GERMINATION INHIBITORS OF SUGAR BEET SEED

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A THESIS

Submitted to the

AMERICAN UNIVERSITY OF BEIRUT

In partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE IN
AGRICULTURE

September 1966

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Date Thesis is presented: September 8, 1966

SUGAR BEET SEED INHIBITORS

AQIL

AN ABSTRACT OF THE THESIS OF

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Title: Germination inhibitors of sugar beet seed.

A germination study was conducted in the seed technology laboratory of the American University of Beirut during 1965-66. Different methods for increasing the germination and vigor of sugar beet seed were tried. Washing the seeds in running water for 24 hours and soaking at 30°C or 40°C for 12 hours improved the germination considerably. The improvement in germination was considered mainly due to leaching out of inhibitors. The oscillating vacuum when applied on sugar beet seeds presoaked for different periods of time decreased the germination as compared to that obtained by soaking alone. Various concentrations of gibberellic acid, indoleacetic acid, kinetin, thiourea, hydrogen peroxide, potassium nitrate and naphthaleneacetic acid were used both for presoaking sugar beet seed and as germination media. The growth promoters neither stimulated germination nor inactivated the toxic substances.

The effect of the water extract of sugar beet seed was studied on various crop seeds and seedlings. The extract inhibited the germination percentage and development of radicles and plumules. The effect was, however, differential and more marked on radicles than on plumules. When low concentrations of the extract were applied on seeds and seedlings of alfalfa, sorghum and oats, there was less inhibition. At high dilutions of the extract a significant increase in germination and growth was found. The stimulation was common in oats as compared to alfalfa and sorghum, and in plumules as compared to radicles.

For determining the location of the germination inhibitors, the seed balls were separated into true seeds and pericarp tissue and their water extracts were prepared and tested for their inhibitory properties. The inhibitors were found to be concentrated in the pericarp tissue and practically absent from the true sugar beet seeds.

ACKNOWLEDGEMENTS

The author is grateful to Dr. Salah Abu Shakra for his encouragement and cooperation throughout the study.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

I. INTRODUCTION

The fact that some sugar beet seeds are dormant and do not germinate readily is well known to growers as well as to seedsmen. Under optimum conditions of temperature and moisture in the laboratory germination takes a long time to complete. In addition to slow germination, the radicles become black in color and often die after emerging from the seed ball and before the final count can be made. As a result the total germination of sugar beet seed as determined in the laboratory is very much reduced. Reduction in the field has also been observed especially under low moisture conditions in the soil. In general, however, germination in the field under favourable conditions of moisture and temperature is relatively better than that in the laboratory. Therefore, laboratory germination tests which are meant to give a good estimate of the germinability of the sugar beet seed in the field are not dependable.

This dormancy in sugar beet seed is attributed to the presence of hard pericarp tissue, high osmotic pressure, and the presence of germination inhibitors. It is generally accepted that the germination inhibitors which are water soluble play the major role in inhibiting

germination. Water soluble extracts of sugar beet seed balls have also been found to inhibit the germination of many other crop and weed seeds. However, very little information is available about the nature of the inhibitory substances, or about their effect on the germination and growth of crop seeds.

Any treatment that stimulates germination and improves the vigor of the seedlings will be considered of great importance for decreasing the germination period in the laboratory and for improving the stand in the field. Such a treatment will not only help in developing a standard germination procedure low in variation among germination tests, but it may also make the laboratory tests more comparable with germination in the field. In an attempt to solve this problem the International Seed Testing Association recommends the soaking of sugar beet seed prior to germination. Some seed technologists prefer washing in running water to soaking. Neither soaking nor washing is yet standarized to result in maximum germination and uniformity among germination tests.

In this study soaking and washing in running water for different periods of time and at different temperatures were compared. Various growth regulators and other chemicals were tested for their effect on germination and vigor. The effect of the inhibitory sugar beet water extract on the germination and growth of many crop seeds

was studied. In addition, a location study of the inhibitory material in the seed ball was conducted.

II. REVIEW OF LITERATURE

The sugar beet seed, because of its manifold problems, has been an item of research for many years. The seed is a multigerm and is often called a seed ball or a glomerule. On germination each seed ball may produce as many seedlings as seven. Through extensive breeding work, however, monogerm seed has been developed and is now replacing the multigerm in many agricultural areas in the world. One major problem of sugar beets, whether the seed is a multigerm or a monogerm, is its slow germination. Many causes of this behaviour have been reported. The available literature concerning these causes and the methods of hastening germination is reviewed below.

Causes of Slow Germination

Hard pericarp tissue. The maternal tissue which covers the true seeds is very hard. The scarification of sugar beet seed has been a common practice to increase germination. Hemicellulose was determined by Lackey (1948) to be the cementing substance holding seed-caps on sugar beet seed balls. The germination was increased when the seed-caps were loosened by treating the seeds with a solution of ten percent sodium phosphate, three percent

hydrochloric acid or three percent sulfuric acid. Snyder (1959c) reported that varieties differ in the degree of tightness of the seed-caps over the seed balls. When the seed balls were notched to expose a portion of each true seed, germination was more rapid than that of the whole seed balls. But when the true seeds were separated from the seed balls, the germination was slower indicating a stimulating effect of the maternal tissue on the germination. Peto (1964) reported that germination of monogerm seed having tight seed-caps was greatly improved by chipping off part of the cap. Similar results were obtained by alternate soaking in water and drying, by treating the seed with enzyme hemicellulase and three percent hydrochloric acid.

Osmotic pressure. The failure of many seeds to germinate while in the fruit, has been attributed to high osmotic pressure of the surrounding juice. Evenari (1949, pp. 159-160) reviewed the work of Duym et al. who reported that the inhibitory action in the germination of sugar beet seed was due to high osmotic pressure. It was reported by Dubetz (1958) that at low moisture levels, the application of fertilizers increases the osmotic pressure of the soil moisture and as a result a significant reduction in germination takes place. Through the use of isotonic solutions, it was soon realized that osmotic pressure was only one factor, and was often playing a minor role in the

inhibition of the germination process. Evenari (1949, pp. 159-160) emphasized that some other factors were also responsible for the inhibition. It was reported that solutions isotonic to grape-juice had less inhibitory action on germination than the grape-juice itself. Stout and Tolman (1941a, pp. 691-693) found that sugar beet seed was tolerant to high osmotic pressure. Using solutions isotonic to the water extract of sugar beet seed 70 percent higher germination was obtained than that in the extract. It was also determined that copper, zinc and lead as well as gums and tannins are present in the extract but not in quantities large enough to cause any appreciable degree of toxicity. According to Snyder et al. (1965) the osmotic pressure is not a major factor in the inhibition of the germination of sugar beet seed. The inhibition was not caused by inorganic substances in the fruit but largely by the presence of toxic organic substances.

Germination inhibitors. Another possible cause of dormancy in sugar beet seed may be the presence of germination inhibitors or some toxic substances which are widespread in nature. The germination inhibitors may inhibit some metabolic pathways but their removal, or a lowering in their concentration may stimulate germination. On the other hand, toxic substances cause irreversible injury to

the tissues. Evenari (1949, pp. 156-184) listed over 100 plant species which contained germination inhibitors. These inhibitory substances were classified into ammonia, ethylene, mustard oils, organic acids, unsaturated lactones, aldehydes, essential oils, phenols, and alkaloids.

It was determined by Tolman and Stout (1940, p. 829) that sugar beet seed balls contain some water soluble substances which produce a toxic effect during germination. These toxic substances retard germination and kill the radicles. Stout and Tolman (1941a,p. 711) worked on the nature of the toxic substances and observed that the toxic effect was largely due to the release of ammonia by enzymatic hydrolysis of the nitrogenous compounds of the extracts. It was found that 0.3-0.4 mg of nitrogen as ammonia were released per ml of solution. This amount was toxic when tested in synthetic solutions. In support to this finding Tolman (1948b) reported that seeds produced in saline soils high in nitrates contained more inhibitors. On the other hand, it was reported by Snyder (1959b) that there were no marked differences in the speed or the percent germination of the seed from sugar beet plants supplied with excess quantities of nitrogen. Rehm (1953, p. 12) divided the toxic principles into two classes: (1) primary inhibitors such as inorganic salts and organic compounds which are present in the dry seeds and can be removed by washing, (2) secondary inhibitors such as free

ammonia which is not present in the seed at the beginning but develops during germination. Rehm (1953, p. 12) confirmed the results of Stout and Tolman (1941a) but considered the bacteria, which usually develop in large numbers on germinating beet seeds, to be responsible for the reduction of nitrate into free ammonia. According to De Kock *et al.* (1950) ammonia plays only a minor role in the factors affecting the germination of sugar beet seed. It was not possible to demonstrate the activity of the enzymes which could liberate ammonia in quantities sufficient to cause inhibition. Miyamoto (1957) also questioned the formation of the minimum toxic concentration of 0.1 mg of nitrogen as free ammonia per ml of solution in the vicinity of seeds during germination.

An unsaturated yellow oil was separated by De Kock *et al.* (1950) from the aqueous extract of sugar beet seed and was referred to as the main factor responsible for the inhibition of the germination of sugar beet seed. The oil was found to inhibit salt uptake, respiration and the activity of the polyphenolase enzyme in the sugar beet root. De Kock and Hunter (1950) reported that this yellow oil was a powerful germination inhibitor when tested on cress (*Lepidium sativum*) and other seeds, with pronounced effect on the radicles. According to Miyamoto (1957), on the other hand, the inhibition of germination is due to the high oxalic acid content in the sugar beet seed. Every

sample tested for oxalic acid contained more than the minimum concentration of water soluble oxalates required to inhibit germination. Snyder et al. (1965) found a high correlation co-efficient (-0.66) between the speed of germination and the soluble oxalates in the seed ball extracts. The correlation was not higher because there appeared to have been additional substances in sufficient concentration to cause inhibition of the germination process in some samples containing little soluble oxalate.

Inhibitory Action of the Beet-seed Extract

Tolman and Stout (1940, p. 829) found that water soluble substances present in the seed balls produced a toxic effect on germinating sugar beet seeds both by retarding germination and by killing the radicles. Stout and Tolman (1941a, pp. 701-704) reported that the toxicity of the water soluble extract from sugar beet seed balls was not specific to sugar beet seed only, but also affected the germination of several other seeds. There was either an inhibitory or a retarding effect on the germination in all the cases when the extract was applied on the seeds of onion, lettuce, radish, cucumber, cantaloup, tomato, sweet corn and beans. However, a differential response to the extract was observed. According to Evenari (1949, p. 158) the germination inhibitors are usually non-specific. The work of Froeschel as quoted by Evenari (1949, pp. 158-

159) indicated that the inhibitors in the fruits of Beta vulgaris and the seeds of Trifolium pratense inhibited the germination of seeds in 28 species belonging to 14 families. A one to four concentration of Beta fruit extract inhibited the germination of seeds of Amaranthus caudatus completely, depressed the germination of Lepidium seeds to four percent, and did not affect the germination of Ipomoea purpurea. The unsaturated yellow oil that was separated from the water extract of the sugar beet seed balls (De Kock and Hunter 1950) inhibited the germination of Lepidium sativum and other seeds with pronounced effect on the radicles. When the inhibited Lepidium seedlings were washed, the germination was stimulated. It was considered that glycolytic reactions continued in the inhibited seeds and when the inhibitor was washed out, abundant energy was available for subsequent growth.

Evenari (1949, p. 153) reported that the weed Agrostemma githago did not germinate in the field where Beta was growing nearby. Possibly Beta excretes a substance which inhibits the germination of Agrostemma seed. Klikoff (1964) reviewed the work of Froschel and Funke that Malandrium and rye do not develop when planted with sugar beets in the soil. The effect of toxin present in sugar beet seed was studied as an autotoxin and as a possible inhibitor of the germination of other seeds. No autotoxicity was observed and none of the tested species

except Poa bulbosa were significantly affected when germinated with sugar beet fruits in the soil.

