Can Hard Seeds in Alfalfa be Genetically Scarified?

Edwin T. Bingham – Chapter 1 and 2
ebingham@wisc.edu

William P. Kojis – Chapter 3
wpkojis@facstaff.wisc.edu

Report to the American Seed Research Foundation for research funds received from September 1991 through August 1994. This report appeared in their bulletin "Search," Summer 1995, Issue 29. All images and tables appear after the main text.

Summary

The answer is yes! Two genetically controlled traits were identified which enhance germination. The first trait (Chapter 1) was white seed coat controlled by the recessive basic color factor gene c2. In homozygous recessive plants, anthocyanin pigmentation is blocked in all parts of the plant and seed coats are white. It was shown that white seed coats are more permeable to water, but it takes seven days to realize an advantage in germination of white seeds. Similarly, a mutable trait that produces white streaks on otherwise tan seeds has the same effect at c2. Thus, although seeds with white sectors in seed coats germinate faster than tan seeds, the trait was not considered as useful as the following trait.

The superior trait (Chapter 2) was a highly water permeable seed coat with normal tan pigment that conditions essentially immediate germination. We have termed this condition "exceptionally fast germination" (efg, and we have learned the following: The frequency of alfalfa plants with efg seed in our nurseries at Prosser, WA, is about 5%, based on collecting and germinating seed of over 2000 individual plants. In this efg category 85-90% of the seeds are swollen and beginning to show radicles 48 hours after placing on germination paper. Interestingly, a few seed lots were identified at a frequency of about one-half of one percent in which all seeds were swollen and showing radicles in 24 hours.

Research on the mechanism of efg and preliminary genetic analysis were based on this efg fastest category. The efg plants for analysis were recovered from the half-sib progeny of 1 0 efg individual seed lots identified among over 2000 seed samples from WA. Electron micrographs of hard and efg seed at 1000X are pretty, but appear similar and do not define the efg mechanism. Progeny of efg plants crossed with each other tend to be efg and efg indicating allelism of the efg system. Self progeny of efg plants tend to be efg indicating efg plants are homozygous or near homozygous for the trait. The half-sib progeny of efg and efg plants segregated. Our preliminary genetic interpretation is that efg tends to be a recessive trait. An efg experimental line with essentially no hard seed is under development.

Finally, several additional experiments (Chapter 3) were conducted on the changes in hard seed content over time using hard seed lots of alfalfa breeding material available on our project. Seed lots with a high hard seed content in the spring following production at Prosser, WA, decreased in hard seed content when stored at room temperatures. Hard seed content decreased more rapidly on germination paper, and decreased most rapidly in soil in the outdoors. However, cold storage maintained the hard seed content over the two years of the test.

Introduction

Alfalfa seed production in the Northwest is on the increase. However, there is a high content of hard seed from this region (E.T. Bingham unpubl. data and perro comm. from seed companies). Hard seed content can be reduced by mechanical scarification, but this impairs seed storageability. Moreover, there is a fine line between unscarified and over scarified which can damage the seed. Hence, a genetic modification that would increase permeability and germination while not affecting seed storability would be potentially useful, and provide basic informa1ion about the hard seed phenomenon.

A review of alfalfa seed germination by Gunn (1972) covers much of what is known about hard seed in alfalfa. Hard seed content is affected by both the environment in which seed is produced and by the genotypes of the piants in populations. Hard seed content usually decreases somewhat over time in storage, but the decrease is unpredictable. This change is affected by the original environment, storage conditions, and genotype.

An observation in 1985 indicated seed with a white seed coat had significantly higher germination and plant establishment than isogenic normal tan seed, (Talbert, 1985). Using this study as the springboard, we began the research on alfalfa seed germination. The research was exciting and quickly expanded to include conventional alfalfa breeding materials. Many interesting and diverse experiments were conducted and reported in three separate chapters.

Literature Review

The seminal paper on hard seeds in alfalfa was written by Lute (1928). Lute established that the cuticle of the alfalfa seed could be stained (i.e. would take up moisture) and concluded that the cuticle was not a barrier to permeability. Lute went on to state: "The impermeable layer in the seed coat is at the outer end of the palisade cells." This has been considered dogma for 65 years (Bolton, 1962; Gunn, 1972; Bass et al. 1988). However, Lute in the same paper also stated: "No mechanical or structural difference can be observed in permeable and impermeable seeds." Hence, neither a structural nor a biochemical mechanism for hard seeds was implied by the early work.

The problem with a completely structural (cellular anatomy) explanation for hard seeds is that the permeability of alfalfa seed coats can change over time, and yet the structure (cellular anatomy) of dry seeds is unlikely to change over time. A change over time, especially in seeds in storage, suggests a chemical involvement. A chemical involvement could still interact with structure and account for the effectiveness of scarification. In other words, permeability could increase by decay of an inhibitory chemical or by bypassing it with a mechanical abrasion. One such chemical involvement in impermeable seed coats of peas was found by Marbach and Mayer (1974). They suggested that high levels of phenolics may render seed coats impermeable to water. They reported a relationship between tan seeds and water impermeability.

In alfalfa, Talbert (1985) also suggested that phenolics, which are involved in the tan seed coat color of normal alfalfa, may be related to impermeable seed coats in alfalfa. Talbert observed that unscarified white seed (low phenolic content) germinated readily on wet filter paper, while tan seed (higher content of phenolics) contained many hard seeds. Further, germination and plant establishment studies in soil pots showed the same relationship. The percent germination of tan seed was significantly lower than percent germination of white seed. Plants with white and tan seed coats in Talbert's study in one case were clones from white and purple chimeral sectors of an original plant. Hence, white and purple seed coats were isogenic except for a mutation at the basic pigment locus. In the other case, they were white and purple

segregates from one plant. White and purple in this case were genetically related. Hence, the effect of seed coat as such was tested.

