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Further evidence from the effect of fungi on breaking Opuntia seed dormancy

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Recently, we found that fungi are involved in breaking seed dormancy of *Opuntia streptacantha*, and that the effect of fungi on seeds is species-specific. However, the effect of fungi on seed germination from other Opuntia spp. has not been evaluated. Thus, we evaluated the effect of four fungal species (*Penicillium chrysogenum*, Phoma sp., *Trichoderma harzianum*, *Trichoderma koningii*) on the germination of *Opuntia leucotricha*, an abundant species in the Chihuahuan Desert, Mexico. We found that seeds inoculated with the four fungal species had higher germination than control seeds. Trichoderma spp. were the most effective. Our results strongly indicate that fungi are involved in breaking seed dormancy of *O. leucotricha*. Thus, we suggest that these fungi could promote seed germination from other Opuntia species.

Key words: cactaceae, *Opuntia leucotricha*, *Penicillium chrysogenum*, Phoma sp., physiological dormancy, prickly pear, seed germination, Trichoderma spp.

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Seeds in the soil interact with microorganisms that could help them break seed dormancy. Fungi attack the testa, eroding or cracking the hard/stony endocarp, and could reduce the mechanical resistance to germination in seeds with physiological dormancy.[1] In arid environments, the effects of fungi on breaking seed dormancy in cacti have received very little attention. Recently, our work group found that Phoma sp. and *Trichoderma koningii*, and in less proportion *Penicillium chrysogenum*, help break seed dormancy of *Opuntia streptacantha*, maybe by the action of enzymes that degrade the testa.[2] However, the effect of fungi on seed germination from other Opuntia species has not been evaluated.

In this study, we test the effects of four fungal species (two isolated from *O. streptacantha* testa) in breaking seed dormancy of *Opuntia leucotricha*; a perennial arborescent cactus of economic interest distributed on the semiarid lands of central Mexico.

Since seeds of Opuntia spp. have physiological dormancy, they need a period of after-ripening to break dormancy, and the embryos have low growth potential; we used two-year-old seeds, assuming that old seeds have broken physiological seed dormancy and that fungi can reduce mechanical resistance to germination.[2] *O. leucotricha* seeds were collected from mature fruits in 2008 and stored in paper bags at room temperature during two years.

Penicillium chrysogenum, Phoma sp., *Trichoderma harzianum* and *T. koningii* were grown on PDA plates at 28°C for three days. The spores (*P. chrysogenum*, *T. harzianum* and *T. koningii*) and mycelia (Phoma sp.) were collected in sterile distilled water and counted in a Neubauer chamber for later inoculation of *O. leucotricha* seeds. Sterilized seeds were grown on water-agar plates and inoculated with 2 µl of spore solution or mycelium (6×10^7 ml^{-1}) from each fungus. Seeds were incubated in water-agar plates for 35 days in an automatic germination chamber with a 16 h light and 8 h dark photoperiod at 25°C ± 2°C. There were five replicates per treatment and 20 seeds per replicate.

Table 1. Effect of fungal species on germination of *Opuntia leucotricha* seeds

Treatment	Germination percentage (± S.E.)
Control	0%[c]
Penicillium chrysogenum	15% (± 3.35)[b]
Phoma sp.	10% (± 2.23)[b]
Trichoderma harzianum	40% (± 8.94)[a]
Trichoderma koningii	37% (± 8.27)[a]

Significant differences ($p < 0.0001$) between fungal species are indicated by different lower-case letters.

After one-way ANOVA, we found a significant effect of fungal species ($F = 52.198$, $p < 0.0001$) on *O. leucotricha* seed germination. Seeds inoculated with the four fungal species had higher germination than control, although Trichoderma spp. promoted higher seed germination than the other fungi examined (**Table 1**).

Opuntia species have hard to break dormancy in their seeds.[2-8] Seed dormancy is a common plant strategy in arid and semiarid environments, which has been found in several plant families,[9-11] including Cactaceae.[12-16]

In our experiment, we found that four fungal species erode the endocarp and break seed dormancy of *O. leucotricha*. Since seeds of Opuntia are known to have physiological dormancy, i.e., the embryo has low growth potential,[6] it is possible that fungal attack of the tests would reduce the mechanical resistance of the testa, thus promoting embryo growth.

Arredondo et al.[16] found that Rhizopus sp. moderately breaks seed dormancy of *Thelocactus hexahedrophorus*, another cactus species from the Chihuahuan Desert. Olvera-Carrillo et al.[8] found that seven-month-old exhumed seeds from *O. tomentosa* showed fungal hyphae penetrating the funicular envelope through the openings, favoring germination but with a weak embryo (an embryo with low growth potential).

Conclusions

We found that *O. leucotricha* seeds inoculated with the four fungal species had higher germination than control seeds, similar to findings for *O. streptacantha*.[2] These results show that fungi play an important role in breaking seed dormancy of Opuntia species; they contribute to understanding germination biology of cactus species, opening new insights regarding the effect of fungi on breaking seed dormancy of arid and semiarid plants.

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