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EFFECT OF ABA PRETREATMENT ON CHLOROPLASTS STRUCTURE IN HYPERICUM RUMELIACUM BOISS. PLANTS SUBJECTED TO CRYOPRESERVATION

D. KOLEVA*, Ts. GANEVA and M. STEFANOVA
Sofia University “St. Kliment Ohridski”, Faculty of Biology, BG - 1164 Sofia, Bulgaria

Abstract


Chloroplast ultrastructure in mature leaves’ cells of in vitro cultured Hypericum rumeliacum Boiss. plants regenerated from cryopreserved shoot tips pretreated with abscisic acid (ABA) for 3, 7 and 10 days periods respectively was analyzed by transmission electron microscopy (TEM). The chloroplasts in the control-unfrozen plants were lenticular with well-developed peristromium and thylakoids. The chloroplasts in regenerants passed 3-day period of pretreatment with ABA were structured almost the same to the control ones while in regenerated after 7 day pretreatment plants, reduced volume of the thylakoids was observed. In 10 day pretreated regenerants TEM revealed round shaped chloroplasts and both grana and stromal thylakoids were destroyed. The structural analysis of the plastid apparatus in regrown H. rumeliacum plants pointed out the 3-day ABA-pretreatment as the most efficient for cryopreservation on sub-cellular level of organization.

Key words: abscisic acid, chloroplasts, cryopreservation, Hypericum rumeliacum
Abbreviations: ABA – abscisic acid; TEM – transmission electron microscopy

Introduction

Preservation of plant material at ultralow temperatures (cryopreservation) is a promising method for long-term conservation and storage of valuable plant genotypes. Although, it is known that freezing could cause damages on tissue, cellular and sub-cellular levels. The undisputable necessity of this method and the problems related to its application are well-founded reasons for many studies to direct their attention to optimizing the cryopreservation protocols (Mycock et al., 2010). There are still many open questions that refer to the structural and functional status of the treated plants (Kaczmarczyk, 2008). One of the steps in the cryopreservation process is the plant material pretreatment that could increase the survival rate and genetic stability (Yordanova et al., 2010). Abscisic acid is routine applied cryoprotectant against ultrastructural damages in the plant material subjected to ultralow temperatures (Haisel et al., 2006; Kaczmarczyk, 2008; Brunakova et al., 2010).

Hypericum rumeliacum Boiss. is a Balkan subendemic species with conservational value for the Bulgarian flora. At the same time number of studies reported accumulation of phenolics and flavonoids with valuable pharmacological properties (Kitanov, 2001; Galati et al., 2008). Danova et al. (2010) reported that in vitro cultured H. rumeliacum plants produce high levels of phytochemical compositions commensurable to the levels in the intact plants.

The aim of the present study was to analyze by TEM the chloroplast ultrastructure in mature leaves’ cells of in vitro cultured H. rumeliacum plants regenerated from cryopreserved shoot tips pretreated with ABA for 3, 7 and 10 days periods respectively.

Material and Methods

The preculture treatment performed to H. rumeliacum was based on 0.076 μM ABA exposure of shoot tips in RMB, liq-

*E-mail: koleva_phd@abv.bg
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uid culture medium for 3, 7 and 10 days periods. Further explants were treated for 20 minutes in LS solution (2M glycerol and 0.4M sucrose) at room temperature. Plant shoot tips were dehydrated in PVS3 (50% w/v sucrose and 50% w/v glycerol) for 90 minutes on ice and finally directly immersed into liquid nitrogen. After one week of storage, thawing was performed in water bath at 40ºC for 1 minute. Tips were rinsed in liquid RMB0.5 containing 1.2 M sucrose. Afterwards shoot tips were cultivated on semi-solid RMB0.5 for regeneration. The transmission electron microscopy was performed approximately 2 months after thawing. Leaf segments (1 mm²) from the middle part of fully expanded leaves were fixed in 3 % glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 12 h at 4ºC. Then the leaf segments were post fixed in 1% KMnO₄ in the same buffer for 2 h at room temperature. After dehydration by increasing concentrations of ethylalcohol (from 25% to 100%), the samples were embedded in Durcupan (Fluka, Buchs, Switzerland) and cross-sectioned with Reichert-Jung (Wien, Austria) ultramicrotome. Observation was performed by JEOL 1200 EX (Tokyo, Japan) electron microscope.

**Results**

The chloroplasts in the control-unfrozen plants were lenticular with well-developed peristromium entirely orientated to the cell wall (Figure 1). The internal membrane system consisted of large-areal grana with different height (4–30 thylakoids) and long stromal thylakoids. Only in few chloroplasts there were small stromal zones where the thylakoids were orientated perpendicular to the longitudinal axis (Figure 1 – arrow).

![Fig. 1. Control plant chloroplast](image1)

![Fig. 2. 3-day ABA-pretreated plant chloroplast](image2)

![Fig. 3. 7-day ABA-pretreated plant chloroplast](image3)

![Fig. 4. 10-day ABA-pretreated plant chloroplast](image4)
The chloroplasts in plants passed 3-day pretreatment with ABA were structured in almost the same way as the control ones (Figure 2). The internal membrane system had comparatively large volume. It consisted of well-developed grana and stromal thylakoids.

The chloroplasts in regenerated after 7-day pretreatment with ABA plants had reduced volume of the thylakoid system than the control ones (Figure 3). The observed grana were not so large-areal and the number of the thylakoids did not exceed 10. The amount of the stromal thylakoids was also small. The thylakoid membranes were well developed and the peristromium was with comparatively large volume.

The chloroplast structure in regenerated after 10-day pretreatment with ABA plants differed greatly from the control ones (Figure 4). They were round shaped. The observation revealed internal membrane system in state of destruction. There were no distinguishable grana and stromal thylakoids in the stroma.

In none of the examined experimental plants, starch grains in the stroma were found.

Discussion

Chloroplasts are the organelles mainly affected by the low temperature conditions (Kratsch and Wise, 2000). There are many records that low temperatures cause destruction and reorganization of the thylakoid system in chloroplasts and deviations in the metabolic transport (Taylor and Craig, 1971; Ristic and Ashwort, 1993; Kratsch and Wise, 2000; Deryabin et al., 2007; Popov et al., 2007). Other cell organelles such as mitochondria and nucleus are much more stable in same experimental conditions (Ishikawa, 1996; Kratsch and Wise, 2000). According to Dellaire et al. (1994) ABA protection ability does not depend on its concentration. The present study revealed that if the structure of the chloroplasts is considered as a criterion for the cryoprotective role of ABA then for H. rumeliacum the duration of the pretreatment is very important. The longer the duration of the pretreatment is, the weaker is the protective effect of ABA and the stronger is the negative effect of the low temperatures along with the other stress factors on the ultra structure of the plastid apparatus.

The TEM analysis supported and completed the results from the histological light microscopy observation of the leaves of H. rumeliacum plants in same experimental conditions (Danova et al., 2009).

Conclusions

The structural analysis of the plastid apparatus in recovered H. rumeliacum plants pointed out the 3-day ABA pretreatment as the most efficient for cryopreservation on sub-cellular level of organization and supported the assertion of the great significance of ABA-pretreatment duration for photosynthetic tissue structuring.

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References


Kaczmarczyk, A., 2008. Physiological, biochemical, histological and ultrastructural aspects of cryopreservation in meri-
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stematic tissue of potato shoot tips. Doctoral dissertation.


