Influence of Exogenous Fatty Acids on Cottonseed Germination

By

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Abstract

Effects of exogenous free fatty acid application on percentage germination and radicle length were investigated with more chill sensitive (Pima S-4) and less chill sensitive (Pima S-5) cottonseed (*Gossypium barbadense* L.) at chilling (14°C) and optimal (34°C) temperatures. Of the organic solvents able to solubilize free fatty acids, aqueous solutions as high as 5% dimethyl sulfoxide did not adversely affect germination at 34°C. Palmitic, oleic and linoleic acids (120 μM) were solubilized in 5% DMSO.

At 14°C, percent germination of fatty acid treated Pima S-5 significantly increased, while no difference was observed for Pima S-4. Conversely, radicle length of fatty acid treated Pima S-4 significantly increased especially with oleate and linoleate, while no difference was detected for Pima S-5. Fatty acid supplementation influenced neither germination nor radicle length for Pima S-4 and Pima S-5 at 34°C.

Introduction

Cotton (*Gossypium* spp.) is a chilling sensitive plant especially during germination and radicle elongation (Christiansen 1963). Differences in seedling emergence under field conditions during low soil temperatures have been correlated with total dry seed unsaturated/saturated fatty acid ratios (Bartkowski *et al.* 1977). Seed having high unsaturated/saturated fatty acid ratios and germinated at chilling temperatures maintain physiological and biochemical functions to a greater degree than seed with low ratios (Clay *et al.* 1976). This relationship appears to be a function of fatty acid moieties of lipids which influence physical and biochemical functions of membranes. Positive associations have been demonstrated between plants whose mitochondrial membranes have high mole percentages of unsaturated fatty acids and the ability of these plants to resist chilling dysfunctions (Lyons *et al.* 1964, Lyons and Raison 1970). The temperature at which a thermal phase-transition occurs in a membrane is related to the proportionality of unsaturated to saturated fatty acid residues. Membranes of plant tissues subjected to chilling temperatures undergo phase-transitions from a liquid-crystalline to a solid gel structure (Kumamoto *et al.* 1971, Overath *et al.* 1976). Insertion of free fatty acids into membranes has successfully altered membrane activities (Ahkong *et al.* 1973, Schramm *et al.* 1967).

In a similar manner, it may be possible to increase cottonseed chilling resistance by exogenous addition of free fatty acids. However, solubility limitations in aqueous solutions can minimize entry into the biological effectiveness of free fatty acids in seed. Therefore, it is desirable to identify organic solvents capable of solubilizing fatty acids without affecting germination adversely. Soaking cottonseed in unlimited water generally reduces seedling emergence in field plantings (Buxton *et al.* 1977). Therefore, the volume of solution applied to seed may be as important as the soaking time period.

The purpose of this study was threefold. First, determine an optimum method for adding an aqueous solution to cottonseed prior to germination without reduction of germination potential. Second, identify organic solvents which do not adversely influence germination and can serve as carriers for free fatty acid supplementation to seed. Third, evaluate the addition of free saturated and unsaturated fatty acids to more and less chill sensitive cottonseed germinated under both favorable and chilling temperatures.

Abbreviations: Fatty acids — $C_{16:0}$, palmitic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid. U/S, unsaturated/saturated; DMSO, dimethyl sulfoxide.

Materials and Methods

Seed. Commercial cottonseed (*Gossypium barbadense* L.), cultivars 'Pima S-4' and 'Pima S-5', were collected from plants grown in replicated yield trials at Phoenix, Arizona, during 1973. Seed were acid delinted and selected for undamaged and

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crack-free seed coats (Buxton and Sprenger 1976). Seed sinking in water at 25°C were considered mature, while floaters were discarded as immature seed.

