Abstract

Within the thioredoxin superfamily, glutaredoxins represent a group of small oxidoreductases, 10-15 kDa in size. They are involved in a large number of cellular processes, play a crucial role in redoxregulation and response to oxidative stress. Conserved cysteins located in the active site motifs participate in disulfide reduction and thus modify target proteins posttranslationaly. According to their active site motif glutaredoxins are classified in three families, the CPYC, CGFS and CC-type glutaredoxins. The CC-type family is land plant specific and expanded dramatically during land plant evolution from two in *Physcomitrella patens* to over 20 in *Arabidopsis thaliana*, coinciding with the evolution of more complex organs. Contrarily, the number of CPYC and CGFS glutaredoxins remained similar in all investigated land plant groups. The *Arabidopsis* mutants *Atroxy1* and *Atroxy1roxy2* reveal a floral phenotype affecting petal initiation and morphogenesis as well as anther differentiation. Furthermore, glutaredoxins are involved in pathogen response and it was shown, that they interact with floral and pathogen-associated TGA-transcriptionfactors in *Arabidopsis*.

Towards understanding the ancestral function of CC-type glutaredoxins, *PpROXY1* and *PpROXY2,* from *Physcomitrella* were characterized in this work. Surprisingly, complementation studies revealed a functional conservation of *PpROXY1/2* and *AtROXY1*. Both *Physcomitrella* CC-type glutaredoxins were able to restore the *Atroxy1* petal phenotype. Tridimensional protein structure prediction showed a similar structure for PpROXY1/2 and AtROXY1. Ectopic expression of *PpROXY1/2* leads to an earlier flowering time and further indicate higher pathogen sensitivity for 35*S::PpROXY2* plants. Thus, overexpression of *PpROXY1* and *PpROXY2* in *Arabidopsis* gave evidence for different functions of PpROXY1/2 in a heterologous system.

RT-PCR analysis revealed a highly dynamic expression pattern of *PpROXY1/2*, as it was already shown for *Arabidopsis* and *Oryza*. The detection of expression of *PpROXY1/2* in young, just initiated tissues such as gametophores was supported by GUS experiments. Summing up, a transient dynamic expression in young, just initiated tissues and organs seems to be a common characteristic of *ROXY* genes in distantly related species.

Pproxy1- and *Pproxy2*-knock-out-lines were produced to investigate the *PpROXY1/2* function in *Physcomitrella*. Using a gene replacement strategy, several single knock-out lines were verified and the phenotypes of 2 lines per gene were further characterized. These studies revealed an earlier gametophore initiation, which was most significant in the *Pproxy1*- and *Pproxy2*-knock-out-lines. As a result of the earlier formation of gametophores, *Pproxy1*-knock-out-lines may have produced longer gametophores if they were not induced to produce reproductive organs, which stopped their length growth.

To summarize, besides exerting redox-regulated functions, CC-type glutaredoxins might have been co-opted during the evolution of land plants to participate in different other processes such as flower development and pathogen response.