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SHORT COMMUNICATION

Antifungal metabolites from *Schinopsis balansae* Engl (Anacardiaceae): isolation, identification and evidences of their mode of action on *Fusarium graminearum* Schwabe

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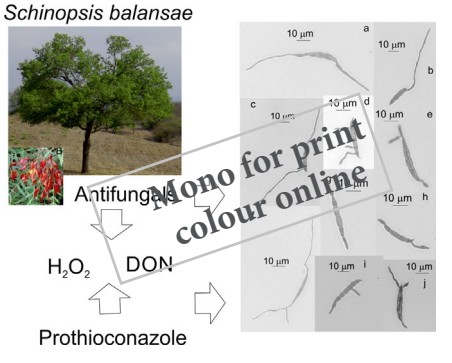
ABSTRACT

An antifungal activity-directed fractionation of leaf constituents from *Schinopsis balansae* on *Fusarium graminearum* yielded a fraction mainly made of a mixture of four 3-*n*-heptadec(en)ylcatechols (PALK). The PALK fraction showed on macroconidia germination a MIC_{100} value of 500 µg/mL which was twofold higher than that required for prothioconazole (MIC_{100} = 250 µg/mL). Sublethal concentrations of PALK modify the morphogenesis in germinating macroconidia, and decreased fungal production of H_2O_2 and deoxynivalenol biosynthesis at early fungal growth. Mixes of PALK and prothioconazole showed a synergic interaction. Our findings suggest that PALK constituents might restrict the adherence of *F. graminearum* to the surface of its hosts and its virulence on susceptible cereals. They deserve further research as additives of azole fungicides against *F. graminearum*.



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1. Introduction

Cereal ear rots caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*) contaminate the grains with deoxynivalenol (DON), a mycotoxin responsible of human and animal toxicoses (Sampietro et al. 2009). DON biosynthesis also is a virulence factor that favours the spread of the ear rot disease (Jansen et al. 2005). Several official agencies established maximum permissible contents of DON in cereals and derived products (FDA 2002). Azole fungicides are applied at flowering for control of the ear rots. Nevertheless, their use led to the development of fungal resistance and other adverse environmental effects (Becher et al. 2010). New fungicides, or additives of commercial ones, able to control fungal growth are needed in order to solve these concerns.

The *Schinopsis balansae* (tribe Rhoeae, Anacardiaceae family) is a native tree from northeast Argentina (Pell et al. 2011). Phytochemistry of this tree has been investigated only regarding its heartwood rich in tannins. Few reports are available about the phytochemistry of the genus *Schinopsis* (Aristimuño Ficoseco et al. 2014; Donati et al. 2015). This work reports an antifungal activity-directed fractionation of leaf constituents from *Schinopsis balansae* on *F. graminearum* and new evidences of the antifungal mode of action of the identified fraction, mainly made of a mixture of four 3-*n*-heptadec(en)ylcatechols (PALK), compared with that of prothioconazole.

2. Results and discussion

Preliminary results obtained by dot blot bioautographies on *F. graminearum* showed the highest diameters of growth inhibition for the dichloromethane and the ethyl acetate extracts (Table S1). TLC bioautographies of these extracts inhibited the fungal growth at *Rf* = 0.70. The GC–MS analysis of this band showed a mix of heptadec(en)ylcatechols (Table S2), which also were found in the urushiol fraction of other species of the Rhoeae tribe (Ma et al. 2012). The urushiol fraction of *S. balansae* differed from that reported for *Sardinella brasiliensis* which contained methyl 6-alk(en)yl-2-hydroxy-4-methoxybenzoates with side chains of 2–19 carbons (Cardoso et al. 2005) and for *Schinopsis lorentzii*, which contained 3-pentadecylcatechol and 3-heptadec-8,11,14-trienylphenol (Aristimuño Ficoseco et al. 2014). Regarding the antifungal activity, it was assayed on macroconidia germination and early hyphal growth, which are the fungal stages targeted by azole fungicides and define the adherence of *F. graminearum* to the surface of its hosts (Semighini et al. 2008). The urushiol fraction (PALK) had a MIC_{100} =500 µg/mL on macroconidia which was twofold higher than that observed for prothioconazole. Macroconidia incubated in water exhibited a bipolar germination (Figure S1a,c,f), with 59 ± 2% (LABI 123), 66 ± 2% (LABI 2), 50 ± 2% (NRRL 28063) and 62 ± 2% (NRRL 26916), in 80 ± 2% (LABI 123), 82 ± 1% (LABI 2), 78 ± 1% (NRRL 28063) and 82 ± 1% (NRRL 26916) of overall germination (Table S3). This pattern was no longer prevalent in macroconidia exposed to rising concentrations of both PALK (i.e. 8 ± 1%, 7 ± 1%, 5 ± 1%, 7 ± 1% in 34 ± 1%, 38 ± 2%, 24 ± 1%, 38 ± 2% at 250 µg/mL) and prothioconazole (i.e. 11 ± 1%, 9 ± 1%, 4 ± 1%, 9 ± 1% in 35 ± 3%, 30 ± 2%, 18 ± 2%, 30 ± 2% at 125 µg/mL). PALK also shifted the germ-tube formation in macroconidia from distal cells towards the middle ones (i.e. 6 ± 1%, 6 ± 1%, 7 ± 1%, 6 ± 1% in 34 ± 1%, 38 ± 2%, 24 ± 1%, 38 ± 1% at 250 µg/mL; Figure S1d) and generated more than one germ tube in a same distal cell (i.e. 5 ± 1%, 5 ± 1%, 6 ± 1%, 5 ± 1% in 34 ± 1%, 38 ± 2%, 24 ± 1%, 38 ± 1% at 250 µg/mL;

Figure S1j). PALK and prothioconazol differed in their impact on DON biosynthesis and H_2O_2 production at early fungal growth (Table S4). At 38 µg/mL and higher concentrations, PALK lowered the accumulation of H_2O_2 with respect to the water controls. It completely suppressed DON biosynthesis at concentrations higher than 38 µg/mL. Prothioconazole increased DON accumulation after induction of an oxidative redox imbalance of the fungal cells, a situation previously reported and expected after a suboptimal application of azoles on *F. graminearum* (Audenaert et al. 2010). The antimycotoxigenic activity of PALK could be due to a direct H_2O_2 scavenging activity of the 3-heptadec(en)ylcatechols, which avoid the oxidative burst required for DON biosynthesis. The differences in antifungal modes of action evidenced for PALK and prothioconazole were also indicated by a synergistic inhibitory interaction observed for their mixtures on fungal growth (FICI = 0.31).

3. Conclusion

The antifungal activity-directed fractionation of leaf constituents from *S. balansae* yielded a mix of 3-heptadec(en)ylcatechols. At sublethal concentrations, they disturbed the morphogenesis of germinating macroconidia, which might affect the adherence of *F. graminearum* to its host surfaces. They also change the redox balance of fungal cells and supressed DON biosynthesis, an important fungal virulence factor. These evidences of antifungal mode of action encourage to investigate the urushiol fraction of *S. balansae* as an additive of azole fungicides against *F. graminearum*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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