Changes in permeabilitiy over time depend on such factors as storage temperature and relative humidity. As reviewed by Bass et al. (1988) loss of hard-seededness varied among cultivars when stored at 5°C and 40% relative humidity in the National Seed Storage Laboratory. However, in all cultivars that had more than 12% hard seeds, the hard seed percentage decreased by about half in 12 years of storage except for 'Travois' which contains much Medicago falcata germ plasm. Travois stored for 0,12, and 23 years contained 41%, 41%, and 36% hard seed.

CHAPTER 1: Experiments Involving Seed Coat Color Materials and Methods

Categories of seed coat color and color patterns are illustrated in Figure 1. Tan is typical of normal, commercial alfalfa seed. Tan, white, and mutable 2 were used in Objectives 1 and 2; tan and tan sectored were used in Objectives 3 and 4.

Objective 1. Determine the effect of the seed coat per se on the development of hard seeds. The procedure utilized a purple flowered, tan seeded revertant on a mutable 2 plant that was identified in the summer of 1991. It is in a different genetic background from the one used by Talbert, 1985. We know from the comprehensive studies by Talbert and Bingham 1986 that purple flowered, tan seeded revertants are chimeras with only the outer cell layer involved in reversion. The germ layer remains mutable 2. A purple revertant sector and a mutable 2 (basically white) were cloned by vegetative cuttings. The purple and mutable are identical in the germ line, and differ in the outer cell layer (the cell layer that determines the flower and seed coat colors) by only the c2-m2 allele which reverted to C2 to permit production of pigment in flower and seed coat. These unique stocks were selfed to produce seed that has the same array of genotypes in the embryos but differ in seed coat color. The two seed types are referred to as Class 1 and Class 2 in Table 1. A quantity of self seed was produced in January of 1992. Seed produced in the greenhouse in the winter is notorious for hard seed. Seed of both seed coat colors was germinated as scarified and unscarified seed. We scarify by rubbing seed between layers of fine sandpaper. One person (E.T.B.) did all scarification as uniformally as possible. Scarified seed all germinated in the 90% range, and only the unscarified results are reported. Germination percent indicated the effect of seed coat per se on development of hard seeds.

Objective 2. Determine the effect of the embryo per se on the developing seed coat and eventual hard seed. At the same time plants were selfed for the tests of seed coat per se, they were crossed with a normal purple-flowered/tan seeded plant (one genotype) from the cultivar 'Magnum'. Use of Magnum was arbitrary; we just happened to be using it in other experiments. Germination tests of combinations which all have the same seed coat color within respective combinations, tested the effect of embryos per se on the developing seed coat and eventual hard seed.
All germination tests were conducted using standard germination paper (Anchor 38 lb. seed germination paper) saturated with distilled water and placed in plastic bags at a constant laboratory room temperature of 72°F. Germination tests involving these genetic stocks involved 50 seeds in each test, replicated twice.

Objective 3. Determine if small white sectors on otherwise tan seed coats are equivalent to scarification in promoting germination. This objective used mutable 5 (c2-m5) that has purple flowers with small white sectors and tan seeds with small white sectors (Bingham and Clement 1989. The mutable 5 line was grown in the summer 1991 and about 10 strong plants with small white sectors in their seed were identified. These plants were used for production of self and cross seed in the greenhouse in January 1992. Germination tests as previously described were

used. The control for this experiment was solid purple flowered/tan seeded segregates identified in the mutable 5 line in the summer of 1991.

Results

The results for the tan versus white seedcoat were the same for isogenic and genetically related materials Table 1. Comparisons of Class 1 versus 2, and 3 versus 4, tested the effect of the seed coat per se on germination. White seed coats were consistently higher in germination comparisons of Class 1 versus 3, and 2 versus 4 tested the effect of the embryo per se. Germination was the same in each seed coat class; hence, the embryo has no effect. Another strong test of the effect of the embryo was conducted with a line that has essentially 100% germination even with greenhouse-produced seed. It was pollinated with WISFAL (a tetraploid Medicago falcata line with 60-70% hard seed). Seed of this line containing the hybrid embryo involving WISFAL germinated the same as self and half sib seed. Once again, the embryo had no effect.

A particularly hard seed lot containing genetically related tan and white seed was produced at Prosser, WA in 1990. This material was placed on germination paper and monitored for two years (see Chapter 3 for procedures). The progression in germination over time is reported in Table 2.

The results in Table 2 provide further evidence that white seeds are more permeable than tan. This seed lot was very hard, and it took a long time for white seed coat to show an advantage, but by the end of the test, white seed germination was almost complete, while tan was a little more than half.

Objective 3 was to determine if small white sectors on otherwise tan seeds was equivalent to scarification in promoting germination. In our early thinking and in the proposal we were not focusing on the time factor. Tan seeds with white sectors are comparable to solid white seeds, but they are not as germinable in a 3-4 day period as mechanically scarified seed. The average of four germination tests with 50 seeds of each type in each test indicated tan 32%, sectored 45%, and scarified seeds of both were near 90%. Hence, the sectored seed germinates better than the tan, but it is not as good as scarified seed.

CHAPTER 2: The Fast Germination Trait Materials and Methods

Once funding was obtained from the American Seed Research Foundation, several ongoing research projects were used to generate information on germination in normal breeding materials. For example, we collected one stem with ripening seeds from each of 3000 plants in our research nurseries in Prosser, WA beginning in 1992. The large numbers of individual plants were tested for germination in Madison, WI, several exceptionally fast germination (efg) lines were identified, and the efg trait was recovered using the half-sib seed and progeny of the original efg seed lots produced at Prosser, WA.

Results

The "exceptionally fast germination" (efg) trait was obtained by germ testing seed from a large number of individual plants. Seed of individual plants was obtained by collecting shoot segments with seed from individual plants (Table 3). Once an efg seed lot was identified (Fig. 2),

the half-sib progeny were grown, intercrossed and individually germ tested (Fig. 3). There is reason to believe that the hard and average segregates are from crosses, and that efg plants are usually selfs. At any rate we were able to recover efg plants relatively quickly.

