Sugar Beet Germination Selection Results in Osmotic Pressure Solutions I. Germination Methodology and Osmotic Selection Effects on Germination of Four Varieties

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A considerable amount of work has been done in Europe on the selection of germinating seed in high osmotic pressure solutions with reports of some remarkable changes in the material selected. Fryxell (2)[2] has reported that the European investigators had increased yields of wheat, buckwheat, barley, beans, tomatoes, timothy, and other crops. Increased resistance to cold, drought, and disease was obtained. In this work sugar and salt were used as osmotic pressure solutions. Investigations have been made in this country by Coe (1) on sugar beets, by Rodger (5) on alfalfa, by Helmerick and Pfeifer (4) and by Powell and Pfeifer (6) on winter wheat. Rodger found that winter hardy alfalfa varieties germinated slower in sugar and salt solutions than non hardy varieties. Helmerick and Pfeifer used mannitol and found varietal differences in germination and early growth of winter wheat. Powell and Pfeifer also used mannitol, and improved drought hardiness in Cheyenne winter wheat.

If this type of selection is effective on sugar beets, it would allow for early testing of large quantities of material in the laboratory, and would not interfere with the usual selection procedures used on growing plants. Accordingly, a project was set up at Rocky Ford, Colorado, in 1955 to determine the effect of osmotic pressure selection on the characteristics of the sugar beet.

Germination Methodology and Results

Preliminary work was conducted with sugar (sucrose) and salt (NaCl) separately and in combination in some 20 different concentrations. Petri dishes were first used but due to lack of humidity control were found to be unsatisfactory. To correct this difficulty, aliminum pans 15½ inches x 10½ inches x 1 inch were obtained in which three germination blotters were placed, to accommodate 200 or more seeds. Since the amount of liquid for germination is very important the procedure developed was to pour 235 ml. of liquid into each pan and allow the blotters to become thoroughly soaked. Following this, the blotters were turned over and pressed flat to eliminate any entrained air. Pans were then tilted slightly for five minutes to allow excess liquid

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[2] Numbers in parentheses refer to literature cited.

to go to the end of the pan after which this excess was drained off. Seed lots were soaked in the germinating liquids for four hours and then spread uniformly on the blotters. Several rubber bands were placed around each pan, and the pan enclosed in a light polyethlene bag of suitable size. The enclosed pans were then placed in a standard germinator at a temperature of 72° F.

The seed used in these experiments was multigerm in type. In preliminary experiments whole seed sized 9-14/64 inch was used, but size of seed was found to be a factor in osmotic germination work. Processed seed sized 7-9/64 inch was used in all experiments reported in this paper.

The results of experiments with various concentrations of sugar, salt, and sugar and salt in combination indicated that three-fourths percent sugar and three-fourths percent salt in combination depressed germination enough so that true early sprouters and true late sprouters could be selected. Germination results in this osmotic solution as compared to tap and distilled water are given in Table 1.

Table 1.—Comparison of Germination Ability of American No. 3 N Processed 7-9/64 Inch in One and One-half Percent Sugar-Salt and Water.

Germination Liquid	No. Seeds in Test	Germination Percent by Hours			
		48	96	168	240
Distilled Water	2000	40.3	81.9	88.0	88.1
Tap Water	2000	39.1	76.9	88.1	88.2
1½% Sugar-Salt	2000	3.5	68.8	78.9	80.2

Table 2.—Germination Results Obtained in One and One-half Percent Sugar-Salt Compared with Distilled Water for Three Sizes of Seed of American No. 3N. (Seven of 19 Counts Given).

