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Table 1—Decrease in viability of seeds and embryos of 20 varieties of alfalfa due to germination at 12 atmospheres of osmotic tension.

Variety	% decrease in viability*		Variety	% decrease in viability*	
	Seeds	Embryos		Seeds	Embryos
CK	100	100	Culver	99	98
Teton	93	73	Nomad	100	89
Rambler	99	71	Narragansett	88	47
Semipalatinsk	99	94	Cody	80	52
Ladak	98	57	Buffalo	87	74
Grimm	96	58	Atlantic	75	49
Rhizoma	99	94	Du Puits	83	51
Vernal	98	72	Williamsburg	85	45
Ranger	95	64	Lahontan	64	22
Cossack	97	74	Arizona Chilean	64	22

* Calculated as: $100 - \frac{\text{No. viable at 12 at.}}{\text{No. viable at 0 at.}}(100)$

Table 2—Increase in viability of 20 varieties of alfalfa germinated at 0 and 12 atmospheres of osmotic tension due to removal of seed coat.

Variety	% increase in viability*		Variety	% increase in viability*	
	0 at.	12 at.		0 at.	12 at.
CK	42	100	Culver	35	100
Teton	340	1,700	Nomad	15	- †
Rambler	22	2,500	Narragansett	9	464
Semipalatinsk	39	500	Cody	8	156
Ladak	5	2,000	Buffalo	8	118
Grimm	20	1,233	Atlantic	9	118
Rhizoma	19	500	Du Puits	9	220
Vernal	10	1,200	Williamsburg	12	300
Ranger	16	650	Lahontan	11	140
Cossack	45	1,100	Arizona Chilean	7	130

* Calculated as: $\frac{\text{No. viable embryos}}{\text{No. viable seeds}}(100) - 100.$

† Nomad embryos viable at 12 at. = 10. Nomad seeds viable at 12 at. = 0.

tension solution than seeds of nonwinter hardy varieties. Some deviations from the trend are evident, as was anticipated.

The decrease in viability of embryos of the nonhardy varieties due to osmotic stress was less marked than comparable values for seeds of the same entries. This considerably extended the range of the data in Table 1 for the embryos as compared to that for intact seeds. The correlation of the decrease values was highly significant, $r = .85$. Better agreement between the winter hardiness ranking and reduction in viability of the seeds was obtained than between hardiness and the effect of stress on the embryos. At present it is not possible to explain why a response partially controlled by a seed structure is more closely associated with a mature plant character, such as winter hardiness, than is the comparable response of developing seedlings.

The effect of removal of the seed coat upon viability in 0- and 12-atmosphere solutions is presented in Table 2. At both tensions, excision of the embryos improved germination but the increase was relatively greater in .5 *M* mannitol. It is apparent that the seed coat exercised more control over germination in some varieties than in others. The increase in viability of Teton at 0 atmospheres and of Rambler, Ladak, and Teton at 12 atmospheres was most evident. These data seem to be associated to some degree with the levels of winter hardiness of the respective varieties.

An interpretation of the responses obtained as they relate to ability of seeds of these alfalfa varieties to germinate under adverse conditions of soil moisture has not been attempted. The performance of seeds of these entries under field conditions of moisture deficits is not believed to be sufficiently well established.

REFERENCES CITED

1. DOTZENKO, A. D., and DEAN, J. G. Germination of six alfalfa varieties at three levels of osmotic pressure. Agron. J. 51:308–309. 1959.
2. ————, and HAUS, T. E. Selection of alfalfa lines for their ability to germinate under high osmotic pressure. Agron. J. 52:200–201. 1960.
3. HEINRICHS, D. H. Germination of alfalfa varieties in solutions of varying osmotic pressure and relationship to winter hardiness. Can. J. Plant Sci. 39:384–394. 1959.
4. RODGER, J. B. A., WILLIAMS, G. G., and DAVIS, R. L. A rapid method for determining winter hardiness in alfalfa. Agron. J. 49:88–92. 1957.
5. RUMBAUGH, M. D., and SWANSON, C. R. Influence of mannitol on germination and seedling development of alfalfa. Rept. Seventeenth Alfalfa Imp. Conf. 68–73. 1960.
6. SCHWER, J. F., TAYLOR, N. L., and KENDALL, W. A. Use of osmotic pressure in evaluation and selection of small-seeded legumes. Agron. Abstr. Nov. 16–20. 1959.

