Effects of Seed Cryopreservation and Priming on Germination in Several Cultivars of *Apium graveolens*

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Seed germination of seven celery cultivars was studied after storage in liquid nitrogen for 1 or 30 d. Cryopreservation was also carried out on pelleted and primed seeds. None of the treatments applied reduced germination percentages. T_{50} (time for germination to reach 50%) significantly decreased in Florida, Utah and Istar cultivars when priming, alone or in combination with cryopreservation, was used.

Key words: *Apium graveolens* L., celery, cryopreservation, liquid nitrogen, pellet, priming.

INTRODUCTION

Seed preservation in liquid nitrogen (LN) can be used for a large number of species of agronomic importance, reducing seed deterioration (Stanwood, 1985). Previous studies on celery (*Apium graveolens* L.) seed storage in LN have shown similar germination percentages to those of control seeds (Styles *et al.*, 1982; Iriondo, Pérez and Pérez-García, 1992).

Celery seeds show low and gradual germination (Thomas, Biddington and O'Toole, 1979). This has great economic implications in horticulture and hence several techniques have been assayed to synchronize and reduce the time of germination. Seed priming (the incubation of seeds in high-osmotic potential solutions prior to germination) has been successfully applied to celery, allowing for a more uniform germination (Salter and Darby, 1976). On the other hand, as in many other horticultural crops, celery seeds are often pelleted for mechanical sowing. This factor should also be considered in seed cryopreservation.

TABLE 1. Final germination (%), mean $\pm$ s.e., after 30 d, of pelleted and non-pelleted seeds of three celery cultivars after storage in liquid nitrogen (LN)

Cultivar	Duration of storage in LN		
	Control*	1 d	30 d
Florida 683			
Non-pelleted	83 $\pm$ 3	83 $\pm$ 1	81 $\pm$ 1
Pelleted	78 $\pm$ 3	66 $\pm$ 3	71 $\pm$ 2
Golden Spartan			
Non-pelleted	88 $\pm$ 1	94 $\pm$ 1	97 $\pm$ 1
Pelleted	97 $\pm$ 1	98 $\pm$ 1	97 $\pm$ 1
Utah			
Non-pelleted	66 $\pm$ 1	59 $\pm$ 1	79 $\pm$ 4
Pelleted	43 $\pm$ 2	43 $\pm$ 3	51 $\pm$ 4

* Control = zero days in LN.

In this work, the effects of seed cryopreservation on the germination of seven celery cultivars, and its possible interactions with seed priming or seed pelleting have been studied.

MATERIALS AND METHODS

The celery seeds used in the experiments were from the following cultivars: 'Florida 683', 'Utah' and 'Golden Boy' from Asgrow Seed Co, USA; 'Golden Spartan', 'Isel' and 'Istar' from Clause Semences Professionnelles, France; and 'Tall Utah 52-70R' from Nunhems, Zaden, The Netherlands. Pelleted seeds of Florida 683, Utah and Golden Spartan were also tested. Seed moisture content was determined using the air oven method with samples being held at 105 °C for 24 h. Moisture content, expressed as percentage of fresh weight, ranged from 5.5 to 8.9% (8.4, 8.9, 7.2, 7.2, 8.6, 5.5 and 7.9%, respectively for the cultivars mentioned above). Moisture contents of pelleted seeds were 4.4, 5.7 and 1.5%, respectively for the three cultivars mentioned.

Celery seeds were placed in Cel-Cult cryovials and introduced by immersion in liquid nitrogen (–196 °C, seed cooling rate of approximately 200 °C min^{–1}) for 1 or 30 d. The warming procedure consisted of letting the samples warm under ambient laboratory temperature (20–25 °C) to equilibrium. The effect of cryopreservation in combination with priming was studied using 15.7 g l^{–1} NaNO₃ or 19.3 g l^{–1} KNO₃ solutions (ψ_{π} = –0.8 MPa). Priming solutions (3 ml) were added to Petri dishes containing 50 seeds on two sheets of filter paper. The seeds were then kept at 25 °C in darkness for 7 d, and subsequently desiccated for 3 d in a chamber with silica gel. Priming was carried out before (Priming + LN) or after (LN + Priming) cryopreservation (1 d in LN).

