### INVESTIGATIONS ON SALT RESISTANCE IN COTTON

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This investigation was carried out to find out the possibility of inducing salt resistance at germination stage by pretreatment of cotton seeds with calcium chloride and gibberellic acid. The pretreatment of cotton seed with calcium chloride solution was found to increase salt resistance as observed by increasing percentage of germination. The pretreatment with gibberellic acid and calcium salts together before sowing in sodium chloride solution only in case of Pak-American Cotton Variety M4 and not in case of Tandojam Desi Cotton Variety No. 1.

In spite of the large number of investigations at present there is much controversy about the possibilities of inducing salt resistance by pretreatment of cotton seeds. Most of the research work on inducing salt resistance in crop plants by pretreatment of seeds has been carried out by the Russians. According to Henckel and Strogonov<sup>I</sup> a considerable increase of salt resistance and productivity was obtained in cotton and wheat by pretreatment of seeds with salt solution. Swollen seeds of cotton were subjected by them to the influence of 3% sodium chloride or Vant-Hoff solution for an hour. After that the seeds were rinsed for 1.5 to 2.0 hours in water and then sown. This gave an increase of 15 to 30% in the production on chloride salted soil. However, in the sulphate dominated soil it was found necessary by them to have pretreatment with magnesium sulphate. The above mentioned methods of inducing salt resistance was tried in wheat without success by Chaudhri and Wiebe.<sup>2</sup> They, however, found that pretreatment of wheat grains with calcium chloride make them salt resistant. Working on the same lines the present investigations were carried out to find the possibilities of inducing salt resistance in some cotton varieties of West Pakistan by pretreatment of cotton seeds with calcium chloride and gibberellic acid.

In the first instance the effect of different concentrations of sodium chloride and calcium chloride on germination was studied. After establishing the favourable effect of calcium salts on the subsequent germination of cotton seeds in sodium chloride solution detailed investigations were carried out to find the possibility of inducing salt resistance at the germination stage by pretreating the seeds with calcium chloride. The effect of gibberellic acid on salt resistance was also investigated. In the course of these investigations it became apparent that during different periods of the year cotton seeds showed variations in their capacity to germinate in salt solution. This phenomenon was investigated by carrying out periodic germination tests.

In the various experiments the seeds of two varieties of cotton namely Tandojam Desi Cotton Variety No. 1 belonging to Gossypium arboreum: and Pak-American Cotton Variety M4 belonging to Gossypium hirsutum Linn. were employed. The seeds were obtained from Agricultural Research Institute, Tandojam, West Pakistan. The various experiments were conducted in thermostat having controlled temperature. The experiments were carried out at 32°C. 25 seeds were kept in each Petridish on a double layer of filter paper and in each Petridish 15 cc. of germinating media was added. The length of about 2 to 3 cm. of the radical was taken as a measure of germination. Standard deviation was determined in various observations given in different Tables and it has been shown after  $\pm$  signs.

### **Effect of Water**

The desirable quantity of water in Petridish having 11.4 cm. diameter in which the cotton seeds were sown was determined by employing different quantities of water and studying its effect upon germination. Seeds were sown in Petridishes having a pair of filter papers. The result of germination in different quantities of distilled water in various Petridishes is given in Table 1.

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|--------|------------|--------|------|----|------|------|-------|-------|-------|
| TIES ( | OF         | DISTIL | LED  | W  | ATER | ON   | GERM  | IINAT | ION   |
| IN     | <b>FAN</b> | NDOJAM | DESI | Co | OTTO | N VA | RIETY | No.   | I     |

| 4  | 000 | 0  |
|----|-----|----|
| AT | 32° | U. |

| S. | Quantity of water per | Germination     |
|----|-----------------------|-----------------|
| No | Petridish             | percentage      |
| Ι. | 40 cc.                | 61.3±6.2        |
| 2. | 20 CC.                | $62.0 \pm 5.03$ |
| 3. | 15 cc.                | $74.6 \pm 0.9$  |
| 4. | IO CC.                | $55.0 \pm 1.3$  |

The results show that 15 cc. of distilled water gave better percentage of germination and in view of this 15 cc. of germinating media per Petridish was used in all the germination tests in various experiments.

