Influence of Seed Pretreatments on Salt Tolerance of Cotton During Germination[1]

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ABSTRACT

One factor which limits crop production in the arid Southwest is the high salinity of soils and irrigation waters; especially during germination and early growth. The purpose of this study was to determine if salt-conditioning seed pretreatments would be effective in increasing the salt tolerance of cottonseed.

Effects of 11 seed pretreatments on cotton (*Gossypium barbadense* L.) germination under saline and nonsaline conditions were studied in the laboratory to determine their usefulness in increasing relative salt tolerance. Salts, phytohormones, and adenosine monophosphate were used in seed pretreatments and their effectiveness was tested by germinating the pretreated seeds in single and mixed salts of NaCl and $CaCl_2$.

Several pretreatments hastened germination under salinity by at least 1 day over nontreated controls. However, soaking in distilled water enhanced germination under saline conditions as much as any other pretreatment. Using original seed weight to determine drying time of seeds after pretreatment was inadequate because of gains or losses of solute during the soaking cycle.

Additional index words: Gossypium barbadense, Gibberellic acid, Kinetin, Indole acetic acid, Adenosine monophosphate.

LONG-STAPLE cotton (*Gossypium barbadense* L.) is an important crop in many hot, arid environments where saline irrigation water is used. We conducted this study to determine if various seed pretreatments, such as have been described with wheat (4, 11), could effectively increase the salt tolerance of a commercial variety of long-staple cotton. Cotton pretreatment has not been studied as extensively as that of wheat and, whereas grains are very salt tolerant during germination and more sensitive during seedling and later growth stages, cotton is sensitive during germination and more tolerant during the later growth stages (2).

Sometimes, costly management practices can minimize soil salinity problems during early plant growth but in some areas saline irrigation waters make salt stress unavoidable. Thus, the potential value of preconditioning seeds to increase germination and seedling development is apparent.

Typically, soil salinity delays normal development of root and shoot extension at germination (1). Prolonging this critical growth period increases chances of seedling damage by pathogenic or environmental factors. Furthermore, critical storage reserves within the seed are slowly depleted, causing decreased seedling survival and vigor. A beneficial and desirable consequence of seed pretreatment would be to maintain normal germination rate in the presence of salt.

In 1883, Will and de Saussure (as cited in 17) used soaking and drying as a seed pretreatment before planting to increase drought tolerance. This technique was applied to increase salt tolerance by pretreating seeds in salt solutions (9). Since these early studies, soaking seeds in solutions that contain salts (4, 10), phytohormones (7) and other chemicals (10) has had many effects on germination and subsequent plant growth and development. Among the beneficial effects noted have been increased germination (4, 10), more rapid radicle elongation (17), more extensive root systems (7), increased yields and greater drought (8), cold (5, 6), and salt resistance (11).

In 1924, Toole and Drummond (19) described prewetting of cottonseed for improving germination in dry climates or of hard seed. They did not dry the seeds and they attributed better germination to a higher seed moisture content. Genkel (8) reported that seed treatment with 3% NaCl (0.5 *N*) increased yields of wheat and cotton 15 to 30% when grown in chloride-salted soils. Similar results have been reported for $CaCl_2$ pretreatments in increasing wheat germination in NaCl medium (4). At low salinities, germination improved even when seeds were dried after pretreatment (11). This is important and essential because seed usually must be dry to be sown by mechanical planters and drills.

MATERIALS AND METHODS

A commercial lot of long-staple 'Pima S-4' cottonseed was used for all experiments. The acid-delinted, fungicide-treated seed tested 85% germination based on official germination methods.

Germination tests were made on blotters soaked in the appropriate solutions and then placed under and over the seeds

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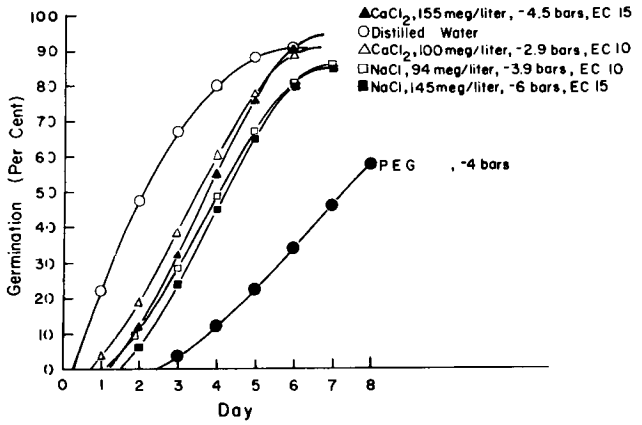

Fig. 1. Effect of single salts and PEG on cottonseed germination.

