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Short Communication

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The Wheat Puroindoline Genes Confer Fungal Resistance in Transgenic Corn

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Abstract

Puroindoline a and *b* (*Pina* and *Pinb*), together make up the functional components of the wheat grain hardness locus (*Ha*) and have antimicrobial properties. The antifungal activity of puroindoline proteins, PINA and PINB, has been demonstrated *in vitro* and *in vivo*. In this study, *Pina* and *Pinb* were introduced into corn under the control of a corn *Ubiquitin* promoter. Two *Pina*/*Pinb* expression–positive transgenic events were evaluated for resistance to *Cochliobolus heterostrophus*, the corn southern leaf blight (SLB) pathogen. Transgenic corn expressing Pins showed significantly increased tolerance to *C. heterostrophus*, averaging 42.1% reduction in symptoms. Pins are effective *in vivo* as antifungal proteins and could be valuable tools in corn SLB control.

Introduction

The puroindolines, *Puroindoline a* and *b* (*Pina* and *Pinb*), are the functional components of the wheat grain hardness locus (*Ha*) and control wheat kernel texture (Giroux and Morris 1998). The antifungal activity of PINs has been demonstrated *in vitro* against several fungal plant pathogens (Dubreil et al. 1998) and *in vivo* in transgenic rice (Krishnamurthy et al. 2001), apple (Faize et al. 2004) and wheat (Luo et al. 2007).

Corn southern leaf blight (SLB), caused by the necrotrophic ascomycete *Cochliobolus heterostrophus* (Drechs.) Drechs. (anamorph = *Bipolaris maydis* (Nisikado) Shoemaker; synonym = *Helminthosporium maydis* Nisikado), is a serious foliar disease frequently found in warm and humid, corn-growing areas worldwide (White 1999). Race O of *C. heterostrophus* is the most common and predominant race in the United States and has the potential to decrease yield by 40% or more (Byrnes et al. 1989). Most SLB genetic resistance is quantitatively inherited, and the gene action is primarily additive or partially dominant and most U.S. germplasm is largely SLB resistant (Balint-Kurti et al. 2006, 2007, 2008).

B73 is a historically important inbred line with high susceptibility to SLB (Carson et al. 2004). Corn does not naturally contain any *Pins* (Zhang et al. 2009). Both *Pina* and *Pinb* were introduced into HiII line, which is a hybrid created between the SLB susceptible inbreds A188 and B73 (Armstrong et al. 1991), and the resulting transgenic lines were backcrossed to B73 and their progeny lines were tested for SLB resistance.

Materials and Methods

Plant materials

The plant materials used in all experiments were chosen from the transgenic lines described by Zhang et al. (2010). Both *Pina* and *Pinb* coding sequences were under the control of a corn *Ubiquitin* promoter and were transformed by biolistic transformation into callus derived from immature embryos of the HiII hybrid (Zhang et al. 2010). The HiII hybrid is created by crossing the two SLB susceptible cultivars A188 and B73 and is used for transformation because it is highly regenerable (Armstrong et al. 1991). Vectors *pBAR184*, *pUbiPina* and *pUbiPinb* carrying the *Bar*, *Pina-D1a* or the *Pinb-D1a* gene, respectively were described in Krishnamurthy and Giroux (2001). The T_0 transgenic lines were pollinated with B73 pollen and advanced to homozygousity to identify T_1-derived lines that were homozygous positive or negative for the transgene locus (Zhang et al. 2010). Two representative transgene-positive homozygous $T_{1:3}$ lines (UP6, UP8) derived from B73, along with their corresponding negative controls (UP6-n, UP8-n), were chosen for SLB test. Both UP6 and UP8 had a single or low copy number integration of the *Pin* genes, and in both events, the transgenes were inherited as single loci. SLB highly resistant (Mo17), moderately resistant

(A697), moderately susceptible (B73) and highly susceptible (A188) inbreds were used as controls. The transgene-positive and transgene-negative T_3 lines from UP6 and UP8 plus the control inbreds were grown in a greenhouse. The greenhouse conditions consisted of a 16 : 8 photoperiod with a daytime temperature of 28°C and a night-time target temperature of 21°C.

