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Inheritance of Hard Seed in Cotton[1]

Joshua A. Lee[2]

ABSTRACT

The purpose of this study was to determine the inheritance of hard or impervious seed coat in cotton (*Gossypium hirsutum* L). A hard seed was defined as any that could resist water uptake for 24 hours at 27 C. The breeding line SP-hard seed was crossed with the nonhard-seeded variety 'Carolina Queen' and various backcross and filial generations were raised. Graphing the relative frequencies of hard and nonhard seed taken from individual plants in the various generations revealed evidence for two genes. Their concerted action determines the level of seed hardness noted in SP-hard seed (i.e. from 50 to > 90%, depending on the environmental conditions under which the seed matured). Under the humid conditions of greenhouse growth, expression of hard seed appeared to be largely recessive than nonhard, but there was evidence that this condition is subject to reversal in an arid environment.

Additional index words: **Gossypium hirsutum L., Impervious seed coat, Germination inhibitor, Chemical scarification.**

GOOD quality seeds of upland cotton (*Gossypium hirsutum* L.) germinate within 5 days if planted in warm, moist, soil whereas the seeds of wild (ruderal) forms of *G. hirsutum* can remain dormant for prolonged periods after being exposed to conditions that ensure prompt germination in domesticated forms. This delay of germination is usually attributed to hardness or impermeability of the seed coat, although chemical inhibitors may also be a factor (3).

Christiansen and Moore (2) compared in detail the anatomy of seeds from hard and nonhard strains of cotton. They found that hard seed has a massive, well organized, chalazal cap, which adheres strongly to the palisade layer of the seed coat as the maturing seed dries. In the center of the chalazal cap is a plug of wax and pectin. This plug is readily dissipated by hydrocarbon solvent soaks or hotwater (27 to 29 C) baths. Presumably the chalazal plug dissolves at a slow rate under natural conditions of repeated weting, no doubt mediated by bacterial or fungal action, or both.

Nonhard seed (characteristic of cultivated cottons) has a smaller, poorly organized, chalazal cap, which only occasionally adheres to the palisade layer. Thus seeds of these cottons imbide freely.

Hard seed is of potential agronomic importance in the various regions where cotton is grown. In arid regions where the crop matures under dry atmospheric conditions, hard seed can cause uneven germination (3). Thus breeders in such areas might wish to eliminate the character from their breeding stocks. In humid environments, hard seed might be of value in preserving seed quality, because seeds sealed from ambient moisture changes suffer less deterioration before harvest and after storage.

Because of this, knowledge of the inheritance of seed hardness could be helpful to breeders in eliminating the character or in introducing it into breeding lines. The purpose of the current study was to determine the inheritance of hard seed coat in the breeding line SP-hard seed, a cotton that commonly displays from 70 to 90% seed hardness under humid conditions of plant growth.

MATERIALS AND METHODS

Because the expression of seed hardness is a conditional character in the day-neutral stocks available for study, an operational criterion that defined hard seed "en masse" was needed before any genetical experiments could be tried. Thus if good quality seeds of an upland cotton are placed in a saturated chamber at 27 C, virtually 100% will imbibe water within 5 hours. On the other hand, if seeds of the experimental line (SP-hard seed) are similarly challenged, a small number will imbibe within the first 5 hours with water uptake virtually ceasing until nearly the 20th hour. After that, more and more of the seeds imbibe water until, after about a week, most of the seeds in the lot will have germinated.

Twenty-four hours is very nearly the 20-hour span noted before renewed imbibition for SP-hard seed lots. Moreover it is a convenient interval for experimental operations of this kind. Therefore, I used the 24-hour incubation period for scoring (i.e. declaring any seed that imbibed water during the interval as phenotypically nonhard and any that survived the test unsaturated as phenotypically hard).