Treatment	Seed Size	No. Seed Tested	No. Seed Balls Per Lb.	Germination Percent by Hours						
				24	36	48	60	120	180	240
Water	7-8/64"	800	49,500	12.8	30.2	60.6	67.6	77.2	77.6	77.6
Sugar-Salt		800		5.6	10.0	24.6	33.2	67.0	71.4	72.8
Difference				7.2	20.2	36.0	34.4	10.2	6.2	4.8
Water	8-9/64"	800	40,100	11.2	35.8	67.8	79.8	86.0	86.8	86.8
Sugar-Salt		800		4.4	13.2	32.6	44.8	78.4	83.4	83.8
Difference				6.8	22.6	35.2	34.6	7.6	3.4	3.0
Water	9-10/64"	800	34,600	6.4	24.6	61.6	73.0	82.8	83.6	83.6
Sugar-Salt		800		2.4	12.4	43.0	57.2	79.4	82.8	83.4
Difference				4.0	12.2	18.6	15.8	3.4	.8	.2

Germination tests of processed seed sized 7-8/64 inch, 8-9/64 inch and 9-10/64 inch were made using one and one-half percent sugar-salt compared with distilled water to determine the effect of size on germination percent (Table 2).

From these data it is evident that the larger processed seed was less affected by the osmotic solution than the smaller. Differences with 9-10/64 inch seed between sugar-salt and water, were consistently less than the two smaller sizes at every count. Therefore seed of 7-9/64 inch was used for test work.

Although the sugar-salt osmotic solution was found to depress germination to a satisfactory degree it was often difficult to obtain many sprouting seeds after 192 hours (late sprouts). The cause of this was assumed to be the toxic effect of the "Cl" ion in salt. Since other investigators (4) (6) had used mannitol with satisfactory results, and since a supply of inositol was available, an experiment was set up to determine the effect of different concentrations of this compound on germination. Seven and one-half percent inositol (approximately 10 atmospheres) appeared to be the most satisfactory (Table 3).

Table 3.—Comparison of Germination in Distilled Water, Sugar-Salt, and Inositol of American No. 3N Processed 7-9/64 inch.

Treatment	No. Seeds Tested	Germination Percent by Hours				
		40	88	136	184	240
Distilled Water	600	32.0	80.0	85.0	86.0	86.5
1½% Sugar-Salt	600	10.5	53.5	73.0	76.5	77.0
7½% Inositol	600	3.0	22.5	37.5	48.0	64.5

This concentration of inositol caused slow but continued germination for a 240 hour period, and therefore inositol at seven and one-half percent concentration was used for all later tests and selection work.

Variety Selection

Materials and Methods

Four American Crystal Sugar Company commercial varieties —No. 2, No. 3N, No. 3 LSR and No. 3S—were chosen for osmotic selection work. These were multigerm varieties, processed and graded to 7-9/64 inch size. Each lot had a potential germination of 85 to 90 percent. Using the technique previously described a 4 percent selection for early sprouts (24-48 hrs.) and a 4 percent selection for late sprouts (192-240 hours) was made from the 4 varieties and planted in 4-inch pots in the greenhouse in the 1955 fall season. Plants were allowed to grow for at least 2 months, after which they were placed in photo thermal induc-

tion (3). The first selections were later transferred to the greenhouse for winter seed production so that field tests could be made in the 1956 summer season. The later selections were transplanted in the field in 8 space isolation groups for 1956 summer seed production and reselection.

In selection, a seed was considered as sprouting when the radicle (or plumule) showed white at the edge of the seed cap. The early selections were labelled "A" and the late selections "B", in each variety. These selections were from one and one-half percent sugar-salt. The reselections for early sprouts in the sprouting selections, and for late sprouts in the late sprouting selections were labelled "AA" and "BB" respectively and were made form seven and one-half percent inositol solution.

Control of pollination in the first selections which were grown in the 1956 greenhouse was accomplished by means of white craft bags. Plants within selections were either bagged together or bags were switched between plants. Bags containing sizeable quantities of seed were bulked by selection for field test. The seed lots produced in the 1956 summer season were from space isolation groups. These lots were reselected for early and late sprouts in inositol in September 1956. Plants were photo thermally induced and planted in small areas in the greenhouse in 1957, and surrounded by polyethlene plastic cages (Figure 1) for seed production. Adequate quantities of reselected seed (AA and BB) were obtained. One unselected parent, American No. 3 N was also increased by this method.