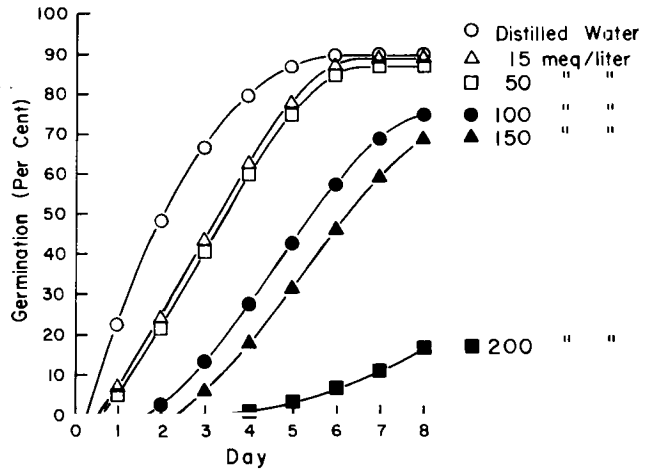

Fig. 2. Effect of mixed salt solutions on cottonseed germination.

in 95- × 95-mm, covered, plastic germination dishes. Twenty-five seeds per dish were used with a minimum of six replications per treatment. Seeds were germinated in the dark in a germinator at 22.5 ± 2 C, were checked daily, and were considered germinated when the radicles were at least 2 mm long.

Salt and Osmotic Treatment Experiments. Nonpretreated Pima S-4 seed was tested for salt tolerance by germination in both single-salt and mixed-salt solutions. In many investigations, only single-salt solutions are used for germination tests. Although single-salt studies are useful in the analysis of ion uptake and exchange mechanisms, mixed-salt solutions more closely represent irrigation waters and, therefore, are included in this study. Solutions used to test the effects of single salts and osmotic pressure were distilled water, 94 and 145 meq/liter NaCl and 100 and 155 meq/liter $CaCl_2$. Isoequivalent amounts of NaCl and $CaCl_2$ at a total concentration of 15, 50, 100, 150, 200, and 300 meq/liter were used in the mixed-salt tests.

An 87-g/liter (—4 bars osmotic potential) polyethylene glycol (PEG) (MW 570-630) solution was also used to test osmotic effects.

Pretreatment Experiments. Preliminary tests were conducted to determine optimum pretreatment time and number of pretreatment cycles. Seeds were weighed before pretreatment and then were immersed in well-aerated pretreatment solutions. After soaking, seeds were allowed to drain for 1 hour on stainless steel screens, then placed on paper towels, and dried at normal room temperature (20 to 22 C) and humidity. Weights were recorded daily for 3 weeks to determine when the seeds were air dry. Water content of the seeds was determined by oven-drying at 90 to 100 C for 16 hours. Aqueous pretreatment solutions used were: 0.1 *N* $CaCl_2$; 0.5 *N* $CaCl_2$; 0.5 *N* NaCl; and 5 m*M* solutions of KH_2PO_4, NaH_2PO_4, NH_4Cl, gibberellic acid (GA), indole-3-acetic acid (IAA), kinetin (β furfuylaminopurine), and adenosine 5′monophosphoric acid sodium salt (AMP). Phytohormones and AMP were commercial preparations.

Testing of Pretreated Seeds for Salt Tolerance. In the final experiment, pretreated and nonpretreated seeds were germinated using three different salt concentrations: 0 (distilled water); 100 meq/liter NaCl; and 150 meq/liter mixed salts of NaCl and $CaCl_2$.