The nonhard-seeded line used in the current work was selected as an inbred line from 'Carolina Queen,' a now obsolete Upland variety. The hard-seeded stock (SP-hard seed) is a line stemming from a cross of 'Glandless 38-6' and the Upland variety 'Coker 100-A.' Depending upon the conditions under which it is grown, SP-hard seed commonly shows 70 to 90% seed hardness, based on the 24-hour survival criterion. Carolina Queen rarely produces any seed that fail to imbibe within 24 hours. Progeny tests have shown that the permeable part of a given lot of seed from SP-hard seed produces the same level of seed hardness in ensuing generations as plants stemming from the impermeable portion. This result is interpreted to mean that the line is homozygous for capacity to produce a high level of phenotypically hard seed.

After the two parents were crossed, the following generations were reared under greenhouse growth (i.e. temperatures ranging from 27 to 35 C during the day and 18 C at night and humidity ranging from 50 to 60%): P_1 (Carolina Queen), P_2 (SP-hard seed), F_1, F_2, BC_1 to P_1 ("B_1"), and BC_1 to P_2 ("B_2").

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[2] Research geneticist, ARS, USDA and professor of crop sci., N. C. State.

At maturity, fruits were allowed to open and dry on the mother plant. After harvest, the seed cotton was dried further at 45 C and acid delinted. Then the seed was redried before storing at subfreezing temperatures.

To assay for permeability, lots of 100 seeds/plant were placed in wet paper towels backed by waxed paper. These "rag-dolls" were then incubated at 27 C for 24 hours in a saturated chamber. At the end of the trial, the "rag-dolls" were removed and the seed lots were read for water uptake as quickly as possible. Any seed in which the coat could be slipped by pressure of the fingers was classed as phenotypically nonhard.

Because hard seed is a material character, one must scan the seeds produced on a given plant, to ascertain the phenotype of that plant. For example, one establishes the phenotypic array of an F_2 population by challenging individual plant-lots of seeds containing F_3 embryos.

RESULTS

The distribution of phenotypes in SP-hard seed (P_2) shows a mode at about 95% (Fig. 1). Because all lots of 'Carolina Queen' inbibed within 24 hours, the results from P_1 are not presented graphically. The distribution of phenotypes in the F_1 (Fig. 2) suggests that under greenhouse growth and perhaps also humid field conditions, the expression of hard-seed is largely recessive to that of nonhard-seed. This conclusion is further supported by the results from the B_1 and B_2 (Figs. 3 and 4). In the B_2, the bar graph is definitely bimodal with approximately equal portions of the

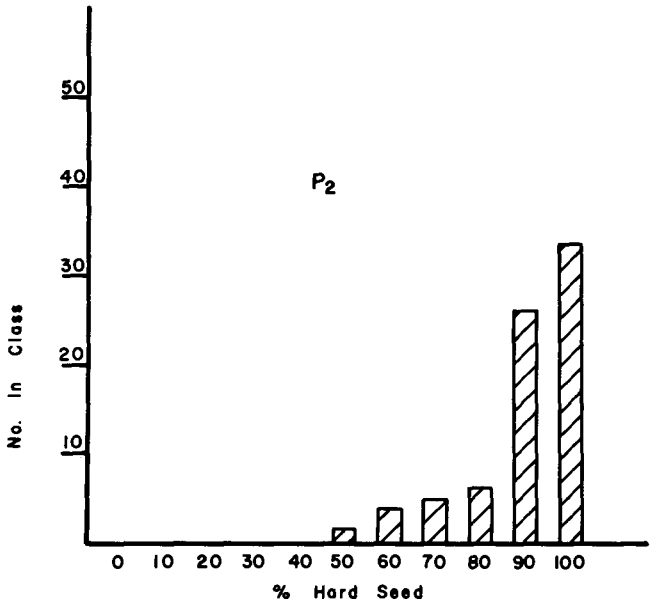

Fig. 1. Distribution of phenotypes in SP-hard seed (P_2).