Imbibition procedure. To determine the optimal amount of water that could be applied to cottonseed prior to germination, 50 seed were placed in 60 × 15 mm Petri plates with aliquots of glass distilled water ranging from 0.5 to 5.0 ml. Seed were stirred initially to insure uniform wetting. Petri plates were sealed and kept in darkness at 23°C. All water was imbibed by the seed within 2 to 2.5 h with the seed coats appearing dry. Seed were germinated at an optimum temperature of 34°C (Arndt 1935) for 40 h in germination paper dolls as outlined by Buxton and Sprenger (1976). Seed were considered germinated when the radicle extended at least 2 cm out of the seed coat. At the end of the germination period radical length of seedlings was determined. The experimental design was a randomized complete block with five replications per treatment.

Organic solvents. To identify organic solvents which would solubilize fatty acids and yet not inhibit seed germination, various organic solvents were similarly applied to cottonseed (2 ml/50 seed and imbibed within 2 to 2.5 h) at concentrations of 1, 3, 5, and 10% in glass distilled water. The compounds were acetic acid, acetone, *m*-cresol, dichloromethane, DMSO, ethanol, glycerol, pyridine, Triton X-100 and Tween 20 (polyoxyethene-20-sorbitan monolaurate). All compounds were reagent grade. Seed were germinated in paper dolls at 34°C and measured as described above. Four replications of each treatment were handled in a randomized complete block design.

Free fatty acid treatment. Cottonseed of Pima S-4 and S-5 were treated with fatty acid solutions (120 μM) in 5% DMSO as described above. This fatty acid concentration approached the upper limit at which palmitic acid dissolves in 5% DMSO. The fatty acids were palmitic, oleic, and linoleic acids. Fatty acids were reagent grade (Supelco, Inc., Bellefonte, PA 16823). Palmitic acid, a saturated fatty acid, and oleic and linoleic acids, unsaturated fatty acids, were used because they represented the principal fatty acids found in cottonseed of *G. barbadense* (Bartkowski *et al.* 1977). Seed were germinated at 34°C for 63 h or 14°C for 14 days. The lower temperature approaches the lower limit at which cottonseed will germinate (Wanjura and Buxton 1972). The experimental design consisted of three replications with each experimental unit having two 50-seed sub-samples arranged in a split plot design. Percent germination and radicle length were measured at the end of each study.

Results and Discussion

The effects of seed soaking injury have been investigated (Eystes 1940, Orphanos and Heydecker 1968, Roos and Pollock 1971). Our results show that germination percentage and radicle length were maximized by soaking 50 cottonseed with 2.0 ml water (Table 1). Water treatments in excess of 2.5 ml per 50 seed reduced germination percentage, although average radicle lengths did not differ significantly. Cottonseed of Pima S-4 and Pima S-5 responded similarly to all water treatments and the data presented were averaged for the two cultivars.

Table 1. *Effect of pregermination soaking of Pima S-4 and Pima S-5 cottonseed in water before germination at 34°C for 40 h.* Since both lines responded similarly to all treatments, the data were averaged over the two cultivars. Each value represents the mean of five replications per treatment and cultivar. Values followed by the same letter in each column are not significantly different at the 0.05 level according to the Student–Newman–Keuls test.

Water imbibed ml/50 seed	Germination %	Radicle length mm
None	66 ab	55.9 a
0.5	60 a–d	56.5 a
1.0	72 abc	53.7 a
1.5	78 ab	62.4 a
2.0	86 a	63.1 a
2.5	58 a–e	61.8 a
3.0	50 b–e	61.4 a
3.5	46 cde	57.4 a
4.0	30 e	59.5 a
4.5	36 de	53.9 a
5.0	28 e	59.5 a

These data indicate that a pregermination soaking with 2.0 ml water per 50 cotton seed optimizes germination potential. Seed index (seed weight per 100) averaged 12.3 for the two cultivars. Buxton *et al.* (1977) observed significant reductions in field emergence when cotton-seed were soaked in unlimited water for varying lengths of time prior to germination. Apparently, the volume of water and method of application to cottonseed appears to be more critical than duration of soaking.