Efforts to Improve Germination

Elimination of pericarp restriction. Lackey (1948) improved the germination of sugar beet seed by loosening the seed-caps. The alkaline solutions used, loosened the seed-caps but depressed the germination. On the other hand, the acidic solutions of three percent hydrochloric acid and three percent sulphuric acid increased the germination more than ten percent over checks treated with distilled water. It was found by Stewart (1950) that the germination of Beta trigyna could be improved by sulphuric acid scarification. Snyder (1959c) succeeded in hastening germination by notching the seed balls so as to expose a portion of the true seed. Peto (1964) applied alternate soaking and drying for loosening seed-caps and got higher germination.

Soaking and washing. It was reported by Skundra and Doxtator (1938) that the Association of the Official Seed Analysts of North America recommended the soaking of beet seeds for two hours at a temperature of 20°C before the germination test. Tolman and Stout (1940, pp. 824-827) compared soaking with washing and found that washing in running water was more effective in removing the toxic substances. Washing beyond six hours gave no apparent advantage. For soaking, the quantity of water used was

very important and washing the samples for five minutes in running water after soaking had a marked effect on subsequent germination. Tolman (1948a)mentioned in a report of the Seed Germination Committee of the American Society of Sugar Beet Technologists that washing the sugar beet seed is more convenient and is a better standard procedure than soaking. The Committee recommended that washing the seed in running water for a period of two hours be made as a standard procedure. An improvement on the washing method was introduced by Rehm (1953, p. 12) where the seeds should be washed in running water for two hours and then disinfected by dipping in 0.5 percent Aretan solution for twenty minutes. The seeds should then be dried without further washing. The disinfected seeds would germinate without the development of bacteria and the formation of free ammonia. According to Stout and Tolman (1941a, p. 711) the beneficial effect of washing or soaking the seeds prior to the germination test is the removal of the water soluble nitrogen fraction of the pericarp tissue. Stout and Tolman (1941b) tried to make the laboratory germination test of sugar beet seed to represent the actual germination in the field. By means of washing, the germination rate was increased and the total germination percentage was raised to a point comparable to the soil germination. It was suggested that further studies should be

made to determine what modifications of the practice or specifications as to the quantity of water in proportion to the sample that may be recommended. Miyamoto (1957) found improved germination by inactivating free oxalates in the sugar beet seed by treating it with a mixture of M/1000 mercuric acetate and 0.5 percent magnesium sulphate.

Application of growth promoters. Growth promoters have been used to increase germination in many dormant seeds. However, their effects on all the seeds have not been established as yet and contradictory results are very common. It was reported by Kahn (1960) that gibberellic acid promoted germination in the dark of Lactuca scariola and Lactuca sativa species which normally require light for germination. Gibberellic acid may be substituted for light and when it is so used counteracts the inhibition by high temperature, infra-red light, and high osmotic pressure. Anderson (1962, p. 739) got higher germination when dormant brown top millet(Panicum ramosum)seeds were germinated in a solution containing 692 mg of gibberellic acid per litre. Similarly Donoho and Walker (1957) obtained high germination of peach seed by the application of gibberellic acid. Peterson (1958) failed to obtain any effect on percent germination or vigor of seedlings from spray applications of gibberellic acid on sugar beet seeds. Similarly Snyder (1959a)could not hasten the germination

of sugar beet seed by soaking it in gibberellin solutions up to a concentration of 10,000 ppm. Hard seededness in legumes is a kind of anatomical dormancy which is usually broken by scarification. Fletcher and Martin (1962) could not replace scarification by breaking the anatomical dormancy of legumes by gibberellic acid treatment.

Salisbury (1957) reported that kinetin promoted the germination of lettuce seeds. Belderok (1961, p. 706) reviewed the work of Pollork and of Moorman and reported that kinetin removes the inhibiting influence exerted by the covering layers of the lettuce seeds. As concluded by Leff (1964), kinetin interacts with light in the promotion of the germination of Grand Rapids lettuce seeds. The interaction was at its optimum when the seeds received low intensity illumination.

Indoleacetic acid stimulates germination only under very specific conditions and gives quite contradictory results at different concentrations. When applied at 10^{-7} M concentration, as reported by Mayer and Poljakoff-Mayber (1963, pp. 97-98), the germination of lettuce seeds was raised from seven percent to 25 percent. Pearse (1948, p. 45) reported that a 0.01-0.001 N solution of indoleacetic acid increased the germination percentage of some old sugar beet seeds when soaked for 24 hours in the solution. The germination of barley seed, however, could not be improved when Lafferty (1940, pp. 26-27)

applied indoleacetic acid in the powder form at the rates of 2-160 ppm by weight of the seed. The plumules were, however, stimulated at high concentrations.

Lafferty (1940, pp. 26-27) also tried to improve the germination of barley seed by applying naphthaleneacetic acid in the powder form at the rates of 2-160 ppm by weight of the seed. The germination was not increased but on the contrary the root growth was rather retarded. According to Barton (1961, p. 578) soaking sugar beet and many other kinds of seeds in solutions of potassium 1-naphthaleneacetate at concentrations of 320.0, 106.6, 35.5, 11.8, 3.7, and 1.2 mg per litre failed to increase the speed or the percentage of germination. Seedling injury was obtained at the high concentrations used.

Thiourea stimulates the germination of many seeds at higher concentrations but inhibits the subsequent growth unless it is removed. Using a 0.25 M thiourea solution as a germination medium, Belderok (1961, pp. 738-739) observed a considerable increase in the seedling vigor of newly harvested wheat seed. Anderson (1962, p. 737) observed that for brown top millet seed, moistening the substrata with one gram per litre thiourea soluion, served both as a fungicide and as a stimulant in breaking dormancy. Mayer and Poljakoff-Mayber (1963, pp. 92-93) reported that thiourea greatly improves the germination of lettuce seeds when they are presoaked in the solution.

The effective concentration, in this connection, was reported to be 0.5 percent by Thompson and Kosar (1939) and 0.5-3 percent by Mayer and Poljakoff-Mayber (1963, pp. 92-93).

Nakamura (1962, pp. 710-724) experimented on the germination of many dormant grass seeds. It was found that a 0.2 percent solution of potassium nitrate broke dormancy in seeds of timothy, Kentucky bluegrass, perennial ryegrass, African millet and Japanese lawngrass. According to Schwendiman and Shands (1943), a 0.2 percent solution of potassium nitrate was equally as effective as prechilling in overcoming delayed germination of freshly harvested seeds of Vickland oats (Avena sativa). Mayer and Poljakoff-Mayber (1963, pp. 90-92) reported that a potassium nitrate solution stimulated the germination of Lepidium verginium, Eragrostis curvula, Polypogon monspelliensis, Agrostis spp. and Sorghum halepense when applied at a concentration of 0.017 percent. The promotion of germination by potassium nitrate is usually light and temperature dependent. Toole and Toole (1941, p. 86) reported that the germination of Digitaria ischaemum was promoted by nitrate solution at low temperature alternations which were otherwise unfavourable for germination. The germination of Eragrostis curvula, as reported by Mayer and Poljakoff-Mayber (1963, p. 92), was stimulated by a 0.2 percent potassium nitrate

solution at constant temperatures between 15 and 30°C in the dark but with no effect at alternate or higher temperatures. On the other hand, Toole (1938, p. 242) reported stimulation of Polypogon germination by potassium nitrate solutions only at alternating temperatures.

Hydrogen peroxide also increases germination of some seeds. Belderok (1961, pp. 706-707) reviewed the work of Pollork and of Moorman who found that hydrogen peroxide promoted the germination of dormant barley to a considerable extent. The germination was stimulated both at optimum water supply and at an excess of water but there was no difference whether the seeds were presoaked or germinated directly in the hydrogen peroxide solution.

The Location of the Inhibitors

Tolman and Stout (1940, p. 820) suggested that the main factors, causing large differences between varieties in the germination rate, are to be found in the pericarp of the seed ball rather than in the true seed. The varietal differences were largely dissipated when naked seeds instead of seed balls were germinated. As determined by Rehm (1953, p. 2), the pericarp tissue contained more nitrate than did the true seed. It was concluded that the pericarp tissue was mainly responsible for the inhibition. Snyder et al. (1965) determined the oxalate contents of whole seed balls, processed seed balls

and the pericarp material removed in processing. The oxalate was found concentrated in the outer pericarp tissue of the seed balls. The extract of processed seed balls did not inhibit the germination of wheat seed. Snyder (1959c) reported that the pericarp tissue showed some stimulatory effect on the germination of naked seeds.

Summary

Dormancy in sugar beet seed is attributed to the tight seed-caps over the true seeds, high osmotic pressure and the presence of germination inhibitors in the pericarp tissue. It is generally believed that the germination inhibitors which are water soluble, play the major role in this dormancy. Soaking or washing the sugar beet seeds in water improved the germination. The water extract of the sugar beet was found to be inhibitory to the germination of other seeds. The nature of the inhibitory material as well as its mode of action on germination and growth are not known.

III. MATERIALS AND METHODS

The research work was conducted in the seed technology laboratory of the American University of Beirut, Lebanon during 1965-66. Thirteen sugar beet varieties were obtained from the Agricultural Research and Education Centre, Beqaa, Lebanon. A preliminary germination test was carried out on these 13 varieties and from these the two varieties Mezzano AU/N and Maribo Auta-poly 7015 were selected for the present study. These two varieties were the slowest in germination and thereby it was expected that these varieties would contain a maximum amount of inhibitory material. Even though the seeds of the variety Mezzano AU/N were fairly uniform in size, the seed lot was divided into three fractions: Small, medium and large. The medium fraction in which the seeds varied from 4.5-6.0 mm in size was used in this study. The seeds of the variety Maribo Auta-poly 7015 were mixed thoroughly and sampling was done by the Boerner divider. The variety Mezzano AU/N was used in the study of methods of increasing germination in the sugar beet seed. The variety Maribo Auta-poly 7015 was used to study the effect of the inhibitors on the germination of other crop seeds and to find the location of the

inhibitors.

Methods of Increasing Germination

Washing the seed in running water. The washing of the sugar beet seeds was done by tying a sample of seed in a piece of linen cloth and then putting it in a glass trough under the running tap water. The temperature of the running tap water was not controlled and it varied from 16-19°C during the study. Six washing times were compared namely 0, 2, 6, 12, 24, and 36 hours. The washed seeds were dried between two blotters for three minutes before they were put to germinate. Duplicates of 100 seeds each were put between folded blotters 20 x 30 cm in size. The seeds were then placed in the Stults germinator at an alternating temperature of 20-30°C. After 84 hours the number of seedlings of the one centimeter and more in size was recorded to indicate the speed of germination. The experiment was repeated four times and the results of the four replications were analysed statistically.

Soaking the seed in distilled water. The volume of the water used for soaking the seed has been found to be very important. In a preliminary experiment it was determined that to get the best possible results, the amount of the water for soaking should be 25 times the weight of the seed. In the present experiments, the soaking was done

in distilled water at two temperatures, 30°C and 40°C. Six soaking times of 0, 2, 6, 12, 24, and 36 hours were compared. When the soaking was over, the seeds were taken out and washed in running tap water for five minutes. The seeds were then dried and germinated following the same procedure used on the washed seeds.

Soaking under oscillating vacuum. Samples of the seed balls were soaked at 40°C for 1/2, 3, 5, 8 and 11 hours. The beakers containing the soaked seed balls were all transferred at the same time to an oven at 40°C where six cycles of oscillating vacuum were applied. Each cycle was composed of eight minutes of vacuum (-15 pounds per square inch) and two minutes of release of vacuum. The seeds were then washed, dried and germinated as in the previous soaking experiment.

