Research Paper

High infestation levels of *Schizotetranychus oryzae* severely affects rice metabolism

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ABSTRACT

High levels of *Schizotetranychus oryzae* phytophagous mite infestation on rice leaves can severely affect productivity. Physiological characterization showed that *S. oryzae* promotes a decrease in chlorophyll concentration and the establishment of a senescence process in rice leaves. Late-infested leaves also present high levels of superoxide radical and hydrogen peroxide accumulation, along with high levels of membrane integrity loss, which is indicative of cell death. To better understand the rice molecular responses to high levels of mite infestation, we employed the Multidimensional Protein Identification Technology (MudPIT) approach to identify differentially expressed proteins. We identified 83 and 88 proteins uniquely present in control and late-infested leaves, respectively, along with 11 and one proteins more abundant in control and late-infested leaves, respectively. *S. oryzae* infestation induces a decreased abundance of proteins related to translation, protease inhibition, and photosynthesis. On the other hand, infestation caused increased abundance of proteins involved in protein modification and degradation. Our results also suggest that *S. oryzae* infestation interferes with intracellular transport, DNA structure maintenance, and amino acid and lipid metabolism in rice leaves. Proteomic data were positively correlated with enzymatic assays and RT-qPCR analysis. Our findings describe the protein expression patterns of late-infested rice leaves and suggest several targets which could be tested in future biotechnological approaches aiming to avoid the population increase of phytophagous mite in rice plants.

1. Introduction

Rice is the staple food for over half of the world's population. Approximately 715 million metric tons of paddy rice, which generates about 480 million metric tons of milled rice, are produced annually in over a hundred countries (Muthayya et al., 2014). Rice is the primary source of calorie intake for an estimated 3.5 billion people worldwide. This is especially the case in the developing world, where it accounts for approximately 50% of the dietary caloric supply and a substantial part of the protein intake, being therefore critical for food security (Muthayya et al., 2014). For this reason, rice production faces the challenge to be enhanced by 50% by year 2030 to meet the growth of the population in rice-eating countries (Ahmadi et al., 2014). This is definitely not an easy task, since the oscillations observed in annual production of this culture derive mainly from abiotic and biotic stresses, which limit plant germination, development, and productivity (Lim et al., 2013; Dametto et al., 2015). The estimates for the potential losses caused by animal pests are around 25% worldwide (Oerke, 2006). One of the main reasons for losses in rice productivity is phytophagous mite infestation (Blasi et al., 2015; Buffon et al., 2016) that can damage rice plants during its entire development, depending on the mite species and infestation level.
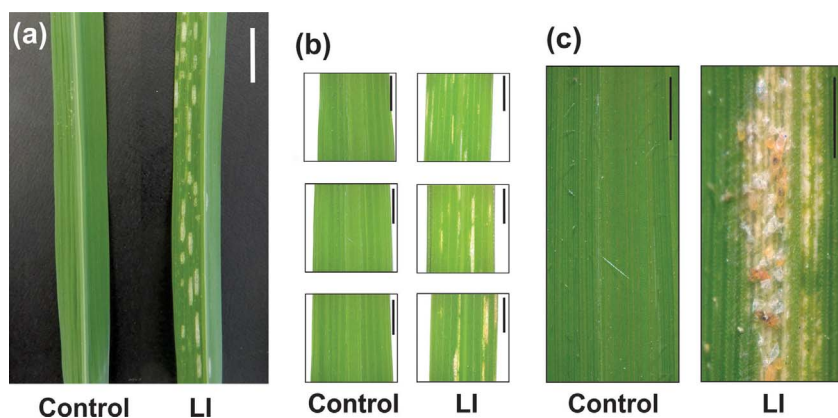
Fig. 1. Visual characteristics of leaves from control and late-infested (LI) leaves (a). Detailed view of these leaves under stereomicroscope (b and c). Bars indicate 1 cm in (a), 0.5 cm in (b) and 0.25 cm in (c).

