Published March, 1977

Cottonseed Germination Related to DNA Synthesis Following Chilling Stress[1]

Willard F. Clay, D. R. Buxton, and Frank R. H. Katterman[2]

ABSTRACT

Identification of cotton (*Gossypium* spp.) seed lots that establish uniform stands has been inconsistent with the standard germination test, especially when soil temperatures are low. To aid in developing better predictive tests, critical biological processes associated with seed germination at low temperatures need to be better understood. This study was conducted to evaluate the relationship between DNA synthesis and seed performance. Pima cottonseed (*G. barbadense* L.) lots were evaluated during 3 years of experimentation for field emergence, germination percentage at both 15 and 25 C, and capacity to synthesize DNA during germination following a chilling stress. DNA synthesis was positively correlated with seed germination percentage at both temperatures, with the closest association occurring at 15 C when a large number of seed lots were evaluated. The DNA test was more consistently correlated with field emergence than percent germination. These results show that capacity to synthesize DNA following chilling is an important process associated with seed germination and emergence during low soil temperatures.

***Additional index words:* *Gossypium barbadense* L., Chilling stress, Seedling emergence, Standard germination test, Deoxyribonucleic acid.**

SEED germination and seedling emergence in many crops, including cotton (*Gossypium* spp.), are adversely affected by low or chilling temperatures. Exposure to chilling may accentuate differences in germination among cotton cultivars which are related to genetic (3) and environmental backgrounds of the parent plants (17, 18). Identification of seed lots that germinate rapidly and establish uniform stands, especially at low soil temperatures, has been inconsistent with the standard germination test (9, 22, 23, 28). In part, this occurs because of the favorable temperatures of the standard germination test compared to field conditions. Genetic lines that germinate well at favorable temperatures often germinate poorly at low temperatures (3).

In order to develop better predictive methods of field performance of seed during low soil temperature, critical biological processes need to be better understood. Exposing sensitive plants to low temperature results in alterations in nucleic acid and protein metabolism (11, 16) and degradation of membrane lipids (29). Plant mitochondria are also known to be sensitive to low temperatures (2, 19).

The capacity to synthesize DNA during low temperature stress has been studied in various plant systems. Low temperature inhibits DNA replication in *Chlorella* (24). Cultivars of wheat (*Triticum aestivum* L.) (10) and applies (*Pyrus malus* L.) (14) adapted to low temperature environments have been shown to efficiently synthesize DNA during chilling stress.

In a previous communication, we demonstrated that chilling damage to cotton seedlings (*G. barbadense* L.) was associated with impaired capacity of DNA synthesizing enzymes to incorporate radioactive precursors into DNA (7). In this paper, we provide evidence that the capacity to synthesize DNA by cotton seedlings following chilling is related to seedling performance during low soil temperatures.

MATERIALS AND METHODS

DNA Synthesis Analysis

Seed were surface sterilized with 1% sodium hypochlorite and germinated for 34 hours at 34 C as previously outlined (8). As established in previous communications, seedlings of *G. barbadense* L. actively replicate DNA between 30 and 36 hours of germination (6, 8, 13). This time period also is a period of sensitivity to chilling damage (4, 7). After 34 hours of germination, seedlings were chilled at 3 C for 5 hours. Chilling at this time has been shown to appreciably reduce DNA replication (7).