Two replicates of 50 seeds each were placed in Petri dishes (7 cm inner diameter) on two sheets of filter paper moistened with 3 ml distilled water. Water was replaced regularly. The

TABLE 2. Final germination (%), mean $\pm$ s.e., after 30 d, and T_{50} values (d, in parentheses) for celery seeds after storage in liquid nitrogen (LN)

Cultivar	Duration of storage in LN		
	Control*	1 d	30 d
Florida 683	74 $\pm$ 1 (7.5)	74 $\pm$ 0 (8.5)	74 $\pm$ 0 (9.0)
Utah	64 $\pm$ 3 (9.0)	64 $\pm$ 0 (10.5)	70 $\pm$ 3 (9.5)
Golden Spartan	93 $\pm$ 1 (7.0)	93 $\pm$ 1 (7.0)	92 $\pm$ 1 (7.0)
Isel	95 $\pm$ 1 (7.0)	96 $\pm$ 0 (8.0)	96 $\pm$ 1 (7.0)
Tall Utah 52-70R	100 (5.0)	94 $\pm$ 0 (5.0)	99 $\pm$ 1 (5.0)
Istar	93 $\pm$ 1 (7.0)	85 $\pm$ 6 (7.0)	88 $\pm$ 1 (7.0)
Golden Boy	78 $\pm$ 1 (7.0)	86 $\pm$ 4 (7.0)	77 $\pm$ 6 (7.0)

* Control = zero d in LN.

seeds were incubated at 25/15 °C day/night, with 16/8 h day/night light regime, and an irradiance of 35 μ mol m⁻² s⁻¹, provided by cool white fluorescent tubes (OSRAM L 58W/20). The number of seeds showing radicle emergence was counted every 2 d and removed from the Petri dishes. The number of days needed to reach 50% of the final germination percentage (T_{50}) was estimated by median values of the germination time (Georghiou, Thanos and Passam, 1987).

Germination percentage after 30 d and T_{50} values were obtained and means were subjected to analysis of variance. Germination percentages were previously submitted to the

arcsine transformation. Comparison of means was made using Duncan's Multiple Range test ($P < 0.05$).

Microphotographs of seeds were taken using a scanning electron microscope HITACHI 2500. Samples were previously metallized with a 40 nm platinum coat.

RESULTS

In all the experiments there were highly significant differences ($P \leq 0.001$) among the germination percentages of the cultivars studied (Tables 1, 2, 3).

In general, non-pelleted seeds had higher ($P \leq 0.01$) germination percentages (Table 1). However, in Golden Spartan pelleted seeds responded better ($P \leq 0.05$) than non-pelleted seeds. In this study, cryopreservation seemed to induce different responses depending on the cultivar. No significant differences among the varying cryopreservation treatments were found in Florida and Golden Spartan. However, in Utah the best response was observed after 30 d in LN.

Similar results were obtained when the cryopreservation of non-pelleted seeds of the seven cultivars was studied (Table 2): there were no significant differences among the germination percentages of the three treatments. No coat damage was detected in observations with the scanning electron microscope (Fig. 1). Overall, a significant increase ($P \leq 0.05$) in the T_{50} value for seeds cryopreserved for 1 d was observed with respect to control seeds (Table 2). However, when results were analysed separately for each cultivar, the differences were not significant.

