FACTORS AFFECTING COTYLEDONAL CRACKING DURING THE GERMINATION OF BEANS (*PHASEOLUS VULGARIS*)

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While studying cotyledonal abnormality an unusual type of injury was observed in snap beans which had been carefully hand-picked and hand-shelled. The injury consisted of one or more transverse breaks which extended partially or completely across the cotyledon, severing the vascular elements, figure 1. Not only was the movement of materials into the seedling impeded by the breaks but the cotyledons remained swollen often preventing the shedding of the seed coat, figure 2. The fissures also provide a seat of infection for decay organisms.

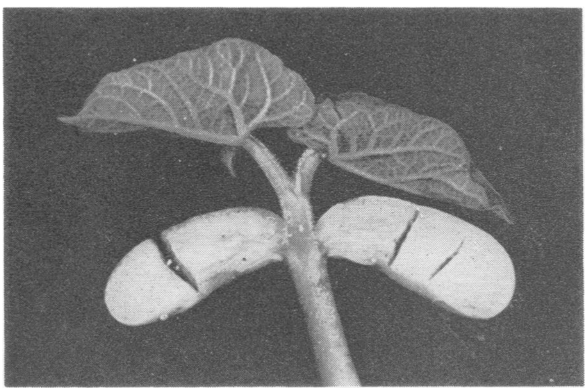

FIG. 1. Seedling showing cotyledonal fissures which developed during germination.

In a recent study of mechanical injury to seed beans (**1**) injury was classified into two categories: visible or external and invisible or internal, of which the latter was detected by a viability test. Transverse cotyledonal breaks were attributed to mechanical injuries during the processing of the seed. SHULL and SHULL (**5**) observed internal cracking of pea cotyledons due to uneven swelling. They explained an apparent increase in the rate of water absorption as due to cavities formed by the breaking but did not relate it to germination. MILLER (**3**) showed by x-ray photographs that lima beans absorb water through the vascular bundle of the hilum. It then goes to the chalaza and around the seed primarily through the vascular elements of the testa. The seed coat was found to be impermeable until wetted from beneath.

The present study investigates whether or not cotyledonal cracking takes place during germination and if so under what conditions. Seed characteristics as they affect cotyledonal cracking and related problems are also considered.

Materials and methods

The experimental beans were grown in the field at Urbana, Illinois, during the summer of 1950. At maturity, the seeds were harvested and shelled by hand, and then kept in the laboratory for about two months. Seven varieties and strains which had shown a variation in cracking of cotyledons were selected. They include Cherokee wax, 49-3 (Eastern States Farmer's Exchange), Commodore, Top Crop, Rival, B1229-1-2-6 (Regional Vegetable Breeding Laboratory) and B1762 (Regional Vegetable Breeding Labora-

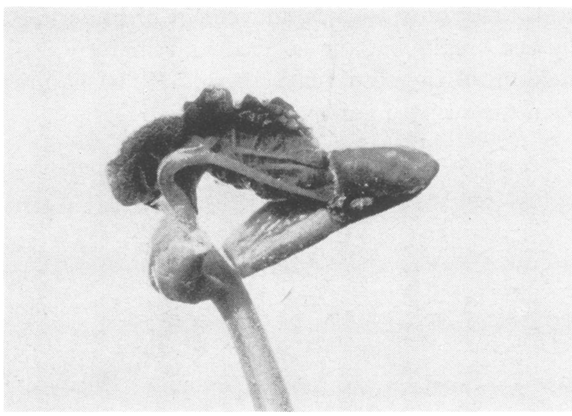

FIG. 2. Seedling with cotyledons broken during germination and seed coat adhering when ends of cotyledons failed to shrink.

tory) with respective percentages of cracking of 0, 1.3, 5.3, 13.5, 15.3, 38.7 and 48.3.

Except where otherwise indicated, germination tests to determine cotyledonal cracking were made using 100 seeds of each treatment in a greenhouse maintained at 65 to 75° F. The seeds were planted in steam sterilized sand and covered about three fourths of an inch deep. The cotyledons were usually observed for cracking as soon as the old seed coats had shed. In a few cases, however, the seed coats were carefully removed for the examination.

