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Research article

Nitrogen and carbon relationships between the parasitic weed *Orobanche foetida* and susceptible and tolerant faba bean lines

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ABSTRACT

The parasitic weed *Orobanche foetida* (Poiret) is an emergent agronomical problem on faba bean in Tunisia. The Tunisian breeding programs for faba bean resistance to *O. foetida* have produced several tolerant lines including the line XBJ90.03-16-1-1-1, which limits both parasite attachments to the host roots and growth of the attached parasites. The present study aims to provide a better understanding of the nutritional relationships between the parasite and this tolerant line in comparison with the susceptible Bachaar genotype. Phloem saps of faba bean were harvested using phloem exudation experiments. The major organic compounds potentially transferred from both faba bean genotypes to the parasite were identified as sucrose, raffinose, stachyose, citrate, malate, asparagine (ASN), aspartate (ASP), glutamine, glutamate, serine, alanine and GABA. However, the phloem exudates of the tolerant line were highly deficient in nitrogen when compared to that of the susceptible line. When attached to roots of the tolerant line, the parasite displayed limited activities of soluble invertases in tubercles, and especially in shoots, suggesting that the low performance of the broomrapes attached to the tolerant line resulted from a reduced capacity to utilize the host-derived carbohydrates. On the other hand, the mechanisms involved in the osmotic adjustment and primary metabolism of the parasite did not differ significantly according to the host genotype: mineral cations, especially potassium and calcium, predominated as the major osmotically-active compounds in both tubercles and shoots; shoots accumulated preferentially hexoses as organic solutes although tubercles accumulated preferentially starch and soluble amino acids, especially ASP and ASN. This suggests an important role for a glutamine-dependent asparagine synthetase (EC 6.3.5.4) in the N metabolism of the parasite.

1. Introduction

Some species of the holoparasitic genus *Orobanche spp.* (broomrape, Orobanchaceae) are serious parasitic weeds causing great losses in many major crops including faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), winter oilseed rape (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.). Infestations are widespread in the Mediterranean basin and Asia Minor where all the harmful broomrapes (*robanche crenata* Forsk, *robanche ramosa* L., *robanche aegyptiaca* Pers. and *robanche cumana* Wallr.) benefit from favorable developmental conditions. *Orobanche foetida* is widely distributed in natural habitats in the Western Mediterranean area (Portugal, Spain, Morocco, Algeria, Tunisia) parasitizing wild herbaceous leguminous plants [36]. Nevertheless, *O. foetida* should be considered as an emergent important agricultural parasite in faba bean in Tunisia [20,21] and on common vetch in Morocco [37].

Some particular traits of broomrapes can be highlighted. Once germinated in response to stimulants secreted by the roots of the neighboring host plants, the parasite attaches to the host vascular system (stage 1) through a haustorium, which serves as both an attaching organ and a bridge for water and nutrient transfer, essentially from the host phloem [15]. Indeed, the host phloem supplies the majority of most nutrients, even minerals such as nitrogen, magnesium and potassium whose fluxes are larger in the host xylem. The extent to which the phloem tissues of host and parasite communicate differs according to the host and broomrape species. Symplasmic connections through interspecific plasmodesmata have been observed between host and parasite phloem in the pathosystem *Vicia narbonensis*–*O. crenata* [10]. However, no

Abbreviations: ASN, asparagine; ASP, aspartate; CWI, cell wall invertase; GABA, γ amino-butyric acid; SAI, soluble acid invertase; SNAI, soluble neutral-alkaline invertase.

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information is available on this phenomenon between *O. foetida* and faba bean. Once connected to the host phloem, the parasite develops primarily a tubercle outside the infected root (stages 2 and 3) which gives rise to a subterranean shoot (stage 4) then to an achlorophyllous and flowering spike after emergence from the soil (stage 5). The parasite competes successfully with the host sink organs for water and nutrients due to a mechanism assuring a higher osmotic pressure compared with the host plant [31,46]. The sink strength of the parasite is based mainly on the immediate cleavage of the host-derived sucrose into glucose and fructose mediated by a putative invertase, thus doubling the osmotic value of the sugar component. Consequently, several studies have emphasized the primary role of hexoses and mannitol in the osmotic adjustment of *Orobanche* [7,31,45]. In addition, excess carbon is massively incorporated into starch [41]. Nevertheless, the mechanisms involved in the osmotic adjustment of broomrapes needs clarification in comparison with chlorophyllous parasitic plants [32,40,42].

Various cultural and chemical strategies have been assayed to control *O. foetida* but without sufficient success [19,21]. Recently, it was shown that the faba bean genotypes carrying *O. crenata* resistance were of major interest in breeding for resistance to *O. foetida* [2]. Thus, the Tunisian breeding line XBJ90.03-16-1-1-1 is partially resistant and highly tolerant to both *O. foetida* and *O. crenata* [1–3]. This breeding line differs from the susceptible cultivar Bachaar by a low stimulatory activity of root exudates for eliciting *Orobanche* seed germination and a delay in parasite attachment, both of which are known to be essential components of faba bean resistance to broomrapes [29,46]. In addition, the performance of the XBJ90.03-16-1-1-1-parasitizing parasite was low once connected to the host vascular tissues, as demonstrated by a significantly slower subterranean growth. A low performance was also observed for *O. ramosa* when attached to winter oilseed rape cultivars showing a high degree of tolerance (Simier, personal communication).

