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Research article

Biochemical and histochemical analyses revealing endophytic *Alcaligenes faecalis* mediated suppression of oxidative stress in *Abelmoschus esculentus* challenged with *Sclerotium rolfsii*

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ABSTRACT

Sclerotium rolfsii is a highly aggressive pathogen that causes huge economic losses, especially in temperate climates. *Alcaligenes faecalis*, particularly in endophytic form, has rarely been used to control this fungus. In this study, endophytic *Alcaligenes* sp. strain BHU 12, BHU 16 (isolated from *Abelmoschus esculentus* leaf) and BHU M7 (isolated from *Andrographis paniculata* leaf) were reported to trigger a wide range of host defenses in Okra plant against the collar-rot pathogen *S. rolfsii*. Endophytic colonization of the strains in ten days old plants was assessed through re-isolation of the rif-tagged strains on rifampicin augmented nutrient agar media. The ability of the endophytic strains to induce systemic defense responses in above-ground organs was assessed by collecting leaf tissues of the Okra plants grown under non-gnotobiotic conditions at different time intervals post seedling bacterization with the endophytic biocontrol agents. The pathogen challenged unprimed plants exhibited flaccidity of the stem and leaves at 48 h post infection (hpi) in contrast to the bioprimed and challenged plants. Biochemical and histochemical analyses explained the above phenomenon as activation of phyto-peroxidases leading to an increased metabolism of the reactive oxygen species (ROS), accompanied by activation of the phenylpropanoid network and a subsequent enhancement in plant phenolics. Interestingly, though the maximum increase in the defense pathways was observed in treatments with native endophytes of Okra plant, yet the enhancement in antioxidant pathway due to *A. paniculata* borne endophytes was also quite significant. Thus, this work clearly demonstrates how Okra plants respond to the “non-hostile” colonization of bacterial endophytes and how induced defense response can contribute to the biocontrol activity of the endophytic strains.

1. Introduction

Sclerotium rolfsii is a disastrous and polyphagous soil-borne pathogen infecting a wide array of hosts (Singh et al., 2013a). Post penetration into the host, the subcuticular hyphae proliferate and disintegrate the cell wall matrix by secretion of oxalic acid as well as several cell-wall degrading enzymes. Calcium released from the disintegrating cells reacts with oxalic acid forming stable calcium oxalate crystals in the necrotic tissue (Heller and Witt-Geiges, 2013; Uloth et al., 2015). Reports are available regarding wilting of the host due to vascular plugging by calcium oxalate crystals (Jain et al., 2015). However, apart from the aforementioned characteristics, the major blow to host plant immunity posed by oxalic acid is due to inhibition of phosphorylated protein kinases responsible for initiation of oxidative burst signaling program (Cessna et al., 2000). Besides, the pathogen also releases hydrogen peroxide (H_2O_2) during its growth and differentiation process resulting in a high concentration of H_2O_2 in its vicinity ultimately leading to apoptotic cell death of host tissues (Cheeseman, 2007; Papapostolou et al., 2014). Thus, in brief, while on one hand oxalic acid released from *S. rolfsii* curbs the normal oxidative burst pathway of infected hosts, the pathogen itself, on the other hand releases large amount of H_2O_2 in host tissues leading to host cell death.

Plants have an inherent counter mechanism of oxalate oxidase

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production which leads to quenching of oxalate crystals for resumption of the normal hypersensitive response pathway (Heller and Witt-Geiges, 2013). Besides, several self defense mechanisms including the production of various antioxidants (like ascorbate) and antioxidative enzymes, such as superoxide dismutase (SOD), peroxidase (PO), etc. also take place in an accelerated fashion throughout the host system upon counter with any form of stress (Mohammadi and Kazemi, 2002; Blokhina et al., 2003; Mittler et al., 2004; Türkan and Demiral, 2009; Loureiro et al., 2012). However, the innate defense systems of the host are readily superseded during pathogen challenge due to rapid and overproduction of ROS resulting in severe tissue damage. On the contrary, intensity of defense responses and stress tolerance is much higher when the plants are bioprimed with beneficial rhizospheric microbes, particularly plant-growth promoting fungi (PGPF) and plant-growth promoting rhizobacteria (PGPR) (van Loon et al., 2006; Shoresh et al., 2010; Jain et al., 2012; Ponce de León and Montesano, 2013; Singh et al., 2013; Bisen et al., 2015; Mishra et al., 2015; Sarma et al., 2015) (Fig. 1).

Irrespective of the various benefits conferred by epiphytes, the endophytes acquire a competitive edge, primarily because, i) the endemic bacteria remain in close contact with their host plants and thereby remain protected from the abiotic and biotic stress conditions in the soil, ii) endophytic bacteria do not affect the indigenous bacterial populations within host plants, unlike epiphytes in soil which cause an exemplar shift in the soil microbial community, and iii) endophytes ensure an augmented response to plant growth as compared to the bacteria restricted to rhizosphere and the root surface (Singh et al., 2013, 2013b; Ray et al., 2015).