Absorption of water was determined by placing seeds in distilled water at the rate of 100 seeds per 150 ml. of water and then removing them at intervals, depending upon the rates of absorption. The seeds were dried quickly between pads of absorbent cotton covered with cheesecloth and weighed. Unless otherwise stated, rates of absorption were measured at 20° C and calculated as percentage increase in weight during the period of observation.

Results

The testae were carefully removed from a large number of seeds of Top Crop which is subject to cotyledonal cracking and Cherokee which is resistant, and none were found with cracks when examined under a stereoscopic microscope. One hundred cotyledons of each variety were then placed on moist filter paper in covered Petri dishes at room temperature. Within an hour marked cracking occurred in the cotyledons of both varieties (fig. 3). Cherokee is resistant to cracking during germination but became susceptible when the seed coats were removed and water could be absorbed freely. This suggested that resistance to cracking depended upon the rate of water uptake and not upon a difference in the imbibitional properties of the cotyledons.

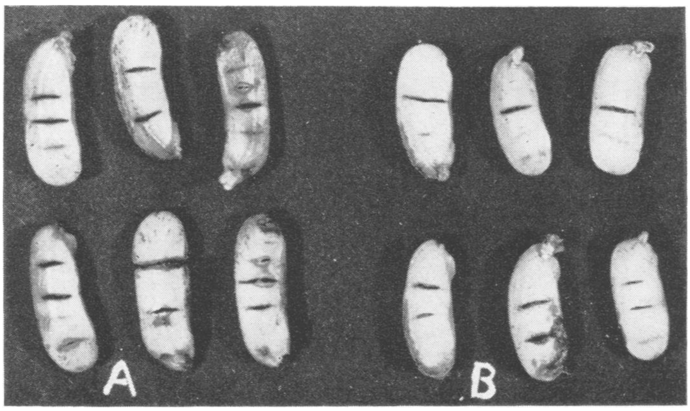

FIG. 3. Cotyledonal fissures resulting from rapid water absorption on moist filter paper after removal of the testae. The resistant variety Cherokee (A) cracked as readily as the susceptible strain 49-3 (B) under these conditions.

To check these points a number of experiments on the absorption of water were made. Seeds of B1229-1-2-6, Top Crop, 49-3 and Cherokee were soaked and the rates of uptake of water measured. After 30 hours the seed coats were carefully removed and the cotyledons observed for fissures. The data (fig. 4) show the rates of absorption by the strains to be in the order of B1229-1-2-6 > Top Crop > 49-3 > Cherokee. As indicated by wrinkling of seed coats the first two began absorbing water within a few minutes after immersion. About three hours were required for all of the seeds of 49-3 to begin and several hours before any of the seeds of Cherokee began, and then the water moved very slowly around the cotyledons of this variety as indicated by wrinkling of the seed coats. Strain B1229-1-2-6 and Cherokee with highest and lowest rates of absorption had respectively highest and lowest percentages of seeds with cracked cotyledons, 84 and zero. Although Top Crop absorbed water more rapidly than 49-3, it had only 59 as compared to 64% of seeds with cracked cotyledons. This is perhaps due to the delayed absorption in 49-3 and to its longer cotyledons, which were subject to greater tensions as a result of differential swelling.

To determine if imbibitional properties of the cotyledons might in part account for the differences in rates of absorption by varieties, the testae were carefully removed from 50 seeds of each Cherokee and 49-3 and the excised cotyledons immersed in water. They were weighed at intervals of 30 minutes for five hours and then observed for cracks. The cotyledons of both Cherokee and 49-3 imbibed water rapidly and were severely cracked, the percentages being respectively 84 and 96. When the testae of the former were being removed, it was noted that they adhered tenaciously to the cotyledons, which may explain the slow wrinkling and movement of water into the seeds of this variety.

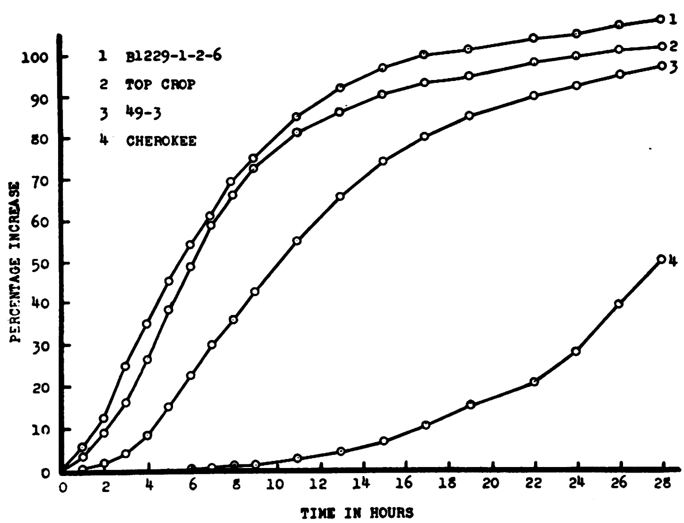

FIG. 4. Rate of water absorption by bean seed as percentage increase in weight.