After chilling, 20 seedlings of each seed lot (radicle length 3 to 4 cm) were washed with 0.1% sodium hypochlorite and distilled H_2O. The seedlings were incubated at 34 C for 90 min with 100 μCi of ^3H — thymidine diluted with 3 ml of distilled H_2O. After incubation, the seedlings were frozen at —70 C and the DNA isolated from excised radicles by a modified procedure of Humphreys (12) and Smillie and Krotkov (20). Radicles were ground in cold grinding medium (0.02 *M* sucrose, 0.025 *M* KCl, 0.01 *M* $CaCl_2$, 0.05 *M* Tris-HCl pH 8.0) and cold 12 *M* $HClO_4$ was added to a concentration of 0.5 *M*. The precipitate was collected by centrifugation and incubated with 0.3 *M* NaOH at 37 C for 18 hours. DNA was precipitated by adding cold 12 *M* $HClO_4$ to 1 *M* and 2 volumes of cold ethanol. The precipitate was washed with cold 0.2 *M* $HClO_4$ and the DNA hydrolized to nucleotides with 0.5 *M* $HClO_4$ at 90 C for 20 min. The nucleotide solution was clarified by centrifugation and the specific activity analyzed as previously described (8). Each experiment contained three replications in a randomized block design.

Field Experiments

Field experiments with Pima cottonseed were conducted in 1973, 1974, and 1975 at the Univ. of Arizona Cotton Center in Phoenix and the Experiment Station at Marana. The soil at Phoenix is Avondale clay loam, a member of the fine-loamy, mixed hyperthermic Torrifluventic Haplustolls. The Marana soil is a Pima clay loam, a member of the fine silty, mixed, thermic Typic Torrifluvents.

In 1973 and 1974, two planting dates were used at each location. In 1975, one planting was made at Marana and one in Phoenix. Plantings were in a randomized block design with five replications in 1973, six in 1974, and eight in 1975. Individual plots consisted of 100 seed planted in 9.4 m of row with rows approximately 1 m apart. Seed rows were capped with about 10 cm of soil. After radicle emergence, the caps were removed so that seedlings emerged through about 4 cm of soil.

The 1973 tests compared five lines from a common environmental background grown at Phoenix in 1972, to maximize genetic differences and to minimize differences due to parent plant environment. The 1974 tests compared four lots of 'Pima S-4' grown under different environmental conditions to maximize differences due to parent plant environment. These lots

[1] Contribution for the Arizona Agric. Exp. Stn. as Technical Paper No. 2642. Supported in part by funding from the SuPima Assoc. of America. Received 29 July 1976.

[2] Research associate, associate professor, and professor, Dep. of Plant Sciences, Univ. of Arizona, Tucson, AZ 85721.

Table 1. Summaries of capacity to synthesize DNA after chilling stress, seedling emergence per 100 seed planted in field experiments, and laboratory germination experiments conducted at 15 and 25 C.

Seed lot	DNA specific activity	% germination 15 C	% germination 25 C	% Field emergence
	cpm/μg			
		1973, Genetic lines		
E-2	478 a*	71 b	85 a	69 a
Pima S-3	344 b	72 b	83 a	67 ab
P-23	286 b	91 a	87 a	64 ab
Pima S-4	202 c	72 b	87 a	58 bc
P-21	185 c	50 c	89 a	60 a-c
		1974, Seed source		
Pima S-4, Marana, AZ	868 a	70 a	96 a	62 a
Pima S-4, Safford, AZ	596 b	64 ab	95 a	54 b
Pima S-4, Salome, AZ	545 b	22 c	84 b	64 a
Pima S-4, Fabens, TX	385 c	32 c	76 b	27 c
		1975, Commercial lots		
Pima S-4, lot 189	621 a-d	56 b-f	90 a	61 a-d
Pima S-4, lot 191	621 a-d	68 a-c	89 a	55 c-g
Pima S-4, lot 190	537 b-e	64 a-e	91 a	58 b-f
Pima S-4, lot 199	534 b-e	70 a-c	83 a	56 c-g
Pima S-4, lot 198	485 d-f	56 b-f	87 a	51 d-h
Pima S-4, lot 192	483 d-f	63 a-e	88 a	50 e-i
Pima S-4, lot 193	474 d-f	50 c-f	86 a	52 d-h
Pima S-4, lot 194	461 e-g	40 f	87 a	46 g-i
Pima S-4, lot 197	391 e-g	48 d-f	88 a	44 h-i
Pima S-4, lot 200	386 fg	44 ef	87 a	40 ij
Pima S-4, lot 196	376 fg	39 f	90 a	44 h-j
Pima S-4, lot 195	333 g	56 b-f	89 a	48 f-j
P-28, lot 5	677 ab	73 ab	89 a	61 a-d
P-28, lot 3	650 ab	66 a-d	89 a	60 a-d
P-28, lot 4	648 abc	75 ab	88 a	63 a-c
Pima S-5, lot 3	679 a	73 ab	94 a	70 a
Pima S-5, lot 6	674 ab	81 a	96 a	70 a
Pima S-5, lot 5	645 ac	79 a	94 a	66 ab
Pima S-5, lot 4	500 cf	70 a-c	87 a	60 b-e

