# BIOCHEMICAL STUDIES ON SEED VIABILITY
# I. MEASUREMENTS OF CONDUCTANCE AND REDUCTION

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(WITH TWO FIGURES)

## Introduction

Previous work (9) on this problem led to the conclusion that a correlation existed between electrical conductivity of seed extracts and seed viability. With the broadening out of the work and an improvement in the method it was hoped that this conclusion would be still further substantiated. The studies here reported therefore deal with further investigations on this and related lines.

Such work, based on permeability, must necessarily be far from complete in its elucidation, since we have as yet no precise knowledge of the phenomenon itself. That permeability exists in seeds is self-evident but no one has yet offered a plausible explanation. It has been known for some time that a definite relation exists between the permeability of the cell membrane and its injury (19), and it might readily be inferred that a variation in degree of permeability would be correlated with similar variations in degree of injury. The degree of permeability may be indicated by measuring the resistance of the solution in which the seeds have been immersed. Many tests have shown that dead seeds are more permeable than viable ones, so that one might naturally expect that a variation in permeability would be correlated with variations in germination. Thus, a series showing variations in germination in increments of two or three per cent., on the range of zero to one hundred, might be worked out. Obviously, this would be more desirable than merely to know that a seed is dead or is viable.

## Historical

### CONDUCTANCE METHODS

The use of conductance methods for measurements in physiological research dates back to the work of EDWARD WEBER (26) in 1836 and DU BOIS-REYMOND (8) in 1849. RANKE (21) in 1865 noticed the decrease in resistance in plant and animal tissues upon death.

BROOKS (2) in 1923 worked with *Laminaria*, yeast, bacteria, and blood cells. He showed that during the progress of heating of *Laminaria* the "net conductance" approached a constant value which he considered indicative of death. With *Bacillus coli*, his results are rather variable.

JOHNSON and GREEN (**12**) have shown that upon death the conductivity of yeast cells increases, this being due both to exosmosis of salts and to decrease in size of cells.

The outstanding investigator in the field of electrical conductivity measurements of permeability in plant cells is OSTERHOUT (**18**), who recommends this method and shows that the results do not vary more than one per cent. from the mean. In a later work (**19**) he pointed out that the time curve expressing the increase in permeability of *Nitella* during the progress of dying is practically the same whether derived from measurements of exomosis or electrical resistance.

### OTHER METHODS

The problem of viability of seeds has been attacked from several different points of vantage. DARSIE and ELLIOTT (**7**), working with PEIRCE in 1914 noticed that the heat of respiration was greater for live seeds than for dead ones. Heat measurements were made under adiabatic conditions, the seeds being placed in silvered Dewar flasks under suitable conditions for germination. These authors claimed that there was a "normal temperature" for each species of plant and that departure from this temperature indicated departures from the best conditions of the organism. Excess of normal temperature indicates infection, while subnormal temperature is indicative of lessened vigor, usually due to increased age. The authors did not make any great claims as to the accuracy of this method for determining viability, but it is evident that seeds of high, low, and medium viability only could be thus determined.

LESAGE (**14**) evolved a method in which seeds were soaked in solutions of KOH of strengths varying from normal to N/682. Non-germinating seeds imparted a color to all solutions, while the viable seeds colored the strong solutions and those down to N/32, but had no noticeable effect upon weaker solutions.

Many workers have attacked the problem of seed viability from the standpoint of enzyme activity. KASTLE (**13**) in his classical work on oxidases, states that peroxidases and catalases are even more widely distributed in living tissues than are oxidases.

Peroxidases attack hydrogen peroxide and liberate atomic oxygen. Their presence may be demonstrated by the fact that they cause bluing of guaiacum. Samples are ground, a drop or two of guaiacum added, and two to three cubic centimeters of neutral hydrogen peroxide introduced. If the peroxidases are present, the guaiacum becomes blue, the intensity of color being proportional to the degree of enzyme action.

McHARGUE (**16**) has applied this peroxidase test to seeds of corn, hemp, tomato, oat, cow pea, soy bean, castor bean, and lettuce. He claims that these seeds, when exhibiting zero germination, displayed no peroxidase reaction. He goes still further and claims that the peroxidase reaction might be used for seed testing and that seeds of high, low, and medium viability might be thus classified.