No physical barrier limiting water and nutrient transfer was detected at the interface between *O. foetida* and the tolerant XBJ90.03-16-1-1-1 line (personal observations). This suggests that nutritional factors, such as host phloem composition, could negatively influence the osmotic adjustment and the resulting sink strength of the parasite towards the host-derived resources. The present study deals with the comparison of the phloem composition of the tolerant XBJ90.03-16-1-1-1 and the susceptible Bachaar lines and the impact of the faba bean line on the levels of the major solutes and invertase activities in the parasite. Other aspects related to C metabolism, including starch levels, have also been investigated.

2. Results

2.1. Impact of parasitism on phloem composition in faba bean

When faba bean plants were free of *Orobanche*, the leaf petioles exuded mainly carbohydrates, especially stachyose and raffinose corresponding to Raffinose Family Oligosaccharide (RFO) compounds, regardless of the genotype (Table 1). Large amounts of hexoses were also detected massively in the phloem exudates, attesting that a high proportion of sucrose was cleaved in the exudates by some contaminant extracellular invertases. Thus, sucrose contents were greatly underestimated in our experiments. The detected hexoses should be interpreted as sucrose. Among the organic acids, malate and essentially citrate were strongly accumulated in the phloem exudates. Finally, the amino acid profiles of the phloem exudates were similar in the two faba bean genotypes, showing seven major amino acids corresponding to the pairs asparagine (ASN)/aspartate (ASP) and glutamine/glutamate, serine, alanine and γ *amino-butyric acid* (GABA). Nevertheless, ASP and ASN were by far the predominant amino acids. It can be noted that the tolerant line displayed higher contents of organic compounds in the phloem exudates than the susceptible line did, suggesting that organic acids and amino acids are loaded more intensively in leaf phloem in the tolerant line.

The phloem composition did not change significantly in response to broomrape attack in the susceptible line. In contrast, parasitism induced a marked decrease in the levels of all the amino acids in the phloem exudates of the tolerant line, which thus contributed to only 6% of the total level of the organic compounds. On the other hand, the carbohydrate and organic acid levels were not affected significantly in phloem exudates of the tolerant line challenged with the parasite.

2.2. Impact of the host genotype on the contents of the main osmotically-active compounds in tubercles and subterranean shoots of broomrape

Regardless of the faba bean genotype, the cell saps from broomrape tubercles and shoots (stage 4) showed equivalent osmolarities (Table 2), to which the organic solutes contributed about 20% (Table 2). Organic acids, mainly malic acid, were scarcely accumulated in both organs in comparison with carbohydrates and amino acids (Table 2). Tubercles accumulated preferentially amino acids, notably the ASP/ASN pair, and this accumulation was more evident in the parasite attached to the tolerant line. In comparison with host phloem exudates (Table 1), glutamine and glutamate were scarcely accumulated in the parasite. Moreover, the subterranean shoots accumulated mainly hexoses, especially in the parasite attached to the susceptible line, which displayed the highest levels of carbohydrates in shoots. Similarly, mannitol was preferentially accumulated in shoots but to a lesser extent than hexoses. In addition, RFO compounds and sucrose were poorly represented in the parasite in comparison with high levels in host phloem exudates (Table 1).

Given that calcium is located in the cell wall of plants, it is difficult to extract from cell liquids. Thus, in the present study, mineral cations were extracted from broomrape powders. Regardless of the host line, mineral cation levels were similar in the tubercles and subterranean shoots of the parasite (Table 3), with a large dominance of potassium, followed by calcium to a lesser extent. Consequently, the parasite displayed high K/Ca ratios, notably in shoots due to an enhanced accumulation of potassium. Theoretically, by taking into account the water status of the FW tissues, contents expressed as $mg\,g^{-1}$ DW could be transformed into intracellular concentrations expressed as mmoles per liter. For example, given that water represented 85% FW in shoots of the parasite attached to the susceptible line, the deduced potassium concentration was estimated at about 290 mM, corresponding to 75% of the total osmolarity (Table 2). Thus, potassium played a major role in the osmotic adjustment of the parasite in both shoots and tubercles, independently of the faba bean line.

2.3. Impact of the host genotype on invertase activity and protein levels in tubercles and subterranean shoots of broomrape

Regardless of the host faba bean line, tubercles and subterranean shoots of the parasite (stage 4) displayed high soluble invertase activities in comparison with insoluble invertase activity (Table 4). The soluble acid invertase (SAI) was almost three-fold more active in shoots than in tubercles while the neutral-alkaline invertase (SNAI) was equally active in both organs. Tubercles and shoots accumulated similar contents of soluble and insoluble proteins, independently of the host genotype.