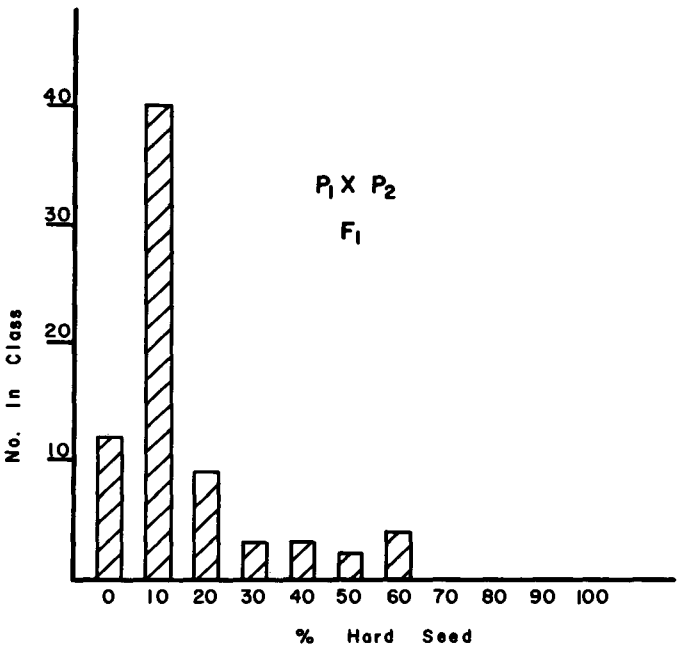

Fig. 2. Distribution of phenotypes in the F_1, Carolina Queen $\times$ SP-hard seed ($P_1 \times P_2$).

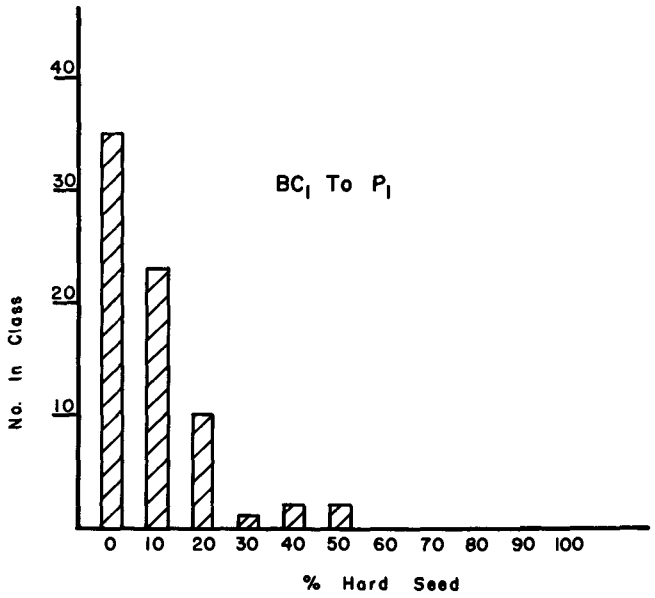

Fig. 3. Distribution of phenotypes in the BC_1 to P_1 (B_1).

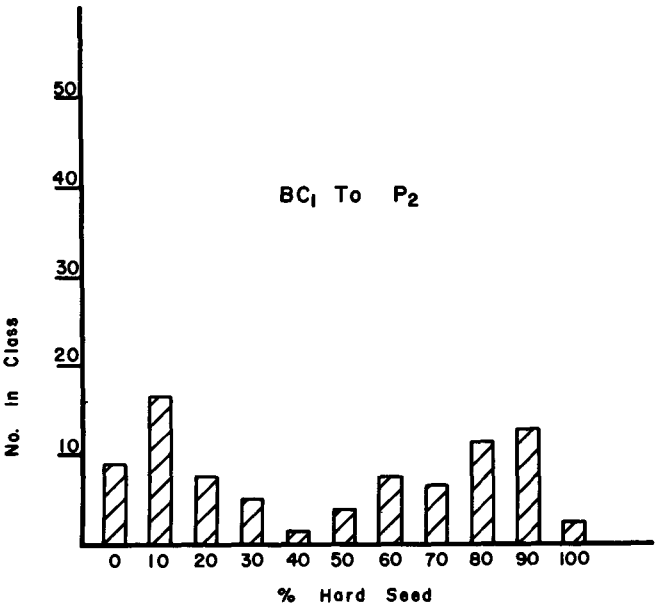

Fig. 4. Distribution of phenotypes in the BC_1 to P_2 (B_2).