On the other hand, BROCQ-ROSSEU and GAIN (**1**) studied the peroxidase activity of seeds ranging from two years to five thousand years old. They found peroxidase activity exhibited in a wheat sample 2000 years old and claim that wheat will retain peroxidase activity 100 years after it loses its ability to germinate.

The enzyme catalase has also been employed for determining viability of seeds. This enzyme attacks hydrogen peroxide and liberates molecular oxygen. A known weight of the powdered sample is mixed with a known volume of neutral hydrogen peroxide and the volume of oxygen evolved is measured. CROCKER and HARRINGTON (**6**) determined the catalase activity of Johnson grass and Sudan grass seeds, of the same sample, before and after they had germinated. It was found that the catalase activity increased with germination, thus paralleling respiratory intensity. With *Amaranthus* seeds no correlation was found to exist between catalase and respiratory intensity, vitality, and age. They conclude that generally there is a close correlation between catalase activity and respiratory intensity, but not a very close correlation between either of these and the vitality of the seed or the vigor of the seedlings.

NĚMEC and DUCHON (**17**) demonstrated a close correlation between catalase activity and viability of seeds, being able to obtain a difference between seeds varying not more than two or three per cent. in germination. These workers employed cereal grains, legumes, and other seeds.

In 1924 MAROTTA and KAMINKA (**15**) found that NĚMEC'S catalase method could not be applied to wheat seeds. SHULL and DAVIS (**24**) have found a relationship between catalase activity and delayed germination in *Xanthium* seeds. There is a decrease in catalase activity in delayed germination. In an earlier work with *Xanthium* seeds, SHULL (**23**) showed that oxygen accelerates germination and that a greater amount of oxygen is absorbed with the seed coats removed. CROCKER (**5**) showed that oxygen increases respiration and in this way initiates germination.

Since catalase is an oxidizing enzyme and believed to participate in some manner in respiration, it may be this connection with vital activities that suggested the large amount of work attempting to correlate catalase with viability.

All of the evidence herein quoted seems to furnish direct confirmation of the conception of enzymatic activity and its relation to vital phenomena

as held by PALLADIN (**20**, p. 173) who says: "Life processes are not to be interpreted simply as enzymatic activity." This writer continues to show how organisms might be killed without destroying the enzymes and that enzyme activity is always exhibited by freshly killed tissues where precaution was not previously taken to destroy the enzymes, and that the only difference between enzyme activity in living tissues and dead ones is that in the former they are organized in their work.

We have thus attempted to review briefly the work that has been done toward shortening the method of determining viability of seeds. The majority of the workers quoted here have sought to correlate enzymatic activity with viability. Though enzymes are originally associated with living organisms, we have attempted to show in this historical review that they are not necessarily to be taken as indices of life in organisms.

Catalase and peroxidases might be classified as respiratory enzymes and for this reason may be thought to be concerned with vital phenomena. On the other hand, both dead and living seeds respire and respiratory measurements would be of little value for determining viability. Again any carbon compound might be oxidized to carbon dioxide and this process thus be taken for one of respiration.

The deeper we penetrate into this problem the more complex it grows. It practically resolves itself into the question of "When is a seed dead?" or it becomes a matter of evolving a physico-chemical means of determining the difference between life and death. Has this been determined even for the higher organisms? We are told that death is gradual even in man.

Whether there be a chemico-physical difference between life and death, it seems that there should be a correlation between viability and permeability of plant cells. It seems reasonable to suppose that non-living cells should be more permeable than living ones and that the salts should leach out more rapidly from the dead cells than from the live ones. The amount of salts diffusing out of the cells of the seeds should then modify the resistance of distilled water in which the seeds were soaking and this change of resistance could thus be measured electrically. An attempt was made to measure this change in resistance and to correlate it with viability of seeds. Suitable apparatus was assembled for making the determination.

## Apparatus and method

With the exception of a few minor changes the apparatus recommended in the paper by HIBBARD and CHAPMAN (**10**) was used. The immersion type of electrolytic cell, the best for this kind of work, was thrust into the liquid in which the seeds were soaking.

