CRYOGENIC PRESERVATION OF COTTONSEED WITH LIQUID NITROGEN

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Abstract

Cottonseed were placed in culture tubes and the tubes were submerged in liquid nitrogen LN_2 (-196ºC) for periods of 3 to 180 days. The seed were then returned to room temperature and evaluated for quality, using the Cool-Warm Vigor Index test and field studies. Storage with LN_2 through 180 days had no significant effect on the germination and vigor of different varieties of cottonseed, and young seedlings from the treated seed showed no more tissue damage than seedlings from control seed. Also, treated and control cottonseed grown under field conditions had similar stands and appeared to produce similar amounts of seed cotton. When germination studies were carried out to determine the best seed moisture levels for LN_2 treatment, the treated cottonseed performed best at moisture levels between 4 and 12.3%. LN_2 treated seed , however, showed a significant drop in quality at moisture levels above 12.3%. Direct immersion of cottonseed into LN_2 often caused severe damage to the cotyledons of young seedlings, and though these seed germinated well on paper towels, they did not have good stands under field conditions. These cryogenic studies indicate that storage above but not directly in LN_2 may be suitable for long-term preservation of cottonseed and cotton germplasm.

Introduction

Liquid nitrogen (LN_2) at -196ºC has the potential of providing indefinite preservation of seed germplasm and has been shown to be effective with the seed of a number of crops (1, 2). It is used for long-term storage of various types of seed at the USDA Seed Storage Laboratory at Fort Collins, CO, but to our knowledge has not been reported to be of use for the preservation of cottonseed. LN_2 also may be helpful in the storage of deteriorated cottonseed and cottonseed infected with microorganisms for long-term studies. The intent of this study was to: 1) screen healthy and deteriorated seed of a number of varieties of cotton for their tolerance to LN_2 cooling and rewarming; and 2) test the viability and vigor of the seed after storage in LN_2 for up to 180 days.

Materials and Methods

Seed varieties Acid-delinted cottonseed of the varieties Acala 1517-88, Acala Maxxa, Coker 320, Deltapine 50, Deltapine 90, Deltapine 5415, Paymaster HS-26, Stoneville 453, and TAMCOT HQ-95 were used. Most of the seed used were of high quality and were obtained from companies or breeders; however, certain lower quality seed samples were obtained from cotton harvested in College Station after periods of weathering.

Seed moisture content Moisture content (wet weight basis) was determined by oven-drying in triplicate 20 gm samples at 105ºC for 48 hr. Cottonseed were placed in desiccators containing Drierite for 8 to 12 days to obtain moisture contents of 2 to 5%. Desiccators containing deionized water or saturated solutions of magnesium acetate or $(NH_4)H_2PO_4$ were used to obtain moistures of 9 to 17%.

Indirect exposure to liquid nitrogen Healthy and deteriorated cottonseed were placed in 25 x 250 mm culture tubes and the tubes submerged in LN_2, to prevent direct contact with LN_2. The seed were then stored near -196ºC for 3 to 180 days. A model 8038 CryoMed portable LN_2 container (47.4 L) with six canisters was used to store the tubes. Upon removal of the tubes from LN_2, the cottonseed were directly transferred to room temperature and allowed to warm for 1 to 2 hr. They were then dried overnight at 40ºC to reduce moisture.

Germination studies The cottonseed were treated with Captan, placed on germination towels, and evaluated for viability and vigor, using the Cool-Warm Vigor Index (CWVI) test. This test consists of two parts: 1) a warm germination test that cycles daily for 16 hr at 20ºC and 8 hr at 30ºC, counted after 4 days; and 2) a cool germination test at 18ºC, counted at exactly the end of 7 days. Germination percentages were added together to give the CWVI. Each germination test was carried out on 200 seed with 25 seed on each germination towel. Thus, the CWVI was determined by studying 400 seed. The CWVI was also determined for control cottonseed of each variety that were not exposed to LN_2.