RESULTS AND DISCUSSION

Effects of Salts and Osmotic Pressures on Germination Decreased germination may be measured as slowing of germination, a lowering of the ultimate germination percentage, or both. Two factors detrimental to seed germination in saline soils are decreased osmotic potential, due to increased solute concentrations, and specific ion toxicities. Germinating seeds can overcome all or part of the osmotic influence through the uptake of some of the surrounding solutes. But they make this osmotic adjustment at the expense of time and risk of specific ion toxicities, as illustrated in Fig. 1. If the germinating seed cannot absorb the solute, they cannot adjust to imposed osmotic influences. For example, with PEG germination was delayed until day 3 and then was slower than for the comparable NaCl treatment (Fig. 1). Maximum germination did not exceed 60%. Seeds in salt solutions apparently adjusted to the osmotic potential and germinated well, even at lower osmotic potentials than that imposed by the PEG solution. Curves for $CaCl_2$ (—4.5 bars) and NaCl (—3.9 bars) show the contributing influences of specific ions. In general, $CaCl_2$ affected germination less severely than did NaCl, whether compared on osmotic, electrical conductivity (EC) or milliequivalent bases. This was probably due to the effect of Ca on membrane stability and ion selectivity (14).

Based on these data, 100 meq/liter NaCl was selected as one of the treatments to test the effectiveness of the seed pretreatments. At this concentration, the germination rate was significantly decreased.

Figure 2 shows that percent germination decreased as isoequivalent concentrations of NaCl + $CaCl_2$ solutions increased. At 300 meq/liter salt (data not shown) no seeds germinated within 9 days. When ungerminated seeds were rinsed with distilled water after 150-, 200-, and 300-meq/liter pretreatment, seeds were still capable of up to 84, 67, and 62% germination, respectively. Thus, at high-salt concentrations, correspondingly more seeds suffered from irreversible salt effects either through toxicity or predisposal to other detrimental effects in their environment. We cannot draw conclusions from mixed-salt treatments that can be applied universally to all field situations. Often specific ion imbalances exist, and when seeds are in soil the total water potential will be greater because matric potential effects are added. Our results do indicate the relative effect of increasing concentrations of mixed salts and provide a guide to selecting concentrations best suited for testing effects of seed pretreatments. In 150-meq/liter mixed salts (NaCl and $CaCl_2$), germination was markedly inhibited but seed viability was unaffected; we selected this concentration as that best for mixed-salt treatment to evaluate seed pretreatments.

Table 1. Summary of mean germination percentages of pretreated cottonseed in distilled water, 100 meq/liter NaCl, and 150 meq/liter mixed salts of NaCl and $CaCl_2$.*

	Distilled water				100 meq/liter NaCl				150 meq/liter (NaCl + $CaCl_2$)				
	Day				Day				Day				
Pretreatment	1	2	3	4	2	3	4	5	3	4	5	6	7
1. $CaCl_2$ (0.1 *N*)	28 a	79 a	85 a	89 a	19 bcd	63 a	82 a	87 a	9 abc	35 ab	55 a	65 a	67 a
2. KH_2PO_4 (5 m*M*)	25 abc	71 abc	81 ab	83 ab	26 ab	56 a	74 a	77 ab	15 a	37 a	52 a	63 a	70 a
3. Dist. H_2O (wet control)	20 bcd	73 ab	81 ab	83 ab	24 abc	58 a	77 a	80 ab	11 ab	31 ab	47 ab	61 ab	68 a
4. Kinetin (5 m*M*)	21 abcd	69 abc	73 bcd	77 bcd	20 abcd	54 ab	73 a	77 ab	8 bcd	27 abc	43 ab	51 bc	62 a
5. NaH_2PO_4 (5 m*M*)	18 cd	67 bc	78 abc	79 abc	19 bcd	64 a	76 a	79 ab	15 a	37 a	51 a	65 a	72 a
6. GA (5 m*M*)	17 de	68 bc	73 bcd	74 bcd	27 a	61 a	72 a	77 ab	9 abc	32 ab	51 a	54 abc	63 a
7. AMP (5 m*M*)	11 ef	61 cd	71 cd	75 bcd	19 bcd	56 a	81 a	83 ab	3 cd	25 bcd	43 ab	56 abc	61 a
8. NH_4Cl (5 m*M*)	26 ab	62 cd	67 de	72 cd	19 bcd	65 a	77 a	82 ab	9 abc	25 bcd	36 bc	48 c	59 a
9. None (dry control)	5 fg	53 de	77 abc	84 ab	5 e	25 c	59 b	72 b	5 bcd	15 cd	31 c	47 c	62 a
10. IAA (5 m*M*)	5 fg	45 e	59 e	67 cd	14 d	45 b	51 b	55 c	5 bcd	16 cd	25 cd	35 d	43 b
11. $CaCl_2$ (0.5 *N*)	0 g	18 f	50 f	71 cd	1 e	5 d	33 c	56 c	1 d	2 e	7 e	18 e	27 c
12. NaCl (0.5 *N*)	0 g	6 g	19 g	34 e	6 e	8 d	21 d	23 d	6 bcd	14 d	19 d	27 de	29 c