* Numbers for each parameter within a year followed by the same letter are not significantly different at the 0.05 level according to the Student-Newman-Keuls Test.

were grown in 1973 at Salome, Marana, and Safford, Arizona, and Fabens, Texas. The seed lots were the courtesy of C. V. Feaster, USDA, and delinted as previously described (3, 4).

The 1975 tests were expanded to include 12 commercial lots of Pima S-4, four of 'Pima S-5', and three of an experimental line P-28. These lots were distributed by the Arizona Cotton Planting Seed Distributors. The seed used in 1973 and 1974 were not treated with a fungicide while the lots used in 1975 were treated with combinations of PCNB (pentachloronitrobenzene), Captan [N-(trichloro--methylthio)-4-cyclohexene-1,2-dicarboximide], and methoxyclor [1,1,1-trichloro-2,2bis(P-methoxyphenyl)ethane].

As seedlings began to emerge from the soil, counts were made two or three times per week until after emergence was complete. Daily maximum and minimum soil temperatures at planting depth were recorded during emergence by averaging the readings from two thermometers. The average daily minimum temperatures for the plantings in Phoenix ranged from 11.3 to 14.3 C for the first plantings of each year and 13.4 to 14.4 C for the second. For Marana, the ranges were 14.1 to 16.6 C for the first and 16.0 to 16.4 C for the second plantings.

Germination Experiments

All seed lots were germinated at both 15 and 25 C between March and July in the same year that the seed were planted in the field. Twenty-five seed were germinated in rolled germination sheets in temperature-controlled water baths (3, 4). Seed were germinated for 4 days at 25 C and 8 days at 15 C and percent germination determined by counting seedlings with undamaged radicles longer than 1 cm. These experiments were in a completely randomized design with 8, 6, and 5 replications in 1973, 1974, and 1975, respectively.

Table 2. Correlation coefficients between DNA synthesis after chilling stress and field emergence and seed germination.

Parameter	1973	1974	1975
Field Emergence	0.95*	0.72	0.90**
% Germination 15 C	0.30	0.74	0.80*
% Germination 25 C	-0.71	0.85	0.51

*,** Significant at 0.05 and 0.01 levels, respectively.

RESULTS AND DISCUSSION

Although the planting dates were normal, all plantings were subjected to at least one daily minimum soil temperature of 7 to 13 C during emergence which is below optimum for cottonseed germination and emergence (25, 26). The low soil temperatures were in the range reported to cause chilling damage (4, 5, 7, 25, 26) and were associated with extended periods required for 50% of total emergence ranging from 11.2 to 27.6 days.

It has been previously reported that seed lot field performance is influenced by both parent plant environment and genetic differences (3, 17, 18). In the test conducted in 1973, lines were studied that had a common environmental background and in 1974, the same genetic line with different environmental backgrounds was used. Differences in seedling emergence data among the Pima lines planted in 1973 and 1974, presented as averages for the plantings in each year, were significant (Table 1) supporting the combined effects of genetic and environmental backgrounds (3, 17, 18). The number of seed lots was expanded in the 1975 tests to include 19 lines. The range in emergence differences was large (40 to 70%) with the differences being significant at the 0.01 level (Table 1).

