Zeitschrift: Veröffentlichungen des Geobotanischen Institutes der Eidg. Tech. Hochschule, Stiftung Rübel, in Zürich

Herausgeber: Geobotanisches Institut, Stiftung Rübel (Zürich)

Band: 112 (1993)

Artikel: Kryokonservierung von "Lemnaceae" = Cryopreservation of "Lemnaceae"

Autor: Sauter, Peter Richard

Kapitel: Summary

DOI: https://doi.org/10.5169/seals-308976

konservierung ebenfalls wichtig. Besonders gut eignen sich auf Agarnährmedien gehaltene Pflanzen. Die Erhöhung der Gefrierresistenz durch eine nitratreiche Ernährung lässt vermuten, dass viele Arten Proteine produzieren, die wahrscheinlich durch Nukleationshemmung des Zellsafts und/oder Membranstabilisierung Schutz vor Gefrierschäden bieten. Nach dem Auftauen ist darauf zu achten, dass den Pflanzen Zucker (Saccharose) zur Verfügung steht, der für die Regeneration der Knospen von Bedeutung ist. Insbesondere wenig gefrierresistente Arten können sich in einer zuckerfreien Nährlösung nicht weiter entwickeln, und die Knospen sterben mit der Zeit ab. Die Überlebensrate wird ebenfalls durch das Kulturalter beeinflusst. Aus jungen und sehr alten Kulturen stammende Pflanzen zeigen eine verminderte Gefrierresistenz.
Samen von *Lemnaceae* weisen einen vergleichsweise hohen Wassergehalt auf, und die verwendeten *Lemna aequinoctialis*-Samen konnten nur dank vorangegangener Lufttrocknung erfolgreich kryokonserviert werden. Die Gefrierschutzmittelkonzentration war bedeutend tiefer, als sie für ganze Pflanzen benötigt wird, und die Lösung kristallisierte bereits während dem Kühlen aus. Bei Einfrierexperimenten mit Turionen von *Spirodela polyrrhiza* gelangen keine positive Resultate.
Die Kryokonservierung ist als Lagermethode für die Unterfamilie *Lemnoideae* grundsätzlich geeignet. Die folgenden acht Arten können in flüssigem Stickstoff bedenkenlos aufbewahrt werden: *Spirodela punctata*, *Lemna gibba*, *Lemna disperma*, *Lemna minor*, *Lemna japonica*, *Lemna obscura*, *Lemna ecuadoriensis* und *Lemna turionifera*. Für die restlichen *Lemnoideae*-Arten, ausser *Spirodela polyrrhiza*, ist eine Flüssig-Stickstofflagerung ebenfalls möglich, sofern eine genügend grosse Stückzahl eingefroren wird. Allerdings sollte bei diesen Arten vorerst jeder Stamm überprüft werden, ob zumindest eine geringe Gefrierresistenz vorhanden ist. Aus der erfolgreichen Kryokonservierung von *Lemna minor* über einen Zeitraum von 21 Monaten kann geschlossen werden, dass kein Vitalitätsverlust während einer Langzeitlagerung zu befürchten ist. Die erarbeitete Kryokonservierungsmethode kann bei *Wolffia*- und *Wolffiella*-Arten, die sehr sensibel auf osmotische Änderungen reagieren, nicht angewandt werden.

SUMMARY

The aim of the present work was to develop a cryopreservation method for *Lemnaceae* (duckweeds), so that whole collections can be stored without great expense of time and work in the future. Of the 34 world-wide known species, 24 were involved in these investigations. Generally vegetative plants of one to several fronds were tested. In addition a few experiments were conducted with turions of *Spirodela polyrrhiza* and seed of *Lemna aequinoctialis*. Unless stated otherwise, the present observations belong always to whole plants.
After thawing living specimens were obtained by all 16 species of the genera *Spirodela* and *Lemna*, which could be cultivated further on and multiplied. But with *Spirodela polyrrhiza* only one pre-experiment was successful, and since then this result was irreproducible. The freezing resistance of each species is very different. This establishment applies also to clones of one species. There is no noticeable correlation of geographical origin of the plants and survival rate.
Fronds, roots, and parts of daughter fronds, which are outside of the pouches, are killed by the addition of the cryoprotectant or by its removal. The pouches seems to play a very important role to the *Lemnaceae* cryopreservation by keeping those parts of tissue, which are inside the pouches, from osmotic shock, supposedly due to the slow penetration of the fluid added into these pouches. Thus buds and young daughter fronds are exclusively available for the actual cryopreservation, but regeneration to new individuals requires no special culture conditions for *Lemnaceae*, in contrast to most other flowering plants. The