PROPERTIES OF DOUBLED HAPLOIDS OF COTTON[1]

James R. Meyer and Norman Justus[2]

A COLLECTION of haploid and doubled haploid cotton plants is being studied at the Delta Branch Experiment Station, Stoneville, Miss. Since the literature contains few references pertaining to the properties of doubled haploids of cotton, it seems desirable to report on this work.

The first monoploid angiosperm described in the literature was reported in *Datura* by Blakeslee et al. (3) in 1922. Cotton haploids have been found as small twin seedlings (6, 2) or as scrawny field plants. Harland (6) wrote that he found a haploid cotton plant in 1920, but it was not identified as such until 1932. He used various techniques (7), including colchicine, but was unable to double the chromosome number in other haploid cotton plants. Beasley (1) developed a colchicine technique for doubling the chromosome number of haploid cotton plants and developed several pure lines from them (2).

Harland (6) discussed possible uses of doubled haploids in genetic and breeding programs. Chase's work with monoploids (4, 5) made their use in a hybrid corn program a definite possibility.

All haploids with a "Z" prefix (Table 1) were obtained from the Texas Agricultural Experiment Station; three of these were obtained as doubled haploids. M-11 was obtained from the Arkansas Agricultural Experiment Station as a haploid. The remaining "M-designated" material originated at the Delta Branch of the Mississippi Agricultural Experiment Station, Stoneville, Mississippi. In general, the haploids were doubled by repeated application of 0.2% colchicine solutions to the meristems of grafted plants until doubled sectors appeared. Original haploid material has been maintained in the greenhouse by grafts and cuttings.

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Table 1—Doubled haploids being maintained at the Delta Branch Experiment Station, Stoneville, Miss.

Gossypium hirsutum		Gossypium barbadense	
Number*	Parental material	Number*	Parental material
Z 99	(Unknown)	Z 101	Sea Island var. Puerto Rico
Z 104	Acala	M-50	Sea Island var. Tz Rv
Z 106	Stoneville	M-51	Sea Island var. Seabrook
Z 108	Acala	M-52	Sea Island var. Seaberry
Z 109	Acala	M-53	Sea Island var. Seaberry
Z 112	(Unknown)	M-54	Sea Island var. Seaberry
M-8	Deltapine-14	M-55	Pima S-1
M-11	Empire	M-56	Pima S-1

* Z numbers obtained from Texas Agricultural Experiment Station, College Station, Texas. M numbers doubled at Delta Branch Experiment Station, Stoneville, Mississippi. M-11 obtained as haploid from Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

Table 2—Mean property values of 7 G. Hirsutum doubled haploids grown in 1956.

Doubled haploid	Number seed per boll	Seed index	Lint index	Fiber length		Tena-city (T1)	Elong-ation	Fiber fineness	
				UHM	Mean			A	D
Z 99	32	13.2	6.5	1.22	1.02	2.04	5.6	462	28
Z 104	33	13.3	7.7	1.13	0.95	1.98	6.9	445	22
Z 106	32	13.1	6.2	1.14	0.94	1.79	6.3	505	40
Z 108	27	12.9	7.7	1.11	0.97	2.20	6.8	417	20
Z 109	27	12.7	7.3	1.13	0.98	2.03	6.8	421	20
Z 112	29	12.0	5.6	1.19	0.97	2.01	5.8	523	38
M-8	32	10.2	6.5	1.10	0.91	1.75	8.4	461	34

Cotton haploids are easily distinguished from normal plants. Haploid cotton plants have thin zigzag stems, short internodes, small leaves, small squares, small flowers, and no pollen (Figures 1, 2, and 3). They usually lack seeds and bolls and may be taller than normal plants in the fall.

The different appearance of haploid and doubled haploid plant parts is illustrated in Figures 1 and 2. A general increase in size occurs in the doubled (2 n) tissue which results in thicker stems, larger leaves with a darker green color, and larger flowers. Pollen and bolls are freely produced on doubled haploids.

In general, the doubled haploid progenies are uniform in appearance. No gross mutations have been observed.

A general description of the doubled haploids listed in Table 2 follows:

Z99 is not very productive, the bolls lack fluff and rot easily;

Z104, Z108, and Z109 are in general rather tall, late, and unproductive at Stoneville.

Z112 is productive; the bolls lack fluff, rot readily, and shatter badly.

Z106 produces good yields of seed cotton and has large non-stormproof bolls. It becomes large and weak-stalked with excessive water and nitrogen.