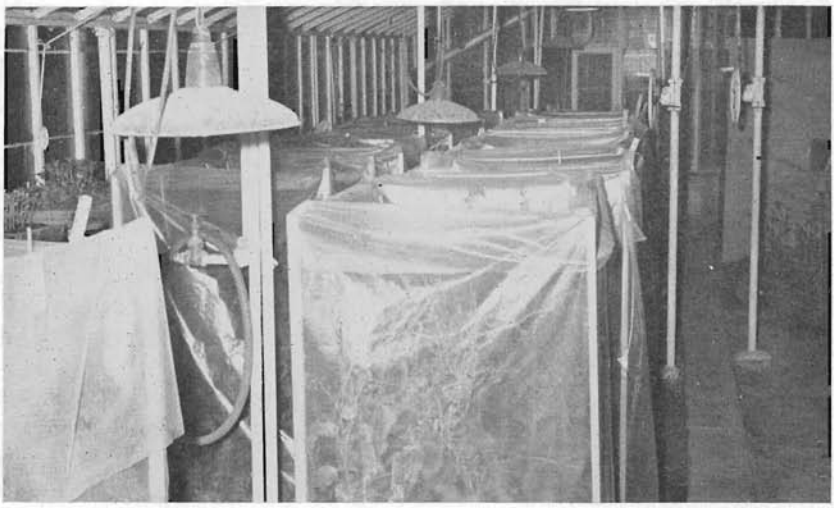

Figure 1.—Plastic "cages" used in the greenhouse for isolation of the second selections of the early and late sprouting groups.

Both seven and one-half percent inositol and distilled water were used to test these progenies for germination. Two hundred seeds of each progeny were used for each pan for each of the germination liquids. Three germination "runs" (or replications) were made with all seed lots. Counts of germinating seeds were made at 12-hour periods starting at 24 hours and continuing to 240 hours.

Experimental Results

The results of the germination tests as an average of the four varieties for each selection, in the two germination liquids is given in Figure 2. Total germination for the early sprouting selections (AA) were higher at all 12-hour periods than the late sprouting selections (BB), not only in inositol but also in distilled water. At the end of 240 hours, differences in favor of the early sprouting selections in inositol and water were 26.7 percent, and 10.8 percent respectively.

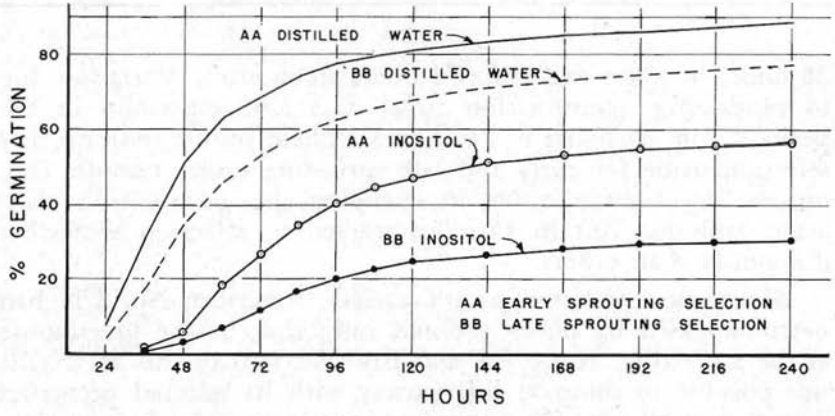

Figure 2.—Average germination percent of two osmotic selections from four varieties in distilled water, and in 7.5% Inositol.

Since each selection of each variety had been tested in the two germinating liquids in three germinator "runs," it was possible to set up a statistical analysis of the germination data obtained. In Table 4 is given F values for 7 of the 19 12-hour germination periods for distilled water and inositol.

The greatest differences found in these statistical analyses were between selections, with highly significant differences being found in all counts except the 36-hour period in inositol. Varieties were not significantly different after 48 hours in water but were highly significant after 48 hours in inositol. The variety-selection interaction was highly significant for all periods after

Table 4.—F Values for Germination Percent in Distilled Water and Seven and One-half Percent Inositol, of Early and Late Sprouting Selections in 4 Varieties of Sugar Beets.

