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A Study of Cotyledonal Cracking in Snap Beans (*Phaseolus vulgaris* L.)

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A STUDY OF COTYLEDONAL CRACKING IN SNAP BEANS

(Phaseolus vulgaris L.)

by

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John L. Morris

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ABSTRACT

A Study of Cotyledonal Cracking in

Snap Beans (Phaseolus vulgaris L.)

by

John L. Morris, Doctor of Philosophy

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Certain varieties of snap bean, Phaseolus vulgaris L., seeds are very susceptible to cracks that develop naturally across the cotyledons during pre-harvest, storage, or germination. This phenomenon is commonly known as cotyledonal cracking and may cause serious yield reductions on plants developing from affected seeds.

Cotyledonal cracking susceptibility of six white and six colored seeded varieties of snap beans were compared. Considerable differences were found in cracking susceptibility, but there was little or no relationship between seed coat color and cracking susceptibility.

An experiment was conducted to determine if a metabolic stress of the plant during the time of pod set could be involved in cotyledonal cracking. Blossoms were tagged on individual plants beginning with the day of first blossom, and tagging was continued for 21 days as blossoms emerged. Individual pods were harvested at maturity and maintained

under controlled conditions throughout a simulated weathering treatment to follow. Seeds of each pod were classified according to the amount of cotyledonal cracking sustained. It was concluded that if a stress were involved, it apparently affected the seed several days after pod initiation and that an increase in cotyledonal cracking was negatively correlated to an increase in the number of pods set during one day.

Simulated weathering tests were made of seeds remaining in the pod and seeds from the same varieties that were shelled. The results indicated that the pod provides about equal cotyledonal cracking protection for all varieties tested. Apparently the pod is not an important cause of cracking resistance in certain varieties of snap beans.

Seed coat permeability was measured and compared for the 12 varieties. A technique was employed by which the bean seed coat served as a semipermeable membrane between a distilled water and a sucrose solution. Sucrose dilution was measured refractometrically and the rate of water penetration calculated. There was little relationship between seed coat permeability and cracking susceptibility among the varieties.

The rate of imbibition and drying for seeds of six varieties having varying degrees of cracking susceptibility was tested. Imbibition and drying conditions were closely controlled and weight changes were recorded at regular intervals during imbibition and drying. Results indicated that

some of the varieties expressing the most rapid moisture changes were also the most resistant to cracking. It was concluded that the rate of change of seed moisture was not the primary factor controlling cotyledonal cracking susceptibility.

Rate of imbibition was tested for two susceptible and two resistant varieties. When the pre-imbibition seed moisture was above 10 percent, all varieties imbibed water freely. When pre-imbibition moisture was below 10 percent, several seeds of resistant varieties became slowly permeable while nearly all seeds of the susceptible varieties imbibed freely. This suggested the possibility that a hard seed tendency of the resistant varieties may be one source of protection against cotyledonal cracking. Preliminary data suggested that the seed coats of susceptible varieties remain permeable even at moisture levels below 10 percent, while many seed coats of the resistant varieties become rather impermeable at low moisture levels.

Microscopic examination of cotyledonal cracks from four different varieties indicated that the splitting occurred across cotyledonal cell walls more rapidly than between cell walls. This suggested that a weakness of the intercellular middle lamella is not responsbile for cotyledonal cracking susceptibility. Further microscopic examination and comparison of the cotyledonal cell structure of two susceptible and two resistant varieties failed to show any structural differences between varieties that could account for differences in cotyledonal cracking susceptibility.

(134 pages)

INTRODUCTION

Cotyledonal cracking in snap bean (Phaseolus vulgaris L.) seed can reduce the potential yield of an affected seedling by as much as 88 percent (Hollis, 1964). Under unfavorable environment stresses, many of the cracked seeds may even fail to produce a plant. This failure may be attributed to a delay in emergence and a consequent depletion of the partial-cotyledonary food reserves before the seedling can emerge above the soil, or to microbial invasion of the cracked region and subsequent seed decay.

Cotyledonal cracking is characterized by naturally-occurring fractures that usually develop the flat, inner surface of the cotyledon or deep within the cotyledon. Cracks are occasionally observed, however, on the outer, rounded side. Occasionally a cotyledon may have cracks developing in all directions in a pattern that resembles shattered glass. The cracks may range from hairline fissures, which are quite minute and detectable only under magnification, to deep, easily observed fractures that cause the cotyledon to break apart when only a slight stress is applied. Cotyledonal cracking is unique from other types of seed injury since it may even occur in warehouse storage several months after harvest (Atkin, 1964). Quite frequently, the cracking becomes much worse after only a short period of warehouse storage. For example, it may occur during the

brief period of 60 days or less between the arrival of the seed in the warehouse and the milling process (Anderson, 1963). Atkin (1961) reported that cotyledonal cracks may occur anytime after seed maturity until the seed is planted and germinates. It is difficult for the seed supplier to predict several months in advance how seeds of a variety that is susceptible to cotyledonal cracking will perform when finally placed in the soil to germinate. Even if seed from such a variety arrives at the grower's field in a crack-free condition. there is no assurance that the emerging seedlings will have unbroken cotyledons attached. McCollum (1953) noted that a high degree of cracking may even occur when seed of a susceptible variety is planted in wet soil.

The cotyledonal cracking weakness also poses a delicate problem for the seed analyst during the course of the germination test. Since certain varieties are quite susceptible to water fracturing, the moisture content of the germinating medium itself may have a considerable effect upon the amount and severity of cracking that is observed in the germinated sample (Moore, 1965a). For this reason, lack of standardization between laboratories in wetting of the medium may result in considerable variance in the evaluation of a single lot of bean seed.

Since the performance of a crack-susceptible variety of bean seed varies, sometimes considerably, under different environmental conditions, it is difficult to adjust the planting rate to insure a good stand. If a higher than normal planting rate is used and ideal growing conditions prevail after

planting, the plant population will be too great to maximize yield and quality. On the other hand, if a normal planting rate is used and growth conditions are unfavorable, an extremely poor stand may result.

Cotyledonal cracking has resulted in considerable losses in time and money to seedsmen and commercial canners. In recent years, the problem has increased considerably with the development of newer, high quality varieties. Because of the seriousness of the problem, the present research has been conducted to determine the cause, or causes, of cotyledonal cracking.

BACKGROUND

The question may come to mind why more attention has not been directed toward solving a problem as serious as cotyledonal cracking. To answer this, we need to review the background and nature of the problem.

Cotyledonal cracking of seeds was first recognized in garden peas in 1932 by Shull and Shull (1932). They noted that after pea seeds were soaked for a certain interval of time, an abrupt increase in the rate of water imbibition occured. They attributed this increased rate of water uptake to cavitations, or cracks, that developed within the cotyledon during soaking. At this time, the small amount of natural cracking that was occurring in snap bean seeds was apparently unnoticed. During the early 1950's, however, cotyledonal cracking of snap beans was becoming a more serious production problem. It was during this same period of time that commercial canners were demanding, and plant breeders were selecting for, snap bean varieties with a high degree of tenderness. Many new varieties were released for production that were characterized by a meaty, low-fiber pod wall and small seeds that had a thinner, more tender seed coat (Moore, 1965b). It should be noted at this point that the results of the present study strongly indicates that other, less obvious differences were also incorporated into the newer selections.

With the development of these newer varieties, germination tests frequently dropped below acceptable standards. The Federal Seed Act of 1939 declared that a bean seedling of a germination test with less than 50 percent of the cotyledonal tissue remaining intact was abnormal. It was necessary, therefore, to include such seedlings with the non-germinable calssification.

In view of the reduction in germinability of many of the newer varieties due to cotyledonal cracking, the Federal Seed Act was revised during the mid 1950's. The original 75 per cent minimum germination requirement of the regulation was reduced to 70 percent for certain varieties in an effort to alleviate the difficulty seedsmen were having in supplying acceptable seed (Atkin, 1957; Anonymous, 1963). Since the rules for seed testing consider a seedling normal if 50 percent or more of the cotyledonal tissue remains on an otherwise normal seedling, a true evaluation of the production potential of such a crop can not be made just because it has exceeded the 70 percent or 75 percent germination requirement.

Atkin (1964) observed that a great economic loss may result from removal of cotyledonal tissue from bean seeds. He noted that the productivity of a bean plant was directly related to the amount of cotyledonal tissue remaining on the emerging seedling. This observation agreed with the earlier data of Waters (1960) who demonstrated that when one-half of the cotyledonal tissue was removed from an emerging

Contender bean seedling, the dry seed yield was only 50 percent of normal. If 25 percent of the cotyledonal tissue remained, the yield was only 5.6 percent of normal. Other reports of stunted growth, delayed flowering and yield reductions have been noted from studies involving the removal of cotyledonal tissue at, or prior to, emergence of the bean seedling (Moore, 1964, McAlister et al., 1951). However, when cotyledonal tissue was removed as much as 4 days after emergence, there was no apparent effect upon plant development (McAlister et al., 1951). Varner (1963) concluded that during the initiation of germination in peas, some factor (or factors) moves from the axis tissues into the cotyledons and exerts a great influence on their metabolism. One can speculate that when a bean cotyledon cracks, the vascular system is severed and this certain factor is prevented from activating the cotyledon to nurture the developing seedling. Perhaps this is the reason why chlorophyl often fails to develop in the outer portion of a severely cracked cotyledon.

Early research by Toole and Toole (1951) as well as others (Atkin, 1957; Anonymous, 1949) indicated that the use of larger, more complex harvesting machines and increased mechanization of processing were partly responsible for the increased injury that lowered germination. Until rather recently, this opinion was still held by the majority of seedsmen and researchers involved. Recommendations were suggested for remodeling combines and processing equipment to reduce the amount of mechanical injury. Some of the suggestions were: (a) use a slower cylinder speed on

the thresher; (b) eliminate all long drops of the seed during its flow through the machinery; (c) install rubber cushioning on all metal surfaces where possible; and (d) handle the packaged seed carefully to avoid long drops. These precautions decreased the amount of injury somewhat, but did not eliminate it entirely and it soon became apparent that other factors were also responsible for cotyledonal cracking.

McCollum (1953), working on the assumption that a factor other than mechanical injury was involved in cracking, treated different hand-harvested varieties of snap bean seed by soaking them in water to simulate conditions of weathering. Seeds that were free of cracks prior to treatment were cracked with various degrees of severity after a period of soaking. He concluded that some of the injury that had been attributed to mechanical causes in the past was actually caused by internal forces of the seed that were induced by a rapid inbibition of water. More recently, the majority of seedsmen and seed research workers have agreed that much of the cotyledonal cracking is a naturally-occuring phenomenon. Anderson (1963) observed that cotyledonal cracking may occur on plants left standing in the field to the extent that the beans are useless for seed purposes. The failure by those involved to recognize cotyledonal cracking earlier is understandable since it is difficult, if not impossible, to tell this injury from true mechanical injury. Often a hand-threshed and a machine-threshed sample must be compared before one can be sure of the difference (Moore, 1965a).

Cotyledonal cracking is believed by many, if not most, seedsmen and seed research workers, to be induced by some kind or some combination of moisture relations within the seed. McCollum (1953) observed that when a crack-susceptible variety was planted in sand with a moisture content of 1.96 percent, 11 per cent of the seed cracked, while 65 percent of the seed cracked if the moisture content of the sand was increased to 13.59 percent. Atkin (1961) believes that cracking can occur only after seed has dried to 10 percent moisture or less. He further contends that the seed must re-absorb water to cause cracking, but that this may even be accomplished in an atmosphere with a relative humidity of about 75 percent.

The necessity of reabsorption of moisture to induce cracking is supported by the work of Clark and Kline (1965). They noted that seeds having a moisture decrease from 12.5 percent down to 6.5 percent over approximately 30 days had no significant increase in cracking over those dropping to 11.5 percent moisture during the same period. This is an indication that the degree of dryness in itself is not responsible for cracking. Drier seed, however, is more susceptible to mechanical injury and may receive fractures in handling. These breaks could be confused with naturally-occurring cotyledonal cracks (Atkin, 1957; Barriga, 1961).

The importance of maintaining a higher moisture level as suggested by Atkin (1961) is emphasized by the fact that White pea-beans are normally harvested and stored at 17 percent to 18 percent moisture in order to prevent cracking of the seed (Dexter, 1955).

The effect that pre-inbibition moisture has upon the degree of cotyledonal cracking in snap bean seed has been demonstrated by McCollum (1953). He reported that seed stored at 56 percent relative humidity had a moisture content of 11.5 percent and expressed the least cracking of any moisture level tested. Seeds stored at 15 percent relative humidity contained 5.7 percent moisture and during imbibition cracked more than the seeds of any other moisture level.

There is some evidence that temperature influences the severity of cotyledonal cracking. McCollum (1953) planted seeds of Rival variety of snap beans in three chambers, each containing sand and maintained at 10, 20 and 30 C respectively. He concluded that cracking was more severe when snap bean seeds imbibed water at the cooler temperatures. This conclusion is supported by Atkin's (1961) research. Clark and Kline (1965) demonstrated that temperature influences the degree of cracking only during the initial period of imbibition. Snap bean seed samples were rolled into wet towels and stored in a germinator for 24 hours at 25 C. Thereafter, the rolled towels were left in the 25 C germinator, but were soaked daily with 10 C water until the germination period was completed. The germination tests were evaluated and the cotyledons were examined for cracks. It was concluded that while the cold water treatment appeared to reduce the germination slightly, there was no evidence that it increased the amount of cotyledonal cracking. Campbell (1966), using mostly newer, white-seeded varieties

of snap bean seed, studied the influence of imbibition temperature on cracking. He was unable to show a higher degree of cracking at lower imbibition temperatures.

Anderssen (1956) reported that temperature during growth of the bean plant influenced splitting in the seeds that were produced. (It is assumed that his seed-splitting is the equivalent of cotyledonal cracking referred to in this investigation.) He observed that plants grown under a constant temperature of 15 C produced no split seeds. However, if plants were grown at 27 C during the day and 22 C during the night, 54 percent of the seeds split. No splitting occurred if the plants were grown in a "hot" room for 10 days after flowering and then moved to a "cold" room. Conversely, no splitting occurred when plants were grown in a "cold" room for 23 days and then moved to a "hot" room. Andersen (1956) concluded that the sensitive period for inducing the splitting is between 11 and 22 days after flowering.

Atkin (1961) has pointed out that earliness of maturity in snap beans and reduced cotyledonal cracking are directly correlated. The earlier maturing varieties, he contends, are seldom subjected to the greater moisture fluctuations that commonly occur in the field later in the Fall. This is in agreement with Green, et al. (1966) who reported that in 3 years of study with soybean seed, there was a definite tendency for seed from later dates of harvest to have more cotyledonal cracking.

While research specialists have learned much about the indirect causes of the cracking and most of them agree it is caused by some natural forces within the seed, they have been unable to agree upon the specific mode of action. In the present research, several of the possible causes have been studied in an effort to resolve some of the disagreement concerning the cause or causes of cotyledonal cracking.

Before going into the various cotyledonal cracking investigations, it should be emphasized that most of the snap bean varieties studied in this research are newer than those used by many of the early investigators. For this reason, inconsistencies between the data of the following experiments and earlier studies should not necessarily be interpreted as contradictory, but may be an expression of differences between the old and newer varieties.

CHAPTER I

CRACK INDEX CLASSIFICATION

Introduction

Seed from 12 varieties of snap beans was used in one or more of the experiments in this thesis study. The objective of these experiments was to correlate physiological and anatomical differences among varieties to differences in their susceptibility to cotyledonal cracking. The crack index classification experiment was designed to expose all 12 varieties of snap beans to simulated weather conditions similar to those that commonly occur during the harvest season. Their crack indices were then calculated and a comparison of relative cotyledonal cracking susceptibility was made.

Literature review

McCollum (1953) and Anderson (1963) noted that snap bean varieties showed marked differences in susceptibility to cracking. Moore (1963) and Atkin (1958) also contend that white-seeded varieties of beans with seed coats that are highly permeable to water tend to crack more easily than the darker-seeded varieties that tend to restrict water uptake.

Methods and materials

Fifty seeds each of 12 varieties of snap beans were randomly selected from samples supplied by commercial seedmen of Idaho. The experiment was designed with five replications and 10 seeds of each variety tested per replication. Each replication was tested on a different day to permit more time for examining the individual cotyledons. The seeds were removed from refrigerator storage and soaked in covered 4 1/2 X 4 1/2 inches plastic boxes containing 4 1/4 X 4 1/4 inches Kimpactissues saturated with 80 ml of water. Ten seeds of each variety were oriented on their sides in each of the boxes and stored for 24 hours in a Percival Model PGC-78 growth chamber adjusted to 10 C. The seeds were removed from the 10 C chamber and dried in a forced-draft oven for 24 hours at 32 C. They were then returned to the sandwich boxes and soaked for 24 hours at 25 C in a second growth chamber. After this period of imbibition, the seed coats were removed and the cotyledons were examined and evaluated for cracking severity. They were classified by a 1 to 5 crack index as illustrated in Figure 1. A crack index of 1 classified cotyledons with only a slight crack; 2 for those with one definite crack; 3 for those with two definite cracks; 4 for those with three definite cracks; and 5 for cotyledons having four or more cracks.

