EFFECT OF DESICCATION AND CRYOPRESERVATION ON THE GERMINATION OF EMBRYONIC AXES AND SEEDS OF COTTON[1]

MARIA ELENA GONZÁLEZ-BENITO[2], JULITA MARÍA FROTA-CHAGAS CARVALHO[3] and CÉSAR PÉREZ[4]

ABSTRACT - The aim of the present work was to explore the feasibility of seed cryopreservation as a tool for cotton (*Gossypium hirsutum* L.) genetic resources conservation. Therefore, seeds of five cotton cultivars (CNPA 4M, CNPA 5M, CNPA Precoce 1, CNPA Precoce 2, Coker 312) weresubjected to desiccation and/or immersion in liquid nitrogen. Germination was not affected by any of the treatments in cultivars CNPA 4M, CNPA Precoce 1 and Coker 312. Desiccation from 9.8% and 15.6% moisture contents to 2.8% and 3.0% decreased seed viability in CNPA 5M and CNPA Precoce 2. However, germination of desiccated seeds improved after freezing. Embryonic axes of CNPA Precoce 2 and Coker 312 withstood cryopreservation when their moisture content was 19.4% or lower. Humidification did not have any effect on germination of Coker 312 seeds; on the other hand, it improved viability of CNPA Precoce 2 desiccated seeds.

Index terms: *Gossypium hirsutum*, liquid nitrogen.

EFEITO DA DESSECAÇÃO E CRIOCONSERVAÇÃO NA GERMINAÇÃO DE EIXOS EMBRIONÁRIOSE SEMENTES DE ALGODÃO

RESUMO - O objetivo do presente trabalho foi explorar a viabilidade da crioconservação de sementes como um método de conservação do patrimônio genético do algodão. Sementes de cinco cultivares de algodão (*Gossypium hirsutum* L.) (CNPA 4M, CNPA 5M, CNPA Precoce 1, CNPA Precoce 2 e Coker 312) foram submetidas a dessecação e/ou imersão em nitrogênio líquido. A germinação não foi afetada por nenhum dos tratamentos nas cultivares CNPA 4M, CNPA Precoce 1 e Coker 312. A viabilidade das sementes das cultivares CNPA 5M e CNPA Precoce 2 foi reduzida quando estas sementes foram dessecadas e o teor de umidade baixou de 9,8 e 15,6% para 2,8 e 3%, respectivamente. Entretanto, a germinação das sementes dessecadas foi aumentada depois da imersão em nitrogênio líquido. Os eixos embrionários das cultivares CNPA Precoce 2 e Coker 312 resistiram a crioconservação quando seu teor de umidade foi igual ou menor do que 19,4%. O umedecimento das sementes da cultivar Coker 312 não teve nenhum efeito na germinação, enquanto que a viabilidade das sementes da cultivar CNPA Precoce 2 foi aumentada pela dessecação.

Termos para indexação: *Gossypium hirsutum,* nitrogênio líquido.

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[2] Eng. Agr., Dr., Dep. de Biologia Vegetal, E.T.S.I., Universidad Politécnica de Madrid, 28040 Madrid, Espanha.

[3] Eng. Agr., Dr., Embrapa-Centro Nacional de Pesquisa de Algodão (CNPA), R. Oswaldo Cruz, 1143, Centenário, CEP 58107-720 Campina Grande, PB, Brazil.

[4] Eng. Agr., Dr., Dep. de Biologia Vegetal, E.T.S.I., Univ. Politéc. de Madrid.

INTRODUCTION

Seed banking at low temperatures and low moisture contents usually allows the preservation of plant material for long periods of time (Cromarty et al., 1982). However, loss of viability can occur with prolonged storage, depending on species (Roberts & Ellis, 1984). It has been claimed that biochemical processes are virtually stopped at ultra-low temperatures (liquid nitrogen, -196ºC), reducing biological deterioration in seed storage (Stanwood, 1985; Pritchard, 1995). Cryopreservation could improve cotton seed longevity and, therefore, be an important tool for the genetic resources conservation of this crop. Stanwood & Bass (1978) reported the high germination percentage of *Gossypiumhirsutum* L. seeds with moisture content lower than 13% after cryopreservation and rapid thawing.