Differences in laboratory germination were significant for the 3 years studied when conducted at 15 C but were inconsistent at 25 C (Table 1). These results, which have been consistently obtained (3), indicate that differences in chilling resistance are enhanced when seed are germinated at low temperatures.

It would be of special significance if physiological responses could be correlated with plant performance under adverse field conditions. Physiological responses in response to chilling (11, 16, 21, 27) and differences in chilling resistance correlated with fatty acid composition (11, 15, 19) have been reported. We have demonstrated that the time period of maximum chilling sensitivity during germination (4, 7) corresponds with maximum DNA synthesis which is associated with the nuclear membrane (6, 8). Subjecting seedlings to a chilling stress during the sensitive period results in a marked reduction in DNA synthesis and DNA polymerase activity (7).

As shown in Table 1, the capacity of germinated seed to synthesize DNA, as measured by the incorporation of ^{3}H-thymidine after chilling stress, differed significantly among the seed lots in each of the 3 years. The range was large for each year with differences significant at the 0.01 level.

Correlations were made to determine if the differences in DNA synthesis during chilling stress were significantly associated with field emergence and labora-

tory germination (Table 2). Correlation coefficients between specific activity of DNA isolated after chilling and field emergence were consistently positive for all 3 years and significant for 1973 and 1975. Correlations with laboratory germination were positive at 15 C and significant for 1975 where the number of entries was large. Correlations with germination at 25 C were inconsistent which has been routinely noted.

Differences in DNA synthesis among seed lots are significantly only when seedlings are subjected to a chill stress (unpublished results). This fact, coupled with correlations of DNA synthesis being significant with germination or field emergence when temperatures are suboptimal (Table 2), indicate that differences in chilling resistance are more reliably detected at lower temperatures. This is important if seed are planted when field conditions are adverse since decisions on planting seed are commonly made on results of standard germination tests conducted at more optimum growth temperatures (8 hours at 20 C and 16 hours at 30 C for 12 days.

Many physiological parameters are probably adversely affected by exposure to chilling; however, the results reported here demonstrate the capacity to synthesize DNA following chilling stress is one physiological parameter closely associated with seedling performance when temperatures are unfavorable. Thus seed lots that have the capacity to complete the replication of nuclear DNA under chilling conditions are apparently better adapted to germinate and emerge when temperatures are unfavorably low.

