

Figure S1 The GUS expression was dependent on the transfer of T-DNA. The right border sequences of pCBEP:GUS were deleted for transient expression. After Agro-infiltration, barley seedlings were incubated in growth room (24 °C, 16-h light/8-h darkness photoperiod) with 1 day of moisturizing treatment; samples were collected and analyzed at 4 dpi. The experiments were repeated three times, and similar results were observed.

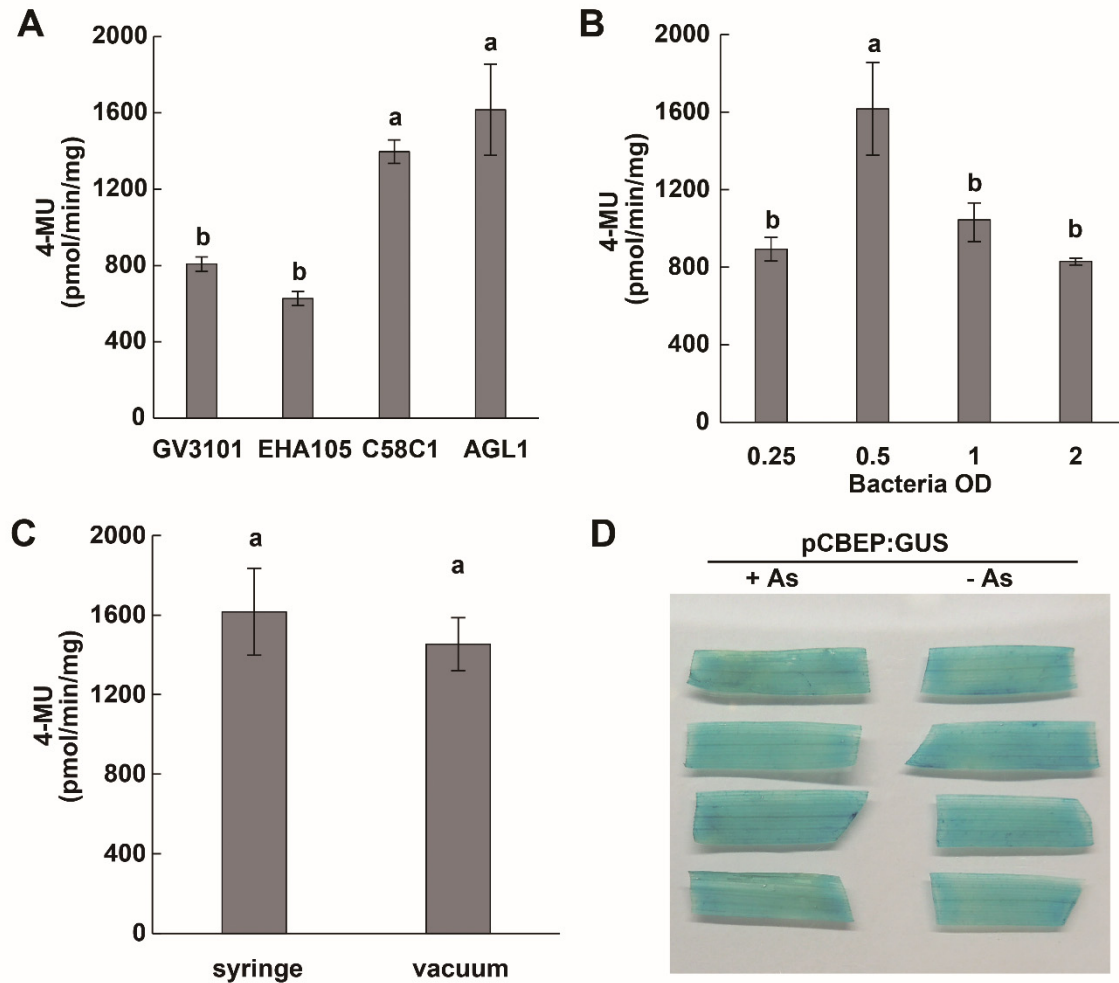


Figure S2 Optimization of transient expression in barley leaves. **A.** Quantification of GUS activity with diverse *Agrobacterium* strains (AGL1, C58C1, GV3101, and EHA105) harboring pCBEP:GUS. **B.** Different concentration (0.25-2 OD₆₀₀) of AGL1. **C.** Agro-infiltration by syringe or vacuum. **D.** With or without acetosyringone (AS) in the infiltration buffer. AGL1 (0.5 OD₆₀₀) harboring pCBEP:GUS was used for Agro-infiltration in the first leaf of barley with 1 day of moisturizing treatment; samples were collected and analyzed at 4 dpi. Data shown were means \pm SD (n = 6). Different letters indicate significant differences at P<0.01. The experiments were repeated three times, and similar results were observed.