It is not necessary to understand the mechanism of efg in order to use it, although it would be interesting to know the mechanism. Thus, we used a scanning electron microscope (SEM) to examine 8-10 individual seeds of three efg plants and a hard seed control. The SEM pictures are pretty, but there is not a great difference between efg seeds (SEM Figs. 1-4) and hard seed (SEM Figs. 5-8). Scanning the lens area at up to 5000X indicated that the cells in the lens of the hard seeded type may be packed a little tighter (SEM Figs. 9-12) but no conclusions were made.

We lost interest in the lens when the following experiments indicated the lens was not the mechanism of efg. First, we determined that germination of efg seed could be blocked by coating seeds with nail polish, wax, epoxy or Vaseline. Vaseline was the eaisest to work with and was used to coat the lens area of efg seeds.

Whereas coating the whole seed prevents quick germination, coating the lens does not. Seeds with the lens blocked germinate as fast as uncoated seeds. No quantitative data are presented because this was a "yes or no" experiment. This experiment indicated that a very permeable seed coat is the mechanism of efg. The lens could be very permeable, but so is the remainder of the seed coat. This conclusion was reenforced by staining seeds with ferrous ammonium sulfate (FAS) (1f3 grain in 1 liter of distilled water). When tan seeds absorb FAS they turn black. It should be mentioned that white seeds do not stain with FAS. Evidently, FAS stains by interacting with phenolics in tan seeds, which are lacking in white seeds.
Tan efg seeds placed in FAS solution or on germination paper saturated with FAS solution, turn black before your eyes within one hour. Most of the staining is in the general seed coat. An example of staining is illustrated in Fig. 4.

The review article by Gunn (1972) contains a picture of an alfalfa seed showing selective stain uptake by the lens. We observed stain uptake by all types of seeds under a dissecting scope. We expected to see this selective uptake at the lens quite often. Uptake at the lens was observed, but by the time it began to spread out from the lens, other areas on the seed coat were staining black. At this point we believe the seed coat as such is at least as important as the lens in water absorption.

Genetic analysis of efg will continue for some time. Anything we report now is preliminary. At this time, we have examined the self progeny of three efg plants. Seed produced in the greenhouse has been used for germ tests. This is considered an "acid-test" because greenhouse seed is notoriously hard. Selfs progeny (12-14 self plants in each family) have all been efg indicating efg plants are homozygous or near homozygous.

The half-sib progeny from open pollination of efg plants have segregated for hard, average, and efg. It is likely that the hard and average segregates are from crosses, and represent dominance or partial dominance over efg. The efg trait appears to be recessive.

A partial diallel of six efg plants examined thus far indicates that efg × efg produces efg. This indicates allelism of the genetic control. The plants that breed true will be used to synthesize an experimental line to test the practical value of efg as soon as possible.

CHAPTER 3: Additional Alfalfa Hard Seed Experiments

William P. Kojis
Agronomy Specialist
Agronomy Department
1575 Linden Drive
University of Wisconsin
Madison, WI 53706-1597

Introduction

Seed increases of project alfalfa lines have been made at Prosser, WA for more than 20 years. At the time we applied for the grant, we had on hand some particularly hard seed lots of experimental alfalfa seed. William P. Kojis, Agronomy Specialist working with the alfalfa breeding project, conducted the experiments reported in this section. The objectives were to study germination over time of hard seed lots and checks, and monitor changes in hard seed content of project lines that may be of general interest.

Materials and Methods

Five alfalfa lines produced at Prosser, WA with differing germination rates were selected to analyze differences in germinability over time. The lines, their germination rates, and other basic information are given in Table 1. Two samples of approximately 100 seeds of each line from each temperature regime were placed on germination paper, which had been soaked in a 0.1 % Captan solution to retard spoilage. The seeded germination papers were stored in a plastic bag at 2rC and kept saturated. The germination tests were scored three days after initiation and once a week thereafter. As the seeds germinated, they were removed from the paper and scored. The non-germinated seeds were also counted, and an initial germination percentage was calculated. Sequential tests were initiated April 12, 1991, and once a month thereafter for the first year. After one year, the tests were initiated every other month.

The same five lines, plus another entry, W71-42 (see Table 1) were planted in flats filled with a 3:1:1 mixture of unsterilized field soil: peat moss: sand. 500 seeds of each entry were scattered on the surface and covered with about 1 cm. of the soil mix. A row of 50 Vernal seeds was planted at one edge of each flat as a check. Ridomil (metalaxyl) at 1/2 teaspoon per pint was hand sprayed on each flat and watered into the soil to reduce damping off. The flats were placed in a soaking basin and watered from the bottom when needed (about every 3 or 4 days). As the seeds germinated and the seedlings emerged, and were positively identified as alfalfa seedlings, they were pulled from the soil and the data recorded. The flats were scored once a week from May through September, when they were moved to an outdoor cold frame to simulate field conditions. The flats were again scored weekly until they were covered with snow. The row of Vernal check seeds were replanted every four weeks to test the soil for germination suitability.

Results and Discussion

The effect of storage at three different temperatures is reported in Table 2. This table shows the initial germination rates (as a percentage average of 2 reps) of the 5 entries. There are 3 different storage temps (+ 25, .0, -15°C) and 16 different initiation dates (once/month for first year, once every two months for the second year). Note that for the first germination test (4/12/91) the germ percentage for each entry should be the same under the three temperatures, since there has not been any storage differentiation at this point. At the second date (5/15) the seeds have been differentially stored for 1 month; at 6/10, 2 months; at 7/11, 3 months; etc. This

table essentially shows how the different storage temperatures affect initial germ percentages over time.

The 5 entries studied can be broken down into two groups - high initial germ rates (Vernal, VSX) and low (20%) initial germ rates (W90-16, 90-18, and 90-19). The high germ types showed either no change in initial germ percentage over time (Vernal) or very small increases (VSX at 0, -15°C). Those entries with low initial germ percentages all had an increase in their germination rates, with the greatest increase at + 25°C, and smaller increases at 0° and -15°C.

