LOW TEMPERATURE (-196°C) STORAGE OF TRUE SEED FROM THE TUBER-BEARING SOLANUM SPECIES

Leigh E. Towill

Abstract

Seeds from several of the tuber-bearing Solanum species were exposed to low temperatures using liquid nitrogen. No differences in final percent germination or in the rate of germination were observed between the control and treated seeds for any species tested. High viability after liquid nitrogen treatment suggests that long-term storage of Solanum seed should be practical and warrants further investigation, especially for the long-term storage of valuable germplasm.

Resumen

Las semillas de varias especies tuberizantes de Solanum fueron expuestas a baja temperatura utilizando nitrógeno líquido. No se observó diferencias en porcentaje de germinación final o en la velocidad de germinación entre el testigo y las semillas tratadas en ninguna de las especies probadas. La alta viabilidad de la semilla después del tratamiento de nitrógeno indica que el almacenamiento de semilla de Solanum por periodo prolongado sería práctico y requiere mayor investigación, en especial cuando se trata de germoplasma valioso necesario de almacenar por largos períodos.

Introduction

At the Inter-regional Potato Introduction Station, Sturgeon Bay, Wisconsin, true seed of species of the tuber-bearing Solanum are stored at 1-2°C after drying them to a moisture content of about 6% over potassium acetate. Seeds are parcelled and stored within sealed moisture-proof laminar polyester envelopes (personal communication, R. Hanneman, Jr., Project Leader, Inter-regional Potato Introduction Project, 3). Data gathered during the past 30 years by various workers suggest that the usable viability period for most potato seed accessions is about 20 years (2, 3, 4). Accessions are replenished when the number of seed for distribution of a given accession drops below about 1,000 or when germination falls below 50%. Seed for the accession usually is increased by sib mating of plants grown from the original source seed (3).

Storage for the IR-1 working seed lot (i.e. that which is regenerated for distribution purposes) under the above stated conditions is usually adequate since replenishment for the reason of low seed number often occurs before

1Cooperative investigation of the U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, and the Wisconsin Agricultural Experiment Station.

2Plant Physiologist, USDA, SEA, AR, Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706.

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viability drops below 50%. However, longer term storage procedures are desirable for source seed lots because this seed is used for regenerating the working seed lot every 10-20 years and is expected to retain the genetic diversity originally present in the accession. Regeneration schemes should minimize loss due to genetic drift. Decline in viability during very low temperatures, such as −196°C (liquid nitrogen, LN2), is argued to be slight over at least several decades due to low kinetic energy of molecules and negligible diffusion characteristics (6). Background irradiation during the storage period is thought to be the major potential source for damage to cells, and, although not well studied in many systems, the conclusion is still that virtually no change should occur over long periods of time (1). The main injuring events in a cryogenic protocol usually occur during cooling to and warming from low temperatures, and typically are associated with hydrated cells in which the manner and location of ice formation become critical. Since seed from orthodox species usually has a low moisture content, these rate factors probably are not important in retaining high survival after low temperature exposure. In many plants, seed having a moisture content less than some critical value shows no injury during exposure to liquid nitrogen or the vapors above liquid nitrogen (5, 7, 8). The moisture content at which freezing injury is first observed varies from species to species but often is within the range of 10-20% (wet weight basis) (7, 8, 9). Rapid temperature changes can cause cracking damage in seed from some species (8). The objective of the following germination experiments was to determine whether seed from the tuber-bearing Solanum species could survive liquid nitrogen exposure and, thus, open the possibility that long-term storage is feasible for these materials.

**Materials and Methods**

All seeds obtained for this study came from the IR-1 Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin and were harvested from fruit collected from species grown in the field or from greenhouse-crossed bulk populations. Seeds were extracted by briefly blending the fruit in water, screening out the large debris, followed by repeated washings in water. Seeds were air dried at room temperature on cheesecloth. Exposure to low temperatures was done either by immersing small glass or plastic tubes containing the seed into liquid nitrogen within a dewar (cooling rate about 460°C/min between −10 and −60°C) or by suspending these tubes in the vapor phase above liquid nitrogen in a Linde X-12 liquid nitrogen refrigerator (cooling rate about 120°C/min). Thawing was accomplished by either suspending the tubes in air at room temperature or briskly shaking

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