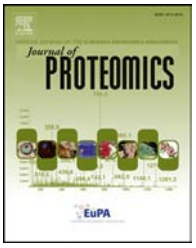
JOURNAL OF PROTEOMICS 98 (2014) 99–111

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



Changes in sugar content and proteome of potato in response to cold and dehydration stress and their implications for cryopreservation

Raquel Folgado[a,b], *Kjell Sergeant*[a], *Jenny Renaut*[a], *Rony Swennen*[b,c,d], *Jean-Francois Hausman*[a], *Bart Panis*[c]

[a]*Environment and Agro-biotechnologies department (EVA), Centre de Recherche Public-Gabriel Lippmann, 41, rue du Brill, L-4422 Belvaux, Luxembourg*
[b]*Laboratory of Tropical Crop Improvement, Division of Crop Biotechnics, KU Leuven, Willem de Croylaan 42 bus 2455, B-3001 Leuven, Belgium*
[c]*Bioversity International, Willem de Croylaan 42 bus 2455, B-3001 Leuven, Belgium*
[d]*International Institute of Tropical Agriculture, POB 10, Duluti, Arusha, Tanzania*

ARTICLE INFO

Article history:
Received 8 July 2013
Accepted 29 November 2013

Keywords:
Cryopreservation
Abiotic stress
Soluble sugars
Heat shock proteins
Cold
Potato

ABSTRACT

The key to successful cryopreservation lies in the induction of tolerance towards dehydration/desiccation and freezing. The accumulation of osmo-active compounds, which can be induced by drought and cold stress, is therefore important. In the present study, three-week old shoots from *in vitro* plantlets of the cultivated potato *Solanum tuberosum* and its frost-resistant relative *Solanum commersonii* were submitted to osmotic stress (by using sucrose) and chilling (6 °C). After 14 days of exposure, shoot tips were sampled in order to gain an insight into changes of the proteome and soluble sugars. Also, the effect of these treatments on growth performance behaviour and on the success of cryopreservation was evaluated. Identified proteins that changed in abundance due to stress were associated with stress response. Additionally, carbohydrate analyses in both species, after exposure to chilling, also indicated species-related differences; this observation could point towards a better-adapted physiological state of the donor plants of *S. commersonii* prior to the cryoprocedure and therefore a better recovery of the meristems.

Biological significance
To our knowledge, this is the first study in which cryopreservation experiments are combined with the observation of the responses to abiotic stress exposure involving the potato species *S. commersonii* and *S. tuberosum*. These two species are known to have a different cold-acclimation behaviour, which seems to be closely related to their tolerance towards cryopreservation. Furthermore, common and differential responses to abiotic stresses were observed in the two species indicating that some pathways could be crucial not only in the plant's response to stress but also in tolerance towards cryopreservation.

1. Introduction

Preserving the agricultural biodiversity is essential to assure the world food productivity, *e.g.* by using plant diversity in breeding programmes to increase or stabilize yields in adverse environmental conditions. Crop diversity should thus be preserved to protect the needs of future generations [1]. In an effort to avoid the loss of genotypes, different methods to preserve biodiversity have been developed [2].

1874-3919/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.11.027

Potato is one of the cultivated plants with the richest genetic diversity worldwide [1]. Apart from commercial potatoes, all *Solanum tuberosum* L. ssp. *tuberosum*, seven other cultivated and 228 wild potato species have been identified [3]. These genetic resources are generally conserved in field collections, tuber collections or as *in vitro* plants. Cryopreservation or storage at ultra-low temperatures, often at −196 °C, the temperature of liquid nitrogen, is an ideal solution for the long-term conservation of biological material [4], since in these conditions, all biochemical and most physical processes are completely arrested. Using cryopreservation, the plant material can be stored for unlimited long periods of time while the stability of the genetic material is preserved and phytopathological and physiological risks usually associated with the maintenance of plant gene banks are drastically reduced [5]. Furthermore, the costs and efforts of maintaining collections of cryopreserved plant germplasm are much lower compared to other methods. Since 1977, when Bajaj reported cryopreservation of potato [6], several cryopreservation procedures have been developed [7], some of them with significant success. However, there is currently still no standard procedure available that results in high post-thaw regeneration frequencies for all described potato species and cultivars.