The effect of growth promoters. The following solutions of the various growth promoters were prepared to study their effect on seed germination: Gibberellic acid 400, 600 and 1000 ppm; indoleacetic acid 5, 10, and 15 ppm; kinetin 0.5×10^{-5} M and 1×10^{-5} M; potassium nitrate 0.2, 0.4, and 0.6 percent; thiourea 0.2, 0.4, and 0.5 percent; hydrogen peroxide 1, 1.5, and 2 percent; and naphthalene-acetic acid 0.01 and 0.02 percent. Two methods were used in this experiment. One method was to soak the seeds for 12 or 24 hours at room temperature. About ten ml of the growth promoter solution was used to soak 50 seed balls

in small petri dishes. After the soaking time, the seeds were taken out and washed for approximately one minute under running tap water to remove the excess of the growth promoter. The seeds were then dried between blotters and germinated as usual in duplicates of 25 seeds between blotters of the size 13 x 30 cm. The second method was to germinate 25 seed balls in duplicates in petri dishes using the prepared solutions of the growth regulators as the moistening solution. In addition to the concentrations that were used above the following were also used in the second method: Gibberellic acid 50 and 100 ppm, indole-acetic acid 1 and 2 ppm, kinetin 0.1 x 10^{-5} M, potassium nitrate 0.1 percent, thiourea 0.1 percent, hydrogen peroxide 0.5 percent, and naphthaleneacetic acid 0.005 percent. Four replications were used in these experiments and results were recorded at the end of 84 hours and also at the end of 132 hours in the second method.

Effect of the Water Extract on the Germination of Different Seeds

The water extract of the sugar beet seed balls was prepared by soaking the whole seed balls for 24 hours in distilled water at 10°C. The extract was prepared by soaking ten grams of seedballs in 100 ml of water. The extract was then filtered through Whatman filter paper No. 4 and was referred to as the whole seed

ball extract of 100 percent concentration. The effect of the water extract on the initial germination was tested on the following field crop seeds: Sunflower (Helianthus annuus), sugar beet (Beta vulgaris), rice (Oryza sativa), cabbage (Brassica oleracea), barley (Hordeum vulgare), cotton (Gossypium americanum), perennial ryegrass (Lolium perenne), vetch (Vicia sativa), wheat (Triticum vulgare), sorghum (Sorghum vulgare), alfalfa (Medicago sativa), and oats (Avena sativa). These seeds were obtained from the Agricultural Research and Education Centre, Beqaa, Lebanon. Duplicates of 20 seeds from each lot were placed on two filter papers in petri dishes which were moistened with three to four ml of the whole seed ball extract. Germination in distilled water was used to serve as the check. An alternating temperature of 20-30°C was used during the germination period of all the seeds. At an arbitrary time when the effect of the extract was obvious, the average radicle length of the seedlings in each petri dish was measured by taking into consideration all the normal seedlings. Four replications were used with each crop to get the average effect of the extract on germination.

In addition to the whole seed ball extract, 50 percent and 75 percent dilutions were prepared by adding distilled water. The effect of the different concentrations was observed on germination of alfalfa, sorghum and oat seeds. In this

experiment, all the healthy plumules and radicles were separated from the germinating seeds, dried gently between two filter papers to remove any surface water, and the fresh weight was determined.

To find the effect of the extract on the subsequent growth of the seedlings about 100 seeds of each crop were germinated between blotters and twenty normal seedlings with radicles about one centimeter in length were used for the test. The seedlings in duplicate were placed on filter papers in petri dishes, moistened with 2.5 ml of the whole seed ball extract, and allowed to grow. Similarly seedlings were allowed to grow in distilled water to serve as the check. After an arbitrary period of further growth, the average plumule and radicle lengths were determined as usual. In addition, the effects of the whole seed ball extract on the fresh weight of the plumules and the radicles of oat, sorghum, and alfalfa seedlings were tested.

The Location of the Inhibitors

In order to determine the location of the inhibitors the seed balls were broken, and the true seeds separated from the pericarp tissue. The water extract of each part was prepared and its effect was then tested on the germinating seedlings of alfalfa and sorghum and on the

germination of true sugar beet seeds. The whole seed balls were ground by the Corona corn mill with grinding disks adjusted to about three mm clearance to ensure coarse grinding. The South Dakota seed blower was used to help in the separation of the true seeds from the pericarp tissue. Final separation of the true seeds and the pericarp tissue was done by hand. Only the healthy unbroken true seeds were selected for germination. The water extracts of the true seeds and the pericarp tissue were prepared in the same way as that of whole seed balls. The effect of the two extracts was tested on the growth of alfalfa and sorghum seedlings with radicles about one cm long as well as on the germination of true sugar beet seeds. Ten seeds or seedlings were put in each petri dish over a filter paper moistened with two ml of the extract. The fresh weights of the plumules and the radicles were obtained after three days. In addition, true sugar beet seeds were washed for six hours and their germination was compared with that of the unwashed seeds.

In these experiments the replications were spread over time and analysis was done according to randomized complete block design (Le Clerg et al., 1962).

IV. RESULTS AND DISCUSSION

This study was conducted at the seed technology laboratory of the American University of Beirut to find out methods and means for improving the germination of sugar beet seed in the laboratory as well as to study the effect of the inhibitory material produced by the seed balls on other crop seeds. In addition an experiment was done pertaining to the location of the inhibitory material in the seed balls.

Methods of Increasing Germination

Washing. The washing of sugar beet seed in running tap water proved quite effective in improving germination. The effect of washing the seeds in running water on germination percentage is shown in Table 1. Zero washing gave very low germination of 2.8 percent. Washing for two hours only raised the germination percentage to 15.8. Further increase in the time of washing was accompanied with an increase in the germination percentage till the maximum germination of 62 percent was obtained by washing the sugar beet seed for 24 hours. This increase in germination with the increase in the time of washing is probably due to the softening of the hard pericarp tissue surrounding the true seeds and the leaching out of inhibitory material

Table 1. Effect on germination of washing sugar beet seed in running water.

Time of washing (hours)	% germination after 84 hours				
	R I	R II	RIII	R IV	Mean
0	1	6	2	2	2.8
2	14	18	16	15	15.8
6	27	33	30	35	31.3
12	42	36	36	45	39.8
24	63	60	59	66	62.0
36	15	17	15	16	15.8

LSD 5% level = 4.1 seedlings
1% level - 5.7 seedlings

Analysis of variance

Source	d.f.	MSS
Treatments	5	1793.77**
Replications	3	14.37
Error	15	7.38
Total	23	

** Significant at 1 percent level.

present in the seed balls. This finding is in line with the work of Peto (1964) that softening of the seed-caps above the true seeds with alternate soaking and drying improved germination. Stout and Tolman (1941a, p. 711) believe that the beneficial effect of washing the seeds prior to germination is due to the removal of the water soluble nitrogen fraction of the pericarp tissue. Stout and Tolman (1941b) tried to make the laboratory germination test of sugar beet seed represent the actual germination in the field. By means of washing, the germination rate was increased and the total germination percentage was raised to a point comparable to the soil germination under favourable conditions of moisture and temperature. In this study washing for 24 hours was needed to get maximum germination. Contrary to this, Stout and Tolman (1941b) reported that six hours of washing was enough to get maximum germination. Tolman (1948a) gave the recommendation to the Seed Germination Committee of the American Society of Sugar Beet Technologists that washing the seeds in running water for two hours be made the standard procedure. The time of washing of the sugar beet seed to get maximum germination, varies from variety to variety depending on the amount of inhibitory material present, hardness of the pericarp tissue and the temperature of the water used for washing. The variety used in this study was selected out of thirteen varieties on the

basis of maximum inhibitory contents. The temperature of the running water was not controlled and varied from 16-19°C during the experimental period. Washing beyond 24 hours decreased the germination percentage (Table 1). This decrease in germination is probably due to the loss of some essential material for the germination processes due to extended washing since it is known that during the washing process seeds imbibe water and as a result hydrolysis of the food reserves and growth begin.

Soaking. Water imbibition by seeds and dissolution of inhibitory substances occur faster at relatively high temperatures. Therefore, soaking the sugar beet seed in water at 30°C and 40°C was expected to speed up germination by hastening the removal of the inhibitory substances. As shown in Table 2, the maximum germination of 59.3 percent was obtained by soaking the seed at 30°C for only 12 hours. This germination is comparable to that obtained by 24 hours washing as shown in Table 4. The change of the optimum time from 24 hours of washing to 12 hours of soaking at 30°C indicates that the temperature of the water used for soaking is an important factor in leaching out the inhibitors and in accelerating the germination activities in the seed. As shown in Table 3, 12 hours of soaking was needed at 40°C to get a maximum germination of 55.5 percent which is comparable to the 59.3 percent

Table 2. Effect on germination of soaking sugar beet seed in water at 30°C.

Time of soaking (hours)	% germination after 84 hours				
	R I	R II	R III	R IV	Mean
0	1	0	0	1	0.5
2	10	9	13	10	10.5
6	41	32	38	40	38.0
12	60	59	63	57	59.3
24	31	28	24	27	27.8
36	12	5	5	6	7.0

LSD between means: 5% level = 3.8 seedlings
1% level = 5.3 seedlings

Analysis of variance

Source	d.f.	MSS
Treatments	5	2002.17**
Replications	3	13.78
Error	15	6.34
Total	23	

** Significant at 1% level.

Table 3. Effect on germination of soaking sugar beet seed in water at 40°C.

Time of soaking (hours)	% germination after 84 hours				
	R I	R II	R III	R IV	Mean
0	1.0	0.0	0.0	0.0	0.3
2	16.5	15.5	18.5	17.0	17.3
6	45.0	44.5	34.0	49.0	43.0
12	60.0	52.0	54.0	56.0	55.5
24	7.0	2.5	0.0	6.0	3.9
36	0.0	0.0	0.0	0.0	0.0

LSD between means 5% level = 14.23 seedlings
1% level = 19.70 seedlings

Analysis of variance

Source	d.f.	MSS
Treatments	5	2303.87**
Replications	3	20.35
Error	15	89.24
Total	23	

** Significant at 1% level.

Table 4. A comparison of the effect of the optimum length of time of washing and soaking on sugar beet seed germination.