A stirring apparatus was set up so that possible error due to hand agitation would be avoided. A weighed amount of seeds was poured into

each of the four clean pyrex beakers containing 100 cc. of liquid. The beakers were then placed in a constant temperature water bath at 25° C. A friction-drive universal motor was connected to the four stirrers so that the four samples should be run simultaneously. The electrodes which were immersed in the beakers were connected to a four-way switch for greater convenience.

The most radical departure from the method of FICK and HIBBARD (**9**) was made in the substitution of dilute $KMnO_4$ solutions for the conductivity water in the measurements of resistance. A number of references have been made above to the work on enzymatic activity as related to viability. KASTLE (**13**) mentions the fact that catalases, which occur in plant extracts and which liberate molecular oxygen from hydrogen peroxide, will reduce other oxidizing agents, among them being $KMnO_4$. While making some preliminary tests for catalase it was noticed that seeds reduced very dilute solutions of $KMnO_4$ at different rates.

The thought occurred that along with electrolytes there were substances of organic nature leaching out of the seed and hence not measurable by conductivity methods as previously used. This being true, the distilled water might be replaced by the $KMnO_4$ solution and the change in resistance noted as the permanganate is reduced.

## Electrical resistance of solutions containing seeds of varying viability

### EXPERIMENT I

Beakers containing 100 cc. of M/20,000 $KMnO_4$ were placed in the water bath, the electrodes inserted and connected, and the stirrers started. The seed samples were then added to each beaker, permitting one minute to elapse between the addition of subsequent samples, in order that the resistance readings might be made in the same order. The quantity of seeds used was one gram of timothy, or 50 peas, or 100 wheat seeds. Resistances were determined and the readings recorded at regular intervals. The results are presented in tables I, II and III. For the sake of brevity the figures which represent the resistance of the original $KMnO_4$ solution plus that of the salts and organic substances leached out of the seeds will hereafter be designated as "solution resistance."

The wheat seeds were obtained from the Farm Crops Department of Michigan State College. The variety was Red Rock and the samples varied in age. One hundred seeds were taken from each sample, after all broken, discolored, and infected seeds had been discarded. These were germinated and the percentage germination found was taken as that of the sample. In the above table four different samples having three different germination percentages were taken for the "solution resistance" tests.

TABLE I

SOLUTION RESISTANCE OF WHEAT IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

| SAMPLE | GERMINATION | SOLUTION RESISTANCE | AVERAGE |
|---|---|---|---|
| | per cent. | ohms | ohms |
| 108 | 86 | 7361 | 7361 |
| 101 | 86 | 7364 | 7364 |
| 100 | 81 | 7241 | 7292 |
| 100 | 81 | 7361 | |
| 102 | 22 | 4663 | 5119 |
| 102 | 22 | 5576 | |

These garden pea samples were obtained from the Michigan Farm Bureau. The variety was Alaska. Those selected for this experiment varied in germination percentages, as may be seen from the table, from 97 to 63 representing samples of varying ages. History of these samples are not known, but the variety was good, and germination was especially high for the most recently harvested ones. The chief idea here as in the

TABLE II

SOLUTION RESISTANCE OF PEAS IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

| SAMPLE | GERMINATION | SOLUTION RESISTANCE | AVERAGE |
|---|---|---|---|
| | per cent. | ohms | ohms |
| 110 | 97 | 12880 | 12985 |
| 110 | 97 | 13090 | |
| 106 | 93 | 16250 | 15945 |
| 106 | 93 | 15640 | |
| 107 | 93 | 16320 | 16145 |
| 107 | 93 | 15970 | |
| 111 | 92 | 16320 | 13355 |
| 111 | 92 | 16390 | |
| 112 | 90 | 11600 | 10800 |
| 112 | 90 | 10000 | |
| 109 | 83 | 9048 | 9729 |
| 109 | 83 | 10410 | |
| 103 | 76 | 10660 | 10535 |
| 103 | 76 | 8051 | |
| 105 | 75 | 11830 | 10072 |
| 105 | 75 | 8315 | |
| 104 | 63 | 5699 | 5518 |
| 104 | 63 | 5337 | |

other seed samples where the samples were not described in detail was to get good samples of different ages and different germination percentages.