M-8 has a good open plant type with a strong stem. The bolls are fluffy and have about the right amount of stormproofness for Mississippi. M-8 is being tested in station strains tests and is being used in the breeding program as a parent. Preliminary data indicate that it is useful as a recurrent parent in a backcrossing program and has possibilities as a commercial variety at this location.

The M-11 haploid of Empire was received in 1957. This plant was extremely hard to "double." Selfed seeds from this material were finally obtained in the summer of 1958. These seeds were sent to the winter cotton garden at Iguala, Mexico, for increase from which a field planting was made in 1959.

The plant and fiber properties listed in Table 2 were obtained from the 1956 haploid nursery at Stoneville. This nursery consisted of one block. Plants of each haploid were grown in a single plot consisting of not less than 8 rows.

Figure 1—Comparative branches of cotton from doubled haploid M-8 (left) and haploid M-8 (right).

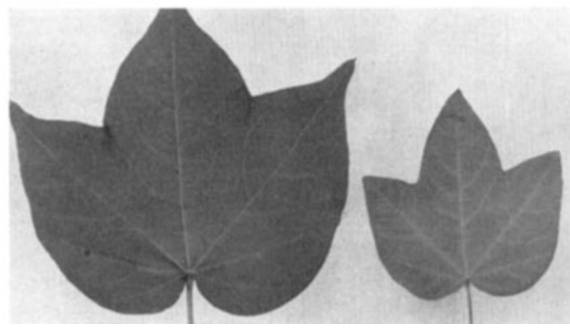

Figure 2—Leaf of cotton from doubled haploid M-8 (left) and haploid M-8 (right).

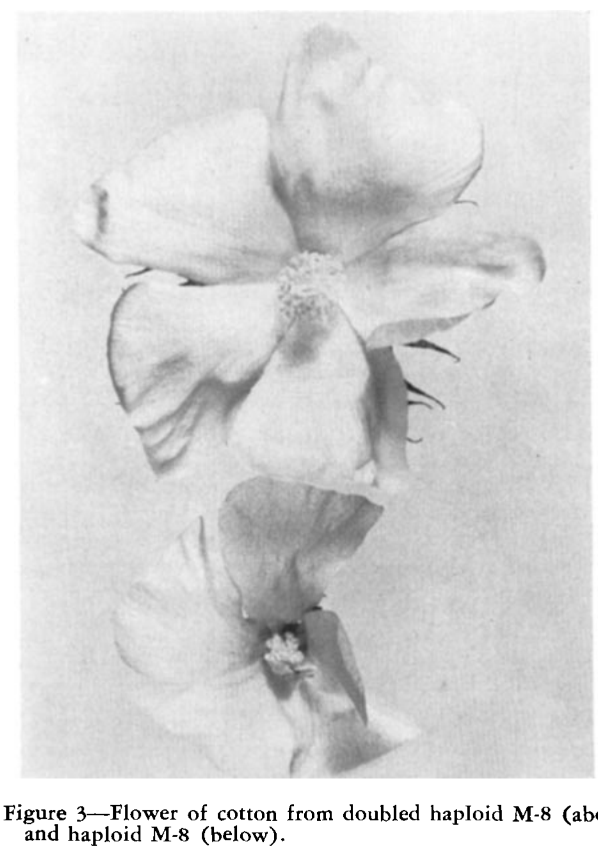

Figure 3—Flower of cotton from doubled haploid M-8 (above) and haploid M-8 (below).

All seed, lint, and fiber-property determinations were made on composite 15-boll samples taken from each row in each plot. Only one boll per plant was harvested. The fiber data are presented as plot averages. The fiber measurements were obtained from samples sent to the USDA Fiber Laboratory at Knoxville, Tenn.

Unfortunately the field design used for obtaining the data in Table 2 did not lend itself to statistical analysis. Studies are underway to determine the variability and combining ability of some of the *G. hirsutum* doubled haploids.

The other 8 doubled haploids are *Gossypium barbadense* and not adapted to Mississippi environmental conditions. In general, the plants are vegetative and somewhat photoperiodic; most of them lack earliness and productiveness.

SUMMARY

A description of eight *G. hirsutum* doubled haploids has been presented. Eight *G. barbadense* doubled haploids have been briefly described. The data presented indicate that the *G. hirsutum* doubled haploids have several contrasting characters and that they could be used in inter-doubled-haploid crosses or used singly in genetics and breeding programs. The agronomically desirable characters in M-8 make it of special value in breeding programs.

