germinability test) were applied and their influences on the viability of the seeds were analyzed.

To study the tolerance to dehydration and LN exposure, seeds were equilibrated at relative humidities (ERH) between 11% and 95% using saturated salts (LiCl, $MgCl_2$, K_2CO_3, $NaNO_2$, NaCl, KCl, KNO_3). Sorption isotherms were determined and modeled. Seed viability was analyzed by germination experiments; seeds were sown in hermetic controlled germination conditions and the percentage of normal seedling was evaluated 4–6 weeks after sowing. Seed desiccation sensitivity was quantified by the quantal response model. Differential scanning calorimetry thermograms between −120 and 100 °C were determined on cotyledon tissue that was previously dehydrated reaching different moisture contents; ice melting enthalpies and unfrozen water contents were measured from these thermograms. In order to analyze the effect of pre-sowing treatment on seed viability, experiments were carried out in a narrower range of equilibrium relative humidities (ERH: 59–85%). All the seeds subjected to conditions of seed desiccation, or seed desiccation followed by LN treatment were either directly sown under germination conditions or subjected to pre-sowing rehydration procedures; the tested procedures were preheating (40 °C during 4 h) and pre-humidification (equilibrium at 100% RH, 20 °C).

In the cases of seeds that were only dried and in seeds that were dried and treated with LN, the tested pre-sowing treatments (pre-humidification or heating) did not significantly improve the viability of the seeds with respect to the control samples ($P < 0.05$); in fact preheating significantly deteriorated the viability of the LN treated seeds.

The survival of *C. sinenis* and *C. paradise* seeds, subjected to cryopreservation in LN was examined and seed desiccation sensitivity following rehydration procedures was quantified. Results showed that in order to reach the maximum viability, the seeds exposed to LN, must be first dehydrated to a range of ERH 69–81%(0.16–0.31 $gH_2O\ g^{-1}$dry basis) for *C. sinensis* and 69–75% (0.09–0.11 $gH_2O\ g^{-1}$dry basis) for *C. paradise.* The limit of hydration for LN *Citrus* seeds treatment corresponded to the unfrozen water content in the tissue, confirming that seed moisture should be reduced to such an extent to avoid the formation of intra-cellular ice crystals during ultra-rapid freezing, thus preserving the integrity of seed tissues.

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26. Invariance of the glass transition temperature of plant vitrification solutions with cooling rate. Aline Schneider Teixeira [1], Miloš Faltus [2], Jiří Zámečník [2], Renata Kotková [2], Maria Elena González-Benito [3], Antonio Diego Molina-García [1], [1] ICTAN (CSIC), Jose Antonio Novais 10, 28040 Madrid, Spain, [2] Crop Research Institute, Prague, Czech Republic, [3] Dept. Biología Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

Glass, the state of matter where molecular mobility is so reduced that most physicochemical processes are virtually detained (including ice formation), is basic for cryopreservation. The glass transition temperature (T_g), a temperature range at which supercoooled liquid becomes glass, is characterized by a change in heat capacity (C_p), evaluated at its inflection point.

Vitrification in cryopreservation protocols is achieved, without sophisticated cooling equipment, simply plunging specimens into liquid nitrogen (LN) after a set of physicochemical treatments increasing cytoplasmatic microviscosity and enhancing tissue resistance to cold and dehydration. Quick cooling is required to achieve vitrification avoiding ice formation. Both T_g and C_p are generally considered dependent on cooling rate (e.g. Angell et al. 82 (1978) J. Phys. Chem., 2622; Debenedetti et al. 410 (2001) Nature 259). The present work endeavors to increase knowledge in this area characterizing the calorimetric glass transition of the most common plant vitrification solutions, under a wide range of cooling rates.

The solutions studied were Plant Vitrification Solutions 1, 2 and 3: (Uragami et al. 8 (1989) Plant Cell Rep. 418; Sakai et al. 9 (1990) Plant Cell Rep. 30; Nishizawa et al. 91 (1993) Plant Sci. 67). Cooling was performed using the calorimeter control (5, 10 and 20 °C min^{-1}), or for higher rates, by quenching closed pans with PVS in LN, either naked (− 5580 °C min^{-1}) or inside cryovials (360 °C min^{-1}). Quenched pans were then transferred to the pre-cooled sample chamber. Glass transition temperature was observed by DSC with a TA 2920 instrument, upon warming pans with solution samples from −145 °C to room temperature, at standard warming rate: 10 °C min^{-1}.