The germination percentages of priming, priming + LN and LN + priming treatments did not differ significantly with the two priming solutions studied (Table 3). Nor were significant differences found when either control or LN

TABLE 3. Final germination (%), mean $\pm$ s.e., after 30 d, and T_{50} values (d, in parentheses) for celery seeds after various combinations of priming and freezing treatments

Cultivar	Treatments					
	NaNO ₃			KNO ₃		
	P	P+LN	LN+P	P	P+LN	LN+P
Florida 683	64 $\pm$ 3 (5.0)	73 $\pm$ 1 (5.0)	70 $\pm$ 1 (5.0)	67 $\pm$ 2 (5.0)	73 $\pm$ 1 (5.0)	67 $\pm$ 4 (5.0)
Utah	74 $\pm$ 3 (6.0)	61 $\pm$ 4 (7.0)	72 $\pm$ 1 (5.0)	68 $\pm$ 1 (6.0)	71 $\pm$ 1 (5.0)	64 $\pm$ 3 (6.0)
Golden Spartan	91 $\pm$ 1 (7.0)	95 $\pm$ 1 (7.0)	92 $\pm$ 1 (6.0)	91 $\pm$ 2 (6.5)	96 $\pm$ 0 (7.0)	94 $\pm$ 0 (6.0)
Isel	94 $\pm$ 2 (5.5)	98 $\pm$ 1 (5.0)	93 $\pm$ 1 (6.0)	94 $\pm$ 0 (5.0)	92 $\pm$ 1 (5.0)	94 $\pm$ 1 (6.0)
Tall Utah 52-70R	93 $\pm$ 1 (5.0)	95 $\pm$ 1 (5.0)	95 $\pm$ 1 (3.0)	97 $\pm$ 1 (5.0)	98 $\pm$ 0 (5.0)	95 $\pm$ 1 (3.0)
Istar	94 $\pm$ 1 (5.0)	93 $\pm$ 1 (5.0)	91 $\pm$ 2 (6.0)	89 $\pm$ 1 (5.0)	97 $\pm$ 0 (5.0)	96 $\pm$ 1 (6.0)
Golden Boy	80 $\pm$ 1 (6.5)	77 $\pm$ 4 (7.0)	86 $\pm$ 2 (6.0)	81 $\pm$ 1 (7.0)	78 $\pm$ 0 (7.0)	82 $\pm$ 1 (6.0)

P = Priming.

LN = 1 d in liquid nitrogen.

P+LN = Priming before LN.

LN+P = Priming after LN.

FIG. 1. SEM micrograph of control (A) and frozen seed (B) of celery cv. Florida 683.

treatments were included. However, significant ($P \leq 0.001$) differences among treatments and cultivar $\times$ treatment interactions were found with the variable T_{50} (Table 3). In all seven cultivars, priming, priming + LN and LN + priming treatments had lower T_{50} values than the control and LN treatments (Tables 2, 3). This difference was significant in Florida, Utah and Istar both in the NaNO_3 and the KNO_3 solutions. The LN + priming treatment gave the lowest T_{50} in Golden Spartan and Tall Utah 52-70R with the NaNO_3 solution and in Tall Utah 52-70R and Golden Boy with the KNO_3 solution (Table 3).

DISCUSSION

Celery seed storage in LN did not reduce germination percentage in any of the cultivars for either periods of time studied, thus confirming results of previous works (Styles *et al.*, 1982; Iriando *et al.*, 1992). Moreover, cryopreservation did not have a negative effect on the germination of pelleted seeds.

The priming, priming + LN and LN + priming treatments did not affect final germination percentage. However, in general, they showed lower T_{50} values than control and LN

treatments. Heydecker and Gibbins (1978) stated that some of the advantages of priming can be lost if seeds are subsequently desiccated. In our experiments, seeds were always desiccated after priming to eliminate the adverse effects of high moisture contents on cryopreservation. In spite of this, the positive effects of priming were still noticeable in some cultivars. In general, the sensitivity of seeds to dehydration increases with the priming incubation time (Heydecker and Gibbins, 1978). In our conditions, the time of priming incubation was short (7 d) when compared to periods of 21 d (at 10 °C) used by Singh, Morss and Orton (1985), also working with celery. Thus, a 7 d pretreatment appears to be insufficient to alter celery seed desiccation sensitivity.

The results obtained in this study confirm that, as has been shown for other horticultural species (Stanwood and Roos, 1979; Styles *et al.*, 1982), seed cryopreservation in LN is a feasible technique for celery seed storage. Furthermore, they show that this technique is, in the case of celery, compatible with other seed pretreatments, of great relevance in horticulture, such as priming and pelleting.

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