Priming with endophytic microbes enhances the host tolerance to oxidative stress principally in two ways that occur concomitantly in accord (Tyburski et al., 2009). The endophytic microbes may gradually release small amounts of ROS into plant cells resulting in the acclimatization of the host cells to ROS through subsequent augmentation of related enzymes and antioxidant pathways (White and Torres, 2010). Since an elevated ROS level is directly related to augmented apoptosis (Overmyer et al., 2003), thus an alternative process is also incorporated which includes the secretion of auxin by endophytic bacteria that increases the production of ROS scavenging enzymes like, catalase, ascorbate peroxidase etc. leading to quenching of the ROS formed. Besides, endophytic microbes are also reported to produce phenols, mannitol, other sugar alcohols and carbohydrates that have been shown to be quenchers of ROS (http://www.sciencedirect.com/science/article/pii/S1754504811000687?np=y Jennings et al., 1998; http://www.sciencedirect.com/science/article/pii/S1754504811000687?np=y Malinowski and Belesky, 2000; http://www.sciencedirect.com/science/article/pii/S1754504811000687?np=y Schulz et al., 2002).

In our previous study, we have demonstrated *in-vivo* colonization potential and plant growth promoting activity of the three

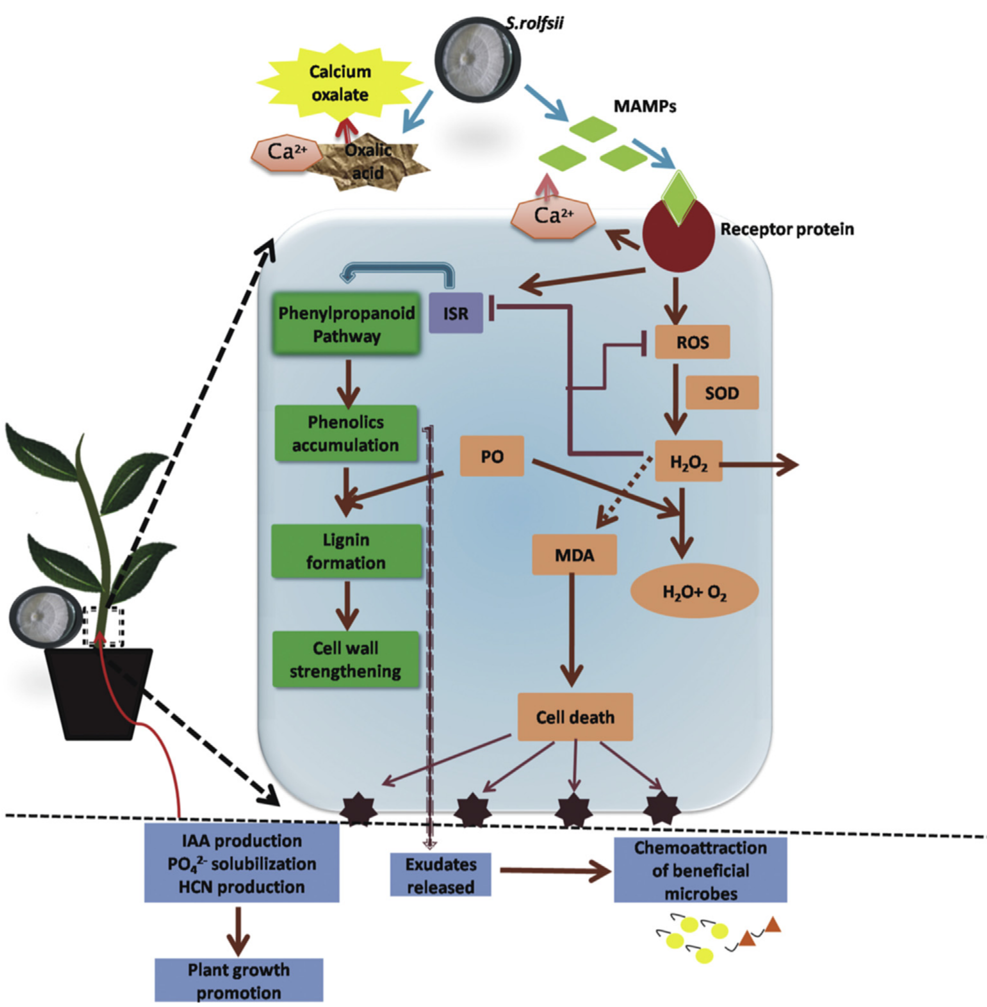

Fig. 1. Systemic representation of oxidative burst pathway initiated by the binding of microbe associated molecular patterns (MAMPs) to receptor proteins on the cell surface. Presence of oxalic acid in unprimed hosts elevates the apoplastic H_2O_2 level which further downregulates oxidative burst and ISR pathway in the cytosol and an elevation in the MDA content finally leading to cell death. Priming leads to an amelioration in the oxidative burst pathway and an enhancement in the ISR pathway.