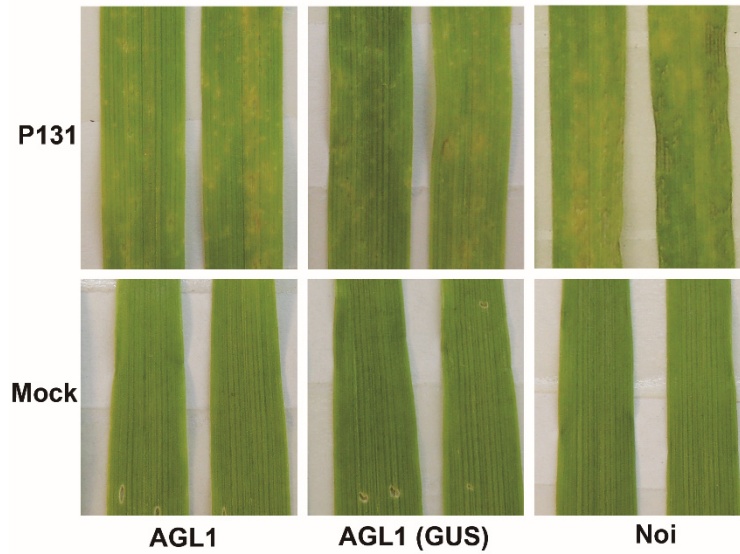


Figure S3 Blast fungus can successfully infect Agro-infiltrated leaves of barley. At 3 d after Agro-infiltration of the AGL1, AGL1 (GUS) or not (Noi), barley leaves were spray-inoculated with *M. oryzae* P131 (1×10^5 spores/mL) and incubated in a dark growth chamber at 25 °C for 24 h, followed by a 16-h light/8-h darkness photoperiod. The plants were sprayed with sterile deionized water as mock. Photographs were taken 3 days after fungal inoculation. The experiments were repeated three times, and similar results were observed.

Table S1. Plasmids used in this study

Plasmids	Relative properties or purpose	Source/References
pIPKb002	Constitutive binary expression vector, Spec ^R	Lu et al., 2016
pER8:DEST	Intermediate vector modified from pER8, inducible expression vector, Spec ^R	Fang et al., 2017
pCBEP:DEST	Intermediate vector modified from pER8 to pCBER DEST, Kana ^R	Fang et al., 2017
pINDEX2	Inducible expression vector, Kana ^R	Ouwerkerk et al., 2001
pTA7002	Inducible expression vector, Kana ^R	Aoyama and Chua, 1997
pCBDEST-transient	Constitutive expression vector, Kana ^R	Chang et al., 2019
pIPKb002:GUS	Transient expression of <i>GUS</i>	This study
pER8:GUS	Transient expression of <i>GUS</i>	This study
pCBEP:GUS	Transient expression of <i>GUS</i>	This study
pCBEP:GUS-Flag	Transient expression of <i>GUS</i> tagged Flag	This study
pCBEP-noRB:GUS	Transient expression of <i>GUS</i> deleting the right border	This study
pINDEX2:GUS	Transient expression of <i>GUS</i>	This study
pTA7002:GUS	Transient expression of <i>GUS</i>	This study
pCBDEST-GUS	Transient expression of <i>GUS</i>	This study
pCBEP:cLUC	Transient expression of cLUC for Split-LUC assay	This study
pCBEP:nLUC	Transient expression of nLUC for Split-LUC assay	This study
pCBEP:cLUC-OsbHLH6	Transient expression of cLUC-OsbHLH6	This study
pCBEP:cLUC-OsJAZ1	Transient expression of cLUC-OsJAZ1	This study
pCBEP:OsMYC2-nLUC	Transient expression of OsMYC2-nLUC	This study
pCBEP:OsNYC3	Transient expression of OsNYC3	This study
pCBEP:OsNUDX21	Transient expression of OsNUDX21	This study
pCBEP:OsMRS2-9	Transient expression of OsMRS2-9	This study
pCBEP:OsAk2	Transient expression of OsAk2	This study
pCBCS	Constitutive binary expression vector, Kana ^R	Chen et al., 2021
pCBCS:OsNYC3	Stable overexpression of OsNYC3 for <i>Arabidopsis</i> transformation	This study
pCBCS:OsNUDX21	Stable overexpression of OsNUDX21	This study
pCBCS:OsMRS2-9	Stable overexpression of OsMRS2-9	This study
pCBCS:OsAk2	Stable overexpression of OsAk2	This study

^Rresistance: Spec, spectinomycin; Kana, kanamycin.