Since the seed had not been grown and harvested under controlled conditions, there was a possibility that some cotyledonal cracking had occurred prior to arriving in our laboratory. Forty randomly-selected

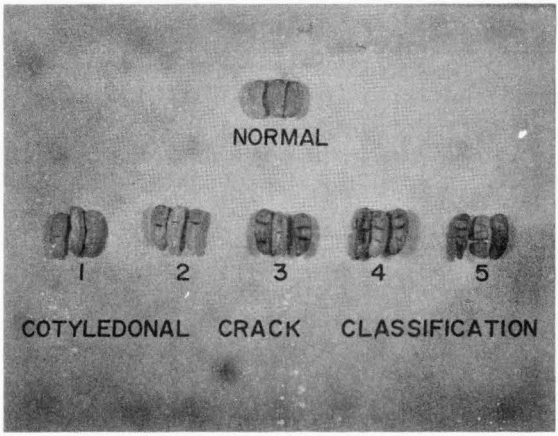

Figure 1. Examples of the five crack index classifications used to rate cracking severity throughout the following experiments.

unsoaked seeds of each of the 12 varieties were slit along the dorsal suture and the two cotyledons split apart. The cotyledons were examined and the cracking severity was scored using the 1 to 5 index system just described.

Results and discussion

Table 1 lists the cotyledonal crack index averages for the 12 varities of snap beans studied in the cotyledonal cracking investigation. A comparison of their average crack indices and respective seed coat colors indicates there is little or no correlation between seed coat color and severity of cracking.

Because there was insufficient time, it would have been impractical to grow and harvest the seed under controlled conditions to obtain crack-free seed. It is for this reason that commercially grown seed lots were used in the experiments. The data of the first column of Table 1 indicate there is a small difference in varietal relationships between the original and induced-crack indices. This difference can be attributed to variations in pre-harvest weather conditions from one variety to another. The length of time the unthreshed seed is left in the field after maturity and differences in season of maturity could account for much of this variation.

Table 1. Cotyledonal cracking index of 12 varieties of snap bean seed that were used in the experiments to follow. Column 1 lists the crack index of the seed as it arrived in the laboratory and column 2 lists the crack indices after simulated weathering at 25 C. The original averages are based on 80 cotyledons and the treated average on 100.

Variety	Original crack index	Induced crack index	Seed coat color
White Seeded Tendercrop	0.580	2.580	white
Tenderwhite	0.525	2.240	white
Tendercrop	0.033	1.990	colored
Harvester	0.260	1.875	white
Wade Bush	0.240	0.980	colored
Earliwax	0.035	0.860	white
Top Crop	0.191	0.610	colored
Corbett Refugee	0.190	0.600	colored
Bountiful	0.015	0.470	colored
Kinghorn Wax	0.050	0.460	white
Improved Higrade	0.000	0.280	white
Improved Landreth Stringless	0.020	0.090	colored

Summary

Data of the crack index classification indicate there is a difference between varieties in susceptibility to cotyledonal cracking. Campbell (1966) studied several of the same varieties and obtained relative crack indices which were in close agreement. This relationship has also been observed by Anderson (1963). There is no clear evidence, however, that white-seeded varieties are more susceptible to cracking than those that are colored. It is true that White Seeded Tendercrop cracked more than any of the other 11 varieties tested. However, Improved Higrade, another white-seeded variety, was one of the most resistant to cracking.

CHAPTER II

RELATIONSHIP OF COTYLEDONAL CRACKING TO POD-INITIATION SEQUENCE

Introduction

Early in the present investigation, it was suggested that cotyledonal cracking may be caused by a nutrient deficiency that occurred in certain varieties during seed development. It was speculated that the deficiency could occur during a short period when the nutrient is vital to the cotyledonal structure. It seemed possible that a genetically controlled inefficiency of certain varieties to assimilate or translocate nutrients that are vital in developing strong cell structures could affect cotyledonal cracking susceptibility. Such a deficiency would probably have been most pronounced when the plant was undergoing its most rapid development, such as during the highest rate of flower formation or during pod initiation and seed development. It was believed that at this time the greatest amount of a particular nutrient would be needed.

This experiment was conducted to learn if the sequence of pod initiation affected the cracking severity of snap bean seed cotyledons.

Literature review

Sun (1956) reported that first flowers of cucumber and bean plants yielded more seed and in most cases the seed germinated better and gave better developed, more productive plants than those formed later. Collander (1941) reported great differences in the relative amounts of nutrients absorbed by various plant species. Snaydon and Bradshaw (1961) have observed similar differences between populations within a species. Myers (1960) suggested that genetically controlled differences exist in the content of various elements within plants and in the physiological processes involved in their uptake, transport and metabolism. Geraline, et al. (1961) have reported that the uptake of calcium, magnesium and potassium by certain single-crosses and inbreds of maize was genetically controlled. Roa and Stokes (1963) have cited an example of a genetically controlled calcium deficiency in tobacco. They observed a high degree of calcium deficiency in tobacco plants that were monosomic for a certain chromosome "H". Weiss (1943) has shown that a single gene controls the iron utilization efficiency of soybeans.

A unique experiment conducted by Anderssen (1956) suggested that cotyledonal cracking was not related to the nutrient uptake of snap bean plants. The above-ground portions of several non-cracking lines of beans were grafted onto the root stocks of bean plants that were susceptible to cotyledonal cracking. The seeds were harvested at maturity and tested for cracking. No cracking of the cotyledons was observed. Anderssen

designed the experiment to determine whether a virus was responsible for cracking. He concluded that a virus was not responsible, but the results at the same time suggested that difference in root absorption and translocation are apparently not involved.

Methods and materials

During the summer of 1964, a randomized block experiment of six replications was planted on the Rogers Brothers Seed Research Farm near Twin Falls, Idaho. Each replication consisted of four single-row plots, 15 feet in length, and each row represented one of four varieties that were included in the experiment. These varieties were: Earliwax which is moderately crack-resistant and reaches canning stage in 56 days; Tendercrop, which is moderately crack-susceptible and reaches canning stage in 55 days; Tenderwhite, which is crack-susceptible and reaches canning stage in 60 days; and Improved Higrade, which is crack-resistant and reaches canning stage in 60 days. Tendercrop is a lavender-mottle colored seed and the other three varieties are white. Prior to blossom, 10 plants per plot were selected at random for the study and tagged for identification. A jewelers string-tie tag with one of 21 different colors or shapes were looped over each blossom on the day of anthesis to identify the sequence of pod initiation. In this experiment, pod initiation and anthesis were synonymous. All plants received the same coding sequence regardless of the date of first blossom. This coding

was based upon the number of days after first blossom that a particular blossom occurred. Each plant was tagged for 21 days following the first blossom on that particular plant. The first blossom of the experiment was tagged on July 7 and tagging continued through August 15. A record was kept of the number of blossoms that were tagged on a particular plant each day. The pods were harvested from the plants after they had matured to the wrinkled stage and prior to complete drying. At this stage of development, the seeds were fully matured and loose in the pod. The peduncles were left attached to avoid breaks in the pods. A record was made of the coding on all pods harvested. This record was later compared to the tagging record for each plant. In some instances, intertwining of plants caused errors in identifying a particular pod with the correct plant. In each case where the daily tagging record did not agree with the harvested pod codes, the data for that particular plant were removed from the experiment. This resulted in an unequal number of plants between varieties in the experimental data. Check samples were shelled from pods of the same developmental stage as the experimental pods and used for moisture checks throughout the experiment. The coded pods were placed in # 4 paper bags and stored in a cool basement, along with the check samples, to avoid rapid drying and to maintain a seed moisture in excess of 10 percent. After 1 week in this storage, the bags of experimental pods and check samples were sealed in metal cans

containing ammonium phosphate to maintain a humid atmosphere and transferred from Twin Falls, Idaho to Utah State University, Logan. The bags of pods were then removed from the cans and placed in 7 C refrigerated storage where proper humidity was maintained by moistening the floor of the storage area whenever the relative humidity was below 60 percent. Moisture content of the check seed samples was tested at least every 7 days with a Steinlite moisture tester. During the 18 week storage following harvest, the moisture content of the check samples ranged between 11 percent and 17 percent. The pods were frequently inspected for mold development and rearranged inside the bags which were left open for aeration.

Eighteen weeks after harvest, the experimental pods were assembled into a randomized-block weathering experiment. The experiment was made up of seven replications of 24 plants each. Each replication consisted of one plant per variety for each of the six field replications, or a total of six plants per variety. The seven weathering experiment replications represented 7 of the 10 plants per field plot. The remaining three plants per plot were used in working out the weathering technique or were reserved for periodical checks for cotyledonal cracking in storage. At no time throughout the course of the experiment were cotyledonal cracks detected in the experimental seeds prior to the weathering treatment.

Prior to simulated weathering, the pods for each replication were transferred from the 7 C storage to a ventilated, constant-temperature room of 21 C where they dried for 7 days. Then the pods were removed from dry storage and prepared for soaking. The pods from one plant of each of the four varieties represented were placed in each of six 3 1/2 X 7 1/2 X 11 inches crisper trays with double layers of paper toweling separating the varieties. The sequence of layering for the four varieties within the boxes was randomized from one box to the next. After all pods were properly placed in the boxes, distilled water pre-equilibrated to 7 C was poured into the boxes until the water level was raised to approximately 1 inch above the pods. The crisper trays were immediately stored in a 7 C cold-room where the pods soaked for 8 hours. Then the water was siphoned from the crisper trays and the pods were transferred to the wire racks of a Percival Model PGC-78 growth chamber where they dried in the oven at 25 C for 7 days.

Check samples of unshelled beans that received the same treatment as the experimental pods were shelled and tested for moisture after each treatment by drying in a forced-draft oven for 48 hours at 100 C. Table 2 lists the average moisture for each variety throughout the entire weathering process and each replication of the experiment.

After drying, the seeds were shelled from the pods and planted in metal flats containing verniculite that had been saturated with water and

Table 2. Average seed moisture of seven check samples, one from each replication, for four stages of simulated weathering in four varieties of snap bean seeds.

Variety	Percent moisture			
	Initial	Soaked in pod 8 hours	Shelled and soaked 4 hours	Dried[a] 7 days
Earliwax	9.80	11.80	11.00	10.81
Improved Higrade	9.09	13.25	13.71	10.77
Tenderwhite	9.20	17.83	50.26	10.84
Tendercrop	9.09	12.93	19.37	10.14

[a] Moisture test taken from seed dried in pod only.

allowed to drain and equilibrate for 2 hours prior to planting. The flats were placed on a four deck wooden rack to germinate at 21 C $\pm$ 2 C. for 7 days. The rack was enclosed with polyethylene to reduce evaporation and after the first day 225 ml of tap water was sprinkled over the vermiculite of each flat to maintain adequate moisture for germination.

At the end of the 7 day germination period, the cotyledons were examined for cracks with the aid of jewelers glasses and scored for cotyledonal cracking severity using the crack index system of classification previously described in Chapter I (Figure 1).

Results and discussion

An analysis of variance for a completely randomized design was carried out for each of the four varieties. Differences in cotyledonal cracking as affected by day of pod initiation were significant at the 1 percent level for Improved Higrade (resistant) and Tenderwhite (susceptible), but were insignificant for Earliwax (resistant) and Tendercrop (susceptible). Greatest cracking occurred in seeds of Improved Higrade that formed the 2nd and the 7th to 9th day after first pod initiation (Figure 3), and in seeds of Tenderwhite that formed the 9th day after first pod initiation (Figure 4). The cracking sensitivity patterns for the four varieties are compared in Figure 6. Although there appeared to be a peak in the cracking index for Earliwax, it was insignificant in the analysis of variance. This may

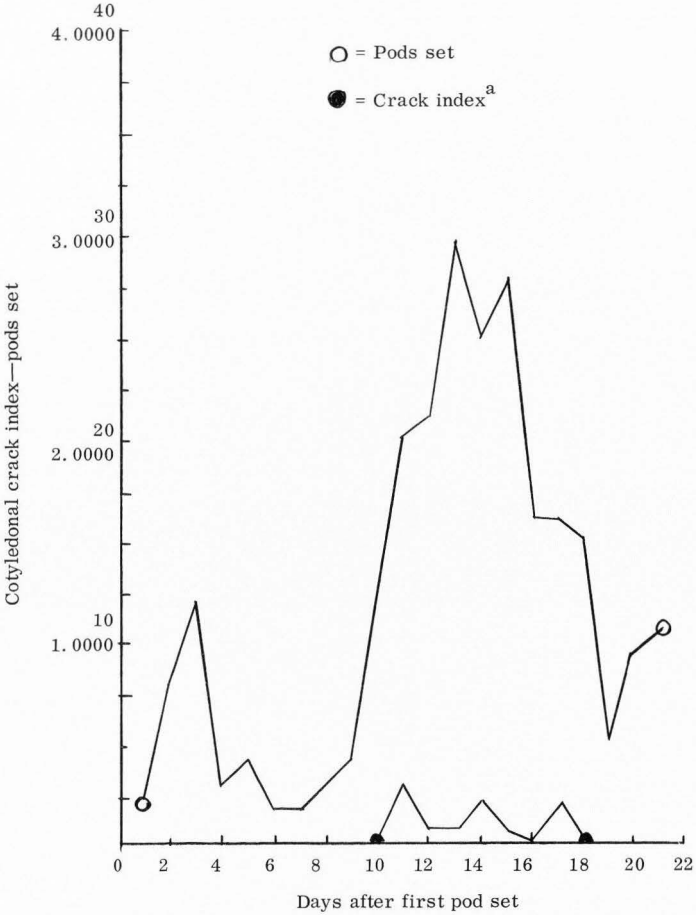

Figure 2. Relationship of cotyledonal cracking severity to number of pods set during 1 day for Earliqax (resistant) snap beans. Day number 1 was the time of first blossom (pod initiation) for each of the 25 plants per variety.

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[a]Crack index values multiplied by 10 for greater accuracy in graphing.

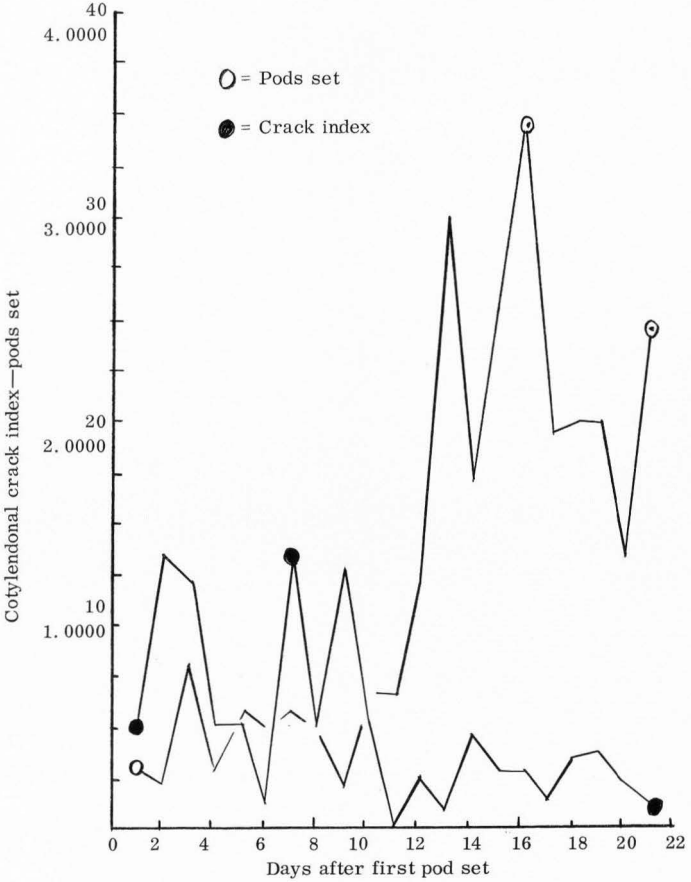

Figure 3. Relationship of cotyledonal cracking severity to number of pods set during 1 day for Improved Higrade (resistant) snap beans.

