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Reactions of *Lotus japonicus* ecotypes and mutants to root parasitic plants

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Summary

Witchweeds (*Striga* spp.) and broomrapes (*Orobanche* spp.) are obligate root parasitic plants on economically important field and horticultural crops. The parasites' seeds are induced to germinate by root-derived chemical signals. The radicular end is transformed into a haustorium which attaches, penetrates the host root and establishes connection with the vascular system of the host. Reactions of *Lotus japonicus*, a model legume for functional genomics, were studied for furthering the understanding of host–parasite interactions. *Lotus japonicus* was compatible with *Orobanche aegyptiaca*, but not with *Orobanche minor*, *Striga hermonthica* and *Striga gesnerioides*. *Orobanche minor* successfully penetrated *Lotus japonicus* roots, but failed to establish connections with the vascular system. Haustoria in *Striga hermonthica* attached to the roots, but penetration and subsequent growth of the endophyte in the cortex were restricted. *Striga gesnerioides* did not parasitize *Lotus japonicus*. Among seven mutants of *Lotus japonicus* (*castor-5*, *har1-5*, *alb1-1*, *ccamk-3*, *nup85-3*, *nfr1-3* and *nsp2-1*) with altered characteristics in relation to rhizobial nodulation and mycorrhizal colonization, *castor-5* and *har1-5* were parasitized by *Orobanche aegyptiaca* with higher frequency than the wild type. In contrast, *Orobanche aegyptiaca* tubercle development was delayed on the mutants *nup85-3*, *nfr1-3* and *nsp2-1*. These results suggest that nodulation, mycorrhizal colonization and infection by root parasitic plants in *Lotus japonicus* may be modulated by similar mechanisms and that *Lotus japonicus* is a potential model legume for studying plant–plant parasitism.

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Introduction

Witchweeds (*Striga* spp.) and broomrapes (*Orobanche* spp.) are obligate root parasitic plants. *Striga* spp., the most damaging parasitic flowering plants, are widespread in the semi-arid areas of Africa, but they also endanger regions of similar climate across the world (Ejeta, 2007). The most economically important *Striga* spp. are *Striga asiatica*, *Striga hermonthica* on cereals including sorghum, millet, upland rice and maize, and *Striga gesnerioides* on cowpea. *Orobanche* spp. predominate in the Mediterranean region and parasitize economically important crops such as tomato, faba bean and sunflower. Both *Striga* and *Orobanche* produce numerous tiny dust-like seeds with limited food reserves. The limited food reserves necessitate the occurrence of germination in close proximity of the host root and that of attachment and penetration within a few days after germination. To curtail germination away from a host root, suicidal germination, *Striga* and *Orobanche* spp. have evolved a requirement for labile germination stimulants with limited persistence and mobility in soils. The first stimulant, strigol, was isolated from cotton (*Gossypium hirsutum*), a non-host (Cook et al., 1966). Since the isolation and characterization of strigol, a series of analogous stimulants, collectively referred to as strigolactones, was isolated from host and non-host plant species (Butler, 1995). Following germination, a specialized organ, the haustorium, is developed for attachment and penetration into the host root. Haustorium initiation, which represents the switch from the vegetative to the parasitic phase, is triggered by a chemical signal that is distinct from the germination stimulants (Ejeta, 2007). The complexity of the parasitization process extends beyond chemical signaling to include the establishment of physical and physiological associations between the host and the parasite. For successful parasitism, which entails withdrawal of resources from the host, a continuum between the host and the parasite must be established. However, the dynamics of the interactions permitting or precluding establishment of the host–parasite continuum are not clearly understood.

Recent investigations employing model plants to study both symbiotic and parasitic associations provided routes to dissection of the complex interactions between plants. Studies on host–parasite interactions using *Arabidopsis thaliana* revealed the importance of up-regulation of defense genes in response to *Orobanche ramosa* attack (Santos et al., 2003) and indicated no significant role for the host flavonoids (Westwood, 2000). Studies employing the legumes *Lotus japonicus* and *Medicago truncatula*, as model plants, provided advanced knowledge on the complex biological interactions between nitrogen-fixing rhizobial bacteria, phosphorus-acquiring arbuscular mycorrhizal fungi (Stougaard, 2001; Oldroyd and Downie, 2004; Kistner et al., 2005), parasitic nematodes (Lohar and Bird, 2003; Weerasinghe et al., 2005; Poch et al., 2007), parasitic plants (Lozano-Baena et al., 2007) and their respective hosts.

The present investigation was undertaken to study reactions of *Lotus japonicus* ecotypes and mutants to the root parasitic plants *Orobanche aegyptiaca*, *Orobanche minor*, *Striga hermonthica* and *Striga gesnerioides*, with the objective of revealing the suitability of *Lotus japonicus* as a model for furthering understanding of parasitism in plants.

Materials and methods

Plant materials and growth conditions

Seeds of Japanese trefoil (*L. japonicus* (Regel) Larsen ecotypes Miyakojima MG-20 and Gifu B-129) were obtained from the National BioResource Project through the Frontier Science Center of Miyazaki University, Japan. Phenotypic characterizations of Nod$^-$ mutants (*nfr1-3*, *castor-5*, *ccamk-3*, *nsp2-1*, *nup85-3*), Hist$^-$ mutant (*alb1-1*), and a hypernodulating mutant (*har1-5*) with Gifu B-129 background were as described by Imaizumi-Anraku et al. (1997), Kawaguchi et al. (2002), Sandal et al. (2006) and Murakami et al. (2006). Seeds of *O. aegyptiaca* Pers., collected from mature plants parasitizing vetch (*Vicia sativa* L.), were supplied by Prof. Julie Scholes, The University of Sheffield, UK. Seeds of *O. minor* Sm. were collected from mature plants parasitizing red clover (*Trifolium pretense* L.) in Japan. Seeds of *S. hermonthica* (Del.) Benth. were supplied by Prof. Abdel Gabar Babiker, Sudan University of Science and Technology. Seeds of *S. gesnerioides* (Willd.) Vatke and cowpea (*Vigna unguiculata* (L.) Walp.) were obtained from Dr. Satoru Muranaka, International Institute of Tropical Agriculture, Nigeria.

Seeds of *L. japonicus*, sown on moist glass fiber paper in Petri dishes, were covered with aluminum foil and incubated in the dark. Seeds of ecotype Miyakojima MG-20 were kept in the dark at 28 °C for 2–3 d, while those of Gifu B-129 and its mutants were kept in the dark at 23 °C for 3–5 d. Seedlings were transferred to test tubes and allowed to grow hydroponically in 40% Long–Ashton nutrient solution (Hewitt, 1966) for 2 weeks. The plants were maintained in growth chambers set at 28 °C and a 16 h photoperiod for Miyakojima MG-20 and at 23 °C and a 16 h photoperiod for Gifu B-129 and its mutants. After 2 weeks, the plants were transferred to Petri dishes filled with rockwool (rhizotrons) and watered with Long–Ashton nutrient solution.