Variation Due to	D/F	F Values for Hours of Germination							Significant F Value, 1%
		36	48	72	96	144	192	240	
Distilled Water									
Replications	2	3.6	11.9	1.3	.1	.1	.0	.0	
Varieties	3	12.7	10.1	.1	.5	.5	.4	1.4	5.6
Selections	1	48.9	111.8	34.8	31.2	51.6	40.8	39.8	8.9
Var. x Sel.	3	5.2	15.6	8.7	8.6	16.1	10.1	12.2	5.6
Error	14								
Total	23								
7½% Inositol									
Replications	2	.0	.1	.3	.9	.1	.1	.0	
Varieties	3	.3	2.7	6.0	36.9	38.3	55.0	83.4	5.6
Selections	1	.2	23.3	51.2	221.1	285.7	415.2	683.3	8.9
Var. x. Sel.	3	.1	.4	3.8	8.2	10.2	18.4	35.5	5.6
Error	14								
Total	23								

36 hours in water and after 72 hours in inositol. Variation due to replication (germination runs) was low, especially in the periods from 96 hours to 240 hours. These results indicate that selection made for early and late sprouting under osmotic conditions had been effective in changing the germination character, and that certain varieties reacted to selection somewhat differently than others.

Since one unselected parent variety, American No. 3 N had been increased by photo thermal induction in the greenhouse at the same time as the AA and BB selections of this variety, it was possible to compare this variety with its selected progenies in germination test. Two hundred seeds were used for germination of each of the three lots in seven and one-half percent inositol and distilled water, the test being made three times in the germinator. The data obtained from counts made every 12 hours is given in Figure 3.

These results show that the parent variety was intermediate in germination percent between the early and late sprouting selections at every 12-hour count. Levels of significance at the 5 percent point show that all six curves were significantly different after 84 hours of germination. Greater differences were observed between the three seed lots in inositol germination than in distilled water at the end of the germination period.

As previously mentioned, the statistical analysis of the data obtained from the tests indicated a significant variety-selection

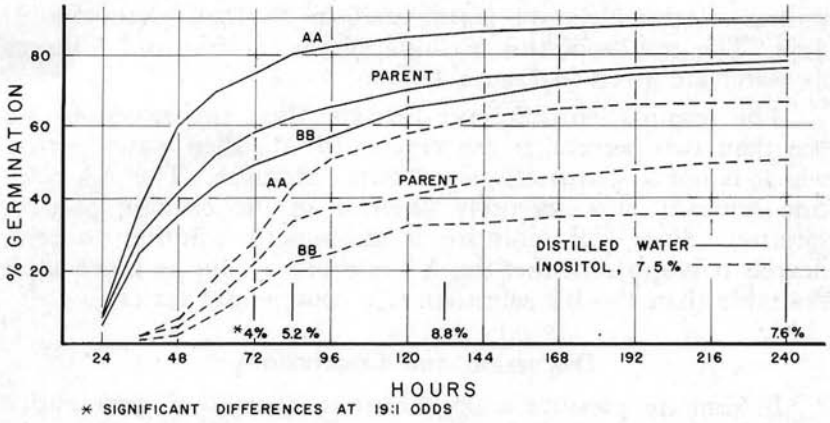

Figure 3.—Germination curves of early and late sprouting osmotic pressure selections (AA and BB) compared with the parent, from tests in distilled water and 7.5% Inositol (Variety—American No. 3 N).

interaction. The variety contributing most to this interaction was American No. 3 S; which had a distilled water germination of 85.0 and 84.3 percent for the AA and BB selections, respectively. In inositol, these selections germinated 46.3 and 20.3 respectively. In an effort to determine the cause of the low inositol germination of the BB selection, all seeds of both selections which had not germinated in 10 days in inositol were