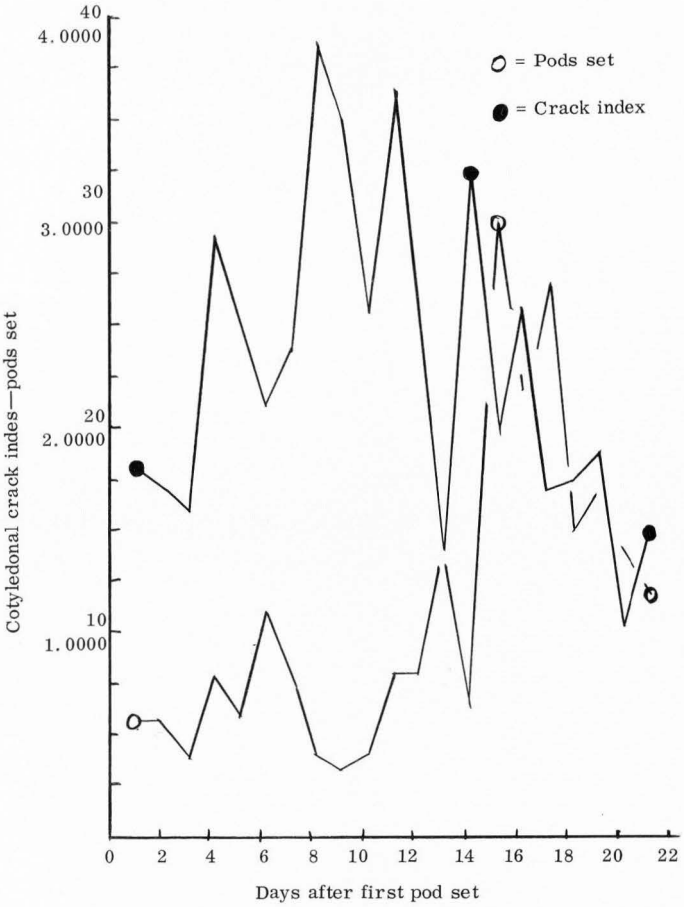

Figure 4. Relationship of cotyledonal cracking severity to number of pods set during 1 day for Tenderwhite (susceptible) snap beans.

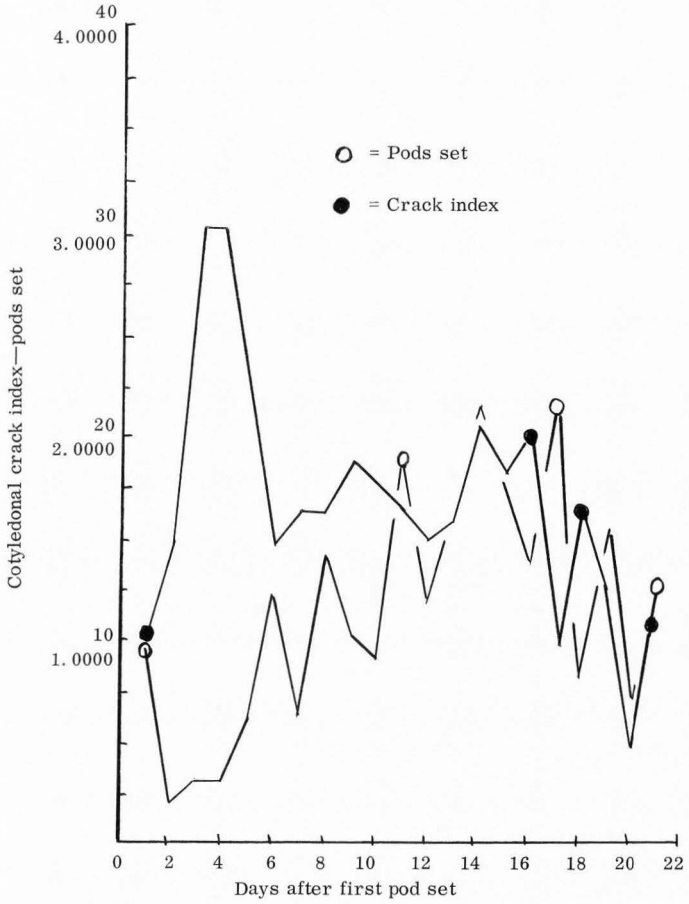

Figure 5. Relationship of cotyledonal cracking severity to number of pods set during 1 day for Tendercrop (susceptible) snap beans.

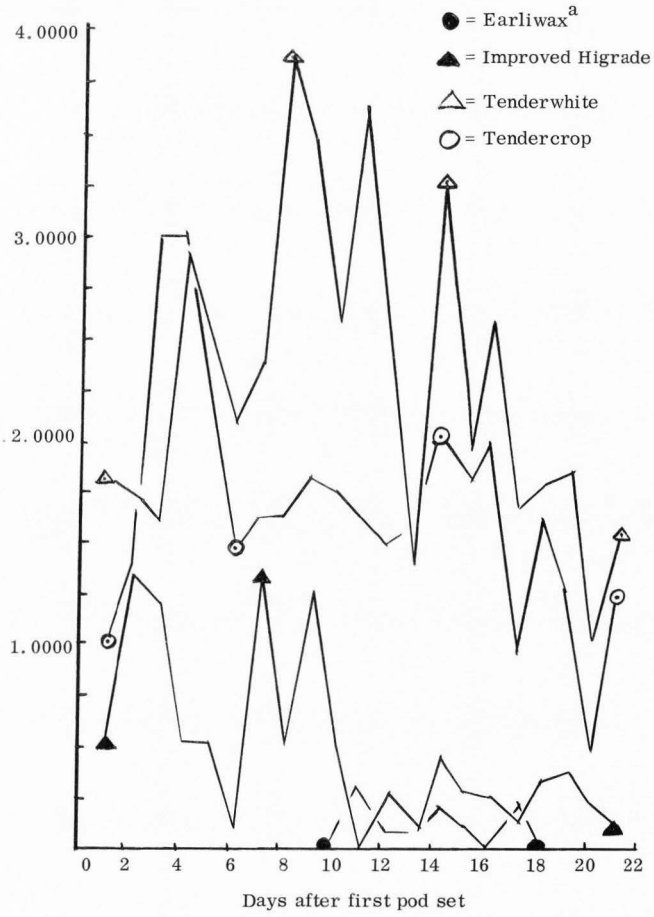

Figure 6. Comparison of average crack indices for 25 plants each of four varieties during a 21 day period.

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[a]Variety A values multiplied by 10 for greater accuracy in graphing.

have been caused by the extremely low incidence of cracking even during the peak period. The crack index values for Earliwax (Table 3) were so small that a multiplication factor of 10 was used to obtain values large enough for graphing. Although a gradual peaking of the cotyledonal cracking index for Tendercrop occurred about the 15th day (Figure 5), then decreased as the 21st day approached, cracking differences between days were statistically insignificant for Tendercrop. This insignificance may have been caused by the wide variation in the degree of cracking between pods initiated during the same day, or by the small number of pods that were analyzed in the data for certain days.

The cotyledonal cracking peaks for Improved Higrade and Tenderwhite (Figures 3 and 4) were significant. Greatest cracking occurred in seeds of Improved Higrade and Tenderwhite about 7 and 12 days prior to maximum pod initiation. This is an indication that cotyledonal cracking is not caused by an increase in the number of pods formed within a short period of time and subsequent metabolic stress. If the cotyledonal cracking and pod set relationship of Improved Higrade and Tenderwhite (Figures 3 and 4) are studied, an inverse relationship tendency can also be seen. The days of greatest pod set were often the time when seeds that cracked least were initiated.

It is interesting to note the small amount of moisture that was imbibed in the soaked-in-pod treatment of all four varieties and in the

Table 3. Average crack index for seed of four varieties of snap beans, starting with first pod set and continuing for 21 days

Day	Earliwax	Tendercrop	Tenderwhite	Improved Higrade
1	0.0000	1.0000	1.8150	0.5000
2	0.0000	1.4075	1.7280	1.3400
3	0.0000	3.0000	1.6067	1.2200
4	0.0000	3.0000	2.9330	0.5000
5	0.0000	2.2075	2.5075	0.5083
6	0.0000	1.4380	2.1000	0.1167
7	0.0000	1.5950	2.3683	1.3275
8	0.0000	1.6140	3.8750	0.4880
9	0.0000	1.8214	3.5000	1.2550
10	0.0000	1.7500	2.5850	0.5025
11	0.0281	1.6267	3.6600	0.0000
12	0.0085	1.4788	2.5483	0.2513
13	0.0081	1.5817	1.3388	0.0909
14	0.0206	2.0455	3.2667	0.4655
15	0.0113	1.8000	1.9329	0.2819
16	0.0000	2.0000	2.6143	0.2633
17	0.0222	0.9538	1.6954	0.1450
18	0.0000	1.6280	1.7511	0.3275
19	0.0000	1.2410	1.8843	0.3688
20	0.0000	0.4450	1.0171	0.2250
21	0.0000	1.2543	1.5167	0.1215

shelled and soaked treatments of all except Tenderwhite (Table 2). When the technique for inducing cotyledonal cracking was worked out prior to the actual experiment, moisture changes within the seed were not measured. The soaking time and temperatures were selected that would induce some cracking in all varieties and at the same time would not crack all seeds of the more susceptible varieties. This balance was necessary to be able to measure increases or decreases in the amount of cracking.

The small amount of moisture change that induced cracking was unexpected. However, this observation is consistent with those of Atkin (1964) in which he observed that even changes in relative humidity could induce cotyledonal cracking. Although data is not presented, it is of interest to note that when check samples of seeds that were stored at 12 percent moisture were soaked and dried just as the experimental samples, no cracking occurred. This also supports the claim of Atkin (1964) and McCollum (1953) that cotyledonal cracking is greatly enhanced when there is less than 10 percent moisture in the seed prior to imbibition.

Summary

There is little evidence from this investigation that indicates metabolic stresses of the growing plant are responsible for cotyledonal cracking. While there was a significant difference in cracking between days that pods were initiated for two of the four varieties, the difference

did not appear to be correlated to cracking susceptibility. One variety that showed a significant difference was susceptible to cracking, while the other was resistant. If a metabolic stress, resulting from the plant's increased nutrient requirement for pod initiation, is responsible for cotyledonal cracking, the stress apparently affects the cotyledon a few days after initiation of the pod in which it is contained. Or, an increase in cracking severity is inversely related to an increase in the number of pods set during a single day and cracking is actually reduced in cotyledons initiated during periods of greater plant stress. It appears unlikely that such a stress is solely responsible for cracking, since a crack-resistant, as well as a susceptible variety, expresses similar pod initiation and cotyledonal cracking relationships. The possibility remains, however, that such a stress could enhance the cracking of the susceptible variety that also has other weaknesses for cotyledonal cracking. Perhaps the crack-resistant variety that did not possess any of these additional weaknesses would not crack as a result of pod initiation stress.

CHAPTER III

EFFECT OF THE POD UPON COTYLEDONAL CRACKING

Introduction

Cotyledonal cracking commonly occurs in the field while the seed is still in the pod. Even samples of seed that appear to develop cracks after harvesting may have been predisposed to cracking while they were in the pod. It seemed possible that varietal differences in cotyledonal crack-susceptibility of snap beans could be caused by differences in their pods. The present experiment was conducted to learn if the bean pod is responsible for cracking resistance in certain varieties.

Literature review

Recent evidence by Puhkalbskaya (1964) indicated that the fiber structure of the inner pod parchment layer has an important effect upon the rate of water penetration and upon the amount of seed cracking. She believes that new varieties should be selected that have fibers with thick cell walls if we are to reduce the incidence of cotyledonal cracking. Selection of varieties that have thicker fiber cell walls would, however, be in direct opposition to the desires of the commerical canners who demand a green bean with extremely low fiber content. On the other hand, Hoffman and Kanapaux (1952) reported that water loss from 10 varieties of green

bean pods at canning stage showed no relationship between dry matter or crude fiber of the pod and water loss. Their results suggested that cotyledonal cracking susceptibility would not be related to differences in pod permeability.

Methods and materials

Twenty plants of each of four varieties were randomly selected from the pod initiation sequence plots of Chapter II. The pods were removed as they began to wrinkle and were stored along with the pods of the pod initiation sequence experiment to maintain them at the same moisture level. Then the seed from 10 plants was shelled from the pods and soaked. The same procedure was followed as for the unshelled seed of Chapter II, except the soaking time was decreased to 4 hours for the shelled seed. The pods from the other 10 plants were also soaked for 4 hours. The pods and seeds were removed from the water after soaking and dried, then the seeds were germinated by the same procedure as used in Chapter II. Oven-dry moisture tests were made from check samples of shelled and soaked seed that was sampled before and after the 4 hour soaking interval. These moisture values are listed in Table 2. After the seedlings emerged, the cotyledons were examined for cracks and the crack indices recorded. The average crack index for the 4 hour soaked in pod and for the shelled and soaked seed were compared to the average crack index for the seed from the 25 plants per variety of Chapter II that were soaked in the pod for 8 hours.

Results and discussion

Table 4 lists the crack index averages for the four varieties of seed that were compared. The same varietal relationship to crack-susceptibility was observed in the shelled and weathered seed as in the seed weathered in the pod. The pod obviously retards cotyledonal cracking as is evidenced by a crack index of the 4 hour shelled and soaked seed that is much greater than for the 4 hour soaked in pod samples.

There appears to be little or no varietal differences in the effect of the pod upon cracking of the cotyledons. If the pod is responsible for the cotyledonal cracking resistance of Earliwax and Improved Higrade the seeds of these varieties should crack considerably more when shelled and weathered. While the cracking index for shelled seeds of Earliwax was slightly higher, the significance is doubtful because of the extremely small amount of cracking from either treatment.

No theory can be offered to explain why the shelled seeds of Tendercrop and Tenderwhite imbibed more water, yet were cracked less severely than the seeds soaked in the pods. When seeds of Earliwax and Improved Higrade are dried to a low moisture, they have a tendency toward hard-seededness. However, they appear to imbibe water normally at a moisture level greater than 11 percent.

Table 4. A comparison of average crack indices among seed samples from four varieties of snap bean seeds weathered in the pod and out of the pod

Variety	Weathered in pod 8 hr[a]	Weathered in pod 4 hr[b]	Shelled and weathered 4 hr[b]
Tenderwhite	2.9580	0.5146	2.2068
Tendercrop	1.6635	0.6850	1.2105
Improved Higrade	0.3720	0.0571	0.0944
Earliwax	0.0131	0.0145	0.0190

[a] 8 hour soak.

[b] 4 hour soak.

Summary

The experimental results indicate that the pod is not responsible for varietal differences in crack-susceptibility. This does not, however, imply that the pod does not have a protective effect against cracking of the cotyledon. Even with the varieties that are highly susceptible to cracking, a reduction in cracking was noted when the seed was weathered in the pod rather than shelled and weathered. Varietal relationships to cotyledonal crack-susceptibility were the same whether the seed was weathered in the pod or shelled and then weathered. The pod had a similar protective effect on all varieties.

CHAPTER IV

SEED COAT PERMEABILITY COMPARISONS

Introduction

This part of the cotyledonal cracking investigation was organized on the premise that the cracking was caused by a rapid influx of water into the seed. It seemed logical that if rapid water uptake caused cracking in susceptible varieties, some kind of barrier must be present in resistant varieties to retard the incoming water. Furthermore, it seemed reasonable to assume that differences in seed coat permeability could be involved. The present experiment was designed to investigate the possible differences in seed coat permeability between cotyledonal crack-resistant and crack-susceptible varieties.

Literature review

Powrie et al (1960) noted that navy bean seed coats have a high capacity for water (76.6 percent) and suggested the possibility that water migrates through the seed coat and hydrates the cotyledonal tissue. McCollum (1953) stated that susceptibility to cracking is associated with seed coat permeability and rapid imbibition of water. He suggested that a rapid rate of water uptake apparently cuased differential swelling of the cotyledons

that induced cracking. As previously cited Moore (1963) and Atkin (1958) contend that white-seeded bean varieties have seed coats that are highly permeable to water and tend to have more severely cracked cotyledons than the darker-seeded varieties that tend to restrict water uptake. Kannenberg and Allard (1964) have noted that white-seeded varieties of lima bean (a) gain and lose water more rapidly; (b) are more easily damaged; (c) germinate more rapidly; and (d) are inferior in emergence to colored-seeded varieties. They further contend that the most striking difference between the two types is the markedly lower lignin content of the whites. Differences in lignin content is believed to be involved with most of the above characteristics.

Shull (1913) contends that the outer layer of the testa cannot function as a semi-permeable membrane. The inner layer of cells or aleuron, and the parenchyma cells of the seed coat, however, do possess osmotic properties. He also believes that the aleurone layer possesses the greater osmotic property. These differences in osmotic potential between the various layers of the seed coat could possibly explain the theory that water movement through the seed coat must be initiated from the inside (Brown, 1931). Once water has saturated the seed coat internally, perhaps then and only then can capillary action move the water from outside in through the seed coat.

Data presented by Denny (1917b) indicated that lipoids, tannins, and pectic substances were factors in determining permeability of seed coats. When some of the tannins and lipoids were extracted by a hot water treatment, permeability of peanut and almond seed coats were increased by as much as 500 percent. Ott and Ball (1943) concluded that the polyuronide and pentosan content of dried bean seed coats was involved in the retention of water. It can be theorized that a deficiency of one or more of these seed coat constituents could be at least partially responsible for differences in the rate of water movement through the seed coats.

Watson (1948) studied leguminous seeds of the Trifoliae and Loteae tribes. He noted a great variation in structure and chemical nature of the seed coats investigated. There was sufficient suberized, cuticularized sub-cuticular and malpighian thickening observed in each species of all those studied to cause a high general rate of impermeability. It was concluded, however, that there was no structural feature causing impermeability that was present in all impermeable and in none of the permeable seeds. This conclusion confirmed an earlier investigation by Coe and Martin (1920). In a study of sweet clover testae, they observed that absorption of water was not prevented by either the cuticularized layer or the structures of the malpighian layer, but by the light line. The light lines of the impermeable testae were usually broader, the

malpighian cells were thicker below the light line, and the cavities in the malpighian cells were smaller. Only an occasional canal crossed the light line in impermeable testae. Steinswat (1966) reported no structural differences between the testae of permeable and impermeable lima bean seeds (Phaseolus lunatus).