The behaviour of seeds of several Brazilian cultivars has been studied after immersion in liquid nitrogen (LN) and desiccation. All of them are early cotton cultivars, important in the production system of cotton, due

to their avoidance of plagues such as boll weevil (*Anthonomus grandis* Boh.) (Bajaj & Gill, 1992). A commercial cultivar (Coker 312) is also included for comparison. In addition, the behaviour of excised embryonic axes was studied in two cultivars. The influence of humidification before placing the seeds to germinate was considered.

The aim of the present work was to explore the feasibility of seed cryopreservation as a tool for cotton genetic resources conservation.

MATERIAL AND METHODS

Five cotton (*G. hirsutum*) cultivars were studied: two perennial (CNPA 4M and CNPA 5M) and two annual cultivars (CNPA Precoce 1 and CNPA Precoce 2) bred by the Embrapa-Centro Nacional de Pesquisa de Algodão (Campina Grande, Brazil), and a commercial one(Coker 312). Seeds had been stored for two years at 5°C and were acid delinted immediately after storage, except for Coker 312 seeds which were acid delinted before storage.

Seeds were desiccated in a chamber with silica gel for 35 days. Moisture content was determined on a fresh weight basis after drying in a 105°C oven for 24 hours (two replicates of 20 seeds each). Desiccated and non-desiccated seeds were wrapped in aluminium foil, plunged into liquid nitrogen (LN) and kept there for one day. Thawing took place at room temperature. Seeds were set to germinate at 25°C with an 18 hour (day)/6 hour (night) photoperiod, in 9 cm Petri dishes with two sheets of filter paper moistened with distilled water. Four to eight replicates of 25 seeds were used per treatment. Germination was studied after five days and arcsin transformed data analysed using Duncan´s multiple range test.

Coker 312 and CNPA Precoce 2 seeds were surface sterilized with 20% commercial bleach (1% NaOCl) for 15 min with continuous stirring, rinsed three times with sterile distilled water and soaked in water for 24 h. Embryonic axes were excised and subjected to desiccation with silica gel under sterile conditions (20 embryonic axes per approximately 20 g silica gel) at 25°C for 0, 30, 60 and 120 min. Moisture content was determined as for seeds but with three replicates of 30 embryonic axes for each cultivar. Desiccated and non-desiccated axes were placed in cryovials and directly plunged into LN. Thawing took place in a 40°C water bath. Axes were cultured on liquid SHs medium (Stewart & Hsu, 1977) on cellulose plugs and incubated as for the seeds. Six replicates of five axes were used per treatment. Embryonic axes germination was studied after two weeks.

The effect of humidification before setting the seeds to germinate was studied in desiccated and non-desiccated seeds of Coker 312 and CNPA Precoce 2. Small incisions were made in seed coats without damaging the embryo axis. Seeds were then placed in a saturated atmosphere for three days. Germination and analysis of data were performed as stated before.

RESULTS AND DISCUSSION

During the desiccation period seed moisture content dropped from 9.8-15.6% to 2.8-3.5% (Table 1). For CNPA 4M, CNPA Precoce 1 and Coker 312 there was not a significant effect of immersion in LN and/or desiccation on germination. Frozen non-desiccated seeds of the cultivarsCNPA 5M, CNPA 4M, CNPA Precoce 1 andCoker 312 did not have their germination reduced significantly in comparison with control seeds. However, cryopreservation had a negative effect on the germination of seeds with the highest moisture content (15.6 %, CNPA Precoce 2). CultivarsCNPA 5M and CNPA Precoce 2 showed sensitiveness to desiccation. However, when desiccated seeds were immersed in LN, an increase in germination index was observed, such that the final percentages were not significantly different to those of non-desiccated control seeds. Stanwood & Bass (1978) observed high germination percentages of cotton cryopreserved seeds with moisture contents lower than 13%. In the present study, seeds with up to 14.7% moisture content also withstood direct freezing in LN. Desiccation improved slightly germination index after cryopreservation only in the cultivar CNPA Precoce 2, although the difference with that of non-desiccated frozen seeds was not statistically different.