* For a given germination condition different letters within columns indicate significant pretreatment differences at the 5% probability level using Duncan's multiple range test.

Water Pretreatments. We compared the germination rates of pretreated seeds with those of nonpretreated seeds (dry controls) and seeds pretreated with distilled water (wet controls).

Pretreatment with distilled water alone significantly increased germination over that of the nonpretreated seeds for 2 days in nonsaline and 4 to 6 days in saline germinating media (Table 1).

Because of the beneficial effect of water pretreatments, we made additional studies to determine optimum pretreatment time and number of pretreatment cycles. Pretreatments as short as 20 min increased germination but a single 4-hour water treatment was optimal. Additional cycles decreased germination percentage, and longer pretreatments (up to 24 hours) stimulated rates of germination but lowered the final germination percentage. Weekly germination tests showed that water pretreatments continued to have beneficial effects under saline germination conditions for at least 12 weeks after the seeds were dried.

The effectiveness of our 4-hour water pretreatment coincides well with the results Christiansen (5) obtained in reducing chilling damage to cottonseed. Chilling protection was attributed to an increase in seed moisture content to 14% or above. While pretreatments do increase seed moisture levels initially, our data indicate that less than 1% moisture remains after 3 weeks. When seeds were dried at normal room temperature and humidity after soaking, original seed weight could not be used to determine the end of the drying period. Our preliminary studies had shown that original seed weight was obtained within 4 days with all other pretreatments (Fig. 3). All pretreated and nonpretreated seeds lost moisture by drying for 2 weeks. After 26 days, final moisture contents for all pretreated seeds were similar (5.0 to 5.3%), and were significantly higher than those of the dry controls (4.5 to 5.0%). A comparison of mean dry weights of pretreated and untreated seed showed that seeds treated with 0.5 *N* salts gained solutes during treatment, whereas seeds receiving any other pretreatment lost solutes. In each case, the net solute change was about 1% of the original seed weight before treatment.

Hormone Pretreatments. Hormones play various roles in relation to salt balance and salt effects in plants, but as pretreatments in cottonseed, none of the hormones used increased germination rate or percentage (Table 1).

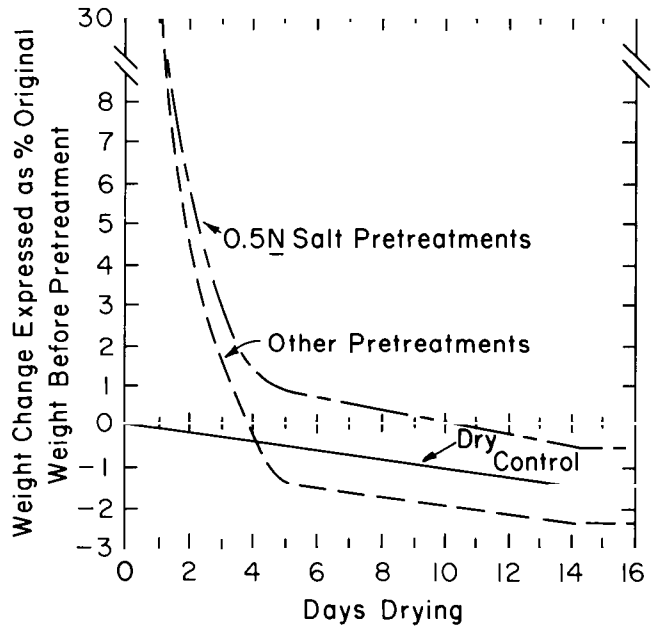

Fig. 3. Weight loss of cottonseed during drying.