Inoculations

The *C. heterostrophus* race O isolates: 2–16 Bm and Hm28 were mixed 1 : 1 and the mixture used as inoculum in this study. Inoculum was prepared from 21-day-old potato dextrose agar medium plates. Conidia were suspended in water with 0.05% agar and ~0.01% TX-100 with a final concentration of 5×10^3⁄ml determined by a hemocytometer. Four leaf stage plants were inoculated according to Zhu et al. (1998). Plants were sprayed at 21 days after planting (~at the 4 leaf stage) until runoff. The plants were then left to dry and placed in a dew chamber at 21°C for 16 h, after which time the plants were returned to the greenhouse.

Ratings and statistical analysis

Disease ratings were taken on each plant using a 0–100% scale, in increments of 5%. The highly resistant control line Mo17 was scored as 10%, and the highly susceptible control line A188 was scored as 100%. Ratings considered the number, size and colour of the lesions. All genotypes were assessed in two replications of twelve plants at 6 days postinoculation (dpi). Transgene-positive lines were compared with their respective transgene-negative controls using *t*-tests.

Results and Discussion

To study the effect of *Pina* and *Pinb* expression on corn SLB race O resistance, we inoculated two *Pina*/–*Pinb* expressing transgenic events and various control genotypes. The highly resistant control Mo17 showed only small, circular chlorotic flecks, while the highly susceptible inbred A188 developed brown necrotic lesions and wilted. The average percent SLB severity on resistant control A679 was 18%, which showed small, chlorotic flecks as expected (Table 1). The average percent SLB severity on susceptible control B73 was 53.05%, which showed brown, necrotic lesions. SLB symptoms began to appear on transgenic-negative controls at 1 dpi. The overall results of the inoculations revealed SLB symptom reduction in both PIN transgenic lines ($P < 0.01$ for UP6 vs. UP6-n, $P < 0.05$ for UP8 vs. UP8-n) (Table 1, Fig. 1).

In this study, we evaluated the antimicrobial activity of PINs in transgenic corn against *C. heterostrophus*. The level of symptom reduction was similar to the level of control by PINs of several rice foliar pathogens (Krishnamurthy et al. 2001) and of wheat leaf rust (Luo et al. 2007).Our results indicate that initial fungal infections were similar between PIN-positive and PIN-negative plants, suggesting that the resistance mechanism is fungal growth inhibition. PINs are effective antifungal proteins *in vivo* and could be a valuable tool in the control of a wide range of fungal pathogens of crop plants. Given that PINs are antifungal proteins and not resistance genes, their effectiveness as disease control agents depend upon several factors. These include the level of PIN expression and tissue specificity, host plant susceptibility and pathogen load.

Table 1
SLB severities for two transgenic events expressing *Pins* and their corresponding negative counterparts expressed as percent of leaf area affected compared to Mo17 (resistant = 10%) and A188 (susceptible = 100%)

Genotype	Disease severity scores[a]	P value[b]
Mo17	10	
A188	100	
A679	18 ± 2.8	
B73	53.05 ± 0.1	
UP6	23.55 ± 2.0	
UP6-n	50.55 ± 4.3	< 0.01
UP8	42.35 ± 0.2	
UP8-n	61.1 ± 8.6	< 0.05

SLB, southern leaf blight; [a]Disease assessments are expressed as mean percent disease severity +/– standard deviations; [b]P values are for comparisons of homozygous-positive vs. homozygous-negative lines.

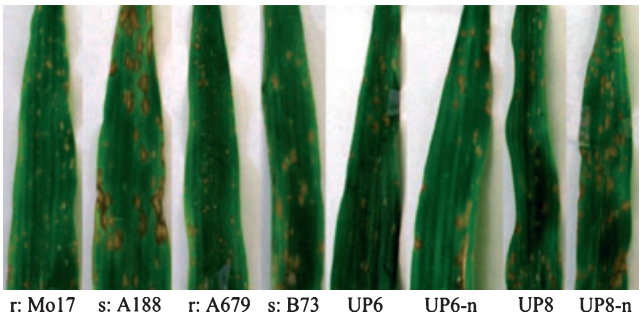

Fig. 1 Infection types of representative leaves of controls and the homozygous-positive transgenic lines and the corresponding homozygous negative lines 6 dpi with the southern leaf blight (SLB) pathogen. r denotes resistant and s susceptible controls

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