TABLE III

SOLUTION RESISTANCE OF TIMOTHY IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

| SAMPLE | GERMINATION | SOLUTION RESISTANCE | AVERAGE |
|---|---|---|---|
| | per cent. | ohms | ohms |
| 58 | 81 | 6340 | 6340 |
| 68 | 70 | 4970 | 4795 |
| 68 | 70 | 4620 | |
| 57 | 54 | 5974 | 5715 |
| 57 | 54 | 5456 | |
| 65 | 0 | 4205 | 4266 |
| 65 | 0 | 4327 | |

Samples 58 and 57 were obtained from Wisconsin. The year these samples were harvested is not known. Sample 65 came from Iowa, harvested in 1912. Sample 68 also came from Iowa, harvested in 1915 from the Agronomy plots.

Greater encouragement was received from the resistance measurements in $KMnO_4$ than from any in conductivity water. In table I the wheat shows true correlation. The viability varies directly with the resistance. In the case of the peas (table II) the samples of from 90 to 97 per cent. germination show high resistance. With but one or two exceptions the rest of the figures for resistance fall in their proper places in the table. The experiment with timothy (table II) is not entirely in accord with the first results.

## EXPERIMENT II

Corn seeds from 8 different samples were next divided into two lots. Lot 1 was placed on a window ledge overnight where the temperature reached as low as 0° F. (Subsequent tests showed that these seeds did not freeze. Hence they will be used as checks against the killed seeds).

Lot 2 was kept in a hot air oven long enough to kill the embryo. The solution resistance of these two lots appear in table IV.

In column 2 of this table, opposite the sample numbers indicated in column 1, one may find the germination percentages for the seeds in the different samples. The solution resistance before and after the heat treatment is shown in columns 3 and 4. Fig. 1 represents the results of column 3 in graphical form.

TABLE IV

SOLUTION RESISTANCE OF LIVING AND DEAD CORN SEEDS

| SAMPLE | GERMINATION | SOLUTION RESISTANCE (50 MIN.) | |
|---|---|---|---|
| | | Lot 1 (alive) | Lot 2 (dead) |
| | per cent. | ohms | ohms |
| 155 | 100 | 19670 | 17395 |
| 156 | 99 | 27410 | 24680 |
| 157 | 98 | 17250 | 14760 |
| 152 | 96 | 16810 | 15000* |
| 150 | 94 | 14650 | 12385** |
| 148 | 70 | 12170 | 12980 |
| 144 | 65 | 14920 | 10556** |
| 154 | 1 | 6449 | 5712 |

* Duplicate discarded. ** Browned by heat.

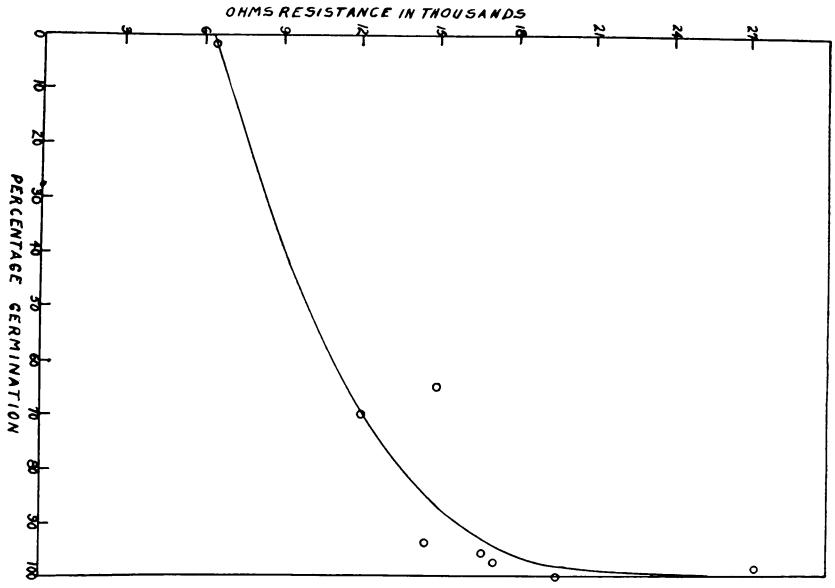


FIG. 1. Graph of relation of solution resistance to viability, from lot 1, table IV.