Treatment	Time of washing or soaking (hours)	% germination after 84 hours				
		R I	R II	R III	R IV	Mean
Washing	24	63	60	59	66	62.0
Soaking						
at 30°C	12	60	59	63	57	59.3
at 40°C	12	60	52	54	56	55.5

Analysis of variance

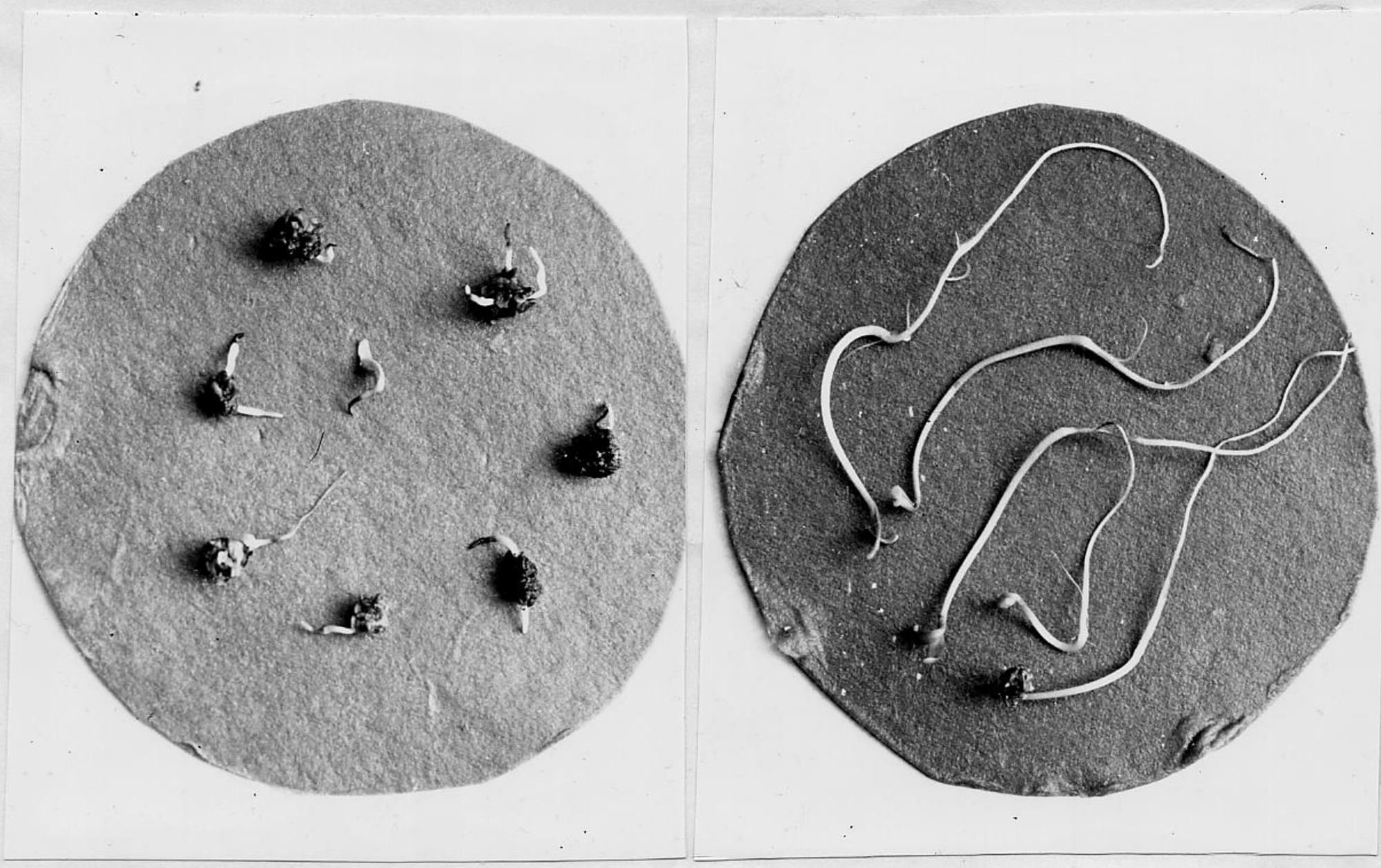
Source	d.f.	MSS	F-ratio
Treatments	2	43.58	Non-significant
Replications	3	8.52	Non-significant
Error	6	9.69	
Total	11		

obtained at 30°C soaking. Only six hours of soaking at 40°C gave a high germination of 43 percent which is not statistically different from that obtained at 12 hours of soaking. One important effect noticeable in Tables 2 and 3 is that when the seeds were soaked for more than 12 hours at 30°C or 40°C, the germination was seriously reduced. Soaking of sugar beet seed was preferable to washing because less time was needed in soaking to get equally high germination. The soaking method has another advantage over the washing in that it is easier to standardize. The temperature as well as the volume of the water are easily controlled in the former but practicably are uncontrollable in the latter. Pearse (1948, p. 44) reported that soaking the sugar beet seed in water only increased the yield of sugar beet roots in the field over that obtained with dry seed. The increase in yield was probably due to the better germination and stand. Tolman and Stout (1940, pp. 824-827), on the other hand, found washing more effective in removing toxic substances as compared to soaking by using only eight times volume of water to weight of seed for soaking. In this study, however, it was found necessary in a preliminary test to use 25 times volume of water to weight of sugar beet seed under controlled temperature for getting relatively good results. Tolman (1948a)also mentioned that washing was more convenient and a better standard procedure than soaking. The results of this study support

the soaking method in as far as the time economy and the ease of standardization are concerned.

In addition to the improvement in germination percentage, the general health of the seedlings was greatly improved. The germination of washed and unwashed sugar beet seeds are compared in Figure 1. Figure 1(a) shows the toxicity effects on the radicles after emerging from the seed. These radicles are short, dark in color and abnormal compared to the washed (Figure 1b) which look healthy and show no signs of discoloration.

Vacuum oscilatting between zero and -15 pounds per square inch was also applied for one hour on seeds soaked for different periods of time at 40°C. The treatment of the oscillating vacuum (vacuum followed by release of vacuum) was expected to help in the leaching out of the inhibitors into the water and consequently resulted in improved germination in a short period of time. Contrary to this expectation, the treatment considerably inhibited the germination as compared to that obtained by soaking alone. The effect of one hour of oscillating vacuum after different times of presoaking the seed at 40°C is shown in Table 5. In all the cases the germination was very low. Soaking for 12 hours at 40°C including one hour of oscillating vacuum gave a germination of 15.5 percent compared to 55.5 percent with no vacuum. This reduction in germination might be due to the influx of inhibitory material from the surrounding pericarp tissue



(a) unwashed sugar beet seeds

(b) washed sugar beet seeds

Figure 1. Germination of washed and unwashed sugar beet seeds.

Table 5. Effect of oscillating vacuum for one hour on the germination of presoaked sugar beet seed.

Time of presoaking (hours)	% germination after 84 hours					
	R I	R II	R III	R IV	R V	Mean
0.5	2.0	5.5	0.5	1.0	2.0	2.2
3.0	10.5	14.0	8.5	6.0	13.0	10.4
5.0	7.5	9.5	8.0	12.0	11.0	9.6
8.0	10.5	14.5	14.5	10.5	27.0	15.4
11.0	12.0	21.0	13.5	13.5	17.5	15.5

LSD between means 5% level = 4.4 seedlings
1% level = 6.0 seedlings.

Analysis of variance

Source	d.f.	MSS
Treatments	4	148.31**
Replications	4	35.64*
Error	16	10.56
Total	24	

* Significant at 5% level
** Significant at 1% level

to the true seeds inside the seed balls.

The results of the washing and soaking at 30°C and 40°C as well as the application of oscillating vacuum on the seeds presoaked at 40°C are compared in Figure 2. The germination curves of the different treatments summarize the optimum times in hours of washing and soaking for maximum germination. The harmful effects of the prolonged treatments are also shown clearly in the diagram.

Growth promoters have been used in many instances to break dormancy and promote germination in seeds. Their application in this study was considered to break the dormancy, increase the germination and vigor of sugar beet seeds. The results of the germination of sugar beet seeds that were soaked in different concentrations of the growth promoters are shown in Table 6. In general, the growth promoters used at different concentrations had no beneficial effect on germination as compared to that of distilled water. Soaking the seeds for six or 12 hours in the different growth promoters had no different stimulating effect on germination than that of distilled water. It can be seen from the data in Table 6, that gibberellic acid, indoleacetic acid, kinetin and potassium nitrate at all concentrations gave relatively similar results to that of water at six or 12 hours of soaking before germination. This indicates that growth promoter solutions had no stimulatory effect on germination more than the effect of

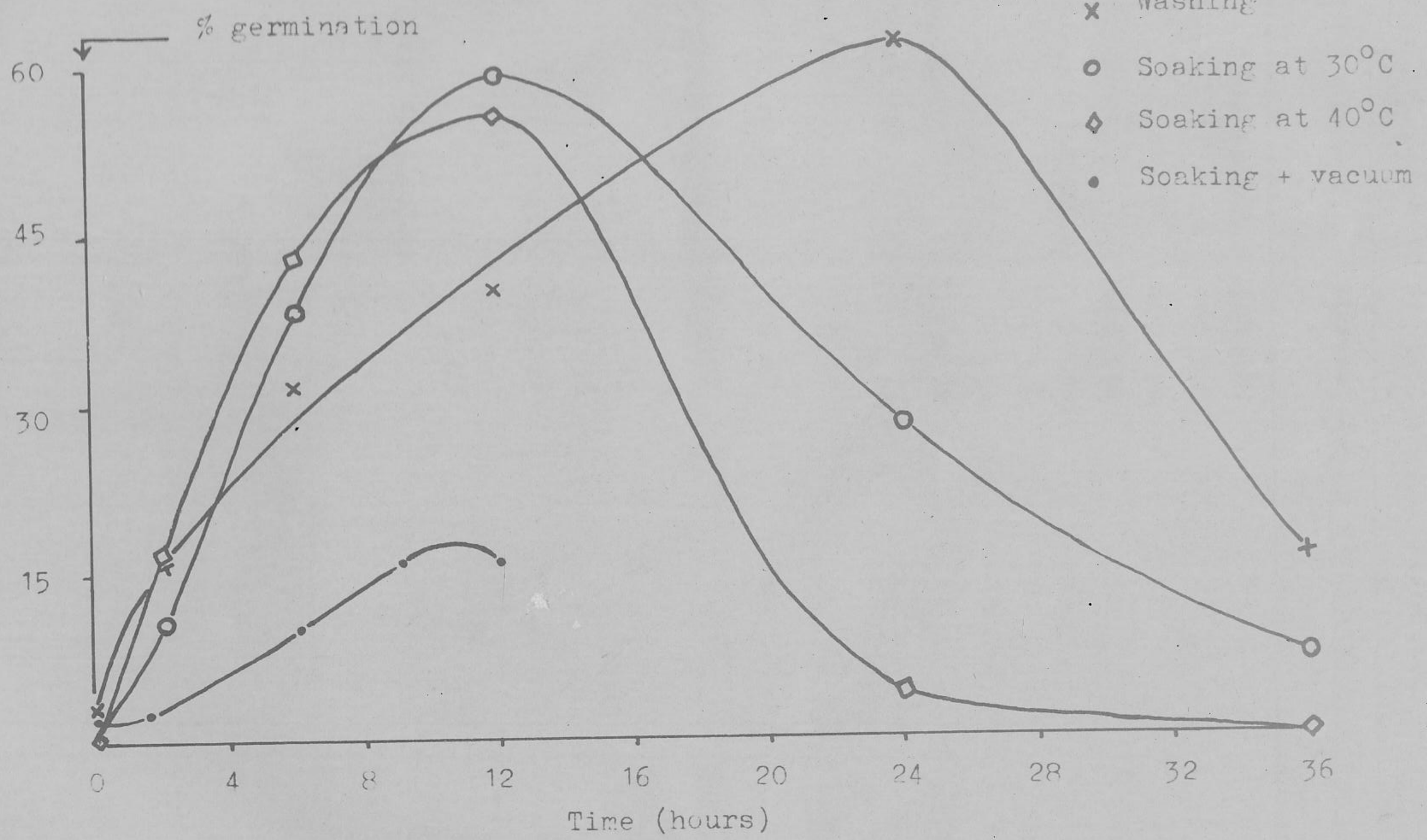


Figure 2. Effect of washing, soaking and oscillating vacuum on the germination of sugar beet seed.

Table 6. Effect of presoaking sugar beet seed in growth promoter solutions on germination.