REFERENCES

1. Bartkowski, Edmund J., D. R. Buxton, Frank R. H. Katterman, and Henry W. Kircher. 1977. Dry seed fatty acid composition and seedlings emergence of Pima cotton at low soil temperatures. Agron. J. 69:37-40.
2. Breidenbach, R. W., N. L. Wade, and J. M. Lyons. 1974. Effect of chilling temperatures on the activities of glyoxysomal and mitochondrial enzymes from castor bean seedlings. Plant Physiol. 54-324-327.
3. Buxton, D. R., and P. J. Sprenger. 1976. Genetic variability for cottonseed germination at favorable and low temperatures. Crop Sci. 16:243-246.
4. ———, ———, and E. J. Pegelow, Jr. 1976. Periods of chilling sensitivity in germinating Pima cottonseed. Crop Sci. 16:471-474.
5. Christiansen, M. N. 1967. Periods of sensitivity to chilling in germinating cotton. Plant Physiol. 43:431-433.
6. Clay, W. F., F. R. H. Katterman, and P. G. Bertels. 1975. Chromatin and DNA synthesis associated with nuclear membrane in germinating cotton. Proc. Natl. Sci. USA 72:3134-3138.
7. ———, E. J. Bartkowski, and F. R. H. Katterman. 1976. Nuclear DNA metabolism and membrane fatty acid content related to chilling resistance in germinating cotton. Physiol. Plant (In Press).
8. ———, F. R. H. Katterman, and J. R. Hammett. 1975. Nucleic acid metabolism during germination of Pima cotton (*Gossypium barbadense*). Plant Physiol. 55:231-236.
9. Comer, L. L. 1968. Evaluation of tests to predict performance of cottonseed (*Gossypium hirsutum* L.) and (*Gossypium barbadense* L.) M.S. Thesis. Univ. of Arizona, Tucson.
10. Das, P. K., and H. K. Jain 1972. Studies on adaptation in wheat. Z. Pflanzenzuecht. 68:18-27.
11. Guinn, G. 1971. Changes in sugars, starch, RNA, protein, and lipid-soluble phosphate in leaves of cotton plants at low temperatures. Crop Sci. 11:262-265.
12. Humphreys, T. Quantitative measurements of RNA synthesis. p. 141-163. *In* Molecular techniques and approaches in developmental biology. M. J. Chrispeels, Jr. (ed.). Wiley and Sons. 1973.
13. Katterman, F. R. H., and W. F. Clay. 1975. Size and specific radioactivity of the [^{3}H]-thymidine triphosphate pool and DNA synthesis in the radicles of germinating cotton. Plant Physiol. 56:523-525.
14. Li, P. H., and C. J. Weiser. 1969. Metabolism of nucleic acids in one-year old apple twigs during old hardening and dehardening. Plant Cell Physiol. 10:21-30.
15. Lyons, J. M., and J. K. Raison. 1970. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiol. 45:386-389.
16. Omran, R. G., C. R. Benedict, and R. D. Powell. 1971. Effects of chilling on protein synthesis and CO_2 fixation in cotton leaves. Crop Sci. 11:554-556.
17. Peacock, H. A., and B. S. Hawkins. 1970. Effect of seed source on seedling vigor, yield, and line characteristics of upland cotton, *Gossypium hirsutum* L. Crop Sci. 10:667-670.
18. Quisenberry, J. E., and J. R. Gipson. 1974. Growth and productivity of cotton grown from seed produced under four night temperatures.
19. Raison, J. K., J. M. Lyons, R. J. Mehlhorn, and A. D. Keith. 1971. Temperature-induced phase changes in mitochondrial membranes detected by spin labelling. J. Biol. Chem. 246: 4036-4040.
20. Smillie, R. M., and G. Krotkov. 1960. The estimation of nucleic acids in some algae and higher plants. Can. J. Bot. 38:31-49.
21. Taylor, A. O., R. Slack, and H. G. McPherson. 1974. Plants under climatic stress. Plant Physiol. 54:696-701.
22. Tekrony, D. M., D. B. Engli, and A. Phillips. 1974. A seed vigor index for evaluating soybean quality and estimating field emergence Am. Soc. Agron. Abstr., p. 94.
23. Vanderlip, R. L., F. E. Mockel, and Halim. 1973. Evaluation of vigor tests for sorghum seed. Agron. J. 65:486-488.
24. Wanka, F., and J. Geraedto. 1972. Effect of temperature in the regulation of DNA synthesis in synchronous cultures of *Chlorella*. Exp. Cell Res. 71:188-192.
25. Wanjura, D. F., and D. R. Buxton. 1972. Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. Agron. J. 64:427-431.
26. ———, ———, 1972. Hypocotyl and radicle elongation of cotton as affected by soil environment. Agron. J. 64:431-434.
27. Yang, J., and G. N. Brown. 1974. Isoaccepting transfer ribonucleic acids during chilling stress in soybean hypocotyls. Plant Physiol. 53:694-698.
28. Yayock, J. Y., H. Jan, and R. L. Vanderlip. 1975. Temperature, time and NH_4Cl concentration in vigor testing of sorghum seed. Agron. J. 67:241-242.
29. Yoshida, S., and A. Sakai. 1974. Phospholipid degradation in frozen plant cells associated with freezing injury. Plant Physiol. 53:509-511.