High germ types showed little or no effect due to different storage temperatures, (Le. Vernal maintained about 80% germ at all 3 temperatures when stored for 18 months), while low-germ types responded to differences in storage temperatures. For these, the longer they were in storage, the higher the initial germ percent (especially at + 25°C). For these low-germ types, storage at high temperatures greatly increased initial germ percentages over time, while storage at low temperatures only slightly increased initial germ percentages. In other words, hard seed content remained unchanged in cold storage.

Table 3 shows the progressive germination in hard seed over time when left on germination paper for up to two years. It also shows the effect of storage for different lengths of time at different temperatures. The first column (eg. 4/12/91) shows the initial germ percent (see Table 1) and the other columns are the progressive germ percentages at 3 mo, 6 mo, 9 mo, 12 mo, etc., until the completion of the study on 4/15/93. Hence, we have 24 months of data for April 91, 18 mo for October 91, 12 mo for April 92, and 6 mo for October 92.
Vernal began with a high initial germ rate, and after about 10 days finished its germination, producing no new germinated seed after this time. This trend held over all initiation dates and storage temperatures, with Vernal having a constant initial germ of about 80% and a final germ about 3% higher. It should be noted that Vernal seed was 10 years old and had been stored in the laboratory at room temperature. All other entries showed a fairly consistent rate of increase in progressive germination. For VSX, W90-16, 18 and 19, it appeared that the seeds could be kept on germ paper indefinitely until all the hard seeds had germinated. Vernal, on the other hand, either germinated rapidly or rotted after about 10 days.

VSX had initial germs in the 65-80% range, and continued a steady increase in the number of seeds germinated over time. It appeared that there were no major differences for the progressive germs at any of the different storage temperatures. W90-16 showed the greatest increase in germinability over time, reaching over 70% germ in some instances. It consistently produced the highest increase in germinability over all storage temperatures. Thus, W90-16 appeared to lose its hard seed content faster than the other entries. W90-18 and 90-19 behaved similarly in all situations, showing a nearly identcial rate of germination, slightly lower than that of W90-16. In most instances, W90-19 was slightly lower in germinability than W90-18, even though their rate of progressive germination was almost the same.

Table 4 shows the progressive germ percent of the seeds planted in soil flats in the laboratory, and then moved outdoors to a cold frame. Note that W71-42 has been added. Initial germ percentages are given in the first column. The second column (11/1/91) shows the progressive germ percent after the first growing season. The third column, (8/1/92) shows the progressive germ percent after about three months of the second growing season. The last column shows the final germ percentage (i.e. no new germination after 8/1/92). This table shows how hard seed responds in soil/field conditions, and the effect of vernalization on hard seed germinability. Initial germ rates in soil were similar to initial germ rates on paper (although W90-16 was considerably higher in soil). During the first growing season, Vernal had its typical

early flush of germination, and stopped germinating after 3 weeks. VSX showed a slow but steady increase in germination. W90-16, -18, & -19 all had high rates of increase, basically tripling the number of seeds germinated over the initial germ percentages. W71-42 had the slowest rate of increase (other than Vernal).

After vernalization in the cold frames over winter, Vernal had no additional seeds germinate, while VSX had a small increase from 82% to 86%. W90-16, 18 & 19 all showed dramatic early season germination, raising their progressive germ to the 80%+ level. W71-42 had a small increase from 64% to 68%. It is likely that the 80% levels for VSX, W90-16, 18, and 19 and 67% for W71-42 represent the total amount of live seed remaining in the soil.

Compared to the germination data from the laboratory study, much more seed germinated in a shorter period of time in the soil. For example, W90-16 reached 67% germ in the soil within 6 months, but needed about 15 mo. to reach 67% germ on paper. After vernalization, the germ percentages of W90-16, 18, and 19 were all 80%, whereas the germ rates did not approach this level even after two years on paper. (For W90-18 & 19 on germ paper, the germ percent was about 50% after 2 years). These data suggest that there are factors in the soil (micro-organisms, ice-scarification, etc.) that cause an increase in hard seed germination in soil.

Summary of Additional Experiments

1. Captan kept the germination papers free of microbial growth for the two years of the progressive germination experiments. We were very impressed. How long would Captan provide control? Perhaps indefinitely?

2. Seed lots with a high hard seed content in the spring following production at Prosser, WA, decreased in hard seed content over time when stored at room temperature. However, seeds were stored for 3112 years before seed lots with a 85% hard seed became 50-70% germinable.

3. Cold storage maintained the hard seed content over the two years of the test.
This has been shown before; see Gunn 1972 cited in another section.

4. The hard seed content decreased much faster when the seed was in the moist environment of germination paper at room temperature.

5. The hard seed germinated faster still when in soil in the outdoors.

Images and Tables:

Chapter 1

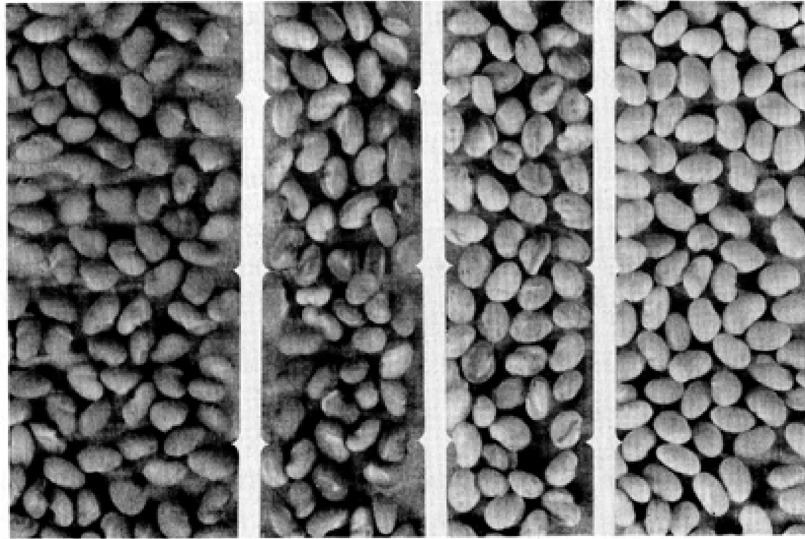


Figure 1. Alfalfa seed coat color patterns. Columns of seed (left to right): Tan seed coat, tan-white sectored (mutable 5), white-tan sectored (mutable 2), and solid white seed.