Methods and materials

A comparison of the rates of water movement through the seed coats of different varieties of snap beans was attempted with a seed coat permeability apparatus designed after the model specifications used by Denny (1917b). In Denny's model, the seed coat served as a semi-permeable membrane between distilled water and a sucrose solution. As water moved into the sucrose, the increase in volume was measured by means of a capillary tube attached to the sucrose reservoir. In preliminary tests using 6 mm diameter sections cut from the center area of each side of the seed coat, reproducible results were not obtainable with Denny's model. This may have been caused by inadequate temperature control and an air lock that always developed within the system. Because of the small area involved, it was decided that a more sensitive model was needed. At the suggestion of Weibe (1965), the refractometric method (Gaff and Carr, 1964) was used to measure water movement by the resulting changes in concentration of a sucrose solution. The Denny apparatus and procedure have been modified to reduce the variables encountered with the original apparatus.

The permeability apparatus was constructed of acrylic plexiglass with an over-all diameter of 62 mm. Sections 1 and 3 are 13 mm thick and section 2 is 3.5 mm thick (Figure 7). Circular grooves were machined into each section and rubber "O" rings inserted to seal the system. Inside diameters of the "O" rings in sections 1, 2, and 3 are 9.5 mm 5.0 mm and 22.0 mm, respectively. A 6 mm hole was drilled in the upper surface of section 1 to form a chamber for the sucrose solution. Slight depressions were machined into the lower surface of section 3 and connectors made of acrylic plexiglass tubing were inserted and cemented. The intake tubing was attached to the center connector "B" and the discharge tubes were attached to the two outside connectors "C" and "D". The three sections of the permeability apparatus were held together by three 5 mm screws. A microscope slide was placed over the sucrose chamber to reduce evaporation.

The complete permeability testing system consisted of the permeability apparatus, a Braun Thermomix II constant temperature circulating pump water bath, and an American Optical ABBE-3L refractometer (Figure 8). Glass distilled water, maintained at 25 C $\pm$ 1 C was circulated through the system at the rate of 400 ml per minute.

Seed coats of 12 varieties of snap beans were tested for differences in permeability. A completely randomized block design consisting of three replications was used. Means were compared according to Duncan (1955).

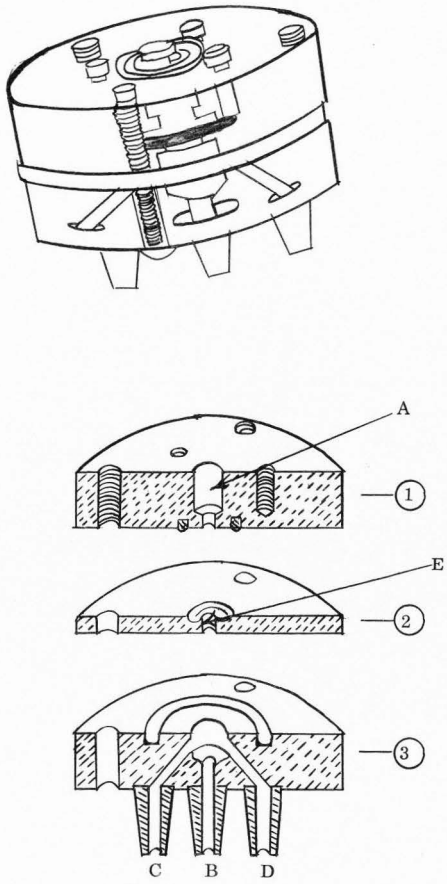


Figure 7. Seed coat permeability testing apparatus. Upper figure illustrates assembled apparatus (actual size). Lower figure is an exploded cross-section of apparatus illustrating A) sucrose chamber: B) water inlet connector; C and D) water discharge connectors; and E) seed coat chamber.

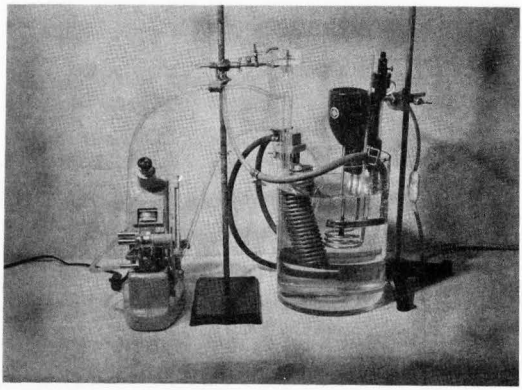


Figure 8. Permeability testing apparatus ready for operation. A constant temperature circulating pump at right circulates water through the permeability block and the refractometer at left.

Three seeds of each variety were randomly selected and placed on a single layer of Kimpak and soaked in 50 ml of glass distilled water in a 4 x 4 inches plastic box. The boxes were then placed in a Percival Model PGC-78 growth chamber maintained at 25 C $\pm$ 2 C for 12 hours. The seed coats were carefully removed and a 6 mm diameter section was cut from the center area of each side with a cork-borer. The sections were dried between blotters to flatten and stored at room conditions for future testing.

Each replication was preceeded by a test with dialysis membrane to determine the amount of variability that could be attributed to the apparatus or to experimental error. Aluminum foil discs were also tested to determine if there were leaks in the system that would cause erroneous measurements. A 25 percent ($\pm$ 0.25 percent) sucrose solution was measured. The 5 and 20 minute interval removals served to stabilize the rate of water movement through the membrane; only the average of the three 40 minute intervals was included in the final data. After each 40 minute interval an aliquot of sucrose was taken from the chamber for testing and absorbent paper was used to remove the remaining solution before a fresh aliquot was added.

Results and discussion

An analysis of variance for a completely randomized design (Snedecor, 1956) indicated a signficant difference in seed coat permeability between varieties at the 1 percent level of signficance.

Differences between replications within a variety were not significant. The permeability means for each of the 12 varieties were compared by Duncan's (1955) multiple range test to find which differences in seed coat permeability were significant. Table 5 lists the permeability value and seed coat color for each of the 12 varieties and Duncan's (1955) comparison of means is diagrammed. With the exception of Corbett Refugee variety, all of the colored seed coats were less permeable than the white ones. The fourth column lists the relative cotyledonal cracking susceptibility of the 12 varieties and is based upon the cracking index study summarized in Table 1. Interestingly, Tenderwhite and Improved Higrade have the most permeable seed coats, yet Tenderwhite is highly susceptible, while Improved Higrade is highly resistant to cracking. Conversely, Tendercrop and Improved Landreth Stringless have the least permeable seed coats, but Tendercrop is quite susceptible to cracking and Improved Landreth Stringless is highly resistant.

It was concluded from this experiment that no correlation exists between seed coat permeability and cotyledonal crack-susceptibility. These results do support previous claims of other investigators that the white see coats of lima and snap beans are more permeable to water than the colored (Atkin, 1958, Kannenberg and Allard, 1964; Moore, 1963). Corbett Refugee variety presents one exception to this pattern of seed coat permeability. The seed coat of this variety, as previously pointed out, is

Table 5. Comparison of seed coat permeability and seed coat color in 12 varieties of snap beans.

Variety	mg water/ mm^2/hour[a]	Duncan's (1955) comparison of means[b] *	Seed coat color	Cracking resistance
Tenderwhite	1.8243		white	low
Improved Higrade	1.3091		white	high
Corbett Refugee	1.2786		colored	moderate
Kinghorn Wax	1.0900		white	moderate
Harvester	0.9857		white	low
White Seeded Tendercrop	0.9714		white	low
Earliwax	0.8971		white	high
Wade Bush	0.8914		colored	moderate
Top Crop	0.8523		colored	moderate
Bountiful	0.8328		colored	moderate
Tendercrop	0.8128		colored	low
Improved Landreth Stringless	0.7828		colored	high
Dialysis (control)	3.1419			

[a] Average of three replications selected on basis of random samples (Snedecor, 1965).

[b] Any two means bracketed by the same line are significantly different. Any two means not bracketed by the same line are not significantly different.

* Significant at 5 percent probability.

significantly more permeable to water than the seed coats of the other colored seed varieties. Perhaps it is significant that the pedigrees of at least three of the more permeable white-seeded varieties may be traced back to Corbett Refugee parentage.

In about 1930, Corbett Refugee originated as a mosiac-resistant selection from a field of Refugee snap beans that was seriously infected with common bean mosaic. During the subsequent years, this selection by Ralph Corbett was used by many bean breeders as a source of mosaic resistance. It seems possible that the character of increased seed coat permeability, as well as mosaic resistance, could have been inherited in the newer varieties.

The permeability values of the snap bean seed coats in this investigation were comparable to the permeability values of several of the seed coats studied by Denny (1917a). He observed that water moved into a 0.5 M (approximately 13 percent) sucrose solution through peanut testae at the rate of from 1.4418 to 2.0596 mg/mm^2/hour. Water moved into a saturated sucrose solution through an almond testa at the rate of 0.8922 mg/mm^2/hour and through a squash seed testa at the rate of from 0.5664 to 0.7899 mg/mm^2/hour. These values are comparable to the permeability range of 0.7828 to 1.8243 mg/mm^2/hour recorded in this experiment.

It had been suggested by Dainty and Ginzburg (1964) that insufficient mixing of the sucrose solution may have resulted in the formation of a more dilute layer immediately adjacent to the seed coat, with a

consequent decrease in osmotic pressure. In Table 6, the 40 minute refractometric readings have been compared to those for 20 minutes. The 20 minute readings were totaled for the 12 varieties and compared to the average of the three 40 minute intervals for all 12 varieties. It was found that the average for the 40 minute intervals was slightly more than twice the value of the 20 minute intervals. This suggests that the osmotic gradient was not affected greatly by the surface dilution.

It is apparent, however, that after the sucrose within the chamber had been diluted sufficiently, a reduction of the osmatic gradient caused a decrease in the rate of water movement through the seed coat. A curve plotted to determine the amount of error caused by this dilution is shown in Figure 9. The straight line represents the rate of water movement through a single thickness dialysis membrane with the 10 minute dilution rate extended for a theoretical 90 minutes. The curved line represents the actual amount of water that moved through a single thickness dialysis membrane after 10, 20, 30, 40, 50, 60, 70, 80 and 90 minute intervals of continuous running. The curve indicates that the permeability rate was not greatly affected until approximately 1.75000 mg/mm^2 of water had diluted the 0.2 ml of sucrose within the chamber. Since the dilution did not exceed 1.2502 mg/mm^2 during any of the tests, the error due to sucrose dilution is believed to be negligible.

Table 6. Average permeability at 25 C for three seed coats each of 12 varieties of snap beans. The diluted sucrose solution was removed from the chamber between each time interval and the refractive index was determined. Only the three 40 minute intervals were averaged and included in the comparative permeability data.

	mg water/mm^2 seed coat				
Variety	5 minutes	20 minutes	40 minutes	40 minutes	40 minutes
Bountiful	0.2753	0.2951	0.5163	0.5163	0.5897
Corbett Refugee	0.4718	0.3392	0.8257	0.8452	0.8650
Earliwax	0.9436	0.3244	0.5897	0.6193	0.6193
Harvester	0.2563	0.5111	0.7077	0.6290	0.6290
Improved Higrade	0.2359	0.3695	0.7864	0.8649	0.8257
Kinghorn Wax	0.4423	0.3145	0.6880	0.7469	0.7274
Landreth Stringless	0.2359	0.2516	0.5307	0.5307	0.4875
Tendercrop	0.3734	0.2064	0.5307	0.5505	0.5307
Tenderwhite	0.3931	0.4521	1.1795	1.2502	1.1991
Top Crop	0.3538	0.2949	0.5505	0.5741	0.5741
Wade Bush	0.3736	0.2949	0.5741	0.5897	0.6094
White Seeded Tendercrop	0.1967	0.2556	0.6487	0.6487	0.6290
X	0.3794	0.2606	0.5419	0.5577	0.5524
	5 min.	10 min.	20 min.	20 min.	20 min.
Dialysis (control)	0.3557	0.5336	1.0671	1.0078	1.0671

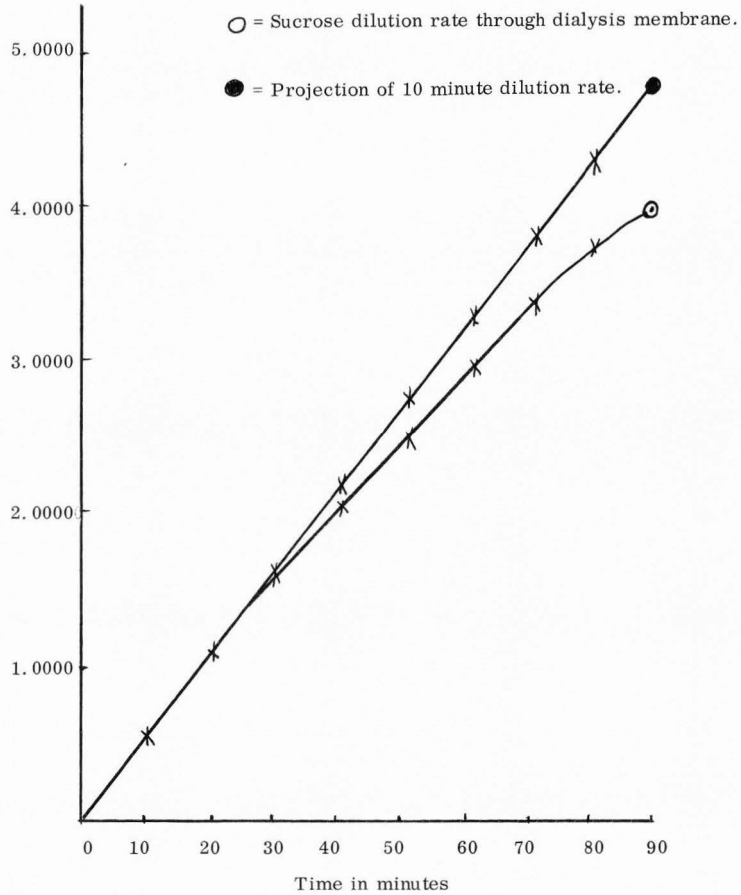


Figure 9. Sucrose dilution curve. Based upon single thickness dialysis membrane and 25 percent sucrose solution at 25 C.

Summary

Results of this experiment do not support the theory that differences in seed coat permeability are directly related to cotyledonal cracking susceptibility. While the seed coat of white-seeded crack susceptible Tenderwhite was considerably more permeable than the other varieties and characterized by cotyledonal cracking susceptibility, colored-seeded Tendercrop had a seed coat with a much lower rate of permeability, yet is cracked nearly as readily as Tenderwhite. On the other hand, the seed coat of Improved Higrade was more permeable than most of the other varieties, but the cotyledons were highly resistant to cracking.

CHAPTER V

RATE OF IMBIBITION AND DRYING STUDY

Introduction

The experimental results of Chapter IV indicated there was little relationship between seed coat permeability and cotyledonal cracking. However, the effect that seed coat permeability had upon the actual imbibition rate of intact seeds was unknown. This experiment was conducted to learn if there was a relationship between the rate of imbibition and cotyledonal cracking susceptibility.

Literature review

Several investigators have suggested a close correlation between rate of imbibition and drying of lima and snap bean seeds to cotyledonal cracking (McCollum, 1953; Atkin, 1958; Powrie, et al., 1960; Moore, 1963; Kannenberg and Allard, 1964). Atkin (1964) observed that cracking may occur as the result of a sudden change in relative humidity where no free water is actually involved. Stiles (1949) studied the water relations of lima beans (Phaseolus lunatur), scarlet runner bean (Phaseolus coccsines), snap bean (Phaseolus vulgaris), and soybean (Glycine max). He noted that (a) cotyledons are not active water-absorbing structures, but do act as water reservoirs; (b) various bean seeds differ in total

amount of water absorbed and in rate of absorption; and (c) seeds apparently possess slight degrees of adaptation to germination in mesic, hydric and xeric conditions. Marshall (1966) reported distinct differences between pea varieties in their capacity for absorbing water. He has attributed this to differences in amylose content of the starch granules.

Methods and materials

Seeds of varieties of snap bean seeds representing various degrees of cotyledonal cracking susceptibility were selected for the experiment. Seeds were selected at random and examined for seed coat breaks with the aid of a dissecting microscope until 15 good seeds of each variety were obtained. Each seed was identified by a small number marked on the tip with india ink. The seeds were weighed to the nearest one-thousandth of a gram on a continuous read-out micro-balance and the fresh weights were recorded. All weights for the experiment were made on the same micro-balance. Seeds of the six varieties were divided into three replications of five seeds each. The replications were started on three consecutive days.