In their natural environment, plants are regularly exposed to stress conditions such as low temperatures, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity. Indeed abiotic stress is the main cause of the reduction in productivity for most major crops worldwide [8] For potato, cold is considered as the major constraint that adversely affects growth and yield in commercial cultivars. In response to these stress factors, various metabolic pathways act in synergy to mitigate the effects of stress. They lead to the adjustment of the cellular environment and increased tolerance, a process known as acclimation [9]. Such biochemical changes can be analysed using different approaches, one of them being proteomics [10]. Few studies on protein changes related to an increasing abiotic stress tolerance in potato have been undertaken until now [11–13]. On the other side exposure of plant material to osmotic stress or frost, prior to cryopreservation, is known to have a positive impact on survival after cryopreservation [4]. Criel [14] and Kaczmarczyk [15] used potato as a model to study the protein abundance associated with cryopreservation using different pre-culture conditions. It is therefore expected that a better knowledge of how plants respond to stress conditions will provide insights into how to improve cryopreservation technologies.

A pre-culture of shoot-tip donor plants in the cold prior to cryopreservation is often used to improve the recovery after cryopreservation for those species that are able to cold-acclimate [16,17]. However, it has been demonstrated that such exposure to low temperatures can also improve cryopreservation results for species that are considered as unable to acclimate [17,18]. Alternatively, exposure to high concentrations of sucrose is used to improve cryopreservation ability in species that are unable to cold-acclimate. Since our study deals with one species that is able to acclimate (*Solanum commersonii*) and another that is not (*Solanum tuberosum*) [11,19], both cold acclimation and dehydration pre-culture conditions were compared.

Here, we present a proteomic study combined with an analysis of soluble sugars to decipher the effects of the stress treatments on *in vitro* plants. Furthermore, cryopreservation was applied on meristems derived from the pre-cultured explants.

2. Material and methods

2.1. Plant material and stress treatments

Ten millimetres sized shoots were excised from vegetatively-propagated three-week-old *in vitro* plantlets of the commercial cultivar *S. tuberosum* L. cv. Désirée and the wild potato *S. commersonii* Dun that were cultured onto Murashige and Skoog (MS) medium (MS salts and vitamins + 0.09 M sucrose) [20] at 22 °C, 16/8 h day/night and a light intensity of 50 μmol m^{-2} s^{-1}) and transferred to the same culture conditions for one week. Then they were exposed for 2 weeks to control conditions (same conditions for light, temperature and culture medium as above) or one of the two following stress conditions. The osmotic stress treatment was provided by MS medium supplemented with 0.21 M sucrose, resulting in a total sucrose concentration of 0.3 M. Plants exposed to the cold treatments were cultivated on MS medium with same light conditions but at constant temperature of 6 °C.

After the treatments, shoots from *in vitro* plantlets were collected and stored at −80 °C. Five biological replicates per treatment, each composed of a pool of 12 shoots grown in the same culture container, were sampled for both protein (8 shoots per sample) and carbohydrate (4 shoots per sample) analyses.

2.2. Morphological study

After 14 days of exposure to the treatments the following growth parameters were recorded, length of shoot and number of leaves. Additionally, fresh and dry weights (FW and DW) were measured to calculate the water content (WC) of the shoots (calculated as % of water that is present in the shoot, WC = (FW-DW/FW) × 100).

The data presented (Fig. 1) are the mean values of at least three independent experiments with 12 explants per treatment. Data were analysed using one-way ANOVA, and comparisons between the mean values were evaluated by the least significant different test at $p < 0.05$. The Kolmogorov–Smirnov test was used to test the normality of the samples, using the SigmaStat software.

2.3. Cryopreservation procedure

2.3.1. Pre-culture and excision of apical meristems

Control, sugar pre-cultured and cold treated shoots (conditions see above) were used as pre-culture before applying the cryoprocedure. After 14 days of exposure to the three conditions, apical shoot-tips of 1 × 0.5 mm were excised under a binocular microscope. The dissected meristem tip contained the apical dome covered by two leaf primordia and protected at the base by stem tissue. The excised meristem tips were left