Table 1. Germination percentages of different seed coat/embryo relationships based on classifications at 7 days on germination paper of 50 seeds per test, replicated twice.

Embryo		Seed Coat	
		Tan	White
Selfed		Class 1 Seeds coat and embryo from same plant	Class 2 Seed coat and embryo from same plant
		- germination percentage-	
Test	1	22	47
	2	18	38
	3	28	45
	4	24	35
Crossed		Class 3 Hybrid embryo with maternal seed coat	Class 4 Hybrid embryo with maternal seed coat
Test	1	23	44
	2	20	41
	3	28	47
	4	25	39
Comparisons.	Class 1 versus 2 and 3 versus 4 tests effect of seed coat per se.		White seed coat germination higher
	Class 1 versus 3 and 2 versus 4 tests effect of embryo per. se.		Germination within seed coat class is the same. Embryo has no effect.

Table 2. Germination percentages of tan versus white seed over a 24 month period on germination paper. One hundred seeds of each class were monitored; replicated three times.

Seed Class	Germination over time				
	4/91 Initiated	6 mo.	12 mo.	18 mo.	24 mo.
	- germination percentage -				
Tan	6	27	39	56	58
White	8	38	67	80	95

Chapter 2

Table 3. Number of individual alfalfa plants with efg and efg seed in four breeding populations.

Entries	Produced in	Year	No. indiv. plts germ tested	No. relatively fast germinaters	No. 1
6040	WA	1992	1065	45	5
WISYN-C	WA	1992	1012	52	3
WISYN-B	WA	1993	940	47	4
WISYN-X	WA	1993	671	25	0

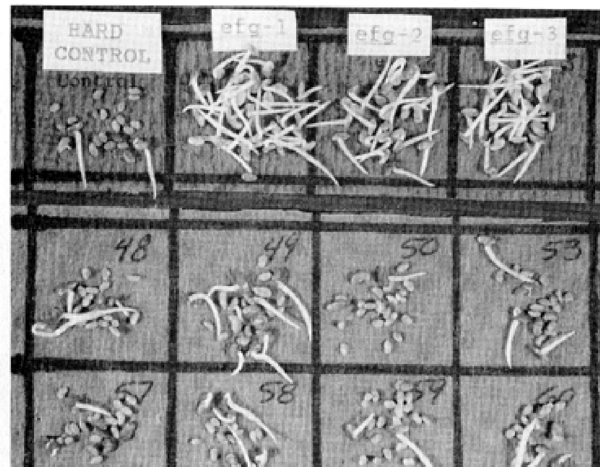


Figure 2. Alfalfa seed germination 45 hours after placing on germination paper. Hard control is a clone which produces hard seed under all conditions tested. The efg 1-3 are typical of the efg trait. Note that all the seeds have germinated and that the radicles are relatively uniform in length. Numbers 48-69 (series not complete) are from experimental population 6040 and are typical of the range in hard seed content of a sample of 6040, and cultivated alfalfa in general. Note the entries 48 and 49, and 58 and 69 have many germinated and swollen seeds. They will be 60-70% at the standard 4 days of a germ test; and, at end of seven days all these entries would have adequate germination.

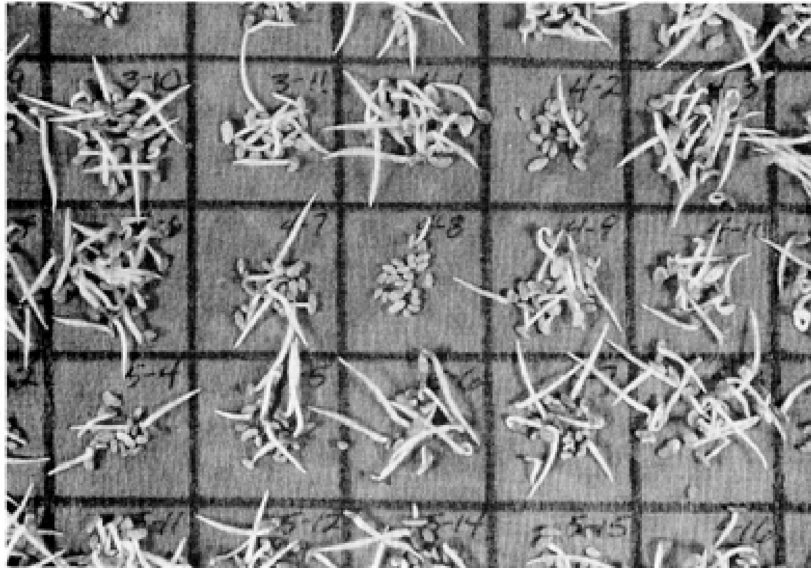


Figure 3. Segregation of half-sib progeny for efg in a sample of efg seed lots from 6040. Numbers 4-1, 4-3, and 4-6 are efg; numbers 4-7, 4-9, and 4-10 are average; and, numbers 4-2 and 4-8 are relatively hard. A similar range can be seen in group 5.

Figure 4. Tan seed of a plant with average germination at 45 hours after germination in water (left group with two radicles showing), and after germination in ferrous ammonium sulfate solution (right group with black, tan and spotted seeds). The black seeds have taken up the solution, the spotted seeds are in the process, and the tan seeds have not started.

SEM

Figures 1-12. Scanning electron microscope (SEM) photomicrographs of alfalfa seeds and the lens areas of seeds at increasing magnification.