Kimpak tissues were placed in six plastic boxes 1 X 4 1/2 X 4 1/2 inches square. The Kimpak was soaked with 50 ml of distilled water and the lids were closed. The boxes were placed in the Percival growth chamber operating at 25 C to equilibrate for 1 hour. Five seeds per variety were placed on their sides in each of the six boxes. Individual

were weighed after imbibing water for 2, 4, 6, 8, 10 and 18 hours and the weights recorded. The seeds were removed from the Kimpak after 18 hours and placed in another six plastic boxes containing dry Kimpak tissues. Paper towels were cut into squares and saturated with distilled water. Two squares of toweling were placed over the soaked seed in each of the dry boxes. The soaked toweling prevented excessively rapid drying and a consequent high incidence of seed coat rupturing. The lids were left off and the boxes returned to the 25 C growth chamber for drying of the seed. During the drying period, the seeds were weighed at 2, 4, 6, 8, 10, 12, 24 and 51 hour intervals and the weights recorded. The seed coat of each seed was examined with the aid of a dissecting microscope and given a crack index of 1 if it contained minute cracks, up to an index of 5 for severe cracks. The seed coats were removed and each cotyledon was examined under the dissecting microscope and the severity of cracking was classified with a 1 to 5 rating as described in Chapter I. The seeds were finally placed in a forced-draft oven and dried at 100 C for 48 hours to obtain an oven-dry weight for use in calculating moisture percentages.

The results were analyzed by a factorial analysis of variance (Snedecor, 1956) to determine if there were differences in the rate of imbibition and drying between varieties or interactions between varieties and time periods.

Results and discussion

A significant difference in the rate of imbibition and drying was found at the 1 percent level of significance between varieties; time intervals and varieties; and time interval interactions. A graphical comparison is illustrated in Figure 10. The imbibition and drying curve for Landreth Stringless was obviously different than for the other varieties, but a Duncan's (1955) comparison of means was made to determine if there were other differences. Comparisons were made for each time interval of imbibition and drying, but Improved Landreth Stringless was the only variety with a significantly different rate of change at time intervals other than the 10 and 18 hour imbibition and 2 hour drying. These comparisons are listed in Table 7 at the 1 percent and 5 percent level of significance. The colored-seeded Improved Landreth Stringless and Tendercrop expressed the slowest rate of imbibition and at the 18 hour interval both were significantly different than the white-seeded Earliwax and Improved Higrade at the 5 percent level. Improved Landreth Stringless and Tendercrop were significantly different than Improved Higrade at the 10 and 18 hour imbibition and the 2 hour drying intervals. The imbibition and drying rate of the colored-seeded Corbett Refugee was not significantly different than Earliwax or Improved Higrade in any comparison.

Table 8 lists the initial, 18 hour imbibition, and 24 hour drying moisture for all 15 seeds of each of the six varieties tested. The 18

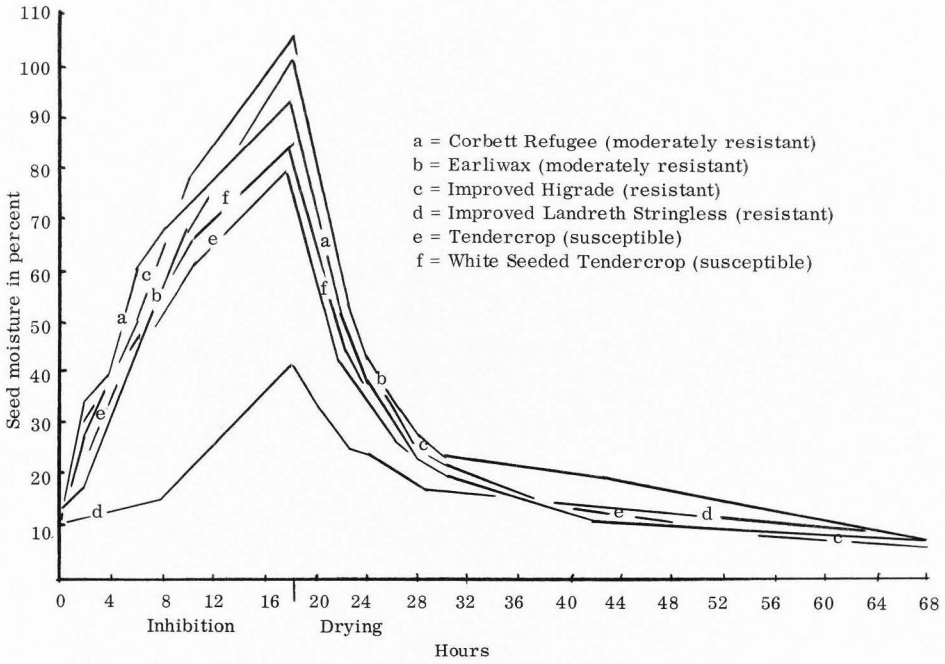


Figure 10. Rate of inhibition and drying comparison for seeds of six snap bean varieties.

Table 7. Duncan's (1955) Test[a] comparing means for percent moisture within seeds of six different varieties of snap beans during imbibition and drying at 25 C

	IH	EW	CR	WT	TC	LS
10 hr. imbibition	78.48	69.30	72.83	65.85	60.29	21.52
					**	
18 hr. imbibition	105.20	103.60	94.90	85.30	79.30	41.30
				**		
2 hr. dry	81.17	79.16	70.64	62.38	59.59	33.06
					**	
10 hr. imbibition	78.48	69.30	72.83	65.85	60.29	21.52
				*		
18 hr. imbibition	105.20	103.60	94.90	85.30	79.30	41.30
			*			
2 hr. dry	81.17	79.16	70.64	62.38	59.59	33.06
			*			

[a]Any two means underscored by the same line are signficantly different. Any two means not underscored by the same line are not significantly different.

*Significant at 5 percent probability.

**Significant at 1 percent probability.

CR - Corbett Refugee

EW - Earliwax

IH - Improved Higrade

LS - Improved Landreth Stringless

TC - Tendercrop

WT - White Seeded Tendercrop

Lettering code for the varieties in Table 7

Table 8. Comparison of imbibition and drying rates at 25 C for 15 seeds each of 6 varieties of snap bean seeds and their relationship to cotyledonal cracking.

			Percent moisture		Crack index	
Variety	number	Initial	18 hours Imbibition	24 hours drying	seed coat	Cotyledon
Corbett Refugee	1	10.17	158.94	9.72	4	0-0
	2	10.83	138.90	9.26	3	0-0
	3	10.40	119.28	10.59	5	1-1
	4	8.77	29.59	10.28	0	0-0
	5	9.37	124.07	8.84	4	0-0
	6	6.97	67.51	10.54	0	0-0
	7	14.98	133.64	15.30	0	0-0
	8	10.21	52.38	12.74	0	0-0
	9	10.66	108.66	10.45	1	2-2
	10	10.57	89.78	12.64	0	0-0
	11	11.06	96.73	12.50	1	0-0
	12	10.92	130.34	9.53	1	0-0
	13	12.60	13.84	12.91	0	0-0
	14	10.44	68.82	14.39	0	0-2
	15	10.88	91.66	12.08	2	0-0
	$\bar{X}$	10.59	94.94	11.45		

Table 8. Continued.

Variety	Seed number	Initial	Percent moisture: 18 hours imbibition	Percent moisture: 24 hours drying	Seed coat	Cotyledon
Earliwax	1	11.18	96.16	9.55	0	0-0
	2	10.78	82.37	13.15	0	0-0
	3	11.14	128.66	12.27	5	0-0
	4	10.17	90.23	11.22	0	0-0
	5	10.80	91.32	11.86	0	0-0
	6	10.94	78.23	15.97	0	0-0
	7	10.64	87.40	14.51	0	0-0
	8	10.98	106.90	14.54	0	0-0
	9	12.11	160.90	10.88	5	1-0
	10	10.80	52.72	15.79	0	0-0
	11	11.55	111.14	11.35	3	0-2
	12	11.21	105.06	13.46	2	0-0
	13	11.36	119.07	14.57	0	0-0
	14	12.15	130.65	13.54	0	2-2
	15	11.53	112.52	12.74	1	0-0
	X	11.16	103.56	13.03		

Table 8. Continued

Variety	Seed number		Percent moisture: Initial	18 hours imbibition	24 hours drying	Seed coat	Cotyledon
Improved Higrade	1		7.11	91.14	8.37	0	0-0
	2		7.95	119.80	9.05	1	0-0
	3		8.29	87.46	10.88	0	0-0
	4		11.58	106.84	15.09	0	0-0
	5		11.24	90.80	13.16	2	0-0
	6		10.54	160.17	10.97	5	0-0
	7		9.31	94.59	12.36	1	0-0
	8		8.35	125.45	10.23	5	0-0
	9		11.72	167.78	12.55	5	0-0
	10		10.30	135.49	9.27	5	0-0
	11		10.18	88.05	12.91	1	0-1
	12		9.34	63.75	14.50	0	0-0
	13		12.66	69.72	19.71	0	0-0
	14		10.22	76.03	14.56	0	0-0
	15		10.57	100.17	13.43	0	0-0
		X	9.96	105.15	12.47		

Table 8. Continued

Variety	Seed number	Percent moisture			Crack index	
		Initial	18 hours imbibition	24 hours drying	Seed coat	Cotyledon
Landreth Stringless	1	10.55	71.13	14.46	0	0-0
	2	10.68	54.24	14.97	0	0-0
	3	10.55	11.70	10.85	0	0-0
	4	10.28	50.27	14.55	0	2-0
	5	10.19	52.60	14.54	0	0-0
	6	10.31	13.43	11.16	0	0-0
	7	11.70	114.77	16.28	0	0-0
	8	9.68	11.28	10.00	0	0-0
	9	10.41	28.50	13.76	0	0-0
	10	10.71	11.95	10.39	0	0-0
	11	10.63	40.89	15.56	0	0-0
	12	10.65	19.14	12.15	0	0-0
	13	10.99	11.83	10.79	0	0-0
	14	12.23	70.16	15.42	1	0-0
	15	10.64	56.81	16.72	1	0-0
		10.68	41.25	13.44		

Table 8. Continued

Variety	Seed number	Percent moisture			Crack index	
		Initial	18 hours imbibition	24 hours drying	Seed coat	Cotyledon
Tendercrop	1	9.39	66.90	12.60	0	0-0
	2	9.86	66.58	13.66	0	0-0
	3	9.25	90.01	11.06	0	0-0
	4	9.86	69.33	12.52	0	2-0
	5	9.21	74.93	11.18	2	3-4
	6	10.31	78.80	13.62	0	0-0
	7	11.11	87.40	13.20	0	0-0
	8	10.69	84.19	12.65	0	2-3
	9	9.32	96.00	11.64	0	0-0
	10	9.98	82.00	11.97	0	2-2
	11	10.80	46.14	12.31	0	0-0
	12	11.63	90.88	13.07	3	0-2
	13	9.51	75.06	12.96	0	3-3
	14	10.66	69.51	14.23	0	0-0
	15	12.11	111.05	11.85	0	2-1
	X	10.24	79.25	12.57		

Table 8. Continued

Variety	Seed number	Percent moisture			Crack index	
		Initial	18 hours imbibition	24 hours drying	Seed coat	Cotyledon
White Seeded Tendercrop	1	9.63	81.70	14.27	0	2-0
	2	9.21	82.52	13.80	0	0-0
	3	8.63	85.93	9.98	3	4-2
	4	9.89	77.44	12.46	3	2-3
	5	8.80	69.31	12.29	0	0-0
	6	8.72	84.72	12.87	0	2-2
	7	11.51	71.61	16.00	2	3-4
	8	10.18	86.88	13.00	0	0-0
	9	13.61	140.42	13.62	0	4-4
	10.	10.11	87.41	11.35	4	0-0
	11	10.71	88.69	10.84	3	0-1
	12	10.72	81.83	13.70	0	0-0
	13	10.13	90.85	13.09	0	0-0
	14	10.26	82.32	12.68	3	2-2
	15	10.62	67.25	14.07	0	0-0
		10.18	85.26	12.95		

hour imbibition data indicate there is considerably more difference in the amount of water imbibed by seeds within a variety for Corbett Refugee and Improved Landreth Stringless than by seeds of the other four varieites. It may also be noted that even though different varieties of seed and different seeds within a variety imbibed greatly varying amounts of water, they all dried to very nearly the same moisture level after 24 hours. In fact, the graph of Figure 10 shows that most of the difference in moisture between seeds is removed after only 6 hours drying. It appears that seeds imbibing the greatest amount of water imbibe more rapidly and dry more rapidly than seeds imbibing less water.

It may be noted that the moisture was not always greatest in seeds with broken seed coats. Some of them may have cracked while drying, and not during imbibition, which could be the explanation for this. In most cases, however, seeds having severely cracked seed coats absorbed the most water. There does not appear to be a direct relationship between degree of seed coat cracking and cotyledonal cracking. For example, in White Seeded Tendercrop, some of the seeds with cracked seed coats also have cracked cotyledons (Table 8), but some with severe seed coat cracks have no cotyledonal cracks. It is believed that these exceptions are too numerous to directly relate rate of imbibition and seed coat cracks to cotyledonal cracking severity.

It is apparent from the extreme variation in the rate of imbibition between seeds of even the same variety that moisture relations must be

considered on an individual seed basis. Many previous studies have involved bulk sample material. If the small (41 percent) increase in moisture for a bulk sample of Landreth Stringless is considered, it may be concluded that the low imbibition rate is responsible for cracking resistance. Yet, the present study indicates that three of the 15 seeds imbibed more than 70 percent moisture and one imbibed 114 percent, but had no cotyledonal cracking. The slowly permeable nature of Improved Landreth Stringless and some other varieties may give protection against cotyledonal cracking. The fact that they resist cracking even when water is imbibed rapidly, however, indicates that other factors are also involved.

Summary

Data of this study indicate that differences in the rate of imbibition and drying are not responsible for differences in susceptibility to cotyledonal cracking. In fact, the crack-resistant Earliwax and Improved Higrade imbibed water and dried faster than the crack-susceptible varieties.

CHAPTER VI

EFFECT OF DESICCATION ON THE SUBSEQUENT RATE OF IMBIBITION

Introduction

Evidence in the literature (Lebedeff, 1947; Hyde, 1954; Honma and Denna, 1962) suggested that certain seeds were less permeable when dried to less than 14 percent moisture. When bean seed grown in southern Idaho matures during the hot, dry months of August and September, seed moistures as low as 9 percent are common. It was theorized that if a variety had a hard seed tendency, it should be less susceptible to seed moisture fluctuations while curing in the field prior to harvest. This experiment was conducted to learn whether the hard seed characteristic could be a factor contributing to the cotyledonal cracking resistance of certain snap bean varieties.

Literature review

Before continuing this discussion of bean imbibition, it may be helpful to briefly review the external anatomy of the seed. Along the inside suture of the seed and at the point of attachment to the pod, is the scar-like hilum (Figure 11). Immediately adjacent to the hilum and toward

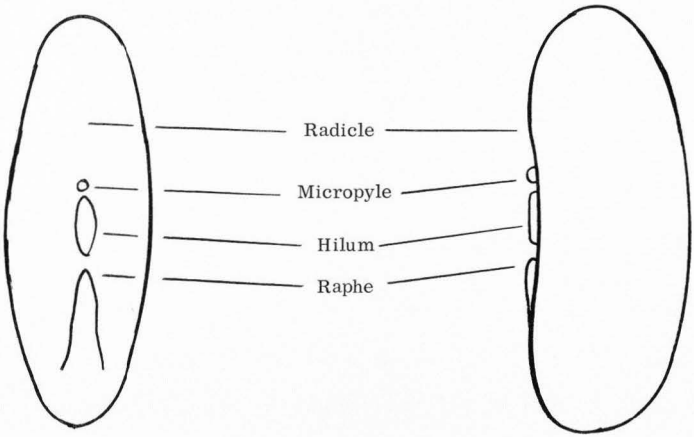


Figure 11. External view of a typical snap bean seed.

one end of the seed is a raised portion which covers the radicle. Between the tip of the radicle and the hilum is a smaller opening which is the micropyle. The pollen tube passes through the micropylar opening during fertilization. It has been noted that in most varieties of snap beans this opening fails to close (Moore, 1965c). At the opposite end of the seed, a low ridge extends from the hilum toward the tip. This is the raphe which contains a network of conducting tissues that fan out into the seed coat. The area of seed coat at the raphe end of the seed is often referred to as the chalazal region.

Distinct varietal differences in the pathway of water entry into seeds of beans (Phaseolus vulgaris) have been observed by Kyle (1959) and Kyle and Randall (1963). The greatest amount of water entered through the micropyle of Great Northern while most of the water passed through the hilum and raphe areas of the Red Mexican bean seed. They further noted that the remaining areas of the seed coat of these two varieties, exclusive of the micropyle, hilum, and raphe areas, were responsible for only 2 percent to 3 percent of the total water intake. Kyle and Randall (1963) concluded that these differences were genetically controlled.