Researchers have obtained conflicting results regarding the effects of organic solvents on germination (Brewer and Wilson 1975, Rao and Khan 1975). In our study, DMSO treated seed did not exhibit decreased percentage germination when compared to the control of water and actually significantly increased germination speed as measured by radicle length over all organic solvents and water (Table 2). Since cottonseed of Pima S-4 and Pima S-5 responded similarly to all treatments, the data was pooled. Though radicle length was significantly less at 10% than at 1, 3, and 5% DMSO, it was still statistically equivalent to or better than any other solvent tested. No other solvent showed such desirable characteristics. Acetone and ethanol achieved high germination percentages but tended to yield lower radicle lengths than DMSO.

DMSO has been employed as a carrier of agronomic and horticultural nutrients and pesticides (Leake 1966). It is completely miscible with water, has very low systemic toxicity and lowers freezing points of aqueous solutions. DMSO penetrates cellular membranes and will associate with most cellular components probably due to its polar

Table 2. *Effect of pregermination soaking of Pima S-4 and Pima S-5 cottonseed in organic solvents before germination at 34°C for 40 h.* Since both lines responded similarly to all treatments, the data were averaged over the two cultivars. Each value represents the mean of four replications per treatment and cultivar. Values followed by the same letter in each column are not significantly different at the 0.05 level according to the Student–Newman–Keuls test.

Solvent	Germination %	Radicle length mm
Water	92 a	61.6 b
1% Acetic acid	88 a	54.2 bc
3% Acetic acid	22 e–i	29.0 e–i
5% Acetic acid	4 hi	28.0 e–i
10% Acetic acid	0 i	0.0 j
1% Acetone	94 a	61.9 b
3% Acetone	92 a	59.7 b
5% Acetone	94 a	64.1 b
10% Acetone	90 a	61.6 b
1% *m*-Cresol	54 bcd	40.4 c–g
3% *m*-Cresol	0 i	0.0 j
5% *m*-Cresol	0 i	0.0 j
10% *m*-Cresol	0 i	0.0 j
1% DMSO	92 a	76.3 a
3% DMSO	92 a	70.0 a
5% DMSO	97 a	72.8 a
10% DMSO	95 a	55.5 bc
1% Dichloromethane	90 a	61.4 b
3% Dichloromethane	84 a	51.8 bc
5% Dichloromethane	48 b–e	41.2 c–f
10% Dichloromethane	26 d–i	37.9 d–h
1% Ethanol	92 a	61.0 b
3% Ethanol	90 a	58.5 b
5% Ethanol	96 a	58.5 b
10% Ethanol	74 ab	42.8 c–f
1% Glycerol	70 abc	44.8 cde
3% Glycerol	74 ab	37.9 d–h
5% Glycerol	66 abc	34.9 d–h
10% Glycerol	46 cde	29.7 e–i
1% Pyridine	44 cde	23.6 hi
3% Pyridine	38 ef	25.2 ghi
5% Pyridine	32 d–h	22.8 hi
10% Pyridine	10 f–i	22.9 i
1% Triton X-100	22 e–i	37.6 d–h
3% Triton X-100	8 ghi	36.3 d–h
5% Triton X-100	8 ghi	43.4 c–f
10% Triton X-100	4 hi	46.0 cd
1% Tween-20	36 d–g	41.4 c–f
3% Tween-20	36 d–g	46.7 cd
5% Tween-20	10 f–i	46.6 cd
10% Tween-20	12 f–i	32.0 d–i

nature. The ability to DMSO to solubilize fatty acids is known (Block 1964). Therefore, DMSO may serve as a carrier of fatty acids to membrane surfaces, which are considered to be a primary site of chilling dysfunction of plant tissue.

Speculation has arisen over the ability of DMSO to replace water in biological membranes (Beger and Hauthal 1977). This dipolar aprotic solvent can accept protons and is involved in the formation of hydrogen bonds. Proteins, a major component of membranes, have been shown to assume a conformation in DMSO with greater flexibility than the native protein, which in turn may enhance a beneficial interaction between lipids and proteins of membranes.

Cottonseed were determined to be more or less chill sensitive based on field emergence studies as outlined by Bartkowski *et al.* (1977). The percentage emergence of cottonseed subjected to low soil temperatures within the range in which chilling damage will occur (Lyons 1973) differed significantly (Pima S-4, 40% vs. Pima S-5, 67%). These values are significantly different at the 0.05 level according to the Student–Newman–Keuls test. This indicates that Pima S-5 cottonseed are considerably less chill sensitive than Pima S-4.