Table S2. Primers used in this study

Primers	Sequence 5'-3'	Purpose
GUS-attb-F	aaaaagcaggctATGTTACGTCCTGTAGAAACCCC	Cloning of <i>GUS</i>
GUS-attb-R	agaaagctgggtTTATTGTTTGCCTCCCTGCTG	
DEST-F	gcctcgacacgtgtaaGACTCTAGCCTCGAGAATCAACA	Cloning of the <i>attR</i> cassette
DEST-R	agatgtacatactegaGATCGACTAGTATCAACCACTTTGT	
pCBEP noRB-F	TGGCGGCCGCTCTAGAACTA	Cloning of pCBEP:GUS without right border for self-ligated
pCBEP noRB-R	AATCCGATCCGCACATGAGG	
attb-c/nLUC-sfiI-F	aaaaagcaggctTACTATTCTAGTCGACCTGCAG	Cloning of cLUC and nLUC containing two different SfiI sites to construct pCBEP:cLUC and pCBEP:nLUC
attb-c/nLUC-sfiI-R	agaaagctgggtCTGGTGATTTTTGCGGACTCTA	
OsMYC2-sfiI-F	attaacaaggccattacggccATGTGGGTTTTGTTATCTCCTCTCCTC	Cloning of <i>OsMYC2</i> from cDNA of rice (Nipponbare) to construct pCBEP:OsMYC2-nLUC
OsMYC2-sfiI-R	aactgattggccgagggcgccccCTCGGCGATGCGGGTGTAGA	
OsHHLH6-sfiI-F	attaacaaggccattacggccATGGACGCCGAGATGGCCA	Cloning of <i>OsHHLH6</i> from cDNA of rice (Nipponbare) to construct pCBEP:cLUC-OsHHLH6
OsHHLH6-sfiI-R	aactgattggccgagggcgccccGCTCAACGGACTGCCGGT	
OsJAZ1-sfiI-F	attaacaaggccattacggccATGGATCTGTTGGAGAAGAA	Cloning of <i>OsJAZ1</i> from cDNA of rice (Nipponbare) to construct pCBEP:cLUC-OsJAZ1
OsJAZ1-sfiI-R	aactgattggccgagggcgccccCTGGGCCTTGCCCTCAGC	
attb-Flag-sfiI-F	aaaaagcaggctTACTATTCTAGTCGACCTGCAG	Cloning of Flag containing two different <i>SfiI</i> sites to construct pCBEP:Flag
attb-Flag-sfiI-R	agaaagctgggtCTGGTGATTTTTGCGGACTCTA	
SfiI-OsNYC3-F	attaacaaggccattacggccGCCACTAGTGTGGCCATGGAAGT	Cloning of <i>OsNYC3</i> from cDNA of rice (Nipponbare) to construct pCBEP:OsNYC3 and pCBCS:OsNYC3
SfiI-OsNYC3-R	aactgattggccgagggcgccccTCTAGATATTACCCATGTGTTG	
SfiI-OsNUDX21-F	attaacaaggccattacggccACAATCAAAGATTTCGATGGCCG	Cloning of <i>OsNUDX21</i> from cDNA of rice (Nipponbare) to construct pCBEP:OsNUDX21 and pCBCS:OsNUDX21
SfiI-OsNUDX21-R	aactgattggccgagggcgccccCAGCGCAGACTGCAGCTTGTTG	
SfiI-OsMRS2-C-F	attaacaaggccattacggccGACCTCGACGGCCATGGACCAC	Cloning of <i>OsMRS2-C</i> from cDNA of rice (Nipponbare) to construct pCBEP:OsMRS2-C and pCBCS:OsMRS2-C
SfiI-OsMRS2-C-R	aactgattggccgagggcgccccCAAGGGGAAAAACCTTCTCTTCTTG	
SfiI-OsAdK2-F	attaacaaggccattacggccGTTAATATGGTGAAGGAACGCC	Cloning of <i>OsAdK2</i> from cDNA of rice (Nipponbare) to construct pCBEP:OsAdK2 and pCBCS:OsAdK2
SfiI-OsAdK2-R	aactgattggccgagggcgccccTGCGCTAGCCACCATTCTGT	

MGActin-qF	GGTCTTGAGAGCGGTGGTATCCATG
MGActin-qR	TGCCAGGGCAGTGATCTCCTTC
Barley actin F	CCTCGGCACACTGGTGTCATGGTA
Barley actin R	AAGGGGCCTCAGTGAGCAACACA
UBQ10-F	GGCCTTGTATAATCCCTGATGAAT
UBQ10-R	AAAGAGATAACAGGAACGGAAACA
β -tubulin-F	CGGTCTCAAGATGTCCTCCAC
β -tubulin-R	AGAAAGCCTTGCGACGGAACA
R-Actin1-F	TGGCATTGCTGACAGGATGAGC
R-Actin1-R	CGTCGTACTCAGCCTTGGCAATC
OsNYC3-qF	ATTACAACCATGGGCGGAGG
OsNYC3-qR	TCCCAGAGAGTTCCCCACAA
OsNUDX21-qF	CATGATGTTCCCCAAGGGCG
OsNUDX21-qR	TCTTGTAGTACCAGCAGCCG
OsMRS2-C-qF	ACATTTCGTGGTGGCCATCTT
OsMRS2-C-qR	GTCCATTTCGAACGCATGTGG
OsAdK2-qF	AAGGTCATGATAGCAGGGGC
OsAdK2-qR	AGCTCGCTTCCCATTCTCAC

Magnaporthe oryzae Actin gene for qPCR

Barley HvActin gene for qPCR

Arabidopsis UBQ10 gene for qPCR

Colletotrichum higginsianum β -tubulin gene for qPCR

Rice *OsActin* gene for qPCR

Rice *OsNYC3* gene for qPCR

Rice *OsNUDX21* gene for qPCR

Rice *OsMRS2-C* gene for qPCR

Rice *OsAdK2* gene for qPCR