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Construction of overexpression vectors of *Magnaporthe oryzae* genes *BAS1* and *BAS4* fusion to mCherry and screening of overexpression strains

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ABSTRACT. The aim of this study was to construct overexpression vectors and selecting strains of the *Magnaporthe oryzae* effectors BAS1 and BAS4. Primer pairs of BAS1, BAS4, and mCherry were designed based on their known nucleotide sequences. The coding sequences of BAS1 and BAS4 were amplified, and the pXY201 plasmid was selected as a template to amplify the mCherry sequence. Fragments of BAS1 and mCherry, and BAS4 and mCherry were ligated into the pCAMBIA1302 vector. The recombinant pCAMBIA-BAS1-mCherry and pCAMBIA-BAS4-mCherry plasmids were transformed into *E. coli* DH5α competent cells. Transformants were screened by PCR, and plasmids from the positive transformants were extracted by enzymatic digestion to obtain pCAMBIA-BAS1-mCherry and pCAMBIA-BAS4-mCherry. The pCAMBIA-BAS1-mCherry and pCAMBIA-BAS4-mCherry plasmids were transformed into protoplasts of rice blast strains and the transformed

strains were screened by PCR using primer pairs against the hygromycin gene. The result showed that the PCR products corresponded with the theoretical sizes. RT-PCR was used to analyze the expression of *BAS1* and *BAS4* in five transformed strains of *BAS1* and *BAS4*, and the result showed that the higher expression level of the two genes was occurred in five transformant strains comparing to wild-type strain A3467-40 (the strain containing *BAS1* and *BAS4*), but there was no difference among the five overexpression strains. The sporulation and spore germination of transformed strains was higher than in wild type strain, and there was no difference in the germination time. Construction of overexpression vectors and strains of *M. oryzae* effectors BAS1 and BAS4 provide reference material for other new effectors.

Key words: *Magnaporthe oryzae*; Effector protein; Overexpression vector; PEG-mediated transformation