Field Studies Captan-treated cottonseed of six varieties that were stored under cryogenic conditions for 75 days were planted in a field at College Station. Each variety had 120 seed that were divided into four plots with 30 seed each, using a random block design. Also, 120 control seed for each variety were included and divided into four plots. The stands for each plot were determined and the plants that grew were evaluated for appearance and yield.

Direct exposure to liquid nitrogen Cottonseed were wrapped in cheesecloth and immersed directly in LN_2 for 3 to 90 days. They were then returned to room temperature, dried overnight at 40ºC , and used in germination and field studies.

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Results

Cottonseed from culture tubes submerged in LN_2 for periods of 3, 90 and 180 days had similar CWVI values as control cottonseed, and the warm and cool germination components of the CWVI values indicated that there was not a significant decline in cottonseed quality over the 180 day period (Table 1). Seedlings from the treated and control groups of cottonseed looked healthy and usually had normal appearing radicles, cotyledons and related plant structures. Cottonseed exposed to the ultra-cold conditions also grew well under field conditions and produced normal appearing plants. The treated and control cottonseed also produced similar stands, and appeared to yield similar amounts of seed cotton.

The moisture content of cottonseed at the time of freezing usually was not a problem, and similar CWVI values were obtained for treated and control batches of cottonseed, containing between 2 and 12% moisture. There was, however, a noticeable decline in the quality of the various cottonseed at moisture levels between 12.3 and 16.6% indicating that these seed had difficulty withstanding the ultra-cold conditions. Thus, it appeared that the best moisture range for preserving cottonseed is probably between 4 and 12%, since below 4%, there sometimes was a drop in both LN_2-treated and control seed quality.

We did not determine if seed storage near -196ºC significantly affects the frequency of fungal contaminants in weathered cottonseed; however, we found that fungi which are common pathogens to cottonseed, i.e., *Fusarium equiseti*, *F. pallidoroseum*, *Alternaria alternata*, and *Cladosporium spp.* can survive in cottonseed 180 days, when the seed are stored near -196ºC. This suggests that cryogenic techniques may be of help in preserving cottonseed in various stages of infection. This could be useful where studies with infected seed take long periods of time to complete.

Our first cryogenic studies were carried out with cottonseed that had been immersed directly in LN_2. Cottonseed immersed directly in LN_2, for periods between 3 and 90 days were damaged; however the damage appeared to be confined to the cotyledons, leaving healthy appearing radicles, hypocotyls, and related structures. Although cotyledons frequently contained only a few small splits, they sometimes were badly torn with more than 50% of the total cotyledonary tissue missing. The cottonseed immersed directly in LN_2 germinated well on germination towels but did not produce good stands in the field.

Conclusions

These studies show that healthy and deteriorated cottonseed can be stored near -196ºC for up to 180 days without a significant loss of quality. This suggests that longer-term storage in LN_2 would probably have little detrimental effect on the seed.

The optimal moisture range for cryopreservation of cottonseed by LN_2 appears to be between 4 and 12%.

Storage of cottonseed above LN_2 is recommended, although the temperature is slightly higher than -196ºC. The USDA Seed Storage Laboratory in Fort Collins also stores seed above LN_2 (at temperatures of approximately -150 to -180ºC). It is likely that direct immersion of cottonseed in LN_2 causes some of the liquid to accumulate in the seed. This trapped liquid is then probably rapidly released as a gas when the seed are removed from LN_2. The rapid degassing of LN_2 trapped in cotyledonary folds of the seed may account for the cotyledonary damage that occurs with direct immersion in LN_2.

The use of LN_2 is helpful for preserving deteriorated and/or infected cottonseed for later studies. However, this method should be used with caution, since ultra-cold conditions affect membranes and may influence cellular processes that are restored after warming.

Disclaimers

Mention of firm names or trade products does not imply that they are ndorsed or recommended by the U.S. Department of Agriculture over firms or similar names not mentioned.

References

Stanwood, P. C. 1980. Tolerance of crop seeds to cooling and storage in liquid nitrogen (-196ºC). J. Seed Technol. **5:** 26-31.