SEM

Figs. 1-4 (60X) are of genotype 495 from 6040 which is efg. The white arrowheads point at the lens (a.k.a. strophiole). The lens appears as a relatively prominent crease in the seed coat of 495.

SEM

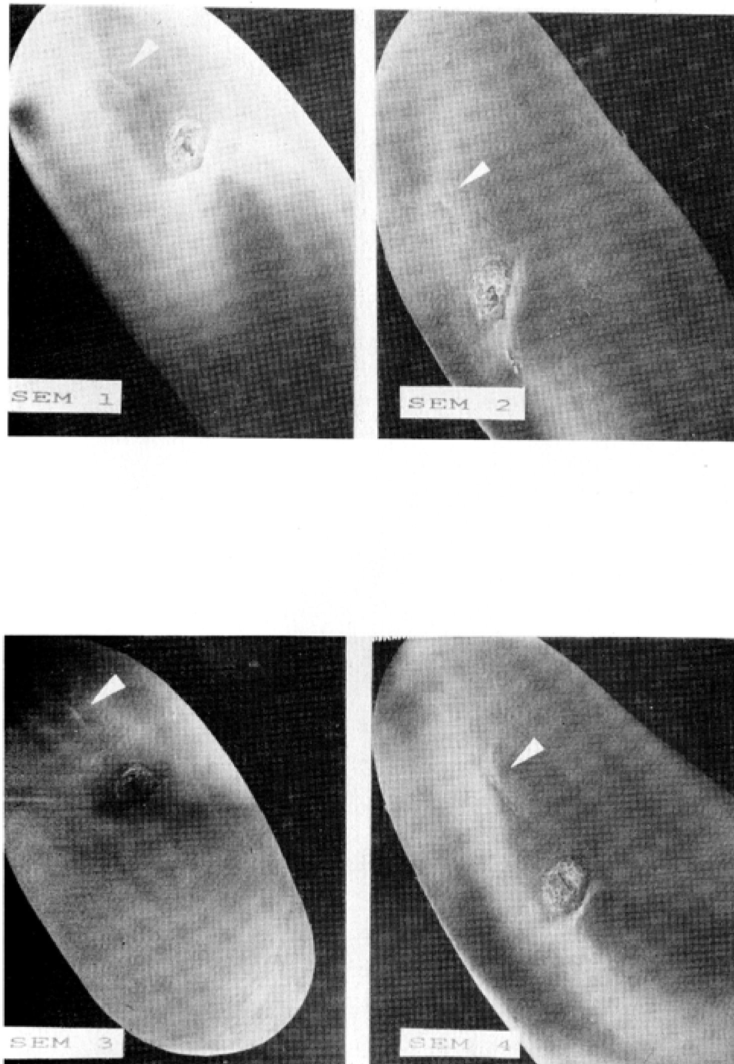
Figs. 5-8 (60X) are of genotype 6-4 ms a cytoplasmic-genic male sterile clone identified in 'Saranac' alfalfa 25 years ago and used in many studies on this and other projects around the world. The seed in Fig. 8 is laying on its side. Genotype 6-4 ms has 60% hard seed when grown in WA and 95% in the greenhouse. The lens area of 6-4 ms is very similar to that of 495 (Figs. 1-4), but not as prominent. At this time we cannot conclude anything because we have seen efg genotypes with seed like 6-4 ms in terms of the appearance of the lens.

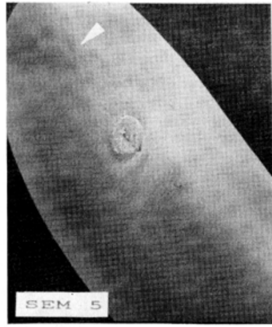
SEM

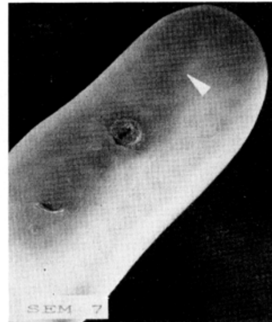
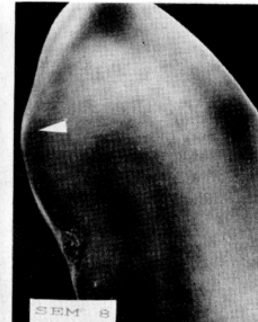
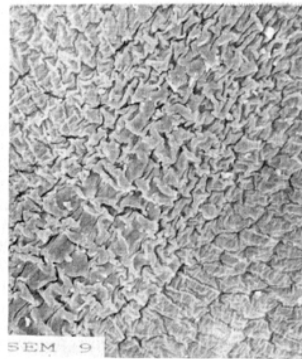
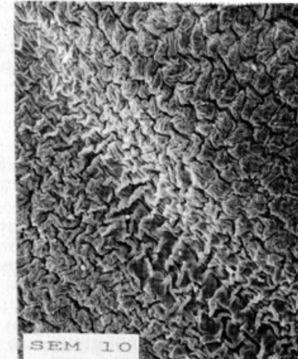
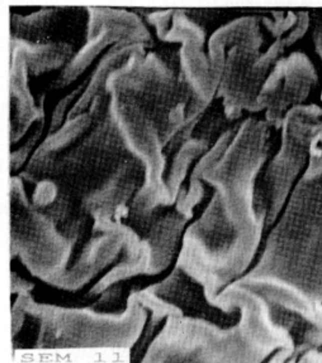
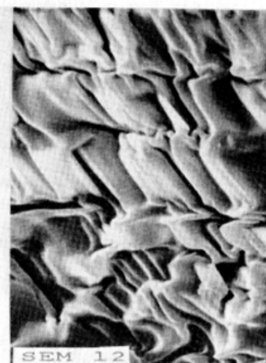
Figs. 9 and 10 (1000X) are of the lens areas of genotypes 495 (Fig. 9) and 6-4 ms (Fig. 10), respectively.

SEM

Figures 11 and 12 (5000X) are of the lens cells of 495 (Fig. 11) and 6-4 ms (Fig. 12) respectively. Genotype 495 lens cells are not packed as tightly as those of 6-4 ms. However, we do not want to draw a conclusion at this time.