### **Periodicity in Salt Resistance**

The cotton seeds were found to differ in their germination capacity in salt solution during different times. To study this regular periodic germination tests of Tandojam Desi Cotton Variety No. I were carried out during the course of this work in 1% sodium chloride solution. The results of these periodic tests are given in Fig. I.

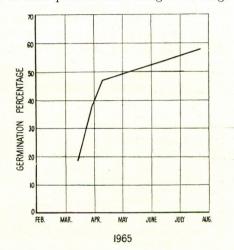


Fig. 1.—Germination of Tandojam Desi Cotton Variety No. 1 during different periods of the year in 1% sodium chloride solution. It shows the average percentage of germination obtained in three days at 32°C.

The results of these periodic tests indicate that cotton seeds do not show good germination immediately after harvest. Their capacity to germinate in salt solution was found to increase gradually with the passage of time. This shows that older seeds are comparatively more salt resistant than the fresher ones. This phenomenon seems to do with annual internal rhythm in seed germination discussed by Buenning.<sup>3</sup> This behaviour of germination put certain limits on the seed germination tests as an indicator of salt resistance. Thus the experiments performed at different periods are not comparable with each other.

## **Effect of different Concentrations**

The seeds of both the cotton varieties were sown in different concentrations of sodium chloride and calcium chloride. The results of germination in different concentrations are shown in Figs. 2 to 5. The results indicate that germination is proportional to the osmotic pressure of the germinating media. Osmotic pressure of 1% sodium chloride is approximately equal to 2% of hydrated

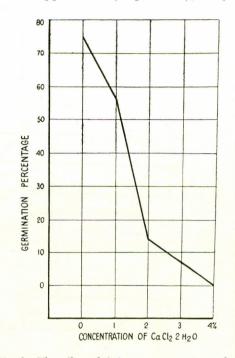


Fig. 2.—The effect of different concentrations of calcium chloride on germination of Tandojam Desi Cotton Variety No. 1. It shows the average percentage of germination obtained in 3 days at 32°C.

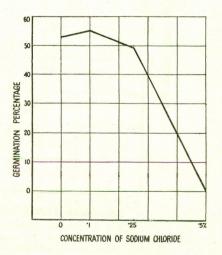


Fig. 3.—The effect of different concentrations of sodium cloride on germination of Pak-American Cotton Variety M4. It shows the average percentage of germination obtained in 5 days at  $32^{\circ}$ C.

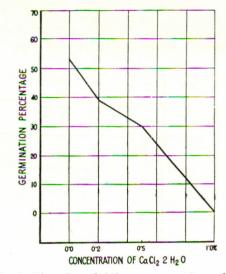


Fig. 4.—The effect of different concentrations of calcium chloride on germination of Pak-American Cotton Variety M4. It shows the average percentage of germination obtained in 5 days at 32°C.

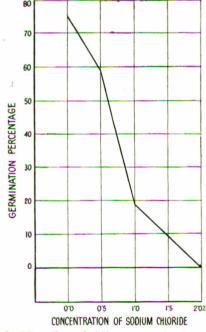


Fig. 5.—The effect of different concentrations of sodium chloride on germination of Tandojam Desi Cotton Variety No. 1. It shows the average percentage of germination obtained in 3 days at 32°C.

calcium chloride. The specific ions of calcium and sodium seem to have very little influence. The salt resistance of Tandojam Desi Cotton Variety No. 1 is much higher than that of Pak-American Variety M4. The Desi Cotton Variety can withstand salts more than double the quantity as compared to the Pak-American Cotton Variety.

# **Effect of Gibberellic Acid**

Gibberellic acid is known to have favourable effects on salt resistance, (Nieman and Bernstein).<sup>4</sup> In view of this its effect on salt resistance in cotton seeds at the germination stage was investigated. The effect of 100 ppm and 200 ppm of potassium salt of gibberellic acid on germination in sodium chloride solution was studied. The effect of gibberellic acid on germination in salt solution is shown in Table 2 in case of Pak-American Cotton variety M4 and in Table 3 in case of Tandojam Desi Cotton Variety No. 1.

