Free polyamine and polyamine regulation during pre-penetration and penetration resistance events in oat against crown rust (*Puccinia coronata* f. sp. *avenae*).

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Summary:
Crown rust is the most important disease of the oat crop (*Avena* spp.), and is caused by the obligate fungal pathogen *Puccinia coronata* f.sp. *avenae* (*Pca*). The important losses caused by this pathogen make rust resistance one of the desired traits in oat breeding programs. However, resistance introduced in new varieties, often based on major genes, is rapidly defeated by the evolving pathogen. Resistance mechanisms engaged prior to the hypersensitive response, such as pre-penetration and penetration resistance mechanisms, are important, as they are mostly non race-specific and are usually under polygenic control, making resistance more durable.

Polyamines are low molecular weight compounds related to various biological processes in plants. In recent years, a wealth of studies has focused on the role of polyamines in plant–environmental stress interactions. However, less is known about the role of these compounds in plant disease resistance, and most of this knowledge is related to the hypersensitive response. The principal objective of this study was to unravel the involvement of polyamines in the oat–crown rust interaction, focusing on mesophyll cell pre-penetration and penetration resistance mechanisms.

With this aim in mind, plants of the resistant cultivar 'Saia' and the susceptible cultivar 'Araceli' were inoculated with *Pca* isolate C0-04, and samples were harvested and fixed at different hours after inoculation. First, a microscopic assessment of fungal development, as well as which resistance mechanisms were engaged following pathogen attack, was performed. Then, the amounts of total and excreted polyamines in control and inoculated oat leaves were quantified using high performance liquid chromatography (HPLC) analysis at different times following inoculation. Additionally, pharmacological approaches were carried out to assess the effects of polyamines on the different resistance responses after rust inoculation. Finally, we studied polyamine regulation in resistant and susceptible oat genotypes by assessing the activity of some of the previously reported key enzymes involved in polyamine biosynthesis (i.e., arginine decarboxylase (ADC)) and in polyamine catabolism (i.e., diamine oxidase (DAO) and polyamine oxidase (PAO)). Expression of the ADC gene was also analyzed, using real-time qRT-PCR.

Our data show that Saia, which is characterized by efficient pre/penetration resistance responses to crown rust, showed increases in polyamine content at crucial times during the crown rust infection process, both intracellularly and excreted to the leaf surface. No change or lower increases were observed in the susceptible cv. Araceli. The early excretion of polyamines upon rust attack coinciding with the time of germ-tube growth and appressorium formation suggests that excreted polyamines might act as chemical signals able to interfere with these

fungal processes during pre-penetration events. This is supported by the increased resistance observed in Araceli after treatment with exogenous polyamines, which reduced appressorium formation. Furthermore, elevated levels of spermidine and spermine, together with increases in cell wall-bound DAO and PAO activities and concomitant increases in the polyamine oxidation product 1,3-diaminopropane, were observed in the resistant cultivar at early stages of the infection process. These results suggest an involvement of polyamines and derived H_2O_2 during penetration resistance, since H_2O_2 is crucial for the formation of papillae. In addition, ADC activity and gene expression in Saia were increased at key time points for the pre-penetration and penetration resistance responses, which point to ADC being a key regulator of the resistance response.

Overall, our data indicate a role for free polyamines during resistance events prior to the hypersensitive response, influencing both germ tube growth and appressorium formation over stomata, as well as the penetration of mesophyll cells. Further involvement of conjugated polyamines or polyamine catabolic products (such as hydrogen peroxide) in penetration resistance still needs to be clarified. However, the already proven role of free polyamines in pre-penetration and penetration resistance responses offers opportunities for breeders to develop varieties with more durable fungal resistance.