Considerable evidence indicated that the nature of the hilum, micropyle and raphe were determinants of the hard seed characteristic. Atkin (1964) contended that the hard seed tendency in certain varieties was a factor in their resistance to cotyledonal cracking. The expression

of this tendency is believed to be closely related to the relative humidity of the atmosphere and the effect it has upon seed moisture. It has been demostrated (Lebedeff, 1947; Hyde, 1954; Honma and Denna, 1962) that the incidence of hard seed within a certain lot increased as the moisture of the seed decreased below a critical level of from 8 percent to 14 percent, depending upon the variety. This phenomenon was considered to be genetically controlled, thus accounting for the differences in response between varieties. James (1949), on the other hand, contended that the character for impermeability in crimson clover was not heritable, or if it was, the heritable factors were masked by environmental effects.

Following a study on the testae of perennial ryegrass (Lolium perenne), Brown (1931) reported that the cuticle layer of the testa retarded water absorption, but the permeability was increased by stretching of the cuticle after seed began to swell. He suggested that water first entered the hilum and began to swell the endosperm. The swelling in turn increased the seed permeability and permitted more water to enter. The swelling and increased water entry sequence progressively increased until the seed was fully imbibed.

Moore (1965c) observed the imbibition of water by lima bean seeds and suggested the following pattern of water entry: water first penetrated the micropyle and hilum, then moved rapidly along the raphe and accumulated in the chalazal area. As the water moved between the

cotyledon and seed coat, it was unequally distributed because of irregularities in the seed coat attachment to the cotyledon. This caused uneven swelling of the seed coat and cotyledon and resulted in stress being directed to the dry, unsoaked areas. Moore (1965c) believed this stress was one of the primary causes of cotyledonal cracking of bean seed.

Atkin (1964) and Moore (1964) observed that the more loosely the seed coat was attached to the cotyledon, the more susceptible the seed was to cracking and crushing injury. They pointed out that the seed of many of the newer snap bean varieties had a more loosely fitted seed coat than was typical of most of the older varieties and were consequently more susceptible to injury.

Methods and materials

Randomly selected 1 pound samples of Tenderwhite, Tendercrop, Earliwax and Improved Higrade snap bean seed were stored in sealed cans containing anhydrous calcium chloride granules. After about 14 days, seeds were selected at random and examined for breaks in the seed coat with a dissecting microscope. Thirty of each variety with crack-free seed coats were obtained for the experiment and identified by a number marked on the tip of the seed coat with india ink. The initial weights were obtained by weighing individual seeds on a continuous-read-out micro-balance and recorded to the nearest ten-thousandths of a gram. Kimpak tissue was cut into squares and placed in plastic boxes 1 X 4 1/2 X 4 1/2 inches

square. Each Kimpac square was saturated with 50 ml of distilled water and the boxes were placed in the Pervical growth chamber to equilibrate to 25 C. After 1 hour, the 30 seeds of each variety were placed on the saturated Kimpak with their sides down and returned to the 25 C growth chamber to imbibe for 15 hours. The seeds were removed from the boxes, lightly blotted with absorbent tissue and weighed. They were returned to the boxes and stored in the growth chamber for an additional 9 hours of soaking, making a total of 24 hours. The seeds were weighed again and then dried at 100 C for 48 hours to obtain an oven-dry weight. Table 9 lists the percent increase in moisture for the 30 seeds of each variety. Table 10 was compiled from imbibition data of Chapter V for the normal seeds and data of Table 9 of this experiment for the desiccated seeds.

The second part of the experiment was carried out to learn if varieties differed in their dependence on the hilum-micropyle region for water entry. Seeds of Earliwax (resistant) and Tenderwhite (susceptible) were randomly selected and treated as described for the seeds used in the first part of this experiment. Twenty crack-free seeds of each variety were prepared for soaking as follows. Ten refrigerated seeds with a moisture content of 12 percent were selected and the hilum, micropyle and raphe of five of the seeds were sealed with petroleum jelly. Ten seeds were adjusted to 8 percent moisture by desiccation and the hilum, micropyle and raphe of five of these seeds were sealed with petroleum

Table 9. Water imbibed in 15 hours at 25 C by seeds of four varieties of snap bean seeds following desiccation.

Seed number	Percent moisture increase, (dry weight basis)[a]			
	Tenderwhite	Tendercrop	Improved Higrade	Earliwax
1	93.0	79.0	109.5	36.8
2	103.0	113.0	88.5	8.1
3	98.0	80.0	104.0	23.7
4	64.0	82.0	92.0	104.0
5	101.0	66.5	96.0	67.0
6	105.0	73.0	105.0	108.5
7	120.0	77.0	24.2	1.4
8	95.0	111.0	99.5	72.0
9	103.5	99.5	74.5	76.0
10	102.0	81.0	94.5	1.6
11	84.0	46.0	102.9	21.0
12	111.0	73.0	96.3	55.0
13	107.0	55.0	106.2	80.0
14	117.0	54.0	66.6	105.0
15	103.0	57.0	4.6	1.7
16	104.0	90.0	35.6	97.0
17	107.0	75.0	100.4	10.7
18	111.0	50.0	23.4	78.0
19	107.0	83.0	83.5	3.1
20	101.0	90.0	12.6	32.0

Table 9. Continued

Seed number	Percent moisture increase, (dry weight basis)[a]			
	Tenderwhite	Tendercrop	Improved Higrade	Earliwax
21	102.0	86.0	97.6	23.0
22	100.0	98.0	115.0	1.1
23	94.0	97.0	102.3	7.1
24	104.0	78.0	9.8	2.7
25	107.0	92.0	125.2	30.0
26	106.0	1.3	4.9	112.0
27	100.0	73.0	86.6	103.0
28	93.0	99.0	131.0	1.5
29	105.0	86.0	93.4	46.5
30	99.0	92.0	75.3	22.0
X	101.6	77.9	78.7	44.38

[a] Based on 7.3, 7.8, 8.2 and 7.7 percent initial moisture for Tenderwhite, Tendercrop, Earliwax and Improved Higrade, respectively.

Table 10. Comparison of imbibition rate at 25 C for desiccated and normal seeds of Earliwax (resistant) snap beans.

Seed number	Percent moisture (dry weight basis)[a]*				
	Desiccated		Normal		
	15 hours	24 hours	2 hours	10 hours	18 hours
1	21.0	68.0	1.4	45.6	85.0
2	55.0	111.0	3.3	42.0	71.6
3	80.0	107.0	32.4	84.8	117.5
4	105.0	115.0	1.0	39.2	80.0
5	1.7	20.0	3.7	49.1	80.5
6	97.0	126.0	3.0	34.3	67.3
7	10.7	52.0	5.9	45.6	76.8
8	78.0	109.0	7.3	62.3	95.9
9	3.1	44.0	19.6	129.5	148.8
10	32.0	91.0	0.8	18.1	41.9
11	23.0	46.0	25.2	80.1	99.6
12	1.1	1.2	0.1	65.3	93.8
13	7.1	38.0	0.2	27.7	107.7

Table 10. Continued

Seed number	Percent moisture (dry weight basis)[a]*				
	Desiccated		Normal		
	15 hours	24 hours	2 hours	10 hours	18 hours
14	2.7	29.0	5.5	87.8	118.5
15	30.0	72.0	1.9	60.7	100.9
$\bar{X}$	36.5	68.61	7.42	58.14	92.39

*[a] The weight of the seed prior to imbibition. The average initial moisture for desiccated seeds was 8.2 percent and 11.3 percent for the normal.

jelly. The seeds were placed on saturated Kimpak tissues in the plastic boxes and stored in the Percival growth chamber at 25 C for 18 hours. Table 11 lists the moisture content for each of the seeds following the soaking treatment.

Results and discussion

The data of Table 9 indicate that many of the seeds of Earliwax became very slowly permeable after the moisture level had decreased to 8 percent. The imbibition of seeds of Improved Higrade appeared to be affected by low pre-imbibition moisture, but to a lesser degree than those of Earliwax. Only an occasional seed of Tendercrop imbibed slowly and seeds of Tenderwhite were apparently unaffected. The frequency of impermeability is almost inversely related to cracking susceptibility in the four varieties. In other words, the varieties with the greatest number of impermeable seeds were also the most resistant to cracking. Since the imbibition of seeds of Earliwax were affected most by disiccation, the data of Table 10 was included to compare them to seeds of the same lot that contained normal pre-imbibition moisture. The 2 hour interval of imbibition for normal seeds (11 percent moisture) was included since they imbibed about the same amount of water during this period as many of the disiccated seeds imbibed after 15 hours. The amount of imbibition during the 10 hour interval was comparable to the amount imbibed by several of the desiccated seeds after 24 hours. The 18 hour interval

Table 11. Seed moisture comparisons for Earliwax (resistant) and Tenderwhite (susceptible) after 18 hours imbibition at 25 C. Comparisons were made with the hilum, micropyle and raphe sealed with petroleum jelly and also with these regions unsealed.

Pre-imbibition moisture	Seed number		Earliwax		Tenderwhite	
			Unsealed	Sealed	Unsealed	Sealed
12 %	1		62.0	27.0	65.0	62.0
	2		51.0	28.0	80.0	67.0
	3		59.0	58.0	65.0	57.0
	4		54.0	53.0	80.0	66.0
	5		61.0	50.0	73.0	77.0
		$\bar{X}$	57.4	43.2	72.6	65.8
8 %	1		28.0	1.4	78.0	77.0
	2		57.0	1.1	69.0	75.0
	3		4.4	1.5	101.0	131.0
	4		6.5	1.2	2.6	---
	5		47.0	2.0	85.0	63.0
		$\bar{X}$	28.6	1.4	67.1	69.2

is representative of the imbibition pattern of normal seeds of Earliwax just prior to germination. Previous tests indicated that shortly after 18 hours imbibition the radicle began to emerge from seeds that imbibe normally.

The data of Table 10 indicate there was approximately a 14 hour delay in imbibition of desiccated seeds of Earliwax. It appeared that most of the seeds of Earliwax began to imbibe water during the 15 hour soaking period; however, the rate of increase appeared to be slower than in normal seeds. The data also indicate that while several of the disiccated seeds of Earliwax expressed a hard seed character after 15 hours imbibition, they were imbibing water rather freely after 24 hours, with the exception of one seed. The tendency for the seeds of Earliwax to become impermeable when disiccated is further evidenced by comparing the seeds of this variety after 18 hours of imbibition (Table 10) to the seeds of Tenderwhite and Tendercrop that had imbibed for only 15 hours (Table 9). The seeds of Earliwax imbibed less water and had greater variability in the amount of water imbibed than was observed in Tenderwhite and Tendercrop. Nearly all of the seeds of Tenderwhite and Tendercrop that had the same pre-imbibition moisture as Earliwax were almost fully imbibed after 15 to 18 hours soaking. If it is assumed that the severity of cotyledonal cracking was affected by the amount and rate of water imbibition of bean seeds, then this hard seed tendency could possibly be involved in the cracking resistance

of Earliwax and Improved Higrade. During the harvest season in southern Idaho, light rain showers are rather common. Heavy dew is also common during the months of Setpember and October and when it occurs, bean pods toward the outer part of the windrow become fairly well soaked. The dews and most of the rain showers soak the bean pods for only a short time and in most cases would not exceed 15 to 20 hours. In such cases, many of the seeds of Earliwax and Improved Higrade may be protected against moisture uptake if the seeds were dried to a moisture content of 8 percent to 10 percent prior to soaking. A study of the seed moisture records for several bean seed crops delivered to one commercial warehouse in southern Idaho indicates that these low moisture levels commonly occur in curing crops of snap beans during September.

There is considerable evidence for the involvement of the hilum, micropyle and raphe in seed imbibition (Brown, 1931; Lebedeff, 1947; Hyde, 1954; Kyle, 1959; Honma and Denna, 1962; Kyle and Randall, 1963; Atkin, 1964; Moore, 1965a). A preliminary study involving Earliwax (resistant) and Tenderwhite (susceptible) suggested that varieties of seed differ in their dependence upon these areas for water entry and that this difference is affected by pre-imbibition seed moisture. Seeds of the two varieties imbibed water nearly as rapidly with the micropyle, hilum and raphe sealed as when they were left open if the pre-imbibition seed moisture was 12 percent or greater (Table 11). However, if the

pre-imbibition moisture was 8 percent or less, Earliwax became practically impermeable with these areas sealed, while imbibition of Tenderwhite appeared to be unaffected. It appeared that the seed coats of Earliwax became impermeable after desiccation and were dependent upon the hilum, micropyle and raphe for the initial entry of water, while the seed coats of Tenderwhite were permeable after desiccation. It should be noted that the seed coats of Earliwax had a tendency to adhere tightly to the cotyledon, while seed coats of Tenderwhite were normally loose. It is believed that this difference is partly responsible for the impermeable character of Earliwax after desiccation. Further evidence for this is suggested by Figures 12 and 13. The hilum illustrated in Figure 12 was sectioned from a seed of Earliwax which imbibed water very slowly. It is however, opened nearly as wide as the hilum sectioned from a seed of Earliwax that imbibed water normally (Figure 13). This suggests that after the seed has been desiccated, even though water enters the hilum, an interval of time is necessary to "soften" the inner lining of the seed coat and cause it to become permeable. This theory is in agreement with the suggestion of Atkin (1964) that seeds with more tightly adhering seed coats are more resistant to cracking.

This part of the study was only preliminary and is not conclusive because of the limited number of seeds tested.

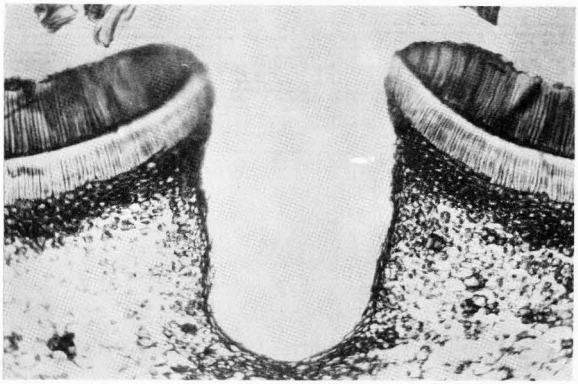


Figure 12. Hilum of a slowly permeable seed of Earliwax after 18 hours inhibition at 25 C. (approximately 300 X).

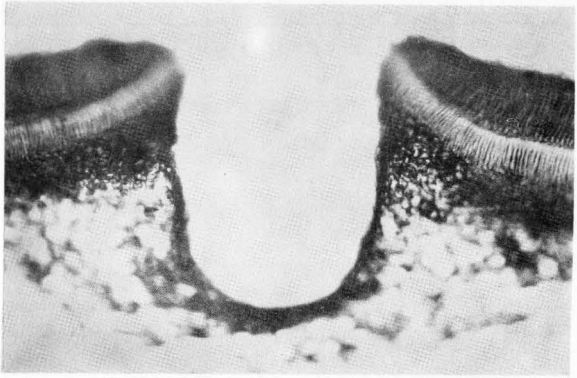


Figure 13. Hilum of a permeable seed of Variety A after 18 hours inhibition at 25 C. (approximately 300 X).

Summary

The data of Table 9 indicate that when the seed was dried to approximately 8 percent moisture, seeds of Earliwax and Improved Higrade had a tendency for hard seededness. The data of Table 11 indicate that a further decrease in the permeability of Earliwax resulted when the hilum, micropyle and raphe areas were sealed with petroleum jelly. It is believed from studying these data that when the moisture was above 10 percent in the seeds of Earliwax and Improved Higrade, the seed coats were as permeable as in the seeds of crack susceptible varieties. When the seeds of Earliwax and Improved Higrade were dried, however, the seed coats became impermeable and the hilum, micropyle and raphe offered the only pathway for entry into the seed. It appeared that the rate of water movement into the seed was regulated by the size of the hilar opening and/or by the tightness of seed coat adherence to the cotyledon. It is possible that this seed coat adherence layer of Earliwax and Improved Higrade was gradually soaked as water entered through the hilum and caused a capillary connection for water to move through the previously impermeable seed coat. When the hilar, micropylar and raphe openings were sealed, water could not enter to initiate this capillary movement. It is also possible that as water moved inside the seed coat and the cotyledon began to swell, the seed coat cuticle stretched and became permeable as suggested by Brown (1931).

This impermeability mechanism of Earliwax and Improved Higrade is not presented as the differentiating characteristic between the crack-susceptible and crack-resistant varieties, but rather as a possible protective mechanism against cotyledonal cracking. Since it was shown in Table 8 that these varieties resist cracking even when water is imbibed normally, other factors are apparently involved in this resistance.

CHAPTER VII

INVOLVEMENT OF MIDDLE LAMELLA IN COTYLEDONAL CRACKING

Introduction

The results of previous experiments in this thesis suggest that seed moisture fluctuations enhanced the expression, but were not the basic cause, of cotyledonal cracking in snap bean seeds. Evidence in the literature suggested that a calcium deficiency in the intercellular pectates of the cotyledons could cause the cracking. The following experiment was conducted to learn if cotyledonal cracks originated in the middle lamella and, if so, to determine whether a calcium deficiency was responsible for the weakness.