## TABLE 2.—EFFECT OF GIBBERELLIC ACID ON Germination of Pak-American Cotton Variety M4 at $32^{\circ}$ C.

| S.<br>No.      | Germination media                      |         |      | Germination<br>percentage |
|----------------|--|---------|------|---------------------------|
| 1.             | Distilled water                        |         |      | $56.2 \pm 0.56$           |
| 2.             | 0.5% sodium chloride                   |         |      |                           |
| 3.             | 100 ppm. gibberellic acid              |         |      |                           |
| 4.             | 200 ppm. gibberellic acid              |         |      | $64.0 \pm 1.80$           |
| 3.<br>4.<br>5. | 0.5% sodium chloride and berellic acid | 100 ppm |      | 35.0+0.35                 |
| 6.             | 0.5% sodium chloride and berellic acid | 200 ppm | gib- | 55.0+1.50                 |

TABLE 3.—EFFECT OF DIFFERENT CONCENTRA-TIONS OF GIBBERELLIC ACID IN SODIUM CHLORIDE SOLUTION ON GERMINATION OF TANDOJAM DESI COTTON VARIETY NO. 1 at 32°C.

| S.<br>No. | Germination media                                  |      | Germination percentage |
|-----------|--|------|------------------------|
| 1.        | Distilled water                                    |      | 66.0 + 3.85            |
| 2.<br>3.  | 1% sodium chloride                                 |      | $58.0 \pm 3.00$        |
| 3.        | 1.5% sodium chloride                               |      | $40.0 \pm 1.80$        |
| 4.<br>5.  | 100 ppm. gibberellic acid                          |      | 59.0 + 0.35            |
| 5.        | 200 ppm. gibberellic acid                          |      | 66.0 + 1.80            |
| 6.        | 1% sodium chloride and 100 ppm<br>berellic acid.   | gib- | 52.0+0.35              |
| 7.        | 1% sodium chloride and 200 ppm<br>berellic acid.   | gib- | 59.0+1.50              |
| 8.        | 1.5% sodium chloride and 200 ppm<br>berellic acid. | gib- |                        |

## **Induction of Salt Resistance**

Experiments on induction of salt resistance in cotton by pretreatment with calcium salts were carried out in view of the success obtained by Chaudhri and Wiebe<sup>2</sup> in wheat.

(a) Effect of Calcium Chloride Pretreatment.—In the initial experiments the seeds of Tandojam Desi Cotton Variety No. 1 were treated with 2% calcium chloride solution for 6 hours, 12 hours and 24 hours and then after washing with distilled water and blotting on filter papers these were sown in Petridishes containing 15 cc. of 1% sodium chloride solution at 32°C. The results of 3 days germination are given in Fig. 6.

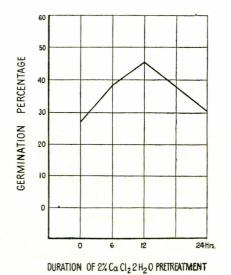


Fig. 6.—The effect of pretreatment of 2% calcium chloride for 6,12 and 24 hours on subsequent germination in 1% sodium chloride solution. Tandojam Desi Cotton seeds after pretreatment were washed in distilled water, blotted on filter papers and then transferred to 1% sodium chloride solution. It is the average

germination obtained in 3 days at 32°C.

The results show that pretreatment for 12 hours with calcium chloride solution gives the highest germination subsequently in sodium chloride solution.

The seeds of Pak-American Cotton Variety M4 were pretreated for 12 hours with 1% and 0.5%calcium chloride solutions in the same way as with the above mentioned Desi Cotton Variety. Then the seeds were grown in 0.5% solution of sodium chloride after washing in distilled water and drying on filter papers at 32°C. The results are given in Table 4.

The results clearly establish the favourable effect of calcium chloride pretreatment on germination in salt solution.

(b) Pretreatment in the Gibberellic Acid.—The seeds of Pak-American Cotton Variety M4 were pretreated with 1% calcium chloride and 200

TABLE 4.—THE EFFECT OF PRETREATMENT WITH CALCIUM CHLORIDE ON PAK-AMERICAN COTTON VARIETY M4.

| S.<br>No. | Pretreatment           | Germination media     | Germination percentage |
|-----------|------------------------|-----------------------|------------------------|
| 1.        | None                   | 0.5% calcium chloride | 21.3±7.00              |
| 2.        | None                   | 1.0% calcium chloride | $2.0\pm1.20$           |
| 3.        | None                   | 0.5% sodium chloride  | $4.0 \pm 3.50$         |
| 4.        | 1% calcium<br>chloride | 0.5% sodium chloride  | $69.3 \pm 0.75$        |
| 5.        | 0.5% calcium chloride  | 0.5% sodium chloride  | $66.6 \pm 1.02$        |

ppm gibberellic acid for 12 hours after washing in the distilled water and blotting them with the filter papers. These were transferred to 0.5%sodium chloride solution (32°C.). The results of germination are given in Table 5.