The samples used here were obtained from the Farm Crops Department of Michigan State College. The variety was Duncan. Eight groups were taken for the test.

### Reduction of potassium permanganate by viable and non-viable seeds

In determining the solution resistance of seeds in potassium permanganate it was noticed that the permanganate was reduced at different rates

by different samples, the end-point being an easily recognized amber color. Resistance measurements taken at regular intervals did not take into account the color changes, being concerned with the changes in solution resistance only.

It was then decided to determine the time-rate of reduction of $KMnO_4$ by the seeds. One hundred corn seeds were ground to the fineness of meal, one-gram samples weighed out and permitted to stand in twenty cubic centimeters of distilled water for an hour. At the end of this time the mixture was filtered, one cubic centimeter of the filtrate drawn off and added to 0.5 cc. of M/800 $KMnO_4$. The time of adding the filtrate to the permanganate was recorded as well as the time when it was completely reduced.

The seeds were ground because the substances reducing the permanganate were dissolved in the water more readily and the process thus hastened. The cells would hardly be destroyed by this coarse grinding since it was performed in a meat chopper. Material must be ground with quartz sand in order to be reasonably sure of rupturing the individual cells. Grinding did not influence the relative rates of reduction by the different samples as was shown in several preliminary tests.

The relation of germination percentages to time-rate of reduction will be found in table V and fig. 2.

TABLE V

RELATION OF GERMINATION PERCENTAGES OF CORN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS (POWDER)

| SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|
| | per cent. | min. |
| 122 | 98.3 | 38.3 |
| 124 | 97.0 | 30.8 |
| 129 | 96.0 | 40.2 |
| 128 | 87.5 | 36.7 |
| 127 | 87.0 | 29.8 |
| 126 | 80.4 | 26.8 |
| 123 | 34.0 | 13.3 |
| 121 | 33.0 | 13.0 |
| 125 | 3.0 | 5.9 |

It was found that, with the exception of but a few of the high-germination samples, there was direct correlation between viability and time of reduction of permanganate. The higher the germination the longer the time required for complete reduction of the permanganate.

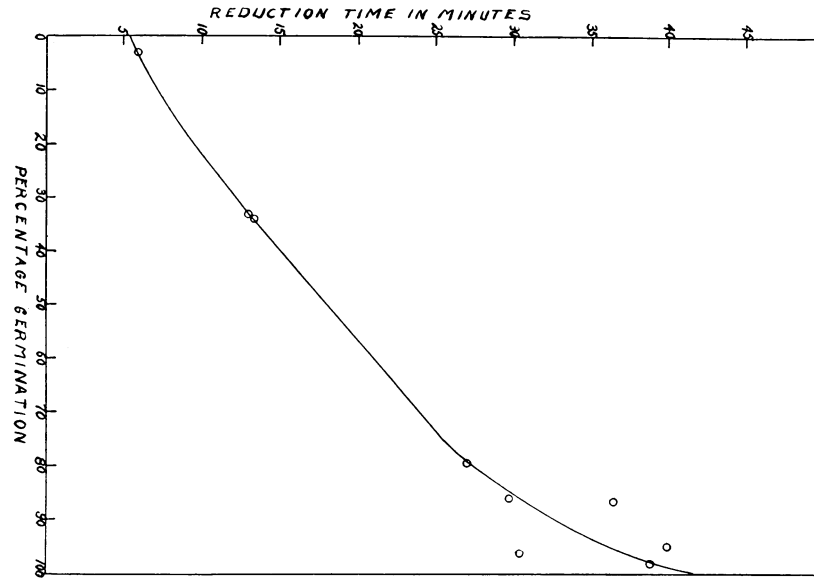


FIG. 2. Graph of relation of time-rate of reduction of $KMnO_4$ to viability, from table V.