Literature review

The function of calcium within the cell structure has been outlined by Chambers and Chambers (1961) and Miller (1957). The middle lamella (a layer of intercellular material that cements together adjacent cells) is dependent upon calcium salts for stiffness and its property of cementing cells together. If calcium is removed from the medium, sodium largely replaces calcium so that the intercellular cement is transformed into dissociated and soluble sodium proteinates or pectates. The cells then

have a tendency to fall apart. Plants grown in calcium-free water, especially at low pH, tend to show root decomposition and cell separation. Morris (1963) observed that snap bean seed produced on ground following sugar beets, without zinc or sulfur fertilizer, had only half as many cotyledonal cracks as seed produced on ground following alfalfa hay or a previous crop of snap beans.

Lunin and Gallatin (1965) reported that the bean plant composition generally reflected the cation composition of the soil. In other words, the cation balance within the plant was essentially the same as the cation relationship of the soil. Similar results were obtained by Van Buren and Peck (1963). They observed that the calcium concentration within the pods of Tendercrop snap beans increased when the calcium level of the nutrient solution was increased. It was also noted that increasing the level of calcium resulted in firmer canned pods that had less tendency to slough and split. De Kock (1964) and True (1922) reported that the potassium and calcium balance within plants has a constancy of product. An increase in one of the cations is accomplished by a proportional decrease in the other.

The effect of various nutrient levels and balances on pea seed was studied by Sayre and Nebel (1930). They concluded that (a) peas grown in high calcium had seed coats with 25 percent taller palisade cells; (b) cells of the cotyledons in low calcium treatments appeared

physiologically older than cells from plants grown in normal calcium; (c) the higher the calcium level in the seed coats, the tougher the peas; (d) as the proportion of potassium increased, the level of calcium decreased and the peas were more tender; (e) the starch grains were larger in the cotyledons of peas that were grown in high calcium solutions; and (f) the cotyledonal cells were slightly larger in cotyledons of peas grown in high calcium. Reeve (1947) obtained similar results from a study of mineral nutrition of peas. He concluded that nutrition had a definite effect upon the texture of the seed coats and appeared to be related to slight changes in the pectic materials.

Methods and materials

Pieces of naturally-cracked cotyledons were prepared for microscopic observation by the paraffin method of Sass (1958). They were killed and fixed in formalin-acetic acid-alcohol (FFA). The pieces were dehydrated by the alcohol method, embedded in paraffin blocks and microtome sections 25 microns thick were cut. They were affixed to the microscope slides and stained with safranin and fast green.

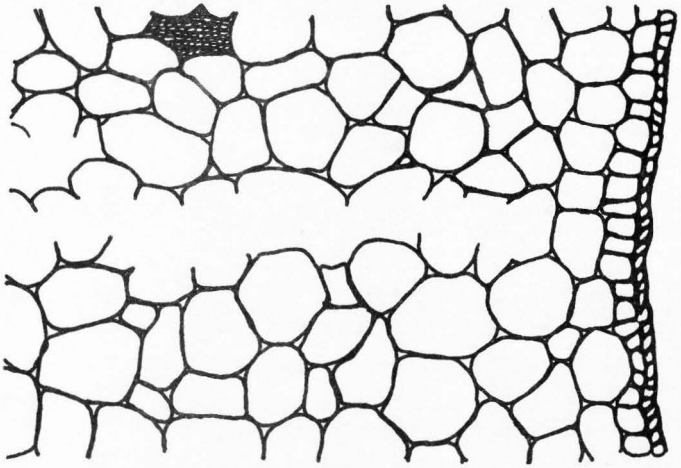
Cotyledonal cracks of Harvester, Tenderwhite, Tendercrop and White Seeded Tendercrop were examined under 100 X and 250 X magnification to determine the pathway of the cracks as they developed across the cotyledons.

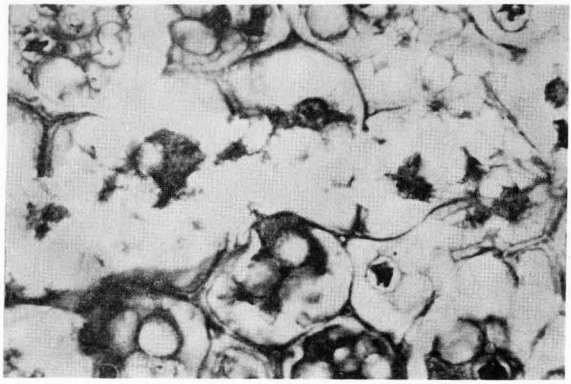
Results and discussion

Figures 14 and 15 illustrate a cotyledonal crack which is representative of those examined in this experiment. Cracking occurred across the cotyledonal cell walls at a much higher frequency than was observed along the intercellular middle lamella.

Summary

If cotyledonal cracking is caused by a structural weakness of the cells, the weakness is apparently in the cell wall rather than the middle lamella. There was little evidence in the literature to indicate that a compositional weakness of the cell wall caused cotyledonal cracking. Therefore, it was concluded that the cracking was caused by a physical force upon the cells.





CHAPTER VIII

RELATIONSHIP OF ANATOMICAL DIFFERENCES TO COTYLEDONAL CRACKING

Introduction

This investigation was conducted to microscopically study the seed coats and cotyledons of crack-resistant and crack-susceptible varieties of snap beans to learn if anatomical differences between varities could explain the differences in cracking susceptibility.

Literature review

Kannenberg and Allard (1964) have pointed out that white-seeded lima beans have thinner seed coats, shorter and broader cells, and fewer cells per unit in the palisade layer than colored seeds. Steinswat (1966) has reported that no anatomical differences were observed between seed coats of permeable and impermeable lima bean (Phaseolus lunatus) seeds.

Atkin (1964) believes that cotyledons of certain varieties are basically more resistant to cracking; however, there has been no direct evidence from previous studies to support this view.

Powrie, et al. (1960) have noted great differences in the size and shape of individual cells throughout the cotyledons of navy bean seeds.

They observed that the epidermal cells on the flat side of the cotyledon were more than three times longer than those of the round side epidermis and varied greatly in length. The hypodermal cells were larger than the epidermal cells of both the flat and round sides.

Experimental results reported by Bils and Howell (1963) indicated that cell division within the cotyledon was essentially completed during the first 2 weeks after flowering and that beyond this the cells increased in size only. Lowenberg (1955) noted that cotyledonal cells of snap beans seed increased from 360,000 to 2,600,000 during development and maturation and that this represented less than three cell generations.

Methods and materials

Mature seeds of Tenderwhite, Tendercrop, Earliwax and Improved Higrade snap beans were cross-sectioned into three parts, killed and fixed in formalin-acetic acid-alcohol (FAA). The sections were dehydrated by the alcohol method, embedded in paraffin blocks and microtome sections of 25 microns thickness were cut (Sass, 1958). Longitudinal and cross sections of each of the four varieties were mounted on slides and stained with safranin and fast green.

Seed coats of the four varieties were studied under the microscope at 100 x and 250 x in search of anatomical variations that could cause differences in permeability. Longitudinal and cross sections of the cotyledons were also examined microscopically and compared for

anatomical differences. Photomicrographs were made of the seed coats and cotyledons with a 35 mm camera attached to the microscope. The photographs were taken at 100 x and 250 x and enlarged to approximately 300 x and 750 x.

Measurements of seed coat thickness and of the cotyledonal cell dimensions were made with the aid of an ocular micrometer inserted into the eye piece of the microscope. Drawings were made by using a Leitz camera-lucida attached to the microscope.

Results and discussion

Seed coats. Photographs of external cell layers of the seed coats from four varieties of snap beans are illustrated in Figures 16 through 19. The nutrient cells and aleurone layer (Figure 20) normally collapse and deteriorate in mature seeds, resulting in seed coats of irregular thickness. There is, however, greater uniformity in the thickness of the palisade and osteosclereid cells. The measurements of these cells are listed in Table 12. The data indicate that Tendercrop had significantly thicker palisade and osteosclereid cells than were found in the more permeable seed coats of Improved Higrade and Tenderwhite and significantly thicker palisade cells than Earliwax, Improved Higrade and Tenderwhite. They also indicate that the order of increasing cell thickness is in direct relation to the order of decreasing permeability of the seed coats for these varieties. The data for Tenderwhite, Tendercrop, and Improved Higrade

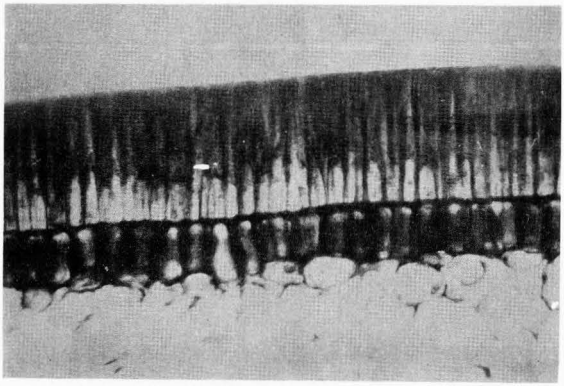


Figure 16. Cross section of Tendercrop (susceptible) snap bean seed coat (approximately 1000 X). Palisade and osteosclereid cells illustrated.

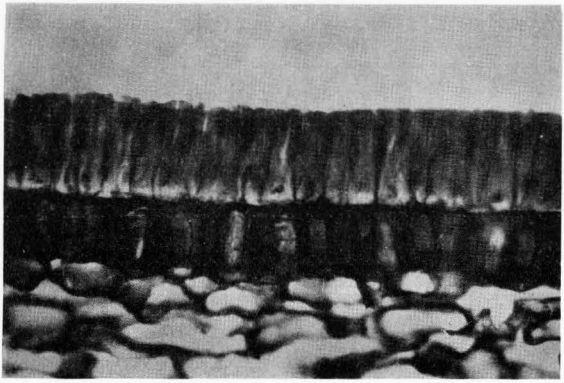


Figure 17. Cross section of Earliwax (resistant) snap bean seed coat (approximately 1000 X). Palisade and osteosclereid cells illustrated.

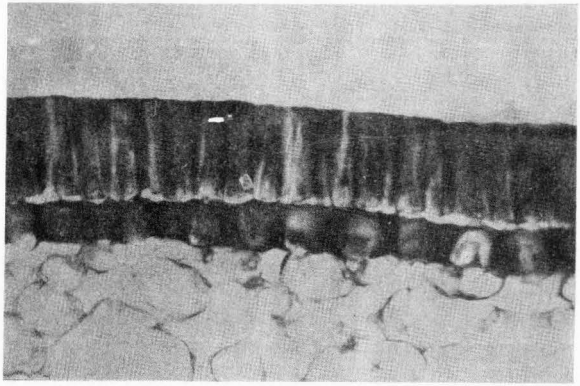


Figure 18. Cross section of Tenderwhite (susceptible) snap bean seed coat (approximately 1000 X). Palisade and osteosclereid cells illustrated.

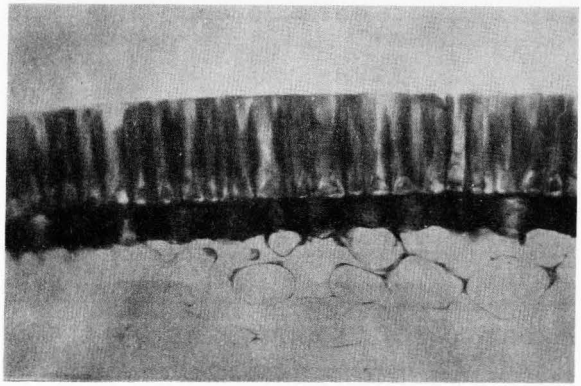


Figure 19. Cross section of Improved Higrade (resistant) snap bean seed coat (approximately 1000 X). Palisade and osteosclereid cells illustrated.

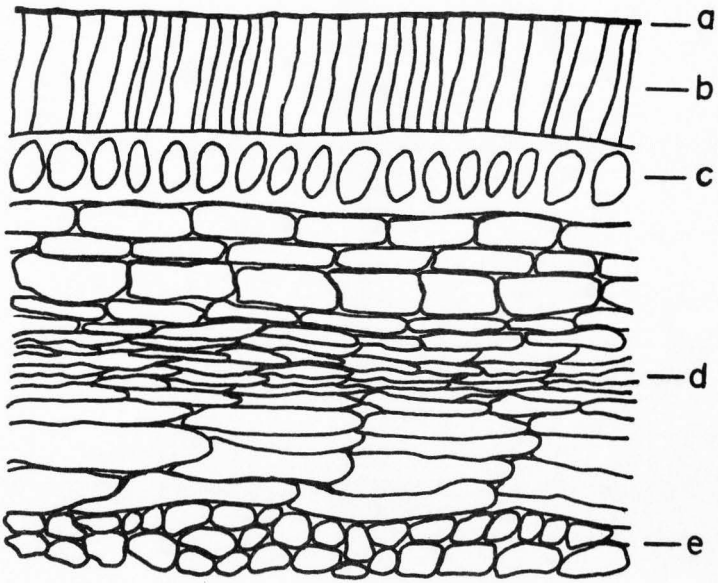


Figure 20. Cross section of a seed coat of Earlwax snap bean which illustrates the typical cell strata.

Section taken from side of seed coat and camera-lucida drawing made at approximately 1200 X.

a - Cuticle;
b - Malpighian, or palisade cells
c - Osteosclereid cells
d - Nutrient cells
e - Aleurone layer (After Watson, 1948)

Table 12. Thickness, in microns, of the palisade cells (column A) and osteosclereid cells (column B) of seeds from four varieties of snap bean seeds. The measurements were taken from both sides of each of five seeds per variety. The means are listed near the bottom of the table with the combined means of the palisade and osteosclereid cells recorded below them.

Seed number	Tenderwhite		Tendercrop		Improved Higrade		Earliwax	
	A	B	A	B	A	B	A	B
1	44	18	50	20	46	12	40	24
	44	16	50	20	48	16	44	24
2	40	16	48	18	44	16	40	24
	44	16	48	18	40	18	40	20
3	44	16	54	20	44	18	40	24
	40	16	54	20	48	16	40	24
4	38	18	56	24	40	18	44	18
	40	18	54	22	44	18	40	20
5	40	18	52	20	44	16	40	28
	40	18	56	20	42	18	40	28
$\bar{X}$	41.4	17.0	52.2*	20.2*	44.0	16.6	40.8	23.4*
$\bar{X}^a$	58.4		72.4		60.6		64.2	

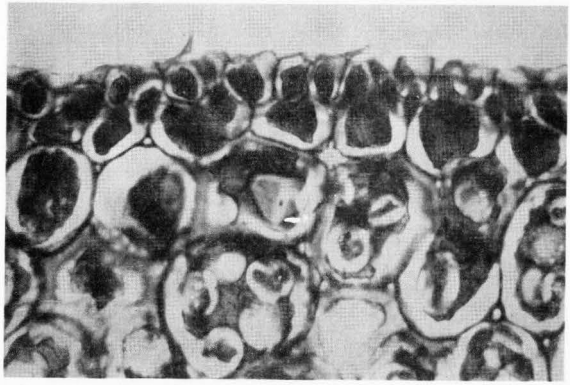
[a] Combined means of palisade and osteosclereid calls.

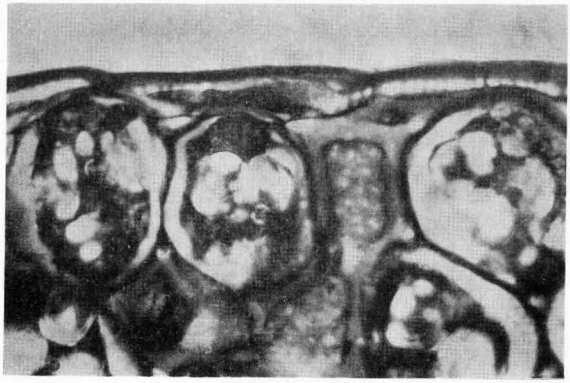
* Significantly different from similar cells of other varieties at 5 percent probability (Duncan, 1955).

seem to suggest that the palisade cell thickness was of primary importance in regulating seed coat permeability. However, the average thickness for Earliwax palisade cells is slightly less than for Tenderwhite which is much more permeable. A closer study reveals that the ostoesclereid cells of Earliwax are significantly thicker than for Tenderwhite and apparently is partially responsible for the lower permeability. The fact that the great difference between the permeability of Earliwax and Tenderwhite was not fully reflected in the thickness of the cells indicates there are other differences involved that are not directly related to thickness of cells. In this regard, compactness of the palisade cells and intercellular differences may be of importance.

Cotyledons. Longitudinal and cross sections of the four varieties of snap bean cotyledons were carefully studied and compared. No characteristics were consistently observed that could account for differences in crack-susceptibility.

Cell shape, size and arrangement were nearly identical in comparisons of longitudinal and cross sections of individual cotyledons in all four varieties. One notable difference was observed in the epidermal cell layer on the flat side of the cotyledons. These cells were small and spherical in longitudinal sections (Figure 21), but rectangular in cross sections (Figure 22). This indicated they were long, cylindrical cells considerably smaller than the internal cotyledonal cells. Since





all other cotyledonal cell structure was quite similar in both planes, and since cracks usually develop across the longitudinal plane, comparisons in this experiment are illustrated only for the longitudinal sections.