individual plant progenies assignable to each peak. This result suggests that most of the difference between hard and nonhard-seed is mediated by a single allelic pair. This conclusion is strengthened by the distribution of phenotypes in the F_2 (Fig. 5). Here again, the curve is bimodal, but the second mode is much more depressed than the first. In both the B_2 and the F_2, the phenotypic array spans the distribution between the parental phenotypes.

Although the generation curves examined thus far suggest that alleles at a single locus determine most of the difference in expression between phenotypically hard and nonhard-seed lots, the distributions are not discrete (i.e. the phenotypes overlap). With such distributions, genes of minor effects could be present. Such genes might be detectable with progeny testing. Accordingly remnant seeds of the B_2 generation were grown out and the plants selfed. The percentage of seed hardness in individual plant progenies ranged from 0 to 95% and lineages derived from the extremes of the distribution tended to breed true. A single plant gave rise to a line that showed from 6 to 40% seed hardness, depending upon the particular trial and another plant's lineage stabilized between 55 and 80% hard seeds. These lines (designated H-21 and H-26, respectively) were crossed with each other and with the P_1; F_2 progenies were reared. The results are presented in Figs. 6 through 8.

In Fig. 6, the distribution of phenotypes suggests that a single, largely recessive allele conditions the level of seed hardness noted in H-26. The case for a single allele in H-21 (Fig. 7) is not nearly as strong. The F_2 stemming from the cross of H-21 and H-26 is similar to that in Fig. 5: the distribution of phenotypes is equally expansive and the general shape of the curve is the same.

There is reason to believe that H-21 harbors a gene of relatively minor effects that acts together with an allele in H-26 to account for the level of seed hardness noted in SP-hard seed. In the trials whose results were graphed in Figs. 6, 7, and 8, the following lines were used as controls: P_1, which produced no hard seed; P_2, which averaged 86% and H-21, 6%; and H-26, 64%.

DISCUSSION

A single allelic pair accounts for most of the expression of seed impermeability in the experimental line of *G. hirsutum,* SP-hard seed. Additionally, there is

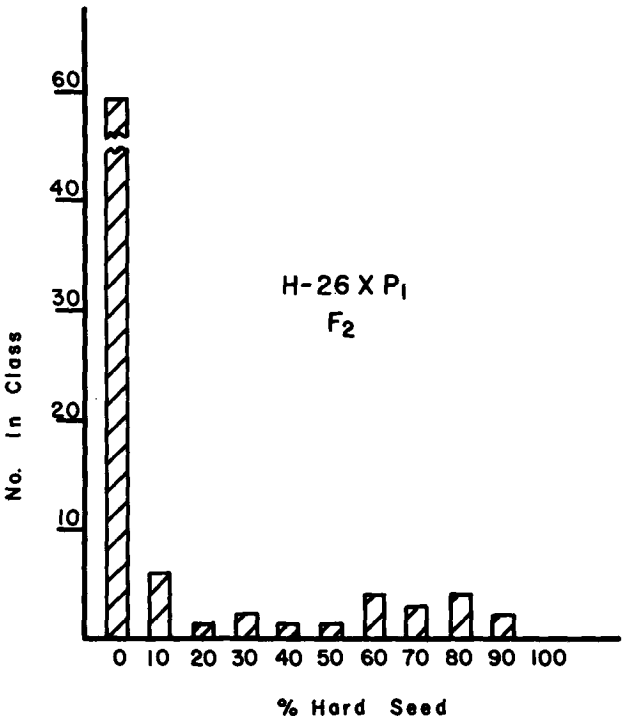

Fig. 6. Distribution of phenotypes in the F_2 (H-26 × P_1).