Glass transitions showed clear and consistent temperature differences among vitrification solutions, related to composition and water content. Roughly, two sets of T_g values were obtained, for PVS1 and 2, at −112 °C and −114 °C, respectively, and for PSV3, at −90 °C. The observed T_g did not significantly change within a wide range of cooling rates (from 5 to 20 °C min^{-1}). The highest cooling rate (5580 °C min^{-1}) increased glass transition temperature significantly, compared to the values at the slowest cooling rates (5–20°C min^{-1}). This change in T_g inflexion (by 1.2 °C min^{-1}) did not influence considerably the glass transition region because the whole transition interval was, on average, 7 °C. However, no significant differences were found between T_g obtained with the highest cooling rate and that with the middle cooling rate (360 °C min^{-1}). In conclusion, T_g of plant vitrification solutions did not significantly change when the cryopreservation methods based on either direct plunging samples into liquid nitrogen or plunging of samples in closed cryovials were used. We can conclude that the T_g of commonly used PVSs did not change with the cooling rates tested.

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27. Physiological–biochemical characteristics of Pisum sativum seedlings after long-term storage of seeds in the permafrost conditions. I.A. Prokopiev, G.V. Filippova, A.A. Shein, E.S. Khlebnyy, Institute of Biological Problems of Cryolithozone SB RAS, Yakutsk, Russia

Loss of seed viability cannot be prevented, this process is irreversible, but it is quite possible to slow the process down if the necessary storage conditions are created. In this context it is very important to develop and use methods of long-term storage of seeds. It is known that some of the main factors affecting the duration of seed storage are temperature and humidity (moisture content). Long-term storage of plant material in the form of seeds is one of the most popular and effective methods to the preservation of most species of the world. Creation of seed banks has significant advantages over other methods for plant preservation *ex-situ*: storage of a large number of samples is simplified, space saving and has relatively low labour costs.

The material for the study were the seeds and seedlings of three cultivars ("Imposant", "Latores" and "Rosol") of pea (*Pisum sativum* L.). Seeds of 1977 harvest were laid down in long-term storage in permafrost in the underground laboratory of the Institute of Permafrost, SB RAS (Yakutsk) to a depth of 12 m. Storage for 34 years was carried out in hermetical glass vessels of 100 cm^3 at a constant temperature of −6.5 ± 0.5 °C. Before laying down the seeds for storage their moisture content did not exceed 5–7%. As a control seeds and seedlings were used from the same cultivars of *P. sativum* 2007–2009 from the collection of N. I. Vavilov Research Institute of Plant Industry.

It is shown that after 34 years of low temperature (−6.5 ± 0.5 °C) storage of three cultivars seeds ("Imposant", "Latores" and "Rosol") of pea (*Pisum sativum* L.) under permafrost conditions, the physiological parameters of seed and seedlings (germination, root length, dry weight), as well as the mitotic activity of roots cells did not differ from control. However, a small increase in the number of abnormal ana-telophase of mitosis in root meristem cells of cultivars "Imposant" and "Latores" was shown in comparison to the control. After long-term storage of three *P. sativum* cultivar seeds balanced work prooxidant-antioxidant systems in a simple compensation regime was observed in the tissues of seedlings. It was noted that long-term storage of seed cultivars "Imposant" and "Latores" caused a decrease of photosynthetic pigment content in the seedling tissue that along with a small increase of the number of abnormal mitoses may be evidence of the beginning of the process of aging of seeds.

In general, it is shown that long-term storage of seeds of three cultivars of *P. sativum* in permafrost conditions enabled the preservation of their viability and can be offered as prospective way to store seeds.

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28. Using Synchroton infrared microspectroscopy to better understand the freezing-resistance of lactic acid bacteria. J. Gautier [1], S. Passot [2], F. Jamme [3,4], S. Cenard [1], F. Fonseca [1], [1] INRA, UMR 782, 78850 Thiverval Grignon, France, [2] AgroParisTech, UMR 782, 78850 Thiverval Grignon, France, [3] Synchrotron SOLEIL, SMIS Beamline, 91192 Gif-sur-Yvette, France, [4] INRA, CEPIA, 44026 Nantes, France

Freezing is commonly applied to preserve the functionalities of concentrates of lactic acid bacteria (LAB). However, it is still a critical step in the production of LAB concentrates, as it affects both the viability and acidifying activity upon thawing. Several environmental factors influence the resistance to freezing of LAB (strain, medium composition, temperature, etc.) but the mechanisms of cell injury during