Cottonseed of Pima S-5 treated with fatty acids and germinated at 14°C had significantly higher germination percentages than 5% DMSO treatments (Table 3). With

Table 3. *Effect of pregermination soaking of Pima S-4 and Pima S-5 cottonseed in 120 µM fatty acid solutions of 5% DMSO on percentage germination and seedling radicle length.* Saturated fatty acid, palmitate ($C_{16:0}$), and unsaturated fatty acids, oleate ($C_{18:1}$) and linoleate ($C_{18:2}$), were studied. Seed were germinated at 14°C for 14 days and 34°C for 63 h. Each value represents the mean of three replications having 50 seed subsamples arranged in a split plot design. Values followed by the same letter in a column are not significantly different at the 0.05 level according to the Student–Newman–Keuls test. C.V., Coefficient of variation.

Treatment	Germination, %		Radicle length, mm	
	Pima S-4	Pima S-5	Pima S-4	Pima S-5
		14°C		
5% DMSO	59.8 a	84.5 b	18.9 b	36.5 a
$C_{16:0}$	69.3 a	91.8 a	22.7 ab	36.5 a
$C_{18:1}$	63.8 a	90.2 ab	25.2 a	38.9 a
$C_{18:2}$	68.7 a	93.8 a	26.4 a	39.4 a
Mean	64.1	88.0	22.3	37.3
C.V. %	14.4	13.7	19.0	12.9
		34°C		
5% DMSO	87.2 a	96.2 a	116.2 a	119.7 a
$C_{16:0}$	91.2 a	96.2 a	112.7 a	114.8 a
$C_{18:1}$	94.5 a	94.3 a	120.5 a	118.5 a
$C_{18:2}$	92.7 a	97.2 a	119.1 a	119.7 a
Mean	90.9	93.8	115.4	120.2
C.V. %	13.0	18.9	11.3	16.1

respect to germination, fatty acid addition did not influence Pima S-4 at 14°C. Pima S-4, which is more chill sensitive and has lower levels of endogenous unsaturated fatty acid than Pima S-5, might be expected to have its germination percentage improved at 14°C when supplied with exogenous fatty acids, especially oleic and linoleic acids. As noted in Table 3, only Pima S-5 responded positively to fatty acid supplementation through increased germination percentages.

However, a preference for saturated or unsaturated fatty acid was not observed. At 34°C, both fatty acid and DMSO treated Pima S-4 and Pima S-5 cottonseed responded similarly as measured by germination percentage and radicle length. Also, relatively low coefficients of variation indicated repeatability of results between experiments.

Radicle lengths were measured for Pima S-4 and Pima S-5 cottonseed treated with fatty acids and germinated at 14 and 34°C (Table 3). Germination studies conducted at 14°C approach the minimal temperature at which cottonseed will germinate and emerge under soil conditions, and the temperature is well within the range capable of inducing chilling injury (Wanjura and Buxton 1972). Fatty acid supplementation of Pima S-4 significantly increased radicle length over water and 5% DMSO treatments. Unsaturated fatty acids, oleic and linoleic, tended to influence radicle length more positively than palmitic, a saturated fatty acid. Fatty acid effectiveness was not demonstrated on radicle length in Pima S-5 germinated at 14°C nor in Pima S-4 or Pima S-5 at 34°C.

Results of this study suggest that the relative effectiveness of fatty acid supplementation may be related to the ability of cottonseed to utilize both exogenous and endogenous fatty acid pools simultaneously at the time of germination and subsequent radicle elongation. The mechanism of fatty acid involvement in more and less chill sensitive cottonseed is not presently understood. Research is now being conducted to clarify this point.

In conclusion, this study has shown a potential for supplying cottonseed with additional free fatty acid via an organic solvent, DMSO, prior to germination to minimize chilling sensitivity. Cottonseed that are less susceptible to chilling injury might be planted at earlier dates, have improved stand potential and be an economic benefit to growers.

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