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Table 1. Cottonseed Cryopreservation Study Using Liquid Nitrogen (LN) for 3, 90 and 180 days

Varieties	Treatmnt (days above LN)	Warm Germ. Test in % (S.E.) *A*	Cool Germ.Test in % (S.E.) *B*	Cool-warm Vigor Index *A+B*	Ratio (C-W Vigor Index for 3, 90, or 180 days divided by that for 0 days)
Deltapine 50	0	94.5 (1.7) A	92.5 (1.8) A	187.0	n.a.
	3	93.0 (2.0) A	85.0 (3.0) B	178.0	0.95
	90	94.0 (1.5) A	89.0 (2.1) A,B	183.0	0.98
	180	94.5 (1.3) A	91.5 (1.6) A	186.0	1
Deltapine 5415	0	96.0 (0.8) A	88.0 (1.9) A	184.0	n.a.
	3	96.5 (1.6) A	72.0 (1.7) C	168.5	0.92
	90	90.0 (2.0) B	79.0 (3.4) B	169.0	0.92
	180	98.0 (0.8) A	87.0 (2.0) A	185.0	1.01
Deltapine 90	0	99.5 (0.5) A	94.5 (1.5) A	194.0	n.a.
	3	99.5 (0.5) A	95.5 (1.6) A	195.0	1
	90	95.5 (1.6) B	88.5 (2.4) B	184.0	0.95
	180	97.5 (1.1) A,B	81.5 (2.5) C	179.0	0.92
TAMCOT HQ-95	0	92.0 (1.9) A	52.5 (2.8) A	144.5	n.a.
	3	88.0 (2.7) A,B	52.5 (4.0) A	140.5	0.97
	90	87.5 (1.9) A,B	48.5 (3.3) A	136.1	0.94
	180	86.0 (1.3) B	57.5 (2.5) A	143.5	0.99
Paymaster HS-26 (1995)	0	91.0 (2.2) A	86.0 (1.7) A	177.0	n.a.
	3	92.0 (1.9) A	84.5 (1.8) A,B	176.5	1
	90	89.5 (0.7) A	78.0 (3.4) B	167.5	0.95
	180	92.5 (1.4) A	87.5 (2.4) A	180.0	1.02
Paymaster HS-26 (1994)	0	95.0 (1.0) A	81.5 (2.7) A	176.5	n.a.
	3	90.0 (2.0) A	81.5 (3.7) A	171.5	0.97
	90	94.0 (1.9) A	80.0 (2.0) A	174.0	0.99
	180	94.0 (2.7) A	80.0 (3.3) A	174.0	0.99
Acala Maxxa	0	93.3 (1.9) A	93.0 (2.0) A	186.3	n.a.
	3	94.0 (1.5) A	90.5 (1.7) A	184.5	0.99
	90	96.0 (0.8) A	91.0 (1.3) A	187.0	1
	180	94.0 (2.4) A	90.0 (1.9) A	184.0	0.99
Acala 1517-88	0	94.0 (2.0) A	78.5 (3.9) B	172.5	n.a.
	3	93.0 (2.1) A	68.0 (2.7) C	161.0	0.93

	90	92.5 (1.6) A	84.0 (2.6) A,B	176.5	1.02
	180	87.5 (3.2) A	87.0 (2.1) A	174.5	1.01
Stoneville 453	0	95.5 (1.2) A	90.0 (2.3) A	185.5	n.a.
	3	91.5 (1.4) A	86.5 (0.7) A	178.0	0.96
	90	91.5 (1.8) A	89.0 (2.4) A	180.5	0.97
	180	95.5 (1.6) A	92.0 (3.2) A	187.5	1.01

1. The cottonseed used in these studies contained moisture levels between 4.4% for Stonville 453 and 8.7% for Paymaster HS-26 (1994).

2. Values for the germination tests are the mean and standard error of eight samples. Data were subjected to ANOVA and Fisher's LSD test. Within treatments of each variety, means followed by the same letter are not significantly different ($P<0.05$).