Pretreatment with auxin (IAA) decreased the germination rate to the level of the dry control or lower. The inhibitory effect of IAA may be due to stimulation of active Cl^- uptake, although this was not verified. Active uptake of Cl^- has been stimulated in oat coleoptiles in response to either auxin treatment or low pH (16). In our experiments, seeds pretreated with GA where also exposed to low pH, and their germination equalled that of the wet controls. Thus, it seems that the inhibitory effects of IAA was operating through some mechanism other than in pH sensitivity. The general stimulatory effects of pretreatments on germination suggest that an inhibitor may be washed out during pretreatment; however, we made no attempt to identify an inhibitor in the pretreatment solutions after use.

Cyclic AMP has been found to be a mediator of hormonal response in plants (3), and either cyclic AMP

(6) or AMP (15) accelerated cottonseed germination. Since plant cell wall phosphatases can hydrolyze nucleotides (13), studies using nucleotides as treatments leave unresolved the interpretation concerning which moiety is producing the observed effect – the entire AMP molecule or the adenine, adenosine, or phosphate-hydrolytic end products. Both inorganic phosphate and ammonium salts are commonly used as fertilizers, and each can enhance germination (10). In 100 meq/liter of NaCl (Table 1), no differences were found between AMP and inorganic phosphate pretreatments. During the first 3 days in nonsaline environment and until day 5 in mixed salts, seed treated with inorganic phosphate germinated significantly better than did AMP-pretreated seed. Mean germination of water-pretreated seed was higher than that of AMP-pretreated seed, but usually lower than that of seed pretreated with inorganic phosphate.

Salt Pretreatments. Only during the first 1 to 2 days did pretreatment with 0.1 *N* $CaCl_2$ significantly increase germination in nonsaline medium over either wet or dry controls (Table 1). Pretreatments with 0.5 *N* salt solutions, as with IAA, significantly reduced both the rate of germination and the final germination percentage. In nonsaline media or in 100 meq/liter NaCl, seed pretreated with 0.5 *N* $CaCl_2$ germinated faster than did those pretreated with NaCl. Final germination percentage was also greater with the 0.5 *N* $CaCl_2$ pretreatment. Again, these results might be indicative of the influence of Ca on membranes (14). In mixed-salt media, however, this pattern was reversed and germination rate of the NaCl-pretreated seed was higher than that of $CaCl_2$-pretreated seed (Table 1), although final germination percentages were not different.

Other workers (4) who used these pretreatments to drought-harden wheat and barley seedlings found germination was enhanced with distilled water and dilute $CaCl_2$ pretreatments. Although Jacoby and Oppenheimer (12) indicated that water pretreatments did not increase drought resistance of sorghum, they noted an increase in resistance to water loss in developing seedlings.

Dry seeds contain no functional membranes until water enters during the early minutes of hydration (18). During this period solutions containing ions, hormones and even proteins may traverse the nonfunctioning membrane and subsequently affect ensuing metabolic processes. Preconditioning seeds with a 4-hour water treatment allows membrane and protein hydration and an initiation of processes such as oxidative phosphorylation, RNA synthesis, polysome formation, and protein synthesis (20). When seeds are redried, membranes again lose their integrity (18). Little is known concerning the reversal of other metabolic activities. The seed evidently retains some advantage that allows more rapid germination in both saline and nonsaline environments upon rehydration. While small amounts of Ca and phosphate improved germination slightly, although nonsignificantly, hormone and other salt pretreatments did not increase germination rate more than did the water pretreatments.

Our studies with cotton provided no evidence that pretreatment increases salt tolerance during germination, as has been found for wheat (4, 11). The beneficial effects seem limited to water pretreatment hastening the germination of seed by 1 day over dry controls (Table 1). Under optimum field conditions this is hardly noteworthy, but under stress the difference may be significant.

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