Growth promoter	Concentration	% germination after 84 hours	
		6 hour presoaking	12 hour presoaking
Distilled water	(check)	31	48
Gibberellic acid	400 ppm	21	41
	600 ppm	22	36
	1000 ppm	30	42
Indoleacetic acid	5 ppm	21	31
	10 ppm	24	38
	15 ppm	33	32
Kinetin	0.5×10^{-5} M	27	34
	1.0×10^{-5} M	39	43
KNO_3	0.2%	31	41
	0.4%	26	35
	0.6%	20	40
Thiourea	0.2%	15	23
	0.4%	22	30
	0.5%	18	12
H_2O_2	1.0%	12	18
	1.5%	15	16
	2.0%	11	17
Naphthaleneacetic acid	0.01%	8	15
	0.02%	11	8

the solution as a medium for dissolving the inhibitory material. Thiourea, hydrogen peroxide and naphthalene-acetic acid had rather inhibited germination at all concentrations. The results of this study are in agreement with Pearse (1948, p. 44) who reviewed the effect of growth regulators on sugar beet seed germination and gave a detailed report of failures to get any response. Peterson (1958) failed to get any effect on percent germination or vigor of sugar beet seed by spray applications of gibberellic acid. Similarly Snyder (1959a) could not hasten germination of sugar beet seed by soaking in gibberellin solutions up to a concentration of 10,000 ppm. According to Barton (1961, p. 578) soaking sugar beet seed in solutions of potassium 1-naphthelene acetate at concentrations of 320.0, 106.6, 35.5, 11.8, 3.7 and 1.2 mg per litre failed to increase the speed or the percentage of germination.

The action of growth promoters on sugar beet seed is further shown in an experiment where seeds were germinated in petri dishes using growth promoter solutions as moistening agents. A wide range of concentrations of growth promoters was used in this study. The results of the germination of the seed in the growth promoter media are shown in Table 7. The dry seeds when germinated in petri dishes using water as a moistening agent showed ten percent germination after 84 hours but this was reduced

Table 7. The germination of sugar beet seed in growth promoter media.

Growth promoter	Concentration	% seedlings after 84 hours			% seedlings after 132 hours		
		Normal	Black tipped	Dead	Normal	Black tipped	Dead
	Unwashed	10	0	0	4	8	4
	Washed (24 hr)	56	0	0	84	0	0
Gibberellic acid	50 ppm	0	0	0	10	12	4
	100 ppm	4	6	0	14	16	4
	400 ppm	2	0	0	6	14	2
	600 ppm	2	0	0	6	16	4
	1000 ppm	2	0	0	8	18	10
Indoleacetic acid	1 ppm	6	0	0	0	12	16
	2 ppm	4	2	0	0	10	18
	5 ppm	2	0	0	2	4	14
	10 ppm	10	0	0	0	8	24
	15 ppm	2	0	0	0	8	18
Kinetin	0.1×10^{-5} M	10	0	0	6	6	16
	0.5×10^{-5} M	4	0	0	8	4	8
	1.0×10^{-5} M	14	2	0	4	4	22
KNO_3	0.1%	0	0	0	0	16	10
	0.2%	0	0	0	10	6	10
	0.4%	0	0	0	4	6	2
	0.6%	0	0	0	0	2	0
Thiourea	0.1%	0	2	0	0	20	20
	0.2%	8	0	0	8	8	12
	0.4%	4	0	0	6	4	12
	0.5%	0	0	0	8	2	6
H_2O_2	0.5%	2	0	0	0	4	12
	1.0%	0	0	0	0	0	2
	1.5%	0	0	0	0	0	8
	2.0%	0	0	0	2	0	10
Naphthalene-acetic acid	0.005%	0	0	0	2	0	16
	0.01%	2	0	0	0	4	8
	0.02%	0	0	0	0	0	6

to four percent after 132 hours due to the toxicity effects on the radicles. When, however, the seeds were washed for 24 hours and then germinated in petri dishes, there was a high germination of 56 percent after 84 hours and 84 percent after 132 hours without any toxicity symptoms. Here again the growth promoters, when used as moistening media, did not stimulate germination. It can also be seen from the data in Table 7 that there was no reduction in the toxicity symptoms on the radicles.

It can be concluded that the growth promoters at the concentrations used in these experiments did not promote germination in any case, inactivate the inhibitors in situ as was expected, and therefore, did not check the toxic effects.

Effect of the Water Extract on Other Seeds

The inhibitory material present in the sugar beet seeds was extracted by soaking the seeds in water for 24 hours at 10°C. The extract was applied on various crop seeds to see its effect on germination, radicle emergence and plumule development. It can be seen clearly in Table 8 that the water extract depressed the germination percentage and the average radicle length of all the crop seeds tested. The effect of the extract was, however, differential in that it was more prominent on barley, alfalfa, sunflowers, perennial ryegrass, cabbage, rice,

Table 8. Effect of the water extract of sugar beet seed on % germination and radicle development of different crop seeds.

Crop seed	Germination time (hours)	Germination (% of check)	Average radicle length (% of check)
Barley	84	1.7	18.2
Alfalfa	72	3.3	12.0
Sunflower	96	4.2	28.6
Perennial ryegrass	144	4.2	11.4
Cabbage	84	10.3	12.9
Rice	204	19.8	14.3
Sugar beet (Inhibitor free)	96	35.4	36.1
Sorghum	72	53.2	17.1
Oats	132	66.7	6.7
Vetch	84	78.4	52.2
Cotton	96	79.5	42.5
Wheat	72	90.9	25.3

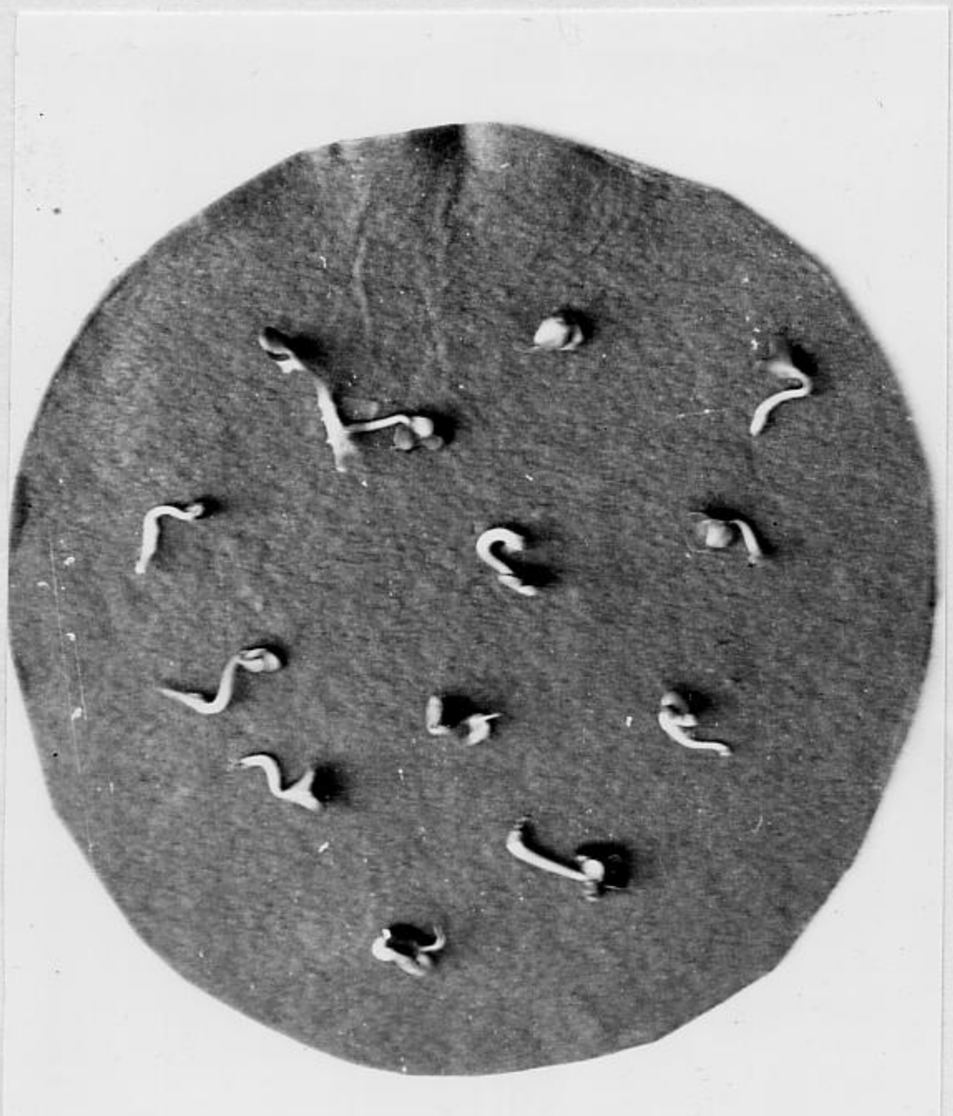
and sugar beets than on sorghum, oats, vetch, cotton and wheat. Its effect on the average radicle length was very high in that the growth was reduced to 50 percent, or less, of the check. The crops tested are listed as follows according to the decreasing effect of the inhibitory material: Oats, perennial ryegrass, alfalfa, cabbage, rice, sorghum, barley, wheat, sunflower, sugar beets, cotton and vetch. The results are in agreement with Stout and Tolman (1941a, pp. 701-704) who reported that the toxicity of the water extract from sugar beet seed balls was not specific to sugar beet seed only but also affected the germination of several other seeds. The effect was either inhibitory to germination or retarding to growth when the extract was applied on the seeds of onion, lettuce, radish, cucumber, cantaloup, tomato, sweet corn and beans. Similarly Evenari (1949, p. 158) reviewed the work of Froeschel who found that the inhibitors in the fruits of Beta vulgaris inhibited the germination of seed in 28 species belonging to 14 families. While reviewing the work of Froeschel, Evenari (1949, p. 158) also reported that one to four concentration of Beta fruit extract inhibited the germination of seeds of Amaranthus caudatus completely, depressed the germination of Lepidium seeds to four percent and did not affect the germination of Ipomea purpurea. In the present study only one to ten concentration of the extract was used, and germination

of all the seeds was depressed. The plumule development on the other hand, was very little affected. The germination of cabbage seed in the sugar beet seed extract as compared to the germination in distilled water is shown in Figure 3. The radicles of the cabbage seedlings show complete inhibition due to the effect of the sugar beet seed extract. The plumules, however, were inhibited to a lesser extent. This observation is similar to that found by De Kock and Hunter (1950) on the germination of Lepidium sativum seed.