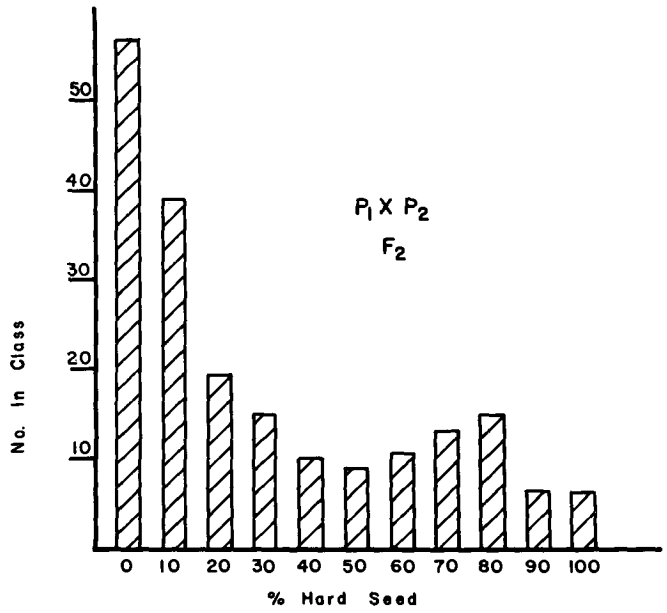

Fig. 5. Distribution of phenotypes in the F_2 (P_1 × P_2).

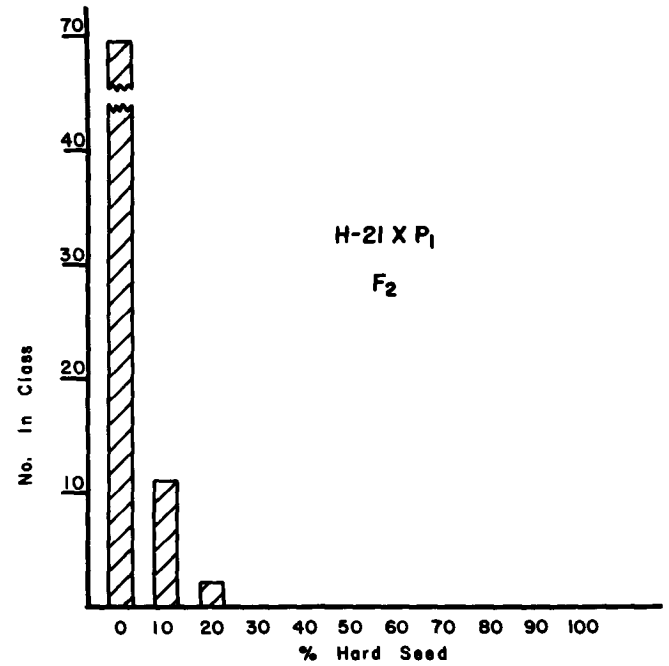

Fig. 7. Distribution of phenotypes in the F_2 (H-21 × P_2).

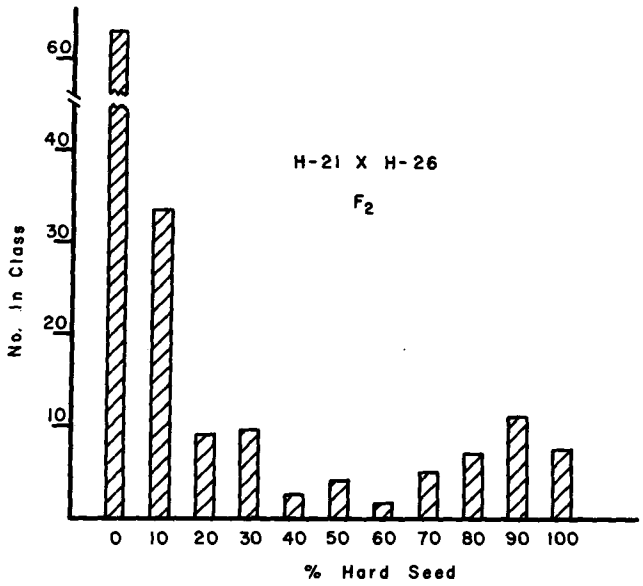

Fig. 8. Distribution of phenotypes in the F_2 (H-21 × H-26).