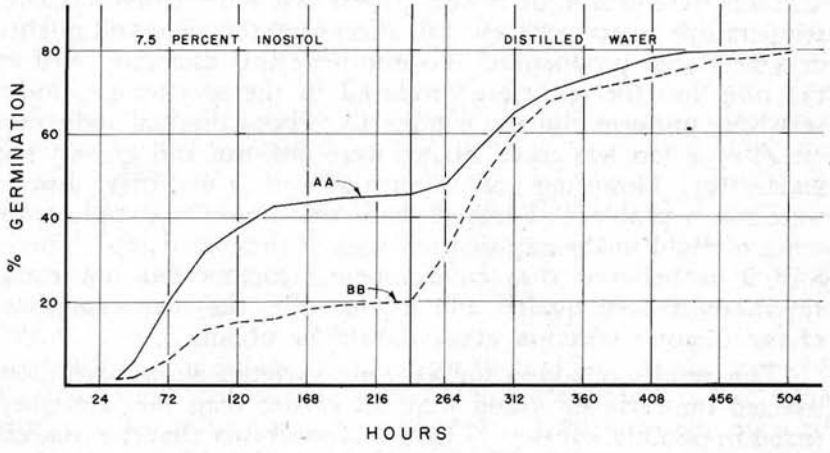

Figure 4.—Germination curves of early and late sprouting osmotic pressure selections (AA and BB) in 7.5% Inositol for 240 hours, followed by distilled water germination for 264 hours (Variety—American No. 3 S).

washed thoroughly, and germinated in distilled water for 11 days. The results of this germination in inositol and followed by water are given in Figure 4.

The germination difference between these two selections was less than two percent at the end of the distilled water period, which is not a statistically significant difference. The AA selection however, was definitely superior in the osmotic pressure solution. Since soil moisture is an osmotic solution to some degree, it is apparent that the AA selection would be much more desirable than the BB selection as a commercial variety.

Discussion and Conclusions

In osmotic pressure study, accurate control of germination conditions is essential. Too little or too much liquid affects germination speed much more in osmotic solutions than in water. Sizeable germination differences have been obtained between 100 seed lots at each end of the same pan due to a slight tilt of the pan in the germinator. This is an example of how important the environmental factors are. Although it was not possible to make counts in a humidity controlled room, this is another factor which is important. A check of 200 multigerm beet seeds in germination at one time usually requires at least five minutes, during which time moisture loss occurs from the germination bed. Nevertheless with the technique developed, it was possible to select the early and the late sprouts with reasonable accuracy.

Another important factor is the environment in which the seed lots were grown. It is well known that soil fertility, climate, temperature, insect pests, etc. can affect both the yield and quality of a beet seed production. To minimize this difficulty, seed of the nine lots for test were produced in the greenhouse under relatively uniform climatic conditions. Photo thermal induction on all nine lots was good. Stands were uniform and growth was satisfactory. Flowering was uniform as well as maturity. Insects were not a problem. Further, there was almost a complete absence of mold in the germination tests of these seed lots. Therefore it is believed that environmental factors did not cause variations in seed quality and in this case, the true expression of the osmotic selection effect should be obtained.

The results obtained for all four varieties show that those selected for early sprouting were all earlier than the late when tested in osmotic solution. There is a possibility that the osmotic selection also affected the germination ability in water since three of the four early sprouting selections were better than the late in distilled water.

If selection were to be continued on the AA types it appears that a higher concentration of inositol would need to be used to obtain further rapid improvement. Continued selection for late sprouting (BB) selection in seven and one-half percent inositol would certainly be more difficult because of the inherent lack of ability of these selections to absorb moisture from osmotic solutions.

Summary

By the use of large aluminum pans, three blotters per pan, polyethlene covers for the pans and a germination cabinet with temperature control, a satisfactory methodology was developed for germination studies of beet seed in osmotic pressure solutions.

Seven and one-half percent inositol (approximately 10 atmospheres pressure) was found to be a better germination depressant than one and one-half percent sugar-salt because of less toxicity.

Selections were made for early sprouting and late sprouting in four commercial varieties. These were reselected, and these selections were grown for seed under uniform conditions in the greenhouse. Isolation was obtained by means of plastic cages.

Early sprouting selections of all four varieties germinated significantly higher than the late when tested in inositol. In distilled water three of the early selections were significantly better. The early and late selections of the fourth variety were equal.

One parent variety (American No. 3 N) was compared against its early and late selections in inositol and in distilled water and was found to be intermediate in germination percent for both germination liquids during the 10-day germination period.

Selections of one variety (American No. 3 S) germinated equally well in distilled water (85.0 and 84.3 percent) but in inositol the early selection germinated 46.3 percent and the late 20.3 percent. The non-sprouting seeds of both selections of the inositol test were washed and retested in water. Germination of the two was within two percent of each other at the end of the water germination period which is evidence that these two selections were different only in germination ability under osmotic pressure conditions.

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