SEM 5

SEM 6

SEM 7

SEM 8

SEM 9

SEM 10

SEM 11

SEM 12

Chapter 3

TABLE 1.
SEED STOCKS USED IN ADDITIONAL GERMINATION EXPERIMENTS, INCLUDING AREA OF SEED PRODUCTION, GERMINATION, AGE OF SEED, AND GENERAL DESCRIPTION.

Entry	Produced In	Unscarified Germ %	Scarified Germ %	Age of Seed	Description
VERNAL	WA	80	94	10 Years	STANDARD CHECK CULTIVAR
W90-VSX	WA	72	90	6 Months	VERNAL STRAIN CROSS WITH COMMERCIAL CULTIVARS
W90-16	WA	11	94	6 Months	EXPERIMENTAL VERNAL x SARANAC (SYN-2)
W90-18	WA	17	87	6 Months	PIONEER 532 x WISFAL (SYN-1)
W90-19	WA	8	86	6 Months	WISFAL x PIONEER 532 (SYN-1)
W71-42	ID	34	82	20 Years	EXPERIMENTAL M. sativa x M. falcata

SEED OF ALL ENTRIES STORED IN LABORATORY AT ROOM TEMPERATURE.

TABLE 2. GERMINATION PERCENTAGE OF ALFALFA SEEDS AFTER STORAGE AT ROOM TEMPERATURE (+25 C), REFRIGERATOR (0 C), AND FREEZER (-15 C)

STORAGE TEMP.	ENTRY	DATES OF GERMINATION TEST																
		START 4/12/91	5/16/91	6/10/91	7/11/91	8/12/91	9/16/91	10/22/91	11/18/91	12/16/91	1/17/92	2/17/92	3/16/92	4/17/92	5/19/92	8/17/92	10/20/92	9/9/94
		GERMINATION PERCENTAGES																
+25	VERNAL	75.8	85.2	78.2	84.1	83.6	81.1	73.4	85.8	82.1	85.3	81.0	83.4	81.9	79.8	80.3	82.5	82.0
	VSX	72.9	70.8	65.9	73.1	73.1	76.1	68.6	77.5	73.7	75.6	72.1	77.1	74.5	75.6	74.2	71.0	75.0
	W90-16	13.2	11.9	6.3	15.0	17.7	25.2	16.4	33.0	35.9	29.5	29.6	34.8	28.5	24.8	37.4	49.2	71.0
	W90-18	14.6	15.7	12.1	18.2	8.4	20.3	16.0	22.2	23.9	22.8	19.9	18.0	27.1	26.4	28.3	34.9	50.5
	W90-19	7.4	7.0	8.2	11.7	9.8	7.2	9.4	10.9	11.5	15.7	12.1	10.9	10.4	15.1	14.2	20.2	48.6
0	VERNAL	80.8	85.6	78.1	84.8	83.3	79.3	77.1	83.0	84.6	88.9	83.7	84.6	80.4	82.8	78.9	78.3	88.0
	VSX	72.0	75.8	67.5	72.4	76.8	78.5	65.5	74.2	72.6	75.7	74.1	81.8	73.9	75.4	79.0	76.9	75.0
	W90-16	11.0	18.3	12.5	10.5	11.3	13.7	5.0	15.8	13.0	12.1	16.3	14.8	14.8	16.2	12.1	15.8	22.5
	W90-18	17.2	18.4	12.1	16.4	14.9	15.9	14.1	20.4	13.7	14.2	17.2	24.5	17.0	18.0	18.1	19.4	16.0
	W90-19	7.6	12.9	7.2	9.9	8.6	7.5	7.7	8.8	12.7	8.7	7.3	8.3	10.4	8.2	13.1	12.2	13.0
-15	VERNAL	83.0	86.4	79.0	82.5	84.9	81.2	71.8	84.8	76.1	86.2	78.2	82.9	81.8	82.1	82.6	84.2	81.8
	VSX	69.8	77.8	72.2	78.6	80.7	80.8	67.2	84.8	78.3	79.0	78.3	81.3	78.2	80.1	79.0	83.8	77.0
	W90-16	8.7	15.1	16.7	15.9	15.1	12.5	10.7	14.2	15.8	14.0	16.7	15.5	17.4	12.6	18.0	12.0	19.5
	W90-18	17.9	19.4	21.6	16.9	16.5	16.1	13.8	18.8	17.5	26.0	19.8	18.9	19.4	20.2	23.0	24.3	20.0
	W90-19	8.7	17.4	8.5	8.7	7.4	10.2	7.0	9.6	10.1	8.5	7.8	10.8	10.5	14.1	7.9	10.8	22.0

TABLE 3.
PROGRESSIVE GERMINATION OF ALFALFA SEEDS LEFT ON GERMINATION PAPER FOR RESPECTIVE NUMBER OF WEEKS

(PROGRESSIVE GERMINATION OF SEEDS STORED AT ROOM TEMPERATURE SINCE HARVEST)

	GERMINATED SEED COUNTS								
ENTRY	INITIATED 4/12/91	+13wks	+26wks	+39wks	+52wks	+65wks	+78wks	+91wks	+104wks
VERNAL	75.6	78.1	78.1	78.1	78.1	78.1	78.1	78.1	78.6
VSX	72.9	79.5	85.6	86.9	87.3	87.8	88.2	89.1	89.5
W90-16	13.2	33.2	46.8	55.5	61.4	65.0	69.5	74.5	75.0
W90-18	14.5	25.8	34.4	38.0	41.6	48.2	53.4	57.9	58.3
W90-19	7.4	15.7	24.0	25.5	28.9	38.2	48.1	48.0	51.5

(PROGRESSIVE GERMINATION AFTER 6 MONTHS IN STORAGE AT RESPECTIVE TEMPERATURES)

