South African Journal of Botany 119 (2018) 132–141

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Biochemical characterization of peroxidases from the moss *Dicranum scoparium*

A.O. Onele [a,b], A. Chasov [a,b], L. Viktorova [a], R.P. Beckett [c], T. Trifonova [a], F. Minibayeva [a,b,*]

[a] Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", PO Box 30, Kazan 420111, Russia
[b] Kazan Federal University, Kremlyovskaya 18, Kazan 420008, Russia
[c] School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg, Scottsville 3209, South Africa

ARTICLE INFO

Article history:
Received 16 April 2018
Received in revised form 29 June 2018
Accepted 16 August 2018
Available online 13 September 2018

Edited by KI Ananieva

Keywords:
Dicranum scoparium Hedw
Bryophytes
Mosses
Desiccation tolerance
Peroxidase isoforms
Superoxide radical

ABSTRACT

Mosses are a convenient model to study stress responses of plants because of their remarkable stress tolerance. Peroxidase (EC 1.11.1.7) activities were tested in three moss species, namely *Dicranum scoparium*, *Hylocomium splendens* and *Pleurozium schreberi* growing together in the same location in a boreal forest. Peroxidase activity in *D. scoparium* was twice as high as in other mosses. Total peroxidase activity in unstressed *D. scoparium* was constitutively high; furthermore, long-term desiccation caused a significant increase in activity after 48 h of drying. Interestingly, when thalli desiccated for a week were rapidly rehydrated, peroxidase activity initially declined and then increased after 2 h rehydration. Diverse anionic and cationic isoforms were detected by native isoelectric focusing and PAGE of both crude extracts and partially purified peroxidases. The ability of peroxidases from *D. scoparium* to produce superoxide radical ($O_2^{\bullet-}$) was confirmed using the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay and in-gel nitroblue tetrazolium chloride (NBT) staining; specific $O_2^{\bullet-}$ producing isoforms were revealed using 2D electrophoresis. Given a quinone and chelated Fe^{3+} *D. scoparium* could produce extracellular hydroxyl radical ($^{\bullet}OH$), and production was increased by desiccation/rehydration stress. The possible roles of peroxidases and quinone reductases in apoplastic $^{\bullet}OH$ production is discussed. Our data demonstrate that *D. scoparium* possesses high constitutive peroxidase activity that can be further increased by desiccation stress. Among the diverse moss peroxidases, some anionic isoforms displayed both pro- and antioxidative activities. These findings suggest that the ability of peroxidases to produce and detoxify reactive oxygen species is an evolutionarily ancient characteristic, important for plant stress tolerance.

1. Introduction

Bryophytes are non-vascular plants represented today by three phyla, namely liverworts (phylum *Hepatophyta*), mosses (phylum *Bryophyta*), and hornworts (phylum *Anthocerophyta*) (Záveská et al. 2015). Bryophytes are believed to be among the first plants to colonize the land (Ponce de León and Montesano 2013). Mosses are currently represented by approximately 10,000–15,000 species that grow in some of the harshest environments on earth such as dry heaths, rock faces, tree trunks and even deserts (reviewed in Dey and De 2012). They exhibit distinctive adaptations which allow them to occupy this wide range of environments, including the ability to tolerate drying out, i.e., they are desiccation tolerant (Proctor et al. 2007). The dominant phase of the moss life cycle is a haploid gametophyte, meaning that any mutations will immediately be visible in the phenotype (Chobot et al. 2008). Furthermore, they respond to some hormones and environmental stimuli in the same way as vascular plants (Cove et al. 1997). These features, taken together with their lack of waxy cuticle, epidermis and well-developed conduction system make mosses good models to study mechanisms of stress tolerance in plants, particularly desiccation tolerance.

Among the mechanisms of desiccation tolerance, regulating the levels of stress-induced reactive oxygen species (ROS) plays a crucial role. ROS such as the superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$) are produced in healthy plants, but their production greatly increases when plants become stressed. Once produced, ROS can attack cellular components by causing damage to lipids, proteins, nucleic acids and activate cell death (Demidchik

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid; DMBQ, 2,6-Dimethoxy-1,4-benzoquinone; IEF, isoelectric focusing; MDA, malondialdehyde; NBT, nitroblue tetrazolium chloride; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide..

* Corresponding author at: Laboratory of Redox Metabolism, Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", Lobachevsky 2/31, Kazan 420111, Russia.
E-mail addresses: chasov@kibb.knc.ru (A. Chasov), minibayeva@kibb.knc.ru (F. Minibayeva).

https://doi.org/10.1016/j.sajb.2018.08.014
0254-6299/