A large number of samples was secured and the corn meal was passed through a twenty-mesh sieve before soaking. As was true in other experiments where a greater number of samples was employed, the results are not nearly so uniform. These results are presented in table VI.

## TABLE VI

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF MEAL (20 MESH)

| SAMPLE | GERMINATION | TIME TO REDUCE | SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|---|---|---|
| | per cent. | min. | | per cent. | min. |
| 139 | 100 | 21.9 | 150 | 94 | 15.9 |
| 142 | 100 | 18.5 | 153 | 93 | 15.5 |
| 156 | 99 | 16.5 | 160 | 93 | 14.1 |
| 146 | 99 | 10.9 | 143 | 83 | 17.9 |
| 147 | 99 | 14.3 | 159 | 79 | 23.5 |
| 140 | 99 | 14.2 | 148 | 70 | 16.5 |
| 157 | 98 | 12.5 | 141 | 71 | 10.3 |
| 152 | 96 | 22.5 | 138 | 65 | 18.3 |
| 158 | 96 | 17.9 | 144 | 65 | 10.9 |
| 151 | 95 | 21.9 | 154 | 1 | 19.0 |
| 145 | 94 | 12.0 | 149 | 0 | 7.5 |

For the purpose of shortening the method, grinding was dispensed with in another experiment and the seeds themselves were soaked in the permanganate. Ten seeds of corn were placed in twenty cubic centimeters of N/1000 potassium permanganate and the time recorded. The time was again recorded when the permanganate was completely reduced. It was found that if a few drops of N/10 oxalic acid were added to the mixture the end point would be closer and colorless instead of amber colored. The results may be found in table VII.

TABLE VII

RELATION OF VIABILITY OF CORN TO TIME-RATE OF REDUCING POTASSIUM PERMANGANATE (WHOLE SEEDS)

| SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|
| | per cent. | min. |
| 146 | 99 | 10.9 |
| 152 | 96 | 22.5 |
| 151 | 95 | 21.9 |
| 143 | 83 | 17.9 |
| 141 | 71 | 10.0 |
| 149 | 0 | 7.5 |

In the six samples included in table VII there is only one (146) which does not occur in the regular order. There is here almost complete correlation between viability and the reduction of potassium permanganate.

Another modification in the method was made. To hasten the time of reaction the seeds were first soaked for 12 hours in water, and 1 cc. aliquots withdrawn for the test instead of using the seeds. One cubic centimeter of this extract was treated with one drop of N/2 $KMnO_4$. The time required for complete reduction was recorded. The results will be found in table VIII.

TABLE VIII

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF WHOLE SEEDS

| SAMPLE | GERMINATION | TIME TO REDUCE | SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|---|---|---|
| | per cent. | min. | | per cent. | min. |
| 155 | 100 | 23.0 | 167 | 81 | 20.5 |
| 161 | 99 | 23.0 | 168 | 65 | 19.0 |
| 162 | 99 | 21.5 | 171 | 25 | 12.0 |
| 163 | 98 | 24.5 | 173 | 2 | 7.0 |
| 172 | 97 | 11.5 | 169 | 0 | 18.0 |
| 164 | 86 | 24.5 | 170 | 0 | 12.0 |
| 166 | 83 | 21.0 | | | |

Here, too, low viability seemed to be consistent with the rapidity of reduction of potassium permanganate, although there is a great amount of non-uniformity in the table. The results in table IX were obtained in the same manner as those in table VIII. Here again the low-germinating seeds were first to reduce the potassium permanganate.

TABLE IX

RELATION OF VIABILITY OF CORN TO REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS OF WHOLE SEEDS

| SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|
| | per cent. | min. |
| 162 | 99 | 39 |
| 163 | 98 | 34 |
| 164 | 86 | 38 |
| 166 | 83 | 37.5 |
| 167 | 81 | 29.5 |
| 168 | 65 | 31 |
| 170 | 0 | 22 |
| 149 | 0 | 12 |
| 154 | 1 | 19 |
| 169 | 0 | 22 |

