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H_2O_2 IN INTERSPECIES SIGNALING: A NEW ROLE IN HOST DETECTION

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Striga asiatica, a parasitic plant of many cereal crops, is characterized by a sensory pathway enabling it to gauge the presence of a host plant. The contact between the parasitic seedling and the host surface induces the oxidation of phenolic components on the cell wall of the latter, producing quinones that signal the induction of parasitization. This signaling event was investigated by laser scanning confocal microscopy and electron microscopy methods, revealing a concentration gradient of H_2O_2 radiating from the meristem of the parasite. The source of H_2O_2 is likely to be a membrane-bound NAD(P)H oxidase, and the presence of the oxidant appears to occur without the cell defense and programmed cell death usually associated with its production. Moreover, the xenognostic quinones downregulate H_2O_2 production, thus avoiding the induction of defensive responses by the host. However, downregulation is not complete, but dose-responsive and fine-tuned so that the level of remaining H_2O_2 is sufficient for generating more xenognostic quinones.

One day-old *S. asiatica* were incubated in a staining solution of dihydrodichloro-fluorescein (H_2DCFH-DA) 10 µM for 3 min. Two rinsing steps in KCl 0.1 mM ensued, and the seedlings were transferred to a custom-made cell and mounted on a Zeiss LSM 510 laser scanning confocal microscope. The accumulation of fluorescence, and its modulation by a series of chemical effectors, was employed to localize and characterize the production of H_2O_2 in the *S. asiatica* seedling[1,2,3]. For SEM, *S. asiatica* seedlings were fixed in 1.25% (v/v) glutaraldehyde/1.25% (v/v) formaldehyde in 50 mM Na-cacodylate buffer, processed by CPD, coated with Au/Pd and imaged on a Topcon DS-130 FESEM at 5kV (Fig 1). Cytochemical localization of hydrogen peroxide on the surface of *S. asiatica* seedlings was carried out for TEM following modified procedure based on the production of cerium perhydroxides[1,2,4]. Seedlings were incubated in 5mM $CeCl_3$ for 2hrs, then fixed in 1.25% (v/v) glutaraldehyde/1.25% (v/v) formaldehyde in 50 mM Na-cacodylate buffer, pH 7.2. Postfixation in 1% (v/v) osmium tetroxide followed, and the samples were subsequently dehydrated and embedded in Embed-812. Selected blocks were thin-sectioned (70- 80 nm) and observed with a JEOL JEM-1210 transmission electron microscope at 80 kV. Confocal analysis staining with H_2DCF-DA revealed constitutive production of H_2O_2 in the epidermal layer of the seedling root meristem (Fig.2). $CeCl_3$ histochemical localization of H_2O_2 showed that secretion of the oxidant accumulated in the interstitia between epidermal cells (Fig. 3). In addition, exposure of the seedlings to xenognostic quinones induced a drop in H_2DCF-DA induced fluorescence and no accumulation of ceric deposits, further confirming a feedback mechanism linking the concentration of quinones to that of H_2O_2.

References:
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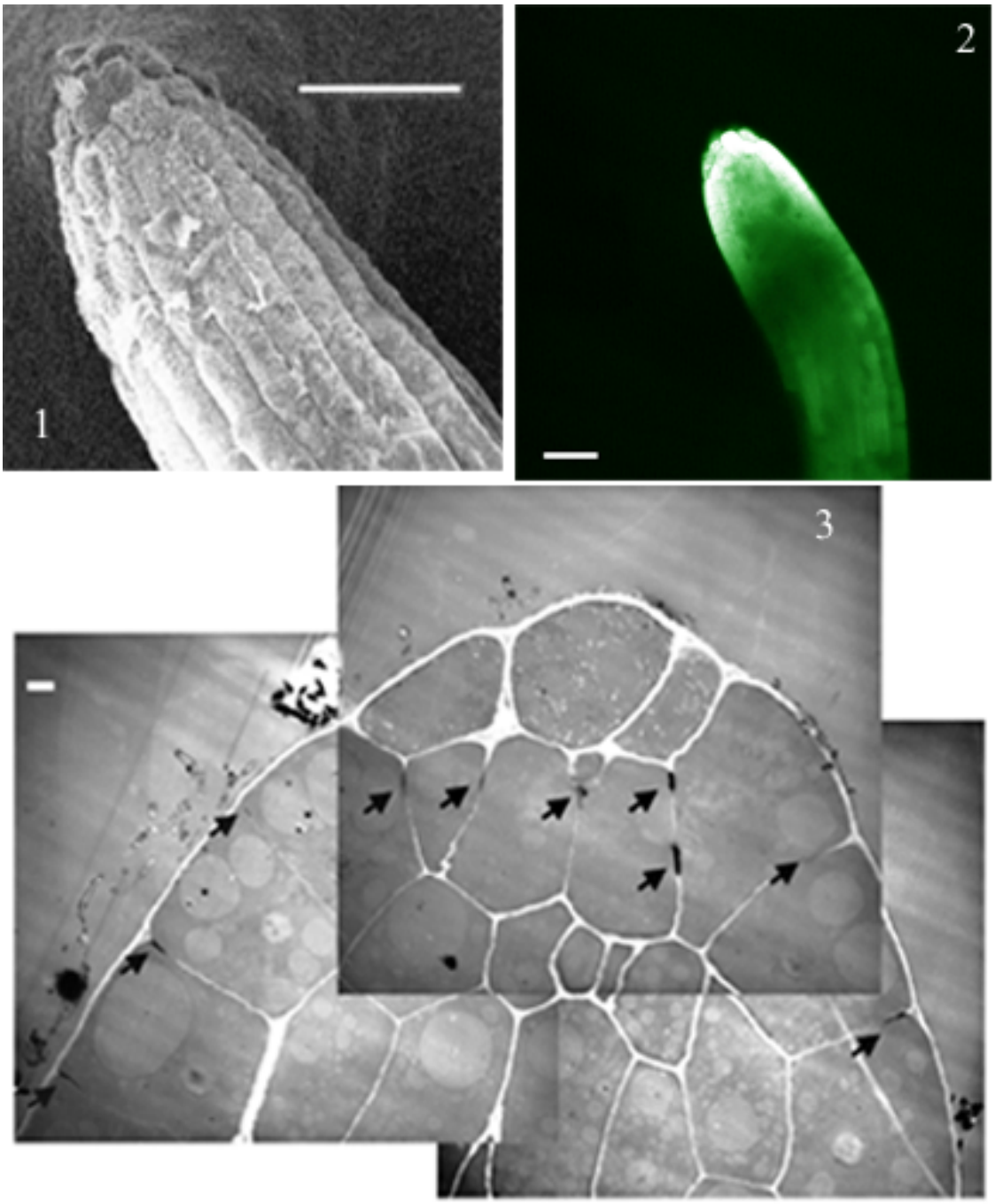

Figure 1. SEM of *S. asiatica* seedling root tip. Bar = 40 µm
Figure 2. In vivo staining of H_2O_2 by DCFH-DA, visualized via laser scanning confocal microscopy. Bar = 50 µm
Figure 3. TEM of seedling root tip section. Notice the $CeCl_3$ deposits accumulating on the spots of H_2O_2 secretion. Bar = 2 µm

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