Since the moisture content of the soil may be expected to affect the rate of absorption and consequently the cracking of cotyledons, three flats were filled with sand having respective moisture contents of 1.96, 3.52, and 13.59%. Each flat was planted with Top Crop and B1229-1-2-6 in alternate rows. The flats were then placed on a greenhouse bench and covered with glass over which paper was laid. After three days the covers were removed and all of the flats were watered uniformly. The data in table I show that lower soil moisture during the initial stage of germination resulted in decreased cotyledonal cracking. In strain B1229-1-2-6 it varied from 11 to 65%, while in Top Crop it ranged from 10 to 32. The data indicate that the severity of cracking may be increased when susceptible varieties are planted in wet soils.

To determine the effect of temperature on the relation between rate of absorption and cracking of cotyledons, Rival beans were soaked and the rates of absorption measured at 10, 20, and 30° C. At the end of seven

TABLE I

COTYLEDONAL CRACKING OF BEANS DURING GERMINATION IN SAND OF DIFFERENT MOISTURE CONTENTS.

Moisture content of sand	Cotyledonal cracking	
	B1229-1-2-6	Top crop
1.96	11	10
3.52	45	18
13.59	65	32

hours the testae were removed and the cotyledons examined for fissures. There was an increase in rate of absorption with temperature but a decrease in cotyledonal cracking. The percentages were 76, 70, and 60 for the respective temperatures. This experiment was repeated with germinating seeds. A one-gallon container filled from a lot of uniformly moist sand and covered to prevent loss of moisture was placed in each of three chambers maintained at 10, 20, and 30° C respectively. As soon as soil temperatures became constant, seeds of the Rival variety were planted in each container. When germination began, the seeds were taken from the sand, the seed coats removed and the cotyledons observed for fissures. The results show the same inverse relationship between cotyledonal cracking and temperature but more strikingly than in the previous experiment. The percentages of seeds with cracked cotyledons were 65, 54, and 28 for the respective 10, 20, and 30° C temperatures. The data indicate that cracking would be increased by low soil temperatures.

Because of the low relative humidities in the laboratory where the beans had been stored since harvest, the cotyledons were rather brittle. Under such conditions the seed might be predisposed to cotyledonal cracking. Therefore, the effect of seed conditioning on subsequent cracking of cotyledons during germination were investigated. Samples of 100 seeds of Cherokee, B1762 and Commodore with two 100-seed lots of Top Crop, five lots in all, were placed in each of six desiccators. Relative humidities of approximately 95, 75, 56, 35, 15, and 0% were maintained in the desiccators by

TABLE II

PER CENT. RELATIVE HUMIDITY OF SEED STORAGE, MOISTURE CONTENT OF BEANS AND COTYLEDONAL CRACKING DURING GERMINATION.

Storage humidity	Moisture content of Top Crop	Cotyledonal cracking			
		B1762	Cherokee	Commodore	Top Crop
95	28.7	37.9	0.0	1.0	0.0
75	16.2	25.8	0.0	0.0	0.0
56	11.5	15.5	0.0	0.0	0.0
35	8.8	37.7	0.0	2.1	3.1
15	5.7	87.8	0.0	2.0	22.4
0	3.3	73.3	0.0	3.2	19.1

L.S.D. at 5% Level, 12.29; at 1% Level, 16.99

means of saturated salt solutions of disodium phosphate, sodium chlorate, calcium nitrate, chromic oxide, lithium chloride and anhydrous calcium chloride. The desiccators were covered but not sealed and placed in a constant temperature chamber maintained at 20° C.