LITERATURE CITED

1. BEASLEY, J. O. The production of polyploids in *Gossypium*. J. of Hered. 31:39–48. 1940.
2. ——————. Hybridization, cytology, and polyploidy of *Gossypium*. Chron. Bot. 6:394–395. 1941.
3. BLAKESLEE, A. F., BELLING, J., FARNHAM, M. E., and BERGNER, A. D. A haploid in the jimson weed, *Datura stramonium*. Science 55:646–647. 1922.
4. CHASE, SHERET S. Techniques for isolating monoploid maize plants. A. J. Bot. 34(10):582. 1947.
5. ——————. Monoploid frequencies in a commercial double cross hybrid maize, and its component single cross hybrid and inbred lines. Genetics 34:328–332. 1949.
6. HARLAND, S. C. The use of haploids in cotton breeding. Indian J. of Gen. and Pl. Br. 15:15–17. 1955.
7. ——————. Recent progress in the breeding of cotton for quality. J. of the Textile Institute 46:172–182. 1955.

A FLOTATION METHOD FOR SEPARATING DEHULLED OATS FROM GRAIN SAMPLES[1]

K. J. Frey, R. L. Grindeland, and H. C. Murphy[2]

THE amount of thresher-dehulled grain in a sample is a source of error in the determination of groat percentages of oat grain. The percent of thresher-dehulled seed in a sample may vary with the variety, the adjustment of the threshing machine, the plumpness of the oat seeds, and possibly other factors. The 29 varieties and strains tested in the North Central States Uniform Oat Nursery in 1959 showed a range in thresher-dehulled kernels of 3.0 to 10.9% (12-station average).[3] The range among strains at one station was 5.3 to 36.0% dehulling. The effect of threshing practices is evidenced by the range in mean percent of dehulled grain among the 12 experiment stations in 1959 of 0.7 to 19.3%. In one variety at one station the thresher-dehulled seed would have raised the groat percentage from its true value of 67.7 to a value of 73.6.

Figure 1—Oat seed being stirred into sugar solution.

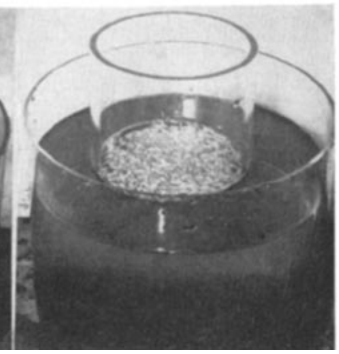

Figure 2—Groats at bottom of solution and oat seeds with hulls floating.

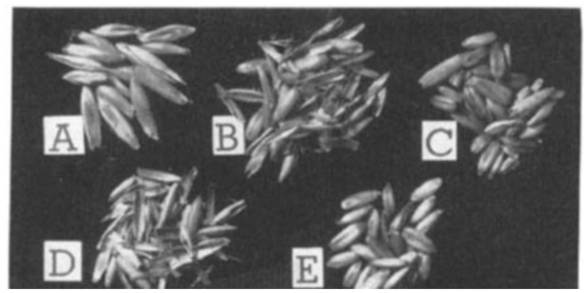

Figure 3—A. Oat sample after flotation; B. Oat sample after being run through the hulling machine; C. Oat sample after being passed over clipper mill; D. Hulls separated by the clipper mill; E. Groat sample after hand picking.

Thresher-dehulled groats increase the apparent groat percentage and bushel weight and decrease the computed grain yields.

Groats can be completely and quickly separated from oat seed with hulls by a flotation method. The items of equipment needed for this procedure are a battery jar about 15 inches in diameter and 12 inches high, a plastic cylinder 6 inches in diameter and open at both ends, and a screen in which to set the plastic cylinder (Figures 1, 2, and 3). The solution used for flotation is made with 450 grams of sucrose per liter of water.

The seeds are poured into the solution inside the plastic cylinder (Figure 1) and stirred vigorously causing the groats to sink to the bottom and seeds with hulls to float on top. If not stirred, the seeds with hulls tend to hold groats on the surface of the solution (Figure 2). The plastic cylinder is lifted slightly, a screen is placed under it, and the floating seeds are lifted with it from the solution. The seeds adhering to the plastic cylinder are washed into the screen and all the seeds are sprayed thoroughly with water to wash off the sugar solution. Failure to wash

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[3] Data from the North Central States Uniform Oat Nursery for 1959 is used with the permission of the cooperating experiment stations.

[4] The experimental oat dehuller used in these studies was loaned to the Iowa Agricultural Experiment Station by the Quaker Oats Company of Chicago, Illinois.