At the conclusion of this experiment on reduction of $KMnO_4$ by corn seeds some additional experiments were conducted with beans. The results (table X) exhibit a moderate amount of correlation. The figures for germination were obtained by selecting fifty normal-appearing seeds, sterilizing them, and testing for germination. This was done because it was learned that these were mixed samples, having been more or less adulterated by the growers. This is quite evident from the results of several different germina-

TABLE X

RELATION OF VIABILITY OF BEAN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$

| SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|
| | per cent. | min. |
| 174 | 100 | 31.0 |
| 175 | 100 | 31.0 |
| 176 | 98 | 25.5 |
| 177 | 98 | 26.5 |
| 178 | 100 | 19.0 |
| 179 | 46 | 38.0 |
| 180 | 0 | 14.0 |

tions. In one set a hundred seeds were selected at random; in another the twenty seeds that had been used in the reduction of the $KMnO_4$ were subsequently germinated. The variation observed in table XI may also be due to the difference in methods applied.

In regard to the bean seed, these were of the Navy variety and were harvested in 1925. The varying germination percentages in these samples were due to selection. The samples showing highest germination were gathered, picked, and selected after harvesting and the rest were left on the ground for several days, exposed to the climatic conditions, being sampled on different days. In this way samples of varying germination percentages were obtained from seeds that had died or were infected to seeds of high or perfect germination.

TABLE XI

GERMINATION TEST OF BEANS

| SAMPLE | GERMINATION | | |
|---|---|---|---|
| | 100 SEEDS | 200 SEEDS SELECTED | 50 SEEDS, SELECTED AND STERILIZED |
| | per cent. | per cent. | per cent. |
| 174 | 90 | 100 | 100 |
| 175 | 89 | 95 | 100 |
| 176 | 85 | 85 | 98 |
| 177 | 76 | 90 | 98 |
| 178 | 27 | 65 | 100 |
| 179 | 15 | 15 | 46 |
| 180 | 0 | 0 | 0 |

As will be shown in the latter part of this paper, the reducing substance has been found to be present in large quantities in the seed coat of the bean. Accordingly, ten bean seeds selected from the seven different samples were soaked over night in distilled water, and the seed coats then removed and placed in 10 cubic centimeters of N/1000 $KMnO_4$, the time for reduction being recorded. The ten seeds, after the coats had been removed, were germinated. The germination percentages and the time-rate of reduction are to be found in table XII. Not only was direct correlation between germination percentages and rate of reduction found, but sample 179, which was out of the expected order as far as time relations were concerned in table X, appeared in its proper place in table XII. This experiment was repeated at a different time with similar results.

An experiment was next performed in which the three parts of the seed (seed coat, cotyledons, plumule and hypocotyl) were treated with the potassium permanganate solution. In this case there were employed 5 seed

TABLE XII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY THEIR SEED COATS

| SAMPLE | GERMINATION | TIME TO REDUCE |
|---|---|---|
| | per cent. | min. |
| 174 | 100 | 26 |
| 175 | 100 | 24 |
| 176 | 100 | 23 |
| 177 | 100 | 23 |
| 178 | 90 | 20 |
| 179 | 30 | 16 |
| 180 | 0 | 1 |

coats, 10 cotyledons, and 10 embryos. The figures for germination are those obtained from the 50 selected and sterilized seeds. The results in table XIII show almost complete correlation in all cases. The seeds germinating above 90 per cent. do not always occur in the proper order; yet they are usually higher in the table than those of low germination.

TABLE XIII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCING $KMnO_4$ BY DIFFERENT PARTS OF SEED

| SAMPLE | GERMINATION | TIME REQUIRED FOR REDUCING | | |
|---|---|---|---|---|
| | | COATS | COTYLEDONS | EMBRYOS |
| | per cent. | min. | min. | min. |
| 174 | 100 | 34.0 | 11.0 | 12.0 |
| 175 | 100 | 30.5 | 6.0 | 11.0 |
| 176 | 98 | 27.5 | 8.5 | 9.0 |
| 177 | 98 | 32.5 | 6.5 | 8.5 |
| 178 | 100 | 30.0 | 7.0 | 10.0 |
| 179 | 46 | 26.5 | 5.0 | 7.0 |
| 180 | 0 | 1.5 | 2.0 | 3.0 |

## Studies of the nature of the substance

It is of interest to know the nature of this substance which reduces the potassium permanganate. REED (**22**) believes that the peroxidases in plant juices, having the power to absorb oxygen from oxygenases, will attack $KMnO_4$ in the same manner. BUNZEL and HASSELBRING (**3**) note that $KMnO_4$ may be reduced to a straw color by peroxides of manganese, and further to a clear solution by organic substances, but they hold that the oxidations are brought about by peroxides of manganese rather than by activated plant peroxidases.