One of the two lots of Top Crop seed in each desiccator was used as a control and weighed initially and then weekly until equilibrium was reached. After 31 days the moisture content of these controls was then determined by drying in a ventilated oven at 105° C. The remaining lots in the six desiccators were planted in sand filled flats in a randomized test with four replications of each treatment. The flats were watered uniformly and as needed until the seed germinated. Then the cotyledons were observed for fissures.

The moisture content of the Top Crop beans as shown in table II ranged from 28.7 to 3.3%. Even after storage at the range of relative humidities,

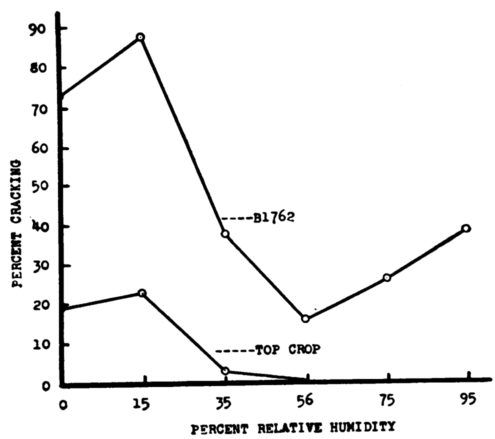

FIG. 5. Effect of storage humidity on cotyledonal cracking during the germination of beans.

all treatments of strain B1762 showed cotyledonal cracking ranging from 15.5 to 87.8% during germination. The percentage of cracking was lowest after storage at 56% and highest after storage at 15% relative humidity. Statistically significant cotyledonal cracking occurred in Top Crop beans after storage at low humidity, but not at the higher ranges and none occurred in Cherokee beans after storage at any of the six humidities. Although the percentage of cotyledons with fissures was low in Commodore beans after storage over anhydrous calcium chloride, the cotyledons tended to break off during germination.

Storage at low humidity caused some of the Cherokee beans to become hard (impermeable to water) and others to germinate slowly and unevenly. The data indicate that seed conditioning in beans with respect to moisture content may be important before planting or making a germination test, but the same conditioning may not be best for all varieties. When the controls of Top Crop beans which had been stored at 95% relative humidity were dried rapidly in a ventilated oven at 105° C, the outer surfaces of the cotyledons cracked badly because of the more rapid shrinkage of the drying surfaces of the cotyledons. This indicates how internal cracking may result from unequal distribution of water in the cotyledons.

Germination was strikingly affected by the moisture content of the seed at the time of planting (fig. 5). Seed of B1762 from the 95% humidity chamber showed some decay, while the seed of Cherokee from this chamber germinated best. On the other hand, after storage over anhydrous calcium chloride germination of the B1762 variety was slow and uneven. Many of

FIG. 6. Germination of bean varieties after storage at different humidities. Varieties: A, B1762; B, Cherokee; C, Commodore; D, Top Crop. Relative humidity: 1, 95%; 3, 56%; 5, 75%; 6, 0%.

these seeds absorbed water but failed to germinate; others remained impervious to water. A similar observation on the effect of storage at low humidity on beans was made by NUTILE and NUTILE (**4**) and GLOYER (**2**).

Summary

Varieties of snap beans showed marked differences in susceptibility to cotyledonal cracking during germination. A high incidence of cracking was found in strains B1762, B1229-1-2-6, Rival, and Top Crop. No cracking was observed in the variety Cherokee except where the testae were broken. Susceptibility to cracking was associated with seed coat permeability and rapid imbibition of water. A rapid rate of water uptake apparently causes differential swelling in the cotyledons. Tensions arise that often result in transverse fissures.

Cotyledonal cracking was found to vary in relation to soil moisture during germination at 20° C, indicating that a high incidence of cracking may be expected when susceptible strains are planted in wet soils.

The relation between water absorption and cotyledonal cracking was altered by temperature. As temperature increased between 10 and 30° C, the rate of water uptake also increased but cracking decreased. More coty-

ledonal breakage may therefore be expected when susceptible varieties are planted in wet soils during a period of low temperatures.

The relative humidity at which seed beans are held for a period before planting may affect their susceptibility to cotyledonal cracking during germination. In the most susceptible strain tested, B1762, the maximum cotyledonal breakage occurred after storage at a relative humidity of 15%, a minimum breakage occurred at 56% and cracking was higher after storage at higher humidities.

From these experiments it seems likely that some of the bean seed injury attributed by other investigators to mechanical means may have occurred during germination.

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