findings of this study with *Lemnaceae* are in good agreement with cryobiological experiments, where until now almost, exclusively young tissue of meristematic origin with cells fit for segmentation was successfully cryopreserved. Buds surviving cryopreservation are sometimes also damaged and not all of them grow out after thawing. Also callus formation and succulent plants can be recognized, which are in many cases not useable for a further cultivation. Specimens of certain species which are slightly damaged tend to produce anthocyanins. A comparatively late recovery after thawing suggests that many buds need a time period for regeneration.

It is assumed that for the successful cryopreservation of *Lemnaceae* the plants were dehydrated by the cryoprotectant before freezing and the cryosolution vitrifies during cooling. During thawing a slight crystal growth can be tolerated in some cases. Glycerol in a concentration of about 50 v/v% is the only one of all cryoprotectants tested which comes up to these demands and is comparatively untoxic at least for *Lemnoideae* species. Though the minimal cooling rate has to be -3°C/min and the samples must be rapidly thawed in a 30°C water bath. *Lemna minor*, by a wide margin the most freeze-resistant species can be cryopreserved very easily in the presence of 50 v/v% glycerol, and a distinct increase of the survival rate seems hardly to be possible. It is recommendable for all other *Lemnoideae* species to avoid any crystal growth even during thawing. This aim can be achieved either by an ultra-rapid cooling and thawing of a 50 v/v% glycerol drop containing the plants or by increasing the glycerol concentration to 60 v/v%, while simultaneously shortening the incubation time for dehydration. Wether protection by glycerol against freezing injuries is only external or if this agent has the ability to penetrate into the cells could not be established.

The kind of cultivation before and after cryopreservation is also important for the survival rate. Plants grown on an agar nutrient medium are especially fitted for a storage in liquid nitrogen. The increase of freezing resistance by a food sources rich in nitrate suggests that many species produce proteins, which offer a protection against freezing damage, probably by inhibiting intracellular nucleation and/or by stabilisation of membranes. After thawing sugar (sucrose), which is important for recovery of the buds, should be available for the plants. Importantly, buds of plants with a low freezing resistance do not regenerate when growing on a sugar-free nutrient solution and die after a certain time. Also the age of culture influences the survival rate. Plants from young or very old cultures show a reduced freezing resistance.

Seed of *Lemnaceae* contains much water and the seeds of *Lemna aequinoctialis* studied could survive cryopreservation only following previous air-drying. The concentration of the cryoprotectant needed was remarkably lower than is necessary for whole plants, and the solution crystallised during cooling. In freezing experiments with turions of *Spirodela polyrrhiza* there was no success.

Cryopreservation is basically suitable as storage method for the subfamily *Lemnoideae*. The following mentioned eight species can be stored in liquid nitrogen without hesitation: *Spirodela punctata*, *Lemna gibba*, *Lemna disperma*, *Lemna minor*, *Lemna japonica*, *Lemna obscura*, *Lemna ecuadoriensis* and *Lemna turionifera*. Storage in liquid nitrogen is also possible for the residual *Lemnoideae* species, with exception of *Spirodela polyrrhiza*, as far as the sample size of frozen fronds is big enough. First of all each clone of these species should to be tested for existence of low freezing resistance. It can be concluded from the successful cryopreservation of *Lemna minor* over a period of 21 months that there is no fear of lost of viability during a long-term storage. The cryopreservation method developed in this work is not applicable for *Wolffia* and *Wolffiella* species, which respond very sensitively to osmotical changes.