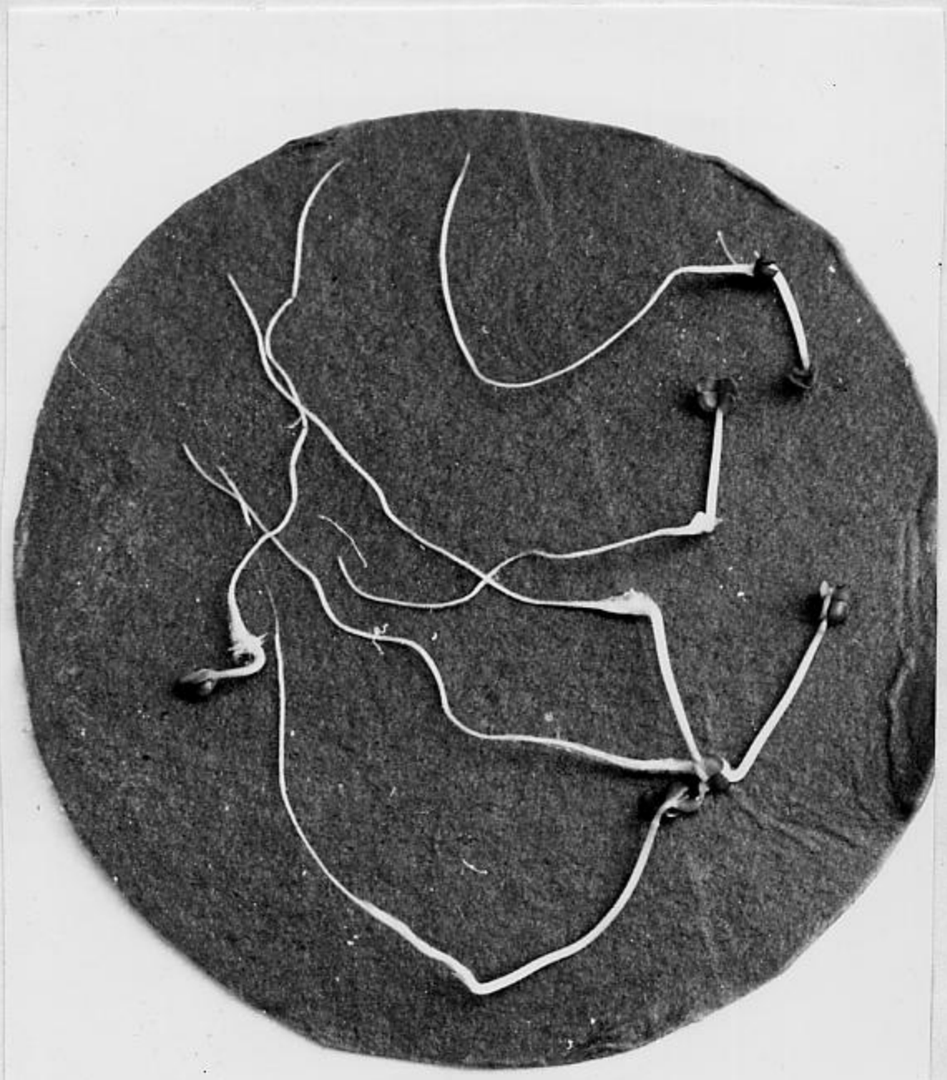
When the extract was applied on the seedlings, it completely stopped the growth of radicles of alfalfa, cabbage, perennial ryegrass, sunflower, sugar beets and vetch indicating a subsequent effect on growth (Table 9). There was a decreasing effect on the radicles of sorghum, oats, wheat, cotton, barley and rice in that order. The effect of the water extract on plumule growth was relatively less compared to that on radicles. The length of the plumules in wheat was about 50 percent and in barley about 70 percent of the check. The rest of the plumules were little affected.

The Dilution Effect of the Extract on Germination

It was shown in the previous experiments that the sugar beet seed extract inhibited the germination of various crop seeds. The inhibition was differential and more



(a) germination in the sugar beet seed extract



(b) germination in distilled water

Figure 3. Germination of cabbage seeds in the sugar beet seed extract and in distilled water.

Table 9. Effect of the water extract of sugar beet seed on growth of various seedlings.

Seedling	Time allowed for growth (hours)	Average radicle length (% of check)	Average plumule length (% of check)
Alfalfa	48	0.0	90.0
Cabbage	48	0.0	90.0
Perennial ryegrass	48	0.0	100.0
Sunflower	72	0.0	100.0
Sugar beet	72	0.0	100.0
Vetch	72	0.0	100.0
Sorghum	72	6.7	100.0
Oats	75	14.3	87.5
Wheat	48	16.7	54.5
Cotton	72	16.7	86.6
Barley	48	50.0	70.6
Rice	120	66.7	100.0

marked on radicles than on plumules. Different dilutions of the extract were prepared and applied on seeds and seedlings of alfalfa, sorghum and oats. Fresh weights of the radicles and plumules were taken as a measure to evaluate the growth regulatory effect of the different dilutions of the extract.

The effect of these dilutions of the sugar beet seed extract on the germination percentage of alfalfa, sorghum and oats is shown in Table 10. The extract did not inhibit the germination of any seed at the high dilution of 75 percent. The full strength of the extract (zero dilution), on the other hand, decreased the germination percentage of all the seeds significantly. The effect was, however, differential at 50 percent concentration, because it decreased the germination of alfalfa and oats and not of sorghum. The decrease in germination of seeds with the increase in concentration of the extract shows that some inhibitory materials present in the extract became effective at high concentration. The reduction in germination percentage was mainly due to the inhibition of radicles that resulted in abnormal seedlings. It can be seen in Table 11 that the radicle weights of alfalfa and sorghum were not reduced by the 75 percent dilution but were highly significantly reduced by the 50 percent dilution. Reduction in the development of oat radicles was obtained only when the oat seeds were germinated in the

Table 10. Effect of various dilutions of the sugar beet seed extract on the % germination of alfalfa, sorghum and oat seeds.

% dilution of the extract	% germination		
	Alfalfa after 144 hours	Sorghum after 120 hours	Oats after 120 hours
Check (water)	90.0	95.0	63.9
75	89.4	97.5	55.0
50	75.6	90.0	46.0
0	8.1	41.3	39.6
LSD 5%	12.02	13.94	11.07
LSD 1%	17.29	20.05	15.93

Table 11. Effect of various dilutions of the sugar beet seed extract on the radicle development of alfalfa, sorghum and oat seeds.

% dilution of the extract	Fresh weight of radicles (mg)		
	Alfalfa after 144 hours	Sorghum after 120 hours	Oats after 120 hours
Check (water)	67.1	122.8	62.5
75	63.0	137.8	81.6
50	26.1	87.4	61.5
0	9.6	30.8	39.6
LSD 5%	22.65	34.67	11.01
LSD 1%	32.57	49.86	15.83

concentrated extract (zero dilution). These results indicate that a minimum concentration of the extract is needed to cause radicle inhibition and that this minimum concentration may not be the same for all the seeds. Another important effect that can be seen in Table 11 is the stimulation of oat radicles at 75 percent dilution. Therefore, the extract not only inhibits radicle development but may also stimulate it at lower concentrations.

This kind of inhibition at high concentrations of the sugar beet seed extract and stimulation at low concentrations is characteristic of many growth regulators. No conclusion can be made at this point concerning the nature of the inhibitors because the crude extract that was used in this study was a mixture of many growth influencing factors.

The effect of the same dilutions of the extract on plumule development is shown in Table 12. The concentrated form (zero dilution) of the extract caused a significant reduction in the plumule development of the sorghum at one percent level, of the alfalfa at five percent level and no significant effect on the oat radicles. No inhibition of the plumules was produced by any dilution of the original concentration. Instead, at 75 percent dilution, the plumules of oats were significantly stimulated at one percent and of sorghum at five percent level. There was stimulation of the plumules of alfalfa at this concentration

Table 12. Effect of various dilutions of the sugar beet seed extract on the plumule development of alfalfa, sorghum and oat seeds.

% dilution of the extract	Fresh weight of plumules (mg)		
	Alfalfa after 144 hours	Sorghum after 120 hours	Oats after 120 hours
Check (water)	144.5	73.6	60.1
75	173.5	100.9	96.1
50	163.6	57.7	71.1
0	93.4	48.0	58.0
LSD 5%	43.84	17.65	15.98
LSD 1%	63.05	25.38	22.98

but it was not statistically significant.

This stimulation effect of the extract might be due to the presence of growth promoting factors that are stimulatory only when present at low concentrations. In a similar study that was done by De Kock and Hunter (1950), it was considered that glycolytic reactions continued in the inhibited seeds and when the inhibitor was washed out, abundant energy was available for subsequent growth. In this experiment, however, the stimulation was due to a specific low concentration of the extract rather than subsequent stimulation after primary inhibition.

The above mentioned dilutions of the extract were also applied on the seedlings of sorghum, alfalfa and oats. The effect on the subsequent growth of radicles is shown in Table 13. The radicles of sorghum were significantly inhibited by the extract diluted to 75 percent. The magnitude of inhibition was found to increase with the decrease in dilution. A significant reduction at the one percent level in the growth of alfalfa radicles was obtained when the seedlings were allowed to grow in a 50 percent dilution of the extract. The radicles of oats were not inhibited by any dilution but on the contrary, their growth was stimulated at the 75 percent and 50 percent dilutions. Radicle development of alfalfa, sorghum and oats was seriously inhibited by the original concentration of the extract. The dilution effect of the

Table 13. Effect of various dilutions of the sugar beet seed extract on the growth of radicles of alfalfa, sorghum and oat seedlings.

% dilution of the extract	Fresh weight of radicles (mg)		
	Alfalfa after 48 hours	Sorghum after 72 hours	Oats after 72 hours
Check (water)	53.1	114.4	94.1
75	49.6	88.8	113.6
50	30.9	75.9	105.9
0	13.5	48.8	68.3
LSD 5%	13.42	12.48	5.42
LSD 1%	19.31	18.91	7.80

extract on subsequent growth of the radicles is almost similar to that on emergence and development (Table 11). These results probably show that the extract affects the growth processes that are essential to seed germination and seedling growth as well.

The effect of different dilutions of the extract on subsequent growth of plumules is shown in Table 14. It can be seen that the growth of plumules was not inhibited in any case at any concentration of the extract. In the previous experiment, however, the full strength of the extract when applied on seeds of alfalfa and sorghum, inhibited the plumule development (Table 12). These data indicate that after germination the plumules may not be inhibited by the same concentration of the extract as that during germination. Instead, the growth of plumules was greatly stimulated in this experiment. The plumules were significantly stimulated in the case of alfalfa at 50 percent dilution; in sorghum at 50 percent and zero dilutions and in oats at 75, 50 percent and zero dilutions.

In conclusion the different dilutions of the extract not only inhibited the germination or growth processes but also resulted in promotion at comparatively lower concentrations. The promotion effect was more common on plumules than on radicles. In general the stimulation of seed germination and seedling growth of oats was more prominent than that of alfalfa and sorghum.

Table 14. Effect of various dilutions of the sugar beet seed extract on the growth of plumules of alfalfa, sorghum and oat seedlings.

% dilution of the extract	Fresh weight of plumules (mg)		
	Alfalfa after 48 hours	Sorghum after 72 hours	Oats after 72 hours
Check (water)	99.0	54.8	187.8
75	111.6	46.9	247.8
50	130.6	76.5	252.0
0	98.5	93.2	241.3
LSD 5%	23.26	10.05	17.63
LSD 1%	33.44	15.21	25.35