In the same way the seeds of Tandojam Desi Cotton Variety No. 1 were pretreated with 2%calcium chloride and 200 ppm gibberellic acid and were grown in 1.5% sodium chloride solution. The results of the experiment are given in Table 6.

TABLE 5.—EFFECT FO GIBBERELLIC ACID AND CALCIUM CHLORIDE PRETREATMENT ON GERMINA-TION IN SODIUM CHLORIDE SOLUTION OF PAK-AMERICAN COTTON VARIETY M4.

| S.<br>No. | Pretreatment | Germination                                  | Germination<br>percentage |
|-----------|--------------|--|---------------------------|
| 1.        | None         | 0.5% sodium chloride<br>0.5% sodium chloride | $2.4 \pm 0.29$            |
| 2.        | 1.0% calcium | 0.5% sodium chloride                         | $54.4\pm1.00$             |

 1.0% calcium chloride 0.5% sodium chloride 57.6±1.30 and 200 ppm gibberellic acid

TABLE 6.—EFFECT OF GIBBERELLIC ACID AND<br/>CALCIUM CHLORIDE PRETREATMENT FOR12 HOURS ON GERMINATION IN SODIUM<br/>CHLORIDE SOLUTION OF TANDOJAM DESI<br/>COTTON VARIETY NO. 1.

| S. I<br>No. I | Pretreatment     | Germination media                                | Germination<br>percentage |
|---------------|------------------|--|---------------------------|
| 1. 2%         | calcium chloride | 1.5% sodium chloride<br>and 1.5% sodium chloride | $32.0\pm1.8$              |

Gibberellic acid pretreatment shows the favourable effect on germination alongwith calcium chloride in sodium chloride solution in case of Pak-American Cotton Variety M4., but the same pretreatment has adverse effect on germination in case of Tandojam Desi Cotton Variety No. 1. It is hard to explain these contradictory results. It is felt that more detailed experiments are needed to arrive at any definite conclusions. The results show that pretreatment with calcium chloride gave better percentage of germination subsequently in sodium chloride than the control. It shows that it is possible to induce salt resistance by pretreating the seeds with calcium chloride and growing them in sodium chloride solution.

This induction of resistance against sodium by calcium salts appear to be based on the well known phenomenon of antagonism between monovalent and divalent cations which have been observed besides others by Hoefler <sup>5</sup> and Viet. <sup>6</sup>

The toxic effects of high quantities of sodium salts are known to be reduced in the presence of calcium salts. This is also known that sodium and calcium salts effect the hydration of the protoplasm in the opposing directions (Hoefler).<sup>5</sup> Thus there seems to be need for a balance between the salts of these two for proper functioning of the cell structures.

#### Discussion

The seeds germination tests in sodium chloride solution show that Tandojam Desi Cotton Variety No. 1. is much more salt resistant than the Pak-American Cotton Variety M4. This agrees with the general impression prevailing with cultivators. Cotton seed germination in sodium chloride solution as a measure of salt resistance was found to have certain limitations. The capacity of cotton seeds to germinate in salt solution differs widely at different periods of the year. Just after harvest cotton seeds give minimum percentage of germination which gradually increases with the passage of time. Thus the results of various experiments performed at different times are not comparable with one another. The increased tolerance to sodium chloride after pretreatment of seeds with calcium chloride is possibly due to maintenance of balance between sodium and calcium cations which keep hydration of the protoplasm within normal limits for growth.

Gibberellic acid was found to have favourable effect on germination in sodium chloride solution only in case of Pak-American Cotton Variety M4. Pretreatment of cotton seeds with calcium chloride and gibberellic acid together also gave better germination in sodium chloride solution in Pak-American Cotton, but it had inhibiting effect in case of Tandojam Desi Cotton Variety No. 1. This investigation opens the possibility of developing pretreatment of cotton seeds for inducing salt resistance. It is too early to predict the success of this pretreatment in increasing crop yields in saline soils under field conditions. There is no substitute to actual field trials for answer to this question.

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