Figures 23 through 26 are camera-lucida drawings of the cotyledon's flat side for Tendercrop, Earliwax, Tenderwhite, and Improved Higrade respectively. The second and subsequent cell layers of the flat side were much larger than the epidermal cells and were circular to elliptical in shape in both longitudinal and cross sections. This suggested that the internal cells were nearly spherical.

Figures 27 through 30 illustrate longitudinal sections of the cotyledon's round side for Tendercrop, Earliwax, Tenderwhite and Improved Higrade respectively. All four varieties have small, spherical epidermal cells along the round side and subsequent layers of cells become progressively larger toward the center of the cotyledon. Similarly, the epidermal and subsequent layers of cells on the round side of the longitudinal sections are of comparable size and shape to those of the cross sections. The cells appeared to be nearly spherical and the outer one to three cell layers were considerably smaller than those toward the center. This could account for the more rapid rate of water penetration along the round side, since the greater number of intercellular spaces could provide more points of entry. All the varieties studied had this characteristic and it apparently had little effect in cotyledonal crack-susceptibility.

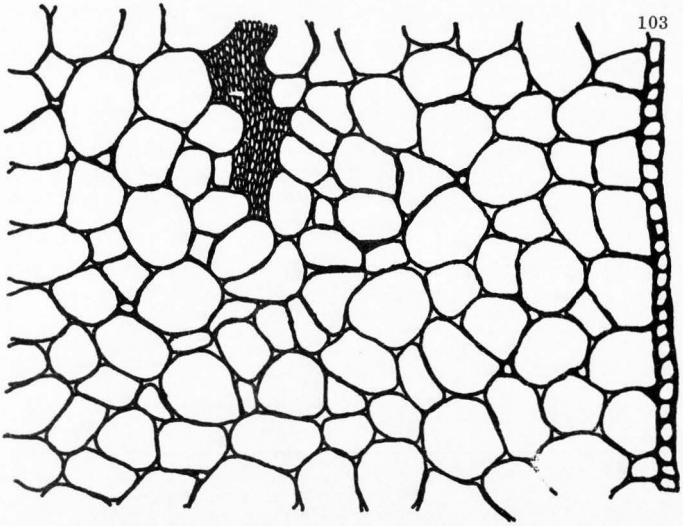


Figure 23. Longtiudinal section drawing from flat side of Tendercrop (susceptible) snap bean cotyledon (approximately 750 X).

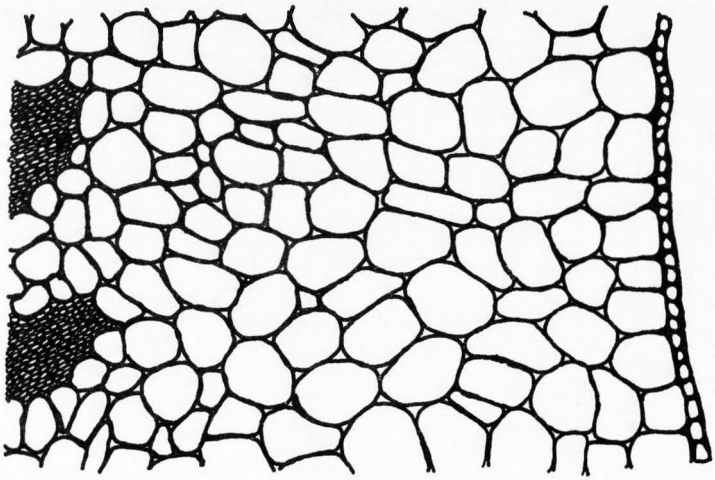


Figure 24. Longitudinal section drawing from flat side of Earliwax (resistant) scap bean cotyledon (approximately 750 X).

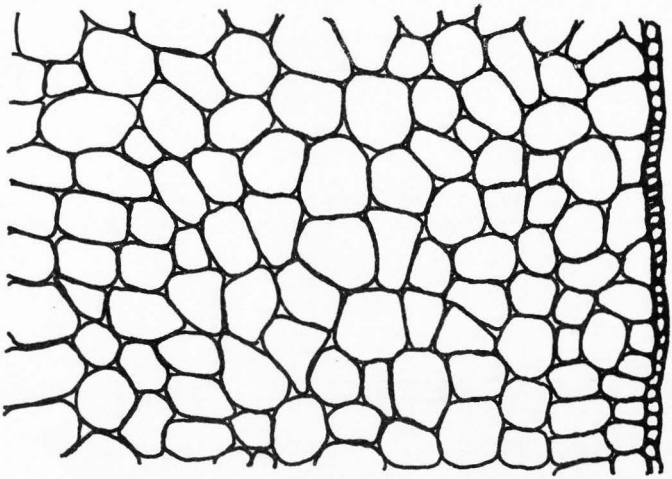


Figure 25. Longitudinal section drawing from flat side of Tenderwhite (susceptible) snap bean cotyledon (approximately 750 X).

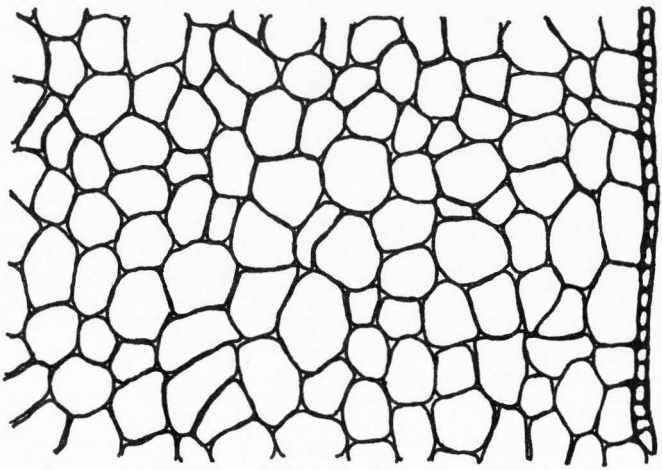


Figure 26. Longitudinal section drawing from flat side of Improved Higrade (resistant) snap bean cotyledon (approximately 750 X).

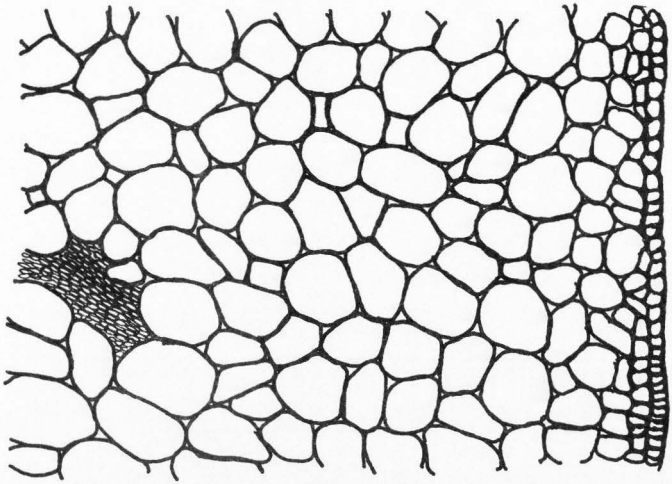


Figure 27. Longtiudinal section drawing from round side of Tendercrop (susceptible) snap bean cotyledon (approximately 750 X).

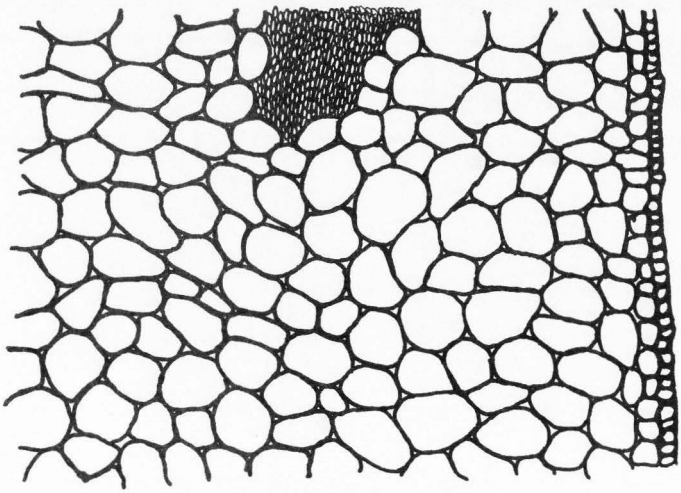
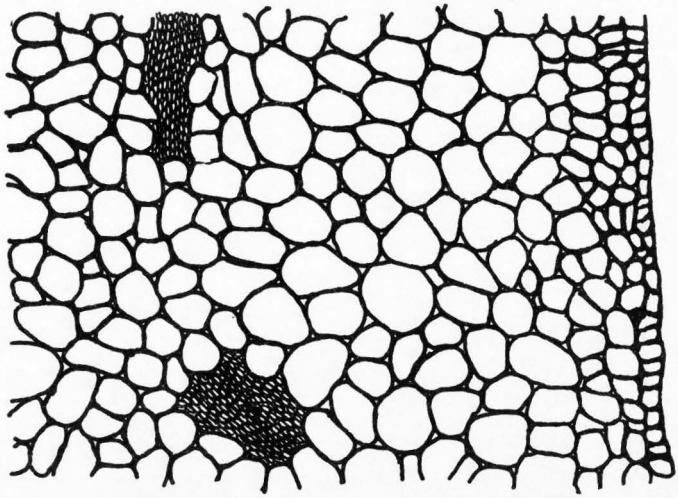
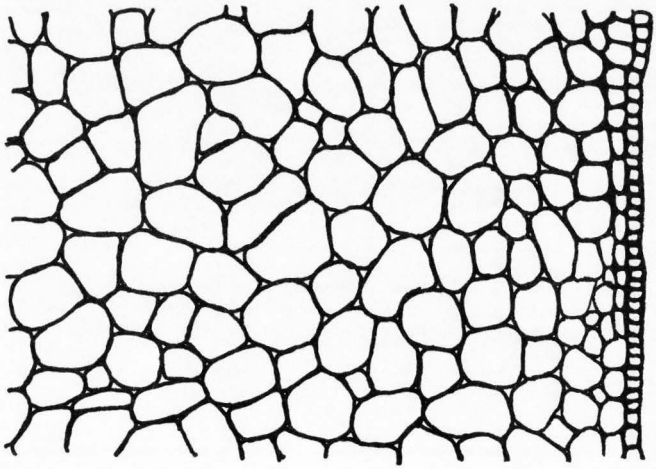


Figure 28. Longtiudinal section drawing from round side of Earliwax (resistant) snap bean cotyledon (approximately 750 X).





Differences in cell size between seeds within a variety were significant at less than 10 percent probability for Earliwax and Improved Higrade and at less than 25 percent probability for Tendercrop. A significant difference was not found between seeds of Tenderwhite (Table 13). It is believed there would be a more significant difference in size of cells between seeds within a variety if more replications were included. The cell size differences among varieties were not significant.

A considerable difference was observed in cell sizes within an individual seed. It is believed that this difference was largely due to sectioning spherical cells on different planes. A cell sectioned near the edge would appear much smaller as a single-plane section than a neighboring cell of the same size sectioned near the center.

Summary

It was concluded that seed coats with thicker palisade and/or osteosclereid cells were less permeable. There was no indication that cotyledonal cell size, shape or arrangement were responsible for differences in crack-susceptibility. Differences in cell size were apparently controlled more by seed size than by variety. No differences in cell shape were detected among varieties. All cotyledonal cells appeared to be nearly spherical except for the epidermal layer of the flat side. Differences in intercellular spaces or cell wall thickness were not detected.

Table 13. Comparison of cotyledonal cell size for seeds of four snap bean varieties.

Seed number	Cotyledonal cell area in microns^2 [a]			
	Earliwax *	Improved Higrade	Tenderwhite	Tendercrop**
1	7752	7662	6868	9307
2	5486	8638	8115	8099
3	6320	5843	5216	6921
4	6320	7131	7945	7265
5	6259	8665	6836	6299
$\bar{X}$	6427	7588	6996	7578

[a] Each value is an average of 10 randomly selected cells from the internal region of the cotyledons. Area obtained by multiplying length times width.

* Significant differences between seeds within a variety at 10 percent probability (Snedecor, 1956).

** Significant differences between seeds within a variety at 25 percent probability.

SUMMARY DISCUSSION

The evidence presented in this investigation does not indicate that cotyledonal cracking of snap bean seeds is directly due to seed coat or pod differences, metabolic stress within the plant, or variation in cotyledonal cell structure. This suggests that cracking may be caused by a biochemically related weakness within individual cells rather than moisture stresses within the cotyledons.

The possibility of an auxin involvement in cotyledonal cracking has been suggested by the research of Sacher (1957) and Glasziou, et al. (1960). Sacher conducted an experiment in which segments of one group of Kentucky Wonder pole bean cotyledons imbibed distilled water and a second group imbibed distilled water and NAA(napthalene acetic acid) auxin. Those that imbibed the auxin remained plump and rigid and those that imbibed water alone became soft and flaccid. The auxin-treated segments imbibed as much water, but observations of hand sections indicated that intercellular spaces were filled with air, while those treated with water were filled with liquids. It appeared that the auxin maintained a selective permeability of the membrane and prevented exosmosis of cellular substances into the intercellular spaces. Auxin involvement in cotyledonal cracking was not studied in the present investigation and remains for future study.

The report by Veiss and Powrie (1959) that starch granules of navy bean seeds began to swell at a temperature of about 60 C suggests a possible theory for cotyledonal cracking. Since the cotyledons of navy beans are composed of approximately 39 percent starch granules at maturity (Powrie, et al., 1960), a doubling of the granule size would cause extreme stress on the cell walls. If this is also true in snap beans, then differences in susceptibility to cracking could be related to different types of starch granules and their response to high temperatures. Reeve (1954a, 1954b, 1954c) found such differences in potato varieties that expressed various degrees of susceptibility to cell rupturing when heated. Lee (1966) has reported that certain types of starch granules may gel and double in size at temperatures as low as 45 C. In this regard, it is of interest to note that Hawthorn, et al., (1966) have recorded temperatures of 60 C inside pea pods curing in the field. If seed of crack-susceptible varieties of snap bean seed contain starch granules characterized by a low gelling temperature, they could be much more susceptible to cracking than varieties containing starch granules with a higher gelling temperature. In this investigation, seeds of crack-susceptible varieties were induced to crack by a drying, soaking and re-drying treatment. The results indicated that the treatment did induce cracking, since the treated samples were cracked much more severely than the pre-imbibition samples. Reeve (1954c) contends that cracks in potato cubes which are produced by

heat induced swelling of starch granules and subsequent cell rupturing may not become apparent until the cubes have been dehydrated and rehydrated. If this is also true in snap beans, seeds that appear to be free of cracks before soaking may, nevertheless, have been predisposed to cracking by high temperatures in the field. When the seeds are dried in the laboratory and rehydrated, the undetected internal fissures may develop into easily observed cracks across the cotyledon. Anderson (1963) observed that crops of snap bean seeds left standing in the field and apparently subjected to only minor moisture changes have expressed severe cotyledonal cracking when hand-threshed samples were inspected. The heat-induced starch gelling and expansion could be the explanation of this phenomenon. No research is cited in which this theory has been tested on snap bean seed. It is possible that a detailed study of varietal differences in starch granule response to temperature and moisture changes would reveal the cause of cotyledonal cracking in certain varieties of snap beans.

SUMMARY

1. There are distinct varietal differences in susceptibility to cotyledonal cracking.
2. If a stress-induced nutrient deficiency of the developing bean plant caused cotyledonal cracking, the deficiency apparently affected the seed several days after initiation.
3. There appeared to be a negative correlation between an increase in the number of pods set during a single day and an increase in cotyledonal cracking in seeds initiated during that day.
4. In some instances, rapid seed moisture fluctuations of less than 10 percent induced cotyledonal cracking in dry seeds of cotyledonal cracking susceptible varieties of snap beans.
5. The bean pod apparently was not responsible for differences in cotyledonal cracking susceptibility, although it did protect the seeds of both susceptible and resistant varieties to some extent.
6. There was little correlation between seed coat permeability and cotyledonal cracking susceptibility. Some varieties with highly permeable seed coats were crack-resistant while some that were the least permeable were crack-susceptible.
7. White seed coats were generally more permeable than colored.

8. Permeability was lower in seed coats that had thicker palisade and osteosclereid cells.

9. Cotyledonal cracking resistant varieties had a greater tendency for hard-seededness than was observed in crack-susceptible varieties.

10. No differences were observed in the cotyledonal cell structure of the varieties studied that could account for differences in susceptibility to cotyledonal cracking.

11. Recommendations for controlling cotyledonal cracking are:

    A. Strive to develop crack-resistant varieties by hybridizing with known crack-resistant lines.

    B. Plant crack-susceptible varieties of snap bean seed at a uniformly shallow depth to promote rapid seedling emergence and uniform seed development and maturity. Seeds on early maturing plants in an unevenly maturing population will become dried and will be subjected to temperature and moisture changes while the later plants are maturing.

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