That the reduction mentioned in this paper is not enzymatic may be demonstrated by first boiling the aqueous extract of seeds for fifteen minutes. The reduction will take place just as readily after boiling.

There is evidence supporting the view that these reducing substances may belong to the group of peptides, acid amides, or amino acids. If the proteins are precipitated by lead acetate and the excess lead removed by means of sodium carbonate, the filtrate will still reduce the potassium permanganate. Furthermore, an aqueous extract of the precipitate brought down by the lead acetate will not produce the reduction unless previously boiled with 10 per cent. HCl solution or incubated with pepsin solution.

Proteoses would remain in the solution after the proteins had been removed with lead acetate. However, phosphotungstic acid brought down no precipitate and it was assumed that in this case there were no proteoses present.

For a long time it was believed that ungerminated seeds contained no protein cleavage products. This view has now been changed by JODIDI (**11**) and co-workers who have found amino acids and peptides to be present in ungerminated kernels of maize, rye, wheat, and oats. BUSHEY (**4**) showed that frosted and "hailed" corn, besides being high in certain proteins, was also much higher in amide content than normal grains.

## Discussion

A very detailed and extensive study of the conductance method as briefly outlined in the paper by FICK and HIBBARD (**9**) revealed that as a test for distinguishing between seeds of high, low, and medium germination it was very good. As a test for distinguishing difference in seeds varying in increments of 2 or 3 per cent. it is worthless and so far no method has yet been devised to obtain this degree of accuracy. There are some facts militating against the use of this method. It is quite expensive to begin with, and assumes that the operator has at least a technical and a more or less complete knowledge of the details of a set up for making conductance measurements.

Furthermore, the method presupposes that the electrolytic content and its nature are the same for different samples of seeds of the same species and that the change in solution resistance would be solely dependent upon the change of permeability in the seed. It is also possible that salts clinging to the seeds would affect the results although they had previously been washed with distilled water in the hope of ridding them of all salts.

The direct measurement of tissues or of any tissue as a measure of permeability has been called in question by STILES (**25**, pp. 180–182).

The work with conductance measurements in solution of $KMnO_4$ rather than distilled water offered more promising results. Though there were a

few discrepancies, resistance for the most part rose with germination percentages. Curves plotted from any of the individual tables indicate as great a degree of consistency as most workers seem to obtain from other methods. Seeds of high germination percentages repeatedly exhibit a proportionately high resistance in solution.

By far the simplest method evolved in these experiments on viability was the comparison of the time element in the reduction of $KMnO_4$ with viability. It is obvious that seeds of low viability exhibit the property of reducing the potassium permanganate in less time than is required by seeds of higher viability. It does not matter whether the seeds of zero germination have been killed by heat, frost, disease, or have died of old age. They are nearly always the first to reduce the permanganate. It is possible to employ this method for determining seeds of high, low, and medium viability, but like other methods mentioned above, it needs considerable refinement before it will show differences in seeds varying 2 or 3 per cent. in germination.

## Summary

1. A review of the literature on the various methods for determining viability of seeds is presented.

2. A new and very simple method of determining viability of seeds is recommended. It is useful for seed testing and, as in other methods mentioned, in classifying seeds of high, low, and medium viability only. It is not acceptable when difference in germination of two or three per cent. is desired, and we have still to hunt for a means of obtaining this degree of accuracy. The method suggested consists in determining the time-rate of reduction in $KMnO_4$ solution in which the seeds are soaking.

3. Proof is furnished that the substance which reduces the $KMnO_4$ is not an enzyme and that it may belong to the group of substances known as amino acids, peptides, and amides.

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