TABLE 1. Effect of desiccation and immersion in liquid nitrogen (LN) for one day on seed germination percentage of several cotton cultivars[1].

Cultivar	Non-desiccated			Desiccated		
	Moisture content (%)	Germination (%)		Moisture content (%)	Germination (%)	
		Control	LN		Control	LN
CNPA 5M	9.8±0.0	91 a	64 ab	2.8±0.3	55 b	65 ab
CNPA 4M	10.3±0.1	74 a	60 a	2.8±0.1	61 a	69 a
CNPA Precoce 1	14.7±0.0	52 a	54 a	3.5±0.1	38 a	50 a
CNPA Precoce 2	15.6±0.4	89 a	56 b	3.0±0.3	58 b	71 ab
Coker 312	13.2±0.2	92 a	83 a	2.8±0.0	86 a	83 a

[1] Means with the same letter, within a cultivar, are not significantly different according to the Duncan's Multiple Range test, $p< 0.05$; moisture content: mean ± SD.

Embryonic axes lost most of their moisture content during the first 30 min of desiccation (Table 2). In the two cultivars studied, frozen non-desiccated embryonic axes did not germinate. Embryonic axes could be cryopreserved without loss of viability at moisture contents of 20% (30 min desiccation) or lower.

Neither desiccation nor humidification had a significant effect on germination of Coker 312 seeds (Table 3). As previously observed, desiccation decreased seed germination percentage inCNPA Precoce 2. Ellis et al. *(*1985) recommended humidification of cotton seeds at ambient temperatures and 95-100% relative humidity before germination. This was confirmed in this study with the cultivar CNPA Precoce 2 as this treatment improved germination after desiccation. However, this pretreatment seemed not to be necessary for Coker 312. According to those authors acid delinting could not totally avoid the problem of hardseededness which is induced in the majority of cotton seeds when dried to 5% or 6% moisture content (Walhood, 1956). However, it has been demonstrated for all cultivars studied, that freezing in liquid nitrogen circumvents the reductions in seed germination associated with seed desiccation.

The results reported here showed that cryopreservation of cotton seeds in liquid nitrogen could be an alternative to conventional seed banking. However, germination after freezing varied among cultivars, probably due to their different moisture contents. The differences among cultivars were reduced when the embryonic axes were cryopreserved, as shown with the two cultivars studied. The humidification of desiccated seeds prior to germination seemed not to be necessary for cryopreserved seeds.

TABLE 2. Effect of desiccation and immersion in liquid nitrogen (LN) for one day on the germination of embryonic axes of two cotton cultivars[1].

Desiccation.	Moisture	Coker		CNPA	
time (min)	content (%)	Control	LN	Control	LN
0	58.7 ± 0.4	97 a	0 b	93 ab	0 c
30	19.4 ± 1.5	100 a	100 a	90 ab	87 b
60	13.6 ± 1.1	100 a	100 a	100 a	93 ab
120	9.5 ± 3.3	100 a	100 a	100 a	100 a

[1] Means with the same letter, within a cultivar, are not significantly different according to the Duncan's Multiple Range test, $p < 0.05$; moisture con-tent: mean ± SD.

TABLE 3. Effect of desiccation and humidification on seed germination percentage of two cotton cultivars[1].

Treatment	Coker 312		CNPA	
	MC (%)	Germination (%)	MC (%)	Germination (%)
Control	7.5	88 a	11.2	90 ab
Humidified	14.8	96 a	14.2	86 bc
Desiccated	2.6	86 a	2.4	67 c
Desi.-humidified	14.7	91 a	13.7	99 a

[1] Means with the same letter, within a cultivar, are not significantly different according to the Duncan's Multiple Range test, $p < 0.05$; MC= moisture content.

CONCLUSIONS

1. Seed storage in liquid nitrogen can be used for cotton germplasm conservation as germination index is not reduced in most cultivars studied.

2. To assure optimal germination index after immersion in liquid nitrogen previous seed desiccation to around 3% moisture content is recommended in all cultivars.

3. Germination index can be improved in some cultivars that show sensitiveness to desiccation (e.g. CNPA Precoce 2) if seeds are humidified previously to sowing.

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