		INITIATED 10/22/91	+13wks	+26wks	+39wks	+52wks	+65wks	+78wks
+25 C	VERNAL	73.4	82.9	82.9	82.9	82.9	82.9	82.9
	VSX	65.6	71.9	72.3	74.1	76.8	77.7	77.7
	W90-16	18.4	45.8	53.8	64.7	73.9	76.1	76.9
	W90-18	18.0	36.4	39.1	47.6	56.0	58.2	60.4
	W90-19	9.4	23.9	29.6	39.8	48.6	51.6	54.0
0 C	VERNAL	77.1	89.9	89.9	89.9	89.9	89.9	89.9
	VSX	65.6	81.5	82.6	84.9	87.8	89.1	89.9
	W90-16	5.0	19.8	28.7	39.5	56.6	61.2	64.3
	W90-18	14.1	30.6	38.3	42.7	47.6	49.6	50.8
	W90-19	7.7	19.5	26.4	34.1	40.5	45.0	49.1
-15 C	VERNAL	71.8	83.3	83.3	83.3	83.3	83.3	83.3
	VSX	67.2	84.0	85.2	87.2	88.0	89.2	89.2
	W90-16	10.7	28.7	34.4	45.1	56.6	60.2	62.3
	W90-18	13.8	33.9	37.1	43.3	49.6	50.4	53.1
	W90-19	7.0	16.1	18.6	25.1	30.7	34.2	37.2

TABLE 3 continued:

(PROGRESSIVE GERMINATION AFTER 12 MONTHS IN STORAGE AT RESPECTIVE TEMPERATURES)

		INITIATED 4/17/92	+13wks	+26wks	+39wks	+52wks
+25 C	VERNAL	81.9	86.6	86.6	86.6	86.6
	VSX	74.5	84.6	88.8	89.6	90.7
	W90-16	28.5	42.3	55.7	58.5	60.4
	W90-18	27.1	37.5	44.6	47.5	47.9
	W90-19	14.8	24.6	34.3	35.2	36.6
0 C	VERNAL	80.4	82.1	82.1	82.1	82.1
	VSX	73.9	79.5	81.1	83.1	83.9
	W90-16	14.8	32.8	43.6	47.6	53.2
	W90-18	17.0	32.1	38.1	41.3	43.6
	W90-19	10.4	27.9	36.0	41.4	44.6
-15 C	VERNAL	81.8	84.8	84.6	84.6	84.6
	VSX	78.2	81.1	85.3	86.8	87.4
	W90-16	17.4	32.2	49.6	55.2	59.6
	W90-18	19.4	28.7	34.8	39.7	43.3
	W90-19	10.5	21.5	30.1	34.0	36.4

(PROGRESSIVE GERMINATION AFTER 18 MONTHS IN STORAGE AT RESPECTIVE TEMPERATURES)

		INITIATED 10/23/92	+13wks	+26wks
+25 C	VERNAL	82.5	83.4	83.4
	VSX	71.0	78.8	80.3
	W90-16	49.2	63.0	66.7
	W90-18	34.9	47.2	49.1
	W90-19	20.2	29.3	35.1
0 C	VERNAL	78.3	82.7	82.7
	VSX	78.9	78.7	80.1
	W90-16	18.6	30.7	34.0
	W90-18	18.4	27.2	34.1
	W90-19	12.2	30.7	34.6
-15 C	VERNAL	84.2	86.6	86.6
	VSX	83.8	86.8	88.1
	W90-16	12.0	28.5	31.6
	W90-18	24.3	33.0	34.3
	W90-19	10.8	18.8	25.0

TABLE 4. PROGRESSIVE ALFALFA SEEDLING COUNTS OF SEEDS LEFT IN SOIL FLATS

ENTRY	5/1/91	11/1/91	8/1/92	11/1/92
	- PERCENTAGE OF SEEDLINGS EMERGED -			
VERNAL	70.8	73.2	73.2	73.2
VSX	64.2	81.2	86.0	86.0
W90-16	22.4	66.8	88.6	88.6
W90-18	13.6	36.4	82.0	82.0
W90-19	9.4	31.4	84.4	84.4
W71-42	55.2	63.6	67.8	67.8

- SEEDS STORED AT ROOM TEMPERATURE SINCE HARVEST
- 500 SEEDS/FLAT (NOT REPLICATED)

References

Bass, L.N., C.R. Gunn, O.B. Hesterman and E.E Roos. 1988. Seed physiology seeding performance, and seed sprouting. Agronomy Monograph 29:961-983.

Bingham, ET. and W.M. Clement, Jr. 1989. Alfalfa transposable elements and variegations. Developmental Genetics 10:552-560.

Bolton, J.L. 1962. Alfalfa. Interscience Publishers, Inc. N.Y. 474 pp.

Gunn, C.R. 1972. Seed characteristics. Agronomy Monograph 15:677-687.

Hyde, EO.C. 1954. The function of the hilum in some Papilionaceae in relation to ripening of the seed. Annuals of Botany 18:242-256.

Lute, A.M. 1928. Impermeable seed of alfalfa. Colo. Agric. Exp. Stn. Bull. 326, 36 pp. Marbach,

I. And Mayer, A.M. 1974. Permeability of seed coats to water as related to drying conditions and metabolism of phenolics. Plant Physiol. 54:817-
820.

Slattery, H.D. 1986. Physiological and gen etic control of seed coat impermeability in subterranean clover. (Trifolium subterranean.). The J. Australian lnst. Agric. Sci. 52:58.

Slattery, H.D., B.J. Atwell and J. Kuo, 1982. Relationship between colour, phenotic content and impermeability in the seed coat of various Trifolium subterranean L. Genotypes.) Ann. Bot. 50:373-378.

Talbert, L.E. 1985. Genetic studies with a mutable allele for authocyauin pigmentation and breeding studies with maximum heterozygosity in alfalfa. University of Wisconsin DA8601127.

Talbert, L.E and ET. Bingham. 1986. Somatic analysis of a mutable trait for anthocyanin pigmentation in alfalfa. Crop Sci. 26:823-827.