Journal of Experimental Botany, Vol. 69, No. 22 pp. 5519–5530, 2018
doi:10.1093/jxb/ery304 Advance Access publication 17 August 2018

RESEARCH PAPER

Poplar miR472a targeting *NBS-LRRs* is involved in effective defence against the necrotrophic fungus *Cytospora chrysosperma*

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Received 7 February 2018; Editorial decision 8 August 2018; Accepted 9 August 2018

Editor: Katherine Denby, York University, UK

Abstract

The hemibiotroph *Colletotrichum gloeosporioides* and the necrotroph *Cytospora chrysosperma* cause poplar foliage and stem disease, respectively, resulting in substantial economic losses. In this study, *Populus trichocarpa* ptc-miR472a was down-regulated in leaves treated with salicylic acid, jasmonic acid (JA) or bacterial flagellin (flg22). Here, ptc-miR472a and a short tandem target mimic (STTM) of miR472a were overexpressed in *P. alba* × *P. glandulosa*, and overexpression lines of miR472a and silenced lines of STTM472a were generated. Compared with the STTM472a and wild type lines, lower reactive oxygen species accumulation was detected in miR472a overexpressing plants treated with flg22, *C. gloeosporioides* or *C. chrysosperma*. In addition, the miR472a overexpressing lines exhibited the highest susceptibility to the hemibiotroph, *C. gloeosporioides*, but the highest effective defence response to the necrotroph, *C. chrysosperma*. The JA/ethylene marker gene *ERF1* was rapidly up-regulated in miR472a overexpressing plants. Furthermore, five phased, secondary, small interfering RNAs (phasiRNAs) were confirmed in the miR472a overexpressing and STTM472a lines, triggering phasiRNAs predicted to enhance *NBS-LRR* silencing. Taken together, our results revealed that ptc-miR472a exerts a key role in plant immunity to *C. gloeosporioides* and *C. chrysosperma* by targeting *NBS-LRR* transcripts. This study provides a new strategy and method in plant breeding to improve plant disease resistance.

Keywords: *Colletotrichum gloeosporioides*, *Cytospora chrysosperma*, microRNA (miRNA), *NBS-LRR*, phasiRNA, *Populus*, redox oxygen species.

Introduction

Plants inhabit environments thronging with infectious microbes that pose frequent threats to their survival. Accordingly, plants have evolved effective defence systems against the microbes. In these systems, resistance (R) proteins have a pivotal role in response to pathogen infections, and can induce oxidative bursts and the expression of pathogenesis-related (*PR*) genes and programmed cell death (PCD) (Jones and Dangl, 2006; Wu *et al.*, 2014; Zou *et al.*, 2018). NBS-LRR proteins, which contain a nucleotide-binding site and leucine-rich repeat domains, are the largest class of known R proteins. NBS-LRR