evidence for a second allelic pair with lesser effects. These two genes act together to produce the level of seed hardness, or impermeability, commonly noted in SP-hard seed.

Under field conditions in North Carolina, variations in rainfall and humidity during the period of seed ripening produce dramatic fluctuations in the level of seed hardness recoverable in a 24-hour incubation test. Thus with SP-hard seed, I have recorded from 50 to > 90% impermeability, depending upon the particular year of the trial. All trials reported herein were performed under greenhouse growth in attempts to reduce this season to season variability. Evidently this objective was realized, because in seven plantings of SP-hard seed in 5 years, no trial averaged less than 80% hard seed nor more than 90%. During the same period one field trial assayed 97% seed hardness for SP-hard seed and an F_1 from crossing SP-hard seed with Carolina Queen showed 75% seed hardness. These values, taken from an exceptionally dry autumn, suggest that the level of seed hardness in an inherently impermeable line rises as humidity falls. Moreover the gain in seed hardness in that particular F_1, as compared with the low values recorded for F_1s grown in the moderately humid greenhouse, suggest that under arid conditions of growth expression, hard seed might shift toward dominance in crosses with permeable stocks.

Rarely have I assayed individual plants of SP-hard seed that produced 100% impermeable seed. Yet some wild, or "house-yard," accessions of *G. hirsutum* commonly produce seed lots that are 100% impermeable and which remain so after days or even weeks of incubation. This superior level of impermeability might be imparted by the presence of additional alleles of minor effects, genes that lose much of their effectiveness when transferred to an essentialy Upland background. Furthermore some "house-yard" cottons (i.e. WH-219 from Nicaragua) have a water-soluble substance about the embryo which inhibits germination, even after the seed coat is removed. I could find no evidence that any such substance persists in the embryos of SP-hard seed, once the seed is well dried.

There are certain problems involved in attempting to use impermeable seed in modern schemes of cotton production: i) one would need to acquire and maintain through breeding a level of impermeability such that the quality of harvested and stored seed is enhanced and maintained over that of current commercial varieties; ii) impermeability must be relieved prior to planting so that there can be uniform germination; iii) cotton producers need to be forewarned that impermeable seed might transform cotton into a potentially troublesome weed that could harbor insect and disease pests.

Work performed by Christiansen and Justus (1) points up the feasibility of using hard seed as a protectant against weathering. There is a need for work on the potential advantages of hard seed in preserving quality of stored seed. The experiments of Christiansen and Justus further suggest that the extreme degree of impermeability characteristic of "house-yard" and wild cottons is not needed. Indeed such a degree of impermeability might be extremely difficult to obtain in the relatively large seeds of upland varieties. Walhood (3) and Christiansen and Moore (2) have developed techniques for relieving seed hardness before planting. However the use of the chemicals and facilities needed would impose an additional cost which hopefully would be offset by enhanced seed quality. The problem of weediness is largely speculative at this time. However various observers have noted that hard seed can remain viable over winter in humid regions and thus germinate and grow in crops other than cotton.

It is doubtful whether any scheme of cotton production could ever tolerate a persistant inhibitor to germination (i.e., as is found in the seeds of certain wild and "house-yard" cottons). Fortunately the breeding lines available, SP-hard seed and 16-B-7 (1, 2), seem to be devoid of any such substance. This fact coupled with a fair degree of seed impermeability, should make the current materials attractive to breeders, should programs on seed impermeability be initiated in the future.

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