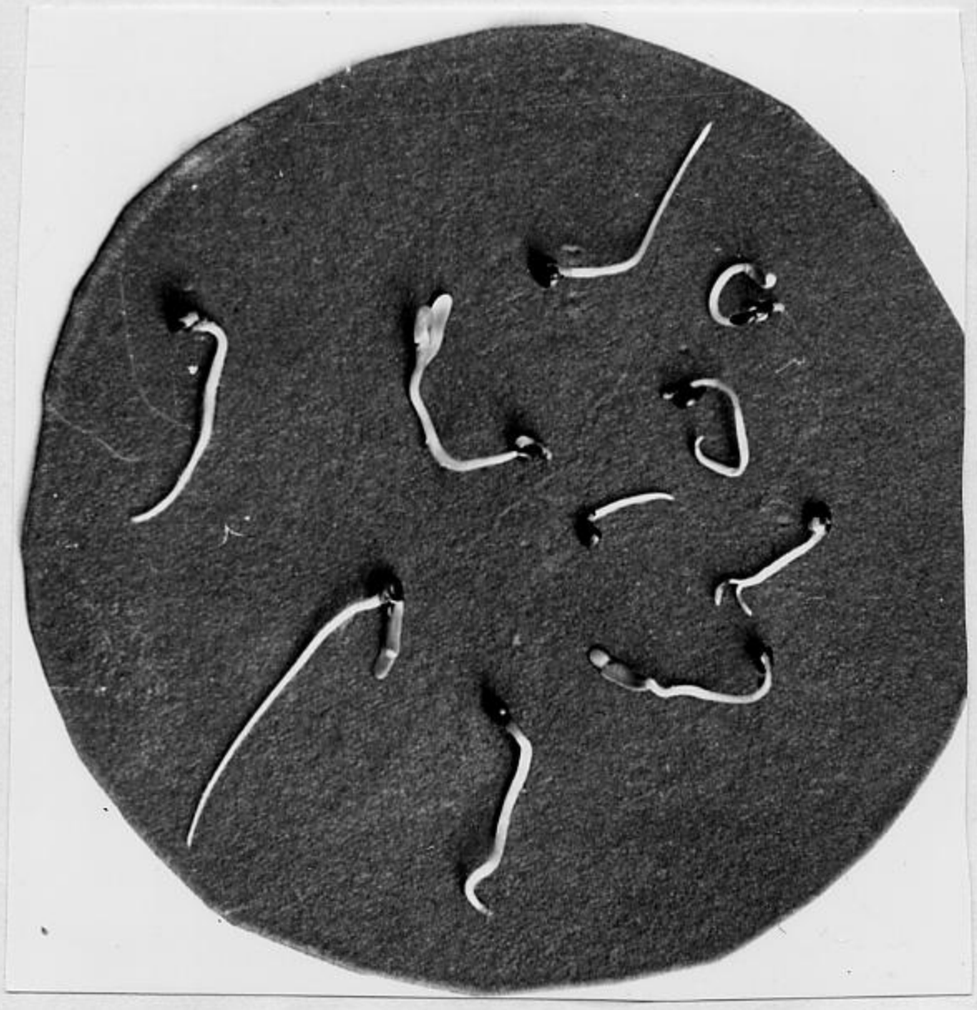
Location of the Inhibitors

In this experiment, the sugar beet seed balls were separated into two parts, the pericarp tissue and the true seeds. The water extracts of the ground seed balls (whole seed balls), pericarp tissue and true seeds were prepared and applied on sugar beet true seeds, and seedlings of alfalfa and sorghum. Radicle development was chosen as the criterion for testing the inhibitory effect of the extracts. It can be seen from Table 15 that the growth of the radicles was not inhibited by the water extract of true sugar beet seeds. The growth of radicles (fresh weight in mg) in the water extract of true seeds was very similar to that in distilled water.

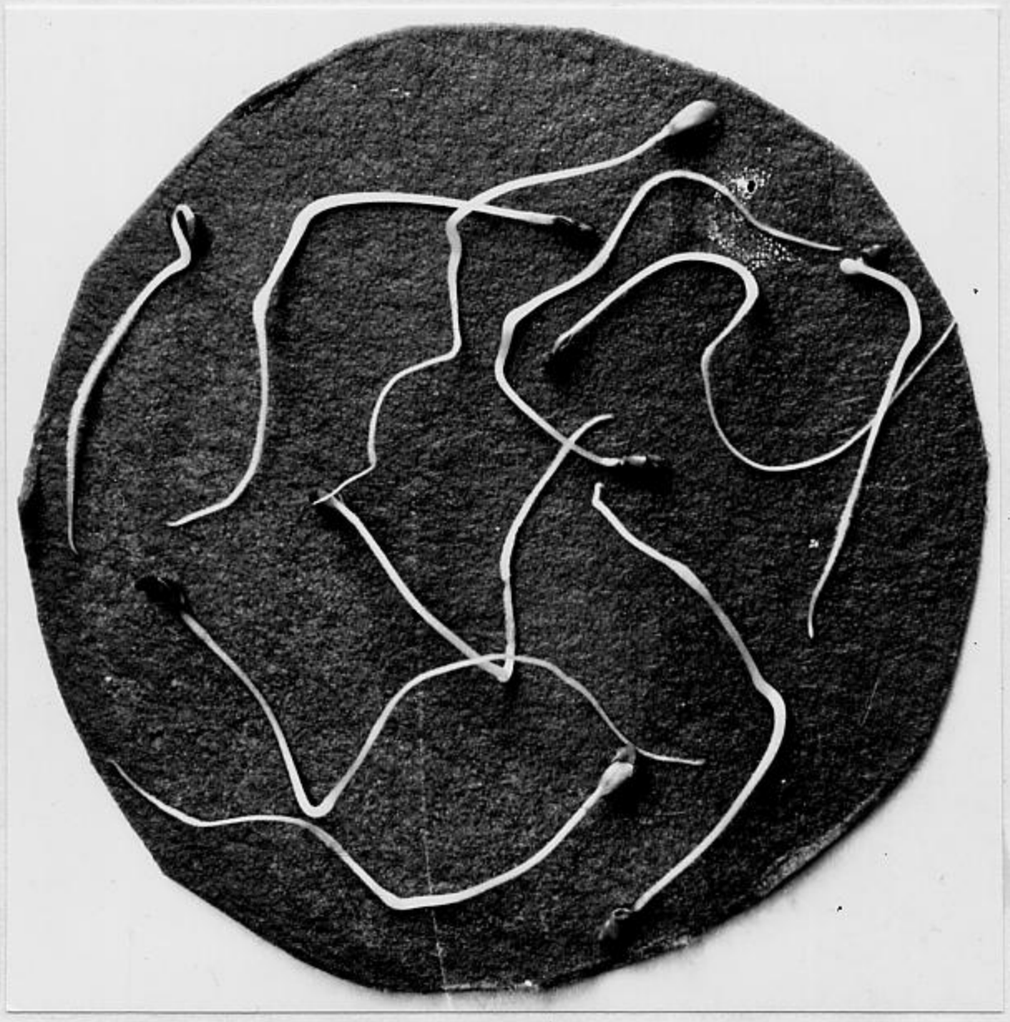
When, however, the water extract of the pericarp tissue was applied on true sugar beet seeds, and seedlings of alfalfa and sorghum, the radicles were greatly inhibited. The magnitude of inhibition was comparable to that caused by the extract of the ground seed balls. These results show that inhibiting materials are concentrated in the pericarp tissue and are practically absent from the true seeds. It can be seen in Figure 4 that the extract of the true sugar beet seed did not inhibit the germination of true sugar beet seeds or the growth of alfalfa and sorghum seedlings. The seedlings look normal and with no signs of toxicity. On the other hand,

Table 15. Effect of different extracts on the germination of true sugar beet seeds, and the growth of alfalfa and sorghum seedlings.

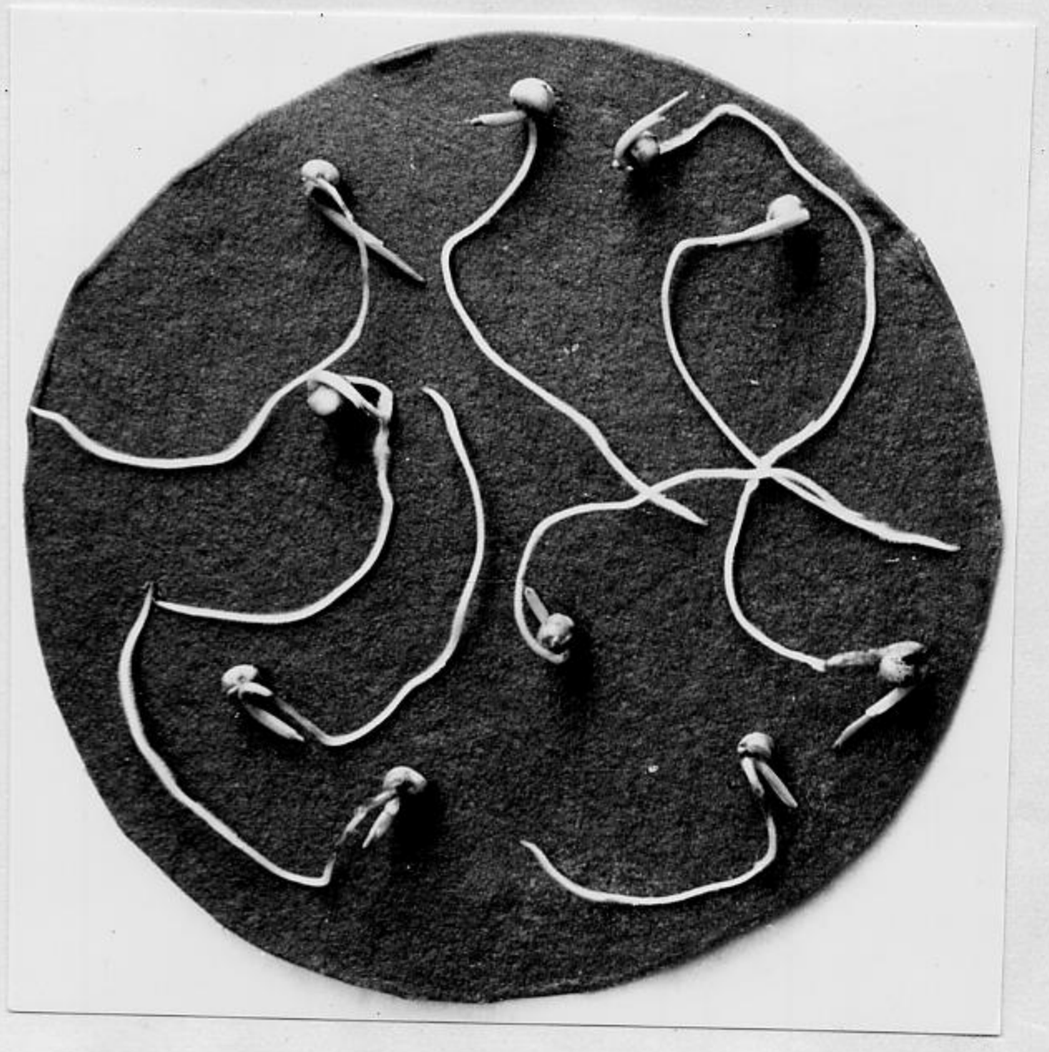
Extract	Fresh weight of radicles after 72 hr (mg)		
	Sugar beet true seeds	Alfalfa seedlings	Sorghum seedlings
Check (distilled water)	20.71	55.69	118.50
Sugar beet true seeds	19.39	49.69	118.19
Pericarp tissue	2.35	13.75	38.13
Ground seed balls	1.40	10.69	33.75