Phytophagous mites of the Tarsonemidae (*Steneotarsonemus furcatus* De Leon and *Steneotarsonemus spinki* Smiley) and Tetranychidae (*Schizotetranychus oryzae* Rossi de Simons and *Oligonychus oryzae* Hirst) families are frequently found infesting rice culture as reviewed by Blasi et al. (2015). The spider mite *Schizotetranychus oryzae* (Acari: Tetranychidae) was first found in Argentina, and has been reported in rice plantations in Brazil and other South American countries (Ferla et al., 2013). Rice leaves infested with *S. oryzae* may present yellowish white elongated areas on the upper side of the leaves that corresponds to mite colonies in different stages of development. These colonies are located on the lower surface of the leaves (Buffon et al., 2016). In order to defend themselves from herbivore attacks, plants employ a wide range of induced defenses, including mostly jasmonic acid- and salicylic acid-dependent (Agut et al., 2015). These defenses result in morphological changes and synthesis of secondary metabolites that cause a decrease in herbivore performance (Alba et al., 2011; de Oliveira et al., 2016). The induction of these plant defenses depends on the ability of the plant to identify and recognize its attackers, and varies with the herbivore species and infestation level or the amount of time since the attack (Kant et al., 2004; Wu and Baldwin, 2009; de Oliveira et al., 2016). For example, in early attack stage plant responses are regulated through phytohormones, whereas late stage responses typically involve the synthesis of phytoalexins and secondary defensive metabolites (Agut et al., 2015). Rice responses to early *S. oryzae* infestation include down-regulation of photosynthetic performance, carbon assimilation and energy production, fatty acid biosynthesis and actin cytoskeleton remodeling. On the other hand, gene expression at the translational level, protein partitioning to different organelles, protein modification and degradation, β-oxidation of fatty acids (essential to jasmonic acid biosynthesis) and cell wall remodeling are up-regulated in response to early infestation (Buffon et al., 2016). It is important to highlight that a small/limited number of mites on rice plants can induce all of these molecular effects. In late stages, where there is a substantial increase in herbivore activity promoted by a larger number of mites infesting rice plants, the responses are still completely unknown.

Even though mite infestations in rice plantations can cause drastic yield reductions of up to 90% (Hummel et al., 2009), most of the plant-mite interaction studies have been limited to the visual effects of the infestation (population growth rate and injury symptoms) or physiological changes (Evaristo et al., 2013; Jaimez-Ruiz et al., 2015). Thus, the molecular mechanisms specifically elicited by mite infestation in rice plants remain virtually unexplored. In recent years, rice proteomics has achieved tremendous progress in development of highly efficient sample preparation methods and physiological and biochemical pathway data analysis under different developmental stages, in various tissues, organs, and organelles, and against biotic and abiotic stresses (Kim et al., 2014). Previously, we detected differentially expressed proteins in rice leaves after *S. oryzae* phytophagous mite early infestation (Buffon et al., 2016). The understanding of rice response to an intense parasitic activity promoted by *S. oryzae* in late infestation on rice leaves is important since the main damages directly affecting rice productivity occurs in this stage. In this work, we focused on the physiological and molecular alterations in rice leaves exposed to high levels of *S. oryzae* infestation, hypothesizing that we could use proteomic approach to identify potential targets to avoid the population increase of phytophagous mite in rice plants. Our findings will be helpful for future biotechnological and molecular breeding efforts that aim to promote mite resistance in rice leaves.

2. Materials and methods

2.1. Plant growth and mite infestation conditions

Plant growth and mite infestation conditions were performed according to Buffon et al. (2016). In all the experiments, we analyzed healthy (control) and late-infested leaves (LI, 60 days after infestation, containing about 177.8 ± 22.4 mites per leaf) (Fig. 1). It is important to highlight that IRGA 424 cultivar is not affected by the means of plant height and tiller number, but some leaves present high infestation levels after only 30 days, and the grain yield is reduced significantly (data not shown).

2.2. Total chlorophyll concentration

Samples containing 100 mg of leaves from rice plants submitted to control or infested conditions were ground in liquid nitrogen and chlorophyll extracted in 85% (v/v) acetone. Chlorophyll *a* and *b* were quantified by measuring absorbance at 663 and 645 nm and the concentrations calculated according to Ross (1974).

2.3. In situ histochemical localization of O_2^- and H_2O_2

In situ accumulation of O_2^- and H_2O_2 was detected by histochemical staining with nitro blue tetrazolium (NBT) and diaminobenzidine (DAB), according to Shi et al. (2010) with minor modifications. For O_2^- detection, leaves of control and late-infested plants were excised and immersed in a 1 mg ml^{-1} solution of NBT in 10 mM phosphate buffer (pH 7.8) at room temperature. Immersed leaves were illuminated for 2 h until appearance of dark spots, characteristic of blue formazan precipitates. For localization of H_2O_2, another set of leaves was sampled and immersed in DAB solution (1 mg ml^{-1}, pH 3.8) in 10 mM phosphate buffer (pH 7.8), and incubated at room temperature for 8 h in the light until brown spots were visible, which are derived from the reaction of DAB with H_2O_2. For both staining methods, leaves were bleached in boiling concentrated ethanol to visualize the blue and brown spots, which were kept in 70% ethanol for taking pictures with a digital camera coupled to a stereomicroscope.