal"	$gg\text{-}D_1D_1D_2D_2$	Yellow	Yellow
H-Gd_1	$GG\text{-}d_1d_1D_2D_2$	Green	Yellow
H-Gd_2	$GG\text{-}D_1D_1d_2d_2$	Green	Yellow
H-Gd_1d_2	$GG\text{-}d_1d_1d_2d_2$	Green	Green
H-Cyt *G*	cytoplasmic	Green	Green

Physiological traits: Five leaf samplings were made every 10 days from 20 to 60 days after flowering in Harosoy lines, and from 30 to 70 days after flowering in Clark lines. The last sampling coincided in both cultivars with approximately 7-10 days before maturity.

Mean ribulose biphosphate carboxylase activities (Rubisco activities) for the 10 isolines showed a steady decrease in activity as maturity was approached. The sharpest decrease in activity took place in normal types, while the slowest decrease occurred in cytoplasmic green types. Genetic green types (d_1d_2) had slightly higher activities near maturity, and cytoplasmic greens were the highest at early stages. Genotypes d_1 and d_2 showed somewhat intermediate values in the last sampling date.

Only the genetic greens had a definite trend of continuously increasing mean specific leaf weights (SLWs) toward maturity in both cultivars. They had, in general, lower SLWs at early sampling dates, and the highest SLWs near maturity.

Total chlorophyll contents (Table 2) decreased linearly through sampling dates in all genotypes in both backgrounds, with the exception of d_1d_2, which first increased and then decreased to their lowest values. Normal types in both Clark and Harosoy had lower contents for all samplings, while types d_1 and d_2 had higher contents early and intermediate values late. Genetic greens had the highest values near maturity, whereas cytoplasmic green types were intermediate between normal and genetic greens, and slightly lower than d_1 and d_2 genotypes at all sampling dates.

Table 2. Total chlorophyll contents (mg/dm^2) of Clark and Harosoy isolines in soybeans (1981). (Means of 6 reps.)

		Sampling dates		
Background	Isoline	1st	2nd	3rd
Clark	Normal	5.2	4.6	3.6
	d_1	6.5**	6.2**	4.6
	d_2	7.4**	6.9**	4.4
	d_1d_2	7.1**	8.6**	6.3**
	cyt-G	6.7**	6.0**	4.2
Harosoy	Normal	7.6	6.1	3.3
	d_1	8.4	7.6**	5.1**
	d_2	8.0	7.6**	5.5**
	d_1d_2	7.9	8.2**	7.9**
	cyt-G	7.9	6.8	4.8**

*Significantly different from "normal" with P (0.05).

**Significantly different from "normal" with P (0.01).

Chlorophyll a contents followed essentially the same pattern as total chlorophyll, but with cytoplasmic greens being closer to normal types. The general trend for chlorophyll b through sampling dates in all lines of both cultivars was similar to that of total chlorophyll and chlorophyll a. Genetic and cytoplasmic greens, however, had the highest contents at the last two sampling dates, d_1 and d_2 genotypes were intermediate, and normal types had the lowest values.

For mean chlorophyll a:b ratios, the difference between cytoplasmic greens and the rest of the genotypes was readily apparent. The former had much lower ratios in both backgrounds, and the differences became more pronounced near maturity.

It is obvious from the results presented that, in chlorophyll-related traits, the green types behaved differently from normal types, and that there is a clearly distinct behavior between genetic and cytoplasmic greens. Also, there could have been some gene dosage effects, as suggested by the intermediate values of d_1 and d_2 types.

However, no analysis nor interpretation of the effects of d_1 and d_2 genes can be made because of the presence of *G* genes, unless we make the assumption that no physiological effects are associated with the *G/g* locus.

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