Germination of true sugar beet seeds



Growth of alfalfa seedlings

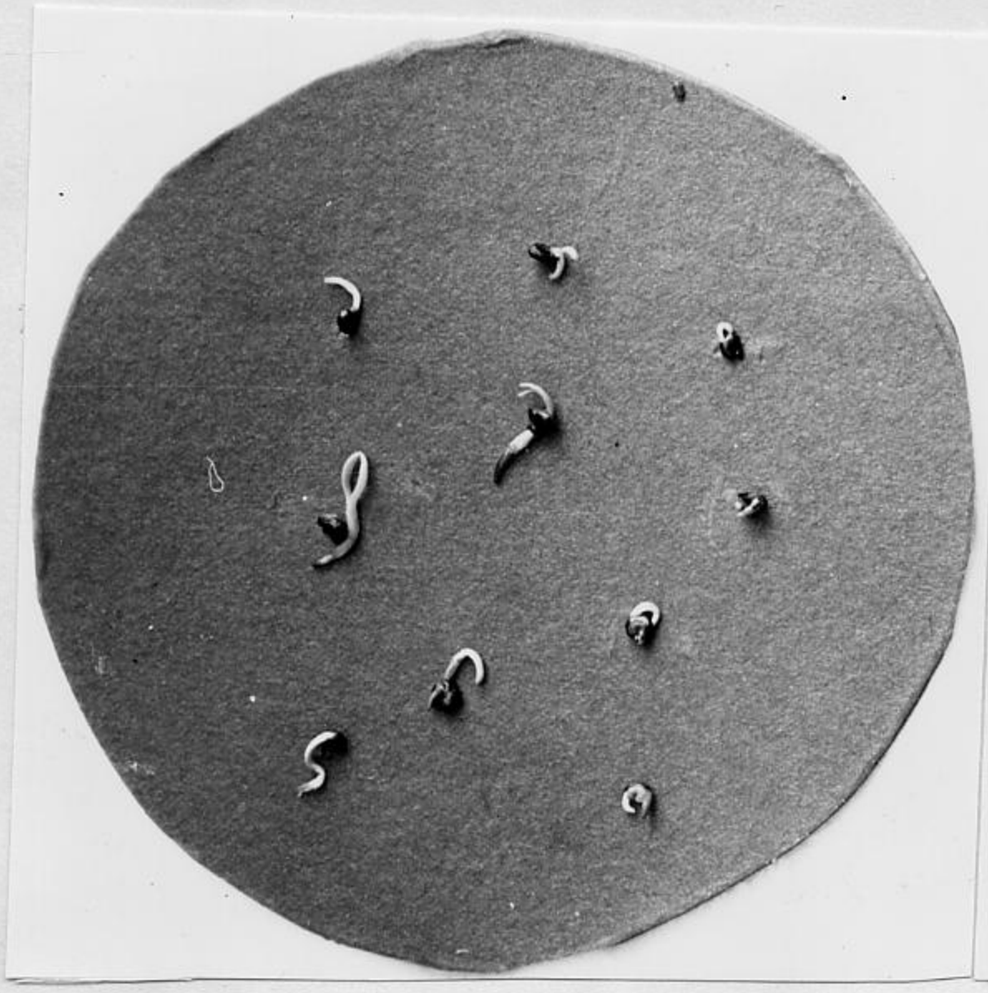


Growth of sorghum seedlings

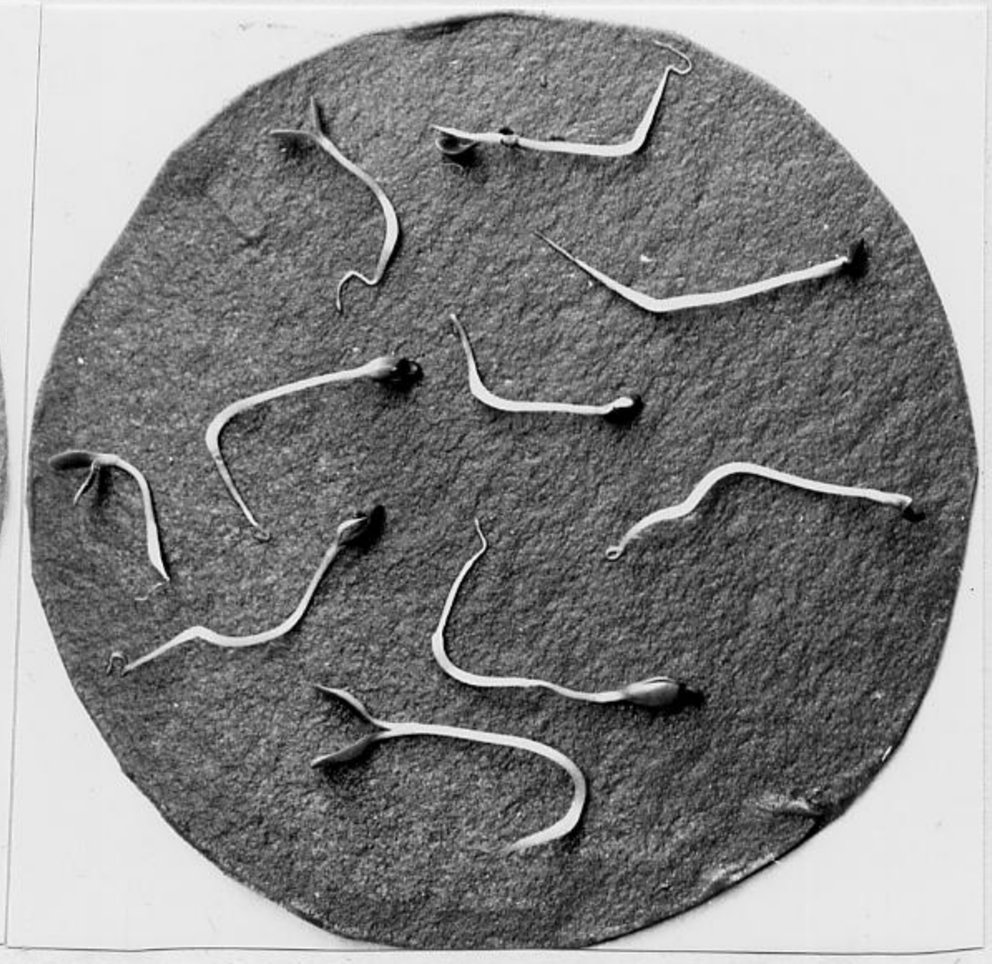
Figure 4. The germination of true sugar beet seeds and growth of alfalfa and sorghum seedlings in the water extract of true sugar beet seeds.

germination and growth processes are inhibited by the pericarp tissue extract (Figure 5) and the seedlings look stunted and abnormal with marked effect on the radicles.

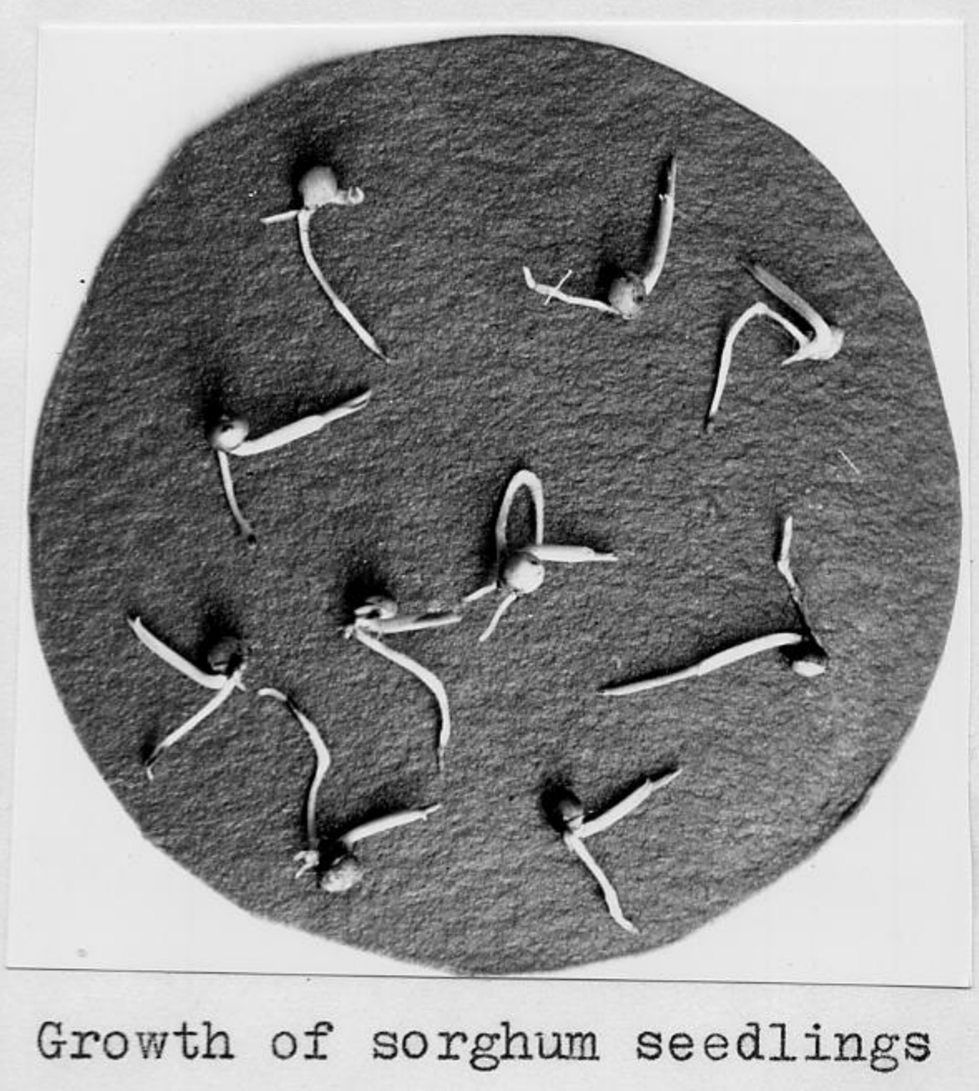
To substantiate these results, the true sugar beet seeds were washed in running tap water for six hours and then germinated in petri dishes. It can be seen from Table 16 that the growth of the radicles and plumules was not improved by washing. As there were no inhibitors in the true seeds, there was no improvement in germination upon washing. The results are in agreement with the findings of Rehm (1953, p. 2) and Snyder et al. (1965) that the pericarp tissue is mainly responsible for inhibition.



Germination of true sugar beet seeds



Growth of alfalfa seedlings



Growth of sorghum seedlings

Figure 5. The germination of true sugar beet seeds and growth of alfalfa and sorghum seedlings in the water extract of pericarp tissue.

Table 16. Effect of washing true sugar beet seeds for 6 hours on radicle and plumule development.

Replications	Fresh weight radicles (mg)		Fresh weight plumules (mg)	
	Unwashed	Washed	Unwashed	Washed
I	19.25	32.00	33.00	33.00
II	20.10	17.50	32.85	35.50
III	23.50	21.00	30.00	35.00
IV	18.00	22.25	33.00	31.50
Mean	20.71	23.19	32.21	33.75

V. SUMMARY AND CONCLUSIONS

A germination study was conducted in the seed technology laboratory of the American University of Beirut, Lebanon, to improve the germination of dormant sugar beet seeds, determine the effect of the beet seed extract on the germination of other crop seeds and find the location of the germination inhibitors in the sugar beet seed balls.

Washing the seed in running tap water, soaking in water at 30° or 40°C, subjecting presoaked seeds to oscillating vacuum (zero to -15 pounds per square inch), and the use of growth promoters were the methods tested to increase the germination and vigor of sugar beet seed. Highly significant improvement in germination was obtained by washing the seeds for 24 hours or by soaking at 30° or 40° C for 12 hours. Washing for 36 hours or soaking for 24 hours decreased the germination considerably. The improvement in germination was considered mainly due to the leaching out of inhibitory substances from the seed balls. The oscillating vacuum when applied on sugar beet seed presoaked for different periods of time decreased the germination as compared to that obtained by soaking alone. Soaking the seeds for six or 12 hours in

various concentrations of gibberellic acid, indoleacetic acid, kinetin, thiourea, hydrogen peroxide, potassium nitrate and naphthaleneacetic acid had no beneficial effect on germination as compared to that of distilled water. Similarly there was no increase of germination or inactivation of inhibitors when the growth promoter solutions were used as germination media.

The effect of the water extract of sugar beet seed was studied on various crop seeds and seedlings. The extract inhibited the germination percentage and development of radicles and plumules. The effect was, however, differential and more marked on radicles than on plumules. When low concentrations of the extract were applied on seeds and seedlings of alfalfa, sorghum and oats, there was less inhibition. At high dilutions of the extract, a significant increase in germination and growth was found. The stimulation was common in oats as compared to alfalfa and sorghum, and in plumules as compared to radicles.

For determining the location of the germination inhibitors, the seed balls were separated into true seeds and pericarp tissue. The aqueous extract of the pericarp tissue inhibited the radicles of true seeds, alfalfa and sorghum. The extract of the sugar beet true seeds did not show any inhibitory properties and there was no improvement in germination of true seeds on washing.

In brief the following conclusions can be made:

1. Maximum germination was obtained when the dormant sugar beet seeds were washed for 24 hours or soaked for twelve hours at 30° or 40°C.

2. The different concentrations of the growth promoters used and the application of the oscillating vacuum on presoaked sugar beet seeds did not improve germination.

3. The inhibitory effect of the sugar beet seed extract was more prominent on radicles than on plumules.

4. At low concentration of the extract, stimulation of plumules and radicles was observed in some seeds.

5. The inhibitory material was found to be concentrated in the pericarp tissue and practically absent from the true sugar beet seeds.

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